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CYTOLOGICAL ASSESSMENT OF CARROT PLANTS OBTAINED IN ANTHER CULTURE

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Anthers of Feria F1 and Narbonne F1 carrot cultivars were cultured in vitro to induce androgenic embryos.To confirm the microspore origin of developed embryos, chromosome counts of root tip meristematic cells weremade for each carrot plant obtained in anther culture. Using phase contrast technique and fluorescence microscopy, cytological changes of microspores during culture leading to proembryo formation were documented in the first days after anther placement on the induction medium. More than 90% of the carrot plants obtained in anther cultures had no haploid chromosomes.

Key words: Anther cultures, embryo, microspore, chromosome number, ploidy.

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PLANT REGENERATION FROM *ONOBRYCHIS SUBNITENS* BORNM. HYPOCOTYL EXPLANTS VIA SOMATIC EMBRYOGENESIS AND ORGANOGENESIS

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An efficient procedure is established here for plant regeneration from hypocotyl explants in *Onobrychis subnitens* Bornm., an important forage legume in Iran. Two types of calli, embryogenic and non-embryogenic, were induced from hypocotyl explants on MS medium supplemented with 2,4-D and BAP at different concentrations. To initiate morphogenesis, embryogenic calli were transferred to MS medium with 0.5 mg·l-1 NAA and 1 mg·l-1BAP. Initiated somatic embryos or adventitious buds developed into plantlets following culture on MS medium without any growth regulators or with 2 mg·l-1 NAA, respectively. All the regenerated plants were normal with respect to morphology and growth characters.

Key words: Hypocotyl culture, *Onobrychis subnitens* Bornm., organogenesis, plant regeneration, somatic embryogenesis.

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HABITAT EFFECTS ON LEAF MORPHOLOGICAL PLASTICITY IN *QUERCUS ACUTISSIMA*

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Phenotypic plasticity acts to increase the performance of plants under stress. Leaf morphological plasticity and its causes in different environments are incompletely understood. We measured the leaf morphological parameters of *Quercus acutissima* Carr. seedlings, including leaf size, leaf shape and venation pattern, assessing the effects of different habitat conditions on leaf morphological plasticity. A field study in forest edge and understory was combined with experiments simulating different light and water conditions. Leaf morphology variations occurred over most of the parameters, and the causes were consistent between the field study and lab experiment. Leaf size decreased with low supply of light and water. Leaf length and width were only affected by leaf area. The leaf petiole did not lengthen under shade stress, suggesting a trade-off relationship between functional tissues and support structures. Leaf shape became narrower in drought and broader in the shade, as reflected in changes in three leaf fractions. Higher vein density played a part in enhancement of mechanical support and water supply. Leaves with more teeth show more active photosynthesis, but are disadvantageous in xeric environments because of higher transpiration. Light was the main factor inducing leaf morphological plasticity. The variations caused by drought were due mainly to the allometry. Our results showed that the leaves of *Q. acutissima* seedlings respond to different habitats with phenotypic plasticity of morphology, suggesting that this is an important mechanism for seedlings to adapt to broader ecological amplitudes.

Key words: *Quercus acutissima* Carr., leaf morphological plasticity, allometric analysis, forest edge, understory, drought, shade, leaf shape, venation pattern.

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SEASONAL CAMBIAL ACTIVITY OF SPRUCE (*PICEA ABIES* KARST.) WITH INDENTED RINGS IN THE PANEVEGGIO FOREST (TRENTO, ITALY)

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Wood with indented rings has long been of interest because it was believed to have special acoustic characteristics and was preferred by the most famous lute and violin makers of the past. In recent years its biological, technical and physical features have become the subject of research. The indentations, which are anatomical anomalies, can be explained by abnormal cambial growth but it is still unclear why and how they are produced. The pinning technique has been used to study the duration and intensity of wood formation of spruce with indented rings grown in the Paneveggio Forest, Italy. The present work describes and discusses the kinetics of cambial activity of trees examined in 2002. Comparison of normal wood and indented xylem showed very similar cambial activity dynamics, characterized by contemporaneous onset and cessation, and by similar trends. Growth rate and final width were the same in each part of the ring. The main differences were not in the timing of xylogenetic processes, but in the morphology of the new cells formed.

Key words: Pinning technique, cambium, indentation, intra-annual wood formation, *Picea abies*, Hazel growth.

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DROUGHT AND EXCESS UV-B IRRADIATION DIFFERENTIALLY ALTER THE ANTIOXIDANT SYSTEM IN CUCUMBER LEAVES

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The combined effects of enhanced UV-B radiation and soil drought on antioxidant enzyme activity were investigated in cucumber leaves. One-month-old cucumber plants (*Cucumis sativus* cv. Dar) were exposed to UV-B irradiation and water deficit alone or combined. Physiological measurements were made in seedlings kept under stress conditions for nine days and then two more days with stresses withdrawn. Generally a decrease in relative water content and an increase in dry weight content were recorded. The more significant changes were observed under drought than under UV-B radiation and or combined UV-B and drought. Both stresses stimulated antioxidant enzyme activity. Superoxide dismutase activity increased earlier (day 2) than guaiacol peroxidase and glutathione reductase activity (days 5 and 7). Elevation of enzyme activities was higher under drought than under UV-B. Combined UV-B and drought functioned synergistically: one of the stresses reduced the effects caused by simultaneous application of the other.

Key words: Glutathione reductase, guaiacol peroxidase, soil drought, superoxide dismutase, ultraviolet-B.

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VASCULARIZATION OF ZYGOTIC AND SOMATIC EMBRYOS OF *ARABIDOPSIS THALIANA*

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Somatic embryos were induced from immature zygotic embryos of *Arabidopsis thaliana* cultured on Gamborg basal medium (B5) supplemented with dichlorophenoxyacetic acid (2,4–D) at 5 μM l-1. The somatic embryos were of unicellular origin. The sequence of divisions and the orientation of newly formed walls resembled the Onagrad pattern of early embryogenesis. Histological studies revealed no connection between the somatic embryos and explant tissue. In contrast to zygotic embryos, in late heart-shaped somatic embryos both sieve and tracheary elements were present. The sieve elements that formed in somatic embryos were characterized by larger plates than normal sieve elements observed in seedlings. Typical features of the tracheary elements in somatic embryos were irregular shape and thickening of the secondary walls.

Key words: *Arabidopsis thaliana*, zygotic embryos, somatic embryogenesis, vascularization.

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MICROSTRUCTURE AND CHEMICAL COMPOSITION OF LEAF CUTICULAR WAXES IN TWO *SALIX* SPECIES AND THEIR HYBRID

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Leaf epicuticular wax morphology and chemical composition of total cuticular waxes were studied in two *Salix* species (*Salix alba* and *S. fragilis*) and their hybrid (*S.* ×*rubens*). A smooth wax layer with small, scattered wax structures covered the adaxial leaf surface in all three taxa, and a crustlike wax layer composed of terminally fused wax filaments was present on the abaxial surface. The leaf cuticular waxes, both epicuticular and intracuticular, were obtained by hot extraction in chloroform and then analyzed by gas chromatography and mass spectrometry. The principal components of the waxes were primary alcohols, fatty acids, aldehydes, *n*-alkanes and wax esters. The qualitative composition of the waxes was quite similar but there were quantitative differences between the taxa. The epicuticular crystalline waxes are composed of very-long-chain aldehyde polymers.

Key words: *Salix*, willow, epicuticular and intracuticular waxes, SEM analysis, chemical analysis, wax extraction.

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GROWTH, ANATOMY AND CHLOROPHYLL FLUORESCENCE OF CORIANDER PLANTS (*CORIANDRUM SATIVUM* L.) TREATED WITH PROHEXADIONE-CALCIUM AND DAMINOZIDE

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This study evaluates the effects of the two gibberellin inhibitors (daminozide, prohexadione-calcium) on certain growth and anatomical characteristics of coriander (*Coriandrum sativum* L.). Both growth retardants were found effective in reducing stem elongation; that response varied with the concentration used. Prohexadione-Ca at 200 mg l-1 reduced height by 38%. Coriander fruits were heavier after the application of prohexadione-Ca, but this did not translate to increased fruit yield or fruit essential oil yield. Prohexadione-Ca induced precocious anthesis (3–5 days earlier). Both prohexadione-Ca and daminozide apparently affected leaf and stem anatomy. Generally, retardant-treated plants possessed thicker leaves, wider stems with more collenchyma tissue, and more vessels in the vascular bundles. Chlorophyll fluorescence measurements disclosed a decrease in the photochemical efficiency of PSII in retardant-treated plants as compared to the controls. The chlorophyll fluorescence parameters Fv/Fm and Fv/Fo can provide a tool for early diagnosis of the use of growth retardant even before any signs of growth retardation are visible in the plants.

Key words: *Coriandrum sativum* L., Coriander, prohexadione-Ca, daminozide, growth, anatomy, chlorophyll fluorescence.

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GENETIC VARIATION IN POLISH POPULATIONS OF *CALLITRICHE COPHOCARPA*

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Callitriche cophocarpa Sendtn. is typically a submerged macrophyte, widespread throughout Poland. It is a monoecious plant with unisexual flowers which are produced only in the floating rosettes. We studied the allozyme variation of 9 populations of *C. cophocarpa* from Polish rivers in order to establish its genetic structure, mode of reproduction, clonal structure, and gene flow between populations. Genetic variation of the examined populations was low. Only 7 of the 12 loci studied were polymorphic. One of the 24 detected multilocus genotypes was widespread, found in 8 populations, and the remaining genotypes were rare. The mean percentage of polymorphic loci within populations was 19.44 and the mean number of alleles per locus was 1.231, with mean values of 0.0219 observed and 0.0421 expected heterozygosity. Fixation indices calculated for each population showed a significant deficit of heterozygotes. The study showed high differentiation between populations, with almost 50% of the total variation in *C. cophocarpa* located between populations. Allozyme studies showed a high contribution of clonal reproduction. The populations of *C. cophocarpa* are isolated from each another, and the existing physical barriers decrease the probability of gene flow between populations and cross-fertilization. Gene flow within rivers is more probable.

Key words: *Callitriche*, water starwort, macrophytes, allozyme, genetic structure, inbreeding, clonal growth.

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METHYL JASMONATE INHIBITS ANTHOCYANIN SYNTHESIS IN SEEDLINGS OF COMMON BUCKWHEAT (*FAGOPYRUM ESCULENTUM* MOENCH)

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Exogenously applied jasmonic acid methyl ester (JA-Me) inhibited biosynthesis and accumulation of anthocyanins in hypocotyls of seedlings of etiolated common buckwheat (*Fagopyrum esculentum* Moench) exposed to light. The phenomenon was observed in experiments with various methods of JA-Me treatment, in whole seedlings and in excised hypocotyls. Even very low quantities of JA-Me taken by seeds during imbibition were enough to inhibit anthocyanin synthesis in buckwheat hypocotyls. This means that there are no significant barriers to the transport and action of JA-Me in buckwheat seedlings, as solute and in gaseous form. Although JA-Me inhibited accumulation of anthocyanins in buckwheat hypocotyls, it had no effect on phenylalanine and tyrosine ammonia-lyase activity. Such JA-Me action suggests that it can act not in the first but in later steps of anthocyanin biosynthesis. JA-Me had no effect on the level of anthocyanins in cotyledons or on hypocotyl growth, but clearly inhibited the growth of main roots of buckwheat seedlings.

Key words: Anthocyanins, *Fagopyrum esculentum*, common buckwheat, hypocotyls, cotyledons, methyl jasmonate.

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ANTIOXIDATIVE RESPONSES IN RADISH (*RAPHANUS SATIVUS* L.) PLANTS STRESSED BY COPPER AND LEAD IN NUTRIENT SOLUTION AND SOIL

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Radish (*Raphanus sativus* L.) is commonly grown in urban and suburban areas where the soil may be polluted with heavy metals such as Cu or Pb. In this study, short exposure of radish plantlets to 0.5 mM Cu or Pb in nutrient solution (two days) in growth chamber conditions elicited an antioxidative response, measured in terms of lipid peroxidation, protein and proline accumulation, and peroxidase and catalase activity. Longer exposure to Cu or Pb when radish was grown outdoors for 50 days in pots filled with field soil with different Cu and Pb content also resulted in higher lipid peroxidation and proline accumulation, and altered protein content and enzyme activity. The tested parameters of radish antioxidative responses to heavy metal stress differed depending on plant part (leaf or hypocotyl) and stress intensity (heavy metal content in growth medium, exposure duration). The reported data show that plants grown in soil from sites where this crop could be cultivated do show an oxidative stress response similar but not identical to that seen under laboratory treatment with heavy metals.

Key words: Catalase, copper, hypocotyl, guaiacol peroxidase, heavy metals, lead, leaf, oxidative stress, proline, *Raphanus sativus* L., radish.

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PREY ATTRACTION IN CARNIVOROUS *GENLISEA* (LENTIBULARIACEAE)

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In this study we test three hypotheses. (1) Secretory hairs in the arms and the distal part of the neck of the carnivorous plant *Genlisea* (Lentibulariaceae) have a different principal function than the digestive hairs in the digestive chamber, that is, prey attraction. (2) Only bacteria and other organisms inside the trap and on the external trap surface lure prey. (3) Substances produced by the plant have a minor influence on prey attraction; more important is trap shape and morphology, because protozoa and microfauna may move to the small interspaces (traps or capillaries) by accidental, nonspecific wandering. We studied the structure of secretory hairs (glands) in the arms and the distal and proximal parts of the trap neck using light, fluorescence and electron microscopy. We tested the hypotheses with several experiments using sterile *Genlisea* traps as well as glass tubes acting as a *Genlisea* trap model, and various organisms as prey (*Blepharisma* sp., *Paramecium bursaria*, *Euglena* sp.). Hairs in the arms and the distal part of the *Genlisea* trap neck represent polysaccharide-proteinsecreting hairs. Prey still moved to cleaned traps without chemical attractants. In the proximal part of the neck the secretory hairs have the same ultrastructure as digestive hairs in the digestive chamber of *Genlisea*. Sterile traps do not need commensals for catching prey. The results of the behavioral experiments reported here support the hypothesis that prey can move to the traps or capillaries by accidental, nonspecific wandering to small objects filled with water. Thus, the complex structure of the *Genlisea* trap with long arms may help catch prey simply by providing a large surface with many small openings which mimic the interspaces between soil particles, and the plant does not need special mediators for prey attraction.

Key words: *Genlisea*, Lentibulariaceae, prey attraction, carnivorous syndrome, carnivorous plants, secretory hairs, ultrastructure, mucilage-secreting hairs, *Paramecium*, *Euglena*.

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RESPONSES OF HYBRID POPLAR TO CADMIUM STRESS: PHOTOSYNTHETIC CHARACTERISTICS, CADMIUM AND PROLINE ACCUMULATION, AND ANTIOXIDANT ENZYME ACTIVITY

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The effect of two cadmium concentrations (10-5 M and 10-4 M) on growth, Cd accumulation and within-plant distribution, proline (Pro) content, and the activity of superoxide dismutase (SOD), catalase (CAT), glutathione-Stransferase (GST) and glutathione peroxidase (GSH-Px) was examined in leaves and roots of poplar plants. Symptoms of Cd toxicity were evident in Cd-treated plants: stunted growth (plant height and biomass), decreased root length, and chlorosis of young leaves. Stem and leaf growth was more affected than root growth. The decreased photosynthetic activity of treated plants may have been due to lowered chlorophyll synthesis. The activity of SOD, CAT, GST and GSH-Px was altered, as was Pro accumulation. The increment of pollutant concentration in the nutrient medium was associated with higher Cd accumulation in plants. Cd in roots was ~40 times higher than in leaves and stems of plants exposed to 10-5 M Cd. Although Cd accumulation in roots was high, the potential use of this hybrid poplar for remediation of Cd-contaminated sites seems low. This is because, apart from the described growth disturbances, translocation of Cd from roots to aboveground parts is low.

Key words: Antioxidant enzymes, biomass production, cadmium, chlorophyll, poplar, proline.

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PHYLOGENY AND CLASSIFICATION OF CHINESE *BUPLEURUM* BASED ON NUCLEAR RIBOSOMAL DNA INTERNAL TRANSCRIBED SPACER AND *rps16*

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Separate and combined analyses of nuclear ribosomal DNA internal transcribed spacer (ITS) and cpDNA *rps16* provided phylogenetic hypotheses and molecular data for the taxonomy of Chinese *Bupleurum* species. The phylogenetic results derived from Bayesian and maximum parsimony analyses supported the monophyly of the *Bupleurum* with strong evidence. The origin of Chinese *Bupleurum* is likely to be through the Middle East and the Caucasus from the Mediterranean region with the basic chromosome number (8→7→6), and polyploidization. Our molecular data are not consistent with other earlier Chinese *Bupleurum* classifications and are consistent with the molecular classification proposed by Neves and Watson. The analyses also provide molecular data to elucidate the taxonomic treatments for *Bupleurum falcatum* from China and Europe.

Key words: Chinese *Bupleurum*, phylogeny, taxonomy, ITS, *rps16*.

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RAPD ANALYSIS POINTS TO OLD WORLD *BROMUS* SPECIES AS ANCESTRAL TO NEW WORLD SUBGEN. *FESTUCARIA*

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The genus *Bromus* subgen. *Festucaria* is a widespread Old World and New World taxon having genomes A, B and L, distinguished cytogenetically. Stebbins (1981) suggested that evolution in *Bromus* went from the small genomes A and B to the large genome L. Thus, Old World species with genomes A and B could be ancestral to New World species with genome L. To test this hypothesis we carried out RAPD analysis of a representative group of species from subgen. *Festucaria*. RAPD band patterns enabled resolution of 13 species after excluding the species-specific bands with high/moderate support. The basal position of *Bromus variegatus* M. Bieb. – an Old World species presumably having a B genome – in relation to some New World species with the genome L confirmed Stebbins' hypothesis of its ancestry in relation to the Old World species. The group of high bootstrap support, *B. cappadocicus* Boiss. et Balansa–*B. erectus* Huds.–*B. riparius* Rehm., with genome A. and a distinctly xerothermic ecological profile, was related to a New World species with genome L, *B. auleticus* Trin. ex Nees, pointing to their presumably common evolutionary history.

Key words: Genomes A and B, genome L, evolutionary migration, historical biogeography, phenetics.