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Cover Page Illustration : Ovules of *Arabidopsis thaliana* WT lar during megasporogenesis and megagametogenesis (See Naumova *et al.*, pp 85-93, for details)

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Rana P. Singh[H] and Pawan K. Jaiwal
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Heterotrophic plastids in non-leaf tissues, especially the fruiting structures, are the main sites for the biosynthesis of plant reserves determining the yield potential of a crop species. However, in contrast to chloroplast, these plastids cannot harvest light efficiently and thus depend on other metabolic pathways such as glycolysis and oxidative pentose phosphate pathway for energy, reducing power and carbon skeletons for synthesizing seed reserves. Studies on the activities of some key enzymes of the photosynthetic carbon reduction (PCR) cycle and C_4 metabolism, rates of $^{14}CO_2$ fixation in light and dark, and initial products of photosynthetic CO_2 fixation conducted with non-leaf tissues of various crops indicated that compared to activities of ribulose 1,5-bis phosphate carboxylase (RuBPcase) and other photosynthetic carbon reduction (PCR) cycle enzymes, the activities of phosphoenol pyruvate (PEP) carboxylase and other enzymes of C_4 metabolism are generally much higher in non-leaf tissues than in the leaf. Short-term assimilation of CO_2 by illuminated fruiting structures produces malate as the major labelled product with less labelling in 3-phosphoglycerate (3-PGA), whereas the leaf shows major incorporation into 3-PGA indicating that the fruiting structures use PEP carboxylase mainly to recapture the respired or photorespired CO_2. However, the fruitwall of *Brassica* seems to be an exception, and assimilates CO_2 via the reactions of the PCR cycle. Based on the information reviewed here, a model depicting carbon assimilation in fruiting structures is proposed.

Mango Malformation : Etiology and Preventive Measures

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Malformation of mango (*Mangifera indica* L.) is causing considerable damage to the mango orchards worldwide. The disease afflicts both young seedlings as well as the flowering trees of mango. The malady has been variously ascribed to be acarological, viral, fungal and physiological in nature. No effective control is known against the disease. The review summarizes the etiology and preventive measures of the disease with special emphasis on the hypothesis which suggests that the disorder may, however, be due to the production of "stress ethylene" by mango plants.

Biosynthesis of Isoprenoids in Higher Plants

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This review focuses on the current understanding in the biosynthesis of isoprenoids in higher plants with special emphasis on the regulation of biosynthesis of isoprenoid C_{15} compounds, sesquiterpenes. It is now evident that in higher plants, two distinct and biochemically different IPP biosynthetic pathways are present; the classical cytoplasmic acetate/mevalonate pathway, and the glyceraldehyde-3-phosphate/pyruvate pathway (Rohmer pathway) limited to the plastidic compartment. The Rohmer pathway is now believed to be responsible for the formation of all plastid-derived isoprenoid compounds in plants, including carotenoids. 3-Hydroxy-3-methyl-glutaryl-CoA reductase (HMGR) appear to be the key regulatory enzyme of IPP biosynthesis in most of the classical cytoplasmic acetate/mevalonate pathway in plants. Sesquiterpenes, which comprise a large and diverse group of phytochemicals originate from the acyclic precursor *trans, trans*-FPP. The conversion of FPP into sesquiterpenes is catalyzed by unique enzymes known as sesquiterpene cyclases or synthases. Sesquiterpene cyclases, and the genes encoding these enzymes, have been characterized from several higher plants. It now seems clear that sesquiterpene cyclases are potential targets of genetic engineering to regulate economically important isoprenoids in higher plants. As a model, biochemical and molecular characterization of the biosynthesis of a sesquiterpene stress metabolite, α-farnesene, in the skin of apples will be discussed.

Biogenic Emission of GHGs from Agricultural Fields

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Methane (CH_4) and Nitrous oxide (N_2O) are the two potential greenhouse gases emanating from the agricultural fields. These gases are radiatively many times more active than the CO_2 and hence contribute immensely to enhanced earth's radiative forcing, despite of their low concentrations in the atmosphere. With the intensification of agriculture, their emission from the agricultural fields has increased many folds in recent years. That is why; India and China in Asia, which have large areas under cultivation, are surmised to be major contributors to global CH_4 and N_2O budgets. However, a few options have been worked out to mitigate CH_4 and N_2O fluxes from the agricultural fields. Such efforts may not necessarily stop the global warming, but certainly help in postponing the danger of climate change.

Engineering Salinity Tolerance in Crop Plants : A Reality

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Soil salinity is one of the most serious factors limiting productivity of agricultural crops. Recent studies on salinity tolerance in crop plants have ranged from identification and molecular characterization of salt stress induced genes/ gene products to the production of salt-tolerant transgenic plants. This review focuses on molecular engineering of salt tolerance in plants in context of our current knowledge of metabolic changes elicited by salt/ drought stress and the progress anticipated in this area by the application of functional genomics. Present engineering strategies rely on the transfer of one or more genes that encode for an important enzyme or a biochemical pathway, participate in signalling pathways or act as transcriptional regulators for coordinate regulation of stress related genes. These gene products protect, either directly or indirectly, against the salinity stress. Various transgenic strategies used so far includes the over-expression of key enzymes/proteins involved in ion/ proton transport, biosynthesis of certain osmoprotectants, scavengers of reactive oxygen species, stress proteins (e.g. late-embryogenesis abundant protein), signalling and control of transcription in model plant species such as *Arabidopsis*, tobacco and rice. The engineered transgenic plants have shown marginal to significantly higher tolerance to salinity stress. To give an example, transgenic salt-tolerant *Arabidopsis*, tomato and *Brassica napus* plants over-expressing *AtNHX1*, a vacuolar Na^+/H^+ antiport, allowed the plants to grow and produce fruits even at 200 mM NaCl, which inhibits the growth of almost all crop plants. Use of such transgenic genotypes, have also been suggested for the reclamation of saline soils. The process of gene discovery is likely to increase several-fold with the application of new, powerful molecular techniques such as high-throughput analysis of expressed sequence tags, cDNA microarrays, genome sequencing, T-DNA or transposon insertional mutagenesis, gain-of-function or mutant complementation, etc. Application of transgenic research to engineer complex salinity tolerance trait involving one or many genes or gene complexes coupled with rapid gene discovery via functional genomics research in plants shall provide effective means for designing salt-tolerant crops.

Partial Purification and Characterization of Calmodulin-dependent NAD^+ Kinase from Potato Leaves

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NAD^+ kinase (ATP: NAD 2'phosphotransferase, EC 2.7.1.23) is a key regulatory enzyme that generates $NADP^+$ from NAD^+, and plays a central role in biosynthetic processes, stress tolerance and antioxidant enzyme function by providing $NADP^+$ which is subsequently reduced through the pentose phosphate pathway. Calmodulin-dependent NAD^+ kinase (CaM-NADK,) was isolated and partially purified from potato leaves (*Solanum tuberosum* L., cultivar ND 860-2). A 4800-fold purification of CaM-NADK was obtained from potato leaves after DEAE-cellulose and calmodulin-affinity chromatography. Due to the instability of the enzyme, further purification with Mono Q-FPLC column chromatography resulted in the loss of its activity. Affinity purified CaM-NADK showed the highest activities at pH 7 and at a temperature of 35^0C. At this pH and reaction temperature of 37^0C, K_m values for NAD^+ and ATP were 37 and 5.9 μM respectively. Partially purified enzyme separated on SDS-PAGE and analyzed after re-naturation showed activity in subunits with molecular masses of 50, 30, and 20 kD. Two isoforms with molecular masses of 247 and 145 kD were identified by size exclusion chromatography.

Cytoembryological Characterization of Apomixis Elements in *Arabidopsis thaliana* Mutants

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Apomixis has been reported in some plant species and is generally associated with polyploidy. There are several components of apomixis, which can be recognized cytologically. Here we report the isolation and characterization of *Arabidopsis thaliana* mutants showing different components of apomixis. The mutants were isolated in the M2 population derived from EMS mutagenic treatment of a conditional male sterile *cer (eceriferum)* line (*cer1*) of *A. thaliana*. At nine days after emasculation, the mutants grown under dry condition (low humidity) showed elongated siliques and enlarged ovules, active embryo sac without degeneration and zygote- like egg cell with dense cytoplasm and a large nucleus. Because of partial ovule sterility, both fertile and degenerated ovules were observed within the same silique, showing normal and abnormal processes of megasporogenesis and megagametogenesis. Cytoembryological analyses revealed that megasporogenesis was affected at different stages. The importance of cytoembryological investigations in the detection of mutants and a better understanding of the regulation of apomixis elements for engineering apomixis are discussed.

Polypeptide patterns and isoenzymes in different populations of *Aconitum heterophyllum* Wall ex Royle, from Garhwal Himalaya

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Polypeptide and isoenzyme patterns, in seeds of *Aconitum heterophyllum* Wall ex Royle, were studied in five populations collected from Garhwal Himalaya. Electrophoretic patterns of seed polypeptides were reproducible and revealed qualitative differences among and within populations of *A. heterophyllum*. Some high and low molecular weight polypeptides were detected as a population specific and these also showed differences in their staining intensity. The zymograms for different enzymes also varied greatly among different populations of *A. heterophyllum*. Sikkim population of *A. heterophyllum* showed one specific band of esterase. All the populations showed similar trend in respect of peroxidase and acid phosphatase isoenzymes.

Isolation and Characterization of NodD$^-$ Mutants of *Mesorhizobium ciceri*

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***Mesorhizobium ciceri*, a symbioint of chickpea (*Cicer arietinum* L.), has very narrow host range and nodulates only the cultivated and wild species of the genus, *Cicer*. Eighteen Nod$^-$ mutants of *M. ciceri* were isolated by random *Tn*5 mutagenesis. Only 11 mutants were complemented for nodulation on chickpea by a cosmid carrying *nod*NMLEFDABCIJ genes *of R. leguminosarum* bv. *viciae* (genes essential for nodulation of pea). Two mutants HN-9 and HN-13 did not show the induction of inducible *nod*ABCIJ promoter in the presence of inducer (naringenin). The transfer of *nod*D gene of *R. leguminosarum* bv. *viciae* in HN-9 and HN-13 restored the induction of inducible *nod*ABCIJ promoter, and confirmed their NodD$^-$ phenotype. The transfer of heterologous *nod*D genes did not extend host range of *M. ciceri* indicating that host specificity of *M. ciceri* may be controlled by the host specific *nod*D gene in the early stages of nodulation or there are certain yet other unknown genes which are independent of *nod*D induction.**

Traits of Nitrogen Use Efficiency for the Selection of *Pennisetum glaucum* in an Environment of Nutrient Limitations

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Yield and nitrogen use efficiency traits were evaluated in the field in 36 families of pearl millet in a nutrient-limited environment. Among the traits, there were significant differences between families for the measured traits: second leaf N content at harvest (LNCH), stover N content at harvest (SNCH) and grain N content (GNC); and for the calculated traits: stover (SNA), grain (GNA) and biomass N acquisition, second leaf N mobilization (NME), N use efficiency for grain production (NUEG) and harvest index for N (HIN). Therefore, SYH and GY are suggested to be the first traits to be used for selection of pearl millet genotypes for stover and grain production under a limited N supply, followed by the LNCH, SNCH and GNC traits, among those selected for yield, improving the stover and grain quality.

Respiratory substrate in *Arabidopsis* plants during carbohydrate starvation

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The possibility of utilization of proteins, under carbohydrate starvation, as alternative respiratory substrates when plants of *Arabidopsis thaliana* WT and mutant TC265 (mutant in starch degradation) were subjected to extended darkness while growing intact and undisturbed, was studied. Prolonged darkness was selected as an approach to starve the plant root of carbohydrate. The results showed that roots lost respiratory capacity after prolonged darkening; their initial carbohydrate and protein contents were only enough to support respiration for 6 - 25 h. Contents of carbohydrate and protein in roots declined. The data suggest that root respiration of *Arabidopsis* was sustained by carbon sources other than carbohydrate plus protein in roots at the start of dark treatment. Possibly, the breakdown of carbohydrate and/or protein in leaves provided respiratory substrates to roots, and this sustained root respiration. The results indicate that the supply of carbohydrate from shoot continues independently of photosynthesis. It is concluded that root respiration was sustained by the utilization of carbohydrate and protein from both root and shoot.

Non-Systemic Induction of Polyphenol oxidase in pea and chickpea after wounding

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Wounding caused by pest and pathogen infection is among the main factors accounting for unpredictable and often severe crop yield losses worldwide. Attempts were made to study the differential expression of polyphenol oxidase (PPO), a presumed anti-herbivore enzyme, in response to wounding in pea and chickpea plants. A dramatic transient increase in PPO activity was observed only in wounded leaves of both the plants following mechanical wounding and insect feeding. No systemic induction of PPO was found in unwounded parts in response to wounding, methyl jasmonate (MeJA) treatment and insect feeding. The non-systemic induction of PPO activity does not support the active defense role of this enzyme against pest and pathogen attack.

In vitro organogenesis and histomorphological investigations in senna (*Cassia angustifolia*) – a medicinally valuable shrub

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A protocol for *in vitro* recurrent shoot production was established for *Cassia angustifolia*, a valuable medicinal plant which is used as laxative and in the treatment of a wide range of human ailments. Among the various seedling derived explants, cotyledonary node showed highest multiplication rate (2.41 shoots per explant) on Murashige and Skoog's (MS) medium supplemented with 1 µM N^6-benzyladenine (BA). Nodal explants of the *in vitro* raised shoots too induced a maximum average number of shoots (2.5) with average shoot length (4.7 cm) on 1 µM BA. Of the cytokinins tried, N^6-benzyladenine was found more effective than kinetin (Kn). The excised shoots were transferred to MS medium augmented with α-naphthalene acetic acid (NAA) for rooting. Nearly 80% shoots organized roots in half strength MS + 10 µM NAA within twenty days. The plantlets have been successfully transferred to soil. Histological examination of *in vitro* raised nodal explants revealed that multiple shoots formed both from pre-existing and *de novo* buds on MS medium containing growth regulators. Morphological characteristics of *in vitro* and *in vivo Cassia* leaf have also been studied. Tissue-culture derived plants had functional stomata on both the surfaces of leaf and scattered wax particles on dorsal surface, whereas non-tissue-cultured plants had normal stomata with dense wax particles on the dorsal surface, the ventral surface showing only abnormal stomata which appeared non-functional.

Comparative Effect of Modes of Gibberellic Acid Application on Photosynthetic Biomass Distribution and Productivity of Rapeseed-Mustard

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An experiment was conducted to compare the effect of two modes of gibberellic acid (GA_3) application (seed-soaking or leaf-applied) on growth, photosynthesis and plant dry mass. Dry mass distribution in leaf, stem and pod and yield characteristics were also studied. GA_3 at a concentration of 0, 10^{-6}, 10^{-5} or 10^{-4} M was applied as seed-soaking for 8 h or applied as foliar spray at 40 d after sowing (pre-flowering). GA_3 spray proved more effective in producing higher photosynthesis rate and dry mass than seed soaking. The plants which received GA_3 as spray showed higher efficiency in translocation of biomass to pods, and thus increasing more pods dry mass, harvest index and seed yield than seed-soaking.

Effect of Sulphur Dioxide on Maize (*Zea mays* L.) var. (Co-1) Seedlings at Lethal Dose 50

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The effect of SO_2 on seedling growth, total chlorophyll, chlorophyll - a and b, carotenoids, stomatal characters, sugar content, starch content, amylase activity and proline content was studied in maize plant (*Zea mays* L. var. Co1) at LD 50 (Lethal dose 50). SO_2 could not affect the seedling growth significantly though it reduced the shoot length. Total chlorophyll, chlorophyll - a and b contents significantly decreased under SO_2 but only minor variations in carotenoids were observed. Stomatal frequency and stomatal index were not affected under SO_2 treatments. However, the size of the stomata was significantly reduced. Starch and sugar contents also decreased over control in the stressed plants. Amylase activity and proline contents were increased due to SO_2 stress in maize seedlings.

High frequency of multiple shoot regeneration in *Mentha piperita* L. – A multipurpose herb

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A rapid and efficient protocol for induction of multiple shoots from leaf and stem explants of *Mentha piperita* L. was developed. Leaf and stem explants were inoculated on MS medium containing different concentrations of 6-benzylaminopurine (BAP) or Kinetin (Kn) either alone or in combination with naphthalene acetic acid (NAA) or indole acetic acid (IAA) growth hormones. MS medium supplemented with BAP (2 mg/ L) induced maximum number of shoots (28.7±0.8). The shoots were rooted on half strength of MS medium supplemented with indole butyric acid (IBA) and rooted plantlets were established in soil as phenotypically normal mature plants.