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Effects of Aluminium Toxicity in Two Cultivars of *Phaseolus vulgaris* with Different Resistance to Aluminium I: Effects on Growth and Lipid Metabolism

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Abstract: The study was carried out to investigate the effects of aluminium (Al) on growth (root length, shoot length, fresh and dry weights of roots and shoots), phospholipids, oils and glycerol content of either sensitive cv. Contender or resistant cv. Giza 3 *Phaseolus vulgaris* seedlings as well as the activities of lipase (EC1.1.31). No changes in growth parameters (root length, shoot length, fresh and dry weights) of Al-resistant cultivars treated with 50 and 100 μM Al, while at 150 μM Al, a rapid decrease in all growth parameters were observed. On the other hand, Al-sensitive cultivar treated with increasing concentration of Al showed significant decreases in all growth parameters determined. Progressive significant decreases in phospholipids, oils, glycerol contents as well as increase in the activity of lipase in Al-sensitive cultivar while in Al-resistant cultivars-treated with 50 and 100 μM Al, no change in the contents of phospholipids, oils, glycerol and lipase activity but a significant increase in these metabolites were observed in Al-resistant cultivar treated with 150 μM Al.

Key words: Aluminum, glycerol, growth, lipase, oils, phospholipids, *Phaseolus vulgaris*

Introduction

Aluminum (Al) toxicity is recognized as an important factor affecting the growth and yield of many cultivated plants in mineral soils with pH's below 5.0, although both species and cultivars within species show substantive differences in their response to the presence of Al in solution (Slaski *et al*., 1996). Al induced alterations in the physical properties of cell membranes, Vierstra and Haug (1978) studied the effect of Al on the physical properties of membrane lipids in *Thermoplasma acidophilum* and found that Al decreased membrane lipid fluidity. Zel *et al*. (1993) reported that Al decreased membrane fluidity in the Al-sensitive fungus, *Aminate mascara* but an increase in membrane fluidity was observed in the Al-resistant fungus, *Lactarius piperatus.* Al has been shown to bind to phospholipids vesicles and induce phase separation of artificial membranes (Zhang *et al*., 1996). Al induced conformational changes in plasma membranes proteins have also been reported in roots of *Triticum aestivum* (Caldwell, 1989).

In Al-sensitive cultivars of wheat, Al inhibits root growth within minutes at low pH (Lindberg and Hakan, 1997). Al is not considered toxic at pH values exceeding 5, but still has some negative effects even at a neutral pH e.g. on the electrical membrane potential difference and on activities of some enzymes in the plasma membrane (Widell *et al*., 1994). To further clarify the immediate effects of Al on growth and certain metabolic changes in two different cultivars of *Phaseolus vulgaris.* The present study compared the effects of Al on the Al-sensitive *Phaseolus vulgaris* cv. contender and the Al-resistant *Phaseolus vulgaris* cv. Giza 3.

Materials and Methods

Seeds of *Phaseolus* with two cultivars, Giza 3 and contender were selected ans surface sterilized by soaking in 10^{-3} M $HgCl_2$ for 3 min. The seeds were washed with sterile water and germinated on Whatman No. 1 filter paper watered with 20 cm^3 of Hoagland nutrient solution (1%-strength) in plastic dishes. When necessary, additional equal amounts of the nutrient solution were added. The germinating dishes of the two cultivars were incubated in the dark at 25°C for 45h. Then 5 uniform germinating seeds were placed in a Perspex plate suspended over a black-painted glass cylinders (600 cm^3) containing either Hoagland nutrient (1/4-strength) solution supplemented with 50 μM, 100 μM and 150 μM Aluminum. All the culture solutions were adjusted at pH 4.5.

The cylinders were placed in a growth chamber adjusted at optimum growth conditions (temperature 28±2°C, light intensity 3000 to 3500 Lux, relative humidity 60 to 70% and continuous aeration from an air pump at a rate of 2L/hr/clinder (Steingrover, 1983).

Sampling of seedlings was made after 5 days for determination of growth parameters as well as for determination of phospholipids, glycerol and oil content and lipase activity.

Determination of phospholipid phosphorus: Phospholipids were determined according to the method of Schneider (1945) which was applied and culbrated by Hasaneen (1981).

Determination of glycerol: Glycerol was estimated by the method of Younis *et al*. (1987).

Determination of oil content: The method adopted for extraction and determination of the oil content of plant tissues was that described by Meara (1955).

Estimation of liapse activity: The soluble enzyme was extracted in chilled acetone (-15°C) and phosphate buffer (pH 7.5) as described to provide maximum enzymatic activity which was determined by the method of Fiore and Nord (1949).

Results and Discussion

Effects on growth and shoot/root ratios: Length of roots and shoots, freash and dry masses of both roots and shoots of resistant cultivars of Phaseolus vulgaris seedlings treated with 50 and 100 μM Al were unchanged as compared with control seedlings (Table 1). On the other hand, all growth

Table 1: Effect of different concentrations of aluminum (All on growth parameters of french bean seedlings (5 d)

concentration Aluminuim [μM]	Root length [cm]	Root fresh mass [g]	Root dry mass	Shoot lenth [cm]	Shoot fresh [g]	Shoot dry [g]	Shoot fresh mass/ Root fresh mass [S/R ratio]
Sensitive (cv. contendor)							
0	3.0±0.02	0.38±0.00	0.011±0.0	7.1±0.02	0.51±0.01	0.019±0.0	1.34
50 [μM]	2.6±0.01**	0.35±0.01**	0.009±0.0**	6.7±0.01**	0.42±0.00**	0.012±0.0**	1.20**
100 [μM]	2.0±0.01**	0.27±0M1**	0.006±0.0**	6.0±0.02**	0.31±0.00**	0.009±0.0**	1.14**
150 [μM]	1.1±0.01**	0.19±0.00**	0.003±0.0**	5.3±0.00**	0.20±0.01**	0.004±0.01**	1.05**
L.S.D. at 5% level	0.15	0.01	0.001	0.36	0.02	0.001	
L.S.D. at 1% level	0.17	0.04	0.002	0.41	0.03	0.002	
Resistant (cv. Giza 3)							
0	4.0±0.02	0.50±0.01	0.015±0.0	9.6±0.02	0.80±0.0	0.025±0.0	1.60
50 [μM]	4.09±0.01	0.50±0.01	0.015±0.0	9.59±0.01	0.80±0M1	0.025±0.0	1.60
100 [μM]	4.08±0.01	0.49±0.0	0.014±0.0	9.58±0.0	0.80±0.02	0.025±0.0	1.81
150 [μM]	3.70±0.03**	0.40±0.01**	0.010±0.0**	8.5±0.01**	071±0.00**	0.019±0.0**	1.77
L.S.D. at 5% level	0.21	0.02	0.001	0.48	004	0.001	
L.S.D. at 1% level	0.29	0.03	0.002	0.53	0.05	0.002	

*p = 0.05,**p = 0.01

Table 2: Effect of different concentrations of aluminum (Al) on phospholipid content lmg phosphorus. 100 g^{-1} dry matter, glycerol content mg glycerol content lmg glycerol. 100g^{-1} dry matter, oil content [mg Oils, 100^{-1} dry matter in french bean seedlings (5 d)

Concentration of Aluminum (μM)	Phospholipids	Glycerol	Oils
Sensitive (cy. contendor)			
0	18.1±0.02	5.1±20.0	40.1 ±0,02
50 [μM]	13.0±0.03**	3.9±0.02*	30.0±0.03**
00 [μM]	10.2±0.01**	4.0±0.02**	25.1±0.06**
150 [μM]	6.1±0.02**	4.2±0.01**	16.9±0.03**
L.S.D. at 5% level	0.9	0.15	2.0
L.S.D. at 1% level	1.2	0.23	3.3
Resistant (cv. Giza 3)			
0	20.6±0.03	4.9±0.01	62.3±0.01
50 [μM]	20.3±0.02	4.85±0.03	62.2±0.01
100 [μM]	20.0±0.02	4.80±0.30	62.0±0.03
150 [μM]	28.3±0.04**	5.85±0.02**	69.3±0.04**
L.S.D. at 5% level	1.03	0.20	3.1
L.S.D. at 1% level	2.1	0.30	4.2

*p = 0.05,**P = 0.01

Table 3: Unit activity determinations of lipase of french bean seedings (5 d) treated with different concentrations of alumnium (Al). The values listed are given as μ mol produced cm^3 oil^{-1} per seedling min^{-1}

Concentration of Aluminum (μM)	Lipase
Sensitive (cv.contendor)	
0	110.00±0.09
50 [μM]	120.00±0.08* *
100 [μM]	131.60±0.07**
150 [μM]	144.3±0.06**
L.S.D. at 5% level	5.5
L.S.D. at 1% level	7.3
Resistant (cv. Giza 3)	
0	119.2±0.03
50 [μM]	119.15±0.03
100 [μM]	119.14±0.06
150 [μM]	134.2±0.05
L.S.D. at 5% level	5.9
L.S.D. at 1% level	7.6

parameters determined showed significant decreases in resistant cultivar (at 150 μM Al) and in sensitive cultivar treated with increasing concentrations of Al (Table 1). SIR ratio of Al-sensitive cultivar showed significant decrease by Al-concentrations, whereas this ratio was significantly increased in Al-resistant cultivar as compared with control SIR ratio (Table 1).

A reduction in root length, shoot length fresh and dry masses of Al-sensitive french bean cultivar contender is often recognized as one of the symptoms of Al toxicity in plants (Strid, 1996). Using these parameters, I was able to detect differences between the Al-sensitive contender and the Al-resistant Giza 3, after 5 days of exposure to Al at 50 and 100 μM. In contrast, Strid (1996) observed that at 50 and 100 μM Al, the growth of wheat plants was reduced compared to controls in sensitive cultivar, the reduction being more evident for roots.

Accordingly, the SIR-ratio increased in the Al-treatments. Furthermore, analysis of root growth in the presence of different concentration of Al-resistant cultivar of wheat and Al-sensitive cultivar exhibited differences in resistance to Al. Exposure to 1 O μM Al had no significant effects on growth of roots of the Al-resistant cultivar P.T. 741. Increasing the Al concentration to 20 μM resulted in a 28% reduction in the rate of root growth (Zhang *et al.,* 1996).

Effects of Al on phospholipids, oils, glycerol and lipase activity: Phospholipids, oils and glycerol contents of sensitive cultivar contender were significantly and progressively decreased by increasing Al concentrations (Table 2). On the other hand, the above contents were significantly unchanged in resistant cultivar Giza 3 treated with Al up to 100 μM, whereas at 1 501 μM Al, all these lipid portions were significantly decreased in resistant cultivar. Lipase activity of sensitive cultivar contender was significantly increased whereas in resistant cultivar Giza 3, lipase activity was unchanged upto 100 μM whereas at 150 μM Al, a significant increased in lipase activity was apparent (Table 3). Zel *et al.* (1993) reported that Al decreased membrane fluidity in the Al-sensitive fungus, *Amonita muscaria* but an increase in membrane fluidity was observed in the Al-resistant fungus, *Lactorius piperatus.* Al has been shown to bind to phospholipid vesicles and induced phase separation of artificial membranes (Akeson *et al.*, 1989). Aluminium induced conformational changes in plasma membrane proteins have also been reported in roots of Triticum aestivum (Zhang *et al.*, 1996).

A decrease in both phospholipids and oil content accompanied with an increase in both glycerol and lipase activity in sensitive cultivar contendor and resistant cultivar Giza 3 (at 150 μM Al) suggested a degradative process due to Al toxicity. In senescing cotyledons of Phaseolus vulgaris degradation of phospholipids was accompanied by

an increase in diacylglycerols, free fatty acids, long chain aldehydes and long chain hydrocarbons (Yao *et al*., 1991a, b). A reduction in phospholipid and an accumulation of free fatty acids could also result from reduced phospholipid synthesis. In spite of this explanation, the observed increase in lipase activity in sensitive cultivar and in resistant cultivar (at 150 μM Al may suggest a degradative process due to Al toxicity. In this study, Al induced changes in growth, phospholipids, glycerol, oils and lipase activity (Table 1-3), may reflect adaptation of the Al-resistant Giza 3 to stress conditions (Zhang *et al.,* 1996).

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