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О РЕЗУЛЬТАТАХ ПОВТОРНОГО ИЗУЧЕНИЯ ПЛАЗМОГЕНЕЗА ВОКРУГ ЯДЕР - СПРЕМИЕВ У *PICEA ABIES* (L.) KARST.

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Введение

Вопрос о цитоплазме мужских гамет у покрытосеменных и голосеменных в литературе поднимался неоднократно. Он и сегодня волнует эмбриологов, цитологов и генетиков. Главные споры идут вокруг покрытосеменных (Гинъяр, 1891; Страсбургер, 1892; Навашин, 1911; Поддубная-Арнольди, 1964; Мюнтцинг, 1967; Чеботарь, 1972, 1985; Батыгина, 1974; Банникова, 1975). Среди эмбриологов нет единого мнения: участвует ли цитоплазма в оплодотворении, точнее в образовании неоплазмы зиготы; имеется ли принципиальное отличие между спермиями одной пары; какова судьба клеточных органелл мужских и женских гамет в и сингамнах постсингамных процессах.

До конца не решен вопрос, обладают ли ядра - спермии хвойных своей цитоплазмой. Нет единого мнения и в вопросе: какие органеллы цитоплазмы спермия, будучи внесенными в яйцеклетку, продолжают свой биогенез. Рассматривая гаметогенез хвойных, как важное звено репродуктивной биологии высших растений, следует ожидать, что дальнейшее углубленное изучение гапло- диплофаз раскроет нам новые факты становления и тенденции эволюции полового размножения растений.

Вместе с тем, как показал анализ литературы (Мошкович, Чеботарь, 1986), спермиогенез, как впрочем и оплодотворение, у большинства голосеменных изучен весьма недостаточно. Приходится констатировать, что главными исследованиями остаются работы, проводимые в конце прошлого и начале нашего века (Hofmiester, 1858; Буйцицкий, 1899; Ferguson, 1901; Miyake, 1903; Козубов, 1982; Мошкович, 1992 и др.). Этим, вероятно, объясняется то, что как в ранних так и в последующих публикациях встречаются диаметрально противоположные мнения. И хотя те и другие исследователи затрагивают схожие вопросы филогении, эволюции и наследования родительских признаков (Hofmeister, 1858; Strasburger, 1892; Chamberlain, 1957; Цингер, Размологов, 1972; Козубов, 1974, 1982; Singh, 1978; Мошкович, 1992), в целом четкое понимание в этих вопросах отсутствует.

Данное сообщение, вероятно, заинтересует изучающих мужской гаметогенез хвойных, в частности у *Picea abies* (L.) Karst., хотя потому, что одному из авторов недавно опубликованной работы (Мошкович, Чеботарь, 1986) пришлось повторно возвращаться к напечатанному и рассказать о весьма существенных дополнительных подробностях в прохождении спермиогенеза. Главное свое внимание сосредоточили на дополнительных подробностях, протекающих в ходе и сразу после деления ядра базальной (сперматогенной) клетки. Как нам кажется, представленный экспериментальный материал по новому раскрывает многие стороны спермиогенеза и оплодотворения в целом.

В подтверждение повторного изучения спермиогенеза у ели обыкновенной и приводим более детальное описание процесса обособления цитоплазмы вокруг сестринских ядер - спермиев. Речь идет о цитоплазмогенезе в ходе деления базальной (генеративной, сперматогенной) клетки. Наши наблюдения старались отразить на тщательно выполненных рисунках, воссоздавая процесс обособления цитоплазмы и поведения сестринских ядер спермиоклеток в прогамной фазы оплодотворения.

Описывая характер и этапы индивидуализации цитоплазмы мужских гамет

у *Picea abies* (L.) Karst., нам хотелось не только привлечь внимание к более тщательному изучению гаметогенеза хвойных вообще, но и найти более полное объяснение тому, какая часть мужской цитоплазмы попадает в яйцеклетку, обратить внимание на всеобщность выдвинутых нами принципов гаметогенеза (Чеботарь, 1972, 1986) с неумолимым постмейотическим отсевом, отбрасыванием, аббревиацией генетически неполноценных (гаплоидных.) структур.

Материалы и методы

Объектами исследования послужили разновозрастные семяпочки *Picea abies* (L.) Karst., зафиксированные через определенное время после свободного опыления. Дополнительный экспериментальный материал был выбран в городском дендропарке и Ботаническом саду г, Кишинева (1984- 1988), возраст деревьев достиг более 15 лет. Фиксирующей жидкостью послужила смесь С. Г. Навашина. Постоянные препараты окрашивались железным гематоксилином и изучались нами в световых микроскопах “Nf, Ng” и “Jenaval” (Karl Zeiss). Повторное изучение проводили с помощью нового более совершенного немецкого микроскопа с использованием объективов 40х и 6Ох иммерсионных - 90х и 100х. Большие увеличения породили трудности другого порядка. Дело в том, что, в отличии от большинства покрытосеменных, спермии хвойных настолько велики, что ядра выходят далеко за поле зрения даже объектива 6Ох. При изучении процесса закладки фрагмопласта - оболочки спермиев - мы руководствовались тем, что плазмалемма представляет собой мелкогранулированную мембрану, мало отличимую от оболочки ядра спермиев. Такие структуры в световых микроскопах невозможно изучить объективами сухой иммерсии. Рисунки выполнены с помощью рисовального аппарата РА- 4.

Результаты исследования

Описание спермиогенеза у ели обыкновенной (*Picea abies* (L.) Karst.) мы начинаем с краткой характеристики попавшего на нуцеллус пыльцевого зерна (ПЗ). Зрелый мужской гаметофит ели обыкновенной, как показали А. М. Мошкович (1986), А. М. Мошкович, А. А. Чеботарь (1986), характеризуется весьма четкой организацией, морфологией и размерами. Нетрудно убедиться, что по форме пыльца округлая, слегка уплощенная, с двумя воздушными мешками. Последние довольно значительны и на их долю приходится ⅔ общего диаметра ПЗ, который составляет 48.3 нм. Базальная клетка (БК), вторая по величине структура ПЗ, в отличие от других клеточных образований, имеет более плотную цитоплазму, практически лишенную вакуолей. При фиксации жидкостью С. Г. Навашина и окрашивании железным гематоксилином состояние плазматических и протоплазматических структур весьма четко различимы. БК отделяется четкой мембраной от клетки трубки (вегетативной), цитоплазма которой менее плотная, содержит крахмальные зерна и многочисленные мелкие вакуоли. Ядро БК довольно крупное (3.1 х 2.8 нм), слегка вытянутой формы с двумя (иногда тремя) ядрышками, со слабыми хроматиновыми глыбками по периферии. Ядро клетки трубки меньше ядра БК, ядрышко одно, крупное, с рыхлой структурой, кариолимфа как бы лишена хроматиновых нитей, равномерно рассеяна мелкими хроматиновыми зернами. Клетка ножка (КН) имеет линзовидную форму, плотно прижата к БК, ее цитоплазма менее плотная, чем у последней, хотя также равномерно зерниста и лишена вакуолей. Ядро овально удлиненной формы с четкой хроматиновой структурой. Отметим также (рис. 1), что от проталиальных клеток (ПК1 и ПК2) виден интенсивно окрашенный остаток плазматического сгустка.

Экзина ПЗ прослеживается даже в световом микроскопе, причем в зоне воздушных мешков она принимает своеобразную (рыхлую) структуру, впрочем, весьма характерную для сосновых. Интина, как видно из представленного рис. 1,

остается в контакте с цитоплазмой клетки трубки, обеспечивая прорастание ПЗ и рост пыльцевой трубки.

Отметим еще тот факт, что до выхода спермиев (Сп) в пыльцевую трубку (ПТ) базальная клетка и клетка ножка образуют как бы общий комплекс (рис. 2), который сохраняется до конца прогамной фазы. По состоянию цитоплазмы, митотической активности ядер, отсутствию оболочки (их разделяют лишь собственные плазмалеммы), БК и КН ведут себя чрезвычайно активно, чем и объясняется активный лизис клеток нуцеллуса на пути пыльцевой трубки.

Прорастание пыльцевого зерна происходит, когда проростковая пора оказывается в непосредственном контакте с нуцеллусом. Другие ПЗ (рис. 3) не образуют функциональные ПТ и вскоре дегенерируют.

В отличии от цветковых, ПТ ели обыкновенной по ширине почти равна диаметру ПЗ и легко "прокладывает" себе путь между нуцеллярными клетками. Ко времени выхода БК-КН из ПЗ длина ПТ равна примерно трем диаметрам ПЗ. К этому времени цитоплазма клетки трубки уж повсеместно заполнена пластидным крахмалом. В целом содержимое растущей ПТ в световом микроскопе создает впечатление пенистообразной массы; она (ПТ) заполнена крахмальными зернами (амилопластами), митохондриями и др. структурами цитоплазмы. Ядро КТ становится двуядрышковым, лопастной формы, с мелкими многочисленными хроматированными глыбками или хлопьевидными образованиями. Лишь небольшая, оптически более плотная, зернистая масса окружает ядро КТ.

Полный выход базальной клетки из пыльцевого зерна виден на рис. 3. Рядом находится клетка ножка. Цитоплазма и той и другой, как и ядра, четко различимы, хотя в целом они как бы повторяют то же ядерно-плазменное соотношение, форму и место расположения ядер в клетке. Однако, такое состояние, как увидим дальше, вскоре меняется. Ядро БК быстро увеличивает свои размеры, сохраняя округлую форму. Оно содержит четыре и более крупных ядрышка. Кариоплазма равномерно заполнена интенсивно окрашенными хроматиновыми нитями (предпрофазное состояние хромосом). Передняя часть клетки вытянута, задняя тупая - в целом БК приобретает яйцеобразную конфигурацию. Подобную форму имеет и клетка ножка, которая возникает, вероятно, также под действием восходящего и нисходящего токов цитоплазмы. Из представленного рисунка 3 можно также заключить, что рядом расположенные клетки нуцеллуса подвергаются интенсивному лизису (разрушению).

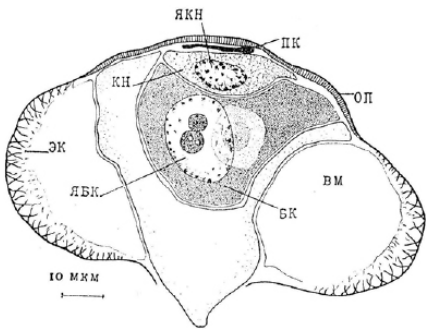

Рис. 1. Пыльцевое зерно (ПЗ) *Picea abies* (L.) Karst. на фазе начала прорастания в микропилярной части семяпочки (5-6 день после попадания ПЗ на нуцеллус семяпочки). Базальная клетка (БК) с плотной зернистой цитоплазмой, крупным ядром (ЯБК) и с двумя ядрышками.

Линзообразная клетка ножка (КН) с удлиненным ядром (ЯКН) заполненным хроматиновым и узелками, образующими сетчато-гранулярную структуру. Вторая проталиальная клетка (ПК2) в виде темного сгустка прижата к оболочке пыльцы (ОП). ВМ - воздушный мешок; Эк - экзина. Ув. 60х, 15х (по Чуботару, 1992).

Мы отметили еще в предыдущей работе, что ядро - клетки трубки (ЯКТ) первым

оставляет ПЗ и всегда находится близко к кончику растущей ПТ. Вслед за ЯКТ идет БК, за ней клетка ножка, которая позже перемешается в переднюю часть БК. Основная цитоплазма КТ с крупными крахмальными зернами расположена в растущей части, включая зону расположения базальной клетки и клетки ножки. Следует отметить, что, войдя в ПТ, элементы базальной клетки заметно растут, причем плотность цитоплазмы и ядра не снижается, в то же время «освободившееся» место в ПЗ занимает одна крупная и много более мелких вакуолей (рис. 4).

Далее проследим за состоянием ядра и цитоплазмы базальной клетки в ходе вступления ее в деление. На рис. 5 можно видеть, как цитоплазма базальной клетки перемещается в переднюю часть (к кончику ПТ), приобретает лопастную или воронкообразную форму, тем самым как бы «проглатывает» клетку ножку. Нельзя забывать и тот факт, что базальная клетка перемещается в сложной системе противоположно двигающихся токов цитоплазмы растущей ПТ, в которой она (БК) стремится к яйцеклетке. Нелишне отметить, и что на протяжении всей прогамной фазы БК и СП сохраняют определенную дистанцию от растущего кончика ПТ. Трудно сказать, какова роль КП в самодвижении БК, т. к. она занимает центр направляющей части БК, на данной фазе (времени) роста ПТ различия между БК и КН еще более чувствительны. И хотя в КН процессы деструкции пока не наступили, ядерно- плазменные соотношения значительно изменились; снизило свою плотность содержимое цитоплазмы и ядра.

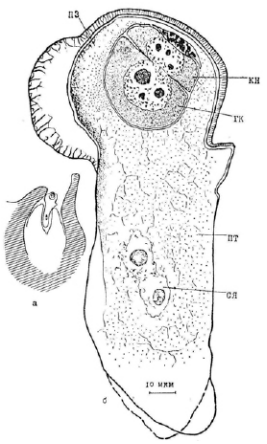

Рис. 2. Рост пыльцевой трубки (ПТ) в нуцеллусе семяпочки на 4-5 день после начала прорастания пыльцевого зерна (ПЗ) *Picea abies* (L.) Karst.

ПТ прямая, довольно широкая, заполнена пластидным крахмалом. Вакуолярный аппарат слабо развит. Сифоногенное ядро (СЯ) переместилось к растущему кончику ПТ. Генеративная клетка (ГК) и клетка ножка (КН) продолжают оставаться на месте; их цитоплазма равномерно зерниста. а - общий вид семяпочки с тем же ПЗ и ПТ. Ув. 5х, 3,5х; б - 90х, 7х (по Чуботару, 1992).

По своей субмикроскопической организации базальная клетка *Picea abies* (L.) Karst. во многом напоминает генеративную клетку цветковых (Чеботарь, 1972). Мы уже указали (Чеботарь, Мошкович, 1986), что изучая ультраструктуру Picea asperata, французский эмбриолог H.Camefort (1978) показал, что цитоплазма БК содержит митохондрии, пропластиды, диктиосомы и редуцированный вакуолярный аппарат. При этом эндоплазматический ретикулюм, как правило, гранулярный, позже агранулярный. Тот же автор, говоря о структуре БК, указывает, что вскоре после деления БК в ее организации наступает определенный спад, упрощение ультраструктурной организации: эндоплазматический ретикулюм редуцируется, исчезают структуры Гольджи, появляются лизосомы, вызывающие лизис клеток микропиле, яйцеклетки, куда устремлена пыльцевая трубка. Автор отмечает так же, что к моменту оплодотворения в цитоплазме спермиевой клетки остаются лишь информационные органеллы: пластиды (пропластиды), митохондрии,

полирибосомы и структуры типа лизосом.

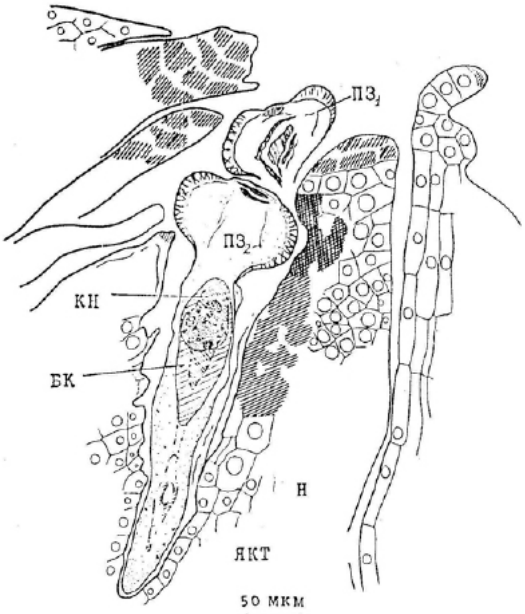

Рис. 3. Два пыльцевых зерна (ПЗ). в микропилярной части семяпочки *Picea abies* (L.) Katst.

Общий вид микропилярной части нуцеллуса семяпочки с растущей пыльцевой трубкой. Базальная клетка (БК) и клетка ножка (КН) спроецированы друг на друга. Вегетативная клетка (клетка трубки) богата пластидным крахмалом, расположенным вокруг БК и КН. Ув. 3,5х, 7х (по Чуботару, 1992).

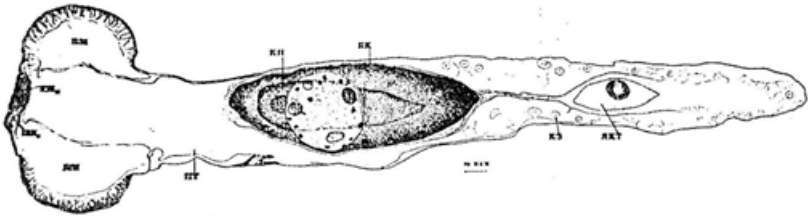

Рис. 4. Пыльцевая трубка (ПТ) той же семяпочки *Picea abies* (L.) Karst., что и на рис. 3.

Сперматогенная базальная клетка (БК) и клетка ножка (КН) имеют одинаковую форму, но разные размеры. Впереди близко к растущему кончику ПТ ядро клетки трубки (КТ) так же окружено небольшим участком цитоплазмы богатым пластидным крахмалом. Ув. 20х, 7х (по Чуботару, 1992).

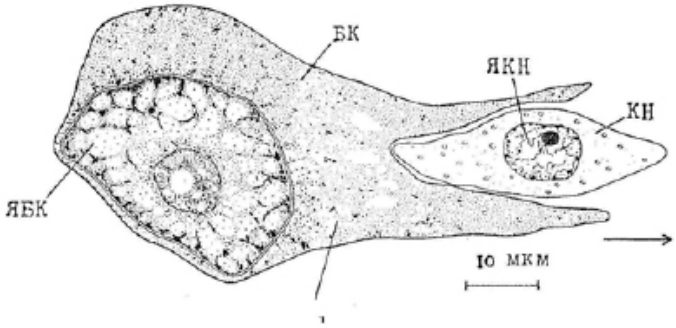

Рис. 5. Базальная клетка (сперматогенная) клетка (БК) и клетка ножка (КН) *Picea abies* (L.) Karst., сблокированные в общем комплексе.

Ядро (Я) и цитоплазма Сп1 по сравнению с Сп2 более значительны ЯБК в ранней профазе деления, цитоплазма амебоидной формы, равномерно зерниста. Клетка ножка оптически менее плотна, с мелкими вакуолями. ЯКН по сравнению с предыдущей фазой уменьшилось и содержит тонкую хроматиновую сетку. Ув. 90х, 7х (по Чуботару, 1992).

Деление БК у *Picea abies* (L.) Karst. начинается за 9-10 дней до «вхождения» спермия в яйцеклетку. Делению БК предшествует перемещение ядра в центр клетки. Ось анафатической фигуры митоза по отношению к длинной оси пыльцевой трубки (см. фото 8а. в работе Мошкович, Чеботарь, 1986) смещена на 130-140°, что вероятно предопределяет разное количество, да и качество, цитоплазмы спермиоклеток. Вместе с тем на завершающем этапе спермиогенеза имеются и другие отличительные моменты. Если кариокинез приводит к образованию двух довольно крупных и мало отличающихся по размеру ядер, переходящих в интеркинез, то цитокинез, как справедливо отмечают и другие авторы (Camefort, 1978), еще на начальных этапах формирования фрагмопласта приостанавливается. Далее небольшие участки фрагмопласта распадаются, а протопласт спермиев до конца остается разделенным шероховатой плазмалеммой. Сказанное можно проследить на примере двух последующих рисунков (рис. 6 и 7), выполненных после тщательного изучения многочисленных срезов и препаратов, отличающихся отличной дифференцировкой окрашенных структур.

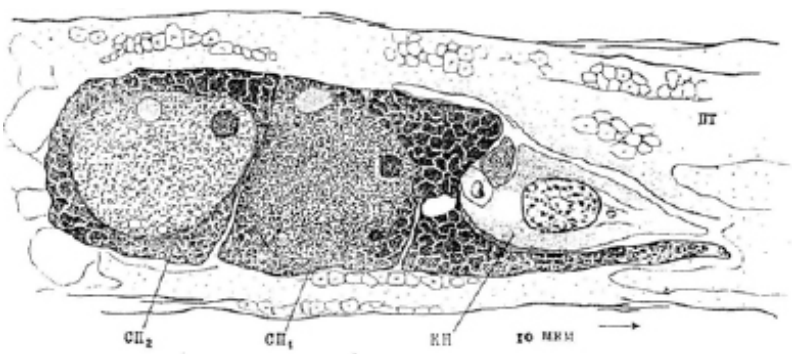

Рис. 6. Участок пыльцевой трубки (ПТ) *Picea abies* (L.) Karst. на фазе завершения деления базальной клетки.

Обособление цитоплазмы вокруг ядер - спермиев завершено. Плазмалемма окружающая протопласт спермиоклеток (СП1 и СП2) хорошо прослеживается. Клетка ножка (КН) вплотную прижата к Сп1, цитоплазма которого полностью окружает ее. Стрелка показывает направление роста ПТ. Ув. 90х, 7х (по Чуботару, 1992).

На рис. 6 видны два ядра – спермев и начало закладки фрагмопласта между ними. По своей форме сестринские ядра полностью отражают состояние цитоплазмы материнской (базальной) клетки. Форма общей цитоплазмы рядом расположенных спермиоклеток соответствует тем процессам движения токов цитоплазмы, которые имеют место в ходе роста пыльцевой трубки (Камия, 1962; Чеботарь, 1964, 1965). Клетка ножка (рис. 5, 6) «пристроена» в передней части спермиоклетки 1 и к этому времени обнаруживает признаки депрессии, ядрышки в ее ядре исчезают, а в кариолимфе проявляются интенсивно окрашенные гетерохроматиновые структуры. Следует указать и на то, что вскоре, по завершению деления БК, в структуре и функции спермиоклеток наступают четкие морфолого-функциональные изменения ядра (см. рис. 6): фрагмопласт как таковой полностью исчезает, его роль выполняет плазмалемма. Спермия отличаются между собой по форме и плотности интенсивности окрашиваемости), структуре и характеру распределения хроматина в кариолимфе, количеству и плотности ядрышек, состоянию и характеру положения плазменной и ядерной мембран (рис. 7). Движущиеся спермиоядра у ели, как впрочем и у цветковых, сохраняют первоначальное положение в растущей пыльцевой трубке. В целом, этот период отличается интенсивным ростом ядер и плазматической массы, сохранением тесного контакта между СП1, СП2 и КН.

Более продвинутое состояние спермиогенеза мы представили на рис. 7а и 7б, где

от фрагмопласта остались лишь следы. Речь идет о возникновении (образовании) плазмалеммы (Пл), которая просматривается на хорошо фиксированных объектах и умеренно плотно окрашена. В данном случае они (Пл) четко прослеживаются как разделяющие общую цитоплазму материнской (базальной) клетки между двумя сестринскими ядрами. С этого момента правильнее говорить о спермиоклетках (СП), как о голых клетках, лишенных оболочек, т. е. о половых клетках, протопласт которых ограничен лишь плазмалеммой.

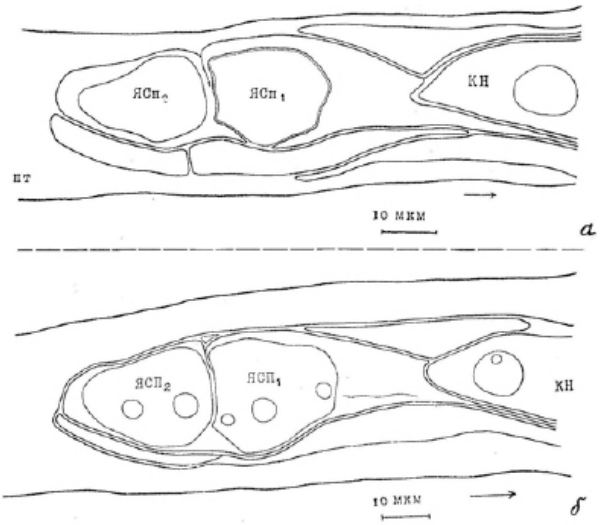

Рис. 7. Два участка последовательно продольного среза одной и той же пыльцевой трубки в зоне размещения спермиоклеток. а - сестринские ядра спермиев (СП1 и СП2) предельно плотно сближенных.

Между ними легко прослеживается плазмалемма. ЯСп1 крупнее, содержит три ядрышка, его протопласт амебоиднообразный охватывает клетку ножку (КН); б - те же спермиоклетки КН на втором срезе. Сплошными линиями обозначены островки цитоплазмы соответственных спермиоклеток. Ув. а и б - 90х, 10х (по Чуботару, 1992).

Из приведенного рисунка 7 нетрудно установить, что сестринские спермиоклетки по своему морфофизиологическому состоянию (строению) легко отличимы: Сп1 крупнее Сп2, цитоплазма его имеет лопастную форму, местами с заметными инвагинациями, равномерно структурирована, интенсивно окрашиваемая. Ядро спермиоклеток, главным образом, округлой формы со многими, разной интенсивности окрашиваемости, ядрышками, микроядрышками и равномерной, хорошо проявляемой хроматиновой сеткой. В целом цитоплазма, и ядро второго спермия отличаются от первого: хотя в кариолимфе обнаруживаем подобные же нитевидные хроматиновые структуры (хромосом). Однако заметьте, последние менее интенсивно окашиваются, тем самым придавая ядру рыхлое состояние; подобное уменьшение плотности, пока, и в меньшей степени, наблюдаем и по отношению к цитоплазме, которая по объему также уступает таковой первого спермия.

Некоторые исследователи, описывая процесс спермиогенеза у *Picea abies* (L.) Karst. (Camefort, 1965), справедливо отметили, что образовавшийся фрагмопласт между двумя спермиями вскоре исчезает, т.е. имеет лишь временный характер. Но нельзя согласиться с тем, что два спермия - ядра тем самым остаются в общем протопласте. Ведь речь идет не о клетках вообще, а о гаметах, голых клетках, протопласт которых, как уже отметили, ограничен лишь плазмалеммой.

Остановимся подробнее на описании других спермио-клеток, на предсингамной фазе развития (рис. 7-10). Эти фазы нами также тщательно были изучены под иммерсией. На их примере нам хотелось также показать целостную систему ПЗ-

ПТ, как основное «требование» прогамной фазы. Удачные продольные срезы через всю систему ПЗ-ПТ (см. рис. 3, 4, 8, 9): ПЗ функциональных пыльцевых трубок на протяжении долгой прогамной фазы сохраняют свою сферическую форму и заполнены большой вакуолью, отходящей в ПТ.

Как видно, возникшие клетки-спермии в результате деления базальной клетки, во многом различаются. Различия между парой спермиоклеток имеют тенденцию нарастать, увеличиваться на протяжении прогамной фазы оплодотворения. Отметим, что впереди БК, близко к кончику ПТ, среди плотно расположенных амилопластов находится ядро клетки трубки. Ядрышко ядра интенсивно окрашивается в то время, как ядро потеряв округлую форму, становится лопастным, местами со своеобразными протуберанцами. В оптически светлой кариолимфе видны мелкие, редко расположенные по периферии хроматиновые глыбки. Рядом (или впереди) спермия-1 располо-жена клетка ножка. Ее размеры уменьшились, ядро приобрело более резкий рисунок, объем цитоплазмы хоть и меньше предыдущего, однако по прежнему интенсивно окрашивается, что свидетельствует о ее физиологической деятельности. Ядра спермиев округлой формы с двумя ядрышками и равномерно зернистой кариолимфой. В целом ядро СП1 по сравнению с СП2 менее интенсивно окрашено, что свидетельствует, вероятно, о завершении постмитотической активности и вхождении в интеркинез. Как уже отметили, ядро СП2 с тремя ядрышками имеет слегка более темный цвет, содержит мелко расположенный по ядру хроматин, а цитоплазма СП2 оптически менее плотная, равномерно окрашена и лишена каких-либо вакуолей. Цитоплазма СП1 оптически более плотная, в ней видны многочисленные темноокрашенные митохондрии и различной формы пропластиды. Темноокрашенные структуры как бы ориентированы по длине оси ПТ. Отметим, что зернистость ядер более выражена, чем у цитоплазмы.

На другом срезе (рис. 8) мы показываем те же спермии, что и на рис. 9. Обращает на себя внимание, что окруженная плазмалеммой цитоплазма спермиоклеток в средней части оптически перекрывает друг друга (нахлестка). Причем у первого спермия она линзообразна. Клетка ножка также линзообразна и смещена с боку от фронтальной части спермиоклетки. Этот рисунок охватывает всю так называемую систему пыльцевое зерно - пыльцевая трубка (Чеботарь, 1965). При этом обращает внимание общее состояние спермиоклетки. Спl крупнее Сп2, цитоплазма окрашена в более интенсивный цвет, в ней видны многочисленные темноокрашенные тела - вероятно митохондрии и пропластиды. По мере завершения прогамной фазы оплодотворения различие между спермиоклетками становятся более явными.

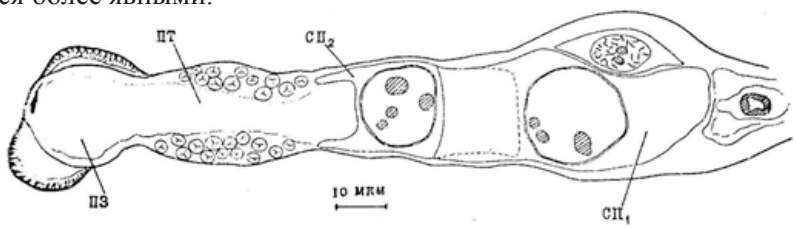

Рис. 8. Обособленные цитоплазмой спермиоклетки (Спl и Сп2) передвинуты к растущему кончику пыльцевой трубки (ПТ). ПТ-ПЗ сохраняют биологически целостную систему.

Основная протоплазма клетки трубки перемещена в растушую часть и вокруг Сп. ПЗ и значительная часть ПТ оптически прозрачна. Цитоплазма спремиоклеток в межядерном пространстве как бы (нахлестом.) перекрывает друг друга. Ув. 20х, 7х (по Чуботару, 1992).

В завершении сказанного мы вынуждены еще раз обратить внимание на две микрофотографии (рис. 10 а, б), приведенные в упомянутой нами публикации (Мошкович, Чеботарь, 1986). Нетрудно заметить, что даже на довольно

посредственно воспроизведенных микрофотографиях видны плазмалеммные разграничения протопласта каждого спермия, т.е. между спермиями - клетками хорошо видны разделительные мембраны.

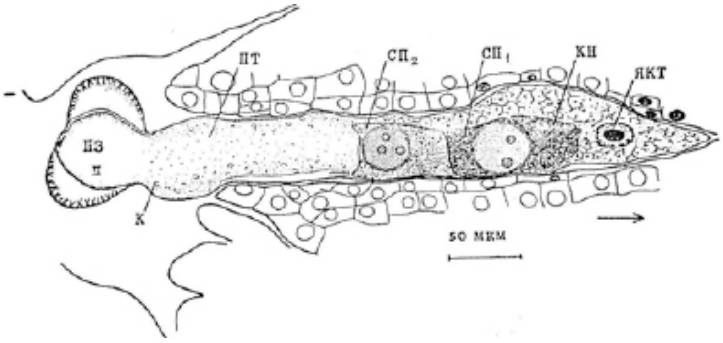

Рис. 9. Медиальный срез спермиоклеток (Сп1 и Сп2) той же пыльцевой трубки, что и на рис. 8. Впереди идущий Сп1 крупнее Сп2. Его цитоплазма более богата цитоплазматиче скими образованиями сохраняет тесный контакт с клеткой ножкой (КН). Ядро клетки трубки (ЯКТ) подвергается деструктивным процессам. В зоне расположения клеток - спермиев пыльцевая трубка (ПТ) заметно расширена и наполнена пластидным крахмалом (К) (по Чуботару, 1992).

Итак, мы просмотрели достаточный материал этой завершающей фазы прогамии и пришли к выводу, что у ели обыкновенной каждый спермий имеет свою цитоплазму, ограниченную лишь плазмалеммой, которая сохраняется вплоть до вхождения ПТ в микропиле. До того, как ПТ достигает последнюю, у второго спермия обнаруживаются признаки дезинтеграции (рис. 10). Интенсивно окрашенная мелкозернистая кариолимфа последнего, как бы съеживаясь, отходит от оболочки; ядрышки ядра скучиваются и уменьшаются в размере; заметно убывает и объем цитоплазмы. По сравнению с предыдущими фазами и у первого спермия количество цитоплазмы заметно уменьшается, в то время как ядро по-прежнему сохраняет свою форму, объем и структуру.

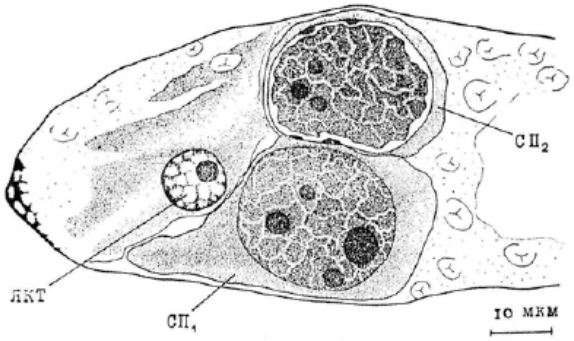

Рис. 10. Участок кончика растущей пыльцевой трубки Picea abies (L.) Karst. вблизи микропилярнога в хода семяночки части яйцеклетки.
Спермиоклетки (Сп1 и Сп2) по морфофизиологическому состоянию различны. Ядро Сп1 крупнее, с большими и малыми ядрышками, Кариолимфа равномерно заполнена зернистыми структурами. Цитоплазма Спl более значительна с вытянутым вперед участком, обнаруживает признаки деструкции. Ядро окружено незначительной частью цитоплазмы. ЯКТ - ядро клетки трубки. Ув. 90х, 7х (по Чуботару, 1992).

Повторное изучение одного из наиболее сложных и спорных вопросов в гаметогенезе хвойных на примере *Picea abies* (L.) Karst. на технически новой основе и на дополнительном материале привели нас к следующему заключению.

Заключение

Поэтапная визуализация спермиогенеза у *Picea abies* (L) Karst. является частью того экспериментального материала, который говорит в пользу существующего мнения (Strasburger, 1879, 1892; Dixon, 1894; Hirase, Coulter, Chamberlain, 1901, 1918; Coulter, 1897 – цит. по Sterling, 1963), а именно - представителей семейства Pinaceae спермии представляют собой клетки, т. е. каждое ядро спермиев окружено собственной цитоплазмой. Таким образом, вслед за указанными авторами мы подтверждаем: мужские гаметы, в данном случае ели обыкновенной, представляют собой самостоятельные клетки. Это мнение не совпадает с утверждением Fergusson (1901) и Camefort (1978) и уточняет мнение, высказанное по этому вопросу в упомянутой работе (Мошкович, Чеботарь, 1986).

Ретроспективный анализ спермиогенеза у покрытосеменных, где в отличие от хвойных эволюционно закреплена функциональная зависимость второго спермия от первого (сестринского, осуществляющего копуляцию - оплодотворение яйцеклетки) и где возникшие спермиоклетки всегда четко разделены и самостоятельно двигаются (перемещаются) в ПТ и зародышевом мешке (с момента их появления и вплоть до вхождения одного из них в яйцеклетку) сохраняют полный контакт. В таком же сблокированном положении проходит продвижение и клетка ножки в ПТ. Она (КН) постоянно занимает одно положение - впереди (или сбоку) первого спермия (см. рис. 6, 7, 8, 9). Отметим, что клетка ножка - сестринская клетка базальной (сперматогенной) клетки и на протяжении всего периода сперматогенеза также находится в тесном сближении с БК.

Поведение клетки ножки и ее морфофункциональная зависимость сперва от базальной, а после от одной из спермиевых (впереди двигающейся) клетки заслуживают внимания. Об особом морфофункциональном взаимодействии КН и первого спермия говорят приведенные наблюдения: КН почти полностью окружена амебоидными выростами базальной клетки (рис. 5), КН плотно «пристроена» сбоку впереди двигающегося спермия (рис. 7), то ли спермий толкает ее, то ли она буксирует спермиоклетки (рис. 6, 7). Однажды, обгоняя двигающуюся базальную клетку (в ПТ), свое положение не меняет до конца прогамного процесса.

Мы не смогли привести фото-рисунки подтверждающие то, что второй спермий, остающийся в районе канальцевых клеток, или между яйцеклеткой и нуцеллусом, или близко к приемной вакуоли, всегда сохраняет вокруг себя небольшой остаток собственной цитоплазмы. У нас сложилось впечатление, что задолго до того, как передний спермий достигает яйцевую клетку, вторая спермиоклетка обнаруживает признаки дезинтеграции (рис. 10), в целом приведшей к ее дегенерации - резорбции. При этом трудно согласиться с бытующим мнением, будто окружающая цитоплазма двух ядер - спермиев в ходе продвижения и вхождения одного из них в яйцеклетку смешивается.

Со временем классической работы С. Г. Навашина (1911), в которой приведены подробности об образовании мужских половых ядер у Lilium martagon, возникла открытая дискуссия между ним, Л. Гиньяром и Э. Страсбургером; С. Г. Навашин оспаривал участие цитоплазмы спермиев цветковых растений в оплодотворении (т.е. в образовании неоплазмы зиготы - А.А.Чеботарь), Этот вопрос, спустя сто лет неоднократно будируется и в наше время. В свое время мы высказывались в пользу утверждения С. Г. Навашина, подтверждая его высказывания в наших светооптических и злектронномикро- скопических исследованиях на примере большой группы цветковых растений (Чеботарь, 1969, 1972; Chebotaru, 1985, 1990). В то время наши исследования хвойных показали, что цитоплазма спермия, участвующего в оплодотворении вслед за его ядром, все же сопровождает его вплоть до яйцеклетки и окружает ядро возникшей зиготы, тем самым принимает участие в образовании ее неоплазмы (Chesnoy, Tonias, 1971; Чеботарь, 1986, 1990; Мошкович, 1992 и др.).

Из выше сказанного следует особенно подчеркнуть и то, что на примере гаметогенеза и спермиогенеза хвойных, как нам представляется, можно сделать другой исключительно важный вывод (- отсеивание второго спермия) это проявление всеобщности явления аббревиации (отсеивания) предгаметных и гаметных образований, закономерно наблюдаемое у высших растений. В этом исследовании мы получаем подтверждение в пользу выдвинутой нами гипотезы о всеобщности закономерности устранении рассевании (аббревиации) генетически неполноценных структур (Чеботарь, 1972, 1984, 1992).

На примере гаметогенеза хвойных, где спермиогенезу предшествуют пять делений гаплоидных структур, где с каждым делением одно из сестринских образований как бы отсекается (отчуждается) от дальнейшего участия в конечном (финальном) результате, легко убедиться, что закономерная аббревиация предгаметных и гаметных образований в гаметогенезе это (не что иное, как) проявление (действие) дарвинского отбора на гаметном уровне. Таким образом, в гаметогенезе (♀ и ♂) происходит закономерное отсеивание, отчуждение генетически нетипичных виду репродуктивных структур. К таким следует отнести и второе ядро-спермий, его цитоплазму, которая, как увидим, подвергается полной деструкции.

Следовательно, отсеивание - аббревиация предгаметных и гаметных образований - явление закономерное в гаметогенезе высших да и низших растений. Как и в предыдущих работах (Чеботарь, 1972, 1984, 1990) мы констатируем: гаметогенезу характерны определенные (стартовые) числа митотических делений, строго повторяющиеся из поколения в поколение. Возможно именно такое число делений гаплоструктур (пять ♀ и пять ♂ у хвойных, два ♂, три ♂ и более у цветковых), закрепленное наследственно, свидетельствует об отработанном механизме эволюционного развития половодеятельной адаптации, став тем закономерным явлением, где число мейотических и митотических делений гаплоидных структур обеспечивает как бы «очищение», освобождение хромосомных носителей наследственной информации от лишнего, генетически атипичного (пассивного) груза, возникшего под влиянием экзогенного воздействия в филогенезе. Иными словами, можно утверждать: закономерное отсеивание - аббревиация структур репродуктивной сферы, проистекающее на стыке перехода спорофит - гаметофит - спорофит, составляет основу (начало, истоки) дарвинского отбора. Здесь формируется, как мы увидели, норма реакции в ходе эмбрионального развития (Чеботарь, 1972, 1985) на фоне жесточайшей конкуренции (борьбы) в ходе змбриоадаптации, бесчисленного количества половых зачатков.

В то же время отсеивание - аббревиацию предгаметных и гаметных образований не следует рассматривать как произвольный, подталкиваемый извне процесс, от генетически детерминированный морфологически саморегулярный.

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CONTRIBUTION TO THE KNOWLEDGE OF VEGETATIVE ORGANS STRUCTURE PF *PAEONIA PEREGRINA* MILL.

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Introduction

The available scientific literature includes also data referring to the structure of vegetative organs from species of *Paeonia*; we refer to papers published the late 19th century [4, 6] and in the 20th century [3, 12]. Some of these data are found in the synthesis on the anatomy of dicotyledons [5, 11] or anatomy of angiosperm in generally [7]. Between the analyzed species are also included *P. peregrina* Mill. (*P. romanica* Brândză), the investigated organs being the stem and the petiole lamina.

A comparative anatomical study on three species of *Paeonia* from Romania Flora (*P. peregrina, P. tenuifolia, P. triternata*) belongs to Georgeta Filipescu (1972), investigating all vegetative organs, emphasizing their common structural features, as well as those that are different; in addition the author reveals adaptive changes caused by environmental conditions in which plants grow.

In this contribution we resume the anatomic study on the species *Paeonia peregrina* Mill., perennial, xeromesophylic plant, protected as natural monument [10]. It is a Balkan species, spread sporadically in the forests, from the steppe and silvosteppe, in the southern part of the country, in clearings, at forest edges, in *Quercetalia pubescentis* association [1, 8, 9, 10].

Materials and methods

Investigated material (underground and aboveground vegetative organs) was collected from natural forest reserve Gârboavele-Galați, in phenophase anthesis, in 2014. In order to fulfill the present study, the vegetal material has been fixed and preserved in 70% ethylic alcohol. The sections were cut with a microtome and a botanical razor. The obtained sections were then colored with iodine green and alaun-carmine. The next stage was mounting the sections in gel. The micrographs were performed by means of a Novex (Holland) microscope, using a Sony (Cyber-shot) camera.

Results

The root. The root system is represented by thin roots, some tuberous, all rich in reserve substances [8].

a. Thin root (Fig. 1: 1-3). At the sectioned level, the structure is secondary, as a result of the activity of both lateral meristems: phellogen and cambium. The phellogen produces a relatively thick blanket of suber (5-6 layers, the external ones undergoing exfoliation) and one of thin phelloderm. The primary cortex is a relatively thick (5-6 layers) amyliferous parenchyma. The central cylinder is very thick, resulted, in large part, from the bifacial activity of cambium. The cambium produced a thin ring of phloem (sieve tubes, companion cells and parenchyma cells) and a very thick area of xylem, with two subzones: one thin axial zone (with a few vessels separated by amyliferous parenchyma cells) and a external zone (with vessels dispersed irregularly, separated by a few cellulosic parenchyma cells and libriform fibers, with external wall extremely thick).

b. Tuberous roots (Fig. 1: 4-6). The structure resembles with the structure of a rhizome. At the periphery a periderm is present; the phelloderm cells are not different from those of thick cortical amyliferous parenchyma. The central cylinder is very thick, almost entirely amyliferous. In the fundamental parenchyma numerous vascular bundles are present, elongated, arranged on a circle and separated by very broad medullary rays, parenchymatous-cellulosic, amyliferous type. All vascular bundles have less primary phloem (sieve tubes, companion cells) and more primary wood (cellulose vessels

and parenchyma cells). The root axis has little xylem vessel, often solitary, dispersed in amyliferous fundamental parenchyma.

The stem (Fig. 2). The cross-sectional contour of the stem (80-85 cm height) is circular, with less prominent ribs.

The epidermis presents very small cells, with very thick and covered by cuticle external wall. From place to place stomata are visible, which protrude slightly over the epidermal cells.

The cortex is relatively thick (25-30 layers), with collenchyma on the outside and parenchymatic cellulosic in rest, the cell size increasing to the central cylinder. From place to place some aerial cavities of irregular shape and different size are observed.

The central cylinder is very thick and includes: a very tortuous ring of vascular bundles and an extremely thick medulla, parenchymatous-cellulosic. The vascular bundles are numerous, very close to each other, with very different sizes; the largest being located next to the stem ribs. All vascular bundles are linked by very narrow medullary rays (sclerified and lignified) and have, at the periphery of the phloem, few thin cordons of sclerenchyma fibers, extremely thick and intensely lignified.

The vascular bundles, especially large ones, show both primary structure (phloem with sieve tubes and companion cells; and xylem with vessels and parenchyma cells) and secondary structure (in phloem we also found parenchyma cells and in wood libriform fibers appear).

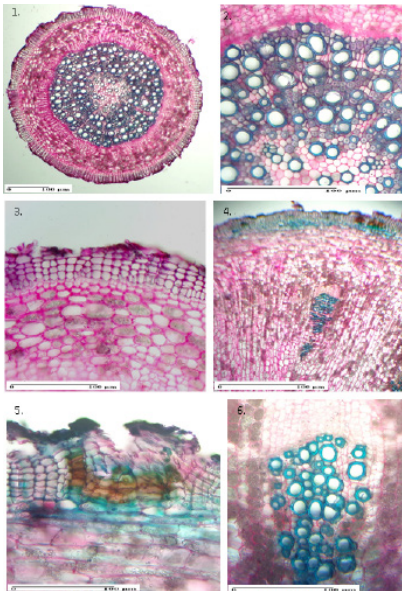

Fig. 1. Cross section through the root.
1. Thin root – overview; 2. Thin root – central cylinder detail, 3. Thin root – exterior layers detail; 4. Tuberous roots – overview, 5. Tuberous roots –– exterior layers detail, 6. Tuberous roots – vascular bundle.

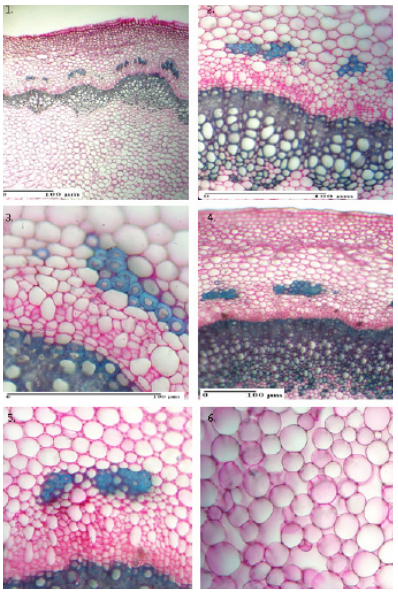

Fig. 2. Cross section through the stem.
1. Median part of the stem – overview, 2-3 – vascular bundles detail, 4. Inferior part of the stem – overview, 5. Inferior part of the stem – vascular bundles detail, 6. Inferior part of the stem – pith.

To the base of the stem (which is much thicker), the ribs are attenuated; the vascular bundles are very large in the primary cortical parenchyma. The entire phloem of all vascular bundles forms a thin ring and the xylem forms a much thicker ring, both rings very tortuous. Many periphloemic sclerenchyma fibers are very close, resulting in 1-3 mechanical cordons at the periphery of each vascular bundle.

The leaf has leaflets obovate-oblong, 2-3 sectate or pinnately lobate, on the upper surface shows craving along ribs [8].

The petiole **(Fig. 3: 1-2)**. The contour of cross-section through petiole is circular-

elliptical, modified by two latero-adaxial ribs, which delineates a relatively wide and deep ditch. The epidermis presents cells with thick external wall and cover by a thin cuticle. The fundamental parenchyma is colenchymatic type under the epidermis and meatic in the rest. In this parenchyma 7-8 vascular bundles are arranged in an arc, the median-abaxial one being very large and the ones from latero-adaxial ridges being very small. In the median bundles parenchyma cells in phloem and libriform fibers in wood are also observed, evidence that the secondary structure is forming. Groups of sclerenchimatic fibers are observed at the periphery of vascular bundles.

The lamina **(Fig. 3: 3-6)**. The epidermis frontally viewed shows large cells, irregular contour, with strongly sinuous sidewalls. The stomata, numerous per unit area, are localized in the lower epidermis, so the leaf is hipostomatic. The epidermal cells next to the ribs are polygonal elongated, with numerous punctuations in the side walls.

In cross-section through lamina, the median rib (and sometimes the lateral one) protrudes very much the underside of the lamina and contains a vascular bundle with primary structure. The epidermis presents isodiametrical cells on median ribs, with different sizes and sometimes tangentially elongated between lateral ribs, always higher in the upper face of the lamina. The mesophyll comprises a low palisade layer of cells on the upper face and many cells of spongy tissue underside, so the lamina has a bifacial heterofacial structure. Some palisade cells presents septa relatively deep from the outer wall to lumen. The vascular bundles from lateral ribs (higher-order) are small and very small, some having only phloem elements. On the analyzed material we did not observed oxaliferous cells, cells mentioned by Filipescu in 1972.

Discussions

P. peregrina is a perennial species, sporadic in our country, showing both thin roots and tuberous roots, with different thickness. If the very thin roots are a primary central cylinder diarch and triarch, the tuberous roots present a secondary structure, with moderate collenchymatosus parenchyma, many cells containing calcium oxalate. Both meristems produce secondary tissues, but cambium produce a lot of secondary xylem. In xylem thickness is observed areas where parenchyma predominates. These areas alternate with areas in which prevail vessels and wood fibers.

Through their structure, many of tuberous roots remember of rhizomes; in their fundamental cellulosic parenchyma is observed numerous vascular bundles radial elongated, with primary structure, arranged in a circle and separated by very large medullar rays, parenchymatous cellulosic, amylipher type; all the vascular bundles present a little phloem and a lot of xylem. In the organ axis there are few vessels, often solitary, separated by a lot of amylipher parenchyma. Such a structure, intermediary between the root structure and rhizome structure, is not recorded in specialized works consulted.

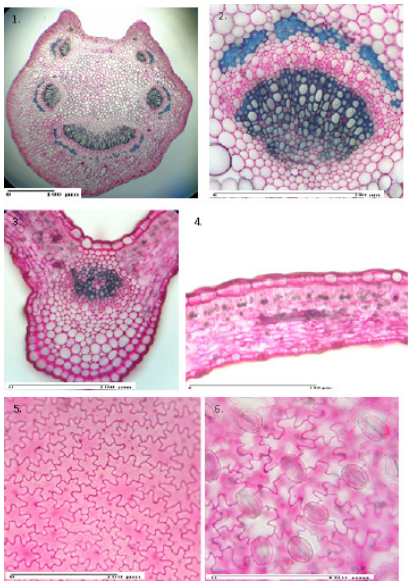

Fig. 3. Cross section through the leaf.
1. Petiole – overview, 2. Petiole – vascular blade, 3. Lamina – median rib, 4. Lamina – overview, 5. Superior epidermis, 6. Inferior epidermis.

Compared to other authors [2, 3], we have not seen brahisclereide at the available material.

The stem presents stomata that prevailing over the epidermis; and at the basal level the two vascular tissue form two concentric rings, very sinuous, the phloemic one being in direct contact with sclerenchyma cords from the periphery of former vascular bundles of primary structure.

The foliar blade is hipostomatic, with bifacial heterofacial structure; the palisade tissue having short cells, many of them being provided with longitudinal septum to superior epidermis, as mentioned by other authors [2, 11]. At the analyzed material did not notice oxaliferous cells (mentioned by Filipescu in 1972).

The histo-anatomical traits of vegetative organs, correlated with living conditions in which plants live, reflect the meso- xerophile character of *Paeonia peregrine* Mill.

Conclusions

In conclusion we can affirm that the secondary structure of thin roots is resulting from both lateral meristems activity (phellogen and cambium); tuberous root structure partially resembles with a rhizome, most part of conductive tissue forms collateral bundles arranged in a circle and separated by medullary rays, very broad, rich in starch granules. The stem shows both primary and secondary structure. The mechanic tissues are represented by hypodermic collenchymas and sclerenchyma (cordons of sclerencyma fibers, libriform fibers and medullary rays). The leaf presents hipostomatic lamina and bifacial heterofacial structure, the palisade tissue being monolayered, comprised from short cells and, here and there, with radial septa on external walls.

Acknowledgements

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THE PRIMERS FROM THE MUDR TRANSPOSON – MOLECULAR MARKERS OF TOMATOES GENOTYPES

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Introduction

Cultivated tomato is one of the most consumed vegetable crops and a well-studied object in genetics, genomics, and breeding [5]. Despite the fact that many tomato varieties differ in productivity, shape, size, color, taste, flavor, stress tolerance, and numerous other traits, very few polymorphisms within the cultivated tomato gene pool have been identified, even using sensitive molecular markers [3]. Thus, although the study of genetic markers for tomato has been conducted over 30 years through various approaches, the problem of identifying new types of molecular markers, allowing genome fingerprinting of different varieties of tomato, is actual [11].

Recent data show that a very rich source of molecular polymorphism and an ideal tool for genome analysis are transposable elements (TE) [1, 2, 7, 9, 10]. TEs are DNA fragments that can move from one genomic location to another by a process called transposition, and they make up a fairly large portion of eukaryotic genomes [6, 4].

The aim of this study was to determine the genetic polymorphism of tomato genotypes – spontaneous forms used in amelioration and perspective breeding, using ET MuDR primers as molecular markers.

Materials and methods

Twenty two tomato genotypes were analyzed – spontaneous forms used in amelioration *Lycopersicon esculentum* var. *racemigerum*, L. *esculentum* var. *cerasiformae*, L. *pruniformae*, L. *esculentum* var. *cheesmaniae* and varieties: Buran, Delta, Elvira, Jubiliar 60/20, Mary Gratefully, Mihaela, Milenium, Nistru, Nota, Perfect peel, Prestij, Rio Grande, Tomis and new hybrids created in IGFPP of AŞM: Rio Grande x Delta, Rio Grande x Nistru, Nota x Buran.

Total DNA was extracted from seedlings by a modified CTAB method [8].

The complete sequence of maize transposable MuDR presented in GenBank consists of 4942-bp, terminates in almost identical 215-bp TIRs (terminal inverted repeats) and carries two convergent, transcribed genes: mudrA and mudrB, separated by only a 225-bp intergenic region [12]. 13 primers: were tested the primers E1 (tgccattatagacgaagagcgg), E 2 (ggcgttggcttctatgatctg), E 3 (aaacagaaaggtgacagcgct) belong to the region TIR of MuDR, the primers N 39 (ttggcgtactcctctcctcg, 5'→3'), N 40 (gtcttcctactgcggctgct, 5'→3'), N 44 (tgtagatggccacaattggatg, 5'→3'), N 42 (aacccagatatgcatggacca, 3'→5'), N 45 (cactccactggcgaaatcaa, 3'→5'), N 46 (ccttgtcggtggtggagaag, 3'→5') belong to the region MudrA of MuDR and D 14 (tcatcatctacggaagggttgtc, 5'→3'), E 10 (tgccaccttgtacctctggaa, 5'→3'), E 7 (tcatctggttgttgcacagga, 3'→5'), D 15 (N 80) (ggtcgtttatctcttcgaacctgt, 3'→5') – to the region MudrB of MuDR. So, depending on primers and the objects of study, the experimental research was divided in three groups (tab. 1).

Tabel 1.The primers' groups and the analyzed objects of study

Number	Primers	Genotypes
I.	TIR	Jubiliar 20/20, Prestij, Milenium, Tomiş, Mihaela, Elvira
II:	MudrA	Jubiliar 20/20, Prestij, Milenium, Tomiş, Mihaela, Elvira, Perfectpeel, Rio Grande
III.	MudrB	*L. esculentum var. racemigerum, L. esculentum var. pruniformae, L. esculentum var.* cheesmaniae, Nota, Nota x Buran, Buran, Delta, Rio Grande x Delta, Rio Grande,Rio Grande x Nistru, Nistru, *L. esculentum var.* cerasiformae

The PCR mixture, in a volume of 25 µl, contained: 66 mM tris-HCl (pH-8,4), 16 mM (NH4)2SO4, 2,5 mM MgCl2, 0,1% Tween 20, 7 % glicerol, 100 µg • ml-1 Bovin Serum Albumin, 0,2 mM dNTP of each, 1,25 U Taq DNA polymerase (Fermantas), 5 pM primer and 5-10 ng DNA.

The PCR included 8 cycles: 95^0C – 1 min, 40^0C – 2 min, 72^0C – 1 min, followed by 35 cycles: 95^0C –30 sec, 65^0C – 1 min, 72^0C – 1 min.

The products of amplification were divided into 1,5 % agarose gel by electrophoresis (5-8 V/cm) in a migration buffer of Tris/borate EDTA with ethidium bromide, viewed in the UV (302 nm), photographed and authenticated using GelAnalyzer 2010 program.

Results and discussions

TIR primers determined the amplification of 2-7 monomorphic and polymorphic fragments, with variable ratio (fig. 1, tab. 2).

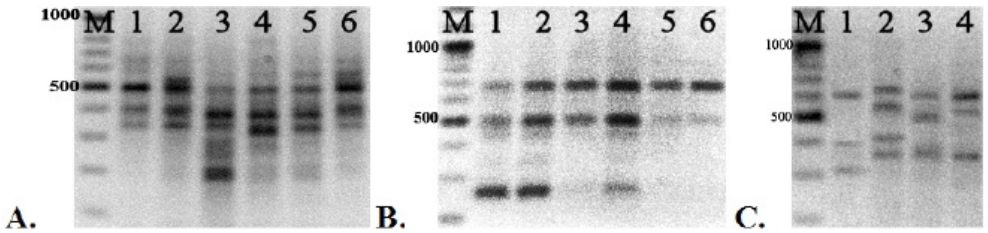

Fig. 1. The electropherogams of the amplification products with homologous primers TIRs E 1 (A), E 2 (B.), E 3 (C).

Jubiliar 60/20 (1), Prestij (2), Milenium (3), Tomiş (4), Mihaela (5), Elvira (6), M. GenRulerT 100bp DNA LadderPlus.

Tabel 2. The lenghth of amplicons of the tomato genotypes identified with TIRs of MuDR primers

Genotype / Primer	Jubiliar 60/20	Prestij	Milenium	Tomiş	Mihaela	Elvira
	bp					
E 1	590 506 403 338	**518** 506 403 338	506 403 338 265 **235** 180	506 403 338 265 180	650 590 506 403 338 265 180	650 590 506 403 338
E 2	680 490 258	680 490 258	680 490	680 490 258	680 490	680 490
E 3	610 **360** 273	**657** **550** **390** 327	610 **489** 327	610 **517** 327	-	-

Analizing the obtained data, we concluded that in case of the E 2 primer, the frequency of polymorphic fragments specific to a unique genotype is zero, also in case of the E 1 primer- 0,22, and E 3-0,77. Also, the E 1 and E 3 primers generated polymorphic specific spectrums 100 %, from the analized genotypes.

MudrA primers determined amplification of the 3-8 fragments (fig. 2, tab. 3). In

case of the N 40 primer for the genotype Jubiliar 60/20 and N 41 primer for RioGRande and Milenium, no fragment was amplified.

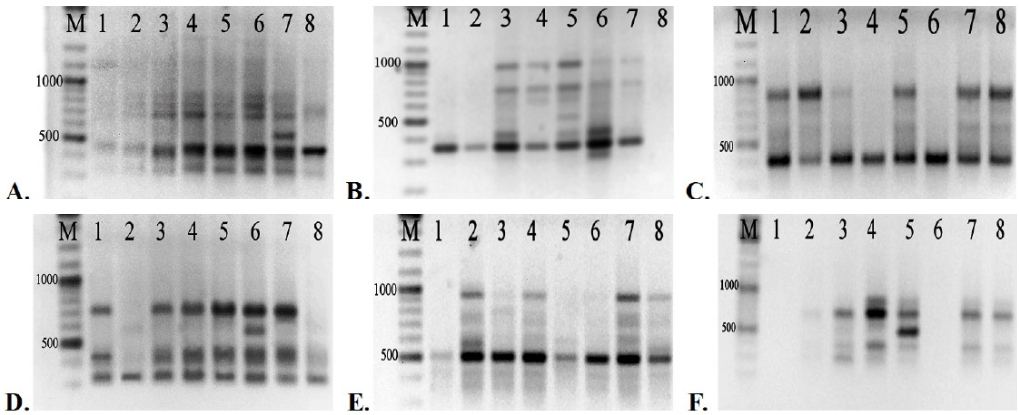

Figure 2. The electropherogams of the amplification products with homologous primers MudrA N 39 (A), N 40 (B), N 42 (C), N 45 (D), N 46 (E), N 41(F).
1. Rio Grande, 2. Perfectpeel, 3. Elvira, 4. Mihaela, 5. Tomiş, 6. Milenium, 7. Prestij, 8. Jubiliar, M. GenRulerT DNA LadderMix.

Tabel 3. The lenghth of amplicons of the tomato genotypes identified with MudrA primers

Genotype / Primer	Rio Grande	Perfectpeel	Elvira	Mihaela	Tomiş	Mile-nium	Prestij	Jubiliar 60/20
	bp							
N 39	**1250** 463 333	845 783 690 463 333	845 783 690 463 333	845 783 690 463 333	845 783 690 463 333	845 783 690 463 333	845 783 690 **550** 463 333	 **705** 463 333
N 40	 355	 355	996 740 505 420 355	996 740 655 355	996 740 655 505 420 355	996 740 **600** 420 355 **335**	996 740 355	-
N 41	-	667	667 **548** 378 **291**	667 378	667 **502** 378	-	667 378	667 378
N 42	813 680 **516** 437	813 680 437	813 437	 437	813 680 562 437	 437	813 680 562 437	813 680 562 437
N 45	665 452 404	 404	665 452 404	665 452 404	665 452 404	665 **562** 452 404	665 452 404	 452 404
N 46	 500 420	931 720 **560** 500 420	931 778 500 420	931 778 720 593 500 420	593 500 	500 420 	931 778 720 593 500 420	931 720 **526** 500 420

In case of primers from MudrA region, the frequency of unique polymorphic fragments that are genotype specific is relatively low. The potential of primers to generate polymorphic spectrums that are genotype specific varies in larger limits.

The N 40 and N 46 primers showed a medium potential of discrimination, generating polymorphic spectrums of 62 and 71 % from analized genotypes. The N 39, N 41, N 45 primers were less effective for differentiation of analized genotypes, generating 12-37 % of polymorphic spectrums that are genotype-specific.

The primers from the MudrB region determined amplification of 7-12 fragments (fig. 3; tab. 4).

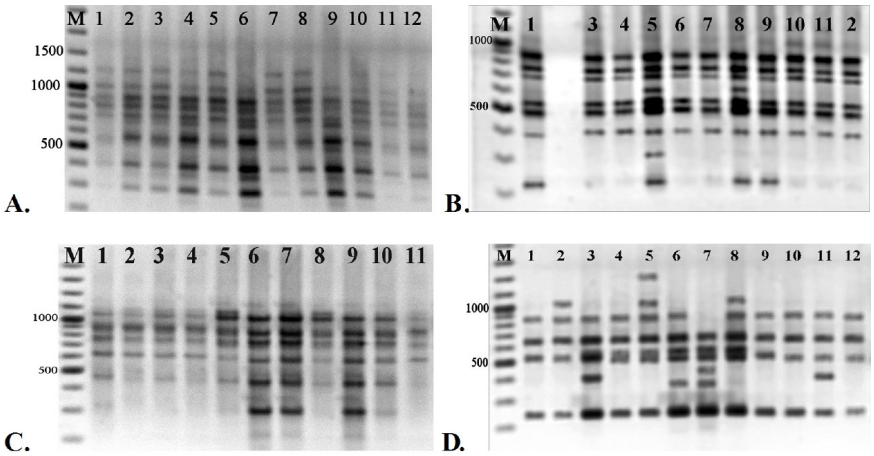

Figure 3. The electropherogams of the amplification products with homologus primers MudrB D 14 (A), D 15 (B), E 10 (C), E 7 (D).
L.esculentum var. racemigerum (1), L. pruneforme (2), L. cheesmaniae (3), Nota *(4),* Nota x Buran *(5),* Buran *(6),* Delta *(7),* Rio Grande x Delta *(8),* Rio Grande *(9),* Rio Grande x Nistru *(10),* Nistru *(11), L. esculentum var. cerasiformae (12). M. GenRulerT 100bp DNA LadderPlus.*

The most amplicons generated by primers from the MudrB region are monomorphic, rarely polymorphic. The frequency of amplicons that are genotype specific varied from 0 (D 14 and E 10 primers) to 0,1 (D 15) and 0,33 (E 7). A small number of polymorphic fragments that are genotype specific determined a small share of polymorphic spectrums that are genotype specific, which varies from 0 % (E 10 primer) to 27 % (E 7).

Most polymorphic spectrums of the Nota x Buran, Rio Grande x Delta and Rio Grande x Nistru hybrids are polymorphic without parental forms spectra.

Usually, identified fragments in the electrophoretic profile of the hybrid are found at least in one parental form. Simultaneously, for the hybrid Nota x Buran and Rio Grande x Delta, fragments amplified are not found at any parental form.

The spontaneous forms of tomatoes analyzed showed especially monomorphic fragments, found in forms created by amelioration. In these forms no unique genotype specific fragment was identified.

Generalizing the presented data, we conclude that all analyzed primers highlight common monomorphic fragments for the analyzed genotypes, so it may be used in taxonomic identification of Licopersicon esculentum. Primers which generated polymorphic spectrums that are genotype-specific may be applied in studying the inter-variety polymorphism of tomatoes.

Tabel 4. The lenghth of amplicons of the tomato genotypes identified with MudrB primers

	Genotype*											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
	bp											
D 14	1227 1000 904 801 710 527 405	1227 1000 904 801 710 661 527 405 298	1227 1000 904 801 710 661 527 405 298	1227 1000 904 801 710 661 527 405 298	1227 1000 904 801 710 527 405	 904 801 710 661 527 405 298 265	1227 904 801 710 527 405	1227 1000 904 801 710 527 405	 1000 904 801 710 661 527 405 298 265	1227 904 801 710 527 405 298	1227 904 801 710 527 405	 1000 904 801 710 527 405
D 15	888 743 686 518 472 380 225	888 743 686 518 472 380	888 743 686 518 472 380	888 743 686 518 472 380	888 743 686 570 518 472 380 290 225	888 743 686 518 472 380	888 743 686 518 472 380	888 743 686 570 518 472 380 225	888 743 686 518 472 380 225	888 743 686 518 472 380	888 743 686 518 472 380	888 743 686 518 472 380
E 10	1093 897 769 613 450 310	1093 897 769 613 450	1093 897 769 613 450	1093 897 769 613 450	1093 897 769 613 450	1093 897 769 613 450 310 210	1093 897 769 613 450 310 210	1093 897 769 613 450	1093 897 769 613 450 310 210	1093 897 769 613 450 310	-	-
E 7	 838 639 516 245	 1083 838 639 516 245	 838 639 516 396 245	 838 639 535 500 245	**1540** 1083 838 639 535 500 245	 838 639 535 500 370 245	 639 535 500 **425** 370 245	**1136** 838 639 535 500 245	 838 639 516 245	 838 639 516 245	 838 639 516 **396** 245	 838 639 516 245

*Note: * - 1. L.esculentum var. racemigerum, 2. L. esculentum var. pruneformae, 3. L. esculentum var. cheesmaniae, 4.* Nota, *5.* Nota x Buran *6.* Buran, *7.* Delta, *8.* Rio Grande x Delta, *9.* Rio Grande, *10.* Rio Grande x Nistru, *11.* Nistru, *12. L. esculentum var. cerasiformae*

Tabel 5. The primers homologous to different regions of MuDR perspective for the tomato genotypes identification

Primers	Genotypes
E 1	Jubiliar 20/20, Prestij (+), Milenium (+), Tomiş, Mihaela, Elvira
E 3	Jubiliar 20/20 (+), Prestij (+), Milenium (+), Tomiş (+)
N 39	Jubiliar 20/20 (+), Prestij (+), Rio Grande (+)
N 40	Prestij, Milenium (+), Tomiş, Mihaela, Elvira
N 41	Tomiş (+), Elvira, Perfectpeel
N 42	Rio Grande (+)
N 45	Jubiliar 20/20, Milenium (+), Perfectpeel
N 46	Jubiliar 20/20 (+),Tomiş, Elvira, Perfectpeel (+)
D 14	Buran, Rio Grande, L. esculentum var. cerasiformae
D 15	Nota x Buran, Rio Grande x Delta
E 7	Nota x Buran (+), Rio Grande x Delta (+), Delta (+), Nistru (+)

(+) - polymorphyc distinguished fragment

Conclusions

We described the DNA- polymorphism of some tomato genotypes using ET MuDR primers.

We concluded that all analized primers generate enough monomorphic amplicons, which can be considerated to distinguish Licopersicon esculentum.

The tomato forms created by amelioration have more pronounced polymorphism than wild forms, where monomorphic amplicons predominate.

We selected the primers E 1 and E 3 homologous to TIR regions of ET MuDR, N 39, N 40, N42, N 45, N 46 homologous to the mudrA region of ET MuDR and D 14, D 15, E 7 homologous to the region mudrB perspective, for the tomato genotypes identification.

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GENETIC FACTORS THAT PREDISPOSE TO CORONARY ARTERY DISEASE

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Introduction

Detection of the genetic factors that cause or predispose to CAD remains the topic of many scientific papers in this field. They were investigated separately or associated in the European population, but the genetic complexity of this disease was not foreseen, new approaches being needed [2, 6].

Studies, aimed at identifying the genes responsible for the heritability of CAD, have uncovered several candidate genes with different roles in vascular biology that are believed to be involved in the pathogenesis of CAD. Of these more important are renin-angiotensin system, endothelial dysfunction and homeostasis genes: ACE gene and angiotensin II type 1 receptor gene (AGT1R), Asp298Glu (A/G) of eNOS and of platelets (PlA1/2) GPIIb/IIIa receptor polymorphisms. Polymorphisms within these system genes have been extensively studied in relation with CAD, however, findings are conflicting [1, 3, 5, 6, 7]. To clarify these data, we studied association of genes polymorphism with conventional risk factors and cardiovascular complications.

The aim of the current study was to assess the association of gene polymorphisms with conventional risk factors and cardiovascular complications patients with CAD.

Material and methods

The case control study was conducted in 2007-2011 and included 405 patients with acute coronary episodes admitted to the *Municipal Clinical Hospital „Sfânta Treime"*, Chisinău. The control group consisted of 290 matched persons without CAD (data used for matching were age, sex, residence and professional activity). Sex-distribution in the study group was uniform, male / female ratio being 2:1, that is two times more males ($P<0.001$). Mean age was $57,93 \pm 0,34$ years, with insignificant variation in the control group ($P>0.05$).

The study was bicentric, case-control, approved by the *National Ethics Committee for Clinical Trials and Drug Development of Ministry of Health of the Republic of Moldova (Nr.331, 03.06.2010). All subjects were native-born citizens and residents of the Republic of Moldova*, had comparable socio-economic status, and were ethnically matched.

Patients were included in the study in the order of their hospital admission, after clinical and enzyme stabilization and obtaining informed consent, this type of patient selection ensuring randomness of the study group.

Criteria for inclusion in the study were the clinical diagnosis of acute Q wave and non-Q-wave myocardial infarction, unstable or exercise angina pectoris, in agreement with recommendations of the *European Society of Cardiology* [4].

Exclusion criteria: hypercholesterolemia (total cholesterol ≥8mmol/L) and secondary hypertriglyceridemia, pacemaker implant with evidence of ventricular preexcitation, atrioventricular conduction blocks (2nd or 3rd degree sinoatrial or atrioventricular block), active liver disease, acute gastrointestinal diseases, severe kidney disease and associated diseases that influence life expectancy.

Standard questionnaires were used to collect data on past and current medical history, examination results, and also personal and demographic data, cardiovascular risk factors, family history of CAD, hemodinamic data; lipidogram, blood glucose level, cardiac enzymes, instrumental investigations- ECG and echocardiography.

Polymorphism of renin-angiotensin system: I/D of ACE gene and A1166C genotype (cytosine or adenine variants, A/C) of AGT1R gene, Asp298Glu (A/G) of eNOS gene and PlA1/2 (A1A2) genotypes of GPIIb/IIIa receptor gene were identified by amplified polymerase chain reaction and restricted fragment length polymorphism in the *Institute of Genetics and Plant Physiology of the Academy of Sciences of Moldova* [3,5].

Data were computer processed by variation, association and descriptive analysis methods. The relationships between the studied phenomena were determined by using simple linear regression, quantitatively expressed by the correlation coefficient "r". For estimating genetic frequencies we used POPULATION GENETIC ANALYSIS by Nei Masatoshi, Director of the I*nstitute of Molecular Evolutionary Genetics*, Diploid Data Set at the Genetics Center, New York University, Langone. Frequency of studied genes loci was calculated with the help of Hardy-Weinberg equilibrium.

Results and discussions

Stratification of coronary patients according to ACE I/D polymorphism confirmed the prevalence of homozygous individuals with risk deletion/deletion (D/D) genotype as compared with the controls (19,64% vs. 11,03%, respectively, $\chi 2=8,77$, $P<0,05$), while genotype II was present in the control group (33,11% vs. 19,64%, $\chi 2$ 13,31, $P<0,01$). There were no significant differences in the number of heterozygous I/D in both groups (60,72% vs. 55,86%, respectively, $p>0,05$). ACE I/D polymorphism genotyping and the estimation of the allele frequency revealed significant differences in the presence of risk D allele in patients with CAD compared with controls (78,65% vs. 61,24%, OR=1,29, $\chi 2=8,77$, $P<0,05$). Compared with those in which this was not present (II), the analysis of the risk factors and clinical manifestations showed that ACE D/D homozygous or ACE I/D heterozygous genotypes in patients with CAD was associated with increased prevalence of hypertension (90,91% and 88,24% vs. 78,18 %), systolic blood pressure (155,32 ± 1,46 mm Hg and 140,5 ± 1,31 mm Hg vs. 125,42 ± 1,36 mm Hg), diastolic blood pressure (95,42 ± 1,35 mm Hg and 90,6 ± 1,28 mm Hg vs. 80,5 ± 1,84 mm Hg) and recurrent angina pectoris (40,00% vs. 34,11% vs. 23,64%, respectively, $P<0,01$).

No statistically significant differences were found between carriers of genotypes I/I, D/D or I/D in terms of degrees of hypertention. Considering the spectrum of risk factors and the clinical presentation according to ACE gene polymorphism recorded in this study, it appears that the presence of D allele and, in particular, homozygous D/D state are associated with blood pressure values exceeding the optimal level [$r_{xy}=0,81$, $P_{(DD-II)}<0,01$]. The carrier of D allele and heterozygous I/D state was associated with recurrence of angina symptoms [$r_{xy}=0,42$, $P(ID-II)<0,05$] and a significantly higher risk of cardiovascular death [$r_{xy}= 0,27$, $P(ID-II)<0,05$].

Genotype frequencies of AGT1R cytosine or adenine variants (A/C) in the group of patients with CAD were: A/A genotype was detected in 72 (25,74%) of the patients, C/C – in 47 (16,78%) and A/C – in 161 (59,28%). In the control group genotype frequencies were: 31(10,69%) C/C carriers, 162 (55,86%) A/C and 97 (33,40%) A/A carriers. No significant differences in the presence of the studied genotypes were found ($P>0,05$).

Genotyping AGT1R A/C polymorphism showed no conclusive differences between the presence of the risk allele C in CAD patients (72,83% vs. 70,71%, $P>0,05$), or non-risk allele A frequency (27,17% vs. 29,29%, $P>0,05$), compared with controls.

Comparative analysis of the characteristics of CAD patients grouped according to A/C polymorphism of AGT1R gene, revealed the association of homozygous C/C state or heterozygous A/C state with increased prevalence of hypertension (95,49% and 89,44% vs. 68,33%, $P<0,05$).

Estimation of the association between clinical determinants and A/C polymorphism of AGTR gene showed that the presence of the risk CC genotype in the coro-

nary patients is associated with increased prevalence of hypertension [r_{xy} = 0.88, P(CC-AA)<0.01] compared with homozygous AA genotype. Analysis of association indices in patients with CAD certify that between the carrier of the D risk allele of ACE gene and the C risk allele of the gene AGT1R was a moderate positive correlation (r_{xy} = 0.58, $\chi 2$= 35.30, P<0.001).

The distribution of Asp298Glu eNOS gene polymorphism frequencies in CAD patients showed no differences between them and control in terms of frequency of A/G genotype (53,21% vs. 57,93%, P>0.05) and risk allele AA frequency (63% vs. 79%, P>0,05). No significant age-related differences were found, but there was a tendency towards accumulation in women (37,84% vs. 24,27%, P=0,06).

Comparative analysis of the characteristics of CAD patients grouped according to Asp298Glu eNOS gene polymorphism, revealed that homozygous state with risk genotype AA or heterozygous A/G state are associated with increased prevalence of hypertension (96,00% and 87,91% vs. 69,64%, P<0,05), with no clear difference in terms of obesity (57,33% and 44,96% vs. 37,50%, P>0,05).

Analysis of clinical manifestations shows that almost 89,33% of the AA genotype carriers had arterial hypertension grade II-III, while such levels of hypertension were found in only 69,64% of the GG carriers and in 85,23% of AG carriers. Analysis of echocardiographic findings showed reduced ejection fraction <50% in more than half of AG genotype (57,05%), the same being also found in patients with genotypes GG and AA (42,86% vs. 46,67%, respectively).

Estimation of the association between clinical determinants and Asp298Glu eNOS gene polymorphism has shown that compared with non-carrier individuals (GG), homozygous AA state and heterozygous carriers (AG) in coronary patients are associated with increased prevalence of arterial hypertension [r_{xy} = 0,84, $P_{(AA-GG)}$<0,01].

Analyzing the frequencies of PlA GPIIb/IIIa receptor genotypes according to the polymorphism detected by MspI enzyme digestion we found that risk haplotype A2A2 was detected in 63 (22,50%) of the patients and 28 (9,66%) controls, the difference being statisticaly significant ($\chi 2$=16.28, P<0.001). Significant age-group differences were not found, but a trend of male prevalence (53,39% vs. 43,24%, P=0,06).

Analysis of A1A2 GPIIb/IIIa polymorphism genotyping revealed that mutant A2 allele tents to be more common in the CAD patients compared with controls (72,85% vs. 70,71%, P=0,06). At the same time, the frequency of recessive A1 alleles in the coronary patients was lower than in the controls.

Platelet membrane glycoproteins play an important role in platelet adhesion and aggregation. The allelic variants for GPIIb/IIIa bind to fibrinogen being the key reaction in the process of platelet aggregation. The presence of PlA2 allele leads to increased functional activity of receptors and is associated with intense adenosine diphosphate (ADP) induced platelet aggregation in vitro.

The analysis of the relationship between the carrier-state of different genotypes and risk factors revealed a significant difference between genotypes A1A1, A1A2 and A2A2 carriers and the prevalence of smoking (48,68% and 53,90% vs. 69,84 %, respectively, P<0,01), and mixed dyslipidemia (59,21% and 75,17% vs. 63,49%, P<0,05). Note the statistically significant difference between groups in terms of the share of old myocardial infarction in the history of the study patients: A2A2 genotype was detected more frequency than A1A1 (20,63% vs. 9,21%, respectively, P<0.05).

Analysis of biochemical characteristics in relation with A1/A2 GP IIb/IIIa gene polymorphism showed that A2/A2 genotype was associated with higher prothrombin levels as compared to A1A1 and A1A2 variants (106,96 ± 0,52% vs. 90,83 ± 0,59% vs. 80,00 ± 1,05%, P<0,05). Signs of grade II and III heart failure were present in 25,53% of A1A2 genotype carriers, 15,87% of A2A2 and 14,47% of A1A1 (P>0,05) genotype.

It is noteworthy that one fourth of the risk A2A2 and A1A2 genotype carriers presented Q wave acute myocardial infarction, compared with A1A1 carriers (28,36%,

22,22% vs. 19,73%, respectively, P<0,05).

It can be said that the presence of the A2 allele and homozygous state A2A2 were associated with the presence of dyslipidemia [r_{xy}= 0,53, $P_{(A2A2\text{-}A1A1)}$<0,05], smoking [r_{xy} = 0.64, $P_{(A2A2\text{-}A1A1)}$<0,01], as risk factors and a high frequency of previous myocardial infarction.

Conclusions

Carrier state of D/D genotype and D allele in ACE gene is a marker of increased risk for CAD and is associated with a high frequency of hypertension and cardiovascular death, being positively correlated with the risk CC polymorphic variant of AGTR1 gene. A2/A2 genotype of GP IIb/IIIa receptor gene is associated with susceptibility to CAD and high frequency of myocardial infarction and dyslipidemia, particularly in smokers.

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HUMAN HEALTH. ROLE OF GENETIC AND EPIGENETIC FACTORS

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Introduction

Actually, genetic and genomic science is redefining the understanding of the continuum of human health and illness [59].

The health definition has evolved over time, at the same time with the development of biology and medicine as has its measurement. Because health cannot be measured directly, a number of variables have been used as indicators of the concept of health. Prior to the mid-1900s, negative indicators such as mortality and disease rates were used with the idea that the lower the rate, the healthier the population. Mortality or morbidity rates continue to be used as broad indicators when comparing populations such as infant mortality rates or rates of specific diseases. However, a view of health as something much broader than the mere absence of disease has led to an evolution in thinking about the framework for health determinants [26].

A report by the Institute of Medicine in Washington (1999) reported explored core concepts of health, proposing a model of determinants that illustrated how individual characteristics (biology and life course, lifestyle and health behavior, illness behavior, personality and motivation, and values and preferences) and environmental characteristics (social and cultural, economic and political, physical and geographic, and health and social care) influence health-related quality of life (symptoms, functional status, health perceptions, and opportunity) [30]. Kaplan and colleagues [35] proposed a framework that "builds bridges between levels rather than attributing primary importance to one level or another." Their multilevel approach to health determinants includes pathophysiological pathways, genetic/constitutional factors, individual risk factors, social relationships, living conditions, neighborhoods and communities, institutions, and social and economic policies as the major forces that affect health [26].

Thereby, health is determined by several factors including genetic inheritance, personal behaviors, access to quality health care, and the general external environment (such as the quality of air, water, and housing conditions). In addition, a growing body of research has documented associations between social and cultural factors and health [5, 44].

The urgency of health problem is also conditioned by the fact that, till now, the „health" phenomenon's definition itself has not been clarified by science and contemporary medicine continues to maintain the nosologic direction. In 2013, Furdui and Ciochina proposed a comprehensive definition of the human organism's health, reflected through the prism of the most important features [65]. According to this definition after its recent review, integral human health is a complex multi-dimensional integral structural, metabolic, informational, physiological, mental and social state of the human organism, wherein its structural, metabolic, informational and physiological matrices are reproduced in ontogenesis according to the organism's genetic development program through the interaction with intrinsic and extrinsic factors: I) in the antenatal period, at the zygote formation stage – that of gametes, at the embryo stage - that of cells and tissues, at the fetus stage - that of tissues and organs; at the stages of zygote, embryo and fetus as integral systems - with the maternal organism; II) in the postnatal period – that of the child with the parents, the environment, and the lifestyle, actions that cause the genetical, physiological and psychological potential's externalization and that: 1) take place in the phylogenetically determined limits of intensity, inter-coordination and integrity of the informational, structural, metabolic and physiological processes, of or-

ganogenesis, of the genesis and functions manifestation of organs and vital systems, of the activity level, rhythmicity, autonomy, their coordination, and 2) ensure a) the achievement of physiological, psychic and social needs at the level, generating sensations of satisfaction, b) perception and identical reflection of the organism's internal and external environment, c) adequate orientation in the environment and adaptation to it, d) conscious and creative realization of daily activities and self-defense, e) opposition to own and others' intentions and actions, that may harm the person oneself, the society or nature, f) lack of pain sensation, g) reproduction of sanogenic offspring.

This definition is based on the fact that integral human health is determined by interrelations between genetic and environmental factors, and the genetic factor constitutes the basis of the organism's sanogenicity development.

Till now, human genetics have included only research regarding the manifestation of hereditary characters or different diseases and no data which would include genetic health determinants have been obtained. Therefore, the existing data as well as the well-established genetic regularities will be used to elucidate the genetic bases of human health.

Nowadays, the importance of genetics is well recognized in single gene related disorders, where mutation to a single gene results in the expression of clinical disease. The inheritance of common diseases is usually polygenic and disease outcome is also dependent on environmental factors [20].

The progress in genomics demonstrate immense promise in early prevention, characterization and prognostication for „many Mendelian diseases for sure, but potentially for chronic diseases as well" [37, 53]. Genomic medicine, one aspect of personalized medicine, „is a way to customize medical care to your body's unique genetic makeup" [22].

Traits determined by a single gene or allele are rare in human beings [21]. The vast majority of human diseases (e. g., cancer, heart disease, and diabetes) are complex traits affected by a large number of genes [13, 51]. Likewise, almost all human traits of interest to social scientists are complex, such as personality, cognition, motivation, and health behaviors. These traits are likely the consequence of many genetic and environmental factors, as well as interactions among them [27, 39, 38]. Therefore, it is important to incorporate multi-genetic and multi-environmental factors in gene-environment interaction (G x E) research on complex social outcomes [43].

Typically, understanding the genetics of human health begins with the identification of genetic variants associated with specific diseases. Epidemiologic studies play a key role in identifying these associations. Assigning causality can be difficult, however, because of the multifactorial nature of most diseases. Even when a genetic mutation conferring increased risk is present, health outcomes may be influenced by a variety of environmental exposures, behaviors, and other genes, and interaction among some or all etiologic factors may occur [42].

The completion of the Human Genome Project in 2003 was a major driver for the current period of biomedical discovery, the pace of which continues to accelerate [16]. Genetic predisposition plays a central role in most common diseases, and is the primary cause of most rare diseases [9]. Enormous advances have been made in the understanding of genetic disease [18], but at the same time remain poorly understood mechanisms through which various factors, including genetic ones, determine human health, rather than manifestation of a disease.

Prenatal Genetic Programming. Handwerger and Aronow [24] argue that the genetic program that directs human placental differentiation is poorly understood. The placenta performs many different functions, including 1) exchange of substrates, gases, and other factors between the maternal and fetal circulations; and 2) synthesis and secretion of protein and steroid hormones, growth factors, and other substances vital for regulation of maternal and fetal metabolism and growth [3]. Most of these biologic

actions occur at the trophoblast layer of the placental villous that is composed of two cell types: syncytiotrophoblasts and cytotrophoblasts [24].

Cytotrophoblast differentiation results from a dynamic genetic program in which some genes within specific functional groups are induced, while others within the same groups are repressed [24].

Blastocyst is stage of pre-implantation embryo development, when murine embryo is composed of 150 compacted blastomeres, arranged in a spherical form [23]. At the blastocyst stage, the cells divide into germ cells that form embryoblast (indoors) and trophoblast (outdoors). Embryoblast cells are pluripotent stem cells, thus giving rise to all the tissues of the body, except those that compose the placenta and fetal envelopes. Trophoblast cells can give rise only to the placenta and the fetal envelopes. Embryonic stem cells, like embryonic germ cell (EG) may also come from the primordial germ cells that form eggs and sperm, can be isolated and cultivated in vitro where continue to multiply and maintain their differentiation capacity [60].

These statements demonstrate high differentiation capacity of embryonic cells in various tissues and organs, based on the same genetic information, and this allows assuming that differentiation can be intentionally controlled and directed by applying of external sanogenic factors at the very well identified stages.

There is an increasing recognition that prenatal development is not simply an unfolding of a genetically determined timetable that is disrupted only in the context of exposure to extreme insults, but rather that the prenatal environment plays a critical role in shaping the developing fetus and contributes to individual differences in development. Normative changes in the prenatal environment, including variations in the exposure to maternal hormones, alter the developmental trajectory and may, in a predictive fashion, adapt the fetus for the postnatal environment. This issue of Zero to Three will consider new research illustrating the importance of prenatal influences such as maternal stress and stress hormones that critically influence the developmental program and the influence these factors have on adaptation to the postnatal world [14].

Most genes are expressed from both parental chromosomes; however, a small number of genes in mammals are imprinted and expressed in a parent-of-origin specific manner. These imprinted genes play an important role in embryonic and extraembryonic growth and development, as well as in a variety of processes after birth. Many imprinted genes are clustered in the genome with the establishment and maintenance of imprinted gene expression governed by complex epigenetic mechanisms. Dysregulation of these epigenetic mechanisms as well as genomic mutations at imprinted gene clusters can lead to human disease [33].

The 'fetal origin of disease' hypothesis proposes that adulthood hypertension, insulin resistance, and dyslipidemia, leading to markedly increased rates of cardiovascular disease and non-insulin-dependent diabetes in adult life, originate through adaptation that the fetus undergoes when the environment (for example: nutrition) in early life is poor, caused by either maternal under-nutrition or placental insufficiency. These functional and structural changes of the newborn develop in likely different time windows, mainly during pregnancy, but also in very early childhood [1]. Hocher [28] proposed that an event occurring during a critical early period of life might permanently alter the organ structure and function in response to environmental factors. Such events may lead to cardiovascular / metabolic and renal diseases in later life.

Novel discoveries in the field of molecular epidemiology that can help explain susceptibility to exposures and disease will be demonstrated using the multifunctional enzyme paraoxonase 1 (PON1) as an example [29].

Paraoxonase 1 is an enzyme involved in oxidant defense by hydrolyzing oxidized lipids [41] and also plays a key role in the detoxification of some organophosphate pesticides [12]. Thus, individuals with low PON1 levels and activities may be more susceptible to organophosphate exposures and oxidative stress, which occurs when there is an excess of damaging reactive oxygen species. PON1 genetic variants and

lower enzyme levels have been linked to adverse health outcomes including oxidative stress-related conditions such as cardio-vascular disease and obesity [25, 6, 17, 40, 57]. Therefore, it is of considerable clinical interest to characterize the protective role of endogenous antioxidant enzymes against the development of obesity and metabolic syndrome (MetS) in children. Previous reviews of PON1 research have shown that age and genetics are key factors associated with PON1 variability and, thus, susceptibility [29].

Monk, Spicer și Champagne (2012) consider that studies of the impact of prenatal maternal distress suggest that this form of early life adversity can lead to neurobiological, behavioral, and psychological consequences for infants. Moreover, this distress can also lead to altered mother-infant interactions during the postpartum period, which has been demonstrated to shift developmental trajectories. Thus, it is evident that development is a dynamic process during which shifts in the experiences of the fetus and infant can have profound consequences. In the case of maternal distress, the psychosocial characteristics of the mother can induce these effects, raising the question of how these effects are achieved. An evolving approach that has been applied to address this question involves exploring the biological mechanisms through which environmental exposures shape the activity of genes within the developing organism. This epigenetic perspective has been a significant breakthrough in linking the psychological and physiological experiences of an individual to mechanistic pathways within cells that either enhance or reduce gene expression with consequences for multiple biological and behavioral outcomes [47].

The same source indicates that the developmental origins of disease risk have been established through epidemiological studies in humans and illustrate the profound impact of early life adversity. During prenatal development, the fetus is particularly vulnerable to the effects of a broad range of environmental exposures, with consequences that can persist into infancy, adolescence, and adulthood. In particular, maternal distress during pregnancy, in the form of exposure to chronic or acute stressors, depression, and/or anxiety, can influence both fetal and infant behavioral and physiological outcome measures.

Epigenetics, human diseases and health. Every cell in the organism carries an identical genome, however, despite the stability of these instructions, the terminal phenotype within an organism is not fixed and deviation is caused by gene expression changes in response to environmental cues.

Although there are many possible causes of human disease, family history is often one of the strongest risk factors for common disease complexes such as cancer, cardiovascular disease, diabetes, autoimmune disorders, and psychiatric illnesses. A person inherits a complete set of genes from each parent, as well as a vast array of cultural and socioeconomic experiences from his/her family. Family history is thought to be a good predictor of an individual's disease risk because family members most closely represent the unique genomic and environmental interactions that an individual experiences [36]. Inherited genetic variation within families clearly contributes both directly and indirectly to the pathogenesis of disease [26].

Genetic factors affect but do not determine human behavior, and their effect depends largely on the environment in which individuals live [56]. As animal and human studies show, changes in environmental conditions can influence expression of genes related to various phenotypes [2, 4, 10, 11, 49, 63].

DNA methylation, histone modification and RNA-associated silencing are the major ways these changes are controlled [34]. These mechanisms affect the transcription rate of certain genes involved in the pathogenesis of cardiovascular diseases. Examples of epigenetically regulated genes involved in the pathogenesis of cardiovascular and metabolic diseases are the genes of the renin-angiotensin system (RAS), the peroxisome proliferator-activated receptors (PPAR) system, or the glucocorticoid receptor

[7, 31].

Different cell types execute distinctive programs of gene expression that are highly responsive to developmental, physiological, pathological and environmental cues [45].

Complex human traits are likely to be affected by many environmental and genetic factors, and the interactions among them [43].

Epigenetic modifications are crucial for gene expression regulation during the cell cycle, development, differentiation, and in response to environmental or biological variations [8]. Epigenetic mechanisms are key regulators of pluripotency maintenance and also of cell fate specification [62].

Epigenetic factors known to cause such direct and indirect effects are well-documented but their exact mechanism has not been accurately elucidated.

Epigenetic information regulates the accessibility of chromatin to transcription factors and thereby coordinates gene expression. Consequently, epigenetic changes influence various biological processes, including cell differentiation, aging, and cancer [61].

Cytosine methylation, one of the best-known epigenetic markers, is often associated with gene silencing and is generally found at CpG dinucleotide sites in vertebrate genomes [61].

The study of gene-environment and gene-gene interactions represents a broad class of genetic association studies focused on understanding how human genetic variability is associated with differential responses to environmental exposures and with differential effects depending on variations in other genes [26].

It is known that the epigenome is susceptible to dysregulation throughout life; however, it is thought to be most vulnerable to environmental factors during embryogenesis, which is a period of rapid cell division and epigenetic remodeling [19, 15]. The normal timetable for reprogramming of methylation of non-imprinted and imprinted genes during early development, begins with the primordial germ cells (PGCs) of each of the parents (F0) through gametogenesis, fertilization, the embryonic period of the offspring (F1), followed by the maintenance of methylation in somatic cells and the development of germ cells that will become F2 [32, 54, 58]. These dynamic stages represent windows of potential vulnerability to epigenetic dysregulation [32]. While the maintenance of imprinted genes throughout the preimplantation period is essential for normal embryonic development, demethylation of other genes is needed to make the genome broadly available to the developing embryo. Thus, after fertilization and prior to implantation, the embryo undergoes genome-wide demethylation, with the exception of imprinted genes (which retain the methylation profile of the parent-of-origin) and some retrotransposable elements [19]. Beginning when the embryo is in the blastocyst stage (starting day 5 post fertilization for humans) and before implantation into the uterine wall (about 7 days post fertilization), methylation patterns in non-imprinted genes are reestablished de novo by the DNA methyltransferases DNMT3a and DNMT3b and their cofactor DNMT3L [54, 50]. DNA methylation patterns are maintained by DNMT1, which restores full methylation to hemi-methylated CpG sites following DNA replication; this maintenance is critical for normal development [54, 52].

The epigenetic impact of postnatal mother-infant interactions has also been explored in both humans and animals and, as has been previously described, may be an important consideration in studies of prenatal adversity. Deprivation of parental care, such as that observed in institutionalized infants, has been found to have broad epigenetic consequences. Among institution reared (since birth) children aged 7-10 years, analysis of blood samples indicates an increased DNA methylation throughout the genome when compared to age-matched children reared by their biological parents [48]. Among the differentially methylated genes are those implicated in brain development, including genes within vasopres-sinergic, serotonergic, glutamatergic, and GABAergic pathways. The epigenetic effects of childhood abuse have also been observed in human

brain tissue [46].

If genes are silent and does not synthesize proteins only when stimulated by environmental factors, through epigenetic factors, it means that not only genes but also epigenetic factors play an important role in the development and phenotypic variability of the body [55]. As the H. Wu and Ye Sun [64], show, epigenetics can explain better how cell differentiation runs and how stem cells turn into differentiated cells.

Thus, now, health is regarded as a unique dynamic process that is specific to each person and depends on a lot of genetic and environmental factors. The specificity of influence on health creation manifests itself lifelong starting with the gametogenesis period. Especially intensively occurs accumulation of the specific indices of sanogenicity in the organism's early development period. The source of genetic information on health creation is obtained from parents at the time of zygote formation and is manifested according to the principles underlying genetics.

The creation and the maintenance of health take place in accordance with the genetic program of the organism's development, the achievement of which depends on the environment. Action of the genetic and environmental factors is dialectical by its nature.

Genes which are included in DNA contain genetic information not only about sanogenic indices of the future organism but also about pathogenic ones. There are approximately 2,000 genetic diseases; most of which are manifested at birth or during human development.

More than 900 indices reflecting sanogenicity and pathogenicity are inherited by autosomal dominant type: polydactyly, brachydactyly, disproportionate dwarfism, hemochromatosis, night blindness, exostoses, etc. More than 800 indices are transmitted by autosomal recessive type: albinism, multiple metabolic diseases such as phenylpyruvic oligophrenia, galactosemia, mucopolysaccharidoses etc. There are about 150 known indicators of the human organism's normogenicity and pathogenicity the inheritance of which is caused by genes located in X and Y sex chromosomes.

The autosomal dominant, the autosomal recessive types of inheritance as well as that coupled with X chromosome and the codominant type are characteristic of monogenic indicators, each of which is encoded on chromomere only by the alleles of one locus. In case of the autosomal dominant inheritance type, if one parent is homozygous according to the gene which controls a dominant index and the other - according to the gene which controls a recessive index, then, within the first generation, all the children, in accordance with the first Mendel's law, will possess only dominant clues. If in one parent the dominant gene is heterogeneous whereas the second parent is homozygous, half of the children will possess the dominant index while the other half – the recessive one. Recessive index is manifested when the gene that controls it is homozygous. If both parents are heterozygous for the same indicator, it is possible that 25% of their children will possess the indicator which is controlled by a recessive gene.

Conclusions

Health of the integral organism is determined by interrelations between genetic and environmental factors. The genetic program constitutes the fundamental basis of the human organism's sanogenicity development. Elucidation of the mechanisms through which genes are expressed and how they are influenced by other genes, proteins and environment will serve as a basis for developing strategies of creation, maintenance and directed strengthening of health. In order to estimate the health level, exploratory research for the identification of genetic markers of sanogenicity is needed.

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PRENATAL DIAGNOSIS AND MEDICAL GENETIC COUNSELING

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Introduction

Nowadays, modern biotechnologies as a contemporary stage of the development of science in general, as well as the exponent of scientific and technical progress, having a deep implication in the traditional perception of the sphere of medical genetics [4, 9]. As in other modern scientific specialties, prenatal cytogenetic diagnosis (PCD) has to solve significant challenges and critical issues related to the prevention of genetic diseases and birth of children with numerical and structural chromosomal abnormalities. Thus, PCD allows diagnosing of cytogenetic pathologies, and, firstly, of chromosomal abnormalities in fetuses before birth.

Diagnosis of chromosomal abnormalities, using all the spectrum of biotechnologies, should be performed in prenatal period [2, 6]. The essence and value of PCD is determined mainly by the information on the genotype and phenotypic manifestations in fetuses and preventing the birth of children with genetic diseases. These issues are analysed from all points of view, taking into consideration the vital prognosis and quality of life. In some situations when severe pathologies that are incompatible with life are diagnosed in the fetus, therapeutic abortion can be used as solution, since the main priority is healthy life without disability. The decision to save the pregnancy is a problem for the couple, for the parents and/or for the future mother. The methods of PCD represents some reliable and widely applied tests, and during the counseling the medical geneticist giving the comprehensive and precise information for the patient about the role, risks and benefits, indications and contraindications of these investigations [10, 13]. During the medical genetic counseling the basic principle is to create the reliable relation between the physician and the patient.

Introducing the new methods of prenatal diagnosis and genetic testing have led to the emergence of a whole range of ethical issues, related to the increase of the misuse of genetic information and discrimination based on genetic characteristics [12]. One of the main principles to correct the negative processes, caused by using the new biomedical technologies in the practice of medical genetics, is correct, comprehensive, authentic information of patients, their relatives, patients from various social and professional groups, the the community and the public as a whole with respect to achievements, capabilities and limitations of modern genetic technologies. In this context, significantly increased the role of medical-genetic counseling since it ensures informing and advising the patient and his family about the hereditary pathology that contribute to making certain decisions about genetic testing and reproductive health. Intervention effect on genes, in patients with genetic hereditary diseases represents one of the largest fields of research of the 21st century [1, 7, 14].

Diagnosis of fetal chromosomal abnormalities should be population-wide through implementing the technologies of prenatal cytogenetic diagnosis. Of invasive prenatal diagnostic methods most commonly administered amniocentesis with fetal karyotype study at 16-18 weeks of gestation. Knowledge of prenatal testing in I and II trimesters of gestation and their carrying out should become a priority. It is known that 5-10% of children with chromosomal abnormalities are born in families of "high-risk groups",

and 95%-90% are born in families that did not have indications for medico-genetic counseling [4, 9]. This is explained by the fact that in any family and in healthy couple, the genetic risk is 3-5%. Anyone can accidentally fall into the category of increased genetic risk, and addressing to specialist and respecting the indications by the pregnant woman justifies the need for prenatal tests. Prenatal screening offers the possibility of early diagnosis of severe fetal pathologies in early stages of pregnancy. At the same time, it allows to select a specific group of pregnant women for invasive prenatal diagnosis, i. e. amniocentesis, using cytogenetics investigation in culture of fetal amniocyte cells [5, 11].

Prenatal cytogenetic diagnosis has an exceptional significance for the medical-genetic counseling since it allow transition from calculations of risk by means of formal genetics to precise individual prognosis for the health of individual child in groups of high genetic risk for chromosomal abnormalities. The selection of pregnant women suitable for prenatal cytogenetic diagnosis is carried out by geneticists conform clinical indications such as: advanced maternal age, historical information concerning the birth of children with chromosomal pathology, parent carriers of balanced chromosomal aberrations or mosaics, ultrasound markers for chromosomal pathology, mutagenic factors which have had an impact on the embryo in the first trimester of pregnancy, history of the birth of children with isolated or multiple congenital anomalies, and couples with previous repeated miscarriages etc. Medical-genetic counseling represents the main topic in the group of indirect methods of investigation of the pregnant woman for the prevention of chromosomal abnormalities [3, 8, 15].

Based on the above-mentioned issues, the aim of the present work consists in highlighting the role of the PCD in identifying chromosomal abnormalities at the early stages of development, while respecting the principles of good bases of prenatal medical genetic counseling. To achieve this goal, were developed the following objectives:

1. As early as possible (up to 16 weks of gestation) diagnosing of possible markers for chromosomal abnormalities in fetuses using of clinical and pedigree analysis, ultrasound, and biochemical markers to determine the indications for cytogenetic analysis (PCD with fetal karyotype study);
2. Studying and respecting aspects of good principles of prenatal medical genetic counseling;
3. Determining the incidence of chromosomal abnormalities in fetuses in prenatal period during 21 weeks of gestation;
4. Evaluating the results of invasive prenatal genetic diagnosis in pregnant women during the first and second trimesters of pregnancy and studying the sharing of fetal chromosomal abnormalities;
5. Developments of algorithms of prophylaxis and genetic diagnosis in pregnant women at risk within the medical genetic counseling.

Materials and methods

During the the investigation we are used the prospective medical genetic counseling, aimed at identifying the target group of 12938 of pregnant women at risk, in which was administered the investigation at the Center of Human Reproduction and Medical Genetics of the Institute of Mother and Child, in the period from 2005 to 2014. Of these, in 4731 (36.6 ± 0.4%) of women have made prenatal cytogenetic diagnosis: amniocentesis and chorion villi sampling. Patients were divided into two clinical groups:

- Group I – 4731 (36,6 ± 0,4%) pregnant women at medium and high genetic risk with positive family and obstetrics history of complications (risk grade > 6 %);
- Group II – 8207 (63,4 ± 0,4%) pregnant women at low genetic risk (risk grade < 6 %).

During the medical genetic counseling of pregnant women was obtained the necessary information concerning the data which allowed the construction of pedigree of each family. Obtaining the data (i. e., family and obstetrical history), studying the labo-

ratory values and the information from genetic analysis allow the calculation of degree of genetic risk in different groups of studied women.

Inclusion criteria for PCD administering to pregnant women were: complicated course of pregnancy with risk factors from obstetrical history, stopping of fetal development, empty embryonic sac, spontaneous abortions in obstetric history, oligo- and polyhydramnios; ultrasound markers of chromosomal abnormalities; advanced parental age (maternal age over 35 years and paternal age over 40 years); the presence in the family of children with mental and physical delay; one of the parents is a carrier of structural balanced chromosomal aberration; the history of birth of children with congenital anomalies, neural tube defects, spina bifida; contact with ionizing irradiation during pregnancy; the use of medicines with teratogenic potential before or after conception etc.

For diagnosis of chromosomal abnormalities in fetuses it is proposed to use the complex methods of prenatal diagnosis, invasive as well as non-invasive, which are complemented to each other.

Ultrasound examination is recommended to all pregnant women to determine the exact term of pregnancy, to determine the fetal development and to diagnose the fetal anomalies. Ultrasonography of the fetus is recommended in stages of pregnancy where this method is most informative for imaging the fetal anomalies and ultrasound markers for chromosomal aberrations, i. e., in 11 – 13 weeks plus 6 days of gestation, at 16 – 18 weeks and in 20 – 21 weeks of gestation.

Prenatal cytogenetic diagnosis, i. e. amniocentesis with fetal karyotype study was conducted in 4731 women at medium and high risk during 16 – 18 weeks of gestation.

Results and Discussion

The indications for medical genetic counseling were determined by general practitioners and gynecologist, conform to the program of obligatory medical insurance UNIC.

Table 1. Distribution of pregnant women by age

Studied groups	Age, years								
	17 – 24 years		25 – 29 years		30 – 35 years		36 – 44 years		Mean age
	abs.	%	abs.	%	abs.	%	abs.	%	M±m %
Group I (n = 4731)	767	16,2	937	19,8	2171	45,9	856	18,1	29 ± 5,6
Group II (n = 8207)	3085	33,2	3273	35,3	1803	28,3	46	3,2	23 ± 4,8

The age of pregnant women at medium and high risk which were included in the study ranged from 17 to 44 years (average 26,1 ± 5,3 years). The term of pregnancy at the time visit to geneticist was from 6 to 22 weeks of gestation (mean value 14 ± 5,1 weeks of gestation).

It is known that there is a correlation between the age of pregnant women and the risk of fetal anomalies. Based on this fact we are divide the overall group of pregnant women to two studied groups by age. At the same time, we note that more than half of the women were aged between 25 – 35 years (65,7% in Group I –and 63,6% in Group II). The same trend was mentioned for both studied groups, the highest share of women studied were aged 25 – 29 years (35,3% in Group I vs. 19,8% in Group II); the second place occupies the number of pregnant women aged from 30 to 35 years, i. e., 28,3% for Group II and 45,9% in Group I, and the lowest share of pregnant women recorded were from 36 to 44 years (18,1% in Group I vs. 3,2% in Group II). The share of pregnant

women aged from 17 to 24 years was 33,2% in Group I and 16,2% in Group II (fig.1).

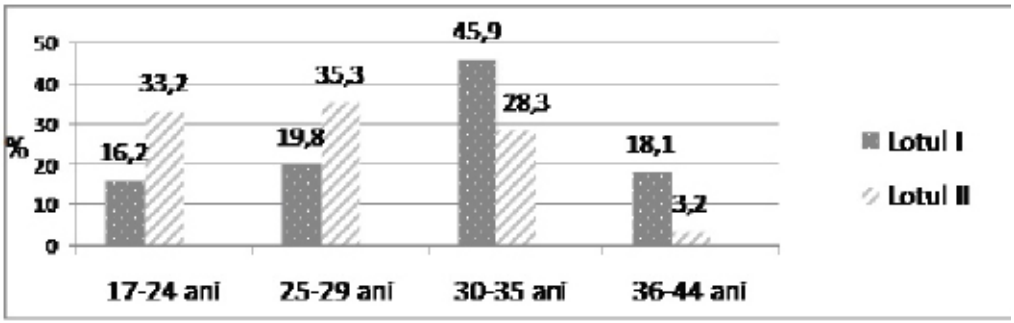

Fig. 1. Distribution of pregnant women by age.

We draw attention to the fact that in healthy families and couples genetic risk is 3 – 5% [1]. Anyone can accidentally fall into the group of high genetic risk, and addressing to geneticist and respecting the indications by the pregnant woman reasoned the need for prenatal genetic tests. Despite these well-known facts, only 11% of the studied women were consulted by the geneticist before conception, and other 8% of women used folic acid before conception. Thus, our data suggests that only 19% of pregnant women have had folic acid supplement in dose of 400 – 800 mcg daily before planning their pregnancies, 57% of pregnant women takes the folic acid supplement during the first trimester of pregnancy and 24% of pregnant women have not given folic acid supplement during pregnancy (fig. 2).

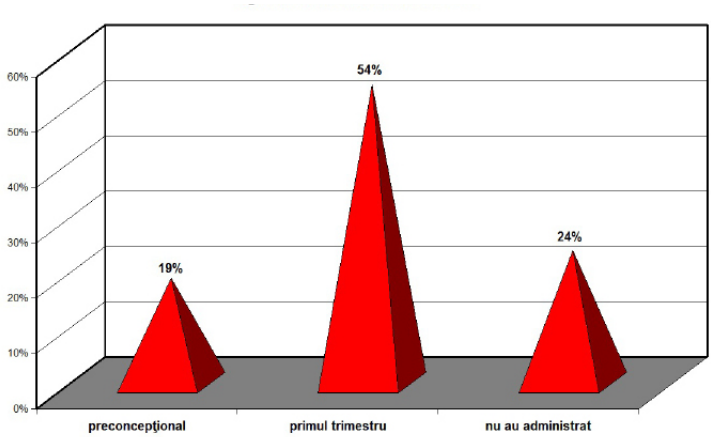

Fig. 2. Folic acid supplementing.

Another important aspect is that administration of prophylactic doses of folic acid before conception prevents occurrence of neural tube defects, spina bifida, etc. [5]. According to data from the literature, the deficiency of active folate in women before conception and during the first trimester of pregnancy can lead to CA of nervous system, anencephaly and other anomalies, including folate dependent CA of cardiovascular system [2, 6].

Ultrasound examination before 22 weeks of gestation allow prenatal detection of markers for chromosomal aberrations and anomalies of different organs and systems of fetus. USG was used in studied pregnant women at informative terms of gestation, i. e., in 12 – 14 weeks of gestation, in 17 – 18 and in 20 – 21 weeks of gestation. We are noted the fact that both in the Group I and in Group II were diagnosed CA in the fetuses which are incompatible with life, 247 cases in Group I and 164 cases in Group II. It

has been demonstrated that fetal ultrasound performed at 11 – 14 weeks of gestation was enable to reveal markers specific for CA in the fetus and markers of chromosomal aberrations in 86% of cases.

Thus, ultrasound examination allow to detect severe fetal pathologies in 12 – 14, 17 – 18 and 20 – 21 weeks of gestation in Group I and II of sudied pregnant women, i. e., in terms of gestation where markers of CA and chromosomal aberrations can be detected.

The indications for invasive methods of prenatal diagnosis were: diagnosed severe fetal anomalies, abnormal values of biochemical parameters (i. e., low or high levels of serum alpha-fetoprotein), and ultrasound markers for chromosomal diseases, such as thickening of neck zone, poorly visualized nasal bones, cystic formations in choroids plexus, cerebral ventricular enlargement, oligo- and polihydramnios, etc.), and in this case the risk for pregnancy was rises up to 0,6%. 4731 (36,6 ± 0,4%) of studied pregnant women passed the invasive prenatal diagnosis procedures, i. e., amniocentesis with karyotype study of the fetus.

Table 2. Methods of prenatal diagnosis, 2005 – 2014

Used invasive method	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	Total
Chorion villi sampling	22	24	19	21	29	27	3	7	14	9	175
Amniocentesis	305	369	434	482	398	389	443	531	616	764	4731
Total	327	393	453	503	427	416	446	538	630	773	4906

In Table 3 shown that during the period from 2005 to 2014 in the Center of Human Reproduction and Medical Genetics, Institute of Mother and Child, were carried out 4906 procedures of cytogenetic investigations, including 175 procedures of chorion villi sampling and 4731 procedures of amniocentesis.

Chromosomal abnormalities are genetic changes that occur as a result of specific chromosomal mechanisms: abnormal segregation of chromosomes in meiosis or mitosis, the aberrant chromosomal recombination, erroneous repairing of chromosome breakage. We are note especially that chromosomal abnormalities can occur spontaneously as a result of the "de novo" mutation.

Diagnosis of chromosomal abnormalities in fetuses by cytogenetic methods with the study of fetal karyotype in amniocyte cell cultures has contributed to reveling aberrations of fetal karyotypes before their birth. In the following table are presented cases of fetal chromosomal abnormalities which were diagnosed during the years 2005 – 2014 in 164 pregnant women (3,5±0,3%).

Table 3 shows that from the total number of studied women the most frequent abnormalities are cases of aneuploidy, of which autosomal trisomies are the most common, i. e. Down syndrome in 75 cases (1,6±0,2 %), Patau syndrome in 9 cases (0,2±0,06%) and Edwads syndrome in 20 cases (0,4±0,09%). Have been diagnosed also gonosomale abnormalities, i. e. Turner syndrome in 8 cases (0,17±0,06%) and Klinefelter syndrome in 10 cases (0,2±0,07%). During the years 2005 – 2014 have been diagnosed prenatal the triploidy in 6 cases (0,1±0,05%) and 31 cases (0,6±0,1%) of other structural chromosomal syndromes.

In situations when is diagnosed numerical or structural chromosome abnormality which is incompatible with the life, the abortion may be a therapeutic option, with legal support, but controversial in ethic terms. During the medical genetic counseling these aspects can be analysed from all points of view, taking into account the vital prognosis and quality of life. The patients were offered medical genetic counseling. The decision to save the pregnancy is depend from the preferences of the couple, of parents and/or mother.

We are note that methods of prenatal diagnostic are considered as safe tests which applied in current practice, and geneticist during the medical genetic counseling give correct and comrehensive information about role, advantages, riscs, indications and contraindications of investigations.

Table 3. Changes of number of cases of fetal karyotype abnormalities revealed by prenatal diagnosis in 2005 – 2014

Chromosomal abnormalities	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	Total/ mean
	abs, (%)	abs, (%)	abs, (%)	abs, (%)	abs, (%)	abs, (%)	abs, (%)	abs, (%)	abs, (%)	abs, (%)	M±m %
Down syndrome	3 (0,9)	7 (1,8)	7 (1,5)	4 (0,8)	5 (1,2)	5 (1,2)	5 (1,1)	10 (1,9)	14 (2,2)	15 (1,9)	75 (1,6±0,2)
Patau syndrome	1 (0,3)	-	2 (0,4)	-	-	2 (0,5)	1 (0,2)	-	1 (0,2)	2 (0,3)	9 (0,2±0,06)
Edwards syndrome	-	4 (1,1)	3 (0,7)	3 (0,6)	1 (0,2)	2 (0,5)	1 (0,2)	3 (0,6)	2 (0,3)	1 (0,1)	20 (0,4±0,09)
Turner syndrome	1 (0,3)	2 (0,5)	1 (0,2)	1 (0,2)	1 (0,2)	1 (0,2)	-	-	-	1 (0,1)	8 (0,17±0,06)
Klinefelter syndrome	2 (0,6)	-	3 (0,7)	2 (0,4)	-	-	1 (0,2)	-	1 (0,2)	1 (0,1)	10 (0,2±0,07)
Triplo-X syndrome	-	-	-	-	-	1 (0,2)	-	3 (0,6)	-	1 (0,1)	5 (0,1±0,05)
Cases of Triploidy	2 (0,6)	-	-	1 (0,2)	1 (0,2)	-	1 (0,2)	-	-	1 (0,1)	6 (0,1±0,05)
Other syndromes	4 (1,2)	2 (0,5)	4 (0,9)	1 (0,2)	2 (0,5)	2 (0,5)	2 (0,4)	5 (0,9)	3 (0,5)	6 (0,8)	31 (0,6±0,1)
TO-TAL	13 (3,9)	15 (3,8)	20 (4,4)	12 (2,4)	10 (2,3)	13 (3,1)	11 (2,5)	21 (3,9)	21 (3,3)	28 (3,6)	164 (3,5±0,3)

Prenatal diagnosis includes non-invasive screening tests, such as ultrasound and biochemical tests as well as invasive diagnostic methods, such as amniocentesis and chorionic villi sampling [10, 3]. Prenatal screening offers the possibility of early diagnosis of severe fetal pathologies at early terms of pregnancy, and allow to select a

specific group of pregnant women for invasive prenatal diagnosis, i. e. amniocentesis, which is one of the most effective medical measures for prevention of genetic pathologies (Down syndrome and other chromosomal aberrations), which is administered for pregnant women with a high genetic risk. Prenatal cytogenetic diagnosis procedures have allowed detection of chromosomal pathologies of fetuses in the early period of intrauterine development (16 18 weeks of gestation), without harming the health of the mother.

The present study allowed us to use and introduce in practice in the framework of medical genetic counseling some algorithms for the prevention and diagnosis of birth defects and chromosomal abnormalities, which are adapted to the condition of our current activity.

Stages of the algorithm presented have facilitated the tactics of medical service for the pregnant women at risk, which were addressed to the medico genetic counseling. Pregnant women have been informed, counseled and investigated for diagnostic purposes on early period of fetal development. During the medical genetic counseling we pointed out that preventive measures taken before conception and during the first trimester of pregnancy which are necessary for preventing the congenital anomalies.

According to its basic principles, the medical genetic assistance, including prenatal diagnosis should be offered to those who need it, according to medical indications, regardless of income levels of patients and from other social and legal conditions. Prenatal diagnosis (PD) is based on the principle of "voluntary". The essence and value of prenatal diagnosis is determined mainly by the information on the genotype and phenotypic manifestations in fetuses and preventing the birth of children with genetic diseases. These issues are explored in all aspects taking into consideration the vital prognosis and quality of life.

Medico-genetic counseling should be administered before the prenatal diagnosis. Geneticist offers the information of women related to the pathology, disease evolution, including the terms of manifestation [3]. After confirming the diagnosis and making the decision of family or couple should respect the right and legal issues in according to the local normative acts of the country. Only parents and by no means health workers, take decision on fate of the fetus. In a case of indication can e used repeated medical genetic investigation in the case of invasive prenatal diagnosis and other non-invasive genetic and laboratory tests. Similarly, the family receives full information and signs the Informed Consent to genetic testing or investigation, and the geneticist is guided by the basic principles and must offer the following family information:

- The precise name(s) and general features of pathologies which may be diagnosed as a result of the PD. Will mention the influence over the future status of the child, parents and members of his family.
- Genetic risk calculation and description of the probability that the child may be affected. The risk can also be expressed as a percentage, proportion or in words.
- Possibility of unfavorable results and sporadic cases of "de novo" mutations. Probability of obtaining the laboratory end ultrasound data informative for the diagnosis and prophylaxis of hereditary pathologies.
- Resources for improvement of child born with genetic pathology, including pharmaceutics treatment and social support, from which parents will benefit.
- Possible measures to solve the problem, if the child will be affected. For example, the birth a child in family or state medical institution, refuse and protection of rights, termination of pregnancy, treatment of fetus during the pregnancy or immediately after the birth.
- Explaining the laws of hereditary transmission of pathologies (Mendelian, complex, "de novo" mutations), and principles of treatment, resistance to symptomatic therapy of hereditary pathologies, as majority of genetic pathologies and chromosomal abnormalities are not treated during prenatal period.
- No genetic tests cannot guarantee the health of children, as well exists some

persons with gene mutations which have not phenotypic and clinical manifestations. Moreover, the specialists can have only partial information about the family and about genetic risk (in situations where one or both spouses are healthy from phenotypic point of view, but are carriers of chromosomal structural mutations, Robertsonian translocations which confer the risk to transmit the gene mutation to offspring, in more severe form).

• Information about existence the programs of non-invasive prenatal diagnosis, and biochemical screening is only first stage of prenatal diagnosis which not allow to make individual precise diagnosis.

• Name and contact address of the specialized institution for the persons in the case of diagnosis of pathology in fetus.

The above principles and aspects of regulation medical and genetic counseling and prenatal diagnosis, having a general character and are not include all the organizational and clinical problems which are present in current practice of geneticist. Of course it is necessary to consider the possibilities of genetic tests that are changing and growing so fast.

The achievements of the modern biotechnologies development offers new opportunities which can influence on human beings. On the one hand, implementation of new biomedical methods open new ways and directions of research, and, on the other hand, presents a real danger not just for physical health, but also for the spiritual human values and for preserving the moral traditions of the society. Such hazard is often accompanied by epochal scientific discoveries, such as, for example, the accomplishments in the field of nuclear physics. Scientific discoveries in itself represents only one tool in the hands of mankind. The future and the fate of humankind depend largely on how we handle our powerful force, as well as biomedical technologies, including genetic technologies.

Conclusions

Methods of prenatal cytogenetic diagnosis, i. e., studying of fetal karyotype, and medical genetic counseling contribute to diminishing the prevalence of chromosomal aberrations in newborns.

Thanks to the methods of PCD it was possible to take the measures for prevention of birth 164 (3,5±0,3%) children with chromosomal anomalies diagnosed during prenatal period before 21 weeks of gestation.

Studying the sharing of fetal chromosomal aberrations before 22 weeks of gestatn in was showed the fact that more prevalent were the cases of Down syndrome – 75 cases (1,6±0,18%), Edwards syndrome – 20 cases (0,4±0,09%) and structural chromosomal abnormalities – 31 cases (0,7±0,12%).

The principles and fundamental values become advisable to follow in current practice within the medical and prenatal genetic counseling in order to rationalize and modernize the medical service as well as by reason of the necessity of readjusting the local medical system to international provisions.

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GENETIC ISSUES IN PEDIATRIC HYPERTROPHIC CARDIOMYOPHATY

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Introduction

Cardiomyopathy is defined by structural and functional abnormalities of thr ventricular myocardium that are unexplained by flow limiting coronary artery disease or abnormal loading conditions [8]. The recent ESC Guideline (2014) adopt the classification system in which all cardiomyopathies are divided by specific morphological and functional criteria and then grouped into familial/genetic and non-familial/non-genetic subtypes, including of the presence of extracardiac diseases. The hypertrophic cardiomyopathy (HCM) is defined by the presence of increased left ventricular (LV) wall thickness that is not solely explained by abnormal loading conditions. This definition is available for adults and children, but no specific etiologic factor [9].

HCM is a common genetic cardiovascular disease with the overall prevalence estimated between 0.02-0.23% in adults [13, 28]. In pediatrics registries, the prevalence of HCM in children is unknown, but population-based studies report an annual incidence of 0.3 to 0.5 per 100,000 [11, 18]. Recent publications indicate that the HCM accounts for 42% of childhood cardiomyopathy and has an incidence of 0.47/100,000 children [6].

The HCM is marked by phenotypic and genotypic heterogeneity. In up of 60% of adolescent and adults with HCM, the disease is an autosomal dominant trait caused by mutation in cardiac sarcomere protein genes (Table1) [12, 25].

Table 1. Casual genes for Hypertrophic Cardiomyopathy

Gene	Symbol	Frequency
Established Causal Genes		
ß-Myosin heavy chain	MYH7	~25%
Myosin-binding protein C	MYBPC3	~25%
Cardiac troponin T	TNNT2	~3%-5%
Cardiac troponin I	TNNC1	~ 3%-5%
α-Tropomyosin	TPM1	~1%
Myozenin 2 (calsarcin 1)	MYOZ2	1:250
Myosin light chain 1	MYL3	Rare
Myosin light chain 2	MYL2	Rare
α-Actin	ACTC1	Rare
Titin	TTN	Rare
Telethonin	TCAP	Rare
Possible causal Genes		
Myosin light chain kinase 2	MYLK2	Rare
α-myosin heavy chain	MYH6	Rare
Cardiac troponin C	TNNC1	Rare
Caveolin 3	CAV3	Rare
Phospholamban	PNL	Rare

Many genes are involved in HCM and all types of mutation have been reported (missense, frameshift, nonsense, splice site, and small deletions and insertions). The two most frequently mutated genes are MYBPC3 and MYH7 [17]. But most of HCM patients are heterozygous for a mutation, in 3-5% of the cases, patients carry two mutations in the same gene, or different genes (digenic). The association of the different mutations is characterized with a more severe phenotype with younger age of onset and more adverse events [12, 20]

The diagnosis of HCM is a clinical one, made on the basis of an increased LV wall: 15 mm or more, or 13 mm or more in relatives of HCM patients. This diagnostic condition is assessed using echocardiography or cardiac magnetic resonance imaging. Management of children with HCM is indispensable to the clinical evaluation of extracardiac manifestations and signs suggestive of etiology can direct specific cases of HCM (Table 2).

Table 2. Examples of signs and symptoms suggestive of specific diagnoses [9]

Symptom/sign	Diagnosis
Learning difficulties, mental retardation	• Mitochondrial diseases • Noonan/LEOPARD/Costello syndrome • Danon disease
Sensorineural deafness	• Mitochondrial diseases (particularly with diabetes) • Anderson-Fabry disease • LEOPARD syndrome
Visual impairment	• Mitochondrial diseases (retinal disease, optic nerve atrophy) • TTR-related amyloidosis (cotton wool type vitreous opacities) • Danon disease (retinitis pigmentosa) • Anderson-Fabry disease(cataracts, corneal opacities)
Gait disturbance	• Friedreich's ataxia
Paraesthesia /sensory abnormalities/ neuropathic pain	• Amyloidosis • Anderson-Fabry disease
Carpal tunnel syndrome	• TTR-related amyloidosis (especially when bilateral and in male patients)
Muscle weakness	• Mitochondrial diseases • Glycogen storage disorders • FHL1 mutations • Friedreich's ataxia
Palpebral ptosis	• Mitochondrial diseases • Noonan/LEOPARD syn (1978) drome • Myotonic dystrophy
Lentigines/café au lait spots	• LEOPARD/Noonan syndrome
Angiokeratomata, hypohidrosis	• Anderson-Fabry disease

However, it should be underscored that in principle, any degree of wall thickness is compatible with the presence of the HCM genetic substrate clinical and genetic testing. Owing to age-dependant penetrance, a negative clinical test does not exclude the possibility of developing HCM at a later age. Patients may develop sudden arrhythmic death, progressive heart failure, and atrial fibrillation. Treatment is based on symptoms, and whether left ventricular outflow tract obstruction, family history and risk for sudden cardiac death are present. Possible modalities are pharmacologic, surgical myectomy or percutaneous alcohol septal ablation, or a combination of therapies, and implantable cardioverter defibrillator (ICD) [12, 14, 24].

The clinical sensivity can by depended on variable factors such as age or family history. At the same time, genetic testing plays an important role in management strategy and prognosis, differentiated by age (Table 3).

In the positive genetic test, the child need at regular cardiological evaluation to detect clinical and echocardiographic signs and to estimate the risk of sudden cardiac

death (SCD). If the genetic tested are negative, relatives without the mutation can be discharged from cardiological follow-up.

Table 3. Proposed Clinical Family Screening with Echocardiography or Cardiovascular Magnetic Resonance (and 12-lead Electrocardiography) for Detection of HCM Left Ventricular Hypertripy *[16]

Age < 12 yrs Optional unless: Malignant family history of premature death from HCM, or other adverse complications Competitive athlete in an intense training program Onset of symptoms Other clinical suspicion of early left ventricular hypertrophy
Age 12-21 yrs ǂ Every 12-21 month
Age >21 yrs Imaging at onset of symptoms, or possibly at 5-yrs intervals at least through midlife< more frequent intervals for imaging are appropriate in families with malignant clinical course, or history of late-onset HCM

Predictive genetic testing of relatives is only possible if a pathogenic mutation has been identified. For risk stratification, there are major and possible risk factors. Risk factors in adult studies are well described. Studies have been inconsistent in children, which may reflect the varying etiologies of HCM. All children with HCM should be guided by the best interests of each child in accordance with international standards for good practice.[4, 5, 7, 22, 25, 27].

Material and methods

A retrospective study was performed on 23 children with HCM, aged before 19 years, hospitalized in Department of Pediatric cardiology of Child and Mother Institute (2008-2012). HCM was defined as primary, inappropriate hypertrophy in non-dilated heart with normal or exaggerate systolic function in the absence of valvular outflow obstruction or underling systemic disease, with absolute wall thickness above +2 SD for age, and exceeding the 95th centile [1, 3, 10]. All subjects underwent detailed assessment that included clinical history (symptoms, when they started, date of diagnosis of the disease, family history data on evolution, past and present therapy, etc.), clinical examination, 12-lead electrocardiogram (ECG) and transthoracic echocardiographic study (2D, M- mode, and Doppler); ECG Holter monitoring. Each clinical case was analyzed with reference to detection the presence of unfavorable risk factors at primary diagnosis.

Results and discussion

The study group included 23 children, aged between 1, 5 month to 17,8 years, male prevalence. The average age of children was 7.2 years and 9(39,1%) are infants. Clinical characteristics of the patient population are listed in Table 4.

It is important to note that nearly half of the children (11 children) were asymptomatic. The most common symptoms were cardiac murmur, palpitations or chest pain or discomfort. Three patients presented symptomatic heart failure due to systolic dysfunction. Symptoms caused cardiac examination family, who confirmed this positive family history in five patients, including cardiac death in young age.

The 12-lead ECG is a fundamental initial diagnostic strategy for early evaluation. In our study only one patient had a normal ECG, but more than 95% of children had the abnormal ECG, preponderant signs of LV hypertrophy (98%). Simultaneously, standard ECG and Holter ECG monitoring are confirmed arrhythmia in some children (7 pts), including 5 children with asymptomatic tachycardia (supraventricular and ventricular),

an infant with asymptomatic Wolff-Parkinson-White (WPW) syndrome.

More recently, clinical suspicion sufficient to trigger genetic testing can be raised by: WPW pattern in PRKAG, important in some glycogen storage diseases [2, 26]. The signs of LV hypertrophy and arrhythmias also considered a marker for early ventricular dysfunction and risk factor for sudden death in children [7, 21, 23].

Table 4. Patients clinical characteristics

Female, n (%)	**9(39)**
Age at diagnosis, yrs	
Cohort mean, yrs (±2SD)	7,2±4,3
Age ≤1 yr , n (%)	9(39,1)
Age >1 and ≤ 5yrs, n (%)	2(8,7)
Age >5 and ≤ 10yrs, n (%)	-
Age >10 and ≤18yrs, n(%)	12(52,1)
Presenting signs and symptoms	
Asymptomatic	11(47,8)
Murmur, n(%)	10(43,5)
Family history of HCM, n(%)	2(8,7)
Family history of sudden death, n(%)	3(13)
Syncope/near syncope, n(%)	2(8,7)
Dyspnea on exertion, n(%)	2(8,7)
Palpitation/chest disconfort, n(%)	5(21,7)
Heart failure, n(%)	3(13)
Echocardiographic data	
Left ventricular morphology	
Asymmetric septal hypertrophy, n(%)	20(87)
Concentric left hypertrophy, n(%)	3(13)
Left ventricular outflow tract obstruction, n(%)	13(56,5)
Septal wall z-score in diastole,	
Mean ±2SD	11,3±4,7
Posterior wall z-score in diastole,	
Mean ±2SD	4,2±2,4
Septal/posterior wall ratio,	3,1±1,9
Mean ±2SD	
Fractional shortening,	
Mean±2SD	45±8,9
Other cardiac anomaly, n(%)	1(2,3)

Echocardiography (2D, M-mode and Doppler) is considerate at key to the noninvasive diagnosis of HCM. This test allow for the morphology, structural abnormalities and hemodynamic disturbances in HCM, some of which have profound prognostic value [1, 3]. All children in our study had normal or supernormal systolic function in obstructive and nonobstructive variants in HCM. Most patients (87%) had a asymmetric form of LV, typically involving the septum. Distribution and extend of LV hypertrophy was diverse. Among the 23 study patients, the basal anterior septum showed the highest average maximal LV septum thickens (range 6,3 to 40 mm), elevated according to BSA and 5 of them have the LV wall thickness more 30 mm. In some time the concentric LV hypertrophy (3pts) explains early cardiac dysfunction and heart failure. Many pediatric studies have demonstrated the importance of the patterns of LV hypertrophy in estimate the risk factors and treatment [14, 15].

Phenotypic expression and presence of family history of HCM genetic consultation conditioning of children included in the study. The risk of SCD in patients with HCM is significantly higher in the 8-16 year age range than in the 17-30 year age range, which is a strong argument for family screening to be carried out at an early age in families with HCM [19, 27] Summarizing the results of the investigations and clinical data, we

identified the presence of adult risk factors for sudden cardiac death. At the same time, according to pediatric publications in HCM in children are discussed and other risk factors, such as signs and symptoms at early age, almost 50% of our cases. The management strategy in HCM provides highlighting risk factors, of our patients who were present: syncope, family history, extreme parietal hypertrophy, left ventricular outflow obstruction output and arrhythmia. We are guided by the recommendations of pediatric studies that have mapped this HCM various diseases in children, including extracardiac involving other systems and organs. According to these descriptions, 3 suspected mitochondrial disease 2 infants with family history of HCM, where CMH has been associated with neurological disorders, muscle and eyes. Patients with multiple conditions and risk factors for SCD and their parents underwent clinical examination, EKG and genetic including blood samples was collected for DNA extraction in order genetic diagnostic reference centers after possibility. The guiding principle is that a genetic or a clinical test in a child should have an impact on management; lifestyle and further clinical screening All HCM patients and relatives should be fully informed by virtue of some from genetic counseling. Certified genetic counselors play an important role by collecting detailed family planning. The multidisciplinary approach has a considerable advantage in everyday assessment of children with HCM.

Study limitation

This study is a retrospective descriptive analysis and has limitations intrinsic to such an analysis. There are a low number of patients, incomplete family history, clinical evaluation was not performed at the same time, imaging tests were not conducted by a similar protocol. The most important limitation is the lack of results of genetic tests which would help in confirming the etiology of children HCM cohort described.

Conclusions

HCM is a complex and heterogeneous disease, pediatric occurs at all ages, it is characterized by marked diversity in clinical presentation, often is asymptomatic, mainly with morphological pattern of asymmetric left ventricular hypertrophy, with or without obstruction. Sometime HCM associated extracardiac manifestations, which the non-sarcomeric etiology confirming in pediatric cases of HCM. All patients with HCM should to take advantage of genetic consultation, important to refine diagnosis and prognosis, and to provide optimal management for families, including multidisciplinary approach.

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ULTRASTRUCTURAL EVALUATION AND HISTOLOGICAL PECULIARITIES OF ANDROGENETIC STRUCTURES OF BARLEY

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Introduction

Androgenisis is based on three crucial events: i) repression of gametophytic development and leads to the dedifferentiation of the cells; ii) restore of cell divisions and of embryonic potential; iii) formation of embryo-like structures. As shown by several experiments, embryogenic development during in vitro androgenesis is induced by the application of different types of abiotic stresses, including heat shock, cold, and starvation, among others [6]. The stress converts a cell destined to produce a male gametophyte, i.e. a pollen grain, into an embryogenic cell that will develop into a sporophyte, i.e. an embryoid [8, 9]. Reprogramming of cellular metabolism can occur through the repression of expression related to starch biosynthesis and the induction of proteolytic genes (e.g. components of the 26S proteasome, metalloprotease, cysteine, and aspartic proteases) and stress-related proteins (e.g. GST, HSP, BI-1, ADH) [6].

It is know that androgenesis can run in two ways: through reprogramming and direct proliferation of microspores or callus induction as indirect result of dediferentiation of somatic cells of anther. In the first case, microspores after the second meiotic phase, proliferate within exine and form multicellular structures, which subsequently lead to embryogenic structures and exine degeneration. In the second, proliferation of somatic cell of anther conducts to the callus formation. Unfortunately, trigger mechanism of dedifferentiation remains unclear and the induction of microsporal embryogenesis is a major task in solving androgenesis [4, 7].

Therefore, the embryogenic microspore is an extremely complex in vitro biological system where cellular responses to abiotic stresses co-exist with the reprogramming towards a new developmental fate and the cessation of the old programme. All these simultaneous changes must be reflected in a dramatic remodeling of cell architecture.

Considering that histological characterization of androgenic structure has been little reported the purpose of work is to study the ultrastuctural aspects of androgenetic structures with different embryogenic capacity for barley cultivars.

Materials and methods

The spring (Galactic, Unirea, Sonor) and winter (Stralucitor) barley cultivars served as biological material.

Donor plants, for culture in vitro of anthers, were grown in controlled conditions, in optimal conditions concerning photoperiod, light intensity, temperature and nutrition, according standards recommended by Cistue et al. [3]. Spikes were collected when most of the microspores were at the mid to late-uninucleate stage. For cytological identification of meiosis stages the anthers were fixed in Carnoy solution (3:1) and stained with aceto-carmine. Collected spikes were rinsed in water with three drops of Tween-20 (0,1%) and under running tap water for 10 min; after that the spikes (60-80 mm long) were sterilized with 70% ethylic alcohol followed by 5,2% sodium hypochlorite (dilution with distillate water 1:1). At last, in laminar airflow hood the anthers were excised and incubated in culture medium.

For induction the dedifferentiation and reclaim sporophytic development were used different schemes of pretreatment and various hormone balances of nutrient medium. According to the obtained data, positive response of anthers to *in vitro* condition was generated by cold pretreatment and Mannitol inanition. The main effective schemes

were found: i) T=4 °C, Mannitol 0.34M, followed by the passage supplemented with 0,17M Mannitol; ii) T=4 °C, during 10-14 days or iii) Mannitol – 0,17M. As induction medium we used three variants: C3 (Jacquard et al., 2003), N6 (Chu, 1978) and FHG (Hunter, 1988). The schemes of pretreatment and the medium composition conducted according [1]. For each variant in medium 25-30 anthers were inoculated in Petri dishes and incubated at 25±2 °C in dark for 14 days.

After 30 days the explants were transferred to dishes with fresh medium and exposed to a constant temperature 25±2 °C, with 16 hours photoperiods and light 2000lux. Every 30 days we performed passages, respecting the aseptic rules.

Embryogenic and non-embryogenic cells population derived under *in vitro* anthers culture were used for cytological examination, using semi- and ultra-thin sections. Samples were fixed with 4% aqueous glutaraldehyde and post-fixed with 1% osmium tetroxide. After dehydration in ascending ethanol concentrations and propylene oxide the material was embedded in a mixture of Epon. Semi-thin sections, produced on ultramicrotome UMTP 3, were stained with methylene blue. Thin sections, were prepared at LKB ultramicrotome and stained in 4% uranyl acetate and in lead citrate. For transmission electron microscopy analysis, the specimens were analyzed with an electron microscope EMB 100 BR.

Results and discussion

According to the previous data was reported that microspore embryogenesis for barley pass via a complex morphogenetic pathway dependent not only on the pretreatment conditions, but also of a lot of interrelated factors, including the genotype peculiarities [1]. As results of optimized technique for initiation *in vitro* embryogenesis for evaluated cultivars (Galactic, Sonor, Unirea and Stralucitor), was established that cold stress (+40 °C) in complex with mannitol starvation generate reorganization involving anther wall cells (tapetum, endothecium) degeneration and microspores reprogramming.

In the anthers with androgenic response as result of dedifferentiation inside microspore, embryonic structures were generated, accompanied further by the exine break and destruction of sterile anther tissues (endothecium and tapetum).

At the first stage it is reported the cell wall differentiated, plasmodesmata formation (Fig. 1). Also at this phase were differentiated the traheids (Fig. 1 B).

These structures are criteria for determination of the tissue and indicate the beginning of differentiation. Cells organelles, such as mitochondria and plastids, are at young stage and are represented by promotocondria and proplastids, with a compact matrix without developed intern membrane system (Fig. 1 C).

In the vacuole cavity has been established minor accumulations of starch (Fig. 1 A, D). In some cases were attested aggregates of lipid compounds precursors of sporopollenin (Fig. 1 C).

This component is a mixture of biopolymers, containing fatty acids, phenylpropanoids, phenolics and traces of carotenoids. Sporopollenin is a major component of the tough outer (exine) walls of pollen grains. It is know that the sporopollenin is built up via catalytic enzyme reactions in the tapetum, and both the primexine and callose wall provide an efficient substructure for sporopollenin deposition. As is reported by Ariizumi and Toriyama [2], the currently accepted understanding of the molecular regulation of sporopollenin biosynthesis, must be review. These new approach would explain the occurrence of these compounds in the cells of microsporal origin.

The nuclei are characterized by an unbalanced distribution of heterochromatin (Fig. 1 E). In the compact peripheral zone predominate active cells with meristemic characteristics. Near the cell wall can be found lomosome-like bodies, vesicular structures involved in wall development (Fig. 1 F).

After several reproductive cycles, the structures derived from microspores with embryogenic potential derived meristematic centres characterized by compact arranged cells with hyperchromatic cytoplasm due to high content of organelles and intracellular

compounds, major centrally located nuclei, lack of central vacuole. Histological studies showed that globular structures originated at sub-marginal region and were covered by the epidermis. The somatic embryos formed independently or very close and appeared to be linked by their basal region [1].

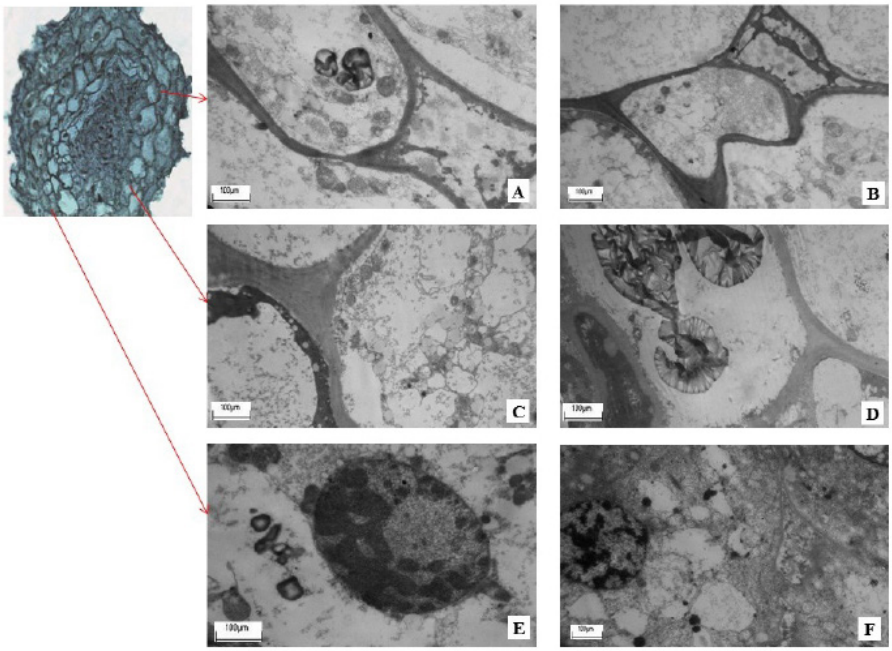

Fig. 1. Ultrastructure of cells at the globular stage. Structural aspect of cells from different zone.
A, B – intern compact zone, C, D – medium layers, E, F – compact zone from periphery.

In pre-embryonary stage are distinguish two areas: the compact and spongy area in which cells lose their adhesion (Fig. 2).

The compact area is characterized by strong adhesions and cells with high metabolic activity, not differentiated vacuome. It is found presence of many mitochondria and proplasdids (Fig. 2 A–E). In the spongy zone, the cells lose contact, had large vacuoles with reduced cytoplasm arranged parietal (Fig. 2 F-H). Heterochromatin is distributed mainly perimembranar (Fig. 2 H). Mitochondria have differentiated internal membrane system. It is also noted the presence of lomosome-like bodies, denoting intense process of cell wall formation.

For both embryogenic structures in interphase are characteristic four morphological types of nuclei:

- nuclei with major areas of heterochromatin distribute balanced on the surface of the nucleus with little diffuse chromatin (Fig. 2 C),
- nuclei with major areas of heterochromatin distributed unbalanced, denoting asymmetric distribution of genetic material (Fig. 2 A, F, G),
- nuclei with small areas of peripheral heterochromatin, indicating a metabolic hyperactivity (Fig. 2 B, H),
- interphase nuclei with typical differential nucleoli (Fig. 2 E).

In late developmental stage, the globular structures that formed embryos, exhibit polarity (apical and basal regions) and tracheary differentiation, which elements were distribute among the meristematic cells. Further sub-cultivation conducted to embryo differentiation or loss of cell adhesion and cell vacuolization [1].

In the embryogenic structures that formed *albino* plants are found cells with reduced cytoplasm, cavity being filled up with macromolecular compounds likely polysaccha-

ride (Fig. 3 D, E). Abundant storage and compounds mobilization probably cause an inhibitory effect on mitochondria, present in a major number (Fig. 3 C). Inactive status of these organelles is confirmed by their compact matrix and undeveloped internal cristae. Also, the plastids are undifferentiated, while the interphase nuclei are typical morphology, showing a high functional level (Fig. 3 A, B).

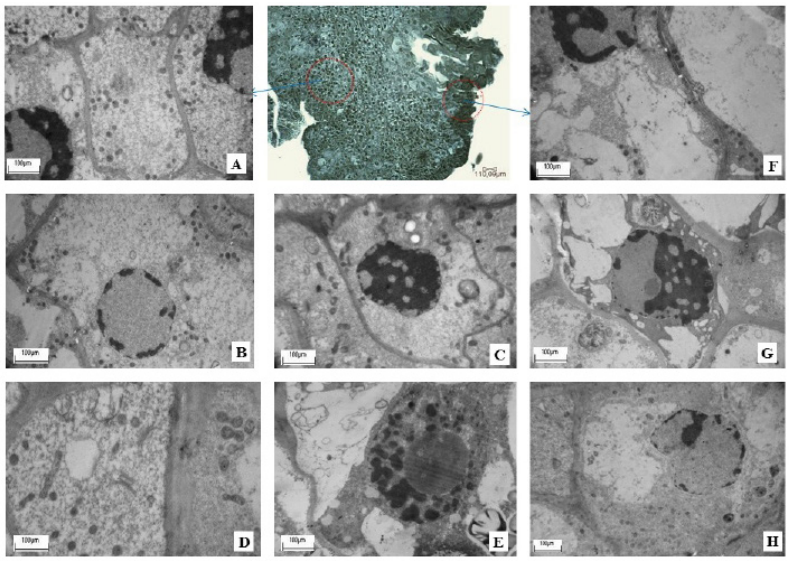

Fig. 2. Structural overview of distinct zone at pre-embryonic stage.
Fine structure of cells from compact zone - A, B, C, D, E; internal structure of cells from spongy zone – F, G, H.

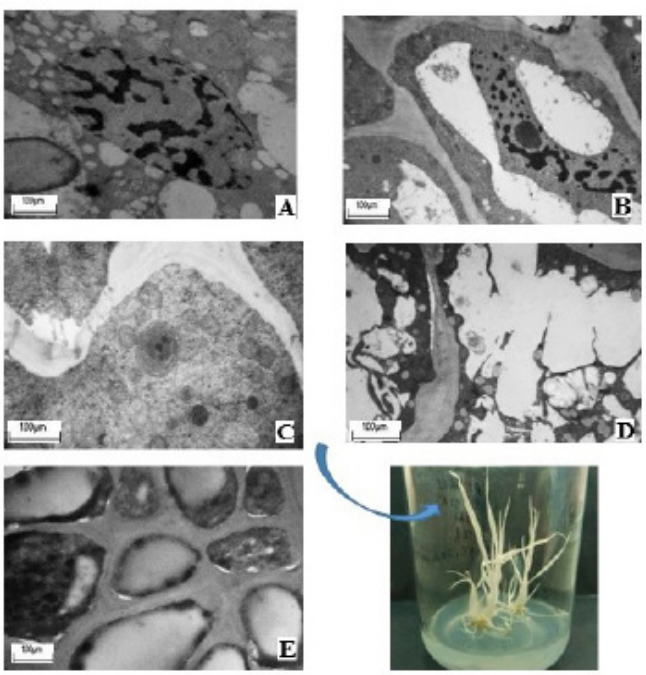

Fig 3. Ultrastructural aspects of embryogenic structure derived *albino* plants.
A- morphological aspect of nucleus; B general aspect of proliferative cell; C- fragment of cytoplasm with mul-

tiple mitochondria; D, E – storage of macromolecular compounds in vacuole cavity.

Specific for embryogenic structures which led to the formation of green regenerants are presence of amyloplasts and accumulation of phenolic compounds. Cells from embryogenic structures are different degree of differentiation of the vacuoles (Fig. 4). Mitochondria are present in a major number. Nuclei are with typical structure and balanced distribution of heterochromatin and distinguished nucleoli (Fig. 4 A, B, D). Nucleolar organization reflects high metabolic activity that is necessary for the synthesis of ribosomal subunits and realization of translational processes. The cell wall is thin. Additional primary starch deposit in plastids is observed insignificant reserves stored in vacuome cavity.

It is known that an important prerequisite for the potency expression is the nutrient substrate, such as starch, which serves as a source of energy for metabolic needs at the initial stage. The accumulation of phenolic compounds can conduct to modification of *in vitro* degradation of IAA by IAA-oxidase. Consequently, the genotype with maximum *in vitro* protection for IAA is regarded as the best genotype for androgenesis [5].

For embryogenic structures that generate green regenerants is characterized the development of the endoplasmic reticulum, presence of plasmodesmata, which proves the relationship between the cells within the tissue.

Based on obtained data were not established specific structural features between evaluated barley cultivars. The embryogenic structures derived from *in vitro* anthers culture present different ultrastructural aspects depending on the proliferation capacity and further development.

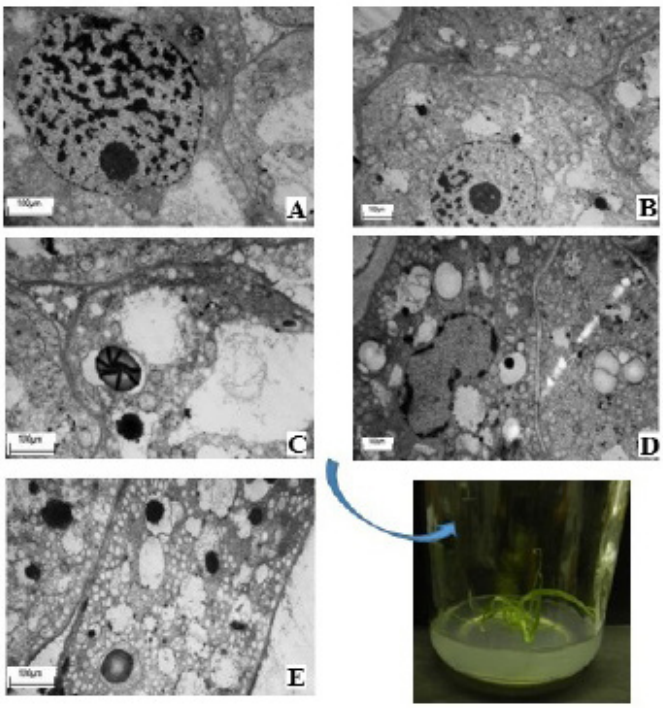

Fig 4. Ultrastructural aspects of embryogenic structure derived green plants.
A - morphological aspect of nucleus; B - general aspect of proliferative cell; C, D, E - structural aspect of proliferative cells fragments, it is certified the presence of amyloplasts and phenolic compounds.

Conclusions

On base of ultrastructural study of the formations derived via microspore embryogenesis were revealed:

- the embryogenic initiation it is marked by the differentiation of plasmodesmata and tracheary elements, while there has been characterized by a high variability of nuclei morphology, expressed in unbalanced chromatin distribution, reflecting a position effect;
- at pre- and embryogenic stages is observed differentiation of vacuoles, plastids. In cells with high proliferative capacity is remarkable presence of mitochondria in an increased number;
- embryogenic tissue, which subsequently generated albino plants are characterized by huge accumulations of starch and polysaccharides;
- in realization of androgenic potential an important role play the organelles of energetic systems: plastids and mitochondria, proving cytoplasmic factors involved in establishing embryogenic capacity.

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EXPRESSION OF SOME GENES IN BARLEY UNDER VIRAL INFECTION

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Introduction

Plants respond to virus infection with a number of structural, physiological and genetic modifications, which lead to disease development. The process of disease development in plants is still not fully understood, but two models have been proposed: the competitive model, where the viruses compete with the host for resources and the interaction model, where the disease develops as a consequence of viral components interfering with host processes [8]. In case of systemic infections the viruses dramatically affect photosynthesis, respiration and carbohydrate synthesis [16; 31].

The contribution of viral infection to the enhancement of somatic and meiotic recombination, resulting in rearrangements that could, potentially, be transmitted to the next generation, has been recognized [5; 20]. According to the previous data, it was established that viral infection can contribute to chromosome breaks [3; 24], modification of chromatin condensation [24], activation of transposing elements [9], cell proliferation and gene expression [14; 18; 29].

Exposure of maize plants to Barley stripe mosaic virus seems to activate transposable elements and to cause mutations in the non-infected progeny of infected plants [20]. The induction by the Barley stripe mosaic virus of an inherited effect means that the virus has a non-cell-autonomous influence on genome stability. The authors reported a threefold increase of homologous recombination frequency in both infected and non-infected tissue of tobacco plants with either tobacco mosaic virus or oilseed rape mosaic virus. A similar increase in DNA recombination was also observed in the progeny of the infected plants, indicating that pathogen-induced recombination can lead to heritable adaptations to environmental stresses [11].

A lot of researches regarding plant viruses, reveal that the character of systemic symptoms of virus infection in plants is determined by the expression of both host and virus genes. The consequences of viral infection are highly variable, thereby leading to a continued lack of understanding of these effects.

The goal of the study was to analyze the expression levels of superoxide dismutase (*SOD*) and ascorbate peroxidase (*APX*) genes, involved in antioxidant metabolism and of pathogenesis-related proteins (*PR-3, PR-5, PR-10*) in barley plants derived from susceptible cultivars to Barley stipe mosaic virus.

Materials and methods

Biological material. The experiments were performed on spring barley (Hordeum vulgare L.) cultivars Galactic, Sonor and Unirea. Seeds were obtained from healthy (mock-inoculated that served as control variant) plants and infected mechanically with barley stipe mosaic virus (BSMV) (virus variant). BSMV is a virus with straight tubular particles, with segments of about 22 nm in diameter and of three length (100-150 nm). The genome is represented by single-stranded RNA molecules, comprising a functionally tripartite genome. The plants at the 2 leaves stage were infected two times at 2-days interval with inoculum of BSMV using carborundum powder. Inoculum was obtained by grinding leaf tissue in distillate water (1:2). Initially, extract was prepared from field-grown barley plants that presented symptoms specifically to those induced by BSMV and showed positive response to negative staining test [15]. The seeds collected from diseased plants served as materials for further evaluation.

The seeds were sown in pots filled with a mixture of soil, peat and sand with six each (three pots for each experimental variant) and grown in controlled conditions (Sanyo chamber). Samples (0,3 – 0,5 g from the middle part of the youngest, but fully developed leaf) at the 3th leaf stage were collected, frozen in liquid nitrogen and stored at -800C for further analysis.

RNA isolation and Reverse Transcription PCR. Total RNA was isolated using *TRI Reagent* (*Applied Biosystems*) according to the manufacturer's instruction. The RNA quantity and quality was assessed spectrophotometric (λ 260/280 nm) and by electrophoresis in MOPS-formaldehyde 1,4% agarose gel.

The samples were treated with *RQ1 DNA-ase* (*Promega*) to remove the residual DNA. First-strand cDNAs were synthesized from 0,6 μg total RNA using Oligo(dT18), random hexamer primers (*Fermentas*) and RevertAid Reverse Transcriptase (Fermentas) according to the manufacturer's instructions.

Real Time PCR analysis. Five genes were selected for investigations (table 1): superoxide dismutase (*SOD*, GenBank: AK252295), ascorbate peroxidase (APX, GenBank: AJ006358) and pathogenesis-related (*PR*) proteins: *PR-3* (GenBank: AJ276226), *PR-5* (GenBank: AJ276225), *PR-10* (GenBank: AJ220734). As reference gene was used α-tubulin (GenBank: Y08490) [33].

Table 1. Characteristics of primers and amplicons

Gene	Sequence	Lenth of specific amplicons, bp
α-tubulin	5′-TCCATGATGGCCAAGTGTGA-3′ 5′-ATGTCGCTTGGTCTTGATGGT-3′	126
SOD	5′-CCGAAGATGAAATCCGCCAT-3′ 5′-CGGCCAATGATTGAATGTGG-3′	126
APX	5′-CGGAGCTTTTGAGTGGTGACA-3′ 5′-CCGCAGCATATTTCTCCACAA-3′	107
PR3	5′-AGTTGGCCTTGACAAGAAGCG-3′ 5′-CGCATAACGTCAAGGACGAAG-3′	104
PR5	5′-ACGACATCTCGGTTATCGACG-3′ 5′-TTATTGCCACTGCAGGCGT-3′	153
PR10	5′-CTAGGGTGTTCAAGACGGA-3′ 5′-CCCTGAGCTTCGCCACACA-3′	122

qRT-PCR was performed with Maxima SYBR Green/ROX qPCR Master Mix (*Fermentas*) on a DTprime Real-time cycler (*DNA-Technology*, Russia) with the following cycling parameters: 10 min at 950 C, 5 cycles of 10 s at 950C, 20 s at 640 C, 40 cycles of 15s at 950 C, 40 s at 600C. The specificity of PCR products was verified by dissociation curve analysis and agarose gel electrophoresis 2% in TAE buffer [26]. The experience was carried out in triplicate independent cDNA synthesis (for each sample of RNA) and in double repetition qPCR (n = 6). The relative expression was calculated via the $2^{-\Delta\Delta Ct}$ method [21].

Statistical analysis of the experimental data was performed according to Clever [7].

Result and discussion

In barley progenies obtained from virus infected plants the activity of *Apx* and *Sod* was in decline in most variants. A no statistically deviation was established only for the expression of *Apx* in cv. Sonor (table 2).

Similarly, no changes in activity of *SOD* were detected in the early response to local lesion-producing Tobacco mosaic virus in tobacco [17]. According to the Clarke et al.

[6], in symptomless virus–plant interactions, the activity of *SOD* decreased. Reactive oxygen species, especially hydrogen peroxide, and callose have similar patterns of induction in plants inoculated with mild and aggressive virus isolates [30].

Table 2. Transcriptional activity of studied genes in barley leaves in healthy and progeny of virus infected plants

Gene	Genotype	Treatment	
		Control	Virus
Sod	Sonor	0,5175±0,0443	0,3543±0,0387*
	Galactic	0,7675±0,0719	0,6317±0,0760*
	Unirea	0,5772±0,0339	0,4643±0,04203*
Apx	Sonor	0,3183±0,0379	0,3327±0,05 49
	Galactic	1,8255±0,4343	0,9937±0,1761*
	Unirea	0,8307±0,0856	0,5433±0,0250*
PR-3	Sonor	0,0947±0,0091	0,0065±0,0012*
	Galactic	0,7102±0,0624	0,1712±0,0137*
	Unirea	0,0297±0,0046	0,0333±0,0065*
PR-5	Sonor	0,197±0,0219	0,0513±0,0053*
	Galactic	0,1638±0,0236	0,7525±0,0665*
	Unirea	0,1065±0,0133	0,3043±0,0286*
PR-10	Sonor	0,0142±0,0017	0,0025±0,0005*
	Galactic	0,0446±0,0044	0,0155±0,0016*
	Unirea	0,0173±0,0029	0,0202±0,0035*

* - significant difference from the control at P<0,05.

Susceptible plants respond to viral infection by activating of some genes, including pathogenesis-related proteins (*PR*) [32]. If initially, it was considered that PR proteins associated with plant response against viruses by local lesion, then after more researches, has been established that PR proteins can be synthesized in tobacco plants systemically infected with tobacco mosaic virus [13]. Subsequently, similar data were found for other hosts which have exhibited sensitivity to various viruses [32].

The results of the present study showed a general up- or down-regulation effect of transcriptional activity of *PR-3* and *PR-10* genes in dependence of genotype. The significant decrease of the *PR3* activity was established for treated variants of cv. Galactic and cv. Sonor (93% for cv.Sonor and 76% for cv. Galactic, respectively less than in the control). A similar tendency was find and for *PR10* activity (fig. 2).

The up-regulation of genes related to pathogenesis was reported for PR5 for all treatment variants. The highest increase (by 4,6 times) was detected for cv. Galactic. According previous cytogenetically studies this genotype present more plasticity. It is know, that the PR-5 is related to stress mediated by jasmonate or ethylene signaling pathway [19]. Their activity in young plant in normal condition is less, but in case of pathogenesis significantly increases [28]. Also, PR10 exhibit ribonuclease, and PR-3 - chitinase activity, which are more specific.

The role of different types of *PR* proteins remains unclear. *PR* proteins with peroxi-

dase activity (type *PR-9*) or ribonuclease (type *PR-10*) is involved in antiviral protection while the *PR-3* promotes infectious process [32].

It is considered that the accumulation of PR proteins depends on the viral strain. The RNA-ase activity was most critical in case of acute symptoms. Similarly, β-1,3-glucanase activity is elevated in soybean plants that experienced serious symptoms against mosaic virus comparative to the symptomless plants [1]. According to the electrophoretic spectrum of *PR* proteins in extracts of Datura stamonium healthy and infected with strains of potato virus X plants were found only quantitative differences, which proved to be dependent on the viral strain vulnerability [cited from 32].

It is known that plant viruses are capable of reprogramming host gene expression provided evidence of a possible connection between this phenomenon and the pathogenicity of viruses by reporting a correlation between the intensity with which infection interrupts the expression of cellular genes ('shut-off') and the severity of viral symptoms [25].

Previous research on the susceptible interaction of potato and PVY_{NTN} showed changes in the cytokinin level in inoculated leaves [10]. In addition, photosynthesis related genes and genes involved in perception, signaling and defence response were shown to be involved in the early response to virus inoculation [2].

Differential gene expression in chlorotic tissues infected with the wild-type pepo strain of *Cucumber mosaic virus* (CMV) and two strains carrying coating proteins mutants with diverse chlorosis severity showed that CMV inoculation appeared to have similar effects on the transcriptional expression profiles of the symptomatic chlorotic tissues, and only the magnitude of expression differed among the different CMVs [23]. Gene ontology analysis with biological process and cellular component terms revealed that many nuclear genes related to abiotic stress responses, including responses to cadmium, heat, cold and salt, were up-regulated, whereas chloroplast- and photosynthesis-related genes (CPRGs) were down-regulated, in the chlorotic tissues.

For susceptible race cultivars to *Rice tungro spherical virus* (RTSV) was found that about 11% and 12% of the genes in the entire genome were differentially expressed. Nearby 30% of the differentially expressed genes (DEGs) were detected commonly for two virus strains (TN1 and TW16) [27]. The authors related that stress response processes were significantly overrepresented in both TN1 and TW16. Evident differences between strains included defense and development-related genes regulated in asymptomatic plants even with a very low level of RTSV, and that the TN1- and TW16-specific gene regulations might be associated with regulation of RTSV accumulation in the plants.

Barley (*Hordeum vulgare* L.) has a physical map of 4.98 Gb, with more than 3.90 Gb anchored to a high-resolution genetic map, projecting a deep whole-genome shotgun assembly, complementary DNA and deep RNA sequence data onto this framework supports 79,379 transcript clusters, including 26,159 'high-confidence' genes with homology support from other plant genomes [22].

Druka et al. [12] described the expression of genes in 15 tissues at different ontogenetic stages. By applying Barley1 GeneChip platform it was described 18,481 gene transcripts present more than twice in at least one tissue. The number of genes expressed in a single tissue varies between 10 189 in the anther and 14 805 in crown. The expression of about 14,943 genes may vary more than 4 times, and the other 3, 538 being constituent.

For evaluated virus-host system were established evident modifications in transcriptional activity of genes including: (1) suppression of genes involved in antioxidant metabolism; (2) activation or suppression of genes for jasmonate synthesis or chitinase activity in dependence of genotype specificity; (3) up-regulation of genes for stress-related transcription factors such as PR10.

According Boyco and Kovalchiuc [4], infection with a compatible virus result in activation of various signals such as small RNAs, what spread systemically from the

place of infection to non-infected tissues, including those that form the gametes. The signal results in a loss or gain of DNA methylation at the specific loci, effect transmitted to the immediate progeny.

The obtained results evolved the complexity of the pathogenic process and reveal that the plant defenses against viral infections are diverse as form.

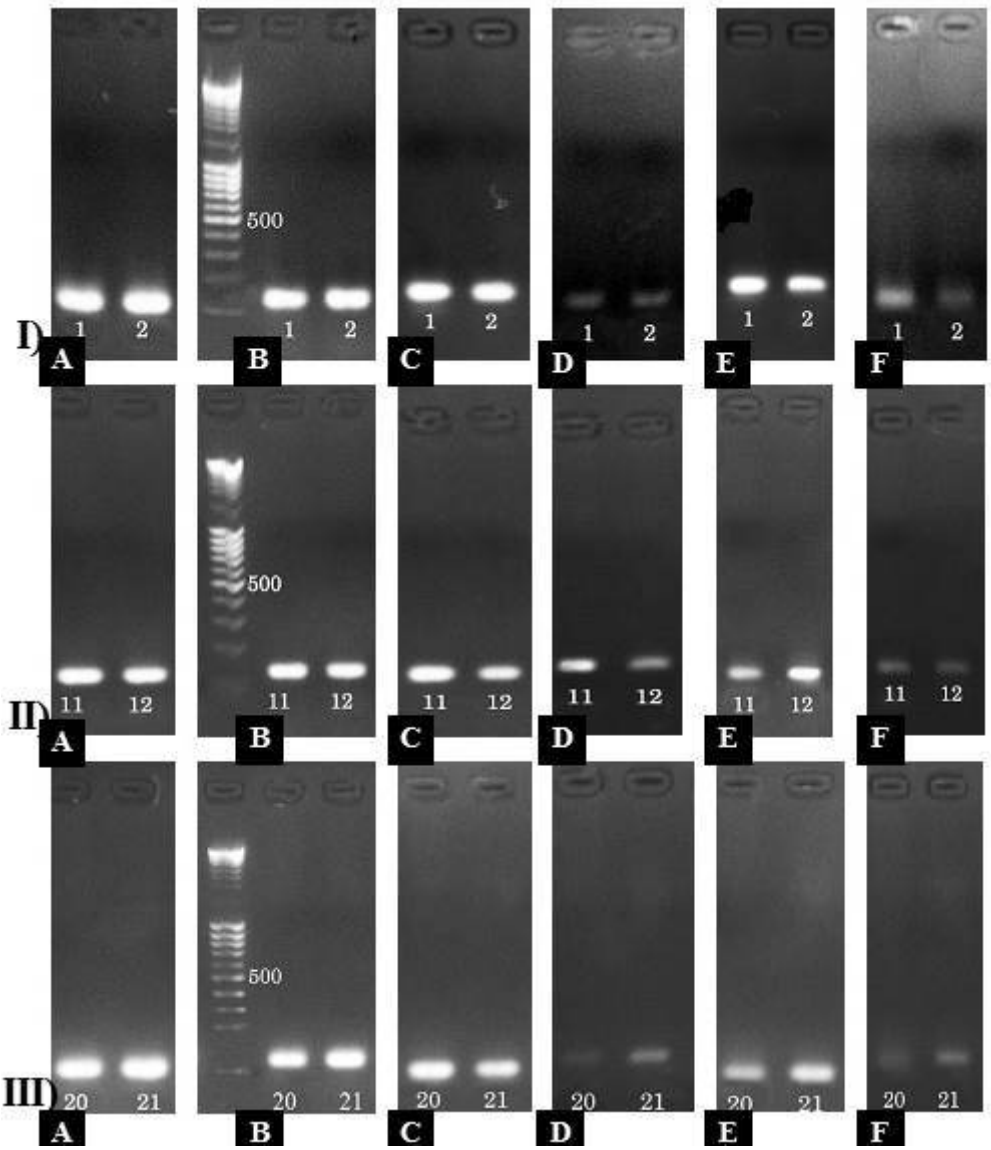

Fig. 1. Electropherograms of amplification products of the primers for gene
A - α-tubulin, B - *Sod*, C - *Apx*, D - *PR3*, E – *PR5*, F – *PR10* in control (1, 11, 20) and virus (2, 12, 21) leaves of barley genotype: I) Galctic, II) Sonor, III) Unirea.

Conclusions

Were established the quantitative deviations in expression levels of some genes in leaves of barley seedlings of cultivars Galactic, Sonor and Unirea obtained from plants infected with barley stripe mosaic virus.

The activities of genes involved in antioxidant metabolism (*APX* and *SOD*) were decreased in most variants of barley virus infected progenies. The expression of the

Apax gene was reduced by about 35-46% for cv. Galactic and Unirea, while the activity of *Sod* gene was less influenced by viral infection, conducting to the diminution by 18 to 31% (for cv. Galactic and Sonor respectively).

The relative expression of genes for pathogenesis-related proteins was significantly modified in studied pathogen-host systems. The obtained data showed up- or down- regulation of *PR3* and *PR10* in dependence of genotype, whereas for *PR5* was reported only the up-regulation effects for all treated variants. Thus, the expression of *PR5* gene was by 2.8 and 4.6 times higher in progeny of cv. Unirea and Galactic obtained from plants infected with BSMV.

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CUANTIFICAREA UNOR EFECTE ALE ULTRAVIOLETELOR LA *PHASEOLUS VULGARIS* L. ÎN VEDEREA IDENȚIFICĂRII DE GENOTIPURI CU DIFERITE CAPACITĂȚI DE UTILIZARE A RADIAȚIILOR SOLARE

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Introducere

Deteriorarea stratului de ozon, cu potențiale repercusiuni asupra vieții planetare, ca urmare a creșterii dozei de radiații UV ce ajung la nivelul solului, este o consecință directă a revoluției tehnico-științifice ce a pus omenirea în fața problemei unor modificări de lungă durată ale mediului ambiant.

Alegerea fasolei, *Phaseolus vulgaris* L., drept "specie țintă" se justifică prin faptul că această plantă reprezintă una dintre sursele principale de proteină, ieftină și de bună calitate (23-33%), o bună parte a cercetărilor actuale având drept scop selecționarea unor genotipuri bogate în proteine, care să înlocuiască progresiv cerealele, capabile să atingă o productivitate optimă în condiții extreme de stres abiotic [7].

În esență, s-a urmărit caracterizarea suportului ereditar pe baza căruia soiurile investigate dau răspunsuri diferite, decelabile la nivel molecular, biochimic și fiziologic. Este cunoscut faptul că fasolea este o plată de zi scurtă și prezintă sensibilitate la iradiere UV-B [5, 6, 7].

Material și metode

Material vegetal: *Phaseolus vulgaris* L. (Linnaeus 1753). În cazul experimentelor în care am urmărit răspunsul la iradierea UV prin sinteza de flavonoizi, fotoreparația, efectele citogenetice ale radiațiilor UV la nivelul meristemului radicular al rădăcinilor primare, am luat în studiu drept material vegetal șase soiuri românești de fasole: *Avans, Ardeleana, Star, Ami, Diva, Vera*, create și omologate în România.

Determinarea conținutului în lignină pe plantule s-a efectuat atât la cele șase soiuri românești, cât si la soiuri sud americane (*Pinto, Arroz,Orfeo, Vilmorin-Negro și Tortola*) [7], iar răspunsul la iradiere, tradus prin sinteza de izoflavonoizi, l-am analizat la Saxa (soi nemțesc) [6, 7].

Câmpurile experimentale, folosite în scopul efectuării observațiilor de natură agronomică (aparținând Stațiunii de Cercetare si Dezvoltare Agricolă Podu Iloaiei – Iași), au fost însămânțate respectând perioadele și tehnicile specifice culturii fasolei, adaptate condițiilor meteo-climaterice și pedologice caracteristice regiunii. În câmp au fost cultivate soiurile: *Avans, Ardeleana, Star, Ami, Diva, Vera, Pinto, Arroz, Orfeo, Vilmorin-Negro* si *Tortola* [4].

Analizele biochimice vizând determinarea masei proaspete și a substanței uscate, a acidității totale, a conținutului în: cenușă, substanțe solubile, flavone, polifenoli, lignină, pigmenți asimilatori (clorofile și caroteni), aminoacizi, proteine, vitamina C, minerale, au avut drept material biologic vegetal plante aparținând soiurilor românești, provenite din semințe germinate în condițiile iradierii cu spectru UV total și care au supraviețuit în număr mare în câmp, si anume: *Avans, Star, Ami, Diva, Vera* [3].

Tehnica și condițiile de iradiere: temperatură constantă 25ºC; sursele de lumină: lămpi UV, conform descrierii Surugiu și Maniu, 2002.

Etape si metode de lucru:

1.Determinarea conținutului de flavonoizi: au fost puse la germinat în total câte 20 de semințe a câte șase soiuri românești de fasole (A*vans, Ardeleana, Star, Ami, Diva* si *Vera*). Pentru toate variantele de tratament, plantulele au fost supuse iradierii, în cutii de plastic acoperite cu filtre Schott tipul WG (WG360, WG320, WG305 în funcție de fiecare caz în parte), timp de 20-40h. Atât probele cât și martorul au fost prelucrate conform metodei de extracție a flavonoizilor, imediat după încheierea iradierii: izolarea și identificarea flavonoizilor prin tehnica cromatografiei pe hârtie, extracția flavonoizilor, citirea rezultatelor.

2.Determinarea conținutului de izoflavonoizi (la soiul nemțesc *Saxa*): s-a folosit metoda de inducere a sintezei izoflavonoizilor, de izolare prin migrare pe placa de cromatografie de sticlă acoperită cu silicagel în strat subțire, extracția de pe placă și dozarea, precum și existența și eficacitatea mecanismului de protecție prin fotoreparatie. Au fost iradiate cu radiații de lungimi de undă diferite (obținute cu ajutorul filtrelor WG360 și Q) frunzele primare aparținând aceleiași plante, timp de 30 minute. Rezultatele (caracteristicile de absorbție ale izoflavonoizilor) au fost citite la spectrofotometrul UVIKON 940, la lungimi de undă specifice, și anume: 342nm și 344nm pentru cumestrol.

3.Determinarea conținutului în lignina la soiurile românești (*Ami, Vera, Ardeleana,Avans, Diva Star*), cât și dintre cele sud americane (*Pinto, Arroz, Orfeo, Vilmorin si Tortola*). Plantulele au fost puse la iradiat în UV/lumină albă, două variante de iradiere: cu filtru WG360 și cu filtru WG310. A urmat: *izolarea compușilor peretelui celular și extracția ligninei, formarea complexului lignină-acid tioglicolic.*

4.Formarea dimerilor la nivelul ADN ca urmare a iradierii UV și fotoreparația prin intermediul ADN–fotoliazei, dovedită experimental prin scăderea conținutului în dimeri la nivelul ADN, determinat prin intermediul testului ELISA [8].

Iradierea UV-B în scopul inducerii dimerizării cu sau fără filtru WG360. Timpii de iradiere au fost de 30 de minute în cazul iradierii UV-B si de 60 de minute în cazul iradierii UV-A.

5.Studiile citogenetice: probele prelucrate prin metoda Feulgen. Au fost puse la germinat în total câte 20 de semințe a câte șase soiuri românești de fasole (*Avans, Ardeleana, Star, Ami, Diva* si *Vera*). Plantulele etiolate, provenite din semințe germinate în întuneric au fost supuse, în funcție de scopul urmărit, iradierii timp de 0,5h; 1,5h; 3h, în boxa cu radiații combinate: UV-B, UV-A și lumină albastră (iradierea UV/lumină albă). Au existat șase variante experimentale (cinci variante de iradiere: WG360, WG320, WG305, WG275, Q și una control). A urmat f*ixarea materialului vegetal, hidroliza, colorarea* cu colorant Carr, *efectuarea preparatelor microscopice și examinarea preparatelor la microscop*. Calcularea indicelui mitotic (IM) s-a efectuat conform formulei: IM= nr. nuclei în mitoza x100/nr. total de nuclei [7].

6.Studiile vizând variația unor parametrii agronomici și biochimici cât si comportamentul în mediul natural al unor soiuri de fasole, germinate în mediu UV-B au presupus: germinarea a câte 100 semințe din fiecare soi (*Avans, Ardeleana, Star, Ami, Diva, Vera, Pinto, Arroz, Orfeo, Vilmorin-Negro și Tortola*), iradierea UV total (timp de 48h), însămânțarea pe loturi experimentale. S-a urmărit: procentul de semințe germinate; timpul corespunzător fiecărei perioade de vegetație; procentul de plante răsărite; masa proaspătă și substanța uscată; aciditatea totală; conținutul în: cenușă; substanțe solubile; flavone; polifenoli; clorofile și caroteni; aminoacizi; proteine; vitamina C; lignină; minerale.

Rezultate și discuții

Determinarea conținutului de flavonoizi: comparând rezultatele obținute prin iradierea în aceleași condiții a plantulelor aparținând acelorași soiuri, variind doar durata de iradiere (40 h si respectiv 20 h) se constată că apar diferențe în răspunsul la stresul iradierii prin sinteza pigmenților ecran. În cazul unei durate mai mari de iradiere, flavonoizii sintetizați în cârja hipocotilară se separă prin migrare în doua benzi, situație neîntâlnita în cazul înjumătățirii duratei de iradiere, ceea ce sugerează o anumită deca-

lare în timp a inducerii sintezei flavonoizilor cu greutăți moleculare diferite [7].

În ceea ce privește influența duratei iradierii UV-B (WG 305nm), s-a observat că există o corelație pozitivă, în cazul soiurilor *Diva, Star, Ami* și *Ardeleana*, în sensul că odată cu creșterea duratei de iradiere crește și conținutul în pigmenți ecran. În cazul soiurilor *Vera* și *Avans*, s-a observat scăderea cantității de pigmenți la o durată de iradiere mai mare, demonstrând că, deși aceste soiuri au capacitatea de a reacționa rapid prin sinteza unei cantități mari de flavonoizi, având rezistență foarte bună în cazul unei iradieri intense dar de scurtă durată, în timp se dovedesc a fi sensibile, comparativ cu celelalte soiuri investigate. Un soi valoros din punctul de vedere al toleranței la iradierea UV-B ar trebui să sintetizeze în timp scurt o cantitate însemnată de flavonoizi și să își mențină această capacitate și la o durată de iradiere mai mare [7].

Comparând cantitățile de flavonoizi sintetizați în frunze cu flavonoizii sintetizați în cârja hipocotilară, la aceleași șase soiuri de fasole, se constată că, în toate situațiile, cantitățile sunt mai mari în frunze, în conformitate cu datele citate din literatură [7].

Determinarea conținutului de izoflavonoizi: cantitatea de 3-arilocumarina sintetizată în frunza supusă iradierii cu spectru UV total este mai mare decât cea sintetizată în frunza martor. Nefiind vorba de variabilitate la nivel individual (frunzele iradiate aparținând aceleiași plante) și nici de influența altor factori externi, cu excepția lungimii de undă a radiațiilor, putem concluziona că sinteza unor cantități mai mari de flavonoizi în frunzele supuse acțiunii nocive a radiațiilor UV reflectă stresul pe care îl reprezintă acest factor pentru plantă, fiind un marker de cuantificare a gradului de lezare la nivel ADN.

Determinarea conținutului în lignină: s-a constatat că la soiul *Ami* conținutul în lignina scade în urma iradierii, la *Vera* rămâne constant, în timp ce la *Avans, Diva, Star* și *Ardeleana* crește. În urma iradierii UV-B, se remarcă o stimulare a sintezei de lignină în cazul soiurilor Pinto și Orfeo, în timp ce la soiurile *Arroz* și *Vilmorin* are loc o inhibare a sintezei. La *Tortola* nu se înregistrează nici o schimbare în ceea ce privește procentul de lignină în peretele celular al plantelor supuse sau nu tratamentului cu UV-B [7].

Formarea dimerilor la nivelul ADN ca urmare a iradierii UV și fotoreparația prin intermediul ADN-fotoliazei: comparând, pentru fiecare soi în parte, cantitățile de ADN dozate la probele martor cu cele obținute la probele supuse iradierii se constata că, la toate cele șase soiuri testate, cantitatea de ADN creste în urma iradierii UV – B. Pentru soiurile *Ami* și *Vera*, s-a constatat că numărul de dimeri nu crește în urma iradierii UV-B și nici nu are loc procesul de fotoreparație (numărul dimerilor de timină nu se reduce în urma iradierii UV-A), ceea ce dovedește că, în cazul acestor soiuri, efectele iradierii UV-B la nivel molecular pot fi letale pentru celule. Numărul mai mare de dimeri apăruți în urma iradierii în cazul acestor două soiuri poate fi explicat prin sinteza unor cantități mai mici de flavonoizi în cârja hipocotilară a plantulelor aclimatizate aparținând acestor soiuri, comparativ cu *Star, Avans, Ardeleana*, în timp ce în cazul soiului *Diva* s-au sintetizat tot cantități reduse de flavonoizi, în urma iradierii cu UV cu lungime scurtă de undă. La *Diva* s-a constatat creșterea numărului de dimeri de timină în urma iradierii UV-B, cât și o ușoară scădere a numărului dimerilor de timină în urma iradierii UV-A, fapt ce demonstrează că a avut loc procesul de fotoreparație. % Rep. = 7,2 % [5, 7].

Studiile citogenetice: cea mai mică valoare a IM, comparativ cu martorul cât și cu toate celelalte variante de iradiere, se întâlnește la varianta de iradiere cu spectru UV total. Inhibarea diviziunii celulare cât și creșterea evidentă a frecvenței aberațiilor în cazul variantei de tratament cu UV cu spectru total (Q), indiferent de soi și indiferent de durata iradierii, dovedește nocivitatea radiațiilor UV-C cu lungime scurtă de undă.

Studiile vizând variația unor parametri agronomici și biochimici cât și comportamentul în mediul natural al unor soiuri de fasole germinate în mediu UV-B au dovedit comportamentul diferit în funcție de soi și chiar de locul de proveniență al fiecăruia în parte, soiurile sud-americane *Pinto, Orfeo, Arroz, Vilmorin, Tortola* nu au

ajuns la maturitate [1, 2, 3].

Concluzii

Sinteza pigmenților ecran este un fenomen care depinde atât de factorii interni (informația ereditară, interacțiunea între celule, organul sau țesutul investigat), cât și de cei externi (lungimea de undă a radiațiilor, durata iradierii, condițiile de germinare).

Sinteza izoflavonoizilor are loc în timp, cantitatea lor fiind cu atât mai mare cu cât leziunile la nivel ADN sunt mai puternice, drept răspuns la stres.

În câmp, apar diferențe în ceea ce privește perioadele de vegetație, în special între soiurile având origini diferite.

Analizele biochimice arată că iradierea UV-B induce un dezechilibru al regimului hidric, crește conținutul în flavone, polifenoli, lignină, minerale și aminoacizi esențiali.

UV a produs leziuni ale ADN, tipul aberațiilor induse fiind destul de variat, procentele de apariție a mutațiilor fiind însă asemănătoare cu a mutațiilor naturale.

Se constată un comportament relativ uniform al soiurilor pentru toți parametrii analizați, diferențele de răspuns fiind minime, astfel încât nici unul dintre soiuri nu poate fi considerat deosebit de rezistent sau de sensibil la iradierea UV.

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PROBLEMELE ACTUALE ÎN AMELIORAREA ŞI ORGANIZAREA PRODUCERII SEMINŢELOR DE LEGUME

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Introducere

Legumicultura în Republica Moldova a fost şi continuă să rămână una din principalele ramuri ale agriculturii, menită să asigure populaţia cu legume în stare proaspătă şi industria de conserve cu materie primă. Importanţa social-economică a acestei ramuri rezultă din ponderea legumelor în alimentaţia populaţiei, utilizarea intensivă a terenurilor şi forţei de muncă, obţinere de producţii sporite precum şi a unor venituri esenţiale. Condiţiile pedoclimatice favorabile, tradiţiile şi experienţa acumulată permit cultivarea a peste 60 specii de plante legumicole şi obţinerea unor recolte bogate precum şi a unui beneficiu impunător la majoritatea culturilor [2]. Drept dovadă servesc indicii dezvoltării legumiculturii înregistraţi în perioada anilor până la independenţă (1982-1992), în care anual, recolta globală de legume depăşea nivelul de 1300 mii tone, inclusiv circa 700 mii tone erau prelucrate şi peste 250 mii tone exportate în stare proaspătă.

Pe parcursul anilor recolta globală s-a redus considerabil (de circa 3 ori), provocând diverse dificultăţi pe piaţa internă şi externă, industria de conserve, fiind asigurată cu materie primă la nivel de 15-20% din potenţialul de prelucrare a condus la dispariţia multor fabrici [4].

Potrivit recomandărilor ştiinţifice pentru o alimentaţie corectă a unei persoane sunt necesare anual 90-110 kg de cartof şi 140-150 kg de legume. Satisfacerea acestor cerinţe întru asigurarea securităţii alimentare necesită producerea a circa 450-500 mii tone de cartof şi 550-600 mii tone de legume.

Suprafaţa de cultivare a legumelor şi bostănoaselor în ultimii 10-15 ani constituie 40-45 mii ha, iar pentru cultivarea cartofului – 23-25 mii ha. În aspectul speciilor legumicole, suprafeţe mai mari au fost cultivate cu tomate (12-14 mii ha), pepeni şi dovleci (8-9 mii ha), ceapă (4-6 mii ha), varză (5-6 mii ha), castraveţi (3,8-4,6 mii ha) şi morcov (3,5 mii ha), iar mai mici - cu sfeclă roşie (2,3 mii ha), mazăre de grădină (1,7 mii ha), ardei şi pătlăgele vinete (1,2-1,5 mii ha).

Structura de cultivare a legumelor înregistrată în ultimii ani este departe de cea optimală, care să corespundă mai pe deplin cerinţelor pieţii, securităţii alimentare, posibilităţilor de export şi anume: tomatele - 20%, mazărea de grădină - 10%, ceapa, varza, ardeiul, şi castravetele - 7-8%, vinetele, morcovul şi sfecla de masă - câte 4-5% [2, 4].

Cauzele principale ale reducerii producţiei de legume în ultimii ani sunt pe lângă lipsa pieţelor sigure de desfacere a producţiei legumicole, decalajul dintre preţurile la factorii de producţie (fertilizanţi, pesticide, combustibil şi lubrifianţi, tehnică agricolă etc.) şi de comercializare a legumelor, discordanţa dintre preţurile de achiziţionare a materiei prime şi preţul de cost al acesteia, precum şi nerespectarea tehnologiilor de cultivare în noile condiţii de producere, neimplementarea pe scară largă a celor avansate, lipsa de asistenţa ştiinţifică în ramură, nesoluţionarea problemelor ce ţin de irigarea culturilor legumicole, colectarea, păstrarea şi comercializarea legumelor.

O situaţie şi mai dificilă se atestă în producerea seminţelor de legume, din cauza insuficienţei seminţelor de categorii superioare (bază şi prebază), care conform Legii despre Seminţe se menţin de către instituţiile de cercetări se multiplică seminţe de categorii necunoscute. La moment nu se efectuează lucrări de ameliorare conservativă şi de menţinere a soiurilor la culturilor de varză, ceapă, rădăcinoase, cucurbitacee, leguminoase şi verdeţuri.

În trecut asistenţa ştiinţifică în legumicultură şi controlul metodic asupra implementării varietăţilor legumicole, măsurilor agrotehnice şi tehnologiilor de cultivare se efectuau de către Institutul de Legumicultură din mun. Tiraspol care mai mult de două decenii nu întreţine legături de colaborare cu Ministerul Agriculturii şi alte instituţii de stat din Republica Moldova.

Dezvoltarea durabilă a legumiculturii necesită un studiu permanent asupra nivelului de producere, creare a soiurilor şi hibrizilor noi, elaborarea şi implementarea unor metode şi tehnologii de producere a seminţelor, răsadurilor şi de cultivare a legumelor, în stare să asigure o producţie cu o valoare biologică şi competitivitate înaltă în condiţii deficitare de apă şi resurse energetice. Este evident faptul că declinul economic, legal de trecerea la economia de piaţă a condiţionat reducerea esenţială a activităţii ştiinţifice în crearea de noi soiuri şi hibrizi, lucrărilor de ameliorare şi producere a seminţelor de categorii superioare (prebază şi bază), de menţinere a calităţilor culturale a soiurilor omologate.

Din lipsă de cadre, sub limita eficienţei se efectuează lucrările de ameliorare şi producere a seminţelor la bostănoase şi zarzavaturi. Totalmente sunt abandonate lucrările de ameliorare la culturile bienale: varză, morcov, sfecla roşie, ceapă şi usturoi. Din lipsa propriilor realizări majoritatea varietăţilor legumicole incluse în ultimii ani în Registrul Soiurilor de Plante sunt de provenienţă străină, la care este problematică organizarea multiplicării seminţelor în condiţiile republicii [8].

Starea actuală şi lipsa asistenţei ştiinţifice corespunzătoare necesită crearea unui centru de cercetare şi extensiune în legumicultura a rezultatelor ştiinţifice, pregătire a cadrelor de calificare superioară, precum şi organizare unor discuţii, seminare, loturi pilot demonstrative, zile deschise, îndrumări şi servicii pe teren, coordonarea programelor de producţie şi posibilităţilor de comercializare a produselor legumicole, inclusiv şi a seminţelor.

Introducerea şi menţinerea în producţie a soiurilor şi hibrizilor valoroşi necesită asigurarea an de an, a unor cantităţii importante de seminţe. În acest scop, procesul propriu zis de ameliorare trebuie continuat cu lucrări speciale de producere şi înmulţire a seminţei în cantităţi cerute de unităţile agricole. Rolul ştiinţei în dezvoltarea legumiculturii precum şi a întregului sector agrar este indiscutabil, în special al biologiei, geneticii şi ameliorării, fiziologiei şi protecţiei plantelor. Dezvoltarea prioritară a ştiinţelor naturii este condiţionată nu numai de necesitatea intensificării producerii şi sporirii eficacităţii agriculturii, dar şi a bunăstării omului.

Actualmente sunt omologate şi incluse în Registrul Soiurilor de Plante al Republicii Moldova 697 soiuri şi hibrizi de le¬gume şi cartof [8]. Cu toate că numărul de soiuri a crescut considerabil nu putem afirma că acestea satisfac pe deplin cerinţele pieţei interne şi externe în ce priveşte asigurarea pe tot parcursul anului a legumelor în stare proaspătă de calitatea şi asortiment necesar.

Soiurile existente [8, 9] au un potenţial productiv înalt, însă se realizează numai la nivel 30-40%, în primul rând, din cauza plasticităţii ecologice scăzute a varietăţilor introduse din alte zonei geografice, rezistenţei slabe la boli şi dăunători. Crearea de noi soiuri şi hibrizi tolerante şi/sau rezistente la agenţii patogeni şi dăunători devine o sarcină primordială [7]. Lipsa soiurilor şi hibrizilor rezistenţi la temperaturi scăzute la speciile iubitoare de căldură (tomate, ardei, vinete, castraveţi, pepeni) impune producea legumelor prin metoda de răsad cu cheltuieli suplimentare de energie, care conduce la majorarea costurilor şi diminuarea competitivităţii producţiei. Lipsa soiurilor extra timpurii cu capacităţi de germinare şi fructificare la temperaturi mai scăzute nu permit înfiinţarea culturilor prin însămânţarea direct în câmp. În lipsa soiuri rezistente la secetă şi cu cerinţe scăzute faţă de umiditate se aplică norme mari de irigare, care de asemenea sunt foarte costisitoare.

Pentru industria de conserve sunt cerute soiuri cu un conţinut ridicat de substanţă uscată, zahăr, vitamine, pigmenţi şi alte calităţi care lipsesc la multe din soiurile omologate. Pentru piaţa internă şi externă se cer legume cu însuşiri atrăgă¬toare la formă,

culoare, gust, aromă, rezistente la transportare, păstrare îndelungată.

O problemă şi mai acută pentru ştiinţă este menţinerea principalelor caractere şi însuşiri ereditare ale soiurilor deja omologate. Autorii (menţinătorul) au obligaţiunea să multiplice şi să furnizeze material de bază pentru categoriile biologice superioare [5, 6]. Însă majoritatea soiurilor omologate locale au fost create în fostul Institut Moldovenesc pentru Legumicultura şi Agri¬cultură Irigată din mun. Tiraspol, unde din motive cunoscute nu se reproduc seminţe de categorii biologice superioare în cantităţi necesare. Aceasta este una din cauzele ce a influenţat scăderea calităţii seminţelor de legume pe piaţa interne. Ieşirea din această situaţie constă în aceea ca Ministerul Agriculturii şi Industriei Prelucrătoare să deter¬mine persoane juridice şi fizice cu pregătirea respectivă (menţinători) care perfecţionând schemele de ameliorare conservativă, tehnologiile de hibridare să asigure obţinerea seminţelor de legume şi material săditor de cartof cu puritate, valoare biologică şi culturală sporită, libere de viroze, agenţi patogeni şi dăunători.

La momentul actual nu dispunem de un sistem şi un control eficient asupra verigilor de producere şi comercializare a seminţelor de legume. Din aceasta cauză piaţa este invadată cu seminţe de puritate biologică şi valoare culturală nesatisfăcătoare, stare fitosanitară inacceptabilă.

În rezultatul privatizării şi parcelării terenurilor agricole, în gospodăriile producătoare de seminţe a devenit imposibilă respectarea asolamentelor şi spaţiilor obligatorii de izolare a culturilor cu polenizare încrucişată. Insuficienţa mijloacelor financiare şi tehnice complică respectarea întocmai a tehnologiilor de cultivare, recoltare, extragere, condiţionare şi păstrare a seminţelor. Ca rezultat, a sporit considerabil volumul seminţelor necondiţionate. Din lipsa unei fabrici de procesare similare celor pentru porumb şi sfecla de zahăr în circuitul comercial seminţele de legume sunt incluse sub formă de semifabricat, cu un potenţial redus de productivitate, uniformitate şi calitate.

Analiza declinului în producerea şi comercializarea seminţelor de legume şi bostănoase confirmă lipsa unui sistem de asigurare a pieţii cu sortimentul şi calitatea necesară a acestora. Luând în consideraţie starea deplorabilă actuală, complexitatea multilaterală la producerea şi comercializarea seminţelor, evident este necesară organizarea unui centru (asociaţie, concern) republican cu funcţii de coordonare (fig.), care în bază de contract cu structurile economice din ramură ar organiza toate etapele de producere, achiziţionare şi comercializare a seminţelor [1, 3].

Ţările cu o agricultură avansată, dispun de regulamente stricte şi norme tehnice de producere, supraveghere, certificare şi comercializare a seminţelor, menite să asigure calitatea respectivă a acestora.

Sporirea competitivităţii seminţelor necesită elaborarea unui complex de măsuri organizatorice, ştiinţifice şi agrotehnice, orientate spre asigurarea pieţii şi posibilităţilor de export cu sortimentul şi calitatea respectivă a acestora. În sistemul de producere a seminţelor trebuiesc bine determinate verigile de producere a categoriilor superioare şi certificate. Particularităţile acestui sistem pot fi deter¬minate de către instituţiile ramurile de cercetări ştiinţifice, fiind apoi, reglementate şi adoptate de către Ministerul Agriculturii şi Industriei Alimentare.

Valoarea biologică şi culturală a soiurilor de legume se manifestă mai deplin la plantele crescute din seminţe de prebază şi bază. Puritatea biologică a varietăţilor trebuie menţinută de instituţiile ştiinţifice cu profil de ameliorare, eliberându-se pentru multiplicare gospodăriilor autorizate în producerea seminţelor de legume pentru consum. Seminţele de bază la cultura tomatelor, pătlăgele vinete, ardei, varză, castraveţi, rădăcinoase şi bostănoase se recomandă a fi reproduse numai o singură dată, la leguminoase: mazărea de grădină, fasole, bob, care se caracterizează printr-un coeficient mic de înmulţire – până la trei ori, iar pentru producţia marfă pot fi utilizate seminţe de a treia reproducere. La cultivarea verdeţurilor se folosesc seminţe de prima şi a doua reproducere.

Controlul calităţii seminţelor în întreprinderile şi cooperativele agricole, gospodăriile

de fermieri şi ţărăneşti, precum şi în organizaţiile de achiziţionare şi comercializare, indiferent de forma de proprietate, îl exercită Agenția Națională pentru Siguranța Alimentelor, care împreună cu producătorii şi comercializatorii poartă răspundere de puritatea, valoarea culturală și comercializarea acestora producătorilor de legume.

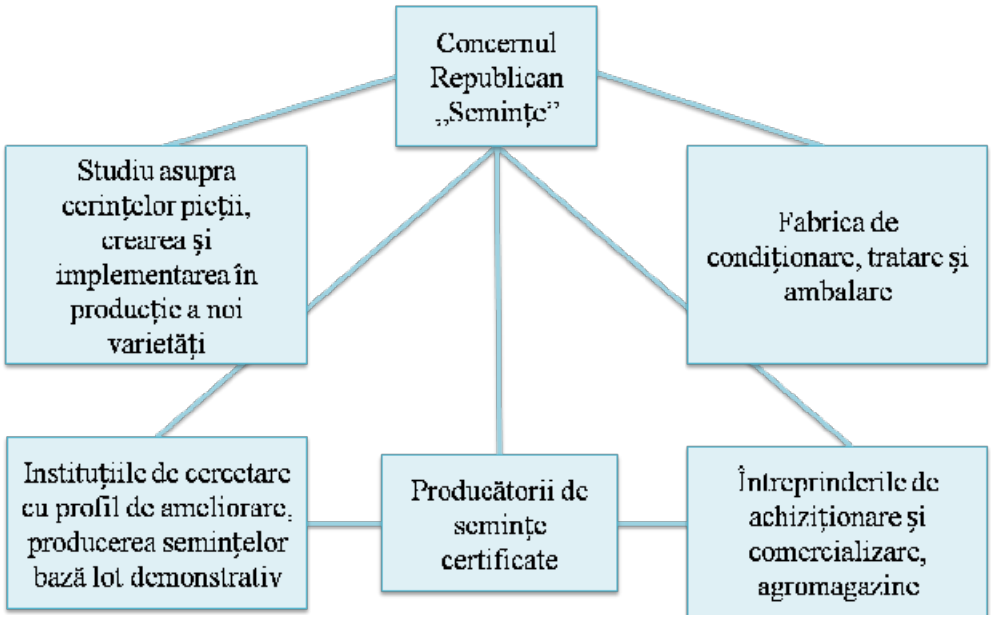

Fig. 1. Structura centrului republican pentru producerea și comercializarea semințelor de legume.

In scopul implementării tehnologiilor avansate, exercitării unui con¬trol eficient, care să contribuie la sporirea calităţii se recomandă concentrarea producerii seminţelor de legume în baza fostelor gospodării şi asociaţii specializate, care actualmente încă dispun de careva bază material-tehnică, specialişti şi experienţă în acest domeniu.

Întru îmbunătăţirea calităţii, evitarea amestecurilor în perioada recoltării, depozitării legumelor, extragerii şi condiţionării seminţelor este raţional ca deţinătorii de licenţe să fie autorizaţi în producerea seminţelor de soiuri concret nominalizate pe parcursul a mai multor ani. Aceasta va conduce la o eventuală specializare şi însuşire mai detaliată a particularităţilor caracteristice soiului dat, reglarea volumelor de producţie precum şi evidenţierea celor mai competitivi producători de seminţe.

Ameliorarea situaţiei la producerea seminţelor de legume ar contribui la excluderea de pe piaţa internă a seminţelor necondiţionate, reducerea pierderilor rezultate din utilizarea acestora, majorarea volumului de producere a seminţelor în valoare de 100-120 mln. lei, perfecţionarea permanentă a specialiştilor, păstrarea genofondului şi bazei material-tehnice, angajarea în muncă a circa 5-7 mii persoane.

Şi mai dificilă este situaţia legată de producerea cartofului pentru seminţe. Cu toate că acest produs ocupă un loc deosebit în alimentaţie şi dispune de mari rezerve de valorificare pe piaţa internă, din lipsa de finanţe cercetările se mărginesc doar la testarea soiurilor de import.

Cauze ce reţin dezvoltarea culturii cartofului în republică sunt multiple, dar principala o constituie lipsa unui sistem de producere a materialului săditor. În situaţia creată are loc un import haotic al cartofului semincer şi alimentar, o mare parte a căruia este de calitate inferioară, de soiuri neomologate şi necorespunzătoare condiţiilor pedoclimaterice locale.

Tradiţiile, experienţa bogată, condiţiile pedoclimatice favorabile sunt premize re-

ale pentru dezvoltarea durabilă şi sporirea potenţialului economic al legumiculturii. Optimizarea suprafeţelor de cultivare a legumelor şi majorarea volumului de producţie până la 1 mln tone anual ar permite încadrarea în muncă a circa 150 mii de persoane. În actualele condiţii economice, un hectar de legume, cu unele excepţii, poate asigura o producţie cel puţin de 150-200 mii lei. Legumicultori cu experienţă produc legume la o rentabilitate de peste 100%. Aceste constatări denotă că investiţiile în legumicultură pot fi recuperate într-un termen mult mai redus în comparaţie cu alte ramuri ale agriculturii.

Cercetările ştiinţifice şi experienţă înaintată dovedesc că în condiţiile noastre recolta posibilă de seminţe la sfecla roşie şi mazărea de grădină este de 1800-2000 kg/ha, la morcov şi varza albă - 1000-1200, la ceapă – 800-1000, la ardei şi castraveţi – 170-180, la tomate – 130-150 kg/ha. Realizarea potenţialului indicat ar satisface pe deplin necesităţile pieţii interne şi posibilităţile de export, în deosebi în ţările CSI, republicile Baltice şi România, în care seminţele din Republica Moldova sunt solicitate de cultivatorii de legume.

În pofida condiţiilor climatice favorabile, importanţei economice şi sociale înalte, producerea seminţelor de legume s-a redus considerabil. În urma reformei agrare şi privatizării gospodăriilor semincere s-a mărit considerabil numărul agenţilor economici care activează în producerea şi comercializarea seminţelor. Mulţi dinte ei, ne dispunând de o bază material-tehnică corespunzătoare, sunt dispuşi să cuprindă un număr cât mai mare de specii şi soiuri, ceea ce deseori complică amplasarea culturilor în spaţiu şi obţinerea seminţelor de calitate.

Întru evitarea amestecurilor în perioada recoltării, extragerii şi condiţionării seminţelor se recomandă a limita la minimum soiurile din aceiaşi specie. Pentru îmbunătăţirea calităţii seminţelor trebuie ca producătorii să fie autorizaţi în producerea seminţelor de soiuri concret nominalizate pe parcursul a mai multor ani. Aceasta v-a conduce la o eventuală specializare şi însuşire mai detaliată a particularităţilor soiurilor, reglare a volumelor de producţie precum şi evidenţierea celor mai competitivi producători şi realizatori de seminţe.

Certificarea culturilor semincere reprezintă un sistem de verificare a ansamblului de măsuri destinate producerii de sămânţă şi material săditor, efectuat sub aspectul purităţii biologice a stării fitosanitare, a modului de aplicare tehnologiilor, care permit identificarea caracterelor de tipicitate în vederea menţinerii şi confirmării pe bază de documente a identităţii, purităţii biologice şi stării fitosanitare a seminţelor şi materialului săditor. Certificarea seminţelor şi materialului săditor prin controlul culturilor semincere în câmp are ca scop menţinerea şi promovarea în producţie a materialului biologic prin soiuri şi hibrizi valoroşi, adoptaţi la condiţiile ecologice de cultură, care să valorifice cu înaltă eficienţă economică potenţialul productiv al solului.

Pentru efectuarea lucrărilor de certificare de o importanţă deosebită este cunoaşterea în detaliu a soiurilor şi hibrizilor supuşi certificării, precum şi cauzele ce pot duce la modificarea structurilor genetice, aspecte ce trebuie să fie posedate temeinic de către toţi specialiştii incluşi în procesul de certificare.

Caracteristicile genetice ale unei populaţii pot fi influenţate în procesul de transmitere a genelor de la o generaţie la alta de anumiţi factori, cum ar fi: mărimea populaţiei, diferenţele de fertilitate â variabilitate a indivizilor, sistemul de împerechere, mutaţia, migraţia genelor, segregările întârziate, selecţia naturală ş.a.

Principalul obiectiv al sistemului şi schemelor de producere şi înmulţire a seminţelor este menţinerea purităţii lor biologice, a capacităţii de producţie, păstrarea sănătăţii seminţelor şi asigurarea la timp a producătorilor agricoli cu sămânţă, care să posede însuşirile originale ale soiului şi hibridului creaţi de ameliorator.

Concluzii

Tradiţiile, experienţa acumulată, condiţiile pedoclimatice favorabile sunt premize reale pentru revitalizarea legumiculturii. Producţia de legume poate fi majorată până la

1 mln. tone anual.

Luând în consideraţie, complexitatea multilaterală la producerea şi comercializarea seminţelor, este necesară organizarea unui centru (asociaţie, concern) republican cu funcţii de coordonare, care în bază de contract cu structurile economice din ramură ar organiza toate etapele de producere, achiziţionare şi comercializare a seminţelor.

Dezvoltarea durabilă a legumiculturii necesită un studiu permanent asupra nivelului de producere, creare a soiurilor şi hibrizilor noi, elaborarea şi implementarea unor metode şi tehnologii de producere a seminţelor, răsadurilor şi de cultivare a legumelor, în stare să asigure o producţie cu o valoare biologică şi competitivitate înaltă în condiţii deficitare de apă şi resurse energetice.

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EVALUAREA ACTIUNII DERIVATILOR POLIANIONICI GLICOZID-FUROSTANOLICI ASUPRA DIVIZIUNII MITOTICE SI A CROMOSOMILOR LA SPECII VEGETALE TEST

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Introducere

Biopreparatele glicozidice naturale, folosite in testările in vivo şi in vitro au fost extrase şi purificate din seminţe de *Lycopersicum esculentum* (ENLE), din frunze de *Digitalis purpurea* (ENDP) şi din seminţe de *Capsicum annuum* (ENCA). Aceste glicozide furostanice naturale conţin ca glucid glucoza, galactoza şi xiloza (ultima în seminţele de ardei iute), gruparea agliconică de natură sterolică fiind reprezentată de neotigogenină (ENLE) sau gitogenină (ENDP şi ENCA).

Bioregulatorii de natura steroid-glicozidică sunt cunoscuţi a avea efecte stimulatoare evidente în procesul de formare a structurilor calusate, în special în cazul unor explante provenite din frunze şi mai puţin în cazul explantelor provenite din hipocotil. Unii dintre acestia pot induce chiar formarea directă de embrioizi somatici, din explante, embrioizi cu potenţial sporit de creştere şi dezvoltare ulterioară a plantelor viguroase.

Plecand de la această premisă, am testat biopreparatele glicozidice din punct de vedere al influenţei asupra procesului de diviziune mitotică şi, implicit, a cromosomilor. In acest scop s-a utilizat următoarea metodologie:

1. folosirea unor plante – test, cu cariotip şi ciclu celular bine caracterizat. Planta test aleasă în acest scop este *Secale cereale.*
2. expunerea carioselor, la acţiunea unor soluţii conţinând biopreparatele glicozidice furostanolice amintite, in diferite concentraţii. Germinarea s-a realizat în soluţiile de derivaţi polianionici glicozid-furastanolici testate.
3. consecutiv germinării, s-a procedat la fixarea materialului.
4. pe materialul fixat s-au efectuat următoarele determinări: stabilirea indicelui mitotic, determinarea procentului de aberaţii cromosomiale în ana-telofaza mitozei.

În general, acţiunea diferiţilor compuşi asupra diferitelor specii de plante, depinde de concentraţia cu care se acţionează asupra materialului genetic. În experimentele efectuate, compararea s-a făcut cu martori care au germinat in aceleaşi condiţii, dar in apă distilată, comparativ cu variantele experimentale la care germinarea s-a realizat in soluţiile testate.

Materiale şi metode

Material biologic: cariopse de secara.

Reactivi: preparate de derivati polianionici glicozid-furastanolici; apă distilată; fixator (alcool-acid acetic 3/1); HCl 1N; HCl 50 %; acid acetic 45 %.

Modul de lucru:

Germinarea. Germinarea s-a realizat în cutii Petri tapetate cu hârtie de filtru, umectată cu apă distilată (pentru variantele martor), sau cu soluţiile de derivaţi polianionici glicozid-furastanolici (in cazul variantelor experimentale). Germinarea a avut loc la întuneric, la 23 – 24 ˚C, până în momentul în care rădăciniţele au atins lungimea de cca. 10 mm. Pentru a respecta aceste condiţii, plăcile Petri au fost plasate în termostat programat la temperatura dorită. Rădăciniţele cu lungimea adecvată au fost obţinute după 48 de ore.

Spălarea, fixarea, hidroliza, colorarea si efectuarea preparatelor microscopice s-au realizat conform metodologiei standard de colorare si observare a cromosomilor la plante (Cimpeanu şi colab., 2002).

Preparatul	Concentratia	Notatie	Observaţie
Tomatozid	0,005%	T	In apă distilată
Tomatozid	0,03%	T	In apă distilată
Moldstim	0,005%	M	In apa distilata
Moldstim	0,03%	M	In apa distilata
Tomatozid oxidat	0,005%	Tf	Oxidat
Tomatozid oxidat	0,03%	Tf	Oxidat

Rezultate

În graficele care urmează sunt reprezentate numărul de celule analizate de-a lungul acestui experiment, atât pentru martori, cât şi pentru probe, în urma vizualizării acestora la microscopul optic. De pe fiecare lamă au fost analizate câte zece câmpuri şi numărate celulele în interfază, profază, metafază, anafază, telofază din totalul de celule.

După cum observăm din graficul de mai sus, la ***Secale cereale,*** derivatul polianionic glicozid-furostanolic T (0,005%) nu influenţează semnificativ celulele aflate în diviziune, acest lucru dovedind o uşoara scădere de la 12,7 (în cazul martorului) la 10,5 (în cazul probei tratate).

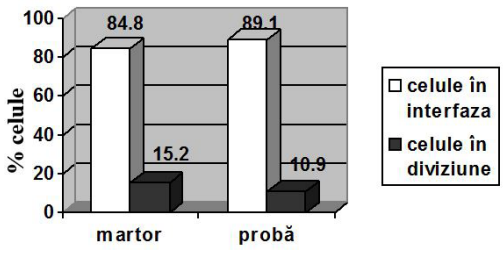

Fig.1. Frecvenţa totală a celulelor în diviziune mitotică la *Secale cereale* in cazul tratamentului cu derivatul polianionic glicozid-furostanolic T (0,005%).

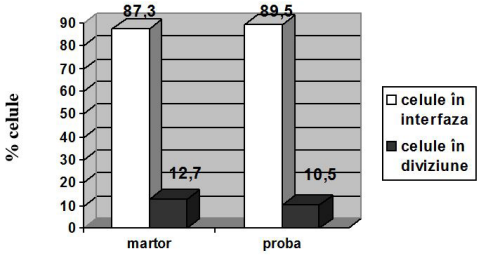

Fig. 2. Frecvenţa totală a celulelor în diviziune mitotică la *Secale cereale* in cazul tratamentului cu derivatul polianionic glicozid-furostanolic T (0,03%).

În cazul derivatului polianionic glicozid-furostanolic T, de concentraţie 0,03%, scăderea procentului de celule in diviziune este mai mare, de la 15,2% (pentru martor) la 10,9% (pentru proba tratată).

Derivatul polianionic glicozid-furostanolic M (0,005%) nu a provocat la *Secale cereale*, scaderi semnificative ale procentului de celule in diviziune mitotică, la concentraţia amintită, comparativ cu varianta martor. Situaţia este similară şi in cazul concentraţiei de 0,03% a soluţiei de derivat polianionic glicozid-furostanolic.

Nu sunt prezentate rezultatele in cazul utilizării derivatului polianionic glicozid-furostanolic Tf (0,005% si 0,03%), deoarece cariopsele nu au germinat in prezenţa acestuia.

Concluzionând, din cele comentate mai sus şi reprezentările grafice, rezultă că soluţiile de derivaţi polianionici glicozid-furostanolici utilizate, T si M, la ambele concentraţii de lucru (0,005% si 0,03%), au un efect bioinhibitor slab asupra diviziunii mitotice a celulelor meristemelor radiculare la Secale cereale.

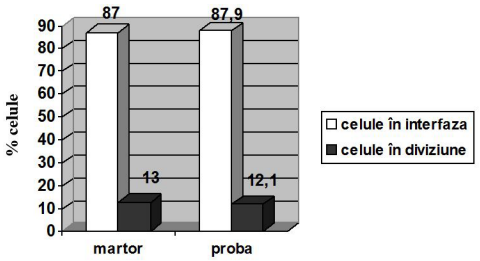

Fig. 3. Frecvenţa totală a celulelor în diviziune mitotică la *Secale cereale* in cazul tratamentului cu derivatul polianionic glicozid-furostanolic M (0,005%).

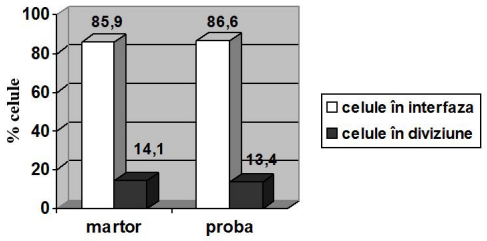

Fig.4. Frecvenţa totală a celulelor în diviziune mitotică la *Secale cereale* in cazul tratamentului cu derivatul polianionic glicozid-furostanolic M (0,03%).

În cazul tuturor derivaţilor polianionici glicozid-furostanolici investigaţi, proporţia A-T aberante a fost superioară celei prezentate de către martorii germinaţi in apă distilată, in cazul acestora din urma, prezenţa aberaţiilor încadrându-se in nivelul normal de mutaţii spontane.

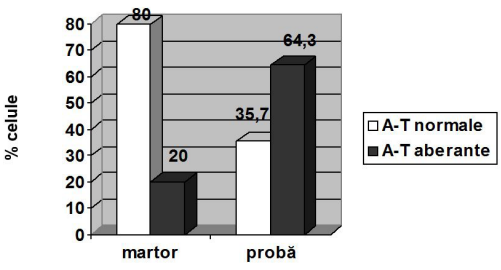

Fig. 5. Proporţia A-T aberante in cazul tratamentului cu derivat polianionic gli cozid-furostanolic T (0,005%).

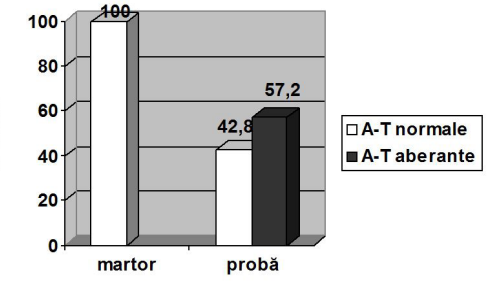

Fig. 6. Proporţia A-T aberante in cazul tratamentului cu derivat polianionic glicozid-furostanolic T (0,03%).

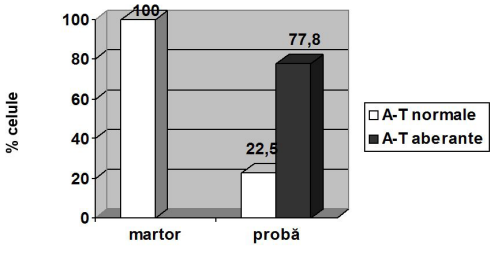

Fig. 7. Proporţia A-T aberante in cazul tratamentului cu derivat polianionic glicozid-furostanolic M (0,005%).

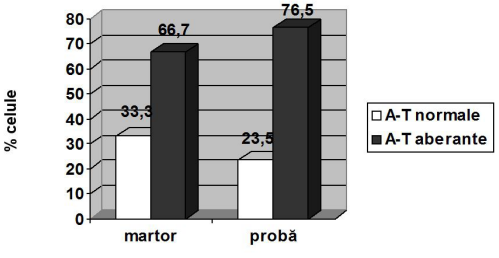

Fig. 8. Proporţia A-T aberante in cazul tratamentului cu derivat polianionic glicozid-furostanolic M (0,03%).

Concluzii

Indicele mitotic la *Secale cereale* prezinta o scădere la variantele de tratament cu derivati polianionici glicozid-furostanolici. Creşterea valorii acestui parametru nu a fost depistată la nici una dintre variantele experimentale. Apariţia de mutaţii structural cromosomiale a fost crescută procentual fata de variantele martor. În general, au fost prezente mai frecvent aberaţii de tip: ana-telofaze cu punţi, punţi si fragmente, fragmente.

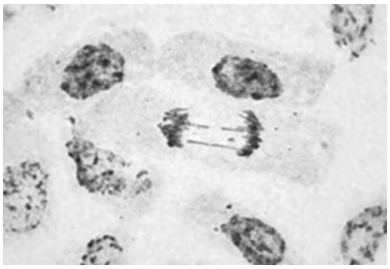

Fig. 9. Ana-telofază cu punte (original).

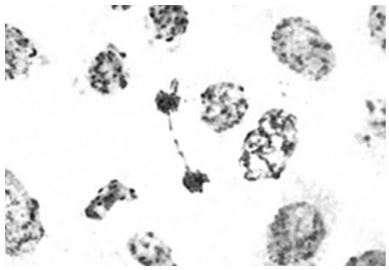

Fig. 10. Ana-telofază cu punte şi fragment (original).

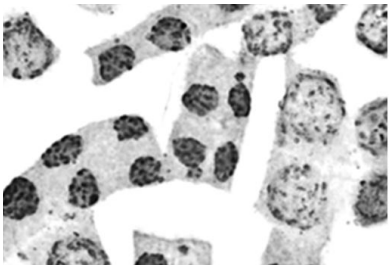

Fig. 11. Ana-telofază cu punţi.

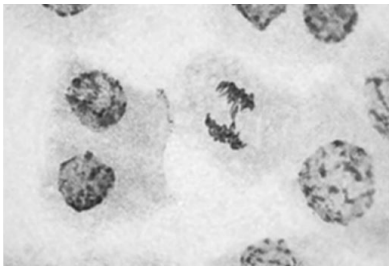

Fig. 12. Interfază cu micronucleu (original).

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EXPRESSION OF GENES INVOLVED IN SCLAREOL BIOSYNTHESIS IN *SALVIA SCLAREA* L.

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Introduction

Sclareol is a labdane-type diterpene alcohol [9] with antibacterial, antifungal properties and growth regulating activity [4], thus being very useful for perfumery and flavoring [8, 14, 22].

This compound has been reported in four plant species from different botanical families: *Salvia sclarea* (*Lamiaceae*), *Cistus creticus* (*Cistaceae*), *Nicotiana glutinosa* (*Solanaceae*) and *Cleome spinosa* (*Brassicaceae*) [4].

Most of the commercially produced sclareol is derived from cultivated clary sage (*Salvia sclarea* L.), which is a pluriannual herb commonly cultivated for its essential oil [1, 6, 19, 11, 21]. The relatively easy farming of this plant and its high sclareol yield has encouraged clary sage producers to begin genetic improvement programs and expand *S. sclarea* L. plantations. According to European Herb Growers Association (Europam), the annual cultivated area of clary sage in France is 5 000 ha and 2 000 ha in Bulgaria and Moldova [16].

Despite the expanded cultivation area of clary sage, the annual production and the sclareol yield is very variable, which is caused by the different synthesis and acumulation capacities of this compound in inflorescences [3]. The success of increase of sclareol content by *S. sclarea* breeding programs could be ensured through ability to regulate the sclareol synthesis metabolic pathway.

It is known that, the biosynthesis of sclareol proceeds in two steps – from geranylgeranyl diphosphate (GGPP) to labda-13-en-8-ol diphosphate and sclareol. The first reaction is catalyzed by labda-13-en-8-ol diphosphate synthase and the second – by sclareol synthase (Figure 1) [4].

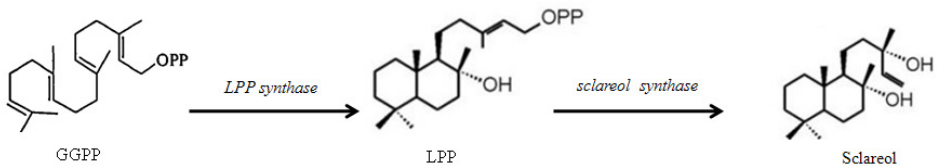

Fig. 1. Biosynthetic pathway of sclareol in Salvia sclarea L.
(GGPP - geranylgeranyl diphopsphate, LPP - labda-13-en-8-ol diphopsphate).

Sclareol synthase has been successfully cloned and functionally characterized and its synthesis pathway had been reconstructed in recombinant *E. coli*, using genetic engineering through overexpression for metabolic biosynthesis of sclareol [23].

Chemical synthesis would seem to be another obvious option for the obtaining of sclareol. However, due to its highly complex structure, an inexpensive synthetic process for this compound is still difficult [15]. So, the analysis of the transcriptional activity of genes involved in the biosynthesis of secondary metabolites, including sclareol, correlated with the selection of valuable parental forms is a current objective of molecular breeding.

The investigation of the molecular basis of the sclareol biosynthesis provides opportunities for further research in a wide range of areas, from structure – function to

genetics of disease resistance, via metabolic engineering of fragrance ingredient precursors.

Materials and methods

Plant material. Twenty-eight genotypes of clary sage, from Aromatic and Medicinal Plants Collection of the Institute of Genetics, Physiology and Plant Protection, ASM, including 13 hybrids and 15 parental forms were evaluated in this study (Table 1). Biological samples for each genotype were collected at 4-6 pairs of leaves stage from five independent plants, grown in field conditions and immediately frozen in liquid nitrogen for further analysis (Figure 2).

Fig. 2. Cultivation in field conditions of clary sage plant material.

Primer design for real-time PCR. The design of primers was performed using **PRIMER3** ***web tool*** (http://primer3plus.com/web_3.0.0/primer3web_input.htm).

The primer pairs for the studied genes were designed based on mRNA sequences: JN133922.1 *Salvia sclarea* (clary) sclareol synthase; JQ478434.1 *Salvia sclarea* (clary) *labd-13-en-8-ol diphosphate synthase* [22] (Table 2). The clary sage actin (NCBI: HM231319.1) was used as a reference gene (Table 2) [25].

RNA isolation. Total RNA was extracted from a bulk of five leaves of each genotype using TriReagent (Ambion, Applied Biosystems), according to the manufacturer's instruction.

These samples were treated with dsDNase (Thermo Scientific) to remove the residual DNA. First-strand cDNAs were synthesized from 0,6 μg total RNA using Oligo(dT18), random hexamer primers (Thermo Scientific) and RevertAid Reverse Transcriptase (Thermo Scientific).

Real Time PCR analysis. qRT-PCR was performed with gene-specific primers (Table 2) and Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific) on a DTprime Real-time cycler (DNA-Technology). The amplification program used for qRT-PCR was: 95 °C for 10 min, 950 C for 15 s and 60 0C for 30 s, 40 cycles of 95° C for 15 s, 60° C for 30 s, and 72° C for 30 s, followed by a melting curve cycle from 60°C to 90° C. All samples were analyzed in three replicates performed in three different runs.

Primer specificity was assessed by agarose gel electrophoresis as well as from melting curve analysis of every reaction. The relative expression was calculated via the $2^{-\Delta Ct}$ method [16].

Quantitative determination of the sclareol by HPLC. Extraction of essential oil from S. sclarea L. was carried out according to the method specified in the European Pharmacopoeia [12, 25]. The fresh plant samples were subjected to hydrodistillation in Ginsberg equipment and kept in freezer compartment prior to be used in the study.

The samples of essential oil were analyzed by HPLC (Gilson: 303 pump series, 803C manometric, 231 injector, RI 131 refractive index detector, Kipp & Zonen BD41 recorder), with the hexane solvents gradient system for elution with 1.0% (v/v) isopro-

pyl alcohol. It was used a column type SGX CN (Prague) 150 x 3,5 mm, mobile phase at a flow rate of 0,7 ml/min and operating pressure of 18 Bar. Detection was done at 270 nm.

Table 1. ***Salvia sclarea*** **L. genotypes used in investigations**

♀ Form *		♂ Form *		Hybrid F1-2 **	
1	S. s. Turkmen/N S_7	15	(K-36 x 0-41) F_2 x 0-19) F_1 x 0-22) B_4 x L-15) F_8	17	[S. s. Turkmen/N S_7 x (K-36 x 0-41) F_2 x 0-19)F_1 x 0-22) B_4 x L-15)F_8] F_1
1	S. s. Turkmen/N S_7	2	(S-1122 528 S_3 x K-50) F_1 x 0-48) F_6]	18	[S. s. Turkmen/N S_7 x (S-1122 528 S_3 x K-50) F_1 x 0-48)F_6] F_1
1	S. s. Turkmen/N S_7	4	(Rubin x S1122 9S3) F_1 x (0-56 x V-24) F_1) F_7	19	[S. s. Turkmen/N S_7 x (Rubin x S1122 9 S_3)F_1 x (0-56 x V-24) F_1) F_7] F_1
3	(V-24-86 809 S_3 x 0-33 S_6) F_7	4	(Rubin x S1122 9S3) F_1 x (0-56 x V-24) F_1) F_7	20	[(V-24-86 809 S_3 x 0-33 S_6) F_7 x (Rubin x S1122 9S_3)F_1 x (0-56 x V-24)F_1)F_7] F_1
3	(V-24-86 809 S3 x 0-33 S6) F_7	9	(S1122 528 S_3 x S. s. TianShan/sud) F_5 x S. s. Tian-Shan/ sud) B_5	21	[(V-24-86 809 S_3 x 0-33 S_6) F_7 x (S1122 528 S_3 x S. s. Tian-Shan/ sud) F_5 x S. s. Tian-Shan/sud) B_5] F_1
3	(V-24-86 809 S_3 x 0-33 S6) F_7	11	Cr. p. 160 S_{11}	22	[(V-24-86 809 S_3 x 0-33 S6) F_7 x Cr. p. 160 S_{11}] F_1
16	M-69 655 S_9	12	(S-1122 528 S_3 x (Rubin x S-786)F_1 x (0-33 S_3 x L-15) F_7) F_7	23	[M-69 655 S_9 x (S-1122 528 S_3 x (Rubin x S-786)F_1 x (0-33 S_3 x L-15) F_7) F_7] F_1
16	M-69 655 S_9	15	(K-36 x 0-41) F_{20-19}) F_1 x L-15) B_5	24	[M-69 655 S_9 x (K-36 x 0-41)F_{20-19})F_1 x L-15) B_5] F_1
16	M-69 655 S_9	13	(M-69 429-82 S_3 x 0-40 S_5) F_7	25	[M-69 655 S_9 x (M-69 429-82 S_3 x 0-40 S_5) F_7] F_1
10	Cr. p. 11 S_{11}	1	S. s. Turkmen/N S7	26	[Cr. p. 11 S_{11} x (S. s. Turkmen/N S_7] F_1
15	(K-36 x 0-41) F_2 x 0-19)F_1 x 0-22) B_4 x L-15) F_8	7	(M-44S_4 x L-15)F_1 x L-15)] B_6	27	[(K-36 x 0-41) F_2 x 0-19)F_1 x 0-22) B_4 x L-15)] F_7 x (M-44S4 x L-15) F_1 x L-15)] B_6] F_2
14	(K-50) F5 x S 1122 (102+113) F_2 x K-43) F_4	6	(0-57S_5 x 0-21S_4) F_8	28	[(K-50)F_5 x S 1122(102+113)F_2 x K-43) F_4 x (0-57 S_5 x 0-21S_4) F_8] F_2
5	(M-55+130 S_4 x (K-44 x L-15) F_2 x 0-47) F_6	7	(M-44 S_4 x L-15)F_1 x L-15) B_6	29	[(M-55+130 S_4 x (K-44 x L-15) F_2 x 0-47) F_6 x (M-44 S_4 x L-15) F_1 x L-15) B_6] F_2
** 1 – 16 clary sage parental forms*				***17 – 29 clary sage hybrids*	

Estimation of sclareol concentration in essential oil extracts from clary sage hybrids was performed by absolute calibration method, using as reference standards sclareol pure solutions. Sclareol concentration was estimated as the product of peak height and half-height length [5].

Data analysis. Statistical analysis of the experimental data was performed according to Dospehov [27]. For calculation of descriptive statistics (arithmetic mean, standard deviation etc.) and Pearson's coefficient of correlation was used Microsoft Excel spreadsheet program.

Table 2. The specific primers used for qRT-PCR

Access number	Nucleotide succession (5'- 3')	Tm	Amplicon, bp
EMBL: JN133922.1	F: GAGCACCAGCAGCGATTAT	56,7	133
	R: GAGAGTTGCTTAGGACGGATTT	58,4	
EMBL: JQ478434.1	F: GACTCCAGAAACAACCCACATT	58,4	138
	R: CCCAGACGACCCTCCACAAGA	63,7	
NCBI: HM231319.1	F: TGGATTTGCTGGAGACGATG	57,3	293
	R: CACGATTGGCCTTGGGATTA	57,3	

Result and discussions

Selection of parental forms is an important first step in any breeding program. The ability to assess accurately genetic differences between parents and subsequently to predict progeny performance could enhance the efficiency of breeding programs [2].

Actually there is a great temptation to use molecular genetics in plant breeding [7, 17]. But, as selection methods based on phenotypic data rely on accurate estimation of breeding values of individuals or groups, similarly, molecular techniques require the accurate estimation of breeding values of alleles from phenotypes [21]. So, molecular genetics does not provide a quick fix to breeders, but rather slightly increased response to selection in part of the genome at a great cost.

Because molecular techniques provide powerful tools to study genetics and physiology of crops, it has been investigated the transcriptional activity of LPPS and SS genes governing the biosynthesis of sclareol in clary sage.

The primary aim of this study was to assess expression levels of SS and LPPS genes in some parent lines and hybrid combinations. In case of the SS no specific product was identified and this gene was excluded from further investigations. Real-Time PCR results were considered only for one gene – LPPS. For this gene a specific DNA fragment of 133 bp was amplified (Figure 3).

The analysis of LPPS transcriptional activity for 15 parental genotypes and 13 hybrid combinations showed that relative expression ranged from 0,2 to 5,8 conventional units. The quantity of transcripts in parental forms was relatively lower compared to clary sage hybrids.

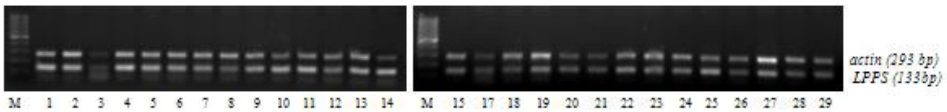

Fig. 3. Gene expression profile obtained using primers for LPPS gene and actin.
(M – 1000 bp Ladder, parental forms: 1 – 15, hybrids: 17 – 29).

In the case of studied hybrids the highest activity of gene was revealed for *([M-69 655 S9 x (M-69 429-82 S3 x 0-40 S5)F7]F1,* followed by *[Cr. P. 11 S11 x (S. s. Turkmen/N S7]F1, [S. s. Turkmen/N S7 x (Rubin x S1122 9S3)F1 x (0-56 x V-24) F1)F7] F1* and *[M-69 655 S9 x(K-36 x 0-41)F20-19)F1 x L-15)B5]F1* but the lowest values were recorded for *[(V-24-86 809 S3 x 0-33 S6)F7 x (S1122 528S3 x S. s. Tian-Shan/sud) F5 x S. s. Tian-Shan/sud)B5]F1* and *[(V-24-86 809 S3 x 0-33 S6)F7 x (Rubin x S1122 9S3) F1 x (0-56 x V-24)F1)F7]F1* (Figure 4, A).

For the parental forms the highest transcript accumulation level of LPPS gene was detected in S. s. Turkmen/N S7, followed by *(S-1122 528 S3 x K-50) F1 x 0-48) F6* while the lowest quantities were observed in case of *Rubin x S1122 9 S3) F1 x (0-56 x V-24) F1 and Cr. P. 11 S11* (Figure 4, B.).

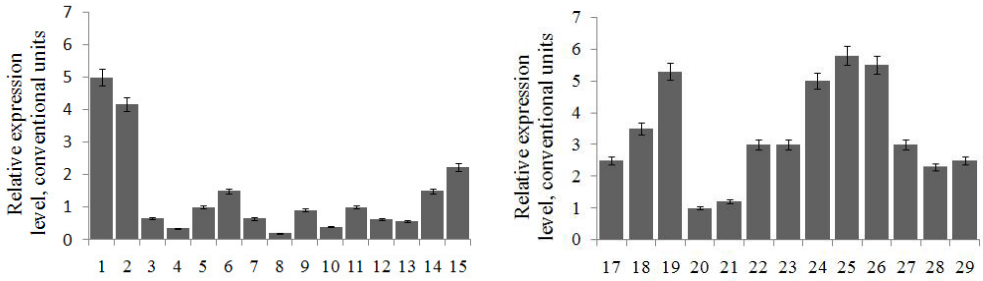

Fig. 4. Relative expression of LPPS gene for hybrids (A) and parental forms (B) .

Results obtained for genetic groups have revealed an association between transcriptional activity of LPPS gene and heterosis effect. Thus, 11 (19-29) of the 13 investigated hybrids have shown quantitative values of the transcriptional activity higher than such in parental forms. In six cases (22 hybrids, 24, 25, 27, 28 and 29) observed differences were significant (Figure 5).

Hybrid vigor is substantial and important for most commercial traits in plants [10, 13, 14]. At genetic level heterotic groups in clary sage have not been well studied or described. The characterization of genetic variability and an estimation of the genetic relationships among varieties are essential to clary sage breeding programs. Thus, these findings could represent a substantial advantage to predict the heterosis expected from crosses at all levels.

The second aim of this study was to estimate correlations between a series of proposed heterotic groups and hybrid performance for sclareol content in clary sage.

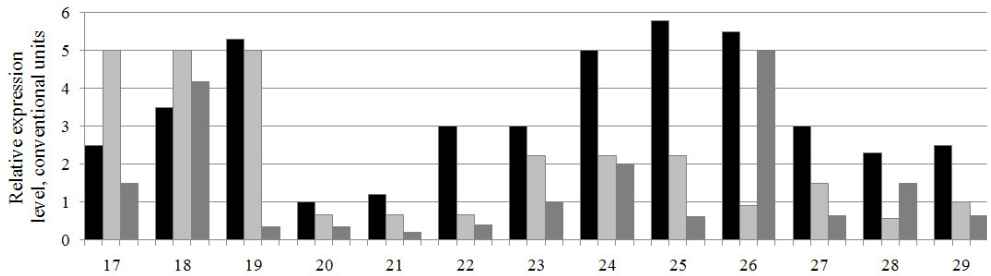

Fig. 5. Relative expression of LPPS gene for clary sage genetic groups
(*the genotypes order on the diagram is as follow: hybrid F1/ F2, ♀ form and ♂ form*)

The results obtained for the biosynthetic capacity show high intrapopulational variability of S. sclarea. Thus, the content of sclareol has varied from 1,9 % (hybrid no. 29) to 10 % (hybrid no. 25) (Figure 6).

The comparative analysis of data obtained for clary sage hybrids, in most cases ascertained a positive correlation (Pearson`s correlation coefficient r = 0,7) between the quantitative parameters which were studied – sclareol content in essential oil and LPPS transcriptional activity (Figure 7).

As example, the highest values of LPPS transcript concentration for the M-69 655 S9 x (M-69 429-82 S3 x 0-40 S5) F7] F1 hybrid correlates with biochemical data. Also, the data for the *[(M-55+130 S4 x (K-44 x L-15) F2 x 0-47) F6 x (M-44 S4 x L-15) F1 x L-15) B6] F2* hybrid, which showed the lowest amount of sclareol in essential oil correlates with the decreased level of transcriptional activity of the analyzed gene (Figure 7).

Summarizing, the obtained data support the hypothesis that LPPS gene transcriptional activity level could be useful in sclareol content prediction in clary sage.

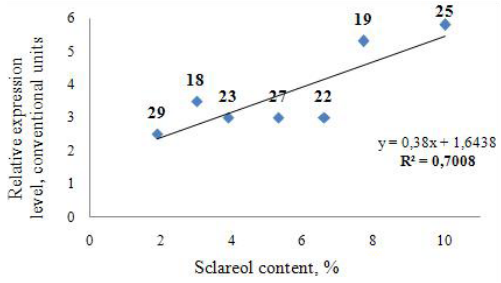

Fig. 6. Sclareol content for clary sage hybrids.

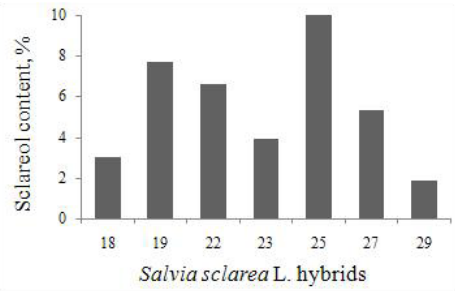

Fig. 7. The correlation between sclareol content and LPPS gene transcript level.

Conclusions

The level of relative expression of the LPPS gene was determined and its involvement in the biosynthesis of sclareol in *Salvia sclarea* L. was demonstrated. Quantitative RT-PCR results revealed six heterotic groups (no. 22, 24, 25, 27, 28, 29), for which the level of LPPS gene transcriptional activity was correlated with heterosis effect.

The results revealed that the highest values of LPPS transcript concentration for the *[M-69 655 S9 x (M-69 429-82 S3 x 0-40 S5) F7] F1* hybrid positively correlates with biochemical data – sclareol content in essential oil. Thus, such hybrid combination can be succesfully used in breeding programs.

The performed study ascertained the opportunity to predict heterosis effect based on the information about the levels of relative expression of genes, involved in secondary metabolites biosynthesis.

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INVENTORYING OF CORNELIAN CHERRY AND HAZELNUT POPULATIONS IN FOREST ECOSYSTEMS OF MOLDOVA IN THE CONTEXT OF THE IN SITU CONSERVATION OF CROP WILD RELATIVES

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Introduction

Under the conditions of global climate change, dramatic degradation of natural resources, growth of human population on the Earth, food shortage and maldistribution of food, the role of crop wild relatives (CWR) as one of stabilizing factors of civilization development significantly increases [19, 3, 5]. CWR of natural flora have the wide spectrum of valuable traits and properties – resistance to draught and limiting temperatures, diseases and pests, increased content of bioactive substances and main nutrituents. Gene pool of these species created during evolution is a valuable source material and is used in various agricultural crop plant breeding [6, 4, 14, 10, 16, 12, 13, 15, 9 et.al.]. Along with cultivated species CWR form a part of plant genetic resources, the diversity of which determines national food security. This fact promotes increase of interest to this component of agrobiodiversity, deepening of studies on various aspects of their evaluation, conservation and use [19].

Laboratory of plant genetic resources of the Institute of Genetics, Physiology and Plant Protection of the Academy of Sciences of Moldova conducts complex studies on inventory, collection, investigation and conservation of genetic resources of crop plants and their wild relatives. Special attention is paid to investigation of natural populations of CWR, search for the germplasm possessing useful properties. Among CWR of fruit crops growing in forest ecosystems of Moldova, an important role belongs to hazelnut (*Corylus avellana* L.) and cornelian cherry (*Cornus mas* L.).

In situ conservation of hazelnut biodiversity represents a challenging issue of high priority in the context of current trend of reduction of areas of pure hazelwoods and decreased presence of this culture in many forest types. On the other hand, share of wild fruit tree-shrub cultures including cornelian cherry increases among the main species used for restoration of forests. For this purpose, for example, more than 2 million seedlings and young plants of cornelian cherry from the material grown in forest tree nurseries in 2002-2008 were planted. This species is of exceptional interest not only due to its role in forest conservancy but also as a fruit and medicine culture [21, 11, 8].

Purpose of investigations was to identify current status of growth and *ex situ* conservation of populations of abovementioned species in different soil-climatic zones of the Republic of Moldova.

Materials and methods

Investigations were conducted in 2007-2014 years in all ecological zones of the republic. Inventorying of cornelian cherry and hazelnut populations was performed in forest ecosystems of more than 30 forest stations and protected natural areas. Positioning of trees was performed using *Garmin eTrex H* – a GPS navigation device. Also some morphobiological characteristics of studied objects were determined – height of trees, tree stem diameter, fructification pattern, phenotypic manifestation of their resistance to environmental stresses, including premature drying.

Results and discussion

Cornelian cherry and hazelnut are important components of forest ecosystems of many countries. Natural habitat area of *Corylus avellana* is rather extensive - from

Mediterranean seaboard of the North Africa to Great Britain and Scandinavian Peninsula, and in the East – to the Ural, Caucasus Mountains, Iran and Lebanon (Thompson et al., 1996). Cultivated form of hazelnut (filbert) is a valuable crop that ranks sixth in production volume among other species of nut plants [2]. In *Cornus* L. genus, which includes about 60 species, the most economically valuable is *C.mas*, occurring in the wild form in the Central and South-Eastern Europe and also in the South-Western Asia. In mountainous areas it grows at a height of up to 1400 m above the sea level [11]. Forest forms of cornelian cherry differ from cultivated ones in that they have irregular fruiting and lower quality of fruits. In many European countries interest to this crop is growing, high-productive varieties have been created and industrial plantations are exploited.

In the territory of Moldova, cornalian cherry and hazelnut occur in all natural zones, more frequently in Codri, Priprutie, in Transnistrian and Tigech highlands, less frequently in southern and northern regions [23, 24, 7, 1]. In the central zone of the Republic the largest forest areas are concentrated (48%). Dominating species here include beech (*Fagus sylvatica*), durmast oak (*Quercus petraea*) and common oak (*Quercus robur*). Among various forest types we should note the beech oak-forests in combination with *Carpinus betulus*, *Fraxinus excelsior, Tilia tomentosa* and *Acer platanoides*, and also common and durmast oak forests with hornbeam, mono-dominant durmast oak forests and etc. Hazelnut and cornelian cherry grow in the undergrowth of said associations along with hawthorn, dogwood, spindle tree, blackthorn, smoke-tree and other species, forming sometimes brushwood of various densities.

It was noted that land topography and vegetation conditions influence on the growth, functional state and fructification of populations of *Corylus avellana* and *Cornus mas*, their resistance to drought and other unfavorable environmental conditions. Plants growing in forest stations located in Edineţi, Soroca, Nisporeni, Straşeni, Calaraşi and some other forest stations were in the best state among natural populations of hazelnut. Biometric measurements of plants were made and showed that height of cornelian cherry bushes varied within 2-7 m, and tree stem diameter was 3-20 cm. Diversity was noted with regard to shape and size of fruits and their taste qualities. Hazelnut bushes were as high as 4-7 m, sometimes up to 10 m, and had 2-5 main stems 2-16 cm in diameter. Some results of positioning of populations of these species and plant morphological data are given in the table 1.

Particular attention should be paid to the issue of intraspecific diversity of cornelian cherry and hazelnut in their natural habitats when determining the degree of genetic erosion under the conditions of dramatic fragmentation and significant anthropogenic pressure on forestlands, characteristic for the Republic of Moldova. On the other hand, survey of mono-dominant and mixed artificial plantations of hazelnut and cornelian cherry in different forest stations (Vadul lui Vodă, Susleni, Anenii Noi, Sîngerei, Chişcăreni, Bobeica, Căzăneşti and etc.) revealed their ineffective use (solely for fruit harvesting) and the absence of any management. However it should also be noted that these sites are ideal "natural laboratories" where valuable genotypes can be found and selected for their reproduction and introduction in the forestry. Selected forms could become valuable sources for traits important for breeding of new productive cultivars and hybrids resistant to environmental stresses.

An important component in the system of conservation of hazelnut and cornelian cherry biodiversity is their conservation in *ex situ* collections in the field gene banks and also using *in vitro* technologies. Thus it is possible not only to increase safety of conservation of accessions and to better safeguard them against the influence of unfavorable environmental factors, but also to carry out more effectively the research works on study of genetic diversity of populations, identification, evaluation and introduction of valuable genotypes in production. Table 2 shows data on the main collections of *Corylus*, held by some research institutions worldwide.

(GF-1, GF-2, GF-3, L-4, L-5, L-6, 4-28). The highest yield of kernels was observed in cultivars Panakhesskiy (51,5%), Cherkesskiy-2 (51,2%) and Ata-Baba (50%).

Table 1. Some geographical areas of distribution of *Corylus avellana* and *Cornus mas* in the forest stations of the National Forestry of Moldova

Forest Stations	Evaluation date	D	H	N	Positioning	
					North	East
***Corylus avellana* L.**						
N.A.Codrii	10.07.08	5	4	1	47°06'42"	28°21'66"
Ocnița	24.08.07	3-5	4	2	48°26'48"	27°35'89"
Chișcăreni	26.06.12	5-9	5-10	P	47°32'20"	28°00'62"
Mândrești	26.08.08	3	3	1	47°28’80"	28°16'78"
NR Plaiul Fagului	28.10.09	1-15	6-8	10	47°17’76"	28°02'90"
NR Plaiul Fagului	30.09.14	7-13	8-15	11	47°07'01"	27°56'33"
Bobeica	27.09.11	3-7	3-4	P	46°55'96"	28°33'70"
Sângerei	27.06.12	5-12	6-8	P	47°34'71"	28°17'97"
Scoreni	28.08.08	2-4	2-3	20	47°05'49"	28°33'74"
Telenești	26.08.08	4-9	4-5	P	47°29’34"	28°23’34"
Telenești	26.08.08	2-8	3-4	12	47°29’24"	28°29’32"
Căpriana	29.08.08	2	3	3	47°07’84"	28°28’72"
Străşeni	05.08.11	10-12	5	1	47°05’75"	28°33’86"
Cimişlia	15.08.13	5-8	3-4	P	46°30’48"	28°45’28"
Călăraşi	29.05.09	3	7	1	47°12’85"	28°18’21"
Călăraşi	29.05.09	12-14	8	2	47°12’88"	28°18’20 "
Băiuş	29.07.13	8-12	6-8	P	46°29’91"	28°2"8’35"
***Cornus mas* L.**						
Nisporeni	30.0911	6	6	1	47°00’16"	28°14’74"
Hîrbovăț	28.05.09	3	3-4	13	46°51’97"	29°23’86"
Hîrbovăț	28.05.09	1,5=3	2-3	21	46°51’97"	29°23’81"
NPA Selişte-Leu	07.11.14	4-6	4	1	47°06’36”	8°05’27”
Sângerei	27.06.12	5-7	4	P	47°36’05"	28°08’57"
Sângerei	27.06.12	3-5	4-4,5	P	47°37’43"	28°09’17"
Criuleni	04.08.11	3-6	4	P	47°16’48"	29°07’06"
Telenești	26.08.08	5	6	38	47°27’91"	28°29’47"
Mândrești	26.08.08	3	3	6	47°28’40"	28°15’68"
Cociulia	25.09.13	3	5	1	46°17’60"	28°22’17"
Ocnița	24.08.07	3-6	3-4	2	48°26’47"	27°35’86"
Căpriana	29.08.08	10	2	1	47°07'89"	28°28'77"
NR Pădurea Domnească	07.10.14	6-10	4	2	47°36'43"	27°23'69"
NR Pădurea Domnească	07.10.14	3-5	5	1	47°35'47"	27°25'75"
Bobeica	27.09.11	12-20	6-10	11	46°55'45"	28°33'85"
Chișcăreni	26.06.12	4-6	3-4	P	47°31'92"	27°59'09"
Străşeni	28.08.08	6,5	5	1	47°06'60"	28°34'58"

Strășeni	05.08.11	22	9	1	47°05' 96"	28°00' 60"
NPA Sărata Galbenă	16.10.14	2-7	4	2	46°42'85"	28°24'64"
Calarasi	29.05.09	4	3	1	47°12'88"	28°18'19"
NR Plaiul Fagului	30.09.14	5-10	4-6	3	47°18'85"	28°05'64"
Zloți	16.08.13	6-8	5	1	46°41'19"	28°54'69"

D – tree stem diameter, cm; H – plant height, m; N – number of plants, pcs.; P - plantation; NR- natural rezervation; NPA - natural protected area

Table 2. World's germplasm collections of *Corylus* [17]

Crop Grouping	**Genus**	**Genebank**		**Accessions**		**Type of accession (%)**				
Nuts,Fruits and Berries		Inscode	Acronym	No.	%	WS	LR	BL	AC	OT
Hazel	*Corylus*	USA026	COR	837	28	13	13	25	48	1
Hazel	*Corylus*	TUR00!	AARI	413	14		100			
Hazel	*Corylus*	UKR046	KPS	188	6				1	99
Hazel	*Corylus*	AZE009	HSCRI	169	6		32	22	46	
Hazel	*Corylus*	ESP014	IRTAMB	120	4		6			94
Hazel	*Corylus*	UZB031	UzRIH-VWM	118	4					100
Hazel	*Corylus*		Others (53)	1153	38	3	9	13	37	39
Hazel	***Corylus***		**Total**	**2998**	**100**	5	23	13	30	29

The latter and the line 4-28 were distinguished by the largest nuts. Selected genotypes were characterized by higher resistance to abiotic and biotic stress factors.

These forms can be introduced in agricultural production. At present, only one hazelnut cultivar (Tonda Gentile Romana) of Italian origin has been regionalized in the Republic of Moldova As to cornelian cherry, 4 cultivars (Corn cu fructe mari, De Baimaclia, De Bucovăț, De Orhei) obtained in the Practical Scientific Institute of Horticulture and Food Technologies were included in the Catalogue of Regionalized Cultivars along with one form as a seedling stock.

Conclusions

Conducted investigations resulted in detection of distribution, description of some morphobiological traits of plants and determination of safety of conservation of 2 species of fruit crop wild relatives – hazelnut and cornelian cherry in forest ecosystems of different soil-climatic zones of Moldova;

It was found that preservation of populations of studied species directly depends on the degree of degradation of the main types of forests of durmast oak, common oak, beech, hornbeam and oak associations and etc. In this regard it is necessary to conduct knowledgeably the required forestry measures to prevent degradation of forest stand structure;

In the works on restoration of forest areas the share of seedlings of hazelnut and cornelian cherry introduced in the structure of forest forming species must be strictly observed;

For the purpose of determination of the degree of genetic erosion, intraspecific diversity of said populations must be studied using various methods, including molecular passportization;

For comprehensive study, guaranteed conservation and sustainable use of cornelian cherry and hazelnut, National collections of these crops must be created with relevant electronic databases.

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BIOCHEMICAL DIVERSITY OF THE *ORIGANUM VULGARE* SSP. *VULGARE* L. AND *ORIGANUM VULGARE* SSP. *HIRTUM* (LINK) IETSWAART GENOTYPES FROM MOLDOVA

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Introduction

Origanum vulgare L. (Oregano) is a herbaceous perennial species from the family of *Lamiaceae* known and used for centuries. Multiple investigations have been carried out in different countries [16, 20, 31, 32, 33] to use and put in value the species. Different biotypes, genotypes, forms, and taxons of Oregano have a strictly local distribution and are distinguished through accentuated morphological and biochemical diversity [11, 19] which is confirmed by the studies on the species, subspecies in the definite areal of prevalence. The relevance of the studies on provenance of Oregano is also determined by the importance of the species as a medicinal, aromatic, culinary, spicy, ornamental, and meliferous plant, supported by the chemical composition – essential oil, flavonoides, vitamins etc. synthesized and accumulated in the aerial part of the plant [34]. Thus, the essential oil [6, 35, 37] and, especially, its major components – carvacrol and thymol, are responsible for the antimicrobial [7, 9, 21], antifungal [8, 24, 40] and antioxidant [7, 22, 25, 27, 37, 41, 42] action, as well as the capacity to inhibit bacterium growth [6, 7, 24, 29, 36, 38]. Oregano essential oil is also successfully employed for its antispastic and antiseptic action [7, 40]. In addition, *O.vulgare* possesses sedative, carminative, emenagogous, diuretically, and other actions. It is also utilized as an aromatizer, preservative in food products [15]. The antimicrobial and antifungal properties of *Origanum vulgare* essential oil are successfully employed for flavouring and preservation, storage of food products [3, 8, 9, 11, 36]. It is known that the decoction of *O.vulgare* possesses antioxidant activity, while its hydroalcoholic extract demonstrates antimicrobial effect [24]. O. vulgare extracts and essential oil are strong candidates to replace synthetic chemicals used by the industry [38]. Six subspecies have been recognized within *O. vulgare* L.: subsp. *vulgare* L., subsp. *glandulosum* (Desfontaines) Ietswaart, subsp. *gracile* (Koch) Ietsw., subsp. *hirtum* (Link) Ietsw. subsp. *virens* (Hoffmanns. & Link) Ietsw, subsp. *viridulum* (Martrin-Donos) Nyman [18].

This work is a continuation of our previous research [13, 14] which includes new genotypes selected for the essential oil content of *Origanum vulgare* L. subsp. *vulgare* and *Origanum vulgare* L. subsp. *hirtum* (Link) Ietswaart. [18].

Materials and methods

The biological material is presented by five genotypes of *O.vulgare* ssp. *vulgare* L. (Ovv) and five genotypes of *O.vulgare* ssp. *hirtum* (Link) Ietswaart (Ovh). The genotypes have been selected from the Aromatic and Medicinal Plant Laboratory collection of the Institute of Genetics, Physiology and Plant Protection. In order to determine the content of essential oil, the samples of fresh herbs, aerial part of the plant, were harvested in the morning hours at the flowering stage. Essential oil was isolated by hydrodistillation for 60 minutes, using the Ginsberg apparatus: 50 g of fresh aerial part per 200 ml of water. The content of the essential oil was recalculated per dry matter. Following distillation, the essential oil was dried over anhydrous sodium sulphate and stored at 4-6 °C. Qualitative and quantitative analyses of the essential oil was conducted using GC coupled with Mass Spectrometry (GC-MS): gas chromatograph -

Agilent Technologies 7890; mass selective detector 5975C Agilent Technologies with a quadruple, capillary column (30m x 0.25mm i.d., film thickness 0.25 μм) at the HP-5ms non-polar stationary phase. The injector and detector temperatures were 250 °C and 280 °C, respectively, using a temperature gradient from T1 = 70° C (2 min), T2 = 200° C (5° C/min) to T3 = 300° C (20° C/min, 5 min). Mobile phase: helium 1ml/min, injected volume of essential oil - 0,03 μl, split rate - 1:100. The identification of the chromatographic peaks was performed with the aid of the software package AMDIS ™, coupled with NIST database. Extraction and total polyphenols determination were carried out by means of procedure according to method described in European Pharmacopoeia with modifications – only total polyphenols were determined, measurements of phenolic compounds not adsorbed by hide powder were not performed. The amount of polyphenols was determined spectrophotometrically, expressed in gallic acid according European Pharmacopeia (5,0:221) with modifications [26].

Results and discussion

The earlier investigations on the assessment of the *Origanum vulgare* L collection have demonstrated the phenotypical diversity corraborated by both the indices of quantitative characters and differences in the content and qualitative and quantitative composition of the essential oil of *O.vulgare* ssp. *vulgare* L. (Ovv) and *O.vulgare* ssp. *hirtum* (Link) Ietswaart (Ovh) [13,14].

The assessment of the promising Ovv and Ovh genotypes in view of the content of the essential oil has confirmed the findings of the precedent years [11,12] that the subspecies *O.vulgare* ssp. *vulgare* is poorer in essential oil than the subspecies *O.vulgare* ssp. hirtum (Table 1) and that this important character varies in the genotypes of the both species considerably. Thus, in the case of the *O.vulgare* ssp. *vulgare* subspecis, the content of essential oil varies from 0,168 % (dry matter) in the Ovv2-38 genotype to 0,360 % (dry matter) in the genotype Ovv7-38. The *O.vulgare* ssp. *hirtum* genotypes are characterized by an elevated content of essential oil, the indices of this character ranging from 2,315% (dry matter) (genotype Ovh7-4) to 4,705% (dry matter), 4,923% (dry matter) in the genotypes Ovh8-40 and Ovh1-78.

A similar difference in the content of essential oil was recorded in Hungary [17] for *O.vulgare* ssp. *hirtum* and in China and Pakistan for Origanum vulgare [15]. Estimation of the essential content in *O. vulgare* (ssp. *vulgare* and ssp. *hirtum*) from the wild flora of Albania also confirms the variability of this character [12]. A very high difference in the content of essential oil (0.1%-1.8%) has been attested in O.vulgare ssp. vulgare in Austria [23]. The variability of this character has also been recorded for the species *O.vulgare* ssp. *glandulosum* (Desf.) Ietswaart (2,5-4,6 %) in Tunisia [27]. It has been also demonstrated that breeding programs have resulted in the development of *O.vulgare* ssp. *hirtum* genotypes characterized by a very high content of essential oil (7-8, 6%) [35].

Table 1. Variability of the essential oil content in the genotypes *Origanum vulgare* ssp. *vulgare* L. and *Origanum vulgare* ssp. *hirtum* (Link) Ietswaart

Origanum vulgare ssp. vulgare		Origanum vulgare ssp. hirtum	
Genotypes	**Essential oil content, % (dry matter)**	**Genotypes**	**Essential oil content, % (dry matter)**
Ovv 2-38	0,168	Ovh 7-40	2,315
Ovv 3-38	0,077	Ovh 8-40	4,705
Ovv 5-38	0,205	Ovh 1-78	4,923
Ovv 6-38	0,239	Ovh 4-78	3,505
Ovv 7-38	0,360	Ovh 6-87	3,540

The *O.vulgare* ssp. *vulgare* stands out for both the content of essential oil and its composition. Qualitative and quantitative analyses performed through GC GC-MS techniques have identified in the essential oil of *O.vulgare* ssp. *vulgare* from 18 components in the genotype Ovv2-38 to 29 components in the genotype Ovv7-38, the identifcation being at a propotion of 92,38%-98,80% (Table 2). Our earlier investigation [13, 14] has revealed forty one components in other genotypes. Some authors have identified forty two components in the *O. vulgare* ssp. *vulgare* population in Lithuania [30] and Germany [2]. In Austria though, a higher number (53) of components has been identified [23].

The concentration of the components identified in the essential oil isolated from the genotypes *O. vulgare* ssp. *vulgare* varies with the genotype. The major component of the essential oil in the genotype of this subspecies is D-germacrene that recorded the values of 26,01%- 33,98% (Table 2). The second major component is β-caryophyllene. This component demonstrated values ranging between 12,16% in the genotype Ovv3-38 and 33,16% in the genotype Ovv6-38. The other component, (+)β-bisabolene is contained at insignificant concentrations in the essential oil isolated from the genotypes Ovv7-38 (6,83%), Ovv5-38 (9,62%), Ovv2-38 (12,65%), and Ovv6-38 (16,04%), while it has not been identified in the essential oil in the genotype Ovv3-38. γ-elemene is present in the essential oil of all the genotypes at a relatively increased concentrations making 3,56%-16,79 %.

The essential oil extracted from all the *O. vulgare* ssp. *vulgare* genotypes assessed contains β-bourbonene, β-caryophyllene, γ-cadinene, γ-muurolene, caryophyllene oxide, β- guaiene, and δ-cadinole, ordinarily, at minor concentrations. The other two components, 1-butanol-3-methyl-acetate and trans-ocimene have been identified in each of the four genotypes at values ranging between 0,83% and 1,88% and 0,57% and 2,81%, respectively. As for the minor components, the presence of thymol (0,32% – 1,45%) has been shown in three genotypes of Ovv5-38, Ovv6-38, Ovv7-38 and that of Carvacrol (0,54% – 3,58%) in the essential oil of the genotypes Ovv3-38, Ovv5-38, Ovv6-38. The earlier assessed genotypes [13, 14] also contained carvacrol and thymol in the essential oil though at more elevated concentrations. Thymol occurs at low concentrations in the oil of our genotypes. A similar value has been attested for this species in Hungary [43]. In total, the presence of thirteen minor components has been demonstrated for the essential oil in each of the three genotypes (Table 2).

Thus, we can conclude that the essential oil major components in the *O. vulgare* ssp. *vulgare* genotypes assessed include Germacrene D (26.01–33.98%); β-caryophyllene (12,16-33,16%); γ-elemene (3,56-16,79%); while β-bisabolene (6,83-16,04) is the major component in four genotyps, their concentrations varying considerably. The chemotypes of *O. vulgare* ssp. *vulgare* are following: 1. germacrene D/ β-caryophyllene/ β-bisabolene; 2. germacrene D/ β-caryophyllene/δ-cadinole/ γ-elemene; 3. germacrene D/ γ-elemene/ β-caryophyllene/ β- bisabolene; 4. β-caryophyllene/ germacrene D/ β-bisabolene; 5. germacrene D/ β-caryophyllene/γ-elemene/β-bisabolene.

These genotypes differ from the earlier assessed ones of *O. vulgare* ssp. *vulgare* [13, 14] in both the number of major compound and their concentrations. For example, germacrene D and β-caryophyllene occur at much more elevated concentrations, while ocimenele (cis- and trans-), on the contrary, at quite low ones. The variability of the number and concentrations of the major components contained in the essential oil of *O. vulgare* ssp. *vulgare* from wild flora in other countries also varies within considerable limits. So, the specimen from wild flora of Lithuania contains in the essential oil the following major components: β-ocimene, germacrene D, β-caryophyllene, and sabinene, each component at different concentrations in the specimen from various localities [30].

The major components of the essential oil isolated from the *O. vulgare* collected in wild flora of Kosovo include sabinene, 1,8-cineole, caryophyllene oxide, β-caryophyllene, p-cymene, α-terpineol, and germacrene D with an accentuated variability in the con-

centration of each component [28]. It should be mentioned that β-caryophyllene in some of our genotypes occurs at much higher concentrations than those recorded in Kosovo.

Table 2. The qualitative and quantitative composition of *Origanum vulgare* ssp. *vulgare* L. essential oil

Nr. pic	Component	Rt	Area %				
		sample	Ovv2-38	Ovv3-38	Ovv5-38	Ovv6-38	Ovv7-38
1	1-Butanol-3-methyl-acetate	3,55	1,65	-	1,88	0,91	0,83
5	Sabinene	5,42	-	1,59	0,62	-	4,16
6	1-Octen-3-ol	5,45	-	-	-	-	-
7	β-Pinene	5,52	-	-	-	-	-
8	3-Octanone	5,64	-	-	-	-	-
9	β-Mircene	5,74	-	-	-	-	0,33
10	3-Octanol	5,82	-	1,68	-	-	-
11	α-fellandrene	6,10	-	-	-	-	-
12	γ-Terpinene	6,37	-	-	-	-	-
13	p-Cymene	6,56	-	-	1,19	0,11	0,54
14	Limonene	6,67	-	-	-	-	0,48
15	Eucalyptol	6,74	-	-	-	-	-
16	trans-Ocimene	6,82	-	1,76	0,57	0,85	2,81
17	cis-Ocimene	7,08	-	1,15	-	1,72	1,50
18	γ-terpinene	7,38	-	-	2,11	0,29	0,78
19	4-Thujanol	7,60	-	-	-	-	-
20	Linalool	8,38				0,37	1,07
21	Camphor	9,46	-	-	-	-	-
22	Borneol	10,18	-	-	-	-	-
23	4-Terpineol	10,48	-	0,75	-	0,49	2,09
24	α-Terpineol	10,82	-	-	-	-	0,73
25	Timol metil ether	12,22	-	-	-	-	-
26	Linalyl acetate	12,51	0,46	-	-	0,57	0,46
27	Timol	13,45	-	-	1,45	0,69	0,32
28	Carvacrol	13,82	-	2,91	3,58	0,54	-
29	2.5-Diethylphenol	13.99	-	-	-	-	-
30	β-Bourbonene	15,97	0,96	2,28	0,79	0,66	1,15
31	β-Caryophyllene	16,86	31,45	12,16	15,02	33,16	13,21
32	β-Cubebene	17,08	0,73	-	0,75	-	0,33
33	Undecadien-2-one,6,10-dimethyl	17.57	0,51	-	0,59	-	-
34	α-Caryophyllene	17,70	1,59	2,10	1,03	1,48	1,60

35	(+)Aromadendrene	17,88	-	1,31	-	0,36	1,06
36	D-Germacrene	18,38	32,29	26,01	33,98	32,78	31,02
37	Humulene	18.60	0,60	-	-	0,51	0,39
38	γ-Elemene	18,75	3,82	6,75	16,79	3,56	8,32
39	(+)β-Bisabolene	18,98	12,65	-	9,62	16,04	6,83
40	γ-Cadinene	19,36	1,40	3,95	0,94	1,06	2,96
41	γ-Muurolene	20,61	2,27	5,64	0,77	1,23	5,65
42	(-)Spatulenol	20.66	0,97	2,23	2,46	-	-
43	Caryophyllene oxide	20,81	2,18	3,52	1,37	0,58	1,54
44	β-Guaiene	22,10	2,27	5,84	1,10	0,75	3,01
45	δ-Cadinole	22,39	2,41	9,11	1,26	0.86	4,37
46	α-Muurolene	23,23	0,45	1,61	-	-	0,45
47	Sclareol	31,26	-	-	0,73	0,49	0,10
No. identified compounds			18	19	22	24	29
Total, identified compounds %			98,66	92,38	98,60	98,80	98,09

In the essential oil of some provenances of *O. vulgare* ssp. *vulgare* in Turkey, the major components include caryophyllene oxide (34.44%), β-caryophyllene (20.40%), and δ-cadinol (7,02%) [4]. The last component, δ-cadinol, has been also recorded in the genotypes *O. vulgare* ssp. *vulgare* assessed by us, its concentration varying from 0,8% to 9,11% (Table2), and in the oil of the earlier assessed genotypes [13,14]. In the other species, *O. vulgare* L. ssp. *viride* (Boiss), ocimene is the major component (35.1%) with the highest concentration [3]. In our *O.vulgare* ssp. *vulgare* genotypes, ocimenes are minor components and they are not present in the essential oil of all the genotypes assessed.

The qualitative and quantitative analyses of the essential oil separated by hydrodistillation from five *O. vulgare* ssp. *hirtum* genotypes have revealed a different number of components, it varying between 18 in Ovh8-40, 25 in Ovh4-78 and Ovh6-87, representing 99,87 to100% of the total essential oil extracted (Table 3). Other researchers have detected from 19 [21] to 56 [33] and even 81 [39, 40] or 103 [25] components.

The major components of the essential oil in all the *O. vulgare* ssp. *hirtum* genotypes evaluated are carvacrol at a concentration varying between 74.63% and 88.13% depending on the genotype. The second major component is γ-terpinene (3,59-10.69%), followed by p-cymene (2,23-5,06%), the rest of the components being minor at concentrations up to 1% or some of them being at concentrations slightly increased in some genotypes as in the case of β-Caryophyllene (1,49-2,10%) and α-terpinene (1,24-1,45%) (Table 3).

It can be concluded following from the above that carvacrol (74.63-88.13%), γ-terpinene (3,59-10,69%), and p-cymene (2,23-5,06%) are the major components in the essential oil of the genotypes *O. vulgare* ssp. *hirtum* assessed. The *O.vulgare* ssp. *hirtum* genotypes are divided into two chemotypes: 1-Carvacrol/γ-terpinene/p-Cymene and 2- Carvacrol/γ-terpinene/p-Cymene/β-Caryophyllene.

Elevated concentrations of carvacrol (70-93%), that is the major component in the essential oil of *O. vulgare* ssp. *hirtum*, have been found by other authors [25, 31, 35, 43] in both *O. vulgare* ssp. *hirtum* and *O. vulgare* ssp. *scabrum* [1]. In the essential oil of *O.vulgare* ssp. *hirtum* from Sicilia, the major components are thymol (24,0-54,4%), γ-terpinene (9,8-30,5%), p-cimene (5,2%) [40]. Thymol is shown to be one of the major components along with carvacrol in *O.vulgare* ssp. *hirtum* growing wild in South-

ern Italy [11, 12, 33]. In other biotypes of Italy, the major components are thymol and alfa-terpineol, or linalyl acetate and linalool [11]. Other samples of Albania have the major components thymol, carvacrol, linalool and thymol in Albania [12]. In O. onites essential oil the major components are carvacrol, thymol and linalool [19]. In the essential oil of our genotypes, linalool is a minor component, but linalyl acetate has not been identified.

It has been found that the content and composition of the essential oil are stable in both *O. vulgare* ssp. *vulgare* and *O. vulgare* ssp. *hirtum* during the whole flowering period, which is the time of harvesting [43]. The highest oil content in *O. onites* is at the full flowering stage [19]. According to the findings published by some authors, the content and composition of the essential oil are not dependent on the cultivation conditions of *O. vulgare* (ssp. *hirtum, creticum, samothrake*) [2]. Other researchers claim that the concentration of the major components (sabinene and ocimene) is dependent on the cultivation conditions [9]

Table 3. The essential oil qualitative and quantitative composition of *O. vulgare* ssp. *hirtum* (Link) Ietswaart

Nr. pic	Component	Rt	Area %				
		sample	Ovh 7-40	Ovh 8-40	Ovh 1-78	Ovh 4-78	Ovh 6-87
1	1-Butanol-3-methyl-acetate	3,55	-	-	-	-	-
2	Origanene	4,48	0,45	0,28	0,40	0,90	0,64
3	α-Pinene	4,64	0,19	0,12	0,17	0,41	0,25
4	Camfene	4,95	0,06	-	0,04	0,14	0,07
5	Sabinene	5,42	-	-	-	0,15	-
6	1-Octen-3-ol	5.45	0,42	0,19	0,16	0,11	0,27
7	β-Pinene	5,52	0.06		0,05	0,11	0,07
8	3-Octanone	5,64	-	-	0,05	-	-
9	β-Mircene	5,74	0,78	0,71	0,78	1,35	0,97
10	3-Octanol	5,82	-	-	-	-	-
11	α-fellandrene	6,10	0,10	-	0,10	0,16	0,13
12	α-terpinene	6,37	0,62	0,90	0,59	1,45	1,24
13	p-Cymene	6,56	2,35	2,45	2,23	5,06	3,56
14	Limonene	6,67	0,17	-	0,18	0,29	0,22
15	Eucaliptol	6,74	-	-	-	0,16	-
16	trans-Ocimene	6,82	-	0,16	-	-	0,10
17	cis-Ocimene	7,08	-	-	0,04	0,11	0,07
18	γ-terpinene	7,38	4,10	8,84	3,59	10,69	9,72
19	4-Thujanol	7,60	0,31	0,15	0,49	0,41	0,36
20	Linalool	8,38	0,20	0,62	0,17	0,17	0,24
21	Camphor	9,46	-	-	-	-	-
22	Borneol	10,18	0,24	0,27	0,24	0,42	0,36
23	4-Terpineol	10,48	0,37	0,28	0,33	0,44	0,33
24	α-Terpineol	10,82	0,10	-	0,09	0,15	0,09

25	Timol metil eter	12,22	0,07	-	-	0,10	-
26	Linalyl acetate	12,51	-	-	-	-	-
27	Timol	13,45	0,24	0,17	0,23	0,20	0,23
28	Carvacrol	13,82	87,14	82,63	88,13	74,63	79,54
29	2.5-Diethylphenol	13.99	0,31	-	-	-	-
30	β-Bourbonene	15,97	-	-	-	-	-
31	β-Caryophilene	16,86	0,98	1,49	0,85	2,10	0,76
32	β-Cubebene	17,08	-	-	-	-	-
34	α-Caryophyllene	17,70	0,15	0,23	0,13	0,17	0,11
35	(+)Aromadendrene	17,88	-	-	-	-	-
36	D-Germacrene	18,38	0,24	0,19	-	-	0,13
37	Humulene	18.60	-	-	-	-	-
38	γ-Elemene	18,75	-	-	-	-	-
39	(+) β-Bisabolene	18,98	0,35	0,34	0,45	0,12	0,23
No. identified compounds			24	18	24	25	25
Total identified compounds, %			99,94	100	99,87	100	99,94

Some studies have concluded that the productivity and content of the essential oil in O. vulgare depend on the techniques of planting material production [5].

Antioxidant action of Origanum vulgare species is supported not only by the essential oil, but also by the polyphenols that contains the plant. From this point of view, the genotypes of O.vulgare ssp. vulgare as well as O.vulgare ssp. hirtum were evaluated in the content of polyphenols. The obtained results demonstrate the variability of the content of these compounds in the genotypes of both subspecies. Rich in polyphenols, expressed as gallic acid (GA) (mg/100g) are genotypes belonging O.vulgare subspecies ssp. vulgaris: from 99,25 ± 1,598 to 166,43± 3,594. The genotypes of O.vulgare ssp. hirtum, synthesizes and accumulate polyphenols from 53,51± 0,684 to 85,59±0,719 mg/100g.

The results should be confirmed that between the essential oil and polyphenols content of O.vulgare subsp. vulgare as well as O.vulgare ssp. hirtum there is a negative correlation: A high content of essential oil correlate with the low polyphenols content.

Conclusion

The diversity of Origanum vulgare ssp. vulgare L. and Origanum vulgare L. ssp. hirtum (Link) Ietswaart genotypes has been confirmed through the essential oil content, qualitative and quantitative components.

The content of essential oil varies between 0,077% and 0,360% in the genotypes O.vulgare ssp. vulgare, and between 2,315% and 4,923% in the O.vulgare ssp. hirtum genotypes.

Qualitative and quantitative analyses performed using GC GC-MS techniques have found from 18 to 29 components in the O.vulgare ssp. vulgaris essential oil, depending on the genotype, the identification ratio being 92,38%-98,80%.

The component number varies between 18 and 25 depending on the genotypes of O. vulgare ssp. hirtum, this constituting 99,87%-100 % of the essential oil.

The major components in the essential oil of O. vulgare ssp. vulgare include Germacrene D (33,98–26,01%); β-Caryophyllene (12,16–33,16%); γ-Elemene (3,82 – 16,79%), while β- Bisabolene (6,83-16,04) is the major component in four genotypes.

The O. vulgare ssp. vulgare genotypes are divided into five chemotypes: 1. germacrene D/β-caryophyllene/β-bisabolene; 2.Germacrene D/β-caryophyllene/δ-cadinole/ γ-elemene; 3. germacrene D/ γ-elemene/ β-caryophyllene/ β- bisabolene; 4. β-caryophyllene/ germacrene D/ β- bisabolene; 5. germacrene D/ β-caryophyllene/γ-elemene/β- bisabolene.

The major components in the essential oil of O. vulgare ssp. hirtum are carvacrol (77,61-85,88%), followed by p-cymene (3,64-9,33%) or γ-terpinene (8,22%) and p-cymene (5,30%).

The O.vulgare ssp. hirtum genotypes are divided into two chemotypes: 1-carvacrol/ γ-terpinene/p-cymene and carvacrol/γ-terpinene/p-cymene/ β-caryophyllene.

The variability of the content of polyphenols in the genotypes of both evaluated subspecies has been demonstrated. Rich in polyphenols, expressed as gallic acid are genotypes belonging O.vulgare ssp. vulgaris: from 99,25 ± 1,598 to 166,43±3,594 mg/100g. The genotypes of O.vulgare ssp. hirtum, synthesizes and accumulate polyphenols from 53,51± 0,684 to 85,59±0,719 mg/100g and O.vulgare ssp. hirtum – from 53,51± 0,684 to 85,59 ± 0,719mg/100g.

The results should be confirmed that between the essential oil and polyphenols content in both species is a negative correlation: A high content of essential oil correlate with the low polyphenols content.

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HERBICIDE RESISTANCE BREEDING IN SUNFLOWER, CURRENT SITUATION AND FUTURE DIRECTIONS

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Introduction

Sunflower is one of the most important oil crops in the world due to higher adaptation capability, mechanization use and preference by customers as vegetable oil. Sunflower areas are not larger in the world because the income is lower due to influenced more from environmental conditions as a summer crop. To increase production, it is important for decreasing of effects of factors reduced seed yield utilizing from higher production techniques in addition to develop higher yielding cultivars [43, 79, 47].

Weeds and broomrape *(Orobanche cumana* Wallr.) parasite exist among the most limiting factors for sunflower production in especially in Eastern Europe and Black Sea Region which have more than 60% of world sunflower planted areas [45]. Broomrape is a parasite affecting sunflower yield severely until 100%. Broomprape is holoparasitic weed (angiosperm) which lacks chlorophyll and dependent on host plant for nutrients and it develops new aggressive races historically against sunflower resistant genotypes. New races of broomrape such as F, G and H other than 5 known races (A, B, C, D and E) were observed in mostly in Balkan and Black Sea region and also in Spain [85, 63, 17, 41, 22, 78, 64, 26, 43, 46]. Therefore, new hybrids should have resistant genes against these new races or Clearfield technology [6, 53, 54, 4, 39, 44, 80, 55,18].

Clearfield system with Imidazolinone (IMI) tolerant hybrids and IMI herbicide as post emergence application control successfully broomrape and common weeds [57, 4, 8, 18,]. Similarly, Express Sun system and Sulfonyl Urea (SU) herbicide resistant cultivars and post emergence SU group herbicide is also used efficiently especially in Central and Eastern European countries. Clearfield and Express Sun technologies were accepted widely in almost all sunflower grower countries with restriction of growing of genetically modified crops due to not being biotech product [83, 40, 51, 52, 86, 23, 24, 33, 34, 37, 15, 44, 67, 45, 47].

The development of IMI herbicide resistance in sunflower. Imidazolinone (IMI) tolerant wild sunflower popula¬tion was discovered firstly in soybean field in Kansas, USA in 1996 [1, 59] then IMI tolerance genes were transferred via backrossing and two IMI (IMISUN-1 and IMISUN-2) tolerant lines were developed firstly in USDA sunflower program in Fargo, ND, US [3]. The IMI herbicides control weeds by inhibiting a key enzyme in the branched chain amino acid biosynthetic pathway, acetohydroxyacid synthase (AHAS; EC 4.1.3.18) also known as Acetolactate synthase (ALS) [83, 9-11].

Clearfield system were introduced to farmers firstly in 2003 in Turkey lately Argentina, USA and other countries [17]. In Clearfield technology, IMI post emergence herbicides (Imazamox (40 g/l)) applied 6-8 leaf stage control efficiently both broomrape and major broadleaf weeds such as *Xanthium strumarium* Wallr. *Chenopodium album* L., *Echinochloa cruss-galli*, *Sinapsis arvensis* L., *Amaranthus* spp., *Solanum nigrum* L., *Datura stramonium* L. ragweed, *Avena* spp. etc. resulting important yield losses in sunflower [16, 54, 65, 42, 18, 51, 36, 52, 49, 27, 21, 15, 44]. After application IMI herbicide, chlorosis could seems depends on applied herbicide amount and application method but it disappear generally in a week [23, 62]. On the other hand, Anastasov H. (2010) indicated that imazamox results considerable changes in the sunflower leaf anatomy, a reduction of stomata number and an increase in the thickness of leaf lamina (blade) after applied at suggested dose as post emergence.

Ahas locus confers resistance to IMI tolerant sun¬flower [12, 50]. The inherit-

ance of IMISUN is additively controlled by two genes, one partially dominant allele *Ahasl1-1* and a modifier gene [59, 12, 38]. However, this IMI trait has lower oil content in the seed due to the wild parent around the resistant gene [74-76].

The second IMI tolerance source known as CL Plus was developed by seed mutagenesis and selection with imazapyr in sunflower [72]. While the Clearfield® system is based on two genes (*Ahasl1-1* and an enhancer, [83]), the CL Plus system, based on the allele *Ahasl1-3* alone or in combination with Ahasl1-1 [71-76]. This new traits present better stability of the herbicide tolerance in different environmental conditions, permit developing new herbicide formulations providing more flexible and reliable weed control, higher oil content, etc. than previous IMISUN trait [71-73, 88, 89, 68, 69]. Clearfield Plus® trait results higher accumulation of biomass after IMI application at the above-ground and root level because of displaying lower inhibition of the AHAS enzyme extracting by IMI [73, 88, 89, 84].

The development of SU herbicide resistance in sunflower. Sulfonyl Urea (SU) herbicide tolerance sunflower were discovered from wild sunflower isolates ANN-KAN and ANN-PUR in Kansas, US, (which is the same field discovered IMI resistance wild population) [2]. SURES-1 and SURES-2 lines were developed with resistance to sulfonylurea herbicides by introgression of mutations, respectively then transferred into elite breeding lines produced from these crossings [58, 25, 60, 31, 32]. The target-site-tolerance is the result of the mutation P197L at the *Ahasl1* locus and the inheritance of this trait is dominant way as exhibiting completely resistance to tribenuron [60, 50, 19, 28, 29, 74-76]. While White *et al*. (2003) mentioned that at least two ALS gene copies existing in these SU sunflower lines, Bruniard and Miller (2001) pointed out three putative ALS genes. However, Miller and Zollinger (2004) indicated that differences in crop injury among SURES lines (*Ahasl-2/Ahasl1-2*) are the result of the presence of modifier genes. Some studies were carried out to determine SU resistance allele specific markers and in vitro techniques in the lab [13, 20].

ExpressSun® technology is the same type of tolerance as SURES obtained by EMS mutagenesis over the line HA89 [82]. Sulfo¬nylurea tolerant sunflower cultivars (ExpressSun technology) were introduced for farmers in 2007 (USA) and using commonly in many countries especially in Eastern Europe [77, 30, 28, 29, 56].

SU herbicides control more weeds and also cheaper than IMI are used widely in sunflower production in the world. However, SU resistant hybrids have the less control on both broomrape and some common weeds such as Xanthium, Cirsium, etc. so they should be combined with broomrape resistance together [23].

Current stiuation herbicide resistance in sunflower. Farmers like this technology due to offering well control on both broomprape and also common weeds but they should wait until 6-8 leaves stage to apply IMI herbicide for efficient broomrape control. These delaying applications result sometimes not well control of already grown weeds. Therefore, combining broomrape resistant genes with IMI resistance in the same hybrid give farmers more options both for application time and amount depending on weed infestation in their fields [41, 44]. Additionally, seed companies also develop new tolerant hybrids every years mostly combining or adding new traits to broomrape tolerance such as downy mildew resistance as well as IMI herbicide resistance together because Clearfield system is one of the best and efficient option to control both broomrape and major broadleaf weeds [23]. Now, sunflower hybrids combined these traits (IMI + Orb, Orb + SU) have started to sell recently and are preferred widely by sunflower growers. However, due to CL Plus and ExpressSun resistant genes developed by chemical mutation it needs provisional contracts to use by sunflower breeders widely [44].

Although Clearfield and SU technologies were used widely, there are some arising problems in the production such as herbicide residue problems and effects on following crops, gene escaping to wild species, weed tolerance, tolerant sunflower cultivars

response to ALS inhibiting her¬bicides, volunteer plants of tolerant sunflower cultivars have lower sensitivity to other ALS inhibiting herbicides compared to conventional cultivars and hard control of these volunteer sunflower plants [40, 71, 74, 87, 81, 7, 66, 70, 44, 45, 33-35].

Future directions of herbicide resistance in sunflower. Furthermore, broomrape and herbicide resistant hybrids combined all three traits (Orb + IMI + SU) will be developed with using IMI and SU resistant genetic material soon in the future. These new hybrids combined these traits present more economical results to sunflower producers as reducing cost and increasing income per area with giving herbicide selection based on broomrape and weeds in their fields [44].

To provide sustainable and durable broomrape management, herbicide tolerance should be incorporated with resistant genes to different broomrape races in order to avoid breaking of resistance rapidly in the following years. New, more reliable, lower cost and rapid screening methods should be added for efficient herbicide tolerance in addition to phenotypic control and tests at V2-V4 stages such as molecular markers, in vitro screening, etc. Some proved methods were also developed such immature embryo [9], seed germination bioassays for screening IMI-tolerance [84, 10, 27] and SU-tolerance [20] and marker assisted selection especially for introgression of genes for herbicide resistance into high yielding sunflower germplasm [50, 13, 71-76]. On the other hand, new herbicide molecules need to develop for efficient weed control in sunflower due to limited selective herbicides for the sunflower and higher cost of herbicide registration [14, 74-76]. Therefore, research studies should be performed to develop new herbicide resistance genes to supply alternative choices, to increase the productivity and the competitive ability in sunflower.

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ANALIZA FACTORIALĂ A SPECIFICITĂŢII CIUPERCII *ALTERNARIA ALTERNATA* (FR.) KEISSLER

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Introducere

Lipsa soiurilor cu rezistenţă durabilă este legată în mare măsură de interacţiunile puternice *plantă x patogen x mediu*. Cele trei elemente sunt în strânsă interdependenţă, formând un sistem patologic care determină orientarea şi caracterul bolii [1]. Una dintre cele mai răspândite şi păgubitoare boli întâlnită în ultimul timp în lume la tomatele de cultură este alternarioza, provocată de fungii genului *Alternaria* Nees [3, 5, 6], fenomenul atestându-se şi în Republica Moldova [8].

Alternaria prezintă un gen de fungi cu numeroase specii, tulpinile cărora manifestă atât saprofitism cât şi parazitism. Conform surselor bibliografice contemporane, dintre speciile Alternaria, *A. alternata* se manifestă în ultimul timp cu frecvenţă şi agresivitate înaltă, ocupând nişele ecologice sau micşorând aria de extindere a speciei *A. solani* (Ell. et Mart.) Sorauer care în multe regiuni prezintă specia de bază la tomate [4]. Specia *A. alternata* are o specializare largă, cauzând diverse maladii la un cerc larg de specii de plante agricole şi tehnice: tomate, grâu, sorg, orz, floarea-soarelui, rapiţă, bumbac, ş.a., cauzând pierderi economice enorme.

A. alternata este o sursă importantă de micotoxine, aşa ca: alternariolul, alternariol monometil eterul, acidul tenuazonic, altertoxina, care au o specificitate pronunţată pentru planta gazdă, dar totodată sunt dependente de condiţiile de mediu [2, 7].

În acest context scopul cercetărilor a constat în elucidarea rolului factorului de genotip al tomatelor, grâului şi soiei la interacţiunea cu filtratele de cultură ale fungului *A. alternata,* izolat din frunze de tomate.

Material şi metode

Cercetările s-au efectuat conform modelului de analiză bifactorială, în cadrul căreia 3 culturi taxonomic îndepărtate – tomate, grâu şi soia (fiecare reprezentate de 4 soiuri) au fost examinate în baza reacţiei plantelor la filtratele de cultură *A. alternata*, izolată din frunze de tomate cu semne de ulceraţii sau brunificări, şi supuse testului ANOVA (pachetul de soft STATISTICA 7). Seminţele/boabele au fost tratate timp de 18 ore cu filtrate de cultură (FC) ale 4 tulpini de *A. alternata* [9], iar în calitate de martor a servit varianta apa distilată. Experienţa s-a efectuat la temperatură optimă: 24-25ºC (6 zile) şi joasă: 14-15ºC (21 zile). În calitate de indici-test ai reacţiei plantelor, au servit importante caractere de creştere şi dezvoltare la etapă timpurie a ontogenezei – lungimea rădăciniţei şi tulpiniţei.

Rezultate şi discuţii

S-a constatat că răspunsul plantelor la cele 4 FC a fost diferenţiat, funcţie de cultură, genotip, caracter şi izolata fungului, acesta încadrându-se în categoriile: lipsă de reacţie, inhibare, stimulare. Ponderea factorului genotipic în sursa de variaţie a creşterii rădăciniţei a constituit 88,9; 70,0; 34,0% şi 89,9; 52,6; 53,9%, respectiv, culturilor de tomate, grâu şi soia, pe fondal de temperatură optimă şi nefavorabilă (tab.1, 2).

În ceea ce priveşte rolul factorului genotipic în sursa de variaţie a creşterii tulpiniţei, valorile au constituit 80,4; 76,4; 17,8% şi 86,0; 67,9; 66,4%, respectiv, culturilor de tomate, grâu şi soia, pe fondal de temperatură optimă şi nefavorabilă (tab.3, 4).

Tabelul 1. Analiza bifactorială a relaţiilor plante de cultură x filtrat de cultură *Alternaria alternata* la temperatura 24-25°C (test-obiect lungimea rădăciniței)

Sursă de variaţie	Grade de libertate	Suma medie a pătratelor	Contribuţia în sursa de variaţie (%)
Tomate			
Genotip	3	12161,1*	88,9*
Izolată A.alternata	4	427,5	3,1
Interacţiuni genotip x izolată A.alternata	12	801,0*	5,9*
Efecte aleatorii	681	296,4	2,2
Grâu			
Genotip	3	25869*	70,0*
Izolată A.alternata	4	6143*	16,6*
Interacţiuni genotip x izolată A.alternata	12	4635*	12,5*
Efecte aleatorii	1648	304	0,8*
Soia			
Genotip	3	1458,0*	34,0*
Izolată A.alternata	4	1892,2*	44,2*
Interacţiuni genotip x izolată A.alternata	12	779,9*	18,2*
Efecte aleatorii	1212	153,9	3,6
*- p≤0,05.			

Tabelul 2. Analiza bifactorială a relaţiilor plante de cultură x filtrat de cultură *Alternaria alternata* la temperatura 14-15°C (test-obiect lungimea rădăciniței)

Sursă de variaţie	Grade de libertate	Suma medie a pătratelor	Contribuţia în sursa de variaţie (%)
Tomate			
Genotip	3	23352,9*	89,9*
Izolată A.alternata	4	1210,2*	4,7*
Interacţiuni genotip x izolată A.alternata	12	1225,5*	4,7*
Efecte aleatorii	1155	186,8	0,7
Grâu			
Genotip	3	16940*	52,6*
Izolată A.alternata	4	3052*	9,5*
Interacţiuni genotip x izolată A.alternata	12	10962*	34,0*
Efecte aleatorii	1717	1276	4,0
Soia			
Genotip	3	3242,2*	53,9*
Izolată A.alternata	4	1672,3*	27,8*
Interacţiuni genotip x izolată A.alternata	12	874,4*	14,5*
Efecte aleatorii	1289	226,8	3,8
*- p≤0,05.			

Tabelul 3. Analiza bifactorială a relaţiilor plante de cultură x filtrat de cultură *Alternaria alternata* la temperatura 24-25°C (test-obiect lungimea tulpiniței)

Sursă de variaţie	Grade de libertate	Suma medie a pătratelor	Contribuţia în sursa de variaţie (%)
Tomate			
Genotip	3	1908,25*	80,4*
Izolată A.alternata	4	76,81	3,2
Interacţiuni genotip x izolată A.alternata	12	272,23*	11,5*
Efecte aleatorii	681	116,47	4,9
Grâu			
Genotip	3	2358*	76,4*
Izolată A.alternata	4	347*	11,2*
Interacţiuni genotip x izolată A.alternata	12	266*	8,6*
Efecte aleatorii	1648	115	3,7
Soia			
Genotip	3	546,4*	17,8*
Izolată A.alternata	4	1815,3*	59,0*
Interacţiuni genotip x izolată A.alternata	12	650,5*	21,1*
Efecte aleatorii	1212	64,8	2,1
*- p≤0,05.			

Tabelul 4. Analiza bifactorială a relaţiilor plante de cultură x filtrat de cultură *Alternaria alternata* la temperatura 14-15°C (test-obiect lungimea tulpiniței)

Sursă de variaţie	Grade de libertate	Suma medie a pătratelor	Contribuţia în sursa de variaţie (%)
Tomate			
Genotip	3	3728,4*	86,0*
Izolată *A.alternata*	4	368,6*	8,5*
Interacţiuni genotip x izolată A.alternata	12	177,7*	4,1*
Efecte aleatorii	1155	60,9	1,4
Grâu			
Genotip	3	14702*	67,9*
Izolată *A.alternata*	4	1799*	8,3*
Interacţiuni genotip x izolată A.alternata	12	4608*	21,3*
Efecte aleatorii	1717	541	2,5
Soia			
Genotip	3	8094*	66,4*
Izolată *A.alternata*	4	833*	6,8*
Interacţiuni genotip x izolată A.alternata	12	2953*	24,2*
Efecte aleatorii	1289	303	2,5
*- p≤0,05.			

Concluzii

Faptul că factorul genotipic în reacţia plantulelor la tulpinile *A. alternata* (izolate din tomate) a prezentat valori mai înalte pentru plantulele de tomate, relevă specificitatea de reacţie mai pronunţată a acestora, comparativ cu grâul şi soia, şi deci – o posibilă specializare a patogenului *A. alternata* pentru tomate. Fenomenul necesită efectuarea unui monitoring continuu al evoluţiei patogenului în scopul menţinerii durabilităţii caracterului de rezistenţă a tomatelor la alternarioză.

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AMELIORAREA PLANTELOR NECESITĂ AMELIORARE

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Introducere

Printre cele mai valoroase și durabile creații ale civilizației umane se află și culturile agricole. Selectarea și ameliorarea culturilor agricole este una dintre primele și cele mai fundamentale valori intelectuale. Agricultura a luat naștere atunci când omul primitiv, vânător și culegător de ceea ce îi oferea natura, a selectat și a plantat semințele acelor plante care le recolta prin eforturi la distanțe mari.

Anume selectarea și ameliorarea plantelor, care îi ofereau sursa de hrană a fost prima etapă (cea de geneză) a agriculturii și a civilizației umane, etapă, care fiind continuată sute și mii de ani s-a încununat cu crearea marelor culturi agricole: grâul, orezul, porumbul, cartoful și a altor multor culturi. În ultimii 200 de ani au fost create doar două dintre marile culturi agricole: sfecla pentru zahăr și floarea soarelui.

Etapa, așa zisă primitivă, care a durat mii de ani, s-a încununat cu un buchet de creații dintre cele mai valoroase – culturile agricole, care și azi servesc civilizația umană, asigurînd nu doar hrana, dar și sursele de energie, de protecție a solului, a sănătății, a frumuseții acestui pământ. Pe parcursul realizării acestei mari și fundamentale opere au fost inventate și perfecționate și metodele genetice de selecție și ameliorare.

Actualmente crearea și ameliorarea culturilor agricole este o știință foarte complexă și prin realizările sale asigură eficientizarea întregului complex de științe și activități în agricultură. Este demonstrat că peste 50% din tot progresul obținut în agricultură în ultimii 50 de ani, se datorează creării și implementării de noi soiuri și hibrizi. În Republica Moldova potențialul noilor soiuri și hibrizi la principalele culturi e de 3-4 ori superior potențialului soiurilor vechi, loclale. Dar nivelul mondial este încă și mai presus.

Actualmente selecția și ameliorarea culturilor agricole se bazează pe realizările științifice în științele biologice (botanica, fiziologie, genetică, fitopatologie ș.a.) și agricole și pe experiența mondială și metodele, eficiența cărora e confirmată cu rezultate reale.

În acest domeniu Moldova a avut și mai are realizări competitive nu doar pe piața locală și regională, dar și pe cea mondială (la unele culturi). Soiurile și hibrizii de grâu, porumb, floarea soarelui, legume, viță de vie și alte culturi erau apreciate și utilizate nu doar în țările CSI dar și în multe alte țări. Hibrizii și-au demonstrat avantajele pe cele mai necuprinse lanuri din Rusia, Belorusia, Kazahstan, asigurînd producții de 2-3 ori mai mari ca cele create în zonele respective (au fost obținute producții de 15,3 t/ha porumb boabe la hibrizii timpurii de porumb în Belorusia și de 18,4 t/ha hibrizii mai tardivi în Turkmenistan, iar recordul la producția de porumb în Moldova este deținut de hibridul M450, la sectorul Zîrnești din r-nul Cantemir și acest record (17,6 t/ha) încă nu a fost depășit de vre-un hibrid străin.

Actualmente în domeniul ameliorării plantelor s-au acumulat mai multe probleme, dar există și soluții posibile, care nu se realizează. În ceea ce urmează vom enumera doar unele dintre acestea.

Probleme și posibile soluții

Resursele genetice reprezintă materialul inițial pentru ameliorare, menținerea, evoluția, și chiar crearea, resurselor genetice cu calități, însușiri speciale, este o necesitate și o problemă de interes comun, și acest domeniu e necesar să fie finanțat din resurse publice.

Dat fiind resursele genetice un material genetic excepțional, absolut necesar, este necesară și o atitudine responsabilă din partea organelor publice, întru menținerea și

evaluarea acestor resurse, în asigurarea accesului amelioratorilor, şi a creatorilor acestor resurse, la utilizarea acestora. Cazuri, când creatorii acestor resurse, care în decurs de zeci de ani au studiat, ba chiar au creat mai multe mostre, clone, linii cu însuşiri valoroase, nu au acces liber în a le utiliza, sunt insuportabile.

Însă nici genofondul creat (existent) nu este utilizat la maximum în programele de ameliorare. Nu este studiat şi utilizat la reala valoare fenomenul heterozisului. La culturile, la care heterozisul a fost studiat şi utilizat (porumb, floarea soarelui, legume, etc.) avem creaţi şi implementaţi hibrizi competitivi, la alte culturi încă ne bazăm pe soiuri, deşi posibilităţi şi exemple de utilizare a heterozisului există chiar şi la culturile autogame (grâu, de exemplu). Timpul soiurilor tradiţionale a trecut. Timpul hibrizilor actuali, tradiţionali, trece. Vine timpul soiurilor hibride cu multiplicare vegetativă, cu fixarea efectului maximal al heterozisului.

Cele mai evidente realizări în genetică şi ameliorare în Moldova au fost obţinute în studierea şi aplicarea androsterilităţii, în ameliorarea şi producerea de seminţe. Şi la porumb, şi la floarea soarelui, cercetările androsterilităţii au fost realizate la cel mai înalt nivel. Efecul economic de la aplicarea acestor realizări doar la porumb pe o suprafaţă de peste 2,2 mln ha (sectoare seminciere) este colosal – a fost asigurată calitatea genetică a seminţelor (peste 2,5 mln tone) şi exclusă castrarea manuală pe o suprafaţă de 2 mln ha, ceea ce a adus pe parcursul anilor 1965-2015 o economie de 16 mln zile-om, în valoare de 320 mln dolari. Un efect economic aproximativ de acelaşi nivel a fost determinat de sporirea calităţii genetice a seminţelor. Iată doar un exemplu ce ne poate da genetica. La obţinerea unor realizări remarcabile în studierea şi utlizarea andrsterilităţii au contribuit acad. A.E. Covarschii, m.c. T.S. Cialîk, m.c. A.F.Palii, d.şt.a. Eugenia Partas şi mulţi alţii – la porumb; acad. Maria Duca şi colaboratorii la floarea soarelui. Însă tema nu este epuizată – androsterilitatea reprezintă mecanismul genetic, care face posibil utilizarea fenomenului de heterozis.

Actualmente majoritatea cercetărilor şi lucrărilor de ameliorare sunt orientate spre îmbunătăţirea culturilor agricole existente. E bine. Dar e puţin.

E necesar crearea de noi culturi, care ar utiliza mai eficient factorii naturali (în primul rând factorul energetic), tehnogeni şi socio-umani. Iată argumentele: actualmente în agricultura Moldovei culturile de câmp utilizează energia fotosintetic activă (EFA) doar în jur de 1% din posibilele 5%. De ce? În primul rând pe terenurile însămânţate cu culturi de primăvară: porumb, sfeclă pentru zahăr, floarea soarelui ş.a. – peste 750 mii ha (50% din terenul agricol însămânţat) în lunile aprilie şi mai – deci cel puţin 60 de zile, energia solară nu este asimilată de aparatul foliar, care e abia în formare. Pentru a exclude acest fenomen sunt necesare culturi noi, care fiind însămânţate de cu toamnă, ar forma aparatul foliar începînd din primele zile de temperaturi pozitive, deci cu 50-60 de zile mai înainte decât culturile actuale. Aceste culturi, cu plantare de cu toamnă, rezistente la temperaturi scăzute, cu creştere rapidă, ar mai fi necesar să aibă o însuşire foarte importantă: să fie multiplicate vegetativ. Înmulţirea vegetativă ar crea posibilităţi de multiplicare extinsă (pe mln de ha) a unor exemplare unicale, obţinute prin încrucişări, modificări genetice şi selecţii individuale.

Crearea unor asemenea culturi este un obiectiv foarte important şi greu de realizat, necesită cercetări fundamentale în genetică şi ameliorare. Poate fi efectuat doar printr-un program special, finanţat din buget. Cine va realiza această idee se va încununa de onoare.

O cultură cu unele asemenea caractere – plantare de cu toamnă, creştere intensivă şi acumulare de biomasă, poate fi creată, prin încrucişăria unor specii din familia Apoaceae. Această cultură energetică ar acumula 4-5% de EFA, comparativ cu 2-2,5% maximum, obţinuţi la sorgul zaharat şi hibrizii de sorg-iarbă de sudan, ar permite obţinerea biomasei începînd cu luna mai (deci cu 60-70 de zile mai timpuriu, comparativ cu cele mai eficiente culturi actuale).

Un alt program de ameliorare ar fi crearea culturilor, care ar utiliza EFA în lunile iulie-august-septembrie, deci în decurs de 90 de zile până la îngheţurile de toamnă!

Această problemă poate avea două soluții (variante) distincte: 1) crearea sau selectarea de culturi cu perioada de vegetație scurtă, semănate după recoltarea spicoaselor, cu rezistență la secetă, creștere intensivă, receptivitate la irigație. O altă soluție pentru acumularea EFA în lunile iulie-septembrie, sau cel puțin iulie-august ar fi promovarea culturilor de toamnă (inclusiv grâu, triticale, culturi oleaginoase, tehnice, furajere, energetice), care ar vegeta și fotosintetiza activ pe durata integrală a perioadei aprilie-septembrie.

E actual un program de selectare şi ameliorare a culturilor energetice, cu o eficacitate fotosintetică de 2-3 ori mai mare,comparativ cu culturile agricole actuale; în special este actuală selectarea şi ameliorarea culturilor, care sintetizează hidrocarburi (componenți care conțin doar atomi de H şi C), introducerea unor asemenea culturi din alte zone (deşi în flora locală există astfel de plante), selectarea şi ameliorarea culturilor de alge, care deasemenea sintetizează şi acumulează hidrocarburi. Aceste lucrări de selectare şi ameliorare a culturilor energetice ar necesita o perioadă mai lungă (5-6 generații) pentru realizare şi ar fi rațional să fie finanțate din buget printr-un program special.

O altă direcție şi posibilitate strategică în ameliorare ar fi crearea culturilor, soiurilor, hibrizilor de culturi cerealiere cu însuşiri şi capacități de acumulare (fixare) a azotului din atmosferă. Această posibilitate poate fi realizată printr-un program special de 8 generații, selectări şi ameliorări pentru fiecare din principalele culturi cerealiere. Ar fi o realizare care ar reduce cu cel puțin 50% consumul de fertilizanți de N.

Ameliorarea actualmente tot mai mult se bazează şi se integrează cu genetica. Baza genetică a creaţiilor noi în ameliorare va rămâne şi pentru viitorul apropiat – hibridizarea şi selecția, androsterilitatea, dar tot mai mult şi mai intens va cuceri teren modificarea genetică, recombinarea OMG.

Programele de ameliorare ar fi necesar să fie orientate mai mult spre obținerea unor hibrizi rezistenți la factorii nefavorabili (în primul rând secetă, temperaturi înalte/scăzute, boli şi dăunători). Toate aceste ameliorări pot fi realizate relativ mai uşor în cadrul cultivarilor existente, utilizînd în primul rând (în special) metodele genetice, inclusiv şi în special modificarea genetică – OMG – transferul însuşirilor necesare, caracterelor ameliorative valoroase de la alte specii, genuri de plante sau animale. Hibrizi de porumb rezistenți la secetă creați şi lansați în ultimul timp de către compania Pioneer au însuşirea de rezistență transmisă de la animale (scorpion).

Deci şi posibilități există, resurse şi mecanisme genetice, necesități acute există, posibilitățile materiale, tehnice, financiare, trebuie identificate, create (inclusiv şi în special resursele financiare, tehnice, intelectuale necesare). Alfel vom fi condamnați la importul continuu a realizărilor în domeniu, ceea ce ne va costa mult mai scump, comparativ cu realizarea unor programe în țară.

Implementarea realizărilor în domeniul geneticii şi selecției. Cele mai mari şi mai prestigioase şi fabuloase descoperiri, realizări ale geneticienilor şi în general a savanţilor, dacă nu se aplică în practică, dacă nu aduc bine societăţii – delicat vorbind rămân doar ca nişte hulubaşi, care se lansează pe la ceremonii, festivităţi, umbra zborului cărora atât doar pot să mângâie, să împace mintea şi sufletul creatorului, să-i creeze o iluzie că şi el a făcut ceva, că nu şi-a irosit viaţa în zadar. Atât doar! Pînă nu se utilizează spre binele societăţii orice realizare ştiinţifică e un capital mort. Implementarea, aplicarea creaţiilor geneticienilor, amelioratorilor, este o sarcină, o etapă, de cea mai mare importanţă! Întârzierea cu aplicarea creaţiilor amelioratorilor şi geneticienilor nu numai că devalorizează, ba chiar în unele cazuri anulează aceste creaţii, dar aduc prejudicii colosale nu numai ştiinţei (descurajează creatorii, devalorizează creaţiile) dar mai ales economiei. Şi aceste cazuri sunt multiple şi în istorie şi în timpul de faţă. Iată doar un exemplu.

Porumbul hibrid, cea mai mare realizare a geneticii secolului XX – în SUA a fost introdus în agricultură începînd cu a.1932, în 1940 ocupa 90% din suprafaţa cultivată

şi a dublat producţia de porumb la hectar. În fosta URSS implementarea a fost lansată cu 33 de ani mai târziu şi de la lansare (1955) până la extinderea pe toată suprafaţa a durat 25 de ani (în Moldova implementarea hibrizilor de porumb a fost finisată către 1963, deci mai înainte cu 17 ani decât în alte regiuni). Întârzierea cu implementarea hibrizilor de porumb a costat – 1 mln de tone de porumb anual la un milion de hectare, în fosta URSS la 20 mln de hectare (echivalentul a 20 mln tone), în 33 de ani de reţinere – 660 mln de tone de porumb. La preţul de 150 dolari tona, suma pierderilor depăşeşte 100 mlrd de dolari sau 3 mlrd anual. Iată preţul aproximativ al ignoranţei, al reţinerii realizărilor genetice doar într-o ţară, deşi mare, dar nu unica. Au pierdut, dar mai puţin, şi toate ţările care au întîrziat cu 10-12 ani. Dar asta a fost demult.

Acum, cea mai mare realizare a geneticienilor în fitotehnie o prezintă organismele modificate genetic, OMG. La multe culturi – soia, bumbac, porumb soiurile şi hibrizii OMG ocupă zeci de mln de hectare, în total peste o sută şaizeci de milioane de hectare în 30 de ţări. Avantajele OMG sunt clare şi demonstrate. Pericolele – mai mult imaginare, mai mult inventate de interesele de concurenţă. Noi, şi nu numai noi – mai multe ţări europene, am inventat multe bariere legislative, economice, informaţionale în calea acestei realizări genetice. Dacă această repulsie faţă de OMG va mai dura, noi vom fi scoşi din cursa pentru piaţă, când se va deschide poarta, când se vor lua baricadele din calea OMG.

Până nu-i tîrziu, pân' încă nu am pierdut şansa, trebuie să revedem atitudinea faţă de OMG, păstrînd prudenţa şi vigilenţa, să admitem potenţa. Parlamentul UE a scos restricţiile la implementarea OMG (în ianuarie 2015). Acum fiecare ţară decide, vom decide şi noi.

Implementarea realizărilor ştiinţifice în genetică, în ameliorare, este un proces complex. Dar iniţiativa trebuie să vină de la creatori, ei trebuie să facă primii paşi, să-şi scoată creaţiile la dans în hora economiei de piaţă, să le vadă cea mai capricioasă doamnă – Economia de Piaţă, care poate respinge azi ceea ce căuta ieri cu lumânarea, şi poate căuta cu înfrigurare ceea ce respingea mai ieri categoric. Aşa-i hora pieţii, aşa-i hora vieţii.

Promovarea e mult mai uşoară dacă este susţinută de conducerea ţării. Există multiple exemple.

Regele Franţei, Ludovic XIV, ca să promoveze cartoful, purta la haină un buchet de flori de cartof. În cinstea cercetătorului Parmantie, în suburbia Parisului au fost ridicate 2 monumente, pe unul fiind reproduse cuvintele regelui:"Credeţi-mă, că va veni ziua când Franţa vă va fi recunoscătoare pentru că aţi dat pâine lumii flămânde.".

Senatul Imperiului Rus în a.1765-1766, la 23 de şedinţe a discutat problemele legate de implementarea cartofului. Parlamentului nostru numai asta nu-i ajunge! Însă nu-i vorba de cartof dar de agricultură în general, de implementare!

Asta a fost demult. Dar iată exemple mai proaspete. Porumbul hibrid a fost implementat în fosta URSS inclusiv şi în Moldova prin efortul conducerii de vîrf a ţării de atunci CC al PCUS, Nikita Hruşciov. Aşa a fost în majoritatea ţărilor europene, şi nu numai în cele comuniste. În Moldova crearea şi implementarea hibrizilor de porumb, floarea-soarelui, crearea A.Ş.P., institutelor şi laboratoarelor de genetică şi ameliorare – toate s-au realizat la decizia şi cu contribuţia categorică a conducerii ţării. Toate acestea au contribuit radical la dezvoltarea cercetărilor de genetică şi ameliorare. Ultima reformare, despre care nu putem spune nimic bun, realizată în 2008, tot este opera conducerii de atunci a ţării. Sperăm că intervenţia conducerii de vârf în problemele cercetărilor de genetică şi ameliorare să fie mai eficientă decât reformarea din 2008.

Activitatea instituţiilor de cercetare de profil agrar în condiţiile economiei de piaţă

- **Etapele şi direcţiile principale de cercetare**

Problemele, rezolvarea cărora necesită aplicarea ştiinţei agrare, sunt multiple şi de o importanţă vitală şi includ: asigurarea securităţii alimentare, energetice, scologice, sociale, demografice, intelectuale precum şi renaşterea satului.

Evoluţia economiei naţionale spre o economie de piaţă este ireversibilă. Activitatea instituţiilor de cercetare trebuie să fie adaptată la condiţiile economiei de piaţă. Capacitatea de a se acomoda adecvat şi operativ la cerinţele pieţii este decisivă în asigurarea succesului activităţii. Acest proces de acomodare este de lungă durată şi include mai multe etape şi componente obligatorii, precum şi o mare diversitate de modalităţi concrete.

Etapele principale ale activităţii Institutului de cercetare-dezvoltare în condiţiile economiei de piaţă sunt următoarele:

1) Aprecierea pieţii; 2) Crearea valorilor intelectuale (soiuri şi hibrizi); 3) Protecţia proprietăţii intelectuale; 4) Aplicarea (utilizarea) produselor intelectuale.

Direcţiile principale ar fi următoarele:

1. Păstrarea şi valorificarea resurselor naturale, genofondului de plante şi animale.
2. Crearea şi aplicarea soiurilor şi hibrizilor de plante şi animale.
3. Producerea de seminţe şi material săditor pentru piaţa internă şi pentru export.
4. Pregătirea cadrelor ştiinţifice.
5. Informatizarea şi instruirea agricultorilor în vederea aplicării realizărilor ştiinţifice.
6. Comercializarea realizărilor ştiinţifice.

- **Modalităţile de finanţare**

Ştiinţa e raţional să fie finanţată de beneficiarii, care aplică rezultatele realizărilor ştiinţiifce. Însă acest principiu nu trebuie absolutizat. În ceea ce priveşte ştiinţa agrară se propun următoarele variante de finanţare:

1. Prin alocarea din buget a 4-5% din impozitul obţinut de la complexul agroindustrial.
2. Prin comercializarea directă a realizărilor ştiinţifice beneficiarilor.

Fondul bugetar de dezvoltare a ştiinţei agrare se repartizează de către Ministerul Agriculturii instituţiilor de cercetare de profil conform contractelor şi proiectelor de cercetare prin concurs. Din acest fond bugetar se finanţează prioritar următoarele direcţii:

1. Protecţia solului, apelor, resurselor naturale;
2. Păstrarea şi evaluarea genofondului de plante şi animale;
3. Informatizarea şi propagarea realizărilor ştiinţifice;
4. Pregătirea cadrelor şi instruirea agricultorilor;
5. Cercetările fundamentale în ştiinţele biologice şi agrare;
6. Cercetările privind argumentarea actelor normative.

Ca excepţie pot fi finanţate din buget şi unele programe de creare a soiurilor şi hibrizilor, care cer o durată de realizare de peste 5 ani. Însă ca regulă aceste direcţii trebuie să fie finanţate prin comercializarea directă a rezultatelor către beneficiari, prin defalcarea a 12-15% din venitul obţinut de la aplicarea acestor realizări.

- **Aprecierea pieţii**

Dat fiind că în condiţiile economiei de piaţă dictează cumpărătorul este necesar ca în primul rând să analizăm şi să apreciem real, în dinamică, piaţa cu toate elementele ei:

1. Ce producem? Soiuri, hibrizi, seminţe, material săditor, recomandări.
2. Volumul pieţii. Cât producem – pentru intern şi pentru export.
3. Pentru cine producem? Cine sunt cumpărătorii – destinatarii.
4. Capacitatea de plată a cumpărătorilor.
5. Preţul de producere şi preţul de realizare.
6. Concurenţii.
7. Legislaţia, licenţierea, restricţiile.
8. Cerinţele faţă de calitatea producţiei.

9. Dinamica pieţii, inclusiv factorii geopolitici.
10. Stimularea, dirijarea, evoluţiei pieţii în direcţia necesară.

- **Crearea proprietăţii (valorilor) intelectuale.**

Despre rolul şi importanţa ştiinţei am putea vorbi mult, cu argumente multiple. Menţionăm doar câteva exemple: 1) crearea şi implementarea hibrizilor de porumb asigură peste 50% din creşterea producţiei la această cultură, obţinută în ultimii 60 de ani, iar reţinerea cu 33 de ani a implementării hibrizilor de porumb în URSS a adus pierderi de peste 100 mlrd dolari; 2) revoluţia verde, soiurile de grâu intensive, au salvat de la foame sute de milioane de oameni.

Crearea valorilor intelectuale este poate cea mai complexă etapă a procesului de cercetare-dezvoltare. Succesul în această activitate depinde de mai mulţi factori şi mai ales de interacţiunea lor. Factorii decisivi care determină succesul în cercetare şi aplicare: 1. Ideea; 2. Informaţia; 3. Cadrele; 4. Tehnica, tehnologia, materialele; 5. Finanţele; 6. Aplicarea (utilizarea) rezultatelor în producţie. Nici un factor separat nu asigură succesul. Numai îmbinarea lor favorabilă asigură succesul.

În linii mari instituţiile de cercetare acoperă mecesităţile principalelor ramuri ale complexului agroindustrial, însă unele modificări ar putea fi operate ca să asigure o funcţionare mai bună, o finanţare şi o dotare materială corespunzătoare. Cea mai gravă situaţie este în pregătirea cadrelor, asigurarea cu tehnică şi materialele, care practic depind de nivelul de finanţare. Dacă ştiinţa agrară (precum şi toată ştiinţa), nu va fi susţinută şi finanţată la nivelul cuvenit, Moldova va fi nevoită să plătească foarte scump pentru realizările ştiinţifice din import (soiuri, hibrizi, rase de animale) ceea ce va agrava şi mai tare criza din economia ţării. Deja în anii 2014-2015 importul de seminţe ne costă de zeci de ori mai mult decât ar fi necesar pentru finanţarea ştiinţei agrare, care ar putea produce tot necesarul în acest domeniu.

- **Protecţia proprietăţi intelectuale**

Protecţia creaţiilor intelectuale e un obiectiv important şi greu de realizat ca şi crearea acestei proprietăţi. Ignorarea acestei axiome de cele mai multe ori duce la devalorizarea realizărilor ştiinţifice, demolarea procesului de cercetare.

Există mai multe metode (procedee) şi posibilităţi de protecţie a valorilor intelectuale, care pot fi clasificate (convenţional) în următoarele clase:

1. Legislativ-administrative – legi, decizii ale guvernului, ministerului, decizii judecătoreşti. Obligaţia de protejare legislativă a proprietăţii intelectuale îi revine statului, dar şi creatorii de valori trebuie să adopte o poziţie activă.
2. Economico-financiare – principalul să fie cointeresaţi şi creatorii şi beneficiarii de produse intelectuale în protecţia proprietăţii intelectuale.
3. Genetice (de origine): a) Produsele intelectuale trebuie să conţină elementele, care nu pot fi reproduse fără acceptul (participarea) autorilor; b) Produsul intelectual trebuie să posede caractere (însuşiri) deosebite, inconfundabile, care ar evidenţia acest produs.
4. Informaţionale – orice informaţie, care poate servi la penetrarea protecţiei valorilor intelectuale trebuie să fie accesibilă numai unui cerc restrâns de persoane. Orice informaţie care poate servi la protecţia creaţiei intelectuale trebuie răspândită pe larg tuturor consumatorilor produsului intelectual.
5. Socio-umane – acest nivel de protecţie a creaţiei intelectuale presupune solidaritatea tuturor creatorilor, excluderea a oricăror forme de discreditare reciprocă a produselor intelectuale, excluderea sub orice formă a preluării produselor intelectuale ale altor creatori. Creatorii trebuie să fie ei înşuşi un exemplu în protecţia valorilor intelectuale ale altor autori.
6. Restabilirea şi respectarea dreptului decisiv al autorilor asupra creaţiilor intelectuale.
7. Combinative sau complexe – nici unul din procedeele (metodele) indicate mai

sus, separat nu asigură integral protecţia proprietăţii intelectuale. Ea poate fi asigurată doar printr-o îmbinare eficientă a mai multor metode şi procedee.

Protecţia trebuie să fie relativă, limitată în spaţiu şi timp, altfel se va stopa progresul tehnico-ştiinţific.

- **Implementarea (comercializarea) creaţiilor intelectuale**

Implementarea (utilizarea) realizărilor ştiinţifice reprezintă o etapă foarte importantă în complexul cercetare-dezvoltare. Această etapă la rândul ei conţine mai multe componente obligatorii:

1. Aprecierea pieţii posibile de implementare.
2. Crearea modelului funcţionabil.
3. Testarea inovaţiei în laborator, instituţie, Comisia de Stat.
4. Înregistrarea în Registul de Stat, patentarea.
5. Organizarea producerii inovaţiei (hibrid, soi, procedee, etc.)
6. Familiarizarea beneficiarilor cu avantajele inovaţiei.
7. Încheierea contractelor de utilizare a inovaţiei (comercializarea).
8. Instruirea beneficiarilor în procesul utilizării inovaţiei.
9. Cointeresarea beneficiarilor în utilizarea inovaţiei.
10. Alte componente inclusiv instruirea cadrelor, care se ocupă de implementare.

Realizarea acestor componente a procesului de implementare poate fi efectuată cu succes de structuri speciale, de specialişti bine pregătiţi în problemele relaţiilor cu beneficiarii. Încercările de a include cercetătorii ştiinţifici ca responsabili de implementare nu este cea mai bună soluţie, ba chiar dimpotrivă – vor fi dereglate ambele procese – şi cercetarea şi implementarea.

În instituţiile de cercetare de profil agrar e necesar să fie realizat un program special de promovare, implementare şi comercializare a creaţiilor intelectuale, care poate fi executat cu succes doar de o structură specializată în domeniul relaţiilor comerciale cu produsele intelectuale.

- **Stimularea activităţii de cercetare şi inovare**

În contextul economiei de piaţă succesul în activităţile de ceretare-inovare este determinat nu doar de idee (baza teoretică), informatizarea şi competenţa cadrelor, utilajul şi tehnologiile moderne, dar în mare măsură de cointeresarea participanţilor la acest proces. Baza legislativă pentru stimularea materială a participanţilor la crearea şi utilizarea valorilor intelectuale, în primul rând la crearea şi implementarea soiurilor, hibrizilor de plante, serveşte Legea nr. 915-XIII din 11.07.96, despre protecţia soiurilor de plante. Articolul 12, p.6 al acestei legi prevede pentru remunerarea autorilor utilizarea a cel puţin 15% din profitul obţinut de la comercializarea soiului, hibridului. Existenţa unei baze legislative este o condiţie necesară, dar nu şi suficientă pentru a stimula autorii. Se mai cer şi bani. Sursele financiare necesare pentru cointeresarea autorilor pot şi trebuie să fie obţinute prin comercializarea creaţiilor intelectuale.

Utilizând modelul expus mai sus fostul Institut ”Porumbeni” a implementat hibrizi de porumb în 10 ţări, în 8 dintre care prezenţa Institutului era permanentă şi vizibilă. Cota hibrizilor ”Porumbeni” în Moldova constituia 95-97%, iar în Belorusia – 65-70%. Producerea de seminţe în 22 ani (1987-2008) a depăşit 1369 mii tone, inclusiv 700 mii tone în Moldova. Sursele obţinute de la comercializarea seminţelor,constituiau baza financiară a modelului de stimulare – fondul de premiere a autorilor constituia anual 1,5-2 mln lei.

- **Împroprietărirea autorilor. Privatizarea creaţiilor intelectuale**

În concepţia impusă de Hotărârea Guvernului nr.761 din 24.06.2008 şi în formula şi structura de organizare şi funcţionare actuală, institutele de profil agrar nu vor putea realiza programe de cercetare competitive. Prin această Hotărâre s-a efectuat divizarea Institutelor de stat în instituţii publice şi gospodării de stat, separarea cercetării

de structurile de producere şi implementare. Această divizare contravine experienţei mondiale şi locale, de integrare a procesului de cercetare şi implementare, chiar însuşi scopului declarat în Hotărâre, care prevede concentrarea resurselor, nu divizarea. Divizarea institutelor în cele două unităţi a condus la scumpirea serviciilor reciproce (cel puţin cu 20% TVA), între aceste două structuri a apărut conflict de interese.

Reforma nu a rezolvat nici o problemă a instituţiilor de cercetare din complexul agroindustrial (problema menţinerii şi atragerii cadrelor, dotării tehnice, comercializării producţiei intelectuale şi altele) dar a creat noi dificultăţi în activitatea de cercetare şi implementare.

În scopul asigurării ţării cu hibrizii performanţi, seminţe de calitate şi tehnologii eficiente pentru piaţa internă şi export, menţinerii şi renovării potenţialului intelectual şi material, asigurării unei protecţii sociale şi sporirii nivelului de trai a colaboratorilor se propune o soluţie bazată pe analiza profundă a experienţei mondiale şi locale să fie restabilite institutele de cercetare-dezvoltare ca întreprinderi de stat şi să fie transformate în societăţi pe acţiuni cu participarea membrilor colectivelor (în favoarea membrilor colectivelor).

Dreptul decisiv asupra creaţiilor intelectuale (soi, hibrid, metodă, clonă, tehnologie, etc.) trebuie să fie acordat creatorilor (autorilor),nu statului.

Argumente în favoarea privatizării institutelor şi creaţiilor intelectuale:

1) Proprietatea privată şi-a demonstrat eficacitatea în toate domeniile, inclusiv şi în domeniile de activitate cercetare-dezvoltare;
2) Instituţiile de Stat de profil în ţările fostei URSS şi lagărului socialist şi-au demonstrat ineficacitatea totală în condiţiile economiei de piaţă;
3) Capitalul privat nu este interesat să facă investiţii în instituţiile de stat;
4) Toată sfera agriculturii, inclusiv producerea, prelucrarea şi comercializarea semînţelor, materialului săditor este privată;
5) Privatizarea va atrage capitalul privat în modernizarea instituţiilor;
6) Transfomarea institutelor în societăţi pe acţiuni ale colectivelor şi acordarea autorilor dreptului decisiv asupra creaţiilor, vor spori interesarea colaboratorilor în obţinerea unor rezultate performante, vor spori salarizarea, protecţia socială, va stopa exodul cadrelor.

E necesară urgentarea privatizării dat fiind că valoarea institutelor scade considerabil (anual cu 20-25%), datorită reducerii cotei produselor intelectuale pe piaţa internă şi externă

Numai revenirea urgentă la o formă eficientă de organizare şi funcţionare a institutelor, care ar garanta cointeresarea creatorilor, respectarea drepturilor autorilor asupra creaţiilor intelectuale, vor asigura menţinerea potenţialului intelectual, genetic şi material, sporirea bunăstării colaboratorilor, continuitatea şi eficacitatea cercetării, creării şi implementării valorilor intelectuale.

Concluzii şi propuneri

Genetica şi ameliorarea prezintă cel mai eficient domeniu de cercetare şi implemetare pentru a asigura eficientizarea agriculturii şi securitatea alimentară, energetică, demografică şi ecologică a ţării. Alocaţiile pentru acest domeniu e necesar să fie radical sporite;

Să fie lansat şi realizat (în 3-5 ani) un program de stat de studiere, ameliorare şi implementare a culturilor energetice;

Să fie aplicate toate modalităţile şi posibilităţile de stimulare materială şi morală a creatorilor de valori în domeniul ameliorării plantelor;

Dreptul de proprietate asupra creaţiilor intelectuale (soi, hibrid, clonă, metodă, tehnologie, etc.) să fie acordat autorilor (cercetătorilor), nu statului.

INSTITUTE OF PHYTOTECHNY "PORUMBENI" ACHIEVEMENTS AND GENETIC PROGRESS IN MAIZE BREEDING

Pintilie Pirvan, Vasile Maticiuc, Silvia Mistret

Institute of Crop „Porumbeni, R. Moldova

Introduction

High potential of production and wide application of maize in many spheres of human activities, places this crop like the most popular cultivated spices in the world.

Thanks to economic importance for a long time, there were taken measures for continuous development of maize crop. Among them it is counted the foundation of separate scientific unit for studding of this crop - Institute of Crop "Porumbeni".

Within forty years Institute has been subject to many changes both the name as statute, but the many objectives generally remains the same. Staring from agro industrial needs it offers the following:

a) development of competitive hybrids of maize and sorghum of different maturity groups and purpose of utilization;

b) development and improvement of technologies for cultivation of hybrids and parental forms;

c) seed production of superior biological categories;

d) processing and keeping of hybrids and parental forms.

e) application of scientific elaborations and advanced experience in maize and sorghum growing.

Institute of Crop "Porumbeni" in order to improve agricultural efficiency on the base of productive hybrids and their high adaptability to biotic and abiotic factors, has evaluated an integral program of scientific research and works in breeding for development and implementation of maize hybrids.(PÎRVAN, P. 2014)

The main directions of this program are based on the activities of the Institute:

a) studding, identification, maintaining and diversity of germplasm.

b) development and identification of inbred lines of maize with high combinability.

c) sevelopment of competitive hybrids of different maturity groups and purpose of utilization.

d) seed production of parental forms and hybrids for Moldova and for export.

e) promotion and implementation of hybrids with high genetic potential.

The germplasm of maize includes: cultivated hybrids, inbred lines which are used like the parental forms, and improved synthetic populations, cytoplasmic male sterility sources possessing certain characters and agronomic traits, special genetic stocks, local unimproved populations and wild spices related to maize. The basic part of Moldavian maize germplasm is kept in Institute of Phytotechny "Porumbeni" and plays the significant role both for breeding programs of inbred lines development and also for improvement and development of new synthetic populations. (CĂBULEA, I,2004).

A very important direction of maize breeding program is the development of inbred lines adopted for a specific climatic conditions of cultivated regions, used to create a new hybrid combinations. There are following stages in inbred lines development: selection of initial breeding material ant it improvement, breeding within and between descendants from different generations of inbreeding according to main valuable agronomic traits, evaluation of combinability.

In order to encourage breeders, collection of foreign and local inbred lines from Institute has been conventionally classified by germplasm groups: European Flint, Canadian dent, Reid, Iodent, Lancaster, BSSS-B37, Minnesota 13, Osterland, Mindsenpustai. In order to develop the initial breeding material for hard maize with flint kernels

and high carotene content there were used inbred lines which came from local populations Portocaliu, Hăngănesc and Cincvantino. (MICU V, 2008)

The biological material for pop corn inbred lines development has included local varieties, American synthetic populations: White Rice, Yellow Pearl, Queens Golden, South American, Argentine, Ladyfinger, Reid, hybrids of Mc Hone Seed Company (USA), BC 503(Yugoslavia), local inbred lines and lines from the world-wide collection and other genetic sources from Evert and Flint convarieties which have endosperm with corneous starch content.

Sugar inbred lines have been developed from local populations in hybrids making crosses with genetically distinct germplasm sources, inclusive 40 samples from USA, Canada, Romania and France.

Development of inbred lines was traditionally base on pedigree and backcross method. In first selfing (S1) generation phenotypic selection has included: percent of germination and intense rate of plant growing in optimal conditions and low temperatures, precocity, drought resistance, high productivity and pollinated capacity, low moisture content during harvesting, cob and plant diseases tolerance, prolonged activity of leaves after physiological maturity (stay green), tolerance at high density and other important traits for mechanical harvesting. Biological maize material for special use (pop-corn, sugar maize, high carotinoid content), have been selected and analyzed according to main grain traits in the laboratory of biochemistry, physiology and biotechnology.

The main criterion in selection of perspective (pilot) inbred lines by the agronomical trait was and remains the general and specific combinability.

In order to appreciate the combinability, of families S4-S5 generations, after a rigorous screening in fist breeding generations, have been crossed with 4-5 testers from alternative germplasm groups. After breeding researches have been selected only 5-6% from the investigated families. The best families with high combinability have been studded and omogienizated in working collection from breeding laboratories, based on them it was created a great number of hybrids.

According to traditional methods the process of development and selection of inbred lines with high combinability takes up to 10 years. There are just several inbred lines given in Table Nr 1 , parental forms of commercial hybrids.

An essential contributions in inbred lines development belong to: professor Simion Musteaţa doctor of science Pantelimon Borozan, Nicholas Vanicovici, Vitalie Mirza, Grigore Pritula, Silvia Mistreţ, Eugenia Partas and others.

Breeding works with maize for special purpose have been finalized with development of 6 inbred lines of Flint type, 6 inbred lines Everta and 9 convarieties of sugar maize (tab. 1.3), which are the parental forms in 15 registered hybrids. Main contribution for development of these inbred lines belongs to a doctor of agricultural science Vasile Maticiuc.

According to current legislation inbred lines can be patented as intellectual property it is no less important achievements compared to commercial hybrids, because development of maize hybrids is based on hybridization between inbred lines. In this way, hybrids as a final product of breeding process can be released in different formulations, depending on cultivation region and purpose of utilization.

The program of hybrids development was based on heterotic patterns appreciated in the world practice, and membership of parental forms for germplasm group of basic inbred lines.

Selection of performing hybrids is based on trial results in different climatic conditions and efficiency of this program was provided by a number of ecological trials and many years of testing. Annually in preliminary trials in the course of many years about 4500-6100 hybrids combinations are studied and are compared with checks (annually 2-3 for testing) and finally 20% -30% of hybrids selected by breeders are tested in Concurs Trials (CT).

Table 1. Inbred lines FAO 170 – 460 which are used in registered hybrids for grain and silage

Nr. d/a	Name of inbred line	Group of maturity FAO	Type of grain	Pedigree	Developed hybrids with original inbred lines
1.	MKP 33	250	Dent	Reid mixt	P 212 CRf, Bemo 182 CRf
2.	MKP 42	250	Dent	Reid mixt	P174 MRf, 175 MRf, 176 MRf
3.	MKP 36	180	Semident	Dent Canadian	Bemo 172 CRf
4.	MKP 41	180	Dent	Dent Canadian	Bemo 172 CRf
5.	AN615/95	200	Flint	Flint European	P 174 MRf, 175 MRf, 176 MRf, 212 CRf
6	MKP55	210	Semident	Lancaster	Porumbeni 270, Alimentar 325
7	MKP 56	220	Dent	Lancaster	Porumbeni 270
8.	MK 276	420	Dent	Iodent	Porumbeni 359 AMRf, 457 AMRf, 375 AMRf,
9.	MK 271	300	Dent	BSSS-B37	Porumbeni 359 AMRf
10	MK 262	330	Dent	BSSS-B37	Porumbeni457, Porumbeni 375
11	MK 267	320	Dent	BSSS-B37	Porumbeni 458
12	MK 396	420	Dent	Iodent mixt	Porumbeni 458 MRf,
13	AS 808	330	Dent	BSSS-B37	Porumbeni 359 AMRf
14.	AS 814	400	Dent	BSSS-B37, Lancaster	Porumbeni 457 MRf, 458 MRf
15	AS 587	460	Dent	Iodent	Porumbeni 461 MRf
16	AS 591	460	Dent	Iodent	Porumbeni 462 MRf
17	AS 585	440	Dent	Iodent	Porumbeni 443 MRf
18	3070	460	Dent	Lancaster	Porumbeni 461, Porumbeni 375, Porumbeni 459

We would like to pay your attention that, selected hybrids after first year of testing in Concurs Trials (CT) are checked in ecological trails in Moldova, Russia, Republic of Belarus and Kazakhstan.

The main breeding traits in the process of selection are: earliness, grain production, green and dry mass, content of dry substance in grain and green mass, root and lodging resistance, diseases and pest tolerance. For the hybrids of special utilization biochemical analysis for qualitative indexes (content of carotene, pro-vitamin, solvable polysaccharides of total sugar and others), this is a component part of breeding process of pilot hybrids combinations. Also we have analyzed kernels consistence of hard maize, volume of expansion of pop corn, taste, aroma and the texture of pericarp of sugar maize.

Thus in 40 years of activity in the Institute there were created tens of thousands of maize hybrids, from which only 1-2% being submitted to Official Trials. Breeding process concomitant with applied researchers had lead to submission to State Committees of different countries over 200 new maize hybrids, 96 of them are registered in Official Catalogs of plant varieties in Moldova, Russia, Republic of Belarus, Ukraine and Kazakhstan.

In the late 70s – early 80s have appeared maize hybrids of a new generation: Moldavian 385 and Moldavian 420.

Table 2. Inbred lines which are used as parental forms in registered hybrids for special purpose

Name of inbred line	Pedigree	Type of grain	Group of maturiity, FAO	Registered hybrids
Mk 195CRf	Local population	Flint	300	Moldovenesc 349 CRf
Mki 3202	Mk195 x Soi Romanian	Flint	350	Porumbeni 393 MRf
Mki 280	Soi Romanian	Flint	390	Porumbeni 348 MRf
Mki 2494	3929 x Os440 Ungaria	Flint	350	Porumbeni 397 MRf
DMki 3312	Mk195 x Mki 280	Flint	350	Porumbeni 397 MRf
Mke 5410	White Rice – SUA	Everta	400	Porumbeni 356 MRf
Mke 4565	Mke 4691 x 346	Everta	400	Porumbeni 396 MRf
Mke 4221	Sintetic MHSC – SUA	Everta	400	Porumbeni 396 MRf
Mke 9408	Sintetic MHSC – SUA	Everta	400	Porumbeni 394 MRf
Mks 9c,mc	Iulius – SUA	sugar	190	Porumbeni 198 su
Mks 3CRf	Extra early – SUA	sugar	190	Porumbeni 198 su
Mks 36/5	346 x Mks 5su	sugar	400	Porumbeni 340 su
Mks 155/4	Mks36/5 x Mks4	sugar	350	Porumbeni 341 su
Mks 7	Elita – SUA	sugar	400	Porumbeni 342 su

Implementation of these hybrids has opened a new epoch in maize growing in Moldova.

Corresponding hybrids have considerably exceeded the hybrids which were produced before them not only in yield but in the process of seed production.

A stage of no less important was the researchers of maize mail sterility. As a result of the research we have received hundreds of analogous of male sterility, maintains of sterility and restorers of fertility, which became a genetic base for development of a new hybrids with seed production based on cytoplasmic male sterility.

In 80s there was created a laboratory for development of maize hybrids for regions with limited temperatures, which had a main goal of implementing the early maturity hybrids in Russia and Republic of Belarus. As a result of fruitful cooperation have been created hybrids of Bemo group: (Bemo 181,Bemo 182) and hybrids: Moldovenesc 215, Moldovenesc 330 which have been considerably expanded in 1990-2000.

The total area of cultivation of hybrids of the brand "Porumbeni" in 1990-2014 in Moldova and in the former CSI countries has constituted over 11 million hectares, including hybrids promoted in recent years – over 2,5 million hectares. New maize hybrids usually exceed 5% of previous hybrids productivity level, which ensures an economic effort of 300lei/ha.

Annually there were produced 10-15 thousand tons of maize seed, being ensured domestic and export market needs. During last 20 years there were produced and commercialized about 250 thousand tons of maize seed which provided planting of more than 10 million hectares of corn. On the base of parental forms of the Institute, in CIS countries there were produced more than 170 thousand tones of seed material. In Moldova currently "Porumbeni" hybrids are grown on the 70-75% of total areas planted with corn. With performance hybrids the obtained success is due to the promo-

tion of scientific results.

Table 3. Commercial hybrids developed in Institute of Crops "Porumbeni"

Name of hybrid	Type of grain	Group of maturity (FAO)	Year of registration in Catalog	Country of hybrid production
Porumbeni 385 MRf	Dent	280	1981	Moldova
Porumbeni 420MRf	Dent	400	1981	Moldova
Moldovenesc 257 MRf	Semident	250	1984	Rusia, Belarus, Moldova
Moldovenesc 215MRf	Semident	210	1986	Rusia, Belarus, Moldova
Moldovenesc291 MRf	Dent	290	1986	Moldova
Bemo 181CRf	Semident	180	1988	Rusia, Belarus
Bemo 182 CRf	Semident	190	1993	Rusia, Belarus
Moldovenesc 425 MRf	Dent	400	1990	Moldova
Porumbeni 295 A CRf	Dent	300	1995	Moldova, Ucraina, Rusia
Porumbeni 212 CRf	Semident	200	1998	Belarus
Bemo 172 CRf	Semident	170	2000	Rusia, Belarus
Porumbeni 348AMRf	Flint	350	2000	Rusia, Belarus, Moldova
Porumbeni 458 CRf	Dent	450	2001	Moldova, Ucraina, Rusia
Porumbeni 459 MRf	Dent	460	2003	Moldova
Porumbeni 457 AMRf	Dent	450	2004	Moldova
Porumbeni 396 MB	Everta	400	2003	Moldova
Porumbeni 176 CRf	Semident	170	2006	Rusia, Belarus
Porumbeni 375 AMRf	Dent	370	2006	Moldova
Porumbeni 461 MRf	Dent	460	2007	Moldova
Porumbeni 374MRf	Dent	370	2013	Moldova
Porumbeni 383MRf	Dent	400	2013	Moldova
Porumbeni 402 MRf	Flint	400	2013	Moldova

Traditionally in Institute are organized Demonstrative Plots , scientific-practical seminars with participation of seed producers and farmers. Scientists from Institute participate with scientific reports at congresses, conferences and symposiums organized by the Academy of Science of Moldova , and with informational reports at seminars in different regions organized annually by MAIA.

Annually Institute of Crop "Porumbeni" participates at local and international exhibitions: Made in Moldova", "Moldagroteh", "Farmer", "Infoinvent", "Proinvent", scientific results of the Institute have been awarded with diplomas , medals of gold and silver. Institute has participated with exhibits at exhibitions held in cities: Minsk, Moscow, Ashkhabad, Cluj-Napoca, Iasi and Bucharest.

For a long times an inseparable part of Institute activity was training of highly qualified stuff.

The results of this activity are demonstrated by supporting 35 PhD dissertations at eight specialties. In this section we have to mention the merit of famous scientists such as: Tikhon Chealik, Borovschi Mihail, Vasile Micu and Semion Musteata. In the frames of institute in 2011 it was opened the doctoral specialty 411.04 " Breeding and Seed production" . At present moment three young scientists are studding here. During last year's seven research-scientists have received title of docent, two of them received right to became a leader of respective specialty.

In 2014 it was held an international scientific conference, dedicated to anniversary of 40 years from Institute foundation, in which was mentioned by the Academy of Science and the Ministry of Agriculture and food Industry contributions of geneticist and breeders - Vasile Micu,Simion Musteata, Grigore Pritula, Vitalie Mirza, Alexandru Rotari, who has been working during four decades and continue to work in Institute and make their essential contributions in order to support and fulfill the tasks facing the staff of the Institute.

The successes obtained by the Crop Institute "Porumbeni" during four decades is the evidence of the integration of scientific researches and breeding process with implementation in production.

For the future we are looking for a new ways of deepening of scientific researches, training of highly qualified personal, implementation of elaborations in production at the level of present requirements, and of course an important factor , conditions of final economic interest.

Conclusion

According to current legislation inbred lines can be patented as intellectual property it is no less important achievements compared to commercial hybrids, because development of maize hybrids is based on hybridization between inbred lines. In this way, hybrids as a final product of breeding process can be released in different formulations, depending on cultivation region and purpose of utilization.

The main result of activity of Crop Institute "Porumbeni" is the development and submission to State Comite more than 250 maize hybrids of different maturity groups and purpose of utilization , among them 96 hybrids are registered in Official Catalogs of Plant Varieties in Moldova, Russia, Republic of Belarus, Ukraine and Kazakhstan.

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IDENTIFICATION OF AMELIORATIVE POTENTIAL OF GRAPEVINE GENETIC RESOURCES

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Introduction

The grapevine (*Vitis* L.) is a crop with a millenary traditions in our republic, has a major contribution to the diversification of the market offering product of high healing property. Socio-economic changes during recent decades, the fragile stability of the environment (including the phenomenon called "Climate Change") advance new requirements for grapevine varieties: increased quality and productivity; plasticity, advanced resistance to adverse environmental conditions, particularly to the abiotic; diverse natural, ecologically production; economic efficiency. It is through this prism is designed the strategy of accumulation, study and use in breeding programs of the diversity of genetic resources, which is part of sustainable development strategy of vitiviniculture, continuous improvement in the assortment is an essential component of food security.

It was mentioned [16, 17], that in case of genetic uniformity of existing assortment (represented mainly by *V.vinifera* L.) are created epiphytotic conditions for different diseases, and another consequence of industrial-scale cultivation of a limited assortment – ignoring of existing genetic diversity both in its wild form (in situ) and in its preserved form (ex situ) or culture (on farm), resulting in "genetic erosion" [1].

The effect of climate change on viticulture has primarily an economic impact: phenological stages can advance with 10-20 days, shifting of optimal climatic conditions for forming of qualitative production from traditional assortment, and the traditional style of produced wines could be changed, generating and social problems [9].

The analysis of developments of climate parameters in Republic of Moldova for different periods of years reveal some trends in their change, and in these conditions it was found that grape production decreased by 17.4% on average in years with extreme temperatures in summer (loss amounts vary between 2.4% and 46.1%) [18].

The practice of genetic improvement confirms that significant progress is determined by the presence of favorable genetic diversity and application of efficient prebreeding and breeding (bio) technologies [16,17]. But cardinal solving of the problem of grapevine protection against biotic and abiotic stress factors can be achieved by creating new varieties, whose resistance is provided by plant genetic constitution, a shining example in this respect was the progress achieved in grapevine breeding in Republic of Moldova [16, 17, 19, 20, 23].

In the present study are indicated some current targets in grapevine amelioration, including assessment of ameliorative potential of genetic resources presented in the grapevine gene pool of the institute.

Materials and methods

The research was performed on the experimental fields of Grapevine Genetic Pool (Genofong), located in the south of the Chisinau city (46 ° 58'39.65 "N 28 ° 46'21.68 and" E, altitude 201 m). Weather conditions of experimental fields correspond to the conditions of wine area Codru. In study were included diverse varieties and elites with various directions of grape use and from diverse ecological-geographic and genetic origin. Each genotype is given by 5-10 stocks. The scheme of planting 3.0 x 1.25 m. Phytotechnical processes applied for experimental sectors are the standards for industrial plantations.

Evaluation and description of genotypes was performed according the List of descriptors for grapevine varieties and Vitis species [4].

The statistical analysis was performed according to the method used in breeding [22] under STATGRAPHICS 5.0 software systems.

Results and discussions

Analysis of F1 generations of interspecific hybrids allowed to establish correlation character between grape quality and resistance to wintering conditions and to pathogen [16, 20, 23]: values of linear correlation coefficients between berry quality and resistance to wintering and between berry quality and resistance to *Plasmopara viticola* in offspring populations are small and insignificant ($r = -0.02 \div 0.14$), which determines that the descendants have inherited these traits independently. This regularity was confirmed by subsequent generations of descendants, involving seedless parental components ($r = -0.08 \div 0.12$). In biological material the high quality of berries, including seedlessness and productivity, resistance to mildew and frost are found in different combinations and are a practical confirmation of the hypothesis concerning the possibility of free combination of these characteristics. This confirms the absence of linkage between genes or complex of genes that determine studied characters or even if genes are present in the same chromosome, their position is far from one another, so that linkage does not occur. Consequently, there are not the genetic barriers for transmission through heredity to hybrid offspring of quality and resistance.

For the first time was argued possibility of creating such a assortment for the Carpathian-Danubian-Pontic geographical niche and succeeded in creating pioneering varieties with traits - quality, including seedless, productivity, early maturation, diverse use and resistance to biotic and abiotic unfavorable factors: Moldova, Codreanca, Pamiati Negrulea, Struguraş, Decabrischii, Urojainâi, Apiren alb, Apiren negru de Grozeşti, Apiren roz, Apiren roz Basarabean and Apiren roz extratimpuriu [16].

Note that the potential of crossingovers (*V.vinifera* x Complex interspecific hybrids) mentioned by Negrul' and Sorial [21, 24] concerning the quality of production and *V.vinifera* appearance, including leaf, and today presents a beneficial biological material to explore, adding other facets such as seedlessness and productivity, exploring in the frame of V.vinifera the resistance to abio- and bio- stress factors of old autochthonous varieties [2, 14, 15, 16].

In general, improving of assortment, especially for table grapes, was determined by crucial moments of social development and by natural disasters [8]: the first (such as, for example, the industrial revolution) formulated requirements to the appearance and commercial qualities of grapes, while natural disasters (invasion of pathogens and pests, environmental pollution, climate change) continually motivates complex genetic improvement - creating variety of diverse use, resistant to bio- and abio- unfavorable factors of the environment.

Actually, 80% of table grapes traded in the world are seedless, and the market in the sequel requests seedless varieties without rudiments [5]. For these reasons, the objectives of the breeding programs, in general, are the following: seedlessness, specific taste and new flavors, most advanced therapeutic qualities, attractive and large berry, bright colors, crisp pulp, lax and uniform bunch, very early or late ripening, transportability, technological quality, resistance to low temperature and pathogens etc. [5, 3, 8, 10, 17].

Wine producers are turning increasingly to the adaptability properties of the variety, and consumers – to quality, personality of wine and breeder's task is therefore to achieve both goals, embodied in the same genotype [13].

Given the complex objectives formulated by actual vitiviniculture, the research activities are directed to assess the diversity of genetic resources - as potential donors of characters in grapevine breeding [7, 10, 12, 17].

In the last decade they were developed technological processes that contribute to improving efficiency of breeding process. It is proposed reducing the length of breeding cycle by applying modern biotechnology [11]: manipulation of culture conditions - re-

ducing juvenile period and accelerating fructification, stimulate germination and seeds growing, stimulate flowering, the application of molecular biology technologies.

In grapevine breeding, in particular of varieties resistant to pathogens, it was demonstrated the advantage of applying marker assisted breeding, compared to traditional phenotypic methods, in predicting accumulation of resistance genes in hybrid progeny [6]. The process of selecting resistant progeny is recommended to be carried out in two stages: phenotypic for selecting plants resistant to infections, at the initial stage and based on molecular markers for selected plants - at the final stage.

The study of genetic potential of characters required for breeding programs enable the creation of new generations of varieties that meet in genotype and allow fruition of becoming climate conditions (phenomenon of "Climate Change"): frequent temperature fluctuations during wintering including severe temperatures, and adverse conditions during the growing season - long drought altering with critical high temperature.

Along with grapevine breeding objectives mentioned above, and other features required for future varieties are: restoration of architectonic from dormant eyes of multiannual wood and possess the ability to provide and harvest. Likewise, it is desirable to provide a specific architecture of genotypes adapted to mechanization processes, early fruiting etc. Such genotypes will serve as prebreeding components for future variety for a viticulture without support or with simplified support, creating more favorable conditions for using the space, sunlight, and other natural resources

Taking into account space factors of breeder activity - the triangle formed by the demands of society, environmental conditions and the potential of the diversity of genetic resources [17], we can specify a number of features and formulate some goals for breeding, specific to these components (see Figure).

The success of this program lies in a wide variety of genetic resources and of selected material, including pre-breeding.

The prebreeding program designed by us, is oriented to the creation of progeny population with different levels of seedlessness, diverse use, including industrial processing, advanced resistance to unfavorable factors of the environment, and represent the main objective in the use of biodiversity. The first results are a number of pioneering genotypes already homologated, some of them patented, which simultaneously serve original biological material for current and future breeding and prebreeding programs [16, 17]. "Explosion" of table grape varieties, obtained recently in Ukraine and Russia with use as parental components of some new varieties created in Moldova, also evaluated in other geographic areas [12] confirmed the value and potential of these resources as prebreeding material.

Following the evaluation and use of genotypes of diverse origin, we find the presence in gene pool of genetic resources that meet in various combinations of quality attributes, resistance, early maturation - components necessary to further improve assortment and exemplified by the fragment shown in the table.

The necessary components for quality of table grapes - large berry, early ripening, muscat flavor are concentrated in varieties Azur, Greaca, Victoria, Antigona, Doci Nimranga, Xenia, Aromat de Iasi, Avgalia, Muscat timpuriu de Bucuresti, Alma-Atinskii rannii, Kardishah, Muscat iantarnâi, Kavkazkii rannii, Kirghizkii rannii, Doneţkii gemciug, Belgradskii rannii, Kievskii zolotistâi, jemciujnâi Muscat, Muscat Kubanschii, Muscat ţitronâi, Prezentabil, Original, Olimpia etc. Some of genotypes simultaneously possesses more of said characters, and in this respect are highlighted the genotypes from Bulgaria, Hungary of which were selected varieties with big berry, advanced resistance to wintering and *Plasmopara viticola.*

With a high resistance to environmental factors are characterized genotypes Nadejda, Elegy, Jemciug ustoicivâi, Teli muscotaly - I, II-159, Naranksizu group, including the early ripening varieties Kavkazkii rannii, Kardishah, Doneţkii gemciug.

Most of genotypes for wine grape included in the study are characterized by an increased resistance to fungal diseases and frost, allowing winemaking in the first decade

of September – Hibernal, Cristal, Cunleany, Drujba etc.

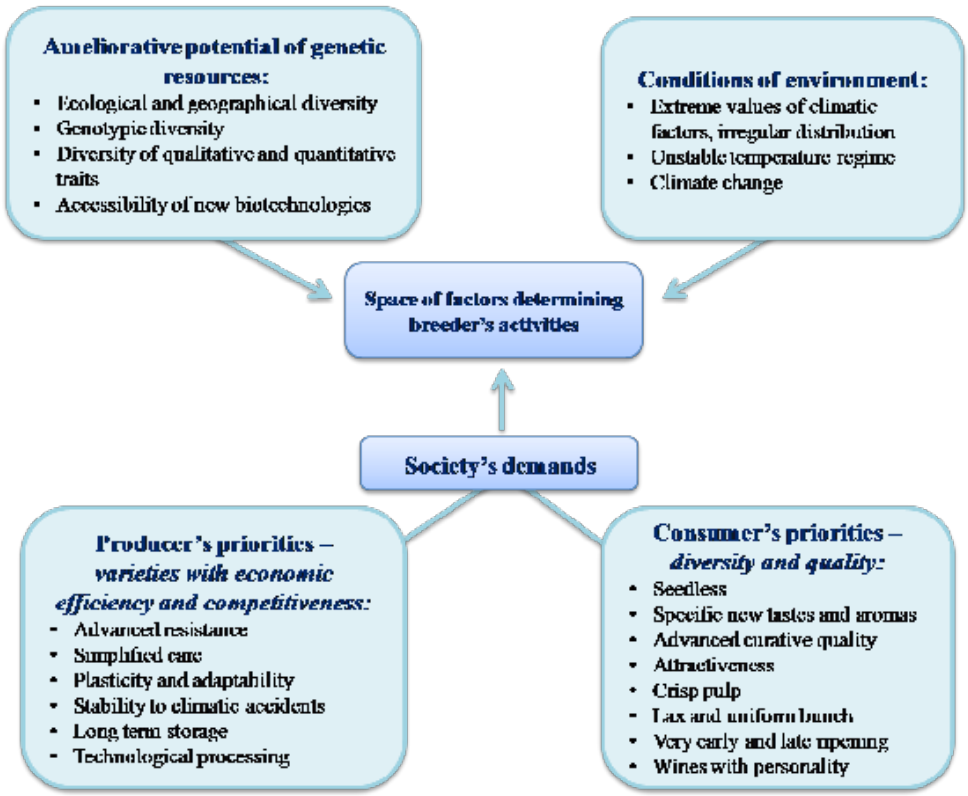

Fig. 1 Space of factors determining breeder's activity

Diversity of seedless genotypes was completed with new sources of seedlessness: Sverhrannii bessemeannâi Magaracia, Kish-mish AZOS, Interlaken, Rusbol, Perlett, Otilia, Himrod – early ripening; Bessemeannâi Melnica, Calina, Kish-mish tairovschii, Mecita, Surpriz – with early-medium ripening; Kish-mish unicalinâi, Romulus, Bessemeannâi Magaraci – medium and Kish-mish Hisrau, Iubilei VIR, Tarnau – with late ripening. Most of them have good adaptability to environmental conditions. Varieties Kis-mis AZOS, Rusbol, Kish-mish unicalinâi, Romulus have a complex resistance to adverse environmental conditions.

We note increased ameliorative potential of new seedless varieties created in Moldova: very early ripening, productivity, high accumulation of sugars, diverse direction of use, including technological processing, advanced or increased resistance to unfavorable factors [16, 17].

Old autochthonous varieties represent a valuable source in creating a sustainable viticulture, because some genotypes are the product of evolution, had high adaptability to abiotic environment, including drought, cold and diseases (Coarnă Neagră, Coarna albă, Grasă de Cotnari, Iordan, Feteasсă Neagră, Rară Neagră and others) [2, 15]. Varieties Frâncuşa, Feteasca alba and Feteasca regala showed a high potential of adaptability to climatic stress in three viticulture zones of Moldova Hills: Cotnari, Iasi and Bujoru Hills [14]. Exploration and cumulation of resistance to abio- and bio- factors in old native varieties (*V. vinifera*) involves the application of the methods of molecular biology. Conjugation of these studies would create a new generation of perspective genetic resources with high adaptability.

Table 1. Diversity of geographical and genetic origin of resources with ameliorative potential (IŞPHTA grapevine gene pool, fragment)

Genotype name	Country of origin	Genetic origin
Antigona	Yugoslavia	Muscat de Hamburg x Galan
Azur	Romania	Coarnă neagră x Cardinal
Avgalia	Russia	Madelein Angevin x Galan
Cabernet severnâi	Russia	(Galan x *V.amurensis*) x polen mixture from V.vinifera x V.amurensis hybrids
Călina	Romania	Braghina x Sultanina
Centennial seedless	USA	Gold x Q25-6
Cristal	Hungary	(Amurskii x Cialoţi Laioş) x Villard blanc
Cunleany	Hungary	(*V.vinifera* x *V.amurensis*) x Caraburnu
Drujba	Bulgaria	Misket Kailishki x (Villard blanc x Muscat de Hamburg)
Favorit	Hungary	Chasselas Victoria x Regina viilor
Hibernal	Germany	Seibel 7053 x Riesling cl.239
Muscat d'Adda	Italy	Muscat de Hamburg (self-pollination)
Muscat Plevenski	Bulgaria	Muscat de Hamburg x Perla de Csaba
Muscat timpuriu de Bucureşti	Romania	Coarnă albă x Regina viilor
Octeabrionoc	Ukraine	Nimrang x Alphonse Lavalle
Original	Ukraine	Ceauş roz x Datier de Saint Vallier
Orion	Germania	Optima (Riesling x Silvaner) x Muler Thurgau) x Villard blanc
Perlette	USA	Regina viilor x Sultanina
Prezentabil	Bulgaria	Pleven x Villard Blanc
Romulus	USA	Ontario x Kiş-mis alb
Schif	Russia	Saperavi severnâi x (Pinot noir x *V.amurensis*)
Xenia	Rumania	Bicane x Muscat de Hamburg

Conclusions

Were established the principles, becomes the basis for formulation and realization of the future breeding programs:

- insignificant coefficient of correlation obtained in multidimensional studies of P and F1 regarding heredity of resistance to some unfavorable factors of environment and of quality of production, including seedlessness and productivity, denote explicit the possibility of free combination in a single genotype of studied characters;
- absence of genetic barrier between hereditary factors determining resistance and quality offers the possibility to create and select a genotypes with advanced resistance and quality.

Practical confirmation of possibility for free combination of studied characters was materialized in revelation, selection and homologation of varieties and elites with advanced resistance to frosts, to downy mildew and high quality: Moldova, Pamiati Negrulea, Urojainai, Struguras et al. The following application in breeding process of formulated principles was completed with the creation, revelation, selection and homologation of seedless varieties and elites with advanced resistance and quality:

Apiren alb, Apiren roz, Apiren negru de Grozesti, Apiren roz extratimpuriu, Apiren roz Basarabean.

Created biological material (varieties, elite, descendants) meets in one genotype: productivity; quality, including seedless; adaptability to stress factors and represents a valuable genetic pool and the potential of these genetic resources may increase and fructify biodiversity of components in prebreeding.

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CHARACTERISTICS OF TOMATO VARIETIES AFTER VALUABLE ECONOMIC FEATURES

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Introduction

Tomatoes represent both an important vegetable food crop and an ideal model for research on cell cultures and tissues *in vitro*. Large and ever-growing needs of agriculture and light industry for quality products in sufficient quantity could not be currently met basing on existing technologies and traditional methods. To resolve this issue the task was set to develop and widely implement new principles and technologies based on molecular and cellular biology achievements [1].

One of the most important issues of improving agricultural crops, including vegetables, was development of new varieties that combined high productivity, increased resistance to unfavourable environmental factors, precocity, big and qualitative fruits.

To accelerate creating such varieties it was necessary to develop and improve technologies and research methods. Traditional breeding programmes applied sexual hybridization of phylogenetically related tomato species. In the synthesis of distant hybrids between crop and spontaneous species there were used various *in vitro* methods and techniques, embryo culture on media culture, embryonic callus culture [2].

The study of the phenomenon for transmitting characteristics from parents to the offspring and changing qualities was a young branch in the biological sciences that might have the right to become "the engine of the contemporary biology".

The data resulting from the tests performed during the study of the various elements and peculiarities of technologies for tomato cultivation enabled the development of fundamental principles and inter-specific hybridization methods qualities, aspects that needed to be taken into account when choosing crossing components and only in this way it was possible to create numerous direct-producer inter-specific hybrids that might combine the satisfactory quality and sufficient crop quantity of species obtained through the *in vitro* development cycle.

Such a manifestation of quantitative characters complicated selection works through the hybridization, as variability induced by environmental factors was maintained in the progeny, whereas improvement success depended on the genetic effect and heritability of the selected character. Success in this area consisted in obtaining distant hybrids with high potential for variability and heritability [3, 4].

Incompatibility of hybrids was the most effective obstacle to changing between species, so it was necessary to develop the methods as a source for genetic basis [5, 6, 7].

In vitro cultures allowed manipulation with the genetic material that was impossible a few decades ago. Thus they opened possibility for obtaining new genotypes to improve the entire gene pool of tomato varieties.

New genotypes required evaluating biological characteristics of complex methods of analysis applied to determine their application value. The results of recent studies [8], demonstrated the necessity of integrating anatomic parameters when studying drought tolerance of tomatoes.

Data on structural genetic variability were practically missed and although the structures were stable over the years and regulated genetically, quantitative structural changes in stressogenic factors activity, including drought, were very informative, especially in tomatoes, because they represented classical biological systems for revealing genetic aspects and adaptive to the factors of the abiotic stress [9].

To achieve the improvement targets it was necessary to know the genetic potential of each variety to highlight and include the improvement genes playing an effective

role in determining the valuable economic characteristics [10].

At the same time there appeared the issue of studying the genetic determinism of adaptive responses of genotypes based on elucidating key genetic factors that determined the variability of quantitative characters depending on adverse environmental conditions [11,12,13].

The issue of removing all causes that might result in the reduction of the agricultural plant crop was of major importance.

One of the main reasons that reduced the crop quantity was the abiotic stress factor that might sometimes decrease production to the level of 70% [14].

A special role among the factors strongly affecting plant yield belonged to droughts. Droughts subject to separate or complex impact of high temperatures and water deficiency was the main limiting factor for agriculture.

Avoiding and minimizing the negative effects of drought required a deep knowledge of resistance mechanisms and genetic control at different stages of plant development [15].

The goal of the current research was to obtain in vitro the new tomato forms with diverse genetic variability, increased productivity, high resistance to drought, cold, diseases and pests.

Material and methods

Investigations were carried out in the laboratory of "Genetics of Plant Resistance" and the experimental fields of the Institute of Genetics, Physiology and Plant Protection of the Moldovan Academy of Sciences (ASM). The following research methods were used: the hybridological method (intra- and inter - specific hybridization); obtaining the intra – and inter - specific hybrids, *in vitro* tissue and cell culture method (sub-culturing, callus induction, embryogenesis and regeneration of seedlings, adapting to *in vivo* conditions; somaclonal variability, histo-anatomic study by classical methods, cyto - and histo-responses chemical processes method description of valuable forms after UPOV requirements.. 30 genotypes (hybrids, lines) which were studied under field conditions served as the object of this study. The research was conducted in two repetitions of 25 plants in each variant including varieties standards (Elvira, Peto 86), evaluation samples were used as a complex of parameters indicated in the UPOV requirements. Histo-anatomic study was performed with the microscope "Micros" (Austria) using classical methods and examining 30 micro-preparations in 4 forms including the check.

Results and discussions

Inter-specific hybridization and biotechnological methods (*in vitro* saving of the immature embryo resulted in developing the tomato collection with valuable quantitative characteristics for improvement.

Inter-specific hybrids of the first generation obtained *in vitro* for long periods under field conditions were identical to the parental forms directly in the morphology of plant shoots, inflorescences, flowers. For them there was characteristic the vigorous bush, creeping with indeterminate growth and strong branching.

Hybrid combinations obtained from crossing with spontaneous forms L.hirsutum, L.chilense and L.peruvianum have exploited yellow - orange, red colours in the F1 generation.

In F2 generation 908 genotypes were grown in the field of which 797 (87,8%) were fertile. The plants were vigorous with many branches, increased leaf number, green leaves were large open and closed and the fruits were red, yellow, yellow-green, green, orange, purple, violet - open, closed purple, violet with anthocyan, lemon yellow, yellow striped with black, yellow with brown stripes of different sizes.. The hybrids exceeded spontaneous paternal forms in fruit size, but did not reach the fruit yield. In F3 generation there were examined 731 plants, of which 660 (90,3%) were found fertile. In F4 generation there were studied 360 hybrid plants. All plants were fertile with

fruits of red colour. Plants' growth and branching were determined and the number of leaves was average Through hybridization and inter-specific biotechnological methods (*in vitro* immature embryo saving) there was created a collection of tomatoes with valuable quantitative characters for improvement..Based on the data obtained were identified new sources of productivity and increased resistance, which were included in the hybridization process and new lines were developed.

Also, the histological and anatomical study there was conducted and basing on screening there were selected bio-morphometric investigations of Iulihirsutian variety and as the check of Elvira, Anatolie, Peto 86 varieties of 30 newly *in vitro* created genotypes (cultivars, lines, hybrids) [16, 17]. The histo-anatomic study of these four genotypes was carried out by using a complex of parameters: the epidermis thickness, cuticle thickness, mesophilic thickness, palisade mesophyll, sponge mesophyll and the correlation between them; number tector of hairs, glandular stomata per unit area; presence or absence of oxalic sandbags and the distribution in mesophilic etc.

Table 1. Histo-anatomical indexes in the foliage apparatus of tomato genotypes

Genotype	Number/ sq. mm					
	Stomata		Glandular hairs		Tector hairs	
	Ep.s.	Ep.i.	Ep.s.	Ep.i.	Ep.s.	Ep.i.
v.Elvira (check)	4,5±0,04	5,6±0,05	1,44±0,01	1,10±0,01	1,1±0,02	1,9±0,02
v. Iulihirsutian	4,7±0,04	5,8±0,03	1,60±0,2	1,30±0,01	1,2±0,01	3,6±0,02
v.Peto 86 check	4,1±0,05	4,6±0,05	1,11±0,01	1,04±0,01	1,2±0,03	1,8±0,02
v.Anatolie	4,1±0,03	4,8±0,04	1,73±0,02	1,20±0,01	2,8±0,03	3,2±0,03

Note: Ep.s. – Moderate, distributed in the middle area of mesophyll between the MP and MS; Ep.i. – lower epidermis;

The results showed that the variety Iulihirsutian - from 0,91 to 0,92 (tab.2) was characterized by maximum photosynthetic activity compared with other analyzed genotypes. Structural peculiarities of these genotypes (increased number of secretory and tector hairs, stomata presence and openness of octiolei, especially on adaxial leaf epidermis, thickness of palisade and sponge mesophyll, and the correlation between them) resulted in histo-anatomical organization of leaf tolerance to drought and a significant contribution in establishing a greater photosynthetic activity of the above-mentioned genotypes.

The analysis of new genotypes showed a correlation between the indexes of productivity, leaf / plant surface and the anatomical ones (glandular hairs density, tectors and the way of distributing the oxalic sandbags). The highest correlation indexes were between productivity and secretory hairs density, productivity value, leaf area and productivity and oxalic sandbags location in the adaxial part, then decrease took place between productivity and the number of leaves tiers, plant height and productivity and the smallest index was between the plant height and leaf area.

Based on the data obtained there were identified new sources of higher productivity and resistance, which were included in the hybridization process and development of new tomato populations (lines and varieties) and submitted to the State Commission for Plant Varieties Testing and two varieties were approved for cultivation in the open field in Moldova.

Iulihirsutian variety was obtained through inter-specific hybridisation of the spontaneous variety *Solanum Lycopersicum hirsutum var glabratum* C.H.Mill. producing small green fruits with glandular hairs and the variety *Solanum Lycopersicum Prizior* with medium-sized red fruits, with undeveloped embryos and ova in in vitro culture. It

was individual selection from F6 generation. It was the variety of determinant growing type, looking normal and healthy, well developed, with 4-6 branches on the main stem rod. The main shoot length was 55-70cm.

Table 2. Histo-anatomical indexes of the foliar apparatus in tomato genotypes

Genotype	MP thickness (mkm) (min;max)	MS thickness (mkm) (min;max)	Ratio MP:MS (min;max)	Oxalic sandbag
v.Elvira (check) early	207-226	275-293	0,75-0,77	Moderate, middle area of mesophyll distributed between the MP and MS
v.Iulihirsutian	249-269	273-291	0,91-0,92	Many primarily in MP, elongated form in MP and spherical when crossing between MP and MS
v.Peto 86 check	202–219	273–290	0,73–0.75	Few, distributed in the MS
v.Anatolie	205-211	260-264	0,78-0,79	Moderate, middle area of mesophyll distributed in the between the MP and MS

Note: MP – mesophyllic palisade; MS - mesophyllic sponge

The length of internodes between I-IV inflorescence was (6, 8, 8, 10) cm. The number of nodes was 8-9. Leaves were ordinary of type 2,27-30cm long and 24-26cm wide. The leaf colour was dark green, embossed. The limb was sectarian and feathery. Leaves position towards the central axis was oriented horizontally. Inflorescence was of intermediate type (tying the 2nd and 3rd). The flower was yellow with a diameter of 2-2,5cm, completely open with 5-6 petals sepals at the level of petals. The inflorescence was simple of 5-7 flowers. The first inflorescence appeared after the 4-5th node, the subsequent - after 1-3rd node. The fruit pedicle was without geniculate articulation. The pedicel length from the point of abscisic to calix (sepals) was 2,5 - 3cm. The fruit curling was absent from the stem. The immature fruits were light green, the ripen ones were dark red. The number of seminal lodges per fruit was 3. The number of seeds per fruit was over 100. The seeds were oval - round with hairs that gave them a silver or gray colour depending on the drawing. The number of seeds per gram ranged from 300 to 370. The fruit weighed 95-100 grams and was round and uniform. The fruits were with fleshy pericarp and inner pulp. In the fruit base there was an average groove. The top of the fruit was flat. The duration of the vegetation period from plant emergence to ripening was 76 days, early variety. The content of dry substances in fruit was 6,24% compared to the check 6,02%, sugar 4,72 to 5,75%, Vitamin C from 45,6 to 57,5 mg /%, titrated acidity 0,35-0,41mg /%. The overall harvest was 59,5- 57,6 t / ha, by 10% higher than that with the standard form, commodity production was 86,9%.

The variety was productive, with high taste qualities, tolerant to drought. It was recommended for growing seedlings, for fresh consumption and industrial processing. It was more resistant to Stolbur than the standard variety Elvira. The variety was productive with high taste qualities and was tolerant to drought. It was recommended for growing seedlings, for fresh consumption and industrial processing. It was more resistant to Stolbur as compared to the standard variety Elvira.

Iulihirsutian variety was created as a result of intra-specific hybridization (Potoc variety x variety Nota). The variety was productive, with high taste qualities, tolerant to drought.. It was recommended for growing seedlings, for fresh consumption and industrial processing. It was more resistant to Stolbur than the standard variety Elvira.

This hybrid was reproduced by in vitro culture as a result of which there were obtained regenerants retarding the improvement process for 2 years. (2 generations). Individual selection was made using F4 generation.

Fig. 1 Variety *Iulihirsutian*

Plants with the determinant type of growth were well developed with 4-6 branches. The main branch length was from 55 to 65 cm. The length of the internodes of the I-IV inflorescence was (4,4, 6,8) cm. The leaves are of common type 1 with the length of 24 to 26 cm. and the width from 16,5 to 19 cm. The leaves' colour was dark green. The limb was sectarian and feathery. The limb was sectarian and feathery. Leaves position towards the central axis was oriented horizontally. The inflorescence of the type (tying the 2nd and 3rd) predominated multi-even. The inflorescence was 8 cm long. The first inflorescence appeared after the 4-5 the node, other appeared after 1-2 nodes. The yellow flower was from 1,7 to 2 cm in diameter, open with 5-6 petals and normal sepals. The number of flowers in the 1st chiorchina was 6.5 and fruits - 5,0 In the 2nd chiorchina there were 6 flowers, 5-fruits, in the 3rd - 6-7 flowers and 6 fruits. The number of flowers The number of fruits on the main stem was 19,5 and flowers 15,6. There were 100 flowers and 85 fruits per plant. The first flower of the inflorescence was missing. The pubescent style was missing. The fruit pedicel lacked the knees. The pedicel length from the point of abscisic up to calix was 1,3-1,5 cm.

Fig. 2 *Anatolie* variety in memory of Prof, Acad. Jacotă

The fruit weighed 65-90 grams, round and slightly elongated, uniform, without wrinkling the stalk. The colour of unripe fruit was green, that of the mature fruit was dark red. The fruit height was 4,84cm, fruit diameter – 4.90 cm. Fruit base there was an average sized groove, the top of the fruit was flat and on some fruits slightly sharp. Pericarp thickness was 0,50 - 0,60cm, the pulp thickness was 3,80-3,95 cm. The number of seminal lodges equalled to 3. The number of seeds per fruit was over 100. The vegetation period lasted for 118 to 125 days, the late variety. Fruits contained the dry matter from 5,3 to 7,0, sugars from 4,9 to 5,1% the ascorbic acid from 35,6 to 59,5%, mg /%. The titrated acidity was 0.32 to 0.35%. The general harvest was from 45,7 to 43,3 t / ha compared to 97% in the check. 94,07% share of fruit as goods. The variety was resistant to low temperatures and and was cultivated through seedlings and seeds.

Conclusions

A collection of tomato with valuable quantitative characters for improvement was created by inter-specific hybridization and biotechnological methods (in vitro saving of the immature embryo)

Iulihirsutian variety and Anatolie variety in memory of Prof, Acad. Jacotă was characterized by maximum photosynthetic activity compared with other analysed genotypes. Structural peculiarities of these genotypes (increased number of secretory and tector hairs, stomata presence and the degree of octiolei openness, especially on adaxial leaf epidermis, palisade and sponge mesophyll thickness and the correlation between them) determined the histo-anatomical organization of leaves with tolerance to drought and a significant contribution to establishing a greater photosynthetic activity of the above-mentioned genotypes.

Productivity indices of the above-mentioned genotypes correlated with the density of glandular hairs, leaf area / plant and oxalic sandbags location in the adaxial part of leaves.

There were identified new sources of higher productivity and resistance that were included in the improvement process and development of new tomato varieties and submitted to the State Commission for Plant Varieties Testing and besides two varieties were approved for cultivation in the open field in the Republic of Moldova.

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GENETIC VARIABILITY OF OIL QUALITY COMPONENTS IN SUNFLOWER AS A FUNCTION OF DEVELOPING HYBRIDS WITH NOVEL OIL

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Introduction

The sunflower is one of the four most important oilseed crops in the world, and the nutritional quality of its edible oil ranks among the best vegetable oils in cultivation. Typically, up to 90% of the fatty acids in conventional sunflower oil are unsaturated, namely oleic (C18:1, 16-19%) and linoleic (C18:2, 68-72%) fatty acids. Palmitic (C16:0, 6%), stearic (C18:0, 5%), and minor amounts of myristic (C14:0), myristoleic (C14:1), palmitoleic (C16:1), arachidic (C20:0), behenic (C22:0), and other fatty acids account for the remaining 10% [30].

Generally speaking, sunflower oil has not been widely used for non-food purposes yet because of its limited supply and higher price as opposed to soybean oil and other industrial oils [13]. The same authors, however, state that, besides the use of sunflower oil in the human diet, this oil can have a wide variety of uses in different industries, such as illumination oil, soaps, cosmetics, pharmaceuticals, emulsifiers, lubricants and greases, drying and semi-drying oils in paints, varnishes and other coatings, plastics and polymers, synthetic rubber manufacture, fat liquors for the leather industry, or substitutes for diesel fuel.

The fatty acids content depends on the genotype well as on environmental factors, especially the location, soil type, precipitation, and most importantly the air temperatures (max. and min.). Oil quality in sunflower (fatty acids and tocopherols) has been subjected to significant genetic changes using induced and spontaneous mutations. The first significant results were obtained by Soldatov (1976), who treated the seeds of the variety VNIIMK 8931 with a solution of dimethylsulfate (DMS), which caused a mutation with a high oleic acid content. Based on this mutation, Soldatov (1976) developed the variety Pervenets, which had an oleic acid content of 75%. Based on this variety, sunflower breeders worldwide developed hybrids and lines with a high oleic acid content (85-92%).

Fernandez-Martinez et al. (2007) point out that a great deal of variation in the levels of all fatty acids and tocopherols in sunflower oil has been developedby searching for natural variations in world collections and to a larger extent by the mutagenesis. When it comes to the most common fatty acids, in the case of high linoleic acid content genetic variability present in the world collections was used, whereas with high levels of oleic, palmitic and stearic acids induced mutations were employed. Also, in the search of sources with a high linoleic acid content wild sunflower species can be used.

Thus, Seiler (1992) determined that a number of wild species of sunflower contain high levels of linoleic acid. Among these, the highest level can be found in *H. porteri* (83.2%). Seiler et al. (2012), on the other hand, recommend certain populations of *H. pumilus* for obtaining high linoleic acid content and some other wild species for obtaining high levels of palmitic and stearic acids.

Using induced mutations, Ivanov et al. (1988), Osorio et al. (1997), Fernandez-Martinez et al. (1997), and Mancha et al. (1994) obtained mutants with a high palmitic acid level. Furthermore, Osorio et al. (1997), Fernandez-Moya et al. (2005), and Mancha et al. (1994) obtained mutants with a high stearic acid content.

Tocopherols are a family of fat soluble antioxidants of great value for both nutri-

tional and technological properties of seed oil. Sunflower seeds mainly contain alpha-tocopherol (95% of the total tocopherols), which has a great vitamin E value but a low *in vitro* activity. Conversely, beta-tocopherol shows more balanced *in vitro* and *in vivo* antioxidant properties [38]. Significant changes in the type and levels of tocopherols were discovered in spontaneous mutations by Demurin (1993) and Demurin et al. (1996).

Velasco and Fernandez-Martinez (2003) studied the variability of tocopherol conent in 952 accessions and in the old Peredovic populations and found two lines that were of interest in this regard - one with a high beta-tocopherol content and the other with high levels gamma-tocopherols. Using induced mutations (a solution of ethyl methane sulfonate (EMS)), Velasco et al. (2004) separated in the M4-5 generation lines with high levels of gamma-, beta-, and delta-tocopherols.

Ayerdi Gotor (2008) pointed out the importance, variability, and possibilities for increasing the levels of tocopherols and phytosterols.

The objective of this paper was to make a review of the genetic variability of oil quality components in sunflower using own results and those of other authors.

Genetic progress achieved in oil quality components in sunflower. Sunflower breeders and geneticists have in the decades past greatly altered the levels of oil quality components, first and foremost the levels of the most common fatty acids and tocopherols using induced and spontaneous mutations as well as using the modern breeding methods (biotechnology at the molecular level). The only case where insufficient progress has been made are phytosterols as minor components that have important impact on oil quality.

The main parameter that determines oil quality is the fatty acid composition and the mutual relationship among fatty acids. The first genetic source with a high oleic acid content was obtained by Soldatov (1976) using induced mutations (treatment of sunflower seeds with a 0.5% solution of dimethyl sulfate). In the M3 generation he selected genotypes with an increased oleic acid content so that by the end of the breeding process a variety called Pervenets was developed that had 78-85% of oleic acid in its oil. The variety Pervenets has been used by breeders worldwide to develop lines and hybrids with a high level of oleic acid. Later on, Andrich et al. (1992) and Ivanov and Ivanov (1992) obtained new mutant lines with a high oleic acid content (Table 1).

Compared with mutant lines with a high oleic acid content, there has been less progress in finding sources of high linoleic acid using mutagenesis. However, sunflower breeders and geneticists have developed a number of lines with a high linoleic acid content using the variability present within populations of cultivated sunflower.

Using mutagenesis lines have been developed that have high levels of palmitic acid. In some instances the level of this acid goes up to 30%, as opposed to 5-6% found in standard sunflower genotypes.

Mutant lines with a high palmitic acid level were developed by Ivanov et al. (1988), Osorio et al. (1997), and Salas et al. (2004). For their part, Fernandez-Martinez et al. (1997) obtained a mutagenic line (CAS-12) that has both a high palmitic acid content and a high oleic levels (Table 1).

Significant results have been achieved in developing mutagenic lines with high levels of stearic acid [22, 10, 11]. The same authors obtained a mutagenic line which is both high stearic and high oleic (Table 1). Three mutagenic lines with medium to high stearic acid content have been developed by Osorio et al. (1995), while Miller and Vick (1999) developed lines that are low in stearic acid (LS-1 and LS-2). These same authors also selected a line (LP-1) which has a low palmitic acid content. When it comes to material low in total saturated fatty acids, Vick et al. (2002) and Seiler (2004) reported obtaining such mutant lines.

In the case of our own breeding program, great attention has been paid to developing lines with different levels of fatty acids and tocopherols.

Table 1. Mutant lines for the most common fatty acids in sunflower oil

Oil type	Lines	References
High oleic	Pervenets G18	Soldatov, 1976 Ivanov and Ivanov, 1992 Andrich et al., 1992
High linoleic	2698-L	Miller and Vick, 2001
High palmitic	275HP CAS-5 CAS-37 HP line	Ivanov et al., 1988 Osorio et al., 1995 Salas et al., 2004 Demurin, 2003
High palmitic-oleic	CAS-12	Fernandez-Martinez et al., 1997
High stearic	CAS-3 CAS-30 CAS-14	Osorio et al., 1995 Fernandez-Moya et al., 2005 Fernandez-Moya et al.,2002
High stearic-oleic	CAS-15	Fernandez-Moya et al., 2005
Low stearic	LS-1 LS-2	Miller and Vick, 1999
Low palmitic	LP-1	Miller and Vick, 1999
Low total saturated acids	RS1 and RS2 NMS 2229	Vick et al., 2002 Seiler, 2004
Medium to high stearic	CAS-8, CAS-4, CAS-3	Osorio et al., 1995

A large number of such lines have been developed. Looking at the variability of fatty acids in our germplasm, the extreme values (min. and max.) in the B lines range as follows: 3.0-11.5% for palmitic acid, 0,6-6,2% for stearic, 20,7->90% for oleic, and 2,6-87,1% (Table 2).

Table 2. Variability of the levels of the main fatty acids in the oil of different sunflower genotypes (%) (Škorić, 2013)

Extreme values	Palmitic	Stearic	Oleic	Linoleic
Min.	3,0	0,6	20.7	2,6
Max.	11,5	6,2	90,2	87,1

High oleic mutant lines are controlled by a certain number of genes. The existance of a single dominant gene, Ol, has been reported by Fick (1984), while Miller et al. (1987) discovered a major gene, Ol, and a gene modifier, Ml. Three complementary genes, Ol1, Ol2, and O3, have been reported by Fernandez-Martinez et al. (1989), whereas Demurin et al. (1996) found the Ol gene with incomplete penetrance determined by genotypic epistatic factors of reversion. Five genes - Ol1, Ol2, Ol3, Ol4, and Ol5 - have been presented by Velasco et al. (2000), while Lacombe et al. (2001) have discovered a high oleic locus, oleHOS, and a suppressor locus, Sup. Also, the mode of inheritance of fatty acid content in mutant lines with high (low) levels of palmitic and stearic acids has been discovered and determined. These findings will not be discussed in detail in the present paper.

Sunflower oils with modified tocopherols. Tocopherols and phytosterols are important minor components of sunflower oil, as they significantly affect oil quality.

Natural tocopherols are present in four isomers: alpha (5, 7, 8-trimethyltocol), beta (5, 8-dimethyltocol), gamma (7, 8-dimethyltocol), and delta (8-methytocol). Standard

sunflower oil predominantly contains alpha-tocopherol (95%) and some beta- (3%) and gamma-tocopherol (2%) as well. The modification of the tocopherol profile through a partial substitution of alpha-tocopherol, with its weak *in vitro* antioxidant action, by other other tocopherol derivatives is an important goal in developing sunflower oil with improved oxidative stability [27].

The discovery by Demurin (1993) that within the sunflower collection there are examples of spontaneous mutations when it comes to tocopherols has made it possible manipulate tocopherol types and contents in the sunflower oil. The first spontaneous mutation occurred in the line LG-15 in which the recessive gene tph1 is present, which in the homozygous form produces 50% alpha- and 50% beta-tocopherol. The second mutation was the recessive gene tph2 found in the line LG-17, which resulted in the expression of 5% alpha- and 95% beta-tocopherol when homozygous. The third was the presence of both tph1 and tph2 in the line LG-24, leading to the manifestation of 8% alpha-, 84% gamma-, and 8% delta-tocopherol in the homozygous state. Lines with different tocopherol types and contents discovered by Demurin (1993) are shown in Table 3.

Using induced mutations, Velasco et al. (2003) obtained mutant lines IAST (over 75% beta-tocopherol) and T589 (up to 48.5% beta-tocopherol). The same authors developed mutant line T2100 with 95% gamma-tocopherol. Velasco et al. (2004) reported developing mutant lines IAST-1 and IAST-540 that contained 95% gamma-tocopherol as well as the line IAST-4, which had over 65% delta-tocopherol (Table 3).

Table 3. Mutant lines high levels of tocopherols (alpha-, beta-, gamma-, and delta-)

Tocopherol	Lines	Percentage of total tocopherol content	References
Alpha	Common sunflower	95	Demurin, 1993
Beta	LG-15 IAST-5 T589	50 >75 30.4-48.5	Demurin, 1993 Velasco et al., 2004 Velasco et al., 2003
Gamma	IAST-1, IAST540 LG-17 T2100 LG-24	>95 95 95 84	Velasco et al., 2004 Demurin, 1993 Velasco et al., 2003 Demurin, 1993
Delta	IAST-4	>65	Velasco et al., 2004

Škorić et al. (2008) have reported part of their resultsbased on which it is possible to develop sunflower hybrids with different oil quality.

To the above results, we should add our own findings on developing male lines (restorers) by crossing the Demurin (1993) lines with high-oil lines. In the segregating generations (F2, F3, and F4), recombinations have occurred with different types and levels of tocopherols. The variability of the newly developed recombinant restorer lines is very great. Thus, in some of the lines the alpha-tocopherol content ranged between 3.7 and 1,282.8 mg/kg oil (ppm). With beta-tocopherol the range was between 0 and 462.8, with gamma- between 0 and 751.8, and with delta- between 0 and 329.8. Looking at the total tocopherol content, great differences were present. The minimum level was only 86.7 in one of the lines, while the maximum level recorded in one of the newly developed lines was as high as 1,970 mg/kg oil (ppm) (Table 4).

In order that the concrete variability of tocopherols in the inbred lines can be seen, we have selected a certain smaller number of the restorer lines for analysis (Table 5). Thus, for example, the line RHA-S-1 had equal amounts of alpha- and beta-tocopherols. Also of interest is the tocopherol composition in the line RHA-S-58, where equal levels of alpha-, beta-, gamma-, and delta-tocopherols (25% each) were recorded. The

line RHA-S-59 is interesting, as it has only gamma-tocopherol. From the breeder's point of view, another interesting line is RHA-S-132, which had 80% gamma- and 20% delta-tocopherol (Table 5).

Table 4. Variability of thocopherol levels in the oil of different sunflower genotypes mg/kg oil (ppm)

Extreme values	Alpha	Beta	Gamma	Delta	Total
Min.	3,7	0	0	0	86,7
Max.	1 282,8	462,8	751,8	329,8	1,970

From the point of view of breeding, also of interest is a group of restorer lines with a high oleic acid content and very low levels of linoleic acid, predominant levels of alpha-tocopherol, mostly a high oil content, and certain tolerance of Phomopsis (Table 6). Among this group of restorers, high oleic acid levels are found in Rus-rf-ol-54, Rus-rf-ol-77, Rus-rf-ol-207, Rus-rf-ol-209, Rus-rf-ol-222, and Rus-rf-ol-242 (Table 6). When it comes to low linoleic acid content, there are two lines with as little as 1% of linoleic acid and six with 2%. Five lines have a very high seed oil content, ranging from 54.7 to 59.13%. Ten of the restorers have less than 50% of oil in their seeds. In any case, all these restorer lines deserve to be crossed with select female lines and tested for their agronomic value in terms of combining abilities (GCA and SCA).

Table 5. Restorer lines with different tocopherol levels (%) (Škorić, 2013)

Line	Alpha	Beta	Gamma	Delta
RHA-S-1	50	50	0	0
RHA-S-9	90	10	0	0
RHA-S-13	20	80	0	0
RHA-S-23	30	Tr.	60	10
RHA-S-25	40	10	40	10
RHA-S-53	60	20	20	0
RHA-S-58	25	25	25	25
RHA-S-59	0	0	100	0
RHA-S-132	0	0	80	20
RHA-S-134	80	20	0	0

Molecular research of components of sunflower oil quality. In the last 10-15 years, major advances have been made in sunflower research at the molecular level aimed at testing oil quality in this crop. The molecular basis of a modified fatty acid content in the seed oil of sunflower has been studied through a QTL and a candidate gene approach by Fernandez-Martinez et al. (2004). According to these authors, a number of sunflower genes coding for enzymes involved in the fatty biosynthetic pathway in seeds have been cloned and their polymorphism studied [28].

As Škorić (2012) collected and analyzed in detail the achievements in the field of molecular research of fatty acids and tocopherols, it is not necessary to repeat these findings in the present paper. Molecular research of sunflower oil quality components should be the subject of a voluminous review paper. What is important to note is the fact that molecular research in this field is taking place on a daily basis and that significant results have been achieved that can help a lot sunflower breeders in their develop-

ment of sunflower hybrids with different oil quality.

Table 6. High-oleic Rf lines with predominant alpha-tocopherol content

No.	Line	Fatty acid composition (%)		Tocopherols (%)		Oil content (%)	Phomopsis (0-5 scale)
		Oleic	Linoleic	Alpha	Beta		
1.	Rus-rf-ol-27	85	4	100	-	47.68	4
2.	Rus-rf-ol-38	89	4	100	-	54.70	4
3.	Rus-rf-ol-39	87	5	100	-	44.87	4
4.	Rus-rf-ol-54	90	2	100	-	51.71	3
5.	Rus-rf-ol-67	89	2	100	-	49.41	4
6.	Rus-rf-ol-68	87	4	100	-	47.88	4
7.	Rus-rf-ol-70	85	5	100	-	43.71	4
8.	Rus-rf-ol-77	91	1	100	-	58.39	4
9.	Rus-rf-ol-78	88	2	100	-	56.00	4
10.	Rus-rf-ol-80	87	4	100	-	45.98	4
11.	Rus-rf-ol-91	88	3	100	-	55.26	3
12.	Rus-rf-ol-94	86	3	100	-	43.55	4
13.	Rus-rf-ol-134	85	6	100	-	43.46	4
14.	Rus-rf-ol-140	84	4	100	-	46.43	4
15.	Rus-rf-ol-142	88	2	100	-	59.13	3
16.	Rus-rf-ol-154	84	4	100	-	53.90	3
17.	Rus-rf-ol-206	88	3	100	-	42.02	4
18.	Rus-rf-ol-207	90	2	100	-	53.46	4
19.	Rus-rf-ol-209	90	1	100	-	52.83	4
20.	Rus-rf-ol-222	90	2	100	-	52.57	4
21.	Rus-rf-ol-242	91	3	100	-	52.57	4

The possibility of developing sunflower hybrids with novel oils. Sunflower breeders and geneticists have developed a large number of inbred lines with different composition of the main fatty acids (oleic, linoleic, palmitic, and stearic) using induced and spontaneous mutations. Of special value are the mutant lines with high levels of the above fatty acids. Also, sunflower geneticists have developed inbred lines with high levels of beta-, gamma, and delta-tocopherols.

The question is why it is important to have all the above sources with different levels of fatty acids and tocopherols. The genes that have been discovered are of great practical importance in developing sunflower hybrids with different oil quality. Thus, Garces et al. (2009) have concluded that the aforementioned variability will enable the development of hybrids with novel oils that can successfully be used in homesteads for preparing high quality foods. Also, it will open up new possibilities in the food industry as well as in non-food technologies (biolubricants and biodiesel).

The new variability of fatty acid and tocopherol contents enables the development of sunflower hybrids with significantly higher oil stability at increased temperatures [30]. Karlović et al. (1997) studied our hybrids using the Rancimat test at 100ºC and reported that the standard oil (linoleic type) was stable for 8 hours, oleic type for 36 hours, and oleic type with a high gamma tocopherol content for over 150 hours. These

findings speak for themselves when it comes to the importance of developing hybrids with a high oleic acid content and high levels of beta- and gamma-tocopherols.

Spanish results are an even better illustration of the importance of developing hybrids with different levels of fatty acids and tocopherols. Using the Rancimat test at 180ºC, Marmesat et al. (2008) state that a sunflower line that has high levels of oleic and palmitic acids and a high level of gamma-tocopherol (HDHPSD-gamma) also has a high degree of stability at frying temperatures. The same authors state that a mixture of HDHPSD-alpha + HOHPSO-gamma tocopherols is an outstanding combination of oils for use in the food industry that ensures not only oxidative stability but also provides the necessary amount of vitamin E.

Besides tocopherols, phytosterols are another minor component of sunflower oil. From the point of view phytosterol content and genetics, the most significant results have been achieved by Ayerdi Gotor (2008). This author analyzed in detail the levels, variability, and types of phytosterols in the oils of sunflower, rapeseed, maize, olive, and palm. Based on the results of that study, the most common component in the oil are beta-sitosterols. The levels of campasterol and stigmasterol, on the other hand, are significantly lower. The same author concludes that beta-sitosterol and gamma-tocopherol are important antioxidants. Ayerdi Gotor (2008) also reports that the stability of phytosterols is signicantly higher than that of tocopherols. Another important contribution by Ayerdi Gotor (2008) is the study of correlations among tocopherols, phytosterols, and fatty acids. She concludes that there are significant positive correlations among the types of tocopherols and phytosterols as well as between tocopherols and phytosterols. Another important finding is the presence of significant positive correlations between oleic acid and alpha-, beta-, gamma-, and delta-tocopherols.

Based on the variability of fatty acids and tocopherols achieved by using spontaneous and induced mutations, it is possible to develop sunflower hybrids with different oil quality (novel oils). Thus, Fernandez-Martinez et al. (2007) conclude that is desirable to work on five new oil types, namely high oleic (suitable for salads and cooking), high stearic-oleic (for the production of healthier margarines) high palmitic-oleic (for high performance frying operations without hydrogenation), high and stable linoleic (applications in the coating industry - a novel therapeutic nutrient), and mid and high oleic combined of *in vitro* antioxidant (usable for biodiesel, deep frying, and biodegradable lubricants). Škorić et al. (2008), for their part, mention at least seven new types of oil in addition to the standard one. Apart from those mentioned by Fernandez-Martinez et al. (2007), these are high oleic (Ol + tph1 genes), high oleic (Ol + tph2 genes), and high oleic (Ol + tph1 tph2 genes). Škorić et al. (2008) conclude that in addition to the aforementioned types of oil it is possible to develop at least 15 other types of hybrid sunflower with altered oil quality, which presents a range of possibilities for both food and non-food uses of sunflower oil. The conclusion of Fernandez-Martinez et al. (2007) is that the combination of several quality traits in a single phenotype will enable the tailoring of specialty oils for specific uses in the food and non-food industries. The novel fatty acids and tocopherol traits are in all cases governed by a reduced number of genes and can be easily managed in breeding programs aimed at developing cultivars incorporating these traits. Škorić et al. (2008) conclude that to define the parameters of the future novel sunflower oils, geneticists, breeders, physiologists, and representatives of the food industry should join forces with medical scientists, nutritionists, and specialists from other fields in a multidisciplinary effort to find the answers to a multitude of questions on future uses of sunflower oil. The sunflower kernel and its novel oil can be used as the basis for a wide range of high-quality final products.

Conclusions

Based on our own results and those of other authors, the following conclusions can be made:

- The oil of common sunflower genotypes ranks among the best vegetable oils in

the world;

- Using induced and spontaneous mutations, mutant sunflower lines have been developed that have high levels of the most common fatty acids (oleic, linoleic, palmitic, and stearic) in their oil. The number of genes for these mutant lines and the mode of inheritance in the F1 and F2 generations have been determined as well. Also, their genetics at the molecular level has been studied to a large extent;
- Induced mutations have been used to develop mutant lines with high levels of beta-, gamma-, and delta-tocopherols. Through spontaneous mutations the genes tph1, tph2, and tph1tph2 have been discovered, which enables great breeding interventions in the genetic manipulation for tocopherol type and content;
- The great genetic variability that has been achieved in the cases of fatty acids and tocopherols makes it possible to develop hybrids with different oil quality (novel oils) for use in both the food and non-food industries;
- Sunflower breeders and geneticists have the task of making the necessary changes of phytosterols in addition to fatty acids and tocopherols, which will increase the possibilities for developing even more diverse sunflower hybrids;
- Some of the novel sunflower oils can be used for medicinal purposes as prevention against cardiovascular disease.

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THE GENETIC CHARACTERISTIC OF YOUNG-HEIFERS OF MOLDOVAN BLACK-MOTLEY TYPE THE POSTERITY OF DIFFERENT BULLS

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Introduction

Using only traditional methods of selection are not enough for a successful selection-blood-stock of work in present time. The selection on productive indication is impossible without studying the genophond of investigated species anal estimation of intraspecific genetical change. One of the directions of using given immunogenetical investigations in selection appears the study of genetical structure of selective population, herd, and lines on markers genes. By investigation of many scientists is demonstrated the reliability of attraction of genetical markers of blood in estimation of the cattle's genotype [1, 2, 4, 8, 9, 10, 11].

The genetical markers of blood's groups appear to be a convenient model for realizing of control proceeding of the selection processes in herds and the estimation of level of selection work on consolidation of herds, lines, blood groups, consolidation of desired indications in the animal's group [14, 16].

The using of groups of bloods opens a wide possibility for detail study of microevolution process in species. They allow to realize a constant control of genophond's changes, to expose the regularity of its change in accordance with direction and concrete particularity of selection-race work and on this base to work out methods of correction of selection, to high its effect and rational using of the race's genophond.

During a long period of time by us is negotiated a constant control of changes in the genophond of Moldovan black-motley type in the herd TES "Maximovca". As for example earlier we informed about the analysis of immunogenetical processes that happened in the herd TES "Maximovca" [18], the monitoring of allelophond [5], immunogenetical structure of bulls - cows and their descendants [6], and also the evaluation of genetical structure of used lines of bulls [7] in given herd.

The aim of this researches was to give a genetical evaluation of joung-heifers of moldovan black-motley type – descendants of different bulls date of birth 2003-2013.

Material and methods

The object of study served joung-heifers of moldovan black-motley type – descendants of 11 bulls: Abiturint 1861 (n=28) and Captain 2354 (n=16) date of birth 2003-200; Abhazian 835 (n=15) and Svet 732 (n=39) - date of birth 2004-2005; Dichii 788 (n=40) - date of birth 2005-2006; Academic 767 (n=13) and Chiparus 79 (n=11) - date of birth 2007-2008; Sinior 7415 (n=67) - date of birth 2008-2009; Meteor 376 (n=15) - date of birth 2011; Caras 656 (n=20) - date of birth 2013 and Vladika 266 (n=11) - date of birth 2007, in all 275 heads. The base for holding investagion served the expertise of materials the reliable origin of the young race date of birth 2003-2013.

The groups of blood were determited by standard sulphur tests with utilization 49 monospecific serum from 9 genetical system. Genotypes of groups of blood and also reliance of animal's origin were exposed by family-genetical analysis according to Miercurev E. [13]. The indicators of genetical distances were subtracted by the formula Serebrovski A.S. [15] and Nei M. [3] on the base of the distribution analysis of the frequencies of the antigens of bloods groups.

Results and discussion

According to the results of hold investigations were exposed the differences in the antigene spectrum at descendants of 11 bulls (table 1). According to AEA locus antigen A2 is exposed at descendants of all bulls with different frequency meeting, antigene Z' is exposed only among descendants of bulls Caras 656, Svet 732, Senior 7415 and Vladika 266 with frequency meeting from 0,0150 (Sinior) till 0,0909 (Vladika).

Table 1. The frequency meeting of antigens at descendants of different bulls

Nr.	Antigens	Bulls										
		Abiturient 1861	Capitain 2354	Abkhazian 835	Svet 732	Dichii 788	Chippers 79	Academic 767	Sinior 7415	Meteor 376	Caras 656	Vladika 266
1	2	3	4	5	6	7	8	9	10	11	12	13
1	A_2	0,78	0,87	0,60	0,77	0,77	0,64	0,92	0,36	0,40	0,70	0,54
2	Z'	0	0	0	0,05	0	0	0	0,01	0	0,05	0,09
3	B_2	0,18	0,68	0,07	0,28	0,62	0,18	0,77	0,59	0,07	0,25	0,36
4	G_2	0,39	0,18	0,27	0,79	0,30	0,63	0,61	0,52	0,93	0,95	0,36
5	G_3	0,43	0,31	0,20	0,45	0,10	0,09	0,15	0	0	0	0
6	I_1	0,07	0,18	0,27	0,05	0,07	0	0	0,10	0,27	0,35	0
7	I_2	0,25	0,25	0,13	0,13	0,20	0,18	0	0,18	0,13	0,35	0,36
8	O_2	0,86	0,87	0,13	0,51	0,82	0,45	0,92	0,64	0,07	0,65	0,18
9	P_1	0	0	0	0	0,05	0,09	0	0,01	0,07	0,05	0
10	P_2	0,03	0,06	0,07	0	0,02	0	0	0	0	0,05	0
11	Q	0	0,18	0	0,05	0,05	0	0	0,03	0	0	0
12	T_1	0	0	0,07	0	0	0,09	0	0	0	0,05	0,18
13	T_2	0	0	0,07	0	0	0,09	0	0	0	0,10	0,18
14	Y_2	0,75	0,56	1,0	0,77	0,32	0,81	0,61	0,70	1,0	0,70	0,82
15	B'	0,32	0,56	0,07	0,05	0,07	0	0,15	0,06	0	0,10	0
16	D'	0,25	0	0,27	0,23	0,17	0,27	0	0,10	0,20	0,10	0,73
17	E'_2	0,25	0,31	0,67	0,64	0,27	0,63	0,46	0,48	0,93	0,75	0,82
18	G'	0,18	0	0	0,15	0,05	0,18	0,08	0,11	0,27	0,15	0,09
19	I'	0	0,31	0,20	0,10	0,05	0,18	0	0,06	0,20	0,10	0,36
20	J'_2	0	0	0	0,02	0	0	0	0,01	0	0,05	0
21	K'	0	0	0	0	0	0	0	0,01	0	0,05	0,09
22	O'	0,18	0	0,0	0,13	0,05	0,09	0	0,13	0,26	0,20	0,82
23	P'	0	0	0,07	0	0	0	0	0	0,06	0	0,18
24	Q'	0,12	0,31	0,53	0,33	0,27	0,36	0,31	0,52	0,93	0,75	0,54
25	Y'	0	0,12	0,13	0	0,15	0,27	0,15	0,03	0	0,05	0
26	B''	0	0,25	0	0,02	0	0	0	0	0	0	0
27	G''	0	0	0	0,02	0,32	0,36	0,15	0,09	0,27	0,05	0,09

28	C_1	0,46	0,37	0,13	0,18	0,72	0,09	0,38	0,45	0,40	0,90	0,54
29	C_2	0,53	0,56	0,27	0,25	0,95	0,18	0,46	0,57	0,40	1,0	0,73
30	E	0,82	0,87	0,47	0,23	0,57	0,81	0,61	0,70	0,80	0,80	0,64
31	R_1	0,03	0,37	0,27	0,18	0,02	0	0	0,01	0	0	0,09
32	R_2	0,28	0,12	0,33	0,26	0,50	0,27	0,15	0,10	0,13	0,40	0,27
33	W	0,68	0,62	0,53	0,36	0,30	0,18	0,31	0,15	0,73	0,65	0,54
1	2	3	4	5	6	7	8	9	10	11	12	13
34	X_1	0,11	0	0,07	0,02	0,05	0,09	0	0,03	0	0	0,18
35	X_2	0,68	0,68	0,93	0,67	0,55	0,82	0,53	0,44	0,33	0,10	0,45
36	C'	0,07	0,06	0	0,05	0,05	0	0	0,07	0	0,35	0,18
37	L'	0,14	0,50	0,27	0,18	0,05	0,18	0,15	0,01	0	0	0,09
38	F	0,93	1,0	0,80	1,0	1,0	0,91	1,0	1,0	1,0	0,85	1,0
39	V	0,21	0,12	0,60	0,18	0,02	0,45	0,15	0,18	0,67	0	0
40	J_2	0	0,18	0,27	0,07	0,20	0,18	0,08	0,27	0,20	0,60	0,64
41	L	0,25	0,37	0,47	0,59	0,62	0,09	0,23	0,24	0,73	0,55	0,09
42	M	0,03	0	0	0	0	0,09	0,08	0,06	0,07	0,05	0
43	S'	0	0,06	0,07	0,13	0,10	0	0,08	0,07	0	0,05	0,18
44	U	0	0,06	0,07	0	0,05	0	0	0,03	0,27	0,05	0,09
45	H'	0,50	0,87	0,53	0,97	1,0	0,74	0,77	0,95	0,73	0,80	0,82
46	U'	0	1,0	0	0,03	0,02	0	0	0,01	0,07	0	0,09
47	H"	0	0,12	0,7	0	0,07	0	0	0,04	0	0	0
48	U"	0	0,06	0	0,02	0,10	0	0	0,01	0	0	0
49	Z	0,07	0,37	0,13	0,28	0,47	0,09	0,23	0,19	0,13	0,25	0,64

In accordance to AEB locus at descendants of all bulls from 25 studied antigens were found out only 6. At the most quantity of antigens were not found at the descendants of bull Academic 767 – 14 (I_1, I_2, P_1, P_2, Q, T_1, T_2, D', I', J'_2, K', O', P', B"), at descendants of bull Dichii 788 on given locus were not exposed the least quantity of 6 antigenes - T_1, T_2, J'_2, K', P', B".

Among the exposed of 6 antigenes (B_2, G_2, O_2, Y_2, E'_2, Q') at descendants of all bulls is noticed a high frequency meeting of antigene Y_2, which varied from 0,3250 (Dichii 788) till 1,0 (Abkhazian 835, Meteor 376).

The antigene B" is exposed only at the descendants of bulls Capitain 2354 and Svet 732, that is affirmed by execution earlier by estimation of genetical structure used lines of bulls in the herd TES "Maximovca" and characteristic for descendants of bulls lines Vis Beak Aidial and Pavni Farm Arlinda Cif [8]. A low frequency meeting of antigens P2, Q, T1, K', J'2, P', peculiar in baze for the all analysed descendants.

According to AEC locus from 10 studied antigens 2 were absent (antigen's R1 and C′) at descendants of bulls Chiparus 79, Academic 767 and Meteor 376, antigen L′ is not exposed at descendants of bulls Meteor and Caras. A high frequency meeting of bearer of antigens E and W is exposed at descendants of al bulls.

According to AEF locus are exposed both antigen's F-V. A high frequency meeting antigenes F is peculiar to descendants of bulls Capitain 2354, Svet 732, Dichii 788, Academic 767, Sinior 7415, Meteor 376 and Vladika 266 - 1,0, at descendants of bulls Abhazian 835 the frequency constituted 0,80. The frequency meeting of antigene V

varied from 0 - descendants of bull Caras till 0,67 – descendants Meteor 376.

According to AEJ locus the antigene J2 is not exposed only at descendants Abiturint 1861, and at the rest of descendants the frequency meeting varied from 0,07 (the descendants of the bulls Svet 732) till 0,64 (the descendants of bull Vladika 266).

According to AEL locus the antigene L is exposed at all analyzed bulls, the frequency meeting varies from 0,09 (the descendants Chiparus 79 and Vladika 266) till 0,73 (the descendants of Meteor 376).

According to AEM locus the antigene M is not exposed at descendants of 5 bulls, at the rest descendants the frequency meeting was low and compied 0,03 - 0,09 – descendants of Abiturient 1861 and Chiparus 79 correspondingly.

According to AES locus from 6 studied antigenes a high frequency meeting is exposed at the descendants of all bulls – bearer of antigene H′, antigenes U', H", U" were absent at many descendants.

According to AEZ locus a low frequency meting of antigene Z possessed the descendants of bull Abiturient 1861 (0,07), but the bull Dichii 788 and Vladika 266 proper a higher frequency meeting – 0,47 and 0,64 correspondingly.

The estimation of saturation of antigene factors estimated at descendants showed, that it is lower at the descendants of bull Academic 767 and Sinior 7415 and makes up accordingly 19,3 and 21,2%, but at descendants Caras 656 and Captain 2354 higher – 28,6 and 29,6%, figure1.

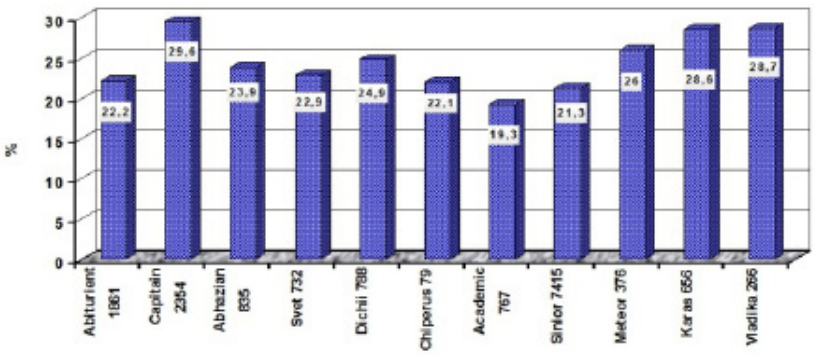

Fig. 1. The saturation of antigens factors of descendants of different bulls.

In the examined period of time were studied the relationships formed between descendants of different bulls (phylogenesis in space and in time), for what was used the method of unsuspended in pairs cluster.

From the results of the analysis of table 2 it is seen, that the least genetical distance is exposed between the descendants of bulls Academic 767 and Sinior 7415 (d = 0,13), and the biggest – between the descendants of bulls Abhazian 835 and Dichii 788 – (d = 0,31), that is confirmed by the picture of dendrogram, figure 2.

As it is seen, the descendants of the estimated bulls make up 4 separate clusters. In the first cluster enter the descendants of bulls Sinior – Academic, the second cluster Abiturient – Captain, the third cluster Abkhazian – Chiparus, the fourth cluster Meteor – Caras. More over the line belonging of bull in the first three clusters is various, only in the fourth cluster the both bulls (Caras, Meteor) belong to the line Vis Back Aidial. The bull Sinior (I cluster), Capitain (II cluster) the same belong to the line Vis Back Aidial. What touches upon descendants combined into the III cluster, then it should be marked, that the bull Abkhazian belongs to the line Montvik Chieftain, but Chiparus to line Pavni Farm Arlind Cif.

How to explain, that the descendants of bulls a various lines turned to be genetical close between breeding stock them and even make up separate clusters?

First, before we carried out a detailed analysis of the use of bulls of various lines in the herd TES "Maximovca" in the period from 1985 till 2005 jears [7].

Table 2. The genetical distances (d) and genetical resemblance (r) between the descendants of bulls used in the herd TES "Maximovca"

	Code	1	2	3	4	5	6	7	8	9	10	11
Academic 767	1	-	0,83	0,72	0,75	0,76	0,83	0,83	0,84	0,84	**0,87**	0,72
Chiparus 79	2	0,17	-	0,78	0,85	0,75	0,83	0,76	0,81	0,76	0,84	0,75
Meteor 376	3	0,27	0,21	-	0,77	0,78	0,77	0,69	0,74	0,70	0,78	0,71
Abhazian 835	4	0,24	0,15	0,23	-	0,69	0,79	0,77	0,78	**0,69**	0,77	0,75
Caras 656	5	0,23	0,25	0,22	0,30	-	0,76	0,73	0,77	0,78	0,80	0,78
Svet 732	6	0,17	0,16	0,22	0,20	0,24	-	0,78	0,81	0,78	0,82	0,75
Captain 2354	7	0,16	0,23	0,31	0,23	0,27	0,22	-	0,83	0,81	0,81	0,72
Abiturient 1861	8	0,16	0,18	0,25	0,22	0,23	0,18	0,17	-	0,80	0,82	0,73
Dichii 788	9	0,16	0,24	0,30	**0,31**	0,21	0,22	0,19	0,20	-	0,84	0,74
Sinior 7415	10	**0,13**	0,16	0,22	0,23	0,19	0,18	0,19	0,17	0,16	-	0,78
Vladika 266	11	0,27	0,24	0,29	0,25	0,22	0,25	0,28	0,27	0,26	0,21	-

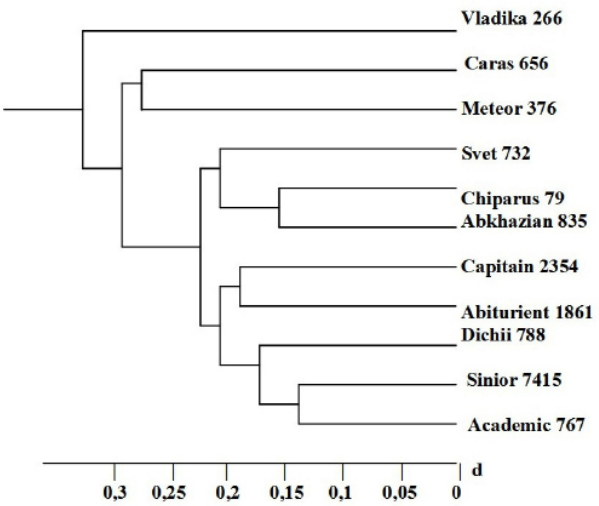

Fig. 2. Microphilogeny relationship between descendants of different bulls in the herd TES "Maximovca".

It was established, that the line of Vis Back Aidial was much more retired in accordance to immunogenetical indicators from all other lines.

In further, beginning with 2005, the consolidation over breeding stock of Moldovan black-motley type were held by bulls of the line Vis Back Aidial, belonged to various branches, as it was foreseen the laying and breeding out the new line with the aim of consolidation of desirable sign (abundant lactic, high percentage of body fat).

Secondly, every two years, he drafted a plan for the consolidation of breeding the

various bulls for breeding stock. Therefore, descendants obtained from the use of the following bulls, inherited in it's of genophond's, both maternal genes and paternal. Dulls Academic (line Rozeiph Sitation) and Chiparus (line Pavni Farm Arlind Cif) were used in individual selection by markers of alleles for the further obtaining of homozygotes calves.

In that way, from the results of the analysis microphilogeny between the descendants of bulls, that belong to different lines (Vis Back Aidial, Rozeiph Sitation, Pavni Farm Arlind Cif and Montvic Chiftane), it follows that in further selection-blood-stock of work with broodstock of moldovan black-motley type in the herd TES "Maximovca" the utilization of bulls of line Vis Back Aidial at a certain time is desirable to stop as the reserve of genetical changes of this population is not isufficient for to further breeding.

Conclusions

The greatest quantity of antigenes is not exposed on AEB locus at the descedants of bulls Academic 767 – 14 (I1, I2, P1, P2, Q, T1, T2, D', I', J'2, K', O', P', B"), Abiturient 1861 – 11 (P1, Q, T1, T2, I', J'2, K', P', Y′, B", G″) and Capitain 2354 – 10 (P1, T1, T2, D', G', J'2, K', O', P', G").

For the increase of genetic change of the population of moldovan black-motley type in the herd TES „Maximovca" is necessary in the plans og selection to use bulls of that lines in wich the genetical distance is the greatest wiht the bulls of line Vis Back Aidial.

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IMPROVEMENT OF TSIGAIE SHEEP BREED OF MOLDOVAN TYPE

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Introduction

Tsigaie sheep's contribution to the economy of the Republic of Moldova is based on a wide range of products required by the local population which are obtained from these animals.

These can include fattened sheep meat, lamb meat and sheep's milk from which cottage cheese and albuminic cottage cheese are made, as well as valuable raw materials for light industry – semi-fine wool and sheepskin.

Breeding of Tsigaie breed of sheep in the Republic of Moldova takes its roots in the southern part of Bessarabia, known as Budjac steppe, the sheep being brought here as far as the 18th century by Bulgarian settlers.

According to the external appearance and morph-productive type (wool-milk), original Tsigaie sheep breed (indigenous type) accounted for some belated animals with relatively reduced production indices.

The average body weight of breeding Rams constituted 65-70 kg, while sheep's – 40-42 kg with a gross production of wool of 3,9-4,2 kg and 2,2-2,3 kg accordingly. Young sheep aged 12-14 months had average mass of 30 kg with a yield of 3,7 kg of wool and length of wool fibers of 8,4 cm with wool quality indices 56-58 [3, 4, 5, 7].

Attempts in the '50 of the last century to improve the breed of sheep Tsigaie in Moldova with the Merino breed, have given negative results. For improving local sheep of Tsigaie type a program has been drawn up by the State which relied on the crossing of the Tsigaie sheep of wool-milk type with Rams-breeders of wool-meat type from „Cernomorskii" farm from Crimea and the Rams specializing in meat-wool from «Roza Luxembourg» farm, Donetsk region (Ukraine). In the period from 1962 until the year 2000 as a result of the work of selection (5 stages) there was created a new type of Tsigaie sheep for wool-meat-milk, and in 2005 it was approved by the State Commission under the name «Elite Type of sheep (Ovis aries L.) Tsigaie Moldovenesc».

The work was completed by growing „in and of itself" of sheep with genotype that includes 10,8% of the blood of indigenous Tsigaie for wool-milk; 48,7% Tsigaie for wool-meat and 40,5% Tsigaie for meat-wool [6].

Tsigaie Moldovenesc sheep possess advantages over standard requirements of Tsigaie breed according to the production of wool by 46,8% on average, body weight by 32,0% and milk production by 57%.

Material and method

The research object were Tsigaie sheep of Moldovan type (Tsigaie Moldovenesc) for wool-meat-and-milk, different groups of sex and age from breeding farm of the Agricultural production Cooperative „Elita-Alexanderfeld", Cahul district.

Testing of sheep in order to continuously maintain and improve the productive qualities of pure breed, was carried out according to the methods and techniques of „Instruction for breeding Tsigaie breed of sheep with elements of selection" [1] and „Guidelines for the evaluation of sheep' products in the Republic of Moldova" [2].

Formation of nuclei of selection in order to obtain the required production capacity with progeny was carried out using the method of independent selective limits.

The main stages of the research and evidence taken into study were:

- record of sheep giving birth calculation of prolificacy;

- individual testing of each youth at weaning (3-3,5 months) by the provisional evaluation after development of body weight and length of woolen strand, with its subsequent ranking according to the scoring scale;
- complete evaluation of youth from the previous year at the age of 12-14 months (outward appearance and constitutional type, body weight, basic characteristics of woolen sheepskin, including quality (fineness) of wool, strand's length, quantity and color of wool oil, curling, homogeneity and expanding of wool, wool production at first cut);
- evaluation of the wool production related to reproductive herd (mechanized shearing with individual evidence of sheepskin through the weighting with precision of 0,1 kg);
- evaluation of the body weight of sheep through individual weighing of all of the flock in the autumn, and classing the flock after this character;
- creating of the selection nuclei and calculation of the difference and intensity of selection by the following groups: breeding Rams, sheep-ewe, ewe-lambs and male lambs of 15-18 months.

Statistical processing of numerical material obtained in the research has been realized according to the existing programs, according to the methods of N. Plohinschii [8] and application of special programmes.

Results and discussion

Prolificacy of Tsigaie breed sheep of Moldovan type as required is minimum 100-120%.

From the results obtained in the research (tab. 1) for the years 2011-2014 shows us that the prolificacy in agricultural production Cooperative „Elite-Alexanderfeld" amounted to 106,4 percent.

At the same time we can mention that 2013 was the most prolific year, as from the 1276 sheep were born 1430 heads of lambs with the percentage of fertility of females of 112,3 %.

Also of lambs obtained in the reference year, 21,5 percent or 308 heads came from twin births.

On the basis of «The recommendations with respect to technology of sheep products in the Republic of Moldova» the evaluation of male lambs and ewe lambs has been carried out at the age of weaning (3,0-3,5 months) depending on live weight and length of fibers, assigning the appropriate points (tab. 2).

Table 1. Results of births

The year	Nr of sheep and ewes that gave birth, heads	Lambs were obtained			Total lambs per 100 sheep and ewes, %
		total heads	including twins		
			heads	%	
2011	1308	1368	120	8,8	104,6
2012	1103	1146	86	7,5	103,9
2013	1276	1430	308	21,5	112,1
2014	1271	1332	122	9,2	104,8
Total	4958	5276	636	12,1	106,4

Thus, in the III-V score points were framed 1367 heads of ewe lambs with body weight between 21,10 to 26,30, wool length between 5,89 to 6,70 cm respectively and 590 heads of male lambs with body weight between 26,01 to 31,46 kg and corresponding length of wool between 6,41-7,58 cm.

This youth was further increased in order to reproduce the breeding herd along with commercializing to other farmers ' households.

Table 2. The results of the evaluation of youth at weaning according to body mass and length of wool fibres

Score	Male lambs				Ewe lambs			
	Heads	%	Body weight, kg	Wool length, cm	Heads	%	Body weight, kg	Wool length, cm
I	396	28,4	20,50 ± 0,08	5,68 ± 0,09	162	8,8	16,61 ± 0,13	5,83 ± 0,18
II	407	29,2	23,34 ± 0,15	5,83 ± 0,08	322	17,4	18,91 ± 0,11	5,57 ± 0,09
III	271	19,5	26,01 ± 0,25	6,41 ± 0,07	561	30,3	21,10 ± 0,12	5,89 ± 0,06
IV	122	8,8	26,46 ± 0,08	7.46 ± 0,12	323	17,5	22,47 ± 0,06	6,59 ± 0,09
V	197	14,1	31,46 ± 0,36	7,58 ± 0,20	483	26,0	26,30 ± 0,30	6,70 ± 0,10
Average	**1393**	**100**	**24,47 ± 0,17**	**6,29 ± 0,10**	**1851**	**100**	**21,92 ± 0,15**	**6,16 ± 0,09**

On the whole of the appreciated herd we can mention that the body weight of male lambs (1393 heads) and ewe lambs (1851 heads) was 24,47 kg and 21,92 kg respectively, and wool length of 6,29 cm and 6,16 cm respectively.

In accordance with the „Evaluation instruction of Tsigaie breed of sheep with elements of selection" in the reference period (2011-2014) there were subject to complex appreciation, 271 heads of male lambs and 1590 of ewe lambs at the age of 12-14 months. The percentage of elite class ewes in the flock constituted 40% and of male lambs 95,9%.

Body weight of evaluated male lambs of the elite class was on average 49,69 ± 0,33 kg. In relation to the minimum requirements of the standard type for body mass which constitutes 45,0 kg, weight of evaluated male lambs is increased by 4,69 kg or 10%.

This index is also greater by 1,0% for ewe lambs (table 3).

Table 3. Results of youth evaluation at 12-14 months, (M + m) 2011-2014

Class	n	%	Body weight, kg	Raw wool, kg	Wool length, cm
Rams					
Elite	260	95,9	49,69 ± 0,33	4,80 ± 0,08	12,65 ± 0,09
Total	**271**	**100**	**49,30 ± 0,34**	**4,77 ± 0,37**	**12,60 ± 0,08**
Ewes					
Elite	648	40,8	40,41 ± 0,11	4,01 ± 0,03	12,44 ± 0,06
Total	**1590**	**100**	**36,14 ± 0,10**	**3,70 ± 0,02**	**12,09 ± 0,03**

In accordance with the requirements of the industry for the production of carpets in the country, the selection focused on wool fibre length that would be no less than 9 cm. In accordance with the previous standards (GOST) for the processing industry, woollen fibre length was for young rams 12,65 ± 0,09 cm and ewes 12,44 ± 0,06 cm, which is higher than the minimum requirements of the type by 15,0% and 38,2% correspondingly.

On the basis of the annual complex evaluation of a selection group there were

selected rams-breeders, sheep, breeding replacement youth. In total during the period 2011-2014 there have been selected (tab. 4) 105 breeding rams with body weight of 80,52 ± 0,74 and woolen production of 6,95 ± 0,24 kg, 3891 sheep with body mass of 57,69 ± 0,09 kg and woolen production of 4.59 ± 0,01 kg, 176 breeding young rams with body mass of 51,25 ± 0,31 kg and woolen production of 6,15 ± 0,10 kg, 224 ewes for breeding with body mass of 42,22 ± 0,18 and wool production of 4,89 ± 0,05 kg.

Table 4. The composition and level of production selected EnterpriseDB sheep (M + m) kg

The year	Rams			Sheep		
	n	Body weight, kg	Raw wool, kg	n	Body weight, kg	Raw wool, kg
2011	21	86,33 ± 1,70	7,28 ± 0,30	1173	59,65 ± 0,20	4,47 ± 0,02
2012	4	82,25 ± 1,00	6,15 ± 0,11	740	55,34 ± 0,10	4,55 ± 0,01
2013	26	76,65 ± 1,28	6,62 ± 0,10	1143	59,05 ± 0,16	4,76 ± 0,03
2014	54	80,01 ± 0,94	7,04 ± 0,17	835	55,19 ± 0,15	4,88 ± 0,02
Total	**105**	**80,52 ± 0,74**	**6,95 ± 0,24**	**3891**	**57,69 ± 0,09**	**4,59 ± 0,01**
	The RAM			**Ewe lambs**		
2011	21	50,85 ± 1,00	6,08 ± 0,19	68	42,01 ± 0,40	4,58 ± 0,09
2012	41	48,68 ± 0,50	5,88 ± 0,14	27	41,11 ± 0,30	4,94 ± 0,18
2013	68	52,67 + 0,59	6,01± 0,10	89	43,02 + 0,29	4,92 ± 0,06
2014	46	51,63 ± 0,54	6,61 ± 0,17	40	41,55 ± 0,35	5,29 ± 0,10
Total	**176**	**51,25 ± 0,31**	**6,15 ± 0,10**	**62**	**42,22 ± 0,18**	**4,89 ± 0,05**

The selection nuclei animals according to the body mass and wool production are more superior according to the standard requirements for the breeding Rams by 0,7% of body mass and 15,8% of the production of wool, that of sheep - after body weight by 15,4% and by 14,8% according to wool production, breeding young rams – by 13,9% according to body mass and by 2,5% according to the production of wool, breeding ewes – by 5,6 % according to body mass and by 8,7% according to the production of wool.

Conclusion

Performance Indices obtained in the result of a task of selection work and well planned improvement of sheep of Tsigaie Breed of Moldovan type created from Tsigaie breed, considerably exceed the minimum requirements of the standard type after body mass production, wool and fibre length.

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СТЕРИЛИЗАЦИЯ ПРИРОДНЫХ ПОПУЛЯЦИИ СЛИВОВОЙ ПЛОДОЖОРКИ *LASPEYRESIA FUNEBRANA* Tr. (Lepidoptera , Tortricidae)

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Введение

Проблемы получения экологически чистой сельскохозяйственной продукции и оздоровления окружающей среды выдвигают необходимость разработки экологически безопасных систем интегрированной защиты растений базирующихся на преимущественном использовании биологических средств, систем направленных на активизацию природных защитных сил агробиоценозов, направленных на снижение затрат энергетических и финансовых средств на проведение защитных мероприятий [6,7,8,9]. Среди биологических методов и безопасных средств защиты растений большое место отводится бирациональным препаратам, созданным на основе биологически активных веществ, выделенных из растений или насекомых и их синтетическим аналогам: препаратам гормональной природы, ингибиторам синтеза хитина - так называемым регуляторам роста, развития и размножения насекомых; феромонам, аттрактантам, репеллентам, детергентам -регуляторам поведения насекомых [2,3,4,5,6,9]; стимуляторам роста и устойчивости растений к болезням и вредителям, растительным экстрактам и маслам, ловушкам с биофизическими аттрактантами [7,8,9];натуральным химическим средствам с пониженной токсичностью [6,7]. Сочетание этих средств защиты растений позволяет снизить экологическую опасность и затраты энергии на 30-40% и более на проведение защитных меролприятий [6]. Разработка таких безопасных систем защиты сливы является основной задачей наших исследований.

Материал и методика

Полевые исследования по химической стерилизации самцов сливовой плодожорки в феромонных ловушках, обработанных безопасным стерилизатором [1% раствор аналога ювенильного гормона Insegar 25 WG (fenoxicarb, 250g/kg), вариант– V2]- были проведены в сливовом саду ООО «Агробрио», сорта Стенлей и Кабардинка, в 2012 на площади 4 гектара. Эталоном [вариант-V1] служил остальной участок сада в 8 га, обрабатываемый химическими инсектицидами по схеме принятой для данной зоны. Стерилизующие феромонные ловушки вывешивали после отлова первых самцов на контрольные феромонные ловушки, которые по три штуки на делянку вывешивали до появления белого бутона, одновременно как на эталоне так и на экспериментальном варианте, для определения фенологических сроков развития вредителя и определения плотности популяций сливовой плодожорки, на обоих вариантах. Ловушки обрабатывали стерилизатором через каждые 15-17дней, феромонные испарители заменяли через каждые 30 дней. На контрольных ловушках клейкие вкладыши заменяли по мере загрязнения, но не реже чем через 15 дней .Оценку фитосанитарной обстановки проводили на обоих вариантах по общепринятым методикам изложенным в кн.«Îndrumări metodice pentru testarea produselor chimice şi biologice de protecţie a plantelor de dăunători, boli şi buruieni în RM / Centrul de Stat pentru Atestarea Produselor Chimice şi Biologice de Protecţie şi Stimulare a Creşterii Plantelor.- Chişinău : S.n. 2002 (F.E.-P.“Tipografie Centrală[1]. Математическую обработку данных проводили по методикам Доспехов 1985.

Таблица 1. Динамика отлова самцов сливовой плодожорки на участке стерилизации в саду ООО «Агробрио», центральная зона Молдовы, 2014

Даты учеты	Число отловленных самцов/ловушку в опыте, по повторностям			В среднем/ лов	Число отловленных самцов/ловушку в эталоне, по повторностям			В среднем/ лов
	1	2	3		1	2	3	
29.04.14	9	2	0	3.6	10	6	3	6.3
30.04.14	3	2	0	1.6	4	2	4	3.3
07.05.14	4	2	0	1.6	23	13	4	13.3
13.05.14	3	4	0	2.3	41	12	3	18.6
20.05.14	5	19	0	8.0	70	30	2	34
27.05.14	15	23	1	13.0	100	41	23	82.0
28.05.14	1	0	0	0.3	18	5	7	10.0
03.06.14	1	0	0	0.3	28	5	1	10.3
10.06.14	0	0	1	0.3	45	9	4	19.3
19.06.14	0	5	0	1.6	21	0	2	7.6
24.06.14	5	2	0	2.3	11	3	2	5.3
02.07.14	0	0	0	0.6	10	3	3	5.3
08.07.14	0	0	0	0	13	2	0	5
15.07.14	2	0	0	0.6	7	1	2	3.6
22.07.14	5	0	2	2.3	25	5	11	13.6
30.07.14	3	2	0	1.6	30	7	3	13.3
05.08.14	0	1	0	0.3	5	4	2	3.6
11.08.14	0	0	0	0	3	2	11	5.3
27.08.14	0	0	0	0	1	0	2	1
03.09.14	0	0	0	0	0	2	0	0.6
всего	**112**			**40, 3**	**707**			**251.3**

Таблица 2. Биологической эффективности метод стерилизация сливовой плодожорки, сливовой сад ООО".Агробрио" центральная зона Молдовы, 2014

Вариант опыта	Число плодов, (шт.)			Биол. эффективность по отношение к эталону, в %
	Обследо-ванных, (шт)	Повреждённых, (шт.)	%	
Первое поколение 1				
V1-Химический эталон	300	2,3	0,66	-
V2-Стерилизация самцов	300	0,6	0,1	84,8
Второе поколение 2				
V1-Химический эталон	300	4.6	2,3	-
V2-Стерилизация самцов	300	2,0	0,66	71,3
Примечание: Эффективность стерилизации по сравнению с химическим эталоном				

Результаты исследований

В сливовом саду АОО "Агробио" с. Бачой, в Центральной зоны Молдовы, на площади 4 га проводил исследование по изучение сезонной динамики лета бабочек сливовой плодожорки на половые феромоны и оценке эффективности метод стерилизация самцов в ловушках, обработанных биорациональным препаратом Инсегар, в снижении плотности популяций сливовой плодожорки. Начало лёта самцов сливовой плодожорки на сигнальные ловушки отмечено 29.04.14 (в среднем 3 особь / ловушку). После чего ловушки обработанные стерилизатором развесели по всему экспериментальному участку из расчёта 10 шт./га. Результаты отлова бабочек на контрольные ловушки на обоих вариантах представлены в таблице 1. Число отловленных бабочек за 5 дней в среднем на ловушку варьировало от 0,3 до 26,0. За период вегетации на эталонном варианте отловлено 655,2 самца, а в среднем на ловушку 218,4. На варианте стерилизации - 203,4 и 67,8 самца. Что свидетельствует о более эффективном снижении численности вредителя на варианте стерилизации чем на эталонном варианте. Это подтвердилось и при анализе плодов на их повреждённость сливовой плодожоркой (табл. 2). Так , процент повреждённых плодов на варианте стерилизации по поколения не превышал 0,3% и 2,0%, тогда как на химическом эталонном варианте он составил соответственно 2 % и 7%. Биологическая эффективность метода стерилизация самцов сливовой плодожорки превысила эффективность эталонного химического варианта по поколениям на 84,8% и 71,3%. Возможно при более высокой численности разница между вариантами была бы менее демонстративной. Опыт необходимо повторить на более обширные площади.

Выводы

Данные отлова самцов на контрольные ловушки показали что плотность популяции сливовой плодожорки на начало опыта была равная. В дальнейшем на химическом варианте она нарастала быстрее чем на варианте стерилизации. За период вегетации на эталонном варианте отловлено на 3 контрольные ловушки 655,5 самца , а на варианте стерилизации -203,4 самца.

Процент поврежденных плодов сливовой плодожоркой по вариантам в первом поколении составил: на эталоне 2%, в опыте 0,3%; во втором поколении - на эталоне 7% и в опыте 2%. Эффективность стерилизация по поколениям составила 85-71.3%.

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MORPHO-ANATOMICAL ASPECTS OF TWO BASIL CULTIVARS PLANTLETS UNDER EXPERIMENTAL HEAVY METAL CONTAMINATION

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Introduction

The pollution of soil with heavy metals is a global concern as a result of the human activities, in particular by the extraction and processing of raw materials, transport, urban areas etc. Although the current technologies for the cleaning of the contaminated areas such as mechanical isolation, pyrometallurgical separation or chemical treatment are effective, they are usually expensive with a high consumption of time and resources. More recently, the use of plants for the metal extraction (phytoremediation) has emerged as a promising alternative in removing the excess of heavy metals from the soil and water [7]. Examples of such species are *Salix alba, Helianthus annuus, Brassica juncea, Arabidopsis halleri* etc. A special interest may be granted to species that grow in substrates with high concentrations of potentially toxic chemicals and also can produce bioactive compounds. This is applicable to compounds that do not change their composition from the presence of the high concentrations of potentially toxic substances from the substrate, and are produced in large quantities such as essential oils [10].

The basil, *Ocimum basilicum* L., belongs to the *Lamiaceae* family and is native to India and China and today is spread in almost all countries around the globe [8]. Some experimental studies demonstrate the ability of basil to remediate the environment polluted with heavy metals, nitrates and pesticides [3]. The essential oil produced by the basil plants is used in traditional medicine, cosmetics, aromatherapy, showing antimicrobial activities, insecticidal, antioxidant, etc. [13, 17].

Cerium ($^{58}Ce_{140,116}$) is the most abundant of the rare Earth elements, about 50 ppm in the Earth's crust and is used in the composition of metal alloys, for the better heat resistance, oxidation and better control shape of steel, polishing and discoloration of glass, auto catalysts, ceramic coatings, polishing precious stones, capacitors, semiconductors, etc. Pollution sources with this element are: cerium ore exploitation and refining, field leakage of coal and plant biofuel, improperly disposed devices [11].

The aim of this paper was to assess the ability of two basil cultivars to germinate and grow under an artificially contaminated substrate with cerium and to sustain any further testing in this regard.

Material and methods

The material used was represented by seeds two basil cultivars (*Ocimum basilicum* L.), Sweet Genovese and Red Rubin, obtained from Seedaholic.com Ireland. Sweet Genovese variety presents a mature height between 35-45 cm, requiring 3-6 days for germination and is green leaves cultivar. Red Rubin cultivar reaches at maturity a height between 60-75 cm, with the leaves and stems of purple pigmentation.

Germination and growth of the plants was carried out in Petri dishes of 9 cm diameter on filter paper (25 seeds per plate, 3 replications per treatment). The treatments consisted in application of different concentrations of Ce (10, 20, 50, 200, 400 mg / l Ce), prepared from the $Ce_2(SO3)_4$ * $8H_2O$, or distilled water for control version, the solutions were applied in a volume of 4 ml / plate. To ensure uniformity in the growth conditions a growth chamber was used (Snijders Scientific) with a photoperiod of 12h: 12h, temperature 22-24 °C, and humidity 60%.

The number of germinated seeds was recorded daily until the 8th day inclusive. The length of root and hypocotyle and the fresh biomass were recorded at 8 days after the experiment installation. For the anatomical analyses, the vegetal material has been fixed and preserved in 70% ethylic alcohol. The sections were cut (roots and hypocotyls of the plantlets) with a microtome and a botanical razor. The obtained sections were then colored with iodine green and ruthenium red. The micrographs were performed by means of a Novex (Holland) microscope, using a Sony (Cyber-shot) camera. For statistical analysis, ANOVA and Tukey tests were used at $p \leq 0.05$.

Results

The number of germinated seeds recorded after 8 days of the experiment is shown in Table 1. It was found that treatment with different concentrations of cerium influenced the number of germinated seeds compared to control, but these differences are not statistically significant. The percentage of germinated seeds was between 84% and 89.32% for Sweet Genovese, 73.32% and 90.64% for Red Rubin.

Table 1. The number of germinated seeds in Sweet Genovese and Red Rubin at different Ce concentration after 8 days

Cerium treatments	0 mg/l	10 mg/l	20 mg/l	50 mg/l	200 mg/l	400 mg/l
Sweet Genovese	22,33±1,45	21±0,88 ns	22±1 ns	22±1,15 ns	22,33±2 ns	21,66±0,88 ns
Red Rubin	18,33±1,45	21±0,88 ns	19,66±0,57 ns	21,66±1.33 ns	21±0,88 ns	22,66±0 ns

The values are means of 3 replication ± standard error (25 seeds / replicate); ns – no statistical significance according to the Tukey Test (p≤0, 05.)

Morphometric evaluation of the plantlets

The length values of the vegetative organs of the basil plantlets are presented in Table 2 and Table 3. Root elongation was reduced between 17,43% and 85,82% comparing with the control plantlets from Sweet Genovese and with 19,31% to 75,87% for Red Rubin at the 20 – 400 mg/l Ce treatments. At 10 mg/l Ce the root length had comparable values with the control for Sweet Genovese and increased values with 14,48% compared to control without a statistical significance in the case of Red Rubin. In Figure 1 are presented the basil plantlets on the Petri dishes after 8 days of cerium treatment. At the concentration of 400 mg/l of cerium, plantlets are visible smaller in length for both cultivars.

Table 2. The root length (mm) of Sweet Genovese and Red Rubin cultivars at different Ce concentration

Cerium treatments	0 mg/l	10 mg/l	20 mg/l	50 mg/l	200 mg/l	400 mg/l
Sweet Genovese	22,56±0,83	22,4±0,74 ns	18,63±1,04 *	18,2±1 **	7,06±0,79 **	3,2±0,51 **
Red Rubin	24,86±1,41	28,46±1,25 ns	20,06±1,28 *	15±0,8 **	8,5±0,77 **	6±0,65 **

The values are means of 3 replication ± standard error (25 seeds / replicate); * - significant statistical differencens ($p < 0,05$); ** - significant statistical differences ($p < 0,01$); ns – no statistical significance according to the Tukey Test

The hypocotyls length of Sweet Genovese was reduced with 20,07% comparing to control at 200 mg/l Ce and with 45,37% at 400 mg/l Ce. The 10 – 50 mg/l Ce treat-

ments did not influence the hypocotyls length. The 200 and 400 mg/l Ce treatments reduced the hypocotyls length of Red Rubin with 21, 98% and 20,70% comparing with the control. For the same cultivar, the 10 mg/l Ce treatment increased the hypocotyls length comparing with the control with 28,75%.

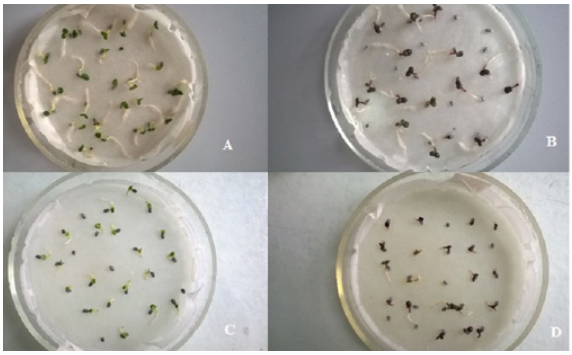

Fig. 1. The morphology of basil plantlets treated with different cerium concentrations.
A – Sweet Genovese Control; B – Red Rubin Control; C – Sweet Genovese 400 mg/ l Ce; D – Red Rubin 400 mg/l Ce.

Table 3. The hypocotyls length (mm) of Sweet Genovese and Red Rubin cultivars at different Ce concentration

Cerium treatments	0 mg/l	10 mg/l	20 mg/l	50 mg/l	200 mg/l	400 mg/l
Sweet Genovese	6,33±0,25	5,66±0,25 ns	6,2±0,30 ns	5,73±0,23 ns	5,06±0,22 **	3,46±0,12 **
Red Rubin	5,46±0,19	7,03±0,23 **	5,63±0,33 ns	4,6±0,16 ns	4,26±0,24 **	4,33±0,16 **

The values are means of 3 replication ± standard error (25 seeds / replicate); * - significant statistical differences ($p < 0,05$); ** - significant statistical differences ($p< 0,01$); ns – no statistical significance according to the Tukey Test

Concerning the biomass the cerium treatments acquired statistical significant differences only at 200 and 400 mg/l Ce for Sweet Genovese. All the other treatments were comparable to the control (Table 4).

Table 4. The biomass (mg) of Sweet Genovese and Red Rubin cultivars at different Ce concentrations

Cerium treatments/ Cultivars	0 mg/l	10 mg/l	20 mg/l	50 mg/l	200 mg/l	400 mg/l
Sweet Genovese	13,61±0,64	15,89±0,69 ns	12,14±0,43 ns	13,27±0,52 ns	10,74±0,57 **	10,08±0,41 **
Red Rubin	12,57±0,58	13,61±0,42 ns	11,53±0,21 ns	13,44±0,61 ns	2,1±0,42 ns	9,41±0,50 ns

The values are means of 3 replication ± standard error (25 seeds / replicate); * - significant statistical differences ($p < 0,05$); ** - significant statistical differences ($p< 0,01$); ns – no statistical significance according to the Tukey Test

The effects of the cerium treatments on the basil plantlets presented a correlation with the investigated morphological parameters depending on the Ce concentrations. The corre-

lations are negative, moderate to strong, for the root and the hypocotyls length and the fresh biomass (Table 5).

Table 5. Pearson correlation coefficient of cerium treatments concentrations and the inve tigated morphological parameters

Cultivar/Parameter	Root length	Hypocotyle length	Fresh biomass
Sweet Genovese	-0,95	-0,97	-0,80
Red Rubin	-0,86	-0,65	-0,90

Anatomical evaluation of basil plantlets under cerium treatments. After the application of the cerium treatments the anatomical structure of the basil plantlets was analyzed and the images of the sections are presented in Figures 1 to 5. The root structure is described in Table 6 and the structure of the hypocotyls in Table 7.

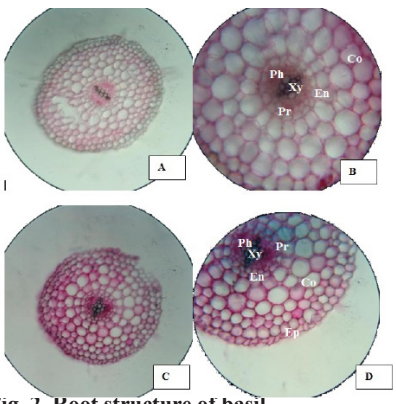

Fig. 2. Root structure of basil plantlets in control.
A – Sweet Genovese (100x); B - Sweet Genovese (400x); C – Red Rubin (100x); D - Red Rubin (400x). (Ep-epidermis, Co- cortex, En- endodermis, Pr- pericyc, Ph- phloem, Xy- xylem)

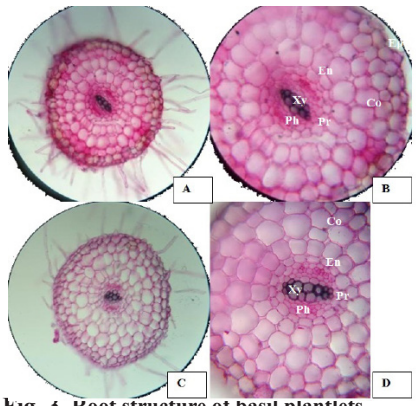

Fig. 3. Root structure of basil plantlets 400 mg/ Ce treatment.
A – Sweet Genovese (100x); B - Sweet Genovese (400x); C – Red Rubin (100x); D - Red Rubin (400x). (Ep-epidermis, Co- cortex, En- endodermis, Pr- pericycle, Ph- phloem, Xy- xylem)

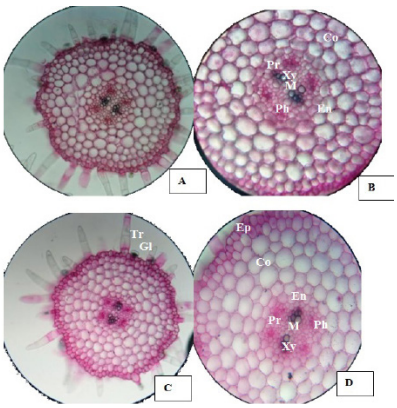

Fig. 4. Hypocotyle structure of basil plantlets in control.
A – Sweet Genovese (100x); B - Sweet Genovese (400x); C – Red Rubin (100x); D - Red Rubin (400x). (Tr- tectorial trichom, Gl- glandular trichom, Ep-epidermis, Co- cortex, En- endodermis, Pr- pericycle, Ph- phloem, Xy- xylem, M- medulla)

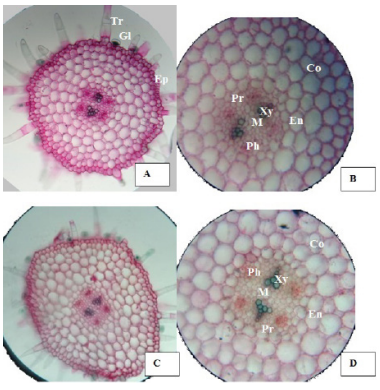

Fig. 5. Hypocotyle structure of basil plantlets in 400 mg/l Ce treatment.
A – Sweet Genovese (100x); B - Sweet Genovese (400x); C – Red Rubin (100x); D - Red Rubin (400x). (Tr- tectorial trichom, Gl- glandular trichom, Ep-epidermis, Co- cortex, En- endodermis, Pr- pericycle, Ph- phloem, Xy- xylem, M- medulla)

Table 6. Anatomical root features of basil plantlets

Treatment / Cultivar	Sweet Genovese	Red Rubin
Control (H2O)	The rhizodermis presents numerous root hairs very long; the cortex is differentiated into three sub areas: exodermis, single ply, having cell walls without suber; cortex is very thin; endoderm cells with very high radial Caspary thickening walls; the stel is diarh type structure, highlighting the two phloem bundles and two xylem bundles;	The structure is similar to the root of Sweet Genovese except that cells of the rhizodermis are smaller, the exodermis cell walls are lightly suberificated, cortex cells are longer and medulla place is occupied by the newest vessels of metaxylem in contact one with other.
10 mg/l Ce	The root hairs are extremely numerous, exodermis has large cells; the cortex is very thick, emerges radial Caspary thickening in their walls; In this way, compared to the control the treatment increase the thickness of the root.	The cortex is thinner with 3 to 4 layers and less visible Caspary thickenings; the root hairs are rare;
20 mg/l Ce	The cortex is thinner.	The root hairs are rare and shorter.
50 mg/l Ce	The root hairs are rare but longer.	The root hairs are rare but longer
200 mg/l Ce	The root hairs are rare	The root hairs are rare
400 mg/l Ce	The root hairs are rare; the cortex is thicker, 4-5 layers, and the number of the xylem bundles is grater 13 to14.	The root hairs are rare, thicker and shorter.

Table 7. Anatomical hypocotyle features of basil plantlets

Treatment / Variety	Sweet Genovese	Red Rubin
Control (H2O)	The epidermis has uniseriated pluricellular hairs, the cortex is thick, the endodermis has cells with visible Caspary thickenings, the stele presents two xylem bundles and the phloem bundles underwent a division and there are four bundles.	The hairs are frequent, with different lengths and very thick, rare glandular trichoms, with a swollen cuticle on the glandular cells, the stele structure is similar to root
10 mg/l Ce	The hairs are numerous and shorter (2-4 cells);	Rare tectorial and glandular trichoms, the xylem bundles have fewer vessels with thinner walls lightly lignified.
20 mg/l Ce	More numerous tectorial trichoms	The hairs with different sizes, rare glandular trichoms, very thin cortex and thicker stel
50 mg/l Ce	Longer hairs and visible short and pluricellular glandular hairs	Numerous hairs and rare glandular hairs
200 mg/l Ce	Very numerous tectorial trichoms and less numerous glandular ones, the stele structure is different because the two xylem bundles are divided and the two halves are rotated with protoxylem vessels next to medulla and metaxylem ones close to cortex.	Rare hairs
400 mg/l Ce	Similar structure to 200 mg/l Ce and the medulla has more cells	The hairs are numerous with different lengths and rare glandular hairs

Discussions

The obtained values indicate that the analyzed basil varieties may germinate in the presence of cerium without being significantly affected. Also, the relatively normal development in terms of morphology of the basil plantlets at concentrations up to 50 mg / l of cerium may indicate the presence of some physico-chemical or physiological mechanisms that allow the plants to reduce the toxicity of this metal. The ability of the basil to grow and develop under contaminated substrate is known for different pollutants. The basil is a tolerant plant, growing in some substrates containing up to of 10-25 mg / kg of Cr, Pb, Ni, Cd, with stimulating effects in regard to certain morphometric parameters and the production and composition of the essential oil [9].

In our study, we found that the cortex of the treated plantlets with a solution of cerium was thicker than that of the untreated plantlets. Also there are more numerous Caspary thickenings and xylem bundles. The anatomical structure of the hypocotyls revealed a stress reaction to the treatment with the cerium solution by thickening of the cortex and an increased number of tectorial trichome on the surface of the epidermis, which has a protective role [15]. These findings are similar with other studies. Gomes et al., 2012 [5] found that the root endodermis and epidermis of Pfaffia glomerata treated with 90 μmol Cd/l was thicker than that of untreated plants and suggests a possible role of the endodermis in limiting cadmium translocation to shoots.

The basil seeds produce mucilage when are in direct contact with water. Such mucilage has a composition high in pectin and uronic acids [2, 14]. The presence of mucilage at the time of germination can thus contribute to adsorption of toxic elements by the compounds stated above, as is the case of copper [6], allowing relative germination and growth in optimal conditions. In the case of chromium, it is considered that the formation of deposits in the periplasmic region of the root cortical cells is a mechanism that maintains a low concentration of this metal in the cytoplasm, contributing to metabolic detoxification [1]. However it is believed that the same mechanism is involved in detoxification of other elements such as Cu, Zn, Co, Ni, Mo. For Cu and Zn, where basil can reduce the toxicity by confining these elements in the cellular wall, process confirmed by radioisotope studies, showing that pectins and uronic acids have a high affinity for these metals [12]. Cerium is a rare metal, found in the Earth's crust in concentrations up to 60 mg / kg. It is considered alongside other rare metals as a possible fertilizer at low concentrations, stimulating germination of rice seed [4]. Although rare metals toxicity is not as high as that of other elements, their increasing use in different industries may increase the risks associated with these metals [11]. Using plants as methods for decontamination of polluted substrates can bring a number of advantages over other technologies, among which reduced costs and increased sustainability. In this respect, particular interest can be given to the plants that can be grown on polluted substrates then use as a raw material source containing no contaminants [7].

Basil falls into this category since the volatile oil is free of contaminants and oil production can be developed under relative optimal conditions in the presence of metals such as Cd (up to 10 mg / l) Cu (up to 150 mg / l) Pb (at concentrations up to 500 mg / l) and As in concentration of 25-100 μM [16].

The obtained values in terms of germination and growth of the analyzed basil varieties in the presence of relatively high concentrations of cerium may indicate that basil is a potential species that can grow in substrates contaminated with this element. Detailed analysis is needed, both physiological and biochemical, to establish this potential.

Conclusions

Genovese and Red Rubin cultivars grow normal in condition of cerium treatments at the concentration of 10, 20 and 50 mg / l and higher cerium concentrations 200 and 400 mg / l exert an inhibitory effect on the growth of the two basil cultivars. The anatomical structure revealed a stress reaction to the treatment with the cerium solution by thickening of the cortex, increased number of tectorial tricome and a decreased number

of root hairs.

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PHYLOGENETICAL APPROACH FOR THE SEARCH OF VALUABLE METABOLIC PRODUCTS IN CYANOBACTERIA

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Introduction

Cyanobacteria are an important group of microorganisms that play crucial role in both terrestrial and aquatic ecosystems. Species are beneficial as a food source, as well as producers of carotenoids, antioxidants and other secondary metabolites.

A phylogenetic approach may be an indispensable tool to detect strains of potential value for the production of various secondary metabolites and to insure the quality and safety of biological products. However, most existing phylogenetic studies lack precision and use mostly rDNA or single gene approaches, thus preventing a more detailed understanding of the cyanobacteria evolutionary trajectories.

Advances in molecular and genome studies have revealed the main genes involved in different stages of secondary metabolites production. Several genes have been identified de novo in triacylglycerol (TAG) biosynthesis pathway and are known to be involved in lipid synthesis (*rbsL, me g6562, accA, accD, dgat g2354, dgat g3280, gat g7063*), production of malic enzyme subunits (ACCase and diacylglycerol acyl transferase), and carotenoid synthesis (*crtB, crtP, crtQ-1, crtQ-2, crtL, crtL-2, crtO, crtR*). The first two gene groups are well studied in relation to biofuel production, while the third group has been widely employed in the pharmaceutical, cosmetics, and food industry for natural dyes and antioxidants.

Carotenoids are a specific metabolite involved in the photosynthesis performed by cyanobacteria. The carotenoids serve as light-harvesting pigments in photosynthesis, and protect the cells against photooxidative damage [4]. Carotenoids play a central role in the deactivation of 3Chl* and 1O2*, and the reduction of reactive oxygen species (ROS) formation due to the thermal dissipation of excess light energy at the level of 1Chl* [6]. In addition carotenoids are able to mitigate the effects of ROS such as superoxide and peroxilipids radicals [3, 11, 14].

In cyanobacteria, various carotenoids have been identified, including the unique ketocarotenoids, echinenone and 4-ketomyxol, and the carotenoid glycosides, myxol glycosides and oscilloldiglycosides (β-carotene, β-criptoxanthin, zeaxanthin, caloxanthin, lutein, lycopene, nostoxanthin, echinenone, canthaxanthin, 3-OH echinenone, deoxymyxol, myxol, ketomyxol, hidroxymyxol, oscillol etc.). Major carotenoids in cyanobacteria include b-carotene, its hydroxyl and keto derivatives, and the carotenoid glycosides [16]. It is well known that the carotenoid composition depends on growth conditions such as light intensity, growth stage, composition of nutrient medium, and intensity of ROS formation [5, 9, 10]. However, the diversity in carotenoid composition might be due to the presence of specific carotenogenesis pathways.

In the biosynthesis pathway of carotenoids, four enzymes convert geranylgeranyl pyrophosphate to *β*-carotene: phytoene synthase, phytoenedesaturase, ζ-carotene desaturase and lycopene cyclase. Four desaturation steps are needed in the conversion from phytoene to lycopene, and two distinct pathways are known among cyanobacteria: the plant type and the bacterial type [16]. The plant-type requires three enzymes: *CrtP* (phytoenedesaturase), *CrtQ* (z-carotene desaturase) and *CrtH* (*cis*-carotene isomerase), and the bacterial type uses only one enzyme, *CrtI* (phytoenedesaturase) to convert phytoene to lycopene. The bacterial type is rarely found in cyanobacteria, and only in strains which retain ancestral properties of carotenoid biosynthesis. These observations

suggest that in the evolution of cyanobacteria species, the gene *CrtI* was replaced by *CrtP* [19]; and that the gene *CrtP* is found throughout species of cyanobacteria, playing an important role in the synthesis of carotenoids. For these reasons, a phylogenetic approach can be used to detect potentially efficient carotenoid producers, as well as identify unknown isolates and suitable primers for screening for useful genes.

Materials and methods

Genes *CrtP* of 14 cyanobacteria were found using BLAST search algorithm [1] of *Anabaena sp.* PCC 7210 *CrtP* from NCBI Nucleotide database. For all samples (excluded uncultured bacterium and identical *Anabaena = Nostoc* PCC 7120 samples) corresponding 16S sequences were found (Table 1). Resulted sequences (15 for *crtP1* and 13 for rRNA small 16S subunit) were combined in correspondent single text files (format fasta) and aligned using MUSCLE 3.5 [2] in Mesquite 3.03 [8]. Alignments were examined visually and ambiguous regions were adjusted manually resulting in 1350 characters from 1910 base pairs alignment total length for *crtP1* and 1479 vs. 1489 nucleotide base pairs for rRNA 16S. Maximum Likelihood (ML) approach was applied to generate phylogenetic trees using GARLI 1.1 [20]. We run 5000000 iterations using GTRGAMMA rates model. Phylogenetic support was assessed by 100 replication of bootstrap analysis using PAUP* 4.0a109 [13].Conserved regions in *CrtP* alignment were found visually based on identity of first and second codon positions with no or some differences (less than in five sequences from 15) in third codon positions.

Table 1. Sequences of *crtP1* gene and rDNA small 16S subunit used for phylogenetic analysis

Species	Strain	*CrtP*		16S rRNA	
		Accession #/ position in genome	Product/ Protein ID	NCBI Reference Sequence	Product
Anabaena sp.	PCC 7120	Y15114	phytoene desaturase CAB56040.1	See *Nostoc* sp.PCC 7120	*Ibid.*
Anabaena cylindrica	PCC 7122	CP003659 Reg. 4412834.. 4414607	phytoene desaturase AFZ59250.1	KM019919.1	16S ribosomal RNA
Anabaena variabilis	ATCC 29413	CP000117 Reg.: 6025392.. 6027184	phytoene desaturase BAB73531.1	NR_074300.1	16S ribosomal RNA
Calothrix sp.	PCC 7507	CP003943 Reg.: 4124330.. 4125464	phytoene phytoene desaturase CAB 56040.1 desaturase AFY 34025.1	NR_102891.1	16S ribosomal RNA
Cylindrospermum stagnale	PCC 7417	CP003642 Reg.: 3596561.. 3597711	phytoene desaturase AFZ25297.1	NR_102462	16S ribosomal RNA
Geitlerinema sp.	PCC 7407	CP003591 Reg.: 1360418.. 1361866	phytoene desaturase AFY65636.1	NR_102448.1	16S ribosomal RNA
Nodularia spumigena	CCY 9414	CP007203 Reg.: 2429160.. 2430283	phytoene desaturase AHJ28780.1	CP007203.2 Reg 1454563… 1456044	16S ribosomal RNA

‚Nostoc azollae'	0708	CP002059 Reg.: 2958611.. 2960379	phytoene desaturase ADI64708.1	NR_074259.1	16S ribosomal RNA
Nostoc punctiforme	PCC 73102	CP001037 Reg.: 3436797.. 3438236	Amine oxidase ACC81307.1	NR_074317.1	16S ribosomal RNA
Nostoc sp.	PCC 7107	CP003548 Reg.: 4841724.. 4843495	phytoene desaturase AFY44694.1	CP003548.1 Reg. 201748… 203226.	16S ribosomal RNA
Nostoc sp.	PCC 7120	BA000019 REG.: 2196420.. 2198212	phytoene desaturase BAB73532.1	NR_074310.1	16S ribosomal RNA
Nostoc sp.	PCC 7524	CP003552 Reg.: 5638496.. 5640281	phytoene desaturase AFY50457.1	CP003552.1 Reg. 2137997… 2139363	16S ribosomal RNA
Oscillatoria acuminata	PCC 6304	CP003607 Reg.: 1499581.. 1500940	phytoene desaturase AFY80941.1	NR_102463.1	16S ribosomal RNA
Rivularia sp.	PCC 7116	CP003549 Reg.: 5251957.. 5253652	phytoene desaturase AFY56622.1	NR_102458.1	16S ribosomal RNA
Uncultured bacterium	clone 66415	KP445989 Reg.: 5912..7700	phytoene desaturase	n. a.	n. a.

n. a. – not applicable

Results and discussions

We conducted an ML analysis of gene *crtP1* on 15 cyanobacteria samples in order to reveal phylogenetic relationships between these taxa. Closely related taxa in which the *CrtP* gene had not been previously described, should contain copies of the gene that vary only slightly. An ML analysis of the gene *CrtP* in 15 taxa of cyanobacteria has shown that the placement of species on a phylogenetic tree does not necessarily correspond to their taxonomical affiliation (Fig. 1). Although *Anabaena* group (three taxa) is basal for the whole tree, it includes one of the *Nostoc* samples (PCC7120) and does not have any significant statistical support. Other Noctoc *CrtP* genes are dispersed all over the tree and do not build any stable clade. Additionally, Nostoc *CrtP* genes are located together with any other gene isolated from this genus. This tree topology might be a result of misidentification, but more likely it reflects some problems in the current taxonomical boundaries of the genus *Nostoc*. We observed similar patterns for *Anabaena* samples (Fig. 1). These two cyanobacteria groups might be artificial and polyphyletic, supported by 16S and *hetR* single gene phylogenies [18], and a genome-wide cyanobacteria phylogeny [12]. Another explanation for the scattered placement of *CrtP* in taxonomically closely related taxa might be horizontal gene transfer. However, more isolates and genes should be included in such an analysis to detect possible horizontal gene transfer events in the evolution of branching filamentous cyanobacteria.

In addition, the topology of *CrtP* and 16S trees is not symmetrical (Fig. 1-2). Similarly, on both trees, *Geitlerinema* and *Oscillatoria* are nested together and this clade has significant bootstrap support. In general, *CrtP* tree has more statistical support compared to rDNA 16S tree, mostly because of more conserved and similar sequences, which produced better alignment. For other taxa that follow some similarity in topology amongst separate clades, sample placement impedes phylogenetic resolution. This might be due to different evolutionary trajectories of *CrtP* and the small ribosomal

subunit. Our trees are also difficult to compare with other phylogenetic schemes of branching filamentous cyanobacteria [Tomitany 2007; Shih et al. 2012] mostly due to sampling quantity. Nonetheless, a common feature to all trees discussed here is wide scale polyphily of various taxa, as shown in data sets of *Anabaena* and *Nostoc* (Fig. 1-2).

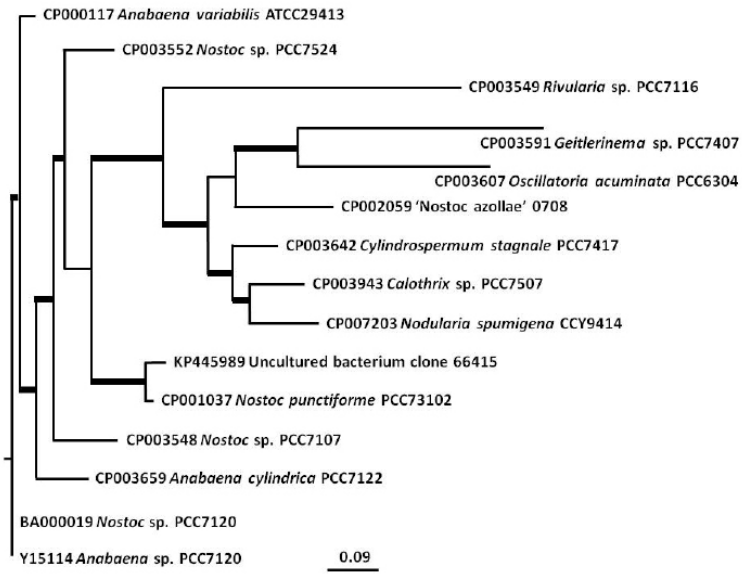

Figure 1. ML tree of crtP1 gene for 15 cyanobacteria isolates. Thick lines show the branches with sufficient (over 70%) bootstrap support.

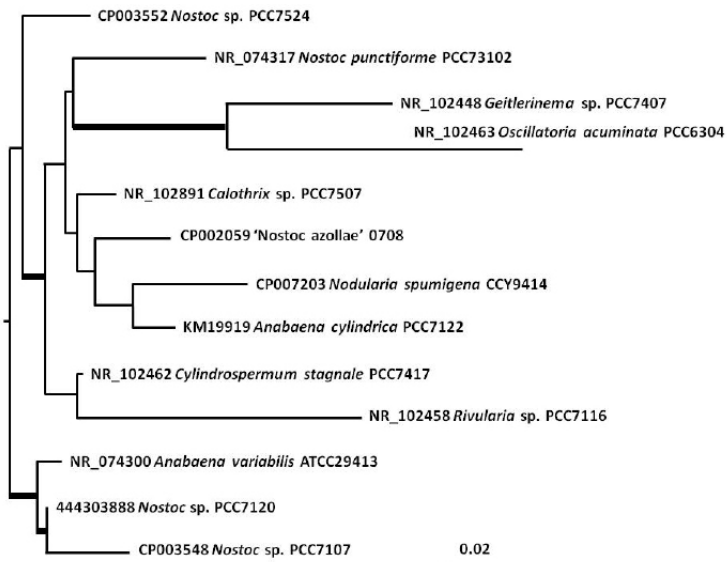

Figure 2. ML tree of 16S rRNA gene for 13 cyanobacteria isolates. Thick lines show the branches with sufficient (over 70%) bootstrap support.

Phylogenetic analysis can be helpful in the identification of unknown isolates. Thus on our tree, the *CrtP* gene of an unknown bacterium (clone 66415, acc. # KP445989)

is nested together with the gene of *Nostoc punctiforme.* These sequences possibly represent the same or closely related species - for example, the sequences of *Anabaena sp.* PCC 7120 (acc. # Y15114) and *Nostoc sp.* PCC 7120 (acc. # BA000019) are identical as Kaneko et al. (2001) suggest; they occupy the same position on the tree [7].

Alignment of amino acid sequences of the genes can also be used for primer designed to screen useful genes for DNA amplification. For the *CrtP* gene we have determined the fragment with total length 1350 nucleotide base pairs (450 amino acids) with no introns and a uniform structure, excluding *Anabaena cylindrica* (strain PCC 7122, genome acc. # CP003659) which contains a small insert of seven amino acids in the beginning of the sequence following the first conserved region. Both starting and ending part of this fragment with length of 39 nucleotide base pairs are well conserved (same amino acids with no changes or minor differences in 3rdcodon positions) are very suitable for primer design. The total length of the fragment allows significant overlap for its amplification using forward and reverse Sanger sequencing (200-300 bp). This fragment contains seven other well conserved regions each with a length over 20 nucleotides that is needed to cover the sufficient primer length (Fig. 3).

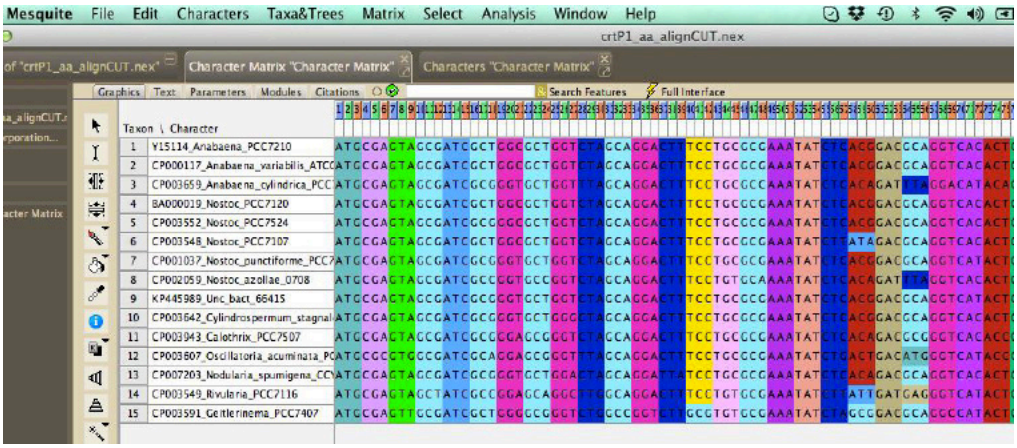

Fig. 3. Alignment of crtP1 gene with starting region (positions 1- 39) quite suitable for primer design.

Thus, phylogenetic analysis of single gene sequences of our selected data set can be useful in three instances: 1) targeting of prospective candidates of useful metabolites through revealing the phylogenetic relationships between them, 2) identification of unknown isolates, 3) screening of targeted genes using PCR methods.

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ON THE EFFECT OF ADVERSE FACTORS IN THE PRESOWING SEED TREATMENT WITH A LOW-FREQUENCY MAGNETIC FIELD

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Introduction

In the development of life throughout the Earth's history, during a long-term evolution, all existing organisms, in one way or another, have fully adapted to the various environmental conditions on our planet [1]. The living organisms have had to adapt not only to the physicochemical conditions, such as temperature, pressure, composition of the atmosphere, light, and humidity, but also to the natural fields of the Earth: geomagnetic, gravitational, electrical, and electromagnetic fields. The living organisms have evolved in an environment characterized by the presence of low-intensity electromagnetic fields, such as the Schumann waves (7.8-0.8 Hz) [2]. Everything that happens in the cells of living organisms is associated with these natural fields.

Within a relatively short historical period, the technogenic human activities have had a significant impact on natural objects and thus dramatically upset the delicate balance between the living organisms and the environmental conditions, which has been formed over thousands of years. This factor has led to many irreparable consequences, in particular, to the extinction of some animals and plants, numerous diseases, a reduction in the average duration of life of people in some regions, and infertility. In recent decades, research into the effect of natural and anthropogenic factors on the human body and other living organisms has been conducted [3].

In modern life, people are constantly being faced with the conditions in which the natural electric field of the atmosphere can be shielded or distorted by metal roofs of houses, reinforced-concrete buildings, vehicles, etc. Electric fields are absent in submarines and spacecrafts, where the plants will play an important role in the regeneration of the gas composition and the replenishment of food products in the future [4, 5]. Therefore, the biological role of natural electric fields in the life of organisms, in particular plants, is an urgent problem.

Along with the natural fields, there are artificial technogenic fields produced by the operation of commercial frequency generators, microwave and EHF devices, transmitters, etc. In addition, the frames of reinforced-concrete buildings partially shield and partially distort natural electromagnetic fields [6, 7] and thus inflict harm to living organisms. This effect is of particular importance to growing organisms which undergo constant cell division and to the regeneration and reproduction processes in mature organisms.

In recent decades, these processes have been the subject of many scientific studies.

It is known that exposure to low-frequency low-intensity fields has beneficial effects on seed germination and plant growth [8, 9]. In our studies of this phenomenon, we used a magnetic field with a flux density of 40-50 μT and a frequency of 1-10 Hz for the pre-germination treatment of dry seeds for 1 h. Positive results were obtained with respect to some parameters, such as seed germination, growing capacity, germination uniformity, yielding capacity, and growing season length. In addition, the differences induced by the energy component in the zone of seed treatment with a field with the above parameters were identified.

Treatment was conducted in buildings made of white ashlar limestone in environmentally safe regions of the city, in open areas in the field, in various parts of an industrial reinforced-concrete building with a high level of electromagnetic interference, and

in a special shielded room. Despite the fact that a magnetic field with fixed parameters was used in all the experiments, results of these experiments were different.

Material and methods

Seeds of wheat of the Odesskaya 51 cultivar were taken for the experiment. Seed germination was conducted in Petri dishes on moist filter paper. The seeds taken as a reference sample were not subjected to any treatment. Ordinary tap water was used for moistening. In the experiments, dry seeds placed in Petri dishes were treated for 1 h; after that, the seeds were moistened and left to germinate.

Experiment 1: treatment and germination in limestone house no. 1 in an environmentally safe region of the city.

Experiment 2: treatment and germination in a room in an industrial reinforced-concrete building on the side facing the television tower located at a distance of 300 m from the building.

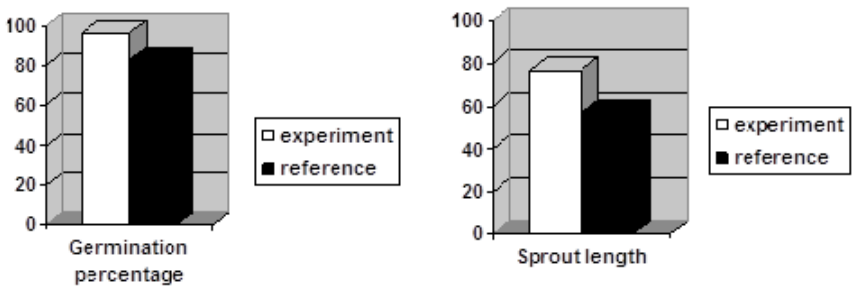
Experiment 3: treatment in a room in an industrial reinforced-concrete building on the side facing the television tower; germination in a grounded metal cabinet.

Experiment 4: treatment in a room in an industrial reinforced-concrete building on the side opposite of the television tower; germination in a grounded metal cabinet.

Experiment 5: treatment and germination in limestone house no. 2 in an environmentally safe region of the city in the zone of action of a constant magnetic field generated by home appliances (column loudspeakers).

Seed germination percentage and sprout length were measured in the experiments.

Results



Fog. 1. Experiment 1.

The "Germination percentage" diagram shows the results of comparative calculation of the percentage of germinated seeds on the 7th day in the experimental and reference samples.

The "Sprout length" diagram shows the results of calculation of the percentage of seeds in which the sprout length is greater than the average sprout length in this experiment.

The results show that, under natural environmentally safe conditions, the seeds in the experimental sample exposed to a positive effect of the magnetic field give better results than the seed of the reference sample.

The "Germination percentage" diagram shows the results of the comparative calculation of the percentage of germinated seeds on the 7th day in the experimental and reference samples.

The "Sprout length" diagram shows the results of calculation of the percentage of seeds in which the sprout length is greater than the average sprout length in this experiment.

The experiment was conducted in the presence of various technogenic fields (elec-

trical power cables, reinforced-concrete building fittings, emissions of television transmitters and retransmitters). The results show that the seeds in the experimental sample were more sensitive to external fields, including harmful technogenic emissions.

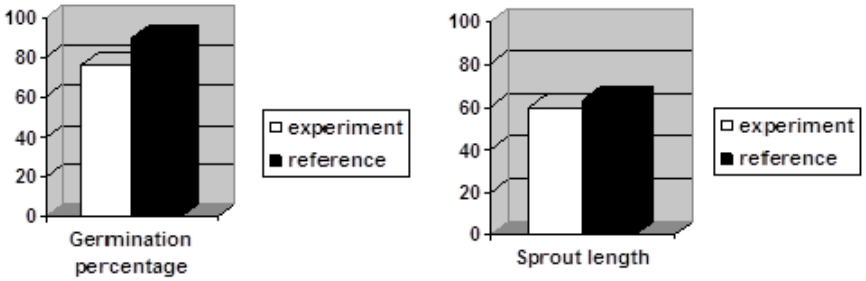


Fig. 2. Experiment 2.

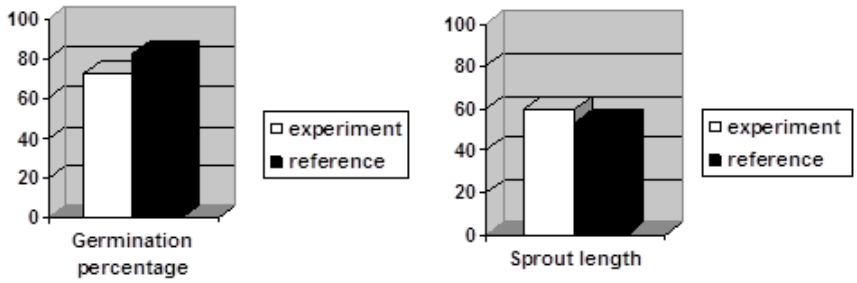


Fig. 3. Experiment 3.

The "Germination percentage" diagram shows the results of the comparative calculation of the percentage of germinated seeds on the 7th day in the experimental and reference samples.

The "Sprout length" diagram shows the results of calculation of the percentage of seeds in which the sprout length is greater than the average sprout length in this experiment.

In the experiment, the magnetic field treatment was conducted with the above parameters in the presence of technogenic fields (similar to Experiment 2), while germination was conducted in a grounded steel cabinet to exclude the impact of any external fields.

The results on the germination percentage were better in the reference sample. The data on the sprout length in the experimental sample were better than in the reference sample.

The "Germination percentage" diagram shows the results of the comparative calculation of the percentage of germinated seeds on the 7th day in the experimental and reference samples.

The "Sprout length" diagram shows the results of calculation of the percentage of seeds in which the sprout length is greater than the average sprout length in this experiment.

In this experiment, treatment was conducted under conditions of a significant weakening of the effect of the television tower, while maintaining all other technogenic fields. Germination was conducted in a grounded steel cabinet similar to Experiment 3.

The results show that there is no difference in the germination percentage of the experimental and reference samples, while the number of sprouts with a length greater

than the average sprout length in the experiment is larger in the experimental sample.

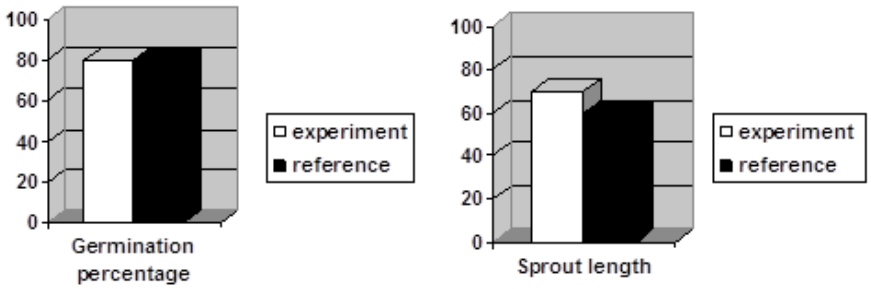


Fig. 4. Experiment 4.

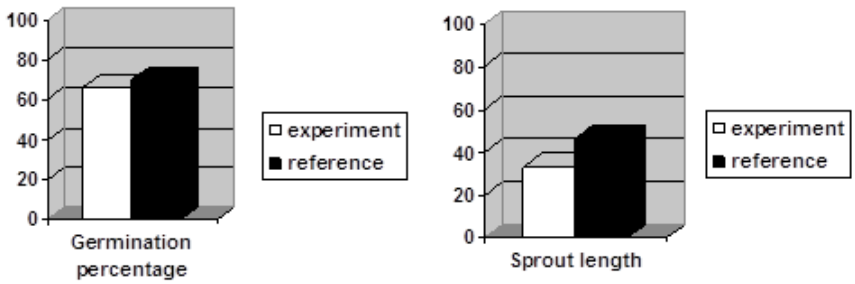


Fig. 5. Experiment 5.

The "Germination percentage" diagram shows the results of the comparative calculation of the percentage of germinated seeds on the 7th day in the experimental and reference samples.

The "Sprout length" diagram shows the results of calculation of the percentage of seeds in which the sprout length is greater than the average sprout length in this experiment.

In this experiment, the effect of a constant magnetic field induced by the magnets of a home audio system was studied. Treatment and germination were conducted in close vicinity (0.7 m) to the magnetic field source. The germination percentage of the experimental sample was lower than that in the reference sample. With respect to the number of seeds with the sprout length greater than the average sprout length in this experiment, the results of the experimental sample were also worse than in the reference sample.

Discussion

To optimize the technique of exposing dry seeds to a low-frequency low-intensity magnetic field, experiments were conducted under different environmental conditions associated with the presence of natural and technogenic electromagnetic fields. In the development of the technique, it was found that the magnetic field treatment of seeds leads to an increase in the sensitivity of the seed cells to external fields. Seed treatment with a low-frequency magnetic field under natural conditions and germination under the same conditions (Experiment 1) give a positive effect in the development of spouts compared to the reference sample because the cells of the germinating seed receive an additional stimulation pulse. In addition, the response to the harmful effects of technogenic fields increases. This fact is illustrated by Experiments 2-5, which were conducted in the presence of various technogenic fields. The development of seeds in

the experimental samples was worse than that in the reference sample. Thus, seed treatment and germination under the same conditions with exposure to technogenic fields (Experiment 2) shows a delay in development in the experimental sample with respect to both seed germination and sprout length in comparison with the reference sample. In the case of treatment in the zone of action of technogenic fields and germination under neutral conditions in a grounded metal cabinet (Experiment 3), the results show that, in the absence of technogenic fields, the experimental sample is superior to the reference sample in the sprout length and inferior in the germination percentage. In Experiment 4, treatment was conducted at a distance from the television tower; this condition led to the same results on the germination percentage in the experimental and reference samples; germination under neutral conditions of a metal cabinet also showed the advantage of the experimental sample. The experiment (seed treatment and germination) conducted under the action of a constant magnetic field generated by a home audio system yielded results identical to those of Experiment 2, where the seeds were subjected to technogenic fields of industrial origin.

These facts suggest that, at the initial stage of development of an organism, in the period of active cell division, the role of the energy component of the environment becomes more significant. Therefore, the absence of any field of artificial technogenic origin is of particular importance in this period.

Taking into account the general laws governing the development of organisms at the cellular level, we can assume that technogenic fields play a particular role for a human body at the moment of impregnation and initial development of the body when the cells of the embryo most actively respond to the surrounding fields.

Conclusions

The exposure of dry seeds to a low-frequency low-intensity magnetic field of natural origin has a positive effect on the development of plants.

Technogenic fields of industrial and domestic origin negatively affect the growth and development of organisms.

It is necessary to take into account the negative impact of artificial technogenic fields on the human body, especially during pregnancy.

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RECENT ACHIEVEMENTS IN MICROBIOLOGICAL PLANT PROTECTION

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Introduction

Plant pests need to be controlled to maintain the quantity and quality of food and feed produced by growers around the world. Different approaches may be used to prevent, mitigate or control of plant pests. Beyond good agronomic practices, growers often rely heavily on pesticides that have contributed significantly to the improvements in crop productivity the past years [6, 8].

However, the environmental pollution caused by use of pesticides, has led to considerable changes in people's attitudes towards the use of pesticides in agriculture. Under such circumstances exploitation of living organisms to reduce the activities of crop pests causing activity of useful micro-organisms seems to be the most appropriate alternative to chemicals. Biological control refers to the purposeful utilization of introduced or resident living organisms to suppress the activities and populations of one or more plant pest [4, 6].

There are the following trends in bio-pesticides development:

» Bio-pesticides are not new they are in use since ages but grower/ industry acceptance happened in last 7 – 8 years.

» Global bio-pesticide market was $ 1.3 billion in 2011, and 63 % of it was microbial basis active ingredients.

» Projected to grow to 3.2 billion by 2017 (15.8 %).

» North America dominates bio-pesticides market with share of 40 %.

» Asia pacific and Europe are expected to be fastest growing market in near future due to stringent regulations for pesticides and increasing demand for organics.

» Overall bio-pesticides represent 2 % of pesticides market.

» Growth is hindered by well established crop protection chemical market, variable efficiency of bio-pesticides, less awareness among growers.

The overwhelming advantages of bio-pesticides are their high selectivity to target pests and safety to non-target and beneficial organisms. In the sustainable intensification of agriculture through green economy, the biopesticides have an immense role. They are amenable to bio-intensive pest management and ideally suited for organic niche products including export-oriented commodities. They can also be tailored to IPM programmes for increased efficacy, higher yield and lower chemical load. These are also effective as pesticide resistance management tools in order to prolong the life span of precious green chemical pesticides. The biopesticide development must also be targeted for integrated cropping systems. They are renewable, sustainable, offer an improved impact profile, and reduce pesticide residues.

Materials and methods

The ultimate success of bio control depends on how well the searching and screening process is done. There is no single way to search or screen. Both depend on the target pathogen, the crop and the cropping system. These isolates are then screened for their activity against the pathogen in laboratory and green house conditions. Any suitable isolate found are then evaluated for their efficacy under the field conditions [8, 9].

The isolates that fail to perform well in the field are again subjected to the in vitro evaluation to ascertain the cause of their failure before they could be rejected.

Testing in laboratory and field experience was carried out in four repetitions ran-

domized respectively, in accordance with the general requirements of experiences of this kind [10].

Results and discussions

The role of useful microorganisms in the biological protection of plants. Plant protection has immensely contributed to the success of Green Revolution and sustained production of food and feed. Due to intensification of

agriculture, loss of biodiversity, reliance on monocropping, and biotic stresses due to pests and pathogens have increased and led to several problems like pesticide residues in food stuff, environmental pollution, disorder of ecological equilibrium. For these reasons, management of pests will continue to play a pivotal role in sustaining production and productivity in agriculture [3, 4].

United States Environmental Protection Agency (EPA) for characterizations of microorganisms, whether indigenous or introduced are an important component of the environment, have proposed the following classification of Bio-pesticides as certain types of pesticides derived from natural materials such as animals, plants, bacteria, and certain minerals:

1. Microbial pesticides – consist of micro-organism (fungi, bacteria, viruses and protozoa) as active ingredient.
2. Biochemical pesticides – plant extracts, pheromones, soaps and fatty acids, natural plant growth regulators. Avermectin, Pyrethrins, Spinocid from natural products but not bio-pesticides.
3. Natural enemies – parasitoids, predators and pathogens of pests.

Bio-pesticides use is directed in:

» First deployed on speciality high value crops, vegetables and greenhouse crops to manage residue.

» Now they are being applied on all type of broad acreage crops (cereals, oilseed, sugar, fiber, forage grains).

» To date 400 plus active ingredients have been registered across globe and 1250 plus products based on these active ingredients have been registered.

» Products based on various *Bacillus thuringensis* strains dominate the market.

» Other major products are based on *Beauveria, Metarhizium, Trichoderma, Bacillus subtilis, Pseudomonas flourescens* and entomopathogenic NPV.

Commercialization of biological preparations. Although the number of bio control products in plant disease management is increasing, these products still represent only 1% of the agricultural control measures while fungicides account for 15% of total chemicals used in agriculture [1, 2, 5]. In recent years many small and large entrepreneurs have entered into the commercial production of bio control agents resulting into the entry of various bio- control products into the world market.

Commercialization of bio-control products is a multi-step process involving a wide range of activities [5, 6, 7]: isolation of micro- organism from the natural ecosystem;

• Evaluation of bio-agent both in vitro and under glass house conditions; testing of the best isolate under field conditions; mass production; formulation; delivery; compatibility, registration and release.

Although the development and approval of a biological preparation activities are needed expensive long, though the world were recorded several biological means (table 1).

Recent Achievements microbiological plant protection in Moldova. Based on the severity of the phytosanitary issues, caused by the action of pests on the background of worsening ecological situation resulting from the application of pesticides to combat them, now the need to develop alternative means of plant protection increases, among which a more important one being the biological products made of various useful microorganisms. Microbiological protection recorded remarkable results in controlling

various pests (pathogenic agents of diseases, insect and mite pests, and weeds), permanently extending the range of the useful agents that are used, as well as the spectrum of the protected crops. There is a growing awareness that microbial pesticides are inherently different from chemical pesticides, with fundamentally different modes of action and that they should therefore be assessed on their own merits and problems and data requirements should be set accordingly. However, the basis for the proposed data requirements needs improved scientific justification.

Table 1. List of bio control products

No.	Bio control agent	Product	Target disease/ organism	Crop	Manufacturer
1	*Ageobacterium radiobacter* strain 84	Galtrol	*Agrobacterium tumefaciens*	Ornamentals, Fruits, Nuts	AgBioChem, USA
2	*Ageobacterium radiobacter* strain K 1026	Nagol	*Agrobacterium tumefaciens*	Ornamentals, Fruits, Nuts	Bio-care
3	*Bascillus subtillus* strain GB34	GB34	*Rhizoctonia, Fussarium*	Soyabean	Gustafon, USA
4	*Bascillus subtillus* strain GB03	Kodiac, companion	*Rhizoctonia, Aspergillus*	Wheat, barley, peas	Growth products, USA
5	*Pseudomonas aureofaciens* strain TX-1	Bio–jet, spot less	*Pythium, Rhizoctonia solani*	Vegetables and Ornamentals in green houses	EcoSoil system
6	*Pseudomonas fluorescence* strain A506	Frostban	Fire blight, bunch rot	Fruit crop, Tomato, Potato	Plant Health Technologies
7	*Streptomycine griseoviridis*	Mycostop	Soil borne pathogens	Ornamentals, Tree seedlings	Kemira Oy, Finland
8	*Trichoderma harzianum* T-22	Root shield, plant shield	Soil borne pathogens	Green house nurseries	Bio works, USA
9	*Trichoderma harzianum* T-39	Trichodex	*Botrytis cinerea*	Most of the food crops	Bio works, USA
10	*Ampelomyces quisquallis* isolate M-10	AQ10	Powdery mildew	Fruits, Ornamentals, Vegetables	Ecogen, USA
11	*Aspergillus flavus* AF36	Alfa guard	*Aspergillus flavus*	Cotton	Circleone globa,USA
12	*Gliocladium catenulatum* strain JI446	Prima stop soil guard	Soil borne pathogens	Vegetables, Herbs, Spices	Kemira Agro Oy, Finland

The scientists of the Institute of Genetics, Physiology and Plant Protection of Academy of Sciences of Moldova, by isolating, identifying and determining biological particularities of various useful microorganisms (viruses, bacteria and fungus) have developed original technological procedures of production and application and submitted for approval some biological preparations effective in controlling pests with the most severe impact on crops. These were submitted for approval or extension of the scope of use of National Council for Approval of Products for Phytosanitary Usage and Fertilizers.

Baculoviral preparation Virin-HSP was elaborated to fight *Helicoverpa armigera* which in recent years recorded expanding the area of spreading, as well as the spectrum

of attacked crops. The product is made on the basis of nuclear polyhedrosis virus with a high degree of specificity of the insect host and has titer of 6 billion polyhedra/g in the form of paste. The preparation has specific action on insects and causes noctuide epizootic phenomena acting on subsequent generations and protecting crops. In the Republic of Moldova it is recommended to protect vegetables (tomatoes, peppers), technical crops (sugar beet, sunflower, and tobacco), and cereals (corn), decorative and medicinal plants. Consumption norm – 0,2 kg/ha.

Paurin - contact bactericide obtained under bacterium *Pseudomonas fluorescens* BKM CP 330 D expected to combat the pathogen *Agrobacterium tumefaciens* Sm. fnd Town. in orchards and grapes, as well as root rots in vegetable crops, soybeans (*Fusarium gibbosum, Rhizoctonia solani, Pytium debaryanum, Alternaria sp., Penicillium sp., Aspergillus sp*.) and in potato (*Fusarium solani, Pectobacterium carotovorum*).

Trichodermin Th-7F SC - fungicide constituted under *Trichoderma harzianum* strain Th-7F (CNMN F-16) expected to combat pathogens in vegetable crops (*Rhizoctonia solani Kuechn, Botrytis cinerea* Pers, *Sclerotinia sclerotiorum* de Bary, *Myrothecium verrucaria, Ascochyta cucumis* (*melonis*) Fautr. Et Roum, *Colletotrichum lagenarium* E. et H., *Fusarium spp., Streptomycetes, Pythium debaryanum* Hesse); decorative (*Rhizoctonia solani* Kuechn, *Botrytis cinerea* Pers, *Sclerotinia sclerotiorum* de Bary; *Fusarium spp., Verticillium dahliae* Kleb.); tobacco (*Fusarium spp., Vericillium dahliae* Kleb., *Thielaviopsis basicola* Ferr, *Pythium debaryanum* Hesse, *Botritys cinerea* Pers); grape-vine (*Botrytis cinerea*) Pers.

Trichodermin SC proposed as liquid fungicide constituted under *Trichoderma lignorum* strain M-10 expected to fight pathogens in sunflower (*Sclerotinia sclerotiorum*), soybean (*Fusarium spp.*), grape-vine (*Botrytis cinerea*), and ensuring high biological, economic and ecological effectiveness. The preparations enhance also the biological indicators of crop development.

Gliocladin SC - fungicide obtained on the basis of the active substance of *Trichoderma virens* strain 3X, expected to fight white rot in sunflower (*Sclerotinia sclerotiorum*), soybean (*Fusarium spp*.), grape-vine (*Botrytis cinerea*). The preparation ensuring high biological, economic and ecological effectiveness.

Prospective directions of crop production by applying useful microorganisms

Induced Systemic Host Resistance. Induced resistance is the most indirect form of antagonism. Induced resistance can be local or systemic. Salicylic acid (SA) and non-expressor of pathogenesis-related genes1 (NPR1) are key players in systemic acquired resistance *Trichoderma harzianum* when inoculated on to roots or on to leaves of grapes provides control of diseases caused by *Botrytis cineria* on leaves spatially separated from the site of application of the bio control agent. Many classes of compounds are released by the *Trichoderma sp*. into the zone of interaction and induce resistance in plants. The first class is proteins with enzymztic or other activity. Fungal proteins such as xylanase, cellulases and swollenins are secreated by *Trichoderma* species. *Trichoderma endochitinase* can also enhance defense, probably through induction of plant defense related proteins [1, 8].

Other proteins and peptides that is active in inducing terpenoid phytoalexin biosynthesis and peroxidase activity in cotton, e.g., the small protein, SM1, which has hydrophobin-like properties, were found to be produced by strains of *T. virens*. Another hydrophobin-like protein produced by T22 that induces both enhanced root development and disease resistance was identified. Another group of proteins that induce defense mechanisms in plants are the products of avirulence-like (Avr) genes.

Infectious diseases of insects. Infectious agents are living units that must invade the insect host in order to initiate an infection. Unlike parasites and predators, pathogens do not always kill the hosts. Infection usually involves reproduction of the agent. The specific characteristics of the infective stages of pathogens greatly influence how

they contact and infect their hosts. The infectious agents responsible for transmission of the pathogen are susceptible to many environmental factors. Survival of the infective stage of insect pathogens outside the host is a major factor in the development of microbial insecticides. Some pathogen species may be very host specific, while others may be able to infect a wide range of insect species. The host range of a pathogen is especially important when considering a non-indigenous pathogen for introduction into a new habitat. Sub-lethal infections are not uncommon and these may include behavioural and developmental changes as well as a decrease in the fecundity of infected adults. Insects are infected by an incredibly large number and diversity of pathogen species. Most insect pathologists believe that there are actually more species of insect pathogens than there are species of insects. The major pathogen groups containing species that infect insects are: viruses, bacteria, fungi, protozoans, microsporidia, and nematodes.

Insect pathogens are used in biological control in at least three different ways: inundative applications, inoculative releases, management of naturally occurring pathogens, and introduction of exotic pathogens as classical biological control agents.

• Inundative applications are those in which insect pathogens are applied in large quantities with the goal of killing as many individuals of the pest population as quickly as possible. Replication of the pathogen in the host and production of additional infectious propagules may be desirable, but is not usually required for microbial insecticides to be effective.

• Inoculative applications are those in which small quantities of insect pathogens are applied or released into an insect host population. The goal is to produce infections in at least a few hosts, which will, in turn, produce numerous infectious propagules that will infect many more susceptible hosts.

• Management of naturally occurring pathogens are important components of the natural enemy complex of many insect species, including pest species. Some groups of pathogens, such as microsporidia, may not always maintain host insect densities below economic thresholds, but they suppress the rapid increase of pest populations. Insects pathogens and are often responsible for the decline of populations that have exceeded the economic threshold. In most cases the major goal for managing naturally occurring insect pathogens is to elicit an epizootic earlier in the season, before the host densities have exceeded the economic threshold. This can be accomplished by inoculative releases of the pathogen or by changing cultural and phyotosanitary practices to promote an epizootic.

Conclusions

Biological control is the best alternative to pests suppression. Bio agents themselves being non-pathogenic to plants need to be formulated in a way that favors the activity and survival of microbe it contains.

The pace of biopesticide research is increasing. From the beginning of 2006 through early April 2015, 4 biological preparations had been registered with National Council for Approval of Products for Phytosanitary Usage and Fertilizers. Many of these are agriculture related and registered for use as such. The value of microbiological means of protection developed by the scientists of the institute does not consist just in their considerable biological, environmental and economic effects, but also their possibility to be included in conventional and organic farming systems.

Achieving maximum efficiency of biological preparations may be registered at the establishment IPM, which is a kind of management using different strategies and techniques such as cultural, biological and chemical in controlling insect pests and diseases in agricultural crops.

In further research and development on biopesticides must be given high priority and agriculturists in general and policy makers in particular must be educated about the dangers of handling and application of chemical pesticides, and importance of sustain-

able agriculture to feed ever growing population.

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SCIENTIFIC AND RESEARCH PRIORITIES OF ACADEMICIAN A.A. ZHUCHENKO

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Academician A.A. Zhuchenko's basic researches in special genetics and ecological genetics of cultivated plants, recombinogenesis, biomonitoring, agroecology, plant breeding, variety testing and seed breeding, as well as on the strategy for adaptive intensification of agriculture have gained worldwide recognition. A.A. Zhuchenko is widely known to scientific communities in Russia and elsewhere as a prominent biologist who created an ecological genetics school (61 doctoral and Ph.D. theses defended under his guidance and supervision); he published 665 scientific papers, including 25 monographs, that have won a high appraisal of global scientific community (for reviews of A.A. Zhuchenko's works see: Gichner T., Biologia plantarum, 1982, Vol. 24, N6. P. 406; Robbelen G., Z. fur Pflanzenzucht. 1983. Bd 91, N1, S.86; Grebenschikov I., Biol. Zen tralblatt. 1984. Bd 103, N4, S.103;see also publications about A.A. Zhuchenko: Rich V. Scientists take share of blame for this year's poor Soviet harvest, Nature: Int. Weekly J. Sci. 1987. Vol. 329, N 6138, P. 382; Zhuchenko Alexander, Who's Who in the world: 9th edition 1989-1990. Wilmette (USA), 1990; Zhuchenko Alexander Alexandrovich, Int. Biogr. Centre: men of achievement. Cambridge, 1991, and many others).

For the first time in contemporary world practice, A.A. Zhuchenko has carried a systems analysis of the adaptive potential of cultivated plants, identified crucial features and qualitatively new mechanisms of adaptive responses of plants during ontogeny and phylogeny, substantiated and formulated the key concepts of plant genetics, ecological genetics of cultivated plants, ecological and genetic foundations of the adaptive system of plant breeding, adaptive crop production, and the strategy for adaptive intensification of agriculture.

Academician A.A. Zhuchenko is among the earliest who advanced special genetics of cultivated plants based on first-time-ever extensive experimental evidence from long-term comprehensive studies on the genus *Lycopersicon* (Tourn.) Mill., including evolution, taxonomy, physiology, embryology, cytology, and biomathematics, examining world plant collections for yield, morphological, physiological and cytological traits, developing lines, forms, mutants, multimarker mutants, cultivars, cultivar genealogy, growing techniques and crop management practices, heterotic hybrids, methodological basis for estimating recombination frequency, combining ability, constructing genetic and cytological maps, refining pot trials, variety trial and seed breeding systems, etc. The monograph *Tomato Genetics* (1973) came to be one of the first books on special genetics of cultivated plants in the world literature, highlighting, for the first time, crucial genetic features of a model plant, such as tomato, both in terms of general and special genetics, and their significance in tackling plant breeding, seed growing, and crop management tasks.

In the latter half of the XX century, many biological disciplines began to undergo the process of ''ecologization''. Ecological genetics (Ford, 1964) came to be one such newly emerging area combining genetic and ecological knowledge. The fourth and final edition of the book on ecological genetics by the British biologist E.B. Ford was published in 1975. The second edition of the book on ecological genetics by German

geneticists K. Stern and P. Tigerstedt appeared in 1974. The fundamental notions dealt with in these and other publications on ecological genetics practically did not differ from those treated in famous works on population genetics. The authors have explored the evolution and adaptation of natural populations in particular ecosystems. The noted American biologist and geneticist R.C. Lewontin, who has made a significant contribution to the development of the mathematical basis of population genetics and theory of evolution, wrote, by way of criticizing the one-sidedness of the approach of the ''ecological genetics'' school that emerged in the 1970s, that in the long run it is not enough to demonstrate that hot summer favors bandless snail shells and that snails with a yellow shell occur in areas with colder winters (Lewontin 1978). The authors of publications entitled ''ecological genetics'' were criticized by eminent geneticists for their attempts to combine genetics and ecology into a single discipline as lacking unity and integrity, particularly for the formal approach taken in combining the above two disciplines and for seeking to give ''ecological genetics'' the status of an independent scientific discipline without having elucidated the fundamental principles and mechanisms of the effect of ecological factors on heritability and variability of characters in higher organisms (Lerner, 1976; and others). Published in 1980 was academician A.A. Zhuchenko's book *Ecological Genetics of Cultivated Plants: Adaptation, Recombinogenesis, Agrobiocenocis* based on extensive experimental evidence on recombinogenesis in *Drosophila*, tomato, *Arabidopsis*, corn, wheat, etc., accumulated at the world's first Institute of Ecological Genetics founded by him, and comprehensive generalized data on adaptation, agrobiocenology, and ecology. For the first time the adaptive potential of cultivated plants is treated as a function of interrelationship of the genetic systems of ontogenetic and phylogenetic adaptation. The author also looks at the resistance of plants to abiotic and biotic stresses, formation of genotypic variability available for selection to act upon (taking recombination system functioning as an example), establishes the relationship between potential productivity and ecological sustainability at the level of species, agrocenosis and agroecosystem, as well as the habitat-forming role of plants and agrocenoses, develops the methodology for increasing the level and broadening the range of genotypic variation in plants through induced recombinogenesis and reduced selective elimination of recombinants.

The academician A.A. Zhuchenko's school (1979-1987) was the first to start extensive practical and basic-research application of remote sensing and control of plants. A.A. Zhuchenko was the first to determine the significance of plant biomonitoring at the level of plant, population and agrolandscape for studying adaptation in the genotype-environment system. For the first time a problem-oriented information-measurement complex comprising a number of modules was constructed for ecogenetic studies and applied research, proper instrumentation and automation of scientific experiments in biology developed, including aerial photography and satellite imagery, with concurrent twenty-four-hour monitoring of the dynamics of readings from sensors recording plant growth and development, photosynthesis, transpiration, water uptake and yield formation of various crops and crop varieties, in phytotrons and in the field (Zhuchenko, Zelikovsky et al., 1981). To this end, the Center for Automation and Metrology (CAM) and the Institute of Ecological Genetics of the Academy of Sciences of Moldova developed and put into operation the first-ever problem-oriented information-measurement complex, BIOTRON, representing an automated research system (ARS BIOTRON). The ARS BIOTRON made it possible to conduct comprehensive, multiparameter studies of the dynamics of plant adaptive responses at the organ, organismal, and population levels under controlled environmental conditions, with automatic processing of the data obtained through application of specialized software packages. The above studies were being carried out under the guidance and supervision of academician A.A. Zhuchenko at the Institute of Ecological Genetics of the Academy of Sciences of Moldova and at the Design Office ''Biopribor'' (Bioinstrument) (research efforts of and scientific papers by scientists such as Z.I. Zelikovsky, Yu.A. Ton, E.I. Kleiman, E.I. Blank, and others) where the first-ever water flow, turgescence, ''leaf-air'' temperature, plant growth, fruit growth and other sensors were developed. All of this made the generation of qualitatively new information on adaptive responses of crop plants under controlled environmental conditions possible. Phytomonitoring came to be a new methodology for continuous, long-term observation of the dynamics

of morphophysiological, biochemical and ecological parameters of a growing or a dormant plant providing a most accurate assessment of the adaptedness of a crop variety to a given environment. The new methods offered a tool for following, in a remote mode, the dynamics of responses of individual crops, crop varieties, forms and genotypes to critical environmental factors affecting plant productivity.

As a result, the academician A.A. Zhuchenko's school has won the highest appraisal of the Presidium of Russian Academy of Sciences (RAS). It has been demonstrated, for the first time ever, that reliable comparative characteristics (patterns) of expression and redistribution of adaptively significant and agronomically important characters across crop species, varieties, hybrids and plant forms can only be obtained through simultaneous multi-parameter (multi-variable) recording of relevant information in problem-oriented modules enabling not only control of variables, such as temperature, humidity, light intensity and mineral nutrition, within a given range of values, but also assessment of the dynamics of key adaptive responses and interrelations of these. At the suggestion of academician B.E. Paton, the above research efforts and research-and-development complex were transformed into an All-Union Biological Research Center within the system of RAS.

In the 1980s, A.A. Zhuchenko's school examined, for the first time, the problem of an ever increasing cost of each additional food calorie which, as demonstrated in his book *Energy Analysis in Agriculture* (1983), came to be a sort of ''cost'' for disruption of biological balance in agroecosystems resulting from genetic uniformity of crop plants at the species, population and organismal levels, as well as changes in the structure (composition) of agrobiocenosis subsystems due to extensive fertilizer and pesticide application. Thus, the doubling of the yield potential of major farm crops calls for a 10-fold increase in inputs of depletable energy, including mineral fertilizers, pesticides, farm equipment, etc. While extensive cropping systems produced 40 to 50 food calories per unit anthropogenic energy, as few as 2–4 food calories, i.e. 10-20-fold less, are normally produced under chemico-technogenic intensification (Zhuchenko et al., 1983). In this paper, the authors examine a strategy for improving agroecosystem productivity through more efficient natural energy resource utilization with special emphasis on the most judicious exploitation of edaphoclimatic conditions in each crop producing area, as well as optimal agroecosystem organization pattern. Moreover, the crucial and most challenging task of crop breeding and management practices involves overcoming or, at least, slowing down the exponential increase in depletable resource inputs per unit yield increase, including food calorie. It is for this reason that a paradoxical situation has developed by early XXI century where a sector relying on most energy-efficient organisms – poikilothermal plants utilizing virtually unlimited and ecologically safe solar and atmospheric (CO2, N, O2) resources – ended up among the most resource- and energy-wasteful and environmentally unsafe sectors. By and large, each succeeding improvement over the attained maximum yielding capacity and total yield, even where the crop is grown on the ''best possible'' soil, tends to become increasingly more input-intensive and ecologically vulnerable, such that the worse the edaphoclimatic conditions, the higher the ''cost'' of each yield increase, with the coefficient of utilization of mineral fertilizers, ameliorants and other chemico-technogenic inputs decreasing, particularly so with increasing rates of application. Concurrently, the extent of environmental pollution is dramatically increased. This calls for increasing emphasis on the capacity of crop plants for more efficient utilization of not only anthropogenic resources (resource- and energy-efficiency coefficients of cultivars) but also not-readily-available soil mineral nutrients and moisture. Note that just three elements – carbon, hydrogen and oxygen – account for 98.5 % of the weight of living organisms, with more than 95 % of a plant's dry matter essentially being the solar energy accumulated during photosynthesis. It is believed that in order to produce 1 g of dry matter, plants utilize an average of 1.5 g of CO2 captured from 2.5 m3 of air (Pal, 1973). Annually synthesized biomass amounts to 180–200 billion tons, of which less than 4 % is used as agricultural produce. And the fact that crop production came to be an energy-wasteful (exponential growth of fossil fuel-based energy inputs per additional unit of yield increase) and most environmentally unfriendly sector (water and wind erosion of soil, destruction of natural landscapes and disruption of the water regime of rivers, pollution of environment with pesticide residues, nitrosamines, etc.), not only locally but

also globally, is at variance with both natural-science laws and common sense. At the same time, bioenergy analysis suggests that in the energy balance of yield formation, even in technologically most intensive agroecosystems, the share of the solar energy is in excess of 99 %. Therefore, according to A.A. Zhuchenko, the real significance of using chemico-technogenic inputs (fertilizers, ameliorants, pesticides, irrigation, etc.) is that of ensuring, with the aid of small anthropogenic energy flows, maximum utilization of solar energy by agrophytocenoses, as well as by their food chains and trophic levels, rather than replacing photosynhesis, respiration and other processes naturally occurring in plants, soil and agrobiogeocenoses (Zhuchenko, 1983, 2010).

A.A. Zhuchenko, for the first time ever, highlighted the importance of evolutionary-genetic, ecological and bioenergetical approaches which is crucial to the shaping of agrobiocenotic genetics as a major branch of ecological genetics of cultivated plants, considering that the accumulated evidence on the genetic nature of ontogenetic and phylogenetic adaptive responses at supraorganismal levels (population, biocenosis, ecosystem, landscape, and even biosphere) is fairly extensive. Therefore, it is no mere chance that studies in autecological and synecological population genetics, phytocenotic and symbiotic genetics and breeding of cultivated plants become increasingly common (Zhuchenko, 1980, 2010).

The main subject of research in ecological genetics of cultivated plants is the relevant adaptive potential of crop plants viewed as a function of its component genetic programs of ontogenetic and phylogenetic adaptation, as well as the effects of their interrelationship. An approach like this, adopted by A.A. Zhuchenko, is primarily due to the dual nature of the adaptation itself attained by organisms through their modificative and/or genotypic variability. Such functional structuring of the adaptive potential can be traced back to works of Darwin, Baur, Darlington, Layzer and others. Note that while in the XIX century the adaptation problem was a central one in biology and synthetic theory of evolution, nowadays it came to be so in economy, technology, politics, etc. as well. As a basis for systematization and analysis of huge data arrays accumulated during biological studies of the adaptive potential of higher eukaryotes, including cultivated plants, A.A. Zhuchenko employed a discrete-systems approach enabling one to initially structure the system functionally into its constituent elements and then, through analysis of realization patterns of the individual system components, as well as interactions of these, to elucidate the mechanisms of functioning of the adaptive system as a whole at various levels of its organization (individual, population, species, cenosis, ecosystem, and biosphere).

A.A. Zhuchenko's basic researches are protected by 24 certificates of authorship and set forth in his notable monographs: *Tomato Genetics* (1973), *Ecological Genetics of Cultivated Plants: Adaptation, Recombinogenesis, Agrobiocenosis* (1980), *Adaptive Potential of Cultivated Plants: Genetic and Ecological Bases* (1988), *Adaptive Crop Production: Genetic and Ecological Bases* (1990), *Strategy for Adaptive Intensification of Agriculture* (1994), *Basic and Applied Research Priorities of Adaptive Intensification of Crop Production in the XXI Century* (2000), *Adaptive System of Plant Breeding: Genetic and Ecological Bases, in two volumes* (2001), *Ecological Genetics of Cultivated Plants* (2003), *Ecological Genetics of Cultivated Plants and Agrosphere Problems: Theory and Practice, in two volumes* (2004), *Resource Potential for Grain Production in Russia: Theory and Practice* (2004), *Adaptive Crop Production (Genetic and Ecological Bases): Theory and Practice, in three volumes* (2008, 2009), *Ecological Genetics as an Independent Scientific Discipline: Theory and Practice* (2010), *Adaptive Strategy for Sustainable Development of Agriculture in Russia in the XXI Century (Genetic and Ecological Bases): Theory and Practice, in two volumes* (2009, 2011), *Mobilization of Genetic Resources of Flowering Plants through Their Identification and Systematization* (2012), *The Role of Flowering Plant Resource Mobilization, Identification and Systematization in Shaping the Adaptive-Integrated System of Agrocenosis, Agroecosystem and Agrolandscape Protection* (2012) and others. In these monographs, the author outlines genetic and ecological bases of the adaptive potential of cultivated plants, unravels qualitatively new effects of the integrated functioning of its component genetic systems of ontogenetic and phylogenetic adaptation, identifies priorities in managing adaptive responses in plant breeding, variety trials and seed growing to be considered in developing comprehensive plant breeding and crop man-

agement programmes, agroecological macro-, meso- and microzoning of agricultural territories, designing adaptive agroecosystems and agrolandscapes, implementing an integrated plant protection programme, switching to an adaptive-innovative strategy for intensification of crop production and agriculture in general.

The global novelty of the key theoretical concepts and practically significant conclusions set forth in A.A. Zhuchenko's monographs lies in the fact that results from basic research into adaptive responses and mechanisms of biocenotic self-regulation in agroecosystems and agrolandscapes, while having been generalized in the synthetic theory of evolution, biocenology, ecomorphology, phytogeography and other disciplines, largely remain outside the crop production theory and practice. Yet, it is the lack of proper basis for and natural-scientific soundness of the agricultural development in the XX century that came to be the prime cause of its global crisis by the turn of the XXI century. The analysis of negative trends in modern global and domestic agriculture performed by A.A. Zhuchenko suggests that these stem from violation of laws and principles of adaptive management of biologically complex ecosystems, such as agrocenoses and agrolandscapes. Seeing the management of adaptive responses of crop species and varieties, as well as other agroecosystem biotic components, as a primary objective of ecological genetics of cultivated plants, he substantiated the advisability of a systems analysis of traditionally separately explored issues, such as adaptation, recombination and agrobiocenosis. He demonstrated that it is the comprehensive approach to studying the above problems that enables efficient use of basic science achievements with a view to enhancing production and environment-improving functions of agrolandscapes. Based on this, he formulated the cardinal ecogenetic principles of a strategy for adaptive intensification of crop production. These are: optimization of the spatiotemporal organization of agrophytocenoses, development of crop varieties and hybrids combining high potential productivity and ecological stability, designing agroecosystems and agrolandscapes on the basis of evolutionary approach (enhanced biodiversity of crop species, their agroecological specialization, exploiting biocenotic self-regulation mechanisms and structures), spatiotemporally adaptive deployment of farm crops in macro-, meso and microzones, adaptive land management, implementing adaptive-integrated plant protection programmes, transition to a strategy of adaptive intensification of agriculture.

Theoretical conclusions of academician A.A. Zhuchenko regarding the adaptive potential of cultivated plants open up fundamentally new possibilities for managing their adaptive responses during both ontogenesis (variety-specific crop management practices, agroecological macro-, meso- and microzoning of an agricultural territory, designing adaptive agroecosystems and agrolandscapes, an adaptive-integrated plant protection programme) and phylogenesis (an adaptive plant breeding system ensuring functional interdependence of stages in the development of new varieties and hybrids, their testing by state organizations, seed growing management, as well as the development of qualitatively new areas of selection (biocenotic, bioenergy-based, symbiotic, edaphic, ecological, design-aesthetic, etc).

The number of A.A. Zhuchenko's supporters and followers is rapidly growing. Many of those, in Russia and elsewhere, who have to do with land, agriculture, biology, genetics, ecology and who care about the destiny of people inhabiting the planet Earth, know him and think highly of him. To us, he came to be a guru to whom we are devoted, whom we follow and whose ideas we are expounding.

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CU DEMNITATE ŞI DRAGOSTE PENTRU PĂMÂNT ŞI OAMENI
Mebru Corespondent Andrei Palii la 75 de ani

Când încercam să determinăm componentele, care influenţează destinul unei persoane, de unde vine succesul în viaţă, putem uşor evidenţia cel puţin trei componente distincte:

» genetică (de la părinţi);

» şcoala (de la învăţători, nu de la treptele şi băncile şcolii, dar şi de la părinţi;

» efortul personal, munca zilnică, an de an, spre a urca treptele vieţii.

În personalitatea multstimatului m.cor. al A.Ş., profesorului universitar Andrei Palii, toate aceste componente au contribuit printr-o interacţiune benefică.

Vine din viţa de ţărani din satul Scorţeni, r-nul Teleneşti (născut pe 1 mai 1940). Dar de unde mai putea să vină, dacă nu din ţărani, din sat, un om, un copil, iubitor de pământ şi de carte. A avut noroc şi de învăţătorii de la şcoala din sat, care i-au cultivat dragostea pentru carte, pentru citire în profunzime.

Fiind eminent din şcoală, a păşit timid, dar sigur, pragul unei vestite (chiar celei mai vestite) şcoli agricole – din Cocorozeni, sat aproape vecin cu satul natal al viitorului academician. Această şcoală agricolă, a dat ţării zeci de oameni de creaţie, academicienii Andrei Lupan, Mihail Lupaşcu, Ilie Untilă, Alexandru Ciubotaru şi alţii; Simion Grosu, Ion Ustian – conducători de stat şi multe alte personalităţi. Aici, în anii de studenţie a viitorului profesor universitar, activa un colectiv de adevăraţi profesionişti devotaţi neamului: Nicolae Lupan, Gavriil Hariuc, Boris Pucalov şi mulţi alţii, care cunoşteau şi iubeau profesia în profunzime, iubeau şi ţineau la copiii veniţi de la sate ca la ai lor. Aici a deprins ţăranul Andrei Palii munca, cartea, respectul pentru colegi şi profesori, omenia venită de la părinţi s-a cristalizat în suflet şi în atitudine.

De la şcoala agricolă din Cocorozeni la Universitatea Agrară – e o treaptă superioară, e o logică să urci o treaptă mai sus. La această universitate (pe atunci în a.1958-63 – Institutul Agricol) a continuat nu doar studiile, dar s-a implicat şi a însuşit metodele de cercetare ştiinţifică, sub conducerea unor profesori eminenţi: M.I. Sidorov, A.E.Covarschii, N.G. Nicolaeva, B.P. Pucalov, G.N. Vanicovici şi mulţi alţii. A deprins cercetarea ca o activitate foarte responsabilă, care cere sinceritate, acurateţe, capacităţi de analiză şi sinteză. Toate aceste calităţi le-a demonstrat cu prisosinţă viitorul profesor universitar. A absolvit universitatea eminent şi a satisfăcut serviciul în armată, a fost invitat şi selectat de către renumitul acad. A.E. Covarschii să fie parte din renumita şcoală ştiinţifică, începând cu doctorantura, apoi continuând cercetările în genetica porumbului în cadrul A.Ş.

Din anul 1965 s-a dedicat cercetărilor ştiinţifice în domeniul geneticii şi ameliorării plantelor, fiind aspirant la Secţia de Genetică a plantelor din cadrul AŞM. Ulterior, fiind ales prin concurs, ocupă funcţia de colaborator ştiinţific inferior (1968 – 1971), iar apoi de colaborator ştiinţific superior (1971 – 1974) în aceiaşi Secţie. În anul 1970 a susţinut teza de candidat (doctor) în ştiinţe agricole în cadrul AŞM. Între anii 1974 – 1975 a fost şef de laborator pentru Ameliorarea calităţii bobului de porumb în Institutul de Cercetări Ştiinţifice pentru porumb şi sorg AŞP „Hibrid".

Din anul 1975 pînă în prezent activează cu succes în cadrul Universităţii Agrare de Stat din Moldova, îndeplinind în diferite perioade, diferite funcţii: docent, prodecan, prof. univ., şef catedră de ameliorare genetică şi biotehnologie a culturilor agricole, decan al Facultăţii de Agronomie. Predă cursurile de Genetică şi Ameliorarea plantelor.

Gradul ştiinţific de Doctor Habilitat în biologie pe specialitatea 03.00.15. – genetica obţine în anul în anul 1984 la Institutul Ucrainean de Cercetări Ştiinţifice în Fitotehnie, Ameliorare şi Genetică „V. Iuriev".

La etapa iniţială de încadrare în lucru ştiinţific A. Palii a efectuat importante investigaţii cu privire la controlul genetic şi utilizarea în practică a diferitor tipuri şi surse de androsterilitate citoplasmică la porumb.

Începînd cu anul 1968 pînă în prezent acordă o deosebită atenţie cercetărilor teoretice şi practice consacrate *studiului şi utilizării variabilităţii genetice în procesul de ameliorare a porumbului pentru calitatea bobului*. Cercetările au fost iniţiate şi, la prima etapă, îndeplinite sub conducerea acad. A. Kovarski.

In urma cercetărilor efectuate împreună cu colaboratorii (m. c. al AŞM T. S. Cialîc, dr. h. V. I. Ţigănaş şi dr. Domnica Ţigănaş) a creat o colecţie de circa 400 analogi linii consangvinizate, ce

conţin în genotipul lor genele *o2, fl2, wx, su2*, care se foloseşte în programele de genetică şi ameliorarea calităţii bobului de porumb, atît la noi în ţară, cît şi în alte instituţii peste hotare. Pentru prima dată a fost descoperită o genă nouă a endospermului – *cfl2*, care condiţionează manifestarea fenotipică a genei *fl2* într-o singură doză şi un conţinut sporit de lizină şi meteonină în bob. Au fost depistate noi surse genetice cu un conţinut înalt de lizină în bob, inclusiv 5, care au în genotipul lor alela *o2* şi *2* – alela *fl2*.

Utilizarea diferitor metode genetice şi de ameliorare a permis crearea unui bogat material iniţial pe baza căruia au fost obţinute cîteva variante de hibrizi speciali de porumb: cu endospermul făinos şi un conţinut sporit de lizină şi proteină în bob; cu endospermul semisticlos sau sticlos şi un conţinut sporit de lizină în proteină; cu endospermul ceros şi un conţinut de 99 – 100% de amilopectină în amidonul din bob, în comparaţie cu 75% amilopectină în amidonul porumbului comun.

Aceste cercetări s-au finalizat prin crearea şi omologarea în Republica Moldova a 6 hibrizi speciali de porumb. Prof. univ. A. Palii este de asemenea coautor a 2 soiuri de grîu durum de toamnă şi 2 soiuri de soia, omologate în republică, dispune de 1 brevet de invenţie.

Dr. h. A. Palii prin cercetările sale, în mod deosebit, contribuie la dezvoltarea geneticii şi ameliorarea plantelor. Rezultatele cercetărilor au fost publicate în **210 lucrări ştiinţifice**, inclusiv o monografie. A publicat lucrări ştiinţifice în editurile din Rusia, Ucraina, Belarusi, Ungaria, SUA, Grecia, Iugoslavia, Japonia, inclusiv în revistele unionale de specialitate: *Genetica, Доклады ВАСХНИЛ, Сельскохозяйственная биология, Физиология растений* ş.a. A participat la diferite Congrese şi Conferinţe republicane şi internaţionale.

Prof. univ. A. Palii manifestă o activitate rodnică în domeniul pregătirii specialiştilor de înaltă calificare. Sub conducerea lui *au fost pregătiţi **2** doctori habilitaţi în ştiinţe şi **10** doctori în ştiinţe, inclusiv **3** cetăţeni din alte ţări. În prezent este conducător ştiinţific a 3 doctoranzi*. A publicat circa **40 lucrări didactico-metodice**, inclusiv trei manuale. În anul 1998 a editat primul manual „Genetica", în limba romînă pentru studenţii facultăţilor de biologie din republică apreciat cu *Premiul Naţional pentru Ştiinţă şi Tehnică (2004)*, iar în a. 2014 – un valoros manual în ameliorarea plantelor, apreciat cu premiul A.Ş. a Moldovei.

Ca o recunoaştere a meritelor sale în cercetările de genetică vegetală şi în activitatea didactică prof. univ. A. Palii devine în anul 1998 academician al Academiei Internaţionale de Ştiinţe Ecologice şi Securitate Vitală (or. S. Petersburg), iar în anul 2000 academician al Academiei Internaţionale în domeniul Învăţămîntului Agrar (or. Moscova), în anul 2007 – m.c. al ASM, iar în 2012 Doctor Honoris Causa al Universităţii de Ştiinţe Agricole şi Medicină Veterinară "Ion Ionescu de la Brad" din Iaşi.

Este decorat cu medalia „*Meritul Civic"* (1993), ordinul *„Gloria Muncii"* (2005), Medalia *„Dmitrie Cantemir"* (2010). Premiul Academiei de Ştiinţe a Moldovei pentru realizări ştiinţifice valoroase ale savanţilor în anul 2014 (martie, 2015).

În diferite perioade, prof. univ. A. Palii activează în calitate de membru al Consiliului Naţional pentru Acreditare şi Atestare, membru al Consiliului Naţional pentru decernarea Premiului de Stat, vice-preşedinte al Societăţii geneticienilor şi amelioratorilor din Moldova, membru al Consiliului Naţional pentru soiurile de plante, membru a Comisiei Naţionale pentru biosecuritate, membru al colegiului de redacţie a revistei „Ştiinţa Agricolă".

Dacă în cazul dat şcolile de profesură şi-au făcut datoria cu cinste, apoi efortul depus depus a fost decisiv în formarea personalităţii m.cor. al A.Ş. Andrei Palii, în formarea unei cariere de succes în ştiinţă, în pedagogie (pregătirea cadrelor) şi în familie. În toate a depus efort, dăruire totală şi a obţinut succes.

Realizările profesorului universitar Andrei Palii în ştiinţă (domeniile geneticii, pregătirii cadrelor) sunt remarcabile, recunoscute şi apreciate. Cei mai mari specialişti în fosta U.R.S.S. din genetica şi ameliorarea porumbului – acad. M.I.Hadjinov, G.S. Galeev, A.E. Covarschii, au susţinut, promovat şi admirat creaţiile tânărului savant. Continuând activtatea în domeniu, a trecut treptele şef de laborator la A.Ş.P. "Hibrid", şef de catedră, decan al facultăţii de agronomie a Universităţii Agrare, profesor emerit, laureat al Premiului de Stat, laureat al premiilor internaţionale.

Cu talentul de profesor adevărat, cu dragoste şi dăruire pentru tineri rămâne şi se manifestă în plină forţă creatoare.

MICU Vasile – *academician al A.Ş.M. şi al academiilor agrare din Rusia, Ucraina, Belorusia, România*
DUCA Maria– *academician al A.Ş.M., rector al Universităţii A.Ş.M.*

ABSTRACTS

UDC: 582.475.2:577.2

ABOUT THE RESULTS OF THE STUDY OF THE PROCESS OF SPERMOGENESIS AT *PICEA ABIES* (L.) KARST. *Ciubotaru Alexandru* // Journal of Academy of Sciences of Moldova. Life sciences. 2015, No 2(326), p. 4-15.

A re-light optical research spermatogenesis *Picea abies* (L.) Karst shows that contrary to the assertions Fergusson (1901) and Camefort (1978) sperm spruce are cells: each core is surrounded by a private limited cytoplasm plasma membrane. At the same time, this study clarifies our opinion expressed in the together published work on this issue (Moszkowicz, Chebotari, 1986). It was also found that the sperm from plazmogenez spruce is completed by the end of the programmed phase of fertilization, ie, by the mioment of the contact with the pollen tube ovule nucellus. Reproached position of the sperm nuclei for the entire period of growth of the pollen tube, creates a single view of the cytoplasm, but large size of male gametophyte spruce and careful microscopic analysis of the same drugs had allowed to clarify the phylogenetic question.

30 references, 10 figures.

Key words: angiospermae, evolution, gamet, gametophyte, gimnospermae, manofilie, phylogeny, pollen grain, sperm cell, sporophyte.

UDC: 582.675.1

CONTRIBUTION TO THE KNOWLEDGE OF VEGETATIVE ORGANS STRUCTURE OF *PAEONIA PEREGRINA* MILL. *Mereacre Anca, Boz Irina, Toma Constantin* // Journal of Academy of Sciences of Moldova. Life sciences. 2015, No 2(326), p. 16-19.

The authors investigated the vegetative organs structure of *Paeonia peregrina* Mill. (P. romanica Brândză), protected as natural monument included in the Critical list of vascular plants from Romania. The structure of tuberous roots reminds of the structure of a rhizome, the conductive tissue forming collateral vascular bundles, disposed on a circle and separated by large medullary rays. The mechanic tissues of the stem are represented by hypodermic collenchyma and sclerenchyma (periphloemic fibers, libriform and medullary rays). The foliar blade presents a bifacial-heterofacial structure, having a single layer of palisadic tissue, consisting of short and occasionally septate cells on the outer side. 12 references, 3 figures.

Key words: anatomy, natural monument, *Paeonia peregrina*.

UDC: 577.2.08 : 631.52

PRIMERII TRANSPOZONULUI MUDR – MARCHERI MOLECULARI AI GENOTIPURILOR DE TOMATE. *Paşa Lilia, Mitin Valentin, Deaghileva Angela, Tumanova Lidia*. // Journal of Academy of Sciences of Moldova. Life sciences. 2015, No 2(326), p. 20-25.

We have studied DNA- polymorphism of some tomato genotypes using primers complementary to the sequence encoding the TIR, mudrA and mudrB regions of maize transposable MuDR. Two primers homologous to TIR region of MuDR, five primers homologous to the mudrA region MuDR and three primers homologous to the region mudrB have been selected as perspective for the tomato genotypes identification. We suggest that all analyzed primers highlight common monomorphic fragments for the analyzed genotypes, so it can be used in taxonomic identification of Licopersicon esculentum. Primers which generated polymorphic spectrums genotype-specific may be applied in studying the inter-variety polymorphism of tomatoes.

12 references, 5 tables, 3 figures.

Key words: molecular marker, MuDR, polymorphism, transposons, tomato.

UDC: 616.12-005.4-08:615.37

GENETIC FACTORS THAT PREDISPOSE TO CORONARY ARTERY DISEASE. *Caproş Natalia, Barbacar Nicolae, Istrati Valeriu, Popescu Victor, Butovscaia Cristina* // Journal of Academy of Sciences of Moldova. Life sciences. 2015, No 2(326), p. 16-29.

The aim of the study was to evaluate the genetic factors that cause or predispose to coronary artery disease (CAD). The case control study was conducted in 2007-2011 and included 405 patients with coronary artery disease and acute ischemic episodes admitted to the Municipal Clinical Hospital „Sfânta Treime“, Chisinău. Insertion/deletion (I/D) genotypes of angiotensin-converting enzyme (ACE) and A1166C polymorphism of angiotensin II type 1 receptor gene, Asp298Glu (A/G) genotypes of the endothelial nitric oxide synthase (eNOS) and PlA1/2 (A1A2) genotypes of GPIIb/IIIa receptor polymorphisms were identified by amplified polymerase chain reaction and restricted fragment length polymorphism. The authors concluded that the carrier of D/D genotype and D allele in ACE gene, being positively correlated with the risk C/C polymorphic variant of angiotensin II type 1 receptor gene was associated with hypertension and cardiovascular death. A2/A2 genotype of glycoprotein (GP) IIb/IIIa receptor gene was associated with susceptibility to CAD and high frequency of myocardial infarction and dyslipidemia, particularly in smokers. 7 references.

Key words: coronary artery, genetic diagnosis, genetic factors.

UDC: 575.17

HUMAN HEALTH. ROLE OF GENETIC AND EPIGENETIC FACTORS. *Furdui Teodor, Ciochină Valentina, Glijin Aliona, Vrabie Valeria, Didilică Ina* // Journal of Academy of Sciences of Moldova. Life sciences. 2015, No 2(326), p. 30-37.

This paper specifies some genetic and epigenetic factors that not only underlie the genesis of different diseases but also ensure human sanogenicity. For the first time, a complex definition of the human health assumed as a basis of sanocreatology has been proposed. Integral human health is determined by interrelations between genetic and environmental factors, and genetic factors constitute the basis of the organism's sanogenicity development. The paper includes a description of particularities and the importance of prenatal genetic programming and the role of epigenetic modifications in determinism of the human organism's sanogenicity. 65 references.

Key words: human health, genetic factors, epigenetics, sanogenity, disease.

UDC: 616:575

PRENATAL DIAGNOSIS AND MEDICAL GENETIC COUNSELING. *Sprincean Mariana, Halabudenco Elena, Barbova Natalia, Egorov Vladimir, Stratila Mihai, Eţco Ludmila, Secrieru Viorica, Nour Veronica, Usurelu Natalia, Sacara Victoria*. // Journal of Academy of Sciences of Moldova. Life sciences. 2015, No 2(326), p. 38-46.

Aim of presented study consists of highlighting the role of the PCD in identifying chromosomal abnormalities at the early stages of intrauterine development, while respecting the practice background of medical genetic prenatal counseling.The data obtained in the PCD and its evaluation allow to carry out prenatal diagnosis of 164 cases of chromosomal abnormalities that amounted 3,5±0,3% from all pregnant women which passed the PCD during the respective period.The PCD (i. e., fetal karyotyping) as well as medical genetic counseling contribute to the reduction the prevalence of chromosomal abnormalities in newborns.

15 references, 3 tables, 2 figures.

Key words: prenatal diagnosis, hereditary congenital malformations.

UDC: 616-053.2:616.127-009.51-085

GENETIC ISSUES IN PEDIATRIC HYPERTROPHIC CARDIOMYOPHATY. *Stamati Adela* // Journal of Academy of Sciences of Moldova. Life sciences. 2015, No 2(326), p. 47-52.

Hypertrophic cardiomyopathy (HCM) is a common genetic cardiovascular disease. It is marked by phenotypic and genotypic heterogeneity. A retrospective study was performed on 23 children with HCM, aged before 19 years, hospitalized in Department of Pediatric cardiology of Child and Mother Institute (2008-2012). All subjects underwent detailed assessment: clinical history and examination, 12-lead and Holter monitoring electrocardiogram (ECG) and transthoracic echocardiographic study. Each clinical case was analyzed with reference to detection the presence of unfavorable risk factors. We are guided by the recommendations of pediatric studies, including extracardiac involving. According to clinical data, 3 infant was suspected for mitochondrial disease. The multidisciplinary approach has a considerable advantage in everyday assessment of children with HCM. 28 references, 4 tables.

Key words: genetic, pediatric hypertrophic cardiomyopathy

UDC: [633.16:631.523]:581.84(478)

ULTRASTRUCTURAL EVALUATION AND HISTOLOGICAL PECULIARITIES OF ANDROGENETIC STRUCTURES OF BARLEY. *Andronic Larisa, Macovei Ecaterina, Smerea Svetlana* // Journal of Academy of Sciences of Moldova. Life sciences. 2015, No 2(326), p. 53-58.

The induction of microspores dedifferentiation and reclaim of sporophytic development is a complex process, dependent by a lot of factors, including pretreatment conditions and the genotype peculiarities. In results of application various experimental variants, distinguished by pretreatment schemes and nutrient media, had been obtained androgenetic positive response for barley cultivars (Galactic, Sonor, Unirea and Stralucitor). The embryogenic structures derived from in vitro anthers culture present different ultrastructural aspects in dependence of proliferation capacity and further development. In realization of androgenic potential, an important role it is found for organelles of energetic systems: plastids and mitochondria, proving cytoplasmic factors involved in establishing embryogenic ability. 9 references, 4 figures.

Key words: androgenesis, anthers, barley, embryogenic structures, in vitro culture, nonmorphogenic structures, ultrastructure.

UDC: 632.3:633.16+633.16:631.52(478)

EXPRESSION OF SOME GENES IN BARLEY UNDER VIRAL INFECTION.

Andronic Larisa, Port Angel, Duca Maria // Journal of Academy of Sciences of Moldova. Life sciences. 2015, No 2(326), p. 59-65.

Impact of viral infection on susceptible host includes a variety of responses affecting struc-

tural aspects, physiological and genetic processes. Using qRT-PCR, it was established quantitative deviations in expression levels of some genes in leaves of barley seedlings obtained from plants infected with barley stripe mosaic virus. The activity of genes studied in the present experiment was clearly influenced by viral infection. In barley progenies obtained from virus infected plants the expression of genes involved in antioxidant metabolism (Apx and Sod) was decreased in most variants. The relative expression of genes for pathogenesis-related proteins was significantly (P<0,05) modified in evaluated barley cultivars (Galactic, Sonor and Unirea). The obtained data showed up- or down- regulation of PR3 and PR10 in dependence of genotype, whereas for PR5 was reported only the up-regulation effects for all treated variants.

33 references, 2 tables, 1 figures.

Key words: antioxidant system, barley stripe mosaic virus, gene expression, pathogenesis-related proteins, susceptibility, viral infection.

UDC: [635.65:631.52]:581.1.036

QUANTIFYING EFFECTS OF ULTRAVIOLET LIGHT ON PHASEOLUS VULGARIS L. IN ORDER TO IDENTIFY GENOTYPES WITH DIFFERENT CAPACITIES FOR USING SOLAR RADIATION. *Băra Csilla Iuliana, Iurea Dorina, Cotenco Eugenia, Vochiţa Gabriela* // Journal of Academy of Sciences of Moldova. Life sciences. 2015, No 2(326), p. 66-69.

The objective of these surveys was to establish the effects of UV radiation (beneficial or harmful) on Phaseolus vulgaris L. in order to identify genotypes with different capacities for using solar radiation. The content of flavonoids, izoflavonoids, lignin was determined, dimer formation at the DNA level was traced as a result of UV irradiation and DNA photolysis photoreparing, cytogenetic studies were carried out and, respectively, studies on the variation of agronomic and biochemical parameters of the germinating bean varieties under natural environment and UV-B environment. There was a relatively uniform response of varieties to all analyzed parameters, the differences in response was minimal, so none of varieties could be considered particularly sensitive or resistant to UV irradiation. 9 references.

Key words: agronomic parameters, effects, genotypes, irradiation, Phaseolus vulgaris L.

UDC: 631.52:635.1/.6(478)

ACTUAL PROBLEMS OF SELECTION AND ARRANGEMENT OF SEED PRODUCTION OF VEGETABLE CROPS. *Botnari Vasile.* // Journal of Academy of Sciences of Moldova. Life sciences. 2015, No 2(326), p. 70-75.

Tradition, experience, favorable soil and climatic conditions are a prerequisite for a real recovery of vegetable production in Moldova. The annual gross harvest of vegetables can be increased to 1 million tons. The planned development of vegetable production requires constant scientific support for the creation of new varieties and hybrids, development and introduction of new methods and technologies for the production of seeds, seedlings and cultivation of vegetable crops, can ensure economically feasible crop levels of high quality harvests under conditions of shortage of water and energy resources. 9 references, 1 figure.

Key words: development, vegetable growing, variety, hybrid, seeds, production, scientific support.

UDC: [581.1:633.4]:631.

EVALUATION OF POLYANIONIC GLICOSIDIC FUROSTANOLIC DERIVATIVES IMPACT ON TESTING PLANT SPECIES MITOTIC DIVISION AND CHROMOSOMES. *Cimpeanu Mirela M., Cimpeanu Cristian S., Csilla Bara Iuliana, Cotenco Eugenia, Iurea Dorina D.* // Journal of Academy of Sciences of Moldova. Life sciences. 2015, No 2(326), p. 76-79.

Glicosidic biopreparations were tested in these studies to reveal their impact on the process of mitotic division and implicitly of chromosomes. It was found that mitotic indices of Secale cereale demonstrated decrease in treatment variants of glycoside-furostanolic polyanionic derivatives. Increase in the value of this parameter was not detected in any of the experimental variants. A structural chromosomal mutations percent increase was determined for the variants studied compared to check variants. Usually aberrations were present more frequently with types of: ana-telophase with decks, bridges and fragments, fragments. references, 12 figures.

Key words: polyanionic – furotanolic glycoside derivatives, Secale cereal.

UDC: 633.8:582.949.27:577.18(478)

EXPRESSION OF GENES INVOLVED IN SCLAREOL BIOSYNTHESIS IN *SALVIA SCLAREA* L. *Duca Maria, Port Angela, Şestacova Tatiana, Martea Rodica, Goncearuc Maria* // Journal of Academy of Sciences of Moldova. Life sciences. 2015, No 2(326), p. 80-86.

Salvia sclarea L. has received attention for its broad range of pharmacological activities and used for fragrances and cosmetics industry. However, little is known at the genetics of the secondary metabolites synthesis in that plant. In this research, the real-time PCR was used to estimation of LPPS and SS gene expression, involved into sclareol metabolic pathway. An

increased transcriptional activity of LPPS gene in hybrids compared with parental forms was identified. Thus, the level of LPPS gene relative expression in most cases correlated with hybrid vigor in clary sage. Moreover, a positive correlation between transcriptional activity of LPPS gene and sclareol content was revealed. Application of molecular breeding approaches could contribute to an easy and quick selection of parental genotypes for high performance hybrid creation.

27 references, 2 tables, 7 figures.

Key words: gene expression, HPLC, LPPS, Real-Time PCR, *Salvia sclarea* L., sclareol.

UDC: [630*18:582.894]:630*165(478)+[630*18:633.898.42]:630*165(478)

INVENTORYING OF CORNELIAN CHERRY AND HAZELNUT POPULATIONS IN FOREST ECOSYSTEMS OF MOLDOVA IN THE CONTEXT OF THE *IN SITU* CONSERVATION OF CROP WILD RELATIVES. *Ganea Anatolie* // Journal of Academy of Sciences of Moldova. Life sciences. 2015, No 2(326), p. 87-91.

The article gives experimental data on in situ inventorying (GPS-positioning) of populations of cornelian cherry (*Cornus mas* L.) and hazelnut (Corylus avellana L.) in forest ecosystems of all soil-climatic zones of the Republic of Moldova, and describes some morphobiological traits of plants. Results and opportunities of conservation of genetic resources of these species in the conditions of ex situ collection are shown and the necessity for deeper investigations of intraspecific diversity of their populations is pointed out. 24 references, 2 tables.

Key-words: cornelian cherry, ex situ conservation, forest ecosystems, hazelnut, in situ conservation, inventorying.

UDC: 633.8:631.527

BIOCHEMICAL DIVERSITY OF THE *ORIGANUM VULGARE* SSP. *VULGARE* L. AND *ORIGANUM VULGARE* SSP. *HIRTUM* LINK) IETSWAART GENOTYPES FROM MOLDOVA. *Goncearіuc Maria, Balmuş Zinaida, Benea Ana, Barsan Victoria, Sandu Tatiana*. // Journal of Academy of Sciences of Moldova. Life sciences. 2015, No 2(326), p. 92-100.

Five genotypes of *O.vulgare* ssp. *vulgare* L. and five genotypes of *O.vulgare* ssp. *hirtum* have been assessed. The diversity of *O. vulgare* ssp. *vulgare*. and *O. vulgare* ssp. *hirtum* genotypes has been confirmed through the essential oil content, qualitative and quantitative components. The content of the essential oil separated by hydrodistillation varies between 0,077% and 0,360% in the genotypes *O.vulgare* ssp. *vulgare*, and between 2,315% and 4,923% in the *O.vulgare* ssp. *hirtum* genotypes. Qualitative and quantitative analyses performed using GC GC-MS techniques have found from 18 to 29 components in the *O.vulgare* ssp. *vulgaris* essential oil, depending on the genotype, the identification ratio being 92,38%-98,80%. The component number varies between 18 and 25 depending on the genotypes of *O. vulgare* ssp. *hirtum,* this constituting 99,87-100 % of the essential oil. The major components in the essential oil of O. vulgare ssp. vulgare include Germacrene D (33,98–26,01%); β-Caryophyllene (12,16–33¸16%); γ-Elemene (3,82 – 16,79%), while β- Bisabolene (6,83-16,04) is the major component in four genotypes. The *O. vulgare* ssp. *vulgare* genotypes are divided into five chemotypes: 1. Germacrene D/β-Caryophyllene/β-Bisabolene; 2. Germacrene D/β-Caryophyllene/δ-(+)Cadinole/ γ-Elemene; 3. Germacrene D/ γ-Elemene/ β-Caryophyllene/ β- Bisabolene; 4. β-Caryophyllene/ Germacrene D/ β- Bisabolene; 5. Germacrene D/ β-Caryophyllene/γ-Elemene/β- Bisabolene. The major components in the essential oil of *O. vulgare* ssp. *hirtum* are carvacrol (77,61-85,88%), followed by p-cymene (3,64-9,33%) or γ-terpinene (8,22%) and p-cymene (5,30%). The *O.vulgare* ssp. *hirtum* genotypes are divided into two chemotypes: 1-carvacrol/γ-terpinene/ p-cymene and carvacrol/γ-terpinene/p-cymene/ β-Caryophyllene. The variability of the content of polyphenols in the genotypes of both subspecies has been demonstrated. Rich in polyphenols are genotypes belonging *O.vulgare* ssp. *vulgaris*: from 99,25 ± 1,598 to 166,43±3,594 mg/100g. The genotypes of *O.vulgare* ssp. *hirtum*, synthesizes and accumulate polyphenols from 53,51± 0,684 to 85,59±0,719 mg/100g. 42references, 3 tables.

Key words: composition, essential oil, *Origanum vulgare* ssp. *hirtum*, genotip, *Origanum vulgare* ssp. *vulgare*, polyphenols.

UDC: [633.854.78:631.8]:631.55

HERBICIDE RESISTANCE BREEDING IN SUNFLOWER, CURRENT SITUATION AND FUTURE DIRECTIONS. *Kaya Yalcin.* // Journal of Academy of Sciences of Moldova. Life sciences. 2015, No 2(326), p. 101-106.

Sunflower grows in summer so it influences greatly more from environmental factors then it could not compete efficiently with other crops (commonly wheat in rainfed areas and corn, sugarbeet, etc in irrigated lands) in the rotation. Therefore, breeders should develop new higher yielding and dry tolerant cultivars and also should find new production techniques to increase production with reducing of effects of environmental factors influenced seed yield. Weeds and broomrape (*Orobanche cumana* Wallr.) exist among the most limiting factors for sunflower production in especially in Eastern Europe and Black Sea Region. Clearfield Technology which

using post emergence Imidazolinone (IMI) herbicides with IMI resistant cultivars presents efficient results both control broomrape and major broadleaf weeds in sunflower production. Furthermore, Sulfonyl Urea (SU) herbicide as post application like IMI is also another method to control weeds efficiently and it is preferred being cheaper than IMI. Farmers prefer commonly this technology because of well control on both broomprape and also common weeds in Eastern Europe. Recent trends in sunflower breeding and production is combining broomrape resistant genes with IMI resistance ((IMI + Orb) and also some other disease resistance such as downy mildew in the same hybrid give farmers more options better control weeds. Future trend is combined all three traits (Orb + IMI + SU) in the hybrids to present more economical results and efficient solutions to control weeds in their fields. 90 references.

Key words: broomrape, control, herbicides, races, resistance, sunflower, weeds.

UDC: [582.28:632.4]:581.083

FACTORIAL ANALYSIS OF SPECIFICITY OF THE FUNGUS *ALTERNARIA ALTERNATA* (FR.) KEISSLER. *Lupaşcu Galina, Grigorcea Sofia, Mihnea Nadejda, Gavzer Svetlana*. // Journal of Academy of Sciences of Moldova. Life sciences. 2015, No 2(326), p. 107-110.

In the article are presented data on the action specificity of *Alternaria alternata* culture filtrate, isolated from the sick plant of tomatoes, on the tomatoes, wheat and soya plant growth. It was established that the responding reaction of the plants was differentiated, being manifested by stimulation or inhibition, and depended from the plant cultivar, variety, fungus strain and temperature conditions. Through factorial analysis it was established that in comparison with wheat and soya, at tomatoes the share of the genotypic factor in the response reaction at the fungus action was higher, that reveals a possible specialization of the pathogen for the host-plant-tomatoes.

9 references, 4 tables.

Key words: *Alternaria* spp., soya, specialization, tomato, wheat.

UDC: 581.5(478)

PLANT BREEDING REQUIRES IMPROVEMENT. *Micu Vasile* // Journal of Academy of Sciences of Moldova. Life sciences. 2015, No 2(326), p. 111-118.

This article highlights main issues and possible solution in plant breeding in free market conditions, including phases and objectives for creating intellectual goods, forms and possibilities of financing, protecting and promoting intellectual goods (races, hybrids, technologies), fostering the authors and other aspects.

Key words: breeding, financing, implementation, privatization, research genetics, stimulation.

UDC: 633.15:631.523/.524(478)

INSTITUTE OF CROP "PORUMBENI" ACHIEVEMENTS AND GENETIC PROGRESS IN MAIZE BREEDING. *Pirvan Pintilie, Maticiuc Vasile, Mistret Silvia.* // Journal of Academy of Sciences of Moldova. Life sciences. 2015, No 2(326), p. 119-124.

The results achieved in the framework of integral research and works to improve the creation and implementation of hybrids of corn over the course of 40 years of activity of the Institute of Crop „Porumbeni". Creating competitive hybrids focuseson inbred lines with high combined capacity, adapted to the specific climatic conditions of cultivation areas. In this context, the final product of the hybrids that process improvement is achieved in different formulas depending on the area of cultivation and the directions for use. Over the course of 40 years of work at the Institute were created tens of thousands of corn hybrids of them just 1-2% being transferred to the official testing. Research were completed by the sending in the State Commissions of the Plant Varieties over 250 new hybrids of corn, of which 96 were included in the Official CATALOG of Plant Varieties of Moldova, Russia, Belarus, Ukraine and Kazakstan. 3 references, 3 tables.

Key words: applied research, hybrid, inbred lines, mayze, breeders.

UDC: 634.8:631.524(86):634.864(06)

IDENTIFICATION OF AMELIORATIVE POTENTIAL OF GRAPEVINE GENETIC RESOURCES. *Savin Gheorghe.* // Journal of Academy of Sciences of Moldova. Life sciences. 2015, No 2(326), p. 125-130.

In the present study are indicated some current targets in grapevine amelioration, including assessment of ameliorative potential of genetic resources presented in the grapevine gene pool of the institute. Are presented some theoretical conclusion concerning the absence of genetic barriers for transmission through heredity to hybrid offspring of quality and resistance and practical realization of this hypothesis – created biological material (varieties, elite, descendants) meets in one genotype productivity; quality, including seedless; adaptability to stress factors and representing a valuable prebreeding components. In Institute's grapevine gene pool are

presented a wide diversity of genotypes with complex characteristics: increased quality and productivity; seedlessness, very early ripening , advanced or increased resistance to unfavorable factors of environment – a necessary basis for future improvement of assortment.

24 references, 1 figure, 1 table.

Key words: breeding, genetic resources, grapevine, resistance, *V. vinifera.*

UDC: [635.64:631.4]:332.36 (478)

CHARACTERISTICS OF TOMATO VARIETIES AFTER VALUABLE ECONOMIC FEATURES. *Siromeatnicov Iulia, Cotenco Eugenia, Calalb Tatiana, Ciobanu Renata.* // Journal of Academy of Sciences of Moldova. Life sciences. 2015, No 2(326), p. 131-137.

Distant hybridization and biotechnological methods (in vitro immature embryo saving and histo-anatomical analyses) have resulted in developing tomato collection with valuable quantitative features for improvement. The Iulihirsutian variety and Anatolie variety in memory of Prof, Acad. Jacotă has been characterized by maximum photosynthetic activity compared with other analyzed genotypes. Structural peculiarities of these genotypes (increased number of secretory and tector hairs, stomata presence and openness of octiolei, especially on adaxial leaf epidermis, mesophyll thickness palisade, spongy and correlated between them) have resulted in histo-anatomical organization with leaf tolerance to drought and a significant contribution to establishing a greater photosynthetic activity of the above-mentioned genotypes. Based on the data obtained there were identified new sources of higher productivity and resistance, which were included in the hybridization process and development of new tomato populations (lines and varieties) and submitted to the State Commission for Plant Varieties Testing and two varieties were approved for cultivation in the open field in Moldova. 17 references, 2 tables, 2 figures.

Key words: hybridization, in vitro culture, histo-anatomical analyses, genotypes, hybrids, lines, varieties.

UDC:633.854.78:631.52

GENETIC VARIABILITY OF OIL QUALITY COMPONENTS IN SUNFLOWER AS A FUNCTION OF DEVELOPING HYBRIDS WITH NOVEL OIL. *Škorić Dragan, Sakač Zvonimir, Demurin Yakov*. // Journal of Academy of Sciences of Moldova. Life sciences. 2015, No 2(326), p. 138-146.

Sunflower is one of the most important oil crops in the world, and its oil is one of the highest quality vegetable oils. Oil quality is determined by the fatty acid composition, tocopherol content and type, phytosterols, carotenoids, and some other compounds. Standard sunflower oil contains linoleic, oleic, palmitic, and stearic fatty acids as well as several other fatty acids that are found in traces. The objective of this paper was to make a review of the genetic variability of oil quality components in sunflower using own results and those of other authors. Standard sunflower oil is linoleic in type, but using induced mutations genotypes have been developed that have high levels of oleic, palmitic, and stearic acids, and the mode of inheritance of these traits (gene number and type) has been determined. Also, some results have been achieved in the study of the mode of inheritance at the molecular level using marker genes. Standard sunflower oil contains predominantly alpha tocopherols (>95%). Using spontaneous mutations the genes tph1, tph2, and tph1tph2 have been discovered that control different levels of alpha, beta, gamma, and delta tocopherols. Also, Spanish researchers used induced mutations to obtain mutants with high levels of beta and delta tocopherols. In one of our own studies, the restorer line RHA-S-59 was found to contain only gamma tocopherol (100%), while 20 other restorer lines had only alpha tocopherol (100%). It has been scientifically proven that phytosterols (campesterol, stigmasterol, and beta-sitosterol) also play an important role in determining oil quality and that different genotypes have different levels of these substances.It has been shown that in genotypes that have high levels of oleic acid coupled with high levels of beta, gamma, or delta tocopherol a certain synergy occurs that increases oxi stability up to 15 times compared to standard sunflower oil. Using the existing genetic variability of components that determine oil quality it is possible to develop sunflower hybrids with novel oil. Thus far, the most has been done in the development of high-oleic hybrids. 39 references, 6 tables.

Key words: fatty acids, oil quality, phytosterols, sunflower, tocopherols.

UDC: 636.22/.28.082.(478.9)

GENETIC CHARACTERISTIC HEIFERS MOLDOVAN TYPE OF BLACK-MOTLEY CATTLE - DESCENDANTS VARIOUS OF BULLS. *Konstandoglo Alexandra, Focsha Valentin.* // Journal of Academy of Sciences of Moldova. Life sciences. 2015, No 2(326), p. 147-152.

In the article are the results of research and monitoring of changes in the gene pool of the Moldovan type black-Motley cattle in the herd STE "Maximovca." Identified differences in antigenic spectrum of blood groups in the offspring of 11 bulls used in the herd during the period 2003 to 2013 years. Among the identified 6 antigens (B2, G2, O2, Y2, E'2, Q') the descendants of all the bulls a there is a high frequency of occurrence of Antigen Y2, which varied from 0,32

(Dichii 788) to 1.0 (Abkhazian 835, Meteor 376). On AEB-locus Antigen B" identified only by descendants of bulls Captain 2354 and Svet 732, as evidenced by the earlier assessment of the genetic structure of the used lines of bulls in the herd STE "Maximovca" and is a characteristic of the descendants of the bulls line Vis Back Ajdiala and Pavni Farm Arlinda Chief. A study of relationship between the descendants of various bulls set the closest were the descendants of the bulls Academician 767 and Signor 7415, the genetic distance is 0,13, and most distant descendants of the bulls Abkhazian 835 and Dichii 788. The study of the relationship established between the descendants of the different bulls showed that the closest were the descendants of the bulls Academician 767 and Signor 7415, the genetic distance is 0,13. The most distant - descendants of the bulls Abkhazian 835 and Dichii 788, the genetic distance is 0,31.

18 references, 2 tables, 2 figures.

Key words: individual selection, blood group, heifers, descendants of the bulls, genetic distance

UDC: 636.32/.38

IMPROVEMENT OF *TSIGAIE SHEEP* BREED OF MOLDOVAN TYPE. *Tofan Ivan, Liuțcanov Petru, Mașner Oleg* // Journal of Academy of Sciences of Moldova. Life sciences. 2015, No 2(326), p. 153-156.

This paper deals with appreciation of the herd of sheep of Tsigaie race of Moldovan type which specializes in wool-meat-and-milk direction of production, at birth, at 3-3,5 months of age, 12-14 months of age. There is presented productivity of animals of different age groups selected in the selection nucleus in relation to the minimum requirements of the standard. According to the animals' body mass and wool production, the animals of selection nucleus are more superior to the official percentage requirements for breeding Rams by 0,7% to the body mass, and by 15,8% to production of wool; while the sheep are superior according to the body weight by 15,4% and according to the wool production - by 14,8%; breeding lambs - by 13,9% and 2,5% according to the body mass and wool production respectively; breeding ewes - by 5,6% according to body mass and by 8,7% according to the production of wool. 8 references, 4 tables.

Key words: breeding rams, Moldavian type, race Tsigaie, sheep.

UDC: 634.22:632.9

STERILIZATION OF NATURAL POPULATIONS OF THE PLUM WORM *LASPEYRESIA FUNEBRANA* TR. (*LEPIDOPTERA, TORTRICIDAE*). *Musleh Mohammed* // Journal of Academy of Sciences of Moldova. Life sciences. 2015, No 2(326), p. 157-159.

Plum moth (Grapholita funebrana Tp) in plum garden the Central Zone of Moldova, an area of 4 hectares traps with sex pheromones. Insecticide analog yuvenilnog insect hormone "Insegar", dose of 0,6 kg / ha.

9 references, 2 tables.

Key words: monitoring chemical sterilization, plum moth sex pheromones.

UDC: 581.1.04:631.526.32

MORPHO-ANATOMICAL ASPECTS OF TWO BASIL CULTIVARS PLANTLETS UNDER EXPERIMENTAL HEAVY METAL CONTAMINATION. *Burducea Marian, Lobiuc Andrei, Rosenhech Elida, Toma Constantin, Zamfirache Maria-Magdalena.* // Journal of Academy of Sciences of Moldova. Life sciences. 2015, No 2(326), p. 160-166.

The present paper investigates the ability of two basil cultivars to germinate and develop in experimental Ce pollution. In higher concentration treatments, anatomical observations of roots indicate reduced number of root hairs, an increased number of hairs, and reduced numbers of glandular hairs in hypocotyls. The treatments did not influenced germination, but the root and hypocotyle length were reduced at higher concentrations (200-400 mg/l Ce). The presence of mucilage at germination may contribute to the absorption of this element from the solutions, as shown for copper. The results justify further evaluation of the phytoremediation potential of the two cultivars.

17 references, 7 tables, 5 figures.

Key words: anatomical structure of plantlets, heavy metal, phytoremediation potential of basil cultivars, pollution of soil.

UDC: 575.86

PHYLOGENETICAL APPROACH FOR THE SEARCH OF VALUABLE METABOLIC PRODUCTS IN CYANOBACTERIA. *Cepoi Liliana, Golan Jacob, Gryganskyi Andrii P.* // Journal of Academy of Sciences of Moldova. Life sciences. 2015, No 2(326), p. 167-172.

Based on *CrtP* gene analysis we showed how phylogenetic approach could be used for detection of potentially efficient carotenoids producers, identification of their unknown isolates and for primer design for the screening of useful genes. ML analysis of the gene *crtP* in 15 taxa

of cyanobacteria has shown that two cyanobacteria groups – Anabaena and Nostoc might be artificial and polyphyletic. More isolates and genes should be included in such analysis to detect possible horizontal gene transfer events in evolution of branching filamentous cyanobacteria. Phylogenetic analysis can be helpful in identification of unknown isolates. So, on our tree *crtP* gene of unknown bacterium (clone 66415, acc. # KP445989) is nested together with the gene of *Nostoc punctiforme* and possibly represents the same or closely related species. For *crtP* gene we have determined the fragment with total length 1350 nucleotide basepairs (450 amino acids) with no intones and pretty uniform structure with small insert of seven amino acids in the beginning of the sequence right after first conserved region. Both starting and ending part of this fragment with length of 39 nucleotide basepairs are well conserved (same aminoacids with no changes or minor differences in 3rd codon positions) are very suitable for primer design.

20 references, 3 figure, 1 table.

Key words: 16S rRNA gene, *CrtP* gene, Cyanobacteria, phylogenetical approach.

UDC: 581.14:573.6.086.83

ON THE EFFECT OF ADVERSE FACTORS IN THE PRESOWING SEED TREATMENT WITH A LOW-FREQUENCY MAGNETIC FIELD. *Sidorenko A., Groisman I., Shibaev A.* // Journal of Academy of Sciences of Moldova. Life sciences. 2015, No 2(326), p. 173-177.

Experiments on the exposure of dry wheat seeds to a magnetic field in a frequency range of 1-10 Hz with a flux density of 40-50 μT have shown that, in the period of active cell division, the cells of plants become more sensitive to external fields. In view of this fact, the seeds of the experimental sample show better results on germination percentage and spout length than the seeds of the reference sample against the background of natural fields under environmentally safe conditions. Under the action of technogenic electromagnetic fields, the experimental seeds showed worse results on germination percentage and sprout length than the reference seeds; this fact proves the enhancement of the negative role of these fields on the cells of the developing organism.Taking into account the general laws governing the development of organisms at the cellular level, we can assume that technogenic fields play a particular negative role for a human body at the moment of impregnation and initial development of the body when the cells of the embryo most actively respond to the surrounding fields. 9 references, 5 figures.

Key words: developing cells, low-frequency low-intensity magnetic field, natural fields, plant seeds, technogenic fields.

UDC: 632.937.1: 579.6.

RECENT ACHIEVEMENTS IN MICROBIOLOGICAL PLANT PROTECTION. *Voloșciuc Leonid, Pânzaru Boris, Lemanov Natalia, Nicolaev Arcadii, Șcerbacov Tatiana, Nicolaev Svetlana, Zavtoni Pantelimon, Moraru Liviu.* // Journal of Academy of Sciences of Moldova. Life sciences. 2015, No 2(326), p. 178-183.

Plant diseases and pest insects are among the main constraints affecting the production and productivity of crops both in terms of quality and quantity. Use of chemicals continues to be the principal activity to mitigate the negative impacts of chemical plant protection. The environmental concerns, health conscious attitude of human beings and other hazards associated with the use of pesticides and using of biological means to control of the crop pests is gaining importance. Biological control involves the use of large spectrum of beneficial organisms and their products (biological active substances, useful microorganisms and viruses, entomophages), that reduce the negative effects of pests and promote positive responses by the plant. In our institute, over several years, a number of commercial products based on different viral, fungal and bacterial antagonists have been registered. These commercial products include, Virin-HSP, on the Nuclear Polihedrosis Virus, as active ingredient, Paurin, on the antagonist bacteria Pseudomonas fluorescens, Trichodermin and Gliocladin, on fungus genus Trichoderma as active ingredient. The value of approved biological means lies not only in the biological, ecological and economic implications, but also the possibility of their inclusion in conventional and organic farming systems.

9 references, 1 tables.

Key words: biocontrol, biopreparation, ecology, Gliocladin, pathogen agent, Paurin, pest, Trihodermin, Virin-HSP.

РЕФЕРАТЫ

УДК: 582.475.2:577.2

О РЕЗУЛЬТАТАХ ПОВТОРНОГО ИЗУЧЕНИЯ ПРОЦЕССА ОБОСОБЛЕНИЯ ЦИТОПЛАЗМЫ (ПЛАЗМОГЕНЕЗА) ВОКРУГ ЯДЕР – СПРЕМИЕВ У *PICEA ABIES* (L.) KARST. *Чеботарь А.А.* // Известия Академии Наук Молдовы. Наука о жизни. 2015, № 2(326), с. 4-15.

Проведено повторное светооптическое исследование спермиогенеза *Picea abies* (L.) Karst и показано, что вопреки утверждениям Fergusson (1901) и Camefort (1978) спермии ели обыкновенной представляют собой клетки: каждое ядро окружено собственной цитоплазмой ограниченной плазмалеммой. В то же время, данное исследование уточняет наше мнение, высказанное в совместно опубликованной работе по данному вопросу (Мошкович, Чеботарь, 1986). Установлено также, что плазмогенез у спермиев ели обыкновенной завершается к концу прогамной фазы оплодотворения, т.е. к моменту контакта пыльцевой трубки с нуцеллусом семязачатка. Сближенное положение ядер спермиев на всем периоде роста пыльцевой трубки, создавало мнение о единой цитоплазме, однако значительные размеры мужского гаметофита ели обыкновенной и тщательный микроскопический анализ тех же препаратов позволили внести ясность в этом филогенетическом вопросе. Библ.- 30, рис.-10.

Ключевые слова: гамета, гаметофит, голосеменные, монофилия, покрытосеменные, пыльцевое зерно, спермиоклетка, спорофит, филогения, эволюция.

УДК: 582.675.1

ВКЛАД В ПОЗНАНИЕ СТРУКТУРЫ ВЕГЕТАТИВНЫХ ОРГАНОВ *PAEONIA PEREGRINA* MILL. *Мереакре Анка, Боз Ирина, Тома Константин.* // Известия Академии Наук Молдовы. Наука о жизни. 2015, № 2(326), с. 16-19.

Авторы исследовали структуру вегетативных органов *Paeonia peregrina* Mill. (P. romanica Brândză), охраняемого как памятник природы и состоящего в критическом списке сосудистых растений Румынии. Структура клубневых корней напоминает структуру корневища, проводящая ткань образует побочные сосудистые пучки, расположенные по окружности и разделеные большими сердцевинными лучами. Механические ткани стебля представлены гиподермической колленхимой и склеренхимой (перифлоэмные волокна, либриформные и сердцевинные лучи). Листовая пластинка представляет двустороннюю-многостороннюю структуру, имеющую один слой палисадной ткани, состоящей из коротких и иногда с перегородками клеток на внешней стороне. Библ.- 12, рис.-3.

Ключевые слова: анатомия, памятник природы, *Paeonia peregrina*.

УДК: 577.2.08 : 631.52

МОЛЕКУЛЯРНЫЕ МАРКЕРЫ ДЛЯ ГЕНОТИПИРОВАНИЯ ТОМАТОВ - ПРАЙМЕРЫ ИЗ ОБЛАСТИ MUDR ТРАНСПОЗОНОВ. *Паша Лилия, Митин Валентин, Дягилева Анжела, Туманова Лидия.* // Известия Академии Наук Молдовы. Наука о жизни. 2015, № 2(326), с. 20-25.

Исследован ДНК полиморфизм некоторых генотипов томата с использованием праймеров, комплементарных последовательностям TIR, mudrA и mudrB областям транспозона кукурузы MuDR. Были выбраны праймеры перспективные для идентификации генотипов томата – два праймера, гомологичные TIR области, пять праймеров, гомологичных mudrA области и три праймера, гомологичные mudrB области MuDR. Показано, что при использовании всех праймеров синтезируется группа ампликонов одинаковой длины, что может быть использовано в таксономической идентификации *Licopersicon esculentum*. Полиморфный спектр специфических ампликонов может быть использован в изучении внутривидового полиморфизма генотипов томата. Библ.- 12, таб.- 5, рис.-3.

Ключевые слова: MuDR, молекулярные маркеры, полиморфизм, томаты, транспозоны.

УДК: 616.12-005.4-08:615.37

ГЕНЕТИЧЕСКИЕ ФАКТОРЫ ПРЕДРАСПОЛАГАЮЩИЕ К ИШЕМИЧЕСКОЙ БОЛЕЗНИ СЕРДЦА. *Капрош Наталья, Барбакар Николай, Истрати Валерий, Попеску Виктор, Бутовская Кристина.* // Известия Академии Наук Молдовы. Наука о жизни. 2015, № 2(326), с. 26-29.

Целью нашего исследования было изучение генетических факторов предрасполагающих к ишемической болезни сердца (ИБС). Нами проведенно исследование типа случай-контроль с 2007 по 2011 в котором было включено 405 пациентов с ишемической болезнью сердца госпитализированных в муниципальной клинической больнице “Святая Троица”, города Кишинева. В качестве предрасполагающих к ИБС генетических маркеров были исследованы с помощью полимеразной цепной реакции I/D генотипы ангиотензин-превращающего фермента (ACE) и A1166C полиморфизм гена ангиотензиновых рецепторов I типа, Asp298Glu (A/G) генотип оксида азота синтазы эндотелия (eNOS) и PLA1/2 (A1A2) генотип гена гликопротеиновых IIb/IIIa рецепторов. Авторы пришли к выводу, что носители D/D генотипа ангиотензинпревращаего фермента положительно коррелирует с полиморфной вариантой C/C гена ангиотензиновых

рецепторов I типа и с артериальной гипертензией. A2/A2 генотип гликопротеиновых IIb/IIa рецепторов ассоциировалось с ИБС, инфарктом миокарда и высокой частотой дислипидемии, особенно у курильщиков. Библ.- 7.

Ключевые слова: коронарные артерии, генетическая диагностика, генетические факторы.

УДК: 575.17

ЗДОРОВЬЕ ЧЕЛОВЕКА. РОЛЬ ГЕНЕТИЧЕСКОГО И ЭПИГЕНЕТИЧЕСКОГО ФАКТОРОВ. *Фурдуй Ф. И., Чокинэ В. К., Глижин А. Г., Врабие В. Г., Дидиликэ И. В.* // Известия Академии Наук Молдовы. Наука о жизни. 2015, № 2(326), с. 30-37.

В работе рассмотрены некоторые генетические и эпигенетические факторы, которые лежат в основе не только генезиса различных заболеваний, но и в обеспечении саногенности человеческого организма. Впервые было предложено комплексное определение здоровья человека, основанное на базе санокреатологии. Здоровье человека в целом определяется взаимосвязями генетических факторов и окружающей среды, а генетические факторы являются основой развития саногенности организма. Статья включает описание особенностей и значимости генетического перинатального программирования и роли эпигенетических изменений в развитии саногенности человеческого организма. Библ.-65.

Ключевые слова: болезнь, генетические факторы, саногенность, человека, эпигенетика.

УДК: 616:575

ПРЕНАТАЛЬНАЯ ДИАГНОСТИКА И МЕДИКО-ГЕНЕТИЧЕСКОЕ КОНСУЛЬТИРОВАНИЕ. *Спринчан Мариана, Халабуденко Елена, Барбова Наталья, Егоров Владимир, Стратила Михаил, Ецко Людмила, Секриеру Виорика, Ноур Вероника, Ушурелу Наталья, Сакарэ Виктория.* // Известия Академии Наук Молдовы. Наука о жизни. 2015, № 2(326), с. 38-46.

Целью представленной работы является освещение роли пренатальной цитогенетической диагностики (ПЦД) в выявлении хромосомных аберраций на ранних стадиях внутриутробного развития, сохраняя основные принципы медико-генетического пренатального консультирования. Данные, полученные с помощью ПЦД и их оценка, позволили провести пренатальную диагностику в 164 случаях хромосомных аберраций, что составило 3,5±0,3% от всех случаев беременности, где была использована ПЦД в течение соответствующего периода. ПЦД (кариотипирование плода), а также медико-генетическое консультирование способствуют уменьшению частоты хромосомных аномалий у новорожденных. Библ.- 15, таб.- 3, рис.-2.

Ключевые слова: пренатальная диагностика, наследственные врожденные пороки развития.

УДК: 616-053.2:616.127-009.51-085

ГЕНЕТИЧСЕКИЕ ПРОБЛЕМЫ ДЕТСКОЙ ГИПЕРТРОФИЧЕСКОЙ КАРДИОМИОПАТИИ. *Стамати Адела.* // Известия Академии Наук Молдовы. Наука о жизни. 2015, № 2(326), с. 47-52

Гипертрофическая кардиомиопатия (ГКМП) является распространенным генетическим заболеванием сердечно-сосудистой системы. Зболевание характеризуется фенотипической и генотипической гетерогенностью. Ретроспективное исследование было проведено для 23 детей с ГКМП в возрасте до 19 лет, госпитализированных в Отделение Детской Кардиологии Института Матери и Ребенка (2008-2012). Все пациенты проходили детальное изучение: история болезни и осмотр, 12-канальное и холтеровское мониторирование электрокардиограммы (ЭКГ) и трансторакальное эхокардиографическое исследование. Каждый клинический случай был проанализирован со ссылкой на обнаружение присутствия неблагоприятных факторов риска. Мы руководствуемся рекомендациями педиатрических исследований, в том числе с экстракардиальным вмешательством. По клиническим данным, у троих младенецев подозревается наличие митохондриальной болезни. Междисциплинарный подход имеет значительное преимущество в повседневном изучении детей с ГКМП. Библ.- 28, таб.- 4.

Ключевые слова: генетическая детская гипертрофическая кардиомиопатия.

УДК: [633.16:631.523]:581.84(478)

УЛЬТРАСТРУКТУРНЫЙ АНАЛИЗ И ГИСТОЛОГИЧЕСКИЕ ОСОБЕННОСТИ АНДРОГЕННЫХ СТРУКТУР У ЯЧМЕНЯ. *Андроник Лариса, Маковей Екатерина, Смеря Светлана* // Известия Академии Наук Молдовы. Наука о жизни. 2015, № 2(326), с. 53-58.

Индукция дедифференцировки микроспор и восстановление спорофитного развития представляет сложный процесс, зависящий от множества факторов, в том числе предварительной предобработки и особенностей генотипа. В результате использования различных экспериментальных вариантов, отличающихся по схемам предобработке и составу питательных сред, был получен андрогенный ответ для сортов ячменя (Галактик, Сонор, Униря и Стрэлучитор). Эмбриогенные структуры, полученные при in vitro культивирование пыльников, представляли различные ультраструктурные аспекты в зависимости от пролиферирующей способности и дальнейшего их развития. В реализации андрогенного потенциала важную роль играют органеллы энергетической системы: пластиды и митохондрий, доказывая участье цитоплазматических факторов в реализации

эмбриогенного потенциала. Библ.- 9, рис.- 4.

Ключевые слова: андрогенез, культура *in vitro*, нон-морфогенные структуры, пыльники, ультраструктура, эмбриогенные структуры, ячмень.

УДК: 632.3:633.16+633.16:631.52(478)

ЭКСПРЕССИЯ НЕКОТОРЫХ ГЕНОВ ЯЧМЕНЯ ПРИ ВИРУСНОЙ ИНФЕКЦИИ. *Андроник Лариса, Порт Анжела, Дука Мария*. // Известия Академии Наук Молдовы. Наука о жизни. 2015, № 2(326), с. 59-65.

Влияние вирусной инфекции у восприимчивых хозяев включают в себя различные ответы, затрагивающие структурные аспекты, физиологические и генетические процессы. Изучение экспрессии генов методом ПЦР в реальном времени, выявило изменение уровня транскрипции в листьях проростков ячменя, полученных от растений, инфицированных вирусом штриховатой мозаики ячменя. Показаны количественные изменения экспрессии изученных генов в зависимости от физиологического статуса растений (здоровые или вирусные). В потомстве ячменя, полученных от инфицированных вирусом растений активность генов, вовлеченных в антиоксидантном метаболизме (Арх и Sod) в большинстве вариантов была понижена. У всех изученных сортах ячменя (Галактик, Сонор и Униря) экспрессия генов, кодирующие белки, связанные с патогенезом (PR), была значительно изменена ($P<0,05$). Результаты исследований показали повышение или понижение, в зависимости генотипа, уровня транскрипции генов, кодирующих PR3 и PR10, в то время как для PR5 установлено возрастание количества транскриптов у всех трех изученных генотипов ячменя. Библ.- 33, таб.- 2, рис.-1.

Ключевые слова: антиоксидантная система, белки связанные с патогенезом, вирус штриховатой мозаики ячменя, вирусная инфекция, восприимчивость, экспрессия генов.

УДК: [635.65:631.52]:581.1.036

КОЛИЧЕСТВЕННОЕ ВОЗДЕЙСТВИЕ УЛЬТРАФИОЛЕТОВОГО ИЗЛУЧЕНИЯ НА *PHASEOLUS VULGARIS* L. С ЦЕЛЬЮ ВЫЯВЛЕНИЯ ГЕНОТИПОВ С РАЗЛИЧНЫМИ ВОЗМОЖНОСТЯМИ ДЛЯ ИСПОЛЬЗОВАНИЯ СОЛНЕЧНОЙ РАДИАЦИИ. *Бэра Ксила Юлиана, Юря Дорина, Котенко Евгения, Войкица Габриела* // Известия Академии Наук Молдовы. Наука о жизни. 2015, № 2(326), с. 66-69.

Целью данных исследований было определение воздействия УФ-излучения (полезного или вредного) на *Phaseolus vulgaris* L. для того, чтобы выявить генотипы с различными возможностями для использования солнечной радиации. Было определено содержание флавоноидов, изофлавоноидов, лигнина, контролировалась образование димеров на уровне ДНК в результате УФ-облучения и фоторепарации фотолизиса ДНК, были проведены цитогенетические исследования и, соответственно, исследования по изменению агрономических и биохимических параметров, как и поведение в естественной среде проращивания сортов фасоли и среды УФ-В. Была установлена относительно одинаковая реакция сортов для всех анализируемых параметров, ответные различия минимальны, и не один из сортов не может считаться особенно чувствительным или устойчивым к УФ-облучению. Библ. -9

Ключевые слова: генотипы, излучение, эффекты, агрономические признаки.

УДК: 631.52:635.1/.6(478)

АКТУАЛЬНЫЕ ПРОБЛЕМЫ СЕЛЕКЦИИ И ОРГАНИЗАЦИИ СЕМЕНОВОДСТВА ОВОЩНЫХ КУЛЬТУР. *Ботнарь Василий* // Известия Академии Наук Молдовы. Наука о жизни. 2015, № 2(326), с. 70-75.

Традиции, накопленный опыт, благоприятные почвенно-климатические условия являются реальной предпосылкой для восстановления овощеводства в Республики Молдова. Ежегодный валовой сбор овощей может быть увеличен до 1 млн тонн. Планомерное развитие овощеводства требует постоянной научной поддержки для создания новых сортов и гибридов, разработки и внедрения новых методов и технологий производства семян, рассады и возделывания овощных культур, способных гарантировать получение экономически оправданных уровней высококачественного урожая в условиях дефицита воды и энергетических ресурсов. Библ. -9, рис. -1

Ключевые слова: развитие, овощеводство, сорт, гибрид, производство, семена, научная поддержка.

УДК: [581.1:633.4]:631.8

ОЦЕНКА ВОЗДЕЙСТВИЯ ФУРАТОНОЛЬНО-ПОЛИАНИОННО ГЛИКОЗИДНЫХ ПРОИЗВОДНЫХ НА ТЕСТИРОВАНИЕ МИТОТИЧЕСКОГО ДЕЛЕНИЯ ВИДОВ РАСТЕНИЙ И ХРОМОСОМ. *Кымпяну Мирела М., Кымпяну Кристиан С., Бэра Юлиана Ксила, Котенко Евгения, Юря Дорина Д.* // Известия Академии Наук Молдовы. Наука о жизни. 2015, № 2(326), с. 76-79.

В данном исследовании были испытаны гликозидные биопрепараты для того, чтобы выявить их воздействие на процесс митотического деления, в частности, хромосом. Установлено, что митотические показатели *Secale cereale* демонстрировали снижение в вариантах обработки гликозидно – фуростанольно – полианионными производными. Рост значения данного параметра

не обнаруживали в каких-либо вариантах экспериментов. Был определен процентный рост структуральных хромосомных мутаций для изучаемых вариантов в сравнении с контролем. Обычно аберрации чаще присутствовали у типов анателофазы с мостами, мосты и фрагменты, фрагменты. Библ. -5, рис. -12

Ключевые слова: Secale cereale, полианионные – фуратонольн - гликозидные производные.

УДК: 632.3:633.811:631.52

АНАЛИЗ ЭКСПРЕССИИ ГЕНОВ УЧАСТВУЮЩИХ В БИОСИНТЕЗЕ СКЛАРЕОЛА У *SALVIA SCLAREA* L. *Дука Мария, Порт Анжела, Шестакова Татьяна, Мартя Родика, Гончарук Мария.* // Известия Академии Наук Молдовы. Наука о жизни. 2015, № 2(326), с. 80-86.

Шалфей (*Salvia sclarea* L.) заслуживает внимания благодаря своей фармакологической активности и используется в парфюмерии, косметической и пищевой промышленности. Тем не менее, для данного вида недостаточно хорошо изучена генетика синтеза вторичных метаболитов. В этом исследовании, ПЦР в реальном времени была использована для оценки экспрессии LPPS и SS генов, участвующих в метаболизме склареола. У гибридов *Salvia sclarea* L. по сравнению с родительскими формами была выявлена усиленная транскрипционная активность гена LPPS. Таким образом, у шалфея уровень относительной экспрессии гена LPPS в большинстве случаев коррелирует с гибридной силой. Кроме того, была выявлена положительная корреляция между транскрипционной активностью гена LPPS и содержанием склареола. Применение методов молекулярной селекции может способствовать быстрому и простому отбору родительских генотипов для создания высокопроизводительных гибридов. Библ.- 27, таб.- 2, рис.-7.

Ключевые слова: LPPS, *Salvia sclarea* L., ВЭЖХ, ПЦР в реальном времени, склареол, экспрессия генов.

УДК: [630*18:582.894]:630*165(478)+[630*18:633.898.42]:630*165(478)

ИНВЕНТАРИЗАЦИЯ ПОПУЛЯЦИЙ КИЗИЛА И ЛЕЩИНЫ В ЛЕСНЫХ ЭКОСИСТЕМАХ МОЛДОВЫ В КОНТЕКСТЕ КОНСЕРВАЦИИ *IN SITU* ДИКИХ РОДИЧЕЙ КУЛЬТУРНЫХ РАСТЕНИЙ. *Ганя А.И.*//Известия Академии наук Молдовы. Науки о жизни. 2015, № 2(326), с. 87-91.

В статье приводятся экспериментальные данные по инвентаризации (GPS-позиционированию) in situ популяций кизила (*Cornus mas* L.) и лещины (*Corylus avellana* L.) в лесных экосистемах всех почвенно-климатических зон Республики Молдова, описаны некоторые морфо-биологические признаки растепий. Показаны результаты и перспективы консервации генетических ресурсов этих видов в условиях коллекции ex situ и отмечена необходимость более углубленных исследований внутривидового разнообразия их популяций. Библ.- 24, таб.- 2.

Ключевые слова: лесные экосистемы, лещина, кизил, инвентаризация, консервация *in situ*, консервация *ex situ.*

УДК: 633.8:631.527

БИОХИМИЧЕСКИЕ РАЗНООБРАЗИЯ МОЛДАВСКИХ ГЕНОТИПОВ *ORIGANUM VULGARE* SSP. *VULGARE* L. И *ORIGANUM VULGARE* SSP. *HIRTUM.* *Гончарук Мария, Балмуш Зинаида, Беня Ана, Бырсан Виктория, Санду Татьяна.*// Известия Академии Наук Молдовы. Наука о жизни. 2015, № 2(326), с. 92-100.

Подтверждено разнообразие генотипов по содержанию, качественного и количественного состава эфирного масла пяти новых генотипов *O. vulgare* ssp. *vulgare* L. и пять новых генотипов *O.vulgare* ssp. *hirtum* (Link) Ietswaart. Содержание эфирного масла, выделенное методом гидродистилляции у генотипов *O.vulgare* ssp. *vulgare* варьирует в пределах 0,077% – 0,360% и между 2,315% и 4,923% у генотипов *O.vulgare* ssp. *hirtum*. Качественный и количественный анализа проведенные методом ГХ, ГХ-МС установили от 18 до 29 компонентов у *O.vulgare* ssp. *vulgare*, которые составляют 92.38-98.80%в эфирного масла. Генотипы *O.vulgare* ssp. *hirtum* в эфирном масле содержат от 18 до 25 компонентов, при идентификации 99.87-100%.

Основные компоненты эфирного масла генотипов разновидности *vulgare* являются: гермакрен Д (33.98–26.01%); β-кариофиллен (12.16–33.16%); γ-елемен (3.82 – 16.79%) и для 4 генотипов – β- бисаболен (6.83-16.04), а генотипы делятся на пять хемотипов: гермакрен Д/ β-кариофиллен/β- бисаболен; гермакрен Д/ β-кариофиллен /δ-кадинол /γ-елемен; гермакрен Д/ γ-елемен/ β-кариофиллен/ β- бисаболен; β-кариофиллен/ гермакрен Д/ β- бисаболен; гермакрен Д/ β-кариофиллен/ γ-елемен/ β- бисаболен; Основные компоненты в эфирном масле *O. vulgare* ssp. *hirtum* являются карвакрол (77.61-85.88%), р-цимен (3.64-9.33%) или γ-терпинен (8.22%) и р-цимен (5.30%). Генотипы этой разновидности делятся на два хемотип: карвакрол /γ- терпинен /р- цимен и карвакрол /γ- терпинен / р-цимен / β-кариофиллен. Содержание полифенолов также варьирует во всех генотипах обеих разновидностях: в *O.vulgare* ssp. *vulgare* – 99.25 ± 1.598 – 166.43±3.594 мг/100г и в *O.vulgare* ssp. *hirtum*: 53.51± 0.684 – 85.59±0.719 мг/100г. Отмечено отрицательная корреляция между содержанием эфирного масла и полифенолов: высокому содержанию эфирного масла соответствует низкое содержание полифенолов. Библ.- 42, табл. - 3.

Ключевые слова: *Origanum vulgare* ssp. *hirtum*, *Origanum vulgare* ssp. *vulgare*, генотип, компоненты, полифенолы, эфирное масло.

УДК: [633.854.78:631.8]:631.55

СЕЛЕКЦИЯ ПОДСОЛНЕЧНИКА НА УСТОЙЧИВОСТЬ К ГЕРБИЦИДАМ, НАСТОЯЩАЯ СИТУАЦИЯ И БУДУЩИЕ ПЕРСПЕКТИВЫ. *Кайя Ялчин.* // Известия Академии Наук Молдовы. Наука о жизни. 2015, № 2(326), с. 101-106.

Подсолнечник произрастает летом, что обуславливает существенно большее влияние факторов окружающей среды на данную культуру, которая, таким образом, не может конкурировать эффективно в севообороте с другими культурами (обычно с пшеницей в богарных районах и кукурузой, сахарной свеклой и т.д. в орошаемых землях). Таким образом, селекционеры должны разработать новые высокопродуктивные и толерантные к засухе сорта, а также должны найти новые методы производства для увеличения урожайности и сокращения воздействия экологических факторов, влияющих на урожайность семян. Среди большинства факторов, ограничивающих производство подсолнечника в особенности в Восточной Европе и регионе Черного моря, сорняки и заразиха *(Orobanche cumana* Wallr.) занимают особое место. Технология Clearfield, которая предполагает использование послевсходовой обработки гербицидами имидазолинона (IMI) с IMI устойчивыми сортами, представляет эффективные решение для контроля заразихи и основных широколиственных сорняков в производстве подсолнечника. Кроме того, применение гербицидов сульфонилмочевины (SU) после IMI, является другим предпочтительным способом эффективного уничтожения сорняков, так как стоимость данных обработок ниже, чем IMI. Фермеры предпочитают обычно эту технологию из-за контроля, как над заразихой, так и над характерными сорняками в Восточной Европе. Последние тенденции в селекции и производстве подсолнечника заключаются в объединении генов устойчивости к заразихе с устойчивостью к IMI (IMI + Orb), а также некоторыми другими видами устойчивости к болезням, например к ложной мучнистой росе, в рамках одного гибрида и дают фермерам больше возможностей лучше контролировать сорняки. Будущее тенденция в сочетании всех трех признаков (Orb + IMI + SU) у гибридов представляет более экономичное и эффективное решение для борьбы с сорняками на полях. Библ.- 90.

Ключевые слова: подсолнух, заразиха, расы, сопротивление, контроль, гербициды, сорняки.

УДК 575.1+635.64

ФАКТОРНЫЙ АНАЛИЗ СПЕЦИФИЧНОСТИ ГРИБА *ALTERNARIA ALTERNATA* (FR.) KEISSLER. *Лупашку Галина, Григорча София, Михня Надежда, Гавзер Светлана.* // Известия Академии Наук Молдовы. Наука о жизни. 2015, № 2(326), с. 107-110.

В работе представлены данные относительно специфичности действия культурального фильтрата гриба *A. alternata*, выделенного из больного растения томата на рост проростков томата, пшеницы и сои. Установлено, что ответная реакция растений была неоднозначной – ингибирование или стимулирование роста и зависела от вида растения, сорта, штамма гриба. Факторным анализом выявлено, что по сравнению с пшеницей и соей, у томата вклад генотипического фактора в ответной реакции на действие гриба был больше, что свидетельствует о возможной специализации гриба к растению хозяина – томату. Библ.- 9, таб.- 4.

Ключевые слова: Alternaria spp., пшеница, соя, специализация, томат.

УДК: 581.5(478)

СЕЛЕКЦИЯ РАСТЕНИЙ НУЖДАЕТСЯ В УЛУЧШЕНИИ. *Мику Василий.* // Известия Академии Наук Молдовы. Наука о жизни. 2015, № 2(326), с. 111-118.

В статье освещаются основные вопросы и возможные решения в селекции растений в условиях свободного рынка, в том числе этапы и задачи создания интеллектуальных товаров, формы и возможности финансирования, защита и продвижение интеллектуальных товаров (расы, гибриды, технологии), стимулирование авторов и другие аспекты.

Ключевые слова: генетические исследования, финансирование, реализация, приватизация, стимуляция.

УДК: 633.15:631.523/.524(478)

ИНСТИТУТ РАСТЕНИЕВОДСТВА «ПОРУМБЕНЬ» ДОСТИЖЕНИЯ И ГЕНЕТИЧЕСКИЙ ПРОГРЕСС В СЕЛЕКЦИИ КУКУРУЗЫ. *Пырван Пинтилие, Матичук Василий, Мистрец Сильвия.* // Известия Академии Наук Молдовы. Наука о жизни. 2015, № 2(326), с. 119-124.

Приведены результаты научных исследований и селекционных работ по создание и внедрение в производство гибридов кукурузы на протяжение 40 лет деятельности Института Растениеводства “Порумбень”. Доказано, что наиболее ценным в создании высокоурожайных гибридов являются самоопылённые линии, сочетающие высокую комбинационную способность и приспособленность к разным климатическим условиям возделывания. На протяжение деятельности Института были созданы десятки тысяч гибридов кукурузы, и только 1-2% из них передавались на официальнее тестирование. Селекционный процесс совместно с прикладными исследованиями закончились передачи в Государственных Комиссий разных стран более 250 гибридов кукурузы, из которых 96 включены в официальные Регистры Молдовы, России, Республики Беларусь, Украины и Казахстана. Библ.- 3, таб.- 3.

Ключевые слова: кукуруза, селекция, гибрид, инбредные линий, прикладные исследования.

UDC: 634.8:631.524(86):634.864(06)

ОПРЕДЕЛЕНИЕ АМЕЛИОРАТИВНОГО ПОТЕНЦИАЛА ГЕНЕТИЧЕСКИХ РЕСУРСОВ ВИНОГРАДА. *Савин Георгий.* // Известия Академии Наук Молдовы. Наука о жизни. 2015, № 2(326), с. 125-130.

В настоящем исследовании описаны некоторые актуальные задачи селекции винограда, в том числе оценка мелиоративного потенциала генетических ресурсов, представленных в институтской коллекции винограда. Представлены некоторые теоретические выводы об отсутствии генетических барьеров для передачи по наследству гибридному потомству качественных признаков и устойчивости и практическая реализация этой гипотезы – созданный биологический материал (сорта, элитные линии, потомки) комбинирует в рамках одного генотипа продуктивность; качественные характеристики, в том числе отсутствие косточек; приспособляемость к стрессовым факторам и представляет собой ценные компоненты преселекции. В институтской коллекции генетических ресурсов винограда представлено широкое разнообразие генотипов с комплексными характеристиками: хорошие качественные признаки и продуктивность; отсутствие косточек, ранний срок созревания, повышенная устойчивость к неблагоприятным факторам окружающей среды – необходимая основа для будущего улучшения ассортимента. Библ.- 24, таб.- 1, рис.-1.

Ключевые слова: селекция, генетические ресурсы, виноград, устойчивость, *V. vinifera.*

УДК: [635.64:631.4]:332.36 (471)

ХАРАКТЕРИСТИКА СОРТОВ ТОМАТА ПО ЭКОНОМИЧЕСКИ ЦЕННЫХ ПРЗНАКОВ. *Сыромятников Юлия, Котенко Евгения, Калалб Татьяна, Чобану Рената* // Известия Академии Наук Молдовы. Наука о жизни. 2015, № 2(326), с. 131-127.

Используя отдаленную гибридизацию, биотехнологические методы (сохранение зрелых зародышей в культуре *in vitro* и гисто-анатомические анализы) создали коллекцию томатов с ценными количественными признаками для селекции. Сорта Юлихирсутиан и Анатолие в память академика А. Жакота характеризуются максимальной фотосинтетической активности по сравнению с другими исследуемые генотипами. Структурные особенности этих генотипов (высокое количество секреторных и тактильных волосков, наличие устьиц и степень обезвоживания, особенно основа эпидермиса на адаксиальной стороне листа, толщина палисадного и губчатого мезофилла и корреляцию между ними) определяет гисто-анатомическую структуру листа толерантный к засухе, имеют значительный вклад в установление фотосинтетической активности изученных генотипов. На основе полученных данных были созданы новые источники продуктивности и устойчивости, которые были включены в процессе гибридизации и создание новых популяций томатов (линий и сортов) и на базе тестирования в Государственной комиссии по сортоиспытанию, были районированы в Республики Молдова. Библ.- 17, таб.- 2, рис.-2.

Ключевые слова: генотипы, гибридизация, гибриды, гисто - анатомический анализ, культура *in vitro*, линии, сорта.

УДК: 633.854.78:631.52

ГЕНЕТИЧЕСКАЯ ИЗМЕНЧИВОСТЬ КАЧЕСТВЕННЫХ КОМПОНЕНТОВ ПОДСОЛНЕЧНОГО МАСЛА В ЗАВИСИМОСТИ ОТ РАЗРАБОТКИ ГИБРИДОВ С НОВЫМИ МАСЛАМИ. *Шкорич Драган, Сакач Звонимир, Демурин Яков.* // Известия Академии Наук Молдовы. Наука о жизни. 2015, № 2(326), с. 138-146.

Подсолнечник является одной из самых важных масличных культур в мире, и его масло является одним из самых лучших по качеству среди растительных масел. Качество масла определяется составом жирных кислот, содержанием и типом токоферола, фитостеринами, каротиноидами, и некоторыми другими соединениями. Стандартное подсолнечное масло содержит линолевую, олеиновую, пальмитиновую и стеариновую жирные кислоты, а также несколько других жирных кислот, которые находятся в следовых количествах. Целью данной работы было создание обзора генетической изменчивости компонентов качества масла в подсолнечнике на основе собственных результатов и результатов других авторов. Стандартное подсолнечное масло относится к линолевому типу, но с помощью индуцированных мутаций были разработаны генотипы, которые имеют высокий уровень олеиновой, пальмитиновой и стеариновой кислот, а также был определен способ наследования этих признаков (число генов и тип). Кроме того, некоторые результаты были достигнуты при изучении наследования на молекулярном уровне с помощью маркерных генов. Стандартное подсолнечное масло содержит преимущественно альфа токоферол (> 95%). С помощью спонтанных мутаций генов tph1, tph2 и tph1tph2 было обнаружено, что последние контролируют различное содержание альфа, бета, гамма и дельта токоферолов. Кроме того, испанские исследователи использовали индуцированные мутации для получения мутантов с высоким уровнем бета и дельта токоферолов. В одном из наших собственных исследований было установлено, что линия восстановитель фертильности RHA-S-59, содержит только гамма-токоферола (100%), в то время как 20 других линий восстановителей содержат только альфа токоферол (100%). Было научно доказано, что фитостерины (кампестерол, стигмастерол и бета-ситостерол), также играют важную роль в определении качества масла и различных генотипов имеют различное содержание этих веществ. Было показано, что в генотипах, которые характеризуются высоким содержанием олеиновой кислоты в сочетании с высоким уровнем бета, гамма или дельта

токоферола возникает определенный синергизм, что повышает устойчивость к окислению до 15 раз по сравнению со стандартным подсолнечным маслом. При использовании существующей генетической изменчивости компонентов, которые определяют качество масла, можно создать гибриды подсолнечника с маслом нового состава. До сих пор, наибольшее внимание уделялось разработке высокомасличных гибридов. Библ.- 39, таб.- 6.

Ключевые слова: жирные кислоты, качество масла, фитостеролы, подсолнечник, токоферолы.

УДК 636.22/.28.082.(478.9)

ГЕНЕТИЧЕСКАЯ ХАРАКТЕРИСТИКА ТЕЛОЧЕК МОЛДАВСКОГО ТИПА ЧЕРНО-ПЕСТРОГО СКОТА - ПОТОМКОВ ОТ РАЗЛИЧНЫХ БЫКОВ-ПРОИЗВОДИТЕЛЕЙ. *Константогло А., Фокша В.* // Известия Академии Наук Молдовы. Наука о жизни. 2015, № 2(326), с. 147-152.

В статье приведены результаты исследований и контроля изменений в генофонде молдавского типа черно-пестрого скота стаде STE ”Maximovca”. Выявлены различия в антигенном спектре групп крови у потомства 11 быков-производителей, используемых в стаде в период с 2003 по 2013 гг. Среди выявленных 6 антигенов (B2, G2, O2, Y2, E’2, Q’) у потомков всех быков-производителей наблюдается высокая частота встречаемости антигена Y2, которая варьировала от 0,32 (Дикий 788) до 1,0 (Абхазиан 835, Метеор 376). По EAB - локусу антиген B” выявлен только у потомков быков Капитан 2354 и Свет 732, что подтверждается проведенной ранее оценкой генетической структуры использованных линий быков-производителей в стаде STE ”Maximovca” и характерно для потомков быков-производителей линий Вис Бэк Айдиала и Павни Фарм Арлинда Чифа. Изучение взаимоотношений, сложившихся между потомками различных быков-производителей показало, что наиболее близкими оказались потомки быков Академик 767 и Синьор 7415, генетическая дистанция равна 0,13, а наиболее удаленными - потомки быков Абхазиан 835 и Дикий 788, генетическая дистанция – 0,31. Библ.- 18, таб.- 2, рис.-2.

Ключевые слова: группа крови, потомки быков, генетическое расстояние, индивидуальный отбор.

УДК: 636.32/.38

СЕЛЕКЦИЯ *ОВЕЦ ЦИГАЙСКОЙ* ПОРОДЫ МОЛДАВСКОГО ТИПА. *Тофан Иван, Люцканов Петр, Машнер Олег.* // Известия Академии Наук Молдовы. Наука о жизни. 2015, № 2(326), с. 153-156.

В статье изложена оценка стада овец молдавского типа цигайской породы, которая специализируется на мясо-шёрстном и молочном направлении производства при рождении, в 3-3,5-месячном и 12-14 месячном возрасте. Представлены результаты продуктивности животных разных возрастных групп, отобранных в племенное ядро на основе минимальных требований стандарта. По массе тела и производству шерсти животные племенного ядра превосходят официальные процентные требования по разведению: для баранов на 0,7 % по массе тела и на 15,8 % по производству шерсти; для овец на 15,4% и 14,8%; для ягнят на 13,9% и 2,5%; для овцематок на 5,6% по массе тела и на 8,7% по производству шерсти соответственно. Библ.- 8, таб.- 4.

Ключевые слова: овцы, бараны производители, Молдавский тип, Цигайская порода.

УДК: 634.22:632.9

СТЕРИЛИЗАЦИЯ ПРИРОДНЫХ ПОПУЛЯЦИИ СЛИВОВОЙ ПЛОДОЖОРКИ *LASPEYRESIA FUNEBRANA* TR. (*LEPIDOPTERA, TORTRICIDAE*). *Муслех Мохаммед*. // Известия Академии Наук Молдовы. Наука о жизни. 2015, № 2(326), с.157-159.

Стерилизация природных популяции сливовой плодожорки в феромонных ловушках (10 шт./га) обработанных не токсичным стерилизатором проводили в сливовом саду ООО « Агробрио» с .Бачёйю Центральная зоны Молдовы, на площади 4 гектара в сравнении с химическими инсектицидами применяемыми хозяйством. Библ.- 9, таб.- 2.

Ключевые слова: половых феромонов сливовой плодожорки, мониторинг химическая стерилизация.

УДК: 581.1.04:631.526.32

МОРФО-АНАТОМИЧЕСКИЕ АСПЕКТЫ ПРОРОСТКОВ ДВУХ СОРТОВ БАЗИЛИКА НА ФОНЕ ЭКСПЕРИМЕНТАЛЬНОГО ЗАГРЯЗНЕНИЯ ТЯЖЕЛЫМИ МЕТАЛЛАМИ. *Бурдуча Мариан, Лобюк Андрей, Росенхеч Елида, Тома Константин, Замфираке Мария-Магдалена* // Известия Академии Наук Молдовы. Наука о жизни. 2015, № 2(326), с. 160-166.

В настоящей работе исследуется способность двух сортов базилика к прорастанию и развитию на фоне экспериментального загрязнения церием (Ce). При обработке высокими концентрациями, анатомические наблюдения корней указывают на уменьшение количества корневых волосков, увеличению числа волосков, и уменьшению числа железистых волосков в гипокотилях. Обработка не повлияла на прорастание, но длина корня и гипокотиля сократились при использовании более высоких концентраций (200-400 мг/л Ce). Наличие слизи при прорастании может способствовать поглощению этого элемента из растворов, как было продемонстрировано для меди. Результаты оправдывают дальнейшие исследования и оценку фиторемедиационного потенциала двух сортов.

Библ.- 17, рис.-5, рисунков -7.

Ключевые слова: анатомическая структура проростков, тяжелый металл, фиторемедиационный потенциал сортов базилика, загрязнение почвы.

УДК: 575.86

ФИЛОГЕНЕТИЧЕСКИЙ ПОДХОД В ОПРЕДЕЛЕНИИ ЦЕННЫХ МЕТАБОЛИЧЕСКИХ ПРОДУКТОВ ИЗ ЦИАНОБАКТЕРИЙ. *Чепой Лилиана, Голан Жакоб, Григанский Андрий* // Известия Академии Наук Молдовы. Науки о жизни. 2015 № 2(326), с. 167-172.

На примере гена *CrtP* показано, каким образом филогенетический анализ может быть применен для поиска потенциальных производителей каротиноидов, для идентификации неизвестных изолятов и для проектирования праймеров с целью поиска полезных генов. ML анализ гена *CrtP 15* таксонов цианобактерий показал что две группы-*Anabaena* şi *Nostoc* могут быть искусственными или полифилетическими. Филогенетический анализ может быть применен также для идентификации неизвестных изолятов. На нашем филогенетическом дереве полученном для гена *CrtP* неизвестный изолят (клон 66415, асс. # KP445989) накладывается на *Nostoc punctiforme* PCC 73102 и следовательно, является тем же штаммом или же очень близким к нему. Для гена *CrtP* был определен участок общей длиной 1350 пар оснований (450 аминокислот), без интронов, с гомогенной структурой и инсерционным участком из 7 аминокислот в начале последовательности, непосредственно после первого консервативного участка. Начальные и концевые участки данного фрагмента длиной 39 пар оснований консервативны (одни и те же аминокислоты или минорные различия в зависимости от третьего основания в кодоне) и подходят для дизайна праймеров. Библ. 20, таблиц -1, рисунков -3.

Ключевые слова: ген 16S rRNA, ген *CrtP*, филогенетический анализ, Цианобактерии

УДК: 581.14:573.6.086.83

ПОБОЧНЫЕ ЭФФЕКТЫ ПРЕДПОСЕВНОЙ ОБРАБОТКИ СЕМЯН НИЗКОЧАСТОТНЫМ МАГНИТНЫМ ПОЛЕМ. *Сидоренко А., Гройсман И., Шибаев А.* // Известия Академии Наук Молдовы. Наука о жизни. 2015, № 2(326), с. 173-177.

Эксперименты по воздействию на сухие семена пшеницы магнитным полем в диапазоне частот 1-10 Гц с плотностью потока 40-50 m T показали, что в период активного клеточного деления, клетки растений становятся более чувствительными к внешним полям. Ввиду этого факта, семена экспериментальных образцов показывают лучшие результаты по всхожести и длине проростка, чем семяна контрольного образца на фоне естественных полей в экологически безопасных условиях. Под воздействием техногенных электромагнитных полей, экспериментальные семена показали худшие результаты по всхожести и длине проростка, чем семена контрольной группы; этот факт подтверждает гипотезу об усилении отрицательной роли этих полей на клетки развивающегося организма.

Принимая во внимание общие закономерности развития организмов на клеточном уровне, мы можем предположить, что техногенные поля, играют определенную негативную роль для организма человека в момент пропитки и первоначального развития тела, когда клетки эмбриона наиболее активно реагируют на окружающие поля. Библ.- 9, рис.-5.

Ключевые слова: низкочастотное малоинтенсивное магнитное поле, семена растений, развивающиеся клетки, природные поля, техногенные поля.

УДК: 632.937.1: 579.6.

ПОСЛЕДНИЕ ДОСТИЖЕНИЯ В МИКРОБИОЛОГИЧЕСКОЙ ЗАЩИТЕ РАСТЕНИЙ. *Волощук Леонид, Пынзару Борис, Леманов Наталья, Николаев Аркадий, Щербаков Татьяна, Николаев Светлана, Завтони Пантелимон, Морару Ливиу.* // Известия Академии Наук Молдовы. Наука о жизни. 2015, № 2(326), с. 178-183.

Основная цель исследовании состояло в анализе результатов полученных в результате разработки и тестирования биологических препаратов предназначенных для борьбы с вредными организмами основных сельскохозяйственных растений. Исследования проведены с применением Вируса ядерного полиедроза Helicoverpa armigera Hbn., антагонистических бактерий Pseudomonas fluorescens BKM CP 330 D, микроскопических грибов *Trichoderma lignorum* штамм М-10 и Trichoderma virens, штамм 3Х). Разработаны оригинальные технологические процессы производства и применения 4 биологических препаратов, зарегистрированных для борьбы с вредными организмами основных сельскохозяйственных культур. Ценность зарегистрированных средств сводится не только в высокой биологической, экологической и экономической эффективности, но и в возможности их использования в системах конвенционного и экологического земледелия. Библ.- 10, таб.- 1.

Ключевые слова: патогенные агенты, вредные организмы, биопрепараты, Вирин-ХСП, Паурин, Trihodermin, Gliocladin, экология, биологическая защита.

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