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SHORT COMMUNICATION

DETERMINATION OF TOXICITY LEVELS OF Al^{+3} IN CHICKPEA (*CICER ARIETINUM* L.) SEEDLINGS

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Seeds of chickpea (*Cicer arietinum* L.) were grown in presence of varying concentrations of $Al_2(SO_4)_3$ Studies indicated that stress was produced at a concentration of 1mM aluminium sulphate. Lower concentrations of $Al_2(SO_4)_3$, through beneficial in the early stages, were also found deleterious at later stages.

Aluminium toxicity is probably the most important factor involved in limiting plant growth in strongly acid soils and mine spoils (McLean, 1976). Both phytotoxic and phytotonic effects have been attributed to aluminium in plants (Roy *et. al.*, 1988). The present paper reports the toxicitity levels of Al^{3+} in chickpea seedlings.

Seeds of chickpea (*Cicer arietinum* L). Co II, obtained from Tamil Nadu Agricultural University, Coimbatore, were divided into 5 groups of 50 seeds each and soaked separately in 1mM. 100 μM, 10μM and 1 μM $Al_2(SO_4)_3$ solutions for 3 hrs. Seeds soaked in distilled water formed the control. Seeds were germinated in the respective solutions on filter paper kept in petri dishes. Fresh weights, starch and soluble sugar contents in cotyledons were determined on the 5th day (Clegg, 1956). Activities of α-amylase (E C 3.2.1.1), aspartate amino-transferase (AST) (EC 2.6.1.1) and alanine aminotransferase (ALT) (E C 2.6.1.2) in cotyledons were also determined on the 5th day, For α-Amylase, the extract of the cotyledons in cold 50 mM acetate buffer (pH 5.8) containing 3mM $CaCl_2$ and 1% PVP was contrifuged at 5000 g. and the supernatant heated at 70°C for 3 minutes followed by another centrifugation at 5000 g. One ml. supernatant was used for

α-amylase assay (Bernfeld, 1955). AST and ALT were extracted with cold 100 mM phosphate buffer (pH 7.4) and estimated colorimetrically using 2, 4-dinitrophenyl hydrazine (Stroev and Makarova, 1989).

After 5 days, seedlings were grown in pot culture as done before (Abitha Devi *et. al.*, 1988). Whole plant fresh weight, number of leaves, fresh weights of individual tissues, water content, calcium and proline contents in plant tissues were determined on predetermined days Ca content in the tissues was measured by subjecting the dry plant tissues to ash, and dissolving it in 0.1 N HCl followed by estimation by the permanganate titrimetry method (Korenman, 1965). Water content in plant tissue was measured as the difference between wet and dry weights.

Results in Table I show that the values for whole plant fresh weight, fresh weights of individual tissues, number of leaves and water content were consistently lowest in 1 mM $Al_2(SO_4)_3$. Chickpea leaves in 1mM $Al_2(SO_4)_3$ group also accumulated maximum amount of proline. Results in Table II show that cotyledon activities of α-amylase were significantly lower and those of AST and ALT significantly higher in the 1mM $Al_2(SO_4)_3$. Both starch and soluble sugar contents in the cotyledons were also higher while the calcium content in root and shoot were significantly lower in the test groups.

Table I. Effect of varying concentration of Al^{3+} on fresh weight, number of leaves, proline and water content in chickpea seedlings

Parameter	Concentration of $Al_2(SO_4)_3$			
	1 μM	10 μM	100 μM	1 mM
Whole plant fresh wt.	117.4±5.5	82.0±1.6	73.3±1.2	59.7± 1.1
Fresh wt. (Shoot)	137.6±4.5	107.2±4.5	75.5±1.4	44.3± 2.1
Fresh wt. (Root)	78.6±1.8	62.5±1.3	85.5±3.1	54.1± 1.2
Fresh wt. (Leaf)	84.8±1.9	77.2±1.4	96.5±2.4	74.4± 2.8
Number of leaves	80.5±4.2	72.5±2.7	88.6±2.3	63.4± 2.3
Water content (Shoot)	137.4±5.2	105.9±5.6	75.3±1.8	45.7± 0.8
Water content (Root)	77.6±2.1	61.8±3.2	85.2±1.6	53.5± 0.9
Proline (leaf)	101.9±2.4	61.6±1.9	60.5±3.1	206.1±12.4

Mean of 6 values±SE.

All values reported are for 14th day and expressed as % of Control.

Table II. Effect of Al^{3+} on the α-amylase, AST and ALT activities, and starch, soluble sugar and calcium contents in Chickpea seedling tissues

Parameter	Control	1mM $Al_2(SO_4)_3$
Cotyledon amylase (mg maltose liberated/15'/g fresh wt.)	2.88±0.08	1.62±0.06
Cotyledon AST (μ mol keto acid formed/min/fresh wt.)	21.1 ±0.6	28.6 ±0.9
Cotyledon ALT (μ mol keto acid formed/min./g fresh wt.)	101.2 ±4.1	172.6 ±5.7
Cotyledon starch (mg/g fresh wt.)	17.8 ±0.4	29.7 ±0.9
Cotyledon soluble sugars (mg/g fresh wt.)	12.2 ±0.4	20.4 ±0.8
Calcium (mg/g dry wt)		
Cotyledon	3.42±0.09	3.24±0.09
Root	8.54±0.23	4.44±0.08
Shoot	6.87±0.28	0.65±0.09

Mean of 6 estimations±SE.
All values reported are for 5th day.
For Ca the values are for 24th day.

Aluminium is known to induce water stress in plants (Krizek and Foy, 1981) which is also noted in the present studies. Aminotransferases (AST and ALT) have been reported to enhance during water stress as a protective measure (Sreenivasulu Reddy *et. al.*, 1990), and this is also supported by the present findings.

Starch is the major food reserve in Chickpea. (Tharanathan *et. al.*, 1987) and α-amylase is involed in its degradation (Juliano and Varner, 1969). Aluminium is known to compete with calcium binding sites (Hanson, 1984). α-Amylase is a calcium-dependent enzyme (Clarkson and Hanson. 1980). In the present study, although, there is no significant difference between the calcium contents of control and test groups, α-amylase activity may be reduced probably because of competition between aluminium and calcium. This may explain for reduced amylase activity and higher starch content in the cotyledons of Al^{3+}-treated seeds. The increase in cotyledon soluble sugars may be due to decerased translocation due to water stress. Hydrolysis of starch is subject to end product inhibition. (Jones and Armstrong, 1971). Thus the accumulation of soluble sugars in the cotyledons would further

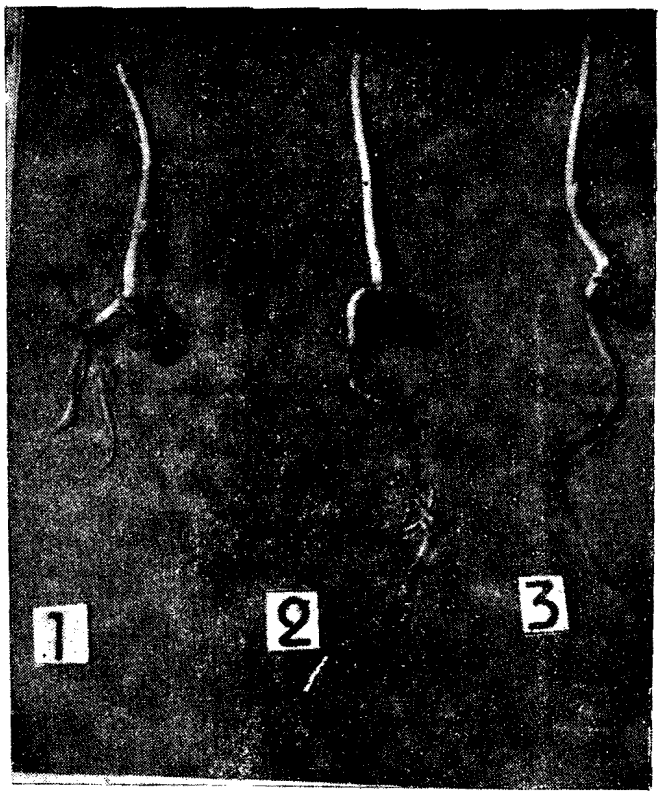

Fig. I. Effect of Aluminium sulphat on root formation in chickpea seedlings
1=10^{-3}M Aluminium sulphate
2=Distilled water (control)
3=10^{-6}M Aluminium sulphate.

suppress amylase activity. Thus aluminum toxicity in chickpea seedlings might be due to a combination of amylase inhibition and induced water stress.

The present studies suggest that stress is developed in chickpea seedlings due to Al^{3+} at a concentration above 100 μM althrough toxicity to lesser extents can be observed at lower concentrations also.

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