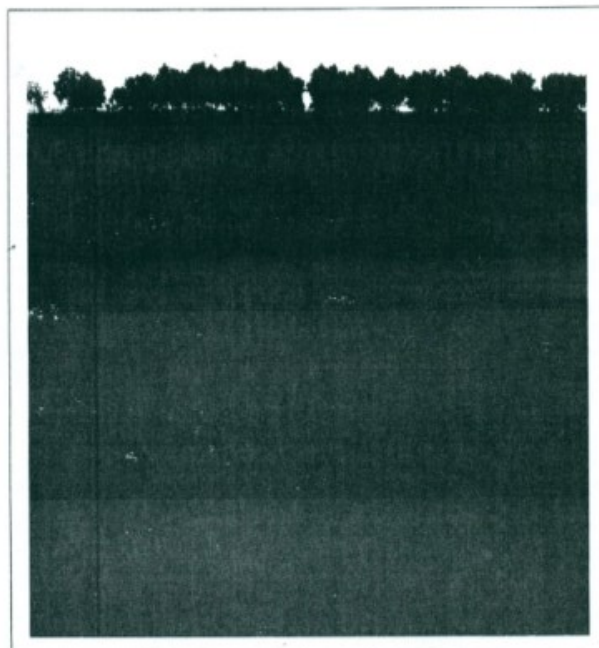


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cells can overcome the damaging activity of the radiomimetic zeocin.

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L1_03

DO ENDOGENOUS CYTOKININS REGULATE TERPENOID BIOGENESIS AND THYLAKOID MORPHOGENESIS IN *ARTEMISIA ALBA* IN VITRO MODEL SYSTEM?

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Aim: In our previous research it was established that terpenoid profile of the essential oils of *Artemisia alba* depends on plant growth regulators (PGR) supplementation and morphological development *in vitro*. These effects were further shown to be related to endogenous cytokinin levels. Here we study the morphology of thylakoid membranes (TM) in order to find possible relation between the obtained dependencies and the status of the photosynthetic apparatus of the plant *in vitro*.

Materials and methods: Shoot cultures of *A. alba* were initiated and maintained as previously described. Different treatments with indole-3-butyric acid (IBA) and benzyl adenine (BA) were applied. Isolated TM were examined by flow cytometry (FCM) and atomic force microscopy (AFM). Levels of endogenous cytokinins were determined by LC/MS chromatography. Terpenoid profile was studied by GS/MS analyses of the essential oils.

Results: FCM revealed co-existence of two TM fractions differing in size and internal structure in all PGR-treatments. AFM further demonstrated that IBA induced the formation of small TM subfraction and small granas (plants with predominant monoterpenoid content in the oils and slightly reduced cytokinin levels), while the combined action of IBA and BA resulted in the formation of large thylakoids and featureless granas (plants with predominant sesquiterpenoids in the oils and a drop of bioactive cytokinins levels).

Conclusion: We demonstrate that PGR influence the endogenous cytokinins level which in turn plays a crucial role in the thylakoid morphogenesis. The fraction of "swollen" thylakoids can be interpreted as indicative of early stage of senescence-like response.

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Keywords: *Artemisia alba* *in vitro* culture, terpenoid biogenesis, endogenous cytokinins, thylakoid membranes, flow cytometry, atomic force microscopy

VARIATIONS IN ANTIOXIDANT CAPACITY DURING PEA VEGETATIVE GROWTH AT HIGH AIR TEMPERATURE

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The purpose of the study was to compare the antioxidant capacities (AOC) of pea plants grown hydroponically at 22°C (normal) and 40°C (high) air temperature by means of AOC assays.

Methods: AOC was determined by HORAC (hydroxyl radical averting capacity), ORAC (oxygen radical absorbance capacity) and F-C (Folin-Ciocalteu) assays which assessed different functions of certain non-enzymatic antioxidants.

Results: Exposure to 40°C suppressed gradually pea growth and development; at the end of exposure pea plants did not expand the upper leaf stages, and did not develop reproductive organs. Changes of opposite pattern occurred in AOC during heat exposure. AOC in the whole plant increased about twofold after short exposure, however, prolonged one influenced negatively AOC of the green organs, particularly leaf and shoot apex AOC were reduced significantly. These responses were confirmed by the three assays though in different magnitude. The heat effect on the potential non-enzymatic antioxidants underlying the used assays will be discussed.

Conclusion: By means of three different *in vitro* assays we established that normally highest AOC was localized in green pea organs (leaves, shoot apex). The AOC values indicated the impacts of certain non-enzymatic antioxidants. The high air temperature affected mostly the AOC of upper leaf stages and shoot apex; pea growth and development were disturbed as well. The AOC is dynamic index; it varies depending on the developmental and environmental conditions, and the used assays are capable to detect the variations.

Key words: *Pisum sativum* L. cv. Ran, heat, growth, antioxidant capacity (AOC) assays.

PROTEIN PROFILING AND ANTIOXIDANT ENZYME ACTIVITY OF ARTEMISIA ALBA CULTURES

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Aim: to assess the impact of exogenously applied plant growth regulators (PGR) on enzyme activities in *A. alba* shoot cultures.

Material and Methods: Protein content of fresh leaf and roots of *A. alba* was measured according to Bradford. Enzymes were estimated by SDS-PAGE, electrophoretic zymography and spectrophotometrically.

Results: The aerals were rich in protein content (~0.01g/g fresh leaf). Application of indole-3-butyric (IBA) acid alone was related to observance of bands in the aerals, corresponding to molecular mass of 24 and 26 kDa, which were absent when combination of IBA and benzyl adenine (BA) was applied.

Application of BA alone increased the protein content in the aerial parts. In roots and callus the protein content (~0.002 g/g fresh tissue) and the variety of protein fractions was lower. The latter samples displayed marked differences in their electrophoretic profile as compared to the aerials. The native PAGE enzyme activity staining, revealed presence of antioxidant enzymes (catalase, superoxide dismutase, ascorbate peroxidase, polyphenoloxidase, peroxidase). Data are in accordance with the activities of antioxidant enzymes obtained spectrophotometrically.

Conclusions: Application of PGR in *A. alba* *in vitro* leads to changes in the electrophoretic profile of samples of aerials and roots of the plant, which is confirmed by the results in determining the activities of antioxidant enzymes. Since relevant changes in polyphenol content and molecular markers of lipid peroxidation and oxidative stress have been obtained, it can be assumed that there is a correlation between enzymatic and non-enzymatic antioxidant protection of the plant *in vitro*.

Acknowledgment: Swiss National Science Foundation in the Framework in the Bulgarian-Swiss Research Programme (BSRP, grant No. IZEBZO_142989; DO2-1153)

Keywords: *Artemisia alba* shoot cultures, antioxidant enzyme activity, SDS-PAGE, native PAGE

P1_07

SCREENING OF THE ANTIMICROBIAL ACTIVITY OF MEDICINAL PLANT CULTURES COMMON TO THE FLORA OF BULGARIA AND TUNISA

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The aim of the present study was to screen the antimicrobial activity of *in vitro* cultivated medicinal plants originating from the flora of Bulgaria and Tunisia.

Material and Methods: *in vitro* cultures of *Hypericum perforatum*, *H. richeri*, *H. rumeliacum* and *Inula britannica* collected from the wild habitats in Bulgaria, as well as *Lavandula dentata*, originating from the experimental field of Biological Agriculture Technical Center in Chott-Mariem, Sousse, Tunisia were initiated by surface sterilization of the stem explants of the plants in benzyl adenine supplemented media. After *in vitro* growth induction stock shoots of the species were maintained in growth regulators free medium. Air dried plant material was subjected to successive ultrasonic extraction with chloroform and methanol. The different extracts were screened for their activity against six references strains pathogen bacteria, using the disc diffusion method.

Results: in this study we found antibacterial activity only in the chloroform extract. The highest activity was founded against *Listeria monocytogenes* by the chloroform extracts of *H. perforatum* and *H. richeri*, followed by the chloroform extracts of *L. dentata* and *Inula britannica*.

Conclusion: *In vitro* culture development of the studied medicinal plants could further be used as a constant and controllable source of raw material for the delivery of antimicrobial agent of the studied species. Further research is in progress to analyze the chemical composition of the various extracts as well as to broaden the study with enriched fractions obtained from these species.

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