Biochemical intake on the chemical composition.
CHAPTER V

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Studies of biochemical effect on the chemical composition

The living organism possesses the ability to withstand specific quantity of essential and non-essential elements present in the environment, and utilize them for their growth and development. These elements are toxic if taken up at a higher concentration than required. In contrast to the essential elements which serve as metabolic precursors for plants, the non-essential elements do not have any known metabolic function. Non-essential elements are grouped together into one major category termed as heavy metals. Heavy metals are the integrated component of the biosphere and are released through industrial effluents and other human activities. With the advancement of science and technology, environmental pollution has become a global problem. Rapid industrialization has led to the accumulation of major atmospheric pollutants like SO$_2$, NO$_x$, CO$_2$, metal ions, etc. Plastic, pesticides, disinfectants, tanneries, electroplating, etc. have also added many metal ions into the ecosystem.
Heavy metals are known to cause oxidative damage and biochemical lesions in plant cells. The heavy metal induced toxicity results in praline accumulation and alteration of various enzymic activities. In addition to these effects, heavy metal ions induce alterations in chlorophyll biosynthesis and electron transport activities, and other enzymes related to photosynthetic process. The in vitro uptake of heavy metals in plants results in interference of growth and metabolism by triggering secondary responses such as oxidative damage by producing highly reactive oxygen species (ROS). Disintegration of biomembranes by lipid peroxidation in a general mechanism of stress induced responses in living systems with the generation of ROS like OH⁻ and O₂⁻ radicals. These ROS are highly active in the hydrophobic environment rather than in hydrophilic aqueous systems. Lead (Pb) and Arsenic (As) are two toxic heavy metals that can cause oxidative damage in plants. Lead toxicity in many plants is known to be associated with inhibition of growth, changes in enzyme activities, leaf chlorosis, reduction in photosynthetic rate and also inhibition of root elongation. Arsenic occurs predominantly in the soil and also in groundwater. Like lead, arsenic can also induce phytotoxic symptoms and can generate ROS. Likewise, other heavy metals like chromium were found to inhibit seed germination, seedling growth and also induce oxidative damage and various biochemical lesions.

The plant hormone ethylene is involved in many aspects of the plant life cycle, including seed germination, root hair development, root
nodulation, flower senescence, abscission, and fruit ripening. Despite its simple two-carbon structure, olefin ethylene is a potent modulator of plant growth and development. The production of ethylene is tightly regulated by internal signals during development and in response to environmental stimuli from biotic and abiotic stresses, such as wounding, hypoxia, ozone, chilling or freezing. To understand the roles of ethylene in plant functions, it is important to know how this gaseous hormone is synthesized, how its production is regulated, and how the signal is transduced. The pathway of ethylene biosynthesis has been elucidated during the last few decades, and the basis for subsequent biochemical and molecular genetic analysis of this pathway has been provided. Morphological changes in dark-grown (etiolated) seedlings treated with ethylene of its metabolic precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), have been termed the ‘triple response’. The exaggerated curvature of the apical hook, radial swelling of the hypocotyl, and shortening of the hypocotyl, and root are unmistakable hallmarks of this ethylene response. Over the past decade, the triple response phenotype has been used to screen for mutants that are defective in ethylene responses. Etiolated Arabidopsis seedlings with minor or no phenotypic response upon ethylene application are termed ethylene insensitive or ethylene-resistant mutants. Mutants have also been identified that display a constitutive triple response in the absence of ethylene. This class can be divided into subgroups based on whether or not the constitutive triple response can be suppressed by inhibitors of
ethylene perception and biosynthesis, such as silver thiosulphate and aminoethoxyvinyl glycine (AVG). Mutants that are unaffected by these inhibitors are termed constitutive triple response mutants, whereas those whose phenotype reverts to normal morphology are termed ethylene-over-producer mutants, which are defective in the regulation of hormone biosynthesis and signalling pathway components in *Arabidopsis* has been established by epistasis analysis using these mutants.

Methane is produced as the terminal step of the anaerobic decomposition of organic matter in flooded soils. Methanogenic bacteria exclusively produce methane in the strict absence of free oxygen at redox potentials of less than -150 mV. In wetland soils, methane is produced by decarboxylation of acetate and by reduction of carbon dioxide. The production of methane during decomposition of organic matter under anaerobic conditions is controlled by the flow of carbon and electrons to the microbial population of methanogens. In addition, the thermodynamic constraints of the *in-situ* reactions involved and changes in the composition of the microbial community affect methane production. Methane emission to the atmosphere is the net difference between methane production and oxidation controlled by methanogens, methanotrophs and ammonium oxidizers.

Neue, H. U., and Yagi have emphasized the aspects related to methane production and its fluxes from lowland rice fields and crop and water management strategies for mitigating methane emissions from
lowlands rice fields. No doubt, land and water management practices can be used for reducing methane production and emission from lowland rice soils but the influence of these management practices on crop productivity need to be considered when evaluating and implementing such practices. Also, application, especially of water management practices may require a good water control that may not always be practically feasible.

The use of chemical agents (compounds or materials) that can inhibit or retard methane production and emission appear attractive for mitigating methane emission from lowland rice fields. The compounds or materials proposed for mitigating methane emission should be effective at reasonable rates of application and should be safe and without any deleterious side effects on soil microbial populations.

Following research on the effects of nitrification inhibitors on nitrification and nitrous oxide production in upland soils, nitrification inhibitors have been evaluated for mitigating methane emission from flooded lowland rice soils. For example, Bronson and Mosier evaluated the effects of nitrification inhibitors on emissions of N₂, CO₂, N₂O and CH₄ from flooded pots in a greenhouse study. It was found that nitrification inhibitor, encapsulated calcium carbide, showed a strong mitigating effect on emissions of N₂ and CH₄. Nitrous oxide fluxes were also reduced by calcium carbide, but the magnitude of losses with urea alone were very small in flooded rice. Carbon dioxide emissions were lower with encapsulated calcium carbide than without it. The effect of
the N rate on methane emissions was variable over the 30 days of study. From the results obtained, it was concluded that encapsulated calcium carbide appears to be an effective tool in reducing emissions of the radiatively active gases, nitrous oxide and methane.

In a micro-plot study in the field, Keerthisinghe et al. found that nitrpyrin and acetylene nitrification inhibitors significantly reduced methane emission in flooded rice. The lowest methane emission rates were observed in the wax-coated calcium carbide treatment. Wax-coated calcium carbide acted as a slow release source of acetylene and produced a sustained effect in reducing methane emission. Application of nitrifying and wax-coated calcium carbide reduced methane emission from 15.4 g CH$_4$ ha$^{-1}$d$^{-1}$ in the control to 5.8 and 2.8 g CH$_4$ ha$^{-1}$ d$^{-1}$, respectively. The reduction in methane emission by calcium carbide was a direct result of slow release of acetylene, which inhibits production of methanogenic bacteria. The mechanism involved for reducing methane emission is also a potent inhibitor of nitrification in aerobic soils.

Perspectives

Methane emission from flooded rice can be mitigated by decreasing methane production, increasing oxidation of methane produced or by reducing the transport of methane through rice plants. Nitrification inhibitors in general affect methane emission by influencing methane production and methane oxidation. The review of recent
litreature indicates that nitrification inhibitors have the ability to reduce methane production and emission from lowland rice soils. Research on the evaluation of a number of compounds or materials indicate that nitrification inhibitors have a wide range in their efficacy to reduce methane production or emission from flooded rice soils. The mechanisms involved in mitigating methane production and emission by nitrification inhibitors are not fully understood. But studies made with some nitrification inhibitors would suggest that in addition to decreasing the populations of methanogens, they also act as flooded soil systems at relatively higher values compared to the control (without addition of nitrification inhibitors). Nitrification inhibitors reduce methane emission by reducing methane production and/or oxidation of methane produced.

The Al-resistant mechanisms can be classified into exclusion or internal sequestration into the vacuoles. The exclusion mechanism prevents Al from entering the symplasm and promotes binding in the cell wall, or removal via root exudation of metal-chelating compounds. Organic acids were reported to play a role both in Al exclusion via release from root and Al detoxification in the symplasm, where organic acids chelate Al and reduce its toxic effects at the cellular level. Root exudation of organic acids has been reported for several plant species, including wheat and corn. Transgenic papaya and tobacco plants containing citrate synthase gene from Pseudomonas aeruginosa displayed increased production and release of citrate that conferred Al
tolerance to them. The present study was aimed at finding out whether external supply of citrate has any alleviating effect on A1 toxicity in seedlings of Indica rice. A1 was reported in induce synthesis of callose (1,3β-glucan) and oxidative stress-mediated peroxidation of membranes in plants. Plant growth, callose and the extent of lipid peroxidation as markers of citrate-mediated alleviation of A1 injury were studied in both A1 and A1 plus citrate-treated seedlings of rice.

Four-day-old seedlings of Suraksha (SUR) and Vikas (VIK) were grown in 1/10 Yoshida's culture culture solution (pH 4.2) containing 80 μ M aluminum and with and without citrate (50-200 μ M) as free acid (Sigma Chemical Co. USA). After four days, root and shoot lengths were measured. Roots and shoots were dried at 70°C for 48 h. The powder was dissolved in dilute acid mixture (HClO₄: HNO₃, 1:1 v/v) and the A1 content was determined using by 8-hydroxyquinoline method (Vogel A.I., 1964). Approximately 100 mg. of root tissue was placed in micro-centrifuge tubes containing 95% ethanol for 1h. Alcohol was subsequently decanted and 1 ml of 1 M NaOH was added to the tubes and root tissue was ground in a pestle and mortar. Samples were incubated in a water bath at 80°C for 15 min and centrifuged at 15,000 g for 5 min. Then the supernatant was incubated with 800 μ l of 0.1% aniline blue, 420 μ l of 1 M HCl and 1180 μ l of 0.1M glycine-NaOH buffer (pH 9.5) for 20 in at 50°C and for 30 min at room temperature.

Nitrogen is a costly input in optimizing crop production, and any limitation directly or indirectly affects the productivity of the crop and

(187)
economy of the farmer. Nitrogen is subject to volatilization, run-off, leaching and denitrification losses in field of rice \((Oryza sativa L.)\). The work done so far has advocated its deep placement, split application and use of slow-release source to increase the N-use efficiency (Ahmed and Sadique 1977). Among the rice varieties the dwarf ones require higher dose of nitrogenous fertilizer than tall ones for maximum productivity. It was therefore felt necessary to study the performance of these and local varieties.

A field experiment was conducted during rainy season 2004 with 4 levels of N(O, 60, 90 and 120 kg/ha) and two varieties muskon and Pro agro-6444, taking N levels as main plot and variety as subplot, in split plot design with 3 replications. The soil was slightly alkaline to normal in reaction, having pH 7.4, organic carbon 0.234%, available N 182 kg/ha, available P 42 kg/ha and available K 210 kg/ha.

The nursery bed (4mx2m) of 6 varieties was sown on 30 June 2004. The crop was transplanted on 23July 2004 in plots with 3 plants/hill. The row-to-row spacing was 20 cm. and plant-to-plant distance 10cm. Before transplanting, the field was furred well, having 3-4 cm. standing water, and the crop was fertilized @ 60, 90 and 120 kg N/ha through urea. Half of N, 40 kg. P/ha through single super phosphate and 40 kg K/ha through muriate of potash were applied as basal dressing. The remaining N was applied as top dressing in 2 equal splits, at tillering and at pancicle-initiation stage.
The crop was harvested on 30 Oct. 2004 at maturity and the plant samples were taken from each plot for chemical analysis. After harvesting, the straw and grain samples were taken separately.

The application of N significantly increased the yield with increase the yield with increase in N level (Table 5.1). Both varieties gave significantly more yield (except straw yield). The interactions of N with varieties revealed significant improvement in yield, up to 90 kg N/ha for all the varieties except. The maximum grain yield was under hybrid variety (3486kg/ha) in muskson (2540 kg/ha). Higher grain yield with increase in N level might be attributed to increase in ear-bearing shoot as well as test weight. The decrease in grain yield at the highest level of N in local variety was owing to lodging of crop. For straw yield, the hybrid variety recorded significantly higher yield than local ones. Higher straw yield is highly associated with dry-matter production. In general the yield level of the varieties was low, perhaps because of alkaline nature of the soil and low organic carbon content.

The application of N resulted in maximum uptake of N, P and K from the soil. The uptake of N, P and K by grain and straw significantly increased with an increase in N level (Fig. 5.1). The local variety showed significantly higher N, P and K uptake than hybrid ones for both grain and straw. The highest uptake of N, P and K was at 120 kg N/ha, followed by 90 kg N/ha and 60 kg N/ha. The N and P uptake significantly increased in the grain, owing to greater supply of N.
Table 5.1

Effect of nitrogen levels on grain and straw yields (kg/ha) in hybrid and local varieties of rice

<table>
<thead>
<tr>
<th>Variety</th>
<th>N₀</th>
<th>N₅₀</th>
<th>N₉₀</th>
<th>N₁₂₀</th>
<th>Mean</th>
<th>Range difference</th>
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<tbody>
<tr>
<td>Grain yield (kg/ha)</td>
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<td></td>
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<tr>
<td>Pro agro-6444</td>
<td>2270</td>
<td>2496</td>
<td>2895</td>
<td>3486</td>
<td>2786</td>
<td>1216</td>
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<td>Muskon</td>
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<td>2175</td>
<td>2540</td>
<td>2348</td>
<td>2232</td>
<td>484</td>
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<tr>
<td>Mean</td>
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<td>2856</td>
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<tr>
<td>SEm±</td>
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<tr>
<td>CD (P=0.05)</td>
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<td>0.798</td>
<td>1.334</td>
<td>1.596</td>
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<td></td>
</tr>
</tbody>
</table>

(190)
Figure 5.1
Effect of different levels of N on uptake of N in different rice varieties