

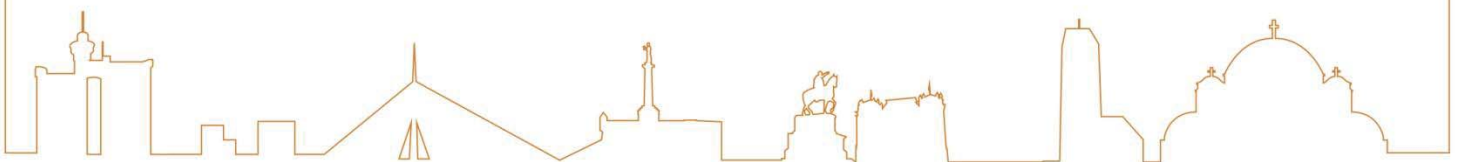
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## KNJIGA SAŽETAKA BOOK OF ABSTRACTS



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## AGROBACTERIUM-MEDIATED GENETIC TRANSFORMATION OF *Viola cornuta*

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**Introduction:** *Viola cornuta* is a valuable perennial ornamental plant. Development of new traits, such as new flower color with classical breeding suffers from many difficulties, which can be overcome using genetic engineering. With aim to develop protocol for *Agrobacterium*-mediated transformation of *V. cornuta*, we used *Agrobacterium tumefaciens* strain LBA4404 harbouring the superbinary vector pTOK233 carried a GUS reporter gene and hygromycin phosphotransferase selectable marker gene.

**Methods:** Hypocotyl explants obtained from seedlings were grown on ½MS medium supplemented with 0.1 mg/l 2,4-D and 2.0 mg/l BA for shoot induction. After two days of pre-cultivation, hypocotyl explants were inoculated in bacterial suspension for 15 min and placed on the same culture medium with addition of acetosyringone 100 µM at pH 5.2. After two days of co-cultivation, explants were transferred on shoot induction medium supplemented with cefotaxime and hygromycin B for selection. Regenerated putative transformants were analyzed by PCR for hygromycin phosphotransferase gene presence and by histochemical assay for β-glucuronidase (GUS) activity.

**Results:** Shoots were obtained within 8 weeks after explants were inoculated with *A. tumefaciens*, with 2.0% regeneration efficiency. PCR analysis confirmed selectable marker gene presence in twelve out of sixteen (75.0%) independently derived putatively transformed lines that were tested. Additionally, all analyzed lines exhibited a notable β-glucuronidase activity that was not present in untransformed plants.

**Conclusion:** This is the first report about *V. cornuta* susceptibility to *A. tumefaciens*. Presented protocol for genetic transformation can be used for further introduction of desirable traits in *V. cornuta* cultivars.

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