

IN VITRO REGENERATION OF THE CROATIAN ENDEMIC SPECIES *IRIS ADRIATICA* TRINAJSTIĆ EX MITIĆ

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Plant regeneration via somatic embryogenesis and organogenesis was achieved in leaf base and ovary culture of the Croatian endemic *Iris adriatica* Trinajstić ex Mitić. Callus induction from leaf base explants occurred in the dark on three media with MS mineral solution containing 4.52 µM dichlorophenoxyacetic acid (2,4-D), 4.83 µM naphthaleneacetic acid (NAA), 0.46 µM kinetin (Kin), 5% sucrose and 200 mg L⁻¹ casein hydrolysate. The media differed only in vitamin and/or proline content. Calli from ovary culture were achieved on MS medium containing 45.25 µM 2,4-D. The mean percentage of callus induction from leaf base explants was 18.9%, with no significant differences between media, and 27.3% from ovary sections. All embryogenic calli were formed on MS media containing 0.45 µM 2,4-D, 4.44 µM benzyladenine (BA) and 0.49 µM indole-3-butyric acid (IBA) under low light intensity (25 µE m⁻²s⁻¹). Transfer of embryogenic calli to hormone-free medium enabled the development of mature somatic embryos on the surface of 6.0% of induced calli produced from leaf base explants and 4.0% of those from ovary sections. Genotype had the main effect on plant regeneration efficiency in *Iris adriatica*.

Key words: *Iris adriatica*, leaf base culture, ovary culture, somatic embryogenesis.

REGENERATION OF PLANTLETS AND TETRAPLOIDY INDUCTION IN *PSEUDOSTELLARIA HETEROPHYLLA*

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This study was aimed at developing an efficient protocol for regeneration of *Pseudostellaria heterophylla* plantlets and induction of polyploidy. Calli of *P. heterophylla* (Miq) from stems, leaves and buds as explants could not differentiate into plantlets. However, young embryo segments used as primary explants produced embryonic calli on MS medium containing 5.0 mg/L 2,4-D and 0.5 mg/L KT. After the embryonic calli with granular protuberances were transferred to MS medium containing 0.5 mg/L BA, they developed shoots and then rooted to form plantlets. Polyploidy was induced when embryonic calli were placed in liquid MS medium containing 0.5% colchicine for 4 days, followed by culturing in solid medium to induce differentiation. Polyploidy was identified by the number of chromosomes and the size of plantlet stomata. The tetraploid plantlets produced larger root tubers than the diploid plantlets.

Key words: Callus, embryonic calli, *Pseudostellaria heterophylla*, regeneration, tetraploidy.

EFFECTS OF SUPPLEMENTAL ULTRAVIOLET-B RADIATION ON GROWTH AND PHYSIOLOGY OF *ACORUS CALAMUS* L. (SWEET FLAG)

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Increased UV-B radiation due to depletion of stratospheric ozone has potentially harmful effects on plant growth and development. The present study uses a field experiment to examine the effect of long-term supplemental UVB radiation at two intensities (+1.8 and +3.6 kJ m⁻² d⁻¹ above ambient) on the growth and physiology of the medicinal plant *Acorus calamus* L. (sweet flag). Plant height and leaf area were inhibited in a dose-dependent manner, with greater inhibition at the higher dose. At the lower dose the net photosynthetic rate increased, with an increase in stomatal conductance and water use efficiency. Stimulation of physiological functions in plants under the lower dose resulted in increased biomass production. At the higher dose, total chlorophyll content showed no marked variation, whereas carotenoids and UV-B-screening pigment flavonoids increased significantly after treatment. Increased flavonoid content under lower exposure correlates well with higher activity of phenylalanine ammonia lyase, a key enzyme of flavonoid biosynthesis. This study clearly showed that the lower dose of supplemental UV-B promoted rhizome growth in *A. calamus*, perhaps due to improved photosynthesis. Plant defense was stronger under the lower dose.

Key words: Ultraviolet-B, *Acorus calamus* L., biomass, growth, pigments, phenylalanine ammonia lyase.

MOLECULAR AND MORPHOLOGICAL EVIDENCE FOR NATURAL HYBRIDIZATION BETWEEN *PRIMULA SECUNDIFLORA* FRANCHET AND *P. POISSONII* FRANCHET (PRIMULACEAE)

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Natural hybridization occurs commonly in plants and blurs their interspecific delimitation. It is unclear whether spontaneous hybridization occurs in natural populations of *Primula* in China. In this study we examined natural hybridization between *P. secundiflora* and *P. poissonii* based on morphological and molecular analyses of individuals with intermediate morphology. Most morphological characters of these individuals were found to be intermediate between the putative parental species, but plant height and corolla tube length were significantly transgressive. Molecular analyses based on nuclear internal transcribed spacer (ITS) additivity and two types of cloned sequences (each corresponding respectively to one of two parental species) clearly suggested that these individuals result from hybridization between *P. secundiflora* and *P. poissonii*. We further used a maternally inherited chloroplast DNA fragment (*rpl16* intron) to trace the maternal composition of the hybrids. Among 26 hybrids, ten (two thrums, eight pins) had the *P. secundiflora* haplotype, while 16 (ten thrums, six pins) possessed the *P. poissonii* haplotype. These results suggest that both parents served as the mother donors of the hybrids. Reciprocal hybridization between the two species seems to be symmetrical rather than unidirectional.

Key words: Hybridization, *Primula secundiflora*, *P. poissonii*, ITS, *rpl16* intron.

EFFECT OF TWO DIFFERENT AMBIENT OZONE CONCENTRATIONS ON ANTIOXIDATIVE ENZYMES IN LEAVES OF TWO TOBACCO CULTIVARS WITH CONTRASTING OZONE SENSITIVITY

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Eight-week-old tobacco (*Nicotiana tabacum* L.) Bel W3 (ozone sensitive) and Bel B (ozone resistant) cultivars were exposed to ozone for two weeks at two sites with differing tropospheric ozone levels in five independent series from May 27 to July 25, 2004. After each exposition, the degree of ozone-caused visible leaf damage and the activity of APX, GuPX, and SOD were examined. Visible leaf damage was observed only in the sensitive cultivar; the resistant one did not exhibit any external symptoms. Three-way ANOVA revealed that the activity of all enzymes varied by exposure site, series and cultivar effects. Significant correlations between GuPX activity in the two cultivars and with the degree of leaf damage to the sensitive cultivar were found. This indicates that GuPX activity in the sensitive as well as in the resistant cultivars track changes in tropospheric ozone levels. The positive correlation between ozone level and APX activity in the resistant cv. Bel B, which did not reveal visible symptoms, indicates that this enzyme may contribute to detoxication of H₂O₂ and alleviation of oxidative damage caused by O₃.

Key words: Tobacco, tropospheric ozone, ascorbate peroxidase, guaiacol peroxidase, superoxide dismutase.

HIGH VARIABILITY OF NUCLEAR DNA CONTENT IN CULTIVARS AND NATURAL POPULATIONS OF *POA PRATENSIS* L. IN RELATION TO MORPHOLOGICAL CHARACTERS

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Kentucky bluegrass is a facultative apomict which is propagated from seeds of variable genetic origins, ploidy levels and nuclear DNA contents. This study analyzes the variability of relative nuclear DNA content among cultivars and natural populations, and examines whether this variability is correlated with morphological traits. Relative nuclear DNA content (an indirect measure of chromosomal variability) was determined in 281 plants from 28 accessions (17 cultivars, 11 populations) using flow cytometry of DAPI-stained nuclei. The same plants were also measured for leaf area and stomatal length. Variation of measured relative DNA content between the studied accessions was very high (5.5-fold). Intra-accession variation was very high in six accessions, even though three of these were cultivars. Relative nuclear DNA content was correlated with stomatal length but not with leaf area. The lack of correlation with leaf area might explain why high intra-accession variability of nuclear DNA content was found in released cultivars that had passed uniformity testing during the registration procedure. We suggest that nuclear DNA content measurement should be made part of the cultivar registration process.

Key words: *Poa pratensis* L., Kentucky bluegrass, flow cytometry, intraspecific variation, relative nuclear DNA content, leaf area, stomatal length, apomixis.

POLLEN MORPHOLOGY OF SOME *CENTAUREA* L., *PSEPHELLUS* CASS. AND *CYANUS* MILLER TAXA

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The pollen morphology of 29 taxa, 24 of which are endemic to Turkey, was investigated in detail by light and scanning electron microscopy. LM examination showed pollen grains to be tricolporate, rarely tetracolporate, isopolar, radially symmetrical, subprolate, spheroidal-subprolate, operculate, tectum perforate, scabrate and microechinate. *P. pecho* Albow. and *P. appendicigera* C. Koch resemble the *Montana* type, *C. pichleri* subsp. *pichleri* Boiss. and *C. pichleri* subsp. *extrarosularis* (Hayek & Siehe) Wagenitz resemble the *Cyanus* type, and *C. pseudoscabiosa* subsp. *pseudoscabiosa* Boiss. & Buhse are consistent with the *Dealbata* type, *C. pestalozzae* Boiss. and *C. carduiformis* subsp. *carduiformis* DC. with the *Scabiosa* type, and the other taxa with Wagenitz's *Jacea* type.

Key words: *Centaurea*, *Cyanus*, *Psephellus*, pollen morphology, LM, SEM.

RAPD ANALYSIS OF GENETIC STRUCTURE IN FOUR NATURAL POPULATIONS OF *TAXUS BACCATA* FROM SOUTHERN POLAND

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This work assessed genetic diversity and genetic structure using random amplified polymorphic DNA (RAPD) variation in 120 individuals of four natural populations of *Taxus baccata* growing in southern Poland (3 in mountains and one in lowland) to obtain basic information on this natural resource. With 9 primers, 185 highly reproducible and clear RAPD bands were obtained. Genetic diversity within populations was relatively high, with percentages of polymorphic bands ranging from 48.65% to 77.30%, averaging 69.59% (Shannon index 0.311). Global AMOVA showed that genetic variation between populations accounted for 26% of total variation, with the remainder (74%) occurring within population. Pairwise ϕ_{PT} values were not correlated with geographic distance. Two groups of populations were distinguished by ANOVA and principal coordinate analysis (PCO) based on a Euclidean metric: those growing in mountains (Nowa Wieś, Cisowa Góra, Zadni Gaj), with higher internal diversity, and those growing in lowlands (Liswarta), with lower internal diversity. The results are typical for an outcrossing, wind-pollinated and long-lived woody species.

Key words: DNA markers, genetic diversity, RAPD, *Taxus baccata* L., yew.

EFFECTS OF Cu^{2+} , CYTOKININS AND JASMONATE ON CONTENT OF TWO FLAVONOLS IDENTIFIED IN ZUCCHINI COTYLEDONS

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This study examined the effects of cytokinins and methyl jasmonate (JAMe) alone and in combination on the growth and flavonol content of zucchini cotyledons cultured in the presence or absence of Cu^{2+} . During greening of cotyledons in intensive diurnal illumination, rutin was identified as the main flavonol compound. Its accumulation was greatly stimulated by phenylurea cytokinin (4PU-30) but reduced by Cu^{2+} . Stimulation by benzylaminopurine (BA) was less. JAMe showed an inhibitory effect, alone and with simultaneous addition of Cu^{2+} or cytokinins. In moderate excess (100 μM and 250 μM CuSO_4), Cu^{2+} enhanced stimulation by 4PU-30 of rutin accumulation; at a higher concentration or with other substances it decreased the rutin level. The other flavonol detected, kaempferol-3-rhamnoside, increased after JAMe or 4PU-30 were added; in the other cases it decreased. The data obtained indicate that cytokinins can increase rutin content in developing *Cucurbita* cotyledons. Rutin content usually decreased under stress induced by excess Cu^{2+} , but Cu^{2+} in moderate excess had a stimulating effect in the presence of higher phenylurea cytokinin levels.

Key words: Copper, *Cucurbita pepo* cotyledons, cytokinins, flavonoids, growth, heavy metals, methyl jasmonate, rutin.

ENDOPOLYPLOIDY PATTERNS DURING DEVELOPMENT OF *CHENOPODIUM QUINOA*

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Patterns of endopolyploidy were studied in embryos and seedlings during early development. Relative nuclear DNA content was measured with DAPI staining and flow cytometry. Somatic tissue of *Chenopodium quinoa* (Chenopodiaceae) revealed extensive endopolyploidization; tissues comprised mixtures of cells with DNA content ranging from 2C to 16C in varying proportions. Endopolyploidy patterns corresponded to the developmental stage and the individual organ. Polysomaty was already present in the radicle of the embryo in the imbibited seed. During seedling development, endopolyploidization took place in many examined organs (roots, hypocotyls, cotyledons) to different extents. The C-value was highest in the differentiated root, where up to 50% of the cell underwent one or two endocycles. Endopolyploidization was not present in nuclei from leaves and the shoot apex.

Key Words: *Chenopodium quinoa*, endopolyploidy, flow cytometry, mean C-level, polysomaty.

LOCALIZATION OF REACTIVE OXYGEN SPECIES DURING SYMBIOSIS OF EARLY CLOVER AND *RHIZOBIUM LEGUMINOSARUM* BV. *TRIFOLII*

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In this work, clover was shown to respond to infection with *Rhizobium leguminosarum* bv. *trifolii* by producing reactive oxygen species. Superoxide radical and hydrogen peroxide were detected in infection threads and nodule primordia. The role of reactive oxygen species in clover-*Rhizobium leguminosarum* bv. *trifolii* symbiosis is discussed.

Key words: Cytochemistry, legume-rhizobium symbiosis, reactive oxygen species, *Rhizobium leguminosarum*, *Trifolium pratense* L.

EFFECTS OF ALUMINUM ON NUCLEOLI IN ROOT TIP CELLS, ROOT GROWTH AND THE ANTIOXIDANT DEFENSE SYSTEM IN *VICIA FABA* L.

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The effects of different concentrations of Al (10 μ M, 50 μ M, 100 μ M) on nucleoli in root tip cells, root growth, antioxidant enzyme activity and malondialdehyde (MDA) content were investigated in hydroponically grown *Vicia faba* L. Aluminum significantly inhibited root growth of *V. faba* treated with 50 μ M and 100 μ M Al. In the nucleolus in root tip cells, some particulates containing argyrophilic proteins were extruded from the nucleus into the cytoplasm, and some were scattered in the nucleus after Al stress. Superoxide dismutase (SOD) activity in leaves and roots exposed to different concentrations of Al was mostly higher than in the control. Seedlings exposed to 100 μ M Al showed significantly higher peroxidase (POD) activity in roots than in the control. POD activity increased much more in roots than in leaves. Catalase (CAT) activity was lower in roots than in leaves. Malondialdehyde (MDA) content in leaves and roots of plants exposed to 50 μ M and 100 μ M Al was significantly higher than in the other groups and the control at 6 to 9 days of treatment. These results suggest that alterations in nucleoli and altered antioxidant enzyme activity and MDA content in *V. faba* can serve as useful biomarkers for detection of Al toxicity. The mechanisms of Al toxicity and tolerance in *V. faba* are briefly discussed.

Key words: *Vicia faba* L., nucleoli, aluminum (Al), antioxidant enzymes, malondialdehyde.

LOW BASE NUMBERS AND DYSPLOIDY IN ANNUAL *HELICHRYSUM* MILL. (ASTERACEAE: GNAPHALIEAE)

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We report one new base number for *Helichrysum*, $x = 5$, found in the South African species *H. indicum* ($2n = 10$), and additional evidence for the recently documented $x = 4$ found in several South African species: *H. litorale* ($2n = 8$), *H. spiralepis* ($2n = 8$) and *H. zwartbergense* ($2n = 8$). Dysploidy is shown as an additional evolutionary trend within the genus, apart from polyploidy which is most extensive and documented. The association of dysploidy processes with the acquisition of an annual life cycle and adaptation to aridity is discussed for these species and other plant groups.

Key words: *Helichrysum* evolution, karyology, karyotype symmetry, polyploidy.

ARE THERE SEED PEDESTALS IN LENTIBULARIACEAE?

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The term "seed pedestal" was introduced recently to describe a structure of placental origin connecting a seed with the placenta. Seed pedestals are widespread in Scrophulariaceae and a few adjacent families, but have not been found in Lentibulariaceae so far. Here their presence is reported for *Utricularia reniformis* from Brazil, and their formation during seed development is described. We observed that the formation of this structure was strictly associated with seed development; seed pedestals were not formed under aborted (unfertilized) ovules.

Key words: Ovule, seed development, placenta, seed pedestal, *Utricularia*, micromorphology, carnivorous plants.

EFFECT OF ETHEPHON AND GIBBERELLIN A₃ ON *AMARANTHUS CAUDATUS* SEED GERMINATION AND α- AND β-AMYLASE ACTIVITY UNDER SALINITY STRESS

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This study assessed the effects of different doses of ethephon and gibberellin A₃ on germination and α- and β-amylase activity in *Amaranthus caudatus* seeds exposed to different levels of salt stress. NaCl at 25 and 50 mM only delayed germination; at 75, 100 and 125 mM it caused 50%, 90% and 99.5% inhibition of *Amaranthus caudatus* seed germination. Both ethephon and GA₃ (0.01, 0.1, 0.3 mM) effectively counteracted inhibition of seed germination under salinity. The stimulatory effect of ethephon appeared earlier, and the seeds were more sensitive to ethephon than to GA₃. Ethephon enabled seed germination in the presence of all NaCl concentrations (75, 100, 125 mM) even after 24 h. GA₃ alleviated inhibition caused by 75 and 100 mM NaCl until 48 h and did not affect reduction of germination caused by NaCl at 125 mM. NaCl (100 mM) reduced α- and β-amylase activity and seed germination after 14 h, and enhanced α-amylase activity after 20 h, although germination was reduced. Ethephon and GA₃ increased α- but not β-amylase activity under salt stress during the first 14 h of incubation.

Key words: *Amaranthus caudatus* seeds, α-, β-amylase activity, ethephon, germination, gibberellin A₃, NaCl.