

## Alleviating effect of citrate on aluminium toxicity of rice (*Oryza sativa* L.) seedlings

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**Seedlings of two Indian rice cultivars (Suraksha and Vikas) differing in aluminium sensitivity were grown in Yoshida's culture solution containing 80  $\mu$ mol aluminium. Growth of seedlings, callose formation, aluminium accumulation and lipid peroxidation were studied in both aluminium-treated and aluminium plus citrate-supplemented seedlings. High callose accumulation seems to be a good marker for screening the cultivars of rice for aluminium sensitivity. Citrate at a concentration of 200  $\mu$ M alleviated the toxic effects of aluminium in both cultivars of rice, mostly by chelating with the metal.**

ALUMINIUM (Al) toxicity is recognized as one of the dominant chemical constraints affecting plant growth in acid soils with pH < 5. Among phytotoxic species,  $\text{Al}^{3+}$  is the most potent and inhibits root growth and uptake of nutrients, which ultimately reduce crop yield. However, the mechanism of toxicity is not well understood<sup>1</sup>. Wild species and cultivars of many crop plants possess strategies to avoid or tolerate Al toxicity. For the selection and breeding of plants resistant to Al toxicity, and for an economical and sustainable approach for improving crop production on acid soils, it is necessary to understand the mechanism of Al stress.

The Al-resistant mechanisms can be classified into exclusion or internal sequestration into the vacuoles<sup>2</sup>. The exclusion mechanism prevents Al from entering the symplast and promotes binding in the cell wall, or removal via root exudation of metal-chelating compounds<sup>3</sup>. Organic acids were reported to play a role both in Al exclusion via release from root and Al detoxification in the symplast, where organic acids chelate Al and reduce its toxic effects at the cellular level<sup>4</sup>. Root exudation of organic acids has been reported for several plant species, including wheat<sup>5</sup> and corn<sup>6</sup>. 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<sup>7</sup>. The present study was aimed at finding out whether external supply of citrate has any alleviating effect on Al toxicity in seedlings of Indica rice. Al was reported to induce synthesis of callose (1,3  $\beta$ -glucan)<sup>8</sup> and oxidative

stress-mediated peroxidation of membranes<sup>9</sup> in plants. Plant growth, callose and the extent of lipid peroxidation as markers of citrate-mediated alleviation of Al injury were studied in both Al and Al plus citrate-treated seedlings of rice.

Four-day-old seedlings of Suraksha (SUR) and Vikas (VIK) were grown in 1/10 Yoshida's culture solution (pH 4.2)<sup>10</sup> containing 80  $\mu$ M aluminium and with and without citrate (50–200  $\mu$ M) as free acid (Sigma Chemical Co. USA). After four days, root and shoot lengths of both control and citrate-supplemented seedlings were measured. Roots and shoots were dried at 70°C for 48 h. The powder was dissolved in dilute acid mixture ( $\text{HClO}_4$  :  $\text{HNO}_3$ , 1 : 1 v/v) and the Al content was determined using Ultra Mass 700 Inductively Coupled Plasma Mass Spectrometer. The amount of Al is expressed in terms of mg/g dry weight of tissue. Callose formed was estimated spectrofluorimetrically, following the procedure of Kaus<sup>11</sup>. Approximately 100 mg of root tissue was placed in micro-centrifuge tubes containing 95% ethanol for 1 h. 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  $\mu$ l of 0.1% aniline blue, 420  $\mu$ l of 1 M HCl and 1180  $\mu$ l of 0.1 M glycine–NaOH buffer (pH 9.5) for 20 min at 50°C and for 30 min at room temperature. Callose content was estimated spectrofluorimetrically, with excitation at 398 nm and emission at 495 nm (Hitachi, 650-10S, Fluorescence Emission Spectrophotometer, Japan). Callose concentration was expressed as Pachyman equivalents (PE) per g tissue fresh weight (mg PE·g<sup>-1</sup> fresh wt).

Lipid peroxidation was measured following thiobarbituric acid (TBA)-reactive substances assay procedure of Heath and Packer<sup>12</sup>. Root tissue (300 mg) was homogenized with pestle and mortar in 3 ml of 0.1% tricarboxylic acid (TCA) solution. The homogenate was centrifuged at 15,000 g for 10 min at 0°C, and 0.5 ml of the supernatant was collected. The supernatant was added to 1.5 ml of 0.5% TBA in 20% TCA. The mixture was incubated at 90°C in a shaking water bath for 20 min. The reaction was stopped by placing the reaction tubes in an ice-cold water bath. The samples were then centrifuged at 10,000 g for 5 min at 0°C and the absorbance of the supernatant recorded at 532 nm. The value for non-specific absorption at 600 nm was subtracted. The amount of malondialdehyde (MDA)–TBA complex (red pigment) was calculated from the extension coefficient 155 mM cm<sup>-1</sup>. All the experiments were repeated two times with identical trends.

Our studies showed that the rice cultivar VIK is more Al-sensitive than the cultivar SUR. This observation was based on growth of roots and shoots under treatment. The extent of Al accumulation, after four days of treatment, in roots and shoots of VIK was 1.98 mg and 0.34 mg/g DW respectively, at an external supply of 600  $\mu$ M Al. In

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contrast, the accumulation of Al in roots and shoots of cultivar SUR was 2.38 mg and 0.41 mg/g DW respectively. With an increase in Al concentration in the medium (50–600  $\mu\text{M}$ ), Al content increased in a concentration-dependant manner in both roots and shoots, but more so in roots than in shoots. The amount of Al accumulated was more in the less-sensitive cultivar compared to the susceptible one (Table 1). Greater accumulation of Al in roots than in shoots might be because of inhibition of Al transport to shoots from roots, as pointed out by Howler and Cadwid<sup>13</sup>. Transport of Al is perhaps not quantitatively linked to Al accumulation in roots. Instead, inactivation of Al in the roots seems to prevent Al transport to the shoots. Alternatively, as suggested by Fageria *et al.*<sup>14</sup>, Al accumulation in plants may be taking place in non-sensitive sites such as cell walls or vacuoles of roots. Lazof *et al.*<sup>15</sup> used secondary ion mass spectrometry and localized Al in the symplasm of soybean roots. Based on our study, we presume that the mere presence of higher or lower levels of Al in rice cultivars SUR and VIK was not crucial for Al tolerance, but its subcellular distribution (including the cell wall) or sequestration and perhaps, the form in which Al was present inside the cell were important, which makes the cultivar relatively Al-tolerant. Al treatment reduced the root and shoot lengths of the control seedlings (without citrate) of SUR (tolerant) by 26 and 21% respectively, and that of VIK by 51 and 23%, res-

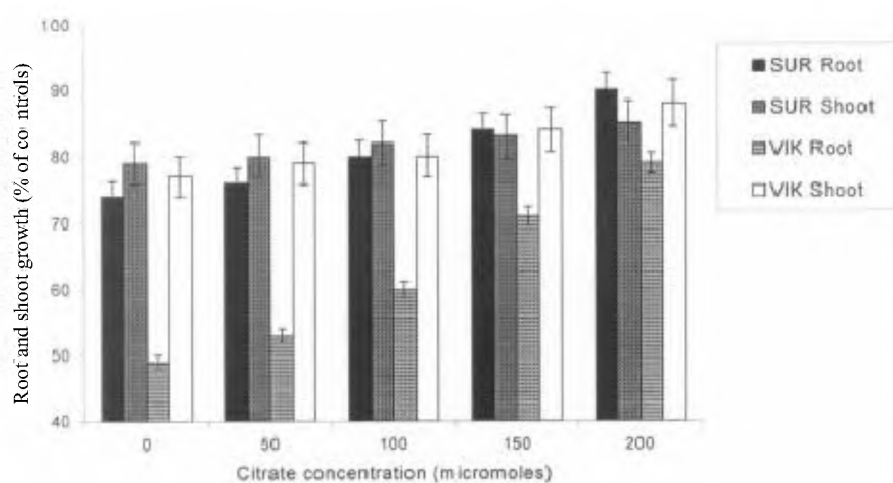
pectively. However, the corresponding seedlings grown in citrate-supplemented solution exhibited better root and shoot growth, particularly at a citrate concentration of above 100  $\mu\text{M}$ . At 200  $\mu\text{M}$  citrate, the root and shoot lengths of SUR and VIK improved by 60% over their respective controls (Figure 1). Al is known to inhibit primarily root growth in several plants, either by inducing changes in cell-wall constituents and increasing rigidity of the root cell walls or by directly interacting with DNA and slowing down cell division<sup>16</sup>. Since roots are the major sites for the synthesis of important phytohormones, especially cytokinins, changes in the root physiology may consequently lead to reduced shoot growth.

Callose levels which were 1.4 mg and 1.8 mg PE/g fresh wt respectively, in control seedlings of SUR and VIK, declined gradually and reached 0.7 mg and 0.8 mg PE/g fresh wt levels in the presence of 200  $\mu\text{M}$  citrate. This revealed that Al toxicity was alleviated significantly by citrate (Figure 2). Either Al toxicity or Al-induced membrane damage may cause the deposition of callose in root epidermal and cortical cells, and consequential changes in cell-wall constituents<sup>17</sup>. It was proposed that 1,3- $\beta$ -glucan synthase located in the plasma membrane gets stimulated by perturbations in membrane properties<sup>18</sup>. In the present study, MDA levels gave an indication of peroxidation of membrane lipids. The MDA levels gradually declined with an increase in the concentration of

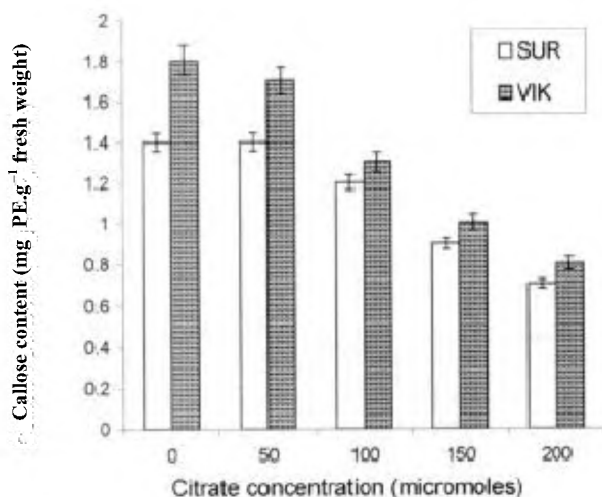
**Table 1.** Concentration-dependent accumulation of Al in roots and shoots of rice cultivars VIK and SUR\*

Cultivar	Aluminium concentration ( $\mu\text{M}$ )											
	Control		50		100		200		400		600	
	R	S	R	S	R	S	R	S	R	S	R	S
VIK	0.45	0.05	1.25	0.20	1.70	0.22	1.82	0.25	1.90	0.27	1.98	0.34
SUR	0.1	0.02	1.25	0.15	1.75	0.23	2.0	0.27	2.15	0.33	2.38	0.41

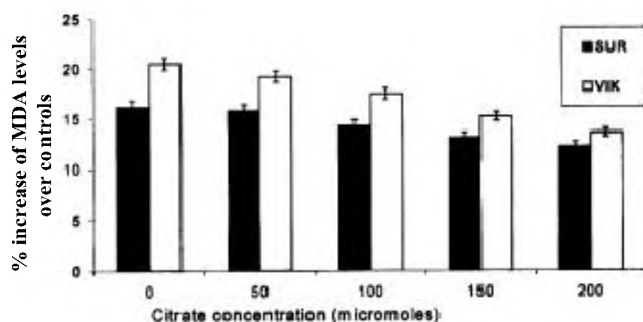
\*Values are means from 10 replicates. R, Root; S, Shoot.



**Figure 1.** Effect of Al and citrate on root and shoot growth in rice cultivars Suraksha and Vikas.



**Figure 2.** Effect of Al and citrate on callose accumulation in Suraksha and Vikas.



**Figure 3.** Effect of Al and citrate on percentage increase of MDA in Suraksha and Vikas.

citrate and a maximum reduction of 25 and 35% (over their controls) as observed in SUR and VIK rice cultivars respectively, at 200  $\mu\text{M}$  citrate (Figure 3). Lipid peroxidation occurs mainly as a consequence of oxidative deterioration of polyunsaturated fatty acids by Al-promoted oxygen free radicals, and is identified as a basic cell-membrane reactive damage in cellular mechanisms<sup>19</sup>.

In this study the ameliorative role of exogenously supplied citrate was confirmed by reduced callose accumulation and decreased MDA levels, leading to improved plant growth in citrate-supplemented rice seedlings compared to their corresponding controls. The ability of citrate to mitigate Al toxicity is consistent with the hypothesis that organic acids may protect cells from Al toxicity through chelation<sup>20</sup>. In the presence of ligands like citrate, Al is chelated and the uptake of chelated Al could be much less than hydroxyl Al. The toxic effects of ligand-bound Al are less than that of free Al. Our results are in concurrence with those of Ownby and Popham<sup>21</sup>, who observed citrate-induced reversal of inhibition of root growth in wheat seedlings. Evidence supporting organic acid-mediated alleviation of Al toxicity was earlier demonstrated in Al-tolerant cultivars of corn<sup>22</sup> and wheat<sup>23</sup>.

From our findings it is concluded that higher levels of citrate effectively alleviate Al-induced toxicity in Indica rice. Other studies by us also revealed that citrate alleviates Al toxicity better than similar organic acids such as malate and oxaloacetate in rice. While citrate is a six-carbon compound, malate and oxaloacetate are four-carbon compounds (data not shown). This may be the reason for the citrate-mediated alleviation of Al toxicity. The effectiveness of the organic anion in reducing Al toxicity is however related to the ability of the anion to complex Al under experimental conditions and in the present study, citrate could be stated to form stable complex with Al, thus making it less toxic.

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## Characterization and origin of silicic and alkali-rich glasses in the Upper mantle-derived spinel peridotite xenoliths from alkali basalts, Deccan Trap, Kutch, northwest India

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**Petrological, textural and chemical characters of glasses found as discrete pockets in the spinel lherzolite xenoliths from Kutch region are discussed. These glasses are silica-saturated and alkali-rich in composition and display sharp contacts with the primary mineral assemblage of the spinel lherzolite. In contrast, the basaltic melt infiltrating the xenoliths exhibits reaction textures. We have attempted to compare these glass compositions with worldwide occurrences. In the absence of any trace-element data, we have used the trace-element data of the Cr-diopsides from the same xenoliths and have discussed the mechanisms of glass formation. The glass formation is attributed to the metasomatic fluids, mostly carbonatitic in composition, which is in agreement with our previous work on these xenoliths. The metasomatism has occurred at a shallow level (7–12 kbar), slightly above the carbonatite stability field.**

STUDY of mantle xenoliths provides significant information about the composition of the mantle and the processes

which impress the mantle composition. The compositions of silicate melts potentially in equilibrium with the earth's mantle have long been of interest to petrologists and geochemists. In recent times, the glasses in the mantle xenoliths have been studied in great detail so as to understand the genesis of silicate melts at mantle depths, and pressure and temperature conditions. Glasses represent such melts, with high silica content (up to 72% SiO<sub>2</sub>) occurring as inclusions and discrete pockets within the mantle xenoliths, and have been reported from a variety of settings. Xenolith glasses have a wide range of major element composition; this has been clearly brought out in the compilation of the glass compositions world over by Draper and Green<sup>1</sup>.

The formation of glasses has been ascribed to a variety of processes, including infiltration of the host lavas<sup>2</sup>, and breakdown of amphibole or phlogopite in response to decompression melting to give rise to silica-undersaturated liquids. These then react with mantle minerals, especially orthopyroxene and crystallize olivine and clinopyroxene, leaving more silica-rich residual liquids<sup>3,4</sup>. The second model includes partial melting of mantle xenoliths at short residence times in crustal magma chambers during ascent to the surface in the host magma<sup>5</sup>. Other models imply formation of silicic melts at mantle depths.

Large group of glasses, however, have remained unexplained by the various processes listed above<sup>1</sup>. These glasses are rich in SiO<sub>2</sub> (> ~ 60 wt%), Al<sub>2</sub>O<sub>3</sub> (18–20 wt%) and alkalis (Na<sub>2</sub>O + K<sub>2</sub>O up to 17 wt%). This prompted several researchers to advocate the hypothesis that such melts could represent a type of metasomatic agent circulating in the upper mantle<sup>6–9</sup>. Experimental investigations have suggested that highly silicic melts could be formed by small degrees of *in situ* partial melting in the upper mantle<sup>10</sup>; such melts are enriched in silica–alumina and alkalis, and depleted in MgO, FeO and CaO. It is also proposed that the silicic, aluminous alkaline melts could be formed by low-degree partial melting of peridotite enriched in alkalis, volatiles and other low melting-temperature components<sup>1</sup>. Recently, on the basis of chemical criteria, the formation of such silica and alkali-rich glasses (with Na<sub>2</sub>O content up to 14 wt%) has been ascribed to the process of carbonatite metasomatism<sup>11</sup>.

In the Kutch mantle xenoliths, the glasses generally occur as pockets or as discrete grains along with the recrystallization of neoblasts of opx and cpx. Majority of these glasses are spongy, dirty green in colour. Chemically, they are SiO<sub>2</sub>–Al<sub>2</sub>O<sub>3</sub>–Na<sub>2</sub>O–K<sub>2</sub>O-rich and TiO<sub>2</sub>–FeO–MgO–CaO–P<sub>2</sub>O<sub>5</sub>- poor types, similar to the set of glasses described from Canary Islands. The Kutch mantle xenoliths which host the silicic glasses are devoid of any hydrous mineral phases; however, they are cryptically metasomatized<sup>12</sup>. The mantle xenoliths are spinel lherzolite in composition; the details of which have been described elsewhere<sup>13</sup>. The silica content of the glasses varies between 55 and 65%. In the light of the various models

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