

THE EFFECTS OF EXCESSIVE EXPOSURE TO COPPER IN BEAN PLANTS

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The present study aimed to identify changes in important physiological events related to Cu, malondialdehyde (MDA), nitric oxide (NO), chlorophyll *a* and chlorophyll *b* content in the antioxidative defense system in bean seedlings (*Phaseolus vulgaris* L. cv. Akman) after Cu treatment. The activity of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) were determined. Cu excess was induced in *Phaseolus vulgaris* (cv. Akman) plants by soaking the roots in 100 μ M CuSO₄ solution for 10 days. Cu content increased in roots, and nitric oxide levels increased remarkably in leaf tissue. Changes in enzyme activity and MDA were observed in root tissue. The highest accumulation of NO was observed in leaf tissue. The study included an assessment of the correlation between heavy metal accumulation in roots, leading to different manifestations of stress, and changes in chlorophyll level. Indications of oxidative stress response were detected by monitoring changes in the activity and content of some components of the antioxidative mechanism. Cu treatment increased the activity of superoxide dismutase, peroxidase and catalase in leaf tissue.

Key words: Superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), nitric oxide (NO), malondialdehyde (MDA), copper, chlorophyll, bean (*Phaseolus vulgaris* L.).

**SEED COAT DEVELOPMENT AND ITS EVOLUTIONARY IMPLICATIONS IN DIPLOID AND
AMPHIDIPOID *BRASSICA* SPECIES**

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Variations in seed coat patterns are successfully employed in the establishment of evolutionary relationships. This research addressed the evolutionary implications of the anatomy of the developing seed coat in amphidiploid *Brassica* species. Light microscopy was used to study the development of seed coat structure in six species (15 accessions): three amphidiploids and their three diploid parents. Four types of epidermis layer, six types of subepidermis and nine types of palisade layer could be recognized during the course of the seed coat developmental process. The types of epidermis and subepidermis layers in diploids and amphidiploids changed similarly during seed development. Although there was little difference in the types of palisade layer among the accessions of diploids and amphidiploids at the early stages, many particular types appeared in these species at middle and later developmental stages. Palisade layer development varied in complicated ways in amphidiploids. Some accessions showed palisade layer types intermediate between the two putative parents, while others resembled only one of the two diploid ancestors. The developmental types of epidermis and subepidermis did not show the relationships between amphidiploids and diploids. However, the development of types of palisade layer apparently can serve as an excellent character indicating the seed coat evolution of amphidiploids.

Key words: *Brassica*, diploids and amphidiploids, seed coat anatomy, seed coat development.

THERAPEUTIC EFFECT OF CYTOKININ SEQUENCE APPLICATION ON VIRUS-INFECTED CATTLEYA TISSUE CULTURES

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The study investigates the chemotherapeutic effect of plant growth regulators in eradicating mixed infection with *Cymbidium* mosaic (CyMV) and *Odontoglossum* ringspot viruses (ORSV) from *Cattleya schönbrunnensis* × *C. leopoldii gutata*. The experiment was designed to test a range of concentrations of 6-benzylaminopurine, kinetin and zeatin added to the basal medium on proliferating *Cattleya* mericlones. The results indicate that to eliminate CyMV from tissue cultures the best protocol for adding plant growth regulators was induction with 3.2 mg·l⁻¹ kinetin added to modified MS medium and then further propagation on 0.2 mg l⁻¹ zeatin. This treatment was advantageous in terms of micropropagation. Micropropagation on basal medium supplemented with 5.0 mg l⁻¹ BA after induction with 0.5 mg l⁻¹ zeatin also effectively eliminated CyMV from cultures of the *Cattleya* hybrid. Infection with ORSV virus persisted in all treatments.

Key words: *Cattleya*, cytokinins, mericlone, *Cymbidium* mosaic virus, *Odontoglossum* ringspot virus.

SCREENING OF *PISUM SATIVUM* (L.) GERMPLASM AGAINST *ERYSIPHE PISI* SYD

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Powdery mildew (*Erysiphe pisi* Syd.) significantly reduces the yield and quality of pea all over the world. Screening of a broad range of germplasm revealed three highly resistant genotypes (Fallon, PS99102238 and PS0010128). eleven (Shawnee, Lifter, Franklin, PS610152, PS810240, PS710048, PS610324, PS810191, CGN3273, CGN3272, and PS9910188) showed symptoms after inoculation but the infection was not severe and recovery was rapid. Powdery mildew caused 86% loss to the germplasm, and the severity of the disease was associated with various phases. The pathogen inhibits seed development in the pod. Severe natural infection is expected to eliminate susceptible germplasm, some of which may have valuable, unique characteristics. The screening data were used to explore the relationship between susceptible and resistant genotypes, and between genetic diversity and geographic patterns. Seed protein assays did not sort genotypes by geographic pattern or disease resistance. It is suggested to transfer genes conferring disease resistance and economic yield to one genotype.

Key words: Gel electrophoresis, pea, powdery mildew, seed protein.

CHARACTERIZATION AND GENETIC DIVERSITY CHANGES IN THE SLOVENIAN COMMON BEAN, ČEŠNJEVEC LANDRACE

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The common bean has been cultivated in Slovenia for centuries, resulting in the development of numerous landraces that are still grown today. The objectives of this study were to define the genetic background and to estimate genetic diversity changes in the traditional Češnjevec landrace of the Slovenian common bean over the last 50 years of cultivation. Fourteen microsatellite loci were analyzed for the presence, number and size distribution of alleles in 231 individuals, representing 67 common bean accessions, including 19 new and five old accessions of landrace Češnjevec collected in the 1950s and stored at the Agricultural Institute of Slovenia (AIS). In factorial correspondence analysis and UPGMA cluster analysis, Češnjevec clustered apart from both Mesoamerican and Andean gene pools. It is suggested that occasional outcrossing, adaptation to particular environmental conditions and strong selection for consumer preferences for seed types could have played a significant role in the evolution of the additional variation in the common bean in this region. Three alleles present in old Češnjevec accessions were undetected in new Češnjevec accessions. The results presented here provide a firm basis for important and informed decisions concerning further conservation strategy and breeding program in Slovenia.

Key words: Common bean, Češnjevec gene pool, genetic diversity, microsatellites.

SEEDLING EMERGENCE IN THE ENDANGERED *JUNIPERUS OXYCEDRUS* SUBSP. *MACROCARPA* (SM.) BALL IN SOUTHWEST SPAIN

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Juniperus oxycedrus subsp. *macrocarpa* is an endangered species in southwest Spain, with seed dormancy as found in other species of the same genus. This study employed different experiments to determine a method to improve the seedling emergence in this species. Three types of seedling emergence trials were performed: (a) untreated seeds under greenhouse conditions, (b) untreated seeds under natural conditions, and (c) treated seeds under greenhouse conditions, with different acids (sulphuric, hydrochloric and nitric) for 10 and 30 min, followed or not by cold stratification for 3 months. In all trials, seeds derived from both mature and immature cones were used to verify which one produced higher seedling emergence. Previously, seed viability was verified and a proper substrate for greenhouse sowing was selected. The best percentage of seedling emergence was obtained in the “a” and “b” trials. In “a” trial, seeds derived from immature cones germinated significantly better than mature ones. Chemical scarification of seeds with or without cold stratification yielded less seedling emergence than the other trials.

Key words: Cupressaceae, *Juniperus oxycedrus* subsp. *macrocarpa*, dormancy, seedling emergence, viability.

**SUCCINATE DEHYDROGENASE AND ACID PHOSPHATASE ACTIVITY
IN *PHASEOLUS LUNATUS* TESTA**

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Succinate dehydrogenase (SDH) and acid phosphatase (AcP) activity in *Phaseolus lunatus* seed testa are demonstrated in enzyme tests, and uptake and transport of vital and indicator dyes such as methylene blue (MB), Congo red (CR) and tetrazolium (TZ) in the seed testa are examined by light and transmission electron microscopy. SDH activity was observed in the vascular bundles (endotesta) and in some cells in endo- and mesotesta. AcP activity was located near cell walls in both meso- and endotesta. In the vascular bundles there was very little AcP activity. Vital and indicator dyes were conducted from the exotesta (hilum) to endotesta. Vesicle mobilization was observed in the mesotesta. Strong enzyme activity in the meso- and endotesta and vesicle mobilization in the mesotesta suggest the potential active role of testa strata in imbibition and the initial nutritional stage of germination.

Key words: *Phaseolus lunatus*, Congo red, succinate dehydrogenase, acid phosphatase, methylene blue, testa, tetrazolium.

DEVELOPMENT AND CYTOCHEMISTRY OF THE EMBRYO SUSPENSOR IN *SEDUM*

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The development of the suspensor in *Sedum acre* L. and *S. hispanicum* L. was investigated using cytochemical methods and light microscopy. After the first division of the zygote, two cells of unequal size are formed: the large basal cell (BC) and the smaller apical one. The basal cell grows enormously and produces haustorial branches invading ovular tissues. The mature differentiated suspensor consist of a large basal cell and 3-4 chalazal cells. Proteins, insoluble polysaccharides, nucleic acids and lipids are localized in the suspensor during different phases of embryo growth. Cytochemical tests showed the presence of high amounts of macromolecules in the suspensor cells, especially during the globular and torpedo-shaped stages of embryo development. The present data indicate that in *Sedum* the suspensor is involved mainly in absorption and transport of metabolites from the ovular tissues to the developing embryo proper.

Keywords: *Sedum acre* L., *S. hispanicum* L., suspensor differentiation, basal cell, cytochemistry.

**ULTRASTRUCTURE OF ENDODERMIS AND STELE CELLS
OF DEHYDRATED *POLYPODIUM VULGARE* L. RHIZOMES**

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Polypodium vulgare L. rhizome cells tolerate water deficit stress to different degrees. This study examined the extent of ultrastructural changes in the endodermis and stelar elements in response to mannitol dehydration treatment. Cytological observations showed that the rhizomes possess structural adaptations to withstand drying by maintaining water inside the stele or activating mechanisms that mitigate stress. There are Casparian strips on the walls of the endodermis, and thicker cell walls of cortex parenchyma cells bordered with endodermal cells. Numerous electron-dense vesicles accumulate in dehydrated endodermal cells, making the organelles not visible. In parenchymatous cells of pericycle and vascular parenchyma cells, only nuclei with slightly condensed chromatin, smaller starch grains and vesicle formation were observed in the cytoplasm after dehydration. Changes in cell membrane ultrastructure were not identified. Incubation of the rhizome in abscisic acid prior to dehydration did not produce ultrastructural changes.

Key words: *Polypodium vulgare*, common polypody, dehydration tolerance, TEM analysis, abscisic acid.

LIGULE ANATOMY AND MORPHOLOGY OF FIVE *POA* SPECIES

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The morphological and anatomical features of ligules of some *Poa* species (members of *Poa pratensis* group: *P. pratensis*, *P. angustifolia*, *P. humilis*; species outside the group: *P. compressa*, *P. alpina*) were studied by light and scanning electron microscopy. They are described in detail, emphasizing interspecific differences and habitat-dependent variation of shape, length and prickle hair density. All ligules studied are membranous, without veins and stomata-like structures, and may have only a few mesophyll-like cells. The *P. pratensis* ligule rarely contains short prickle hairs. Short leaflike prickle hairs occur densely on the ligule of *P. angustifolia*. The ligule of *P. humilis* is densely covered by long prickle hairs. The long prickle hairs of *P. compressa* end in elongated, curved apices. There are no prickle hairs on the ligule of *P. alpina*. The density of hairs on the abaxial surface is habitat-dependent for all species studied. Ligule anatomy in the *P. pratensis* group is quite uniform, leading them to be grouped as closely related species, but the fine differences identified are useful for their identification.

Key words: Ligule, *Poa pratensis* group, *Poa compressa*, *Poa alpina*, prickle hair.

DROUGHT STRESS TOLERANCE AND THE ANTIOXIDANT ENZYME SYSTEM IN *CTENANTHE SETOSA*

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We studied the relationship of the antioxidant enzyme system to drought stress tolerance during leaf rolling in the leaf, petiole and root of *Ctenanthe setosa* (Rosc.) Eichler. Chlorophyll and carotenoid content and the chlorophyll stability index decreased in the early period of drought stress but increased in later periods, approaching the control level as leaf rolling increased. Relative water content decreased, while the root:shoot ratio increased during drought stress. Lipid peroxidation also increased and then declined in the same drought period, contrary to photosynthetic pigment content. Superoxide dismutase (SOD) activity did not significantly change in leaves. In the petiole and root, however, it decreased in the early drought period but increased later. Glutathione reductase (GR) activity did not significantly change in the leaf and petiole versus the control, but increased in root. Peroxidase (POD) activity increased in the leaf and petiole but decreased in the root. A peroxidase isoenzyme activity band present in the control leaves did not appear in leaves exposed to 32 days of drought, but in the later periods that activity increased. Tolerance of drought stress apparently is closely associated with the antioxidant enzyme system as well as leaf rolling in *C. setosa*.

Key words: *Ctenanthe setosa*, chlorophyll, lipid peroxidation, superoxide dismutase, glutathione reductase, peroxidase, isoenzyme.

HISTOLOGICAL AND SEM STUDIES ON ORGANOGENESIS IN ENDOSPERM-DERIVED CALLUS OF KIWIFRUIT (*ACTINIDIA DELICIOSA* CV. HAYWARD)

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Regeneration in endosperm-derived callus of *Actinidia deliciosa* cv. Hayward was documented by SEM combined with histology. Two kinds of callus, morphogenic and nonmorphogenic, were observed. Morphogenic callus consisted of compact cell clusters with epidermis-like tissue covered with a mucilaginous or continuous membranous layer, which partially disappeared, turned into fibrils, or became damaged. Regenerating shoots consisted of the apex and primordial leaves. Abnormal structures were also formed, frequently arrested in development. PAS reactions indicated that the mucilaginous layer and network present in intercellular spaces contains polysaccharides. Nonmorphogenic callus consisted of weakly attached cells without a covering membranous layer.

Key words: *Actinidia*, endosperm culture, regeneration, SEM, histological analysis.

**EMBRYO ULTRASTRUCTURE IN *ORIGANUM MAJORANA* L.(LAMIACEAE)
AFTER SEED CONDITIONING**

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Cytological changes in quiescent or germinated embryos after matriconditioning with Micro-Cel E or osmoconditioning with polyethylene glycol (PEG) were studied in comparison to quiescent or germinated untreated embryos of marjoram (*Majorana hortensis* L.). The sequence of changes related to embryo activation was identical in untreated and conditioned samples, although conditioned embryos underwent activation earlier. In those embryos the degradation of protein and lipid bodies, accompanied by vacuolation and accumulation of starch grains in amyloplasts, was observed even in nongerminated samples, whereas in control embryos the same ultrastructural changes did not occur until germination. The changes in ultrastructure occurred first in the root cap and proceeded towards the shoot meristem. In cotyledons, few symptoms of activation were detected regardless of the treatment. The appearance of Golgi structures in the root cap identified the radicle protrusion stage of germination.

Key words: Embryo ultrastructure, marjoram, matriconditioning, osmoconditioning, priming.

**FATTY ACID PATTERNS OF WASTE PARTS OF TURKISH
PISTACIA VERA L. TREE**

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The fatty acid composition of waste products of *Pistacia vera* (pistachio tree) grown in Turkey was assayed. The waste products and various parts of the tree were classified as fresh leaves (FL), dried leaves (DL), stem (ST), branches (BR), fresh skin of natural woody shell (unprocessed) (FSN), fresh kernel (FK), and skin of processed woody shell (SP). Gas chromatography-mass spectrometry data showed FSN, FL, DL and ST to be rich sources of fatty acids. In particular, FL contains a remarkable amount of linolenic acid ($30.4 \pm 3.28\%$).

Key words: *Pistacia vera*, Anacardiaceae, pistachio, fatty acid, GC-MS.

SOMATIC EMBRYOGENESIS FROM BROCCOLI STIGMAS IN TISSUE CULTURE

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The factors regulating callus proliferation and bud regeneration from stigma tissues are not sufficiently understood. To study the regenerative capacity of pistil elements, pistil of broccoli was cultured. Under simple culture conditions, stigmas with style from broccoli pistils undergo somatic embryogenesis on Murashige and Skoog basal medium. Callus initiation occurred on basal medium supplemented with BAP $4 \text{ mg} \cdot \text{l}^{-1}$, 2,4-D $1.6 \text{ mg} \cdot \text{l}^{-1}$, casein hydrolysate $250 \text{ mg} \cdot \text{l}^{-1}$ and sucrose $30 \text{ g} \cdot \text{l}^{-1}$. Proembryo induction was observed after two callus subcultures. Calluses with globular embryos were cultured on basal medium with BAP $2 \text{ mg} \cdot \text{l}^{-1}$, IAA $1 \text{ mg} \cdot \text{l}^{-1}$ and sucrose $40 \text{ g} \cdot \text{l}^{-1}$ for development, maturation and germination of somatic embryos. A population of somatic embryos was maintained on medium containing BAP $1 \text{ mg} \cdot \text{l}^{-1}$ and NAA $2 \text{ mg} \cdot \text{l}^{-1}$ only. Adding NAA to the basal medium containing BAP considerably enhanced root formation. After acclimatization, all plantlets developed well and produced phenotypically normal flowers.

Key words: *Brassica oleracea* L. var. *italica* subvar. Cymosa, plant regeneration, stigma culture, somatic embryogenesis.