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L3_03

ANTIMUTAGENIC EFFECT OF *PAPAVER RHOEAS* L. EXTRACT ON TEST-SYSTEM *SACCHAROMYCES CEREVISIAE*

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Aim: To evaluate the antimutagenic effect of *Papaver rhoeas* L. extract on yeast *Saccharomyces cerevisiae* strain D7ts1.

Material and Methods: The *S. cerevisiae* D7ts1 diploid strain was used as a tester for simultaneous quantitative determination of reversion, crossingover and gene conversion in nDNA in order to determine possible mutagenic or antimutagenic effect of *Papaver rhoeas* L. extract. The tested concentrations were in the range from 0.25 mg/ml up to 10 mg/ml). Zeocin- 100µg/ml was used as a standard mutagen.

Results: All tested concentrations of *Papaver rhoeas* L. extract lead to decreasing the mutagenic and genotoxic action of zeocin. The most pronounced effect is found at concentration 5mg/ml: the levels of gene conversion decrease two times, the levels of reverse mutation- 5 times and the levels of mitotic crossingover 3 times. The survival frequency is increased two times in comparison with zeocin. The concentration 10mg/ml has been able to increase slightly levels of gene conversion, reverse mutation and mitotic crossingover caused by zeocin, while the survival frequency is decreased.

Conclusions: The extract of *Papaver rhoeas* L. in a concentration 5mg/ml possesses the best antimutagenic effect.

Keywords: 3-6 yeast, papaver, antimutagenic

Acknowledgements: This study is funded under the project ДНТС/ Словакия/01/1.

L3_04

IN VITRO CULTURE OF *SIDERITIS SCARDICA* CULTIVAR SOFIA 2 FOR THE PRODUCTION OF POLYPHENOLIC COMPOUNDS

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Aim: To establish shoot cultures of the commercial cultivar Sofia 2 of the Balkan endemic medicinal plant *Sideritis scardica* and evaluate the biosynthetic potential for the production of polyphenolic compounds *in vitro*.

Material and Methods: Shoot cultures were initiated by sterile germination of seeds of Sofia 2 cultivar (SOM 3157). Modifications consisted of different supplementations of benzyl adenine (BA) and 1-Naphthaleneacetic acid (NAA). Exhaustive ultrasonic extraction at 40 °C was combined with overnight maceration at room temperature till full discoloration of the solvent (80 % EtOH). Phenolics and flavonoids were assayed colorimetrically.

Results: Plant growth regulators free (PGR-free) medium resulted in a low multiplication index (1-2 axillary shoots per explant), showed to be unfavorable for long-term maintenance (over 4 weeks) and required more frequent subculture in order to avoid necrosis of explants of cultivar Sofia 2. Addition of 0.2 mg/l BA and 0.02 mg/l NAA resulted in a slight stimulation of the multiplication rate, and

allowed longer sub-culture periods. PGR treatments also resulted in elevation of the phenolic compounds levels. This was however accompanied by a slight decrease of the flavonoids in the samples. Comparison of polyphenolic contents with the samples from *in situ* derived material showed preservation of the biosynthetic capacity of the plant *in vitro* and moreover, elevation of the proportion of the flavonoids expressed as a ratio of the total amounts of lower molecular phenolic compounds.

Conclusions: Possible auto-toxic effect of the high polyphenolic levels *in vitro* might explain the difficulty to cultivate the plant in PGR-free medium. Further research is in progress for the optimization of an *in vitro* system with high biomass generation capacity together with the preservation of the high biosynthetic potential.

Keywords: Sideritis cultivar Sofia 2, shoot culture, polyphenolic compounds, plant growth regulators

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L3_05

ANTIOXIDANT BIOLOGICAL ACTIVITY OF BULGARIAN HONEY AND ROYAL JELLY ON THE ORIGIN OF REACTIVE OXYGEN SPECIES

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Aim: To measure the antioxidant capacity of honey and royal jelly, produced in Bulgaria, by principally new test for determination the biological activity of antioxidants with natural origin.

Material and Methods: A newly developed test- Ty1antiROS for measuring the antioxidant capacity of Bulgarian honey and royal jelly using as a test system *Saccharomyces cerevisiae*.

Results: The mobility of Ty1 transposon in *Saccharomyces cerevisiae* was found to vary proportionally to the level of ROS generated in cells which provides the possibility to determine antioxidant activity of different products by changes in a cellular process instead of using chemical reactions. The study of royal jelly and honey with the newly developed Ty1antiROS test reveals an inverse exponential dependence of antioxidant activity on increased concentrations. This dependence can be transformed to proportional by changing the source of ROS: Instead of cell-produced to applied as hydrogen peroxide. The different test-responses are not due to excess of added hydrogen peroxide as evidenced by the exponential dependence found by usage of *yap1Δ Saccharomyces cerevisiae* tester cells accumulating cell generated ROS.

Conclusions: The activity of antioxidants to oxidative radicals may depend on the origin of ROS and this activity is elevated for cell-generated ROS compared to ROS added as reagents in the assay. This research can be useful and applicable in the fields of pharmacology, agriculture, medicine, organic farming, food industry etc.

Keywords: Ty1antiROS test, Ty1 transposition, exponential, proportional test response

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BIOCHEMICAL AND MOLECULAR CHARACTERISTICS OF TWO CULTIVARS *PHASEOLUS VULGARIS* L. UNDER DROUGHT STRESS

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Aim: to compare drought tolerance of two cultivars of *Phaseolus vulgaris* L. using some biochemical and molecular markers for oxidative stress.

Material and Methods: Seeds from two cultivars *Phaseolus vulgaris* L. (Dobrudjanski ran and Dobrudjanski 7) were sterilized following routine procedure and grown on SG medium under standard conditions in phytocamera to the phase of the third leaf. Plants were separated into three groups - control (not treated) and treated with 8% and 16% polyethylene glycol (PEG - MW 10 000) for 24h. Four markers for oxidative stress - malondialdehyde (MDA), hydrogen peroxide (H₂O₂), proline (Pro) and heat shock protein (HSP70B) were analyzed.

Results: Dobrudjanski 7 was characterized with higher constitutive levels of Pro and HSP70B and lower levels of MDA and H₂O₂ comparing with Dobrudjanski ran. The constitutive levels of Pro were similar in both cultivars. Drought led to the accumulation of higher content of MDA and H₂O₂ in both cultivars. The most pronounced stress response was obtained for Dobrudjanski ran measured as Pro and HSP70B.

Conclusions: Our preliminary results suggest that cultivar Dobrudjanski 7 is more drought resistant comparing with Dobrudjanski ran. Possible mechanisms involved are discussed.

Keywords: biochemical and molecular markers, drought stress, *Phaseolus vulgaris* L.

Acknowledgements: This study was funded by the projects DDVU_02/87: “Complex morphometric, physiological, biochemical and molecular assessment of drought tolerance in Bulgarian common bean genotypes (*Phaseolus vulgaris* L.)” and “Biochemical and molecular markers of drought tolerance in Bulgarian common bean genotypes” – scientific cooperation between RAS and BAS.

POLYPHENOLICS STIMULATION BY VITAMIN AND PLANT GROWTH REGULATORS IN *INULA BRITANNICA* IN VITRO CULTURE

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Aim: To establish shoot cultures of medicinal plant *Inula britannica*, Asteraceae and optimize *in vitro* culture system for the production of antioxidant polyphenolic compounds in controlled conditions.

Material and Methods: *in vitro* culture was initiated by axillary buds formation of surface sterilized stem explants of *I. britannica*, collected from its wild habitat in Bulgaria. Culture induction medium was 0.5 mg/l benzyladenine (BA) supplemented. Long-term maintenance was in plant growth regulators (PGR) free Murashige and Skoog (MS) basal medium. For optimization of polyphenolics

production vitamin supplementation (MS vs. Gamborg – G5), as well as PGR (BA and 1-Naphthaleneacetic acid - NAA) were experimented.

Results: G5 vitamins generally increased both phenolic and flavonoid contents in *I. britannica* aerial parts *in vitro* as compared with the MS formulation, both in PGR-free and PGR-supplemented media. With the increasing of BA concentration (from 0.2 mg/l to 0.7 mg/l) in both vitamin supplementations there was a raise in the phenolics and flavonoid levels, as this effect was more profound in the MS media. However, with the raise of BA concentration a drop of the proportion of flavonoids from the total amounts of phenolics was recorded in both vitamin supplementations. The supplementation of 0.1 mg/l NAA in combination with BA further increased the levels of polyphenolics in a dose dependent manner.

Conclusions: The obtained results are fully in agreement with the physiological role of vitamins B (higher in G5 formula) in the plant organism and provide useful tool for optimization of polyphenolic production in *I. britannica* *in vitro*. Further research is in progress to evaluate the biochemical and physiological parameters of the interrelations between vitamin and PGR in order to obtain optimal biomass and secondary metabolite production in controlled conditions.

Keywords: *Inula britannica* shoot cultures, vitamins, plant growth regulators, phenolics, flavonoids

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P3_02

PROTECTIVE ROLE OF PRELIMINARY TREATMENT WITH H₂O₂ AGAINST THE TOXIC ACTION OF THE HERBICIDE PARAQUAT ON THE PHOTOSYSTEM INTEGRITY IN PEA THYLAKOIDS

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Aim: This investigation focuses on the protective role of hydrogen peroxide against paraquat toxic effect on the photosynthetic apparatus.

Material and Methods: Pea plants were grown and treated with hydrogen peroxide (H₂O₂) and paraquat (PQ) as in Moskova et al. (Compt.Rend.Acad.Bulg.Sci. 60, 2007, 1101). Thylakoid membranes were isolated according Harrison&Melis (Plant Cell Physiol. 33, 1992, 627). Low temperature fluorescence spectra were measured upon 436, 472 and 515 nm excitation.

Results: The 77K fluorescence emission and excitation spectra of thylakoid membranes isolated from pea plants treated with 2.5mM H₂O₂ and 0.2mM PQ alone and in combination were investigated. The characteristic ratio of fluorescence maxima F₇₃₅/F₆₈₅ was strongly influenced by PQ treatment. The inhibition of energy distribution to PSI after 5h PQ treatment was evident upon both chl **a** and chl **b** excitations, while for excitation in the carotenoid region the effect was pronounced after 2h. Pretreatment with H₂O₂ reduced the later changes. PQ led to expansion of the absorption cross-section of PS I and caused reduction in the energy transfer within PSII supercomplex revealed by the fluorescence ratio F₆₉₅/F₆₈₅. The PSII related changes were less pronounced in case of H₂O₂ pretreatment.

Conclusions: Data show that treatment with H₂O₂ had slight effect on the energy distribution within PSII complex and as expected PQ acted as an inhibitor of PSI functionality. Possible role of H₂O₂ as a protector against herbicide damages on the level of photosynthetic membranes was proposed

Keywords: Oxidative stress, thylakoids, photosystem integrity, 77K fluorescence

Acknowledgements: This study was supported by the National Science Fund of the Bulgarian Ministry of Education and Science, Project DMU 03-60 /12-15.

Keywords: *Papaver rhoeas*, anticlastogenic effect, plant test-system, zeocin

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P3_05

THE CITRININ BIOSYNTHESIS BY ASCOMYCETE FUNGI AS ADAPTIVE MECHANISM: *IN SILICO* STUDY

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Aim: Citrinin possesses antibiotic, bacteriostatic, antifungal and antiprotozoal properties that increase strain adaptability in natural habitats. Applying bioinformatics approach, the evolutionary conservatism of genes involved in citrinin biosynthesis (a regulatory gene (*ctnA*) and a citrinin polyketide synthase gene (*pksCT*)) was investigated in phylogenetic distant citrinin producing fungi (*Monascus*, *Aspergillus* and *Penicillium*).

Material and Methods: The relevant data for the protein sequences were downloaded from the MGD (www.bcrf.firdi.org.tw) and GeneBank (www.ncbi.nlm.nih.gov/genbank/) and BLASTp alignment was performed.

Results: Obtained results showed high identity between *M. purpureus* IFO 30873 strain polyketide synthase gene and its orthologous in *M. pilosus* JCM 22613, *P. chrysogenum* Wisconsin 54-1255 and *A. oryzae* RIB40 strains (45 %, 34 % and 37 %, respectively). Similar analyses were performed with the regulatory gene *ctnA* and respective orthologs in *Aspergillus oryzae* and *Penicillium chrysogenum* strains were identified. The detected similarities vary between 31% and 40. When BLASTp analysis was run against *M. pilosus* genome no significant hit was returned, suggesting that regulatory activator was absent or was significantly different in its protein sequence. Analysis of intracellular localization of proteins, encoded by both genes (*pksCT* and *ctnA*), revealed that in all studied species both products are localized in cytosol. However, all of them possess one or several additional signals, allowing their targeting to different subcellular compartment (EPR, nucleus, etc.).

Conclusions: The lack of citrinin production by *Monascus* fungi decreases their adaptability potential in natural conditions. From biotechnological point of view this is an advantage for production of different secondary metabolites (pigments, monacolins) having a wide application in food, cosmetic and pharmaceutical industries.

Keywords: citrinin, bioinformatics, *ctnA*, *pksCT*

P3_06

RELATIONS BETWEEN ENZYMATIC AND NON-ENZYMATIC ANTIOXIDANT DEFENCE INVOLVED IN POLYPHENOLICS PRODUCTION OF ARTEMISIA ALBA *IN VITRO*

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Aim: to study the physiological and biochemical parameters related to polyphenolics biosynthesis in *A. alba* shoot culture.

Material and Methods: supplementations of indole-3-butyric (IBA) acid and benzyl adenine (BA) were experimented in shoot cultures of *A. alba*. Phenylalanine ammonia lyase (PAL EC 4.3.1.24), glutathione reductase (GR EC 1.8.1.7), ascorbate peroxidase (APX EC 1.11.1.11), catalase (CAT EC 1.11.1.6), guaiacol peroxidase (GPOX EC 1.11.1.7) were estimated spectrophotometrically; electrophoretic profile - by 10% SDS-PAGE; malondialdehyde and hydrogen peroxide - spectrophotometrically; phenolic and flavonoid contents - colorimetrically in 80 % ethanolic extract.

Results: Increased IBA supplementation resulted in a drop of polyphenolics and elevation of antioxidant enzymes CAT, GPOX, GR and APX in the aerial parts. As a result lower MDA and H₂O₂ were detected in both aerials and roots in these media. On the other hand, when compared the aerials and roots of the PGR-free control, roots exhibited significantly lower GPOX. The electrophoretic profile also confirmed the generally lower enzymatic activity in roots. However, the elevated polyphenolics, as well as the increased Apx resulted in a drop of MDA and H₂O₂ in the roots as compared with aerials.

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P3_07

HABITAT PREFERENCES AND THE IMPACT OF ABIOTIC FACTORS ON THE ACTIVITY OF *CRYPTOPS ANOMALANS* NEWPORT, 1844 (CHILOPODA: SCOLOPENDROMORPHA) IN NORTHEAST BULGARIA

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Aim: Investigation of the habitat preferences and the impact of the abiotic factors on the activity of *Cryptops anomalans* in areas with varying degrees of human influence: urban and suburban parks, natural forest and open habitats in Shumen and the Shumen Plateau, Northeastern Bulgaria.

Material and Methods: The material was collected at 8 sites with Barber traps, that were reported on a monthly basis in the period May 2007-May 2009.

Results: The results of LSD analysis of the numerical properties of the species indicate differences between the urban habitats and the natural habitats of the Shumen Plateau, most significant in comparison with the Bukaka reserve and Open meadow (respectively $p = 0,009$ and $p = 0,014$; $p = 0,014$ and $p = 0,021$). Highest in absolute terms, although without statistical significance, is the coefficient of Pearson-Brave for air humidity (-0.567). To model the dependence of terrestrial activity of *C. anomalans* on the observation period, monthly based, one applies a regression model described by the equation: $Y = b_0 + b_1X + b_2X^2 + b_3X^3$.

Conclusions: *C. anomalans* exhibits typical for the most centipedes seasonal dynamics, significantly influenced by the degree of humidity (Auerbach 1951, Blower 1955, Kaczmarek 1979, Lewis 1981), which was also supported by the present study. The species is active from March to October, with a peak in the activity in spring (March-May) associated with an increase in the rainfall, the temperature and the humidity.

Keywords: *Cryptops anomalans*, correlation, regression model, analysis of variance.

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