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**Cover Page Illustration :** Schematic structure of the chimeric NADH:NR formed by joining the N-terminal region of AtNR2 to the C-terminal region of ZmNR1 (See Campbell, pp 31-38, for details)

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# Physiology and Molecular Biology of Plants

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# Phytoremediation of Trichloroethylene using Hybrid Poplar

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The use of plants for the cleanup of contaminated sites is a relatively new idea, replacing the conventional remediation methods that are comparatively expensive and may be aesthetically displeasing. When considering phytoremediation as a method for cleaning up certain contaminants, it is important to choose the right plants for the right contaminants. Poplars, being phreatophytes, have been chosen by many researchers for the phytoremediation of contaminants, in particular organic compounds that are in deep underground water and in the soil. Trichloroethylene (TCE), a potential carcinogen commonly found in ground water, presents a potential health concern. Current research and advances in the phytoremediation of TCE using hybrid poplars are discussed.

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## Nitric Oxide and Nitrate Reductase in Higher Plants

Jose R. Magalhaes<sup>#1</sup>, Filomena, L. I. M. Silva<sup>1</sup>, Ione Salgado<sup>2</sup>, Osvaldo Ferrarese-Filho<sup>3</sup>, Peter Rockel<sup>4</sup> and Werner M. Kaiser<sup>5</sup>

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Nitric oxide (NO) gains increasing attention as a general signaling compound in plant development and plant pathogen interactions. Possible sources for plant NO production are briefly considered, and various methods for detecting NO production and emission from plants and plant tissues are summarized. We then focus on the role of assimilatory nitrate reductase (NR) in higher plants as one important source of NO. We show the capacity of NR for NO production and describe the regulatory properties of NR in context with observed complex patterns of NO production by plants in response to environmental factors and stress conditions. Using a nitrate reductase defective double mutant *NIA1 NIA2*, it was demonstrated that the emission of NO in *Arabidopsis* originated via nitrate reductase.

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# Metabolism of Nitrogen Dioxide in Plants — Assimilation, Dissimilation and Novel Nitrogen Metabolites

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In this review we will focus on the following aspects of the metabolism of nitrogen dioxide in plants.

- (1) The diversity of the capability to assimilate nitrogen dioxide among 217 taxa of naturally occurring plants. More than a 600-fold variation was observed between the highest (*Eucalyptus viminalis*) and the lowest (*Tillandsia ionantha* and *T. caput-medusae*) species.
  - (2) Assimilation of nitrogen dioxide in transgenic *Arabidopsis* plants, in which nitrate reductase, nitrite reductase and glutamine synthetase genes are engineered. The flux control coefficient, which is a measure of the effect of change in a single enzyme activity on the flux, of NiR for NO<sub>2</sub> assimilation was estimated to be about 0.4. The flux control coefficients of NR and GS were close to zero. Thus, NiR appear to be a controlling enzyme in NO<sub>2</sub>-assimilation by plants.
  - (3) Dissimilatory reduction of nitrate and nitrogen dioxide by plants is described. A transgenic tobacco plant whose NiR activity was reduced to less than 5% by the expression of NiR cDNA in an antisense orientation was found to convert nitrate to nitrous oxide. About 0.01% of nitrate nitrogen was converted to nitrous oxide. The emission of nitrous oxide was stimulated when nitrite was added. In addition, the wild-type tobacco as well as the transgenic tobacco were found to convert nitrogen dioxide to nitrous oxide.
  - (4) About 30% of total nitrogen derived from nitrogen dioxide taken up into *Arabidopsis* leaves was found to be recovered in neither Kjeldahl nitrogen nor inorganic nitrogen fraction. This nitrogen was designated as unidentified nitrogen (UN), and the formation of UN of similar amounts was observed in all other plants examined. UN-bearing compounds appeared to be novel nitrogen metabolites formed from nitrogen dioxide in plants.
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# Higher Plant Nitrate Reductase Biochemistry

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The biochemistry of plant nitrate reductase (NR) has been studied now for more than 50 years. These studies have resulted in a detailed understanding of most the structure and function of the enzyme. Most recently, the kinetic mechanism of NR was extended to include pre-steady-state kinetic analysis, which revealed that the long held ideas on the rate-limiting events in NR catalysis are only partially correct. Electron transfer within the enzyme has always been considered rate-limiting, but this is not completely correct. It is now clear that the enzyme is limited by a combination of steps including electron transfer and nitrate reduction at the enzyme's nitrate-reducing active site. In addition, the nitrate binding site has been shown to involve two arginine residues which are located near the invariant cysteine, which is bound to molybdenum in the enzyme's active site. These new results bring greater understanding to the biochemistry of NR. The prospects for employing NR for environmental clean up of nitrate pollution is discussed in light of new advances in NR biochemistry.

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## *In vitro* shoot organogenesis and plant regeneration from cotyledonary node and leaf explants of pigeonpea (*Cajanus cajan* L. Millsp).

N. Dolendro Singh, L. Sahoo, Sonia and Pawan K. Jaiwal<sup>H</sup>

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Simple, efficient and rapid plant regeneration from cotyledonary nodes (without cotyledons) and primary leaf segments has been achieved in pigeonpea. Cotyledonary node explants excised from 16 h water soaked seeds produced a maximum of 5-6 shoots per explant in 85 % of the cultures on MS medium containing 10  $\mu$ M BAP. Among various cytokinins, BAP was the most effective. Leaf segments developed 9-10 shoots per segment in 62.5 % of the cultures on the MS medium supplemented with BAP (10  $\mu$ M) and NAA (1.0  $\mu$ M). Substitution of BAP with other cytokinins and NAA with IAA did not improve shoot regeneration. The shoots were rooted on MS medium supplemented with IBA (2.5  $\mu$ M) and the plantlets were successfully established in soil. Histological studies revealed that the shoots developed adventitiously from the peripheral layers of the meristem developed at explants. The explants were found compatible with *Agrobacterium tumefaciens*-mediated genetic transformation.

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# Role and Regulation of Glutamate Synthases in Higher Plants

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Nitrogen in higher plants is assimilated to amino acids primarily through the synthesis of glutamine and glutamate. The incorporation of ammonium nitrogen into glutamine and then to glutamate involves the sequential action of enzymes glutamine synthetase (GS, EC,6.3.1.2) and glutamate synthases (GOGAT, E.C. 1.4.1.13 and E.C.1.4.7.1), the latter often considered to be the rate limiting enzyme. The operation of this so called GS-GOGAT pathway in ammonium assimilation has been demonstrated by employing various biochemical techniques such as tracing the path of <sup>15</sup>N (labeled NH<sub>4</sub>Cl), by using specific inhibitors of GS and GOGAT, and by examining ammonium assimilation in mutants lacking GOGAT. Two molecular forms of GOGAT, namely Fd specific: and NADH: specific have been demonstrated to be active in almost all tissues of the plants. Both are monomeric proteins and contain FMN and 3Fe-4S cluster as prosthetic groups. The genes for both the species of GOGAT have been cloned and characterised. They are usually upregulated by light and by nitrogen supply and are also regulated by many other environmental and plant factors, for example, by seasonal variations, growth regulators, salinity, water stress, pollutants, plant age etc. Genetic manipulation of the expression of enzyme for qualitative improvement of crops through altered nitrogen assimilation efficiency in the near future, may be an important aspect of the enzymology of GOGAT.

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# **Ureide Metabolism in Nitrogen Fixing Tropical Legumes - A Review**

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Ureides, allantoin and allantoic acid, are key organic molecules for transporting and storing reduced nitrogen in legumes of tropical origin. These compounds in nodules are synthesized from currently delivered photosynthates and products of recent nitrogen fixation via purine synthesis followed by their oxidation and hydrolysis; the pathway of purine synthesis being similar to that operative in several animals and micro-organisms. Metabolic reactions leading to the formation of ureides are compartmentalized both at cellular and sub-cellular levels in nodules. A novel pathway of ureide catabolism that releases 4 NH<sub>4</sub><sup>+</sup>, 2 CO<sub>2</sub> and glyoxylate independent of urease action, has been discussed. Possible significance and consequences of ureide based nitrogen metabolism in tropical legumes have also been discussed.

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## **Recycled Agrowaste and Modified Industrial Byproduct with Halophiles for Improved Yield of Wheat (*Triticum aestivum* L.) in Saline Soil**

**D.P. Patil, M.V. Kulkarni, V.L. Maheshwari<sup>#</sup> and R.M. Kothari**

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Agricultural productivity is severely affected by soil salinity. Use of halophilic isolate *Halococcus* sp. strain MS 2, isolated from marine habitat along with recycled agrowaste (soil conditioner, SC) and modified industrial byproduct (plant growth regulator, PGR) appear promising to restore the fertility of saline soil for improving yield of wheat (*Triticum aestivum* L.). Selection of salt tolerant crop also adds to reclamation efficiency. While SC ensures sustained availability of moisture through its water holding capacity and provides organic carbon, halotolerants eradicate physiological constraints in growth of test crop. PGR provides amino acids based nutritive supplementation for growth and sustenance of halophiles as well as test crop. Alleviative effects of these above inputs are reflected in increasing productivity and growth rate of wheat and reducing soil salinity.

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# **Response of Symbiotic Nitrogen Fixation to Drought and Salinity Stresses**

**R. Serraj**

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The symbiotic nitrogen fixation in legume nodules has been shown to be extremely sensitive to water deficits and salinity stress, which could result in decreasing N accumulation and yield of legume crops under these conditions. The effects of salt stress have been investigated on several legume-rhizobium symbioses. Nitrogenase activity was substantially inhibited by sodium chloride (NaCl), and this inhibition was associated with a significant decrease in plant growth and N content. The effect of salinity on nitrogenase activity has been associated with changes of the nodule permeability to oxygen diffusion. A large genetic variation has been found among legume species and cultivars in the N<sub>2</sub> fixation sensitivity to salinity. Drought sensitivity of N<sub>2</sub> fixation has been compared among several grain legume species. Those species that transport ureides from the nodules were found to be much more drought sensitive than those that transport amides. It was concluded that a feedback mechanism involving ureide level may control N<sub>2</sub> fixation under drought. Consistent with this observation, the drought tolerance of N<sub>2</sub> fixation in soybean was associated with low concentrations of ureides in plant tissues. Regardless of the physiological mechanisms for the inhibition of N<sub>2</sub> fixation, there is now strong evidence that legume species and cultivars present a large spectrum of genetic variability and can thus, be selected for decreased sensitivity of N<sub>2</sub> fixation to salinity and drought stress.

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# Supplemental Nitrogen Applied during the Senescence on Two Rice Varieties : Evaluation of Nitrate Reductase and Glutamine Synthetase Activities and Crude Protein

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An experiment was made to study the effect of supplemental spray applied N (80kg/ha) on the activities of nitrate reductase (NR) and glutamine synthetase (GS) and on the accumulation of grain proteins by two rice varieties: IAC-47 an improved variety, and Piaui a land race. N was applied at 10 and 20 days after anthesis (DAA). Plants were collected since 5 DAA until 28 DAA, at 5 days intervals. Nitrate reductase (NR) and glutamine synthetase (GS) activities were determined in the flag leaf and 2<sup>nd</sup> leaf. NRA increased in both varieties after N-application and later on decreased to values close to the control. GS activities of N-treated plants had a small increase in the 10 DAA treatment. GS activities of control plants in both varieties decreased from 700 nmoles/g fw/ min at 5 DAA to on average 200 nmoles/g fw/ min at 28 DAA. That means a 70% decrease since the onset of anthesis. Piaui plants had a higher protein-N than IAC for both N sprayed plants and controls. However, we should notice that N-protein increases relative to the controls was higher for IAC (21,3%) than for Piaui (14,7%) plants. It looks like a higher proportion of the amino-N in the shoots was transferred to grains in IAC-47 than in Piaui plants.

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# Metabolic Changes in Soybean Plants in Response to Waterlogging in the Presence of Nitrate

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Soybean (*Glycine max* L. cv. IAC17) plants grown in hydroponics with nitrate to the V5 stage presented increases in soluble sugars, amino acids and protein in root tissues during simulated waterlogging (hypoxia) of the root system, while polysaccharides showed a transient increase. Much of the increase in amino acids was due to alanine which also increased dramatically in the xylem sap. Endogenous nitrate of the roots showed a gradual decrease and could account for most of the increase in amino acids and protein of the roots. It is concluded that nitrate was the main source of N for alanine formation and amino acids in general, while phloem transport of carbohydrates appeared to be the source of carbon for the observed increases in sugars, amino acids and protein. On return to normoxia after 5 days under hypoxia, all parameters measured returned to pre-hypoxic levels.

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## Biochemical Studies of Nitrate Tolerant Mutants of *Azospirillum lipoferum*

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Two spontaneous azide resistant mutants (S-8 & S-10) of *A.lipoferum* were studied for acetylene reduction, nitrate reductase (NR), ammonia excretion and crop response. Specific nitrogenase activity of mutant S-8 & S-10 measured at different concentrations of KNO<sub>3</sub> was higher than parent culture A-5. Increase of nitrate concentration inhibited acetylene reduction activity (ARA). Growth was slower under stationary conditions as compared to shaking conditions in 2.0 mM KNO<sub>3</sub>. Mutants expressed better NR activity as compared to the parent both under shaking as well as stationary conditions. N balance studies showed that in the initial stages of incubation, dissimilatory and denitrification dominated, but in later stages DNRA and assimilatory pathway were followed. Plant growth parameters of pearl millet under pot house conditions were positively affected by these two mutants even at 120 kg N ha<sup>-1</sup>

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# **Role of green manure (*Sesbania rostrata*) and biofertilizers (Blue-green algae and *Azotobactor*) in rice-wheat cropping system in state of Uttar Pradesh, India**

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**Agronomic studies on individual and integrated application of BGA biofertilizer and green manure with graded levels of inorganic nitrogen under field conditions and possible utilization of these organisms to meet at least a part of the nitrogen requirement of rice were carried out during Kharif season at agriculture farm of the Regional Institute of Rural Development, Bakshi-Ka-Talab, Lucknow. Application of BGA @ 12.5 kg/ha in combination with 90 kg N/ha as urea recorded favourable results in all the three rice cultivars (Saryu-52, Swarna and Jaya) and it compared with grain and straw yield at recommended dose of 120 kg N/ha. The response of BGA in integration with green manure (*Sesbania rostrata*) was very much pronounced at 30 kg N/ha and it was comparable to individual application of inorganic nitrogen at 90 and 120 kg N/ha while maximum yield was recorded at N<sub>60</sub>. Residual effect of green manure and BGA was also observed in wheat crop with *Azotobactor* biofertilizer at different dose of fertilizer N (i.e. 0, 30, 60, 90 and 120 kg/ha). Maximum yield was recorded at N<sub>90</sub>+*Azotobactor*. This proved that at least 30 kg N/ha may also be saved with increase in yield in wheat crop with the use of *Azotobactor* in the same field where green manure and blue green algae biofertilizer were used during Khraif paddy. Thus, it may be concluded that use of green manure and biofertilizers (Blue green algae and *Azotobactor*) in rice-wheat cropping system can save at least 90-120 kg N/ha/year with increased yield of both the crop.**

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## Development of an *In Vitro* Assay System for Screening of Anticancer Plants for Anti-telomerase Activity

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Extracts of four indigenous plants viz. *Saraca indica* (bark), *Ocimum sanctum* (leaves), *Cynodon dactylon* (stem and leaves) and *Withania somnifera* (whole plant powder), known to have anticancer activity, were assayed for their anti-telomerase activity using vero cell line-based non-radioactive TRAP assay system developed in our laboratory. Vero cells proved to be a suitable alternative to laboratory animals for screening of anticancer drugs including plant extracts. *Saraca indica* and *Withania somnifera* extracts were found to have cytotoxic effects on vero cells. Cytotoxic effects of *Withania somnifera* were more severe and morphological changes appeared as early as 24 hours post treatment. *Cynodon dactylon* and *Ocimum sanctum* did not have any cytotoxicity even up to 72 hours post treatment. Apoptotic changes were seen in in vero cells treated with *Saraca indica*, *Withania somnifera* and *Cynodon dactylon*. Out of the four plant extracts assayed, only lowest concentration (0.01 mg/ml) of *Ocimum sanctum* extract had progressive telomerase inhibitory effect with +1 level telomerase at 24 hours post treatment,  $\pm$  level activity at 48 hours post treatment and no telomerase activity at 72 hours post treatment. Vero cell line-based assay system coupled with conventional polyacrylamide gels and non-radioactive TRAP assay proved to be an efficient *in vitro* assay system for screening of plant extracts for anti-telomerase activity.

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## Effect of Sodium Fluoride on Pollen Physiology in *Triticum aestivum* L.

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Pollen physiology in wheat (*Triticum aestivum* L.) plants grown in soil supplemented with sodium fluoride (25, 50, 100 and 200 mg/kg) was undertaken. The plants grown in the soil with the addition of higher concentrations of NaF exhibited a marked reduction in their pollen fertility and *in vivo* pollen germination associated with the reduction in the quantity of free proline in their anthers.

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# **Gus expression as a novel system for optimization of *Agrobacterium*-mediated transformation procedure in a Basmati rice variety, Pusa Basmati 1**

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The gene cassette encoding  $\beta$ -D-glucuronidase gene was used to optimize *Agrobacterium* – mediated transformation procedure in a commercially important Basmati rice variety Pusa Basmati 1. Effects were studied of several critical factors including initiation of embryogenic calli, duration of co-cultivation of rice tissues with *Agrobacterium* and acetosyringone concentration in co-cultivation media, to improve the transformation efficiency. *Agrobacterium* strain LBA4404 containing the super virulent binary vector such as *pTOK233* and *pSB1* + *pCAMBIA1201* were used. Mature seed scutella tissues produced several small (5-30) highly embryogenic calli after five to six weeks involving one subculture. The highest frequencies of transient gus expression and of production of stable Hyg<sup>R</sup> and Gus<sup>+</sup> calli were obtained when calli were co-cultivated with *Agrobacterium* LBA4404 strains for a period of three days and by using acetosyringone concentration of 100  $\mu$ M in co-cultivation media. Any change in the co-cultivation period and acetosyringone concentration adversely affected the transformation efficiency. The Hyg<sup>R</sup> and Gus<sup>+</sup> calli were obtained at a frequency of 61 and 77% respectively for LBA4404 (*pTOK233*) and LBA4404 (*pSB1* + *pRKJ2*) strains. Plants were regenerated from the selected calli using improved regeneration media containing hygromycin and cefotaxime. About 45% of the plants showed gus expression in the root tip explants, thus confirming the presence and expression of the transgene.

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