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(22nd SPPS Meeting)

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Anatomical and micromorphological investigations of *Artemisia absinthium* L. (*Asteraceae*) from Serbia

PP3-10

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In this work anatomical and micromorphological investigations of vegetative organs of *Artemisia absinthium* L. (*Asteraceae*), wild-growing in Serbia, were conducted. The aim of this study was to examine the general anatomy and micromorphology, as well as to find possible valid taxonomic characters. Microscopic slides were prepared following the standard histological procedures. Triarch type of the vascular bundle is present in primary root structure, whereas typical secondary growth occurs in older roots. Also, large secretory ducts, with a brownish content, are present in the cortex. The stem is polygonal in shape and characterized by collateral vascular bundles. Clearly visible endodermis layer is noticed. The largest parenchyma cells occur in the pith. Also, small secretory ducts occur in the cortex and in the pith of the stem. Petiole has ellipsoidal shape, with similar anatomy to the stem. Concerning leaf anatomy, the isolateral palisade structure is observed. On the surface of all aerial vegetative organs, numerous morphologically variable T-shaped nonglandular, as well as very prominent, large glandular trichomes, with brownish content, were found. All of the data may be considered as possible taxonomic characters which could help in species identification and infrageneric taxonomy of the genus *Artemisia*. Thus, these findings are of importance for future anatomical, micromorphological and phytochemical investigations of this and related species.

Keywords: *Artemisia absinthium*, *Asteraceae*, anatomy, micromorphology

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Cryopreservation of *Viola cornuta* shoot tips using vitrification procedure

PP3-11

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Cryopreservation represents a suitable method for long term storage of different plant genetic resources. The aim of this study was to develop protocol for cryopreservation of *Viola cornuta* shoot tips using one step freezing method with chemical dehydration of tissue with modified Plant Vitrification Solutions (PVS2 or PVS3). Shoot tips (1-2 mm) of two-week cold acclimated shoots were cultured on ½MS medium with 0.3 M sucrose for one day before treatment with loading solution (2 M glycerol, 0.4 M sucrose) for 30 min. Osmotic dehydration with PVS2 solution (30%

glycerol, 15% ethylene glycol and 15% DMSO in liquid ½MS medium with 0.4 M sucrose) were tested at 0 °C or 24 °C. Osmotic dehydration with PVS3 (50% sucrose, 50% glycerol in liquid ½MS medium) were tested at 24 °C for 45 min. After the treatment the explants were directly immersed in liquid nitrogen (LN) for at least one day. Re-warming was performed at 42 °C in water bath for 2 min. After re-warming, the PVS solutions were replaced with unloading solution containing 1.2 M sucrose for 20 min. Re-warmed shoot tips were cultured on ½MS medium with 0.1 mg L^{-1} BAP. We observed that PVS2 solution is cytotoxic for *V. cornuta* shoot tips and cannot be used for cryopreservation. However, cryopreservation with PVS3 solution was successful, where 71.9-100% shoot tips survived treatment before immersion to LN and 31-40% survived after re-warming from LN. Regrowth of cryopreserved shoot tips with new well-formed leaves was obtained after four weeks of culture.

Keywords: horned pancy, Plant Vitrification Solution, PVS2, PVS3

This study was supported by the Grant No. TR31019 from the Ministry of Education, Science and Technological Development of the Republic of Serbia.

Towards *ex situ* conservation of rare and endangered moss *Tayloria splachnoides*: biotechnical approach

PP3-12

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Tayloria splachnoides is an uncommon moss species rarely found in high mountains of central Europe and in Scandinavia. It is red-listed in many European countries: Finland, Norway, Sweden, Austria, Czech Republic, Poland, Slovakia, Switzerland, Bulgaria, Romania and Slovenia. *In vitro* culture of an accession from Slovakian High Tatra Mountains was established with the aim to study massive micropropagation of this widely threatened species. The effect of plant growth regulators, different media types, and sugar content were tested to obtain well developed gametophores. Index of multiplication and secondary protonema diameter were measured. According to the results achieved, it can be emphasized that the best media type for *T. splachnoides* micropropagation was sugar- and plant regulators-free Murashige and Skoog medium, at 18 °C, and 16/8 light/dark condition. Considering protonema diameter, KNOP medium enriched with sucrose (7.5-15 mg L-1) was the most appropriate. In contrast, BCD enriched with sucrose had the opposite effect, i.e. decreasing the secondary protonema diameter. KNOP medium enriched with cytokinin BAP (0.1 μM) combined with auxin IBA (0.1 μM) clearly induced the largest secondary protonema diameter. Gametophore appeared only on KNOP medium supplemented with plant growth regulators, but no clear pattern can be inferred, which implicates no clear agents in bud induction on secondary protonema. Further investigations are urgently needed and in progress.

Keywords: conservation, rare, moss, development, propagation

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