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SHORT COMMUNICATION

EFFECT OF ARSENIC ON NITROGEN ASSIMILATORY ENZYMES IN GERMINATING RICE SEEDS

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SUMMARY

The effect of arsenic on activities of enzymes associated with nitrogen assimilation viz. nitrate reductase, nitrite reductase, glutamine synthetase, glutamate dehydrogenases and aminotransferases was observed in endosperms as well as embryoaxes of seeds of two rice cvs. Malviya-36 and Pant-12 germinated under 25 and 50 μM As_2O_3 in the medium. Results suggest that inhibition in the activity of key NO^-_3 assimilatory enzymes limit the assimilation of NO^-_3 by growing embryoaxes thereby leading to reduction in vigour of seedlings, while increased activity of glutamate dehydrogenase and aminotransferase appears to provide adaptational significance to the seeds germinating under As-polluted conditions.

***Key words*: Aminotransferases, glutamate dehydrogenase, glutamine synthetase, nitrate reductase, nitrite reductase, *Oryza sativa*.**

Arsenic (As), a heavy metal pollutant is a potential contaminant of groundwater especially in Asian countries such as Bangladesh, China, Taiwan and India (Abedin *et al.* 2002). It accumulates in soil due to As contaminated water-irrigation. As is readily taken up by rice plants, thereby eliciting toxic effects (Schmoger *et al.* 2000, Abedin *et al.* 2002). The reduction of NO^-_3 to NO^-_2 is catalyzed by the enzyme nitrate reductase (NR) (Srivastava 1980). Nitrite is subsequently reduced to NH_4^+ by nitrite reductase (NiR) (Shah and Dubey 2003). The glutamine synthetase (GS) and glutamate synthase (GOGAT) act in conjunction and serve as main route of NH_4^+ assimilation in plants under normal growth conditions (Lea and Ireland 1999). An alternative pathway for assimilation of NH_4^+ into glutamate is mediated by glutamate dehydrogenase (GDH) (Kumar *et al.* 2000). Among aminotransferases, aspartate aminotransferase (AspAT) is involved in the synthesis of aspartate from oxaloacetate (Dubey and Pessarakli 2002) whereas alanine aminotransferase (AlaAT) is involved in the synthesis of alanine from pyruvate and glutamate (de Sousa and Sodek 2003). Germination and early seedling growth have been regarded as critical phases of rice plants which are greatly influenced under stressful conditions (Dubey 1994). The present investigation was undertaken to examine the influence of increasing levels of As_2O_3 *in situ* on the activities of N assimilatory enzymes in the different components of germinating rice seeds in order to get an insight of As induced possible alterations in the process of nitrogen assimilation in rice.

Seeds of rice (*Oryza sativa* L.) cvs. Malviya-36 and Pant-12 were surface sterilized with 0.1% sodium hypochlorite solution and then imbibed in water for 24 h. Seeds were treated with uniform quantities of deionized distilled water (control) or As_2O_3 solutions of 25 μM and 50 μM concentrations. Seeds were germinated for 5 days at 28 ± 1°C in a B.O.D. cum humidity incubator maintaining a regular cycle of 12 h light (40-50 μmol m^{-2} s^{-1} irradiance) followed by dark period. Starting with 24 h

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imbibed seeds (0 h of germination), endosperms and embryoaxes were excised at 24 h intervals upto 120 h and all the estimations were conducted in triplicate. NR enzyme activity was extracted and assayed following the method of Hageman and Flesher (1960). NR specific activity is expressed as nmol nitrite produced min^{-1} mg^{-1} protein. NiR activity was extracted and assayed according to the method of Joy and Hageman (1966), and specific activity is expressed as nmol of nitrite reduced min^{-1} mg^{-1} protein. For GS activity assay, endosperms/embryoaxes (200 mg) were homogenized in 5 ml of 0.1 M Tris-HCl buffer (pH 7.5) containing 0.5 mM EDTA and 1 mM $MgCl_2$ at 4°C and assayed following procedure of Boyer *et al.* (1959). Specific activity of enzyme is expressed as µmol of γ-glutamyl hydroxamate formed min^{-1} mg^{-1} protein. Extraction and assay of aminating (NADH-GDH) and deaminating (NAD^+ - GDH) glutamate dehydrogenase were done according to the method of Sukalovic (1990). Enzyme specific activity is expressed as nmol NADH oxidized or NAD^+ reduced s^{-1} mg^{-1} protein. The enzymes AlaAT and AspAT were assayed according to the method of Malik and Singh (1980). Enzyme specific activity of AlaAT and AspAT are expressed as nmoles of pyruvate formed min^{-1} mg^{-1} protein and nmoles of oxaloacetic acid min^{-1} mg^{-1} protein, respectively. Though the activities of GDH, AlaAT and AspAT were assayed in both the rice cultivars, due to almost similar trend observed, data related to only cv. Malviya-36 have been reported. In all enzyme preparations, protein content were estimated according to the method of Lowry *et al.* (1951) using bovine serum albumin (BSA, Sigma) as standard.

Results indicate that in germinating rice seeds, arsenic inhibits nitrate reductase, nitrite reductase and glutamine synthetase, whereas enhances the activities of glutamate dehydrogenases and aminotransferases. A significant reduction in the growth of rice seedlings was observed under 50 µM As in the growth medium and localization of absorbed arsenic was more in roots than in shoots (Jha and Dubey 2004). With 50 µM As_2O_3 *in situ*, about 51 to 63 percent inhibition in the activity of NR and about 44 to 52 percent inhibition of NiR activity as well as about 53 to 59 percent inhibition in GS activity was observed in endosperms as well as embryoaxes at 72 h of germination (Fig. 1a-c). This suggests that arsenic in concentration of 50 µM can significantly inhibit the activities of NR and NiR enzymes in germinating rice seeds leading to decreased

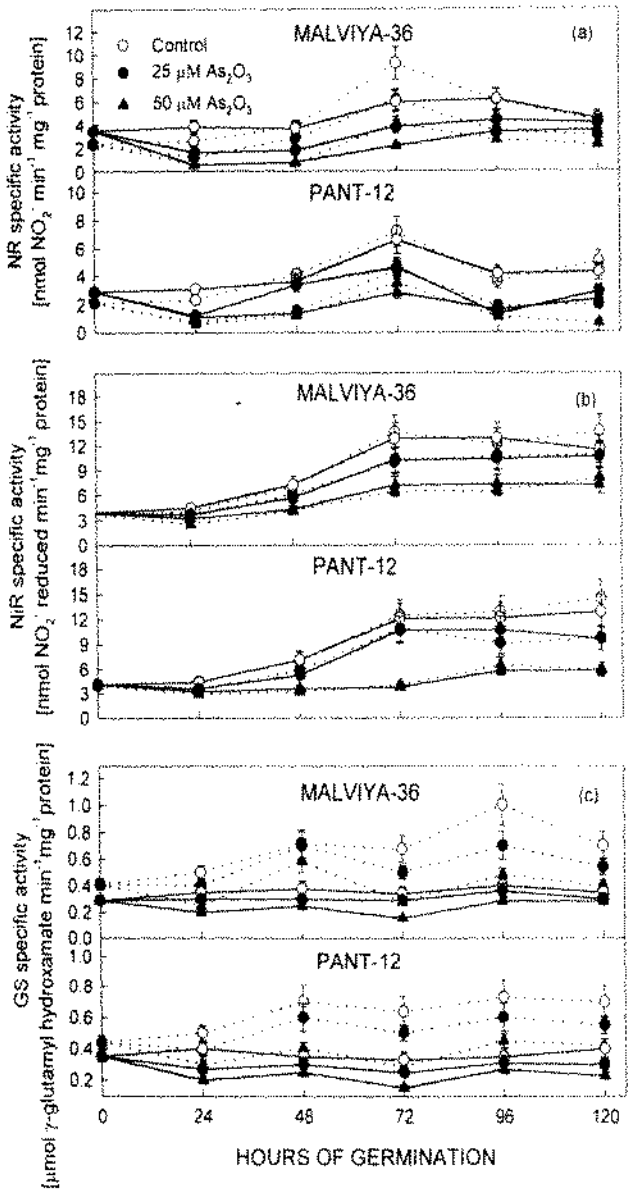

Fig. 1. Specific activities of (a) nitrate reductase (b) nitrite reductase and (c) glutamine synthetase in embryoaxes (----) and endosperms (——) of rice *cvs.* Malviya-36 and Pant-12 at increasing hours of germination under 0 (control), 25 and 50 µM As_2O_3. Values are means based on three independent determinations and bars indicate standard deviations

rate of NO^-_3 reduction in plants growing in arsenic polluted environments. Similar reduction in the activities of NR, NiR and GS was reported in As-stressed rice seedlings (Jha and Dubey 2004). Inhibition of GS activity due to As suggests that GS/GOGAT pathway of ammonia assimilation is adversely affected under arsenic toxicity

conditions. The activities of both aminating and deaminating GDH increased in embryoaxes as well as endosperms due to *in situ* As treatment (Fig. 2a). Upto 22 per cent increase in the activity of aminating-GDH and about 15 to 84 per cent increase of deaminating-GDH was observed in endosperms and embryoaxes at 50 μM As_2O_3 treatment compared to controls at 72 h of germination in rice cv. Malviya-36. Almost similar trend was noted for another rice cv. Pant-12. This indicates the role of GDH pathway in assimilation of NH^+_4 in plants growing in As-polluted environment. Similarly under 50 μM As treatment in rice seedling (Jha and Dubey 2004) as well as under Cd toxicity in soybean plants (Balestrasse *et al.* 2003) increased GDH activity was noticed. With increase in concentration of As_2O_3 in the germination medium, a concomitant increase in the activities of aminotransferases was observed (Fig. 2b). Under 50 μM As treatment about 30 to 34 per cent increase in the activity of AlaAT and upto 32 to 100 per cent increase in AspAT activity was observed at 72 h of germination. Increase in aminotransferase activities in soybean under hypoxia (de Sousa and Sodek 2003) as well under 200 μM Cd treatment (Balestrasse *et al.* 2003) and under 50 μM arsenic treatment in rice seedlings (Jha and Dubey 2004) has been observed. Elevated aminotransferase activity provides adaptive significance to plants growing under stressful conditions possibly through synthesizing more amino acids under such conditions (Dubey and Pessarakli 2002). The present study thus suggest that As toxicity *in situ* inhibits the activities of nitrate assimilatory enzymes NR, NiR and GS whereas the activities of GDH and aminotransferases are enhanced. However, though inhibited activities of key N assimilatory enzymes would limit the reduction of NO^-_3 in germinating rice seeds, but increased GDH activity may play important role in assimilation of NH^+_4 into organic compounds. The enhanced activities of aminotransferases could further provide adaptational potential to the seedlings by synthesizing more amounts of amino acids under As toxicity.

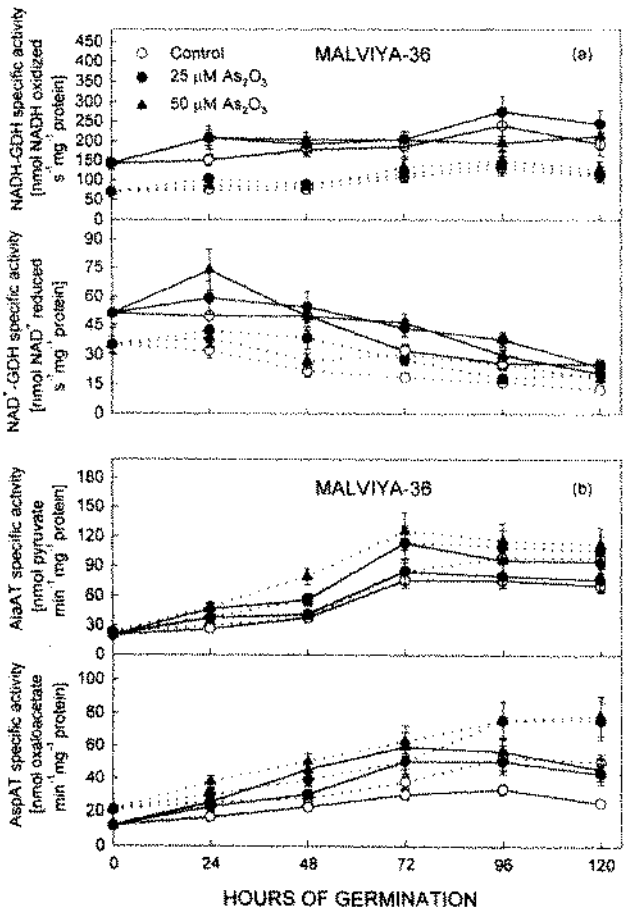

Fig. 2. Specific activities of (a) aminating (NADH-GDH) and deaminating (NAD^+-GDH) glutamate dehydrogenase (b) alanine and aspartate aminotransferase in embryoaxes (----) and endosperms (——) of rice cv. Malviya-36 with increasing hours of germination under 0 (control), 25 and 50 μM As_2O_3. Values are means based on three independent determinations and bars indicate standard deviations

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