

RESEARCH ON CATECHOLASES, LACCASES AND CRESOLASES IN PLANTS. RECENT PROGRESS AND FUTURE NEEDS

TADEUSZ ANISZEWSKI^{1*}, REINHARD LIEBEREI², AND KRZYSZTOF GULEWICZ³

¹Research and Teaching Laboratory of Applied Botany, University of Joensuu,
FIN-80100 Joensuu, Finland,

²Unit of Applied Ecology and Crop Science, Biocenter Klein Flottbek and Botanical Garden,
University of Hamburg, Ohnhorststrasse 18, D-22609 Hamburg, Germany,

³Laboratory of Phytochemistry, Institute of Bioorganic Chemistry, Polish Academy of Sciences,
ul. Noskowskiego 12, PL-60100 Poznan, Poland

*e-mail: Tadeusz.Aniszewski@joensuu.fi

Received February 13, 2008; revision accepted May 30, 2008

Polyphenol oxidases (PPOs) reveal a range of forms and occur in all plants and crops. PPOs are comprised of three enzymes (catecholase, laccase, cresolase) with very different activities and specificities. Cresolase has a dualistic form (cresolase is only in plants and tyrosinase is only in animals and microorganisms). Very often in the literature the generic word "PPO" is used inappropriately as one enzyme. This should be avoided in future studies, as clear systematics and correct nomenclature of PPOs are needed for proper research. PPOs have different substrate specificities and typical inhibitors, and they catalyze hydroxylation and oxidation processes in plants. Pigment formation in cells and cellular systems is affected by active PPOs. Catecholases, laccases and cresolases are encoded by nuclear genes of plants. Various PPO DNA sequences have been found, and PPOs occur in multiple gene families. The protective potential of PPOs in plants and enhanced herbivory resistance is debated, and the final evidence has not yet appeared. The activity of PPOs in germination is recognized, but its mechanism is still not clear. Seed testa coloration in *Arabidopsis thaliana* is effected by laccase and not by catecholase. The TT10 gene encoding laccase in the *Arabidopsis* seed testa has been isolated. *Arabidopsis* genome analysis led to the identification of 16 other putative laccases and their genes, named *AtLAC1* to *AtLAC17* according to their position in the genome. Challenging areas of research for the future are seed testa PPOs and their mobilization in endosperm and micropylar regions, and PPOs as a part of the plant defense system and immunity.

Key words: Plant polyphenol oxidase, catecholase, laccase, cresolase, coloration, plant enzyme.

PHOTOSYNTHESIS AND METABOLITE LEVELS IN DEHYDRATING LEAVES OF *REAUMURIA SOONGORICA*

YUBING LIU^{1*}, TENGGUO ZHANG², AND JIN WANG¹

¹Shapotou Desert Research & Experiment Station,
Cold and Arid Regions Environmental and Engineering Research Institute,
Chinese Academy of Sciences, Lanzhou, 730000, PR China
²Life Science College, Northwest Normal University, Lanzhou 730070, PR China

*e-mail: ybliu13@163.com

Received August 8, 2007; revision accepted January 5, 2008

Reaumuria soongorica (Pall.) Maxim., a perennial semishrub, is able to survive almost complete tissue dehydration when water is withheld from it, and then the stem can rehydrate on rewatering. In this work, a series of experiments were conducted to characterize the drought-induced changes in two-year-old *Reaumuria soongorica*. The plants were subjected to dehydration by withholding water for 15 days. Net photosynthetic rate (P_n), maximal photochemical efficiency of photosystem (F_v/F_m) and the activity of ribulose-1,5-bisphosphate carboxylase (RuBPCO) were significantly decreased under drought stress, but phosphoenolpyruvate carboxylase (PEPCase) activity increased in the leaf extracts. Content of chlorophylls and carotenoids had no marked variation. Zeaxanthin, the xanthophyll cycle pigment, increased during drying. Plants exposed to drought showed accumulation of sucrose, with lower soluble protein content than in the control plants. The SDS-PAGE protein profile showed a 52 kD polypeptide disappearing under progressive drought stress, but no drought-induced protein occurred. All these findings indicate that the metabolic network systems of *Reaumuria soongorica* have a robust regulation capability for management of severe drought stress.

Key words: Gas exchange, chlorophyll fluorescence, ribulose-1,5-bisphosphate carboxylase, phosphoenolpyruvate carboxylase, sucrose, resurrection plant.

HISTOPATHOLOGY OF *DAUCUS CAROTA* L. ROOT CELLS TREATED WITH TOXIC METABOLITES PRODUCED BY *ALTERNARIA RADICINA* AND *A. ALTERNATA*

KRYSTYNA TYLKOWSKA^{1*}, AGNIESZKA BAGNIEWSKA-ZADWORNA²,
JADWIGA GRABARKIEWICZ-SZCZĘSNA³, DOROTA SZOPIŃSKA¹, HANNA DORNA¹,
AND ELŻBIETA ZENKTELER²

¹Department of Seed Science and Technology, Poznan University of Life Sciences,
Baranowo, ul. Szamotulska 28, 62–081 Przeźmierowo, Poland

²Department of General Botany, Adam Mickiewicz University,
ul. Umultowska 89, 61–614 Poznań, Poland

³Department of Chemistry, Poznan University of Life Sciences,
ul. Wojska Polskiego 75, 60–625 Poznań, Poland

*e-mail: kwtylk@poczta.onet.pl

Received September 4, 2007; revision accepted May 25, 2008

Vascular storage parenchyma cells of carrot roots were treated with methanol solutions of radicinin and *epi*-radicinol produced by *Alternaria radicina* and with alternariol and alternariol methyl ether produced by *A. alternata* at concentrations of 25 μ g/ml and 250 μ g/ml, as well as culture filtrates of both fungi. Cell ultrastructure was observed by TEM. No visible changes were noted after treatment with 25 μ g/ml toxin solutions. The most extensive plication of cell membranes, and sometimes also cell walls, and the formation of numerous vesicles in the cytoplasm, was observed in cells treated with the higher concentration of toxins. Plasma membrane withdrawal and vesiculation, microvacuole formation, and accumulation of plastoglobuli in chromoplasts also occurred. No changes in the structure of endoplasmic reticulum and dictyosomes were noted. The responses of cell structures to particular toxins were nonspecific. Treatment with culture filtrates from *A. radicina* resulted in the occurrence of osmiophilic, electron-dense substance in the cytoplasm and plastoglobuli. All alterations induced by filtrates were more extensive than those resulting from toxin solutions, but membrane integrity was not disturbed after any of the treatments.

Key words: Alternariol, alternariol methyl ether, *epi*-radicinol, radicinin, ultrastructure, carrot root.

RAPD ANALYSES OF SOME WILD *TRITICUM* L. AND *AEGILOPS* L. SPECIES AND WHEAT CULTIVARS IN TURKEY

SÜLEYMAN CENKCI, MUSTAFA YILDIZ*, MUHSIN KONUK, AND YASIN EREN

*Department of Biology, Afyon Kocatepe University,
03200 Afyonkarahisar, Turkey*

*e-mail: mustafa_yildizus@yahoo.com

Received October 31, 2007; revision accepted May 30, 2008

Phylogenetic relationships among wild *Triticum* and *Aegilops* species, bread wheat (*Triticum aestivum*) cultivars, and durum wheat (*T. turgidum*) cultivars were investigated using random amplified polymorphic DNA (RAPD) technique. Fourteen RAPD primers generated 328 polymorphic bands in 22 wheat species/cultivars which have the same or different genomes. DNA fragment size ranged from 290 bp to 2570 bp. In the RAPD analysis, wild *Triticum* and *Aegilops* species clustered together and were separated from all other wheat cultivars based on their genome constitution. *T. monococcum* and *T. boeoticum* were closer to *Aegilops* species than to other wheat cultivars. *T. turgidum* cultivars were genetically less diverse than *T. aestivum* cultivars. RAPD markers specific to the D and U genomes were detected. There was a weak correlation between RAPD data and pedigree records of the cultivars sharing common ancestor(s). The results suggest that RAPD analysis can be used to distinguish wild *Triticum* and *Aegilops* species, and wheat cultivars. In addition, RAPD technique can be used to develop genome-specific markers.

Key words: *Triticum*, *Aegilops*, wild wheat species, durum and bread wheat cultivars, RAPD analysis, phylogenetic relationships, genomes.

CHROMOSOME NUMBERS AND KARYOTYPES OF *ALLIUM PRZEWALSKIANUM* POPULATIONS

CHENGQI AO^{1,2*}

¹Key Laboratory of Arid and Grassland Ecology of the Ministry of Education,
Lanzhou University, Lanzhou 730000, China

²School of Life and Environmental Sciences, Wenzhou University,
Zhejiang 325027, China

*e-mail: aocq@wzu.edu.cn, aocq@tom.com

Received November 2, 2007; revision accepted December 30, 2007

In this study, somatic chromosome counts were determined in 302 individuals from 43 *Allium przewalskianum* populations; 90 were diploids ($2n = 16, 16 + 1B, 16 + 2B$) and 212 were tetraploids ($2n = 32$). Of the 43 populations, five were selected for karyotype analysis. Among them, the diploid plants have two karyotypes: $2n = 2x = 14m + 2st$ (2SAT) and $2n = 2x = 8m + 6sm + 2st$ (2SAT). The tetraploid has one: $2n = 4x = 28m + 4st$. Mixoploidy, Robertsonian translations, and B chromosomes were reported for the first time. In combination with previous chromosome data, the present study reveals a uniform basic chromosomal number ($x = 8$) and uniformity of karyotypes (Stebbins's 2A type), indicating that speciation through polyploidization is less likely in *A. przewalskianum*, despite the highly diversified habitats it occupies.

Key words: *Allium przewalskianum*, chromosome number, karyotype, B-chromosome, Robertsonian translocation.

ANATOMICAL AND POLLEN CHARACTERS IN THE GENUS *EPILOBIUM* L. (ONAGRACEAE) FROM NORTHEAST ANATOLIA

SERDAL MAKBUL¹, ZAFER TÜRKMEN², KAMIL COSKUNÇELEBI*³ AND OSMAN BEYAZOGLU³

¹Rize University, Department of Biology, 53100, Rize, Turkey

²Giresun University, Department of Biology, 28100, Giresun, Turkey

³Karadeniz Technical University, Department of Biology, 61080, Trabzon, Turkey

*e-mail: kamil@ktu.edu.tr

Received November 5, 2007; revision accepted February 10, 2008

Anatomical and palynological features of *E. algidum* Bieb., *E. palustre* L., *E. ponticum* Hausskn., *E. confusum* Hausskn., *E. hirsutum* L. and *E. montanum* L. collected from NE Anatolia were examined and evaluated by numerical analysis in order to determine the taxonomic value of the observed internal peculiarities. Features related to pollen shape and ornamentation, idioblast distribution, number of palisade parenchyma rows and the presence and distribution of sclerenchyma fibers were found to be important in separating the examined taxa. Principal component analysis showed that the anatomical characters are more important than the palynological ones in explaining the total variation among the examined taxa.

Key words: *Epilobium*, numerical analysis, anatomical characters, pollen, Turkey.

EFFECTS OF CALCIUM CHLORIDE ON METABOLISM OF SALT-STRESSED *DIOSCOREA ROTUNDATA*

CHERUTH ABDUL JALEEL*, RAGUPATHI GOPI, MUTHIAH GOMATHINAYAGAM
AND RAJARAM PANNEERSELVAM

*Stress Physiology Lab, Department of Botany, Annamalai University,
Annamalainagar 608 002, Tamil Nadu, India*

e-mail: abdul79jaleel@yahoo.co.in

Received November 5, 2007; revision accepted March 15, 2008

In a pot culture experiment, the effect of calcium chloride (CaCl_2) as an ameliorating agent on sodium chloride (NaCl) stress was studied in *Dioscorea rotundata* plants. Plants were raised in pots and exposed to salinity stress (80 mM NaCl) with or without 5 mM CaCl_2 . NaCl-stressed plants showed decreased protein and total sugars, and increased free amino acid and proline content. When NaCl treatment was combined with CaCl_2 , overall plant metabolism was altered, with increased antioxidant enzyme activity, paving the way for partial amelioration of oxidative stress caused by salinity.

Key words: Antioxidant enzymes, calcium chloride, *Dioscorea rotundata*, proline, protein, sodium chloride.

PICLORAM-INDUCED SOMATIC EMBRYOGENESIS IN LEAVES OF STRAWBERRY (*FRAGARIA ANANASSA* L.)

GONA KARIMI KORDESTANI¹ AND OMID KARAMI^{2*}

¹*Department of Microbiology, Kordestan Islamic Azad University, Kordestan, Iran*

²*Department of Biotechnology, Faculty of Agriculture, Bu-Ali Sina University, Iran*

*e-mail: hiva@basu.ac.ir

Received November 12, 2007; revision accepted January 30, 2008

This is the first report of somatic embryogenesis from leaves of two strawberry cultivars (Selva and Comarosa) cultured on MS medium containing picloram. Maximum embryogenesis was induced using 2 mg/l picloram. Globular stage embryos developed into cotyledonary ones after transfer to hormone-free media containing 2%, 4%, 6%, 8% and 10% (w/v) sucrose. Increasing sucrose concentrations in culture media enhanced somatic embryo development. Cotyledonary somatic embryos were converted to plantlets after transfer on MS medium containing GA₃, and maximum conversion was achieved with 1 mg/l and 2 mg/l GA₃. Plantlets were capable of continuous growth under greenhouse conditions.

Key words: Strawberry, somatic embryogenesis, leaves, picloram, conversion.

INFLUENCE OF TEMPERATURE AND ABSCISIC AND GIBBERELIC ACIDS ON POLYAMINE BIOSYNTHESIS IN EUROPEAN BEECH (*FAGUS SYLVATICA* L.) SEEDS DURING DORMANCY BREAKING

KAZIMIERZ KRAWIARZ AND ZOFIA SZCZOTKA*

*Institute of Dendrology, Polish Academy of Sciences
ul. Parkowa 5, 62–035 Kórnik, Poland*

*e-mail: szczotka@man.poznan.pl

Received November 20, 2007; revision accepted March 15, 2008

The effects of the exogenous growth regulators abscisic and gibberellic acids (ABA and GA₃) on the activity of arginine (ADC) and ornithine decarboxylases (ODC) during dormancy breaking were studied in beech seed. During cold-stratification at 3°C, ADC and ODC activity increased rapidly starting from week 7 in embryo axes and week 8 in cotyledons. At 15°C, ADC activity was higher than ODC activity in embryo axes until week 7 and in cotyledons until week 8. Exogenous growth regulators clearly affected ADC and ODC activity. In embryo axes, ADC activity reached its maximum under the influence of GA₃ between weeks 4 and 8. In the control variant (water temp. 3°C), enzymatic activity was moderately high, peaking in week 9 when a high proportion of seeds already showed germinability. In cotyledons the influence of GA₃ on ADC activity was noticeable particularly during the first and last weeks. In the control variant the pattern of changes in the activity of this enzyme was similar but at a much lower level. ABA in both organs clearly inhibited ADC activity, but particularly at the end of the experiment. ODC activity in all variants of the experiment was higher in embryo axes than in cotyledons. The dynamics of change in ODC activity were similar to the changes in ADC activity in embryo axes and in cotyledons.

Key words: European beech seeds, abscisic acid, gibberellic acid, arginine decarboxylase, ornithine decarboxylase, dormancy breaking.

VASCULAR SYSTEM WITHIN DEVELOPING ROOT NODULES OF *LUPINUS LUTEUS* L. PART 1. JUVENILE STAGE**

BARBARA ŁOTOCKA*

*Department of Botany, Warsaw University of Life Sciences,
Nowoursynowska 159, 02-776 Warsaw, Poland*

*e-mail: BŁotocka@gmail.com

Received December 27, 2007; revision accepted February 15, 2008

The ontogeny and (ultra)structure of vascular tissue in *Lupinus luteus* L. root nodules were studied by light and transmission electron microscopy in juvenile nodule primordia up to the 11th day after inoculation. Vascular meristem originated from centripetally dedifferentiated root cortical parenchyma, endodermis and pericycle. The vascular trace was formed between bacteroid tissue initials and the root stele. In the trace's proximal part, cambial strands connecting the vascular trace and root cambium were formed. In the distal part, non-anastomosing vascular bundles started differentiating from the trace at the end of the juvenile stage. In lupine, the formative stage of the indeterminate root nodule vascular system was shown to be unique within the legumes.

Key words: Endodermis, *Lupinus luteus* L., phloem, root nodule, root nodule development, transfer cells, vascular meristem, vascular tissue, xylem.

VASCULAR SYSTEM WITHIN DEVELOPING ROOT NODULES OF *LUPINUS LUTEUS* L. PART 2. DIFFERENTIATED NODULES**

BARBARA ŁOTOCKA*

*Department of Botany, Warsaw University of Life Sciences,
Nowoursynowska 159, 02-776 Warsaw, Poland*

*e-mail: BLotocka@gmail.com

Received December 27, 2007; revision accepted February 20, 2008

The development and (ultra)structure of vascular tissue was studied in *Lupinus luteus* L. mature root nodules 25 and 60 days after inoculation. In the proximal part of the nodule vascular trace, extensive growth took place to match the secondary growth of the root. Development of bacteroid and vascular tissues was correlated. The non-anastomosing nodule vascular bundles elongated and branched due to the activity of nodule lateral meristems, thus forming an extensive vascular network within deep lobes of bacteroid tissue. The vascular trace and vascular bundles differed mainly in the location and ultrastructure of transfer cells and parenchymatous cells, in which the inner membrane of the nuclear envelope formed tubular invaginations within the dense chromatin. Development of the vascular system in the collar root nodule of lupine differed from that of cylindrical indeterminate nodules.

Key words: Cell nucleus, *Lupinus luteus* L., phloem, root nodule, transfer cells, vascular meristem, vascular tissue, xylem.

CDNA LIBRARY PREPARATION FROM A SINGLE WHEAT KERNEL IMRE TAKÁCS^{1*}, DÁVID KÖSZEGI², BEÁTA BARNABÁS¹

¹*Agricultural Research Institute of the Hungarian Academy of Sciences,
H-2462, Martonvásár, POB. 19, Hungary*
²*IPK Gatersleben, Correnstrasse 3, 06466 Gatersleben, Germany*

*e-mail: taki@mail.mgki.hu

Received August 7, 2007; revision accepted January 10, 2008

A simplified cDNA cloning protocol was elaborated, easily scalable to very different sizes of plant tissue. The mRNA fraction was extracted from a single wheat kernel with oligo dT magnetic beads. The solid-phase mRNA was transcribed into single-strand cDNA by reverse transcription. Then DNA tags were incorporated into the ds cDNA fragments prior to PCR amplification. The amplified DNA was ligated to a T overhang cloning vector and some of the resulting clones were sequenced. These sequences were identified by BLASTN search in wheat EST databases.

Key words: Wheat kernel, cDNA library, solid-phase PCR, DNA sequencing, EST.