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ALUMINUM TOXICITY IN TOMATO. PART 2. LEAF GAS EXCHANGE, CHLOROPHYLL CONTENT, AND INVERTASE ACTIVITY

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ABSTRACT: The effect of aluminum (Al) toxicity on leaf gas exchange, leaf chlorophyll content, and sucrose metabolizing enzyme activity of two tomato cultivars (*Lycopersicon esculentum* Mill. 'Mountain Pride' and 'Floramerica') was studied to determine the mechanism of growth reduction observed in a related study (Simon et al., 1994, Part 1). Plants were grown in diluted nutrient solution (pH 4.0) with 0, 10, 25, or 50 μM Al for 16 days. Leaf gas exchange was reduced 2-3 fold in both cultivars as Al concentration increased. Gas exchange of 'Mountain Pride' was more sensitive to Al toxicity than 'Floramerica', agreeing with growth responses observed. Reductions in carbon dioxide (CO_2) assimilation rate appeared to be due to nonstomatal factors in 'Floramerica', but stomatal and non-stomatal limitations in 'Mountain Pride'. Chlorophyll content of leaves was not affected by Al. Acid invertase (AI) and neutral invertase (NI) activity of roots responded consistently to Al concentration in both cultivars. Root AI and NI activity decreased to a greater extent for 'Mountain Pride' than for 'Floramerica'.

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INTRODUCTION

Poor adaptation of plants to acidic soils with high aluminum (Al) content has spurred research targeted at improvement of Al tolerance in crops (Foy, 1988; Howeler, 1991). The physiological bases of Al toxicity and tolerance have been studied extensively in higher plants in an effort to expedite breeding programs (Foy, 1988; Taylor, 1988b). Aluminum toxicity resulted in changes in carbon dioxide (CO_2) assimilation rate and chlorophyll content, and modified the activity of numerous regulatory enzymes in plants (Haug and Shi 1991; Taylor, 1988a; Taylor, 1988b; Roy et al., 1988). However, the relationship between physiological responses of plants to Al stress in both growth or yield remain unclear (Haug, 1984; Haug and Shi 1991; Taylor, 1988a).

Two tomato cultivars were studied in Part I of this series that differed in their sensitivity to Al (Simon et al., 1994, Part I). This study was designed to determine whether the observed growth reductions in the Al-stressed tomato plants resulted from a decline in CO_2 assimilation rate per unit leaf area (A) or a reduction in total leaf area, or both. Also, we examined the influence of Al stress on chlorophyll content and invertase enzyme activity to determine if these factors contributed to the growth response and the differential Al sensitivity of 'Mountain Pride' and 'Floramerica' as observed in Part 1.

MATERIALS AND METHODS

Plant Material and Aluminum Treatment

Tomato plants were grown in a growth chanmber and cultured in diluted nutrient solution (pH 4.0) treated with 0, 10, 25, and 50 μM of Al (as $AlCl_3$) as described previously (Simon et al., 1994, Part 1).

Gas Exchange Measurements

Carbon dioxide assimilation (A) and transpiration (E) were measured by enclosing the entire tomato plant canopy in a 42-L semi-closed gas exchange chamber (Rieger and Motisi, 1990; Rieger, 1992). Environmental conditions were: air temperature, 26.3±1.1°C; dewpoint, 15±1°C; CO_2 concentration, 350±20 μL/L; and photosynthetically active radiation (PAR), 1200-2000 $\mu mol/m^2/s$. Gas exchange measurements were made at the end of the 16-day period of Al exposure on three plants per treatment per cultivar. Leaf area was

determined following gas exchange measurement using a Li-3000 leaf area meter (Li-Cor, Lincoln, NE) (Simon et al., 1994, Part 1).

Chlorophyll Determination

The chlorophyll content of the tomato leaves was determined 16 days after the first Al treatment. Four fully developed leaves (3rd and 4th from top) were collected from the middle section of shoots on each plant. Sixteen leaf discs (0.06-0.13 g, 7-mm diameter) were punched and immersed in 5 mL of N,N-dimethylformamide (Moran and Porath, 1980). After storing the samples in darkness at 4°C for 24 hours, the absorbance of the pigment extract was determined photometrically at 603 nm, 647 nm, and 664 nm. The chlorophyll "a" and chlorophyll "b" content of the leaves was calculated on a fresh weight basis (Moran, 1982). Chlorophyll determinations were repeated three times per treatment.

Invertase Enzyme Extraction and Assays

Freshly harvested tomato roots were used for enzyme extraction following the procedure described by Xu et al. (1989). One to three g of roots were powdered with a mortar and pestle in liquid nitrogen (N_2). The extraction solution was then added to the powdered tissues at a ratio of 3 to 1, volume (mL) to weight (g). One percent (weight to volume, w/v) insoluble polyvinylpyrrolidone (PVP) and 1% (w/v) Dowex-1 (chloride form) were also added to homogenize the powdered tissues. The extraction solution contained 200 mM Hepes/NaOH (pH 7.8), 3 mM Mg acetate, 5 mM dithiothreitol (DTI), 1% (w/v) soluble PVP-40, and 5% (v/v) glycerol. The homogenate was passed through one layer of Miracloth and centrifuged at 34,000 g for 20 min at 4°C. The supernatant was then desalted on a Sephadex G-25 column using a wash buffer of 25 mM Hepes/NaOH (pH 7.5), 2.5 mM Mg acetate, 2.5 mM DTT, and 10% glycerol.

All enzyme assays were performed immediately after extraction to avoid any loss of activity. The assays for soluble acid invertase [EC 3.2.1.26; AI], and neutral invertase [EC 3.2.1.26; NI] were according to an amended procedure (Xu et al., 1989); AI and NI were assayed with 25 mM and 100 mM of sucrose at pH 5.0 and 7.0, respectively. The incubation time was 15 min at 25°C, and the reaction was ended by boiling for seven min. All enzyme activities measured were linear relative to incubation time and plant extract amount. Protein contents in

extracts were measured as described by Bradford (1976). Enzyme activity assays were repeated one to four times per treatment for each cultivar.

Statistics

Data were analyzed by linear regression ad orthogonal contrast (SAS, 1988).

RESULTS AND DISCUSSION

Effect of Aluminum on Gas Exchange Parameters and Chlorophyll Content in Tomato Leaves

Leaf gas exchange in 'Floramerica' was less sensitive to Al stress than for 'Mountain Pride' (Table 1) which is in agreement with the differential sensitivity in growth response to Al between these two cultivars (Simon et al., 1994, Part 1). In 'Mountain Pride', CO_2 assimilation rate (A), transpiration rate (E), and leaf conductance (g) were significantly reduced at the 25 and 50 μM Al treatment levels.

Intercellular CO_2 concentration (Ci) of the 'Mountain Pride' leaves was the same for the 0 and 25 μM levels of Al, suggesting that the decline in A at 25 μM Al was not caused by the reduction in g observed in this treatment. The lower Ci at 50 μM Al suggests that stomatal closure was at least partially responsible for the reduction in A in this treatment for 'Mountain Pride'. Therefore, stomatal closure at 25 μM Al may have been a secondary effect of Al stress, the result only after carboxylation capacity of the leaves was affected.

In 'Floramerica', only the 50 μM Al level significantly reduced leaf gas exchange (Table 1). No change was recorded in Ci among Al treatments, yet over a 2-fold decrease was found in A at 50 μM Al, suggesting a non-stomatal limitation to A with 'Mountain Pride'.

Growth (Simon et al., 1994, Part 1) and A of the two cultivars were similarly affected by Al. In the absence of Al, 'Mountain Pride' had higher A and growth than 'Floramerica', and both A and growth of 'Mountain Pride' were reduced by Al stress more than for 'Floramerica'. In 'Mountain Pride', differences in dry weight between the 0 and 10 μM Al treatments appeared to be due to lower leaf area per plant in the 10 μM treatment, since A was not different between these treatments. However, a combination of reduced leaf area and assimilation capacity resulted in growth reduction at 25 and 50 μM Al. In 'Floramerica', plant dry weight was affected only when A was reduced (50 μM Al only), and leaf area was

Table 1. Gas exchange parameters for two tomato cultivars grown in nutrient solution containing Al for 16 days. A = CO_2 assimilation rate; E = transpiration rate; WUE = water use efficiency; g = leaf conductance to water vapor; C_i = intercellular CO_2 concentration.

Treatment (μM Al)	A (μmol/m^{2}s)	E (mmol/m^{2}s)	WUE (x 10^{-3})	g (mmol/m^{2}s)	C_i (μl/l)
'Mountain Pride'					
0	14.8	3.7	3.9	317	277
10	14.2	3.8	3.7	350	288
25	5.4	2.2	2.6	153	280
50	5.3	0.9	6.1	59	182
Prob > F	0.0008	0.0001	0.1951	0.0008	0.0345
r^2	0.73	0.84	0.18	0.73	0.41
Contrasts:					
0 vs. 10	ns	ns	—	ns	ns
0 vs. 25	*	**	—	*	ns
0 vs. 50	**	**	—	**	*
'Floramerica'					
0	12.0	3.2	3.8	196	260
10	11.8	3.6	3.3	228	265
25	12.2	3.8	3.2	270	281
50	5.2	1.7	3.1	75	262
Prob > F	0.0011	0.0151	0.0884	0.0276	0.7265
r^2	0.67	0.46	0.26	0.40	0.01
Contrasts:					
0 vs. 10	ns	ns	—	ns	—
0 vs. 25	ns	ns	—	**	—
0 vs. 50	**	**	—	**	—

Gas exchange data are means of 3 replications. Orthogonal contrast analysis: ns, *, ** represent P>.05, P<.05, P<.01, respectively.

Table 2. Effect of Al concentration on leaf chlorophyll content of two tomato cultivars grown in nutrient solution containing Al for 16 days.

Treatment (μM Al)	Chlorophyll a	Chlorophyll b	Chl a + b
	($mg \cdot g^{-1}$)		
	'Mountain Pride'		
0	1.28[z]	0.40	1.68
10	1.28	0.43	1.70
25	0.84	0.29	1.12
50	1.03	0.33	1.36
	ns[y]	ns	ns
	'Floramerica'		
0	1.09	0.32	1.41
10	1.34	0.43	1.76
25	1.03	0.33	1.36
50	1.02	0.30	1.32
	ns	ns	ns

[z] Chlorophyll data are means of 3 replications.

[y] Linear regression analysis: ns, *, ** - $P > 0.05$, < 0.05, < 0.01, respectively.

reduced at lower Al concentrations than A. This suggests that leaf expansion or leaf production rate was more sensitive to Al than photosynthesis or leaf conductance. Furthermore, the mechanism of growth reduction in tomato may be cultivar dependent, and appears to be linked to photosynthetic capacity in the Al-tolerant 'Floramerica'. The relationship between growth reduction and photosynthesis may have practical importance in breeding for Al-tolerant tomato cultivars.

Water use efficiency (WUE) was not affected by Al in either cultivar, suggesting that stomatai closure reduced E in portion to the reduction of A (Table 1).

Aluminum had no influence on leaf chlorophyll content of either tomato cultivar (Table 2). This is in contrast to reports of either increased chlorophyll content in *Scenedesmus* algae (Greger et al., 1992) or decreased chlorophyll in sorghum and wheat (Ohki, 1986) in response to Al. However, reductions in chlorophyll in these species occurred at nutrient solution Al concentrations above those used in the current study (> 75 μM).

Our results confirm that Al can reduce A and E of leaves, yet suggest that a reduction in A is not necessarily prerequisite for growth reduction. Most of the Al was found in the roots (Simon et al., 1994, Part 1), suggesting the action of Al on leaf gas exchange was indirect. Since chlorophyll content was not affected by Al, the non-stomatal limitations to A observed must have been the result of reduced function of photosynthetic components other than chlorophyll.

Effect of Aluminum on Invertase Activity in Tomato Roots

Sucrose synthase [EC 2.4.1.13; SS], pyrophosphate-dependent phospho-fructokinase [EC 2.7.1.90; PPi-PFK], and ATP-dependent phosphofructokinase EC 2.7.1.11; ATP-PFK] activities were similar among Al treatments in roots and leaves of both cultivars, and no significant differences were observed in AI and NI activities in the leaves (data not shown).

Of the five enzymes assayed, only AI and NI activity showed consistent responses to Al in the roots of both cultivars (Table 3). AI activity from 'Mountain Pride' was more sensitive to Al at 25 and 50 μM Al than that of 'Floramerica', which is in agreement with the response for gas exchange and growth. Similarly, NI activity in 'Mountain Pride' roots decreased to a greater extent than NI in 'Floramerica' roots (Table 3).

Aluminum activity has been associated with pea (*Pisum sativum* L., Lyne and ap Rees, 1971) and corn (*Zea mays* L., Hellebust and Forward, 1962) root cell elongation and expansion. No definitive role of NI in plants has been suggested and most plant tissues surveyed did not have much NI activity, except in some that have high AI activity (Lyne and ap Rees, 1971; Hellebust and Forward, 1962). In the first study, root length decreased with increasing Al (Simon et al., 1994, Part 1), so the decreased AI and NI activities observed here with increasing Al supports the role of invertases in tissue elongation.

In 'Mountain Pride', root AI and NI activities were highly correlated (r^2 = 0.97) with photosynthesis, although the correlations were nonsignificant for

Table 3. Effect of Al concentration on specific activities of acid invertase and neutral invertase enzymes from roots of two tomato cultivars grown in nutrient solution containing Al for 16 days.

Treatment (μM Al)	Specific activity (nmol min^{-1} mg protein^{-1})	
	Acid Invertase	Neutral Invertase
	'Mountain Pride'	
0	153	58
10	145	46
25	39	20
50	21	15
Linear regression[z]:		
Prob > F	.0100	.0230
r^2	0.76	0.68
	'Floramerica'	
0	150	57
10	93	33
25	61	40
50	55	31
Linear regression:		
Prob > F	.0009	.0281
r^2	0.69	0.40

[z] Analysis performed on means of pooled tissue samples for 'Mountain Pride' due to insufficient quantity of tissue for enzyme activity assays for high Al treatments.

'Floramerica'. Since AI and NI activities are indicators of sink strength, this suggests that A in leaves was closely associated with sink strength of roots for the Al-sensitive 'Mountain Pride'. Lack of sucrose utilization in Al-stressed plants may have resulted in leaf starch accumulation, and a reduction in A through feedback inhibition (Gucci and Flore, 1989).

Sung et. al. (1988,1989) and Xu et al. (1989) identified two suites of enzymes; those that adapt their activities to the developmental/environmental

changes, and those that do not. The "adaptive enzymes" include SS, AI, and PPi-PFK, which can have up to a 20-fold change in activity within a few days (Sung et al., 1988; Sung et al., 1989). NI and ATP-PFK enzymes are examples of "maintenance enzymes" which do not have definitive fluctuations in activity in response to developmental changes and environmental stresses. In this context, the adaptive enzymes to Al stress in tomato were AI and NI, and the maintenance enzymes were SS, PPi-PFK, and ATP-PFK. The reasons for the apparent role reversal of SS, PPi-PFK, ATP-PFK, and NI enzymes in tomato compared to plants studied by Sung et al. (1988,1989) are unclear.

The growth, gas exchange, and sucrose metabolizing enzyme activity of Al-stressed tomato may be interrelated as follows: Al accumulates in roots where tissues are damaged, resulting in reduced sucrose utilization. This, in turn, causes leaf carbohydrate accumulation and feedback inhibition of photosynthesis. Concomitant with root growth reduction may be a reduction in number and area of leaves, since reduced water supply, nutrient uptake, and growth regulator production from Al-stressed roots would not support normal shoot growth (Pan et al., 1988). Thus, the primary, direct effects of Al stress on tomato roots warrant further study, perhaps through reciprocal grafting of tolerant and intolerant genotypes.

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