

# BOOK OF ABSTRACTS

## 3rd International Conference on Plant Biology (22nd SPSS Meeting)



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BELGRADE

**Serbian Plant Physiology Society**

**Institute for Biological Research "Siniša Stanković", University of Belgrade**

**Faculty of Biology, University of Belgrade**

**3<sup>rd</sup> International Conference  
on Plant Biology  
(22<sup>nd</sup> SPPS Meeting)**



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## Allelopathic effect of apple hairy root exudates on the germination and seedlings growth of *Arabidopsis thaliana* *in vitro*

PP2-8

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In the previous study, phenolic acids and polyphenolic flavonoids were identified as putative allelochemicals in apple cv. Golden Delicious hairy roots growth medium. In order to examine their allelopathic potential, the effect of hairy root exudates on the germination and seedling growth of the test species *Arabidopsis thaliana* was elucidated. *Arabidopsis* seeds were cultivated in Erlenmeyer flasks containing 25 mL of liquid medium in which apple hairy roots were cultivated for 4 weeks. The effect of apple hairy root growth medium on *Arabidopsis* was evaluated after 5 and 10 days of seed cultivation. Apple hairy root exudates had no significant effect on *Arabidopsis* germination rate, while growth characteristics of seedlings were significantly affected both after 5 and 10 days of treatment. The roots of treated seedlings were significantly shorter (35.1%), with lower number (74% decrement) of lateral roots compared to control after 10 days of treatment. The growth and morphology of *Arabidopsis* shoots and leaves have been affected too. Thus, the shoots of treated seedlings were almost twice shorter compared to control after 5 days of treatment. At the 10<sup>th</sup> day, this distinction became more pronounced, when two fully expanded leaves were visible in treated seedlings, while control had completely developed rosettes with six leaves. In addition, leaves of treated seedlings were smaller and pale in colour compared to control. qPCR expression analysis of genes involved in regulation of the cell cycle showed reduction of transcript levels in roots of treated seedlings, with the highest expression decrease detected for *CDKA1* and *CYCB2;4* cyclin.

**Keywords:** allelopathy, allelochemicals, cell cycle genes, phenolic acids, polyphenolic flavonoids

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## Transgenic hairy root *in vitro* culture system of apple as a tool for allelopathic studies

PP2-9

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Since the investigation of plant allelopathic interactions in the rhizosphere is difficult to perform because of its high complexity, microbial and environmental factors interactions, the generation of autonomous root culture could be appropriate system for the studies of allelopathic

potential of apple (*Malus x domestica* Borkh.). Thus, we have focused our efforts on the generation of transformed hairy-roots in apple cv. Golden Delicious using the 15834 strain of *Agrobacterium rhizogenes*. The agrobacteria inoculation by dipping the basal cut off end of the stem of *in vitro* grown apple shoots into bacterial suspension proved to be the best option for transformation, providing efficiency of 39.3%. Molecular characterization of hairy roots by RT-PCR analyses confirmed their transgenic nature. *In vitro* conditions for apple hairy root growth and maintenance were optimized using different mineral solutions and auxins. The liquid half-strength MS medium with the addition of IBA at 0.025 mg L<sup>-1</sup> was chosen for the initial stimulation of growth. For their prolonged cultivation and maintenance, the alternation of IBA-containing and IBA-free media is recommendable. Fast-growing lines continuously maintained in IBA-free medium were used for further research. Potential allelochemicals including phenolic acids (chlorogenic, caffeic, syringic, p-coumaric and ferulic), glycosilated flavonoids (phloridzin, rutin, quercitrin, isoquercitrin, kaempferol-3-glucoside) and flavonoid aglycons (floretin, quercetin and naringenin) have been detected by UHPLC/DAD/(+/-)HESI-MS/MS analyses in the growth medium, in which these transformed roots were maintained for 4 weeks, as well as in the root tissue itself. The obtained hairy-root cultures offer a complementary system for studying and validating the allelopathic potential of apple roots.

**Keywords:** *Agrobacterium rhizogenes*, allelopathy, allelochemicals, growth conditions, hairy roots.

*This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. OI173015).*

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## Does ABA pretreatment affect ecophysiological status of bryo-halophyte *Entosthodon hungaricus* during salt-stress?

PP2-10

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*In vitro* culture of a peculiar moss halophyte *Entosthodon hungaricus*, which can be found exclusively on salty grasslands, was established with the aim to study the effect of exogenous ABA treatment of the moss gametophores prior to their growth in NaCl enriched environment. Axenic *in vitro* growth environment was established to avoid synergistic or antagonistic effects of other varying factors and to achieve fully controlled conditions. ABA is known to influence the activation of certain molecular mechanisms and expression of adequate ABA-responsive genes in different vascular plants under dehydration and salt stress.

*E. hungaricus* gametophores were grown for 72 hours on medium containing ABA (50 µM), and afterwards transferred to different levels of NaCl (0-500mM) for 21 days. The survival rate, index of multiplication, content of photosynthetic pigments, total phenols and antioxidative capacity were recorded. The results obtained clearly showed that exogenous ABA resulted in higher survival rate in all treatments. However, ABA had no influence on new gametophore production, as expected. However, in higher salt concentrations, secondary protonema appeared in ABA pretreated plants