

**CADMIUM ACCUMULATION AND ITS EFFECTS ON GROWTH AND GAS EXCHANGE
IN FOUR *POPULUS* CULTIVARS**

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The effects of different concentrations of Cd²⁺ (10, 50 and 100 μM) on the growth of four *Populus* cultivars (*Populus* sp. cv Zhonglin No. 46, *P.* sp. cv Langfang No. 4, *P.* sp. cv Qingyang, *P.* sp. cv Xiaoyeyang) and Cd²⁺ ion uptake were investigated. Cd accumulation in roots, shoot stems, young leaves and mature leaves, and bark and wood of cutting stems was analyzed using ICP-AES. Leaf gas exchange and photosynthetic parameters (net photosynthetic rate, stomatal conductance, transpiration rate) were measured in the first fully expanded leaf from the top shoot at the end of the treatment period. The lower Cd concentration (10 μM) had no inhibitory effect on root growth in the four cultivars. Root growth was significantly inhibited at 100 μM Cd. Among the investigated cultivars, *P.* sp. cv Qingyang showed stronger inhibition of root growth. Cd accumulation increased significantly with increasing Cd concentration and with time in all organs of the *Populus* cultivars. Cadmium concentrated mainly in the roots, and was higher there than in aerial parts. Cd concentrations were significantly higher in bark than in wood. Under 50 μM and 100 μM Cd stress, Cd content in shoot stems was highest in *P.* sp. cv Langfang No. 4, followed by *P.* sp. cv Xiaoyeyang and *P.* sp. cv Zhonglin No. 46. The potential of the cultivars for phytoremediation of Cd is briefly discussed.

Key words: *Populus*; Cd, net photosynthetic rate, stomatal conductance, phytoremediation.

**RETICULATE EVOLUTION OF HIGH-ALPINE *ACONITUM* (RANUNCULACEAE) IN THE
EASTERN SUDETES AND WESTERN CARPATHIANS (CENTRAL EUROPE)**

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The chromosomal and molecular PCR-ISSR+RAPD pattern of high-mountain *Aconitum* sect. *Aconitum* in the Sudetes and Carpathians were analyzed to test whether the taxon has common markers in these two mountain systems. In the Sudetes the taxon forms an autopolyploid chromosome complex ($2n = 32$), and allopolyploid in the neighboring Western Carpathians. The chromosome Giemsa C banding pattern of the allotetraploid Carpathian *A. firmum* was found to be common to both Sudetic autopolyploid *A. plicatum* and diploid *A. variegatum*. The Sudetes are geologically older than the Carpathians, and it is argued that an ancient Sudetic taxon may have contributed to the genome of the Carpathian taxon. The Quaternary glaciations and corresponding range extensions of alpine floras may have facilitated their secondary contact(s). This is supported by a molecular ISSR+RAPD pattern that points to introgression between the Sudetic *A. plicatum* subsp. *sudeticum* and Carpathian *A. firmum* subsp. *maninense*.

Key words: C-banding, heterochromatin, historical biogeography, hybridization, ISSR, phenetics, polyploidy, RAPD.

THE EFFECT OF PHYSICAL MEDIUM STATE ON ANTHOR CULTURE RESPONSE IN POLISH CULTIVATED OAT (*AVENA SATIVA* L.)

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The effect of solid, liquid and double-layer W14 induction media on androgenic response and plant regeneration from 15 F₃ generations of hexaploid oat hybrids was investigated. Embryo-like structures (ELS) were obtained from eight genotypes (average 1.4/100 anthers), of which six showed response on media in all physical states. The frequency of embryo induction (6.6/100 anthers) was highest in genotype CHD1780/05 on solid medium. Plants were regenerated from only two genotypes: CHD1780/05 (2.2 plants/100 anthers) and CHD1989/05 (1.3 plants/100 anthers). A total 35 plants (22 of CHD1780/05, 13 of CHD1989/05) were regenerated only from ELS obtained on solid medium.

Key words: *Avena sativa*, anther culture, medium state, androgenic plants, embryo-like structures.

**OXIDATIVE STRESS ENZYME ACTIVITY IN *LEMNA MINOR* L.
EXPOSED TO CADMIUM AND LEAD**

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Duckweed (*Lemna minor*) is an aquatic plant used in phytotoxicity tests for xenobiotic substances. This study assessed whether ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and superoxide dismutase (SOD) in *L. minor* may be used as bioindicators of stress caused by heavy metals. The plants were cultivated on Knopp's medium spiked with cadmium (0.001–1.0 mM) and lead (0.01–1.0 mM). Cadmium showed higher toxicity than lead in *L. minor*. At lower lead doses (0.01–0.1 mM) growth was even slightly stimulated. Both heavy metals (0.001–0.05 mM Cd, 0.01–0.5 Pb) brought about chlorosis and modified the enzymes of the antioxidative system. GPX showed the highest increase in response to increased cadmium content in the medium and cadmium bioaccumulation. APX activity was affected slightly, but SOD activity was not correlated with cadmium or lead accumulation. Of the antioxidative enzymes analyzed in *L. minor* test plants, only GPX proved useful as a biochemical stress indicator of heavy metal pollution.

Key words: *Lemna minor*, ascorbate peroxidase, cadmium, guaiacol peroxidase, lead, superoxide dismutase.

Abbreviations: APX – ascorbate peroxidase; GPX – guaiacol peroxidase; SOD – superoxide dismutase.

CALLUS INDUCTION AND PLANT REGENERATION IN PROPAGATION OF WHEAT HYBRIDS WITH INTRODUCED A^M (*T. MONOCOCCUM*) OR R (*S. CEREALE*) GENOME

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This study assessed the regeneration potential of interspecific and intergeneric wheat hybrids with the A^m genome of diploid wheat (*T. monococcum*) introduced, in comparison to wheat hybrids with the R genome of rye, in the propagation of F₁ hybrids in vitro. Tetraploid hybrids with the AA^mBD and ABDR genotype formed callus tissue from rachides with significantly higher frequency than from peduncles. The exception was the triploid A^mDR genotype, which formed callus from rachides and peduncles with similar frequency. Callus with the AA^mBD genotype showed the highest regeneration ability (37.1%), and the ABDR genotype the lowest (6.2%). Plant regeneration from peduncle explants was twice more frequent than from rachis explants. Plant regeneration from peduncles and rachides of the A^mDR F₁ hybrid was intermediate (12.3%). The ability of young regenerants to start independent development was the most important factor determining the effectiveness of in vitro propagation. Multiplication of callus and plant regeneration was shown to be a promising method of the maintaining and vegetatively propagating the hybrids AA^mBD and A^mDR expressing strong incompatibility barriers.

Key words: *T. monococcum*, *S. cereale*, *T. aestivum*, immature inflorescence, callus culture, plant regeneration.

**GLANDULAR HAIR ULTRASTRUCTURE AND ESSENTIAL OILS IN
SATUREJA SUBSPICATA VIS. SSP. *SUBSPICATA* AND SSP. *LIBURNICA* ŠILIĆ**

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Satureja subspicata spp. *subspicata* and *S. subspicata* spp. *liburnica*, collected during plant development, were studied comparatively with regard to the ontogenesis of their essential oils, chemical composition and glandular structure. The phytochemistry of essential oils isolated by hydrodistillation was analyzed, and 23 free volatile compounds were identified in all oils. The oils contained the monoterpene phenols carvacrol and thymol in all phenological stages. The major component of both subspecies' oils was α -pinene, especially in the flowering period (52.9%, 42.6%), characterizing these plants as having the α -pinene chemotype.

The glandular structure development of these xerophytic subspecies showed many ultrastructural changes preceding, during and after secretion. Metabolic changes were evident in the disc in cells in the pre-secretion stage, when the plant begins to produce terpenoids. The secretion gland head cells underwent a number of ultrastructural changes, among them the formation of a boundary wall. These changes resulted in an increase of surface tension and the accumulation of free volatile compounds in the subcuticular space of the gland head. In the post-secretion stage, all head cells began lysing and the basal cell was the only compact part of the gland, producing tannins as metabolic reaction to environmental stress.

Key words: Essential oil, glandular hair, *Satureja subspicata* Vis. ssp. *subspicata*, *S. subspicata* Vis. ssp. *liburnica* Šilić, ultrastructure.

INDUCTION OF AUTONOMOUS ENDOSPERM DEVELOPMENT IN OVULES OF UNPOLLINATED PISTILS OF *ARABIDOPSIS THALIANA* VAR. LANDSBERG CULTURED IN VITRO

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The paper reports induction of autonomous endosperm (AE) development in ovules of unpollinated pistils of *Arabidopsis thaliana* var. Landsberg cultured in vitro. The basic nutritive medium was hormone-free Murashige and Skoog (MS) medium with 6% sucrose added. Unpollinated pistils were also cultured on MS medium supplemented with growth regulators: naphthylacetic acid (NAA; 0.1 mg l⁻¹) combined with benzylaminopurine (BAP; 2 mg l⁻¹) and 2,4-dichlorophenoxyacetic acid (2,4-D; 20 mg l⁻¹ or 40 mg l⁻¹). AE development was induced on all the media used, in 7.8% of the cultured ovaries (9 ovules in 9 ovaries). The frequency of AE induction was highest on MS medium with 20 mg l⁻¹ 2,4-D (12.5%), and lowest on MS medium containing NAA and BAP (3.6%). On the hormone-free MS and MS with 40 mg l⁻¹ 2,4-D the AE induction frequency was 6.25% and 8.70%, respectively. The number of AE nuclei ranged from 2 to 9, depending on the length of culture and the medium used. Cellularization and differentiation for the characteristic region of endosperm typical in wild-type *Arabidopsis thaliana* was never observed. AE probably originated from the secondary nucleus, as indicated by nuclear size and structure. Medium containing a high concentration of synthetic auxin (40 mg l⁻¹ 2,4-D) induced initiation of antipodal cell divisions, whereas in planta antipodals degenerated when the female gametophyte of *Arabidopsis* matured. Induction of parthenogenetic development of the egg cell was not observed.

Key words: *Arabidopsis*, in vitro culture, unpollinated ovaries, autonomous endosperm (AE).

QUANTIFICATION OF DAIDZEIN, GENISTEIN AND FATTY ACIDS IN SOYBEANS AND SOY SPROUTS, AND SOME BIOACTIVITY STUDIES

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We used reversed-phase High Performance Liquid Chromatography (HPLC) to analyze the amounts of daidzein and genistein, well-known isoflavonoid aglycones, in methanolic extracts (80%) prepared from soy sprouts and also two types of soybeans (*Soja hispida* L.), one purchased at the local market and the other one cultivated in Turkey. Some commercially sold preparations containing either soy extract or soy isoflavones were also analyzed by HPLC for their daidzein and genistein content. Three oils obtained from the same soybean and soy sprout samples were analyzed for fatty acids by GC-MS. Several in vitro biological activities of the soybean oils were examined, including anticholinesterase, antioxidant, antibacterial, antifungal and antiviral activity. The soy sprouts were much richer in genistein (232.1 µg/g) and daidzein (177.0 µg/g) than the soybean samples. The cultivated soybean sample also showed higher genistein (3.771 µg/g) and daidzein (3.366 µg/g) levels than the soybean sample of market origin (2.971 µg/g and 2.579 µg/g, respectively). The soybean oils were found to be quite rich in essential fatty acids, and the soy sprout oil also contained essential fatty acids in appreciable amounts. The soybean oil of market origin had a notable antiviral effect against *Herpes simplex* as well as antifungal activity against *Candida albicans* at 8 µg/ml.

Key words: *Soja hispida*, soybean oil, soy sprout, daidzein, genistein, fatty acids, bioactivity.

PATTERNS OF PECTIN EPITOPE EXPRESSION DURING SHOOT AND ROOT REGENERATION IN ANDROGENIC CULTURES OF TWO WHEAT CULTIVARS

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Spatiotemporal patterns of expression of pectic epitopes during organogenesis were compared in androgenic cultures of two wheat cultivars: Apollo and Kaspar. The LM8 epitope (against xylogalacturonan) was found in the cell walls of loosely attached peripheral cells of Apollo and Kaspar callus. The LM8 binding signal was much stronger in Kaspar than in Apollo culture, and was localized in conspicuous extracellular material covering almost all surface cells of Kaspar callus. The LM8 epitope was also expressed in extracellular strands and layers that coated the root caps of adventitious roots in the two cultivars studied. The JIM7 epitope (against methyl ester-rich pectins) was not detected in Kaspar culture, but the JIM5 epitope (against methyl ester-poor pectins) was abundant in the cell walls lining the intercellular spaces of callus parenchyma. The LM5 epitope (against galactans) was scattered in the epidermis of shoot buds and in inner parenchymatic cells of Kaspar and Apollo callus; the LM6 epitope (against arabinans) was not found in either of the cultivars. The type and location of particular pectin epitopes are discussed in relation to their possible function in wheat androgenesis.

Key Words: *Triticum aestivum*, wheat, androgenesis, cell wall, extracellular surface matrix network (ECMSN), immunolabelling pectins.

VASCULAR ENDODERMIS IN ROOT NODULES OF *LUPINUS LUTEUS* L. (FABACEAE)*

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The development of endodermis ensheathing the vascular system of root nodules was examined in *Lupinus luteus* L. Within the system, the vascular endodermis was found to attain different developmental stages. Close to the nodule meristems, nodule vascular bundles were surrounded with endodermis with a Casparian band (first developmental stage). In the oldest part of the nodule vascular system, that is, the nodule vascular trace together with its first range ramifications, vascular endodermis in the third developmental stage (not reported previously for the root nodule vascular system) was observed, with a secondary cell wall.

Key words: *Lupinus luteus* L., yellow lupine, Casparian band, endodermis, root nodule.

**EFFECTS OF *pssB* MUTATION ON SURFACE POLYSACCHARIDES AND SYMBIOTIC PHENOTYPE OF
RHIZOBIUM LEGUMINOSARUM BV. *TRIFOLII***

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The *Rhizobium leguminosarum* bv. *trifolii* *pssB* gene encodes a protein with inositol monophosphatase activity (I-1-Pase) which shares sequence similarity with a family of diverse prokaryotic and eukaryotic enzymes. A *pssB::lacZ-Gm^r* mutant (Rt12A) that does not synthesize I-1-Pase exhibits a wide range of pleiotropic phenotypic alterations. These changes include establishing non-nitrogen-fixing symbiosis with clover, doubled production of exopolysaccharide (EPS) with elevated content of the high molecular weight (HMW) form, and EPS viscosity significantly higher than in the wild type strain. Moreover, changes in the LPS I form containing the O antigen were found by SDS-PAGE analysis. The absence of fucose, 2-O-methyl-6-deoxyhexose and 3-N-methyl-3,6-dideoxyhexose, and the appearance of two other 6-deoxyhexoses, confirmed changes in the LPS O polysaccharide of the *pssB* mutant. These alterations indicate that the *pssB* mutation greatly affected not only EPS processing but also biosynthesis of the O antigen of LPS. Light and electron microscopy revealed that clover nodules infected with the *pssB* mutant accumulated significantly increased quantities of starch throughout the nodule and overproduced empty membranous structures, suggesting a defect in bacterial release into plant cells.

Key words: Exopolysaccharide, lipopolysaccharide, inositol monophosphatase, PssB protein, *Rhizobium leguminosarum*.

**THE GIANT EXTRA-FLORAL NECTARIES OF CARNIVOROUS *HELIAMPHORA FOLLICULATA*:
ARCHITECTURE AND ULTRASTRUCTURE**

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Extra-floral nectaries commonly occur in carnivorous plants, forming pitfall traps to attract nectar-feeding insects. Although they are not connected with pollination, extra-floral nectaries promote the reproductive functions of carnivorous plants by increasing the supply of animal-sourced nutrients and thereby increasing the plant's vigor. Our main purpose here was to study the functional ultrastructure of the giant nectaries in *Heliampora*, focusing on nectar production and secretion. We wanted to determine whether specialization of the shape and structure of *Heliampora* nectar spoons has an influence on nectary structure. *Heliampora folliculata*, with its unique nectar storage chamber, may also have specialized giant nectaries differing from other species in the genus. In *Heliampora folliculata* the largest nectaries occur in a nectar storage chamber. Regardless their size, the nectaries have similar ultrastructure. Key features of these cells are ER-sheathed leucoplasts and vacuoles with large osmiophilic phenolic inclusions. The former is characteristic for cells producing monoterpenes; indeed, the giant nectaries produce volatile compounds and may have a function similar to osmophores. Nectary cells are isolated from ordinary parenchyma cells by cutinized walls lacking plasmodesmata (endodermis). Symplastic transport is possible only between nectary cells and special parenchyma cells that have wall thickenings. Between them are many plasmodesmata; thus the nectary is a symplastic and apoplastic field. These specialized parenchyma cells are similar to the flange cells described in parasitic plants. Why has a special spoon with a nectar chamber evolved in *Heliampora folliculata*? One answer given is that it protects nectar against being washed away by frequent rainfalls so that the plant produces less nectar and saves energy. Also, when nectar is not easily accessible the insects have to spend more time near the trap entrance to look for it, and they are more likely to be trapped. Regardless of the shape and structure of *Heliampora* nectar spoons (pitcher appendages), giant nectaries apparently have the same architecture throughout the genus. So far as is known, pollinator-prey conflict does not exist in *Heliampora*; nectaries in this genus are formed only for nectar-feeding prey.

Key words: *Heliampora*, Sarraceniaceae, extra-floral nectaries, nectar, carnivorous plants, ultrastructure, symplastic field, osmophores, leucoplasts, carnivorous syndrome, tepui.

SALT STRESS MITIGATION BY CALCIUM CHLORIDE IN *VIGNA RADIATA* (L.) WILCZEK

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This work assesses the ameliorating effect of calcium chloride on sodium chloride-stressed plants of *Vigna radiata* (L.) Wilczek. Plants were treated with solutions of 100 mM NaCl, 100 mM NaCl with 5 mM CaCl₂, or 5 mM CaCl₂. Groundwater was used for irrigation as the control. Plants were harvested randomly 30 and 50 days after sowing. NaCl and CaCl₂-stressed plants showed reduced growth as indicated by decreased root length, stem length, total leaf area and dry weight. Proline and glycinebetaine content and the activity of the antioxidant enzymes superoxide dismutase, ascorbate peroxidase and catalase were increased under treatment with NaCl alone and CaCl₂ alone. When CaCl₂ was combined with NaCl, CaCl₂ altered the overall plant metabolism to ameliorate the deleterious effects of NaCl stress and increased the vegetative growth of the plants.

Key words: Sodium chloride, calcium chloride, amelioration, growth, antioxidant enzymes.

Abbreviations: APX - ascorbate peroxidase; CAT – catalase; CaCl₂ - calcium chloride; DAS - days after sowing; GB – glycinebetaine; NaCl - sodium chloride; PRO – proline; SOD - superoxide dismutase.