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Effects of Aluminium Toxicity in Two Cultivars of *Phaseolus vulgaris* with Different Resistance to Aluminium II: Effects on Protein, Nucleic Acids and Certain Respiratory Enzymes

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Abstract: This paper describes the changes in protein content, nucleic acids (RNA and DNA) content as well as the activity of glucose-6-phosphate dehydrogenase (G6PDH) and glyceraldehyde-3-phosphate dehydrogenase (G3PDH) in seedling (5-d-old) of sensitive-cultivar contender and in tolerant-cultivar Giza 3 treated with increasing concentrations of aluminium. Rapid increase in G6PDH (EC 1.1.1.49) and G3PDH (EC 1.1.1.40) activities were observed in Al-resistant cultivar, while no change in the activities of the two enzymes were observed in Al-sensitive cultivar during 5 days of germination. Rapid decreases in protein, RNA and DNA contents in sensitive-cultivar and in resistant-cultivar (at 150 μM Al), were observed in seedlings treated with Al. On the other hand, significant increases in protein, RNA and DNA contents were observed in resistant cultivar treated with 50 and 100 μM Al. These results suggest that rapid induction of G6PDH and G3PDH in the Al-resistant cultivar by Al may play a role in the mechanism of Al resistant, possibly by regulation of the pentose phosphate pathway and alteration in either lipid composition of plasma membrane (El-Saht, under publication), or alteration in nucleic acids and protein contents.

Key words: Aluminium (Al), glucose-6-phosphate dehydrogenase (G6PDH), glyceraldehyde-3-phosphate dehydrogenase (G3PDH), nucleic acids (DNA and RNA), proteins, *Phaseolus vulgaris*

Introduction

Enhanced activity of cytosolic enzyme has been reported as a possible internal Al-resistant mechanism (Slaski *et al.*, 1996).

Slaski *et al.* (1996) reported that Al cause rapid changes in the concentrations of metabolites and in the activities of several enzymes from the pentose phosphate pathway in wheat and rye (Secale cereals).

Glucose-6-phosphate dehydrogenase (G6PDH) is a key enzyme which catalyzes a non-equilibrium reactions, and thus regulates flux of carbon through the pentose phosphate pathway (Copeland and Turner, 1987).

Aluminum ions, like other metal ions show a selective association with polynucleotides cellular nucleic DNA and RNA (Karlisk *et al.*, 1989). The interaction with DNA was reported to affect physicochemical properties and biological functions such as cell division, cell elongation and synthesis of DNA and RNA (Wallace and Anderson, 1984). In the preceding study (El-Saht, under publication) I found that Al at concentrations of 50 and 100 μM altered growth and lipid composition as well as lipase activity of Al-sensitive cultivar of *Phaseolus vulgaris* while has no effect on Al-resistant cultivar, at low and moderate concentrations while inhibit growth of both sensitive and resistant cultivars, at high levels (150 μM Al). I extended the investigation to examine the protein, RNA and DNA contents as well as the behaviour of the two key respiratory enzymes (G6PDH and G3PDH) of both Al-sensitive and Al-resistant cultivars incubated in Al. The results, in general, provide further evidence that alteration in RNA, DNA and protein contents as well as rapid induction of G6PDH and G3PDH in the Al-resistant cultivar by Al may play a role in the mechanism of Al-resistant.

Materials and Methods

Time course experiment: Homogeneous seeds of Al-sensitive french bean (*Phaseolus vulgaris* L. cv. contender) and Al-resistant french bean (*Phaseolus vulgaris* L. cv. Giza 3), were used. The procedures of sterilization of seeds and germination of seedlings as well as the experimental set- were the same as previously described by Steingro (1983) and El-Shat (under publication).

Samples for determination of protein, nucleic acids (R and DNA) as well as the activities of both G6PDH a G3PDH were taken of seedlings after 5 days from sowi

Determination of nucleic acids: DNA was measu colourimetrically by the method of Sadasivam Manicham (1992) and RNA was measured by the metho Sadasivam *et al.* (1975).

Determination of protein: Protein content was determi spectrophotometrically using a double be spectrophotometer according to the method adopted Bradford (1976).

Assay of respiratory enzyme activity: For the extractio G6PDH and G3PDH in the present study, the acetone d powdered method of Younis *et al.* (1991) was adopted. to technical difficulties I decided to use the follow chemical methods in the present study.

Glyceraldehyde-3-phosphate dehydrogenase (G3PDH): method used was that of Aeneas *et al.* (1991). It is ba on the fact that NADPH and NADH absorb light wavelength 340 nm, whereas NADP and NAD do not.

Glucose-6-phosphate dehydrogenase (G6PDH): This enz is active with either NAD or NADP as coenzyme, hence change in absorption at 340 nm with time is a measur the course of the reaction. In a spectrocolourimeter t 1.5 cm^3 of Tris-buffer, 0.2 cm^3, of NAD, 0.1 cm^3, 1.5 cm^3 of enzyme preparation and 1.05 cm^3 $MgCl_2$, 0.1 cm^3 of enzyme preparation and 1.05 cm^3 dist. water are added in turn. Readings at 340 nm taken before and at 15-second intervals after mixing w 0.05 cm^3 of glucose-6-phosphate (10 μM) solution (You *et al.*, 1991).

For each of the two enzymes tested, unit activity w calculated and from this specific activities units per mg of protein was obtained. The protein was determined spectrophotometrically at 260 and 280 nm (Colwick and Kaplan, 1955).

Results and Discussion

Effects of Al on protein and nucleic acids: There was an increase in protein content of the resistant french bean cultivar Giza3 treated with 50 and 100 μM Al whereas a significant decrease in protein contents of this cultivar was observed upon treatment with 150 μM Al (Table 1). Protein content of the sensitive cultivar contender treated with increasing concentrations of Al showed significant progressive decreases as compared with control (Table 1).

Table 1: Effect of different concentrations of aluminum (Al) on protein content [mg. 100 g^{-1} f. mass] of Al-resistant cultivar and Al-sensitive cultivar of *Phaseolus vulgaris* seedlings

concentrations of Aluminum [μM] in Hogland solution	Protein
Resistant	
0	52.8±0.1
50	63.1±0.2**
100	72.7±0.1**
150	37.1±0.1**
L.S.D. at 5% level	2.6
L.S.D. at 1% level	3.8
Sensitive	
0	51.2±0.2
50	48.3±0.3**
100	40.2±0.1**
150	34.9±0.2**
L.S.D. at 5% level	2.5
L.S.D. at 1% level	3.7

*P=0.05, **P=0.01

Table 2: Effect of different concentrations of aluminum (Al) on DNA and RNA content [mg. 100 g^{-1} dry mass] of Al-resistant cultivar and Al-sensitive cultivar of *Phaseolus vulgaris* seedlings

Concentrations of Aluminum [μM] in Hoagland solution	DNA	RNA
Resistant		
0	19.6±0.1	58.3±0.2
50	27.1±0.2**	66.7±0.3**
100	34.3±0.2**	69.3±0.2
150	16.2±0.1**	49.7±0.1**
L.S.D. at 5% level	0.97	2.9
L.S.D. at 1% level	1.41	4.3
Sensitive		
0	18.1±0.1	56.2±0.1
50	16.0±0.2*	50.1±0.2
100	11.3±0.2**	42.3±0.1**
150	7.6±0.3**	32.2±0.2**
L.S.D. at 5% level	0.92	2.8
L.S.D. at 1% level	1.11	3.9

*P=0.05, **P=0.01

Proteins are implicated as possible measures of defense in Al toxicity (Putteril and Gardner, 1988). Therefore, the increased protein content in resistant cultivar Giza 3, were due to increased incorporation and not an artifact caused by a reduction in the Al-stressed seedlings (Table 1). The decreased protein content in Al-sensitive cultivar contender may be associated with an Al-toxicity in such seedling.

RNA and DNA contents of Al-sensitive cultivar contender showed progressive significant decrease by increasing Al-concentrations (Table 2). On the other hand, treatment of resistant cultivar with 50 and 100 μM Al lead to significant increase in both RNA and DNA contents, whereas at 150 μM Al, a significant decrease was observed (Table 2). Al-induced cDNAs were identified in the Al-sensitive wheat Warigal that showed homology to a proteinase inhibitor cDNA sequence (Somera *et al.*, 1996).

Effect of Al on respiratory enzymes (G6PDH and G3PDH): A rapid increase in the activities of G6PDH and G3PDH were observed in resistant cultivar treated with increasing concentrations of Al up to 100 μM whereas at 150 μM Al this cultivar showed significant decrease in G6PDH and G3PDH activities (Table 3). No change in the activities of G6PDH and G3PDH were observed in sensitive cultivar treated with increasing concentrations of Al up to 100 μM. A rapid decrease in the activities of the two enzymes detected were observed in sensitive cultivar treated with high concentrations of Al (150μM) (Table 3).

An increase in the activities of G6PDH and G3PDH seems to be a common response of plants to excess of metals, including Cd, Zn and Cu (Van-Assche and Clijstere, 1990). When the specific activities of the two enzymes were assayed in root tips of Al-treated wheat seedlings, increases in the activities of both, enzymes were observed in the Al-resistant genotype. In the Al-sensitive cultivar no changes in the enzyme activities were observed (Slaski *et al.*, 1996).

The rapid increase of G6PDH and G3PDH activities in the Al-resistant *Phaselous vulgaris* Giza3 appears to reflect an induction of enzyme synthesis. Van-Assche and Clijstrs (1990) reported that Zn-stimulated glutamate dehydrogenase activity in leaves of *Phaseolus vulgaris*.

Table 3: Effect of different concentrations of aluminum (Al) on unit activity determinations of G6PDH [units. mg $protein^{-1}$] and G3PDH [units. mg $protein^{-1}$] of Al-resistant cultivar and Al-sensitive cultivar of Phaseolus vulgaris seedlings

Concentrations of Aluminum [μM] in Hoagland solution	G6PDH	G3PDH
Resistant		
0	26x10^{-3}±0.02	431x10^{-3}±0.1
50	36x10^{-3}±0.01**	469x10^{-3}±0.2**
100	48x10^{-3}±0.02**	476x10^{-3}±0.4*
150	20x10^{-3}±0.01**	420x10^{-3}±0.5**
L.S.D. at 5% level	1.3	21.5
L.S.D. at 1% level	2.8	37.1
Sensitive		
0	20x10^{-3}±0.01	420x10^{-3}±0.3
50	20x10^{-3}±0.02	420x10^{-3}±0.2
100	20x10^{-3}±0.01	420x10^{-3}±0.4
150	13x10^{-3}±0.02**	381x10^{-3}±0.3**
L.S.D. at 5% level	1.1	21.1
L.S.D. at 1% level	2.9	36.6

*P=0.05, **P=0.01

It is possible that the rapid induction of G6PDH and G3PDh in Al-resistant *Phaseolus vulgaris* cultivar plays a role in mediating resistant to Al. This induction precedes several well-known structural adaptations in root cells which are believed to play a role in survival under conditions of Al-stress. Since G6PDH is key enzyme of the pentose phosphate pathway, I can hypothesize that this pathway might contribute to Al-resistant by providing precursor of cofactors for other biosynthetic routes. For example, NADPH, generated by pentose phosphate pathway is a factor limiting incorporation of acetyl-CoA into fatty acids, which is required in lipid synthesis (Slaski *et al.*, 1996). In this study, Al induced changes in phospholipids, glycerol, oils and lipase activity may reflect adaptation of the Al-resistant Giza3 to stress conditions (Zhang *et al.*, 1996).

The present results suggested that Al stress affects expression of each of DNA and RNA differently. As previously mentioned (El-Saht, under publication), the activities of enzymes (lipase, G6PDH and G3PDH) contributes to the increment of tested activities by Al treatment, moreover, the induction of RNA and DNA levels by Al ion stress may indicate that some part of the increment of lipase (El-Saht, under publication), G6PDH and G3PDH activities are dependent on transcriptional level.

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