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ГЛАСНИК

ИНСТИТУТА ЗА БОТАНИКУ И БОТАНИЧКЕ БАШТЕ УНИВЕРЗИТЕТА У БЕОГРАДУ

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Ovaj broj „Glasnika Instituta za botaniku i botaničke bašte Univerziteta u Beogradu" posvećen je četrdesetpetogodišnjici naučnog i pedagoškog rada dr Mirjane Nešković, redovnog profesora Biološkog fakulteta, Univerziteta u Beogradu. Autori su želeli da svoje radove posvete profesorki, koja je u različitim fazama njihovog života i rada uticala na njihovo školovanje, opredeljenje za nauku, izbor naučnih problema i dalji tok istraživanja. Posvećujući joj ovaj broj časopisa, autori, kao i članovi Instituta za botaniku i botaničke bašte "Jevremovac" i uredništvo Glasnika, nadaju se da će to biti potsticaj biljnim fiziolozima i istraživačima u srodnim oblastima za dalja traganja u naučnom radu.

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КРИССОХРАНЕНИЕ ГЕНОФОНДА РАСТЕНИЙ И ТКАНЕЙ *IN VITRO DIOSCOREA BALCANICA И D. CAUCASICA*

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Popov, A.S., Vokova, L.A., Ćulafić, Lj. (1995): *Cryopreservation of in vitro plants and plant tissue genofond.* – Glasnik Instituta za botaniku i botaničke baške Univerziteta u Beogradu, Tom XXIX, 1 - 8.

Cryopreservation of plant tissues (meristem, organogenic and embryogenic calli) provides their storage at - 196°C (liquid nitrogen) for idefinitely long time.

At present a cryobank of Timiryazev Institute of Plant Physiology, Moscow (Russia) includes 23 lines of 15 plant species, which stem meristem were successfully *in vitro* cultured upon a long time cryoprservation.

The calli of the endemo-relict species *Dioscorea balcanica* Košanin and *D. caucasica* Lipsky are also maintained in liquid nitrogen in the above cryo-bank. They were introduced into a culture at the Institute for Biological Research, Belgrade (Yugoslavia). Dimethyl sulfoxide (DMSO, 7%) and trehalose were used as cryoprotectors applying freezeng programme of EPK (Russia) at the rate of 0,33°C/min to -30°C, 10°C/min to -60°C and after that the ampules containing frozen tissue were rapidly

dipped into the liquid nitrogen. After several months of storage at -196°C, organogenic and embryogenic calli were thawed in a water bath at 40°C and further culture under *in vitro* conditions using the corresponding nutrient media for plant regeneration. No differences in growth between these samples and cultures permanently grown at 25°C were observed.

This method is an indispensable part of a procedure for the preservation of rare and endemic plant species through *in vitro* culture.

Key words: *Dioscorea caucasica* Lipsky, *Dioscorea balcanica* Košanin, cryopreservation, *tissue culture, plant regeneration.*

Ključne reči: *Dioscorea caucasica* Lipsky, *Dioscorea balcanica* Košanin, kriokonzervacija, kultura tkiva, regeneracija biljaka.

В условиях неограниченного роста потребностей человечества и ухудшения экологической обстановки уже давно понята опасность, угрожающая генетическим ресурсам растений: культурных, лекарственных, исчезающих, эндемичных. Традиционные способы хранения этих ресурсов недостаточны. Культуры апексов побегов *in vitro* сохраняю генотипы, а культуры клеток и тканей могут сохранить полезную части генома, регенерировать растения иногда даже через 2-3 года, но генетически нестабильны. Поддержание таких коллекций неэкономично.

Наиболее надежным по генетической стабильности и неограниченным по длительности способом хранения генофонда является криосохранение. Чтобы избежать губительных перестроек кристаллов льда температуры должны быть ниже -130°C, что удобно обеспечивать с помощью жидкого азота (-196°C).

Проблему криосохранения генофонда растений легче всего решать путем глубокого замораживания семян и пыльцы, то есть достаточно сухих обьектов. Криобанки семян уже существуют, в том чиле и в Институте физиологии растений РАН в Москве.

Но совсем иная ситуация, если надо полностью сохранить данный генотип, как в случае материнских и гибридных форм и растений, размножаемых только вегетативно или имеющих рекальцитратные семена, которые не могут быть высушены без потери жизнеспособности. Поэтому наиболее универсальный способ криосохранения генофонда - глубокое замораживание апексов побегов и эмбрионов. Кроме того, для научных, промышленных и патентных целей необходимо долговременное хранение клеточных штаммов *in vitro.*

Во всех таких случаях мы имеем дело с паренхимными зрелыми клетками растений, специфика которых, как и клеток *in vitro* - большие размеры, сильная вакуолизация, обилние воды - создает значительные трудности для процедуры криосохранения. Следовательно, исследования криорезистентности клеток растений *in vitro* являются важнейшими для проблемы криосохранения.

МЕХАНИЗМЫ ПОВРЕЖДЕНИЙ КЛЕТОК

Ниже приведены некоторые литературные и наши данные о механизмах криорезистентности клеток растений.

Прежде всего надо избежать роста внутри клеток кристаллов льда больших 0.1 μм, которые разрушают структуры клетки. То есть нужно значительно уменьшить обьем возмножного образования льда, значит необ-

ходима серьезная дегидратация. Но, с другой стороны, такая дегидратация вызвивает очень сильное сжатие протопласта, которое, если действует достаточно долго, тоже повреждает клетку.

Процедура криосохранения клеток и апексов состоит из ряда этапов, начиная с подготовки (предварительное культивирование в специальных условиях) и кончая рекультивированием после оттаиваня и регенерацией растений. Важнейшее значение имеет этап замораживания. На каждом этапе механизмы криорезистентности имеют свою специфику, а успех определяется их интегрированным взаимодействеием.

На этапе подготовки мы сначала использовали холодовое закаливание, как наиболее естественный процесс повышения морозостойкости зимующих растений умеренного климата. Его мы применили для клеточных суспензионных культур *Panax ginzeng* (Попов et al., 1982), мутантные штаммы которого имею недостаточную исходную криорезистентность. На клетках штамма Ж-2 было проведено закаливание в течение 3 недель как с добавлением (до 20%), так и без добавления сахарозы в среду (Федоровский et al., 1993). Выживаемость после жидкого азота увеличавалась в процессе закалки почти в 3 раза, но только при добавлении сахарозы. Отношение сухого веса к сырому также увеличивалось, но гораздо меньше - на 60%. Без добавления сахарозы - никакого увеличения не было. Уровень внутриклеточных растворимых сахаров возрастал на 70% при добавлении сахарозы, а без добавления, наоборот, уменьшался: по-видимому сахара усиленно расходовались на поддержание метаболизма и жизне способности клеток при сниженной до 4^{o}C температуре.

Следовательно, 1 - увеличение выживаемости в процессе закаливания требует обязательного добавления сахарозы и связано с ростом сухого вещества и внутриклеточных сахаров. 2 - однако, этот рост существенно меньше, чем увеличение выживаемости и, поэтому, значение закаливания для криорезистентности не исчерпывается увеличием сахаров. По-видимому, существуют и другие механизмы.

Для двух более теплолюбивых женьшеней: *P. quinquefolius* и *P. japonicus* закаливание было неэффективным и для них применили предварительное культивирование с маннитолом, что всега приводило к возрастанию количества сухого вещества в клетках (то есть к дегидратации), но не всегда - к увеличению сахаров.

Иной способ подготовки был применен для клеток *Dioscorea deltoidea*, чей исходный, „дикий" штамм Д-I имел совершенно недостоточную криорезистентность и его предварительно культивировали с добавлением низких концентраций некоторых аминокислот (Волкова et al., 1982; 1984). Такая подготовка значительно увеличавала выживаемость после жидкого азота и приводила к накоплению в этих клетках сахаров в соответствии с эффективностью данной аминокислоты. Известно, что сахара, связывая воду, являются одними из важнейших веществ клеток, определяющими их водоудерживающую способность (Самыгин, 1974). Их увеличение, следовательно, уменьшает сжатие протопласта во время необходимой и неизбежной сильной дегидратации. Этот механизм защиты обнаружен давно как один из важных компонентов холодового закаливания (Туманов, 1979) и работает в цитоплазме.

Максимальное накопление сахаров в клетках Д-I было примерно в 2 раза, а выживаемость возрастала в 5-6 раз: с 5-6 до 30% (при предкультивировании с аспарагином), то есть существенно больше. Что также свидетельствует, как и в случае клеток женьшеня, о действии каких-то других, кроме накопления сахаров, механизмов увеличения криорезистентности.

Для проверки этого препроложения мы использовали также другой способ подготовки клеток *Dioscorea deltoidea* и *Medicago sativa* штаммов Д-I и Л-I, соответственно: инкубацию при 10°С в течение 20-24 часов (Попов et al., 1991). На клетках двух столь разных видов было показано увеличение криорезистентности в результате такой инкубации в 2-3 раза, причем для *D. deltoidea* - на двух очень разных по своей исходной устойчивости штаммах: Д-I и мутантном ДМ-0,5. Самое интересное - это синергизм двух способов подготовки: оказалось, что инкубация при 10° усиливала влияние предкультивирования с аспарагином и выживаемость клеток обеих штаммов достигала 50-54% (около 2/3 от контроля: исходная жизне-способность культур клеток *D. deltoidea in vitro* примерно 70-75%).

Следовательно, механизмы действия этих способов подготовки различны, что подтвердили результаты определения сахаров после инкубации суспензий штамма Д-I, так как никакой разницы с контролем не было. Количество растворимых внутриклеточных сахаров было 61.6 и 60.8 μg/ml для контрольной и охлажденной культур соответственно в первом субкультивировании и 54.9 и 54.1 μg/ml - во втором (через несколько месяцев). Значит, инкубация при 10°С в течение суток не является закаливанием в узком смысле, как понимал этот процесс Туманов, а запускает какой-то другой механизм увеличения криорезистентности.

Вероятно этот механизм связан с изменениями клеточной мембраны, которая является главной мишенью при замораживании (Steponkus, 1984; Попов, 1993). Интересно, что культивирование клеток *Rauwolphia serpentina* при 10°С увеличивало ненасыщенность липидов и текучесть плазмалеммы (Yamada et al., 1980). Фундаментальное изучение процессов холодовой адаптации растений *Secale cereale* cv. Puma на изолированных протопластах позволило Степонкусу и соавторам обосновать существование некоторых механизмов гибели клеток при замораживании вследствие повреждения клеточной мембраны. Первый - это действие внутриклеточного льда. Его можно избежать, если обеспечить достаточную дегидратацию тем или иным способом: замораживанием в режиме „ветрификации" (быстрое осмотическое отнятие воды за счет сверхвысокой концентрации растовра криопротектантов), подсушиванием в потоке стерильного воздуха (оба эти способа сопровождаются быстрым замораживанием) или медленным замораживанием с инициацией кристаллизации раствора (Бутенко, Попов et al., 1983; Keefe et al., 1984). При использовании последнего способа, криомикроскопа и раствора диметилсульфоксида (ДМСО) мы наблюдали клетки *Dioscorea deltoidea* Д-I без льда вплоть до -27°С (Волкова et al., 1984), что косвенно подтверждает витрификацию (переход воды в аморфное твердое состояние) цитоплазмы и при медленном заможивании в присуствии ДМСО. Но именно сильная дегидратация приводит к самому важному механизму гибели клеток растений вследствие перехода липидов клеточной мембраны из ламеллярной в гексагональную инвертированную фазу с образованием мицелл вместо бислоя

(Степонкус, 1984). Этот переход происходит постепенно, приводя к деструкции плазмалеммы, и клетка погибает через некоторое время в период наибольшего сжатия.

Однако, выводы Степонкуса были соформулированы в результате опытов только на одном сорте однога вида. Поэтому необходимо было убедиться в их справедливости для клеток растений других видов. Мы разработали новый флуориметрический метод, однозначно определяющий (при наличии соответствующих контролей и строго стандартной постановке), процент клеток с серьезными, деструктивными повреждениями клеточной мембраны (Попов et al., 1992).

С помощью этого метода мы сопоставили действие замораживания по нашей программе с влиянием медленного сжатия протопластов клеток при 2°С в растворах с сильной осмотичностью, соответствующей той, которая возникает при -30, -40°С (Федоровский et al., 1992; 1993). На 5 клеточных штаммах, принадлежащих столь разным видам, как *Panax ginseng* С.А. Меу и *Dioscorea deltoidea* Wall., и отличающихся по своей исходной криорезистентности, была показана очень близкая корреляция между крио- и осморезистентностью. Значит, деструктивные повреждения клеточной мембраны опре- деляются не низкой температурой, а исключительно дегидратацией.

ПРАКТИЧЕСКЕ ДОСТИЖЕНИЯ

В практическом плане в Институте физиологии растений в Москве для культур клеток и апексов *in vitro* были разработаны методы подготовки к криосохранению, способ и автоматическое устройство для инициации кристаллизации раствора криопротектантов при медленном замораживании и рекультивирование; 23 клеточных штамма 15 видов возобновили рост после жидкого азота и сохранили все свои основные свойства; 17 штаммов хранятся постоянно и их восстанавливают в растущем состоянии, когда они необходимы. Меристемы *Solanum tuberosum, Digitalis lanata, Chamomilla recutita,* 22 сортов *Fragaria x ananasa,* клетки моркови и картофеля регенерировали растения.

КРИОСОХРАНЕНИЕ *Dioscoria balcanica* и *D. caucasica*

Изложенные выше и другие наши исследования послужили исходной базой для разработки криосохранения редких эндемичых видов рода *Dioscorea: D. balcanica* Košanin и *D. caucasica* Lipsky, культивирование *in vitro* каллусных тканей которых с последующей регенерацией растений и микроклональное размножение было разработано в Институту биологических исследований им С. Станковича в Белграде (Грубишич, Чулафич, Боевич-Цветич, 1991). Грозящее полное исчезновение этих видов является, к сожалению, ярким примером скорейшей необходимости использовать все средства - и традиционные, и нетрадиционные - для спасения и их генофонда, и их самих. Их клеточные штаммы *in vitro* представляют интерес также и для биотехнологии. Первые опыты, показавшие возобновление роста органогенных каллусных тканей данных видов после криосохранения, уже опубликованы (Чулафич et al., 1994). Там же приведены подробности методики, включавшей следующие основные моменты.

Расения регенерированные из органогенного каллуса пересажени в почу в оранжереи.
а. - *Dioscorea balcanica*
б. - *Dioscorea caucasica*

Органогенный каллус брали через 3-4 недели после пересадки, когда он содержал наибольшее число зеленых зачатков почек, измельчали, охлаждали на льду и постепенно добавляли холодный раствор криопротектантов. В предварительных опытах наилучшие результаты были получены со смесью 7% ДМСО и 5% трегалозы, поэтому в настоящей работе использовали именно этот раствор. Эту суспензию встряхивали 5 мин и переносили в ампулы, которые выдерживали 1-1.5 часа при 4-6°С и помещали в камеру программного замораживателя растительных клеток ЗРК-I (СССР), когда температура в ней снижалась до 0-4°. При температуре в ампулах -4.4°С автоматически происходила инициация кристаллизации раствора в ампулах и последующая стабилизация температуры в течение 20 мин. Дальнейшее замораживание шло со скоростью 0.33°С мин до -30°, - 10.00°С/мин до приблизительно -60°С и ампулы быстро погружали в жидкий азот, где хранили несколько дней или месяцев.

Оттаивали ампулы в водяной бане при 40°С со встряхиванием. Извлеченные из них каллусы помещали на бумажные фильтры, расположенные на поверхности агаризованной среды в чашках Петри. Чашки помещали в темноту при 25°. Через несколько часов фильтры с тканью переносили в новые чашки и оставляли на ночь в тех же условиях. Утром фильтры онова переносили на

новые чашки на среду с агарозой и помещали в обычные условия с освещением. Спустя несколько дней перенос фильтров иногда повторяли, а после достаточного возобновления роста каллусной ткани - пересаживали ее на среду для регенерации.

Растения *Dioscorea balcanica* и *D. caucasica* в культуре *in vitro* с успехом регенерированны после хранения их каллусных тканей в жидком азоте. В повторных независимых опытах каллусы обеих видов также возобновили рост после оттаивания, причем не только органогенные, но и эмбриогенные: прозрачные глобулярные агрегированные структуры, полученные из органогенных тканей на среде с 2.4-Д (I mg/l). Состояние и рост эмбриогенных каллусов после криосохранения лучше, чем органогенных. В настоящее время эти ткани пересажеы на среду для регенерации, проводятся наблюдения за их развитием и исследуются хромосомные наборы в клетках и каллусов, и кончиков корней. Продолжается также хранение в жидком азоте ампул с тканями этих исчезающих эндемичных видов, замороженных в тех же успешных опытах. Преимущество криопротектантной смеси ДМСО с тргалозой по сравнению с сахарозой неудивительно в свете данных о том, что трегалоза, состоящая из двух глюкозных остатков, лучше стабилизирует мембраны, замещая в них воду и образуя мостики между соседними головками фосфолипидов (Crowe et al., 1988). Таким образом, положено начало спасению названных видов с помощью криосохранения их тканей в жидком азоте.

ЛИТЕРАТУРА

Бутенко, Р.Г., Попов, А.С., Волкова, Л.А., Диттрих, Б., & Лукнер М.(1983): Жизнеспособность клеток растений при разных режимах глубокого замораживания. – Цитология *25*(10): 1191-1196.

Волкова, Л.А., Попов, А.С., Носов, А.М. & Бутенко, Р.Г. (1982): Сохранение биосинтетического потенциала клетками диоскореи (*Dioscorea deltoidea* Wall.) после криогенного хранения. - Доклады Академии Наук СССР *265*(2): 504-506.

Волкова, Л.А., Попов, А.С. & Самыгин, Г.А. (1984): Влияние аминокислот на суспензионную культуру клеток диоскореи дельтовидной и ее возобновление после хранения при -196°С. - Физиология растений *31*(4): 632-638.

Попов, А.С. (1993): Некоторые механизмы криоповреждения клеток *in vitro* и особенности их криосохранения. - Физиология растений *40*(3): 485-496.

Попов, А.С., Волкова, Л.А., Гонзалез, А.М.Т., Диттрих, Б., Донат, П., Донец, Н.Б. & Черняк, Н.Д. (1991): Криосохранение клеточных штаммов и меристем растений: влияние подготовки и криопротекторов. - Биология культивируемых клеток и биотехнология растений. - Наука, Москва.

Попов, А.С., & Федоровский, Д.Н. (1992): Повреждения плазмалеммы клеток диоскореи, культивируемых *in vitro*, в процессе их криосохранения. - Физиология растений *39*(2): 335-343.

Попов, А.С., Черняк, Н.Д. & Бутенко, Р.Г. (1982): Возобновление суспензионной культуры клеток женьшеня *Panax ginzeng* C.A. Meyer после глубокого замораживания. - Доклады Академии Наук СССР *262*(3): 765-768.

Самыгин, Г.А. (1974): Причины вымерзания растений. - Наука, Москва.

Туманов, И.И. (1979): Физиология закаливания и морозостойкость растений. - Наука, Москва.

Федоровский Д.Н. & Попов, А.С. (1992): Повреждения плазмалеммы различных штаммов диоскореи дельтовидной при криосохранении. - Физиология растений *39*(3): 592-598.

Федоровский, Д.Н., Черняк, Н.Д. & Попов, А.С. (1993): Исследование повреждений плазмалеммы клеток женьшеня настоящего при криосохранении. - Физиология растений *40*(1): 117-123.

Грубичич, Д., Чулафич, Л. & Боевич - Цветич, Д. (1991): Регенерация диоскореи кавказской (*Dioscorea caucasica* Lipsky) и диоскореи балканской (*D. balcanica* Košanin) в культуре *in vitro*. - Физиология растений *38*(5): 1018-1022.

Чулафич, Л., Грубишич, Д., Вуичич, Р., Волкова, Л.А. & Попов, А.С. (1994): Соматический эмбриогенез *in vitro* диоскорей кавказской и балканской и криосохранение их органогенных каллусных тканей. - Физиология растений *41*(6): 929-934.

Crowe, J., Crowe, L., Carpenter, J., Rudolph, A., Wistrom C., Spargo, B. & Anchrordoguy, T. (1988): Interactions of Sugars with Membranes. - Biochim. Biophys. Acta, Rev. Biomembranes *947*(2): 367-386.

Keefe, P. & Henshaw, G. (1984): A Note on the Multiply Role of Artificial Nucleation of the Suspending Medium During Two-step Cryopreservation Procedures. - Cryo-Letters *5*(1): 71-78.

Steponkus, P. (1984): Role of the Plasma Membrane in Freezing Injury and Cold Acclimation. - Annual Revue of Plant Physiology *35*: 543-584.

Yamada, Y., Hara, Y., Katagi, H. & Senda, M. (1980): Protoplast Fusion. Effect of Low Temperature on the Membrane Fluidity of Cultured Cells. - Plant Physiology *65*(6): 1099-1102.

Rezime

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KRIOKONZERVACIJA GENOFONDA BILJAKA I TKIVA *IN VITRO* *DIOSCOREA BALCANICA* I *D. CAUCASICA*

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U uslovima neograničenog rasta potreba čovečanstva i poremećaja ekološke ravnoteže u prirodi, već davno je shvaćena opasnost koja ugrožava genetičke resurse: kulturnih, lekovitih i izčezavajućih, endemičnih biljaka. Tradicionalni načini očuvanja tih resursa su nedovoljni. Tako je shvaćena neophodnost kriokonzervacije genofonda ovih biljaka u tečnom azotu (-196°C). Tako dobijena „**kriobanka**" ima poseban značaj za vegetativno razmnožavanje izabranih vrsta, roditeljskih hibridnih formi, i biljaka sa rekalcitrantnim semenima. Istraživanja na vrstama *Panax ginseng* i *Dioscorea deltoidea* dala su mogućnost čuvanja ćelijskih linija ovih vrsta u tečnom azotu i pokazala da je glavni problem dehidratacije ćelija. Danas se u kriobanci Instituta za fiziologiju biljaka K.A. Timirjazeva u Moskvi čuvaju 23 linije od 15 biljnih vrsta čiji su meristemi stabla vraćeni sa uspehom u kulturu *in vitro* posle dugotrajne kriokonzervacije.

Kalusi endemo-reliktnih vrsta *Dioscorea balcanica* Košanin i *D. caucasica* Lipsky, koje su uvedene u kulturu *in vitro* u Odeljenju za fiziologiju biljaka Instituta za biološka istraživanja „Siniša Stanković" u Beogradu, čuvaju se u tečnom azotu u ovoj kriobanci. Kao krioprotektant korišćen je 7% dimetilsulfoksid (DMSO) sa 5% trehalozom, a zamrzavanje je vršeno po programu 3PK-I (SSSR) brzinom 0.33 C/min do -30°C, zatim 10.00 C/min do 60°C a zatim su ampule brzo spuštane u tečni azot. Posle višemesečnog čuvanja na temperaturi -196°C organogeni i embriogeni kalusi su otapani u vodenom kupatilu na 40°C a zatim gajeni u *in vitro* uslovima na hranljivim podlogama za regeneraciju biljaka. Regeneracija je bila uspešna u istoj meri kao i kod kalusa koji su permanentno rasli na 25°C.

Ova metoda je neophodni sastavni deo postupka za očuvanje retkih i endemičnih biljnih vrsta putem metode kulture *in vitro*.

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Review

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NITROGEN ASSIMILATION IN MAIZE

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Hadži-Taškovć Šukalović, V. Vuletić, M. and Vučinić, Ž. (1995): *Nitrogen assimilation in maize.* – Glasnik Instituta za botaniku i botaničke bašte Univerziteta u Beogradu, Tom XXIX, 9 - 17.

A review of the nitrogen assimilation in maize is given, with special emphasis on the differential uptake and metabolic strategies employed for the incorporation of the nitrate and ammonium. The data presented show that for optimum growth and development maize requires both forms of nitrogen to be present in the soil. Under stress conditions (low temperatures, drought or low pH and aluminium toxic soils) such different uptake and metabolic pathways express themselves in a differential response, making it possible to alleviate some of the unfavourable effects of stress conditions by feeding maize plants in the field with an appropriate nitrogen fertiliser mixture. In the future, breeding of plants with specific nitrogen assimilation pathways, as a way to battle non-optimal environmental conditions, could be envisaged.

Key words: nitrogen assimilation, photosynthesis, nitrate, ammonium, ion uptake by roots, *Zea mays* L.

Ključne reči: asimilacija azota, fotosinteza, nitrat, amonijum, usvajanje jona od korena, *Zea mays* L.

INTRODUCTION

Among the mineral nutrients nitrogen is unique in that the plant can take it up as the positively charged ammonium ion (NH_4^+) and /or the negatively charged nitrate ion (NO_3^-). Both nitrogen forms are available in the soil to a smaller or greater extent. Whilst ammonium can be produced in the soil as an intermediate of the urea metabolism and the degradation and oxidation of other organic matter, nitrate is the dominant form of soil nitrogen available to plants under default (normal) conditions. This is due to the fact that under natural conditions ammonium is quickly converted to nitrate as the result of the ubiquitous distribution of soil micro-organisms, capable of metabolic utilisation of the free energy contained in the process of ammonia oxidation. However, in agronomy practice, mankind has devised ways of supplying ammonium to the agriculturally important plants, altering the NO_3^-/NH_4^+ ratio and keeping it far from the natural equilibrium.

Nitrogen assimilation involves the uptake of nitrogen from the soil by the root system, the metabolic conversion of the absorbed nitrate form in the root and/or the shoot, transport to the shoot, and its incorporation into different organic forms in the cell metabolism. Most plants can use either nitrate or ammonium as a source of nitrogen. However, the degree of effectiveness of these forms on plant growth and nutrient uptake when both sources of nitrogen are available is dependent on plant species and $NH_4^+ : NO_3^-$ ratio (Errebhi & Wilcox, 1990). Maize plants require both nitrogen forms for maximal growth (Schrader et al., 1972). If the source of nitrogen is nitrate, it may be reduced in the root or transported to the shoot where it can be processed.

Maize is the plant with which contemporary breeding in the last 70 years has achieved the greatest results, the average yield of grain increasing more than 500% from the yield achieved for centuries before. Of course, such yields are possible only when high energy input agronomy practice is applied, and adequate water, light and high temperatures are available. The photosynthetic process, as far as it is known, is the same amongst the ancient varieties selected by nature in different environments and the new hybrid varieties of maize being planted throughout the conr belt region of the world. It is common knowledge that the ancient varieties are not capable of utilising extra fertiliser (especially nitrogen) when provided to such plants, as opposed to the selected and improved hybrids, capable of utilising up to 200 kg of fertiliser per hectare per year, resulting in such a spectacular increase in growth, yield and utilisation of the efficient C_4 type of photosynthetic process. However, no consistent physiological, biochemical or molecular biology explantation has been offered to explain what is known as *„good recombination characteristics"* and *„heterosis"* amongst the breders. The logical explantation would be that the new varieties of maize have an altered nitrogen assimilation pathway, capable of taking up and transforming the nitrogen provided by human endeavour, when sufficient energy is available, but this has yet to be proved.

AMMONIUM AND NITRATE UPTAKE BY THE ROOT

Ammonium uptake systems, defined as energy-dependent and carrier-mediated in algae, fungi and bacteria (Kleiner, 1981), in higher plants are relatively less studied. In rice roots ammonium influx is biphasic and mediated by two discrete transport systems (Wang et al., 1993). At low ammonium concentrations (below 1mM) influx is mediated by saturable high affinity transport system with high Q_{10} and significant sensitivity to metabolic inhibitors. At higher concentrations ammonium

influx shows a linear response due to low-affinity transport system, being much less responsive to metabolic inhibitors and temperature. Mechanism of high-affinity transport which appears to be an active process in roots of rice (Wang et al., 1994) is unknown. The effects of CCCR, ATPase inhibitor (DES) and respiratory inhibitors (KCN + SHAM) confirm the dependence of these processes on metabolic energy and indicate the involvement of H^* transport (direct or indirect). It was suggested (Wang et al., 1994) that passive entry of ammonium might occur in the low-affinity transport system (specific channel for NH_4^+ or a shared cation channel).

The uptake of NO_3^- appears to be mediated by at least two distinct systems in higher plants. The first is an inducible high-affinity active transport system, which has a low K_m for NO_3^-, shows Michaelis-Menten saturation kinetics, is sensitive to metabolic inhibitors and regulated according to the plant nitrogen status. Nitrate induced pH dependent transient depolarisation of membrane potential, followed by a repolarisation, and observed on different plant objects (Ulrich, 1987), was explained by the operation of a NO_3^-/H^+ symport mechanism with excess protons, and subsequent stimulation of proton pump. In maize roots a similar electrical response, which displayed nitrate-inducibility, pH dependence, as well as sensitivity to plasma membrane ATPase inhibitors, was closely correlated to nitrate uptake characteristics (McClure et al., 1990a, b). The second transport system is a constitutive, low-affinity transport system, operating at higher NO_3^- concentrations, with linear kinetics and lower sensitivity to metabolic inhibitors. Although it has some characteristics that would be expected of a passive, channel mediated transport system, recent results suggest that this system might also be active (King et al., 1992).

The plant plasma membranes contain redox systems involved in trans-plasma membrane electron transport from internal electron donors, such as NAD(P)H, to specific electron acceptors, often accompanied by proton extrusion from the cell. A possible involvement of constitutive plasma membrane-bound nitrate reductase in redox activities in membrane was indicated by immunological correlation between nitrate reduction activity and reduction of extracellular electron acceptors (Jones & Morel, 1988). They proposed a model in which plasma membrane-bound nitrate reductase reduces extracellular electron acceptor and intracellular nitrate and also acts as a trans-plasma membrane proton pump.

Since it was proposed that such a nitrate reductase could be also responsible for nitrate transport across plasma membranes (Butz & Jackson, 1977), the hypothesis was supported or disputed by different authors. Experiments with barley genotypes lacking the nitrate reductase gene demonstrated the independence of nitrate uptake and nitrate reductase activity (Warner & Huffaker, 1989). On the other hand, the existence of nitrate reductase activity localised in the plasma membrane, in addition to soluble cytoplasmic nitrate reductase, in different plant objects including barley and maize root (Ward et al., 1989), and inhibition of nitrate uptake and plasma membrane-bound nitrate reductase of barley roots by same antibodies (Ward et al., 1988), indicates a possible relationship between NO_3^- transport and NO_3^- reduction in the plasma membrane. This plasma membrane-bound nitrate reductase activity in maize roots exhibited two different activities (NADH and NADPH-dependent), both being constitutive and insensitive to ammonium, contrary to the soluble cytoplasmic nitrate reductase with low constitutive activity (De Marco et al., 1994).

METABOLIC CONVERSION OF NITROGEN FORMS AND ROOT-SHOOT INTERACTIONS

Nitrogen taken up from the soil can be stored in the root, incorporated into organic molecules in the root, or transported to the shoot and there stored or incorporated into organic matter. The process of nitrogen incorporation into organic matter is among the most energy-intensive processes in plant. All of the organic forms of nitrogen must be derived from NH_4^+. Conversion of one molecule of NH_4^+ to glutamate requires two electrons and one ATP. In the case of nitrate serving as the nitrogen source, it must be first converted to NH_4^+. This reduction of nitrate to ammonium is a two reaction step, NO_3^- reduction to NO_2^- requiring two electrons and catalysis by nitrate reductase (NR EC 1.6.6.1 and 2), and NO_2^- reduction to NH_4^+ requiring six electrons and catalysis by nitrite reductase (NiR EC 1.7.99.3). Thus, the uptake and incorporation of nitrate by plants is a much more energy-demanding and costly process. Therefore, plants expend less energy for NH_4^+ assimilation. Enzymes required for nitrate reduction and for the assimilation of ammonium ion are found both in the root and shoot (Oaks & Hirel, 1985).

When ammonium is the source of nitrogen, it is incorporated into the amide nitrogen of glutamine, glutamine then being exported to other parts of the plant (Oaks, 1992). In such a case the nitrate uptake system(s), and the nitrate and nitrite reductases, with their high energy demands, would be bypassed. When nitrate is the dominant ion taken up by the plant, the plant root responses to such an increase of environmental NO_3^- are the induction of enhaced NO_3^- uptake system(s) and induction of enzymatic activities to catalyse the reduction of NO_3^- to NH_4^+ (Jackson et al., 1986; Larson & Ingemarsson, 1989). Also, increased availability of NO_3^- induces the system for the assimilation of reduced nitrogen, the transport of NO_3^- to the shoot, proliferation of the root system, changes root to shoot ratios and enhances root respiration (Granato & Raper, 1989; Bloom et al., 1992).

In the case of nitrate reduction occurring in the leaf, the whole process is linked directly to the photosynthetic assimilation process. It was shown that C_4 plants (amongst which is maize) have the enzymes of nitrogen reduction and amination distributed between the two types of photosynthetic cells and chloroplasts, the high energy demand components (reductases) being localised in the mesophyll cell (Moore & Black, 1979). Maize has been known for a long time to be an *efficient nitrogen utilising species*. Such a characteristic was based on the capability of maize to achieve a greater total dry weight to nitrogen ratio, when compared to other agriculturally important plants. It was only with the discovery of the C_4 pathway, when it was shown that the most prominent leaf enzyme, ribulose bisphosphate carboxylase/oxygenase (in C_3 plants accounting for 50% of the leaf protein), due to the efficient functioning of the photosynthetic process of such plants can be reduced in quantity, thus altering the total dry weight/nitrogen ratio (Hatch, Osmond & Slatyer, 1971) that an explantation for such greater efficiency was offered. The energy for nitrate reduction in nonchlorophyllous tissues comes from oxidation of carbohydrates or organic acids.

Under conditions of limited external nitrate concentrations (without ammonium present), higher nitrate reductase is detected in maize leaf than in root tissue (Oaks & Hirel, 1985), but with increasing nitrate concentrations the component of nitrate becoming reduced in roots also increases. Thus the proportion of nitrate reduced in maize roots was shown to be about 37% of the total nitrate taken up (Van Benish-

chem et al., 1989), the remainder being reduced in the shoot tissue. Other authors have shown that the partitioning of nitrate assimilation between root and shoot and relative concentrations of nitrate and reduced nitrogen in xylem sap, in maize plants, showed substantial proportion of nitrate assimilation in the shoot (up to 90%) (Andrews, 1986). Our results (Hadži-Tašković Šukalović & Baoguo, 1996) demonstrated that changes of NO_3^- concentration in nutrient solution from 2.5 mM to 10.9 mM increased the specific activity of the nitrate reductase for 170% in root and 50% in leaf tissue of 15 days old maize. The ratio of leaf to root nitrate reductase activity being 5.7 in low NO_3^- solution and decreasing to 3.2 in high NO_3^- solution. This suggests that growth of plants in high nitrate concentrations alters the proportion of nitrate reduced in the shoot by enhancing the root capacity to reduce NO_3^-. Such observations could be explained by a metabolic shift to a different mechanisms for the uptake of nitrate ions.

Nitrogen from ammonium is processed primarily in the roots (Lewis, 1986). When ammonium is the available source of nitrogen, it is incorporated into glutamine in the root mainly in a reaction mediated by glutamine synthetase (GS EC 6.3.1.2). Glutamine is the dominant form of transport to the other parts of the plant. Synthesis of glutamine requires glutamate, ammonium and ATP in a reaction with GS. This reaction takes place in root tissue under normal conditions, assuming that glutamate is generated from glutamine and 2-oxoglutarate, an intermediate of TCA cycle in a reaction mediated by glutamate synthase, (GOGAT EC 1.4.7.1) as discovered by Lea and Miflin (1974). As a result of this NH_4^+ - induced metabolic activity in the root, which could deprive other tissues of carbon skeletons and energy resources required for growth under high NH_4^+ conditions and thereby result in NH_4^+ toxicity. On the basis of organic nitrogen transported to the shoot, it was suggested that the GS/GOGAT system alone may not be sufficient to assimilate NH_4^+ in roots (Handa et al., 1984) and glutamate dehydrogenase (GDH EC 1.4.1.2) activity in maize roots has been reported with NH_4^+ assimilation (Handa et al., 1985; Oaks et al., 1980), expecially when high NH_4^+ concentration is available for plant growth. Aminating function of GDH which uses 2-oxoglutarate, NH_4^+ and NADH in high ammonia conditions is considered to be involved in tissue detoxification. Recent results have shown that ammonium isomerization of GDH molecule occurs with high NH_4^+ concentration (above 5 mM) to form the hexameric structure of enzyme (Osuji & Madu, 1995). This is a critical reaction step in the synthesis of glutamate. Many authors reported the amination function of GDH for numerous plant tissues (Yamaya & Oaks, 1987; Zhang-Qiang et al., 1992; Osuji & Cuero, 1992). Our unpublished results (Hadži-Tašković Šukalović & Vuletić) on maize root mitochondria isolated from 15-days old plants grown on 10.9 mM NO_3^- ± 7.2 mM NH_4^+ demonsrated also an amination role of GDH induced by high NH_4^+ level present in nutrient solution. Increased GDH activity indicate that mitochondria could be the place of glutamate synthesis and therefore, may be involved in detoxification of excess of NH_4^+. As the result of the requirement of carbon skeleton and energy for NH_4^+ assimilation and biosynthetic purposes, mitochondria exhibited intensified TCA cycle activity and also increased phosphorilative and non-phosphorilative activity which could provide a mechanism for the turn-over of the TCA cycle. Glutamate synthesis in mitochondria by GDH would function as an amino donor in transamina-

tions inside mitochondria to provide aspartate (in a reaction with oxaloacetate) or alanine (in reaction with pyruvate), as well as for the formation of glutamine in a reaction with GS outside mitochondria (Oaks, 1992).

Uptake of NO_3^- in maize is not inhibited in the presence of NH_4^+, but assimilation of NO_3^- into organic nitrogen is retarded by NH_4^+. When both nitrogen forms are absorbed, NH_4^+ is used preferentially for synthesis of amino acids and protein (Schrader et al., 1972). Assimilation of nitrate or ammonium is dependent on carbohydrate metabolism. In root tissue, a significant provision of carbon required for amino and organic acids synthesis is derived from phosphoenolpyruvate (PEP) carboxylation (Arnozis et al., 1988; Cramer et al., 1993). The requirements of NH_4^+ assimilation cannot be fully satisfied by the endogenous supply of 2-oxoglutarate because of the intensified amino acid synthesis, and therefore higher rates of dark fixation of dissolved inorganic carbon provides the carbon skeleton for both, amino acid and organic acid synthesis. In nitrate assimilating plants, the products of PEP carboxylation are preferentially diverted to organic acid synthesis. According to Cramer et al. (1993), the capacity of plants to assimilate NH_4^+, especially under limiting supply of respiratory 2-oxoglutarate, is determined by the capacity of such plants to provide the necessary carbon skeleton in the roots. A balanced ammonium-nitrate assimilation may induce a desirable organic acid content in plant tissue.

NITROGEN ASSIMILATION IN STRESS CONDITIONS

Instead of internal control, the uptake and assimilation of two nitrogen containing ions in maize are regulated also by external conditions. Concentration of nitrogen, pH, temperature and light all influence nitrogen uptake, transport to the shoot and assimilation (Jackson & Volk, 1992).

Nitrate assimilation is more affected than NH_4^+ assimilation in all stress conditions, independent of the stress origin. Many studies indicate that the leaf nitrate reductase is an extremely susceptible enzyme to environmental changes. Decreased leaf nitrate reductase activity was reported in the conditions of low light intensity (Li & Oaks, 1995), high temperature and drought (Amos & Scholl, 1977), low temperature (Bakker & Van Hasselt, 1982; Hadži-Tašković Šukalović & Zarić, 1991), or aluminium toxicity stress (Hadži-Tašković Šukalović et al., 1993). Hadži-Tašković Šukalović & Baoguo, (1996) reported a strong negative effect of aluminium on the maize leaf NADH-nitrate reductase activity. The reduction of activity was dependent on the maize genotype analysed. In some cases, more than 70% of enzyme specific activity was lowered during aluminium stress. An opposite effect was detected in roots. NADH-nitrate reductase specific activity was slightly decreased or even stimulated. Therefore, it was evident that aluminium stress changed proportions for nitrate reduction between shoots and roots. The same authors demonstrated that bifunctional NAD(P)H-nitrate reductase activity was significantly elevated in roots, suggesting that this enzyme plays a more important role in NO_3^- assimilation under aluminium stress and therefore in aluminium tolerance.

Low temperatures decrease the NO_3^- transport to the shoot and increase the NO_3^- concentration in the root tissue (Kafkafi, 1990), but aluminium stress limits the NO_3^- uptake (Durieux et al., 1995) and decrease NO_3^- concentration in maize roots (Hadži-Tašković Šukalović & Baoguo, 1996; Mihailović et al., 1995). Under unfavourable external conditions, NH_4^+ is a better source of nitrogen,

because it is quickly metabolised in the root tissue, and translocation of metabolites to the shoot is less affected by root temperature. It is in this context that the activity of enzymes involved in the incorporation of NH_4^+ to organic compounds are not affected, or even stimulated in different stress conditions.

Miranda-Ham & Loyola-Vargas (1994) reported the resistance of maize root glutamine synthetase during water and salt stress. Hadži-Tašković Šukalović et al. (1990) found that aluminium stress did not affect the GS activity, but even increased GDH activity in roots of many maize inbred lines subjected to stress. In order to survive unfavourable conditions, many plants absorb more NH_4^+ than nitrate (Shaviv, 1990).

CONCLUSIONS

According to Kafkafi (1990) NH_4^+ can serve as a good source of nitrogen as long as sugar reserves and supply are available in the root, and opposite, the conditions when high consumption of sugart takes part in the root, NO_3^- is a better source of nitrogen for the plant. The adaptability of maize plant to change the proportion of NO_3^- or NH_4^+ rates of uptake under stress conditions, gives a mechanism for improvement of crop yield by adequate supply of appropriate nitrogen nutient.

It is obvious from the data presented that maize plants have a versatile and adaptable nitrogen uptake and metabolism system. Under conditions of stress, or through human endeavour and varying fertiliser application, when different forms of nitrogen are supplied to plants, its cellular mechanisms are capable of performing a switch and metabolic adaptation so as to be able to take up as much nitrogen as possible.

The importance of studying nitrogen assimilation Olsen (1986) emphasised with words: *„Controlled nitrogen (ammonium-nitrate) nutrition is the largest and most significant laboratory proven potential for increased crop growth that has not been demonstrated on a field scale"*. We would like to add the word *„yet"*.

REFERENCES

Amos, J.A., & Scholl, R.L. (1977): Effect of growth temperature on leaf nitrate reductase, glutamine synthetase and NADH-glutamate dehydrogenase of juvenile maize genotypes. – Crop Sci. *17:* 445-448.

Arnozis, P.A., Nelemans, J.A. & Findenegg, G.R. (1988): Phosphoenolpyuruvate carboxylase activity in plants grown with either NO_3^- or NH_4^+ as inorganic nitrogen source. – J. Plant Physiol. *132:* 23-27.

Andrews, M. (1986): The partitioning of nitrate assimilation between root and shoot of higher plants. – Plant Cell Environ. *9:* 511-519.

Bakker, N. & Van Hasselt, P.R. (1982): Photooxidative inhibition of nitrate reductase during chilling. – Physiol. Plant. *54:* 414-418.

Bloom, A.J., Sukrapanna, S.S. & Warner, R.L. (1992): Root respiration associated with ammonium and nitrate absorption and assimilation by barley. – Plant Physiol. *99:* 1294-1301.

Butz, R.G. & Jackson, W.A. (1977): A mechanism for nitrate transport and reduction – Phytochem. *13:* 409-417.

Cramer, M.D., Lewis, O.A.M. & Lips, S.H. (1993): Inorganic carbon fixation and metabolism in maize roots as afected by nitrate and ammonium nutrition. – Physiol. Plant. *89:* 632-639.

De Marco, A., Jia, C., Fischer-Schliebs, E., Varanini, Z. & Lütge, U. (1994): Evidence for two different nitrate-reducing activities at the plasma membrane in roots of *Zea mays* L. – Planta *194:* 557-564.

Durieux, R.P., Bartlett, R.J. & Magdoff, F.R. (1995): Separate mechanisms of aluminium toxicity for nitrate uptake and root elongation. – Plant and Soil *172:* 229-234.

Errebhi, M. & Wilcox, G.E. (1990): Plant species response to ammonium-nitrate concentration ratios. – J. Plant Nutr. *13:* 1017-1029.

Granato, J.C. & Raper, C.D. (1989): Proliferation of maize *(Zea mays* L.) roots in response to localized supply of nitrate. – J. Exp. Bot. *40:* 263-275.

Hadži-Tašković Šukalović, V., Lazić-Jančić, V., Vuletić, M. & Denić, M. (1990): The effect of aluminium on the nitrogen assimilation enzyme activities in maizie. – Arch. Sci. Biol. *42:* 155-165.

Hadži-Tašković Šukalović, V. & Zarić, Lj. (1991): Study of effect of low temperature on some nitrogen assimilation enzymes in the maize leaf. – J. Sci. Agric. Res. *52:* 133-142.

Hadži-Tašković Šukalović, V., Lazić-Jančić, V. & Vuletić, M. (1993): Effect of aluminium on nitrate reductase activity in maize genotypes differing in Al-tolerance. – Proced. II Simpozium on chemistry and the environment, 9-13 June, Vrnjačka Banja.

Hadži-Tašković Šukalović, V. & Baoguo, L (1996): NADH and NAD(P)H nitrate reductase activities of maize exposed to aluminium stress. – Plant Physiol. & Biochem. (Special issue): 157.

Handa, S., Waren, H.L., Huber, D.M. & Tsai, L.Y. (1984): Nitrogen nutrition on seedling development of normal and opaque-2 genotypes of corn. – Canad. J. Plant Sci. *64:* 885-894.

Handa, S., Waren, H.L., Huber, D.M. & Tsai, L.Y. (1985): Nitrogen nutrition and N-assimilation in maize seedlings. – Canad. J. Plant Sci. *65:* 87-93.

Hatch, M.D., Osmond, C.B. & Slatyer, R.O. Eds. (1971): Photosynthesis and Photorespiration. – Wiley Interscinece, New York.

Jackson, W.A. & Volk, R.J. (1992): Nitrate and ammonium uptake by maize: Adaptation during relief from nitrogen suppression. – New Phytol. *122:* 439-446.

Jackson, W.A., Pan, W.L., Moll, R.H. & Kamprath, E.J. (1986): Uptake, translocation and reduction of nitrate. *In* Biochemical Basis of Plant Breeding. Vol. 2 (C. Neyra, ed.), pp. 73-108. – CRC Press, Boca Raton.

Kafkafi, U. (1990): Root temperature, concentration and the ratio NO_3^-/NH_4^+ effect on plant development. – J. Plant Nutr. *13:* 1291-1306.

King, B.J., Siddiqi, M.Y. & Glass, A.D.M. (1992): Studies of the uptake of nitrate in barley. V. Estimation of root cytoplasmic nitrate concentration using nitrate reductase activity-implications for nitrate influx. – Plant Physiol. *99:* 1582-1589.

Klainer, D. (1981): The transport of NH_3 and NH_4^+ across biological membranes. – Biochim. Biophys. Acta *639:* 41-52.

Lambers, H., Simpson, R.J., Beitharz, V.C. & Dalling, M.J. (1982): Growth and translocation of C and N in wheat *(Triticum aestivum)* grown with split root system. – Physiol. Plant. *56:* 421-429.

Larson, C.M. & Jngemarsson, B. (1989): Molecular aspects of nitrate uptake in higher plants. *In* Molecular and Genetic Aspects of Nitrate Assimilation (eds. Y.R. Kinghorn and J.L. Wray). – Oxford Scinece Publishers, New York.

Lea, P.J. & Miflin, B.J. (1974): Alternative route for nitrogen assimilation in higher plants. – Nature (Lond.) *251:* 614-616.

Lewis, O.A.U. (1986): Plants and Nitrogen. Edward Arnold, London.

Li, X-Z. & Oaks, A. (1995): The effect of light on nitrate and nitrite reductases in *Zea mays.* – Plant Sci. *109:* 115-118.

McClure, P.R., Kochian, L.V., Spanswick, R.M. & Staff, J.E. (1990): Evidence for cotransport of nitrate and protons in maize roots. I. Effects of nitrate on membrane potential. – Plant Physiol. *93:* 281-289.

McClure, P.R., Kochian, L.V., Spanswick, R.M. & Staff, J.E. (1990): Evidence for cotransport of nitrate and protons in maize roots. II. Measurement of NO_3^- and H^+ fluxes with ion-selective microelectrodes. – Plant Physiol. *93:* 290-294.

Mihailović, N., Hadži-Tašković Šukalović, V., Vučinić, Ž. & Bogdanović, M. (1995): Response of nitrate concentration and assimilation in maize leaves to the presence of aluminium and different ion forms of nitrogen. – Proceedings of the I Regional Symposium „Chemistry and the environment", 25-29. September, 1995, Vrnjačka Banja.

Miranda-Ham, M.L. & Loyola-Vargas, V.M. (1994): Glutamate dehydrogenase and glutamine synthtase activities in maize under water and salt stress. – Fyton *56:* 7-15.

Moore, R. & Black, C.C. (1979): Nitrogen assimilation pathways in leaf mesophyll and bundle sheath cells of C_4 photosynthesis plants formulated from comparative studies with *Digitaria sanguinalis* (L.). – Platn Physiol. *64:* 309-313.

Oaks, A., Stulen,J., Jones, K., Winspear, M.J., Misra, S. & Boessel, J.L. (1980): Enzymes of nitrogen assimilation in maize roots. – Planta *148:* 477-484.

Oaks, A. & Hirel, B. (1985): Nitrogen metabolism in roots. – Ann. Rev. Plant Physiol. *36:* 345-365.

Oaks, A. (1992): A re-evaluation of nitrogen assimilation in roots. – Bio Science *42:* 103-111.

Olsen, S.R. (1986): Using soil and fertilizer chemistry to improve corn productivity. – Potash and Phosphate Institute, Maximum Yield Corn Research Roundtable, November 1985, St. Louis, Mo.

Osuji, G.O. & Cuero, R.C. (1992): Regulation of ammonium ion salvage and enhancement of the storage protien contents of corn, sweet potato, and yam tuber by N-(carboxymethyl)chitosan application. – Agric. Food. Chem. *40:* 724-734.

Osuji, G.O. & Madu, C.W. (1995): Ammonium ion-dependent isomerization of glutamate dehydrogenase in relation to glutamate synthesis in maize. – Phytochem. *39:* 495-503.

Schrader, L.E., Domska, D., Jung, P.E. & Petterson, L.A. (1972): Uptake and assimilation of ammonium-N and nitrate-N and their influence on the growth of corn *(Zea mays* L.). – Agron. J. *64:* 690-695.

Shavir, A., Hazan, O., Neumann, P.M. & Hagin, J. (1990): Increasing salt tolerance of wheat by mixed ammonium nitrate nutrition. – J. Plant Nutr. *13:* 1227-1239.

Ullrich, W.R. (1987): Nitrate and ammonium uptake in green algae and higher plants: mechanisms and relationship with nitrate metabolism. *In* Inorganic Nitrogen Metabolism (Eds. W.R. Ullrich, P.J. Aparicio, P.J. Syrett, F. Castillo). – Springer-Verlag, Berlin Heildelberg.

Van Bensichem, M.L., Nelemans, J.A. & Hinnen, M.G.J. (1989): Nitrogen cycling in plant species differing in root reduction of nitrate. – Colloq. on plant nutrition, Wageningen.

Wang, M.Y., Siddiqi, M.Y., Ruth, T.J. & Glass, A.D.M. (1993): Ammonium uptake by rice roots. II. Kinetics of $^{13}NH_4^+$ influx across the plasmalemma. – Plant. Physiol. *103:* 1259-1267.

Wang, M.Y., Glass, A.D.M., Shaff, J.E. & Kochian, L.V. (1994): Ammonium uptake by rice roots. III. Electrophysiology. – Plant Physiol. *104:* 899-906.

Ward, M.R., Tischner, R. & Huffaker, R.C. (1988): Inhibition of nitrate transport by anti-nitrate reductase IgG fragments and the identification of plasma membrane associated nitrate reductase in roots of barley seedlings. – Plant Physiol. *88:* 1141-1145.

Ward, M.R., Grimes, H.D. & Huffaker, R.C. (1989): Latent nitrate reductase activity is associated with plasma membrane of corn roots. – Planta *177:* 470-475.

Warner, R.L. & Huffaker, R.C. (1989): Nitrate transport is independent of NADH and NAD(P)H nitrate reductases in barley seedlings. – Plant Physiol. *91:* 947-953.

Yamaya, J. & Oaks, A. (1987): Synthesis of glutamate by mitochondria. – An anaplerotic funciton for glutamate dehydrogenase. – Physiol. Plant *70:* 749-756.

Zhang-Qiang, S., Jolivet, J. & Dizengremel, P. (1992): Effect de l'alimentation azotée sur le matabolisme du feuilles de Soja. – C.R. Acad. Sci. (Paris). Ser. III, *314:* 565-571.

Rezime

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ASIMILACIJA AZOTA KOD KUKURUZA

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Daje se pregled mehanizama asimilacije azota (sa posebnim osvrtom na kukuruz), razmatrajući različite puteve usvajanja i metaboličkih transformacija za ugradnju nitritnog i amonijačnog jona. Podaci pokazuju da je za optimalan rast i razvoj kukuruza poželjno prisustvo obe forme azota u zemljištu. U uslovima stresa (niska temperatura, suša ili niski *pH* i aluminijum toksična zemljišta) takvi različiti putevi usvajanja i metabolizma azota nalaze ekspresiju u različitim odgovorima biljke, omogućavajući da se neki od štetnih efekata stresa uklone prihranom biljaka kukuruza u polju odgovarajućom kombinacijom azotnih formi pri đubrenju. U budućnosti, selekcija biljaka sa specifičnim putevima asimilacije azota bi mogla da se pokaže kao jedan od pristupa borbe protiv neoptimalnih uslova spoljašnje sredine.

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EKOFIZIOLOŠKA ISTRAŽIVANJA BILJAKA U SRBIJI

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The paper presents the main problems and trends in the studies of physiological plant ecology during the last three decades in Serbia. It gives a brief survey of the most important aspects of environmental physiology of plants, such as plant water relations, photosynthesis, respiration and dry-matter production, analysed through the ecophysiological parameters of the great number of plants from the principal ecosystems of the region: forest, meadow, agricultural and urban ecosystems. On the basis of the obtained data the general achievements and the future prospects in the theoretical and practical work in physiological plant ecology have been discussed.

Key words: physiological plant ecology, hydrature, transpiration, water potential, water content, photosynthesis, respiration, light compensation point, primary production.

Ključne reči: fiziološka ekologija biljaka, hidratura, transpiracija, vodni potencijal, sadržaj vode, fotosinteza, disanje, kompenzaciona tačka svetlosti, organska produkcija.

UVOD

Fiziološka ekologija biljaka predstavlja važnu sponu između ekologije i fiziologije biljaka. Ona je posebna i veoma značajna disciplina proizašla iz fitoekologije, koja na konkretan i dokumentovan način objašnjava složenu uzajamnu zavisnost životnih procesa u biljci i uslova sredine u kojoj biljka živi. Fiziološka ekologija biljaka proučava fiziološke procesa biljnih vrsta u prirodnim, manje-više neizmenjenim, uslovima njihovih staništa, težeći da, s jedne strane, njihovu ekologiju objasni odgovarajućim fiziološkim specifičnostima i adaptacijama, i, s druge strane, da njihove fiziološke procese (njihov karakter, intenzitet i dinamiku) objasni uticajem konkretnih spoljašnjih uslova na staništu i adaptivnim mogućnostima biljke (J a n k o v i ć, K o j i ć, 1975).

Zadaci fiziološke ekologije biljaka su kompleksni i konkretni. Predmet njenog ispitivanja su prevashodno fiziološki procesi u odgovarajućim prirodnim uslovima staništa, ali, istovremeno, i valorizacija faktora spoljašnje sredine koji specifično deluju na te procese, kao i morfološko-anatomske i druge karakteristike biljaka koje određuju ili posebno usmeravaju pojedine fiziološke procese. Uopšte uzev, predmet fiziološke ekologije biljaka je utvrđivanje specifičnih interakcija i funkcionalno-strukturnih odgovora biljaka na odgovarajuće konkretne uslove spoljašnje sredine.

Fiziološka ekologija biljaka se, od početka 20. veka postepeno razvijala kao samostalna naučna oblast iz potreba da se prevaziđu razlike u pristupu izučavanju između fizilogije i ekologije biljaka. Kako navodi W a l t e r (1960) fiziologija biljaka je zbog svoje velike specijalizacije više upućena na detaljno proučavanje pojedinih životnih funkcija, ili na analizu pojedinačanih efekata spoljašnje sredine na određeni fiziološki proces, te ona, obično, ne obuhvata biljku kao celinu. Ekologija biljaka, naprotiv, polazi od toga da biljka predstavlja jedinstven organizam i da promene faktora sredine utiču na reakciju biljke u celini, ali, pri tome, najveću pažnju poklanja prilikama na staništu, kao i prostornim i vremenskim cenotičkim odnosima između biljaka u okviru biljnih zajednica. Stoga, fiziološka ekologija biljaka predstavlja nov naučni pristup, u okviru koga se, po pravilu, proučavaju životni procesi biljke kao jedinstvenog organizma, na staništu, u promenljivim uslovima spoljašnje sredine, a ne u kontrolisanim uslovima u laboratoriji. Na staništu deluje kompleks faktora spoljašnje sredine koji utiču ne samo na pojedine funkcije, već biljka na njih reaguje kao celina. Najčešće promene intenziteta i kvaliteta delovanja jednog faktora povlače za sobom promene drugih. Ispitivanje uticaja pojedinačnih faktora, u prirodnim uslovima, dosta je teško, te je stoga u ekofiziološkim proučavanjima često potrebno paralelno, dopunsko, korektivno praćenje određenih fizioloških procesa biljaka u kontrolisanim uslovima. Pri tome, valja imati u vidu da biljke u prirodi nikad ne žive izolovano, već u određenim kompetitivnim odnosima u okviru odgovarajućih biljnih zajednica i ekosistema u celini.

Fiziološka ekologija biljaka, međutim, podrazumeva istraživanja na različitim nivoima organizacije biljnih sistema. Ona se, samo uslovno, iz praktičnih razloga, mogu razdvojiti na ekofiziološka proučavanja na subćelijskom i ćelijskom nivou, na nivou organizama (ili pojedinih organa), kao i na proučavanja na ceno-populacijskom odnosno cenotičkom nivou (W a l t e r, 1982; B i e b l, 1962; K r e e b, 1974; L a r c h e r, 1978; L e v i t, 1972; i drugi).

Proučavanja na subćelijskom i ćelijskom nivou do sada su, kod nas, malo primenjivana u rutinskim ekofiziološkim eksperimentima. Ovaj nivo istraživanja više je vezan za laboratoriju i omogućava komparativna objašnjenja rezultata ekofizioloških proučavanja na terenu. Bitna pitanja fiziološke ekologije biljaka istraživana su i

analizirana, pre svega, na nivou organizama, kao i na conotičkom ili ekosistemskom nivou. Po svojoj sveobuhvatnosti, a samim tim i značaju za razumevanje ekoloških problema u prirodi, istraživanja u okviru ekosistema predstavljaju suštinska, najbitnija naučna traganja u fiziološkoj ekologiji biljaka.

Koji su glavni pravci, osnovni segmenti, bitni parametri ekofizioloških istraživanja biljaka? Za funkcionisanje ekosistema, za opstanak živog sveta – organska produkcija u suštini ima najveći značaj. Otuda, jedan od osnovnih trendova u fiziološkoj ekologiji biljaka je usmeren u pravcu rešavanja onih ekofizioloških problema od kojih direktno ili indirektno zavisi produkcioni proces, odnosno primarna organska produkcija.

Fotosinteza odnosno **fotosintetski režim** (pojam pod kojim Walter podrazumeva sve elemente fotosintetskog procesa, kao i fiziološke, biohemijske i ostale činioce od kojih zavisi fotosintetska aktivnost biljaka) nalazi se u središtu ekofizioloških proučavanja. Interesantan problem, sa ekološkog aspekta, predstavlja, pre svega, **produktivnost fotosinteze** i **intenzitet fotosinteze.** Kao parametri ekofizioloških razmatranja u tesnoj vezi sa problemima fotosinteze su i **intenzitet disanja** (od čega zavisi konačni saldo organske produkcije), **kompenzaciona tačka,** i dr. Organska produkcija biljaka i biljnog pokrivača u celini, kao konačna rezultanta svih elemenata fotosintetskog režima, kao i svih ostalih relevantnih fizioloških i drugih pokazatelja, u funkciji konkretnih ekoloških prilika, često se, u ekologiji, na kraju analizira kompleksno da bi se sagledala konkretna veličina, dinamika ili kvalitet biomase određenog ekosistema.

Jedan od važnih zadataka fiziološke ekologije biljaka je da utvrdi pod kojim se uslovima vrši proces fotosinteze, glavni činilac organske produkcije biljaka. Osnovni spoljašnji faktori koji utiču na fotosintezu su koncentracija ugljen-dioksida u vazduhu i svetlosni uslovi. Promenom ova dva faktora nastaju variranja u intenzitetu fotosinteze. Različite biljne vrste ne reaguju na isti način, te je bilo potrebno da se pronađe neko merilo koje će ukazati na diferencijalno ponašanje biljaka u promenljivim uslovima sredine. U vezi s tim, fiziološka ekologija biljaka bavi se određivanjem tzv. **kompenzacione tačke.** Postoje dve kategorije ovog parametra: kompenzaciona tačka svetlosti i kompenzaciona tačka CO_2, a njihovim ispitivanjem bavio se veliki broj autora (Lieth, 1958, 1960; Pavletić, Lieth, 1958; Regula, Pavletić, 1968; Semihatova, 1988 i drugi). U našoj zemlji ovim istraživanjima na velikom broju biljaka iz različitih ekosistema pažnju su posvetili naročito Kojić, Janković i njihovi saradnici (Janković, Kojić, 1969; Kojić, 1968; Kojić et al., 1974, 1989, 1990, 1992, 1995).

Jedno od najistaknutijih mesta u programima istraživanja u okviru fiziološke ekologije biljaka zauzima **vodni režim** s obzirom na krucijalni značaj vode za životne procese biljaka. Najčešće se, do današnjih dana, za karakterizaciju vodnog režima koristila analiza četiri parametra: **hidrature, potencijala vode, hidratacije** i **intenziteta transpiracije.** Više od pola veka, od 1931. godine, kada se pojavilo čuveno delo Hajniriha Valtera o fiziološko-ekološkom značaju hidrature (Walter, 1931), jedno od centralnih mesta u ekofizioloških proučavanjima zauzimao je problem hidraturnih odnosa tkiva, određivan na osnovu osmotskih vrednosti ćelijskog soka biljaka. U tom smislu dobijeni su brojni i značajni rezultati kako u inostranstvu tako i u našoj zemlji, o čemu je mnogo pisano i u svetu i kod nas. Na osnovu brojnih podataka o dnevnim i sezonskim kolebanjima i maksimalnom opsegu promena osmotskog pritiska ćelijskog soka ekološki različitih biljaka načinjeni su „osmotski spektri" određenih ekoloških grupa biljaka, kao što su livadske, listopadne drvenaste, tvrdolisne, mediteranske, četinarske vrste, korovske, gajene i druge biljke (Walter, 1960).

Međutim, poslednjih godina opada interes za istraživanja hidraturnog aspekta vodnog režima biljaka, a sve više dobija na značaju izučavanje **potencijala vode** ili ukupnog **vodnog potencijala** (Ψ). Ukupni vodni potencijal, kao relevantni pokazatelj vodnog bilansa neke biljke, uključuje osmotski potencijal, potencijal turgora i potencijal matriksa.

Brojni autori su (Slatyer & Taylor, 1960; Slatyer, 1967; Kremer, 1960; Walter & Kreeb, 1970; Oertli, 1971; Levitt, 1972) detaljno i kritički objašnjavali koncept i teorijsku osnovu vodnog potencijala kao najadekvatnije mere stanja vode u biljci. Termodinamički vodni potencijal (Ψ) je, prema definiciji Slejčera i Tejlora (Slatyer & Teylor, 1960), jednak razlici u slobodnoj energiji po jedinici zapremine između vode koja je u biološkom sistemu vezana, odnosno pod pritiskom (matričkim, hidrostatičkim i osmotskim) i čiste, slobodne vode na atmosferskom pritisku. U nekom sistemu uopšte, u biljci ili u zemljištu, hemijski potencijal vode je smanjen u odnosu na hemijski potencijal čiste vode delovanjem različitih sila (vezivanje vode za makromolekule silama adsorbcije, vezivanje vode za rastvorene organske supstance osmotskim silama, vezivanja silom kapilarnosti ili silom gravitacije), čime je oslabljena sposobnost takve vode da u datom sistemu obavi rad. Apsolutna vrednost hemijskog potencijala nije koristan pokazatelj, već je značajno termodinamičko stanje vode u bilo kojoj tački sistema izraženo kao razlika između njenog trenutnog hemijskog potencijala i potencijala čiste vode. Prema tome, vodni status biljke najkorektnije se može odrediti upoređivanjem stanja vode u ćeliji sa stanjem čiste vode, pri čemu se razlika izražava kao potencijalna energija vode. Ova energija vode koristi se kao hidroelektrični potencijal i kao hemijski potencijal za biohemijske (u biljkama) i hemijske (u zemljištu) procese. Na osnovu ovih procesa ostvaruje se primarna produkcija biljaka i ukupnog biljnog pokrivača. Sa ovog aspekta posmatrano, za bilo koju tačku kontinuum-sistema „zemljište-biljka-vazduh", značajno je termodinamičko stanje vode, odnosno njena slobodna energija ili potencijal da izvrši rad, a ne trenutna ukupna količina vode u biljci. Veličina slobodne energije ili hemijski potencijal vode u nekom delu sistema određuje se količinom energije na jedinicu mase ili zapremine što je ekvivalentno $J \cdot kg^{-1}$, što se faktorom konverzije može izraziti u barima (ranije atmosferama), odnosno MPa.

Komponente vodnog režima zemljišta su gravitaciona voda koja se posle padavina postepeno spušta u dublje slojeve zemljišta pod dejstvom sile zemljine teže, i kapilarna voda koja zaostaje posle oticanja gravitacione vode (poljski kapacitet zemljišta). Na osnovu kapilarne vode, na različite načine, određenim silama vezane vode, formira se vodni potencijal zemljišta. Nasuprot tome, vodni potencijal vazduha je u direktnoj zavisnosti od relativne vlažnosti vazduha.

Vodni potencijal biljne ćelije ima dve osnovne komponente: a) **osmotski potencijal** i b) **potencijal turgora.** Osmotski potencijal potiče od osmotski aktivnih jedinjenja u ćelijskom soku (šećeri, organske kiseline, joni) koja osmotskim silama smanjuje hemijski potencijal vode (ove osmotski aktivne materije u ćelijksom soku, u ranijoj interpretaciji, određivale su veličinu hidrature biljaka). Bez obzira na činjenicu da se vodni potencijal danas koristi kao osnovni parametar vodnog režima, postoje i izvesne kritičke primedbe na njegov apsolutni značaj (Zimmermann, 1978; Kramer, 1988 i drugi), zbog čega neki istraživači (Passioura et al., 1988, Schulze et al., 1988 i drugi) predlažu nove eksperimentalne pristupe i teorijska objašnjenja vodnih odnosa biljaka.

Pored pomenutih najbitnijih elemenata vodnog režima biljaka postoji izvestan interes u ekofiziološkim istraživanjima i za neke druge karakteristike vode u ćeliji (vododržeća sposobnost, frakcioni sastav vode, vodni deficit i dr.).

Poseban značaj ima kompleksno proučavanje i analiza vodnog režima biljaka u vezi sa otpornošću i mogućnostima prilagođavanja na izuzetno nepovoljne, odnosno ekstremne uslove spoljašnje sredine (suša, zaslanjenost zemljišta, mraz i sl.). Fiziološka ekologija biljaka u uslovima stresa, odnosno, proučavanje fizioloških i biohemijskih reakcija na ekstremne, stresne uslove poslednjih decenija je u žiži interesovanja kako bljnih ekologa, tako i fitofizioloģa u svetu, a i u našoj zemlji.

U našoj zemlji istraživani su, s jedne strane, određeni ekofiziološki problemi na većem broju ekološki različitih biljaka (životnih formi), dok je, s druge strane, veći broj problema (kompenzaciona tačka, vodni režim, produkcija) proučavan na istoj biljci u okviru idioekoloških studija. Uopšteno gledano, istraživanja ekofizioloških parametara sistematično su obuhvatila veliki broj vrsta – cenobionata karakterističnih biljnih zajednica različitih područja naše zemlje. Na osnovu svega toga, sumirajući sva dosadašnja istraživanja i držeći se ekosistemskog principa, ekofiziološki problemi se danas mogu kompleksno razmatrati i mogu se dati ekofiziološke karakteristike glavnih terestričnih ekosistema Srbije.

Posle Drugog svetskog rata više naših naučnih institucija, a posebno Institut za biološka istraživanja „Siniša Stanković" uz stručnjake iz drugih naučnih ustanova, Instituta za botaniku Biološkog odseka PMF, odnosno Biološkog fakulteta, Katedre za botaniku Poljoprivrednog fakulteta, Veterinarskog fakulteta, sa Biološkog odseka PMF-a iz Prištine, organizovano su obavljala ekofiziološka proučavanja na različitim lokalitetima širom Srbije i cele Jugoslavije u okviru različitih ekosistema. I danas se takva istraživanja nastavljaju u tri snažna centra: Odeljenju za fiziološku i biohemijsku ekologiju biljaka Instituta za biološka istraživanja „Siniša Stanković", Institutu za botaniku i botaničkoj bašti „Jevremovac" Biološkog fakulteta u Beogradu i Katedri za botaniku Poljoprivrednog fakulteta u Zemunu. Međutim, valja istaći da su posebni ekofiziološki problemi u sferi interesovanja istraživača u okviru institucija kao što su Institut za kukuruz „Zemun polje", Institut za ratarstvo i povrtarstvo u Novom Sadu, Katedra za botaniku Farmaceutskog fakulteta u Beogradu, Biološki odsek Prirodno-matematičkog fakulteta u Prištini.

EKOFIZIOLOŠKA PROUČAVANJA BILJAKA – CENOBIONATA ODREĐENIH EKOSISTEMA

Dugogodišnja praksa istraživanja u oblasti fiziološke ekologije biljaka sprovedena je, pre svega u okviru najstarije (aktivne preko tri decenije) i najmasovnije Beogradske ekofiziološke škole. Glavni rezultati proučavanja ekofizioloških problema dobijeni su praćenjem velikog broja biljaka cenobionata glavnih terestričnih ekosistema naše zemlje, kao što su: (a) šumski ekosistemi, (b) livadski, pašnjački i stepski ekosistemi, (c) agroekosistemi i (d) ubrani ekosistemi.

Uopšte uzev, u našoj zemlji je osnovna pažnja posvećena ekofiziološkim proučavanjima biljaka u okviru šumarskih ekosistema. U raznovrsnim šumskim zajednicama, na Fruškoj gori, Avali, Prokletijama, Šari, Jastrepcu, Maljenu, Ceru i drugim mestima, ekofiziološkim proučavanjima obuhvaćene su sve životne forme biljaka šumskih fitocenoza (drveće, žbunovi, zeljaste biljke), kao i stelja.

U fitocenozama zeljastih biljaka izvođena su relativno opsežna ekofiziološka istraživanja, koja su se prevenstveno odnosila na cenobionte livadsko-stepske vegetacije, zatim na reliktne i endemične vrste, kao i na druge kategorije posebno interesantnih biljaka (poikilohidrične vaskularne biljke, halofite, lekovite biljke i druge).

Nažalost, mora se konstatovati da su relativno malo proučene biljke iz livadskih ekosistema, široko rasprostranjene od nizijskih do planinskih regiona, od izrazito vlažnih do veoma kserotermnih staništa.

Ekofiziološka proučavanja u okviru agrarnih ekosistema imaju dugu tradiciju, s obzirom na potrebe unapređivanja poljoprivredne prakse (pravovremeno i adekvatno zalivanje, dodavanje mineralnih đubriva, itd.). Objekti raznovrsnih ekofizioloških eksperimenata su cenobionti agrofitocenoza, kako gajene biljke, tako i korovi. Doduše, neki eksperimenti sa biljkama iz agrofitocenoza, strogo uzevši, nemaju pravo ekofiziološko obeležje, jer se odnose, pre svega, na analizu uticaja različitih agrotehničkih i hemijskih mera na visinu i kvalitet prinosa gajenih biljaka i potiskivanje korovskih vrsta.

Najčešće su, u okviru ekosistema naše zemlje, pručavani sledeći ekofiziološki problemi na osnovu analize pojedinačnih parametara relevantnih za monitoring fizioloških procesa u uslovima spoljašnje sredine:

– vodni režim (hidratura, intenzitet transpiracije, vodni potencijal, ukupni sadržaj vode, vodni deficit),

– fotosinteza (intenzitet fotosinteze, fotosintetski režim, količina i dinamika hlorofila),

– disanje (uzajamno dejstvo sa faktorima spoljašnje sredine),

– kompenzaciona tačka (uglavnom kompenzaciona tačka svetlosti, retko kompenzaciona tačka ugljendioksida),

– organska produkcija (bruto i neto primarna produkcija, odnos biomase nadzemnih i podzemnih delova biljke).

Vodni režim

Kao što je već istaknuto, vodni režim predstavlja centralni problem fiziološke ekologije biljaka, s obzirom da fiziološki procesi zavise od vodnih odnosa ili vodnog stanja ćelija, tkiva, organa, tačnije, biljnog organizma u celini. Stoga se, takoreći nezaobilazno, ekofiziološka istraživanja odnose na analizu ukupnih ili pojedinačnih procesa vodnog režima biljaka.

Šumski ekosistemi. – Različiti parametri vodnog režima, ispitivani su, pojedinačno ili kompleksno, kod velikog broja vrsta drveća, žbunastih, polužbunastih i zeljastih biljaka u okviru 11 šumskih zajednica iz različitih visinskih pojaseva i sa različitih lokaliteta naše zemlje (Avala, Fruška gora, Cer, Maljen, Jastrebac, Šar planina, Prokletije).

Među ispitivanim šumskim zajednicama posebno je značajna asocijacija *Quercetum frainetto-cerris* Rudski, koja u termofilnom brdskom području predstalvja klimatogenu zajednicu. Ispitivanja vodnog režima najzastupljenijih i u cenotičkom smislu najvažnijih vrsta u ovoj šumskoj fitocenozi sprovođena su na različitim lokalitetima od strane većeg broja autora (Janković et al., 1967; Kojić & Janković, 1967; Pejčinović et al., 1984; Gligorijević et al., 1984; Popović et al., 1986 i drugi). Posebno je značajno da je, na osnovu brojnih pojedinačnih ispitivanja osmotskih vrednosti ćelijskog soka biljnih vrsta, prvi put u našoj zemlji, za ovu asocijaciju izrađen **osmotski spektar** kao sintetski pokazatelj hidrature, odnosno vodnog režima jedne šumske zajednice (Kojić & Janković, 1967). Utvrđeno je da *Quercus frainetto,* hrast kseroterminijeg karaktera, jedan od edifikatora ove asocijacije, intenzivno transpiriše, sa značajnim variranjima srednjih dnevnih vrednosti (1,48 do 7,43 mg/g/min). Kod ovog hrasta ustanovljena je relativno mala količina vode u listovima (57 do 67%),

što je praćeno velikim variranjem osmotskog pritiska ćelijskog soka (12-43 bara). Druga edifikatorska vrsta hrasta, *Quercus petraea*, mezofilnog karaktera, odlikuje se nižim intenzitetom transpiracije (apsolutni maksimum je oko 8 mg/g/min) i sličnim vrednostima drugih parametara vodnog režima (količina vode, osmotski pritisak ćelijskog soka) kao kod vrste *Quercus frainetto*.

Brdska šuma hrasta kitnjaka – asocijacija *Quercetum montanum* Černj. et Jov. u ekološkom smislu ima prelazni karakter između kserotermofilnih hrastovih šuma i mezofilnih bukovih šuma. U raznim sastojinama ove zajednice posebna pažnja je posvećena proučavanju vodnog režima hrasta kitnjaka *(Quercus petraea)*. Važno je konstatovati da se u ovoj zajednici hrast kitnjak odlikuje većim intenzitetom transpiracije (srednje vrednosti se kreću od 2,59 do 8,19 mg/g/min) nego u asocijaciji *Quercetum frainetto-cerris*. Česte vrste u kitnjakovim šumama su *Tilia argentea* i *Crataegus monogyna* koje se karakterišu intenzivnom transpiracijom (dnevni maksimum čak do 24,09 mg/g/min), kao i *Carpinus betulus*, koji se, kako su istraživanja pokazala, odlikuje niskim intenzitetom transpiracije (maksimum oko 10 mg/g/min), umerenom količinom vode u listovima (53-70%) i visokim vrednostima osmotskog pritiska (11 do 45 bara) (Popović, 1991).

U hrastovim šumama pažnja je posvećena i proučavanju vodnog režima zeljastih biljaka, posebno trava *(Poaceae)*, pri čemu je utvrđeno da postoje velike interspecijske razlike i široke adaptivne mogućnosti pojedinih biljaka iz ove familije u održavanju pozitivnog vodnog balansa u promenljivim uslovima različitih šumskih zajednica (Dimitrijević, 1984).

U hrastovo-grabovim šumama (as. *Querco-Carpinetum sensu lato)* proučavan je vodni režim većeg broja zeljastih i drvenastih vrsta. U prizemnom spratu zeljastih biljaka, proučavanja vodnog režima obuhvatila su široko zastupljene vrste *Melica uniflora, Stellaria holostea* i *Mercurialis perennis*. Posebno se *Melica uniflora* odlikuje intenzivnom transpiracijom (sa dnevnim maksimumom do 30,5 mg/g/min), velikim variranjem količine vode u listovima (18 do 86%) i znatnim kolebanjima osmotskih vrednosti ćelijskog soka (10 do 37 bara). *Stellaria holostea* spada u kseromezofite čiji vodni režim varira u zavisnosti od tipa šuma. U čistoj hrastovoj šumi intenzitet transpiracije (dnevni maksimum preko 18 mg/g/min) i osmotski pritisak su veći (6 do 27 bara) nego u hrastovo-grabovoj šumi, u kojoj se ova vrsta odlikuje izuzetno velikom količinom vode (čak i do 90%). *Mercurialis perennis* u hrastovo-grabovoj šumi umereno transpiriše (maksimalno do 12,60 mg/g/min), sadrži veliku količinu vode u listovima (70-80%), dok je osmotski pritisak ćelijskog soka u granicama karakterističnim za većinu zeljastih vrsta listopadnih šuma umerenih predela (10 do 19 bara) (Popović, 1973, 1991, 1993).

Šume tipa *Querco-Carpinetum* često se u Srbiji nalaze i u pojasu kitnjakovih, odnosno sladunovih šuma, na mestima koja su usled lokalnih orografskih prilika vlažnija i termički umerenija (Janković et al., 1984). Hrast kitnjak *(Quercus petraea)* u ovim uslovima, kako su istraživanja pokazala, odlikuje se umerenim intenzitetom transpiracije (maksimalna vrednost iznosi 12,5 mg/g/min), nižim nego u čistoj kitnjakovoj šumi.

Šume tipa *Fagetum montanum sensu lato* nalaze se iznad pojasa hrastovih šuma, a njima se u izvesnoj meri pridružuju i šume bukve i jele (as. *Abieto-Fagetum)*. Bukva *(Fagus silvatica)*, dominantna vrsta ovih zajednica, odlikuje se ujednačenim i uravnoteženim vodnim režimom, koji se karakteriše niskim intenzitetom transpiracije (dnevni maksimum ne prelazi vrednosti od 6 mg/g/min), umerenom količinom vode u listovima (53-73%), kao i osrednjim vrednostima osmotskog pritiska ćelijskog soka u

listovima (8 do 21 bara). Četinarska vrsta, jela *(Abies alba)* ima još niže vrednosti intenziteta transpiracije (maksimum do 3,45 mg/g/min), manju količinu vode u četinama (svega 50-60%), ali veće osmotske vrednosti ćelijskog soka (12 do 26 bara) u odnosu na listopadne bukve (Popović et al., 1988).

Poseban istraživački interes sa ekofiziološkog aspekta predstavljale su efemeroide. Ove biljke umerenog klimata, čija se vegetacija odvija u relativno kratkom prolećnom periodu čine posebnu ekološku grupu. One izgrađuju ranoprolećnu sinuziju u šumskim fitocenozama. Ispitivanja osnovnih parametera vodnog režima efemeroida u hrastovo-grabovoj (as *Querco-Carpinetum serbicum* Rudski) i bukovoj šumi (as. *Fagetum montanum* Jov.) na Jastrepcu i Maljenu pokazala su da ove biljke intenzivno transpirišu, da imaju visoku i stabilnu količinu vode u listovima i visok nivo hidrature tkiva lista (Janković, 1971; Popović, 1973). U hrastovim šumama efemeroide su relativno intenzivno transpirisale (srednje dnevne vrednosti su bile od 2,2 do 17,6 mg/g/min, sa maksimalnom vrednošću od 22,24 mg/g/min). Snažna transpiracija efemeroida i izrazite dnevne varijacije ovog parametra vodnog režima objašnjavaju se intenzivnom svetlošću (drveće u šumi još nije olistalo), obiljem vlage i povoljnom temperaturom na staništu, pa, prema tome, i stanjem stoma koje su, u datim opštim uslovima, široko otvorene od ranih jutarnjih časova tokom celog dana (Betke et al., 1965). Količina vode u listovima efemeroida bila je veća nego kod drugih ekoloških grupa biljaka i kretala se od 78 do 89%. Posebno visok sadržaj vode konstatovan je kod vrste *Corydalis solida, Scilla bifolia* i *Ranunculus ficaria* na Fruškoj gori (oko 93%). Osmotski pritisak ćelijskog soka efemeroida sličan je onom kod mezofita koje žive na staništima dobro snabdevenim vodom (Matuskiewicz et al., 1953; Pisek, 1956; Gessner, 1956; Walter, 1968), sa vrednostima, najčešće, oko/do 10 bara.

U visokoplaninskoj zajednici *Pinetum heldreichii-Seslerietum autumnalis* M. Jank. et R. Bog. na Ošljaku (Šarplanina), na visini od oko 1600 m.n.v. praćena je dinamika vodnog režima drvenastih i žbunastih vrsta *Pinus heldreichii, Juniperus intermedia, Vaccinium myrtillus,* kao i zeljastih biljaka *Luzula luzulina, Brachypodium silvaticum, Euphorbia amygdaloides, Sesleria autumnalis* i *Scabiosa columbaria.* Među ispitivanim biljkama *Pinus heldreichii* i *Juniperus intermedia* su se odlikovali niskim intenzitetom transpiracije, malom količinom vode u listovima, umerenim osmotskim pritiskom i malim kolebanjima bilokojeg parametra vodnog režima. Nasuprot njima, druge drvenaste, kao i zeljaste biljke ove zajednice ispoljavale su više ili manje labilan vodni režim, zavisno da li su pripadale grupi sa intenzivnim ili umerenim vrednostima transpiracije, količine vode u listovima i osmotskog pritiska ćelijskog soka (Janković et al., 1975). Uopšte uzev, u ekofiziološkim ispitivanjima visokoplaninske šumske vegetacije posebna pažnja je bila usmerena na istraživanja funkcionisanja na staništu endemoreliktnih borova *Pinus heldreichii* i *P. peuce* od strane Jankovića i saradnika. Proučavanju vodnog režima ovih borova na Šarplanini i Prokletijama u okviru zajednica *Pinetum heldreichii-Seslerietum autumnalis* M. Jank. et R. Bog., *Pinetum peucis typicum* M. Jank., *Wulfenio-Pinetum peucis* Blečić et Tatić i *Ajugo-Pinetum peucis* M. Jank. et R. Bog. (Janković et al. 1987). Oba endemo-reliktna bora odlikovala su se stenohidričnim tipom vodnog balansa, veoma slabom transpiracijom (kod munike prosečno 0,898 mg/g/min, a kod molike 2,547 mg/g/min), malom količinom vode u listovima (55-60%), kao i umerenim vrednostima osmotskog pritiska (kod munike oko 20 bara, a kod molike oko 15 bara).

Livadski i drugi ekosistemi zeljastih biljaka. – Prema rezultatima brojnih istraživača, među zeljastim biljkama koje nastanjuju livadske, livado-stepske ekosisteme i različita staništa na kamenjarima, u našoj zemlji, postoje sve vrste prelaznih ekofizioloških tipova u odnosu na vodni režim – od izrazito hidrostabilnih do ekstremno

hidrolabilnih biljaka. Najbrojnije među njima su brojne vrste iz familije trava i različite dikotiledone zeljaste biljke svih prelaznih ekoloških grupa od kserofita, preko mezofita do higrofita.

Trave se uspešno razvijaju na ekološki veoma različitim staništima, od vlažnih do vrlo suvih, stepskih. S obzirom na to one ispoljavaju značajnu funkcionalnu raznovrstnost u odgovoru na vodni režim staništa. U umerenim klimatskim uslovima, gde je uglavnom dobra snabdevenost vodom, kako su rezultati istraživanja pokazali, trave *(Festuca montana, F. drymeia, Melica uniflora, Dactylis glomerata, Poa nemoralis, Brachypodium silvaticum)* odlikuju se relativno velikom količinom vode u listovima (maksimum 75 do 82%), transpiracijom obično umerenog intenziteta, povećanom u ranim jutarnjim časovima, a smanjenom tokom podnevnih sušnih uslova (sa vrednostima koje variraju od 2,0 do 30,0 mg/g/min). Karakterišu se i velikim dijapazonom promena osmotskih vrednosti ćelijskog soka, koje su često dosta visoke (6 do 30 bara). Dakle, sve graminoidne vrste umerenih predela su više ili manje hidrolabilne. S druge strane, trave stepskih livada i stepsko-peščarskih zajednica tipa *Chrysopogonetum pannonicum* L. Stjep.-Ves., *Festucetum vaginatae* L. Stjep.-Ves., *Andropogoneto-Euphorbietum pannonicae* R. Bog. *(Stipa joannis, S. capillata, Festuca valesiaca, F. pseudovina, F. vaginata, Koeleria glauca, K. gracilis, Chrysopogon gryllus)* su hidrostabilne. Ove kserofitne trave imaju redukovanu, nisku i ujednačenu tranpsiraciju (često imaju stalno ili povremeno uvijene listove). Osmotske vrednosti ćelijskog soka najčešće variraju između 15 i 35 (ponekad čak i do 49) bara, što ukazuje na njihov poseban konstitucionalni ekološki tip biljaka – stipakserofite (Stjepanović-Veselićić, 1959; Janković et al., 1979; Stevanović, 1980; Blaženčić, 1982; Dimitrijević, 1984).

U livadskim i livadsko-stepskim zajednicama dominantni cenobionti su, pored trava, brojne zeljaste cvetnice vrlo različitih ekofizioloških karakteristika. Na relativno sušnim i osunčanim staništima posebno se ističu biljke sa dobro razvijenim korenovim sistemom, koje se lako snabdevaju vodom, tako da bez ograničenja transpirišu, sadrže znatnu količinu vode, a imaju umereno visoke osmotske vrednosti ćelijskog soka. Ove biljke pripadaju, s obzirom na svoje strukturno-funkcionalne odlike, malakofilnim kserofitama, sa dobro izraženim periferijskim zaštitama (dlake, kutikula, uvučene stome), i dobro razvijenim dubokim korenovim sistemom, pomoću koga se snabdevaju vodom sa većih dubina. Među njima treba pomenuti: *Astragalus onobrychis* (transpiracija 0,4-37,5 mg/g/min, količina vode u listovima 69-86%, osmotski pritisak 10-35 bara), *Astragalus dasyanthus* (transpiracija 2,7 do 46,5 mg/g/min, količina vode 70-78%, osmotske vrednosti 12-22 bara) i *Echinops banaticus* (osmotski pritisak ćelijskog soka 13-25 bara) i druge. Ove biljke, najčešće sa anizohidričnim karakteristikama vodnog balansa, obično lako prolaze kroz period suše na staništu (Deliblatska peščara), a u najekstremnijim uslovima, tokom godine, ne trpe veći deficit vode u tkivima (Stjepanović-Veselićić, 1959; Stevanović, 1980).

U livadskim ekosistemima ili na otvorenim prostorima degradiranih šumskih zajednica ispitivan je vodni režim brojnih zeljastih vrsta koje se odlikuju tipičnim mezofitnim strukturno-funkcionalnim odlikama *(Stellaria holostea, Trifolium montanum, Coronilla varia, Orlaya grandiflora)* ili karakteristikama na osnovu kojih se mogu svrstati u prelazne ekološke grupe od mezofita ka kserofitama *(Fragaria vesca, Peucedanum oreoselinum, Laserpitium siler, Euphorbia gerardiana* i dr.). U uslovima dobre snabdevenosti vodom ove biljke, poput mezofita, kao pripadnici prelazne grupe kseromezofita, transpirišu bez ograničenja u skladu sa mikroklimatskim promenama (srednje dnevne vrednosti intenziteta transpiracije su obično između 1,0-17,0, a

ponekad čak i 26,0 mg/g/min). Količina vode u listovima je najčešće oko 65-85%. Ovaj strukturno-funkcionalni tip biljaka odlikuje se osmotskim vrednosima koje se najčešće kreću izmedu 10 i 20 bara, a kod kseromezofita čak i do 40 bara (Stjepanović et al., 1968; Matijašević, 1969; Janković, 1971; Pavlović et al., 1978; Blaženčić, 1982; i dr.).

Prolećne geofite stepskih livada *(Adonis vernalis, Paeonia tenuifolia, Pulsatilla vulgaris, Iris pumila)* i temrofilnih šuma i kamenjara *(Paeonia corralina, Asphodeline liburnica),* u ekološkom pogledu su uglavnom heliofite do poluskiofite, sa tendencijom održavanja hidrostabilnih vodnih odnosa. Mnoge od ovih biljaka su kserotermni relikti. Odlikuju se znatnom količinom vode u listovima (najčešće 65-75%), umerenom, ali relativno ujednačenom transpiracijom (prosečno oko 10,0 mg/g/min), kao i relativno visokim osmotskim vrednostima, koje, takođe, malo variraju tokom dana (13 do 20 bara) (Stevanović, 1980; Popović et al., 1983; Đorđević et al., 1992).

U različitim tipovima livadskih zajednica izučavan je vodni režim većeg broja jednogodišnjih i višegodišnjih lekovitih biljaka, s obzirom na značaj i vezu vodnih odnosa ovih biljaka i njihovih farmakoloških stojstava. Pojedine lekovite biljke, kao što su *Mentha piperita* ili *Plantago major,* sa osmotskim vrednostima između 9 i 16 bara, uglavnom naseljavaju staništa sa relativno povoljnim vodnim režimom. Druge, pak, kao što su *Teucrium montanum, Salvia officinalis* ili *Thymus glabrescens,* naseljavaju često i staništa sa deficitom vlažnosti, ali se odlikuju malim dnevnim amplitudama parametara vodnog režima i osmotskim vrednostima karakterističnim za kserofite (10 do 24 bara) (Ćorović et al., 1968; Živanović, 1977; Pavlović et al., 1978; i dr.).

Izuzetno veliki značaj imaju ekofiziološka proučavanja endemičnih i reliktnih biljaka, kojima obiluje naša zemlja (Stevanović, 1990). Ove biljke obično naseljavaju zaklonjena mesta, klisure i kanjone, kamenita krečnjačka i serpentinska staništa, na različitim nadmorskim visinama. Dosadašnja ekofiziološka proučavanja obuhvatila su relativno mali broj zeljastih endemičnih ili reliktnih biljaka. Kompleksna istraživanja obavljena su samo na kserotermnim reliktima panonske nizije *(Adonis vernalis, Paeonia tenuifolia, Pulsatilla vulgaris, Iris pumila, Astragalus dasyanthus),* kao i na mediteranskim reliktima koji nastanjuju kontinentalne predele *(Paeonia corralina, Asphodeline liburnica),* kao i endemoreliktnim vrstama roda *Ramonda* (Stevanović, 1989). Vrste *Ramonda serbica* i *Ramonda nathaliae* odlikuju se izrazito hidrostabilnim vodnim režimom, transpiracijom slabog intenziteta (1,1-9,3 mg/g/min), umernom do značajnom količinom vode u listovima (68 do 79%), kao i niskim vrednostima osmotskog pritiska (6 do 18 bara), u stanju pune hidratacije listova. Pored pomenutih biljaka, parcijalno (samo neki parametri i sporadično) proučavan je vodni režim kod izvesnog broja zeljastih endemičnih ili endemoreliktnih biljaka krečnjačkih i serpentinitskih terena.

Dosadašnjim ispitivanjima vodnog režima jednogodišnjih i višegodišnjih zeljastih biljaka malo su obuhvaćene vrste planinskih, brdskih i dolinskih livada, kao i vrste drugih primarnih ili u manjoj meri antropogeno izmenjenih zeljastih zajednica. U tom pogledu značajni rezultati dobijeni su u poslednje vreme u ispitvanju vodnog režima (transpiracija i osmotske vrednosti ćelijskog soka) važnijih i karakterističnih vrsta brdsko-planinskih livadskih zajednica Rudnjanske visoravni i Radočela (Kojić et al., 1992). Ispitivanjima je obuhvaćeno 19 vrsta iz asocijacije *Danthonietum calycinae,* 17 vrsta iz zajednice *Agrostietum vulgaris* i 15 vrsta iz asocijacije *Koelerietum montanae.* Dnevna dinamika transpiracije, kao i najveće i najmanje vrednosti, pokazuju individualnu varijabilnost, kako u različitim, tako i u okviru iste biljne zajednice. Osmotske vrednosti vrsta iz zajednice *Danthonietum calycinea* varirale su između 7,96 i 22,30 bara,

u zajednici *Agrostietum vulgaris* amplituda osmotskih vrednosti bila je između 7,17 do 16,84 bara, a u zajednici *Koelerietum montanae* između 9,79 i 20,02 bara.

Agroekosistemi. – Vodni režim gajenih i samoniklih biljaka u agroekosistemima, odnosno u agrofitocenozama, predstavlja izuzetno značajan problem primenjene fiziološke ekologije biljaka. Zbog toga su ispitivanja vodnog režima gajenih i drugih biljaka u agrarnom biotopu, kao i vodnog režima takvog staništa bila u žiži interesovanja istraživača i stručnjaka na polju primenjene ekologije. Ne ulazeći u sve detalje koji se odnose na ovaj problem, može se konstatovati da su hidratura i vodni potencijal dominirali kao dva osnovna parametra vodnog režima u istraživačkim zahvatima koja se odnose na biljke iz agrofitocenoza.

Proučavanje hidrature, odnosno određivanje osmotskih vrednosti ćelijskog soka, od velikog je značaja za poljoprivredne biljke, jer pruža relevantne podatke o toku osnovnih fizioloških procesa od kojih zavisi organska produkcija, odosno prinos gajenih biljaka i njihov kompeticijski status u odnosu na samonikle, odnosno korovske vrste. U tom smislu naročito su instruktivni podaci koje iznosi K o j i ć (1987), a koji se odnose na komparativno praćenje osmotskih vrednosti ćelijskog soka, odnosno hidraturno stanje tkiva listova većeg broja korovskih i gajenih biljaka u istoj agrofitocenozi (u usevu kukuruza i u usevu šećerne repe). Cilj ovih istraživanja bio je utvrđivanje tipa vodnog režima (hidrature) koji uspostavljaju korovske biljke u poređenju sa gajenom biljkom u istim uslovima staništa. Hidraturni odnosi u usevu kukuruza isptiivani su kod korovskih vrsta *Sonchus oleraceus, Setaria viridis, Solanum nigrum, Cirsium arvense* i *Chenopodium album,* dok su u usevu šećerne repe analizirane osmotske vrednosti ćelijskog soka kod vrsta *Stachys palustris, Solanum nigrum, Bidens tripartitus* i *Carduus acanthoides.* Rezultati ovih istraživanja su pokazali da su osmotske vrednosti ćelijskog soka redovno bile niže kod korovskih biljaka u odnosu na gajene vrste (kukuruz i šećerna repa). Tako je, na primer, osmotski pritisak ćelijskog soka vrste *Sonchus oleraceus* iznosio 7,06 bara, *Setaria viridis* 9,02 bara, *Solanum nigrum* 10,77 bara, *Cirsium arvense* 11,15 bara, *Chenopodium album* 12,27 bara, dok je u isto vreme osmotski pritisak kukuruza bio 13,77 bara. Slična situacija konstatovana je i u usevu šećerne repe. Korovske biljke, u manje-više istim uslovima, uspostavljaju povoljniji vodni režim, odnosno zadržavaju povoljnije hidraturno stanje tkiva od gajenih biljaka sa kojima zajedno rastu. Slične zaključke K o j i ć (1987) je izneo i za agrofitocenozu pšenice: osmotski pritisak ćelijskog soka listova korovskih vrsta ovde iznosio je kod *Cirsium arvense* 15,38 bara, *Lathyrus aphaca* 16,40 bara, *Vicia tetrasperma* 17,01 bara, *Sambucus ebulus* 19,52 bara, *Aristolochia clematis* 20,65 bara, *Consolida regalis* 23,17 bara, dok je kod pšenice iznosio 25,08 bara. Komparativna istraživanja hidraturnih odnosa korova i gajenih biljaka ukazuju na određenu strukturno-funkcionalnu prednost korovskih biljaka u odnosu na gajene biljke.

Analiza vodnog režima biljaka u agroekosistemima sve se više zasniva na praćenju veličine i dinamike vodnog potencijala u različitim delovima biljke i utvrđivanju odnosa promene vodnog potencijala i količine abscisinske kiseline (ABA) u listovima, korenu, stablu, odosno ksilemu i floemu. Ovim problemima se kod nas intenzivno bave P e k i ć i S t i k i ć (1987), prateći stanje i promene vodnog potencijala raznih linija i sorata (kultivara) kukuruza, s posebnim osvrtom na uzajamnu uslovljenost stresnih uslova vodnog deficita (suše) i hormonskog statusa otpornih i osetljivih sorti gajenih biljaka.

Pored već istaknutih razlika između korovskih i gajenih biljaka u pogledu hidraturnih vrednosti K o j i ć i saradnici (1994) daju pregled komparativnih istraživanja i drugih parametera vodnog režima vrsta u agrofitocenozama (ukupan sadržaj vode,

intenzitet transpiracije). I u ovom slučaju utvrđeno je da postoji razlika koja ide u prilog korovskim biljkama u odnosu na gajene vrste. Ukupan sadržaj vode, kako u toku dana tako i u toku sezone, konstantno je veći kod korova nego kod gajenih biljaka. Dakle, u istoj agrofitocenozi, na istom staništu, sve analizirane korovske vrste raspolagale su većom količinom vode u listovima u odnosu na jedinke gajene biljke, što, načelno posmatrano, ukazuje na prednost koju imaju korovi u obavljanju fizioloških aktivnosti. Analogni rezultati dobijeni su komparativnim proučavanjem intenziteta tranpsiracije korovskih i gajenih biljaka. Kojić i saradnici (1994) su utvrdili da su intenzitet transpiracije, kao i ukupna količina transpirisane vode, znatno veći kod korovksih nego kod gajenih biljaka. Do sličnih rezultata su došli i neki drugi autori (Plavšić-Gojković et al., 1984; Dubravec, 1984; Ristić, 1988; Grupče et al. 1987; Kojić i Ajder, 1991). Takav trend u prometu vode korova i gajenih biljaka ukazuje da fiziološki procesi, odnosno ukupni metabolizam korovskih biljaka, u krajnjoj liniji ima za posledicu intenzivniju organsku i odgovarajuću prednost u korišćenju dostupnih resursa u agroekosistemu u celini.

Na osnovu iznetih činjenica, dolazi se do zaključaka koji dopunjuju saznanja o korovima kao veoma specifičnoj kategoriji biljaka agrarnih ekosistema. Korovi su u toku procesa evolucije u agroekosistemu, u stalnoj borbi sa intervencijama čoveka (antropogeni faktor) i drugim faktorima spoljne sredine, stekli takve adaptivne osobine koje im omogućavaju konkurentsku sposobnost u odnosu na gajene biljke. Ekofiziološki parametri ukazuju na mogućnost korovskih biljaka da svoj vodni režim uspostave i održavaju na kvalitetnijem nivou u odnosu na gajenu biljku kao edifikatorsku vrstu u agrofitocenozi, i na taj način se zadrže na obradivim i od strane čoveka održavanim prostorima.

Urbani ekosistemi su izuzetno ekološki specifični, ali je, sa ekofiziološkog stanovišta, značajan uticaj različitih zagađenja (atmosferski polutanti, smog, dim, čađ i prašina, kao i teški metali, otpad i toksične substance u zemljištu) na osnovne procese i strukturu biljaka. Razni zagađivači u vazduhu i zemljištu narušavaju fine strukture i funkcije vitalnih organa biljaka, naročito listova i korenova (Seinfeld, 1975). Efekti zagađivanja se ispoljavaju na raznim nivoima građe individualnog organizma (subćelijskom, ćelijskom, u tkivima ili organima), ali i šire zahvataju i narušavaju biološke sisteme (populacijske, specijske ili cenotičke). Pod uticajem zagađivača, u urbanim sredinama, nastaju strukturne (morfološke, anatomske, histološke) i fiziološko-biohemijske promene kod biljaka. Fiziološke promene se obično javljaju pre vidljivih strukturnih promena i oštećenja i mogu imati kritičan značaj za biljke (Wolfend et Mensfield, 1991). Kako se fiziološko-biohemijske reakcije biljaka na različite vrste zagađivanja relativno rano pojavljuju, mogu se koristiti za detekciju očuvanosti ili narušenosti (fiziološko-biohemijski monitoring) urbane sredine.

Kompleksna ekofiziološka istraživanja u gradskim sredinama u nas ređe su obavljana nego u okviru prirodnih ekosistema. Ipak, dosadašnji podaci daju opštu sliku o karakteristikama vodnog režima biljaka u uslovima gradske sredine. Tako, istraživanja na području Beograda pokazuju da se ruderalna zeljasta vegetacija u urbanim uslovima odlikuje povoljnim vodnim odnosima (Popović et al., 1988). U najčešćoj urbanoj fitocenozi *Lolio-Plantaginetum majoris* Beger, devet najzastupljenijih vrsta karakterisale su se veoma intenzivnom transpiracijom (dnevni maksimum iznosio je čak i do 32,70 mg/g/min), relativno visokim sadržajem vode koji malo varira (71 do 83%) kao i umerenim vrednostima osmotskog pritiska (8 do 16 bara), što ukazuje na pozitivan vodni balans i dobre produktivne uslove ruderalnih biljaka na staništima u gradskoj sredini.

Stanje vodnog režima drvenastih biljaka u urbanim uslovima praćeno je određivanjem hidrature kod 30 vrsta drveća i žbunova na zelenim površinama u neposrednoj blizini većih saobraćajnica u Beogradu. Utvrđeno je da najveći broj vrsta ima osmotski pritisak ćelijskog soka u listovima između 15 i 20 bara. Od toga odstupaju samo *Lycium barbarum* (sa izrazito malim osmotskim vrednosima, do 6 bara) i *Tamarix tetandra* (sa vrednosima osmotskog pritiska do 23 bara).

Mitrović (1992) daje podatke o ispitivanju vodnog režima drveća *Acer pseudoplatanus, Acer platanoides* i *Acer campestre* u parku Kalemegdan. Umereno visoka transpiracija (maksimum do 19,5 mg/g/min), stabilna količina vode u listovima tokom dana i osmotske vrednosti karaktersitične za listopadne vrste drveća Evrope (8 do 23 bara) govore o dobroj adaptiranosti ovih vrsta na uslove urbane sredine.

Posebno su značajna ispitivanja vrste *Ginkgo biloba,* značajne reliktne vrste, koja su izvedena u uslovima Botaničke bašte u Beogradu (Stevanović, Janković, 1982, 1983). Utvrđena je transpiracija umerene jačine, bez podnevnih depresija, kao i relativno velika količina vode u listovima, praćena ujednačenim osmotskim vrednostima (13-21 bara). Prema rezultatima ovih istraživanja proizilazi da je *Ginko biloba* hidrostabilna vrsta, koja uspeva da tokom dana održi povoljan vodni balans, stabilizuje vodu u protoplazmi, uz istovremeno izbegavanje izraženije fluktuacije količine vode i osmotskog pritiska ćelijskog soka u listovima, otporna i veoma vitalna u urbanim uslovima.

Fotosinteza

Fotosinteza kao ekofiziološki parametar uopšte, a posebno u vezi sa organskom produkcijom biljaka i biljnog pokrivača, predstavljala je jedan od ključnih problema proučavanja saradnika Odeljenja za fiziološku i biohemijsku ekologiju biljaka Instituta za biološka istraživanja „Siniša Stanković". Ispitivanja na terenu obavljena su, najpre, konduktometrijskom metodom, koji se zasniva na elektroprovodljivosti rastvora baze koja apsorbuje CO_2 (Voznesenskij, 1959), aparatom konstruisanim u Institutu za biološka istraživanja (1976). To su bila prva, pionirska istraživanja fotosinteze u prirodnim uslovima u našoj zemlji. Danas se istraživanja intenziteta fotosinteze sprovode standardnim metodom uz primenu aparata LI-COR 6200.

Konduktometrijskom metodom proučavan je intenzitet fotosinteze kod većeg broja biljnih vrsta cenobionata zajednice *Chrysopogonetum pannonicum* Stjep.-Ves. Zbog velikih i naglih promena gotovo svih faktora od značaja za fotosintezu (svetlost, temperatura, vlažnost) konstatovane su i velike promene intenziteta fotosinteze, od vrlo niskih do izrazito visokih vrednosti. Najniži nivo fotosinteze utvrđen je kod *Crataegus monogyna* (maksimum do 27 mg CO_2/g/h), a zatim kod vrsta *Paeonia tenuifolia* i *Anemone pulsatilla* (maksimum do 42 mg CO_2/g/h), dok se većina drugih biljaka ove zajednice odlikuje intenzivnom fotosintezom (do 78 mg CO_2/g/h) (Janković et al., 1975).

Kompleksni rezultati dobijeni su primenom savremene LI-COR aparature, odnsono LI-COR infracrvenog gasnog analizatora kojim se simultano beleži nivo fotosinteze (odnosno disanja u mraku), nivo transpiracije, intracelularnu koncentraciju CO_2 u listovima, atmosfersku vlažnost, temperaturu vazduha i lista, intenzitet svetlosti. Na osnovu svih ovih vrednosti, korišćenjem multipne i parcijalne regresije, može se utvrditi zavisnost fotosintetskog procesa od faktora spoljašnje sredine.

Praćenje fotosinteze LI-COR aparatom kod većeg broja jedinki hrasta kitnjaka različitog vitalnog statusa, dalo je veoma značajne rezultate (Popović et al., 1989)

indikativne za stepen oštećenja u datim uslovima spoljašnje sredine. Biljke sa najmanjim stepenom vitalnsoti imale su vrlo nisku fotosintetsku aktivnost, čak samo 1,45 mol CO_2, povezanog sa prekomernim gubitkom vode u kritičnim momentima. Povećani vodni deficit u listovima narušava fotosintetski metabolizam ovih biljaka, što dovodi do smanjene asimilacije CO_2. Kod najvitalnijih biljaka intenzitet fotosinteze je znatno veći (2,98 mol CO_2), ne ispoljava se podnevna derpesija zbog povećanog intenziteta svetlosti i relativno visoke koncentracije intracelularnog CO_2 (295 mol), koji je akumuliran u periodu kada su stome bile otvorene (P o p o v i ć et al., 1989, 1990; K n a p p et al., 1990).

U proučavanju fotosinteze korišćene su i druge metode. Promene u fotosintetskoj aktivnosti analizirane su brzo i bez oštećenja merenjem hlorofilne fluorescencije primenom aparata „Plant Stress Meter" – biomonitor AB. Ovom metodom praćena je hlorofilna fluorescencija i određivana fotosintetska aktivnosti bukve *(Fagus silvatica),* i to u kulturi sa smrčom, kao i u prirodnoj bukovoj šumi. Utvrđena je veća fotosintetska aktivnost bukve u prirodnim sastojinama u odnosu na sađene sastojine tokom cele vegetacijske sezone (P o p o v i ć et al., 1994).

U ekofiziološkim proučavanjima fotosinteze, kako u prirodnim, tako i u agroekosistemima poseban značaj ima određivanje netofotosinteze, intenziteta fotosinteze, a naročito produktivnosti fotosinteze. W a l t e r (1960) je detaljno proučavao **pruduktivnost fotosinteze** jer je ovaj parametar smatrao najvažnijim u ukupnom **fotosintetskom režimu.** Valter definiše produktivnost kao „priraštaj suve supstance na jedinicu lisne površine u jedinici vremena", za razliku od netofotosinteze koja predstavlja razliku između same fotosinteze i istovremeno proteklog disanja. Na osnovu komparativnih proučavanja produktivnosti fotosinteze utvrdio je značajne razlike kod većeg broja gajenih biljaka. Za ispitivane biljke prosečna vrednost produktivnosti fotosinteze, izražena u gramima suve supstance na 1 m^2 lisne površine za 7 dana iznosi:

– suncokret	10,3 g/m^2/7 dana
– šećerna repa	7,6 g/m^2/7 dana
– kukuruz	6,5 g/m^2/7 dana
– mak	4,8 g/m^2/7 dana
– pasulj	4,7 g/m^2/7 dana

Iz iznetih podataka se vidi da je produktivnost fotosinteze suncokreta čak 100% veća nego kod pasulja ili maka.

K o j i ć (1987) je proučavao produktivnost fotosinteze gajenih biljaka kod dve sorte (kultivara) pšenice (bankut i bezostaja) na plodnom poljoprivrednom zemljištu tipa černozem u okolini Beograda. Visokorodna sorta (kultivar) pšenice bezostaja pokazala je nižu fotosintetsku produktivnost od stare domaće sorte bankut. Prosečna produktivnost fotosinteze, izračunata za ceo vegetacioni period, kod bezostaje iznosi 3,2 g/m^2/7 dana, a kod bankuta 4,0 g/m^2/7 dana. Ovi rezultati su pokazali da opšti nivo organske produkcije ne zavisi samo od intenziteta, odnosno produktivnosti fotosinteze, već i od drugih faktora (rashodovanje materija, odnos fotosinteze i disanja, transformacije i translokacije produkovanih jedinjenja, i drugog).

Uticaj abiotičkih i biotičkih faktora na proces fotosinteze gajenih biljaka, posebno pšenice i kukuruza, proučavan je i u našoj zemlji. Krstić (1981) je detaljno analizirao uticaj spoljašnjih faktora na fotosintezu pšenice. Kojić i Pekić (1981) su proučavali uticaj vodnog režima na fotosintetski proces i organsku produkciju pšenice, pri čemu su posebno pratili parametre vodnog režima kao što su oblici vode i njihova dinamika u organima pšenice, intenzitet transpiracije, transpiracioni koeficijenat i potrošnju vode, vodni deficit, vodni potencijal, hormonalno regulisanje vodnog režima u vezi sa produkcionim procesom pšenice, kao i karakteristike hidraturnih odnosa pšenice u vezi sa organskom produkcijom.

Najzad, valja konstatovati da je veliki broj istraživača agronomske orijentacije proučavao direktan uticaj raznih agrotehničkih i hemijskih mera, kao i efekat faktora spoljašnje sredine na visinu i kvalitet proizvoda gajenih biljaka, bez posebne analize fizioloških procesa, presudno značajnih za organsku produkciju.

Disanje

Disanje, tačnije, intenzitet disanja kao ekofiziološki parametar, od koga bitno zavisi i produktivnost fotosinteze, relativno retko je, u našoj zemlji, proučavan neposredno u uslovima staništa. Prema Rubinu (1976) razni ekološki činioci, a pre svega temperatura, vlažnost, svetlost, mineralni režim, kao i atmosferske prilike utiču na celokupan promet materija u biljci, a u tom kontekstu i na disanje. Kao rezultat delovanja svih tih faktora, a svakako i genetičkih svojstava, intenzitet disanja je karakterističan i specifičan za pojedinačne biljne vrste. U okviru agrarnih ekosistema problem disanja je detaljno proučavan kod korovskih biljaka (Kojić, 1968, 1987). Na osnovu rezultata tih istraživanja, Kojić svrstava korovske biljke u tri grupe:

I – korovske vrste niskog intenziteta disanja (do 1,00 mg CO_2 na 1 g suve supstance za 1 čas)
II – korovske vrste srednjeg intenziteta disanja (od 1,00 do 2,00 mg CO_2/1g/1h)
III – korovske vrste relativno visokog intenziteta disanja (preko 2,00 mg CO_2/1g/1h)

U prvu grupu dolaze sledeće korovske vrste: *Chenopodium album* (0,08 mg CO_2/1g/1h), *Sambucus ebulus* (0,11 mg CO_2/1g/1h), *Chenopodium bonus-henricus* (0,15 mg CO_2/1g/1h), *Sinapis arvensis* (0,23 mg CO_2/1g/1h), *Achillea millefolium* (0,26 mg CO_2/1g/1h).

U drugu grupu spadaju sledeće korovske biljke: *Thlaspi arvense* (1,05 mg CO_2/1g/1h), *Cirsium arvense* (1,11 mg CO_2/1g/1h), *Capsella bursa-pastoris* (1,14 mg CO_2/1g/1h), *Bromus sterilis* (1,18 mg CO_2/1g/1h), *Chelidonium majus* (1,32 mg CO_2/1g/1h), *Ballota nigra* (0,53 mg CO_2/1g/1h), *Lamium album* (0,55 mg CO_2/1g/1h).

U treću grupu mogu se uključiti sledeće korovske vrste naših predela: *Convolvulus arvensis* (4,99 mg CO_2/1g/1h), *Polygonum aviculare* (6,04 mg CO_2/1g/1h) i *Stellaria media* (7,04 mg CO_2/1g/1h) (Kojić, 1987).

Kako su ekološka proučavanja disanja biljaka u drugim (prirodnim) ekosistemima (šumskim, livadskim, stepskim) kod nas malo obavljana, ne postoje značajni rezultati koji bi omogućili uopštena razmatranja i zaključivanja.

Kompenzaciona tačka

Kompenzaciona tačka svetlosti predstavlja značajan ekofiziološki parametar koji ukazuje na uslove pod kojima se obavlja proces fotosinteze, i sa ovim procesom povezana organska produkcija. Kompenzaciona tačka svetlosti je relativno često proučavana u našoj zemlji, posebno u šumskim i agrarnim ekosistemima. Nasuprot tome, kompenzaciona tačka ugljen dioksida, praktično, uopšte nije bila predmet ozbiljnih pručavanja i analiza.

U okviru šumskih zajednica pručavana je kompenzaciona tačka svetlosti kod većeg broja cenobionata (K o j i ć, et al., 1995), pre svega iz hrastovih i hrastovo-grabovih šuma. Ispitivanjima su bile obuhvaćene 23 vrste iz različitih šumskih spratova, pri čemu su utvrđene najveće vrednosti kompenzacione tačke, zabeležene, najčešće, početkom vegetacijskog perioda. Vrednost kompenzacione tačke svetlosti kod šumskih vrsta najčešće je od 400 do 500 luksa, sa izraženim sezonskim variranjima koja se kreću između 200 i 4980 luksa.

U agrarnim ekosistemima detaljno je proučavana visina kompenzacione tačke svetlosti korovskih biljaka (K o j i ć, 1987). Utvrđeno je da svetlost na staništima ovih biljaka varira od 310 do 1000 luksa tokom sezone a da one, najčešće, kompenzacionu tačku postižu pri svetlosnom intenzitetu od 500 do 600 luksa. Istraživanja K o j i ć a (1987) su pokazala da listovi različite starosti, na istoj biljci, postižu kompenzacionu tačku pri različitom intenzitetu svetlosti. Najmladi listovi imaju znatno višu kompenzacionu tačku (trpe intenzivnu svetlost) u odnosu na listove srednje starosti ili one najstarije. Poređenjem različitih fenoloških stadijuma iste biljke, utvrđeno je da listovi srednje starosti uspostavljaju kompenzacionu tačku pri najmanjoj jačini svetlosti.

Organska produkcija

Organska produkcija pojedinačnih biljaka i biljnog pokrivača u celini, u svojoj osnovi, jeste krajnji cilj i konačni efekat fizioloških procesa, pa, prema tome i jedna od osnovnih konstanti pri interpretaciji i reazliciji programa ekofizioloških proučavanja. Suštinski cilj istraživanja organske produkcije i svih pratećih relevantnih fizioloških procesa jeste – utvrđivanje bilansa materije i energije, kao i razrada naučnih osnova za povećanje produktivnosti i racionalno korišćenje pojedinih biljaka i biljnih zajednica u celini.

Istraživanja organske produkcije u okviru šumskih ekosistema naše zemlje bila su brojna i svestrano osmišljena. Značajni rezultati su postignuti kako u okviru proučavanja produkcije biomase i relevantnih činilaca toga procesa u zajednici *Festuco montanae-Quercetum petreae* M. Jank. na Frušoj gori (P o p o v i ć et al., 1984), tako i pri analizi dinamike stvaranja nadzemne biomase biljaka prizemnog sprata i količine stelje u hrastovim zajednicama na Jastrepcu (P o p o v i ć et al., 1986).

Istraživanja su pokazala da je ukupna biomasa nadzemnih delova biljaka u prizemnom spratu zajednice *Festuco montane-Quercetum petraeae* M. Jank. na Fruškoj gori, u toku vegetacijskog perioda, bila u granicama od 442 do 1032 kg/ha. Ukupan broj individua biljaka varirao je od $4{,}26 \times 10^4$ do $5{,}23 \times 10^4$ ind/ha. Najveću lisnu površinu postigla je vrsta *Festuca drymeia* (4860 do 7338 m^2/ha), dominantna, cenotički značajna biljka prizemnog sprata ove zajednice. Ukupna lisna površina svih vrsta zeljastih biljaka

maksimalno je dostizala vrednost od 13209.10 m^2/ha, dok je indeks lisne površine bio 1,32 ha/ha. Sve ove vrednosti govore da se ovde radi o velikoj primarnoj organskoj produkciji biljaka prizemnog sprata ove šumske zajednice.

Dinamika produkovanja nazdemne biomase biljaka prizemnog sprata i količine stelje proučavana je u pet hrastovih zajednica različitog stepena degradacije na Jastrepcu (Popović et al., 1986). Količina biomase se kretala između 155 i 515 kg/ha, a stelje od 3096 do 7392 kg/ha. U skladu sa promenama količine stelje kretao se i energetski ekvivalent (2,2 x 10^6 do 8,58 x 10^6 J/ha).

Organska produkcija livadskih biljnih zajednica proučavana je od strane većeg broja autora i na više lokaliteta. Istraživanja obavljena na Velikom Jastrepcu i Maljenu su dobar primer produkcionih odnosa ovakvih ekosistema.

Na Jastrepcu je praćena količina biomase nadzemnih delova biljaka i njihov energetski ekvivalent u dve livadske zajednice, i to *Trifolio-Cynosuretum cristati* i *Agrostio-Chrysopogonetum grylli*. Mezofilna zajednica *Trifolio-Cynosuretum cristati* u periodu maksimalnog razvića biljnog pokrivača postizala je veliku količinu biomase, čak do 7147 kg/ha, sa energetskim ekvivalentom pojedinih vrsta od 1511 do 1759 x 10^4 J/gr. Brdska termofilna livadska zajednica, *Agrostio-Chryosopogonetum grylli,* odlikovala se manjom količinom nadzemne biomase, koja je dostizala vrednost od 4957 kg/ha (Jovanović et al., 1986).

Na Maljenu su vršena uporedna ispitivanja karakteristika zemljišta i produktivnosti nadzemne biomase u dve kserotermne travnjačke zajednice - *Poo molinieri-Plantaginetum holostei* i *Koelerietum montane*. U prvopomenutoj zajednici količina obrazovane biomase kretala se između 1256 i 4562 kg/ha, sa energetskim ekvivalentom od 13504 do 18739 KJ. U zajednici *Koelerietum montane* količina biomase varirala je od 5070 do 10049 kg/ha, a energetski ekvivalent je bio između 14496 i 20039 KJ (Popović et al., 1988).

U okviru agrarnih ekosistema, u zajednicama gajenih biljaka, na njivama, organska produkcija je tradicionalno praćena, kako u kvantitativnom, tako i u kvalitativnom smislu, ali su se ta ispitivanja odnosila, uglavnom, na reproduktivne organe ili druge delove biljke koje čovek koristi za ishranu ili tehnološku preradu. U ovim slučajevima, organska produkcija je valorizovana pretežno u funkciji primene agrotehničkih i hemijskih mera, kvaliteta zemljišta i sličnog.

Kompleksni problemi organske produkcije kao rezultante delovanja abiotičkih i biotičnih faktora na određenom staništu bili su predmet studiranja i analiza brojnih autora, kako u inostranstvu, tako i u našoj zemlji (Boysen-Jensen, 1932; Weck, 1960; Lieth, 1962, 1972; Janković i Kojić, 1975 i drugi). U tom smislu značajni su pokušaji proučavanja i karakterizacije potencijalne produktivnosti biljaka, odnosno biljnog pokrivača na osnovu bioekoklimatskih pokazatelja izraženih u tzv. CVP - indeksu (Paterson, 1956; Weck, 1960). Na tom problemu kod nas su radili Janković i Kojić (1975) i Kojić (1987). Posle višegodišnjih proučavanja, a na osnovu kompleksne valorizacije spoljašnjih faktora, pre svega klimatskih, Janković i Kojić su, u gore navedenim radovima, prvi u nas, dali preliminarnu kartu produktivnosti biljnog pokrivača naše zemlje. Osim toga, koristeći iskustvo koje su stekli u proučavanjima, analizama i razmatranjima skupnog delovanja ekoloških faktora na organsku produkciju biljaka i biljnog pokrivača nekog predela u celini, Janković i Kojić su pokušali da daju svoj doprinos poboljšanju metodskog prilaza sagledavanju potencijalnih mogućnosti organske produkcije biljaka, odnosno biljnih zajednica ili biljnog pokrivača u celini.

Ekofiziologija stresa

Problemi ekofiziologije stresa, odnosno, odvijanja fizioloških procesa u uslovima spoljašnje sredine čije je delovanje na biljke obično znatno izvan granica optimuma, danas je u žiži interesovanja fitofiziologa, ekologa i ekofiziologa.

Prema podelama koje daje Levitt (1972), stresni faktori spoljašnje sredine mogu biti biotički (parazitizam, kompeticija, dejstvo patogena, herbivora, gaženja, kao i antropogeni stres kojim su obuhvaćeni svi efekti polutanata u atmosferi, zemljištu i vodi nastali aktivnošću čoveka) i abiotički, odnosno mnogobrojni nepovoljni i ekstremni fizičko-hemijski faktori staništa kao što su: nedostatak vode (stres suše), suvišak vode (stres plavljenja i nedostatka kiseonika), intenzivno zračenje, nedostatak svetlosti, izuzetno niska ili visoka temperatura, nedostatak ili suvišak soli i minerala (hemijski stresovi uslovljeni toksičnom koncentracijom jona, gasova, ili soli) ili mehanički stres (vetar, snežni nanosi). Dva stresna faktora, međutim, imaju poseban značaj i najčešće su analizirana i kod biljaka naše zemlje. To su nedostatak vode (stres suše) i nepovoljan sadržaj soli u zemljištu (stres soli) i, u vezi sa tim, adaptivni odgovori *kserofita na vodni deficit i halofita na toksično dejstvo velikih količina soli.*

Suša (vodni deficit ili nedostatak vode) predstavlja period bez dovoljne količine padavina i zbog toga redukovane količine vode u podlozi, praćene intenzivnom evaporacijom. Suša je često neizbežan, a svakako najteži problem za razvoj biljnog pokrivača, *posebno intrigantan u poljoprivrednoj biljnoj proizvodnji.* Ceo tok poremećaja procesa u biljci zbog nedostatka vode u uslovima suše zavisi od karakteristika staništa (fizičko-hemijskih svojstava zemljišta, temperature i vlažnosti vazduha) i od karakteristika same biljke - njenih adaptivnih reakcija (Levitt, 1958; Walter, 1960; Larcher, 1983, 1995). Stoga, sa ekofiziološkog stanovišta, može se reći da su biljke u stanju vodnog deficita kada je turgidnost njihovih tkiva manja od maksimalne. Ovakvo stanje nastupa kada proces transpiracije nadjača usvajanje vode iz zemljišta, prouzrokovano (a) smanjivanjem vlage u zemljištu (zemljišna suša), (b) povećanjem temperature vazduha (temperaturni šok), (c) smanjenjem vlažnosti vazduha, ili (d) povećanjem intenziteta strujanja vazduha (vazdušna suša).

Na osnovu višegodišnjih ekofizioloških istraživanja problema nedostatka vode na poljima kukuruza, uzimajući u obzir i rezultate drugih autora, Pekić (1989) konstatuje da je otpornost prema suši rezultat delovanja skupa adaptivnih mehanizama *kojima se ili povećava usvajanje vode, ili smanjuje njeno odavanje.* Pri vodnom deficitu javljaju se kod kukuruza adaptivne reakcije koje se, ili (a) neposredno indukuju u uslovima suše, ili (b) postoje bez obzira na neposredno delovanje suše. Kod kukuruza adaptacije koje nisu neposredno indukovane sušom čine skup morfoloških i anatomskih odlika korena i lista karakterističnih za vrstu kojima se obezbeđuje određeni stepen kseromorfnosti biljke. Adaptacije koje se indukuju u uslovima suše, međutim, mogu biti fiziološke (osmotsko prilagođavanje i stomaterna regulacija), ali i određene morfofiziološke modifikacije u listu i korenu. Centralno mesto u pokretanju ovih adaptacija ima hormon abscisinska kiselina (ABA) kao regulator fizioloških procesa.

U najnovije vreme problemom stresa soli i adaptacijama halofitskih biljaka detaljno se bavila Dajić (1996) ispitujući stepen halofitnosti određenih biljaka u zajednici *Puccinellietum limosae* (Rapcs.) Wend. na slatini (solončak izrazito alkalne reakcije) u Rusandi (Banat, severoistočna Jugoslavija). Analizom vodnog režima (dnevna i sezonska dinamika transpiracije, sadržaj vode, osmotski potencijal) i hemijskog sastava vegetativnih organa (sezonska dinamika sadržaja Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, PO_4^{3-} i ukupnog sadržaja šećera u korenu, stablu, listu i izdanku u celini)

najzastupljenijih vrsta pomenute zajednice utvrđen je visok stepen otpornosti prema solima koji se ostvaruje preko adaptivnih mehanizama kao što su isključivanje soli na nivou korena, održavanje visokog i konstantnog K/Na odnosa u različitim oganima biljke, zadržavanje jona u korenu i stablu, ekskrecija soli kod vrste *Atriplex tatarica, Atriplex litoralis, Aster tripolium* i *Puccinellia limosa*, kao i akumulacije i aktivne kompartimentacije soli u vakuole ćelija mezofila, što podrazumeva i mehanizme sukulencije kod vrsta *Suaeda maritima, Camphorosma annua* i *Salsola soda*.

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Imajući u vidu dosadašnje rezultate i iskustvo, valjalo bi nešto reći i o budućnosti. Perspektive daljeg razvoja fiziološke ekologije biljaka u nas trebalo bi da idu u pravcu proučavanja ponašanja biljaka u ekstremnim uslovima spoljašnje sredine (ekofiziologija stresa), i to, pre svega, suše i visokog sadržaja soli u zemljištu (ekofiziološki problemi halofita). Istovremeno, ekofiziološka istraživanja molekularnih osnova adaptacije biljaka omogućila bi identifikaciju tolerancije stresnih uslova spoljašnje sredine i primenu novih tehnologija 21. veka. Pored toga, ekofiziološka proučavanja biljaka su već, a u budućnosti će biti još više u funkciji zaštite i unapređenja životne sredine (problem sušenja šuma), i, s tim u vezi, funkcionalno povezana sa ekološkim monitoringom. Intenziviranje i produbljivanje ekofizioloških proučavnja endemičnih, reliktnih i retkih biljaka naše flore doprineće pravovremenom i svrsishodnom očuvanju florističkog diverziteta naše zemlje. Ekofiziološka proučavanja cenobionata agrarnih ekosistema, posebno gajenih biljaka, treba da omoguće razumevanje i racionalnu primenu agrotehničkih i hemijskih mera u cilju povećanja organske produkcije i očuvanja spoljašnje sredine. Uz sve to, trebalo bi da teče i aktivnost na razradi novih i usavršavanju postojećih metoda istraživanja toka i intenziteta fizioloških procesa biljaka u prirodnim uslovima.

ZAKLJUČAK

Fiziološka ekologija biljaka svojim istraživačkim ciljevima i zadacima ne samo što povezuje ekologiju i fiziologiju biljaka, već omogućuje sinhronizovan i objedinjen multidisciplinarni pristup u analizi biljaka na terenu. Ova naučna disciplina podrazumeva poznavanje anatomskih, morfoloških i filogenetskih karakteristika biljaka, svih nivoa njihove raznovrsnosti, ali i osnova mikroklimatologije, pedologije, geomorfologije i geografije, pa i šumarske i poljoprivredne prakse. Ekofiziološkim pristupom se zadire u suštinu opstanka biljaka u određenoj sredini, sagledava način strukturno-funkcionalnog prilagođavanja, širenje ili, eventualno, potiskivanje i išče-zavanje vrsta na određenim staništima.

Dosadašnja istraživanja u našoj zemlji bila su brojna i, takoreći do poslednjih godina, nisu zaostajala za svetskim trendovima. Isticala su se kompleksnim pristupom u okviru kojeg se moglo sagledati višestruko uzajamno delovanje organizama i spoljašnje sredine.

Ekofiziološka istraživanja obuhvatila su brojne biljke iz šumskih zajednica brdsko-planinskog regiona, livadskih i stepskih zajednica, zatim, gajene i korovske vrste iz agroekosistema, ruderalne biljke iz urbanih fitocenoza, kao i sađene i samonikle vrste sa „zelenih površina" u gradu. Najčešće su istraživani vodni odnosi biljaka na osnovu parametara vodnog režima (transpiracija, hidratura, vodni potencijal, sadržaj vode, vodni deficit), fotosinteza, kompenzaciona tačka svetlosti i organska produkcija biljaka u prirodnim ekosistemima, agroekosistemima i urbanim ekosistemima.

Opštom povoljnom ekonomijom vodom odlikuju se šumski ekosistemi naše zemlje, naročito termo-mezofilne hrastove zajednice brdskog pojasa. U ekstremnim

uslovima vodnog režima staništa opstaju biljke u stepskim (livado-stepskim) zajednicama, na kamenjarima, u klisurama i kanjonima, kao i na slatinama. Ispitivanja vodnog režima biljaka iz urbanih ekosistema ukazala su na poremećaje izazvane zagađivanjima vazduha i zemljišta u uskom centru grada.

Naročito opsežna ekofiziološka istraživanja obavljena su u okviru agroekosistema, gde su uporedo analizirani dinamika vodnog režima, fotosinteze, disanja i produkcije gajenih i korovskih biljaka. Značaj ovih istraživanja ogleda se i u unapredivanju agrotehnološke prakse (adekvatno navodnjavanje, dodavanje mineralnih đubriva).

Posebna pažnja, u poslednje vreme, sve više se poklanja istraživanjima molekularno-biohemijskih i ultrastrukturnih mehanizama adaptacije na stresne uslove spoljašnje sredine (suša, povećana koncentracija soli) kako kod autohtonih, tako i kod gajenih vrsta (kukuruz), a posebno kod nekih enedemičnih, reliktnih (*Ramonda* sp.) ili ugroženih biljaka naše flore.

LITERATURA

Alekseev, A.M. (1948): Vodni režim rastenii i vlijanie na nevo zasuxi. Gotgozizdat, Kazan.

Benet-Clark, T.A. (1959): Water relations of cells. Plant physiology: A treatise. Acad. press, vol. 2, 105-191.

Betke, N., Haas, O. & Stocker, O. (1965): Über den Wasserund Photosynthesehaushalt einiger Frühjarsgeophyten. Flora. *156:* 8-49.

Biebl, R. (1962): Protoplasmatische Oekologie der Pflanzen. Wasser und Temperatur. Protoplasmatologia, *12*, 1, Springer Verlag, Wien.

Blaženčić, Ž. (1982): Ekološka studija morfofizioloških adaptacija nekih kserofitnih trava (*Poaceae*) u stepskim fragmentima Fruške gore. Zbornik za prirodne nauke Matice srpske, *63*: 13-60.

Boysen-Jensen, P. (1932): Die Stoffproduction der Pflanzen. Gustav Fischer Verlag, Jena.

Ćorović, M., Stjepanović, L., Nikolić, R., Pavlović, S., Živanović, P. (1967-1968): Uporedna ispitivanja visine osmotskih vrednosti, transpiracije i količine etarskog ulja kod nekih vrsta iz familije *Labiatae*. - Gl. Bot. z. Bgd., *4*(1-4): 19-26.

Dajić, Z. (1966): Ekološka studija halofitske zajednice *Puccinellietum limosae* (Rapcz.) Wend. Doktorska disertacija, Biološki fakultet Beograd.

Dimitrijević, J. (1984): Dinamika transpiracije vrsta iz familije *Poaceae*. Ekologija, *19*, 1, 17-24.

Dimitrijević, J.& Popović, R. (1986): Dinamika nadzemne biomase biljaka prizemnog sprata i količine stelje u hrastovim zajednicama na Jastrepcu. Arhiv biol. nauka, *37*: 1-4.

Dubravec, K. (1984): Dinamika i intenzitet transpiracije listova nekih vrsta korova u voćnjaku Zeline. Fragmenta herbologica Jugoslavica, *13:* 2, 67-73.

Đorđević, L., Stevanović, B.& Janković, M.M. (1992): Water relations of the species *Paeonia corralina* Retz. and *Asphodeline liburnica* (Scop.) Rchb. from thermophylic oak community *Quercetum montanum* Čer. et Jov. *27*(1): 11-19.

Gessner, F. (1956): Der Wasserhaushalt der Hydrophyten und Helophyten. Handbuch der Pflanzenphyisiologie, 3.

Gligorijević, S., Kojić, M., Hoxha, E., Pejčinović, D. (1984): Sadržaj ukupne, slobodne i vezane vode u listovima nekih vrsta hrastova u zajednici *Quercetum-frainetto ceris scardicum* Krasnići kod Kijeva (Metohija). Zbornik radova III Kongresa ekologa Jugoslavije, Sarajevo, 247-252.

Grupče, R.& Grupče, Lj. (1987): Rashod vode transpiracijom pšenice i korova u Skopskoj kotlini. Fragmenta herbologica Jugoslavica, *16: 1-2, 103-111.*

Janković, M.M. (1971): Ekofizioloških karakteristike vodnog režima vrste *Stellaria holostea* u zajednici *Festuco-Quercetum petreae* M. Jank. na Fruškoj gori. Ekologija, *6*, 2, 259-273.

Janković, M.M.& Kojić, M. (1969): The compensation point of light in some plants of the forest community *Festuco-Quercetum petreae* on the mountain Fruška gora. Ekologija, *4*: 2, 131-139.

Janković, M.M.& Kojić, M. (1975): Savremeni problemi fiziološke ekologije biljaka sa osvrtom na rezultate Odeljenja za fiziološku ekologiju biljaka Instituta za biološka istraživanja u Beogradu. Glasnik instituta za botaniku i Botaničke bašte Univerziteta u Beogradu, 1-4, 1-30.

Janković, M.M., Popović, R.& Dimitrijević, J. (1967): Osnovne karakteristike i dinamika transpiracije nekih značajnih biljaka u biocenozi *Quercetum confertae-cerris* Rudski na Avali kod Beograda na osnovu posmatranja u 1964. godini. Ekologija, *2*, 1-2, 81-105.

Janković, M.M., Popović, R.& Dimitrijević, J. (1975): The first results of the studies of plant photosynthesis intensity in the physiologie. Meet. Yug. Soc. Plant Physiol., Stubičke Toplice, Acta bot. croat., *34*, 185.

Janković, M.M., Popović, R., Dimitrijević, J., Stefanović, K.& Karadžić, B. (1984): Ekofiziološke karakteristike biljaka i uslovi staništa bukovih šuma na Jastrepcu. VII Kongres biologa Jugoslavije, Budva, 232.

Janković, M.M., Popović, R., Dimitrijević, J.& Stevanović, B. (1987): Prilog poznavanju ekofiziologije endemoreliktnih balkanskih borova *Pinus heldreichii* i *Pinus peuce*. - Gl. Inst. bot. Bgd., *21:, 5-16.*

Janković, M.M., Popović, R. & Matijašević, B. (1975): Neki rezultati fiziološko-ekoloških proučavanja munike na Ošljaku, Šarplanina. Međunarodni simpozijum o munici, Dečani, 159-170.

Janković, M.M., Popović, R., Stefanović, K. & Dimitrijević, J. (1979): Ekofiziološke karakteristike biljaka i uslovi staništa u zajednici *Chrysopogonetum pannonicum typicum* u Deliblatskoj peščari. Zbornik radova II Kongresa ekologa Jugoslavije, Zadar-Plitvice, *I*: 567-585.

Jovanović, R., Stefanović, K., Popović, R.& Dimitrijević, J. (1986): Prilog poznavanju livadskih ekosistema na području Velikog Jastrepca. Glasnik Instituta za botaniku i Bot. bašte Univ. u Beogradu, *20*: 8-31.

Kojić, M. (1968): Der Lichtkompensationspunkt bei Blättern verschiedene Altern. Bulletin de l'inst. et du jard. botaniques de l'univers de Beograd, *3:, 1, 179-188.*

Kojić, M. (1987): Fiziološka ekologija kulturnih biljaka. Izd. Naučna knjiga, Beograd.

Kojić, M. (1987): Prilog proučavanju produktivnosti fotosinteze nekih sorata pšenice u poljskim uslovima. Arhiv za poljoprivredne nauke, *69:, 63-69.*

Kojić, M. & Ajder, S. (1991): Ekofiziološka proučavanja u agrofitocenozi pšenice. Zbornik radova Simpozijuma „Nedeljko Košanin i botaničke nauke", Ivanjica, 107-114.

Kojić, M. & Janković, M.M. (1967): Über die Hydraturvehältnisse einiger Arten der Thermophyllen Walsgesellschaften von *Quercus conferta* und *Quercus cerris* auf der Avala bei Beograd. Berichte d. Deutch Botan. Gesellschaften, *80*: 2, 71-79.

Kojić, M., Janković, M.M. & Popović, R. (1974): The compensation point of light in some plant species of the forest communities *Festuco-Quercetum petraeae* M. Jank. and *Querco-Carpinetum serbicum* Rudski on the mountain Fruška gora. Archiv biol. sci., *26:, 1 29-38.*

Kojić, M., Mrfat-Vukelić, S., Dajić, Z., Ajder, S., Stošić, M. & Lazarević, D. (1989): Livadska vegetacija Rudnjanske visoravni i Radočela - fitocenološka i ekofiziološka studija. Izd. Medicinske komunikacije i Institut za krmno bilje iz Kruševca, Beograd.

Kojić, M., Pekić, S. (1981): Vodni režim i organska produkcija pšenice. Fiziologija pšenice, Izdanje Srspke akademije nauka i umetnosti, Beograd, 21-38.

Kojić, M., Popović, R. & Mitrović, M. (1989): Kompenzaciona tačka svetlosti nekih drvenastih vrsta u urbanoj sredini. Naučni skup „Josif Pančić i prirodne nauke", Srpska akademija nauka i umetnosti, Beograd.

Kojić, M., Popović, R.& Mitrović, M. (1990): Uporedna analiza kompenzacione tačke svetlosti nekih biljnih vrsta u šumskim zajednicama na Fruškoj gori u uslovima gradske sredine. Naučni skup „Nedeljko Košanin i botaničke nauke", Ivanjica.

Kojić, M., Popović, R.& Mitrović, M. (1992): Uticaj zagađenosti vazduha na kompenzacionu tačku svetlosti nekih drvenastih biljaka (in press).

Kojić, M., Popović, R.& Mitrović, M. (1995): Kompenzaciona tačka svetlosti i njen ekološki značaj. Glasnik Instituta za botaniku i Botaničke bašte Univerziteta u Beogradu, *28*: 71-80.

Kojić, M., Stojanović, J., Ognjanović, R.& Marković, A. (1994): Vodni režim korova i njegove specifičnosti u odnosu na gajene biljke. Acta herbologica. Vol. *3*, No. 1, 19-31.

Kreeb, K. (1974): Oekophysiologie der Pflanzen. Gustav Fischer Verlag, Jena.

Krstić, B. (1981): Uticaj abiotskih i biotskih činilaca na fotosintezu pšenice. Fiziologija pšenice. Izd. Srpska akademija nauka i umetnosti, Beograd.

Larcher, W. (1978): Oekologie der Pflanzen. Ulmer Verlag, Stuttgart.

Levitt, J. (1972): Responses of plants to environmental stress. Ed. Kozlovski. T.T., Academic press, New York.

Lieth, H. (1958): Grenzen und Anwendungsmöglichkeiten der colorimetrischen CO_2 Bestimmungen. Planta, *51:, 705-721.*

Lieth, H. (1960): Über den Lichtokompensationspunkt der Landpflanzen. Planta, *54*, 530-576.

Marijašević, B. (1969): Prilog poznavanju hidrature kod *Fragaria vesca* L. - Ekologija, *4*(2), 141-147.

Matuszkiewicz, W.B. & Kranikowksa-Sznajder, B. (1953): Observacije nad wartose osmoticzne roslin zielnych w zespolach lesnych Bialowieskiego Parku Narodowego. Ekologia polska, *1:*, 7-45.

Mitrović, M. (1992): Ekofiziološke i morfoanatomske karakteristike vrsta drveća u uslovima grada Beograda. Magistarski rad, PMF Biološki fakultet, Beograd.

Passioura, J.B. (1988): Response to dr P.J. Kramer's „Changing concepts regarding plant water relations". Plant, Cell and Environment, Vol. *11*, No 7: 565-568.

Paterson, S.S. (1956): The forest area of the world and its potential productivity. Univers. Royal, Goteborg, *216*.

Pavletić, Z., Lieth, H. (1958): Der Lichtkompensationspunkt einiger immergrunner Pflanez im Winter und im Frühjahr. Berichte d. Deutch. Botan. Gesell., *71, 309-314.*

Pavlović, S., Stjepanović, L., Kuznjecova, G., Klajn, E. & Jančić, R. (1978): Prilog proučavanju *Peucedanum arenarium* W. et K. sa Deliblatske peščare. Glasnik prirodnjačkog muzeja, B, *33*, 79-94.

Pejčinović, D., Hoxha, E., Kojić, M. & Gligorijević, S. (1984): Sadržaj ukupne, slobodne i vezane vode u listovima nekih vrsta hrastova u zajednici *Quercetum frainetto-cerris scardicum* Krasnići kod Kijeva (Metohija). Zbornik radova III Kongresa ekologa Jugoslavije, Sarajevo, 247-252.

Pekić, S. (1989): Kukuruz i suša - ekofiziološka osnova otpornosti kukuruza prema suši. Izd. Naučna knjiga, Beograd.

Pekić, S. & Quarrie, S.A. (1987): Abscisic acid accumulation in lines of maize differing in drought resistance: A comparasion of intact and detached leaves. Journ. of Physiology, *127: 203-217.*

Pisek, A. (1956): Der Wasserhauschalt der Meso- und Hydrophyten. Handbuch der Pflanzenphysiologie, *3:, 825-853.*

Plavšić-Gojković, N., Dubravec, K., Gaži-Baskova, V. & Borišić, B. (1984): Komparativna istraživanja veličine i hoda transpiracije kod listova kukuruza i njegovih najučestalijih korova. Zbornik radova II Kongresa o korovima, Osijek, 189-199.

Popović, R. (1973): Neke ekofiziološke karakteristike vodnog režima efemeroida u zajednici *Querco-Carpinetum serbicum* Rudski na Fruškoj gori. Glasnik Instituta za botaniku i Botaničke bašte Univerziteta u Beogradu, *8:, 1-4, 57-69.*

Popović, R. (1991): Ecophysiology of plant species along a vegetational gradient. I. Water regime of some tree and shurb species in different forest communities. Archiv biol. sci., *43*, 3-4, 67-101.

Popović, R. (1992): Ecophysiology of plant species along a vegetational gradient. II. Water regime of some herbaceus species from different forest communities. Archiv biol. sci., *44*: 1-2, 1-22.

Popović, R. (1992): Ekološka studija hidraturnih odnosa nekih značajnih biljnih vrsta u zajednici *Querco-Carpinetum serbicum* na Fruškoj gori. Glasnik Instituta za botaniku i Botaničke bašte Univerziteta u Beogradu, *7*: 1-4, 1-8.

Popović, R. (1993): Ecophysiology of plant species along a vegetational gradient. III. Water regime of the most significant Chamaephytes and woody lianes (Phanerophytae Scandentiae). Archiv biol. sci., *45: 1-2, 1-8.*

Popović, R., Dimitrijević, J. & Janković, M.M. (1983): Ekofiziološka istraživanja vegetacije Deliblatske peščare. I. Dinamika i intenzitet transpiracije i količina vode u listovima biljaka livadsko-stepske i šumske zajednice. Ekologija, *18*: 1, 15-42.

Popović, R. & Janković, M.M. (1978): Prilog poznavanju ekološkog aspekta fotosinteze biljaka Deloblatske peščare. Treći simpozijum Jug. društva za fiziol. biljaka, Izola, *21.*

Popović, R., Janković, M.M., Stefanović, K.& Dimitrijević, J. (1986): Ekofiziološke karakteristike antropogene smrčeve šume na staništu bukve na Jastrepcu. Zbornik radova „Čovek i biljka", Matica srpska, 383-390.

Popović, R., Karadžić, B. & Mitrović, M. (1989): Effects of forest destruction on water regime characteristics of sessile oak *(Quercus petraea* Liebl.). Congr. „The biology of forest destruction", Budapest, *13.*:

Popović, R. & Karadžić, B. (1990): Karakteristike procesa fotosinteze hrasta kitnjaka u zavisnosti od stepena vitalnosti jedinke. Simpozijum „Nedeljko Košanin i botaničke nauke", Ivanjica, *32*.

Popović, R., Janković, M.M. & Dimitrijević, J. (1984): Površina listova biljaka kao pokazatelj primarne organske produkcije zeljastog sprata zajednice *Festuco montanae-Quercetum petreae* na Fruškoj gori. Arhiv biol. nauka, *35:* 3-4.

Popović, R., Stefanović, K., Janković, M.M., Karadžić, B. & Blaženčić, Ž. (1988): Uslovi staništa i vodni režim biljaka bukovih šuma na Maljenu. IV Kongres ekologa Jugoslavije, Ohrid, *104*.

Popović, R. & Stefanović, K. (1988): Analiza biomase nadzemnih delova biljaka livadskih zajednica na Maljenu. IV Kongres ekologa Jugoslavije, Ohrid, 459.

Popović, R., Stefanović, K., Stevanović, B., Janković, M.M. & Karadžić, B. (1988): Ekofiziološka ispitivanja ruderalne zajednice *Lolio-Plantaginetum majoris* u užem području Beograda. IV Kongres ekologa Jugoslavije, Ohrid, 495.

Regula, L. & Pavletić, Z. (1968): The compensation point of light in some mediterranean and submediterranean plants grown under condition of moderate middle-european climate. Ekologija, *3:, 1, 1-6.*

Ristić, Z. (1983): Dnevna dinamika intenziteta transpiracije i sadržaja vode u listovima *Lathyrus tuberosus* i *Symphytum officinale*. Fragmenta herbologica Jugoslavica, *12:* 2, 45-53.

Schulze, E.D., Lange, O.L. & Koch, W. (1988): Ecophysiological investigation on wild and cultivated plants in the Negev desert. Oecologia. *9:* 317-340.

Seinfeld, J.H. (1975): Air pollution - physical and chemical fundamentals. Me Graw-Hill inc., London.

Semihatova, O.A. (1988): Ratio of respiration and photosynthesis in plant productivity. Photosynthesis and plant productivity, 98-108.

Slayter, R.O. & Taylor, S.A. (1960): Terminology in plant-soil water relations. Nature London, *187:* 922-924.

Stevanović, B. (1980): Ekološka studija vodnog režima nekih značajnih zeljastih biljaka u zajednici *Chrysopogonetum panonicum* Stjep. - Ves. na Deliblatskoj peščari. Doktorska disertacija, Prirodno-matematički fakultet, Beograd.

Stevanović, B. (1986): Ecophysiological characteristics of the species *Ramonda serbica* Panč. and *Ramonda nathaliae* Panč. et Petrov. Ekologija, *21: 2,* 119-134.

Stevanović, B. (1989): Water relations of *Ramonda serbica* Panč. and *Ramonda nathaliae* Panč. et Petrov. in different habitat conditions. Glasnik Instituta za botaniku i Botaničke bašte Univerziteta u Beogradu, *23:* 47-55.

Stevanović, B. (1990): Značaj fiziološke ekologije biljaka za poznavanje životnih uslova endemičnih i reliktnih biljaka. Ekologija i geografija u rešavanju problema životne sredine. Posebno izdanje Srpskog geografskog društva, knj. *69:* 66-71.

Stevanović, B. & Filipi-Matutinović, S. (1992): Survey of ecophysiological investigations in the states of the former Yugoslavia. I. Plant water relations. Ekologija. *27:* 2, 1-33.

Stevanović, B.& Janković, M.M. (1982): Prilog poznavanju hidrature vrste *Ginkgo biloba* L. Ekologija, *17:* 2, 109-118.

Stevanović, B.& Janković, M.M. (1983): Prilog poznavanju vodnog režiam *Ginkgo biloba* L. Ekologija, *18:* 2, 107-120.

Stikić, R. (1987): Uticaj suše na ponašanje stoma kod kukuruza. Doktorska disertacija, Poljoprivredni fakultet, Beograd.

Stjepanović-Veselićić, L. (1959): Oekologische Untersuchungen der osmotischen Werte einiger Pflanzenarten der Sandbodenvegetation. Glasn. Prirod. muzeja, B, *14*: 157-184.

Stjepanović, L., Ćorović, M.& Pavlović, S. (1968): Visina osmotskih vrednosti i količina etarskog ulja kod *Laserpitium siler* L. u zavisnosti od različitih faktora. - Gl. Prir. muz. B., *23:* 27-37.

Tranquillini, W. (1959): Die Stoffproduction der Zirbe an der Waldgrenze während eines Jahres. Planta, *54:* 130-151.

Voznesenskii, V.L. (1971): Konduktometričeski pribor dlja izmerenija fotosinteza i dihanija rastenii v polevih uslovijah. 2 izdanie, Nauka, Moskva.

Walter, H. (1931): Die Hydratur der Pflanze und ihre physiologisch-oekologische Bedeutung. Gustav Fischer Verlag, Jena.

Walter, H. (1968): Die Vegetation der Erde in oeko-physiologischer Betrachtung. Berlin.

Walter, H. (1960): Die Hydratur der Pflanze als Indikator ihres Wasserhaushalt. Handbuch der Pflanzenernährung und Düngung, *1:* 7, 1-42.

Walter, H. (1960): Einfhürung in die Phytologie, III, 2nd edition. Standortslehre. Eugen Ulmer, Studgart. Pp. 238-258.

Weck, J. (1960): Klimaindex und forstliches Produktionspotential. Forstarchiv, *31:* 7, 101-105.

Summary

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ECOPHYSIOLOGICAL STUDIES OF PLANTS IN SERBIA

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Physiological plant ecology not only bridges plant ecology and plant physiology but also deals with synchronized and integrated multidisciplinary approach to plant analysis in the fieldwork. This scientific discipline demands the knowledge of morphological and physiological characteristics of different levels of plant organization, equally with phylogenetic relations and ecological properties of plants, but also requires the fundamental informations of microclimatology, pedology, geomorphology and geography of the hibitats, as well as of forest and agricultural menagement. The ecophysiological approach generates the new insights on the interactions of the plant with the environment, recognizes the essence of plant adaptive strategies (structural and functional), their distribution on Earth or disappearance in disturbed ecosystems.

Investigations carried out in Serbia so far were numerous and until recent years did not lag behind the world studies. There, the ecophysiological examinations were concerned with a great number of trees, shrubs and herbaceous plants from forests of montane and mountain regions, meadows and steppe communities, then cultivated plants and weeds from agroecosystems, ruderal species from urban phytocoenoses as well as planted and spontaneously grown species from „green areas" in the towns. The most frequently monitored ecophysiological processes were water relations, photosynthesis, light compensation point and organic production of plants in mentioned ecosystems from different parts of Serbia. Recently, the special attention is paid on ecophysiological adaptations of endemic, relic or endangered plants of this region, as well as on molecular or biochemical responses of plants to multiple environmental streesses (drought, salty soil, pollution).

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Original scientific paper

SNEŽANA BUDIMIR[1], RADMILA VUJIČIĆ[2], LJUBINKA ĆULAFIĆ[3]

DEVELOPMENT OF SOMATIC EMBRYOS AND EMBRYOGENIC CAPACITY IN *PICEA OMORIKA* (PANČ.) PURK. CULTURE*

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Budimir, S., Vujičić, R., Ćulafić, Lj. (1995): *Development of somatic embryos and embryogenic capacity in Picea omorika (Panč.) Purk. culture.* – Glasnik Instituta za botaniku i botaničke bašte Univerziteta u Beogradu, Tom XXIX, 43 - 50.

In *Picea omorika* seedling explant culture, after induction on medium supplemented only with cytokinin, cells from epidermal or subepidermal cotyledon layers gave rise to embryogenic tissue. Somatic embryos seems to be of single cell origin. Embryogenic tissue was induced on explants from five elite genotypes (seed families). One genotype with an induction frequency of 19% was superior to other four tested. On abscisic acid-containing medium somatic embryos underwent maturation. During precotyledonary stage of development starch accumulation from suspensor to the apical dome was observed. On the cotyledonary stage distinct meristemous were well defined and hypocotyl-shoot axis was formed.

* Dedicated to Prof. Mirjana Nešković on the occasion of her 45 th anniversary of scientific work

Key words: *Picea omorika* (Panč.) Purk., somatic embryogenesis, genotype, embryo development, abscisic acid.

Ključne reči: *Picea omorika* (Panč.) Purk., somatska embriogeneza, genotip, razviće embriona, abscisinska kiselina.

INTRODUCTION

Serbian spruce, *Picea omorika* (Pančić) Purk. is a Tertiary relic species, endemic to the Balkan Peninsula. At present times it resides on a limited number of about 30 localities distributed along the central part of the Drina river (Fukarek, 1951). *Picea omorika* is usually reproduced by seeds and only a few reports indicate the possibility of vegetative reproduction by grafting or cuttings (Gajić, 1994). The regeneration of *Picea omorika* can be also achieved by application of tissue culture methods (Budimir & Vujičić, 1990).

One of the most effective method of *in vitro* vegetative reproduction in conifer species, is by somatic embryogenesis (Sutton et al., 1993). The somatic embryogenesis is a process by which somatic cells of an explant are able to undergo all developmental stages of a zygotic embryo. One of the advantages of vegetative reproduction via somatic embryogenesis are well defined shoot and root apical meristems. In order to achieve a successful induction of somatic embryogenesis, a detailed protocol must be worked out for each species. The efficiency of method can further be enhanced using a phenotype with proven high potential for somatic embryogenesis.

Results which allow practical application of somatic embryogenesis, have already been achieved for a number of conifer species and in particular species of *Picea* (Tautorus, Fowke & Dunstan, 1991; Attree & Fowke, 1993; Vujičić & Budimir, 1995). Somatic embryogenesis in *Picea omorika* has been reported by Budimir & Vujičić (1992). It was demonstrated that somatic embryogenesis in the culture of Serbian spruce was induced when cytokinin, as a sole growth factor was present in the nutrient medium. The induction frequency of somatic embryogenesis was found to vary between 0-8%, most probably because a mixture of seed from various trees was used for obtaining seedling-derived explants.

The aim of the present study was to investigate the effect of genotype on the induction frequency of somatic embryogenesis, using seed families of known origin.

MATERIAL AND METHODS

Plant material

Seeds of *Picea omorika* used in the experiments collected from natural population were stored at 4°C for 18 months, the seeds collected from five open-pollinated elite trees were stored at 4°C for 48 months. The seeds were washed in running tap water for 24 h, surface disinfected with 30% H_2O_2 containing a drop of Tween 20 for 30 min, and then rinsed three times with sterile water.

The seeds were placed to germinate in Petri dishes on the medium containing 2 g l^{-1} glucose and 6 g l^{-1} agar. Shoot apices bearing 4-8 mm long cotyledons (shoot explants) were excised under sterile conditions. Ten explants per dish were placed horizontally on the surface of the culture medium.

Culture medium

Von Arnold & Eriksson (1981) modified basal medium (BM), consisting of salts, vitamins and sugars (90 mM sucrose), was used. Amino acids were omitted. Media were solidified with 7 g l^{-1} agar. The pH of the medium was adjusted to 5.7 prior to autoclaving for 25 min, at 115°C.

For the induction of somatic embryogenesis, explants were grown on BM with 22.5 μM benzyladenine (BA) for 2 weeks and then transferred to growth regulator-free medium for 4 to 6 weeks. Embryogenic tissue that developed on the explant was isolated and grown on BM to which 9 μM 2,4-dichlorophenoxyacetic acid (2,4-D) puls 4.5 μM BA and 30 mM sucrose were added. Cultures were kept in darkness. Subculturing to a fresh medium was carried out every two weeks.

For further embryo development parts of embroygenic tissue were transferred to Petri dishes on filter paper (Whatman No 2) supports on the surface of the solidified BM with 12 μM abscisic acid (ABA) for 4 to 8 weeks. Single mature embryos with developed cotyledons were transferred to half-strength BM without growth regulators for root growth.

Culture conditions

The seeds were germinated and all cultures maintained at $25 \pm 2^{\circ}C$ under a 16 h photoperiod, at a photon flux density of 5.1 μmol m^{-2} s^{-1} provided by white fluorescent tubes (Tesla, Pancevo, 65W, 4500 K), unless stated differently.

Microscopy

(a) Fresh material – small pieces of embryogenic tissue were placed on a glass slide, stained with 0.5% acetocarmin, and pressed gently with a cover glass. The entire preparation was then observed and photographed under Jenamed, Carl Zeiss photomicroscope.

(b) Paraffin – tissue was fixed in formalin-acetic acid-ethanol (FAA) for 24 h, dehydrated in graded ethanol, and embedded in paraffin wax at $57^{\circ}C$. Sections 5 μm thick were stained with haematoxylin.

(c) Plastic – tissue was fixed in 3% phospahte-buffered glutaraldehyde, pH 7.2, for 2 h, and postfixed in 2% phosphate-buffered OsO_4 for 2 h. Samples were dehydrated in graded ethanol, and embedded in Araldite (Serva, Heidelberg). Sections 1 μm thick were stained with methylene blue.

RESULTS AND DISCUSSION

Origin of somatic embryos

In the shoot apex culture of *Picea omorika* the cotyledon elongation was the only morphologically observable response, after two weeks on the induction BM supplemented with 22.5 μM BA. Within the following three weeks on hormone-free medium, whole explant or only its basal region become swollen with nodular appearance. Histological analysis of the cotyledon confirmed that first cell divisions occurred in epidermal and a few subepidermal layers. Anticlinal cell divisions were frequently present in epidermal layers, while in subepidermal layers (Fig. 1) mostly periclinal divisions occurred. Further cell divisions resulted in formation of a callus tissue, which caused disruption of epidermal/subepidermal layers, the neighbouring cells being loosely associated. By the end of fourth week the white glossy, mucilaginous, embryogenic tissue with polarized structures and small cell aggregates was found on the disrupted epidermal tissue (Fig. 2). The cells (Fig. 3), probably formed after an equal division of single superficial cells, are believed to be initials of a somatic proembryo. One of the two cells enlarged first and then divided transversely, which resulted in an

early filamentous somatic proembryo formation (Fig. 4). Further cell divisions of the upper cell, the embryo initial cell, resulted in an immature embryo formation. Immature embryos consist of small group of apical, densely protoplasmic cells, the apical dome, supported by a highly vacuolated and elongated suspensor cells (Fig. 5).

Similar results were obtained by Nagmani, Becwar & Wann (1987) in *Picea abies* and *Picea glauca*. The authors suggested that the origin of somatic embryos can be traced to singel cells in hypocotyl region, of immature embryos and that the first unequal cell division resulted in an early proembryo with the embryonal and suspensor initial. However, Mo & Von Arnold (1991) observed that in seedling explants of *Picea abies*, embryogenic structures could differentiate from epicotyl, hypocotyl and cotyledons. The authors found that embryos originated from nodules that were formed either from epidermal, subepidermal or cortical cells. Some embryogenic cultures might also differentiate directly from single epidermal cells.

Effect of genotype on induction frequency

Seeds collected from five elite trees were used in order to determine the effect of genotype on embryogenic capacity. Seeds originated from a parent tree were designated as seed family. The induction of embryogenic tissue formation was achieved as described above. The frequency of induction obtained among seeds from different families, varied in the range of 1-19%. Seed family No 2, with induction frequency of 19% was significantly superior to the other four families tested. The induction frequency among these four seed families varied in the range of 1.3-6.3% (Table 1).

Tab. 1. – Genotype effect on induction of somatic embryogenesis in Picea omorika

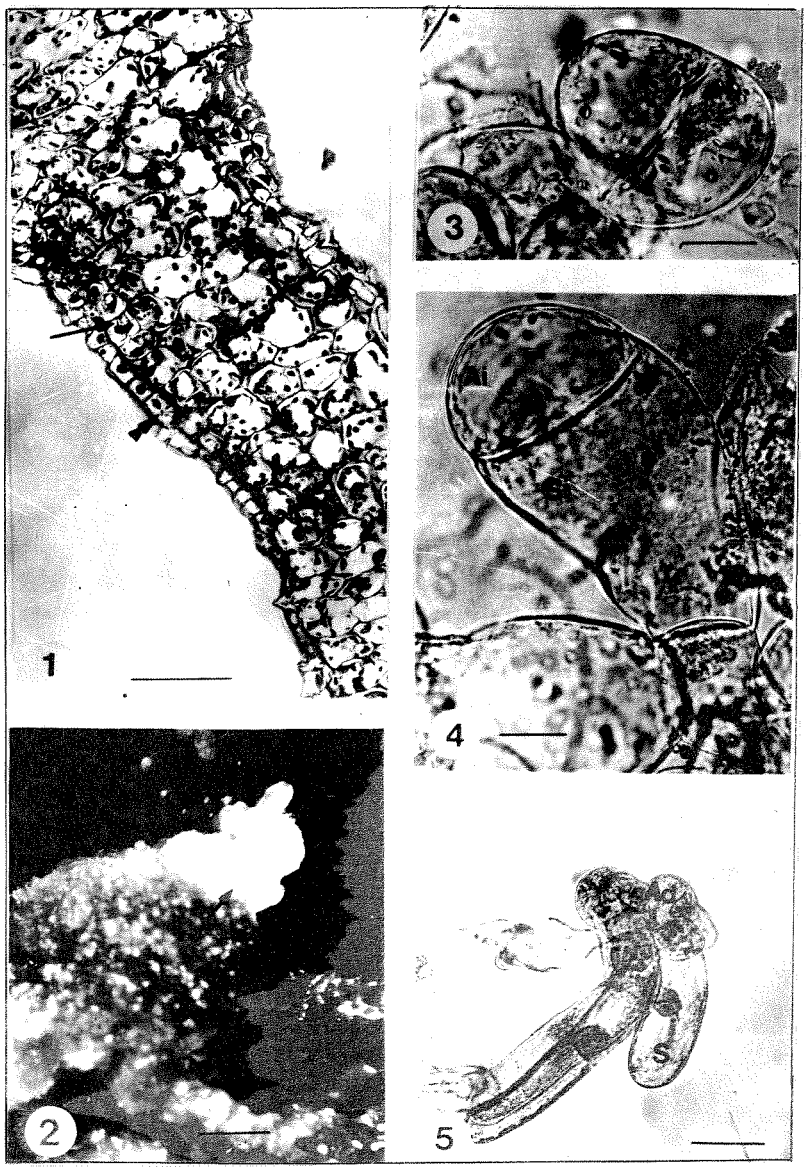
Seed family	No. of explants cultured	Induction frequencies (%)
1	80	6.3
2	107	18.7*
3	125	1.6
4	96	5.2
5	154	1.3

*Induction frequency is significantly different based on confidence intervals on the level $P < 0.05$.

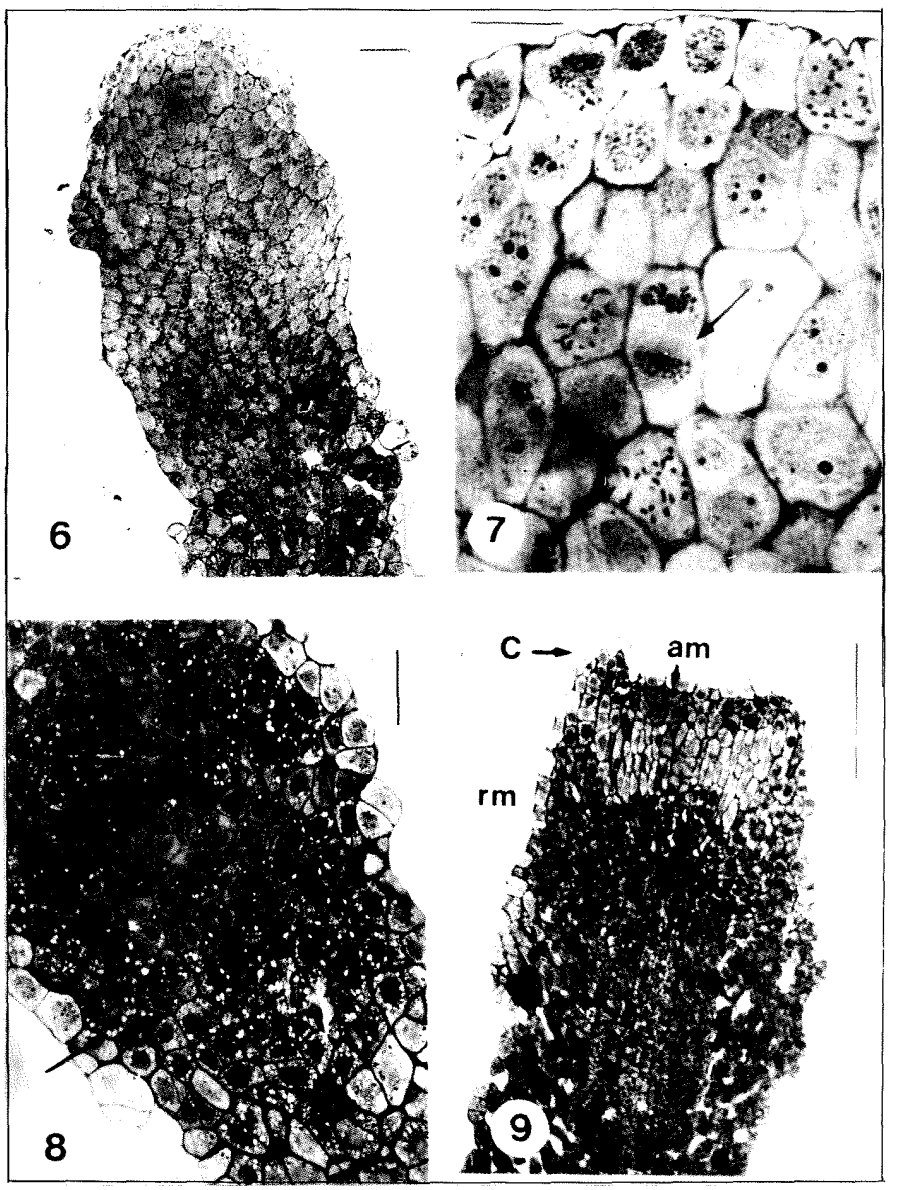
The varied induction ability demonstrated by different genotypes, suggests that in *Picea omorika*, genetic component is implicated in the capacity for embryogenic tissue formation. Cheliak & Klimaszewska (1991) also reported that in *Picea mariana*, among 20 genotypes tested, 85% gave rise to somatic embryogenesis. The induction frequencies varied from 0-17%. Jain, Dong & Newton (1989) suggested that genotype, controlled pollination and pre-conditioning of explants are of tremendous importance in the initiation and enhancement of somatic embryogenesis.

Development of somatic embryos

Isolated embryogenic tissue proliferated when grown on the medium supplemented with 9 μM 2,4-D and 4.5 μM BA (Budimir & Vujičić, 1992). Histochemical analysis of embryogenic tissue with acetocarmin, showed the presence of numerous filamentous, immature embryos. The mechanism of embryo multiplication



Figs. 1. – Longitudinal section of cotyledon with anticlinal cell divisons (double arrowhead) in epidermal, and periclinal cell divisions (arrowhead) in subepiermal cell layers. Bar = 100 μm; 2. – Embryogenic structures (arrow) protruding from the basal part of the cotyledon. Bar = 1 mm; 3. – Two-celled somatic proembryo. Bar=20 μm; 4. – Filamentous somatic proembryo with apical initial (Ai) and suspensor ini-tial (Si). Bar = 20 μm; 5. – Immature somatic embryo with defined apical dome (Ad) and suspensor (S). Bar = 50 μm



Figs. 6. – Longitudinal section of an embryo at the precotyledonary stage. Bar = 50 μm; 7. – Dividing cells (arrow) in the apical dome of somatic embryo. Bar = 50 μm; 8. – The basal part of somatic embryo with starch grains (arrow) in the cells. Bar=50 μm; 9. – Longitudinal section of an embryo at the cotyledonary stage; c - cotyledons, am - apical meristem, rm - root meristem. Bar = 20 μm.

is still unclear. The authors observed that embryos could arise by the mechanism similar to the cleavage polyembryogenesis in zygotic embryos (data not shown). With regular two-week subculturing the embryogenic potential of the tissue was retained for more than two years.

Further development of somatic embryos was observed after exposure to abscisic acid (Budimir & Vujičić, 1992). When grown on the medium containing 12 μM of abscisic acid, single somatic embryos were enlarged, and protruded from mucilaginous tissue. Histological examination of precotyledonary embryos (Fig. 6) showed that embryo enlargement was the result of intensive cell divisions. Cell divisions were observed in the apical dome as well as in the suspensor, the most frequent divisions, however, were found in the apical dome (Fig. 7). A gradient of accumulated starch grains from the suspensor towards the apical dome was prominent on longitudinal sections of the embryo. The cells in the suspensor region contained an abundance of large starch grains (Fig. 8). Similarly in *Picea glauca* culture, starch accumulation began at the proximal zone of the suspensor, continued to the peripheral regions and in the developing embryo. After polysaccharide accumulation, lipid and protein deposition was observed (Joy et al., 1991).

In *Picea omorika* culture structural differentiation of embryos occurred at the cotyledonary stage of embryo development (Fig. 9). Shoot apex with defined shoot meristem attained slightly convex shape. Provascular elements could be distinguished bellow the apex. The root initial consisting of meristematic cells was formed at the opposite side of the shoot apex. Root initial gave rise to cells that divided transversally forming a massive root cap.

An inhibory effect of abscisic acid (12 μM) on embryo multiplication, and at the same time the stimulatory effect on embryo maturation, was also described for a number of other conifer species (Becwar, Noland & Wann, 1987; Von Arnold & Hakman, 1988; Webster et al., 1990) as reviewed by Vujičić & Budimir (1995).

The present results show that the induction of somatic embryogenesis from embryonic (seedling) explants is possible in a range of genotypes of Serbian spruce. The somatic embryos seem to differentiate from single superficial cells of cotyledons. Our studies are now focusing on somatic embryogenesis induction in explants isolated from more mature plant material.

REFERENCES

Attree, S. M. & Fowke, L. C. (1993): Embryogeny of gymnosperms: advances in synthetic seed technology of conifers. – Plant Cell Tiss. Org. Cult. *35*: 1-35.

Becwar, M. R., Noland, T. L. & Wann, S. R. (1987): A method for quantification of the level of somatic embryogenesis among Norway spruce callus lines. – Plant Cell Rep. *6*: 35-38.

Budimir, S. & Vujičić, R. (1992): Benzyladenine induction of buds and somatic embryogenesis in *Picea omorika* (Pančić) Purk. – Plant Cell Tiss. Org. Cult. *31*: 89-94.

Cheliac, W. M. & Klimaszewska, K. (1991): Genetic variation in somatic embryogenic response in open-pollinated families of black spruce. – Theor. Appl. Genet. *82*: 185-190.

Fukarek, P. (1951): Present distribution of Serbian spruce (*Picea omorika* Pančić) and some results concerning the stands (in Serbian). – J. B. Biol. Inst. (Sarajevo) *3*: 141-198.

Gajić, M. (1994): Vegetative propagation. *In* Serbian spruce - *Picea omorika* (Pančić) Purkyne on the Teritory of the National Park Tara (Eds. M. Gajić, D. Vilotić, D. Karadžić, Lj. Mihajlović, V. Isajev). pp. 57-62. – National Park Tara, Bajina Bašta - Institute for Forest Research, Beograd.

Jain, S. M., Dong, N. & Newton, R. J. (1989): Somatic embryogenesis in slash pine (*Pinus elliottii*) from immature embryos cultured *in vitro*. – Plant Sci. *65*: 233-241.

Joy, R. W., IV, Yeung, E. C., Kong, L. & Thorpe, T. A. (1991): Development of white spruce somatix embryos: I Storage product deposition. – *In vitro* Cell. Dev. Biol. *27P*: 32-41.

Mo, L. H. & Von Arnold, S. (1991): Origin and development of embryogenic cultures from seedlings of Norway spruce (*Picea abies*). – J. Plant Physiol. *138*: 223-230.

Nagmani, R., Beewar, M. R. & Wann, S. R. (1987): Single-cell origin and development of somatic embryos in *Picea abies* (L.) Karst. (Norway spruce) and *P. glauca* (Moench) Voss. (white spruce). – Plant Cell Rep. *6*: 157-159.

Sutton, B. C. S., Grossnickle, S. C., Roberts, D. R., Russell, J. H. & Kiss, G. K. (1993): Somatic embryogenesis and tree improvement in interior spruce. – J. For. *91*: 34-39.

Tautorus, T. E., Fowke, L. C. & Dunstan, D. I. (1991): Somatic embryogenesis in conifers (1991). – Can. J. Bot. *69*: 1873-1899.

VonArnold, S. & Eriksson, T. (1981): *In vitro* studies of adventitious shoot formation in *Pinus contorta*. – Can. J. Bot. *59*: 870-874.

Von Arnold & Hakman, I. (1988): Regulation of somatic embryo development in *Picea abies* by abscisic acid (ABA). – J. Plant Physiol. *132*: 164-169.

Vujičić, R. & Budimir, S. (1995): Somatic embryogenesis and plant regeneration in *Picea omorika*. *In* Somatic embryogenesis in woody plants 3 (Eds. S. Jain, P. Gupta, and Newton, R.) pp. 81-97. – Kluwer Academic Publishers, Netherlands.

Webster, F. B., Roberts, D. R., Mc Innis, S. M. & Sutton, B. C. S. (1990): Propagation of interior spruce by somatic embryogenesis. – Can. J. For. Res. *20*: 1759-1765.

Rezime

SNEŽANA BUDIMIR[1], RADMILA VUJIČIĆ[2], LJUBINKA ĆULAFIĆ[3]

RAZVIĆE SOMATSKIH EMBRIONA I EMBRIOGENI KAPACITET U KULTURI *PICEA OMORIKA* (PANČ.) PURK.

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U kulturi isklijalih biljaka *Picea omorika*, nakon indukcije na hranljivoj podlozi sa citokininom, ćelije epidermalnog i subepidermalnih slojeva kotiledona formiraju somatske embrione. Somatski embrioni su verovatno jednoćelijskog porekla. Nezreli somatski embrioni imaju diferenciran embriogeni region, građen od meristemskih ćelija i region suspenzora građen od izduženih, vakuoliziranih ćelija. Embriogeno tkivo je indukovano na eksplantatima poreklom od pet različitih genotipova (familija semena). Jedina familija semena sa frekvencijom indukcije od 19% bila je izrazito bolja od ostale četiri ispitivane familije. Kada se embriogeno tkivo izoluje sa eksplantata i dalje gaji na hranljivoj podlozi sa auksinom i citokininom dolazi do formiranja novih somatskih embriona. Embrioni mogu nastati procesom koji je označen kao „cleavage" poliembriogeneza. Na hranljivoj podlozi sa abscisinskom kiselinom somatski embrioni sazrevaju. Za vreme prekotiledarnog stupnja razvića uočeno je da dolazi do akumulacije skroba i to prvo u ćelijama suspenzora, a zatim i u ostalim delovima embriona. Na kotiledonarnom stupnju razvića apikalni meristemi postaju uočljivi, a duž ose embriona počinju da se diferenciraju vaskularni elementi.

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A ROLE OF CAROTENOIDS IN PHOTOTROPISM OF *ARABIDOPSIS THALIANA* SEEDLINGS

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A carotenoid deficient mutant of *Arabidopsis thaliana* (Am 45-3) was used to investigate the role of carotenoids in phototropism and adaptation. The mutant seedlings appeared pale and contained about 2.5-3% of the amount of carotenoids present in the WT when grown in light. Phototropism of pale seedlings in response to unilateral BL pulse was similar to that of WT seedlings except that the amplitude of the response was lower. Pale seedlings retained their ability to undergo desensitization by BL irradiation as a part of adaptation. These seedlings also exhibited RL-induced enhancement of phototropism. Enhancement appeared to be associated with an increase of carotenoid content. These data are consistent with the conclusion that carotenoids are not the photoreceptor pigments for phototropism or desensitization, although the presence of carotenoids affects the amplitude of phototropism and mechanism for enhancement in *A. thaliana*.

Key words: *A. thaliana*, phototropism, carotenoids, blue light, red light

Ključne reči: *A. thaliana,* fototropizam, karotenoidi, plava svetlost, crvena svetlost

INTRODUCTION

Blue light induces many physiological responses including phototropism in plants (Kaufman, 1993). The identity of the photoreceptor pigment for phototropism has not yet been elucidated and it was recently suggested that mulitple photoreceptor pigments may mediate this process in *Phycomyces* and *Arabidopsis thaliana* (Galland and Lipson, 1987; Konjević et al., 1989). In addition, the existence of photoreceptor pigment was postulated that could control desensitization of *Arabidopsis* seedlings by the BL to a subsequent unilateral photostimulation (Poff et al., 1994).

Because of similarity of absorption spectra of carotenoids, and the action spectrum for phototropism, carotenoids were suggested to be the photoreceptor pigment mediating this process (Curry, 1969). However, absorption spectrum of β-carotene lacks the peak at about 370 nm present in the action spectrum for phototropism (Curry, 1969). Moreover, a mutant of *Phycomyces* that has less than $1x10^5$ of the WT carotenoid content still displayed normal sensitivity to phototropic stimulation (Presti et al., 1977). In addition, corn plants treated with a carotenoid synthesis inhibitor exhibit first and second positive phototropism similar to the response of untreated plants, the only difference being the amplitude of the response (Vierstra and Poff, 1981; Piening and Poff, 1988). These lines of evidence argued against the carotenoids as the chromophores of the photoreceptor for the BL in phototropism. A flavoprotein is now thought to be a photoreceptor pigment for phototropism as well as for other BL mediated processes (Song, 1984).

Adaptation in phototropism has been described in maize (Iino, 1988), *Phycomyces* (Galland, 1991) and *A. thaliana* (Janoudi and Poff, 1991). Irradiation of plants by light induces a change in their sensitivity and/or responsiveness to a subsequent irradiation (Galland, 1991; Janoudi and Poff, 1991). In maize both RL and BL are capable of inducing desensitization in phototropism (Iino, 1988). In *Arabidopsis* only BL can desensitize seedlings to a subsequent BL pulse (Janoudi and Poff, 1991). No attempt has yet been made to characterize the pigment responsible for desensitization in *Arabidopsis* seedlings.

A mutant strain of *A. thaliana* that has a pale phenotype was used to examine its carotenoid content and test for possible effect of decreased carotenoid levels on responses to BL. A mutant with a lower carotenoid content than the WT should be a good tool to test for carotenoid function in the BL absorption for phototropism or adaptation.

MATERIALS AND METHODS

Mutant population

A mixed population, Am 45-3, consisted of about 1/6 of seedlings that appeared pale and 5/6 that were normally pigmented. This fits the expected distribution if the pale seedling is a homozygous recessive which is incapable of maturing and setting seed (Chi-square test on the expected ratio of 5 normally pigmented : 1 pale seedling has

given probability value of 0.5). Two assumptions had to be made to allow comparison of observed ratio of two phenotypes in the Am 45-3 population with the predicted ratio 5:1. First, that the seeds obtained initially were progeny of a heterozygous plant and; second, that dominant homozygous and heterozygous plants have the same ability to set seeds. High probability value obtained in chi-square test is consistent with the hypothesis that Am 45-3 population probably consists of pale seedlings as homozygous recessive mutants, and normally pigmented seedlings representing a mixture of plants that are homozygous dominant and heterozygous for this genetic locus. Further in the text, the latter batch of seedlings is reffered to as normally pigmented, while carotenoid deficient mutants are reffered to as pale seedlings.

Growth conditions

Seedlings used in experiments for measurement of phototropism and gravitropism were grown as described previously (K h u r a n a et al., 1989) with one difference. Seedlings with the pale phenotype were grown for 4 h longer (43 h) than the normally pigmented seedlings (39 h) in order to bring them to approximately same size. Difference in pigmentation between two phenotypes of etiolated seedlings was obvious and easy to score. Pale plants that were light grown for 4 months also had a distinctive phenotype. Although these plants in culture looked morphologically similar to WT plants, except being smaller in size, their stems always shriveled quickly after development of flowers which made it impossible to do any crossing experiment. These seedlings were grown on Murashige-Skoog 1X medium (Gibco BRL) supplemented with 3% (w/v) sucrose. Because the pale seedlings died under the light conditions in which the WT-Col seedlings were grown, the following procedure was used for their culture. Forty hours after they germinated in darkness, pale seedlings were selected from the mixed population according to the colour of their cotyledons and placed into plastic containers with nutrient medium. These containers were then covered with a green plexiglas (Rohm Gmbh Plexigas gs, DIN 4102-B2) that had low transmittance throughout the visible part of the spectrum.

Light sources

White light that was used for growth of seedlings and to potentiate germination (60 μmol $\cdot$ $m^{-2}.s^{-1}$) was obtained from GE (Cleveland, OH) DeLux Cool-white fluorescent tubes. The BL source consisted of a projector equipped with a Sylvania (GTE Products, Danvers, MA) 300 W ELH tungsten halogen lamp and 450 nm interference filter with a half-band width of 10 nm (PTR Optics Waltham, MA). Red light (0.6 μmol $\cdot$ $m^{-2}s^{-1}$) used for pre-irradiation of seedlings was obtained from one gold fluorescent tube (GTE, Sylvania) wrapped with red cellophane (Highland Supply Corp., Highland, IL). This source provides radiation from 560 to 720 nm with maximum output at 620 nm. The duration of actinic BL was controlled with a Uniblitz shutter (Vincent Associates, Rochester, NY). Fluence rates were measured with a Li-Cor (Lincoln, NE) LI-190 SA in combination with a LI 1000 Datalogger.

Measurement of curvature

Phototropic and gravitropic curvature of hypocotyls were measured as described by K h u r a n a et al., (1989), except that a curvature was allowed to develop for 80 min in phototropism experiments.

High Performance Liquid Chromatography Analysis

HPLC analysis was performed as previously described (R o c k , 1991), with the spectrophotometer set at 436 nm for detection of carotenoids. Identification of carotenoids was done according to retention times of peaks on chromatograms. The area under the relevant peaks of absorbance on the chromatograms of the tested carotenoids was used as an indicator of their amounts. Quantification of individual carotenoids was done from the standard curves generated in the laboratory.

RESULTS

HPLC analysis

The content of 10 carotenoids was compared in light grown WT-Col and pale Am 45-3 seedlings. Chromatography revealed that the carotenoid content in the WT-Col plants was approximately 35-40 times higher than that in the pale mutant plants (Fig. 1. a and b). The amount of β-carotene was approximately 2.5 times higher in the WT-Col plants than in the pale plants when expressed as percentage of total carotenoids while the amounts of lutein and antheraxanthin were lower when expressed on the same basis (Fig. 1. a and b).

Relative quantities of individual carotenoids as a percentage of total amount of 9 tested carotenoids in the RL-irradiated seedlings were not different from those in the etiolated seedligns of WT-Col (Tab. 1). However, RL-irradiated WT-Col seedlings had approximately 40% higher quantities of carotenoids than etiolated seedlings (Tab. 2).

Tab. 1. – The quantity of individual carotenoid as percent of total amount of nine tested carotenoids in etiolated (-RL) and red light- irradiated seedlings (+RL) of WT-Col. Values in the table are means ± 1 SE; n=2.

	- RL (%)	+ RL
β-carotene	4.1 ± 1.9	4.7 ± 0.5
lutein	42.7 ± 0.4	42.1 ± 1.5
zeaxanthin	0.6 ± 0.0	1.2 ± 0.6
antheraxanthin	3.2 ± 0.5	3.2 ± 0.1
lutein epoxide	7.7 ± 0.9	8.0 ± 0.2
all-trans violaxanthin	29.4 ± 0.5	28.5 ± 0.1
9 cis-violaxanthin	3.6 ± 0.5	3.5 ± 0.5
13 cis-violaxanthin	3.6 ± 0.8	3.6 ± 0.8
9 cis-neoxanthin	5.3 ± 0.1	5.1 ± 0.0

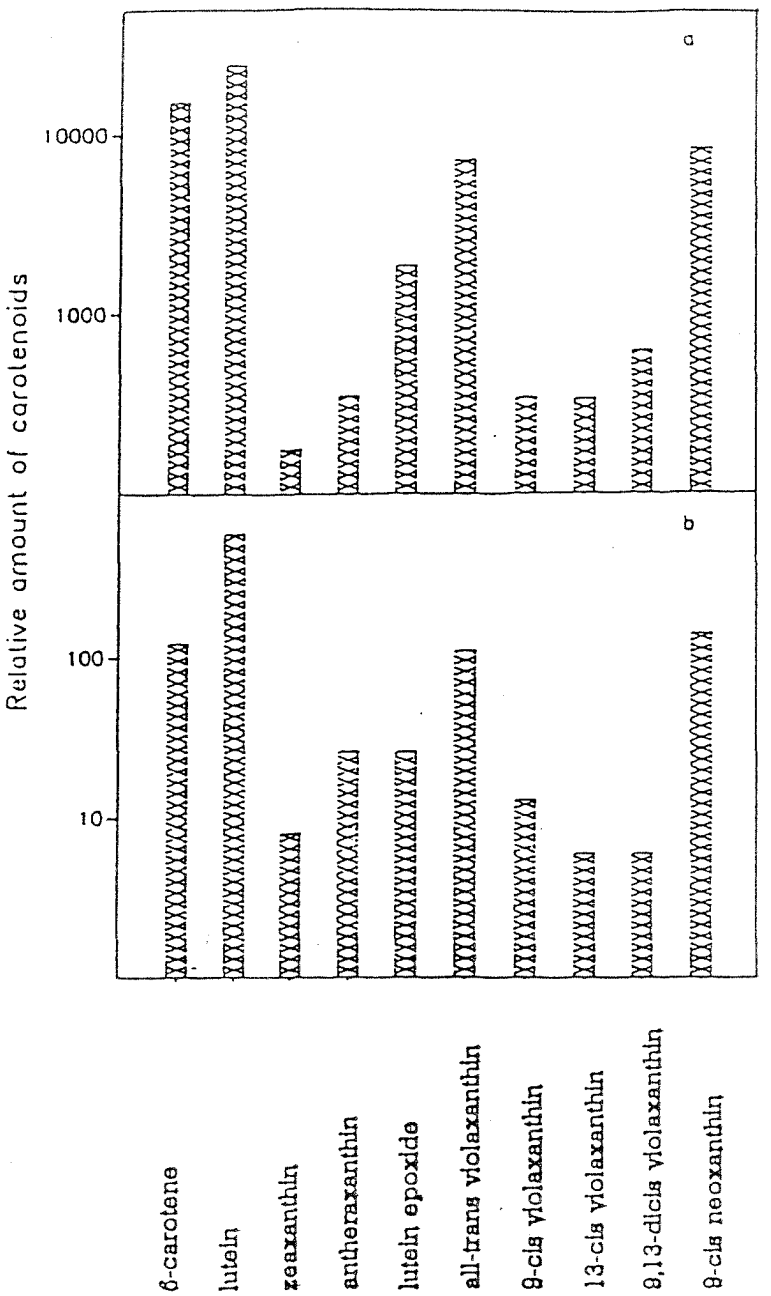

Fig. 1. – The relative amounts of 10 tested carotenoids in light grown (WT-Col)-(a) and pale-(b) seedlings

Tab. 2. – The quantity of individual carotenoids in etiolated (-RL) and red light-irradiated (+RL) seedlings of WT-Col. Values in the table are means ± 1 SE; n=2.

	- RL (mg · gFW^{-1})	+ RL
β-carotene	0.076 ± 0.011	0.097 ± 0.046
lutein	1.367 ± 0.008	2.014 ± 0.062
antheraxanthin	0.144 ± 0.008	0.205 ± 0.026
all-trans violaxanthin	1.707 ± 0.045	2.590 ± 0.047
9-cis violaxanthin	0.072 ± 0.013	0.106 ± 0.012
9-cis neoxanthin	0.287 ± 0.018	0.412 ± 0.002

Fluence-response relationships

The fluence-response relationships for induction of first positive phototropism for the etiolated seedlings from Am 45-3 population with the normally pigmented phenotype and the WT-Col seedlings were similar (Fig. 2. a and b). Fluence requirements for initiation of phototropism as well as for maximum response and descending arm of the curve corresponded very well in these two batches of seedlings (Fig. 2. and b). Based on these data it was decided to further compare responses of pale seedlings to BL and responses of the normally pigmented seedlings to BL assuming that the normally pigmented seedlings have wild type ability to perceive BL. The fluence-response relationship for first positive phototropism to a BL pulse was also measured for pale seedlings (Fig. 2. c). Response of pale seedlings was lower than that of both WT-Col and normally pigmented mutant seedlings (Fig. 2. a, b and c).

Irradiation of seedlings with the RL for 1 hour resulted in higher amplitude of response to a subsequent BL pulse (Fig. 3). This RL-induced enhancement of phototropism was used in all following experiments as the higher curvature was easier to score. The fluence-response curve describing first and second positive phototropism to the BL of the RL pre-irradiated seedlings was measured by varying the duration of pulses at constant fluence rate (Fig. 3). For the normally pigmented mutant seedlings threshold for the first positive response was about 0.01 μmol·m^{-2} and for second positive the threshold was about 100 μmol·m^{-2} (Fig. 3.a). The region of the maximum for the first positive response shows two distinctive peaks followed by the descending arm (Fig. 3. a). The pale seedlings exibited similar response to the unilateral BL pulses after the RL pre-iradiation (Fig. 3.b). The fluence requirements for initiation of the first and second positive responses as well as the fine structure for first positive phototropism corresponded well to those of the normally pigmented seedlings (Fig. 3.a). The major difference between the responses of these two batches of seedlings was the amplitude, with pale seedlings exhibiting a lower response than that of the normally pigmented seedlings (Fig. 3).

The effect of a desensitizing irradiation pulse was examined for both phenotypes from Am 45-3 population (Fig. 4. a and b). Seedlings were first irradiated for 1 hour with RL, and then were given a BL pulse from above followed within 2.5 min by a unilateral BL pulse of 0.3 μmol · m^{-2} to induce phototropism. By varying the desensitizing BL from above a fluence-response relationship for desensitization was measured. The fluence-response curves for desensitization in both phenotypes are similar (Fig. 4). As the fluence of BL administered from above increases, the response to the

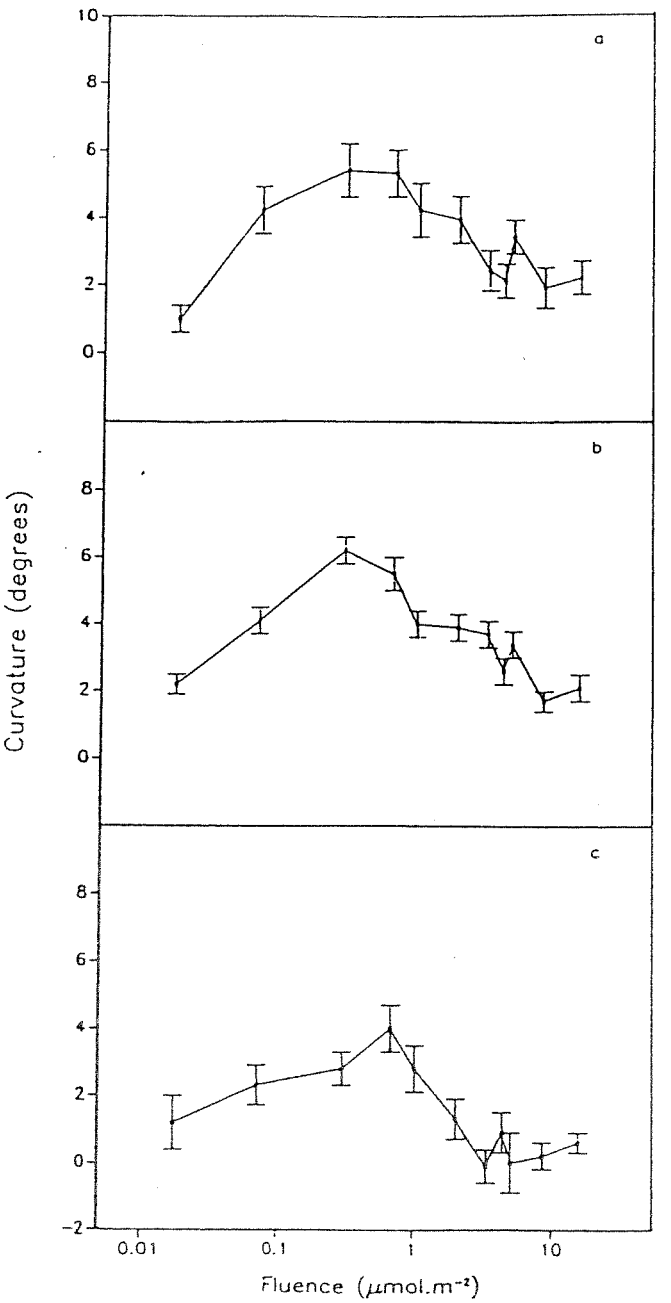

Fig. 2. - Fluence-response relationship for induction of first positive phototropism of etiolated: WT-Col-(a), normally pigmented-(b) and pale-(c) seedlings

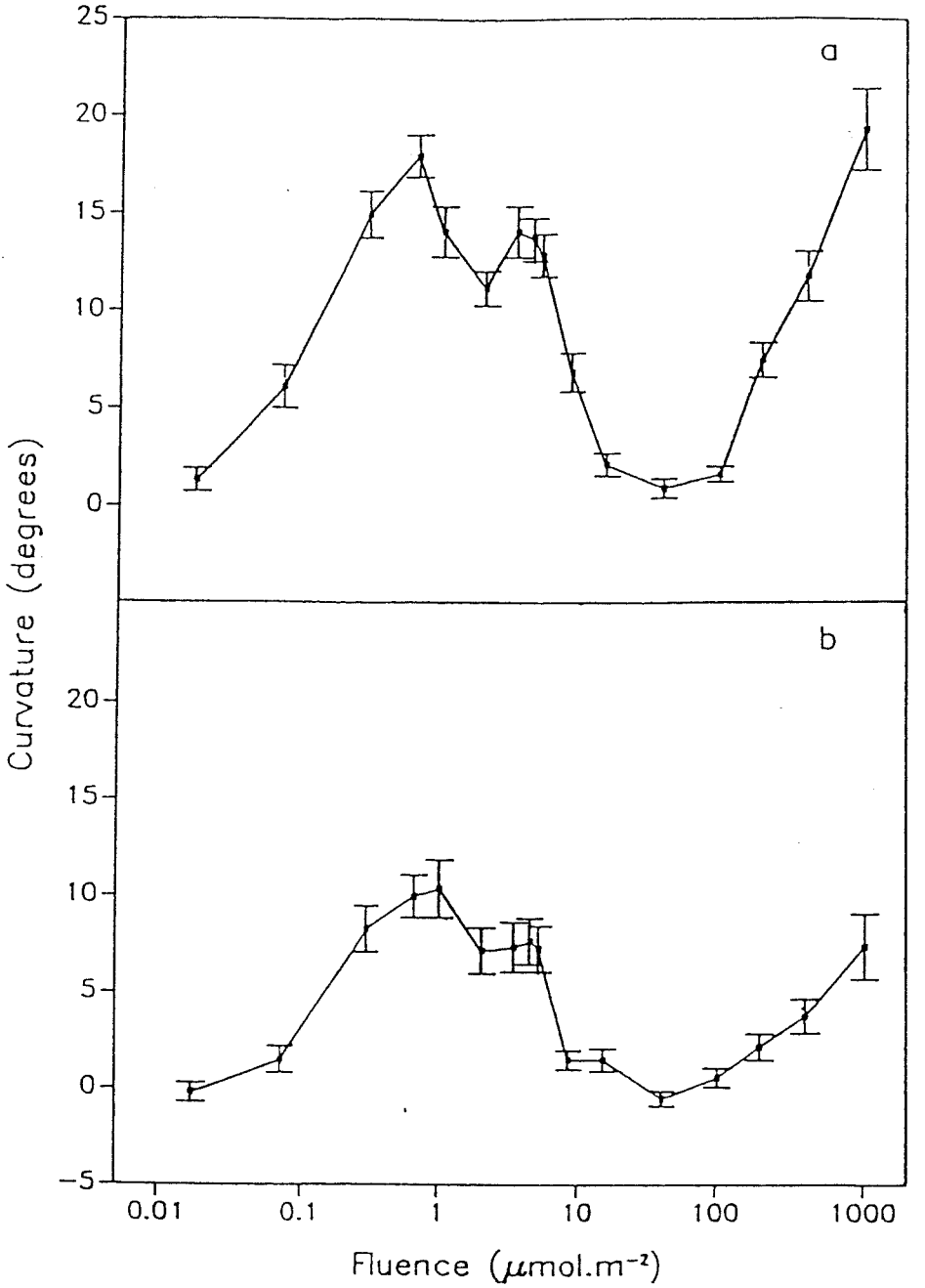

Fig. 3. – Fluence-response relationship for induction of phototropism of RL pre-irradiated: normally pigmented-(a) and pale-(b) seedlings; n>60; vertical bars represent ± 1 SE

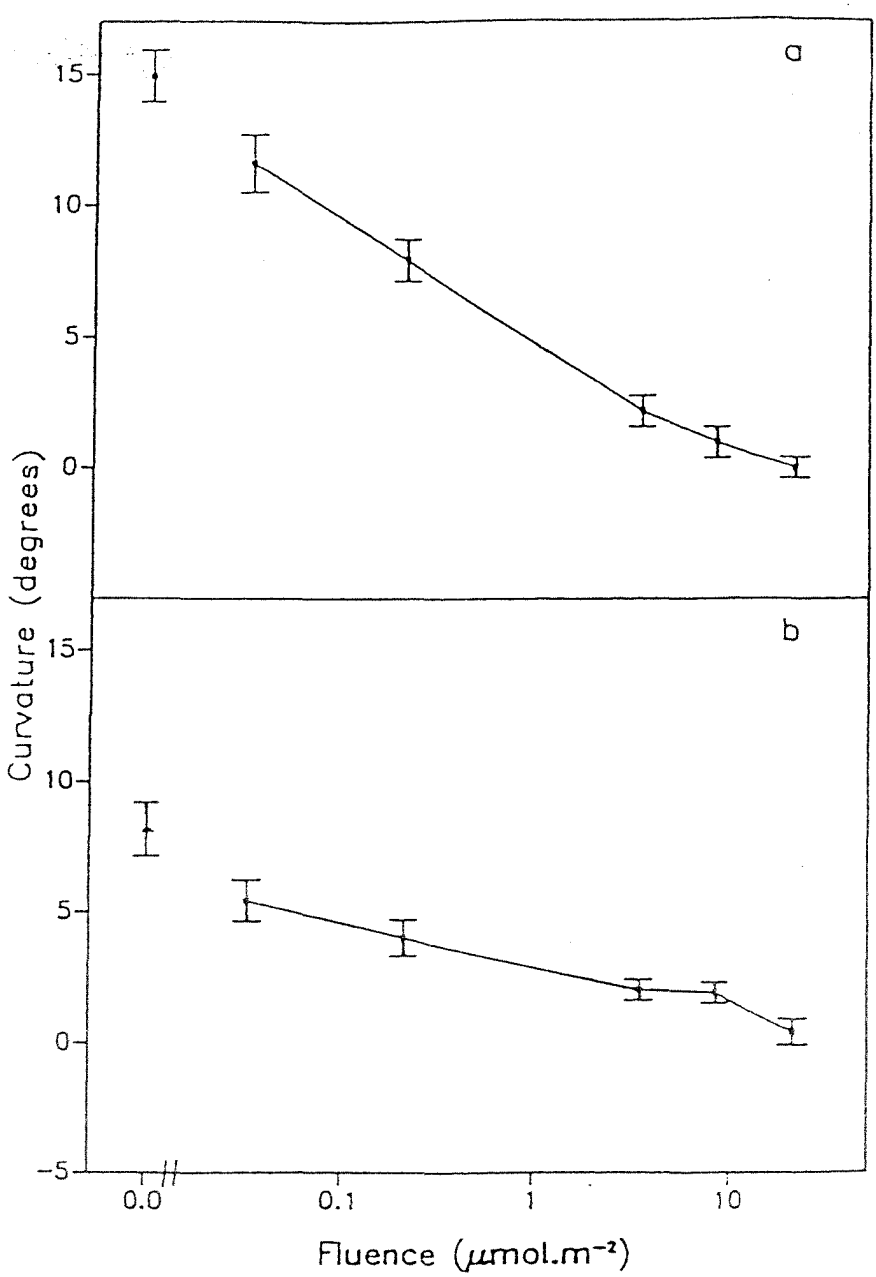

Fig. 4. – Fluence-response relationship for induction of phototropism following the BL pulse from above: normally pigmented-(a) and pale-(b) seedlings; n>60; vertical bars represent ± 1 SE

subsequent unilateral pulse decreases. Even the lowest fluence of BL applied from above (0.033 μmol · m^{-2}), in both batches of seedlings induced desensitization. The highest fluence of BL applied from above (21 μmol · m^{-2}) completely desensitized seedlings to the subsequent unilateral BL pulse (Fig. 4). Pale seedlings in this type of experiment again exhibited a lower amplitude of response than the normally pigmented seedlings (Fig. 4).

Gravitropism measurements

The ability of hypocotyls of pale seedlings to exhibit curvature was tested by exposing them to the constant 90 degrees gravity stimulation for 2 hours. Their response was not different from the response exerted by normally pigmented seedling (Table 3) to the same stimulus, suggesting that the mechanism of differential growth in pale seedlings was not impaired.

Tab. 3. – Gravitropic response to constant 90 degrees stimulation for 2 hours of two mutant phenotypes both RL pre-irradiated. Values in the table are means ± 1 SE; n>60

	degrees of curvature
normally pigmented phenotype	12.8 ± 0.8
pale phenotype	12.6 ± 1.2

DISCUSSION

The results of this study indicate that carotenoids do not play a major role in BL-induced phototropism and desensitization in *A. thaliana*. Although the level of 10 carotenoids in pale mutant seedlings was 35-40 times lower than in WT-Col plants they retained a WT sensitivity to unilateral BL and their phototropic response was only about two times lower in amplitude (Figs. 1. and 3).

The fluence requirements for the initiation of first and second positive phototropic response as well as for induction of two peaks and descending arm in the range of first positive phototropism were similar in pale seedlings and normally pigmented seedlings (Fig. 3. a and b). If any of the tested carotenoids were responsible for perception of unilateral BL that results in curvature, then some (or all) of the features of the fluence-response curve would be expected to move towards higher fluences according to the decrease in the amount of pigment(s).

Although carotenoids probably are not the photoreceptor pigments for phototropism in *Arabidopsis* they do affect the amplitude of the phototropic response (Figs. 1, 3 and 4). In order to detect the direction of light stimulation and respond phototropically, the seedling must detect the difference in absorption of light on its lighted and shaded side. If carotenoids were a screening agent across the seedling then their absence or decreased presence would result in lack of, or decrease in the light gradient. Results describing lower response to unilateral BL of the pale mutant seedlings when compared to the response of the normally pigmented seedlings (Fig. 3) are consistent with such a role of carotenoids.

The data describing the diference in levels of carotenoids in etiolated and RL-irradiated seedlings of WT-Col seedlings also support the theory that carotenoids may play a role as screening agents. One of the possible ways of enhancing the

phototropism could be by increasing the levels of carotenoids in the tissue, resulting in the steeper light gradient across the seedling. Higher levels of carotenoids are detected in the RL pre-irradiated WT-Col seedlings than in the etiolated WT-Col seedlings (Table 2). These results can be correlated with larger phototropic response of RL pre-irradiated normally pigmented mutant seedlings when compared to the etiolated WT-Col seedlings (Figs. 2. a and 3.a). Although these two strains differ genetically this difference has not affected their first positive phototropic response (Fig. 2. a and b). Moreover, seedlings of the Estland ecotype showed the same difference in magnitude of phototropism with and without RL pre-irradiation (J a n o u d i and P o f f, 1991). Enhancement of phototropism by RL may be due to the increased carotenoid content of the tissue (Figs. 2. a and 3. a).

However, there is a discrepancy between ratios of carotenoid levels in two different phenotypes and amplitudes of their responses to unilateral BL pulses. The explanation for this disagreement in the case of decrease of carotenoid content may be that carotenoids are not the only compounds that absorb the light from the blue part of the spectrum. When levels of carotenoids are very low, backscatter of light or the other molecular species such as pterins might prevent the disappearance of light gradient across the seedling which would result in complete absence of the response. Another explanation could be that the ratio of carotenoids in light grown pale vs. normally pigmented plants is not a good indicator of the ratio in etiolated tissue. Therefore the light gradient across the seedling established due to a presence of some molecular species that absorbs incident light although required, will not necessarily determine the magnitude of phototropism.

Pale mutant seedlings also retained the ability to undergo desensitization. Adaptation in phototropism of *Arabidopsis* seedlings includes: desensitization, refractory period, recovery and enhancement (J a n o u d i and P o f f, 1991). The complex shape of fluence-response curve for phototropism has been proposed to be due to adaptation (P o f f et al., 1994). Fluence requirements for desensitization overlap with fluences that induce enhancement. Thus plant is responding to the BL as a stimulus that induces and enhances phototropism and simultaneously causes desensitization. Therefore, response of a plant to a phototropic stimulation is a function of all components in both induction and adaptation of phototropism. If carotenoids were responsible for the perception of light that induces desensitization, pale seedlings would exhibit threshold for desensitization changed corresponding to the changed amount of carotenoids. Since similar fluence requirements for desensitization were exhibited by the pale and normally pigmented seedlings (Fig. 4) suggesting that carotenoids probably are not responsible for mediation of this process.

The quantities of individual carotenoids as a percentage of total amount of carotenoids have not changed due to 1 hour of RL (Table 1). These results indicate that the possible action of carotenoids in enhancement of phototropism is not mediated by single molecular species which would require the increased amount of that pigment.

Some carotenoids are known to be synthesized in plant organs in the absence of light (B r i t t o n, 1988). However, light is known to induce a large increase in carotenogenesis (B r i t t o n, 1988). For example, Oelmuller and Mohr have reported an increase in β-carotene in milo seedlings grown for 72 hours under RL as opposed to the plants grown in darkness (O e l m u l l e r and M o h r, 1985). We have measured the difference in carotenoid content between pale seedlings and the WT-Col seedlings in light grown tissue and related this difference to the magnitude of phototropism of etiolated seedling. The assumption is that the inductive effect of white light on

carotenogenesis is equal in both tested phenotypes. This is supported by the fact that the pale seedlings retain their sensitivity to BL (Fig. 3) and responsiveness to RL (Figs. 2. c and 3. b).

In summary, we report that seedlings with the pale phenotype from the population of mutant Am 45-3 have 35-40 times lower level of 10 tested carotenoids and exhibit two times lower phototropism than the normally pigmented seedlings from the same population. Pale seedlings retained their abiltiy to: undergo desensitization by BL, exhibit RL-mediated enhancement of phototropism and respond gravitropically in the WT fashion. Red-light-mediated enhancement of phototropism may be in part due to the increase of carotenoid content in the tissue. On the basis of these data it is concluded that carotenoids are not photoreceptor pigments for phototropism or desensitization in *A. thaliana* although they appear to affect the amplitude of phototropic response.

REFERENCES

Britton, G. (1988): Biosynthesis of carotenoids. *In* Plant Pigments (T.W. Goodwin, ed). pp. 133-182. – Academic Press, London.

Curry, G.M. (1969): Phototropism. *In* The physiology of plant growth and development (M. B. Willkins, ed.) pp. 245-273. – McGraw-Hill, New York.

Galland, P. & Lipson, E. D. (1987): Blue-light reception in *Phyconyces* phototropism: Evidence for two photosystems operating in low- and high-intensity ranges. – Proceedings of National Academy of Sciences USA *84*: 104-108.

Galland, P. (1991): Photosensory adaptation in aneuaral orgnaisms. – Photochemistry and Photobiology *54*: 1119-1134.

Iino, M. (1988): Desensitization by red and blue light in phototropism in maize celeoptiles. – Planta *176*: 183-188.

Janoudi, A. & Poff, K. L. (1991): Characterization of adaptation in phototropism of *Arabidopsis thaliana*. – Plant Physiology *95*: 517-521.

Kaufman, L. S. (1993): Transduction of blue-light signals. – Plant Physiology *102*: 333-337.

Khurana, J. P., Best, T. R. & Poff, K. L. (1989): Influence of hook position on phototropic and gravitropic curvature by etiolated hypocotyls of *Arabidopsis thaliana*. – Plant Physiology *90*: 376-379.

Konjević, R., Steinitz, B. & Poff, K. L. (1989): Dependence of the phototropic response of *Arabidopsis thaliana* on fluence rate and wavelength. – Proceedings of National Academy of Sciences USA *86*: 9876-9880.

Oelmuller, R. & Mohr, H. (1985): Carotenoid composition in milo (*Sorghum vulgare*) shoots as affected by phytochrome and chlorophyll. – Planta *164*: 390-395.

Piening, C. J. & Poff, K. L. (1988): Mechanism of detecting light direction in first positive phototropism in *Zea mays* L. – Plant, Cell and Environment *11*: 143-146.

Poff, K. L., Janoudi, A., Rosen, E. S., Orbović, V., Konjević, R., Fortin, M. C. & Scott, T. K. (1994): The physiology of tropisms. *In Arabidopsis* (E. Meyerowitz and C. R. Somerville, eds.). – Cold Spring Harbor Laboratory Press, Cold Spring Harbor-New York.

Presti, D., Hsu, W. J. & Delbruck, M. (1977): Phototropism in *Phycomyces* mutants lacking b-carotene. – Photochemistry and Photobiology *26*: 403-405.

Rock, C.D. (1991): Ph. D. Thesis. Michigan State University, East Lansing.

Song, P. S. (1984): Photophysical aspects of blue light receptors: The old question (Flavins versus Carotenoids) re-examined. *In* Blue light effects in biological systems. (H. Senger, ed.). – Springer, Berlin.

Vierstra, R. D. & Poff, K. L. (1981): Role of carotenoids in the phototropic response of corn seedlings. – Plant Physiology *68*: 798-801.

Rezime

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ULOGA KAROTENOIDA U FOTOTROPIZMU KLIJANACA *ARABIDOPSIS THALIANA*

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Mutant *Arabidopsis thaliana* (Am 45-3) koji je deficijentan u karotenoidima je korišćen za ispitivanje uloge karotenoida u fototropizmu i adaptaciji. Klijanci mutanta su bledi i sadrže oko 2.5-3% količine karotenoida koji su prisutni u divljem tipu koji je gajen na belom svetlu. Fototropski odgovor bledih klijanaca na plavu svetlost je bio sličan odgovoru divljeg tipa sem što je amplituda odgovora bila manja. Bledi klijanci zadržali su svoju sposobnost desenzitizacije plavom svetlošću kao elemenat adaptacije. Ovi klijanci su takođe pokazali povećanje fototropskog odgovora indukovano crvenim svetlom. Povećanje fototropizma je izgleda povezano sa povećanjem količine karotenoida. Naši rezultati su u saglasnosti sa zaključkom da karotenoidi nisu fotoreceptorni pigmenti za fototropizam ili desenzitizaciju iako prisustvo karotenoida utiče na amplitudu fototropizma i mehanizam njegovog povećanja kod *A. thaliana*.

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Original scientific paper

RADMILA STIKIĆ, SOFIJA PEKIĆ, ZORICA JOVANOVIĆ, LORA LJUBOJEVIĆ, LJILJANA PROKIĆ

GENOTYPIC DIFFERENCES IN THE RESPONSE OF MAIZE TO EXOGENOUS ABSCISIC ACID

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Stikić, R., Pekić, S., Jovanović, Z., Ljubojević, L., Prokić, Lj. (1995): *Genotypic differences in the response of maize to exogenous abscisic acid.* – Glasnik Instituta za botaniku i botaničke bašte Univerziteta u Beogradu, Tom XXIX, 65 - 76.

Effect of ABA on root and shoot growth and stomatal conductance was investigated in maize lines, selected for ABA content, using two different systems. In the first experiment seedlings were grown in Petri dishes with ABA added in substrate (1 and 10 mmol m^{-3}). Results from this experiment showed that exogenously applied ABA inhibited growth of both coleoptile and root but depending on concentration and genotype. The biggest effect of ABA treatment on root to shoot ratio was evidenced in high-ABA parent line Polj-17 being 1.6 fold bigger than in low-ABA parent F-2. Those differences between progeny lines 167 B1 (high-ABA) and 83 A5 (low-ABA) were less expressed. In the second experiment solution of synthetic (±) ABA was fed to part of the root in aim to manipulate xylem and leaf ABA content without changes in plant water relations. These results showed that the increase in endogenous ABA content (xylem and leaf) reduced leaf elongation rate (LER) and stomatal conductance in all investigated lines. These results also showed existence of genotype differences in sensitivity to ABA and proved that the most sensitive reaction to ABA had high-ABA parental line Polj-17 in both experimental systems.

Key words: Maize *(Zea mays* L.), abscisic acid (ABA), genetic variation, different sensitivity to ABA

Ključne reči: Kukuruz *(Zea mays* L.), abscisinska kiselina (ABA), genetsko variranje, različita osetljvost na ABA

INTRODUCTION

Many results indicate that plant growth is the most sensitive plant process to water stress, especially in the initial phases when water deficit induces drop in turgor pressure demonstrating that leaf growth could be reduced without any changes in the leaf water status (Michelena & Boyer, 1982; Passioura, 1988; Saab & Sharp, 1989; Gowing et al., 1990). It become evident that changes in root environment modify, not only amount of water moving from root to shoot (hydraulic signal), but also the production of some chemicals (non hydraulic signal). Research of the nature of this chemical proved that development of water deficit sensed by root system induces production of abscisic acid (ABA) as a signal molecule (Davies et al., 1987; Zhang, Shurr & Davies, 1987). Moving through transpiration stream into leaves, ABA markedly influences the growth and development of shoot (Trewavas & Jones, 1991). Recent studies have provided evidence that the effect of exogenously applied ABA induces leaf growth inhibition (Zhang & Davies, 1990 a, b).

It is well know that different kinds of stresses (drought, low and high temperature, salt, nutrients and waterlogging) increase ABA content in the leaves. Increasing ABA content leads to many changes in plant physiology, which in general, make the plant better adapted to environmental stresses (Quarrie, 1991). Selection on the basis of ABA accumulation capacity was done with a spring wheat genotypes. Field trials with plants from this selection program showed that high- ABA genotypes significantly outyilded low-ABA genotypes in a water limited conditions (Innes, Blackwell & Quarrie, 1984). A similar selection programe has been carried out with maize to produce recombinant inbred lines having significantly different leaf ABA content under field conditions (Pekić & Quarrie, 1988). For the parental lines in this selection programe (Polj 17 and F-2) it has been previously shown to differ consistently in the responses of a range of traits to drought stress under both cabinet and field conditions (Pekić & Quarrie, 1987; Quarrie, 1991). These response include ABA content and yield: Polj 17 contents more ABA and is more drought resistant than F-2. Recent measurement done with progeny plants didn't prove such marked differences in the field conditions, except at flowering time (Pekić et al., 1995). Therefore, it is still missing enough information to give reliable picture about physiological consequences of genetic variation in ABA content. However, investigation of this problem is in a progress and preliminary results indicate that there are differences in rooting behaviour amongst genotypes. Therefore to elicit response of those plants comparable to that caused by root sourced ABA in drying soil, we repeated Zhang's and Davies's (1990a) ABA feeding maize roots experiment. The aim of this experiment was to determine whether genetic variation in leaf ABA content was reflected in growth and stomatal responses to externally applied ABA.

Since our previous experiments (Stikić *et al.,* 1991) based on techniques developed by Sharp et al., (1988) revealed differencesinin root and shoot growth rates at low water potential between parental lines, we, also, wished to test for genetic variation of growth responses to ABA at the seedlings stage in the progeny.

MATERIALS AND METHODS

Genotypes

The genotypes of maize (*Zea mays* L.) used for this work consisted of the inbred lines Polj 17 (high-ABA) and F-2 (low-ABA) and progeny from the cross between Polj 17 and F-2: high-ABA line 167 B_1 and low-ABA 83 A_5. The two inbred parental lines had previously been shown to differ by up to four-fold in leaf ABA content under field conditions (Pekić & Quarrie, 1988). The recombinant lines were classified into high-ABA and low-ABA lines according to leaf ABA content in the F4 generation. The difference in leaf ABA content means between these two populations and, also, between high and low ABA lines used in this work is about four times (Pekić et al., 1995).

Experiment with young seedlings

Seeds were germinated in wet vermiculite in dark at 25°C. Ten seedlings of each genotype with radicles 3 cm long were placed into plastic Petri dishes (diameter 12 cm) with vermiculite saturated with water (control) and different (±) ABA solutions (1 and 10 mmolm^{-3}). Petri dishes were sealed with plastic sheets, placed vertically into dark at 25°C for 28^h. Coleoptile and root length was quickly recorded at dim green light by marking the lid of the Petri dish.

ABA-feeding experiment with plants in 3 leaf stage

Seeds of investigated lines were germinated in John Innes No 2 compost in a greenhouse, with temperature varying between 22-28°C (day) and 12-15°C (night). At the one leaf-stage were selected for uniformity and transferred in the growth cabinet, where they remained under the constant conditions (day and night temperatures 25 and 18°C, PAR 200 mmol m^{-2}s^{-1} and photoperiod 16^h). In these conditions plant were grown until three-leaf stage, and than transferred (with attached soil) into 80 mm diameter pots from which the bottom were removed and replaced with a piece of plastic mesh (5 mm diameter holes). Pots were inserted into plastic beakers which were blackened and contained 100 cm^3 Hogland's nutrient solution. Plants remained in these conditions (approximately 10 days) until substantial amount of roots (about 20% of the whole mass) was established outside the pot and dangling in the nutrient solution.

ABA-feeding was done with a following ABA concentrations: 10, 50 and 100 mmol m^{-3} (synthetic (±) ABA, Lancaster Synthesis, Morecambe, UK) previously shown to affect growth and stomatal conductance in maize leaves (Zhang & Davies, 1990a). Starting 3 days before and continuing 2 days after ABA-feeding, length of two elongating leaves on each intact plant was measured (by ruler) every 24^h. At the same time (10^h) measurements of conductance of the abaxial leaf surface were done with a porometer (AP-4 Delta T Devices LTD). Xylem exudates and leaf ABA samples were collected after 48^h of ABA-feeding from the youngest mature leaves. Xylem exudate was collected after shoots were detopped as have been described by Zhang and Davies (1990a). After freeze drying ABA content was measured by RIA test (Quarrie et al., 1988). For each treatment 4-5 plants per genotype were used. Growth rate and conductance were calculated as a means of two leaves per plant. Because of the variation in the leaf elongation rate (LER) and stomatal conductance (gs) among genotypes these results are presented as percentages of control values. These percentages were calculated differently for LER and stomatal conductance. Leaf

elongation rate is presented as percentage of the elongation rate before treatments were given. Control values used in these calculations were the average rates of leaf growth during whole experimental period, while control values for stomatal conductance were from measurements done last day of experiment (48^h after ABA feeding).

RESULTS

Experiment with young seedlings

Tab. 1 illustrates the effect of two treatments on coleoptile shoot and root growth in four genotypes based on calculation of the growth rate in percentage of the growth rate in control plants and root to shoot length ratio. Results show that in all genotypes: a) ABA inhibited growth of both organs, b) effect of both concentrations was more expressed on coleoptile than on root growth (especially in parental line Polj 17), and c) effect of the higher ABA concentration was more expressed and, consenquently, induced increase in root to shoot ratio. The biggest effect of ABA treatment on root to shoot ratio was evidented in high-ABA parent (the increase of 1.2 and 1.6 fold for two ABA treatments in comparison to control). Genotipic differences in growth responses to ABA were more expressed between high and low-ABA parents, comparing to high and low-ABA lines. Thus root/shoot ratios at lower and higher ABA concentration were 0.7 and 1.6 times bigger in line Polj 17 comparing to F-2.

Tab. 1. – Effects of ABA on young seedlings shoot and root growth rate (in % of controls) and root to shoot ratio (R/S) based upon seedlings lengths in investigated maize lines

	ABA ($mmol/m^3$)	Polj-17	F-2	$167B_1$	$83A_5$
Shoot	0	100 ± 16	100 ± 14	100 ± 19	100 ± 14
	1	78 ± 18	96 ± 8	89 ± 16	83 ± 11
	10	52 ± 14	80 ± 11	79 ± 16	81 ± 10
Root	0	-100 ± 8	-100 ± 13	- 100 ± 9	- 100 ± 14
	1	- 92 ± 18	- 101 ± 20	- 90 ± 19	- 88 ± 12
	10	- 82 ± 9	- 92 ± 18	- 86 ± 15	- 78 ± 11
R/S	0	1.755	1.473	2.514	1.889
	1	2.058	1.385	2.198	2.029
	10	2.755	1.692	2.617	1.824

Experiment with ABA-fed root

The effect of ABA-feeding on leaf elongation rate (LER), stomatal conductance and xylem and leaf ABA content are presented on tab. 2. Values of LER were expressed as percentages of the rate before treatments because measurements of this parameter showed that there were marked differences in growth habit between investigated lines. Thus, growth rate in controlled condition varied in a range of 1.28 (line F-2) to 2.2 cm per 24^h (line $167\ B_1$). Similarly variation of stomatal conductance values under control conditions was between 58.7 (line $167\ B_1$) and 73.6 mmol $m^{-2}s^{-1}$ (line 83

A_5). Expressing those results in relative, instead absolute values, allowed to distinguish the effect of applied ABA and genotipic differences between investigated lines.

Tab. 2. – Effects of ABA feeding on leaf elongation rate (LER), stomatal conductance (Gs), leaf ABA content and xylem ABA content in investigated maize lines

	ABA (mmol/m3)	Polj-17	F-2	167 B_1	83A_5
LER (% of control)	0	100 ± 11	100 ± 6	100 ± 6	100 ± 4
	10	70 ± 8	72 ± 6	71 ± 9	69 ± 9
	50	55 ± 6	62 ± 12	57 ± 11	61 ± 7
	100	49 ± 5	43 ± 5	52 ± 9	61 ± 5
Gs (% of control)	0	100 ± 12	100 ± 12	100 ± 8	100 ± 4
	10	61 ± 8	48 ± 4	55 ± 11	71 ± 11
	50	55 ± 7	47 ± 7	52 ± 8	48 ± 12
	100	43 ± 4	36 ± 5	51 ± 7	35 ± 5
Leaf ABA (nmol/gdw)	0	1.55 ± 0.3	1.29 ± 0.2	1.21 ± 0.1	1.46 ± 0.3
	10	1.89 ± 0.1	1.93 ± 0.4	3.91 ± 0.7	3.23 ± 0.6
	50	2.27 ± 0.5	2.21 ± 0.1	4.19 ± 0.2	4.33 ± 0.4
	100	6.82 ± 0.7	3.33 ± 0.4	6.80 ± 1.0	5.78 ± 0.4
Xylem ABA ($\mu mol/m^3$)	0	9.30 ± 0.7	11.39 ± 2.0	5.96 ± 0.9	4.90 ± 0.7
	10	14.92 ± 3.0	26.70 ± 5.0	98.20 ± 23.0	25.40 ± 3.0
	50	25.10 ± 4.0	37.60 ± 6.0	116.23 ± 25.0	50.84 ± 4.0
	100	131.66 ± 9.0	137.30 ± 23.0	140.40 ± 20.0	161.07 ± 26.0

The ABA treatment caused decrease in a leaf growth and stomatal conductance in all investigated lines and this effect was proportional to ABA concentration in feeding solution. For example, 100 mmol m^{-3} ABA induced LER and stomatal reduction varying in different genotypes between 43-61% and 35-51% respectively.

Xylem and leaf ABA content increased with increasing concentration of applied ABA indicating that those changes were sufficient to account for observed changes in LER and gs. Maximal values of xylem ABA estimated in 100 mmol m^{-3} ABA solution were ranging from 6 to 140 mmol m^{-3} (line 167 B_1) and from 49-161 mmol m^{-3} (line 83 A_5). Variation between investigated lines was also expressed on the leaf ABA content since these values varying between 1.3 to 3.3 nmol g DW^{-1} (line F-2) and from 1.2 to 6.8 nmol g DW^{-1} (line 167 B_1). Comparing to xylem ABA increase (mean increase 20 fold) those changes were less expressed in all investigated lines (mean increase 4 fold).

Relationship between LER versus the xylem and leaf ABA content is presented on Fig. 1. (A and B). From these figures it can be seen that LER declined as xylem and leaf ABA concentration increased. However, points corresponding to different genotypes indicated different growth response to ABA. In the leaves of the parental lines Polj 17 and F-2 the response of LER to both xylem and leaf ABA had a biphasic character. Initial increase (up to cca 30 mmol m^{-3}) of xylem ABA was followed by a fast reduction of LER until a plateau of approximately 60% of LER was reached. When the values of stomatal conductance were plotted against xylem and leaf ABA content similar graph to LER was obtained (Fig. 2A and B). This figure also confirmed genotipic differences in stomatal sensitivity to both xylem and leaf ABA content. Data also showed that, not only LER but, also, stomata of high-ABA parental line had the most sensitive response to xylem ABA.

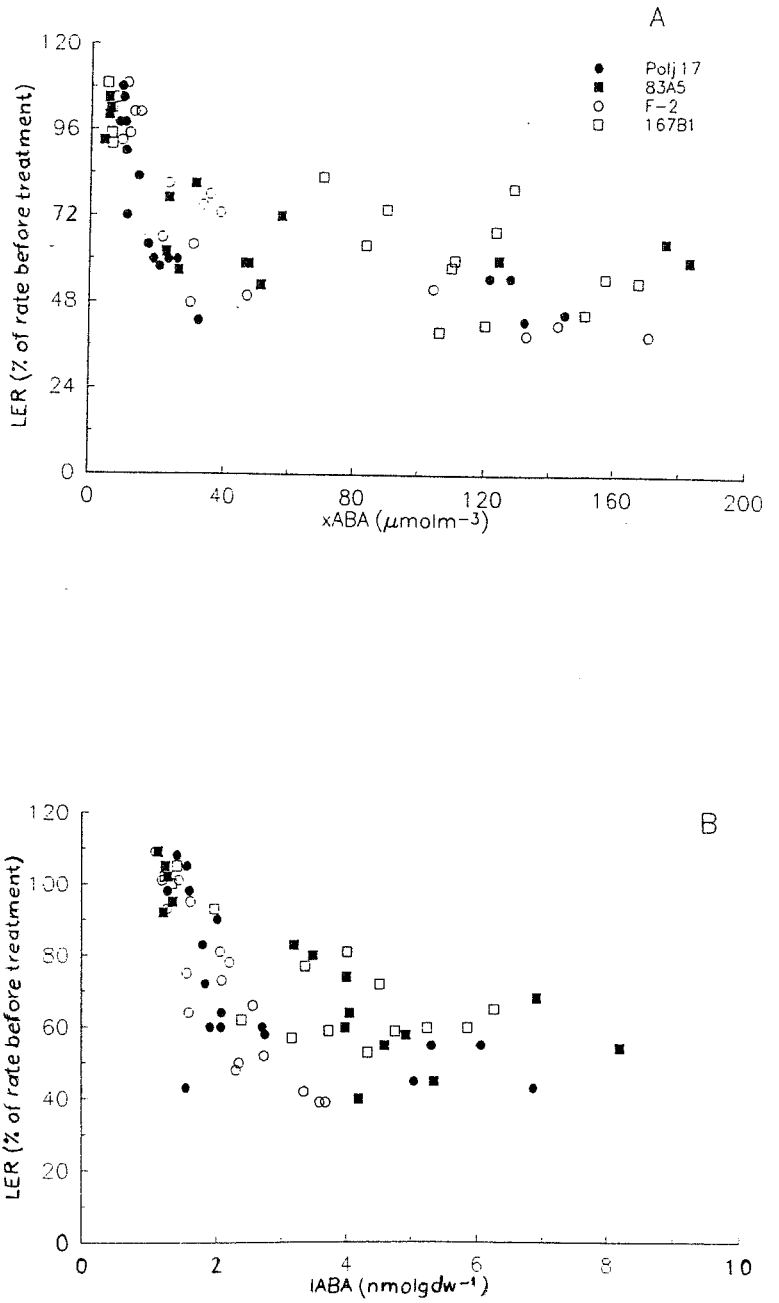

Fig. 1. – Relationship of leaf elongation rate (LER) to xylem (A) and leaf ABA content (B) in investigated maize lines Polj 17 (•), F-2 (O), 167 B1 (□), and 83A5 (■). Each point represents coupled values corresponding to one leaf

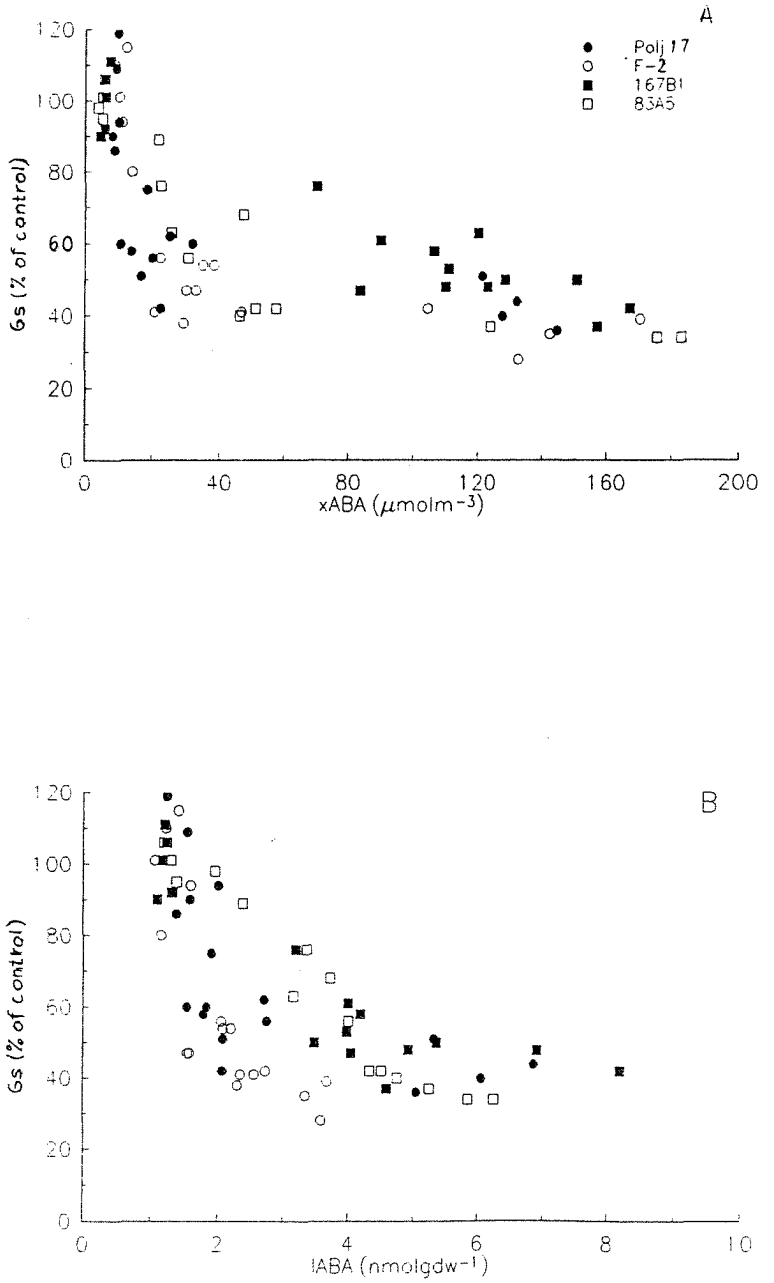

Fig. 2. – Relationship of stomatal conductance (Gs) to xylem (A) and leaf ABA content (B) in investigated maize lines Polj 17 (•), F-2 (O), 167B1 (□), and 83A5 (■). Each point represents coupled values corresponding to one leaf

Comparison of linear regression lines (Tab. 3) for this initial phase between 2 parental lines showed that slopes of LER vs xylem ABA was 2.531 in line Polj 17 and 1.261 for line F-2, while correlation coefficients were similar (0.895** and 0.802**). Differneces in slopes for LER vs leaf ABA between parental lines were less expressed (26.88 for Polj 17 and 31.25 for F-2), while correlation coefficient was more significant in line F-2 (0.80**) than in line Polj 17 (0.58*). The decline of LER vs xylem or leaf ABA in the leaves of progeny lines was more gradual comparing to parental lines and linear for the whole range of ABA values (correlation coefficient varying between 0.76** and 0.86**). Comparison of slopes for both regression (LER vs, xABA and LER vs 1ABA) showed that high-ABA line had smaller slope of LER vs xylem ABA (0.32) than low-ABA line (0.77) while there was no significant difference in the slopes of LER vs leaf ABA.

Tab. 3. – Correlation coefficients (r) and slopes (a) of linear regressions in maize lines

	Polj-17	F-2	$167B_1$	$83A_5$
LER vs xABA				
r	0.89**	0.80**	0.86**	0.80**
a	2.53	1.26	0.32	0.77
LER vs 1ABA				
r	0.58*	0.80**	0.79**	0.76**
a	26.88	31.25	8.25	8.36
Gs vs xABA				
r	0.64*	0.82**	0.94**	0.77**
a	2.02	1.87	0.39	0.35
Gs vs 1ABA				
r	0.59*	0.77**	0.85**	0.96**
a	30.64	43.85	10.09	15.79

*and **indicate the level of significance of correlation coefficient (at $P_{0.05}$ and $_{0.01}$ respectively)

DISCUSSION

The results presented in this paper confirmed that exogenous ABA treatment can modify growth both of the seedling and leaves and to change stomatal reaction in different maize lines. Our results for relative root and shoot growth response of young seedlings can be compared to data obtained in experiment with plants at similar stage of development grown at low water potentials (Stikić et al., 1991). Thus, mean elongation rate for roots grown in ABA solution was about 1.33 mm h^{-1} what is similar to root growth rate found by Stikić et al., (1991) at different water potentials: 1.2 mm h^{-1} at -0.0025 MPa and 0.6 mm h^{-1} at -0.52 MPa. From the results of Saab et al. (1990) differences in ABA content in the root and shoot may be expected to affect relative growth rates under growth conditions. Measurements of endogenous ABA content in roots and shoots of several maize lines (among them Polj 17 and F-2) in experiment done by Stikić et al. (1991) revealed genotipic differences in root and shoot growth responses to endogenous ABA. In only few genotypes under certain drought treatments, both root growth and shoot growth responded to ABA as sug-

gested by Saab et al. (1990) leading to the conclusion that genotypes may differ in the sensitivity to ABA. Genotipic variability in growth responses to exogenous ABA presented in this paper confirms that possibility. Thus, the most striking effect of ABA on line Polj 17, in comparison to F-2 and other lines can be attributed to its greatest sensitivity to ABA if we assume that endogenous ABA content do not differ from F-2 according to data from similar experiment (Stikić et al., 1991).

Experiments done in the field and laboratory conditions have proved that feeding of ABA to the part of an intact plant may be a convenient way to manipulate endogenous ABA content and monitor physiological consequences (Zhang & Davies, 1990a; Tardieu, Zhang & Davies, 1993). Experiments done by Zhang & Davies (1990a) provided evidence that increased ABA content in xylem sap of ABA-fed maize plants was root sourced and responsible for restriction of leaf growth and stomatal closure. Taking the results of these experiments as a reference for the results obtained from our ABA feeding we can confirm large genotipic differences in LER and gs response to applied ABA. For example 60% of gs reduction in Zhang and Davies's (1990a) experiment (done with John Innes F1 maize hybrid) was accompanied by an increase in xylem ABA content of aproximately 60 mmol m^{-3}. Data from our experiment (Fig. 2A) showed that for similar gs reduction xylem ABA concentration varyed between genotypes in a range of 11 to 90 mmol m^{-3}. Different stomatal sensitivity to xylem ABA have been proved in several studies and led to a model of stomatal behaviour in which the effect of xylem ABA is mediated by leaf water status (Tardieu & Davies, 1993). However, in the ABA-feeding experiments the xylem ABA mimics the root signal in drying soil and affects shoots independently from the effects of leaf water status. Thus, observed differences in stomatal sensitivity of our lines could not to be due to water potential differences. Wolf, Jeschke and Hartung (1990) studding long distance transport of ABA have shown that part of ABA could be exported via phloem to the roots and than reciculated to the aerial parts of the plants. However, investigation of effect of girdling on ABA export from the leaves of parental lines Polj 17 and F-2 their progeny didn't prove differences in phloem ABA transport (Pekić et al., 1995). Differences found by measurements of some leaf anatomical characteristics, such as xylem vessel area, between parental lines (Ristić & Cass, 1991) and their progeny (our unpublished data) inderectly indicate possible genotipic differences in hydraulic conductivity of transpiration stream. Differences in chemical composition of xylem sap (ion content and pH) can also alter sensitivity of stomata to ABA as have been proposed by Shurr and Golan (1990). Zhang and Davies (1990a) results indicated that leaf ABA showed a relatively insensitive response (compared to xylem ABA content) to applied ABA. Similarly to Zhang's and Davies's data (1990a) our results indicate that feeding plants with 100 mmol m^{-3} ABA solution increase ABA content in the leaves maximally up to 6 fold, and in the xylem sap up to 30 fold.

Results of Gowing, Jones and Davies (1993) indicated that conductance of cherry leaves fed by ABA in pulses was more influenced by the amount of ABA entering the leaf than by absolute xylem ABA concentration. This indicates that stomata perceive a local concentration or apoplastic ABA content which is in dynamic equilibrium between the rate of ABA arrived via xylem and rate of ABA removal by partitioning into cells as a consequence of pH gradients (Hartung & Slovik, 1991).

The inhibiting effect of applied ABA on the growth of different plant parts is still contraversal. Quarrie and Jones (1977) in a study of the effect of exogenous ABA

in wheat growth reported that reduction of a leaf size was a result of an inhibitory effect on both cell division and cell expansion. Results of Van Valkenburgh and Davies (1983) indicated that ABA reduced cell wall extensibility, possibly by inhibiting proton pumping throughout the plasmalemma into the apoplast (Chen & Kao, 1988). However, work of Munns and King (1988) refused that concept of ABA signal and proposed that, at least in wheat, drought induced increase in an another compound with antitranspirant activity. More recent results of Munns and Sharp (1993) suggested that ABA is responsible for only part of the regulation of leaf growth. They proposed that ABA moved in complexed form through the xylem (ABA-adduct) and that this form is physiologically ineffective and have to be metabolized in free form to affect some leaf processes.

In our ABA-feeding experiment the most sensitive growth and stomatal reaction to applied ABA was established for high-ABA parent Polj 17. For this line it has been previously shown to be resistant in many drought related traits comparing to other parental F-2 line (Pekić & Quarrie, 1978, 1988; Quarrie, 1991; Pekić et al., 1995). Root morphology also markedly differed between those lines both at the seedling (Stikić et al., 1991) and the maturity stage when the greater number of nodal roots produced by Polj 17 was associated with a significantly higher root pulling force than F-2 (Lebreton et al., 1995). Higher sensitivity of shoot growth and stomata to increased endogenous ABA may be an adaptive response, particularly in condition when mild stress induces synthesis of ABA as a root signal. Retardation of leaf area development and partial closure of stomata together with the increase of R/S ratio could be of some benefit in terms of water conservation. This „strategy" may allow Polj 17 to avoid or delay transition to more severe stress and to enable growth even at reduced rate. Such a hypothesis is supported by the field measurements of leaf area and plant height which indicate that effects of drought is less expressed in Polj 17 comparing to another lines (unpublished data). Since investigated lines differ markedly in leaf ABA content under field condition (Pekić et al., 1995), future work with ABA-fed plant under field conditions will provide further evidence on genotipic differences in sensitivity to ABA, their consequence for the overall drought response, and possible role of differential sensitivity to ABA as a regulatory mechanism of plant adaptation to water stress.

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REFERENCES

Chen, C.T. & Kao, C.H. (1988): Proton secretion in rice leaves. – Botanical Bulletin Academia Sinica *29*: 315-320.

Davies, W.J., Metcalfe, J.C., Shurr, U., Taylor, G. & Zhang, J. (1987): Hormones as chemical signals involved in root to shoot communication of effect of changes in the soil environment. *In* Hormone Actionin Plant Development a Critical Apraisal (M.B. Hoad, J.R. Jackson, J.R. Lenton & R. Atkin, eds.), pp. 201-216. – Butterworths, London.

Gowing, D.J.G., Jones, H.G. & Davies, W.J. (1990): A positive root-sourced signal as a indicator of soil drying in apple (malus x dome Borkh). – J. exp. Bot. *41*: 1535-1540.

Gowing, D.J., Jones, H.G. & Davies, W.J. (1993): Xylem transported abscisic acid. The relative importance of its mass and its concentration in the control of stomatal aperture. – Plant Cell Environ. *16*: 453-459.

Hartung, W. & Slovik, S. (1991): Physicochemical properties of plant growth regulators and plant tissues determine their distribution and redistribution: Stomatal regulation by abscisic acid in leaves. – New Phytol. *119*: 361-382.

Hsiao, T.C. (1973): Plant responses to water stress. – Ann. Rev. Plant Physiol. *24*: 519-570.

Innes, P., Blackwell, R.D. & Quarrie, S.A. (1984): Some effects of genetic variation in abscisic acid accumulation on the yield and water use of spring wheat. – J. Agri. Sci. (Camb) *102*: 341-351.

Michelina, V.A. & Boyer, J.S. (1982): Complete turgor maintenance at low water potentials in the elongating regions of maize leaves. – Plant Physiol. *69*: 1145-1149.

Munns, R. & King, R:W. (1988): Abscisic acid is not the only stomatal inhibitor in the transpiration stream. – Plant Physiol. *88*: 703-708.

Munns, R. & Sharp, R.E. (1993): Involment of abscisic acid in controlling plant growth in soil of low water potential. – Aust. J. Plant Physiol. *20*: 425-437.

Passioura, J.B. (1988): Root signals control leaf expansion in wheat seedlings growing in drying soil. – Aust. J. Plant Physiol. *15*: 687-693.

Pekić, S. & Quarrie, S.A. (1987): Abscisic acid accumulation in lines of maize differing in drought resistance: a comparison of intact and detached leaves. – J. Plant Physiol. *127*: 203-217.

Pekić, S. & Quarrie, S.A. (1988): Abscisic acid in leaves of inbred lines and F1 hybrids of maize growing in the field and its relationship to diurnal and seasonal trends in leaf water potential. – Ann. Bot. *61*: 669-678.

Pekić, S., Stikić, R., Tomljanović, L., Anđelković, V., Ivanović, M. & Quarrie, S.A. (1995): Characterization of maize lines differing in leaf abscisic acid content in the field. I Abscisic acid physiology. – Ann. Bot. *75*: 67-73.

Quarrie, S.A. (1991): Implication of genetic differences in ABA accumulation or crop production. *In* Abscisic acid Physiology and Biochemistry (W.J. Davies & H.G: Jones, eds.), pp. 227-243. – Bios Scientific Publishers, Oxford, UK.

Quarrie, S.A. & Jones, H.G. (1977): Effects of abscisic acid and water stress on development and morphology of wheat. – J. exp. Bot. *28*: 192-208.

Quarrie, S.A., Whitford, P.N., Appleford, N.E.J., Wang, T.L., Cook, S.K., Henson, I.E. & Loveys, B.R. (1988): A monoclonal antibody to (s)- abscisic acid: Its characterisation and use in radioimmuno assay for measuring abscisic acid in crude extraxts of cereal and lupin leaves. – Planta *173*: 30-339.

Ristić, Z. & Cass, D.D. (1991): Leaf anatomy of *Zea mays* L. in response to water shortage and high temperature: A Comparison of drought – resistance and drought-sensitive lines. – Bot. Gaz. *152*: 173-185.

Saab, I.N. & Sharp, R.E. (1989): Non-hydraulic signals from maize roots in drying soil: Inhibition of leaf elongation but not stomatal conductance. – Planta *179*: 466-474.

Saab, I.N., Sharp, R.E., Pritchard, J. & Voedberg, G.S. (1990): Increased endogenous abscisic acid maintains primary root growth and inhibits growth of maize seedlings at low water potential. – Plant Physiol. *93*: 1329-1336.

Schurr, U. & Gollan, T. (1990): Composition of xylem sap of plants experiencing root water stress a descriptive study. *In* Importance of root to shoot communication in the responses to environmental stress (W.J. Davies & B. Jeffcoat, eds.), pp. 201-214. – British Society for Plant Growth Regulation, Monograph 21, Bristol.

Sharp, R.E., Silk, W.K. & Hsiao, T.C. (1988): Growth of the maize primary root at low water potentials. I Spatial distribution of expensive growth. – Plant Physiol. *87*: 50-57.

Stikić, R., Tomljanović, L., Pekić, S. & Quarrie, S.A. (1991): ABA production and distribution during seedling drought stress of inbred maize lines differnig in leaf ABA content. – J. exp. Bot. Supp. *42*: 15.

Tardieu, F. & Davies, W.J. (1993): Integration of hydraulic and chemical signalling in the control of stomatal conductance and water status of droughted plants. – Plant Cell Environ. *16*: 341-349.

Tardieu, F., Zhang, J. & Gowing, D.J.G. (1993): Stomatal control by both (ABA) in the xylem sap and leaf water status: a test of a model for droughted or ABA fed field grown maize. – Plant Cell Environ. *16*: 413-420.

Trewavas, A.J. & Jones, H.G. (1991): An assessment of the role of ABA in plant development. *In* Abscisic acid Physiology and Biochemistry (W.J. Davies & H.G. Jones, Eds.), pp. 169-188. – Bios Scientific Publishers, Oxford, UK.

Van Volkenburg, E. & Davies, W.J. (1983): Inhibition of light stimulated leaf expansion by abscisic acid. – J. exp. Bot. *34*: 835-845.

Wolf, O., Jeschke, W.D. & Hartung, W. (1990): Long distance transport of abscisic acid in salt stressed *Lupinus albus* plants. – J. exp. Bot. *41*: 593-600.

Zhang, J., Shurr, U. & Davies, W.J. (1987): Control of stomata behaviour by abscisic acid which apparently originates in roots. – J. exp. Bot. *38*: 1174-1181.

Zhang, J. & Davies, W.J. (1990a): Changes in the concentration of ABA in xylem sap as a function of changing soil water status can account for changes in leaf conductance and growth. – Plant Cell Environ. *13*: 277-285.

Zhang, J. & Davies, W.J. (1990b): Does ABA in xylem control the rate of length growth in soil-dried maize and sunflower plants. – J. exp. Bot. *41*: 1125-1132.

Rezime

RADMILA STIKIĆ, SOFIJA PEKIĆ, ZORICA JOVANOVIĆ, LORA LJUBOJEVIĆ, LJILJANA PROKIĆ

GENOTIPSKE RAZLIKE U REAKCIJI KUKURUZA NA EGZOGENU ABSCISINSKU KISELINU

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U radu je ispitivan uticaj ABA na rastenje korena i izdanaka i na provodljivost stoma kod linija kukuruza selekcionisanih na sadržaj ABA u dva eksperimentalna sistema. Rezultati prvog ogleda sa mladim klijancima pokazali su da egzogeno dodata ABA inhibira rastenje i koleoptila i korena i to u različitoj meri zavisno od koncentracije i genotipa. U ogledu sa starijim biljkama (u fazi 3-eg lista) rastvor ABA je dodavan u deo korenovog sistema sa ciljem da se modifikuje sadržaj egzogene ABA u listu i ksilemu pri optimalnom vodnom režimu. Rezultati ovog ogleda su pokazali da je povećanje sadržaja ABA u listu-ksilemu dovelo do redukcije brzine rastenja lista i stomatalne provodljivosti kod svih linija, kao i da postoje genotipske razlike u osetljivosti ovih procesa na ABA. Najveća osetljivost na ABA konstatovana je kod linije Polj 17 u oba eksperimentalna sistema.

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Original scientific paper

RUDOLF KASTORI, NOVICA PETROVIĆ, IVANA ARSENIJEVIĆ MAKSIMOVIĆ

EFFECT OF LEAD ON THE ACTIVITY OF SOME ENZYMES OF NITROGEN METABOLISM IN SUGAR BEET *(BETA VULGARIS* L.)

Faculty of Agriculture and Institute of Field and Vegetable Crops, Novi Sad

Kastori R., Petrović N., Arsenijević Maksimović I. (1995): *Effect of lead on the activity of some enzymes of nitrogen metabolism in sugar beet (Beta vulgaris L.).* – Glasnik Instituta za botaniku i botaničke bašte Univerziteta u Beogradu, Tom XXIX, 77 - 84.

The subject of the study, a solution culture pot experiment, was the effect of lead on nitrate accumulation, lead content, the activity of some enzymes of nitrogen metabolism (nitrate reductase, glutamine synthetase, and glutamate dehydrogenase), the content of chloroplast pigments and the dry matter mass of young sugar beet plants (*Beta vulgaris* L., hybrid NS-Hy 11). The plants were treated with 10^{-5} or 10^{-3} M lead solutions. The lead concentrations inhibited NR, GS and GDH activity and reduced chloroplast pigments content, but not the nitrate concentration in the leaves. Lead content significantly increased with an increase in lead concentration in the nutrient solution, especially in the root, while the dry matter mass of the above-ground plant parts and the root decreased.

Key words: *Beta vulgaris* L., lead concentration, nitrates, nitrate reductase, glutamine synthetase, glutamate dehydrogenase, chloroplast pigments, dry matter

Ključne reči: *Beta vulgaris* L., koncentracija olova, nitrati, nitrat-reduktaza, glutamin-sintetaza, glutamat-dehidrogenaza, pigmenti hloroplasta, suva masa

INTRODUCTION

The availability of heavy metals in the soil depends on natural processes, especially on lithogenic and pedogenic ones, but also on anthropogenic factors (Filipinski and Grupe, 1990).

Anthropogenic factors, such as industrial activity and mining (Woolhouse, 1983), sewage disposal (Zurera-Cosano and Moreno-Royas, 1990), traffic (Sommer and Stritesky, 1976) etc, are the main factors responsible for an increase in the concentration of heavy metals in the soil.

Heavy metal-induced stress causes various direct and indirect effects on practically all physiological processes in plants (Woolhouse, 1983). The primary toxicity mechanisms of heavy metals alter the catalytic function of enzymes (Van Assche and Clijsters, 1990; Petrović et al., 1990), damage cellular membranes (Tu and Brouillette, 1987) and inhibit plant growth (Láng et al., 1995). These changes cause numerous secondary effects such as inhibition of photosynthesis (Lang et al., 1995) and mineral nutrient uptake (Nunes et al., 1995), hormonal imbalance and water stress (Barcelo et al., 1986; Kastori et al., 1993), different structural and ultrastructural changes (Vasquez et al., 1987), etc.

A considerable number of authors think that heavy metals primarily inhibit enzyme activity and/or cause structural changes in proteins, since they have the ability to interact with proteins, especially structural ones, through their sensitive SH- or histidil groups (Rauser, 1993). Having in mind that the enzymes of nitrogen assimilation contain amino acids rich in sulfur, we thought it would be worthwhile to investigate the effect of lead on the activities of some enzymes of nitrogen metabolism.

MATERIAL AND METHODS

Experiments with young sugar beet plants *(Beta vulgaris* L., hybrid NS-Hy 11) were conducted in semi-controlled greenhouse conditions. Having been allowed to germinate in the vermiculite, the seedlings were transferred into solution-culture pots, on 1/2 Hoangland's solution, which has the following composition [mM]: 2.5 $Ca(NO_3)_2$; 2.5 KNO_3; 1.0 KH_2PO_4; 1.0 $MgSO_4 \cdot 7H_2O$ and [μM]: 21.3 B; 4.6 Mn; 0.38 Zn; 0.052 Mo; 0.15 Cu and 8.95 Fe as $Fe_{(III)}$NaEDTA. Three weeks after this, the plants were grown alternatively on 1/2 Hoangland's solution and in the presence or 10^{-5} of 10^{-3}M Pb, each turn lasting 24 h. The plants were grown in this manner for twelve days, after which symptoms of the excess of lead became clearly visible. They were then picked and the above-ground plant parts and roots separated from one another. The dry matter mass of each organ was determined after drying the samples at 70°C to constant weight. Nitrate reductase (NR) activity was determined *in vivo,* in phosphate buffer (pH 7.4) (Hageman and Reed, 1980). The activity of the *in vitro* transferase reaction of glutamine synthetase (GS) and NADH-dependant glutamate dehydrogenase (GDH) was determined in a common leaf extract according to Coombs and Hall (1982). The lead concentration was established using atomic absorption spectrophotometry. The nitrate content was determined by spectrophotometric assay with phenoldisulfic acid. The levels of chlorophyll *a, b* and carotenoide content were determined spectrophotometrically in the acetone extract of freshly harvested leaves, using molar extinction coefficients according to Holm (1954) and von Wettstein (1957).

The results were statistically processed by calculating the lowest statistically significant differences (LSD).

RESULTS AND DISCUSSION

The dry matter mass of the above-ground plant parts and roots decreased with the increase in lead concentration in the nutrient solution (Fig. 1). The mass of the above-ground plant parts was more negatively affected by lead than the root mass. This was indicated by the ratio of the dry matter mass of the above-ground plant parts and the dry matter mass of the root in the presence of the lead concentrations studied. This ratio was 4.44 in the check tretment, and 3.79 in plants grown in the presence of higher concentrations of lead (10^{-3}M). This suggests that the above-ground plant parts of sugar been are much more sensitive to the presence of higher lead concentrations then the sugar beet root, which is confirmed by the concentration of lead in the above-ground plant parts and in the root. The concentration was much higher in the root, the mass of which decreased to a smaller extent, than in the above-ground plant parts (Fig. 2). Therefore, the deposition of Pb in the root may be considered a form of self-detoxification on the part of plants. Different compounds and ions are involved in the precipitation of Pb within plants, which is thus rendered metabolically inactive (Kneer and Zenk, 1992).

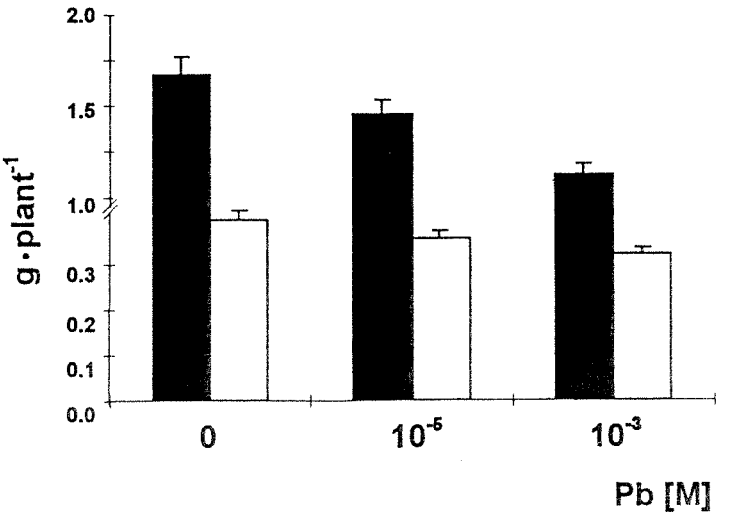

Fig. 1. – The effect of different lead concentrations on dry matter mass of the above-ground plant parts (■) and roots (□) of young sugar beet plants.

The level of chlorophylls *a* and *b* and carotenoids decreased in the presence of lead in the nutrient solution, whereas the chlorophyll *a*: chlorophyll *b* ratio increased (Fig. 3). In maize (Gašić et al., 1992) and cucumber (Láng et al., 1995) Pb displayed similar effect on chloroplast pigments content. A significant number of authors are of the opinion that heavy metals inhibit chlorophyll biosynthesis by causing a decrease in

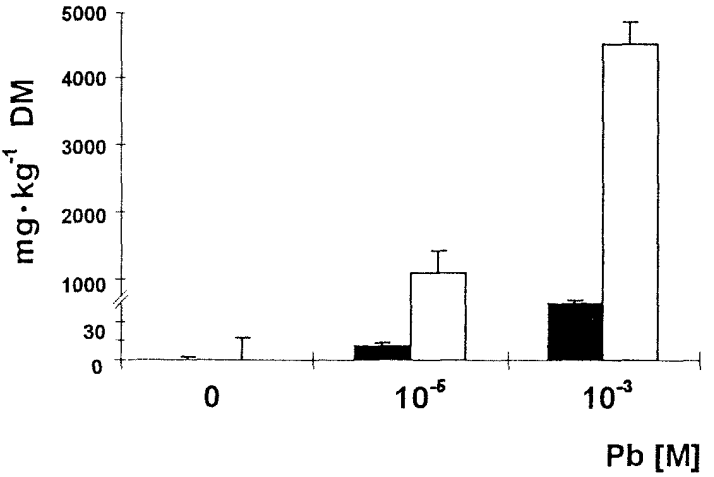

Fig. 2. – The effect of different lead concentrations on lead content in the above-ground plant parts (■) and roots (□) of young sugar beet plants

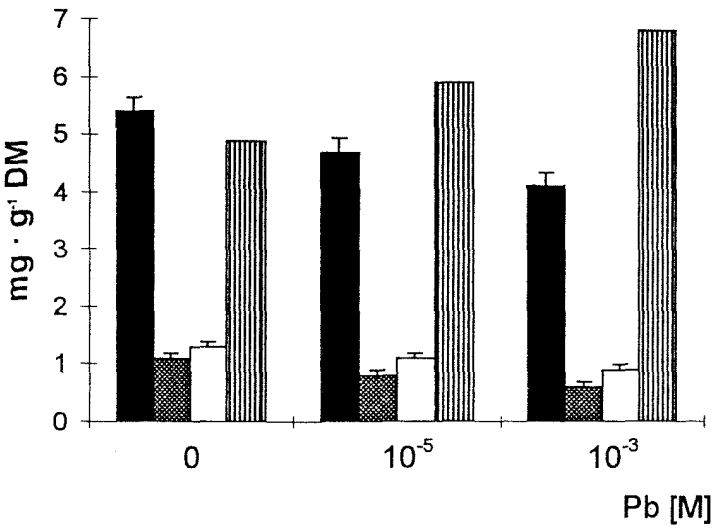

Fig. 3. – The effect of different lead concentrations on the content of chloroplast pigments in young sugar beet plants: chlorophyll *a* (■) chlorophyll *b* (▩), carotenoids (□) and chlorophyll *a*: chlorophyll *b* ratio (▥)

the sum of reducing equivalents and ATP synthesis. This way, altering the activity of RUBP-carboxylase, they indirectly effect the uptake and metabolism of CO_2 (L á n g et al., 1995).

The nitrate content in the leaves of sugar beet significantly increased with an increase in lead concentration in the nutrient solution (Fig. 4). Nitrate content may increase not only as a result of an excessive application of nitrates – it can also be caused by numerous internal and external factors, such as environmental pollution with heavy metals, which may also act as indirect inhibitors of photosynthesis and respiration (V a l e and U l m e r, 1972). Under such conditions the energy pool and the sum of reductive equivalents necessary for nitrate assimilation are reduced. On a number of occasions it has been shown that the majority of non-essential heavy metals inhibit the activity of nitrate reductase, the key enzyme in the process of nitrate reduction (B u r z y n s k i and G r a b o w s k i, 1984; P e t r o v i ć et al., 1990; K a s t o r i et al., 1996). Besides, it is likely that Pb activates the transfer of one part of the nitrate from the „metabolic pool" into the „reserve pool" (O a k s et al., 1989).

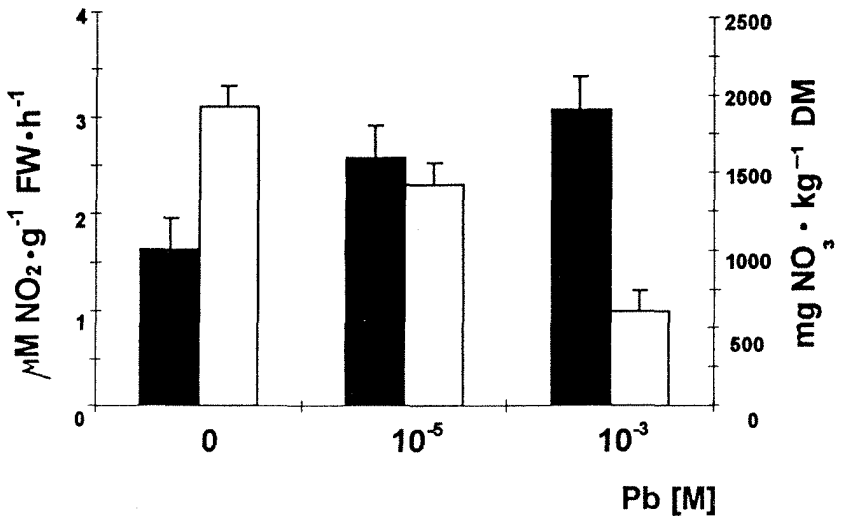

Fig. 4. – The effect of lead on NRA (□) and nitrate content (■) in the above-ground parts of young sugar beet plants

NR activity in the leaves decreased with an increase in Pb concentration (Tab. 4). Similar results were obtained for *Pisum sativum* and *Helianthus annuus,* indicating that Pb has a direct effect on enzyme synthesis (S i n h a et al., 1988). According to B u r z y n s k i and G r a b o w s k i (1984), lower lead concentrations in the nutrient solution affect NR activity indirectly, probably through water stress, whereas the high concentrations directly affect the proteins that build the enzyme structure.

A study of the Pb effect on the activity of nitrogen assimilation enzymes in the leaves of *Pisum sativum* L. showed that NR was most sensitive to the presence of this metal (S i n h a et al., 1988).

GDH activity in leaves was inhibited at both of the Pb concentrations applied, thus inhibiting the reductive amination of α-ketoglutarate as well (Fig. 5). These results are in agreement with previous observations regarding Pb-induced GDH inhibition in maize (Kastori et al., 1993) and Cd-induced inhibition in *Phaseolus vulgaris* (Van Assche and Clijsters, 1990).

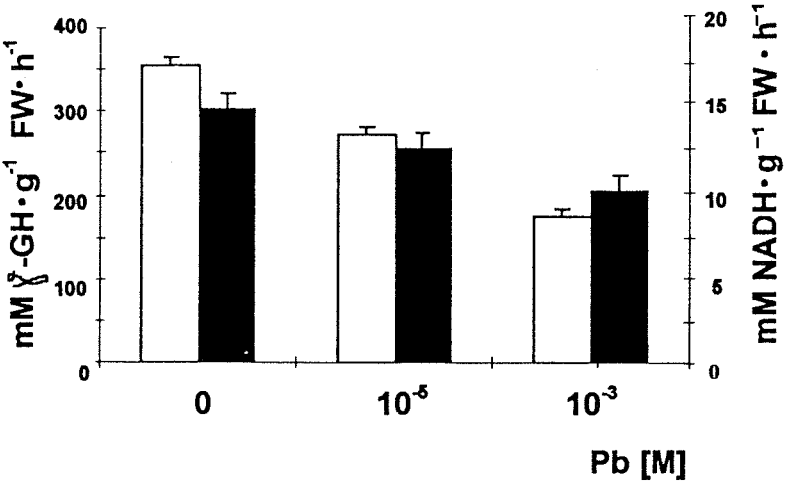

Fig. 5. – The effect of lead on GS (■) and GDH (□) activity in the above-ground part of young sugar beet plants

The presence of Pb had quite similar inhibitory effects on GS activity as well (Fig. 5). Results obtained with lead are similar to those obtained with Cd, which was also shown to inhibit GS activity in sugar beet (Popović et al., 1996).

In the majority of crops GS is present in two isozyme forms: GS_1 and GS_2. It was suggested that isozyme GS_2, which is present in chloroplasts, plays a role in the primary ammonia assimilation and that its activity primarily depends on the amount of light absorbed and the intensity of ATP synthesis (Mc Nally et al., 1983). With respect to this, it can be assumed that the inhibition of GS activity in the presence of lead is likely a results of decreased ATP synthesis (Láng et al., 1995). According to the results of our study, it can be concluded that, either directly or indirectly, lead strongly affects primary nitrogen assimilation. At the same time, the possibility that lead also affects the uptake of nitrogen ions can not be excluded, either (Burzynsky and Grabowski, 1984).

CONCLUSIONS

An increase of the lead concentration in the nutrient solution especially increased the lead content in the leaves, whereas the dry matter mass of both the above-ground plant parts and the root decreased.

The level of chloroplast pigments (chlorophylles *a* and *b* and carotenoids) decreased, but the chlorophyll *a*: chlorophyll *b* ratio was augmented following an increase in lead concentration in the nutrient solution.

The activity of nitrate reductase, glutamine synthetase and glutamate dehydrogenase in lead-treated plants significantly decreased, while the nitrate content significantly increased.

As the enzymes of nitrogen assimilation contain amino acids rich in sulfur, our results support hypothesis after which, in the first place, heavy metals effect functioning of SH-groups and S-S bonds of enzymes.

REFERENCES

Barceló, J., Cabot, C. & Poschenrieder, Ch. (1986): Cadmium-induced decrease of water stress resistance in bush bean plants *(Phaseolus vulgaris* L. cv Contender). II. Effects of Cd on endogenous abscisic acid levels. – J. Plant Physiol. *125*: 27-34.

Burzynski, M. & Grabowski, A. (1984): Influence of lead on NO_3 uptake and reduction in cucumber seedlings. – Acta Soc. Bot. Pol. *53*(1): 77-86.

Coombs, J. & Hall, O.D. (1982): Techniques in bioproductivity and photosynthesis. – Pergamon Press, Oxford.

Filipinski, M. & Grupe, M. (1990): Verteilungsmuster lithogener, pedogener und antropogener Schwermetalle in Boden. Z. Pflanzenernärh. Bodenk. *153*: 69-73.

Gašić, O., Kastori, R., Petrović, N. & Štajner, D. (1992): Effect of lead on some biochemical and physiological parameters in corn *(Zea mays* L.). – Agric. Mediteranea *122*: 257-261.

Hageman, R.H. & Reed, A.J. (1980): Nitrate reductase from higher plants. – Methods enzymol. *69*: 270-276.

Holm, G. (1954): Chlorophyll in barley. – Acta Agron. Scand. *4*: 457-471.

Kastori, R., Petrović, M. & Petrović, N. (1992): Effect of excess lead, cadmium, copper and zinc on water relations in sunflower. – J. Plant Nutr. *15*: 2427-2439.

Kastori, R., Petrović, N. & Petrović, M. (1996): Effect of lead on water relations, proline concentration and nitrate reductase activity in sunflower plants. – Acta Agron. Hungarica *44*: 21-28.

Kastori, R., Petrović, N., Gašić, O. & Štajner, D. (1993): Effect of lead on nitrate accumulation and nitrate assimilation enzymes in maize *(Zea mays* L.). – Zbornik Matice srpske za prirodne nauke (Novi Sad) *84*: 27-36.

Kneer, R. & Zenk, H.M. (1992): Phytochelations protect plant enzymes from heavy metal poisoning. – Phytochemistry *31*: 2663-2667.

Lang, F., Sarvari, T., Szigeti, Z., Fodor, F. & Cseh, E. (1995): Effects of heavy metals on the photosynthetic aparatus in cucumber. *In* Photosynthesis: From Light to Biosphere. Vol. IV (P. Mathis, ed.) pp. 533-536. – Kluwer Academic Publishers, Netherlands.

Nunes, M.A., Matos, M.C., Azinheira, H.G.M. & Quartin, V.L. (1995): Indirect effects of aluminium on photosynthesis and mineral concentrations in *Triticales. In* Photosynthesis: from light to Biosphere, Vol. IV (P. Mathis, ed.) pp. 713-716. – Kluwer Academic Publishers, Netherlands.

Oaks, A., Stulen, I., Jones, K., Winspear, M.J., Misra, S. & Boesel, I.L. (1989): Enzymes of nitrogen assimilation in maize roots. – Planta *148*: 477-484.

Petrović, N., Kastori, R. & Rajčan, I. (1990): The effect of cadmium on nitrate reductase activity in sugar beet *(Beta vulgaris). In* Plant Nutrition – Physiology and Application (M.L. van Beusichem, ed.) pp. 107-109. – Kluwer Academic Publishers, Dordrecht-Boston-London.

Popović, M., Kevrešan, S., Kandrač, J., Nikolić, J., Petrović, N. & Kastori, R. (1996): The role of sulfur in detoxication of cadmium in young sugar beet *(Beta valgaris* L.) plants. Biologia plantarum (in press).

Rauser, E.W. (1993): Metal-binding peptides in plants *In* Sulfur nutrition and sulfur assimilation in higher plants. (J.L. de Kok et al., eds) pp. 237-249 – SBP, Academic Publishing, the Hague.

Sinha, S.K., Srivastava, H.S. & Mishra, S.N. (1988): Effects of lead on nitrate reductase activity and nitrate assimilation in pea leaves. – Acta Soc. Bot. Pol. *57*: 457-463.

Sommer, G. & Stritesky, A. (1976): Gefäsversuche zur Ermittlung der Schadegranzen von Cadmium, Kupfer, Blei und Zink in Hinblick auf den Einsatz von Abfallstoffen in der Landwirtschaft. – Landw. Forsch. *29*: 88-100.

Tu S.I. & Broullettes, J.N. (1987): Metal ion inhibition of cotton root plasma membrane ATPase. – Phytochemistry *26*: 119-128.

Valle, B.L. & Ulmer, D.D. (1972): Biochemical effects of mercury, cadmium and lead. – Ann. Rev. Biochem. *41*: 91-128.

Van Asshe, F. & Clijsters, F. (1990): Effects of metals on enzyme activity in plants. – Plant Cell Environ. *13*: 195-206.

Vazquez, M.D., Poschenrieder, Ch. & Barcelj, J. (1987): Chromium VI induced structural and ultrastructural changes in bush bena plants *(Phaseolus vulgaris* L.). – Ann. Bot. *59*: 427-438.

von Wettstein, D. (1957): Chlorophyll-letale und der Submikroskopische Formwechel der Plastiden. – Exp. Cell Res. *12*: 427-506.

Woolhouse, H.W. (1983): Toxicity and tolerance in the response of plants to metals *In* Encyclopedia of Plant Physiol., New Series, Vol. 12 C, pp. 246-289. – Springer-Verlag, New Nork-Heidelberg-Berlin.

Zurerea-Cosano, G. & Moreno-Royas, R: (1990): Lead and cadmium content in fresh and cloned asparagus. J. Food Qual. *13*: 225-232.

Rezime

RUDOLF KASTORI, NOVICA PETROVIĆ, IVANA ARSENIJEVIĆ MAKSIMOVIĆ

UTICAJ OLOVA NA AKTIVNOST ENZIMA METABOLIZMA AZOTA U ŠEĆERNOJ REPI *(BETA VULGARIS* L.)

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Ogledi su izvedeni u polukontrolisanim uslovima, u staklari, na mladim biljkama šećerne repe (hibrid NS-Hy 11). Nakon naklijavanja u vermikulitu, ponici su preneti u posude za vodene kulture i gajene tri nedelje na 1/2 Hoangland-ovom hranljivom rastvoru. Biljke su nakon toga naizmenično (po 24 sata) gajene na Hoangland-ovom hranljivom rastvoru i u prisustvu 10^{-5} ili 10^{-3}M Pb u toku 12 dana, odnosno do pojave jasno vidljivih simptoma suviška olova.

Proučavano je dejstvo različitih koncentracija olova na aktivnost enzima metabolizma azota: nitrat-reduktaze, glutamin-sintetaze i glutamat-dehidrogenaze u nadzemnim delovima biljaka. Pored toga, ispitivano je dejstvo olova na sadržaj olova, nitrata i pigmenata hloroplasta, kao i na masu suve materije nadzemnog dela i korena.

Na osnovu dobivenih rezultata može se zaključiti da se pri povećanju koncentracije olova u hranljivom rastvoru njegova koncentracija naročito značajno povećala u korenu, dok se masa nadzemnog dela i korena smanjila.

Sadržaj pigmenata hloroplasta (hlorofila *a, b* i karotenoida) se smanjio, a odnos hlorofil *a* : hlorofil *b* povećao sa povećanjem koncentracije olova u rastvoru.

Aktivnost nitrat-reduktaze, glutamin-sintetaze i glutamat-dehidrogenaze se značajno smanjila, a sadržaj nitrata značajno povećao u biljkama tretiranim olovom.

Imajući u vidu da enzimi asimilacije azota sadrže aminokiseline bogate sumporom, dobiveni rezultati potvrđuju pretpostavku da teški metali prvenstveno utiču na funkcionisanje SH- grupa i S-S veza u enzimima.

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UDC 588.7:582.739(497.11)
Original scientific paper

RADOMIRKA NIKOLIĆ, NEVENA MITIĆ

EFFECT OF CYTOKININS ON SHOOT REGENERATION FROM ROOT EXPLANTS OF BIRDSFOOT TREFOIL *(LOTUS CORNICULATUS* L.)

The Agricultural Institute „Serbia", Centre for Agricultural and Technological Research, Zaječar, Yugoslavia

Nikolić, R., Mitić, N. (1995): *Effect of cytokinins on shoot regeneration from root explants of birdsfoot trefoil (Lotus corniculatus L.).* – Glasnik Instituta za botaniku i botaničke bašte Univerziteta u Beogradu, Tom XXIX, 85 - 91.

The effect of three different cytokinins KIN, 2iP and BA at concentration 0.2 and 0.5 mg l^{-1} on shoot regeneration from root explants of domestic birdsfoot trefoil *(Lotus corniculatus* L.) cv. Bokor has investigated. The significant differences in shoot regeneration and development were found among the cytokinins tested. The high frequency of shoot production was achived on the medium containing BA at 0.2 mg l^{-1}. About 1000 plants were obtained from one root explant on the same medium after 120 days of culture. The plantlets were regenerated directly from root segments or via callus, depending upon the type of cytokinin used. KIN and 2iP have provoked spontaneously root formation.

Key words: birdsfoot trefoil, cytokinins, root explant, shoot regeneration

Ključne reči: žuti zvezdan, citokinini, eksplantati korena, regeneracija pupoljaka

INTRODUCTION

Lotus corniculatus L. (birdsfoot trefoil) is a tetraploid (2n = 24) (W e r n s m a n et al., 1964) perennial forage legume. It has softer stems, lower cellulose content and more carbohydrates. This legume good grows on poor, acid and salt soils. Because of its good nutritive composition and the other biological characteristics, today, birds foot trefoil is wide-spread forage crop in the world.

This culture technology has been applied successfully to *L. corniculatus.* Fertile plants were regenerated from calli of different explants: internode segments (O r s h i n s k y et al., 1983; O r s h i n s k y & T o m e s, 1985; S w a n s o n & T o m e s, 1980), anthers (N i i z e k i & G r a n t, 1971), leaves (M a r i o t i et al., 1984) and nodes (T o m e s 1979; P u p i l l i et al., 1992). The plants also were obtained directly from roots (W e b b et al., 1986; R y b c z y n s k i & B a d z i a n, 1987) and leaf explants (W e b b et al., 1986).

In this study we investigate effect of three different cytokinins on shoot regeneration and development from root explants of birdsfoot trefoil cv. Bokor. This cultivar was produced by a polycross method in the Centre for Agricultural and Technological Research in Zaječar and it is well adapted to climate conditions of the Timočka Krajina region. The cultivar Bokor has good chemical composition (19.5% albumens, 19.4% cellulose, 3.2% oil and 103.3 mg/kg β-carotene), stem height (50-55 cm) and it regenerates very fast after mowing.

The aim of this investigation is to establish efficient regeneration system to be applied *in vitro* selection and genetic transformations methods in plant improvement programs.

MATERIALS AND METHODS

Plant material

The seeds of birdsfoot trefoil *(L. corniculatus* L.) cv. Bokor, were surface sterilized in 20% sodium hypochlorite solution (20 min) and washed three times with sterile destilated water. They were aseptically germinated on a 0.45% agar (SIGMA) MS (M u r a s h i g e & S k o o g, 1962) medium, at 25 ± 2°C under $16/8^h$ photoperiod. Non-meristematic root segments 5 mm long were excised from 6 days old seedlings.

Culture media and conditions

A basal medium of MS supplemented with 3% sucrose, 0.45% agar (SIGMA) and (in mg l^{-1} each): glycine 2, nicotinic acid 0.5, B_1 1 and B_6 1 was used.

Different cytokinins, KIN, 2iP and BA at concentration 0.2 and 0.5 mg per liter each were added in the media and pH was adjusted to 5.8 prior to autoclaving. Root explants were grown in 100 ml erlenmayer flasks, containing 40 ml of the media. The cultures were transfered every 20 days to the same, fresh medium. The regenerated plantlets (2-3 cm height) were rooted on hormon free medium.

All cultures were kept at 25 ± 2°C under white fluorescent light (47 μmol $m^{-2}s^{-1}$), in a 16^h day period.

RESULTS

The shoot regeneration from root explants of cv. Bokor was compared to media containing various cytokinins (KIN, 2iP and BA) at 0.2 and 0.5 mg per liter. The type of cytokinin affected shoot regeneration and differentiation. The plants were obtained directly or indirectly via callus.

The shoots were formed directly from root explants which cultured on medium containing KIN (0.2 and 0.5 mg l^{-1}) 15 days after culture initiation (Fig. 1). The number of produced shoots increased during the culture on the same media. No calli formation was observed on media consisted KIN. The average number of shoots per explant was lower and similar on both media (0.2 and 0.5 mg l^{-1} KIN) (7.1 and 6.2 shoots after 60 days of culture, Tab. 1 and Tab. 2). Obtained plants had very elongated internodes (Fig. 2). The average height of plants was 7.3 to 7.6 cm and did not depend upon KIN concentration. The most of plants formed well developed roots on these media.

Tab. 1. – Effects of three different cytokinins at concentration 0.2 mg l^{-1} on shoot regeneration from root explants of cv. Bokor

Cytokinin	No. explants	Callus formation (%)	Shoot regeneration (%)	Average No. of shoots per explant*	Average plants height (cm)
KIN	25	0	8 (32)	7.1	7.3
2iP	17	13 (76)	3 (23)	6.4	6.7
BA	22	20 (91)	17 (85)	62.0	3.6

*The data were recorded after two months of culture.

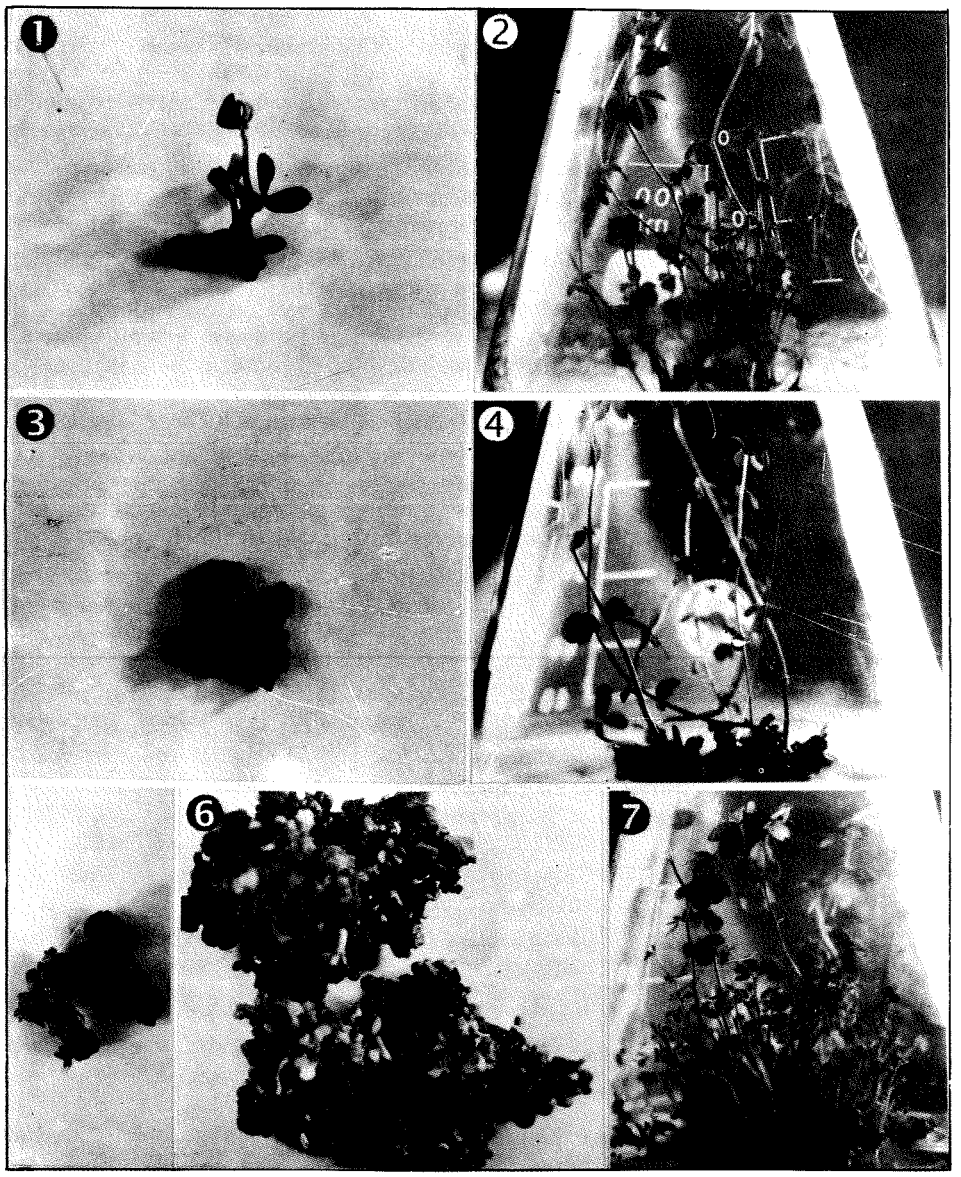
Tab. 2. – Effects of three different cytokinins at concentration 0.5 mg l^{-1} on shoot regeneration from root explants of cv. Bokor

Cytokinin	No. explants	Callus formation (%)	Shoot regeneration (%)	Average No. of shoots per explant*	Average plants height (cm)
KIN	30	0	14 (47)	6.2	7.6
2iP	26	8 (31)	5 (63)	5.5	7.6
BA	36	26 (72)	20 (77)	41.0	2.8

*The data were recorded after two months of culture.

In contrast with the root explants on KIN media, on media supplemented with 2iP and BA, they produced shoots indirectly, via callus stage. Calli initially were observed on both ends of root parts (Figs 3 and 5).

However, further regeneration process was different on 2iP and BA media. Calli, produced on 2iP media, had lower organogenic abilities and they have grown very slow in comparison to the calli on BA media. The average number of shoots from callus per explant was 6.4 on 0.2 mg l^{-1} or 5.5 on 0.5 mg l^{-1} 2iP. The regenerated plantlets had long internodes (6.7 to 7.6 cm stem height) (Tab. 1 and Tab. 2) (Fig. 4), and roots formation were observed to, as well as the plantlets on KIN media.



Figs. 1. – Birdsfoot trefoil shoot regenerated directly from root explant on KIN (0.2 mg l^{-1}) 15 days after culture initiation; 2. – Elongated plantlets 60 days old on KIN (0.2 mg l^{-1}); 3. – Organogenic callus formed from root segment on 2iP (0.2 mg l^{-1}) medium after 15 days of culture; 4. – Plantlets with long internodes on 2iP (0.2 mg l^{-1}); 5. – Calli formed on root segment on BA (0.2 mg l^{-1}); 6. – Organogenic callus 20 days old on medium with BA; 7. – Multiple plantlets on BA (0.2 mg l^{-1}), 60 days after culture initiation

BA was the most effective among the cytokinins tested. On media with BA well developed organogenic, green calli were produced. They formed buds after 20 days of culture (Fig. 6). The shoots number were increased rapidly with the time of culture. A lot of buds were regenerated from calli obtained on medium with 0.2 mg l^{-1} BA (62.0, Tab. 1). BA at this concentration was more effective on shoot regeneration frequency than BA 0.5 mg l^{-1} (41.0, Tab. 2), and on an average 250 regenerants were produced from one root explant after 90 days of culture. The regenerated plantlets had short internodes and lower height than the plantlets obtained on KIN and 2iP media (Fig. 7). Rooting was not found and elongated buds were rooted on MS medium lacking plant growth regulators. No abnormal plantlets were observed on all used media. Only cultures which growed on medium with 0.5 mg l^{-1} BA produced red coloured pigment in the medium.

DISCUSSION

Niizeki & Grant (1971) first obtained the plants of birdsfoot trefoil in *in vitro* conditions, using antheres as a explants. The cultivar Bokor was regenerated erlier in *in vitro* culture (Nikolić, 1995) from apical buds of the seedlings and the whole plants. The plants were obtained from calli on medium which was initially formulated for alfalfa (Saunders & Bingham, 1972).

In the present study, the parts of roots had very good organogenic ability. These explants regenerated shoots directly, or indirectly via callus, depend upon the type of cytokinin used. Only KIN has favourized direct organogenesis, in contrast with the 2iP and BA.

Rybczynski & Badzian (1987) first reported of *L. corniculatus* plant regeneration from non-meristematic root segments. The organogenesis was direct, without callus formation, on hormone free medium. So, no cytokinins needed for shoot regeneration from roots, but the number of buds per one explant was small.

By using low concentration of cytokinins in the media, we found higher shoot regeneration per explant and the shoot number was increased with the time of culture. In this case BA at concentration 0.2 mg per liter, was the most satisfactory on buds multiplication than KIN and 2iP. The other investigaters used also low BA concentration for shoots regeneration through calli from internodes (Orshinsky et al., 1983) and from the apical shoot or node (Tomes, 1979; Pupilli et al., 1992) reported that BA was most effective cytokinin than KIN, and the number of shoots produced per single node of three *Lotus* spp. increased during the culture on BA medium. Our results also confirmed theirs observation that the shoots never rooted on media containing BA.

All these results indicate that the percentage of cultures which produced shoots were not influenced by the presence of cytokinin in the culture medium. However, the addition of a cytokinin, such as a BA, is necessary to increase the number of shoots produced. Using BA (0.2 mg l^{-1}), it would be possible to produce about 1000 plants from a single root explant within 4 months of culture.

Therefore, *in vitro* propagation of *L. corniculatus* by root culture can be efficient regeneration plant system to apply the *in vitro* selection and genetic transformation methods (Webb, 1986; Armstead & Webb, 1987; Petit, 1987) in birdsfoot trefoil breeding programs. By using these methods, it is possible to obtain cell lines and plants with improved characteristics (resistant to disease, herbicides, salt tolerant etc.).

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Original scientific paper

DRAGOLJUB GRUBIŠIĆ, ZLATKO GIBA, RADOMIR KONJEVIĆ

SEED GERMINATION OF *GENTIANA CRUCIATA* L.*

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Grubišić, D., Giba, Z., Konjević, R. (1995): *Seed germination of Gentiana cruciata L.* – Glasnik Instituta za botaniku i botaničke bašte Univerziteta u Beogradu, Tom XXIX, 93 - 100.

Seeds of *Gentiana cruciata* are light requiring. Germination in darkness can be induced by gibberellic acid and N-substituted phtalimide AC-94,377. Gibberellin-induced germination takes place in a broade temperature range (10-30°C) while hypocotyl elongation is more sensitive to temperature with an optimum at 19°C. Additional percent of germination of light-induced seeds can be obtained by gibberellic acid and AC-94,377 application but not by fusicoccin. Light-induced germination is potentiated by potassium nitrate. However, the adition of potassium nitrate two weeks after the onset of imbibition is ineffective while the same treatment with gibberellic acid brings about an additional increase in germination. Prolonged imbibition in darkness at 24°C decreases, while at 4°C increases, percent of germination of light-induced seeds.

Key words: *Gentiana cruciata,* germination, light, plant growth substances

* Dedicated to Prof. Mirjana Nešković on the occasion of her 45th universary of scientific work

Ključne reči: *Gentiana cruciata,* biljne supstance rastenja, klijanje, svetlost

INTRODUCTION

Gentiana cruciata is Eurasian species which grows on dry meadows and pastures, on sunny slopes, among bushes, and at forest edges up to 1500 m altitude mainly at carbonate substrates. Like some other species of this genus, it contains bitter compounds and for that reason it can be used as an alternate source of pharmacologically important substances. In a popular medicine it is used as substitute for *Gentiana lutea.* Specific bitter substances are found in all tissues of the plant and thus, whole plants are collected, during blossoming, and used as a drug – *Gentianae cruciatae herba.* The fruit contains numerous small seeds of ellipsoidal shape, up to 1.5 mm long with fine net like seed coat of a dark color. In many species of *Gentiana* genus, the embryo is poorly

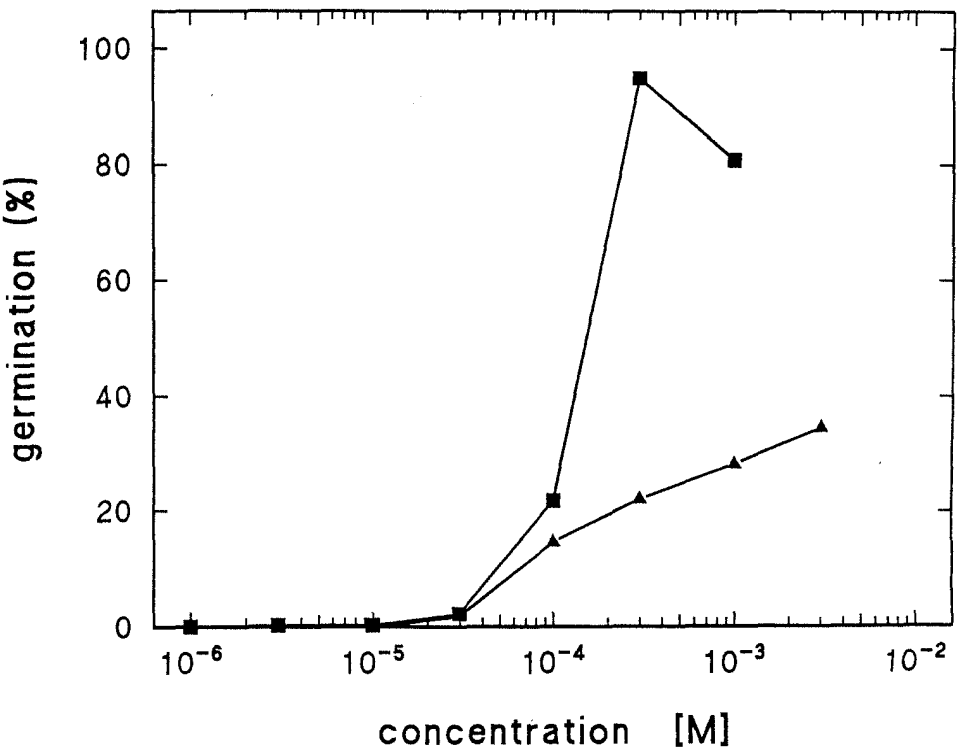

Fig. 1. – The effect of gibberellic acid and AC-94,377 on the germination of *Gentiana cruciata* seeds in darkness
Seeds were imbibed in GA_3 (squares) or AC-94,377 (triangles) solutions and left in darkness to germinate. Germination was scored 2 weeks after the onset of imbibition.

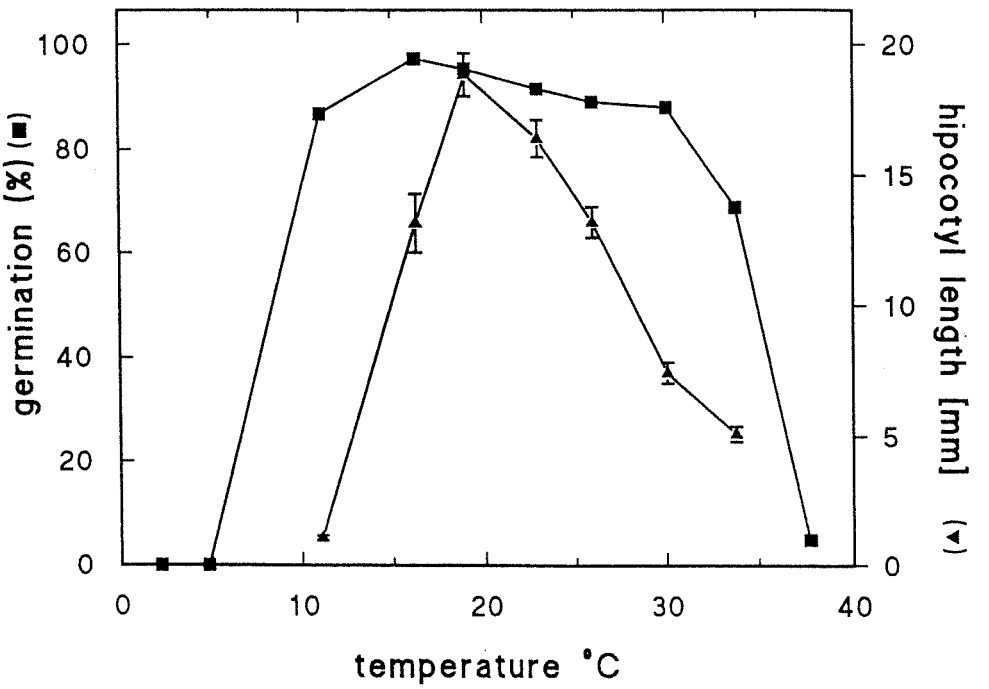

Fig. 2. – The effect of constant temperatures on GA_3 induced seed germination and hypocotyl elongation
Seeds were germinated in 1 mM GA_3 solution (squares) and left in darkness at indicated temperature. Germination was scored 3 weeks after the onset of imbibition. For hypocotyl elongation (triangles) seedlings germinated in light at 25°C were transferred to corresponding temperatures and grown for additional period of time. At the end of this period, hypocotyl length was measured. Each point represents the mean value for 40 plantlets.

developed and some species require prolonged chilling to germinate (Nikolaeva et al., 1985). According to Kinzel (1913) seeds of *G. cruciata* are light-requiring. In the present paper we have tested temperature requirements in differnet phases of germination, as well as the effects of gibberellic acid, N-substituted phtalimide AC-94,377 and fusicoccin on the germination of *Gentiana cruciata* seeds.

MATERIAL AND METHODS

Seeds of *Gentiana cruciata* were collected in October 1991 at the slopes of mountain Koritnik near the village Krstec, Serbia, Yugoslavia. Plant material was

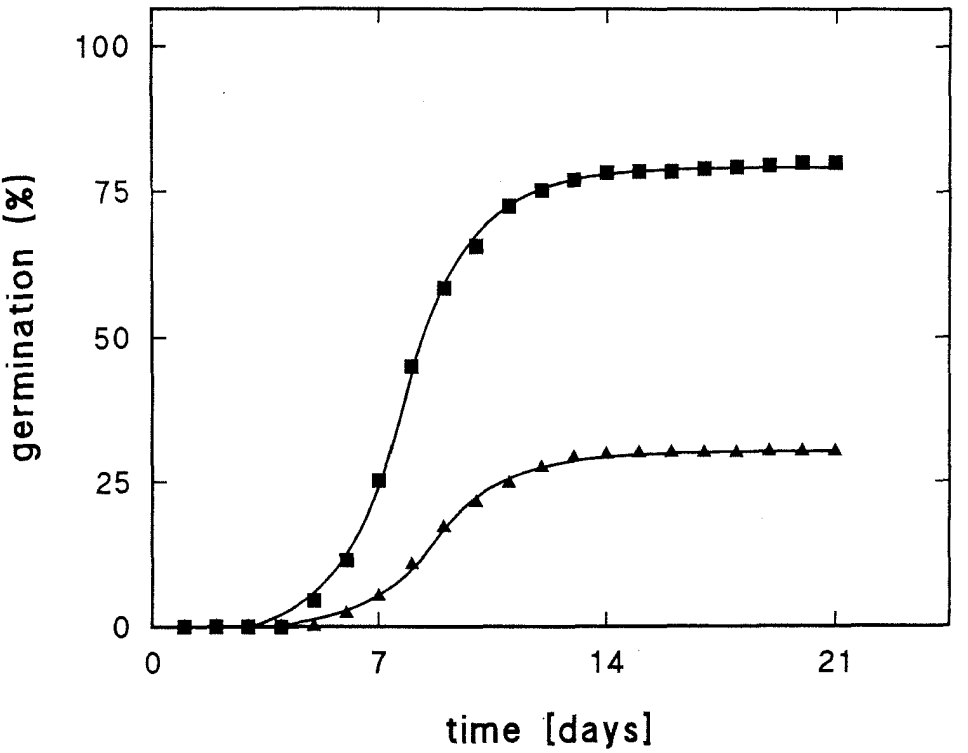

Fig. 3. – The effect of KNO_3 on light-induced germination of *Gentiana cruciata* seeds Seeds were germinated either in distilled water (triangles) or in 10 mM potassium nitrate solution (squares) in light. Germinated seeds were counted every day after the onset of imbibition.

taxonomically identified and saved in the Botanical Garden of the University of Belgrade. Lots of 100 seeds were sown in 6 cm diameter petri dishes containing 2 ml of distilled water or a substance to be tested and kept in darkness for 3 days at 25 or 3°C. Seeds were germinated a) in light; b) in light in the presence of gibberellic acid, AC-94,377 or potassium nitrate; and c) in darkness in the presence of gibberellic acid or AC-94,377. The germination temperature was 25°C, except in experiments where the influence of temperature was tested. In these experiments seeds were kept in darkness at different constant temperature in a termostat with temperature gradient (Autofrigor A.G., Zürich, Switzerland). White light was obtained from fluorescent tubes Tesla (20 W, 4500 K) at fluence rate 23,5 μmol $m^{-2}s^{-1}$ and red light from fluorescent tubes Philips (TL 20/15) combined with 3-mm plastic Röm & Hass filter No. 501 at fluence rate 5,5 μmol $m^{-2}s^{-1}$. Gibberellic acid was purchased from the Sigma

Company and AC-94,377 was obtained from American Cyanamide Company. All experiments were repeated 3 times with four replicates. The data are means of pooled results, and standard errors are not shown since they never exceeded 3%. Specific experimental protocols are given in figure legends.

RESULTS AND DISCUSSION

Preliminary experiments confirmed that *Gentiana cruciata* seeds require light to germinate (Kinzel, 1913). Germination in darkness can be induced by gibberellic acid and N-substituted phtalimide AC-94,377 (Fig. 1). It is well known that exogenous compounds can modify temperature requirements for germination (Reynolds and Thompson, 1971). In blueberry seeds the range of optimal temperatures is wider for GA_3- and AC- than light-induces seeds (Giba et al., 1993). The high percent of germination of *G. cruciata* seeds induced by gibberellic acid was obtained at range of

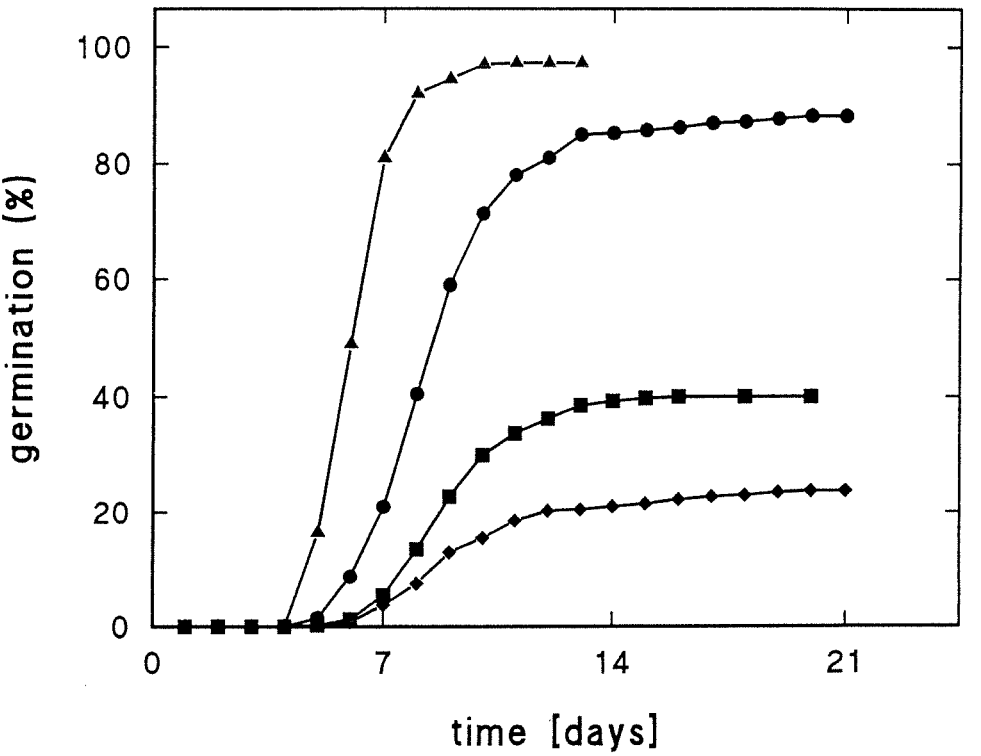

Fig. 4. – The effect of GA_3 AC-94,377 and fusicoccin on light-induced germination of seeds
Seeds were germinated in 1 mM GA_3 solution (triangles), 1 mM AC-94,377 (circles), distilled water (squares) or in 0.01 mM fusicoccin. Germinated seeds were counted every day.

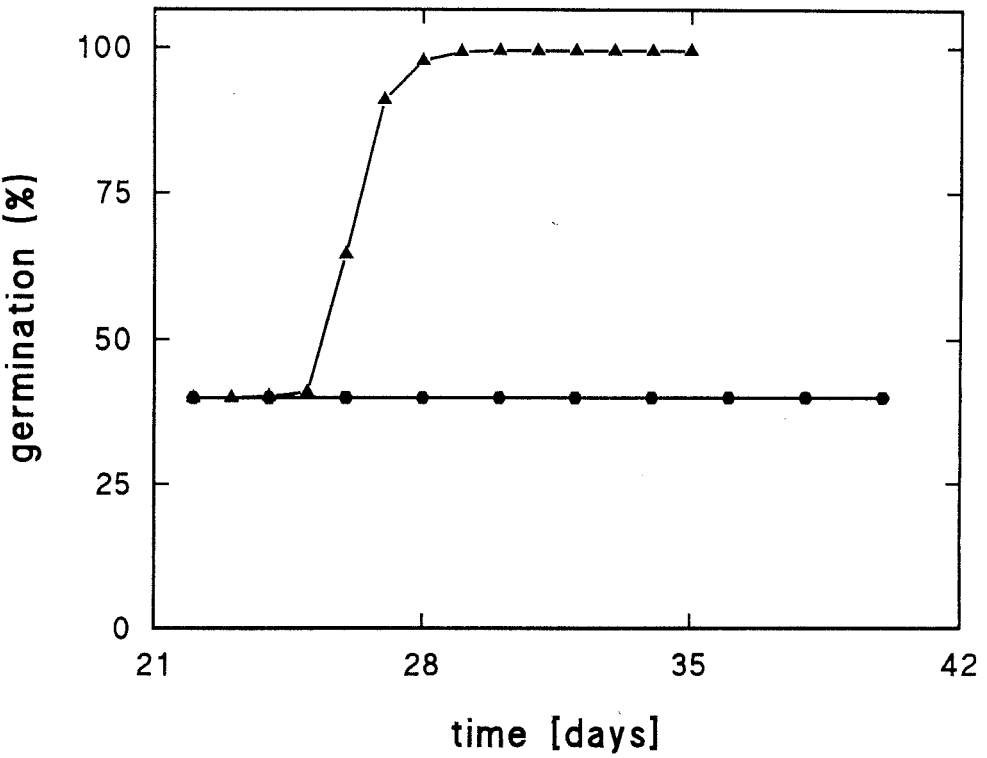

Fig. 5. – The effect of delayed application of GA_3 and KNO_3 on light-induced germination
Seeds were imbibed and germinated in light and water for 3 weeks. At the end of this period they were supplemented either with 1 mM GA_3 (triangles) or 10 mM KNO_3 (circles) and left in light for indicated period of time. Germinated seeds were counted every day.

temperatures between 10 and 30°C (Fig. 2). In *G. lutea* seeds temperature for high germination ranged from 19 to 24°C (unpublished data). Hypocotyl elongation of germinated *G. cruciata* seedlings was more sensitive to tremperature with an optimum at 19°C (Fig. 2). Continuous irradiation with red light induced germination up to 30% after 14 days. Further irradiation did not increase percent germination. Incubation of seeds in potassium nitrate solution, from the onset of imbibition, potentiated light-induced germination (Thompson, 1969). However, in both cases the plateau was reached after two weeks (Fig. 3). When the seed were incubated in gibberellic acid or AC-94,377 from the onset of imbibition, rate and percentage of germination were increased in light. Fusicoccin did not have this effect (Fig. 4). The same effect of gibberellic acid is evident even in GA_3 was added when the maximum of germination

induced by light was reached (21 days). Interestingly, potassium nitrate which stimulates germination when added from the onset of imbibition (Fig. 3) did not show the same effect if its application was delayed like in case of GA_3 (Fig. 5). Thus, stimulatory effect of KNO_3 was obvious only if it was present from the onset of light, while GA_3 additionally induced germination in light regardless of when it was administered. Similar difference between GA_3 and potassium nitrate effects was found in the germination of *Paulownia tomentosa* seeds (Grubišić and Konjević, 1990).

Percent of light induced germination of *Gentiana cruciata* seeds was determined by previous imbibition history. If the seeds were imbibed in darkness at 4°C for different period of time prior to transfer to light, number of germinated seeds was increasing

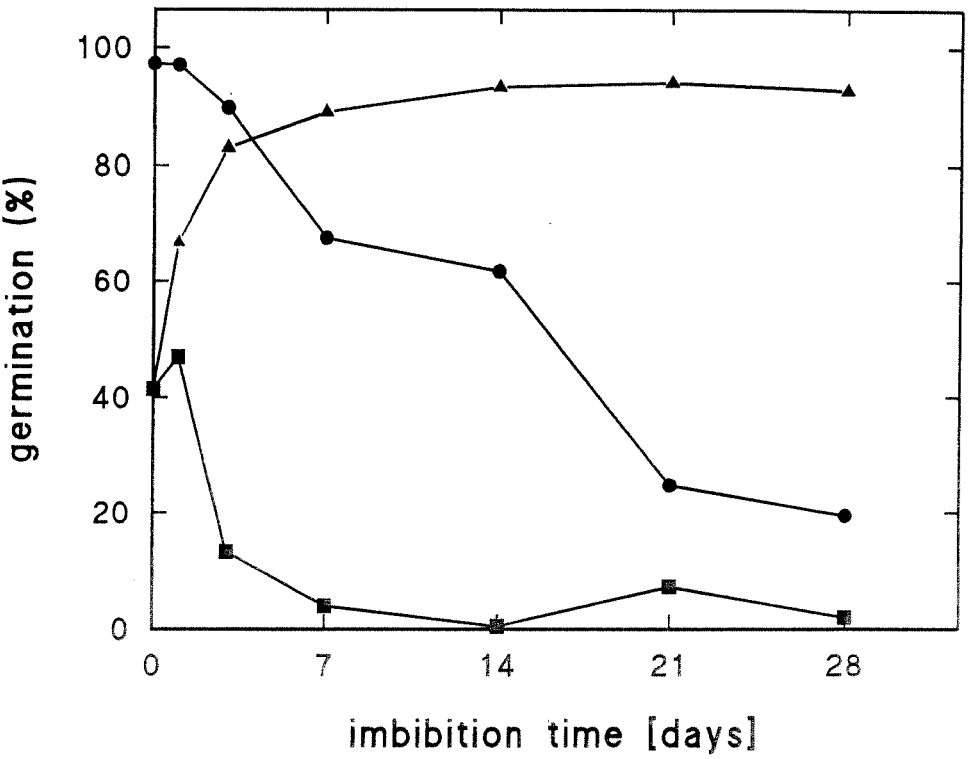

Fig. 6. – The effect of the imbibition time at constant temperatures on subsequent germination at 25°C
Seeds were imbibed in darkness at 4°C (triangles) or 25°C (squares) for indicated period of time and then transferred at 25°C to red light. Germination was scored after 3 weeks. Another set of seeds (circles) was imbibed in darkness at 25°C for indicated period of time, transferred at 4°C and left in darkness for two weeks. After that period, seeds were transferred to red light at 25°C and germination was scored 3 weeks later.

reaching the maximum after two weeks. On the countrary, the increasing time of imbibition at 24°C gradually decreased percent germination ultimately inducing scotodormancy after two weeks. Subsequent exposure of these seeds to low temperature for two weeks, prior to transfer to light, could overcome dormancy. The efficacy of low temperature treatment was inversely proportional to the time of imbibition at 24°C (Fig. 6).

ACKNOWLEDGMENT

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REFERENCES

Giba, Z., Grubišić, D. & Konjević, R. (1993): The effect of white light, growth regulators and temperature on the germination of blueberry *(Vaccinium myrtillus* L.). – Seed Sci. & Technol. *21*: 521-529.

Grubišić, D. & Konjević, R. (1990): Light and nitrate interaction in phytochrome-controlled germination of *Paulownia tomentosa* seeds. Planta *181*: 239-243.

Kinzel, W. (1913): Frost und Licht als beeinflussende Krafte bei der Samenkeimung, Stuttgart, 187S.

Nikolaeva, M.G., Rasumova, M.V. & Gladkova, V.N. (1985): Reference book on dormant seed germination, (M.F. Danilova, ed), pp. 158-160. – Nauka Publishers, Leningrad.

Raynolds, T. & Thompson, P.A. (1971): Characterization of the high temperature inhibition of germination of lettuce *(Lactuca sativa)*. – Physiol. Plant. *24*: 544-547.

Thompson, P.A. (1969): Effects of after -ripening and chiling treatments on the germination of species *Gentiana* at different temperatures. – J. Hort. Sci. *44*: 343-358.

Rezime

DRAGOLJUB GRUBIŠIĆ, ZLATKO GIBA, RADOMIR KONJEVIĆ

KLIJANJE SEMENA *GENTIANA CRUCIATA* L.

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Institut za botaniku Biološkog Fakulteta i
Institut za biološka istraživanja „Siniša Stanković", Beograd, Jugoslavija

Semena *Gentiana cruciata* ne klijaju u otsustvu svetlosti. Indukcija klijanja u mraku se postiže giberelnom kiselinom i N-supstituisanim ftalimidom AC-94,377. Klijanje indukovano giberelinom se odvija u širokom temperaturnom opsegu (10-30°C) dok je izduživanje hipokotila osetljivije na temperaturu sa optimumom na 19°C. Procenat klijanja svetlom indukovanih semena se može povećati primenom giberelne kiseline i AC-94,377, ali ne i fuzikokcina. Klijanje indukovano svetlošću je pospešeno nitratima. Međutim, dodavanje nitrata dve nedelje posle početka imbibicije ostaje bez efekta mada isti tretman giberelnom kiselinom dovodi do povećanja procenta klijanja. Produžena imbibicija u mraku na 24°C smanjuje, dok na 4°C povećava procenat klijanja svetlom indukovanih semena.

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Originalni naučni rad

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STOJANOVIĆ JOVANKA[1]

UTICAJ SORTNOSTI I SKLADIŠNIH TEMPERATURA NA DOZREVANJE I DORMANCIJU PŠENICE *TRITICUM AESTIVUM* L.

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Stefanović, M., Svilkić, B., Topuzović, M., Stojanović, J. (1995): *Effects of sort differences and storage temperatures on after ripening and dormancy of wheat kernels of Triticum aestivum L.* – Glasnik Instituta za botaniku i botaničke bašte Univerziteta u Beogradu, Tom XXIX, 101 - 105.

The effect of the two storage temperatures on the after ripening of the two sorts of wheat (Florida 302 and KG-65S) has been examined.

The differences between the two sorts of wheat have emerged as a results of the temperature effect on the after ripening and the germination has been examined at the begining of the experiment as well as after 45 and 90 days.

Key words: Florida 302, KG-65S, sort differences, storage temperatures, afterripening, dormancy

Ključne reči: Florida 302, KG-65S, sortne razlike, temperatura skladištenja, postžetveno sazrevanje, dormancija

UVOD

U periodu produžene žetve, zbog dužih kišnih perioda i visoke vlažnosti, zrna nekih sorata pšenice *Triticum aestivum* proklijavaju dok su još na klasu. To negativno utiče na prinos i tehnološku vrednost pšenice (Popović, 1984).

Ova pojava je manje izražena kod više dormantnih sorti u odnosu na manje dormantne. Zbog toga se prilikom setve posebna pažnja poklanja ne samo izboru sorata već i njihovoj usaglašenosti sa klimatskim faktorima sredine.

Otpornost dormantnih sorti na prevremeno proklijavanje uslovljena je postojanjem unutrašnjeg bloka u klijanju, koji nestaje nakon određenog perioda mirovanja u određenim uslovima skladištenja. Vreme skladištenja zrna kod žitarica poznato je pod imenom postžetveno dozrevanje.

Dormancija i dozrevanje su genetička svojstva koja određuju sortne odlike, tj. sortnost. Zbog sortnih genetičkih razlika skladišne temperature dozrevanja mogu ispoljiti različito delovanje na oba procesa (Toole, 1923; George, 1967).

MATERIJAL I METODE

U radu je ispitivan efekat temperatura dozrevanja na prekidanje dormancije kod sorata pšenice Florida 302 i KG-65S. Uzorci ispitivanih sorti su uzeti odmah posle žetve, jula 1994. god. sa oglednog polja u Institutu za strna žita u Kragujevcu.

Sortne razlike u strukturi klasa i boji zrna su bile osnovni kriterijumi za izbor materijala. Iako obe sorte imaju beli klas, sorta Florida 302 poseduje klas sa prisutnim osjem i belim zrnom, dok sorta KG-65S ima klas bez osja i zrno crvenkaste boje.

Ovršena zrna ispitivanih sorti su podeljena u dve grupe i držana na konstantnim temperaturama dozrevanja od 5°C i 20°C u periodu od 90 dana. U tom intervalu ispitivano je klijanje na 10, 20 i 30°C i to odmah posle žetve, posle 45 dana posle 90 dana. Utvrđivana je srednja vrednost procenata klijalih semena i standardna greška (SE_{max} = 1,7) u tri istovremeno postavljena ogleda.

Na osnovu dobijenih rezultata, koji su prikazani tabelarno, ocenjivan je efekat skladišnih temperatura na dozrevanje ispitivanih sorata.

REZULTATI RADA I DISKUSIJA

Dormancija i dozrevanje su genetička svojstva sortnosti, a oba procesa su kontrolisana i faktorima spoljašnje sredine. Od genetičkih faktora, sortne razlike u strukturi klasa (osje, plevice, boja i dr.) i odlike zrna (veličina, oblik i boja) znatno mogu ispoljiti efekat na jačinu dormantnog stanja i dozrevnaja (Wellington, Durham, 1958).

Od spoljašnjih faktora, temperatura skladištenja značajno određuje kako dužinu tako i brzinu dozrevanja zrna, odnosno prekidanje dormancije (Larson, 1936).

Neke dormantne sorte žitarica klijaju samo u uskom temperaturnom rangu, dok su van njega inače dormantne. Takve sorte poseduju takozvanu relativnu dormanciju (Vegis, 1964).

U ovom radu je ispitivan efekat temperatura na klijanje zrna dve dormantne sorte pšenice (Florida 302 i KG-65S) kako u periodu žetve (Tab. 1) tako i tokom dozrevanja (Tab. 2).

Tab. 1. – Klijanje zrna pšenice u periodu žetve
Germination of wheat grains during harvest

Temperatura klijanja (°C) Temperature of germination	Florida	KG-65S
	Klijanje % Germination	
10	45	91
20	1	77
30	0	1

Tab. 2. – Uticaj temperature skladištenja i dužine dozrevanja na klijanje zrna pšenice
Effect of temperature storage and length afterripening on germination of wheat grains

Temperatura skladištenja (°C) Temperature of storage	Temperatura klijanja (°C) Temperature of germination	Florida		KG-65S	
		Dužina skladištenja (dani) Length of storage (days)			
		45	90	45	90
		Klijanje (%) Germination			
5	10	59	80	95	92
	20	13	39	91	92
	30	4	38	0	66
20	10	81	93	97	94
	20	25	70	62	99
	30	3	62	56	96

U Tab. 1. rezultati pokazuju da obe sorte poseduju relativnu dormanciju jer bolje klijaju na izabranoj najnižoj temperaturi (10°C), kroz period od 12 dana trajanja ogleda, dok je dormancija ispoljena na višim temperaturama (20 i 30°C). Sorta Florida 302 je bila dormantnija od KG-65S, jer je njeno klijanje bilo više inhibirano ne samo na temperaturi od 10°C, već je potpuna inhibicija klijanja ispoljena i na temperaturi od 20°C. Mada uslovno, ovakav podatak nije saglasan sa činjenicom da su sorte sa pigmentisanom semenjačom (crvena zrna) dormantnije od onih sa belim zrnima (Bewley, Black, 1982).

Odmah posle žetve, kroz period od 90 dana, ispitivano je dejstvo dve izabrane temperature skladištenja (5 i 20°C) na dužinu dozrevanja zrna ispitivanih sorti. U intervalima posle 45 dana i posle 90 dana, uzorci su istovremeno postavljeni da klijaju 12 dana na tri temperature. Rezultati su dati u Tab. 2.

Očigledno je da postoje značajne razlike u delovanju dve izabrane skladišne temperature, pri čemu je skladišna temperatura od 20°C bila efikasnija u klijanju zrna obe sorte, u odnosu na nižu temperaturu od 5°VC. Njihov efekat je bio srazmeran dužini dozrevanja, ali obrnuto srazmeran sa temperaturama klijanja.

Pored toga, postoje značajnije razlike među sortama prema efektu skladišnih temperatura na dužinu dozrevanja. Dok je za sortu Florida 302 za prekidanje dorman-

cije bilo potrebno dozrevanje od 90 dana na temperaturi od 20°C, kod sorte Kg-65S je bilo dovoljno samo 45 dana i to na temperaturi skladištenja od 5°C.

Podaci u ovom radu su potvrdili činjenicu da među ispitivanim sortama postoje razlike ne samo u postžetvenoj dormanciji, već isto tako i u specifičnom efektu temperature u kontroli dormancije i postžetvenog dozrevanja. Iz podataka se vidi da su za dozrevanje pšenice povoljnije više temperature skladištenja, dok je klijanje bilo efikasnije na nižim temperaturama.

ZAKLJUČAK

– Ispoljeni efekti dve skladištene temperature na dozrevanje su bili različiti. Brzina dozrevanja zrna svih ispitivanih uzoraka bila je veća na temperaturi od 20°C, nego na 5°C.

– Bez obzira na temperaturni rang skladištenja dozrevanje je direktno zavisno od dužine skladištenja.

– Efekti tri klijajuće temperature (10, 20 i 30°C), su pokazali da su semena manje dormantna na nižim temperaturama dok je dormancija više ispoljena na visokim temperaturama.

– Od svih ispitivanih uzoraka sorta Florida je bila najviše dormantna, a KG-65S najmanje dormantna.

LITERATURA

Bewley, J.D. & Black, M. (1982): Physiology and Biochemistry of Seeds. – Springer Verlag, Berlin, Heidelberg.

George, W.D. (1967): High Temperature Seed Dormancy in Wheat *(Triticum aestivum* L.). – Crop Sci. *7*: 249-253.

Larson, A.H. Harvey R.B. & Larson, J. (1936): Length of dormant period in cereal seeds. – Agr. Besearch *52*: 811-836.

Popović, A. (1985): Isključivanje zrna na klasu, uticaj sorte i klimatskih faktora. – Semenarstvo *10-11*: 264-271.

Toole, E.H. (1923): Progres report on the germination of dermont heat. – Proc. Ass. Ott. Secd. Anal. *14*: 80-83.

Vegis, A. : Dormancy in higher plants. – Ann. Rev. Plant Physiol. *15*: 185-224.

Wellington, P.S. & Durham, V.D. (1958): Varietal differences in the tendency of Wheat to sprout in the ear. – Empire. J. Exptl. Arg. *XXVI*, 47-54.

Summary

STEFANOVIĆ MILENKO, SVILKIĆ BILJANA, TOPUZOVIĆ MARINA, STOJANOVIĆ JOVANKA[1]

EFFECTS OF SORT DIFFERENCES AND STORAGE TEMPERATURES ON AFTER RIPENING AND DORMANCY OF WHEAT KERNELS *TRITICUM AESTIVUM* L.

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The post-harvest ripening of cereal is a mechanism enabling the existence of dormancy in kernels and a condition for its cease. The dormancy and the after ripening are the temperature depending processes changing in the function of time.

The kernels of the examined sorts immediately after the harvest are less dormant if the germination takes place at the temperature of 10°C while the relative dormancy appears at higher temperature of germination (20 and 30°C).

The after ripening period at the chosen storage temperatures differs and depends on time and temperature.

Although, in dependence on time, the higher storage temperature is more efficient in after ripening and dormancy cease with the both sorts, there are considerable sort differences refering to the effect of the storage temperature on the after ripening duration. For the dormancy cease of the Florida 302 the after ripening lasts 90 days at 20°C while the germination needs 45 days of the after ripening even at 5°C.

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Original scientific paper

SLAĐANA JEVREMOVIĆ, LJILJANA RADOJEVIĆ

IN VITRO PLANT REGENERATION FROM STEM SEGMENTS OF SEVERAL CULTIVARS OF CHRYSANTHEMUM *(CHRYSANTHEMUM MORIFOLIUM* RAMAT.)

Institute for Biological Research „Siniša Stanković", Belgrade

Jevremović, S., Radojević, Lj. (1995): *In vitro plant regeneration from stem segments of several cultivars of chrysanthemum (Chrysanthemum morifolium Ramat.* – Glasnik Instituta za botaniku i botaničke bašte Univerziteta u Beogradu, Tom XXIX, 107 - 114.

Plant regeneration of *Chrysanthemum morifolium* Ramat. *cvs.*: „Fanshine Improved", „Pink Snowdon", „Klondike", „Yellow Spider", „Rivalry", „Crimson Robe", „Bronze Mundial" and „Tom Pierce" using nodal and internodal segments was obtained. Stem segments were cultured on MS mineral solution (Murashige and Skoog, 1962) containing 3% sucrose, 0.7% agar and (in mgL^{-1}): inositol 100, nicotinic acid 10, B_1 30, adenine sulphate 80 and tyrosine 100. This basal medium was supplemented with varying concentrations of indole 3-acetic acid (IAA, 0.1-0.5 mgL^{-1}) and benzyl aminopurine (BAP, 1 mgL^{-1}). Shoot multiplication takes place also on the same medium. Shoots yeild (19.2-90.0%) and average number of shoots *per* explant (2.4-8.6) were affected by the cultivar and medium. Micro shoots were on basal hormone free medium with 1% sucrose and 1/2 MS mineral solution successful rooted. Microplants 10-12 cm tall were transferred into pots, during spring, after 5-6 months plant flower evocation was observed in all plants.

Key words: *Chysanthemum morifolium* Ramat., micropropagation, stem segments culture
Ključne reči: *Chysanthemum morifolium* Ramat., mikropropagacija, kultura segmenata stabla

INTRODUCTION

Chrysanthemum morifolium Ramat. *(Asteraceae)* is a complex hybrid derived from several species that grow wild in China and Japan. This species is one of the three most important cut flowers in the world. Chysanthemums grown from seed are heterogeneous and are usually propagated by cuttings (Cathey, 1968). *In vitro* plant regeneration of Chrysanthemum has been reported earlier by Hill (1968), Roest and Bokelmann (1975), Sangwan et al., (1987), Lu et al., (1990), Bhattacharya et al., (1991) and others using different explants and media. There was no reports about *in vitro* tissue culture of chrisanthemum cultivars that we used in our experiments. This paper describes protocol for plant regeneration of 8 cultivares of chrysanthemum that has been commercially cultivated in our country.

MATERIALS AND METHODS

Stems of *Chrysanthemum morifolium* Ramat. *cvs.* „Fanshine Improved" („FI"), „Pink Snowdon" („PS"), „Klondike" („K"), „Yellow Spider" („YS"), „Rivalry" („R"), „Crimson Robe" („CR"), „Bronze Mundial" („BM") and „Tom Pierce" („TP") were prepared for tissue culture by method that were previously reported Radojević et al. (1987).

Nodal and internodal stem explants (0.3-0.5 cm) of flowered shoots were cultivated on A and B medium. Basal medium (BM) contained MS mineral solution (Murashige and Skoog, 1962), 3% sucrose 0.7% agar and (in mgL^{-1}): inositol 100, nicotinic acid 10, B_1 30, adenine sulphate 80 and tyrosine 100. This basal medium was supplemented with two concentrations of indole 3-acetic acid (IAA) and benzyl aminopurine (BAP). Medium A was BM medium supplemented with IAA 0.1 mgL^{-1} and BAP 1.0 mgL^{-1} and medium B was BM with IAA 0.5 mgL^{-1} and BAP 1 mgL^{-1}, pH 5.8. Shoot multiplication was on same media.

Shoot rooting was on C, D and E medium. The C medium contained MS mineral solution (Murashige and Skoog, 1962), 1% sucrose 0.7% agar and (in mgL^{-1}): inositol 100, nicotinic acid 10, pantotenic acid 10, B_1 2, B_6 1, adenine sulphate 80 and tyrosine 100, pH 5.8. Medium D was same as C only with 1/2 MS. Medium E was as medium C supplemented with NAA 0.02 mgL^{-1}. Rooted shoots of chrysanthemum were grown in greenhouse since flowering.

RESULTS AND DISCUSSION

Stem segments of chrysanthemum cultivars used in this work have different morphogenetic responses. Internodal segments first formed callus and then adventitious shoots (Fig. 1). Nodal segments develops axially buds while at the cutting sides callus appeared and then adventitious shoots are formed axailly (Fig. 2).

Morfogenetic responses of segments on A and B medium are represented in Tab. 1. It is evident from experimental results that the greatest morfogenetic response give *cv.* „CR" (90%) and the lowest 19.2 *cv.* „FI" on medium A (Fig. 3). Multiplication of shoots was by formation of axially and adventitious buds. Index of mutliplication is

represented also in Table 1. Medium A was better for propagation *cvs.* „FI", „PS", „K", „YW" and „TP" but, medium B was better for *cvs.* „R", „CR" and „BM" (Fig. 4, Fig. 5). W a n b u g u and R a n g a n (1981) has been reported that BAP in low concentrations induced multiple shoots and the presence of auxins did not significantly enhance the morfogenetic response. Previously, E a r l e and L a n g h a n s (1972) observed that higher concentrations of cytokinins favoured multiple shoot development. We used as cytokinin BAP in high concentration 1 mgL^{-1} and only in combination with auxin (IAA, 0.5 mgL^{-1}) desirable results are obtained (R a d o j e v i ć et al., (1994). L u et al. (1990) used NAA as auxin in multiplication medium and concentration of 1 mgL^{-1} was more effective than lower and higher concentrations (0.2, 0.5 and 2.0 mgL^{-1} NAA). Our results suggests that higher concentrations of IAA (0.5 mgL^{-1}) significantly increase multiplication (3.6) index of shoots only in *cv.* „R" on medium with 0.1 mgL^{-1} IAA to 8.6 on medium with 0.5 mgL^{-1} IAA. L u et al. (1990), after hormone treatment transferred explants to hormone free shoot elongation medium. In our case, this transfer is not needed, because plantlets grow fine on these combinations of auxins and cytokinin.

Tab. 1. – Effect of medium composition on morfogenetic response and shoot multiplication of chrysanthemum

CULTIVAR	MEDIUM (hormones in mgL^{-1}) A= BM + 0.1 IAA + 1.0 BAP B = BM + 0.5 IAA + 1.0 BAP	% of exsplants that forms shoots	multiplication index
"FI"	A	19.2	3.4 ± 1.4
	B	20.0	3.1 ± 0.5
"PS"	A	40.0	2.8 ± 0.6
	B	66.7	2.3 ± 0.2
"K"	A	50.0	3.4 ± 0.1
	B	70.0	2.8 ± 0.1
"YW"	A	50.0	4.4 ± 0.6
	B	30.0	3.6 ± 1.3
"R"	A	20.0	3.6 ± 1.2
	B	30.0	8.6 ± 2.3
"CR"	A	90.0	2.9 ± 0.3
	B	66.7	3.3 ± 1.0
"BM"	A	20.0	3.0 ± 0.7
	B	60.0	3.8 ± 2.2
"TP"	A	30.0	2.5 ± 0.2
	B	36.4	2.4 ± 0.4

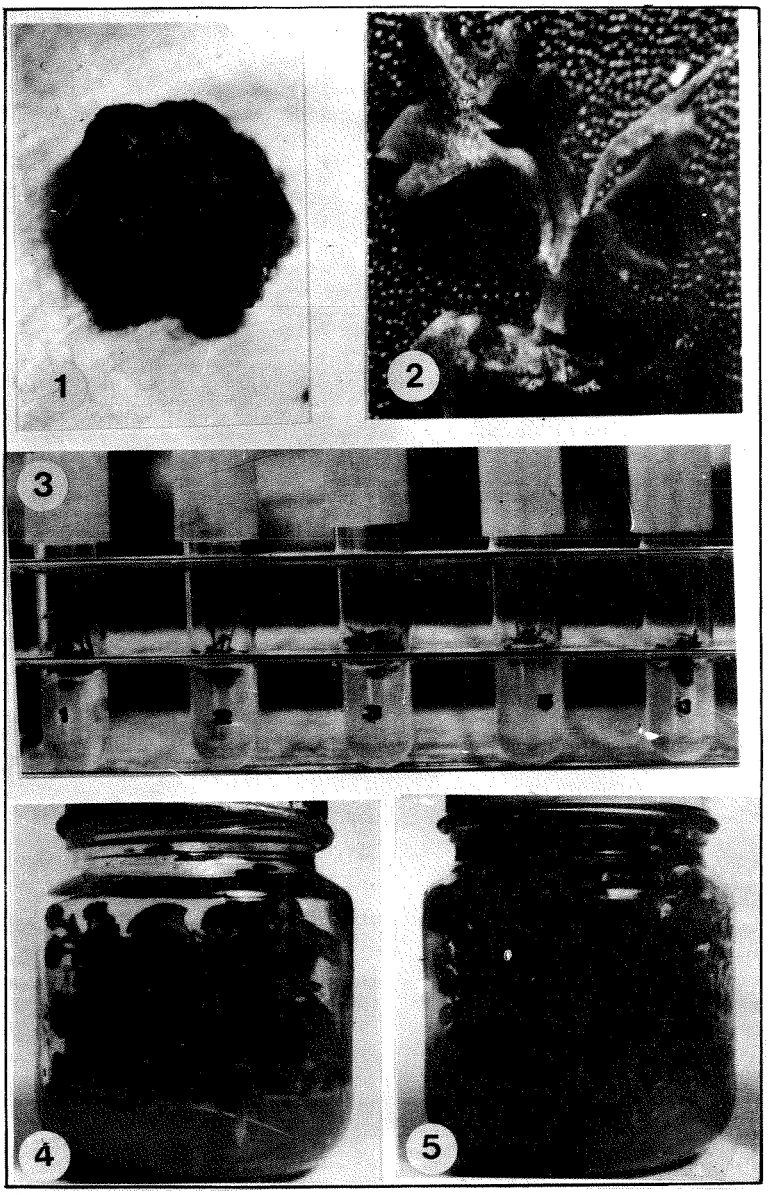
Microshoots (3-5 cm) were cultivated on rooting medium (C-E). The results obtained from three different rooting media (represented in Tab. 2) showed that number and length of roots depended of cultivar and medium. Rooting was the best

on D medium with 1/2 MS where number and length of roots was highest in all cultivars (Fig. 6). This results are similar as those described by B h a t t a c h a r y a et al. (1990) where half strenght MS medium was better than White's modified media. Medium supplemented with 0.02 mgL^{-1} NAA was proved to be less effective than MS hormone free medium.

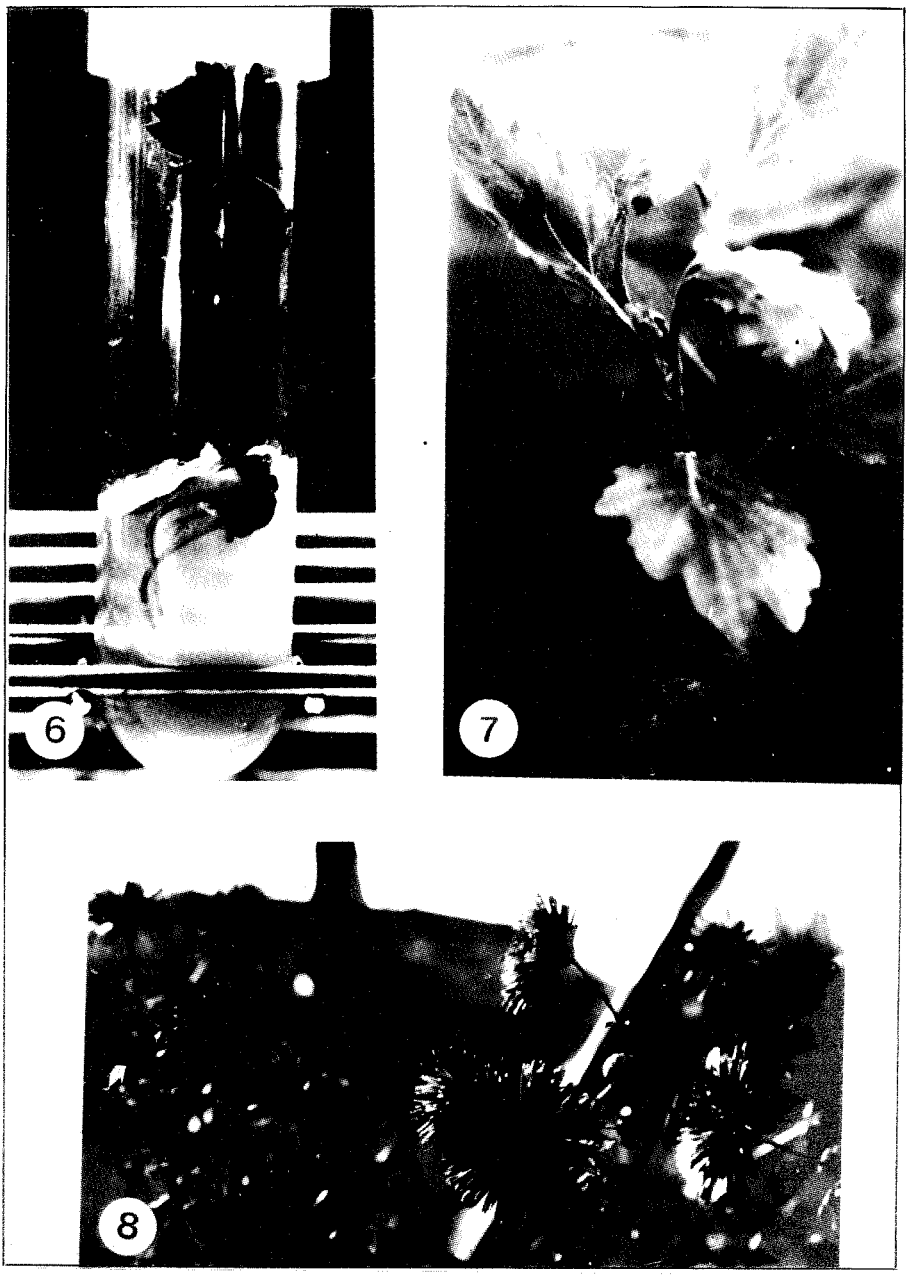
Tab. 2. – Chrysanthemum rooting on indicated media

CULTIVAR	MEDIUM (hormone in mgL^{1}) C = MS; D = 1/2 MS; E = MS + 0.02 NAA	Average N° of roots *per* plant	Average length of roots (mm)
"FI"	C	3.5 ± 2.5	72.3 ± 17.4
	D	4.3 ± 1.9	175.7 ± 63.9
	E	4.1 ± 2.4	82.3 ± 42.8
"PS"	C	7.4 ± 1.9	67.3 ± 27.3
	D	12.6 ± 3.5	120.4 ± 20.8
	E	9.5 ± 3.2	97.5 ± 20.6
"K"	C	2.6 ± 1.3	91.7 ± 56.9
	D	6.8 ± 3.2	123.8 ± 40.1
	E	5.1 ± 2.8	110.5 ± 48.8
"YW"	C	3.4 ± 1.0	52.6 ± 16.7
	D	3.5 ± 1.2	117.5 ± 23.8
	E	2.0 ± 0.8	75.5 ±15.1
"R"	C	1.0 ± 0.3	6.0 ± 2.1
	D	3.2 ± 1.1	82.2 ± 17.2
	E	1.2 ± 0.5	8.3 ± 2.3
"CR"	C	4.4 ± 2.5	61.5 ± 28.4
	D	5.7 ± 2.2	133.3 ± 29.3
	E	4.6 ±2.8	56.5 ± 22.2
"BM"	C	6.4 ± 3.5	16.2 ± 9.6
	D	4.8 ± 2.2	151.7 ± 8.5
	E	3.3 ± 2.4	32.4 ± 19.8
"TP"	C	8.7 ± 2.4	48.3 ± 10.5
	D	16.5 ± 2.6	75.8 ± 51.8
	E	8.4 ± 2.7	62.0 ± 31.5

Microplants about 10-12 cm tall were transferred into pots, during spring. Acclimatisation was achieved in *cv.* „FI"100% *cv.* „PS" 95%, *cv.* „K" 100%, *cv.* „YW" 98%, *cv.* „R" 96.5%, *cv.* „CR" 95.3%, *cv.* „BM" 98% and *cv.* „TP" 99% (Fig. 7). Plant development was normal and phase-change was induced after 5-6 mounts as seedling plantlets (Fig. 8). Phenotypic characters were the same as the donor explants.



Figs. 1.–5. – Micropropagation of *Chrysanthemum morifolium* in stem segments culture: 1. – Calus formation of internodal segments *cv.* „TP" 7-days in culture; 2. – Axially bud of *cv.* „TP" developed on internodal segment 7-days in culture; 3. – Stem segments of chrysanthemums *cvs.* „FI" (1), „PS" (2), „K" (3), „YW" (5) and „BM" (13) one mounth after initiation of culture; 4. – Shoot multiplication of *cv.* „Tom Pierce" on A medium (BM + 1 mgL^{-1} IAA + 1 mgL^{-1} BAP); 5. – Shoot multiplication of *cv.* „Rivarly" on B medium (BM + 0.5 mgL^{-1} IAA + 1 mgL^{-1} BAP)



Figs. 6. - 8. – Micropropagation of *Chrysanthemum morifolium* in stem segments culture: 6. – Shoot rooting of chrysanthemum *cv.* „TP" on D medium (MS 1/2); 7. – Acclimatized plant of *cv.* „FI"; 8. – Flowered plants of chrysanthemum *cv.* „K" in greenhouse

In present paper, we reported efficient, plant regeneration protocol of 8 cultivars of chrysanthemum by culture of stem segments. In conclusion, on this paper a new and efficient micropropagation protocol for *cv.* „FI", *cv.* „PS", *cv.* „K", *cv.* „YW", *cv.* „R", *cv.* „CR", *cv.* „BM", and *cv.* „TP" cultivars are achieved, being, the method also a new alternative for measurement the propagation procedure for *cv.* „FI", *cv.* „PS", *cv.* „K", *cv.* „YW", *cv.* „R", *cv.* „DR", *cv.* „BM" and *cv.* „TP" which has been previously described. The large numbers of shoots produced *per* explant and uniformity of regenerating plants make this system an ideal tool for chrysanthemum propagation, and a promising system for cryopreservation and the genetic manipulations.

REFERENCES

Bhattacharya, P., Dey, S., Das, N. & Bhattacharya, B. Ch. (1990): Rapid mass propagation of *Chrysanthemum morifolium* by callus derived from stem and leaf explants. – Plant Cell Rep. *9*: 439-442.

Cathey, H.B. (1969): *Chrysanthemum morifolium* (Ramat.) Hemsl. *In* The induciton of Flowering (I.T. Evans, ed) pp. 269-290, Mac Millan of Australia.

Earle, E.D. & Langhans, R.W. (1974): Propagation of Chrysanthemum *in vitro*. I. Multiple plantlets from shoot tips and the establishment of tissue cultures. – J. Amer. Soc. Hort. Sci. *99*: 128-132.

Hill, G.P. (1968): Shoot formation in tissue cultures of *chrysanthemum* „Bronze Pride". – Physiol. Plantarum *21*: 386-389.

Lu, C., Nugent, G. & Wardley, T. (1990): Efficient, direct plant regeneration from stem segments of chrysanthemum (*Chrysanthemum morifolium* Ramat. *cv.* Royal Purpure). – Plant Cell Rep. *8*: 733-736.

Murashige, T. & Skoog, F. (1962): A revised medium for rapid growth and bioassays with tobacco cultures. – Physiol. Plantarum *15*: 473-497.

Radojević, Lj., Đorđević, N. & Mančeva, L. (1987): Clonal multiplication of gerbera by *in vitro* culture of different tissues. – Sym. Florizel 87 Arlon – Belgium. Plant micropropagation in Horticultural Industries. pp. 218-223.

Radojević, Lj., Marinković, N., Zdravković-Korać, S. & Jevremović, S. (1994): Primena različitih postupaka *in vitro* kulture u mikropropagaciji aftičke ljubičice, hrizanteme i karanfila. – Savremena poljoprivreda *42*(6): 117-127.

Roest, S. & Bokelmann, G.S. (1975): Vegetative propagation of *Chrysanthemum morifolium* Ram. – Sci. Hort. *3*: 317-330.

Sangwan, R.S., Detrez, C. & Sangwan-Norrel, B.C. (1987): *In vitro* culture of shoot tip meristems in some higher plants. – Acta Hortic. *212*: 661-666.

Wambugu, F.M. & Rangan, T.S. (1981): *In vitro* multiplication of pyrethrum (*Chrysanthemum cinerariaefolium* Vis.) by micropropagation. – Plant Science Letters *22*: 219-226.

Rezime

SLAĐANA JEVREMOVIĆ, LJILJANA RADOJEVIĆ

REGENERACIJA BILJAKA PRIMENOM KULTURE *IN VITRO* SEGMENATA STABLA KOD NEKOLIKO KULTIVARA HRIZANTEME (*CHRYSANTHEMUM MORIFOLIUM* RAMAT.)

Institut za biološka istraživanja „Siniša Stanković", Beograd

Proučavana je regeneracija biljaka *Chrysanthemum morifolium* Ramat. cvs.: „Fanshine Improved", „Pink Snowdon", „Klondike", „Wellow Spider", „Rivalry",

„Crimson Robe", „Bronze Mundial" i „Tom Pierce" u kulturi nodalnih i internodalnih segmenata stabla. Eksplanti su gajeni na MS hranljivoj podlozi sa mineralnim rastvorom Murashige i Skoog, (1962) 3% saharozom, 0,7% agarom i (u mgL^{-1}): inozitol 100, nikotinska kiselina 10, B_1 30, adenin sulfat 80 i tirozin 100, indol 3-sirćetna kiselina (IAA, 0,1-0,5 mgL^{-1}) i benzil aminopurin (BAP, 1 mgL^{-1}). Umnožavanje izdanaka je postignuto na istoj MS hranljivoj podlozi. Morfogenetski odgovor eksplantata (19,2-90,0%) kao i prosečan broj izdanaka po eksplantatu (2.4-8,6) zavisili su od kultivara i hranljive podloge. Najbolje oživljavanje „mikro" izdanaka je bilo na MS podlozi bez hormona sa 1% saharozom. Biljke, veličine 10-12 cm, odgajane su u uslovima staklare. Aklimatizacija „mikro" biljaka se odvijala u proleće i iznosila je 95-100% u zavisnosti od sorte. Posle 5-6 meseci biljke hrizanteme su cvetale i imale su istu boju cveta kao biljke donori.

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Original scientific paper

JOVANKA MILJUŠ-ĐUKIĆ, DRAGAN VINTERHALTER,
BRANKA VINTERHALTER, MILICA ĆALOVIĆ, SLAVICA NINKOVIĆ

IN VITRO PROPAGATION AND AGROBACTERIUM- MEDIATED TRANSFORMATION OF POTATO CV. DESIREE

Institute for Biological Research „Siniša Stanković", Belgrade, Jugoslavija

Miljuš-Đukić, J., Vinterhalter, D., Vinterhalter, B., Ćalović M. and Ninković, S. (1995): *In vitro propagation and Agrobacterium- mediated transformation of potato cv. Desiree.* – Glasnik Instituta za botaniku i botaničke bašte Univerziteta u Beogradu, Tom XXIX, 115 - 121.

Shoot cultures of cv. Desiree were established from five different sources and were maintained on MS medium, without phytohormones. The addition of adenine sulfate provided higher number of internodes. One of five clones was virus-free, according to ELISA test. That clone, designed as PKB, was used for transformation. Transformed roots were obtained by inoculating shoot segments with *Agrobacterium rhizogenes* A4M70 GUS. Hairy roots appeared in 90% of explants. The transformation was confirmed by assaying the activity of β- glucuronidase enzyme.

Key words: *Solanum tuberosum* L., potato, micropropagation, *Agrobacterium rhizogenes,* β-glucuronidase.

Ključne reči: *Solanum tuberosum* L., krompir, mikropropagacija, *Agrobacterium rhizogenes*, β-glukuronidaza.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important food crops in the world. Some genetic characteristics of potato including polyploidy, self-incompatibility and high heterozigosity make application of classical breeding methods difficult in this species. Techniques of genetic engineering via *Agrobacterium* mediated transformation offer interesting oportunities according to which certain useful traits can be directly introduced into economically important potato cultivars.

It is well known that soil bacteria *Agrobacterium tumefaciens* and *A. rhizogenes*, can induce appearance of crown galls and hairy roots in many plant species. Bacteria can transfer specific regions of their Ti plasmids, (T-DNA) into the genome of plant cells (Hoemaka et al., 1984). This natural gene-transferring system, was exploited to transform many plant species, including potato (Zambryski et al. 1983., An et al., 1986). In recent years, many reports have been dedicated to transformation of various potato cultivars.

Ooms et al., (1983) were first to report transformation of potato cv. Moris Bard. They studied the appearance of tumors which appeared after wounding and inoculation of potato shoots with *A. tumerfaciens*. Galls (tumor) tissue could regenerated shoots and roots. Shoots manifested lysopine dehydrogenase activity (LpDH) which is a positive sign of transformation. When these shoots were grafted uppon stems of normal plants they could form stolons and within three months tubers. Among the shoots regenerated from galls authors detected a number of shoots with abnormal morphology and chromosome number. In the further study, the same authors (Ooms et al., 1987), refined transformation method with *A. tumefaciens* and obtained morphologicaly normal transgenic plants of cv. Desiree.

Ooms et al., (1985) also studied transformation of cv. Desiree with *A. rhizogenes* and showed that Ri plasmids also can be used as a vector to introduce genes via Ri T-DNA into potato. Shoots inoculated with the bacteria developed abundant roots which were further cultured and studied. Shoots were regenerated from callus which developed on root cultures. Transformed plants distincly differed from untransformed potatoes. Growth of the transformed plants was more vigorous but the final size of plants and tuber yield were similar.

Shahin and Simpson (1986) developed a transformation system in which leaf discs were cocultivated with disarmed (non oncogenic) LBA4404 *A. tumefaciens* contained binary vector pARC8 with Nos/Npt gene. Transformed nature of plants was assayed by neomycin phospho-transferase II activity.

Tuber slices were used as explants for potato transformation with *A. tumefaciens* in work of Sheerman and Bevan (1988). Shoots appeared within 4 weeks without intervening callus stage. Shoots were first rooted and then screened for transformants on medium supplemented with kanamycine. They worked with Desiree cultivar and out of 200 independant transformants only one was morphologicaly different from parental types. At the same time Stiekama et al., (1988) also presented a study on transformation of Bintje and Desiree using disarmed binary *A. tumefaciens* LBA 4404/ pBi121 vector system. Explants for inoculation with bacteria were tuber discs from which shoots were efficiently regenerated and rooted. In this case, transgenic plants were assayed with GUS test.

Besides the experiments of potato transformation with *A. tumefaciens* strains, different *A. rhizogenes* strains were used too. De Vries-Uijtewaal et al., (1988)

studied transformation of monohaploid and diploid genotypes with *A. tumefaciens* LBA1020 and *A. rhizogenes* LBA 9402, both containing the Ri1855 plasmid. Transformation efficiency was generaly higher in diploids than monohaploids. Hanish ten Cate et al., (1988) obtained transformed root lines of Bintje and Desiree with the LBA 9402 and AR 15834 using leaf segments and tuber discs as explants. Shoots were induced from roots but even more from compact green callus adjoining roots. However only 10% of transformed root lines could regenerate shoots. Transgenic Ri-Desiree plants were all uniform and corresponded to the normal phenotype whilst Ri-Bintje plants showed a pattern of phenotypic variation.

Beside those authors, Visser et al., (1989) transformed potato using a binary vector in virulent *A. rhizogenes* strains (AM8703/ pRi1855 and pBi121). The transformation efficiency was much higher with *A. rhizogenes* then *A. tumefaciens*.

De Block (1988) transformed Desiree, Bintje, Berolina and Russet Burbank by co-cultivation of leaves in bacterial suspension of C58C1 strain carring *nptII* and *bar* genes. The *bar* gen codes for the enzyme phosphinotricin acetyltransferase (PAT) which inactivates herbicide phosphinotricin (glufosinate). Thus transformed plants were resistant to commercial herbicide Basta at high concentration (20 l/ha). Almost recently Figuera Filho et al., (1994) reported upon transformation of several Brazilian potato cultivats with *A. tumefaciens* carrying the pGV1040bar confering the resistance to phosphinotricin herbicides.

Newell et al., (1991) transfered potao virus X and Y coat protein genes into Russet Burbank, using *A. tumefaciens* pMON 9809 vector containing the PVX coat protein gene. The levels of PVX coat protein in transformed shoots were detected by an ELISA assay.

One of the last reports on potato transformation was Nadolska-Orczyk et al., 1995 who reported successfull transformation of 12 polish potato cultivars. The authors used well known strains of *A. tumefaciens* LBA 4404/ pBi121 and C58C1/ pVU104, carrying the NPT and GUS genes. They regenerated plants from leaf explants, which rooted well.

In our country Desiree is the leading potato cultivar presenting 80% of the total potato production. We therefore decided to use cultivar Desiree as a standard model system in our investigation on potato. In this paper we present preliminary research on the use of *in vitro* methods and *Agrobacterium* mediated transformation in the breeding of new, improved potato cultivars.

For transformation studies we used *Agrobacterium rhizogenes*, strain A4M70GUS, which induces hairy roots. This strain carries gene coding for enzyme β-glucuronidase, whose activity can be detected histochemically in the transformed tissues.

MATERIAL AND METHODS

Plant culture: Shoot cultures of cv Desiree were established from etiolated tuber sprouts. Surface sterilization was performed for 20 minutes in 10% solution of commercial bleach containing 4-6% NaOCl. MS (Murashige & Skoog, 1962) medium which was used in all experiments was supplemented with 3% sucrose. Subculturing was performed by segmentation at regular 3-4 week intervals. Segments containing single axillary bud were at least 5 mm long. Cultures were maintained in 100 ml wide neck Erlenmayer flasks or 150 ml blod transfusion bottles. Both types of culture vessels were stopped with cotton wool plugs. Clone PKB which we used for transfor-

mation studies was virus-free according to ELISA test (Sigma enzyme immunoassay kit for potato virus A, M, S, X, Y, detection). Test was performed in Center for potato, Guča by Dr D. Milošević.

Conditions in the growth room: Photoperiod was 18/6 hours light to darknes, provided by cool white fluorescent lamps, irradiance 5.0-7.2 Wm^{-2} and temperature 25 $\pm 2^{o}C$.

The transformation procedure: The *A. rhizogenes*, A4M70GUS (Tepfer, M. and Delbart, C. F. 1987) was maintained on agar (1,5%) solidified YEB medium (Van Larebeke et al., 1977), with antibiotic neomycine. The density of bacterial suspension was about 10^8 bacteria/ml. The 2 cm long shoot segments were inoculated by wounding with a sterile needle, shortly dipped in the bacterial suspension. The explants were then left on the same media in the growth room, and after 2 days transferred to a medium supplemented with cefotaxime, 100 mg/l. Culture were screened after four weeks. About 50 pieces of potato shoots were inoculated in one experiment.

GUS assay: Roots were cut from the stems and β-Glucuronidase enzyme activity was detected, using X-gluc at pH 7.0, after overnight incubation at 37°C (Jefferson et al., 1987).

RESULTS AND DISCUSSION

Establishment and maintenance of shoot cultures

Shoot cultures of cv. Desiree were established from five different sources. Only one of them, designated clone PKB, was found to be virus free. This clone was used in further experiments, while the others was eliminated. Clone PKB from the beginning had more sturdy shoots than the other four introductions.

In a preliminary study on the maintenance of potato shoot cultures we showed that addition of cytokinins to the medium offers no improvement in standard growth parameters. Thus on the hormone free medium the mean number of internodes per explant was 8.6 ± 0.2 and the shoot length 81.6 ± 2.9 mm. Only the addition of adenine sulfate was benefitial providing higher number of internodes per explant 9.7 ± 0.2 and somewhat longer shoots 89.4 ± 2.5 mm at 100 mg/l. Growth of shoot cultures on the hormone free medium was stable and here was no visible variation in growth pathern of successive subcultures.

In transformation studies on potato in which shoots were used as explants for inoculation (Ooms et al., 1983, 1985, 1987 and others) shoots were previously cultured on the hormone free medium. In contrast, the use of leaf and tuber tissue requires presence of both cytokinin and auxin type growth regulators.

Transformation

The appearance of hairy roots after transformation was fast. First root could be observed in the inoculation zone within ten days. This hairy roots could be easily distinguished from adventitious roots which developed on the cut end of shoot explants. Production of roots was not accompanied by formation of callus.

After four weeks on the cefotaxime supplemented transformation medium development of hairy roots was registered in 90% explants i.e 45 out of 50 inoculated shoots.

To investigate the ability of hairy roots for individual growth they were excised and re-cultured on the same type medium. Roots not only elongated well but also produced numerous laterals which enabled us to establish true root cultures. Spontaneous regeneration of shoots was not recorded.

Rapid growth associated with high branching of roots obtained after inoculation of plants with *A. rhizogenes* is a good preliminary indication of successfull transformation (Dobigny, A. et al., 1995). The hairy roots tested for GUS activity were coloured uniformly blue (Fig. 1, Fig. 2). This can be accepted as a proof for positive transformation of potato roots.

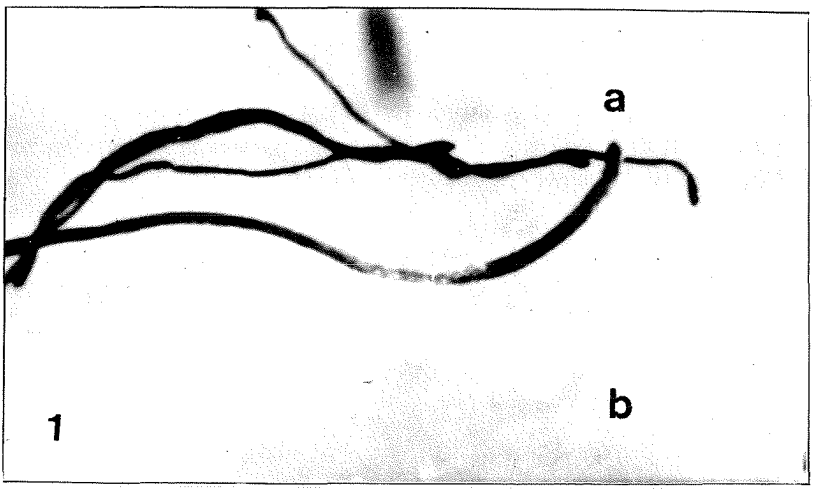

Fig. 1. – Detection of β-glucuronidase activity by histological assay: transformed root becomes blue (a), while control root remains white (b).

Fig. 2. – Phenotypes of root lines: most of the transformed root are highly branched and exhibited a high growth rate

GUS positive roots were fixed in FAA fixative and prepared for hystological investigation which we plan to perform in due course.

The *A. tumefaciens* strain A4 is agropine type, whose Ri plasmid contains two fragments of T-DNA, a TL-DNA carrying the *rol* genes and a TR- DNA carrying genes encoding for opine and auxine synthesis. So, the presence of TL- DNA in transformed tissues can not be detected only by opine marker, and that is the reason we did not performed that analysis.

In this study, a protocol for genetic transformation in potato using a *A. rhizogenes* A4 M70GUS was successfully developed. This transformation method can be used as a routine method for introducing foreign genes into local potato cultivars.

ACKNOWLEDGEMENT

The authors express their gratitude to Dr. P. Landre, Universite P. et M. Curie VI, Paris for kindly providing the culture of *Agrobacterium rhizogenes strain A4M70GUS*. This work was supported by the Ministry of Science and Technology of Serbia, contract No. E0 5 - 8.

REFERENCES

An, G., Watson, B.D. & Chiang, C. C. (1986): Transformation of tobacco, tomato and potato and *Arabidopsis thaliana* using a binary Ti vector system. – Plant. Phyiol. *81*: 301-305.

DeVries - Uijtewaal, E., Gilissen, L. J. W., Flipse, E., Sree Ramulu, K. & De Groot, B. (1988): Characterization of root clones obtained after transformation of monohaploid and diploid potato genotypes with hairy root inducing strains of *Agrobacterium*. – Plant Science, *58*: 193-202.

De Blosk, M. (1988): Genotype-independent leaf disc *transformation* of potato (*Solanum tuberosum*) using *Agrobacterium tumefaciens*. – Theor. Appl. Genet. *76*: 767-774.

Dobigny, A., Ambroise, A., Haicour, R., David, C., Rossignol, L. & Sihachakr, D. (1995): Transformation of potato using mannopine and cucumopine strains of *Agrobacterium rhizogenes*. – Plant Cell, Tissue and Organ Culture *40*: 225-230.

Figuera Filho, S. E. Figueiredo, L. F. A. & Monte - Neshich, D. C. (1994): Transformation of potato (*Solanum tuberosum*) cv. Mantiqueira using *Agrobacterium tumefaciens* and evaluation of herbicide resistance. – Plant Cell Reports *13*: 666-670.

Hanischten Cate, Ch. H., Ennik, E., Roest, S., Sree Ramulu, K., Dijkhuis, P. & de Groot, B. (1988): Regenaration and characterization of plants from potato root lines transformed by *Agrobacterium rhizogenes*. – Theor. Appl. Genet. *75*: 452-459.

Hoekama, A., Hooykaas, P. L. & Schilperoort, R. A. (1984): Transfer of the octopine T-DNA segment to plant cells modified by different types of *Agrobacterium* Tumor or Root inducing plasmids. Generality of virulence systems. – Journal of Bacteriology *158*: 383-385.

Jefferson, R. A., Kavanagh, T. A. & Bevan, M. W. (1987): GUS fusions: Ω-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. - EMBRO J. *6*: 3901-3907.

Murashige, T. & Skoog, F. (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. – Physiol. Plant. *15*: 473-497.

Nadolska - Orczyk, A., Milkowska, L., Palucha, A., Czembor, P. & Orczyk, W. (1995): Regeneration and transformation of polish cultivars of potato. – Acta Soc. Bot. Poloniae *4*: 335-340.

Newel, C. A., Rozman, R., Hinchee, M. A., Lawson, E. C., Haley, L., Sanders, P., Kaniewski, W., Tumer, N. E., Horsch, R. B. & Fraley, R. T. (1991): *Agrobacterium* - mediated transformation of *Solanum tuberosum* L. cv. Russet Burbank. – Plant Cell reports *10*: 30-34.

Ooms, G., Karp, A. & Roberts, J. (1983): From tumour to tuber; tumour cell characteristics and chromosome numbers of crown gall-derived tetraploid potato plants (*Solanum tuberosum* cv. „Maris Bard"). – Theor. Appl. Genet. *66*: 169-172.

Ooms, G., Karp, A., Burrell, M. M., Twell, D. & Roberts, J. (1985): Genetic modification of potato development using Ri T-DNA. – Theor. Appl. Genet. *70*: 440-446.

Ooms, G., Burrell, M. M., Karp, A., Bevan, M. & Hille, J. (1987): Genetic transformation in two potato cultivars with T-DNA from disarmed *Agrobacterium*. Theor. Appl. Genet. *73*: 744-750.

Shahin, E. A. & Simpson, R. B. (1986): Gene transfer system for potato. – Hort. Science *21*: 1199-1201.

Stiekama, W. J., Heidekamp, F., Louwerse, J. D., Verhoeven, H. A. & Dijkhuis, P. (1988): Introduction of foreign genes into potato cultivars Bintje and Desiree using an *Agrobacterium tumefaciens* binary vector. – Plant Cell Reports *7*: 47-50.

Sheerman, S., Bevan, M. W. (1988): A rapid transformation method for *Solanum tuberosum* using binary *Agrobacterium tumefaciens* vectors. – Plant. Cell Reports *7*: 13-16.

Tepfer, M. & Delbart, C. F. (1987): *Agrobacterium rhizogenes* as a vector for transformating higher plants. – Microbiol. Sci. *4*: 24-28.

Van Larebeke, N., Genetello, C. H., Hernalsteens, J. P., De Picker, A., Zaenenl., E., VanMontagu, M. & Schell, J. (1977): Transfer of Ti plasmids between *Agrobacterium* strains by mobilisation with the conjugative plasmid RP4. – Molec. Gen. Genet. *152*: 119-124.

Visser, R. G. F., Jacobson, E., Witholt, B. & Feenstra, W. J. (1989): Efficient transformation of potato (*Solanum tuberosum* L.) using a binary vector in *Agrobacterium rhizogenes*. – Theor. Appl. Genet. *78*: 594-600.

Zambryski, P., Joos, H., Genetello, C., Leemans, J., VanMontagu, M. & Schell, J. (1983): Ti plasmid vector for the introducing of DNA into plant cells without alteration of their normal regeneration capacity. – EMBO, J. 2: 2143-2150.

Rezime

JOVANKA MILJUŠ-ĐUKIĆ, DRAGAN VINTEHRALTER,
BRANKA VINTERHALTER, MILICA ĆALOVIĆ, SLAVICA NINKOVIĆ

IN VITRO PROPAGACIJA I TRANSFORMACIJA KROMPIRA CV. DESIREE POMOĆU *AGROBACTERIUM RHIZOGENES*

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Kulture pupoljaka cv. Desiree uspostavljene su iz pet različitih klonova i održavane na MS medijumu bez fitohormona. Primećeno je da dodavanje adenin sulfata dovodi do povećanja broja internodija. Jedan od pet klonova, označen kao PKB, koji je korišćen za transformaciju, bio je slobodan od virusa, prema ELIZA testu. Transformisani korenovi su dobijeni inokulacijom sa *Agrobacterium rhizogenes* sojem A4M70GUS. Korenovi su se pojavili na 90% eksplantata. Transformacija je potvrđena testom za određivanje aktivnosti β-glukuronidaze.

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Original scientific paper

MILICA ĆALOVIĆ, BRANKA VINTERHALTER, DRAGAN VINTERHALTER

IMPROVED PLANT REGENERATION FROM MATURE EMBRYO DERIVED CALLUS OF WHEAT (*TRITICUM AESTIVUM* L.)

Institute for Biological Research „Siniša Stanković", Belgrade

Ćalović, M., Vinterhalter, B. and Vinterhalter, D. (1995): *Improved plant regeneration from mature embryo derived callus of wheat (Triticum aestivum L.)*. – Glasnik instituta za botaniku i botaničke bašte Univerziteta u Beogradu, Tom XXIX, 123 - 128.

Compact, yellowish and smooth callus of five wheat cultivars was obtained by culturing mature (ripe) embryos on MS (M u r a s h i g e and S k o o g, 1962) medium with 2.0 mg/l 2,4-D. Callus developed from the embryo axis and not from scutellum which turned brown. Differentiation took place on MS medium uppon decrease of 2.4-D concentration in the medium to 0.2 mg/l. Regeneration of whole plantlets from this callus was low ranging from 0 to 7% of explants irrespectivelly of the orientation of the embryo on the medium. However, the exscision of scutellum from the embryo significantly increased the percentage of explants (embryos) which regenerated plantlets. Depending on genotype within range from 9 to 22%. We believe that this increase of the regenerative ability of mature embryos is an important first step which will finaly enable mature embryos to be used as starting material in various *in vitro* techniques aimed at breeding new wheat cultivars and genetic engineering.

Key words: mature embryo culture, scutellum, somatic embryogenesis, *Triticum aestivum* L., wheat.

Ključne reči: kultura zrelih embriona, skutelum, somatska embriogeneza, *Triticum aestivum* L., pšenica.

INTRODUCTION

Wheat is one of the oldest and most important food crops in the world. It is a cereal grass of the Gramineae (Poaceae) family, genus *Triticum*.

During the past 20 years, numerous attempts have been made to obtain wheat tissue cultures which possess the capacity for efficient plant regeneration as a basic prerequisite for crop improvement programs. However, only a small number of plants have been obtained from cultures initiated from mature embryo and differentiated tissues (Shimada et al., 1969; Dudits et al., 1975; Bhojwani and Hayward, 1977; Chin and Scott, 1977). It has been showed that only calli derived from immature embryo scutellum manifest high-frequency regeneration of plants (Shimada, 1978; Shimada and Yamada, 1979; Gosch-Wackerle et al., 1979; Ozias-Akins and Vasil, 1982; Sears and Deckard, 1982; Lazar et al., 1983). Since the most responsive explant source – immature embryo is available only in a very short period of the year, then a procedure based on the use of mature embryos would be wellcome.

MATERIALS AND METHODS

Seeds of five cultivars, *Triticum aestivum* L.: Jugoslavija, Lepenica, Sana, San Pastore and Bankuty were surface sterilised sequentially with 95% ethanol (3 min), 20% commercial bleach (4-5% sodium hypochlorite) (20 min) and 0.2% mercuric chloride (15 min) and thoroughly washed with sterile destilled water. Mature embryos were excised under a binocular microscope. In treatment A, mature embryos of all five cultivars were placed on medium with axial side up (scutellum in contact with medium), in treatment B with the axial side down (scutellum exposed) and in C scutellum was removed and discarded.

The induction medium consisted of MS inorganic salts, 30 g/l sucrose, 100 mg/l inositol, 2.0 mg/l glycine, 0.5 mg/l B6, 0.5 mg/l nicotinic acid, 0.4 mg/l B1 and 2.0 mg/l 2,4-D. The medium was adjusted to pH 5.8 with NaOH, solidified with 0.64% agar, and autoclaved at 114°C for 20 min. Tissue cultures were cultured in plastic sterile Petri dishes (15 x 90 mm), each dish contained 40 ml medium and 8 explants or calli.

Temperature in the growth room was 25 ± 2°C, photoperiod 16/8 hours light to darkness and irradiance 33.5-46.5 μmol $m^{-2}s^{-1}$ provided by 65W 4500°K white fluorescent lamps.

After 28 d of incubation explants forming callus were transferred to differentiation medium supplemented with 0.5 mg/l 2,4-D. After next 28 d explants were transferred again to the medium where the hormone was reduced to 0.2 mg/l.

RESULTS AND DISCUSSION

Mature embryos of *Triticum aestivum* cultured on MS supplemented with 2.0 mg/l 2,4-D showed the initiation of callus within a week and at the end of 4 week, slowly proliferating, compact, smooth surfaced and yellowish calli were obtained. In all three experimental treatments (A, B, C) callus formation was associated with embryo axis, whilst scutellum, if present, turned brown and deteriorated within 1-2 weeks. According to histological investigations performed by Ozias-Akins and Vasil (1983a) in mature embryo callus arises from tissues within and near the procambium of the axis,

whilst in immature embryo callus is formed from parenchyma cells of the scutellum. Their results are in accordance with our findings that in mature embryos scutellum is not required for callus proliferation.

The frequency of callus induction in all five cultivars was similar and generally high, ranged from 89% to 100% (Tab. 1). Such a small variation indicate that the genotype plays no important role in the frequency of callus induction. This result is consistent with the report of O'Hara and Street (1978) and is in contrast with that of Lazar et al. (1983).

Tab. 1. – Frequency of callus induction, embryogenic callus formation and total number of regenerated plants from five Triticum aestivum cvs.: Jugoslavija, Lepenica, Sana, San Pastore and Bankuty after 8 weeks on MS medium with decreased 2,4-D (A – mature embryos with scutellum in contact with medium, B – mature embryos with scutellum that is not in contact with medium and C – scutellum-less mature embryos)

Treatment	Cultivar	Embryos	Calli		Embryogenic calli		Regenerated plants
		No	No	(%)	No	(%)	No
A	Jugoslavija	45	40	(89)	0	(0)	0
	Lepenica	45	42	(93)	1	(2)	2
	Sana	45	40	(89)	2	(5)	3
	San Pastore	45	43	(95)	1	(2)	4
	Bankuty	45	41	(91)	3	(7)	23
B	Jugoslavija	45	43	(95)	1	(2)	5
	Lepenica	45	45	(100)	1	(2)	8
	Sana	45	43	(95)	1	(2)	4
	San Pastore	45	44	(97)	1	(2)	2
	Bankuty	41	41	(100)	2	(5)	7
C	Jugoslavija	45	43	(95)	4	(9)	48
	Lepenica	60	57	(95)	10	(17)	108
	Sana	60	57	(95)	9	(16)	99
	San Pastore	60	59	(98)	13	(22)	73
	Bankuty	45	41	(91)	7	(17)	53

The obtain plant regeneration calli were transferred to media with decreased concentration of auxin. After 3-4 weeks of incubation some calli extensively produced only roots and others exhibited localised nodular area from which evantualy somatic embryos developed. Thus in wheat plant regeneration was coupled with somatic embryogenesis.

Appart from normal somatic embryos, often multiple shoots formed as a result of precocius germination of the primary embryo (Fig. 1 and 2). This is a common situation in wheat previously described by Ozias-Akins and Vasil (1982, 1983b).

In this case first the scutellum of the primary somatic embryo enlarges (leaffy scutellum structure) and then in its base numerous shoots appear. According to Ozias-Akins and Vasil (1982) multiple shoot formation from somatic embryos result from the absence of apical dominance.

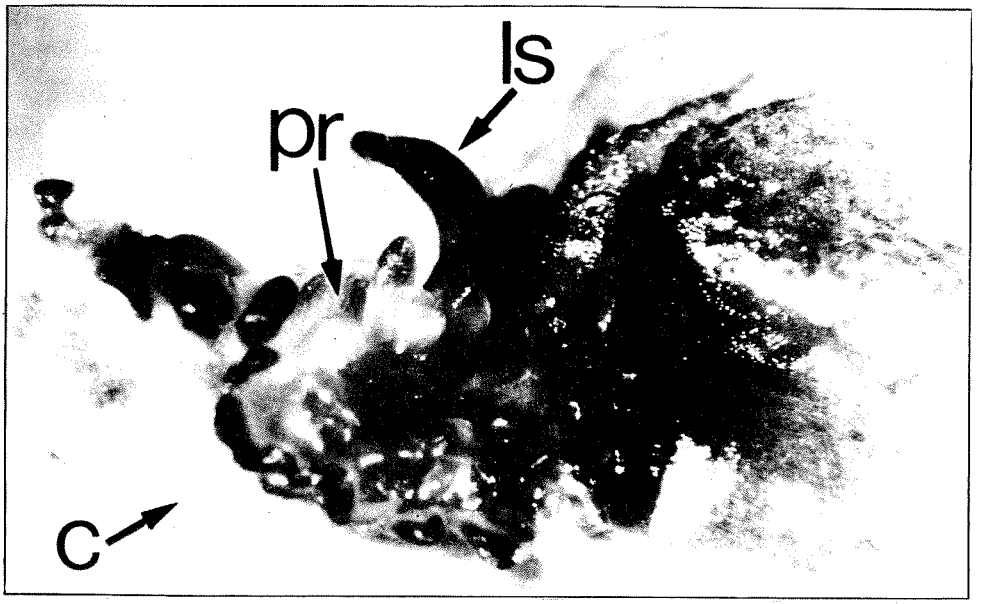

Fig. 1. – Callus derived from mature embryo with numerous shoot primordia after 4-8 weeks on regeneration medium (*pr* – shoot primordia, *ls* – leafy scutellum, *c* – callus)

The results of three treatments for callus formation and plant regeneration are presented in Table 1. The induction frequency of embryogenic callus of all 5 cultivars was significantly higher (≈ 10 times) when scutellum-less embryos were used (treatment C) instead of whole embryos (treatments A and B) as primary explants. In the third group genotypic variation could be observed since embryogenic calli production ranged from 9% (Jugoslavija) to 22% (San Pastore). Regenerative potential as indicated by the frequency of embryogenic callus formation obtained in this way was significantly increased in comparison to previously reported results of 0,4-3,2% (Lazar et al., 1983), 0-4% (Shimada and Yamada, 1979) and 7% (Chin and Scott, 1977).

Regenerative efficiency of the embryogenic callus of the cultivaras was very low in treatments A and B (with exception in cultivar Bankuty). This was not the case with calli derived from mature embryos without scutellum where we observed originated single plantlets and multiple shoot formation in evidently higher number. In cultivar Lepenica, 108 was a total number of regenerated plantlets and in cultivars Lepenica and Sana 34 plantlets were obtained from a single callus.

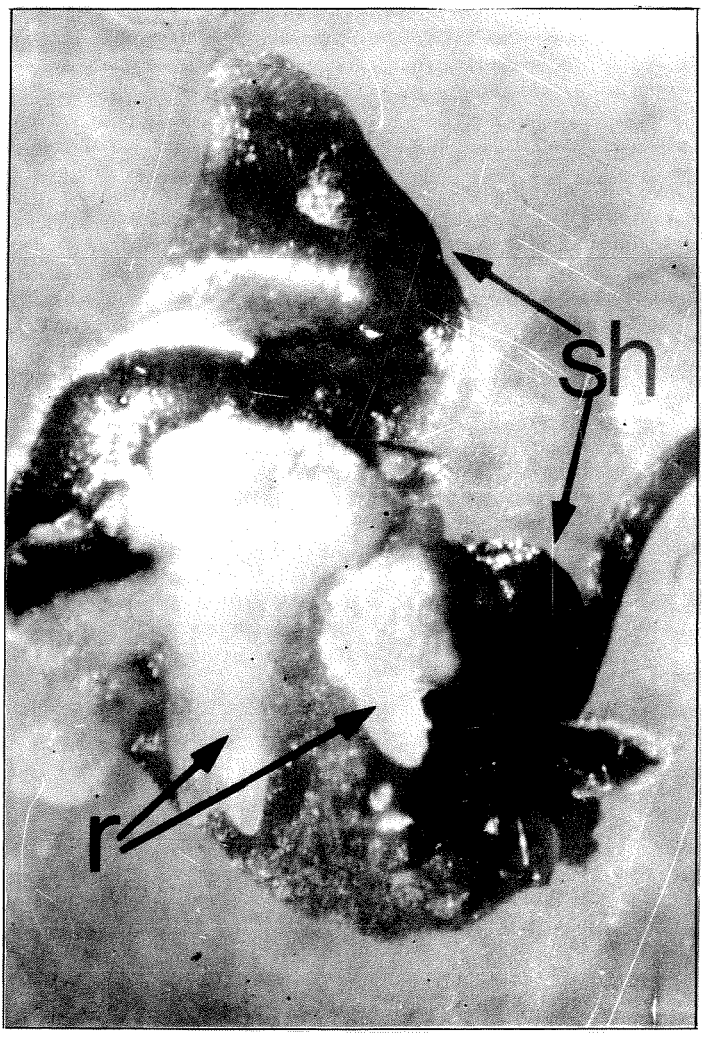

Fig. 2. – Regeneration of plantlets in callus derived from mature embryos (*r* – root, *sh* – shoot)

Results of this study show that we have improved the regeneration system for *Triticum aestivum* which enables a higher frequency of plant regeneration from callus obtained from mature embryos. With this findings and the main advantage of mature embryo explants – the availability thought the whole year, we open up the possibility of utilising this technique for studies related to wheat breeding program.

REFERENCES

Bhojwani, S.S. & Hayward, C. (1977): Some observations and comments on tissue culture of wheat. – Z. Pflanzenphysiol. *85:* 341-7.

Chin, J.C. & Scott, K.J. (1977): Studies on the formation of roots and shoots in wheat callus culture. – Ann. Bot. *41:* 474-81.

Dudits, D., Nemet, G. & Haydu, Z. (1975): Study of callus growth and organ formation in wheat *(Triticum aestivum)* tissue cultures. – Can. J. Bot. *53:* 957-63.

Gosch-Wackerle, G., Avivi, L. & Galun, E. (1979): Induction, culture and differentiation of callus from immature rachis, seeds and embryos of *Triticum*. – Z. Pflanzenphysiol. *91:* 267-78.

Lazar, M.D. Collins, G.B., & Vian, W.E. (1983): Genetic and environmental effects on the growth and differentiation of wheat somatic cell cultures. – The Journal of Heredity *74:* 353-7.

Murashige, T. & Skoog, F. (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. – Physiol. Plant. *15:* 473-97.

O'Hara, J.F. & Street, H.F. (1978): Wheat callus culture: the initiation, growth and organogenesis of callus derived from various explant sources. – Ann. Bot. *42:* 1029-38.

Ozias-Akins, P. & Vasil, I.K. (1982): Plant regeneration from cultured immature embryos and inflorescenses of *Triticum aestivum* L. (wheat): evidence for somatic embryogenesis. – Protoplasma *110:* 95-105.

Ozias-Akins, P. & Vasil, I.K. (1983a): Callus induction and growth from the mature embryo of *Triticum aestivum* (wheat). – Protoplasma *115:* 104-13.

Ozias-Akins, P. & Vasil, I.K. (1983b): Improved efficiency and normalisation of somatic embryogenesis in *Triticum aestivum* (wheat). – Protoplasma *117:* 40-4.

Sears, R.G. & Deckard, E.L. (1982): Tissue culture variability in wheat: callus induction and plant regeneration. – Crop Science *22:* 546-50.

Shimada, T., Sasakuma, T. & Tsunewaki, K. (1969): *In vitro* culture of wheat tissues. I. Callus formation, organ redifferentation and single cell culture. – Can. J. Genet. Cytol. *11:* 294-304.

Shimada, T. (1978): Plant regeneration from the callus induced from wheat embryo. – Japan. J. Genetics *53:* 371-4.

Shimada, T. & Yamada, Y. (1979): Wheat plants regenerated from embryo cell cultures. – Japan. J. Genetics *54:* 379-85.

Rezime

MILICA ĆALOVIĆ, BRANKA VINTERHALTER, DRAGAN VINTERHALTER

POSPEŠIVANJE REGENERACIJE BILJAKA U KALUSU POREKLOM OD ZRELIH EMBRIONA PŠENICE *(TRITICUM AESTIVUM* L.)

Institut za biološka istraživanja „Siniša Stanković", Beograd

Kultivisanjem zrelih embriona 5 različitih kultivara pšenice na MS (Murashige i Skoog, 1962) medijumu sa 2.0 mg/l 2,4-D dobili smo žućkast, gladak kalus kompaktne konzistencije. Kalus se obrazovao od osnove embriona a ne od skuteluma koji je zadobijao braon boju i propadao. Diferenciranje je usledilo nakon smanjenja koncentracije 2,4-D u medijumu na 0.5 mg/l, a potom na 0.2 mg/l. Nezavisno od načina orijentacije embriona u odnosu na površinu podloge (skutelum okrenut ka i od površine) samo 0-7% kalusa regeneriše cele biljke dok je taj procenat značajno veći u grupi gde je skutelum na samom početku odstranjen i odbačen sa primarnog eksplantata (embriona) i u zavisnosti od genotipa iznosi 9-22%. Smatramo da ovo povećanje regenerativne sposobnosti zrelih embriona kultivisanih bez skuteluma otvara interesantan put korišćenja zrelih embriona u različitim *in vitro* tehnikama koje se koriste u genetičkom inžinjeringu i oplemenjivanju biljaka.

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Original scientific paper

BRANKA VINTERHALTER, DRAGAN VINTERHALTER, SNEŽANA BUDIMIR

IN VITRO PROPAGATION OF *JANKEA HELDREICHII* BOISS. *(GESNERIACEAE)*

Institute for Biological Research „Siniša Stanković", Belgrade

Vinterhalter, B., Vinterhalter, D. and Budimir, S. (1995): *In vitro propatation of Jankea heldreichii Boiss. (Gesneriaceae).* – Glasnik Instituta za botaniku i botaničke bašte Univerziteta u Beogradu, Tom XXIX, 129 - 135.

Shoot cultures of *Jankea heldreichii* were established from seeds aseptically germinated on hormone-free MS medium. Various explants including cotyledone and hypocotyle fragments, axillary buds and whole shoots were transfered to MS medium supplemented with 5.0 mg/l BA and 0.1 mg/l IBA for shoots induction. Shoots regenerated directly without intervening callus on types of explants. Most responsive were cotyledone fragments and whole shoots manifesting 80% and 100% shoot regeneration respectively. Shoot cultures were maintained on medium with BA decrased to 0.2-0.5 mg/l BA and rooted on medium with 0.5 mg/l IBA.

Key words: *in vitro*, propagation, shoot cultures, *Jankea heldreichii.*

Ključne reči: *in vitro,* razmnožavanje, kulture izdanaka, *Jankea heldreichii.*

INTRODUCTION

Jankea heldreichii Boiss. is an endemic and relic species of Balkan Peninsula. Nowadays it can be found only on limestone of Olympus mountain (Greece). It belongs to *Gesneriaceae* family which since tertiary is represented in Europe with only few species. Two of them *Ramonda serbica* and *R. nathalie* are native to Serbia. Family *Gesneriaceae* contains genera with species which are propagated as decorative plants. Here belong *Saintpaulia* (african violet), *Streptocarpus* and *Gloxinia*. *Jankea heldreichii* is a small rosette forming plant with thick, hairy, grey-green leaves. Investigation presented here were started wtih the general aim to develop a method suitable for vegetative propagation as an aid in protection of this species.

MATERIAL AND METHODS

Seeds of *J. heldreichii* were collected near village Petra at mountain Olympus in Greece (Stefanović et al., 1992). Seeds were surface sterilized for 30 minutes in 20% commercial bleach (containing 4-5% NaOCl) and then thoroughly rinsed in autoclaved water. Seeds were aseptically germinated on hormone free medium supplemented with 3% sucrose, 0.7% agar, MS (Murashige & Skoog, 1962) vitamins and 1/2 WPM (Lloyd & McCown, 1981) mineral medium. Preparation of medium and conditions in the growth room were same as previously described (Vinterhalter & Vinterhalter, 1994). After germination various explants including fragments of cotyledons, hypocotyls and leafs, apical buds even whole plants were transferred to medium for shoot induction. This medium supplemented with 5.0 mg/l BA and 0.1 mg/l NAA was prepared with MS or WPM salts. Shoot cultures were maintained on media supplemented with 0.1-0.5 mg/l BA and 0.05-0.1 mg/l IBA. Rooting was performed on media supplemented with 0.5 mg/l IBA. In some experiments after initial exposure to the rooting medium supplemented with IBA, shoots were transferred to hormone free medium.

For histological examination material was fixed in FAA (formalin/acetic acid / ethanol), dehidrated in graded ethanol and embedded in paraffin wax at 57°C. Sections 5 μm thick were stained with haematoxylin, observed and photographed under photomicroscope (Jenamed, Carl Zeiss).

RESULTS AND DISCUSSION

Seeds of *J. heldreichii* are fotoblastic same as seeds of *Ramonda* species (Stefanović et al., 1992) and they quickly loose the ability ot germinate. Seeds which we used were fresh providing a nearly 100% germination. Also the method for surface sterilization which was employed provided a high percentage of healthy uncontaminated seedling which was usually higher then 90%.

Among the various explants tested the highest shoot proliferation was obtained in whole plants explants (100%) and cotyledon fragments (80%). Shoots were produced in 50% of leaf explants and 20% of hypocotyl explants. Medium prepared with MS salts was superior in comparison to WPM media.

For long-term maintenance of shoot cultures it was necessary to decrease BA concentration from 5.0 to 0.5-0.1 mg/l. Prolonged exposure to high cytokinin concentration resulted in vitrification and fasciation of cultures and it was therefore considered to be deterimental. Cultures subcultured from shoot induction medium grew well and even multiplied for some time on hormone-free medium, probably due to cytokinin accumulated in plant tissue. Actually, the cytokinin requirement of shoot cultures was small and after the first year spent in *in vitro* conditions it was around 0.2 mg/l BA. The requirement for auxins was also low, IBA at 0.05-0.1 mg/l gave best results in combination with BA.

Characteristic feature of *J. heldreichii* is that apart from axillary buds produced in leaf axils, leaves and leaf pedicels often produce adventitious buds directly on their surface.

Effect of BA on the production of new shoots is presented in Table 1. It is evident that 0.1-1.0 mg/l BA exerts little effect on shoot multiplication via axillary buds which already has a maximum at 0.1 mg/l (5.32). However, BA strongly affects the induction of adventitious buds with maximum at 0.5 mg/l BA on which 67.47% of single isolated leaves produce adventatious buds. At BA concentrations higher than 0.5 mg/l pedicel fail to elongate and cultures appear as small leafy balls. Growth of such cultures is poor since they loose contact with the medium.

Tab. 1. – Effect of 0-1.0 mg/l BA and 0.05 mg/l IBA on formation of axillary and adventitious shoot buds. Subculture duration 30 days

BA (mg/l)	No. of cultures	axillary shoot buds	multipl. index	adventitious buds	
				% of leafs	buds per leaf
0	70	257	3.67 ± 0.2	1.38	1
0.1	59	311	5.27 ± 0.22	25.42	15
0.2	54	268	4.92 ± 0.29	39.06	25
0.5	61	217	3.50 ± 0.25	67.47	42
1.0	55	212	3.85 ± 0.18	67.27	37

J. heldreichii shoot cultures sometimes perish from necrosis associated with gradual browning of the medium. Necrosis appears first in the outer leaf whorld of the rossete. Browning which is usually observed on very soft media seems to be triggered by low pH value of the media (less than 5.8). At pH higher than 5.8 leaves are dark green and media remains translucent.

On media supplemented with auxins apart from rooting of cultures, leaves which were in contact with medium proliferate adventitious buds on the adaxial side and roots on the abaxial side of lamine (Fig. 1 and 2). This morphogenic response of leaves was not observed on hormone-free medium. We thus concluded that in *J. heldreichii* auxin apart from rooting affects also adventitious bud formation. In a treatment in which

single isolated leaves were placed on medium with 0.5 mg/l IBA adventitious buds developed in 13.8% of explants whilst rooting was 100%. Effect of auxins concentration and duration on root length and mean number of roots per explant is presented in Tab. 2.

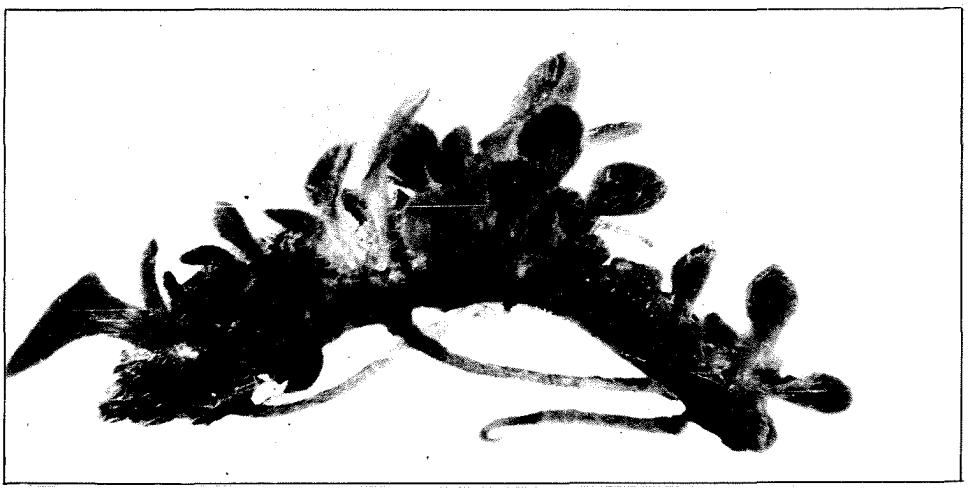

Fig. 1. – Regeneration of adventitious shoot buds on the adaxial and roots on the abaxial side of leafs cultured on MS medium with 0.5 mg/l IBA

Tab. 2. – Effect of the duration of auxin treatment (0.5 mg/l IBA) on root formation and length

auxin treatment days	explants	No of roots	Root length (mm)
9	32	8,0	7.84
12	36	9.11	7.08
16	22	8.5	6.5
19	34	7.67	5.55
30	100	many	2-3

Rooted plants were planted in a mixutre of peat and sand in which they successfully adapted (Fig. 3). However potted plants could not be maintained for more than three months in the glasshouse where they gradually perished from unknown reasons. We assume that the combination of high temeprature and humidity prevailing in our glasshouse was not suitable for this mountain species.

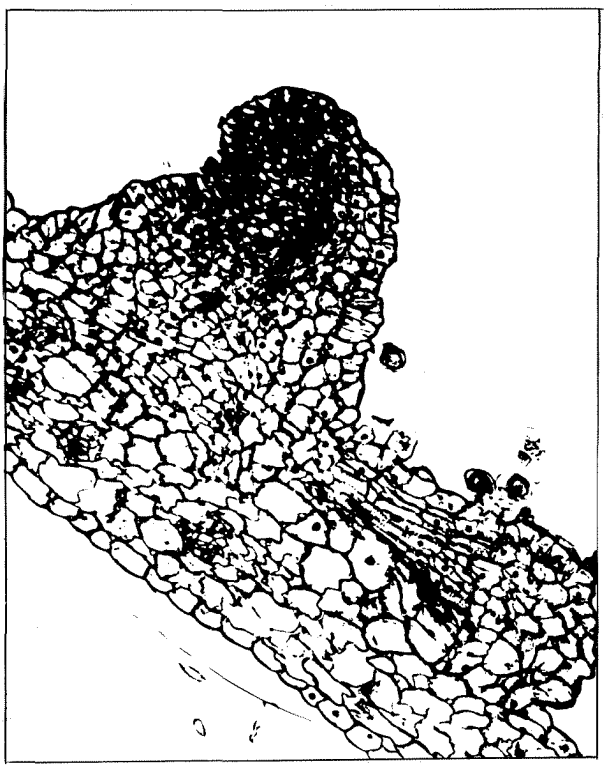

Fig. 2. – Transection through a leaf on medium supplemented with IBA. On the adaxial leaf side an adventitious shoot but is visible in longitudinal section with differentiated vascular elements

Propagation procedure which we employed for *J. heldreichii* was similar to procedures devised for african violet and related species in which induction of shoots was performed by addition of cytokinins to the medium. In some procedures induction medium is supplemented with high cytokinin concentrations (Peck & Cumming, 1974; Start & Cumming, 1976) whilst in others low cytokinin concentration are applied from the beginning of the propagation procedure. However, there are reports (Cooke, 1977; and Radojević et al., 1984) in which shoot multiplication medium apart from cytokinins contains also high concentration of auxins.

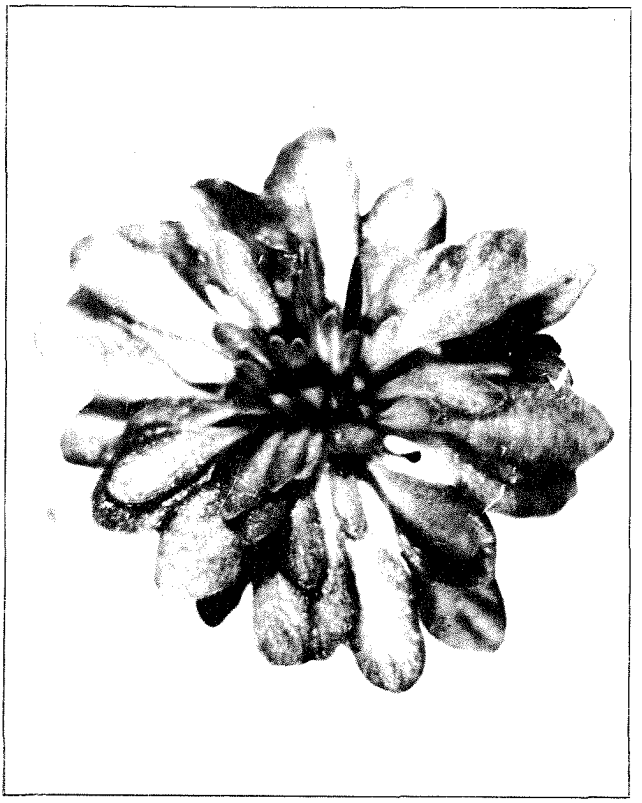

Fig. 3. – Plant propagated *in vitro,* arangement of leaves in the rossete – photographed from above

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REFERENCES

Cooke, R.C. (1977): Tissue culture propagation of african violets. – Hort Science *12:* 549.

Lloyd, G. & McCown, B. (1981): Commercially-feasible micropropagation of mountain laurel Kalmia latifolia by use of shoot tip culture. – Proc. Int. Plant. Prop. Soc. *30:* 421-427.

Manojlović, M., Vinterhalter, B. & Vinterhalter, D. (1992): *In vitro* propagation of *Catalpa ovata* G. Don and *Capalia bignonioides* Walt. – Bull. Inst. et Jard. Bot. Univ. Beograd *26-27:* 7-14.

Murashige, T. & Skoog, F. (1969): A revised medium for rapid growth and bioassay with tobacco tissue cultures. – Physiol. Plant. *15:* 473-497.

Peck, D.E. & Cumming, B.G. (1984): *In vitro* vegetative propagation of cape primrose using the corolla of the flower. – Hort Science *19:* 399-400.

Radojević, Lj., Vapa, Lj., Borojević, K. & Joksimović, J. (1984): Vegetativno razmnožavanje *in vitro S. iontantha* i *P. hortorum.* – Savremena poljoprivreda (Novi Sad) *32:* 421-480.

Start, N.D. & Cumming, B.G. (1976): *In vitro* Propagation of *Saintpaulia ionantha* Wendl. – Hort Science *11*: 204-206.

Stefanović, M., Grubišić, D. & Konjević, R. (1992): Seed germination of Balcan endemic species from the family Gesneriaceae. I. Effect of light and temperature. – Arch. Biol. Sci. *44*: 61-66.

Rezime

BRANKA VINTERHALTER, DRAGAN VINTERHALTER, SNEŽANA BUDIMIR

IN VITRO RAZMNOŽAVANJE *JANKEA HELDREICHII* BOISS. *(GESNERIACEAE)*

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Početni eksplantati za uspostavljanje kulture izdanaka *J. heldreichii* Boiss. bili su delovi sterilno isklijalog sejanca (hipokotil, kotiledon, epikotil) ali i cele biljke bez korena. Podloga za indukciju pupoljaka sadržala je MS mineralnu i vitaminsku kombinaciju, 3% saharoze, 0.7% agara a od hormona visoku koncentraciju BAP od 5.0 mg/l i NAA 0.1 mg/l. Optimalna podloga za kulturu rozeta sadrži BAP 0.1-0.5 mg/l i IBA 0.1 mg/l. Na ovoj podlozi pored razvoja bočnih pupoljaka na rubnim listovima rozete indukuju se i adventivni pupoljci. IBA u koncentraciji 0.5 mg/l pored 100% ožiljavanja izdanaka indukuje i pojavu pupoljaka na licu listova pa je neophodno 10-15 dana nakon tretmana sa IBA izdanke prebaciti na podlogu bez hormona. Na taj način sprečava se dalja multiplikacija izdanaka i omogućava izduživanje začetih korenova. 0.5 mg/l IBA indukuje adventivne pupoljke i kod 13.8% izolovanih listova. Adaptacija ožiljenih biljaka u uslovima staklare bila je teška zbog specifičnih uslova u kojima biljka živi u prirodnom staništu.

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DRAGAN VINTERHALTER[1], IVANA TODOROVIĆ[2], BRANKA VINTERHALTER[1]

IN VITRO CULTURE AND PROPAGATION OF *PUERARIA HIRSUTA* (THUNB.)

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Vinterhalter, D., Todorović, I. and Vinterhalter, B. (1995): *In vitro culture and propagation of Pueraria hirsuta (Thunb.).* – Glasnik Instituta za botaniku i botaničke bašte Univerziteta u Beogradu, Tom XXIX, 137 - 142.

Explants collected from a specimen grown in Belgrade Botanical garden, comprising lateral buds, single node segments, and pulvinii were cultured on a modified MS (Murashige & Skoog, 1962) medium supplemented with 0-1.0 mgl^{-1} BA and NAA. Explants produced only fast growing callus in which shoot differentiation could not be induced. Highest callus proliferation was registered on media with 1.0 mgl^{-1} BA and 1.0 mgl^{-1} NAA. From a plant growing near Dubrovnik, 60 large seeds were collected and aseptically germinated on MS medium with 0.5 mgl^{-1} BA and 0.1 mgl^{-1} IBA. Only two seeds germinated but both shoot explants perished after subculturing. Another seed reacted with a two month delay, producing callus which differentiated shoots. From this shoots a clone of shoot cultures was established and maintained on medium with 0.1 mgl^{-1} BA and NAA. Shoots rooted on hormone free medium could be successfully planted *ex vitro* and further cultured in glasshouse.

Key words: *in vitro,* propagation, callus, shoot cultures, compound leaves, *Pueraria hirsuta* (Thunb.).

Ključne reči: *in vitro,* razmnožavanje, kalus, kulture izdanaka, složeni listovi, *Pueraria hirsuta* (Thunb.).

INTRODUCTION

Pueraria hirsuta Thunb., member of the legume group is a sub-tropical semi-hard liana, which survives winter in the continental climate of Belgrade region. Due to the absence of specific pollinators, plant fails to develop viable seeds in Belgrade. *P. hirsuta* is characterized by very large compound leaves consisting of three individual leaflets, each equipped with an individual pedicel and a prominent pulvinus. These compound leaves can perform complex movements including sun tracking (whole leaf) and spatial rearrangement of lamina position (individual leaflets) induced by changes in irradiance. *P. hirsuta* has no suckers or tendrils but it can climb using other trees for support. It is a fast growing plant in which the main runner elongates more then 40 cm/per day under favorable conditions (D. Vinterhalter, unpublished). In the climate of Belgrade region *P. hirsuta* can be used as fast growing ornamental plant which can provide shade for gardens, veranda (porches), loggia, balconies, etc. Vegetative propagation can be performed by runner layering but this is a slow and often unreliable method. Therefore, *in vitro* propagation of this species has been investigated with intention to find a simple and fast procedure for propagation. Special attention was paid to the development of compound leaves and beginning of sun tracking movements.

The legume group contains a number of species which are difficult to regenerate and propagate by *in vitro* methods. Among them are species from genera *Phaseolus* (Allavena, 1983), *Glycine* (Newell & Luu, 1986; Hammat et al., 1986; Grant, 1984) and *Lathyrus* (Gharyal & Maheshwari, 1983). In some species there is a strong influence of genotype on the success of regeneration/propagation as for instance in *Phaseolus vulgaris* (Martins & Sondhall, 1984) and *Trifolium pratense* (Campbell & Tomes, 1984). In leguminous trees regeneration and propagation is difficult (Lakhsmi Sita et al., 1986) and these species have been characterized as recalcitrant (Tomar and Gupta, 1988).

MATERIAL AND METHODS

Material used for investigation was collected from two trees, one growing in the Belgrade Botanical garden and the second in Mlini (Dubrovnik) obtained as a cutting from the plant in arboretum Trsteno (Dubrovnik). Flowers of the plant from Mlini are visited by bumblebees and large seeds develop in some pods. These seeds were not observed to germinate under normal conditions. Sampling was performed in 1986-1987 and the resulting shoot cultures were maintained until autumn 1991.

Seeds and various plant explants were surface sterilized for 20 min in 10% commercial bleach containing 4-5% NaOCl and then thoroughly rinsed in autoclaved water. Medium contained Murashige and Skoog (1962) inorganic salts, vitamins and inositol was modified by increasing vitamin B1 concentration to 0.4 mgl^{-1} and providing 2% sucrose and 0.64% agar. Medium pH was adjusted to 5.8 prior to autoclaving which was performed for 20-25 minutes at 114-115°C.

Callus induction was performed in Ø 20 x 100 mm test tubes, and aseptical seed germination and rooting in Ø 18 x 180 mm test tubes. Shoot cultures were maintained in 100 ml wide neck Erlenmeyer flasks. All culture vessels were closed with cotton wool plugs.

Conditions in the growth room were: photoperiod 16/8 hours light to darkness, provided by cool white fluorescent lamps, irradiance 5.0-7.2 Wm^{-2} and temperature 25 ± 2°C.

RESULTS AND DISCUSSION

First round of investigation (1986) was started with explants collected from the plant growing in Belgrade Botanical garden. A total of 73 explants (21 lateral shoot buds, 36 node segments and 16 leaf pulvini) were placed on medium supplemented with 1.0 mgl^{-1} BA and 0.1 or 1.0 mgl^{-1} NAA.

All primary explants produced brownish-green callus which was very soft and friable. Callus proliferation was much better on media with 1.0 than on 0.1 mgl^{-1} NAA. It develop from cut surfaces quickly engulfing the whole explant. Most callus was produced on nodal explants and least on pulvini. Only four out of 73 explants were rejected due to contaminations.

Reaction of explants comprising lateral shoot buds was unusual. Axillary buds failed to elongate. In some explants development of vitrified leaves was promoted. Development of axillary but and leafs could not be followed due to rapid proliferation of callus which engulfed the whole explant.

In explants consisting of node explants callus proliferation was so fast that the first subculture was performed only a week after the explants were placed on midium. Subsequent subcultures were also short, not exceeding three week intervals.

Attempts to induce differentiation and organogenesis in this callus were not successful. Various concentrations of BA and NAA same as KIN and IAA or IBA were tried non of which rendered a reliable protocol for differentiation of either shoots or roots. Only two shoots differentiated one on media supplemented with 1.0 mgl^{-1} BA and 0.1 mgl^{-1} NAA and the second on media with 1.0 mgl^{-1} BA and 0.05 mgl^{-1} IBA. Both shoots could not be further multiplied on same type of media. Furthermore, leaves growing on these shoots in contact with medium became vitrified and proliferated masses of undifferentiated callus.

After a year further cultivation of this callus clone was stopped. Two main observations were made:

– fast proliferating, undifferentiated callus was friable. It appeared both on media with high and low hormone concentrations. Thus medium containing 0.2 mgl^{-1} both BA and NAA enabled rapid callus prolifetration same as medium with 1.0 mgl^{-1} BA and 2.0 mgl^{-1} NAA.

– on media supplemented with 0.1 mgl^{-1} BA and auxins at low concentration (- $\approx$ 0.1 mgl^{-1} IBA or NAA) callus manifested signs of organ differentiation including; slow growth rate, appearance of green colour, change of consistency from soft and friable to hard and compact, and finally formation of various green surface structures. In case of *P. hirsuta* these structures did not develop further into shoots.

Second round of investigation was performed with seeds collected from the plant growing in Mlini. From a sample containing 200 fresh seeds 60 largest were surface sterilized and placed on media with 0.5 mgl^{-1} BA and 0.1 mgl^{-1} IBA. After three weeks two seeds germinated but plants after subculturing failed to grow further. Aseptic germination was prolonged with intention to obtain callus. After two months a culture was observed in which the seed proliferated callus from which a single shoot differentiated and continued to grow after subculturing. From this explant a clone of shoot cultures was established.

BA and IBA concentration were varied until medium supplemented with 0.1 mgl^{-1} of both BA and IBA was chosen as optimal. Growth of shoot cultures was not as fast as callus proliferation. Optimal source of explants for shoot multiplication were

6-12 mm long shoots joint into shoot clusters, Fig. 1. Individual, elongated shoots were not suitable for shoot multiplication. Stocks with shoot cultures of *P. hirsuta* were maintained continuously for four years.

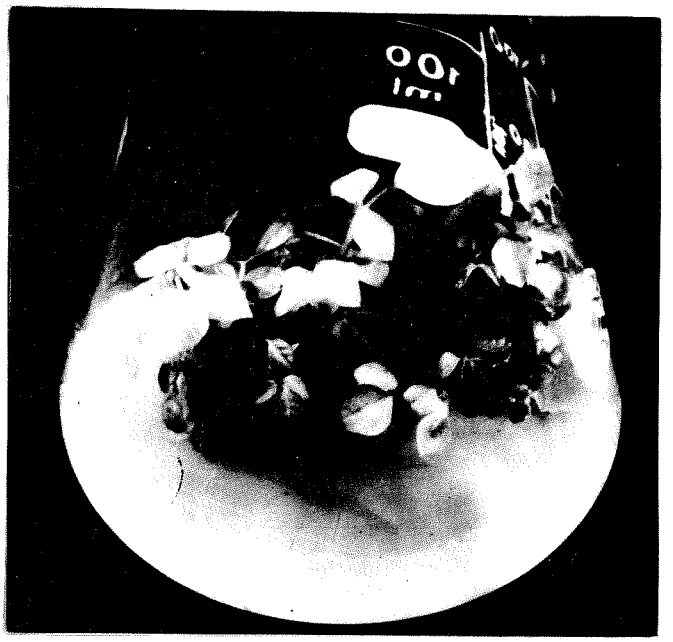

Fig. 1. – Shoot clusters comprising large and small shoots with compound leaves of various size

Rooting was performed on medium containing 0.1 mgl^{-1} IBA or on hormone free medium. Roots were long and slender. Plants were sensitive and difficult to adapt. They had a strong tendency to loose leaves upon transfer *ex vitro*, requiring a rest period to resume growth and form new leaves.

Perhaps the most important finding was that the complex leaf structure was unhindered by *in vitro* conditions. This means that leaves were always compounds, three independent leaflets could be observed even on very small leaves. In other plant species with compound leaves often a reduction in number of leaflets occur as for instance in carob. In this species usually only the first pair of leaflets develop (Vinterhalter & Vinterhalter, 1994). True, compound leaves in carob develop later after adaption.

During *in vitro* culture leaves were indifferent to light. The ability to track the light source developed only after the successful adaptation of plantlets to *ex vitro* conditions (Fig. 2). However, it is interesting to note that leaves formed *in vitro* were morphologicaly complete and that reasons for which leaves fail to perform sun tracking *in vitro* need to be investigated further.

Fig. 2. – Addapted plantlets, arangement of leaflets in the same plane – beginning of suntracking movements

Although shoot cultures of *P. hirsuta* were obtained and maintained for four years in our laboratory, we still consider this species as recalcitrant for *in vitro* propagation. It was difficult to find explants from which shoot cultures could be established. There were also problems which accompanied adaptation and further growth of adapted plants.

REFERENCES

Allavena, A. (1983): Legumes. Beans *(Phaseolus). In* Handbook of Plant Cell Culture. Vol. 2 (W.R. Sharp, D.A. Evans, P.V. Ammirato, Y. Yamada, eds.), pp. 137-168. - MeMillan, New York – London.

Campbell, C.T. (1984): Establishment and multiplication of red clover plants by *in vitro* shoot tip culture. – Plant Cell Tiss. Org. Culture *3:* 49-57.

Gharyal, P.K. & Maheshwari, S.C. (1983): Genetic and physiological influences on differentiation in tissue cultures of a legume, *Lathyrus sativus.* – Theor. Appl. Genet. *66:* 123-126.

Grant, J. (1984): Plant regeneration from cotyledonary tissue of *Glycine canescens* a perennial wild relative of soybean. – Plant Cell Tiss. Org. Culture *3:* 169-173.

Hammatt, N., Davey, M.R. & Nelson, R.S. (1986): Plant regeneration from seedling cotyledons, leaves and petioles of *Glycine clandestina.* – Physiol. Plant. *68:* 125-128.

Lakshmi Sita, G., Chattopadhyay, S. & Tejavathi, D.H. (1986): Plant regeneration from shoot callus of rosewood *(Dalbergia latifolia* Roxb.). – Plant Cell Reports *5:* 266-268.

Martins, I.S. & Sondahl, M.R. (1984): Multiple shoot formation from shoot apex cultures of *Phaseolus vulgaris* L. – J. Plant Physiology *115:* 205-208.

Murashige, T. & Skoog, F. (1969): A revised medium for rapid growth and bioassay with tobacco tissue cultures. – Physiol. Plant. *15:* 473-497.

Newell, C.A. & Luu, K.T. (1985): Protoplast culture and plant regeneration in *Glycine canescens* F.J. Herm. – Plant Cell Tiss. Org. Culture *4*: 145-149.

Tomar, U.K. & Gupta, S.C. (1988): *In vitro* plant regeneration of leguminous trees (*Albizia* spp.). – Plant Cell Reports *7*: 185-188.

Vinterhalter, B. & Vinterhalter, D. (1994): Effect of light and carbohydrate nutrition on expansion of leaf area and lenticel hypertrophy in shoot cultures of carob (*Ceratonia siliqua* L.). – VIII Intern. Cong. Plant Cell Tiss. Org. Cult. in Florence. p. 167.

Rezime

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KULTURA *IN VITRO* I RAZMNOŽAVANJE *PUERARIA HIRSUTA* (THUNB).

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Pueraria hirsuta (Thunb.) se u botaničkoj bašti „Jevremovac" u Beogradu gaji kao retka tropska vrsta koja u odsustvu odgovarajućih oprašivača ne obrazuje mahune sa klijavim semenom. Obzirom da se vegetativno razmnožavanje vrši samo putem položnica to je istražen postupak za *in vitro* razmnožavanje ove vrste. Različiti tipovi eksplantata uključujući bočne pupoljke, segmente izdanaka i pulvinusa su kultivisani na modifikovanoj MS (Murashige i Skoog, 1962) podlozi u koju su dodati 0-1.0 mgl^{-1} BAP i NAA. Aktiviranje i rast bočnih izdnaka nisu dobijeni, eksplantati su obrazovali samo kalus iz kojeg se izdanci nisu mogli diferencirati. Najveća proliferacija kalusa bila je na podlogama sa 0.2 mgl^{-1} BAP i NAA odnosno na podlozi sa 1.0 mgl^{-1} BAP i 2.0 mgl^{-1} NAA. Oko 200 semenki prikupljeno je sa jednog primerka biljke u blizini Dubrovnika od čega je 60 najkrupnijih izdvojeno i postavljeno na aseptično isklijavanje na MS podlogu sa 0.5 mgl^{-1} BAP i 0.1 mgl^{-1} IBA. Samo dve semenke su proklijale, ali su obe biljke propale nakon pasažiranja. Nakon dva meseca još jedna semenka iz ove grupe je reagovala i produkovala kalus iz kojeg se zatim diferencirao jedan izdanak. Ovaj izdanak je uspešno pasažiran i od njega je formiran klon kultura izdanka koji je zatim uspešno održavan 4 godine na podlozi sa 0.1 mgl^{-1} BAP i NAA. Izdanci ožiljeni na podlozi bez hormona su uspešno adaptirani u staklari.

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FACTORS AFFECTING *IN VITRO* ROOTING OF *CRYPTANTHUS BROMELIOIDES* (OTTO & DIETR.)

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Todorović, I. and Vinterhalter, D. (1995): *Factors affecting in vitro rooting of Cryptanthus bromelioides (Otto / Dietr.)*. – Glasnik Instituta za botaniku i botaničke bašte Univerziteta u Beogradu, Tom XXIX, 143 - 148.

Shoot cultures of a decorative clone of *Cryptanthus bromelioides* Otto & Dietr. were maintained *in vitro* on MS (Murashige and Skoog, 1962) medium supplemented with BAP 0.5-1.0 mgl^{-1} and 0.1-0.2 mgl^{-1} NAA. Characteristic of this species is poorly developed root system same as in other epiphytic bromeliads. On the standard rooting medium comprising 0.5 mgl^{-1} IBA root length was only 9.4 mm with 3.8 roots per explant. We therefore investigated the effect of factors and conditions which are know to affect and improve root initiation and elongation. Among various factors including auxins IBA and NAA, light, inorganic nutrition, activated charcoal, phloroglucinol, ancymidol, fusicoccin, riboflavine and ascorbic acid the most effective was activated charcoal which 2.5 times increased the root length.

Key words: *in vitro*, propagation, rooting, shoot cultures, *Cryptanthus bromelioides*, epiphyte.

Ključne reči: *in vitro*, razmnožavanje, ožiljavanje, kulture izdanaka, *Cryptanthus bromelioides*, epifit.

INTRODUCTION

Cryptanthus bromelioides member of the *Bromeliaceae* familiy is a small plant with leaves arranged in a rosette. Like other bromeliads *C. bromeliodes* is an epiphyte and its root system is poorly developed. The poor root growth of bromeliads can be also obsrved under conditions of *in vitro* culture. Thus for instance in *Aechmea fasciata* Baker., the mean root length after 5 weeks of rooting on hormone free medium was only 12.45 mm (Vinterhalter & Vinterhalter, 1994). However addition of 1% activated charcoal to the medium more than doubled the length of roots in *Aechmea*. We therefore investigated *in vitro* rhizogenesis of *C. bromelioides*, with the aim to enhance rooting parameteres supplementing the medium with various rooting cofactors.

There is a number of studies dedicated to *in vitro* propagation of bromeliad species. *Aechmea fasciata* was propagated by Zimmer and Peiper (1974, 1975), Jones and Murashige (1974) and Ziv et al., (1986); *Tillandsia, Guzmania* and *Vriesea* by Mekers (1977) and Mekers and Van Onsem (1983); *Cryptanthus* by Davidson and Donnan (1977) and Mathews and Rao (1982); *Quesnelia* (Hosoki and Asahira (1980) and *Ananas* Mathews and Rangan (1979, 1981), Zepeda and Sagava (1981).

MATERIAL AND METHODS

Shoot cultures used in this investigation were introduced and successfully propagated *in vitro* as *Ananas comosus* L. Plants 1-2 years old were examined and reclassified according to Saakov (1983) as a decorative clone of *Cryptantus bromelioides* Otto & Dietr. (*C. acaulis* Lindl).

Shoot cultures were maintained on MS (Murashige & Skoog, 1962) medium supplemented with 0.5-1.0 mgl^{-1} BA and 0.1-0.2 mgl^{-1} NAA. Shoot culture stock was subcultured at 6-8 week intervals. The duration of rooting treatments was 4 weeks. Each treatment contained 25-30 replicates and was repeated at least twice. Shoots excised for rooting treatments were 35-40 mm long. Root length was measured as the length of the longest root on the rooted plant. Shoot cultures were maintained in 100 ml Erlenmeyer flasks or 125 ml blood transfusion bottles. Rooting treatments were performed in Ø 20 x 100 mm test tubes. All culture vessels were closed with cotton wool plugs. Medium pH was adjusted to 5.8 prior to autoclaving which was performed for 20-25 minutes at 114-115^{0}C.

Conditions in the growth room were: photoperiod 16/8 hours light to darkness, provided by cool white fluorescent lamps, irradiance 5.0-7.2 Wm^{-2} and temperature 25 $\pm$ 2^{0}C.

Adaptation of rooted plants was performed in glasshouse, plants were transferred in peat based substrates (mixture of peat, sand and humus). During adaptation plants were weekly sprayed with fungicides (containing 0.3% Captan).

RESULTS

The first group of treatments was performed with the aim to investigate the effect of auxins on rooting. IAA and NAA were applied i concentration 0-2.0 mgl^{-1} and shoots left in continuous contact with the rooting medium, Tab. 1. Rooting with auxins was nearly 100% efficient. Roots were short, the maximum root length (11.4 mm) was registered on hormone-free and medium with lowest IBA concentration - 0.1 mgl^{-1}.

Root length decreased with the increase of auxin concentration. IBA concentrations under 0.5 mgl^{-1} provided slender roots with numerous hairs whilst at higher IBA concentrations roots were thick and hairs were absent. In treatments with NAA roots were even shorter than on IBA supplemented media. The longest root 5.3 mm was registered on media with 0.1 mgl^{-1} NAA.

Tab. 1. – Effect of NAA and IBA on root elongation and the number of roots per rooted plant

Auxin, mgl^{-1}	Root length. mm ± SEM		Roots per rooted culture ± SEM	
	IBA	NAA	IBA	NAA
0	11.36 ± 0.8	11.73 ± 0.7	2.83 ± 0.3	2.70 ± 0.2
0.1	11.37 ± 1.0	5.27 ± 0.5	3.44 ± 0.2	3.72 ± 0.3
0.2	9.43 ± 0.4	nm	3.13 ± 0.2	nm
0.5	9.33 ± 0.6	4.91 ± 0.4	3.80 ± 0.3	5.00 ± 0.4
1.0	8.60 ± 0.9	3.73 ± 0.4	4.86 ± 0.7	6.61 ± 0.7
2.0	4.46 ± 1.0	3.10 ±0.2	6.73 ± 1.0	5.31 ± 0.4

nm - not measured

The mean number of roots per explant increased with auxin concentration reaching maximum 6.7 on 2.0 mgl^{-1} IBA and 6.6 at 1.0 mgl^{-1} NAA.

Since IBA enabled better elongation than NAA, all furhter experiments were performed with IBA supplemented media. Concentration 0.5 mgl^{-1} IBA was considered to be optimal providing moderately high values for both root elongation and roots per rooted explant parameters.

In the next experiment concentration of MS inorganic salts in the medium was decreased, Table 2. Treatments contained IBA either at 0.1 or 0.5 mgl^{-1}.

Tab. 2. – Effect of inorganic nutrition on root elongation and the number of roots per rooted plant

MS inorganic salts, %	Root length, mm ± SEM		Roots per rooted explant ± SEM	
	IBA 0.1 mgl^{-1}	IBA 0.5 mgl^{-1}	IBA 0.1 mgl^{-1}	IBA 0.5 mgl^{-1}
100	8.3 ± 0.6	8.6 ± 0.7	2.7 ± 0.2	4.3 ± 0.2
50	10.0 ± 0.9	11.6 ± 0.9	3.2 ± 0.2	2.7 ± 0.3
20	11.2 ± 0.7	13.6 ± 0.9	3.3 ± 0.3	3.8 ± 0.4
10	10.5 ± 0.9	14.1 ± 0.7	2.8 ± 0.2	3.4 ± 0.4
0	9.5 ± 0.5	9.6 ± 0.7	2.7 ± 0.2	3.0 ± 0.3

On both media root length increased with decrease of concentration of inorganic salts. Maximum for lower IBA concentration was reached at 1/5 and for higher IBA concentration on media containing 10% MS inorganic salts. Interesting results were obtained for the number of roots per rooted culture. At lower IBA concentration maximum was at 1/5 MS inorganic salts same as the maximum for root elongation. On

the higher IBA concentration (0.5 mgl^{-1}) values decreased with the concentration of inorganic salts. Rooting percentage in these treatments varied from 96,6 do 100%. On salt-free media roots were dark and thin, thread-like.

Next group of treatments was performed with the aim to evaluate the light requirement for rotting of *C. bromelioides*. Both light and dark treatments contained 0.5 mgl^{-1} IBA and full strength MS inorganic salts (Tab. 3). In light the mean number of roots per explant was higher and roots were longer than in dark treatment. Plants in dark treatments were etiolated and their shoot were longer (56.1 mm) than in plants cultured in light (40.1 mm).

Tab. 3. – Effect of light on root elongation and the number of roots per rooted plant

Treatment	Root length, mm ± SEM	Roots per rooted explant ± SEM	Shoot length, mm ± SEM
light	8.68 ± 0.3	4.46 ± 0.2	40.65 ± 0.7
darkness	6.68 ± 0.3	3.58 ± 0.2	56.14 ± 1.2

Finally, a number of substances which are known as cofactors of rhizogenesis were investigated, all on medium supplemented with 0.5 mgl^{-1} IBA and full strength MS inorganic salts, Tab. 4.

Tab. 4. – Effect of rhizogenesis cofactors on root elongation and the number of roots per rooted plant

Cofactor	concentration	root length. mm ± SEM	roots per rooted culture ± SEM
control		9.33 ± 0.6	3.8 ± 0.3
charcoal (activated)	1%	24.64 ± 0.2	4.1 ± 0.2
ancymidol	0.8 mgl^{-1}	7.60 ± 0.4	8.9 ± 0.7
fusicocin	10^{-10}M	7.00 ± 0.8	9.8 ± 1.0
phloroglucinol	162 mgl^{-1}	3.83 ± 0.2	6.9 ± 0.4
ascorbic acid	1 mgl^{-1}	4.63 ± 0.5	8.0 ± 0.7
riboflavine	1 mgl^{-1}	10.86 ± 1.3	4.0 ± 0.3

The most potent cofactor was 1% activated charcoal which increased root length 2.5 times in comparison to the control. Mean number of roots was not much higher than in the control. In contrast to activated charcoal other cofactors increased the number of roots per rooted explants and decreased root elongation.

Ancymidol, fusicocin and ascorbic acid more than doubled the number of roots per rooted explant. Phloroglucinol also increased root production but strongly inhibited root elongation same as ascorbic acid. Ancymidol and fusicocin moderately decreased root length.

Riboflavin slightly increased root elongation and roots per rooted plant above the values registered in the control.

Adaptation of plants rooted on IBA supplemented medium was very good, usually over 90% efficient. Adaptation of plants rooted on NAA supplemented medium was less efficient. After some time plants attain the characteristic rosette growth habit.

DISCUSSION

Our investigation showed that the optimal relation between root length and number was obtained on media with 0.5 mgl^{-1} IBA and 1/10 strength MS inorganic salts. Among various rhizogenesis cofactors activated charcoal significantly increased root length. Ancymidol and fusicocin did not affect much root elongation but increased the number of roots per explant. Phloroglucinol and ascorbic acid were inhibitory to root elongation but increased the production of roots. Riboflavine showed no marked effect on rooting parameters. Adaptation was much better if plant were rooted on IBA than on NAA supplemented media.

The obtained results are in accordance with results obtained with *Aechmea fasciata* (Vinterhalter and Vinterhalter, 1994) in which 1% activated charcoal was found to significantly increase root elongation.

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REFERENCES

Davidson, S. E. & Donnan, A. (1977): *In vitro* propagation of *Cryptanthus* spp., - Proc. Fla. State Hort. Soc. *90*: 303-304.

Hosoki, T. & Asahira, T. (1980): *In vitro* propagation of bromeliads in liquid culture. - Hort Sci. *15*: 603-604.

Jones, B. J. & Murashige, T. (1974): Tissue culture propagation of *Aechmea fasciata* Baker and other bromeliads. – Proc. Intern. Plant Propag. Soc. *24*: 117-126.

Mathews, V. H. & Rangan, T. S. (1979): Multiple plantlets in lateral bud and leaf explant *in vitro* cultures of pineapple. – Sci. Hortic. *11*: 319-328.

Mathews, V. H. & Rangan, T. S. (1981): Growth and regeneration of plantlets in callus cultures of pineapple. – Sci. Hortic. *14*: 227-234.

Mathews, V. H. & Rao, P. S. (1982): *In vitro* plant regeneration in lateral bud explants of *Cryptanthus bromelioides* Var. Tricolor M. B. Foster. - Plant Cell. Rep. *1*: 108-110.

Mekers, O. (1977): *In vitro* propagation of some Tillandsioideae (Bromeliaceae). – Acta Hortic. *78*: 311-317.

Meekers, O. & VanOnsem, J. G. (1983): *In vitro* propagation of *Vriesea* cultivars in comparison with other ornamental *Bromeliaceae*. – Acta Hortic. *131*: 125-130.

Murashige, T. & Skoog, F. (1969): A revised medium for rapid growth and bioassay with tobacco tissue cultures. – Physiol. Plant. *15*: 473-497.

Saakov, S. G. (1983): Оранжерейные и комнатные растений и уход за ними. – Nauka, Liningrad

Vinterhalter, B., & Vinterhalter, D. (1994): True-to-the-type *in vitro* propagation of *Aechmea fasciata* Baker. – Sci. Hortic. *57*: 253-263.

Zepeda, C. & Sagawa, Y. (1981): *In vitro* propagation of pineapple. - HortSci. *16*: 495.

Zimmer, K., Pieper, W. (1975): Further investigation on growing *Aechmea in vitro*. – Gartenbauw. *40*: 129-132.

Zimmer, K. & Pieper, W. (1976): Methods and problems of clonal propagation of bromeliads *in vitro*. – Acta Hortic. *64*: 25-29.

Ziv, M., Yogev, T. & Krebs, O. (1986): Effects of paclobutrazol, and clormequat on growth pattern and shoot proliferation of normal and variant *Aechmea fasciata* Beker plant regenerated *in vitro*. – Isreal J. Bot. *35*: 175-182.

Rezime

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ISTRAŽIVANJE FAKTORA KOJI UTIČU NA *IN VITRO* OŽILJAVANJE VRSTE *CRYPTANTHUS BROMELIOIDES* (OTTO& DIETR.)

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Kulture izdanaka dekorativnog klona *Cryptanthus bromelioides* (Otto / Dietr.) su održavani *in vitro* na MS (M u r a s h i g e and S k o o g , 1962) podlozi uz dodatak 0.5-1.0 mgl^{-1} BAP i 0.1-0.2 mgl^{-1} NAA. Karakteristika ove vrste je slabo razvijen korenov sistem slično kao i kod drugih epifitnih bromelija. Tako na standardnoj polozi za ožiljavanje koja sadrži 0.5 mgl^{-1} IBA dužina korena bila je svega 9.4 mm dok je broj korenova po eksplantatu bio 3.8. Zbog toga su istraživani faktori za koje je poznato da utiču na inicijaciju i izduživanje korena. Između različitih faktora uključujući tu auksine IBA i NAA, svetlost, mineralnu ishranu, aktivni ugalj, floroglucinol, ancimidol, fuzikokcin, riboflavin i askorbinsku kiselinu najveći efekat ispoljio je aktivni ugalj koji je u koncentraciji 1% povećavao izduživanje korena 2.5 puta.

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PLANT REGENERATION OF *IRIS HALOPHILA* PALL. AND *IRIS SIBIRICA* FANCH. BY SOMATIC EMBRYOGENESIS AND ORGANOGENESIS

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Subotić, A. and Radojević, Lj. (1995): *Plant regeneration of Iris halophila Pall. and Iris sibirica Fanch. by somatic embryogenesis and organogenesis.* – Glasnik Instituta za botaniku i botaničke bašte Univerziteta u Beogradu, Tom XXIX, 149 - 155.

Plant regeneration was achieved by somatic embryogenesis and organogenesis in *Iris halophila* Pall. and *Iris sibirica* Fanch. Somatic embryogenesis was induced after seven days by growing emryo explants on B_0 nutrient medium based on MS supplemented with (in mg/l): 2,4-D 5.0, KN 1.0, CH 250.0 and Pro 250.0. Further development of embryogenic callus and differentiation of somatic embryos occurred on B_5 nutrient medium (MS, 2,4-D and KN, 1.0 mg/l, each). The embryogenic potential depended on both the species and the composition of the nutrient medium. For *I. halophila* on B_5 nutrient medium it was 79% and for *I. sibirica* on B_1 it went up to 57%. In addition to NEC, EC and OC also formed on the both nutrient media. During the OC growth of both species on C_3 nutrient medium consisting of MS, BAP and GA_3 (1.0 and 0.1 mg/l, respectively) adventitious buds were induced. Shoot multiplication in *I. halophila* (89%) and *I. sibirica* (98%) was achieved on C_2 medium supplemented with BAP and NAA (1.0 and 0.1 mg/l, respectively). Somatic embryos germination and shoot rooting was attained in MS mineral

solution containing KN 3.0, IAA 1.0 and GA_3 0.1 (mg/l). Regenerants of both species were successfully acclimated under greenhouse conditions.

Key words: *Iris halophila* Pall., *Iris sibirica* Fanch., adventitious buds, embryogenic callus, embryo culture, organogenesis, somatic embryogenesis, somatic embryos.

Ključne reči: *Iris halophila* Pall, *Iris sibirica* Fanch, adventivni pupoljci, embriogeni kalus, kultura embriona, organogeneza, somatska embriogeneza, somatski embrioni.

Abbreviations: AB - adventitious buds; AS - adenine sulphate; CH - casein hydrolysate; 2,4-D - dichlorophenoxyacetic acid; IAA - indole-3-acetic acid; IBA - 2-indolebutyric acid; KN - 6 - furfurylaminopurine; BAP - 6-benzylaminopurine; GA_3 - gibberellic acid; MS - Murashige and Skoog mineral solution; NAA - α-naphtaleneacetic acid; NEC - nonembryogenic callus; EC - embryogenic callus; OC - organogenic callus; Pro - l-proline; SE - somatic embryos; Tyr-l-tyrosine.

INTRODUCTION

The Iris genus includes a great number of species, many of them being important for horticulture and pharmaceutical industry. Similar to most of monocot plants with rhizomes, irises are propagated vegetatively, each individual producing a maximum of some ten plants per year. During the last twenty years *in vitro* propagation of the species was intensified in different ways: using callus in *I. hollandica* (Hussey, 1976), regneration from callus and somatic embryogenesis in *Iris spp.* (Meyer, Fuchigami & Roberts, 1975; Reuther, 1977), somatic embryogenesis by *in vitro* culture of mature *I. pumila* and *I. setosa* embryos (Radojević, Sokić & Tucić, 1987; Radojević & Subotić, 1992), root culture in *I. pseudocorus, I. setosa* and *I. versicolor* (Laublin, 1991) and leaf culture and immature inflorescence culture in *I. pallida* and *I. germanica* (Jéhan et al., 1994).

In the present work the effect of nutrient media composition, hormones and/or amino acids on plant regeneration through somatic embryogenesis and organogenesis were studied using explants from mature zygotic embryos of *I. halophila* and *I. sibirica*.

MATERIALS AND METHODS

Seeds of *I. halophila* Pall (2n = 44) and *I. sibirica* Fanch. (2n = 28) were obtained from the collection of the Moscow Botanical garden. Sterilization and isolation of seeds were performed as described previously (Radojević & Subotić, 1992). For induction of somatic embryogenesis MS solution containing sucrose 5%, agar 0.5% and (in mg/l): inositol 100.0, Pro 250.0, CH 250.0, nicotinic acid 5.0, pantothenic acid 10.0, B_1 vitamin 2.0, 2,4-D 5.0 and KN 1.0 (B_0) was employed. Nutrient media for embryogenic callus (EC) development and somatic embryos (SE) differentiation (B_1 - B_5) are listed in Table 1. For organogenic callus (OC) and adventitious bud (AB) induction and shoot multiplication, (C_0 - C_3) were applied (Table 1). Rooting of the shoots was achieved in MS liquid medium supplemented by IAA (1.0 mg/l) and KN (3.0 mg/l). Cultures were grown at 25 ± 2 C°, 16h / 8h photoperiod (fluorescent lamps "Tesla", Pančevo, 65W, 4500 K) and regenerants rooted. Plantlets were grown in a mixture of sand and soil (3 : 1) in a greenhouse.

Tab. 1. – Composition of nutrient media for regeneration of Iris halophila and I. sibirica

Nutrient media (ingredients in mg/l)
B_0 : MS + 2,4 - D 5.0 + KN 1.0 + Pro 250.0 + CH 250.0
B_1 : MS + 2,4 - D 1.0 + KN 1.0 + Pro 250.0
B_2 : MS + 2,4 - D 1.0 + KN 1.0 + Pro 250.0 + CH 25.0
B_3 : MS + 2,4 - D 1.0 + KN 1.0 + Pro 25.0 + CH 250.0
B_4 : MS + 2,4 - D 1.0 + KN 1.0 + CH 250.0
B_5 : MS + 2,4 - D 1.0 + KN 1.0 + Pro 250.0 + CH 250.0
C_0 : MS + IAA 0.1 + BAP 1.0 + Tyr 100.0 + AS 80.0
C_1 : MS + IBA 0.1 + BAP 1.0 + Tyr 100.0 + AS 80.0
C_2 : MS + NAA 0.1 + BAP 1.0 + Tyr 100.0 + AS 80.0
C_3 : MS + GA_3 BAP 1.0 + Tyr 100.0 + AS 80.0

RESULTS

Somatic embryogenesis

Mature zygotic embryos of *I. halophila* and *I. sibirica* after seven days of growth on B_0 medium for somatic embryogenesis induction, expressed a different morphogenetic potential. During this period, tiny, yellow-pale structures appeared in *I. halophila*, while no callus or nodules were formed on zygotic embryos of *I. sibirica*. After three weeks, explants of both species grown on B_5 nutrient medium with reduced concentration of 2,4-D from 5.0 to 1.0 mg/l developed three types of calli as a result of different differentiation pathways: NEC, EC, OC (Fig. 3. a and b). In embryogenic cultures of both species, the number of SE was increasing parallel to the duration of subculture. Somatic embryos were differentiated mainly at the surface of embryogenic nodules representing a part of EC or, rarely, they were developed adventitiously at the already present SE. In most *I. halophila* cultures SE were developed to globular and heart-shaped stages and there were far less embryos with lateral scutellar notches and in the coleoptile stage (Fig. 3. c). In certain cultures of *I. halophila* grown on B_5 nutrient medium, a simultaneous maturation and germination of SE into plantlets occurred. During a prolonged growth the roots were spontaneously developed.

The highest embryogenic response was obtained in *I. halophila* grown on B_5 medium and in *I. sibirica* grown on B_1 medium (Fig. 1, values represent the means ± S.E. of results from three replicates). In order to increase the biomass of EC and number of SE, embryogenic calli were further cultured on B_0 - B_5 nutrient media. The highest values for fresh callus mass of both iris species from approx. 1600 to 2500 mg, were obtained by growing the EC on B_2 medium.

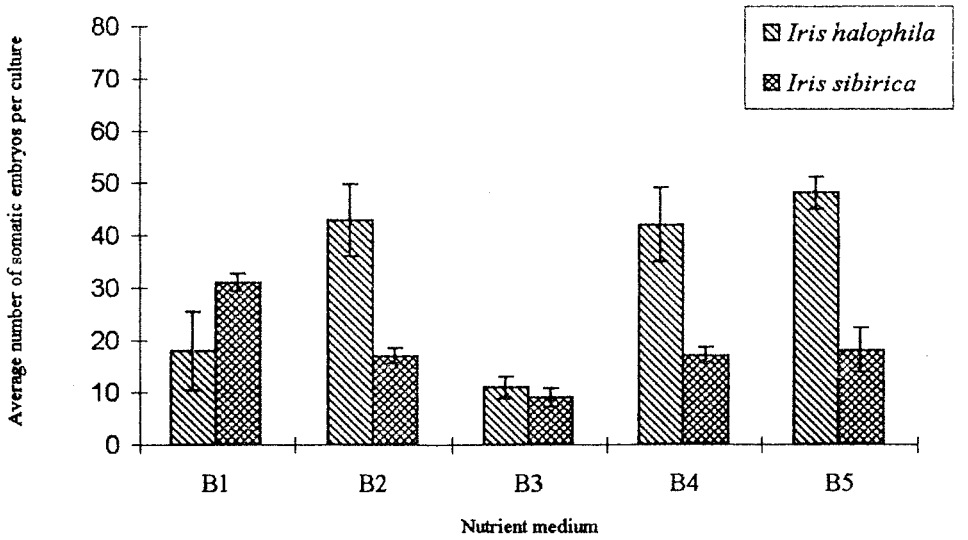

Fig. 1. – Influence of nutrient composition of somatic embriogenesis in *Iris halophila* and *I. sibirica*

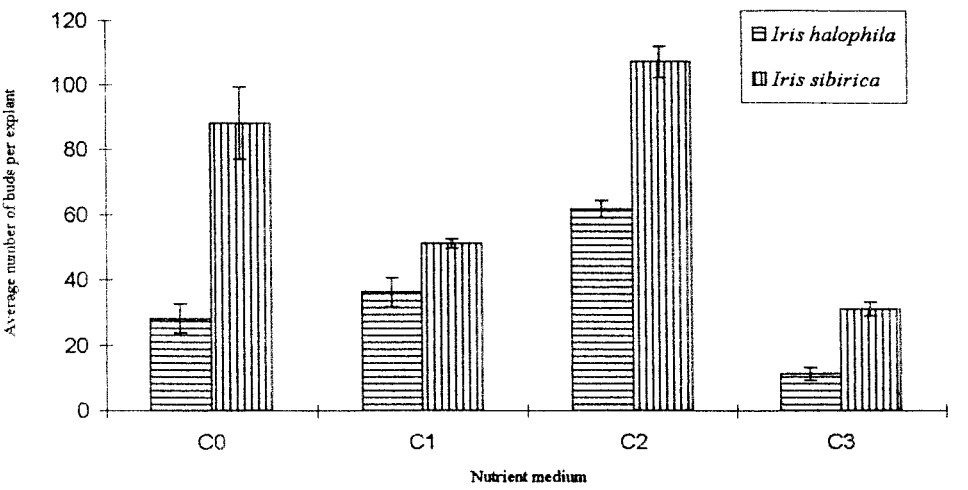

Fig. 2. – The effect of nutrient medium composition on adventitious bud multiplication of *Iris halophila* and *I. sibirica*

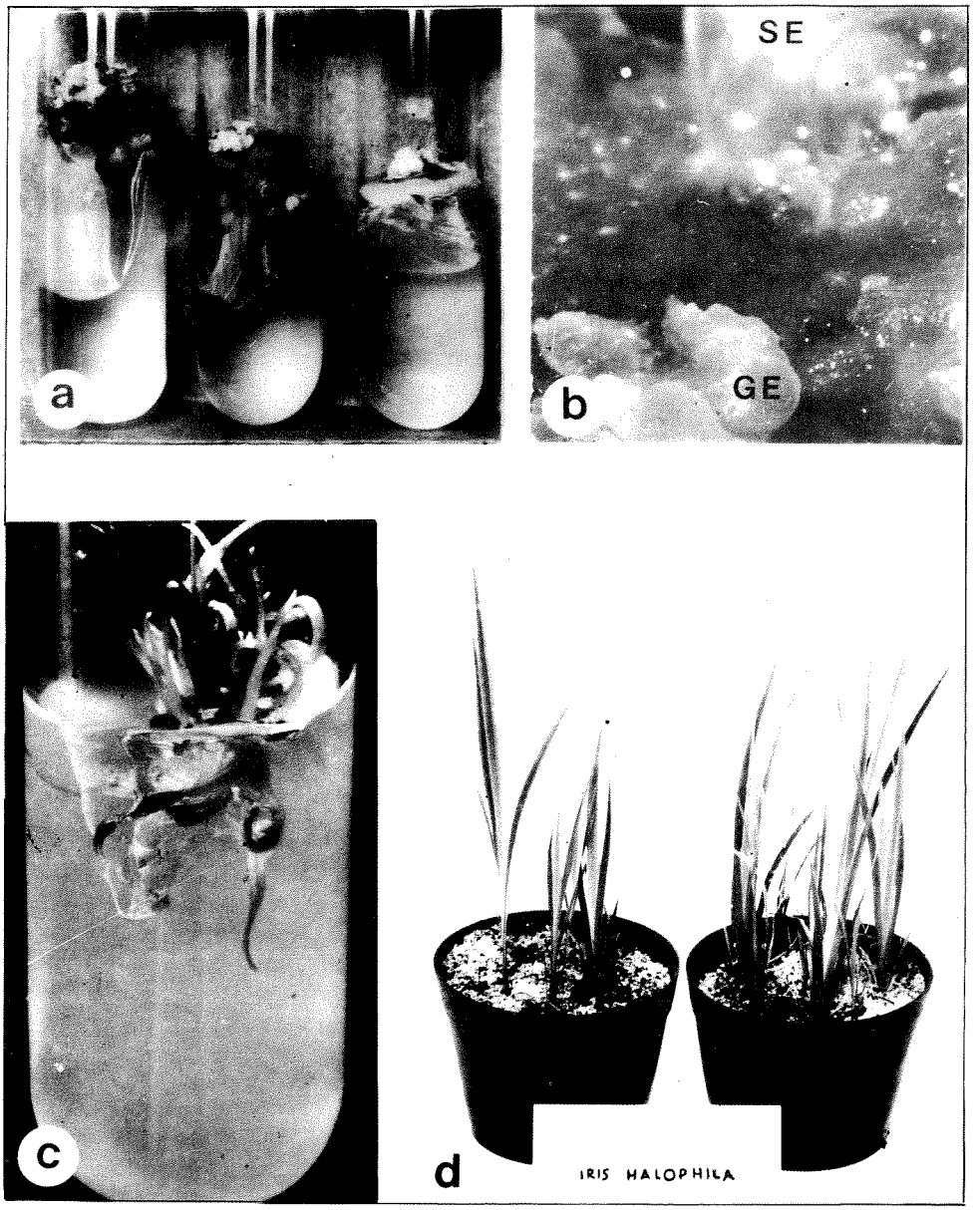

Fig. 3. – Somatic embryogenesis and organogenesis in the culture of zygotic *I. halophila* and *I. sibirica* embryos; a) – Cultures of *I. halophila* and *I. sibirica* on B_5 nutrient medium after weeks of culture; b) – EC of *I. halophila* (a detail) in an early (GE) and a late (SE) stage of development; c) – Multiplication of *I. halophila* after three subcultures; d) – Acclimatized *I. halophila* plants.

Organogenesis

Organogenesis in *I. halophila* and *I. sibirica* species was expressed after preculture of mature zygotic embryos and their growth on B_0 medium with a high concentration of 2,4-D and KN. Green organogenic callus of both iris species was observed already within the first three weeks of culture on C_3 medium. During furhter subcultures, on the same medium, AB (Fig. 3, d) was developed from OC. In order to get a higher number of regenerants the influence of different media (C_0-C_3) on the AB development was examined (Fig. 2). Most of *I. halophila* shoots were developed on C_0 nutrient medium. In *I. sibirica*, the highest number of AB, and thus the highest multiplication was achieved on C_2 medium. The lowest values of multiplication for both iris species were obtained when grown on C_3 nutrient medium. Number of rooted shoots grown in a D nutrient medium was found to be 92% and 96% for *I. halophila* and *I. sibirica*, respectively. Acclimation of plants was about the same for both iris species (Fig. 3, e).

DISCUSSION AND CONCLUSION

Regeneration of plants was achieved by somatic embryogenesis and organogenesis of the two iris species by the culture of mature zygotic embryos. For induction of both processes the presence of high auxin concentration, (2,4-D 5.0 mg/l) is required. The stage of induction in the culture of zygotic embryos is necessary for expression of morphogenetic potential is previously shown for *I. pumila* and *I. setosa* (Radojević et al., 1987; Radojević & Subotić, 1992). A decrease of auxin concentration to 0.1 mg/l in the cultures of the two iris species led to differentiation of three calli types, NEC, EC and OC. Isolation and separation of these calli enabled two pathways of regeneration to proceed. Medium containing 2,4-D and KN (1.0 mg/l) and supplemented with 250.0 mg/l of each CH and Pro (B_5) was found to be the most suitable for furhter EC growth and SE differentiation. Stimulatory action of Pro on somatic embryogenesis has been observed previously in maize (Armstrong & Green, 1985) and in different *Iris spp.* (Radojević et al., 1987, 1992; Jehan, 1994).

For the initial stages of the OC growth and the AB differentiation, C_3 nutrient medium was the most convenient. The best shoots multiplication of the both iris species was obtained on C_2 medium supplemented with BAP and NAA. These results are in accordance with the data of Hussey (1976) who obtained 50 to 100 shoots from a single initial explant of *Iris spp.* It is obvious that different growth regulators influence prominently the number of shoot produced per culture. Germination of somatic embryos and rooting of the AB was successfully achieved in D liquid nutrient medium with KN, IAA and GA_3 as previously described for *I. setosa* (Radojević & Subotić, 1992). This protocol for plant regeneration of both *Iris* species, through somatic embryogenesis and organogenesis, could be used for large scale production of regenerants.

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REFERENCES

Armstrong, C. L. & Green, C. E. (1985): Establishment and maintenance of friable, embryogenic maize callus and involvement of L-proline. – Planta *164*: 207-214.

Hussey, G. (1977): *In vitro* propagation of *Narcissus*. – Ann. Bot. *49*: 707-719.

Jéhan, H., Courtois, D., Ehert, C., Lerch, K. & Ptiard, V. (1994): Plant regeneration of *Iris pallida* Lam. and *Iris germanica* L. *via* somatic embryogenesis from leaves, apices and young flowers. – Plant Cell Rep. *13*: 671-675.

Laublin, H., Saini, S. & Cappadocia, M. (1991): *In vitro* plant regeneration via somatic embryogenesis from root culture of some rhizomateous irises. – Plant cell Tiss. Org. Cult. *27*: 15-21.

Meyer, M. M. Jr., Fuchigami, L. H. & Roberts, A. N. (1975): Propagation of tall 'bearded' Irises by tissue culture. – Hort Sci. *10* (5): 479-480.

Murashige, T. & Skoog, F. (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. – Physiol. Plant. *15*: 437-497.

Radojević, Lj. & Subotić, A. (1992): Plant regeneration of *Iris setosa* Pall. through somatic embryogenesis and organogenesis. – J. Plant Physiol. *139*: 690-696.

Radojević, Lj., Sokić, O. & Tucić, B. (1987): Somatic embryogenesis in tissue culture of iris (*Iris pumila* L.). – Acta Hortic. *212*: 719-723.

Reuther, G. (1977): Embryoide Differenzierungsmuster im Kallus der Gattungen *Iris* und *Asparagus*. *Ber. Deutsh.* – Bot. Ges. *90*: 417-437.

Rezime

ANGELINA SUBOTIĆ, LJILJANA RADOJEVIĆ

REGENERACIJA BILJAKA *IRIS HALOPHILA* PALL. I. *IRIS SIBIRICA* FANCH. PUTEM SOMATSKE EMBRIOGENEZE I ORGANOGENEZE

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U kulturi zigotskih embriona *Iris halophila* i *I. sibirica* postignuta je regeneracija biljaka procesom somatske embriogeneze i organogeneze. Sukcesivnim delovanjem hranljivih podloga, sa različitim auksinima i citokininima, indukovani su neembriogeni (NEC), embriogeni (EC) i organogeni (OC) kalusi, a zatim somatski embrioni (SE) i adventivni pupoljci (AB). Somatska embriogeneza je indukovana na MS mineralnom rastvoru sa (u mg/l): 2,4-D 5.0, KN 1.0, CH 250.0 i Pro 250.0 (podloga B_0). Posle sedam dana gajenja na indukcionoj podlozi eksplantati su preneti na podlogu za diferencijaciju i razviće somatskih embriona, sa smanjenom koncentracijom 2,4-D 1.0 mg/l (podloga B_5). Na ovoj podlozi posle 2-3 nedelje gajenja pojavili su se somatski embrioni na svim razvojnim stadijumima. Razviće organogenog kalusa dobijeno je gajenjem eksplantata na C_3 hranljivoj podlozi sa (u mg/l): BAP 1.0, GA_3 0.1, Tyr 100.0 i AS 80.0. Multiplikacija izdanaka kod obe vrste najuspešnija je njihovim gajenjem na C_2 hranljivoj podlozi. Ožiljavanje zrelih somatskih embriona i izdanaka odvijalo se na MS hranljivoj podlozi sa KN 3.0, IAA 1.0 i GA_3 0.1 (mg/l). Regeneranti, obe vrste, su aklimatizovani na uslove staklare.

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Original scientific paper

ALEKSANDAR TUCOVIĆ, VASILIJE ISAJEV

DIMORFIZAM I FUNKCIJE CVETOVA I CVASTI PAJASENA

Šumarski fakultet, Beograd, Jugoslavija

Tucović, A., Isajev, V. (1995): *Ailanthus dimorphism and functions of flowers and inflorescences.* – Glasnik Instituta za botaniku i botaničke bašte Univerziteta u Beogradu, Tom XXIX, 157 - 165.

The data on the characteristics of tree sexuality in the local populations of ailanthus in Serbia are very scarce. This paper describes sexual dimorphism, inflorescence variability, records the rare phenomenon of neoteny, which are at least partly essential for the competition and aggressiveness of trees of heaven on the sites of urban, industrial, and tourist settlements.

Key words: ailanthus, sexual dimorphism, inflorescences, neoteny, outbreeding, inbreeding

Ključne reči: pajasen, polni dimorfizam, cvasti, autbriding, inbriding

UVOD

Drveće karakteriše neobična složenost grade i funkcija stabala, ogromna raznovrsnost unutar i između populacija i očigledna adaptivnost na veoma promenljive faktore spoljašnje sredine. Svako od ovih svojstava zahteva različite metode istraživanja, što obezbeđuje informacije različitog karaktera. Varijabilnost se ispoljava i u potomstvima većeg broja roditeljskih stabala, kao i u potomstvu jednog roditeljskog para, stvarajući materijal za prirodno i plansko odabiranje. U zavisnosti od faktora koji uslovljavaju varijabilnost organizma istu možemo razvrstati u dve osnovne grupe: (1)

modifikacije, i (2) genotipske varijacije. Modifikacije su uslovljene prvenstveno faktorima spoljašnje sredine, a genotipske varijacije prvenstveno genetičkim prestrojavanjima. Do danas su, pored kombinacione promenljivosti, najbolje proučene genotipske varijacije, koje su vezane za hromozome i gene, a označene su kao mutacije, što ne znači da se time iscrpljuju svi mogući izvori promenljivosti. U ovom radu razmotrićemo promenljivost i funkcionalnost cvetova i cvasti pajasena.

MATERIJAL I METODE

Kao objekat istraživanja odabrana su gajena stabla pajasena *(Ailanthus altissima Swingle)* i odgajena half sib potomstva odabranih semenskih stabala. Pajasen pripada rodu *Ailanthus Desf.* (Familija *Simarubacae L.C.Rich),* koji obuhvata 8 (Jovanović 1985) ili 12 do 15 vrsta (Krüssmann, 1965; Matikašvili, 1970; Vukićević, 1973, 1987), autohtonih u istočnoj Indiji, jugoistočnoj Aziji, Indoneziji i severnoj Australiji. Cveta posle listanja, krajem maja i početkom juna. Cvetovi su sitni beličasti ili zelenkasto žuti, u uspravnim, terminalnim metlicama, dugim 10-30 cm. Cvetovi su jednopolni ili dvopolni, neprijatnog mirisa, imaju žlezde u vidu diska, sa nektarom. Čašičnih listića 5, vrlo mali, u donjem delu srasli, tamnozeleni. Kruničnih listića 5 -6, zelenkastožuti, 2,5 do 3,5 mm dugi, u donjoj polovini kovrdžavo dlakavi. Prašnika 10. Tučak sa 5 oplodnih listića stubićima srasli. Rađa svake godine, počev od 5-te godine starosti. Plod je pljosnata, izdužena krilata orašica, do 5 cm duga i oko 1 cm široka, svetlocrvenkasta ili smeđa. Plodovi sazrevaju tokom septembra i oktobra. Kao pionirska vrsta upotrebljava se u pošumljavanju.

Fenofaze cvetanja i plodonošenja utvrđene su terenskim osmatranjima u intervalima od 7 dana. Analiza cvetova i cvasti obavljena je na 10 muških i 10 dvopolnih stabala i obuhvatila je po 50 (25 prostih i 25 složenih) cvasti tj. ukupno 500 muških i 500 dvopolnih cvasti. Pojava autogamije utvrđena je pomoću pojedinačnih, prostorno izolovanih stabala sa dvopolnim cvetovima-cvastima. Analiza roda obuhvatila je ukupan broj plodova na stablima iskazanim u hiljadama i u kilogramima za stabla različite starosti od 4-te do 41. godine starosti. Kvantitativni podaci o cvetovima biometrijski su obrađeni tj. utvrđene su granice varijabilnosti (minimum-maksimum), srednja vrednost ($\bar{x}$), standardna devijacija (S), varijacioni koeficijent (V), greška srednje vrednosti ($S_{\bar{x}}$), greška standardne devijacije (S_S) i greška varijacionog koeficijenta (S_V).

REZULTATI I DISKUSIJA

Rezultati obavljene analize cvasti stabla pojasena, odgajenih u cenozama drveća i žbunja gradskih, industrijskih i turističkih naselja izneti su u tablicama 1 i 2. Stabla pajasena karakterišu jednopolni- muški i dvopolni- muško-ženski cvetovi. Stabla pajasena obilno cvetaju, odmah nakon listanja, krajem maja i tokom juna meseca. Za osmatrana stabla karakteristično je ponovljeno drugo cvetanje, početkom i tokom avgusta. Muški cvetovi su na posebnim muškim stablima, a dvopolni na rodnim tzv. semenskim stablima tj. razdvojeni na posebnim stablima. Svojstvo stabla sa muškim cvetovima je da obavlja samo funkciju oprašivača- muškog roditelja, a stabla sa dvopolnim cvetovima funkciju i jednog i drugog roditelja. Na dvopolnim stablima funkcionalni su i muški i ženski reproduktivni organi. Naime, prostorno izolovana dvopolna stabla obilno plodonose, kao i stabla odrasla u lokalnim populacijama. Učestalost muških i dvopolnih stabala u lokalnim populacijama ostvaruje se u veoma različitim kombinacijama (od 0:100 ili 100:0). Odnos polova u lokalnim populacijama pajasena karakterističan je za poseban vid polnog dimorfizma, koji karakteriše različiti

odnos muških i dvopolnih stabala; kod dvodomih vrsta javljaju se, u različitim kombinacijama, muška i ženska stabla. Jednopolan tip cvetova na stablima uslovljava ujednačen razvoj muških i dvopolnih cvetova, kao i zametnutih plodova u svim delovima cvasti ili krošnji stabala pajasena. Plod pajasena je pljosnata, izdužena, krilata orašica, do 5 cm duga i oko 1 cm široka.

Tab. 1. – Biometrijska svojstva prostih A. muških i B. dvopolnih cvasti stabala pojasena
Biometric properties of simple A) male, B) bisexual ailanthus inflorescences

Broj stabala Number of trees	Dužina u cm Length in cm		Širina u cm Width in cm		Broj bočnih grana Number of branches		Broj cvetova Number of flowers	
	od min do max	$\bar{x} \pm S_{\bar{x}}$ $S \pm S_s$ $V \pm S_v$	od min do max	$\bar{x} \pm S_{\bar{x}}$ $S \pm S_s$ $V \pm S_v$	od min do max	$\bar{x} \pm S_{\bar{x}}$ $S \pm S_s$ $V \pm S_v$	od min do max	$\bar{x} \pm S_{\bar{x}}$ $S \pm S_s$ $V \pm S_v$
A. Muške cvati - Male inflorescences								
10	10	24.05 ± 2.18	4	12.55 ± 1.98	5	9.00 ± 0.08	50	258.00 ± 5.09
		3.44 ± 1.53		3.13 ± 1.40		1.24 ± 0.06		83.50 ± 3.63
	32	14.33 ± 0.64	23	25.04 ±1.56	12	13.78 ± 0.62	550	31.20 ±1.44
B. Dvopolne cvasti - Bisexual inflorescences								
10	14	21.78 ± 1.50	5	13.16 ± 1.39	5	10.00 ± 0.10	1	117.00 ± 3.67
		2.37 ± 1.40		2.20 ± 0.98		1.66 ± 0.07		58.00 ± 2.59
	28	10.87 ± 0.48	19	16.68 ± 0.74	13	16.66 ± 0.74	300	49.23 ± 2.20

Tab. 2. – Biometrijska svojstva složenih A. muških i B. dvopolnih cvasti pajasena
Biometric properties of compound A) male, B) bisexual ailanthus inflorescences

Broj stabala Number of trees	Dužina u cm Length in cm		Širina u cm Width in cm		Broj prostih cvasti Number of simple inflorescences		Broj cvetova Number of flowers	
	od min do max	$\bar{x} \pm S_{\bar{x}}$ $S \pm S_s$ $V \pm S_v$	od min do max	$\bar{x} \pm S_{\bar{x}}$ $S \pm S_s$ $V \pm S_v$	od min do max	$\bar{x} \pm S_{\bar{x}}$ $S \pm S_s$ $V \pm S_v$	od min do max	$\bar{x} \pm S_{\bar{x}}$ $S \pm S_s$ $V \pm S_v$
A. Muške cvati - Male inflorescences								
10	19	34.22 ± 0.41	20	36.56 ± 0.44	3	7.00 ± 0.11	100	1868.00 ± 42.40
		6.54 ± 0.29		6.95 ± 0.31		1.80 ± 0.08		670.00 ± 29.60
	47	19.13 ± 0.85	58	18.99 ± 0.85	13	25.71 ± 1.15	3200	25.86 ± 1.60
B. Dvopolne cvasti - Bisexual inflorescences								
10	14	32.26 ± 0.35	12	38.22 ± 0.41	2	9.00 ± 0.14	100	876.00 ± 26.78
		5.62 ± 0.25		6.57 ± 0.29		2.28 ± 0.10		360.00 ± 16.07
	49	17.40 ± 0.78	50	17.20 ± 0.77	13	25.33 ± 1.13	800	41.09 ± 1.83

Muški cvetovi pajasena grupisani su u muške proste, ± uspravne cvasti, i složene (zbirne) ± uspravne, horizontalne i retko viseće metličaste cvasti (Tabs. 1A i 2A, Fig.

1). Dužina plodnih cvasti varira od 10 do 32 cm sa srednjom vrednošću od 24.05 ± 2,18 cm (Tab. 1A). Širina cvasti po stablima varira od 4 do 23 cm sa srednjom vrednošću za 10 analiziranih stabala od 12,55 ± 1,98 cm. Broj bočnih grana u prostim muškim cvastima je najvarijabilnija osobina sa prosečnom vrednošću za 250 cvasti od 9 ± 0,08 bočnih grana. Minimalan broj bočnih grana je 5 a maksimalan 12. Broj cvetova po stablima varira od 5 do 550 sa srednjom vrednošću od 258 ± 5,09 cvetova po stablu. Složene (zbirne) cvasti se sastoje od prosečno 7 ± 0,11 prostih cvatsi. Minimalan broj prostih cvasti je 3 a maksimalan 13. Ukupna srednja dužina zbirnih muških cvasti je 34,22 ± 0,41 cm, širina u proseku 36,56 ± 0,44 cm a broj cvetova varira od 100 do 3200 a u proseku 1680 ± 42,40 cvetova po cvasti (Tab. 2A). Muški cvetovi su sa višestruko intenzivnijim mirisom od dvopolnih. Jedan deo muških stabala u lokalnim populacijama cveta po drugi put, početkom avgusta meseca. Pri drugom cvetanju raste broj prostih u odnosu na zastupljenost zbirnih cvasti. Složene cvasti obrazovane avgusta meseca su kraće, uže i sa manje bočnih grana u prostim cvastima. Nakon osipanja muških cvetova samo na jednom stablu od nekoliko stotina osmatranih, uočena je pojava dvopolnih cvetova tj. mozaičnih cvasti izgrađenih od dominantno muških i malog broja dvopolnih cvetova (Fig. 2). Postojanje dvopolnih cvetova u muškim cvastima ovog stabla uočljivo je usled brzog rasta budućih plodova. Sušenje osovina cvasti nakon opadanja muških cvetova dovodi do sušenja obrazovanih, retkih plodova, koji opadaju zajedno sa osovinama muških cvasti. Sakupljanjem i setvom zelenih plodova sa ovakvih mozaičnih stabala, moguće je u uslovima laboratorije odgajati vitalne sadnice pajasena.

Fig. 1. – Prosta (1A, levo) i složena (1B, desno) cvast pajasena
Simple (1A, left) and compound (1B, right) male inflorescence of ailanthus

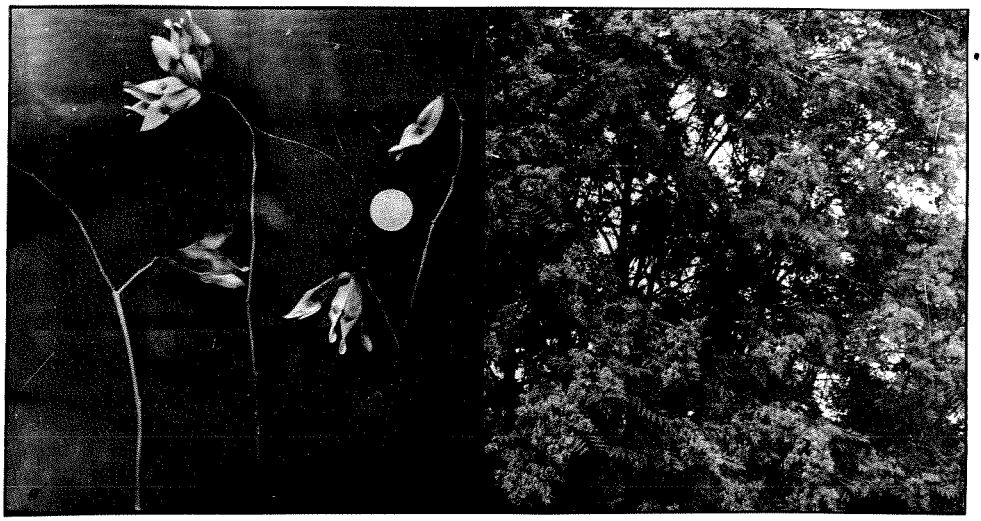

Fig. 2. – Delovi složene muško-dvopolne cvasti pajasena na retkim ± u skupinama krilatim orašicama u sazrevanju (2A, levo). Krošnja dvopolnog stabla pajasena sa brojnim ± uspravnim, ± horizontalnim i visećim složenim plodnim cvastima (2B, desno) pajasena

Parts of mosaic male-bisexual inflorescence with rare æ gathered winged ripening fruits (2A, left). Bisexual ailanthus crown with numerous æ upright, horizontal and drooping, compound fruiting inflorescences (2B, right)

Dvopolni cvetovi pajasena grupisani su u dvopolne (muško-ženske) uspravne proste i složene, uspravne, ± horizontalne i retko viseće cvasti (Tabls. 1B i 2B). Dužine prostih cvasti po analiziranim stablima variraju od 1 do 28 cm sa srednjom vrednošću za 250 cvasti od 21,78 ± 1,50 cm (Tab. 1B). Srednja širina prostih cvasti varira od 5 do 19 cm sa srednjom vrednošću od 13,16 ± 1,39 cm. Broj bočnih grana varira od 5 do 13 sa srednjom vrednošću od 10 ± 0,10. Srednji broj dvopolnih cvasti varira od 1 do 300 sa ukupnom srednjom vrednošću od 117 ± 3,67 cvetova. Od četiri analizirana svojstva prostih dvopolnih cvasti najpromenljiviji je broj cvetova na šta ukazuju i vrednosti za standardnu devijaciju i varijacioni koeficijent. Složene dvopolne cvasti sastoje se od više, obično 4 do 6 prostih cvasti. Ukupna dužina im je 32,26 ± 0,65 cm, širina u proseku 38,22 ± 0,41 cm a ukupan broj cvetova 876 ± 26,78 cvetova (Tab. 2B). Eventualno prisustvo mozaičnih cvasti nije utvrđeno, verovatno zbog brzog opadanja muških cvetova nakon oprašivanja kao i zbog velike retkosti ove pojave. Analizirane složene muške i dvopolne cvasti sastoje se od prostih, pojedinačnih cvasti. Prema karakteru rastenja one su zatvorenog tipa, tj. primitivnije građe, što svedoči o srodnosti ove vrste sa drugim vrstama roda, rasprostranjenim u jugoistočnoj Aziji, Indoneziji i Australiji (Jovanović, 1987; Vukićević, 1973 i dr.). Izuzetno, pri neoteniji, cvetovi pajasena pojavljuju se pojedinačno i terminalno na klijancima (Fig. 3) starosti od 33 dana (Tucović, Isajev, Orlović, 1996).

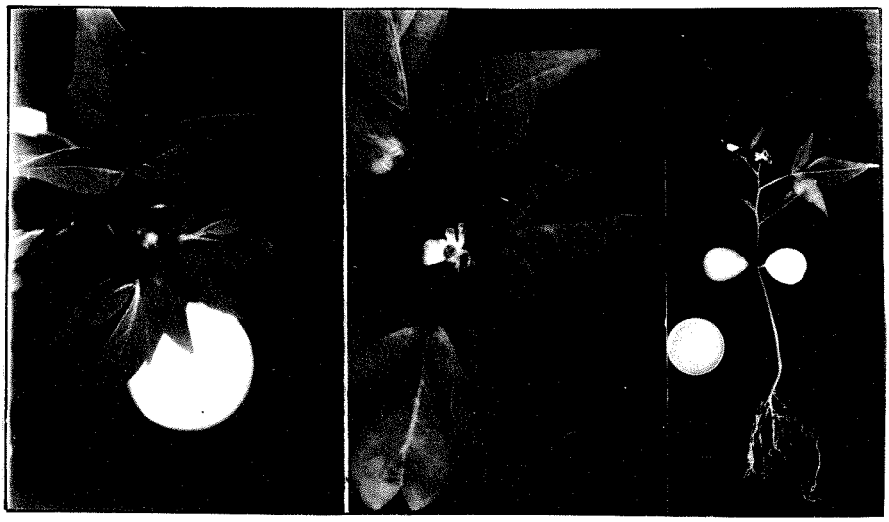

Fig. 3. – Rano cvetanje pajasena u uzrastu klijanaca. Pojedinačni, terminalni cvetni pupoljak (3A, levo) i muški cvet na klijavcu (3B). Opšti izgled klijanca pajasena u uzrastu od 33 dana (3C, desno)

Early flowering ailanthus seedling. Solitary terminal flower bud (3A, left) and male flower on a seedling (3B); general appearance of ailanthus seedling 33 days old (3C, right)

Muška i dvopolna stabla pajasena u različitim kombinacijama sa brojem semenskih stabla u lokalnim populacijama, obezbeđuju sve tipove oprašivanja pajasena: od stranog oprašivanja (autbridinga) do ukrštanja u većem ili manjem srodstvu (inbridinga). Proste i zbirne muške cvasti doprinose stranom oprašivanju, a složene plodne cvasti dorpinose uspešnom rasejavanju plodova – semena, konkurentnsoti i ekspanzivnosti stabla pajasena u cenozama gradskih, industrijskih i turističkih naselja (Fig. 4). Strano oprašivanje (između muških i dvopolnih stabala, između dvopolnih stabala) kao i oprašivanje u srodstvu (između susednih stabala, polusrodnika i samooprašiva-njem), brz rast, obilan rod (Tab. 3), raznovrsnost potencijalnih staništa, omogućuju optimalno prevođenje potencijalne genetičke promenljivosti u slobodnu promenljivost, dostupnu prirodnoj selekciji. Velika slobodna promenljivost klijanaca, mladih a i odrasnih stabala bar delimično objašnjavaju konkurentnost i ekpanzivnost stabala pajasena u cenozama drveća u gradskim naseljima kao i uspešan rast i opstanak i na ekstremnim nalazištima u nas.

Proučavanje faktora koji kontrolišu razviće potencijalnih osobina biljke jedan je od najznačajnijih zaataka savremene botanike, sa aspekta fundamentalne nauke kao i zbog osobina biljaka koje su na različite načine neophodne i korisne. Usled toga, aktuelne probleme hormonalne regulacije polnog dimorfizma biljaka u nas istraživalo je više autora (Ćulafić i Nešković, 1974; 1975; Ćulafić, 1984. i drugi). Pajasen je pogodna vrsta za eksperimentalna izučavanja uzroka polne diferencijacije, hormonalne regulacije polnog dimorfizma i dr. kod drveća s obzirom na rano cvetanje, izražen polni dimorfizam, građu cvasti, brz rast, obilnost roda i konkurentnost stabala na nizijskim i brdskim staništima Srbije.

Tab. 3. – Procena obilnosti roda dvopolnih stabala iskazana u zavisnosti od uzrasta matičnih stabala

Assessment of seed crop abundance of bisexual trees of heaven depending on age

Broj stabala Number of trees	Starost Age	(minimalan) srednji (maksimalan) broj plovoda u hiljadama (minimum) mean (maximum) number of fruits in 000			Srednji rod po stablu u kg Mean seed crop per tree in kg
20	4	(0)	1.5	(3)	0.03
20	5	(1)	4.0	(8)	0.12
20	6	(1)	16.0	(26)	0.50
20	10	(12)	100.0	(154)	3.38
20	15	(30)	80.0	(180)	2.66
10	20	(52)	150.0	(180)	5.00
10	25	(83)	200.0	(270)	8.66
10	30	(75)	180.0	(290)	6.00
5	35	(103)	200.0	(300)	6.66
5	41	(80)	160.0	(210)	5.33

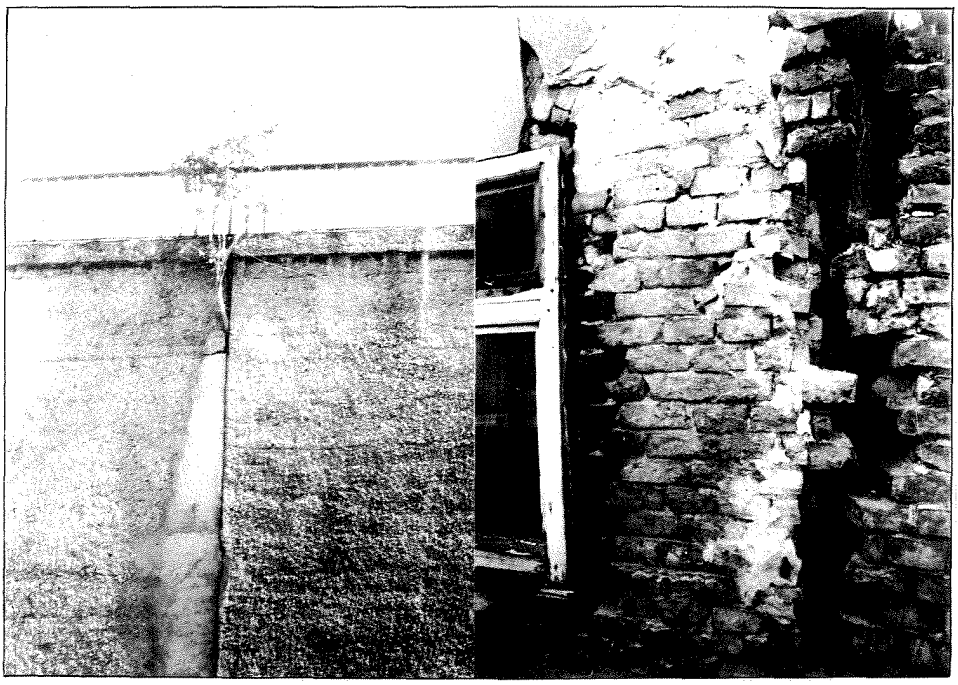

Fig. 4. – Muško stablo pajasena sa prostim cvastima odraslo na potpornom zidu mosta „Bratstvo i jedinstvo" na Savi (4A, levo)

Male ailanthus with simple inflorescences, growing on the retaining wall of the bridge „Bratstvo i jedinstvo" on the river Sava (4A, left)

ZAKLJUČAK

U radu se iznose novi podaci o specifičnom polnom dimorfizmu u lokalnim populacijama pajasena (muška i dvopolna stabla pajasena u različitim odnosima), o svojstvima prostih i zbirnih cvasti i evidentira retka pojava pojedinačnih, terminalnih cvetova na klijancima starosti od 33 dana. Autori opisuju dva tipa muških i dva tipa dvopolnih cvasti, koje se javljaju na različitim stablima. Složene muške i dvopolne cvasti su u osnovi jednostavne građe, sastoje se od 2 do 13 prostih cvasti, zatvorenog tipa. Proste cvasti su uglavnom uspravne, a složene uspravne, horizontalne i retko i viseće. Mozaične muško-dvopolne cvasti su vrlo retke a evidentirane su po prvi put samo na jednom od velikog broja posmatranih stabala pajasena u Srbiji. Proste i zbirne muške cvasti doprinose stranom oprašivanju, a dvopolne stranom oprašivanju i samooprašivanju. Složene plodne cvasti uslovljavaju uspešno rasejavanje semena – plodova sa krilatim dodacima, prilagođenih anemohoriji, a smanjena konkurentnost u urbanoj sredini uslovljava ekspanzivnost stabala pajasena u cenozama gradskih, industrijskih i turističkih naselja.

Pajasen je pogodna vrsta za eksperimentalna izučavanja uzroka polne diferencijacije u lokalnim populacijama, hormonalne regulacije polnog dimorfizma i dr. kod drveća, s obzirom na rano cvetanje, izražen specifičan polni dimorfizam, građu cvasti, brz rast, obilnost roda, ekspanzivnost i konkurentnost stabala na nizijskim i brdskim staništima Srbije.

LITERATURA

Chulafich, Lj. (1978): Sex exspression *in vitro* culture. Hormonal regulation of plant ontogenesis. – Nauka, Moscow.

Ćulafić, Lj. & Nešković, M. (1974): Indole auxin in spinach plants grown in long and short days. – Biol. Plant. *16*(5): 359-363.

Ćulafić, Lj. & Nešković, M. (1975): A study of auixne and gibberellins during shoot development in *Spinacia aleracea* L. Arch. Biol. Nauk. *8*: 19-27.

Giperborejski, S. (1968): Pasen (ajlant, *Ailanthus Desf.)*. Šumarska enciklopedija, 2, pp. 204-205. – Zagreb.

Jovanović, B. (1987): Dendrologija. – Naučna knjiga, Beograd.

Krüssmann, G. (1965): Die Laubgehölre. – Paul Parey, Berlin-Hamburg.

Matikašvilli, I.V. (1970): Sem. *Simarubaceae. Lindl.* Dendroflora Kavkaza. SSSR 5, pp. 32-35.

Tucović, A., Isajev, V. & Orlović, S. (1996): *Ailanthus (Ailanthus altissima Swingle)* neoteny and possible physiological aouses. – The 10th FESP Congress. Sept. 9-13.1996. Firenze, Italy.

Vukićević, E. (1973): Fam. *Simarubaceae L.C. Rich. In:* Flora SR Srbije, *V* (M. Josifović, ed.) pp. 61-62. – SANU, Beograd.

Vukićević, E. (1987): Fam. *Simarubaceae D.C.* Dekorativna dentrologija. pp. 456-457. – Naučna knjiga, Beograd.

Summary

ALEKSANDAR TUCOVIĆ, VASILIJE ISAJEV

AILANTHUS DIMORPHISM AND FUNCTIONS OF FLOWERS AND INFLORESCENCES

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The paper gives the new data on the specific sexual dimorphism in the local tree of heaven populations (male and bisexual trees of heaven in varying ratios), on the properties of simple and compound inflorescences, and also a rare phenomenon of

solitary, terminal flowers on 33-day-old seedlings has been reported. Two types of male and two types of bisexual inflorescences occurring on different trees have been described. Compound male and bisexual inflorescences have basically a simple structure, consisting of 2 to 13 simple inflorescences, of the closed type. Simple inflorescences are mainly vertical, and compound ones are: vertical, horizontal and rarely drooping. Mosaic, male-bisexual inflorescences are very rare and have been observed for the first time only on one, out of a great number of the observed trees of heaven in Serbia. Simple and compound male inflorescences contribute to outbreeding, bisexual ones to outbreeding and self-pollination, and compound fruiting inflorescences to successful dissemination of seeds – fruits, competition and aggressiveness of trees of heaven in our country in the plant communities of urban, industrial and tourist settlements.

Tree of heaven is a suitable species for experimental study of the causes of sexual differentiation in local populations, hormonal regulation of sexual dimorphism etc. in trees, considering its early flowering, an expressed specific sexual dimorphism, inflorescence structure, fast growth, abundant yield, aggressiveness and competition of trees in lowland, hilly and upland sites in Serbia.

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JELENA BLAŽENČIĆ

FLORISTIČKE KARAKTERISTIKE MAKROFITSKE VEGETACIJE SAVSKOG JEZERA KOD BEOGRADA (SRBIJA, JUGOSLAVIJA)

Institut za botaniku i botanička bašta „Jevremovac"
Biološkog fakulteta u Beogradu

Blaženčić J. (1995): *Floristic characteristics of the macrophytic vegetation in Lake Savsko near Belgrade (Serbia, Yugoslavia).* – Glasnik Instituta za botaniku i botaničke bašte Univerziteta u Beogradu, Tom XXIX, 167 - 173.

A comparative analysis of the flora of Lake Savsko recorded at present and ten years ago has been performed. Significant differences in the floristic composition as well as in the spatial distribution of the populations have been identified in these ten years. Out of 24 species recorded in total, 17 have been noticed for the first time at this locality. Most species are widespread in slow-running and stagnant waters of Serbia. *Nitella mucronata* represents a new species in the flora of Serbia.

Key words: reservoir, macrophytes, lake overgrowing.

Ključne reči: vodojaža, makrofite, zarastanje jezera.

UVOD

Savsko jezero, plitka mikroakumulacija izložena intenzivnom antropogenom uticaju, u litoralnom delu lako obrasta makrofitskom vegetacijom. U slobodnoj vodi jezera često se javlja masovno razviće algi. To u velikoj meri narušava osnovne namene

ovog jezera, a to su snabdevanje Beograda vodom i njegovo korišćenje kao reprezentativnog i atraktivnog sportsko-rekreacionog centra.

Da bi jezero funkcionisalo u skladu sa namenom neophodno je njegovo stalno održavanje i periodično generalno čišćenje. Jedna od takvih akcija preduzeta je 1983. godine, kada je i urađena obimna limnološka studija o povećanju biomase u jezeru i efektima njenog suzbijanja (Janković et al., 1983).

O uzrocima koji dovode do eutrofikacije i zarašćivanja Savskog jezera, problemima koji iz toga proizilaze i o merama koje treba preduzeti da se ono održava u granicama projektovanih namena ukazuje niz autora (Obušković, 1978, 1979, Kalafatić et al., 1984, Janković & Janković, 1987).

Budući da je od čišćenja Savskog jezera prošlo više od deset godina i da je ponovo aktuelan problem zarašćivanja, interesovalo nas je da uporedimo prethodno sa sadašnjim stanjem u pogledu flore koja se nalazi u jezeru i njenog prostornog rasporeda, ustanovimo eventualne razlike i iste protumačimo.

MATERIJAL I METODE

Proučavanja flore i prostornog rasporeda populacija makrofita u Savskom jezeru na Ada Ciganliji, obavljena su u junu i julu 1994., februaru i aprilu 1996. godine. Istraživanja su vršena iz čamca metodom poprečnih i uzdužnih transekata. Uzorci su uzimani duž transekata na svaki metar dubine. Sakupljeni materijal je fiksiran i konzerviran u 4% formaldehidu i čuva se u zbirci Instituta za botaniku Biološkog fakulteta u Beogradu (BEOU!).

Na jezeru su registrovani osnovni ekološki faktori: temperatura, pH i providnost vode, fizičko svojstvo dna i dubina sa koje je materijal uziman. Temperatura je merena digitalnim termometrom tipa DT1, proizvodač „Dalmacija", providnost je određivana Secchi-jevim diskom prečnika 25 cm, a reakcija vode (pH) pehametrom. Materijal je sakupljan posebno konstruisanim grabilima (Blaženčić & Blaženčić, 1991).

Determinacija vrsta izvršena je na osnovu ključeva Gollerbah & Krasavina (1983), Josifović (1970-1977), Komarov & Ellin (1934), Hegi (1965).

REZULTATI

Karakteristike biotopa

Na južnoj strani Ada Ciganlije, nekadašnjeg ostrva, a sada poluostrva na reci Savi, koje je od centra Beograda udaljeno samo 4 km, nalazi se Savsko jezero (Fig. 1). Leži na nadmorskoj visini od 70 m. Okruženo je šumom vrbe (*Salix alba* L.), bele topole (*Populus alba* L.), hrasta lužnjaka (*Quercus robur* L.) i dr.

Mikroakumulacija Savsko jezero formirana je kao vodoprivredni i sportsko-rekreativni objekat 1967. godine pregrađivanjem rukavca reke Save.

Jezero ima oblik blago savijene kifle. Dugačko je 4,2 km. Prosečna dubina jezera je 4.5 m, a maksimalna 12 m. U najvećem delu široko je oko 200 m. Prostire se na površini od 86 ha. U njemu se nalazi oko 4 x 10^6 m^3 vode. Vodom se snabdeva prepumpavanjem iz Save, preko taložnika.

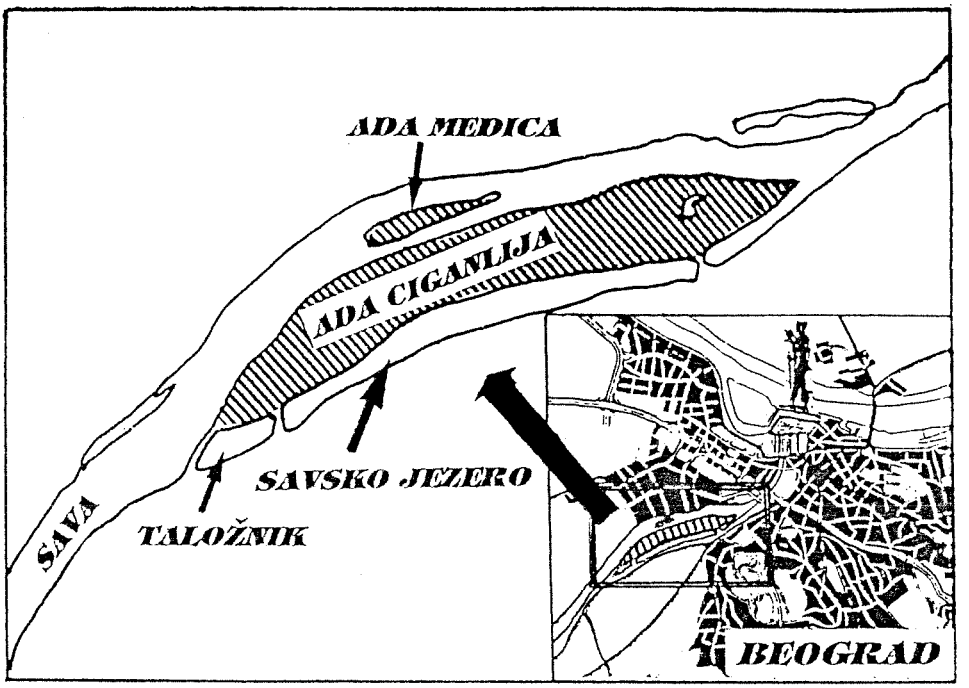

Fig. 1. – Položaj Savskog jezera u Beogradu
Position of Lake Savsko in Belgrade

Dno Savskog jezera je po svojim fizičkim osobinama različito. Veći deo priobalnog dela je šljunkovit. U ostalom delu jezerskog basena dno je muljevito - peskovito, muljevito i glinovito. Po konzistenciji dno varira od rastresitog do čvrstog.

Temperatura vode od površine do dubine od 5 m, tj. u zoni gde se nalazi makrofitska vegetacija, uglavnom je ujednačena. U aprilu razlika u temperaturi između površinskog sloja (9.2°C) i na dubini od 5 m (7,8°C) iznosila je 1,4°C, a između površine i 4 m dubine razlika je samo 0,5°C. U junu temperaturna razlika, između površine i 5 m dubine iznosila je 0,9°C, dok je u julu i na površini i na dubini od 3 m dubine izmerena ista temperatura od 27°C.

U toku zime jezero je često zaleđeno. Led se ponekad zadržava i u dužem vremenskom periodu i dostiže debljinu 20-30 cm, koliko smo izmerili u februaru 1996. godine.

Providnost vode veoma varira kako sezonski, tako i na različitim mestima u jezeru. U proleće voda je bistra i sa izuzetnom providnošću od 4,7 m. Početkom juna providnost opada na 2,2 - 2,6 m, da bi u julu u blizini obale iznosila samo 0,8 m, a u centralnom delu 1,2 m.

Voda Savskog jezera je bazne reakcije (pH 8,2 - 8,72).

Floristički sastav vegetacije

U makrofitskoj vegetaciji Savskog jezera dominantnu ulogu imaju vaskularne biljke, a samo sporadično javljaju se i makroskopske končaste ili složenije građe alge - pršljenčice (*Charophyta*).

Vegetacija Savskog jezera predstavljena je emerznim, flotantnim i submerznim biljkama.

Emerzne i flotantne biljke zadržale su se u severoistočnom i jugozapadnom delu jezera. Tu se razvijaju u vidu potkovice i pružaju duž obala prema centralnom delu jezera, gde se ubrzo gube, jer su severozapadna i jugoistočna obala u najvećem delu uređene kao kupališta.

U zoni **emerznih** biljaka konstatovane su sledeće vrste:

Schoenoplectus lacustris (L.) Palla*

- mala populacija u severoistočnom delu jezera u blizini taložnika, na dubini od 0,5 m.

Bolboschoenus maritimus (L.) Palla* - u jugoistočnom i severozapadnom delu jezera na dubini od 1 m.

Typha latifolia L.* - u severozapadnom, delu jezera na dubini od 1 m.

Polygonum amphibium L.* - oko jezera na dubini od 0,5 m.

Alisma plantago-aquatica L.* - u jugoistočnom delu jezera na dubini do 0,5 m.

Butomus umbellatus L.* - na jugozapadnoj i severozapadnoj obali jezera, na dubini do 0,5 m.

Eleocharis palustris (L.) Roem. et Schult.* - malobrojna populacija u severoistočnom delu jezera u blizini taložnika.

Mentha aquatica L.* - malobrojna populacija u jugoistočnom delu jezera na dubini do 0,5 m.

Kao emerzna i zona **flotantnih** biljaka je redukovana i diskontinuirana. Malobrojni predstavnici biljaka plivajućih listova zabeleženi su mestimično oko celog jezera, a nešto više su zastupljeni u njegovom severozapadnom i jugoistočnom delu.

Potamogeton natans L. - u severozapadnom delu jezera na dubini 1-1,5 m.

Potamogeton nodosus Poir.* - mestimično oko celog jezera, na dubini 1,5 do 2 m.

Salvinia natans (L.) All.* - nađen samo jedan primerak u blizini tornja.

U Savskom jezeru je najrazvijenija i vrstama najbogatija zona **submerznih** biljaka. One se nalaze od same obale pa do dubine od 6 m.

Batrachium circinatum (Sibth.) Spach.* - konstatovan na više mesta u blizini obale.

Batrachium trichophyllum (Chaix.) Van den Bosch* - konstatovan na više mesta u blizini obale.

Myriophyllum spicatum L. - od priobalnih plićaka do dubine od 5 m, oko celog jezera. Dominantna vrsta. Gradi kontinuiranu zonu na dubini između 2 - 4 m. U zoni krocanja (*Myriophyllum spicatum*) u toku leta izmerena je temperatura 27°C, a u toku zime sasvim dobro se održava, u vidu dobro razvijenih podvodnih livada u kojima leže „uspavani" šarani, pri temperaturi od +2°C do +5°C.

* - nova vrsta za lokalitet

Potamogeton pectinatus L. - mozaično obrasta priobalne delove jezera (do 1 m dubine), na mestima gde su posečene emerzne biljke („krčevine").

Potamogeton pusillus L.* - konstatovan na istim mestima kao i *Potamaogeton pectinatus*.

Potamogeton lucens L.* - naden samo jedan primerak na južnoj strani jezera, preko puta tornja, na dubini od 1,5 m - 2 m.

Potamogeton crispus L. - konstatovan na južnoj obali jezera, preko puta tornja, u zoni šume ispred plaže, na dubini od 1,5 m - 2 m.

Najas minor Pers. - konstatovan na istim mestima kao i *Potamogeton pectinatus*.

Najas marina All. - vrlo retka u priobalnim delovima jezera, brojnija u dubljim jezerskim zonama. Mozaično raspoređena oko celog jezera. Gradi donju granicu rasprostranjenja makrofitske vegetacije na dubini od 6 m.

Ceratophyllum demersum L., - naden samo jedan primerak.

Chara contraria Br. ex Kütz.* - konstatovana u severozapadnom delu jezera, preko puta tornja, u zoni šume ispred plaže, na dubini od 1 m, sa *Potamogeton nodosus*.

Chara globularis Thuill.* - na istom mestu kao i *Chara contraria*, ali na mikrostaništu u nešto dubljoj vodi - 2 m, zajedno sa *Potamogeton crispus* i *Nitella mucronata*.

Nitella mucronata (A. Br.) Mig.* - kao *Chara globularis*.

DISKUSIJA

Na Savskom jezeru, od tri tipične vegetacijske zone, dobro je razvijena samo zona submerznih biljaka. Ostale dve, zona emerznih i zona flotantnih biljaka, veoma su rudimentirane i nalaze se samo na mestima koja nisu uređena za kupače.

Floristićkom analizom makrofitske vegetacije konstatovano je prisustvo 24 vrste od kojih 8 pripada emerznim, 3 flotantnim i 13 submerznim biljkama. Među submerznim makrofitama nalaze se i 3 vrste makroskopskih algi iz razdela *Charophyta*.

Uporednom floristićkom analizom sadašnjeg sa stanjem od pre deset godina koje su opisali Janković & Janković (1987) ustanovljene su značajne razlike. One se ispoljavaju kako u broju zabeleženih vrsta (ranije 12, sada 24), tako i u odnosu na sastav vrsta koje grade makrofitsku vegetaciju. U oba floristička spiska zajedničko je samo 6 vrsta: *Potamogeton natans* L., *Potamogeton crispus* L., *Potamogeton pectinatus* L., *Myriophyllum spicatum* L., *Najas minor* Pers. i *Najas marina* All. Na lokalitetu Savsko jezero prvi put je zabeleženo prisustvo 17 vrsta, od kojih su 16 široko rasprostranjene u sporotekućim i stajaćim vodama Srbije (u spisku obeležene znakom *). Za razliku od predhodnih, vrsta *Nitella mucronata* (A. Br.) Mig. je veoma retka u flori Srbije. Zabeležena je još samo u jezeru kod Blaca u blizini Prokuplja (Blaženčić J. & Ž., 20. 08. 1986, BEOU!).

Zapaženo je da pre deset godina nije zabeležena ni jedna emerzna vrsta, a sada ih ima osam. Iz te činjenice i mesta gde se razvijaju, na neuređenim delovima jezera, može se zaključiti da bi se ova zona razvila oko celog jezera da priobalni deo nije pod kontrolom i intervencijom čoveka (pošljunčavanje i uklanjanje vegetacije). Slično tumačenje može se dati i za odsustvo zone flotantnih biljaka. Umesto ranije prisutnih lokvančića (*Nymphoides flava* Hill.) i sočivice (*Lemna minor* L.) sada se kao najbrojnija među flotantnim biljkama javlja vrsta *Potamogeton natans* L.

U zoni submerznih biljaka apsolutnu dominaciju i edifikatorsku ulogu ima vrsta *Myriophyllum spicatum* L. Pre deset godina ta uloga je pored ove vrste, pripadala i

vrstama *Ceratophyllum demersum* L. *Myriophyllum verticillatum* L., i *Potamogeton crispus* L. (Janković & Janković, 1987). U toku naših istraživanja vrstu *Myriophyllum verticillatum* L. nismo našli, od vrste *Ceratophyllum demersum* L. našli smo samo jedan zakržljali primerak, a *Potamogeton crispus* L. konstatovan je samo na jednom staništu sa malim brojem individua.

Da bi smo razumeli navedene promene nužno je da ukažemo i na podatke o vertikalnoj distribuciji makrofita u Savskom jezeru. Submerzne biljke naseljavaju dno od obalskih plićaka do dubine od 6 m. Međutim, kontinuirana zona submerznih biljaka oko jezera u kojoj je osnovni graditelj vegetacije vrsta *Myriophyllum spicatum* L., nalazi se na dubini između 2 m i 4 m. U zoni do 2 m dubine biljke mozaično obrastaju dno i u pogledu svoje produkcione uloge nisu u sadašnjem trenutku toliko značajne za jezero. Ispod 4 m dubine mozaično se javljaju fragmenti populacija *Myriophyllum spicatum* L. i *Najas marina* All. sve do donje granice rasprostranjenja makrofita na dubini od 6 m.

Pre deset godina na donjoj granici rasprostranjenja makrofita najčešće se nalazila vrsta *Ceratophyllum demersum* i to na različitim dubinama od 3.5 do 8.0 m (Janković & Janković, 1987). Povlačenje donje granice rasprostranjenja makrofita sa 8 m, pre deset godina, na 5 - 6 m, danas, iz zone *Ceratophyllum* u zonu *Myriophyllum spicatum* - *Najas marina* predpostavljamo da je nastalo zbog smanjenja transparencije jezerske vode.

Veća biološka raznovrsnost makrofitske flore Savskog jezera ostvarena u proteklom desetogodišnjem periodu, kao i promene u prostornom rasporedu populacija još jedna su potvrda biološke fleksibilnosti i ekološke adaptivnosti živog sveta na promene uslova životne sredine.

LITERATURA

Blaženčić, J. & Blaženčić Ž. (1991): Makrofite Vlasinskog jezera. - Glasn. Prirodnjačkog muzeja u Beogradu *46*: 71-85.

Golterbah, M. M. & Krasavina, L.K. (1983): Opredelitel presnovodnyh vodoroslej SSSR, *14* - Narovye vodorosli - CHAROPHYTA. - Nauka, Leningrad.

Hegi, D.G. (1965): Illustrierte Flora von Mitteleuropa *1*. - München.

Janković, M. M., Janković M., Martinović - Vitanović V., Jakovčev, D. & Kalafatić, V. (1983): Studija o povećanju biomase u jezeru Ada Ciganlija i efektima njenog suzbijanja. - Elaborat, 1-104, Beograd.

Janković, M., M. & Janković, J. M. (1987): Prilog poznavanju i rešavanju problema eutrofizacije i zarašćivanja Savskog jezera (Ada Ciganlija) kod Beograda. - Glasn. Inst. za bot. i bot. bašte Univ. u Beogradu *16*: 1-41.

Josifović M. ed. (1970-1977): Flora SR Srbije *1-8*. - SANU, Beograd.

Kalafatić, V., Martinović - Vitanović, V. & Jakovčev, D. (1984): Problem sanacije Savskog jezera. - Vodoprivreda *16*, 88-89 (2-3): 246-248.

Komarov, V. L. & Ilin, M.M. eds. (1934): Flora SSSR *1*. - Leningrad.

Obuškovič, Lj. (1978): Dinamika fitoplanktona i ekoloških uslova u Savskom jezeru kod Beograda. - Magistarski rad. Biološki fakultet, Univerzitet u Beogradu.

Obuškovič, Lj. (1979): Pojava vodenog cveta vrste *Stephanodiscus astrea* var. *minutula* (Kg.) Grun. u Savskom jezeru kod Beograda. - Biosistematika *5* (2): 127-128.

Summary

JELENA BLAŽENČIĆ

FLORISTIC CHARACTERISTICS OF THE MACROPHYTIC VEGETATION IN LAKE SAVSKO NEAR BELGRADE (SERBIA, YUGOSLAVIA)

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The Lake Savsko reservoir is situated only 4 km away from the centre of Belgrade. Since its foundation in 1967 as a water-supply unit and a sporting and recreational centre, it has been subjected to significant anthropogenic influence. Its littoral is being permenently overgrown by the macrophytic vegetation (Janković et al., 1983., Kalafatić, et al., 1984, Janković & Janković, 1987).

Comparing the data on the flora and on the spatial distribution of species and their populations recorded 10 years ago with the present situation, including the results of actual measuring and descriptions of relevant ecological conditions, the authors wanted to identify the eventual changes in the state of the lake.

According to the results of this investigation, significant changes have occurred regarding the number of species living in the lake (24 species living now instead of 12 in 1987) as well as the composition of the species in the present macrophytic vegetation. Only 6 species recorded in 1987 are present today: *Potamogeton natans, P. crispus, P. pectinatus, Myriophyllum spicatum, Najas marina* and *Najas minor*. The remaining 17 species, the majority of which are widespread throughout Serbia in calm slow-running and stagnant waters, are recorded for the first time in Lake Savsko. *Nitella mucronata* is a new species in the flora of Serbia.

Out of three dominant species in the lake, recorded in 1987, only *Myriophyllum spicatum* has remained, while *Ceratophyllum demersum* and *Myriophyllum verticillatum* disappeared.

The authors presume that the small transparency of water (0,8 - 2,6 m during the vegetative period) caused the dislocation of macrophytes to the lower limit (from 8 m in 1987 to 6 m in 1996). The other factors, such as the resistance to the low temperatures of water, reproduction rate, competition with other species, are hard to define without a supplementary examination. The species *Myriophyllum spicatum* survives extremely well during the winter period under the ice, being adapted to low water temperatures that range from +2 to +5°C.

However, the *Ceratophyllum* zone, which represented the lower limit of the macrophytic vegetation between the depths of 6 and 8 m, disappeared during the last 10 yeary. At present, the lower limit of the macrophytic vegetation is edified by *Myriophyllum spicatum* at depths of 2 to 4 m. Within depth ranges from 4 to 6 m, only the populations of *Najas marina* appear mosaically, occasionally associated with *Myriophyllum spicatum*.

Regarding the lake overgrowing, nowadays it is evident that, for the last 10 years, the lake has not become overgrown in total, as it was predicted (Janković & Janković, 1987). On the contrary, the zone of the macrophytic vegetation has been reduced and, as a result, the lake bottom has become wider. This situation represents the effect of an action of the lake-cleaning service and arrangement of the lake banks along the bathing-place. On the other hand, low values of the water transparency influenced also the shift of the lower limit of the macrophytic distribution to a depth of 6 m.

GLASNIK INSTITUTA ZA BOTANIKU I BOTANIČKE BAŠTE UNIVERZITETA U BEOGRADU
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Original scientific paper

MIRKO CVIJAN, BRANKA TODOROVIĆ, VESNA JOKSIMOVIĆ

THE LICHENS AS BIOINDICATORS OF AIR POLLUTION IN THE TOWNS OF MALI ZVORNIK AND ARANĐELOVAC (YUGOSLAVIA)

Institute of Botany and Botanical Garden „Jevremovac", Faculty of Biology,
University of Belgrade

Cvijan, M., Todorović, B., Joksimović, V. (1995): *The lichens as bioindicators of air pollution in the towns of Mali Zvornik and Aranđelovac (Yugoslavia).* – Glasnik Instituta za botaniku i botaničke bašte Univerziteta u Beogradu, Tom XXIX, 175 - 186.

The results of the study of lignicolous flora of lichens found in Mali Zvornik and Aranđelovac town are presented here.

By examining the collected samples, the presence of 15 genera with 33 species and 12 genera with 29 species were established in Mali Zvornik and Aranđelovac, respectively.

On the basis of the distribution of determined taxa and by using qualitative scale for air pollution, three zones (with two subzones) in terms of air pollution were established in Mali Zvornik, and only two zones in Aranđelovac.

Key words: air, pollution, lichens, bioindication, Mali Zvornik, Aranđelovac

Ključne reči: vazduh, zagađenje, lišajevi, bioindikacija, Mali Zvornik, Aranđelovac.

INTRODUCTION

The papers concerned with the study of lichens and the correlation of their development with air pollution, are numerous (see S a v i ć et al., 1996). Unfortunatelly, papers dealing with lichens occurring in Serbia are scarce. However, in the past few years, there has been a surge of interest for the investigation of urban lichens as bioindicators of air pollution (C v i j a n et al., 1992; S t a m e n k o v i ć, 1992; M i l i ć, B l a ž e n č i ć, 1993; C v i j a n, S t a m e n k o v i ć, 1996).

The results of the study of lignicolous flora of lichens found in two small Serbian towns, in contrast to previously investigated big ones with very high air pollution, are presented here. By using bioindication values of numerous species, adequate zones in terms of air pollution (especially with sulphurdioxide) have been established.

MATERIAL AND METHODS

Samples of lichenes were collected from wooden surfaces of various type, mainly bark, from the urban territory of Mali Zvornik (May-August 1992) and Aranđelovac (spring 1992).

The analysed samples were gathered from 59 and 91 points in Mali Zvornik and Aranđelovac, respectively. The points were precisely marked on the maps of Mali Zvornik and Aranđelovac along with the data on the type of surface the lichens were collected from.

The collected material was examined in the Institute of Botany and Botanical Garden „Jevremovac" in Belgrade by using the available literature (see S a v i ć et al., 1996).

RESULTS AND DISCUSSION

MALI ZVORNIK (Litlle Zvornik) is a small town in Podrinjska area, with small number of inhabitants. It lies on the right bank of the Drina river (Fig. 1). The town area has very heterogeneous relief, the average altitude of which is 160 m. The climate is moderately continental with average annual temperature of 10°C (apsol. max. 34, apsol. min. -16°C) and average annual precipitation of 1025 mm. The west and north-west winds prevail and also south-west in the spring. However, the influence of the Drina river and great differences in the altitude between various points within the town area, are very importnat.

Although Mali Zvornik has prerequisites for good quality of air, still it is polluted. Namely, there are many boiler plants for heating of individual houses and big buildings. Some factories, especially lime-kiln, also use oil and coal for heating and for normal working process round the year. There is also mechanical pollution brought about by small parts of dust particles from the quarry „Bučevski potok" and from the stone cube production plant at the locality Radalj. The main communication routs passing through the greatest part of the urban area of Mali Zvornik, and heavy transit traffic have also harmful impact on the air quality.

By examining the collected smaples, the presence of 15 genera with 33 species was established (Tab. 1).

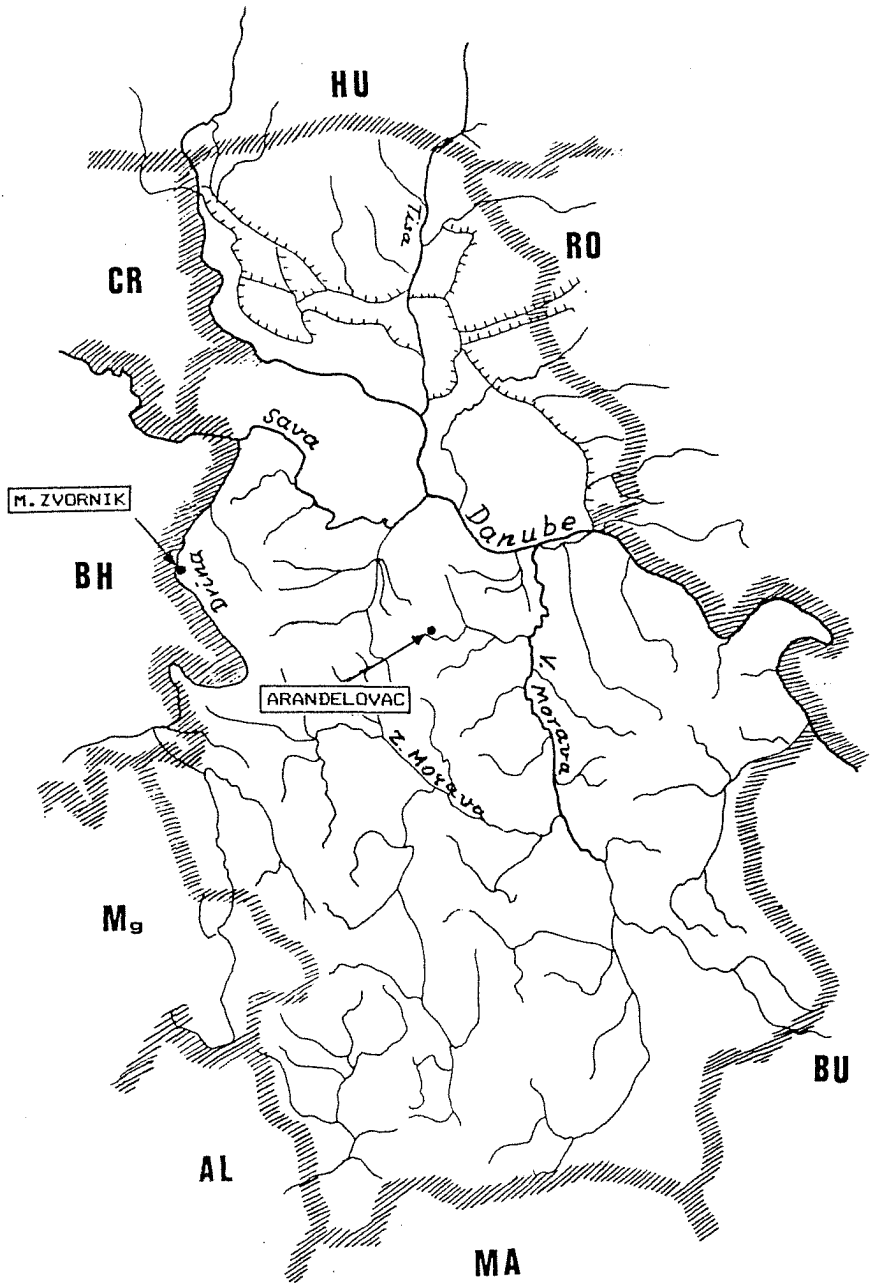

Fig. 1. – The investigated towns in Serbia
Istraženi gradovi u Srbiji

Tab. 1. – The determined lichen taxa from urban territory of investigatrd towns
Određeni taksoni lišajeva sa urbanog područja istraženih gradova

Nos.	Taxa	presence	
		M. Zvornik	Aranđelovac
1.	*Arhonia dispersa* (Schrad.) Nyl.	+	
2.	*Arthonia radiata* (Pers.) Ach.	+	
3.	*Arthothelium sardoum* Bagl.		+
4.	*Buellia punctata* (Hoffm.) Massal.	+	+
5.	*Caloplaca aurantiaca* (Lightf.) Th. Fr.	+	+
6.	*Caloplaca luteoalba* (Turn.) Th. Fr.	+	
7.	*Candelariella xanthostigma* (Pers.) Lett.	+	+
8.	*Cladonia fimbriata* (L.) Fr.	+	
9.	*Evernia prunastri* (L.) Ach.	+	+
10.	*Graphis elegans* (Sm.) Ach.	+	
11.	*Graphis scripta* (L.) Ach.	+	+
12.	*Hypogymnia physodes* (L.) Ach.	+	+
13.	*Lecanora campestris* (Schaer.) Hue	+	
14.	*Lecanora carpinea* (L.) Vain.	+	+
15.	*Lecanora chlarona* (Ach.) Nyl.		+
16.	*Lecanora chlarotera* Nyl.	+	
17.	*Lecanora intumescens* (Rebent.) Rabenh.	+	+
18.	*Lecanora subfusca* (L.) Ach. em. Hue	+	+
19.	*Lecanora* sp.		+
20.	*Lepraria aeruginosa* (Wigg.) Sm.	+	+
21.	*Lepraria* sp. Ach.	+	
22.	*Opegrapha atra* Pers.	+	
23.	*Parmelia caperata* (L.) Ach.	+	+
24.	*Parmelia dubia* (Wulf.) Schaer.	+	
25.	*Parmelia fuliginosa* (Fr.) Nyl.	+	+
26.	*Parmelia perlata* (Huds.) Ach.		+
27.	*Parmelia quercina* (Willd.) Vain.		+
28.	*Parmelia saxatilis* (L.) Ach.	+	+
29.	*Parmelia scortea* Ach.		+
30.	*Parmelia sulcata* Tayl.	+	+
31.	*Pertusaria albescens* (Huds.) Chosisy et Wern.	+	
32.	*Pertusaria isidioides* (Schaer.) Arn.	+	
33.	*Physcia aipolia* (Ehrht.) Hampe	+	+
34.	*Physcia ascendens* Bitter	+	+
35.	*Physcia caesia* (Hoffm.) Hampe	+	
36.	*Physcia grisea* (Lamk.) Lett.		+
37.	*Physcia leptalea* (Ach.) DC.		+
38.	*Physcia orbicularis* (Neck.) Poetch. em DR.	+	+
39.	*Physcia pulverulenta* (Schreb.) Hampe.	+	+
40.	*Physcia stellaris* (L.) Nyl. em Harm.		+
41.	*Physcia tenella* (Scop.) DC.	+	+
42.	*Xanthoria fallax* (Hepp) Arn.		+
43.	*Xanthoria parietina* (L.) Beltr.	+	+
	Total:	34	30

The individuals of determined lichens were gatherd from the bark of varous trees (Tab. 2).

Tab. 2. – The investigated points in Mali Zvornik with determined lichen taxa
Istražene tačke u Malom Zvorniku sa taksonima lišajeva koji su određeni

Nos. of sampl.	Points of sampling	Types of surface	Nos. of taxa from Tab. 1.
1.	Radnička street	*Aesculus hippocastanum* L.	1, 4, 13, 20, 30, 28, 33, 34, 36,38, 41, 43
2.	"	*Juglans regia* L.	4, 13, 17, 34, 43
3.	"	*Acer pseudoplatanus* L.	33, 34
4.	"	*Prunus domestica* L.	4, 34
5.	"	*Prunus avium* L.	30, 34
6.	"	*Thuja orientalis* L.	34
7.	settlement Vile	*Juglans regia* L.	2, 4, 5, 6, 14, 16, 22, 30, 34, 38, 43
8.	"	*Populus nigra* L.	1
9.	"	*Quercus robur* L.	34
10.	"	*Malus sylvestris* Miller	8, 9, 23, 33, 34, 38, 43
11.	"	*Betula pendula* Roth.	34, 43
12.	"	*Tilia argentea* Desf.	1, 11
13.	"	*Prunus domestica* L.	12, 13, 24, 34, 38, 43
14.	"	*Morus nigra* L.	34, 38, 41, 43
15.	"	*Acer campestre* L.	4
16.	"	*Fraxinus excelsior* L.	22
18.	"	*Prunus avium* L.	23, 30
19.	Bučevački brook	*Robinia pseudoacacia* L.	38, 43
20.	"	*Juglans regia* L.	11, 22, 43
21.	"	*Cornus mas* L.	32,
22.	distribution plants	*Juglans regia* L.	38, 43
23.	Miloša Gajića street	*Prunus avium* L.	11
24.	"	*Morus nigra* L.	38, 43
25.	"	*Prunus domestica* L.	7, 34, 43
26.	Zvezdara	*Tilia argentea* Desf.	2
27.	"	wooden enclosure	1
28.	"	*Prunus avium* L.	38, 43, 28, 7
29.	"	*Fraxinus excelsior* L.	1
30.	"	*Quercus* L.	7, 24, 28, 31, 34
31.	"	*Robinia pseudoacacia* L.	1
32.	" (margin of forest)	*Robinia pseudoacacia* L.	38
33.	Maršal Tito street	*Populus nigra* L.	4, 34, 43
34.	"	*Juglans regia* L.	34, 43
35.	"	*Prunus avium* L.	7, 30, 34, 38
36.	"	*Tilia argentea* Desf.	7, 24, 34, 38, 43

37.	"	*Sambucus nigra* L.	38, 43
38.	"	*Taxus bacata* L.	7, 34, 38, 43
39.	"	*Robinia pseudoacacia* L.	4, 35
40.	"	*Fraxinus ornus* L.	39, 43
41.	"	*Thuja orijentalis* L.	34, 43
42.	"	*Aesculus hippocastanum* L.	33, 38, 43
43.	"	*Acer negundo* L.	34, 38, 43
44.	"	*Fraxinus excelsior* L.	34, 38, 43
45.	" park	*Salix alba* L.	6, 34, 38, 43
46.	Drinska street	*Prunus domestica* L.	34
47.	pontoon area	*Populus nigra* L.	34, 43
48.	"Progres" DMB area	*Populus nigra* L.	25, 30, 34, 43
49.	"	*Juglans regia* L.	4, 18, 38, 43
50.	"	*Fraxinus excelsior* L.	7, 25, 30, 34, 43
51.	"	*Prunus cerasus* L.	30, 34, 43
52.	"	*Prunus avium* L.	4, 34
53.	"	*Populus nigra* L.	41
54.	Medical station area	*Robinia pseudoacacia* L.	21, 38
55.	schoolyards	*Fraxinus ornus* L.	38, 43
56.	lime-kiln surrounding	*Corilus avelana* L.	10
57.	"	*Prunus domestica* L.	30, 34, 38
58.	"	*Alnus glutinosa* (L.) Gaet.	38, 43
59.	bank of the Drina river	*Salix alba* L.	38

On the basis of the distribution of determined taxa and by using qualitative scale for air pollution (Hawksworth, Rose, 1970) three zones (with two subzones) in terms of air pollution, were established:

1. „lichen desert" zone (SO_2 concentation over 125 $\mu g/m^3$ of air),
2. „Struggle" zone with:
   a) inner part (SO_2 concentration about 100 $\mu g/m^3$ of air),
   b) outer part (SO_2 concentration about 60 $\mu g/m_3$ of air), and
3. „Normal" zone (SO_2 concentration below 40 $\mu g/m^3$ of air).

1. **„Lichen desert" zone.** It is situated in the small suburban part of the town (the river side and margins of the forest). High SO_2 level in the air is influenced by lime-kiln operation.

Potential „lichen desert" zone is the area which surrounds the quarry „Bučevski potok", where only the rare individuals of no-bioindicator species of *Graphis scripta* and *Opegrapha atra* were found. In this case, qualitative and quantitative pooring of lichens is influenced by the dust form the quarry (at the distribution plants, close to the quarry, dust concnetration is 2.59 times over the maximal concnetration allowed).

2. **„Strugle" zone**. This zone is divided into two parts.

The inner part of the „struggle" zone encircles a part of the settlement Vile. The high air pollution is influenced, in the first place by the transit traffic. The subzone has not quite clear boundaries because of the lack of adequate surface for the lichen development (mostly unhitewashed tress).

This subzone also encircles a part of the forest north of lime-kiln „Kamenita devojka". Only the species *Parmelia sulcata, Physcia ascendens, Xanthoria parietina,* which tolerate average SO_2 concentration of about 70 $\mu m/m^3$ of air, survive here.

Somewhat better quality of air in this subzone is influenced by the nearness of the River and forest (higher level of humidity, circulation of air along the river), and by north-west wind.

The outer part of the „struggle" zone encompasses the greatest part of the investigated area. The lichens, occurring close to distribution plant, are most poorly developed due to the dust from the quarry. On the contrary, the best qualitative and quantitative composition of lichen flora is in the urban part of the town, along the river.

3. **„Normal" zone.** It is situated, primarily, in the eastern part of the settlement Vile. Vile is near the forest and far from the central traffic communication routes. It is very rich in orchards. In addition to lichen species, which are susceptible to air pollution, there are some highly developed lichen species which are not bioindicators.

It should be noted that this zone is very close to the quarry. But, between Vile and the quarry there is the forest at the higher altitude than the quarry itself so that the winds and vertical circulations carry off the dust over the hill in the direction S-SE. In this way the air quality at Vile is protected.

ARANDELOVAC, including Bukovička Spa, is situated 75 km south of Belgrade (Fig. 1), at the altitude of 270 m but the difference between the highest and lowest points of the urban area is about 200 m.

The town is small with many prerequisites for good quality of air, especially great amount of green surfaces. Namely, in the territory of Arandelovac, the parks and the other green surfaces have about 60.22 ha (about 23 m^3/inhabitant).

The climate is moderately continental with average annual temperature of 10.8°C (average temperature in January is -0.2°C, in July 20.6°C) and average annual precipitation of 753.5 mm. West and north-west winds prevail in spring and autumn, though south-east and north-east winds are not without influence.

The primary sources of air pollution are the boiler plants for heating of buildings and houses (52 are in the urban area, and 7 in the very center of the town), traffic (local and transit) and industry.

By examining the collected samples, the presence of 12 genera with 29 species was established (Tab. 1). Most of them were gatherd from the bark of various trees (Tab. 3).

Tab. 3. – The investigated points in Aranđelovac with determined lichen taxa
Istražene tačke u Aranđelovcu sa tkasonima lišajeva koji su određeni

Nos. of sampl.	Points of sampling	Types of surface	Nos. of taxa from Tab. 1.
1.	Maršal Tito street	*Tilia argentea* Desf.	19, 34, 38, 43
2.	"	*Tilia platyphylla* Scop.	19, 38, 43
3.	"	*Populus nigra* v. *italica* (Much.) Duroi	19, 34
4.	Partizanska street	*Populus nigra* v. *italica*	19, 38
5.	"	*Juglans regia* L.	19, 34, 38, 43
6.	"	*Robinia pseudoacacia* L.	4, 34, 38, 43
7.	"	*Castanea sativa* Mill.	14, 38, 43
8.	"	*Pyrus domestica* Medic.	34, 38, 43
9.	M. Matijaševića street	*Juglans regia* L.	7, 38, 43
10.	Kosmajaska street	*Malus* sp.	38, 41, 43
11.	Vojina Gajića street	*Tilia platyphylla* Scop.	15
12.	"	*Populus nigra* v. *italica*	19, 38
13.	Prote Isakovića street	*Tilia platyphylla* Scop.	15, 19, 34, 38, 43
14.	"	*Robinia pseudoacacia* L.	4, 34, 38, 43
15.	"	*Fraxinus excelsior* L.	19, 34, 40, 41, 43
16.	cemetary "Risovača"	*Tilia argentea* Desf.	11, 29, 38, 39, 43
17.	"	*Prunus avium* L.	4, 34, 41
18.	"	*Robinia pseudoacacia* L.	29, 40
19.	"	*Juglans regia* L.	11, 34, 40, 43
20.	Bregalnička street	*Pyrus domestica* Medic.	34, 39
21.	Narodnih heroja street	*Prunus armeniaca* L.	34, 38, 43
22.	"	*Pyrus domestica* Mecid.	4, 7, 30, 34, 36, 43
23.	"	*Malus* sp.	34, 38
24.	Nenada Žakule street	*Prunus domestica* L.	4, 30
25.	Sl. Penezića street	*Robinia pseudoacacia* L.	34
26.	"	*Populus nigra* v. *italica*	4, 19, 34, 38, 43,
27.	"	*Juglans regia* L.	38, 41
28.	"	*Acer campestre* L.	34
29.	"	*Fraxinus excelsior* L.	34, 43
30.	Lomina street	*Tilia argentea* Desf.	4, 7, 19, 34, 38
31.	"	*Pyrus domestica* Medic.	4, 36
32.	"	*Fraxinus excelsior* L.	7, 36, 42
33.	I. Milutinovića street	*Fraxinus excelsior* L.	7, 34, 36, 38, 39, 42, 43
34.	"	*Tilia argentea* Desf.	36, 38, 42, 43
35.	"	*Prunus domestica* L.	34, 37, 40, 41
36.	"	*Populus nigra* v. *italica*	34, 38

37.	"	*Prunus avium* L.	30
38.	"	*Quercus* L.	34, 36, 40
39.	"	*Populus tremula* L.	4, 34
40.	Dušana Dugalića street	*Robinia pseudoacacia* L.	4, 7, 18, 30, 34, 37, 38
41.	"	*Juglans regia* L.	4, 38, 41, 43
42.	"	*Tilia argentea* Desf.	5, 12, 26, 30
43.	"	*Prunus domestica* L.	12, 30
44.	"	*Morus alba* L.	41
45.	Vojvode Stepe street	*Pyrus domestica* Medic.	34, 38, 41, 43
46.	"	*Prunus domestica* L.	30, 37, 42
47.	"	*Robinia pseudoacacia* L.	4, 34, 38
48.	"	*Quercus* L.	3, 7, 34, 36, 38,
49.	"	*Juglans regia* L.	34, 38, 43
50.	Ivo Lola Ribar street	*Acer campestre* L.	34, 37, 38
51.	"	*Fraxinus excelsior* L.	34, 43
52.	"	*Tilia platyphylla* Scop.	34, 38
53.	"	*Robinia pseudoacacia* L.	4, 12, 29
54.	"	*Quercus* L.	25, 29, 38, 41
55.	"	*Populus nigra* v. *italica*	34, 38
56.	Tanjugova street	*Tilia platyphylla* Scop.	29, 38, 39
57.	"	*Juglans regia* L.	4, 5, 7, 19, 38, 43
58.	"	*Populus nigra* v. *italica*	29, 38, 43
59.	Moše Pijade street	*Tilia argentea* Desf.	38, 40
60.	"	*Juglans regia* L.	38, 43
61.	"	*Salix* L.	30, 38
62.	"	*Prunus domestica* L.	19, 30, 38, 41
63.	"	*Malus* Mill.	38, 43
64.	"	*Acer platanoides* L.	34, 38
65.	"	*Populus nigra* L.	17, 19, 38, 43
66.	"	*Pyrus domestica* Medic.	34, 37, 38
67.	Milana Ilića street	*Populus nigra* v. *italica*	34, 38
68.	"	*Fraxinus excelsior* L.	4, 19, 28, 34, 38, 40, 43
69.	"	*Populus tremula* L.	4, 7, 19, 30, 34, 38, 43
70.	Milana Savkovića street	*Populus nigra* v. *italica*	4, 7, 19, 34, 38, 43
71.	29. Novembra street	*Populus nigra* v. *italica*	19, 34, 38
72.	"	*Pyrus domestica* Medic.	34, 36, 38
73.	M. Blagojevića street	*Pyrus domestica* Medic.	28, 34, 37, 38, 41
74.	"	*Juglans regia* L.	19, 34, 37, 38
75.	I Šumad. odreda street	*Salix* L.	34, 38

76.	"	*Robinia pseudoacacia* L.	30, 34, 38
77.	JNA street	*Populus nigra* v. *italica*	7, 19, 34, 38, 41
78.	"	*Robinia pseudoacacia* L.	34, 38, 41
79.	"	*Acer campestre* L.	34, 36, 38, 41
80.	"	*Fraxinus excelsior* L.	4, 25, 38
81.	"	*Tilia platyphylla* Scop.	19, 38
82.	Vožda Karađorda street	*Pyrus domestica* Medic.	17, 19, 34, 38
83.	"	*Populus nigra* v. italica	34, 38, 41
84.	"	*Tilia argentea* Desf.	34, 38, 40, 43
85.	"	*Robinia pseudoacacia* L.	19, 34, 43
86.	Lenjinova street	*Malus* Mill.	34, 38
87.	"	*Tilia platyphyla* Scop.	34, 40
88.	"	*Fraxinus excelsior* L.	34, 38, 40
89.	"	*Morus alba* L.	33, 34
90.	"	*Juglans regia* L.	33, 34, 37, 38
91.	The slopes of mt Bukulja	*Quercus* L.	4, 9, 12, 20, 23, 27, 29, 30, 33, 36, 37, 41, 43

On the basis of the distribution of the determined taxa, two zones, in terms of air pollution, were established:

1. „Struggle" zone and

2. „Normal" zone.

1. **„Strugle" zone.** This zone encircles the central part of the town where the most abundant are *Buellia punctata* and *Lecanora* sp. mixed with *Physcia tenella, Ph. ascendens, Ph. orbicularis* and *Xanthoria parietina.* The thalli of the individuals of the two last mentioned species were frequentlly more or less damaged.

On the basis of the distribution of lichen taxa and quality of their thalli, it could be concluded that sulphurdioxide concentration in this zone never exceeds 100 $\mu g/m^3$ of air and is usually significantly bellow this value.

2. **„Normal" zone.** This zone encompasses the „struggle" zone. The results of our study show that sulphurdioxide concentration in this zone is always bellow 70, and at the outskirts of the town always bellow 50 $\mu g/m^3$ of air.

CONCLUSIONS

The results of the study of lignicolous flora of lichens found in two small Serbian towns are presented here.

The samples were collected from 59 and 91 poits in Mali Zvornik and Arandelovac, respectively.

MALI ZVORNIK (Little Zvornik) lies on the right bank of the Drina river. Although Mali Zvornik is a little town with small number of inhabitants, the air is polluted.

By examining the collected samples, the presence of 15 genera with 33 species was established.

On the basis of the investigations, three zones (with two subzones) in terms of air pollution were established.

Zone of „lichen desert" (SO_2 concentration over 125 $\mu g/m^3$ of air) is situated in the small suburban part of the town (the river side and margins of the forest). High level of SO_2 is influenced by lime-kiln operation. Potential „lichen desert" zone is the area which surrounds the quarry „Bučevski potok", where only the rare individuals of no-bioindicator species were found. The pooring of lichens is influenced by the dust from the quarry.

„Struggle" zone is divided into two parts.

The inner part (SO_2 concentration about 100 $\mu g/m^3$ of air) encircles a part of the settlement Vile. The high level of air pollution is caused, in the first place by the transit traffic. The subzone has not quite clear boundaries because of the lack of adequate surface for the lichen development. This subzone also encircles a part of the forest north of lime-kiln „Kamenita devojka".

The outer part (SO_2 concentration aobut 60 $\mu g/m^3$ of air) encompasses the greatest part of the investigated area.

„Normal" zone (SO_2 concentration below 40 $\mu g/m^3$ of air) is situated, primarily, in the eastern part of the settlement Vile.

ARANĐELOVAC, including Bukovačka Spa, is situated 75 km south of Belgrade. The town is small with many prerequisites for good quality of air, especially great amount of green surfaces.

By examining the collected samples, the presence of 12 genera with 29 species was established.

On the basis of the investigations, two zones in terms of air pollution were established.

„Struggle" zone encircles the central part of the town.The results of the study show that SO_2 concentration in this zone never exceeds 100 $\mu g/m^3$ of air and is usually singificantly bellow this value.

„Normal" zone encompasses the „struggle" zone. The results of the study show that SO_2 concentration in this zone is always bellow 70, and at the otuskirts of the town always bellow 50 $\mu g/m^3$ of air.

REFERENCES

Cvijan, M., Szabados, K. & Savić, S. (1992): Bioindikacija aerozagađenja na području grada Beograda pomoću lignikolnih lišajeva. – Elaborat, Institut za botaniku Biološkog fakulteta Univerziteta u Beogradu.

Cvijan, M. & Stamenković, S. (1996): Bioindication of air pollution in Niš area by use of lichens. – Ekologija *31*(1): 151-157.

Hawksworth, O.L. & Rose, F. (1970): Qualitative scale for estimation of sulphur dioxide air pollution in England and Wels using epiphytic Lichens. – Nature *227*: 145-148.

Milić, M. & Blaženčić, J. (1993): Epifitski lišajevi grada Beograda. – Glas. Inst. bot. i Bot. bašte Univ. u Beogradu *24-25*: 83-96.

Savić, I., Popović, R., Kataranovski, D., Kataranovski, M. & Cvijan, M. (1996): Osnove metodologije u istraživanju uticaja faktora životne sredine na životinjski i biljni svet. – „Ekosistem i zdravlje", Dani zavoda '95, XIV stručna konferencija, Odeljenje medicinskih nauka SANU, pp. 47-96, Beograd.

Stamenković, S. (1992): Bioindikacija aerozagađenja lignikolnim lišajevima na području grada Niša. – Magistarski rad, Univerzitet u Beogradu.

Rezime

MIRKO CVIJAN, BRANKA TODOROVIĆ, VESNA JOKSIMOVIĆ

LIŠAJEVI KAO BIOINDIKATORI ZAGAĐENJA VAZDUHA U MALOM ZVORNIKU I ARANĐELOVCU (JUGOSLAVIJA)

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Univerzitet u Beogradu

U radu su prikazani rezultati istraživanja lignikolne flore lišajeva u mestima Mali Zvornik i Aranđelovac.

Uzorci lišajeva sakupljeni su sa 59 tačaka u Malom Zvorniku, odnosno sa 91 tačke u Aranđelovcu. Gotovo bez izuzetka podloga sa koje su skidani uzorci lišajeva bila je kora drveća.

Obradom sakupljenih uzoraka, u Malom Zvorniku je utvrđeno prisustvo 15 rodova lišajeva sa 33 vrste, a u Aranđelovcu 12 rodova sa 29 vrsta.

Na osnovu distribucije lišajeva, te korišćenjem kvalitativne skale Hawksworth-Rous-a, utvrđeno je prisustvo i raspored odgovarajućih zona aerozagađenja, pre svega sumpordioksidom, u oba istražena mesta. U Malom Zvorniku je utvrđen niži kvalitet vazduha što se ogleda u postojanju zone „lišajske pustinje" koja nije nađena u Aranđelovcu, dok su zona „borbe" i „normalna" zona, kao zone okarakterisane nižim koncentracijama sumpordioksida, nađene u oba mesta.

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MORPHOLOGICAL AND CHEMICAL VARIABILITY OF THE POPULATIONS OF THE *ALYSSUM MARKGRAFII* SCHULZ (*BRASSICACEAE*)

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Janaćković P., Tešević V. (1995): *Morphological and chemical variability of the populations of Alyssum markgrafii Schulz (Brassicaceae)*. – Glasnik Instituta za botaniku i botaničke bašte Univerziteta u Beogradu, Tom XXIX, 187 - 198.

Morphological and chemical variability of populations of *Alyssum markgrafii* Schulz on the territory of the Republic of Serbia was analyzed using the scanning electronic microscope (SEM) and high performance liquid chromatography (HPLC). Achieved results proved that there was some variability in length and width of leaves among populations analyzed. Also, variability of the heigh of the plants matched literary data and the number of trichomes on the square millimeter at both sides of the leaf, especially the back side of the leaf, did not show much variability, but the trichomes at the back side of the leaf were more branchy than those on the front side and their branches were narrower. On the basis of the chromatogram of the leaf ethanol extract and the photograph of the same in the UV spectrum, the presence of the flavonoids was estimated. In addition, a considerable resemblance among the populations in their qualitative structure was observed, particularly among the major components of the flavonoids. The importance of the micromorphological para-

meteres and the qualitative composition of the flavonoid components as taxonomic markers in delimitation of *A. markgrafii* and related species from the *Odontarrhena* section was also discussed.

Key words: *Brassicaceae, Alyssum markgrafii,* trichomes, flavonoids, trichomes, flavonoids, SEM, HPLC, taxo-nomy.

Ključne reči: *Brassicaceae, Alyssum markgrafii* trihomi, flavonoidi, trihomi, flavonoidi, SEM, HPLC, ta-ksonomija.

INTRODUCTION

Alyssum L. (*Brassicaceae*) genus includes about 170 species; its areas are confined to the territory of southwest Asia, eastern Mediterranean, south and southeastern Europe.

Species were classified in the following six sections: *Aurinia* (Desv.) Meyer, *Alyssum (Eualyssum)* Gris., *Psilonema* (Meyer) Hook, *Gamosepalum* (Haussk.) Dudl., *Odontarrhena* (Meyer) Koch., and *Meniocus* (Desv.) Hook (Ančev, 1991).

There are 64 species in Europe, and 32 of them (47%) are European endemics (P. W. Ball et al., 1964). This high percentage of the continental endemics is closely connected to the flora of the eastern Mediterranean, or, more accuratelly, to the southeastern areas of the Balkan peninsula. On this area genus is represented with about 45 species, out of witch 21 is endemic to the Balkans, with the areas limited to the territory of Crete, Greece, Albania, Yugoslavia and Bulgaria. There is 17 species of this genus in the Republic of Serbia (Diklić, 1972).

A. markgrafii is a perennial, herbaceous plant and belongs to the section of *Odontharrena* (Meyer) Koch. It populates areas in limestone, serpentine, most frequently in mountain region. It has been spread in Yugoslavia and Albania (Diklić, 1972).

In systematics revisions of different taxa, micromorphological characters of the leaf surface, fruits and seeds were often ignored or only seldom mentioned, regardless of their stabiltiy (Davis et Heywood, 1963). The use of the scanning electronic microscope (SEM) gave new possibilities in researching microcharacters in plants (Marin, 1989). Nowadays, a greater importance is given to the application of scanning electronic microscope for the analysis of micromorphological characters in taxonomy researches (Hardin, 1979 a and b; Hardin and Gensel, 1982; Husain et al., 1989, 1990; Ančev, 1991; Marin et al., 1994).

Taxonimical significance of the trichome micromorphology within the family of *Brassicaceae* has also been emphasized with *Bornmuellera dieckii* Degen (Marin et al., 1993), as well as with the genus of *Alyssum* L. (Ančev, 1991).

Diversity regarding morpho-anatomic aspect, as well as the way of life in a particular area is reflected in the chemical structure of the plants. Different groups of chemical compounds are from chemotaxonomical aspect more or less typical for certain taxonomic categories. 5-methylthiopentylglucosinolate was identified in this way as taxonomic character of the *Alyssum* L. genus (Hasapis et al., 1981). Than followed isothiocyanates, nitriles and epithiobutanes with *Alyssum minimum* Willd (Lockwood, Afsharypuor, 1986). This substances are the reason for application of the *Alyssum minimum* Willd in the traditional medicine. Flavonoid glucuronoides in *Alyssum minimum* were also researched from chemotaxonomic aspect (Aafsharipuor et Lockwood, 1986). Considering chemotaxonomic aspect,

flavonoids possess a wide range of use on different levels within families (Harborne et Turner, 1984; Husain et al. 1989). The presence of alkanes on the leaves of *Bornmuellera dieckii* Degen and *Alyssum markgrafgii* Schulz (Marin et al. 1993) within family of *Brassicaceae* was researched.

An analysis of the variability of morphologic characters of the vegetative organs was done in this study, with an emphasis on the trichomes on the leaves in ten populations of the *Alyssum markgrafii* Schulz species on the following localities: Brdani, Semedraž, Stragari, (these localities are in the vicinity of Gornji Milanovac), Dobre strane near Kraljevo, Ozren near Sjenica, Sevce near Brezovica and Brezovica itself (Fig. 1). All the localities have serpentine basis. Also, a comparative chemical analysis of the leaf ethanol extract from the same populations was made in order to estimate interpopulation variabilities of the phenolic compound composition in leaves and its taxonomic importance.

MATERIALS AND METHODS

Herbarium materials gathered on the localities of Brdani, Semedraž, Stragari, Dobre strane, Ozren, Sevce and Brezovica (Fig. 1) were used for morphological, micromorphological and chemical investigation. Material used in this analysis was stored in a herbarium of the Institute of Botany and Botanical Garden „Jevremovac", Faculty of Biology, University of Belgrade.

Height of plants, length and width of leaves were measured with a standard elastic ruler. Number of trichomes on the square millimeter on the both sides of the leaf along the main nerve where the leaf is the widest, was estimated using the stereo magnifying glass magnified for 56 x and the net of 1 mm^2 surface in the eyepiece.

The measured results were treated according to the following formulas:

$$X = \frac{\Sigma (Xi \times Fi)}{N}$$

$$Di = Xi - X$$

$$\sigma = \frac{\sqrt{\Sigma (Fi \times Di)}}{N}$$

$$CV = \frac{\sigma}{X} \times 100\%$$

X - average value, Σ - sum, Xi - value of the class, Fi - frequency of the class, Di - average value deviation, N - number of specimens, σ - standard deviation, CV - variation coefficient.

During micromorphological analysis the samples of the leaves were attached to special metal cylinders and than evaporated with 30 nm thick layer of gold-paladium (80:15) in JEOL JEE 4B vacuum evaporator and analyzed on JEOL JSM T.35 scanning electronic microscope.

Ethanol extract from the leaves was acquired as follows: 0.05 of the leaves from each population was crushed in a ball mill and than 50 ml of 70% EtOH (ethanol) was added. Each sample was than reboiled for 2 min to inactivate the enzymes. Than the samples were left for 24 hours for extraction. Ethanol extract was strained through a filter paper and evaporated until dried in a vacuum evaporator (Harborne, 1984).

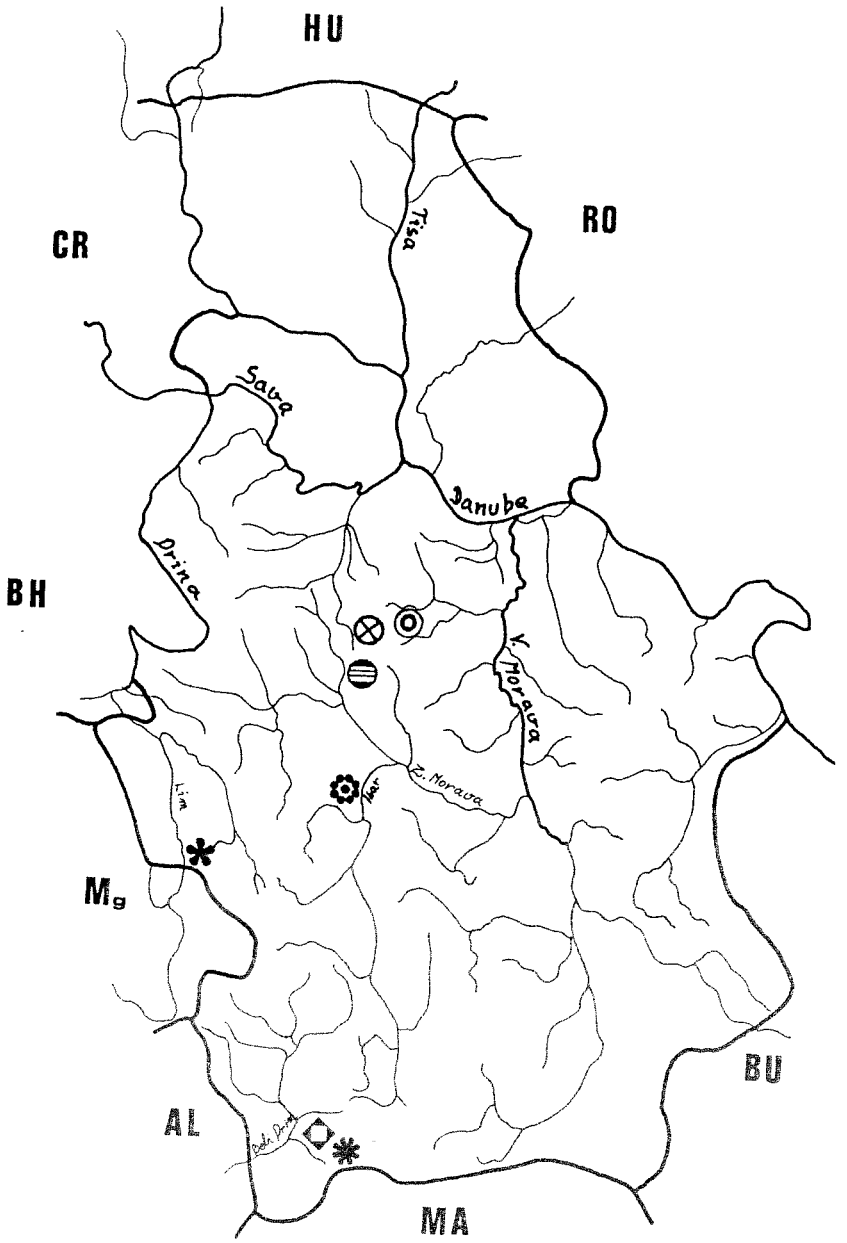

Fig. 1. – The localities of the analyzed populations of *Alyssum margrafii* Schulz
◎ Stragari; ⊗ Brdani; ⊜ Semedraž
✿ Dobre strane; ✱ Ozren; ◇ Sevce;
✱ Brezovica;

In order to remove physical and indissoluble admixtures, samples were refined with the membrane filter of the 3 μm pore size, dissolved in 2 ml MeOH (methanol), filtered again and than diluted with another 2 ml MeOH.

Samples prepared in this way were chromatographed with a reverse-phase HPLC chromatography. OKTADECIL = Si 100 3 YM column of the 125 x 4.6 mm dimension and 3 μm particle size was used. Best separation was achieved with the gradient of MeOH and H_2O eluents at the increase of MeOH concentration from 30% to 70% in a 30 min period. Detection was done with the UV Polychrom 9060 detector at the maximum absorption of 254 nm.

RESULTS AND DISCUSSION

Morphological characteristics of the vegetative organs (such as length and width of leaves, leaf index - ratio btween the length and width of leaves, height of the plant, number of trichomes on the square millimeter on both sides of the leaves), micromorphological characteristics of leaves, as well as the ethanol extract of leaves for ten populations of *A. markgrafii* on the above mentioned localities, were analyzed in this study.

Length of leaves differs from one populations to another although not considerably. Average value of length of leaves is the largest in A_1 population (21 mm) and the smallest in A_9 population (9.4 mm) (Fig. 2).

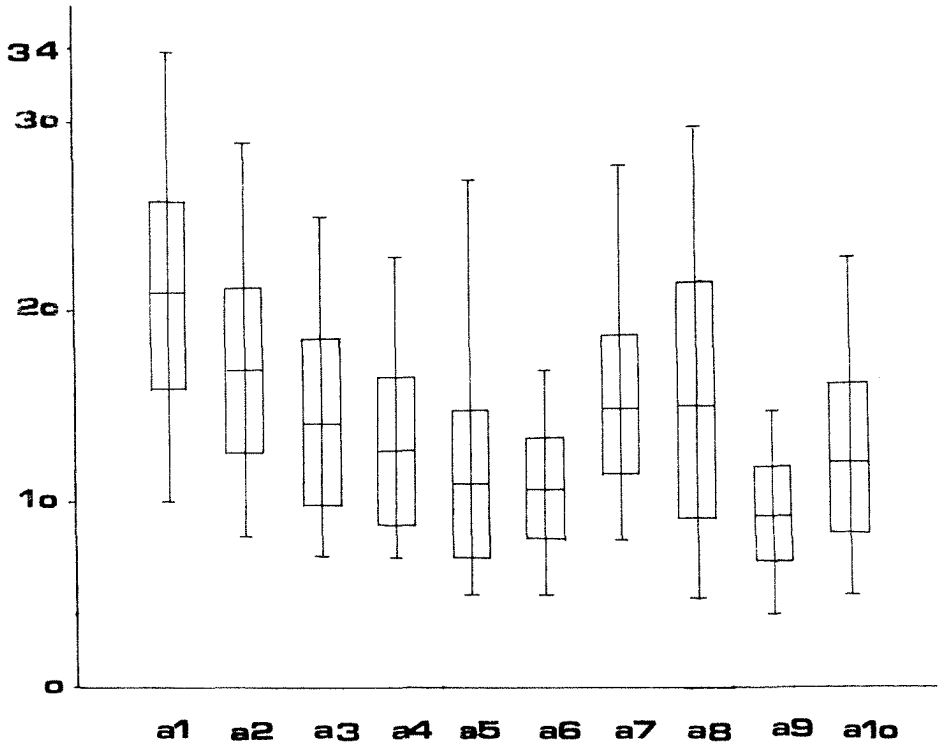

Fig. 2. – Variabiltiy of length of the leaves.

Average value of width of leaves in all population is within the range of 1.8 - 3.1 mm (Fig. 3).

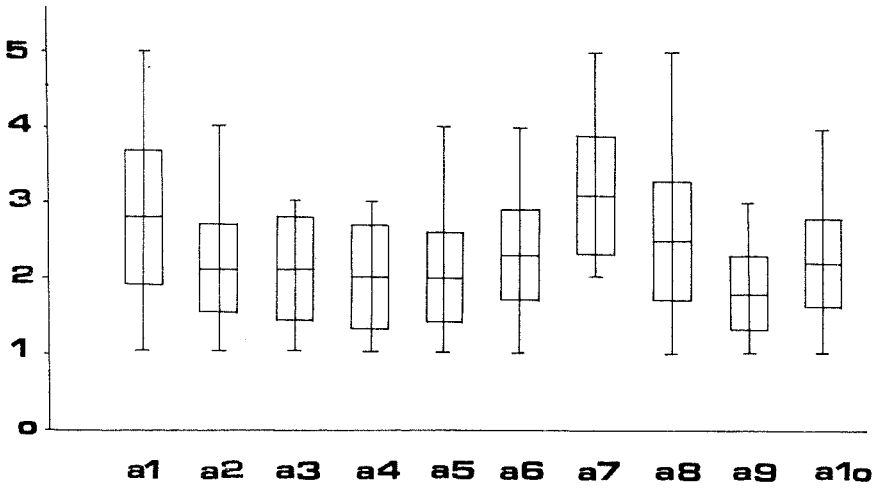

Fig. 3. – Variability of width of the leaves.

Leaf index presents the ratio between length and width of leaves and unites this two morphological characteristics. Population A_2 (8) has the largest average value of leaf index, while the smallest one belongs to A_6 population (4.5). Leaf index shows a greater improtance in interpretation of variability of these populations compared to length and width of leaves in separate (Fig. 4).

Height of plants is very variable. A_1 population has the largest average height value (47.9 cm) and A_9 population has the smallest one (17.9 cm) (Fig. 5).

A_6 population has the largest number of trichomes on the front side of the leaf (7.4/mm^2) and A_8 and A_9 populations have the smallest number of them (3.8/mm^2) (Fig. 6).

A_8 population has the largest number of trichomes on back side of the leaf (29.9/mm^2) and A_2 population have the smallest number (21.6/mm^2) (Fig. 7).

Larger number of trichomes on the back side of the leaf than on the front side is connected to self protection of the plant. Trichomes protect the plant from excessed transpirations as well as from phytophagous insects. Parts of the plant with a small number of trichomes or those parts not having them at all, perianth for example, are often attacked and damaged by phytophagous insects. It should be mentioned that some phenolic compounds in leavers are, besides trichomes, probably the reason why phytophagous insects rarely attack the leavers of these plants (M a r i n et al., 1993). Small number of trichomes as well as their poor branching on the front side of the leaf is connected to more intensive photosynthesis of the leaf (A n č e v, 1991).

Analyzing micromorphological characteristics of the leaf surface in *A. markgrafii*, the presence of dual and tripartite branching trichomes was noticed (Fig. 8, a-d). Relief wartlike bulgers are visible at the trichome surface both on the front and back side of the leaf.

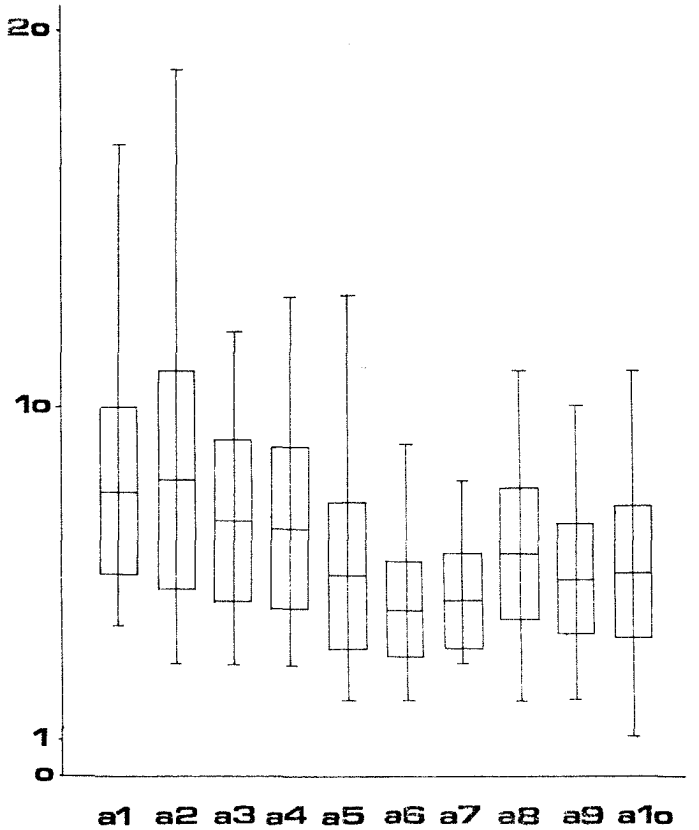

Fig. 4. – Leaf index.

Species from *Odontarrhena* section that were analyzed before, have relatively similar morphological characteristics of trichomes (Ančev, 1991). The trichomes of species from *Odontarrhena* section have a narrow central part of the trichome, more or less furrowed and scatered or evenly covered with warts, unevenly conical as in *A. bertolonii* Desv., or hemispheric in *A. borzaeanum* Niar. The warts gradually become smaller to the top of the branch. Only in *A. murale* Wik ssp. *pichlerii* (Vel.) Stoj. et Stef. trichomes have poorly furrowed central part. Starlike trichomes are most frequently covered with conical or semispheric warts of different size, as in *A. bertolonii* Desv., *A. obtusifolium* Steve ex DC. and *A. murale* Wik ssp. *pichlerii* (Vel.) Stoj. et Stef. As a rule, more massive warts take the central part of the trichome.

A. markgrafii, which had not been analyzed from this aspect by now, has somewhat different trichomes than other members of this section. *A. markgrarfii* trichomes have both fewer and thinner branches and less prominent wartlike bulgers than in other related species from this section.

It could be said that trichomes are, with all their micromorphological characteristics, a stable morphological character of taxonomic importance.

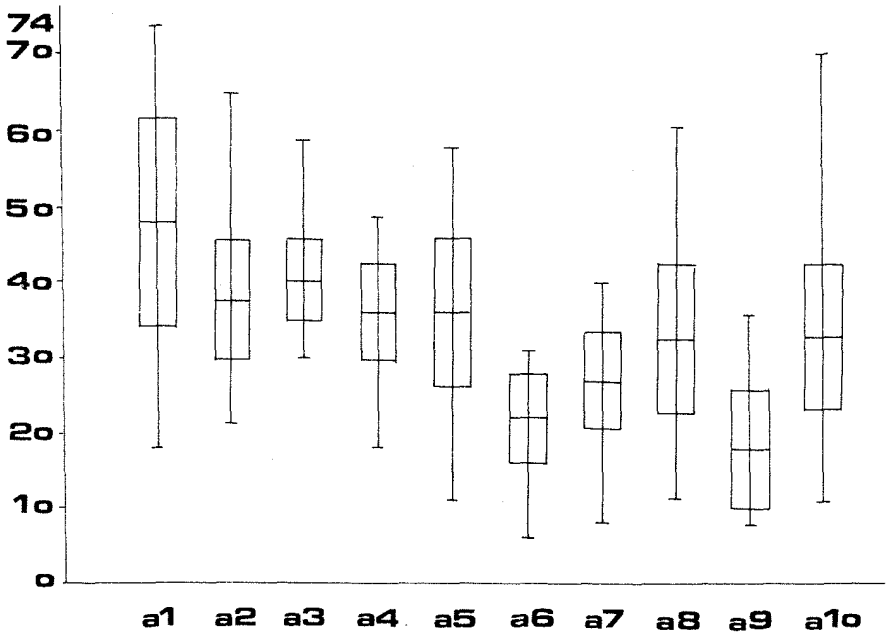

Fig. 5. – Variabiltiy of heigh of the plants.

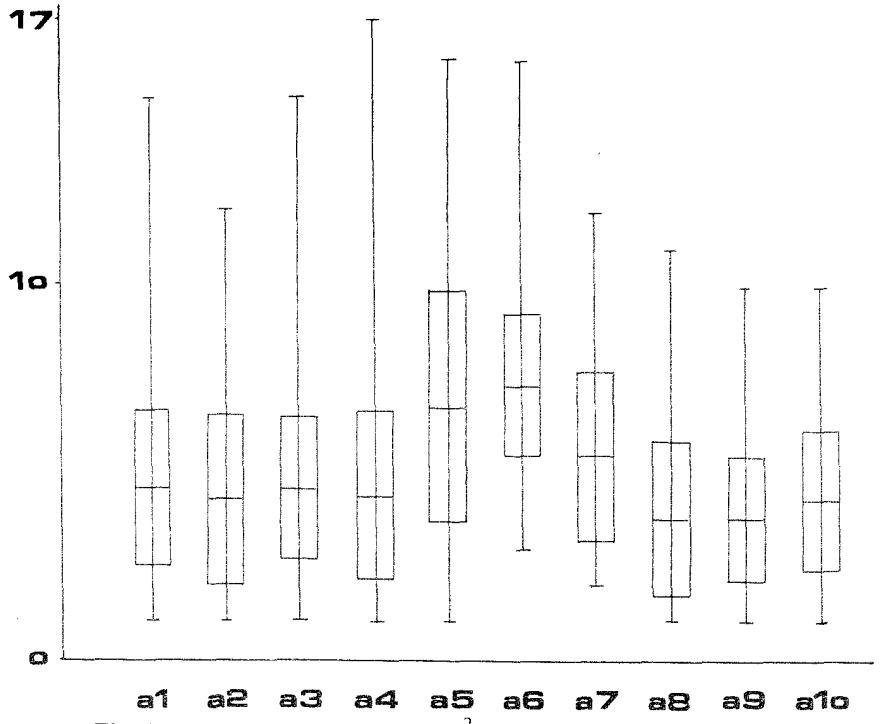

Fig. 6. – Number of trichoms/mm^2 on the front side of the leaf.

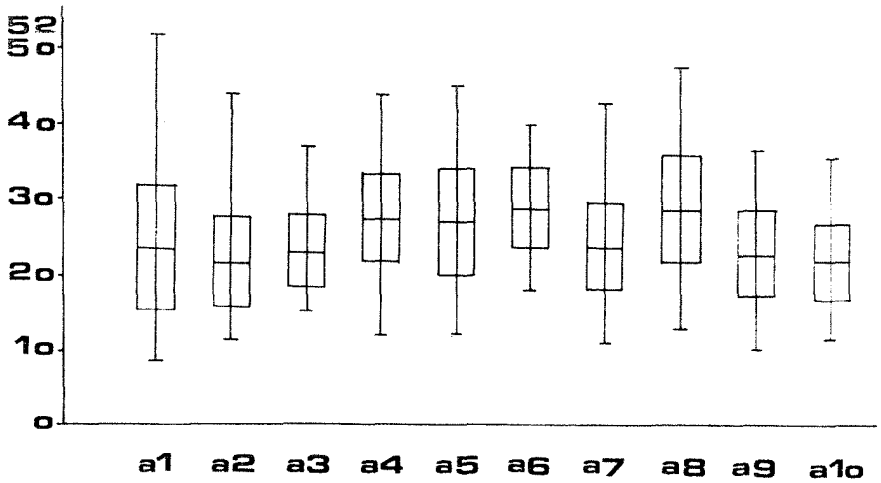

Fig. 7. – Number of trichomes/mm^2 on the back side of the leaf.

On the chromatogram of the leaf ethanol extract of analyzed populations (Fig. 9), peaks from I - XIII, represented more or less in all populations, were noticed. Quantitative differences can be seen in the presence or absence of particular peaks in all populations. Namely, only A_2 population has all the peaks, while A_1 population does not have peaks IX, XI and XII; A_3 does not have peaks VII and XI; A_4 does not have peaks II, XI, XI and XII; A_5 does not have peaks III, VI, VII, XI and XII; A_6 does not have peaks VIII and XII; A_7 does not have peak XII; A_8 does not have peaks VIII and XII; A_9 does not have peaks IV, VII, VIII, IX, X and XIII and A_{10} does not have peaks III, VI and XI. According to chromatograms, all populations have peaks I and V.

Calculating the percentage of the surfaces with treated peaks (I-XIII) on and with corresponding retention times, certain qualitative differences between treated populations were established (Chart 1).

Chart 1. – Qualitative composition of the phenolic compounds

Rf													
P	2.7	4.7	9.0	15.8	17.1	18.8	21.5	23.4	25.1	28.3	30.5	32.6	34.3
A1	1.3	0.3	0.5	1.3	3.3	9.2	3.4	74.1	-	3.5	-	-	3.4
A2	9.9	2.9	1.5	12.9	13.6	30	6.9	2	1.9	0.2	0.2	1.9	16.1
A3	5	0.4	0.4	4	12.4	45.7	-	3.6	2.3	1.6	-	4.4	19.9
A4	4.4	-	2.2	2.6	10.6	-	41.3	4.8	5.4	2.2	-	-	26.1
A5	4.6	5.7	-	6.1	58.6	-	-	-	2.1	-	-	-	22.5
A6	5.9	0.4	4.7	1.4	7.3	0.6	27	-	5.6	4.1	2.2	-	40.3
A8	5.2	1.3	17.6	6.7	9.2	1.3	24.2	-	7	3.7	2.6	-	27.8
A9	2.9	10	0.7	-	12.9	11.6	-	-	-	-	-	61.8	-
A10	6.6	7.9	-	0.8	0.7	-	45.2	2.3	9.8	2.4	-	7.1	17
PEAK	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII

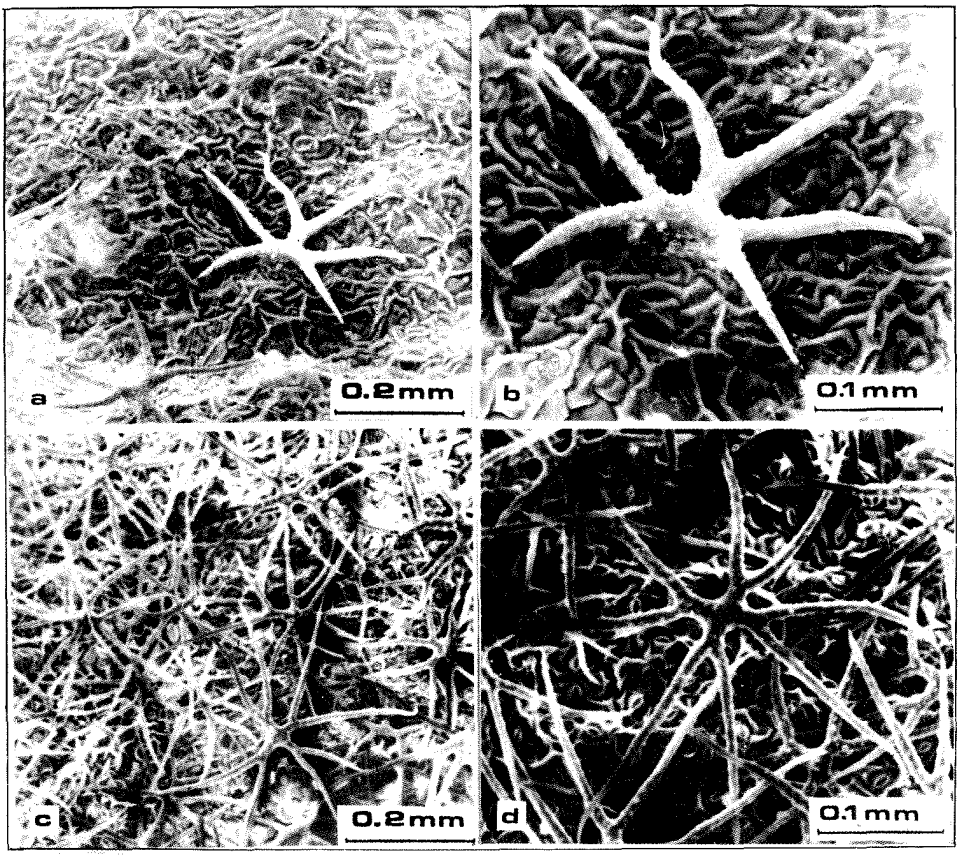

Fig. 8. – SEM of the leaves
a-b. Trichomes on the front side of the leaf
c-d. Trichomes on the back side of the leaf

On the basis of UV specters analysis of the treated peaks with the help of HPLC, the presence of phenolic compounds in leaves, probably flavonoids was established.

Regardless of the acquired quantitative differences in composition of flavonoids in different populations, the qualitative composition of major components in particular, is stable and could be used as a taxonomic character compared to other species of the *Alyssum* genus.

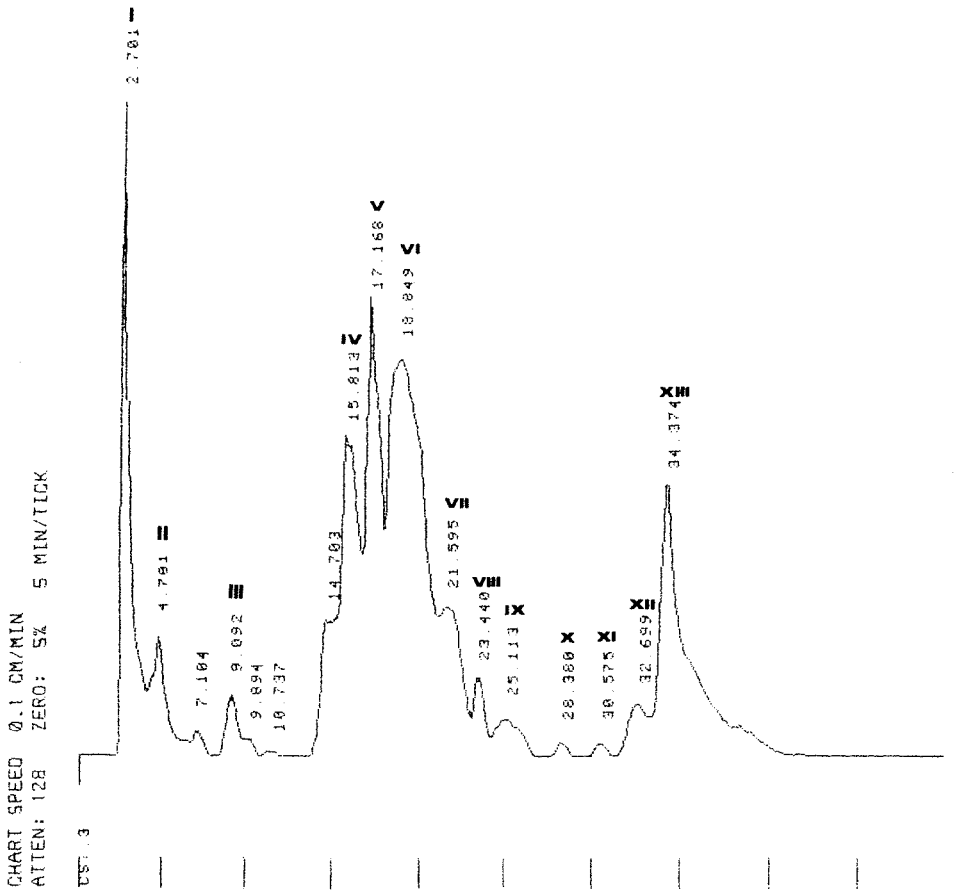

Fig. 9. – The chromatogram of the leaf ethanol extract

REFERENCES

Ančev, E. M. (1991): Rod *Alyssum* L. v Blgarskata flora. - Glas. Bot. Inst. BAN *1113*: 85-118.

Afsharypuor, S. & Lockwod, G. (1986): Flavonoid glukuronides from *Alyssum minimum*. - J. Nat. Prod. *49* (5): 844-5.

Ball, P. W. et al. (1964): *Alyssum* L. *in* Flora Europaea I (ed. Tutin, T. G. et al.). - Cambridge.

Davis, P. H., Heywood, V. H. (1963): Principles of angiosperm taxonomi. - Oliver and Boyd, Edinburg and London.

Diklić, N.: (1972): Rod *Alyssum* L. *in* Flora SR Srbije III (ed. Josifović, M.). - SANU, Beograd.

Harborne, J. B. (1984): Pytochemical Methods (2nd ed.). - Chapman et Hall, London- New York.

Harborne, J. B. & Turner, B. L. (1984): Plant Chemosystematics. - Academic press, London - Orlando.

Hardin, J. W. (1979a): Patterns of variation in foliar trichomes of Eastern Nort American *Quercus*. - Amer. J. Bot. *66*: 575.

Hardin, J. W. (1979b): Atlas of foliar surface features in woody plants. I. Vestiture and trichome types of Eastern North *Quercus*. - Bull. Torrey Bot. Club *106*: 313.

Hardin, J. W. & Gensel, W. H. (1982): Atlas of foliar surface features in woodi plants. IV. *Rhododendrons* (*Ericaceae*) of Eastern North America. - Bulletin of the Torrey Bothanical Club *109*: 189.

Hasapis, X., Macleod, A., & Moreau, M. (1981): Glucosinolates of nine *Cruciferae* and two *Caparaceae* species. - Phytochemistry *20* (10): 2355-2358.

Husain, S. Z., Marin, P. D., Diklić, N. & Petković, B. (1989): Micromorfological and Phytochemical Studies in two new endemic *Nepeta* (*Lamiaceae*) species in Yugoslavia. - Pak. J. Bot. *21* (2): 210-217.

Husain, S. Z., Marin, P. D., Šilić, Č., Qaiser, M. & Petković, B. (1990): A micromorphological study of some representative genera in the tribe *Saturejaea* (*Lamiaceae*). - Bot. J. of the Linean Soc. *103*: 59-80.

Lockwood, G. & Afsharypuor, S. (1986): Comparative study of the volatile aglukones of glucosinolates from *in vivo* and *in vitro* grown *Descurainia sophia* and *Alyssum minimum* using gas chromatography - mass spektrometry. - J. Chromatogr. *356* (3): 438-40.

Marin, P. D. (1989): Hemotaksonomska i mikromorfološka studija rada *Microweria* L. i srodnih rodova iz tribusa *Satureineae* (*Lamiacea*). Doktorska disertacija, Univerzitet u Beogradu.

Marin, P. D., Petković, B., Vajs, V. & Tešević, V. (1993): Morphological and phytochemical analysis of *Bornmuellera dieckii* Degen (*Brassicaceae*). - OPTIMA, VII Meeting, 18-30 July 1993, Borovetz, Bulgaria.

Marin, P. D., Petković, B. & Duletić, S. (1994): Nutlet sculpturing of selected *Teucrium* species (*Lamiaceae*): a character of taxonomic significance. - Pl. Syst. Evol. *In press*.

Markham, K. R. (1982): Techniques of flavonoid Identificacion. - Academic Press, London.

Rezime

PEĐA JANAĆKOVIĆ[1], VELE TEŠEVIĆ[2]

MORFOLOŠKA I HEMIJSKA VARIJABILNOST POPULACIJA VRSTE *ALYSSUM MARKGRAFII* SCHULZ (*BRASSICACEAE*)

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U ovom radu izvršena je analiza varijabilnosti morfoloških karaktera vegetativnih organa kod deset populacija vrsta *Alyssum markgrafii* Schulz (*Brassicaceae*) na području Republike Srbije, sa posebnim akcentom na trihome na listovima. Sem toga, izvršena je i analiza listova pomoću skening elektronskog mikroskopa (SEM), kao i uporedna hemijska analiza etanolnog ekstrakta iz listova pomoću tečne hromatografije (HPLC). Dobijeni rezultati su pokazali da: dužina i širina listova ne pokazuju veliku varijabilnost između istraženih populacija; varijabilnost visine biljaka ispitanih populacija kreće se u granicama od 17.9 - 47.9 cm, što se poklapa sa literaturnim podacima; broj trihoma po mm^2 na licu, a naročito na naličju lista ne pokazuje veliku varijabilnost, a trihomi na naličju lista su razgranatiji od istih na licu i imaju uže grane. Na osnovu mikromorfološke analize listova može se zaključiti da su trihomi stabilan karakter i da se mogu koristiti kao dodatni parametar u delimitaciji *A. markgrafii* i srodnih vrsta u okviru sekcije *Odontarrhena*. Na osnovu hromatograma etanolnog ekstrakta iz listova uočena je velika sličnost između populacija. Ovi preliminarni hemijski parametri upućuju na mogućnost primene flavonoida, kao taksonomskih karaktera, s obzirom na malu varijabilnost između populacija.

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RADOVI MONOGRAFSKOG ILI REVIJSKOG KARAKTERA

MONOGRAPHY AND REWIEV ARTICLES

Nešković, M. (1955): Kultura biljnih tkiva i organa (Plant tissue and organ culture). – Nauka i priroda 8, 59-65.

Nešković, M., Jelaska, S. (1980): Kultura tkiva kukuruza. – *In:* Fiziologija kukuruza (Tissue culture of maize. – *In*: Physiology of maize), Posebno izd. SANU, Beograd 51, 251-274.

Nešković, M. (1981): Kultura tkiva pšenice. – *In*: Fiziologija pšenice (Tissue culture of wheat. *In*: Physiology of wheat). Posebno izd. SANU, Beograd 53, 213-232.

Nešković, M. (1982): Značaj kulture tkiva i ćelija u oplemenjivanju biljaka. – *In*: Teorijski i praktični aspekti kulture tkiva biljaka (The significance of plant cell and tissue culture in plant breeding. *In:* Theoretical and practical aspects of plant tissue culture). - Izd. Jugoslovensko društvo za biljnu fiziologiju, Novi Sad, 3-20.

Nešković, M. (1983): Genetski aspekti reakcije biljaka na hormone. *In*: Prirodni i sintetički regulatori rastenja biljaka (Genetic aspects of plant response to hormones. *In*: Natural and synthetic plant growth regulators). - Izd. Jugoslovensko društvo za fiziologiju biljaka, Beograd, 34-51.

Nešković, M., Konjević, R., Naunović, G., Grubišić, D. (1983): Fiziologija razvoja viših biljakja: fitohrom i giberelini (Physiology of plant development: phytochrome and gibberellins). – Zbornik radova Simp. Multidisciplinarne nauke i njihova uloga u naučno-tehničkom progresu, Centra za multidisciplinarne studije, Univerzitet u Beogradu, 125-132.

Nešković, M., Srejović, V., Vujičić, R. (1986): Buckwheat. – *In*: Biotechnology in Agriculture and Forestry 2, Crop I, Y.P.S. Bajaj ed., Springer-Verlag, Berlin, Heidelberg, 579-593.

Nešković, M., Ćulafić, Lj. (1988): Spinach *(Spinacia oleracea* L.) *In*: Biotechnology in Agriculture and Forestry, Crops II, Y.P.S. Bajaj ed., Springer-Verlag, Berlin, Heidelberg, Vol. 6, 370-384.

Nešković, M., Ćulafić, Lj., Vujičić, R. (1995): Somatic embryogenesis in buckwheat *(Fagopyrum* Mill) and sorrel *(Rumex* L.). – *In*: Biotechnology in Agriculture and Forestry, Bajaj, Y.P.S. ed., Springer-Verlag, Berlin, Heidelberg 31, 412-427.

Nešković, M., Miljuš-Đukić, J., Ninković, S. (1995): Transformation in buckwheat *(Fagopyrum esculentum).* – *In:* Biotechnology in Agriculture and Forestry, Bajaj, Y.P.S. ed., Vol. 34, Plant protoplasts and Genetic Engineering VI, Springer-Verlag, Berlin, Heidelberg, 176-187.

PUBLIKOVANI NAUČNI RADOVI

PUBLISHED SCIENTIFIC PAPERS

Nešković, M. (1955): Pokušaj gajenja pupoljaka *Arundo donax* u sterilnoj kulturi (An attempt to grow *Arundo donax* buds in aseptic culture). – Arhiv biol. nauka 7, 39-45.

Nešković, M. (1966): Interaction of roots, gibberellic acid and light in the promotion of stem growth in peas. – Bull. Inst. Jard. Bot., Univ. Belg. 2, 35-43.

Simić, D., Nešković, M. (1966): Proliferation of isolated *Acer pseudoplatanus* embryos. – Bull. Inst. Jard. Bot.,Univ. Belg. 2, 45-48.

Nešković, M. (1967): Deistvie sveta i gibberellovoj kysloty na rost meždouzlij goroha. – Fiziol. rastenii (Moskva) 14, 1006-1010.

Nešković, M., Burnett, D. (1968): Identifikacija triptofana i indolsirćetne kiseline u ekstraktima stabla graška (Identification of tryptophane and indole acetic acid in pea stem extracts). – Arh. biol. nauka 18, 221-227.

Nešković, M., Ćulafić, Lj. (1968): The influence of light on the content of growth substances in pea shoots. I. Effect of red light in the extractable indole auxins. – Bull. Inst. Jard. Bot., Univ. Belg. 3, 255-262.

Cvetković-Muntanjola, M., Nešković, M. (1968): Studies on the effect of some substances as possible substitutes of light in the sporulation of *Aspergillus flavus* 28-A. – Bull. Inst. Jard. Bot., Univ. Belg. 3, 35-41.

Cvetković-Muntanjola, M., Nešković, M., Ćulafić, Lj. (1968): Morphogenic response of *Aspergillus aureolatus* Munt. – Cvet. & Bata to different carbohydrates and light. – Bull. Inst. Jard. Bot., Univ. Belg. 3, 29-34.

Muntanjola-Cvetković, M., Nešković, M. (1972): Physiological properties of the Cladosarum-like mutant 22B of *Aspergillus aureolatus*. – Arch. Mikrobiol. (Berlin) 84, 141-152.

Muntanjola-Cvetković, M., Nešković, M. (1972): A Cladosarum-like mutant of *Aspergillus aureolatus* that is temperature sensitive and osmotic remedial. – Genetika (Beograd), 4, 305-312.

Nešković, M., Radojević, Lj. (1973): The growth of and morphogenesis in tissue culture of *Spinacia oleracea* L. – Bull. Inst. Jard. Bot., Univ. Belg. 8, 35-37.

Srejović, V., Nešković, M. (1973): The presence of gibberellin-like substances in germinating embryos of different plant species. – Arh. biol. nauka 25, 107-117.

Nešković, M., Konjević, R., Ćulafić, Lj. (1974): Changes in the metabolism of gibberellins induced by light. – *In*: Biochemistry and Chemistry of Plant Growth Regulators, K. Schreiber, H.R. Schutte, G. Sembdner eds. Halle 91-97.

Nešković, M., Konjević, R. (1974): The non-reversible effects of red and far red light on the content of gibberellin-like substances in pea internodes. – J. Exper. Bot. 25, 733-739.

Nešković, M., Sjauš, T. (1974): The role of endogenous gibberellin-like substances and inhibitors in the growth of pea internodes. – Biol. Plant 16, 57-66.

Ćulafić, Lj., Nešković, M. (1974): Indole auxins in spinach plants grown in long and short days. – Biol. Plant. (Prag) 16, 359-365.

Bata, J., Nešković, M. (1974): The effect of gibberellic acid and kinetin on chlorophyll retention in *Lemna trisulca* L. – Z. Pflanzenphysiol. 73, 86-88.

Nešković, M., Radojević, Lj. (1975): Nutritional and hormonal requirements of Meteor pea callus tissue. – Acta Bot. Croat. 34, 43-51.

Damjanović, Z., Vujaklija, G., Nešković, M. (1975): Stimulation and inhibition of organ growth of *Avena sativa* by continual and intermittent light. – Arh. biol. nauka 26, 105-108.

Ćulafić, Lj., Nešković, M. (1975): A study of auxins and gibberellins during shoot development in *Spinacia oleracea* L. – Arh. biol. nauka 26, 19-27.

Radojević, Lj., Vujičić, R., Nešković, M. (1975): Embryogenesis in tissue culture of *Corylus avellana*. – Z. Pflanzenphysiol. 77, 33-41.

Konjević, R., Petrović, J., Nešković, M. (1976): Differential effect of red light on the nucleic acid biosynthesis in cell organelles of *Pisum sativum* L. – Biochem. Physiol. Pflanzen 169, 187-190.

Vujičić, R., Radojević, Lj., Nešković, M. (1976): Orderly arrangement of ribosomes in the embryogenic callus tissue of *Corylus avellana* L. – J. Cell Biol. 69, 686-692.

Ćulafić, Lj., Nešković, M. (1976): Studies on endogenous hormones and inhibitors in dioecious plant *Rumex acetosella* during vegetative growth and development of floral structures. – Acta Univ. N. Copernici, Biol. XVIII, (Torun) 37, 19-26.

Jablanović, M., Nešković, M., Grubišić, D., Konjević, R., Filipović, R. (1977): The distribution of endogenous hormones in dormant apple seeds. – Acta Biol. Med. Exper. (Priština) 2, 97-99.

Jablanović, M., Nešković, M. (1977): Changes in the endogenous level of auxins and cytokinins in axillary buds of *Pisum sativum* L. in relation to apical dominance. – Biol. Plant 19, 34-39.

Nešković, M., Petrović, J., Radojević, Lj., Vujičić, R. (1977): Stimulation of growth and nucleic acid biosynthesis at low concentration of abscisic acid in tissue culture of *Spinacia oleracea*. – Physiol. Plant. 39, 148-154.

Vujičić, R., Radojević, Lj., Nešković, M. (1979): Regular alignement of membranebound ribosomes in embryogenic tissue of *Paulownia tomentosa*. – Z. Pflanzenphysiol. 91, 53-56.

Naunović, G., Nešković, M. (1979): Rapid responses to light and gibberellic acid in etiolated pea stems. – Photochem. Photobiol. 29, 1173-1175.

Naunović, G., Nešković, M., Grubišić, D. (1980): Relationship of light and growth regulators in the phototropic reaction in dwarf pea stems. – U: „Photoreceptors and Plant Development", J. De Greef ed. Antwerpen Univ. Press, 393-397.

Ćulafić, Lj., Nešković, M. (1980): Effect of growth substances on flowering and sex expression in isolated apical buds of *Spinacia oleracea* L. – Physiol. Plant. 48, 588-591.

Petrović, J., Kidrič, M., Vacić, D., Nešković, M. (1980): Nucleic acids of the chrysanthemum *(Chrysanthemum turner)* leaves during transition to flowering. – Arh. biol. nauka 32, 175-178.

Radojević, Lj., Landre, P., Nešković, M. (1980): Isolement de trois souches tissulares a partir d'embryons immatures *d'Acer negundo* L. – Z. Pflanzenphysiol. 99, 191-198.

Filipović, R., Nešković, M., Jablanović, M., Hoxha, Y., Grubišić, D., Konjević, R. (1981): Examination of endogenous growth substances in the apple fruits during development. – Acta Biol. Med. Exper. (Priština) 6, 17-21.

Kidrič, M., Petrović, J., Nešković, M. (1981): Comparative studies of nucleic acids from vegetative and reproductive apices of chrysanthemum *(Chrysanthemum turner)*. – Period. Biol. 83, 407-413.

Srejović, V., Nešković, M. (1981): Regeneration of plants from cotyledon fragments of buckwheat *(Fagopyrum esculentum* Moench.). – Z. Pflanzenphysiol. 104, 37-42.

Bata, J., Nešković, M. (1982): Effect of external factors on growth and morphogenesis in *Lemna trisulca* L. in axenic culture. I. Plant hormones. – Bull. Inst. Jard. Bot., Univ. Belg. 15, 1-9.

Bata, J., Nešković, M. (1982): Effect of external factors on growth and morphogenesis in *Lemna trisulca* L. in axenic culture. II. Light conditions. – Bull. Inst. Jard. Bot., Univ. Belg. 15, 11-13.

Naunović, G., Nešković, M. (1982): Effect of monochromatic irradiation on gibberellin content and apical bud opening in etiolated peas. – Bull. Inst. Jard. Bot., Univ. Belg. 15, 15-19.

Pekić, S., Nešković, M. (1982): Influence of phytochrome on the content of endogenous hormones in *Lemna aequinoctialis* during the long night period. – Bull. Inst. Jard. Bot., Univ. Belg., 15, 21-28.

Ćulafić, Lj., Grubišić, D., Nešković, M. (1982): Endogenous gibberellin-like substances and inhibitors in callus tissue of *Spinacia oleracea* L. – Bull. Inst. Jard. Bot., Univ. Belg., 15, 29-35.

Srejović, V., Nešković, M. (1983): Formation and anatomy of buckwheat organogenic callus tissue *in vitro*. – Bull. Inst. Jard. Bot., Univ. Belg., 17, 45-50.

Ćulafić, Lj., Konjević, R., Nešković, M. (1983): Flowering of *in vitro* grown spinach shoots in the presence of herbicide Sandoz 9789. – Biol. Plant. 25, 155-157.

Vujičić, R., Radojević, Lj., Nešković, M. (1983): Ultrastructural study of somatic and zygotic embryos of *Corylus avellana*. – *In*: Proc. Atti del Convegno Internazionale sul Nocciuolo, Avellino, Italy, 275-178.

Radojević, Lj., Nešković, M., Vujičić, R., Kovoor, A. (1983): *In vitro* vegetative propagation of *Corylus avellana*. – *In*: Proc. Atti del Convegno Internationale sul Nocciuolo, Avellino, Italy, 283-281.

Ćulafić, Lj., Nešković, M., Vujičić, R. (1984): Two morphogenic patterns of plant regeneration in *Rumex acetosella* callus tissue. – *In*: Proc. Inter. Symp. Plant Tissue and Cell Culture. Application to Crop Improvement. Olomouc 161-162.

Babić, V., Nešković, M. (1984): Propagation of three blackberry cultivars from small apical buds *in vitro*. – J. Hort. Sci. 59, 183-185.

Grubišić, D., Ćulafić, Lj., Nešković, M. (1984): *In vitro* vegetative propagation of *Cercis siliquastrum* L. – Bull. Inst. Jard. Bot., Univ. Belg., 18, 77-80.

Nikolić, Đ., Knežević, T., Penčić, M., Nikolić, N., Nešković, M. (1985): Regeneracija biljaka u tkivnoj kulturi nezrelih embriona kukuruza (Plant regeneration in tissue culture of immature maize embryos). – Arh. za poljoprivredne nauke 46, 115-128.

Nešković, M. (1985): Vegetativno razmnožavanje belošljive (*Prunus domestica* L.) u kulturi *in vitro* (Vegetative propagation of plum (*Prunus domestica* L.) in *in vitro* culture). – Arh. za poljoprivredne nauke 46, 375-380.

Nešković, M., Vujičić, R., Srejović, V. (1985): Differential responses of buckwheat leaf cells to growth substances stimulating cell division. – Ann. Bot. 56, 755-760.

Srejović, V., Nešković, M. (1985): Effect of gibberellic acid on organogenesis in buckwheat tissue culture. – Biol. Plant. 27, 432-437.

Grubišić, D., Nešković, M., Konjević, R. (1985): Changes in light sensitivity of *Paulownia tomentosa* and *P. fortunei* seeds. – Plant Sci. 38, 13-16.

Spasenoski, M., Nešković, M. (1985): *In vitro* vegetative propagation of *Actinidia chinensis* Planch. from juvenile and adult plant segments. – Bull. Inst. Jard. Bot., Univ. Belg. 19, 7-13.

Stefanović, M., Grubišić, D., Nešković, M. (1986): Effect of light and growth substances on seed germination in *Ramonda serbica* Panč. – Bull. Inst. Jard. Bot., Univ. Belg. 20, 1-5.

Savić, R., Spasić, M., Milijić, S., Nešković, M. (1986): Regeneracija biljaka u tkivnoj kulturi lucerke *(Medicago sativa* L., sorta Zaječarska 83) i osobine regeneranata u prirodnim uslovima (Plant regeneration in tissue culture of alfalfa (*Medicago sativa* L., CV. Zaječarska 83) and regenerants properties under field conditions). – Arh. za poljoprivredne nauke 47, 273-283.

Ćulafić, Lj., Budimir, S., Vujičić, R., Nešković, M. (1987): Induction of somatic embryogenesis and embryo development in *Rumex acetosella* L. – Plant Cell Tissue and Organ Culture 11, 133-139.

Nešković, M., Vujičić, R., Budimir, S. (1987): Somatic embryogenesis and bud formation from immature embryos of buckwheat *(Fagopyrum esculentum* Moench). – Plant Cell Reports 6, 423-426.

Ćulafić, Lj., Samofalova, A., Nešković, M. (1987): *In vitro* organogenesis in two dioecious species, *Rumex acetosella* and *R. acetosa* L. (Polygonaceae). – Plant Cell Tissue and Organ Culture, 11, 125-131.

Konjević, R., Grubišić, D., Nešković, M. (1987): The effect of norflurazon on phototropic reaction in *Phaseolus aureus* Roxb. seedlings. – Photochem. Photobiol. 45, 821-824.

Grubišić, D., Konjević, R., Nešković, M. (1988): The effect of some growth regulators on light-induced germination of *Paulownia tomentosa* seeds. – Physiol. Plant. 72, 525-528.

Ninković, S., Miljuš-Đukić, J., Nešković, M. (1988): *In vitro* performance of *Medicato sativa* L. cv. Zaječarska 83. Direct Somatic Embryogenesis, Cell and Protoplasts Culture. – Bull. Inst. Jard. Bot., Univer. Belgrade, 22, 27-35.

Jelić, G., Ćulafić, Lj., Kapor, S., Nešković, M. (1988): Endogenous cytokinins in the vegetative and reproductive phases of development in the dioecious plant *Rumex acetosella* L. – Plant Growth Regulation, 7, 53-58.

Nikolić, Đ., Knežević, T., Bogićević, M., Šurlan, G., Nešković, M. (1989): Androgeneza nekih genotipova kukuruza primenom kulture tkiva *in vitro* (Androgenesis of some maize genotypes by tissue culture *in vitro*). – Genetika 21, 45-53.

Naunović, G., Nešković, M. (1989): Effects of substances affecting apoplastic and cytoplasmic calcium concentration in rapid pea stem growth responses. – Bull. Inst. Jard. Bot., Univ. Belg. – 23, 27-34.

Konjević, R., Grubišić, D., Nešković, M. (1989): Growth Retardant-Induced Changes in Phototropic Reaction of *Vigna radiata* Seedlings. – Plant Physiol. 89, 1085-1087.

Nešković, M., Vinterhalter, B., Miljuš-Đukić, J., Ghalawenji, N. (1990): Micropropagation of recessive determinante genotypes of buckwheat *(Fagopyrum esculentum* Moench.) as an alternate approach to uniform seed production. – Plant Breeding 105, 337-340.

Nešković, M., Vinterhalter, B., Miljuš-Đukić, J., Ninković, S., Vinterhalter, D., Jovanović, V., Knežević, J. (1990): Susceptibility of buckwheat *(Fagopyrum esculentum* Moench.) to *Agrobacterium tumeafaciens* and *A. rhizogenes.* – Fagopyrum (Buckwheat Newsletter) 10, 57-61.

Bohanec, B., Nešković, M. (1991): Attempts at haploidy induction in buckwheat *(Fagopyrum esculentum* Moench.) – Proc. Intern. Conf. Buckwheat Breeding, Miyazaki Univ., Japan, 11-15.

Naunović, G., Nešković, M. (1991): Differential effects of calmodulin antagonists on indolacetic acid- and gibberellic acid-induced biphasic growth responses. – Biol. Plant. 33, 75-80.

Vinterhalter, B., Nešković, M. (1992): Factors affecting *in vitro* propagation of quince *(Cydonia oblonga* Mill.) – J. Hort. Sci. 67, 39-43.

Miljuš-Đukić, J., Ninković, S., Nešković, M. (1992): Differential response to growth substances in transgenic buckwheat *(Fagopyrum esculentum* Moench.) clones. – Arh. biol. nauka 44, 197-202.

Miljuš-Đukić, J., Nešković, M., Ninković, S., Crkvenjakov, R. (1992): Agrobacterium mediated transformation and plant regeneration of buckwheat *(Fagopyrum esculentum* Moench.) – Plant Cell Tissue and Organ Culture 29, 101-108.

Zdravković-Korać, S., Nešković, M. (1993): Somatic embryogenesis in spinach *(Spinacia oleracea* L.). – Arch. Biol. Sci. 45, 57P-58P.

Dragićević, I., Nešković, M. (1993): Responses of buckwheat *(Fagopyrum esculentum* Moench.) dwarf mutants to light and gibberellic acid. – Arch. Biol. Sci. 45(3-4), 53P.

Ivić, S., Nešković, M. (1993): Morphogenic effects of a chimeric isopentenyl transferase gene in transformed tobacco *(Nicotiana tabacum* L.) and buckwheat *(Fagopyrum esculentum* Moench.) tissues. – Arch. Biol. Sci. 45(1-2), 19P-20P.

Bohanec, B., Nešković, M., Vujičić, R. (1993): Anther culture and androgenetic plant regeneration in buckwheat *(Fagopyrum esculentum* Moench.) – Plant Cell Tissue and Organ Culture 35, 259-266.

Jovanović, V., Dragićević, I., Miljuš-Đukić, J., Nešković, M. (1993): Plant regeneration from hairy roots of *Fagopyrum esculentum*. – Arch. Biol. Sci. 45(3-4), 55P.

Ninković, S., Miljuš-Đukić, J., Nešković, M. (1995): Genetic transformation of alfalfa somatic embryos and their clonal propagation through repetitive somatic embryogenesis. – Plant Cell, Tissue and Organ Culture, 42, 255-260.

Miljuš-Đukić, J., Nešković, M. (1995): The effect of Cycloheximide on self-incompatible pollen germination in buckwheat *(Fagopyrum esculentum* Moench.). – Arch. Biol. Sci. 47(3-4), 17P-18P.

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Nešković, M. (1978): Regulacija razvića viših biljaka. *In*: Savremeni pristup prirodnim naukama, Izd. Kolarčev narodni univerzitet, Beograd, 39-46.

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Josifović, M. ed. (1970-1979): Flora SR Srbije I-IX. – SANU, Beograd.

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