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COVER PAGE ILLUSTRATIONS

Front Page (above) : *Populus tremuloides* floral developmental Stages 1 through 6.

(below) : ClustalW Alignment of predicted amino acid sequences of *PTM1* and *PTM2*
with AP1 and BpMADS3. (see Cseke *et al.*, pp 187-196, for details)

Back Page : Magnified view of the Flower of *Frerea indica* (see p. 265-268 for details)

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Professor H.S. Srivastava Memorial Young Scientist Best Paper Award

*Nominations are invited from those who are below the age of 35 years and have published their original research paper(s) in *Physiol. Mol. Biol. Plants* Vol 9 (2003) for the above award. The nominations including copy of paper(s) published, a certificate for age, a brief note on the significance of work presented in this paper and a certificate about the actual contribution of the author nominated by the corresponding author/group leader can be submitted to Editor-in-Chief, PMBP before Nov. 2003. The award will be announced at the annual meeting of Professor H.S. Srivastava Foundation for Science and Society, Lucknow (India) in March of each succeeding year. The awardee will be honoured with a citation, a medal and amount of Rupees one thousand in cash.*

Professor H.S. Srivastava Medal for Significant Scientific Contribution in Plant Sciences

Nominations for the scientists above the age of 35 years are invited for this award which will be given to an active researcher once in three years. The research focused on distinct advancement in any area of plant biology and biotechnology for the upliftment of science and society will be considered for this award. The awardee will be honoured with a citation, medal and an amount of Rupees eleven thousand in cash. The CV of the nominated scientist alongwith the proof of research contribution submitted for the consideration and a brief note indicating its impact on science and society can be submitted any time to the Secretary, Professor H.S. Srivastava Foundation for Science and Society, Lucknow, India.

Physiology and Molecular Biology of Plants

Volume 9 Number 2 (June, 2003)

Contents

Opinion

- Regulation of Atmospheric CO₂ and O₂ by Photosynthetic Carbon Metabolism 157-161
Arun Goyal

Review Article

- Paclobutrazol: Its Physiological Effects and Biochemical Functions during Plant Stress 163-173
Sibdas Ghosh, Jon N. Penterman, Ewa Madey, Lisa M. Baird, Jessica Marie Frederick, Demetrius J. Cummings, Faith Hall-Glenn, Anthony De Giacomo, Sherry Volk, Erwin B. Dumbroff
- Regulation of Key Biosynthetic Enzymes of Aspartate Family Amino Acids in Plants 175-186
V.K. Kochhar, S. Kochhar and S.S. Rao

Research Article

- MADS-Box Genes In Dioecious Aspen I : Characterization of *PTM1* and *PTM2* Floral MADS-Box Genes 187-196
Leland J. Cseke, Banalata Sen, Namritha Ravinder, David F. Karnosky and Gopi K. Podila
- A MADS-Box Gene Specifically Expressed in the Reproductive Tissues of Red Pine (*Pinus resinosa*) is a Homologue to Floral Homeotic Genes with C-Function in Angiosperms 197-206
Jun-Jun Liu, A.K.M. Ekramoddoullah and Gopi K. Podila
- Agrobacterium tumefaciens* — Mediated Transformation of *Leucaena leucocephala* - a Multipurpose Tree Legume 207-216
Smita Rastogi and U.N. Dwivedi
- Transient Expression of Foreign Genes in Mature Wheat Embryo Explants following Particle Bombardment 217-223
Sailesh Gopalakrishna, Purnima Singh and Nagendra K. Singh
- Structural and Immunological Relationships Between two new Lectins Isolated From the Legume Seeds of *Vatairea macrocarpa* and *Parkia platycephala* and other Plant Lectins 225-232
L.R. Bomfim, I.M.A. Rocha, H. Debray, V.M. Ceccatto, S.V.F. Madeira, B.S. Cavada and M.V. Ramos
- The Four Protein Fractions in Chickpea Seeds (*Cicer arietinum* L.) 233-237
Ranjeet Singh and N.K. Matta
- Effect of Paclobutrazol on Regularity of Bearing in Mango (*Mangifera indica* L.) 239-248
V.K. Singh and Achal Singh

Short Communication

- Inheritance Analysis of Photosynthetic Characteristics in Bean (*Phaseolus vulgaris* L.) 249-253
R. Bressan-Smith and M.G. Pereira
- Effect of leaf age on transpiration and energy budget in *Ficus glomerata* Roxb. 255-260
Suman Chandra
- In-situ* Foliar Nitrogen Determination in Hybrid Poplar Plantations using a Minolta SPAD-502 Chlorophyll Meter 261-264
Michael J. Young, William E. Berguson, and Neil D. Nelson
- Conservation Status and *In Vitro* Multiplication of *Frerea indica* Dalz., an Endemic and Endangered Plant from Western Ghats of Maharashtra, India 265-268
N.S. Desai, P. Suprasanna and V.A. Bapat
- Isoperoxidases are Lined with Differentiation in Callus Cultures of *Cajanus cajan* L. 269-272
A.K. Verma, Amit Vikram, A.K. Gaur and Laxmi Chand
- Salt Stress Influences Lipid Peroxidation and Antioxidants in the Leaf of an Indica Rice (*Oryza sativa* L.) 273-278
S.K. Panda and M.H. Khan
- Productivity Enhancement by Putrescine in Wheat (*Triticum aestivum* L.) 279-282
Sunita Gupta, M.L. Sharma, N.K. Gupta and Arvind Kumar
- Ecophysiological studies of *Cyperus iria* L. in transplanted rice under mid hill conditions of Himachal Pradesh, India 283-285
K.P. Singh and N.N. Angiras
- Effect of Nitrate Presoaking of Okra (*Abelmoschus esculentus* L.) Seeds on Growth and Nitrate Assimilation of Seedlings 287-290
Bandana Bose and Manish Kumar Pandey
- In vitro* Seed Formation in *Panicum sumatrense* Roth ex roem. et Schult (Little millet) 291-294
K. Vasanth, A. Lakshmi Prabha, M. Jeyakumar and N. Jayabalan

Regulation of Atmospheric CO₂ and O₂ by Photosynthetic Carbon Metabolism

Arun Goyal

Department of Biology, College of Science & Engineering; Department of Biochemistry & Molecular Biology, School of Medicine; and Center of Cell and Molecular Biology, University of Minnesota Duluth, Duluth, MN 55812, USA

Photosynthesis affects the global O₂ level in two main ways. It produces O₂ during CO₂ fixation, yet at the same time the photorespiratory part of photosynthesis limits the O₂ level to a fixed ratio for CO₂ to O₂, as long as the CO₂ concentration is low. When the O₂ level rises, O₂ inhibition or photorespiration shuts down CO₂ fixation and O₂ production. In this manner, the photosynthetic process regulates itself and also limits the atmospheric CO₂ and O₂ concentrations to given levels that can be used by Rubisco in photosynthesis. It is not known how fast the atmospheric O₂ level will increase with current CO₂ increases. Because the O₂ pool is approximately 700 times larger than the CO₂ pool, it would take a long time for the O₂ level to increase significantly. Increasing CO₂ has increased the CO₂ to O₂ ratio to current levels, the rate of net photosynthesis, and has raised the permissible atmospheric O₂ level. With such a complex system balancing the CO₂ to O₂ ratio, experimental testing of the effect of the atmospheric O₂ level on plants is needed. It is also important to re-evaluate the proposals of computer modelers (who are normally not experts in photosynthesis) that the transient man-made increase in atmospheric levels of CO₂ is causing greenhouse effects and global warming.

Paclobutrazol: Its Physiological Effects and Biochemical Functions during Plant Stress

Sibdas Ghosh^{1H}, Jon N. Penterman², Ewa Madey³, Lisa M. Baird⁴, Jessica Marie Frederick¹, Demetrius J. Cummings¹, Faith Hall-Glenn¹, Anthony De Giacomo¹, Sherry Volk¹, Erwin B. Dumbroff^{3,5}

¹*Department of Natural Sciences and Mathematics, Dominican University of California, San Rafael, California 94901, USA;* ²*Department of Plant Microbial Biology, University of California-Berkeley, Berkeley, California 94720, USA;* ³*Department of Biology, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada* ⁴*Department of Biology, University of San Diego, San Diego, California 92110, USA;* ⁵*Kennedy-Leigh Centre for Horticultural Research, Faculty of Agriculture, Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel*

Paclobutrazol (PBZ) induces a myriad of morphological, anatomical, physiological and biochemical changes in plants via significant reduction of hydroxylation reactions required for gibberellin and sterol biosynthesis. Although the efficacy of PBZ can be variable and is highly dependent on treatment conditions, it has been used with marked success in horticulture, forestry and with some agronomic species. Some of the more striking changes induced by PBZ include internode compression resulting in significant reductions in height growth and consequent production of more compact plants that have darker green foliage, higher chlorophyll and carotenoid contents, higher photosynthetic efficiencies, significant changes in the relative levels of endogenous plant growth regulators, delayed leaf senescence, increased resistance to environmental stress, and an enhanced capacity to scavenge and control the production of damaging species of active oxygen. The use of PBZ as a probe during fundamental studies of physiological and biochemical events has also shown promise in efforts to enhance our understanding of sub-cellular events associated with growth and stress-response phenomena in plants.

Regulation of Key Biosynthetic Enzymes of Aspartate Family Amino Acids in Plants

V.K. Kochhar[#], S. Kochhar and S.S. Rao¹

National Botanical Research Institute, Lucknow - 226 001, India

The essential amino acids such as lysine, threonine and methionine derive most of their carbon from L-aspartate and are often referred to as the aspartate family, which also includes leucine. Since these along with five other amino acids are not synthesised by mono gastric animals including man but are required for their proper functioning, they are called essential amino acids. Lysine, threonine and methionine are important as one or more of these amino acids are lacking in both cereals and legume based diets hence attempts are being made to enhance the contents of these amino acids in these plants. An understanding of biosynthetic pathways for these amino acids particularly of their biosynthetic enzymes and their regulation is important for any serious attempt to raise their levels in the crop plants. This article presents a review of the regulation of biosynthetic enzymes of lysine, threonine and methionine including cloning of their genes. The biosynthetic pathways in bacteria have contributed considerably in elucidating these pathways in plants and the bacterial genes are being used for improving the essential amino acids levels of plants, therefore, these pathways have been referred to whenever required.

MADS-Box Genes In Dioecious Aspen I : Characterization of *PTM1* and *PTM2* Floral MADS-Box Genes

Leland J. Cseke¹, Banalata Sen², Namritha Ravinder¹, David F. Karnosky² and Gopi K. Podila^{1#}

¹*Department of Biological Sciences, The University of Alabama in Huntsville, Huntsville, AL 35899, USA*

²*School of Forestry, Michigan Technological University, Houghton, MI 49931, USA*

Populus tremuloides Michx. (trembling aspen) is the most widely distributed dioecious tree species in North America and one of the most ecologically and economically important tree species. However, due to the long juvenile period and overall lack of knowledge surrounding the molecular events of aspen catkin development, selective breeding programs have been problematic. As part of an effort to fill this void, two MADS-box genes, *PTM1* and *PTM2*, were isolated from male and female catkins of adult trees. Based on sequence comparisons and phylogenetic analysis, *PTM1* and *PTM2* share high homology to the *API/SQUA*-class of MADS-box genes from both herbaceous and woody species. Expression analysis showed that, like *API* and *SQUA*, *PTM1* and *PTM2* were both expressed exclusively in floral tissues. Consistent with the floral meristem identity function of *API* and *SQUA*, *PTM1* and *PTM2* were expressed in very early stages of reproductive tissues, and this expression continued throughout floral development. Expression levels of *PTM1* and *PTM2* were different in the two sexes, suggesting that these two genes may have a role in the development and maintenance of the sexes in aspen.

A MADS-Box Gene Specifically Expressed in the Reproductive Tissues of Red Pine (*Pinus resinosa*) is a Homologue to Floral Homeotic Genes with C-Function in Angiosperms

Jun-Jun Liu¹, A.K.M. Ekramoddoullah and Gopi K. Podila^{2H}

¹Pacific Forestry Center, Canadian Forest Service, Natural Resources Canada, 506 West Burnside Road, Victoria, BC, V8Z 1M5, Canada; ²Department of Biological Sciences, University of Alabama, Huntsville, AL 35899, USA

One cDNA clone, *PMADS2*, encoding putative a MADS box protein (transcription factor), was isolated and characterized from immature female cones of gymnosperm red pine (*Pinus resinosa*). *PMADS2* encoded a single polypeptide of 222 amino acids, which shared high similarity ranging from 57% to 99% with members of AG/PLE subfamily from species of angiosperms as well as other gymnosperms. Southern blot analysis showed that *PMADS2* was a single copy gene in the genome of red pine. Northern blot analysis demonstrated that *PMADS2* was expressed only in immature male and female cones, and the transcript was not detectable in vegetative tissues of needles, stems, roots, and shoots. The analyses of sequence similarity and expression pattern suggest that *PMADS2* may be a red pine homologue to members of AG/PLE gene subfamily.

Agrobacterium tumefaciens - Mediated Transformation of *Leucaena leucocephala* - a Multipurpose Tree Legume

Smita Rastogi and U.N. Dwivedi^H

Department of Biochemistry, University of Lucknow, Lucknow - 226 007, India

An *Agrobacterium tumefaciens* mediated transformation protocol for *Leucaena leucocephala*, a multipurpose tropical tree legume was developed. A binary vector, p35SGUSINT, harboring a kanamycin resistance gene encoding neomycin phosphotransferase (*npt II*), as selection marker and the β -glucuronidase (*gus/uidA*) reporter gene interrupted with an intron, was introduced into *A. tumefaciens* strain for plant transformation. Shoot regeneration was achieved on full strength Murashige and Skoog (MS) medium supplemented with 6-benzyl aminopurine (20.9 μ M; 5 mg l⁻¹) and naphthalene acetic acid (5.37 μ M; 1 mg l⁻¹), while rooting was induced in a half strength MS medium containing indole 3-butyric acid (14.76 μ M; 3 mg l⁻¹) and kinetin (0.232 μ M; 0.05 mg l⁻¹). Putative transformants were selected on basal shoot regeneration medium containing 25-50 mg l⁻¹ kanamycin depending on the cultivar. The evidence for expression of the *npt II* and *uidA* genes was established by their continued growth in the presence of otherwise lethal dose of kanamycin and by histochemical and fluorogenic *gus* positive assays of leaf and stem sections of these transformants, respectively. The analysis of transformants by Southern hybridization confirmed the integration of *uidA* gene into plant genome. These transformed plants of *L. leucocephala*, transferred to soil and grown in the green house, were phenotypically normal bearing normal flowers and fruits, though some exhibited precocious flowering and fruiting.

Transient Expression of Foreign Genes in Mature Wheat Embryo Explants following Particle Bombardment

Sailesh Gopalakrishna¹, Purnima Singh^{1#} and Nagendra K. Singh¹

¹Department of Molecular Biology and Genetic Engineering, College of Basic Science and Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar - 263 145, India

So far all the published reports on transgenic wheat have utilized immature embryo explants for the production of transgenics. However, mature embryos being available throughout the year can be the most convenient explant for transformation. For comparison, immature embryos of wheat cv. UP 2338 were co-transformed using 'Biolistic' approach with plasmid vectors pBC-17 and pEmuKON carrying genes for anthocyanin pigmentation and kanamycin resistance, respectively. On an average 83 purple coloured spots were observed per embryo. Transformants surviving selection on kanamycin were recovered at a frequency of 1.89 percent. In contrast, mature embryos of a responsive genotype CPAN 3004 were bombarded separately with vectors pBC-17 and pDM 803 carrying genes for resistance to herbicide 'Basta'. Transient expression assays detected an average of 89 blue spots per embryo with pDM 803 and 211 purple spots per embryo with pBC-17. Eight putative transformants resistant to selection against Basta were recovered with an efficiency of 2.2 percent, which is comparable with the transformation efficiency using immature embryos.

Structural and Immunological Relationships Between two new Lectins Isolated From the Legume Seeds of *Vatairea macrocarpa* and *Parkia platycephala* and other Plant Lectins

L.R. Bomfim¹, I.M.A. Rocha¹, H. Debray², V.M. Ceccatto³, S.V.F. Madeira⁴, B.S. Cavada¹ and M.V. Ramos^{4#}

¹Departamento de Bioquímica e Biologia Molecular, UFC; ²Université des Sciences et Technologies de Lille;

³Universidade Estadual do Ceará; ⁴Departamento de Biologia, Universidade Federal do Ceará, Campus do Pici, Cx. Postal 6033, Fortaleza-Ceará, Brasil - 60.451-970

Structural and immunological relationships between the two legume lectins from seeds of *Vatairea macrocarpa* [VML] and *Parkia platycephala* [PPL] and other lectins were investigated through double immunodiffusion in plates and Enzyme-Linked Immunoabsorbent Assay [ELISA]. In addition, amino acid sequences of both lectins were submitted to hydrophobic and hydrophilic cluster analysis [HCA]. Rabbit antibodies anti-VML fully recognized VML and poorly other lectins, including some ribosome inactivating lectins. Rabbit antibodies anti-PPL interacted similarly with PPL and the closely related lectin from *Parkia discolor*. Furthermore, anti-PPL antibodies weakly recognized many other lectins excepting VML which exhibited considerable identity with PPL. This result was further investigated by sequence alignment and revealed some structural regions within both lectin sequences that could represent common antigenic epitopes. Under a phylogenetic perspective, comparison of the amino acid sequences of PPL and VML with those of many other plant lectins previously studied to construct a phylogenetic tree, places VML among classical legume lectins while PPL is grouped together with the non-legume jacalin and jacalin-related lectins. Thus, PPL may represent a new structural fold within the legume lectin family somehow similar to that observed in jacalin and jacalin-related lectins.

The Four Protein Fractions in Chickpea Seeds (*Cicer arietinum* L.)

Ranjeet Singh and N.K. Matta

Department of Botany, Kurukshetra University, Kurukshetra - 136 119, India

Four seed protein fractions of *Cicer arietinum* L. were analysed for their polypeptide patterns on SDS-gels, relative proportion and amino acid content. The bands of molecular weight 57 kDa, 53 kDa, 42.5 kDa represented the legumin-like polypeptide pairs and those of molecular weight 67 kDa, 64 kDa, 57 kDa, 52.5 kDa, 50 kDa, 39 kDa, 26 kDa, 20 kDa and 16.5 kDa the vicilin-like polypeptides. Albumin polypeptides also exhibited the occurrence of inter-and intra polypeptide disulphide linkages; glutelins and prolamins contained very few polypeptides. Relative proportion of globulins in ten chickpea lines varied between 58.8% and 66.5% and was the highest of four protein fractions; like other legumes, prolamins were the lowest with proportion ranging from 2.3% to 4.5%. Whereas prolamins were the richest in three limiting amino acids-methionine, cysteine and tryptophan, globulins had the lowest content of these.

Effect of Paclobutrazol on Regularity of Bearing in Mango (*Mangifera indica* L.)

V.K. Singh[#] and Achal Singh

Central Institute for Subtropical Horticulture (ICAR), Rehmankhera, Lucknow – 227 107, India

Paclobutrazol (PP₃₃₃) a plant growth retardant was applied in mango cvs. Chausa, Dashehari and Langra as soil drench @ 2, 4, 6 and 8 g a.i. / tree in the month of September during 1997 – 2000. The maximum flowering with more hermaphrodite flowers, fruits set per panicle, fruit number and fruit yield per tree were recorded in 4 g a.i. treated Dashehari tree whereas 6 g a.i. per tree of PP₃₃₃ was found more effective in cvs. Chausa and Langra. Multiple axillary flowering on previous year's fruited stump ("Off" year shoot) and cauliflory were observed in treated trees particularly in those trees which received PP₃₃₃ @ 6 and 8 g a.i. per tree. In untreated trees, such shoot remained vegetative. In treated trees the fruit yield was well distributed in all the directions of the tree i.e. east, west, north and south. However, the higher fruiting was observed in east-south direction. Net photosynthetic rate was increased with the increase in concentration of paclobutrazol (PP₃₃₃) but there was no change at higher concentration of PP₃₃₃. The treatment also improved the fruit quality in terms of total soluble solids (TSS), total acidity, total chlorophyll, total carotenoids, α -amylase and peroxidase activity. The relation of paclobutrazol on the water use in terms of soil moisture content, gas exchange attributes of Dashehari mango was also studied. That indicated that the paclobutrazol has capacity to retain the moisture to some extent and the trees are less sensitive to fluctuation in water supply and may be better able to withstand drought conditions.

Inheritance Analysis of Photosynthetic Characteristics in Bean (*Phaseolus vulgaris* L.)

R. Bressan-Smith^{a#} and M.G. Pereira^b

^aSetor de Fisiologia Vegetal; ^bSetor de Genética Aplicada, Universidade Estadual do Norte Fluminense, RJ, Av. Alberto Lamego, 2000, CEP: 28013-600, Campos dos Goytacazes, RJ, 28015-620, Brasil

Selection of genotypes with high photosynthetic rates may provide the development of bean lines (*Phaseolus vulgaris* L.) with high yield through breeding programs. Segregating populations were obtained from the cross of a common bean line (BAC-6) and a snap bean cultivar (HAB-52). The net photosynthetic rate, stomatal conductance, photosynthetic pigments content and leaf total soluble protein content were evaluated enabling the estimation of means and variances in order to obtain the heritability, as well as additive effects and dominance deviation. In BAC-6, the net photosynthetic rate and stomatal conductance were 17 % and 44 % higher than in HAB-52, respectively. In the F₂ population, a large variation in the amplitude of net photosynthetic rate and stomatal conductance was observed. The same occurred for the remaining characteristics. For most characteristics the broad sense heritability showed to be medium to high (> 0.50). The elevated differences on the physiological parameters between progenitors, associated to the expressive amplitude variation of the segregating population, as indicated by the elevated heritability values, indicate strong influence of genetic factors on photosynthetic traits. Consequently, the differences in photosynthetic efficiency of the genotypes could be explored in breeding programs.

Effect of leaf age on transpiration and energy budget in *Ficus glomerata* Roxb.

Suman Chandra[#]

High Altitude Plant Physiology Research Center, H.N.B. Garhwal University, Srinagar Garhwal - 246174, Uttarakhand, India

This study was conducted to assess energy exchange characteristics and the effect of leaf age on transpiration and energy budget per unit leaf area in the seedlings of *Ficus glomerata*. Study reveals that this species maintains leaf temperature slightly below the temperature of the surrounding air throughout the hot summer months and therefore a negative convective energy flow (i.e. gain of the energy from surrounding air by the convection of heat) was found in the leaves of this species. This indicates that *F. glomerata* would be able to survive in considerably high air temperature and therefore, would reduce the heat load of surrounding air by cooling down the atmosphere around it. The most of the energy absorbed by the leaves was lost by re-radiation, transpiration and conduction across two leaf surfaces which may be considered one of the cause of lower leaf temperature than the temperature of the surrounding air and negative flow of the convection and conduction energy. In general, temperature difference between leaf to air, across leaf surfaces, transpiration rate, energy lost by transpiration and conduction across two leaf surfaces, energy gained by convection and boundary layer thermal conduction and total leaf energy absorption increased, and leaf temperature and energy re-radiation decreased with leaf age. Increased rate of water vapour transfer in old leaves coincides with increasing energy lost by transpiration, which clearly showed that the older leaves or old canopies of this species play much more important role in cooling down the atmosphere around it. Based on the results obtained, this species is identified as an 'under-temperature' species in which transpiration and total leaf energy absorption increased with the leaf age. On the basis of correlation coefficients, it is concluded that leaf age affects more significantly the rate of the transpiration and energy budget characteristics in *F. glomerata* rather than leaf dimensions.

In-situ Foliar Nitrogen Determination in Hybrid Poplar Plantations using a Minolta SPAD-502 Chlorophyll Meter

Michael J. Young^b, William E. Berguson^{a#}, and Neil D. Nelson^a

^aNatural Resources Research Institute, University of Minnesota Duluth, 5013 Miller Trunk Highway, Duluth, Minnesota, 55811-1442 USA.; ^bInternational Paper, P.O. Box 38, Alexandria, Minnesota, 56308, USA

Modern, intensively managed hybrid poplar operations rely on annual assessments of nitrogen (N) availability to maximize net primary productivity. Traditional methods for determining N availability in forest crops include soil NO₃-N and tissue N testing. Although both are useful for predicting sufficiency status, they are expensive, in terms of both time and cost. We evaluated the Minolta SPAD-502 leaf chlorophyll meter for in-situ determination of foliar N concentrations in hybrid poplar plantations. The SPAD meter accurately predicted foliar N for three hybrid poplar clones and can be used for rapid and accurate field assessment of leaf nitrogen status.

Conservation Status and *In Vitro* Multiplication of *Frerea indica* Dalz., an Endemic and Endangered Plant from Western Ghats of Maharashtra, India

N.S. Desai, P. Suprasanna and V.A. Bapat[#]

Plant Cell Culture Technology Section, Nuclear Agriculture & Biotechnology Division,
Bhabha Atomic Research Center, Mumbai – 400 085, India

Frerea indica Dalz. is a herbaceous, perennial monotypic genus of family Asclepiadaceae, found in the restricted localities of Western Ghats of Maharashtra, and is one of the 12 most endangered plant species in the world (IUCN). The species is a comely succulent with star shaped flowers having great scope for domestication as an indoor plant. Thus the species warrants introduction and *in-vitro* propagation. The species has been successfully multiplied *in vitro* for the first time on MS media supplemented with different concentrations of BA (4.4-8.9 μM), NAA (5.4-10.7 μM) and Kn (9.3 mM). The medium supplemented with BA (8.9 μM), NAA (10.7 μM) and Kn (9.3 mM) showed 92% response of shoot multiplication. Further normal rooting was observed on the medium supplemented with IBA (4.7 μM) and rooted plantlets were acclimatized in the green house.

Isoperoxidases are Lined with Differentiation in Callus Cultures of *Cajanus cajan* L.

A.K. Verma, Amit Vikram, A.K. Gaur and Laxmi Chand[¶]

Department of Biochemistry, College of Basic Sciences and Humanities
G.B. Pant University of Agriculture and Technology, Pantnagar – 263145, India

Callus cultures were established from leaf discs of *Cajanus cajan* L. var. Bahar in modified Murashige and Skoog's medium supplemented with 1.0 mg/L BAP and 0.1 mg/L IAA. Calli were grown on the same medium which gave rise to plantlets after second sub-culture. The calli (friable, compact and regenerating) were maintained on the same medium up to seven months. Proteins extracted from these calli were studied for isoperoxidases and isoesterases by native polyacrylamide gel electrophoresis (PAGE). Remarkable differences in banding patterns were observed for isoperoxidases with little differences in isoesterases. It shows that these variations might be due to new synthesis or it may be due to post-transcriptional modifications. These enzyme activities might be playing an essential role in differentiation of the callus tissues.

Salt Stress Influences Lipid Peroxidation and Antioxidants in the Leaf of an Indica Rice (*Oryza sativa* L.)

S.K. Panda[¶] and M.H. Khan

Plant Biochemistry Laboratory, School of Life Sciences, Assam (Central) University, Silchar - 788 011, Assam, India

The influence of salt stress on the pigment, proline, peroxide content, lipid peroxidation (MDA) and on three antioxidative enzyme activities of an indica salt sensitive rice variety's leaf was investigated. A decrease in chlorophyll and carotenoid contents were seen in NaCl treated plants, whereas CaCl₂ treatment showed a promotive role. Proline accumulation was quite significant in case of both the salt treatments. An uniform increase in total peroxide content and MDA level was recorded in salt stressed rice leaves. The activities of catalase (CAT; EC 1.11.1.6); guaiacol peroxidase (GPOX; EC 1.11.1.7) and superoxide dismutase (SOD, EC 1.15.1) showed an increase in rice leaves under salt treatment.

Productivity Enhancement by Putrescine in Wheat (*Triticum aestivum* L.)

Sunita Gupta[#], M.L. Sharma¹, N.K. Gupta and Arvind Kumar

*Department of Plant Physiology, ¹Department of Biochemistry, Rajasthan Agricultural University,
SKN College of Agriculture, Jobner, Jaipur - 303 329 India*

The effect of exogenously applied putrescine was studied in two wheat varieties namely, C-306 (drought tolerant) and HD-2329 (widely cultivated) under non stressed and water stressed conditions. Putrescine ($10^{-5}M$) was applied as seed treatment (ST), spray (SP) and combination of seed treatment and spray (ST+SP). Putrescine increased grain yield, biological yield and seed weight index under all the conditions. Among physiological attributes chlorophyll content and transpiration rate increased and leaf temperature decreased by putrescine. The results suggest that physiological effects of putrescine are similar to cytokinins.

Ecophysiological studies of *Cyperus iria* L. in transplanted rice under mid hill conditions of Himachal Pradesh, India

K.P. Singh[#] and N.N. Angiras

Department of Agronomy, CSK HP Krishi Vishvavidyalaya, Palampur – 176 062, India

A field experiment was carried out during the wet season of 2000 and 2001 to find out the threshold level of yellow nutsedge (*Cyperus iria* L.). The treatment included six densities of *Cyperus iria* L. i.e. 0, 30, 60, 90, 120 and 150 plants/m². HPU-2216 rice variety was used in the study. The weed plant density of 30/m² can be considered the threshold level for transplanted rice, as it causes the minimum loss of 6.57% in grain yield, above which control measures are to be undertaken.

Effect of Nitrate Presoaking of Okra (*Abelmoschus esculentus* L.) Seeds on Growth and Nitrate Assimilation of Seedlings

Bandana Bose[#] and Manish Kumar Pandey

Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University,
Varanasi - 221 005, India

Percent germination, radicle length, change in fresh weight, absolute water content and water uptake of okra (*Abelmoschus esculentus* L.) seeds were higher with soaking of the seeds in different nitrate containing salts like Mg (NO₃)₂, Ca (NO₃)₂ and KNO₃ during germination. Seed dry weight decreased with the increasing time of germination and that was more pronounced in nitrate treatments. The higher level of electrical conductivity (EC) and nitrate content were noticed in nitrate treated seeds. Nitrate reductase activity of the cotyledons of 24 h nitrate soaked seeds was more as compared to distilled water treated seeds. However, among nitrate salts, Mg (NO₃)₂ showed higher response for most of the parameters.

In vitro Seed Formation in *Panicum sumatrense* Roth ex roem. et Schult (Little millet)

K. Vasanth, A. Lakshmi Prabha, M. Jeyakumar and N. Jayabalan[#]

Plant Biotechnology Unit, Department of Plant Science, Bharathidasan University,
Tiruchirappalli - 620 024, India

In vitro inflorescence formation and seed setting have been induced in plants regenerated from shoot tip explant of *Panicum sumatrense* (fam. Poaceae) used as a food crop in South Asia. The multiple shoot (maximum 15 shoots per explant) was induced on MS basal medium supplemented N₆-benzylaminopurine (BAP, 1.33 μM) and Kinetin (KIN, 0.92 μM) in 68% of cultures. The shoots were transferred to MS basal medium for elongation for fifteen days and plantlets were established in culture tubes in which they set seeds on MS supplemented with BAP (8.88 μM), NAA (2.69 μM) and GA₃ (1.156 μM). The cluster of seeds ranged from 5-8 in single branch of spikelet.
