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SHORT COMMUNICATION

INFLUENCE OF ALUMINIUM ON PLANT GROWTH, ALUMINIUM ACCUMULATION AND EXCRETION OF OXALIC ACID BY SEEDLINGS AND CULTURED HAIRY ROOTS OF *SESBANIA ROSTRATA* L. - A GREEN MANURE CROP

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The influence of aluminium (Al) on different growth parameters in a leguminous green manure crop *Sesbania rostrata* L (TSR-1) grown in hydroponic culture is reported here. Growth based on fresh and dry weights was unaffected by Al upto 750 µM, while at 1000 µM Al, there was a decline in both fresh and dry weights. The root length was not affected upto 1000 µM, whereas shoot length showed a decline at 1000 µM of Al. The Al content in root, stem and leaves showed a sharp enhancement upto 1000µM of Al. Analysis of root exudates of seedlings grown in hydroponics and hairy roots grown *in vitro* showed secretion of oxalic acid under Al stress.

Key words: **Al tolerance, green manure crop, hairy root culture, oxalic acid, *Sesbania rostrata* L.**

Aluminium is known to cause phytotoxicity to plants in acidic soils and is a major constraint in crop productivity. Even at low concentrations, Al can cause considerable damage to plants because of its high binding affinity for many metabolically important elements (Haug 1984) and is known to inhibit cation transport across the plasma membrane (Huang *et al*. 1992). Many plants which are tolerant to Al are known to secrete organic acids such as malic acid, citric acid and oxalic acid, which can chelate Al^{3+} and prevent its entry into roots (Delhaize and Ryan 1995)

Sesbania rostrata L. (TSR-1), a green manure crop developed in Bhabha Atomic Research Centre, Mumbai, is a photoperiod - insensitive mutant producing stem nodules which is grown in a wide range of soils including acidic soils and is used as green manure crop. Using this green manure crop, many physiological parameters have been studied including phytoremediation of Pb, U and Cd. (Ramani *et al*. 1990). In the present study, the influence of Al on growth of *Sesbania rostrata* (TSR-1) grown in hydroponics was studied. The results of secretion of organic acids by plants grown in hydroponic condition and also in axenically grown hairy roots are presented.

Seeds were germinated and grown in 3L Steinberg's nutrient solution (Steinberg 1953). The solution pH was adjusted to 4.0 and the plants grown under continuous aeration with 12 h photoperiod (17,000 Lux fluorescent light) at 22± 2°C for 20 days. Aluminium sulphate [$Al_2(SO_4)_3.16H_2O$] at different concentrations (0, 100, 250, 500, 750 and 1000 µM) were added to nutrient medium at pH 4.0 with three replications of each treatment. At the end of 20 days, root and shoot lengths were recorded. Average of four plants per treatment was used. Plants were harvested and partitioned into roots, leaves and stem. Roots were washed thrice with cold 0.05 mM $CaSO_4$ to remove any Al adhering to the root surface and fresh weight of roots, leaves and stem

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recorded. The tissues were later dried at 70ºC for 48 h, wet-ashed using 5:1 HNO_3-$HClO_4$ mixture and the levels of Al in the acid extracts determined using Atomic Absorption Spectrometer (GBC 932, Australia).

Germinated seedlings were subjected to different concentrations of Al at pH 4.0 (10 plants per treatment). Root exudates were collected and centrifuged for 15 min. at 5500 g. The supernatant was passed through 0.2µm filter (Millipore India) and organic acids were analyzed by HPLC (ANATEK, JASCO, Japan). The standard organic acids (malic acid, citric acid, oxalic acid and succinic acid) were used as control. Samples were separated on C_{18} symmetry column 4.6x250mm (ANATEK, JASCO, Japan) using mobile phase of HPLC grade 2% MeCN/Aq H_3PO_4 (0.1%) at pH 2.0 with flow rate 1 ml min^{-1} and detection was at 210 nm. The retention time of the samples were compared with that of the standard acids and co-chromatography was done along with oxalic acid standard to confirm the results.

Axenically grown hairy roots induced by *Agrobacterium rhizogenes*, due to their aseptic nature without the confounding effects of microorganisms can be used for confirmation of secretion of organic acids. Hence hairy roots were induced in *S. rostrata* using *A. rhizogenes* and hairy roots were cultured under sterile conditions and secretion of organic acids in root exudates studied. For induction of hairy roots, seeds of *S. rostrata* were sterilized with 70 % ethanol for 30 sec followed by 0.1% mercuric chloride for 4 min. The seeds were rinsed 5 times with sterile water and grown *in vitro* on MS (Murashige and Skoog 1962) basal medium supplemented with 3% sucrose and 0.8% agar at pH 5.8. Ten day old seedlings were used for infection with *Agrobacterium rhizogenes* strain MTCC 532 (Institute of Microbial Technology, Chandigarh, India). The infected plants were further grown on the same medium for another 2-3 weeks. Hairy roots induced at the site of infection were excised and individually cultured on MS solid medium supplemented with cefotaxime at 500 mg l^{-1}. After two subcultures, the roots were transferred to MS liquid medium with reduced concentration of cefotaxime (250 mg l^{-1}) and in subsequent cultures, cefotaxime was eliminated. To study the organic acid secretion, hairy roots (2-3 gm) were grown in 50 ml milli-Q water supplemented with 500 µM $Al_2(SO_4)_3.16H_2O$ in 250 ml flasks and kept in a shaker at 50-60 rev/min. At the end of 3 days, samples were drawn out for analysis. The organic acids were separated on Supelcogel' H ion exchange column (Dimension: 25 cmx4.6mm, packing type: sulfonated polystyrene divinylbenzene, Ionic form: hydrogen) using 0.15% Phosphoric acid (HPLC grade) as mobile phase with flow rate 0.1ml min^{-1} and detected by variable detector at 210 nm.

Effect of different concentrations of Al on fresh and dry weights indicated that the fresh weight of root, leaf and stem were not affected at 100 and 250 µM of Al, while at 500 µM, there was an enhancement in fresh weight. At 1000 µM of Al, the fresh weight of root, leaf and stem showed a sharp decline (Fig. 1a). A similar trend was seen for dry weight also (Fig. 1b). Root length

Fig. 1. Effect of Al on growth of *S. rostrata* on fresh weight (a) and dry wt. (b) basis. Vertical bars denote ± SE, (n = 3)

of different Al treated plants of *S. rostrata* remained unaffected at all Al concentrations tested. Enhancement in shoot length was observed at 500 µM of Al while a slight decline in shoot length at 1000µM of Al was noted (Fig. 2). There was a sharp increase in Al content in roots, stem and leaves of *S. rostrata*, with an increase in concentrations of Al (> 1000 µg gm^{-1}) (Fig. 3). Higher concentrations (more than 90%) of Al were found to be located in roots as compared to stem and leaves.

Fig. 2. Effect of Al on root length ▪ and shoot length . Vertical bars denote ± SE, (n = 3).

Fig. 3. Aluminium content (µg g^{-1} dw) in root ⎓ stem ⎓ and ⎓ leaves

The root exudates, when analyzed for secretion of organic acids, showed the presence of oxalic acid (Rt-3.0 min. compared with standard 0.5mM oxalic acid Rt-2.99 min.) at all concentrations of Al tested, while in control without treatment, secretion of oxalic acid was negligible (Fig. 4). Hairy root cultures of *S. rostrata* treated with Al secreted 1.5 times higher levels of oxalic acid in response to 500 µM Al as compared to control (Fig. 5). The root exudates did not show the presence of other organic acids.

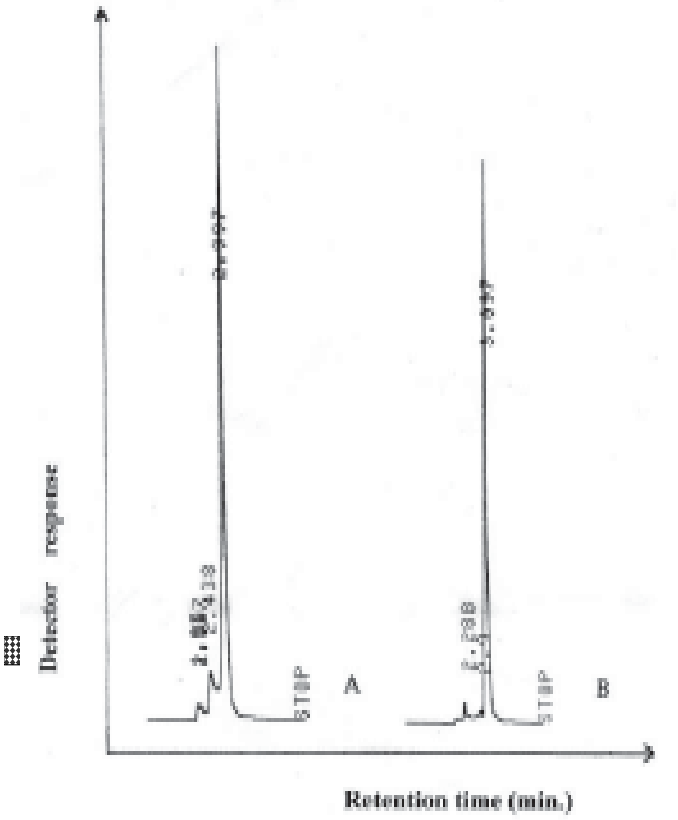

Fig. 4. (A) Std. oxalic acid, (B) Rhizosecretion by seedlings in hydroponic culture under Al stress. HPLC conditions- column: C_{18} symmetry column, mobile phase: 2% MeCN/Aq H_3PO_4 (0.1%), flow rate: 1 ml min^{-1}, detection at 210 Dm

Plants have different mechanisms for Al acquisition. Some plant species have the ability to exclude Al entry into the root apex and root hairs (apoplasmic mechanisms), while other plants have regulatory mechanism wherein they secrete organic acids and form complexes with Al and as Al-organic acid chelate, they enter into the cell (symplasmic mechanisms). Aluminum accumulating species detoxify internal Al by formation of Al-organic acid complexes. The intake of Al by the roots is followed by the formation of 1:3 Al-oxalate complexes in the root cells (Ma *et al.* 1998). The efflux of oxalate and the internal chelation of Al by oxalate are likely to represent two related mechanisms that confer

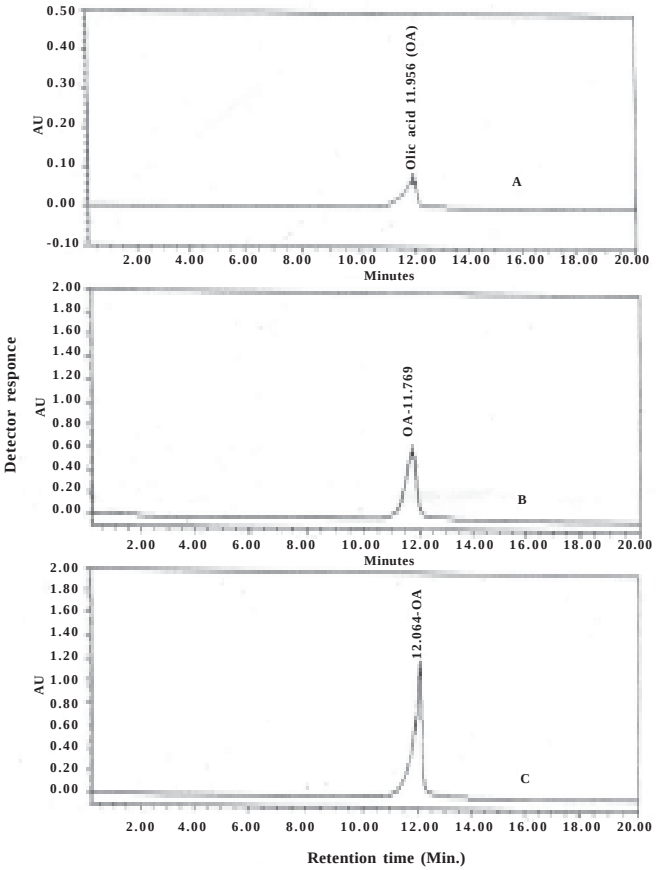

Fig. 5. (A) Std. oxalic acid, (B) Rhizosecretion by hairy roots without Al treatment (control), (C) Rhizosecretion by hairy roots with Al treatment (experimental), HPLC conditions-column: Supelcogel' H ion exchange column, Mobile phase: 0.15% ortho-phosphoric acid, flow rate: 0.1 ml min^{-1}, detection at 210nm

Al tolerance. Secretion of oxalic acid by roots showed an enhancement by Al treatment of *S. rostrata* plants. Since Al accumulation in *S. rostrata* was high, it is likely that Al may form a complex with oxalic acid and the Al-oxalic acid complex may be taken up by the plant roots. Most of the Al in *S. rostrata* was located in roots, while leaves and stems contained lower concentrations of Al. Further studies are needed to understand the role of oxalic acid in Al accumulation in *S. rostrata*.

Results of the present study indicate that Al tolerance in *Sesbania rostrata*, as measured by growth parameters could partly be explained by Al induced release of oxalic acid from roots. This Al induced release of Al chelating compounds could be an important adaptive mechanism, permitting survival of plants in Al toxic soils.

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