

Serbian Plant Physiology Society

Institute for Biological Research „Siniša Stanković“, University of Belgrade

**1st International Conference
on Plant Biology
20th Symposium of the
Serbian Plant Physiology Society**

Programme and Abstracts



Hotel PATRIA, Subotica, June 4-7, 2013

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is) were resequenced, labeled as CeAGP1 through CeAGP5, annotated and uploaded to the GenBank (accessions KC733882 through KC733886). Searching GenBank protein database using translated centaur sequences as queries revealed that CeAGP1, 2 and 4 are fasciclin-like proteins with the FAS1 domains, belonging to the Fasciclin superfamily. CeAGP3 and CeAGP5 also have homology with other plant AGPs, but have known conserved domains. While CeAGP2, 3 and 5 appear to be complete coding sequences with 461, and 202 amino acids respectively, CeAGP1 and 4 are partial sequences with more than 225 and 335 amino acids respectively. The sequenced transcripts differ in the degree of homology with other known plant AGPs. The alignments of novel putative AGPs with published plant AGPs, as well as recognized protein domains are presented. Expression of all 5 genes was confirmed in both leaves and roots by RT-PCR. The sequenced centaur AGPs will be evaluated as potential molecular markers for early developmental stages ofomatic embryogenesis in centaur.

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Vector construction for promoter analysis in chicory and fluorescence evaluation by agroinfiltration

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Chicory (*Cichorium intybus* L.) is rich in sesquiterpene lactones, compounds known for their bitter taste and medicinal properties. Most enzymes involved in the biosynthetic pathway of these secondary metabolites have recently been discovered and characterized. The first step in their biosynthesis is catalyzed by germacrene A synthase (GAS), in chicory present in two forms – long and short, and several P450 mono-oxygenases. So far, promoters of these genes have not been studied, and little is known about the spatial and temporal regulation of their expression. To address this issue, four vectors for plant transformation containing promoter-reporter gene fusions were designed and constructed by Gateway cloning, including one for GAS long, one for GAS short, and one for the cytochrome P450, germacrene A oxidase. As a marker for co-transformation, DsRED, a red fluorescent protein, was used, while the studied promoters were inserted to drive GFP/GUS fusion, to allow for visualization of promoter activity. Integrity and function of the constructs were checked by agroinfiltration in lettuce (*Lactuca sativa* Cv. Olof) – a transient transformation assay. Infiltration was performed with *Agrobacterium tumefaciens*, carrying the promoter constructs. Transformation success was checked five days after infiltration by fluorescent stereomicroscopy, and both DsRED and GFP were detected, indicating that the chicory promoters were active in lettuce. DsRED had strong and uniform fluorescence in all samples, but GFP fluorescence varied among plants infiltrated with different constructs. The GAS long promoter had longest expression, followed by the P450 and the two rather weak GAS short promoters. The fluorescence was visible only in the infiltrated parts of the leaves, in tissues between leaf veins, but not in the veins themselves. Both abaxial and adaxial leaf sides were fluorescing. There were no differences observed between spatial distribution of DsRED and GFP: all infiltrated parts showed both markers. Since these vectors were con-

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Application of CdSe nanoparticles in plant biology research

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Quantum dots (QDs) are semiconductor nanoparticles that are widespread in biology as fluorescent markers. The application of QDs as markers of the cells or their cell walls (CW) for plant bio-imaging would be advantageous because of their small size, brightness, independence of emission on the excitation wavelength, and stability under relatively harsh environments. They also have excellent photo stability [1].

In a plant cell the CW is a first target place for external agents. We studied interaction of CdSe QDs with CWs isolated from a conifer – *Picea omorika* (Panč) Purkyne branch. Binding of CdSe QDs was followed by using fluorescence microscopy, fluorescence and FT-IR spectroscopy. The aim of the study was to see whether the QDs induce structural changes in the CW, and to find out which kind of interactions between QDs and CWs occur. The isolated CW is an appropriate object for study of the interactions with nanoparticles.

The results obtained in this study show that the CdSe QDs linked primarily to cellulose and lignin in the cell wall of *P. omorika*. QDs are linked with lignin mainly through interaction with the C-C and C = C branched chains. The interaction of QDs with cellulose is accomplished through OH groups. Structural redistribution in the cell wall, as a result of interaction with QDs, is significantly dependent on the presence of water in the cell wall. The presented results also show that the QDs are suitable for homogeneous labeling of CW structure. The results have an implication on the use of the QDs in plant bioimaging.

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The role of DIMBOA in maize biotic stress resistance – presence of DIMBOA biosynthesis *bx1* gene in NS inbred lines

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DIMBOA (2-4-dihidroksi-7-metoksi-1,4-benzoksazin-3-on) is a secondary metabolite in grasses which belongs to benzoxazinoid class of chemical compounds and have a protective role against bacteria, fungi, insects and other pests. It is present in many species of *Poaceae* family, including maize, wheat and rye. In maize,

this benzoxazinoid has proved to possess allelopathic activity and provide resistance against aphids (*Rapalosiphum padi*, *R. maydis*), corn borers (*Ostrinia nubilalis*, *O. furnacalis*, *Sesamia nonagrioides*, *S. exigua*, *Diatraea grandiosella*) and fungi (*Diplodia maydis*, *Setosphaeria turcica*). DIMBOA is formed only when plant tissues are damaged by interaction of benzoxazinoid glucosides from vacuoles with specific enzymes glucosidases released from plastids. The concentration of DIMBOA decreases during the vegetative growth and as plants mature, hence the greatest resistance against pests and diseases in early developmental phases. Biosynthesis of DIMBOA is regulated by nine *bx* genes (*bx1-bx9*), however, the polymorphism within *bx1* has the largest effect of DIMBOA content. The dominant allele form of *bx1* gene provides plants with resistance against pest, whereas plants with the recessive allele are susceptible. To assess the presence and variability of *bx* gene in maize breeding material, a set of 96 divergent maize inbred lines developed at the Institute of Field and Vegetable Crops in Novi Sad was screened with a functional SSR marker. The fragment size of PCR products was obtained by capillary electrophoresis using Applied Biosystems 30130 Analyzer. The aim of the study was to identify the source of disease and pest resistance in early development phases of maize and examine the possibility of application of the microsatellite for selection of genotypes in maize breeding process.

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Antioxidative enzyme activities during *in vitro* morphogenesis from leaf explants of *Centaurium erythraea* Rafn.

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In this study we examined changes in activities of antioxidative enzymes, superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) during *in vitro* morphogenesis in leaf culture of *Centaurium erythraea* Rafn. Enzyme activities were estimated during somatic embryogenesis and organogenesis at weekly intervals up to four weeks, using both gel and spec enzyme assays. Somatic embryogenesis and organogenesis were achieved on Murashige and Skoog (MS) basal medium supplemented with 2,4 -dichlorophenoxyacetic acid 2,4 D (0.2 mg/l) and N-(2-chloro-4-pyridil)-N'-phenylurea CPPU (0.01 and 0.5 mg/l) in 16 h light and dark conditions. Measurement of SOD, CAT and POD activities during morphogenesis in light and dark conditions demonstrated that there were differences in isoenzyme patterns and activities. The excision of the leaf explants and their subsequent cultivation in the induction culture medium resulted in the tissue oxidative stress and increase in SOD and CAT activity. Our data suggest the involvement of antioxidative enzymes during morphogenesis from leaf explants of *C. erythraea*.

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