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Changes caused by aluminum in protein and carbohydrate contents in the apex of maize seminal roots

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ABSTRACT. Acid soils contain aluminum that hinders the development of nontolerant plants due to a decrease in roots mitotic activity. This work determined changes caused by aluminum in protein and carbohydrate total contents in the apex of maize seminal roots. Seedlings from tolerant and nontolerant hybrids were grown in nutrient solution containing four aluminum concentrations. Although there was no interaction between genotypes and aluminum concentrations, aluminum levels caused different effects on protein and carbohydrate contents. In the presence of aluminum, changes were induced in both, even though degree of the response seemed to be independent from any ion concentration. Total protein contents were similar in the two genotypes. Although the two hybrids were similar in the absence of aluminum, the nontolerant hybrid accumulated a greater quantity of carbohydrates in the presence of aluminum. Carbohydrate contents in seminal roots seemed to be an expression of nontolerance that differentiates the maize genotypes.

Key words: aluminum tolerance, maize, proteins, carbohydrates.

RESUMO. Alterações induzidas pelo alumínio nos conteúdos de proteínas e carboidratos no ápice de radículas de milho. Os solos ácidos contêm alumínio que dificulta o desenvolvimento de plantas sensíveis pela diminuição da atividade mitótica nas raízes. Neste trabalho objetivou-se evidenciar alterações, induzidas pelo alumínio, nos conteúdos de proteínas e carboidratos no ápice da radícula de milho. Plântulas de dois híbridos, um tolerante e um sensível, foram desenvolvidas em solução nutritiva com quatro concentrações de alumínio. Não houve interação entre genótipos e concentrações de alumínio, mas os níveis de alumínio influenciaram diferentemente os conteúdos de proteínas e carboidratos. A presença do alumínio induziu alterações nas duas características, mas a magnitude da resposta pareceu ser independente da concentração do íon. A resposta para proteínas não diferiu nos dois genótipos. Apesar da semelhança dos dois híbridos na ausência de alumínio, o sensível acumulou maior quantidade de carboidratos na presença do alumínio. O conteúdo de carboidratos em radículas expostas ao alumínio pareceu ser uma expressão da sensibilidade que discrimina genótipos de milho.

Palavras-chave: tolerância ao alumínio, milho, proteínas, carboidratos.

Acid soils contain soluble aluminum available to plants, and its toxic effect hinders the agricultural exploitation of these areas with nonadapted species (Foy *et al.*, 1978; Lopes, 1984). In Brazil, these acidic soils characterize the open lands called *cerrado*, which almost represent one fourth of Brazilian territory. Aluminum has various phytotoxic effects on the metabolism of tolerant plants. Several studies have shown that metabolic and structural damages are serious and frequently result in severe decrease in productivity (Bennet and Breen, 1991; Delhaize and Ryan, 1995; Kochian, 1995).

Multiplicity of effects causes difficulties in the identification of primary response of nontolerant plant exposed to aluminum. An easily recognizable effect is a decrease of mitotic activity in root meristem. Consequently, the plant forms only surface roots which do not penetrate the deepest layers of the soil. Deficient development of roots decreases uptake of soil nutrients and, at the same time, the plant becomes less tolerant to long periods of droughts (Foy *et al.*, 1978).

The causes of different tolerance levels in plants are still unknown and various suggestions have been forwarded to explain the fact (Foy *et al.*, 1978; Rauser and Curvetto, 1980). Urrea-Gomes *et al.* (1996) suggested that different mechanisms may be involved in aluminum tolerance of maize. One of them may be the release of organic acid by root apex, that would join to aluminum in the rizosphere (Pellet *et al.*, 1995). Tolerance may not be attributed to only one physiological factor but it may be the result of various concomitant physiological processes.

One of the difficulties in aluminum tolerance studies is the criterion adopted to differentiate tolerant and nontolerant genotypes. The most frequently used criteria in investigating tolerance in grasses are root characteristics, especially its length (Garcia *et al.*, 1979; Magnavaca, 1982; Rhue and Grogan, 1976; Silva and Furlani, 1976). In maize, there is practically no displacement of aluminum towards the aerial part (Magnavaca, 1982; Rasmussen, 1968). This statement reinforces the conclusion reached by Silva and Furlani (1976) and Magnavaca (1982), namely, that dry weight of top parts of maize seedlings grown in the presence of aluminum in nutrient solution is not a good attribute for tolerance studies. Thus, it is recommendable that genetic studies of tolerance may be based on the level of aluminum toxic effects on seminal roots, as a whole or in part.

There are indications that aluminum is associated with variations in the accumulation of some protein fractions in roots and coleoptile of maize and wheat (Huttova *et al.*, 1998; Mistrik *et al.*, 1997; Richards and Gardner, 1994; Snowden *et al.*, 1995). Using two-dimensional electrophoresis, Basu *et al.* (1994) detected two membrane polypeptides in wheat. Their synthesis was induced specifically by aluminum. It has been inferred that these two polypeptides may play a relevant role in aluminum tolerance. However, stress induced by aluminum did not cause any difference in the protein electrophoretic patterns of maize (Prioli, 1987) and wheat (Basu *et al.*, 1994), either in nontolerant or in tolerant genotypes.

It has been demonstrated that aluminum toxicity results in an intense deposition of (1,3) β-glucan or callose in dicotyledon roots (Massot *et al.*, 1999; Wissemir *et al.*, 1987), as well as in monocotyledon roots, such as wheat (Schreiner *et al.*, 1994) and maize (Horst *et al.*, 1997). Aluminum stimulates accumulation of greater quantities of callose in nontolerant genotypes as compared with tolerant ones. This fact suggests that such a characteristic may be useful as an indicator of aluminum tolerance in plants. In all plant groups, nontolerant roots exposed to aluminum become thick and fragile (Foy *et al.*, 1978). Since protein and carbohydrates are among the most abundant root components, it is plausible to suppose that changes in their quantities and rates would be influenced by aluminum. Such possible changes seem to be related to the root tissues changes induced by aluminum toxicity.

This work shows that aluminum causes modifications in total carbohydrate contents in the apex of maize roots, which could be useful as an indicator to discriminate tolerant and nontolerant maize genotypes.

Material and methods

Plant materials. In this study, two F_1 hybrids of maize were used. They were obtained by crossing inbred lines from the Germoplasm Bank of the Department of Genetics and Evolution, State University of Campinas, SP, Brazil. These inbred lines were evaluated and characterized as aluminum tolerant and nontolerant by Prioli (1987). The tolerant hybrid, F_1(L922 x L902), was the result of a cross between inbred lines L922 (also called Cat100-6) and L902, both highly tolerant to aluminum toxicity. These two lines were derived from Cateto, a flint endosperm autochthonous race adapted to the Southern Atlantic Coast of South America. The nontolerant hybrid, F_1(Ast214 x L16-B), was obtained from crosses between inbred lines Ast214 and L16-B, both nontolerant to aluminum toxicity. These two inbred lines were derived from Tuxpeño, a dent race adapted to the coastal region of the Gulf of Mexico.

Nutrient solution and aluminum levels. A modified nutrient saline solution suggested by Clark (1975) was used, prepared with distilled and deionized water. The nutrient solution consisted of: 3.43 mM $Ca(NO_3)_2.4H_20$, 1.27 mM NH_4NO_3, 0.55 mM KCl, 0.56 mM K_2SO_4, 0.83 mM $Mg(NO_3)_2.6H_2O$, 32.33 μM KH_2PO_4, 61.51 μM $FeSO_4$, 47.29 μM EDTA, 8.28 μM $MnCl_2.4H_2O$, 23.1 μM H_3BO_3, 2.14 μM $ZnSO_4.7H_2O$, 0.56 μM $CuSO_4.5H_2O$, 0.75 μM $Na_2MoO_4.2H_20$. This solution has nitrate/ammonia equilibrium so that pH remains stable throughout the experiment period, completely avoiding corrections with acids and bases. Aluminum was added to the nutrient solution as a double salt $KAl(SO_4)_2.12H_2O$, and the pH was adjusted to 4.0, according to need. Aluminum final concentrations were zero, 2.25, 4.5 and 6.75 μg/mL of Al^{3+}. As demonstrated by Prioli (1987), 4.5 μg/mL of aluminum is the adequate concentration of this ion for discrimination of tolerant and nontolerant maize genotypes; therefore, the amplitude and spacing of this established gradient are suitable for genotype evaluations.

Seed germination and growth room. Seeds were germinated on paper, in a dark room at controlled temperature of 26 ±1°C. Seedlings, with seminal roots of approximately 2 cm, were transferred to floating polystyrene plates (styrofoam). These plates were placed on nutrient solution with and without aluminum, in plastic containers. Seminal roots remained immersed in the solution, and oxygenation was maintained by continuous aeration. The volume was kept constant by daily addition of distilled and deionized water. Temperature of growth room was kept at 26 ±1°C, with 14 hours light and 10 hours dark photoperiod. Light intensity at seedlings level reached approximately 250 $\mu E.m^{-2}.s^{-1}$.

Plant measurements and calculated variables. Seminal root tips (1 cm) were collected from seedlings after seven days in nutrient solution. Protein and carbohydrate total contents were measured in mg/g tissue respectively by the Bradford method and by the colorimetric method, as suggested by Dubois *et al.* (1956).

Experimental design and statistical analysis. Experiment followed completely randomized design with three replications and factorial system. Factors consisted of genotypes and aluminum, with 2 and 4 levels respectively. The experimental unit consisted of 10 seedlings. Variance analysis (Steel and Torrie, 1980) and linear regression plateau were performed with Saeg software (Euclides, 1993) were used.

Results and discussion

Protein and carbohydrate contents in apexes of seminal roots of aluminum-tolerant and aluminum-nontolerant F_1 hybrids are shown in Table 1. Results from analysis of variance, including all aluminum concentrations, are shown in Table 2. Within each genotype, changes due to variation in aluminum concentrations were symmetrical, both in total protein and total carbohydrate contents (Figure 1, A and B). In the absence of aluminum, root tips of both genotypes were similar in total protein content, as well as in total carbohydrate content. In the presence of aluminum, there was a decrease of total protein content in root tips of both tolerant and nontolerant plants. The decrease in total protein was approximately the same in all three aluminum levels, and did not differ significantly between the two genotypes. On the other hand, the presence of aluminum induced an increase of carbohydrate contents in the tolerant and nontolerant genotypes, independently of ion concentration in the nutrient solution.

Table 1. Averages of total protein and carbohydrate contents (mg/g tissue) in the apex of seminal roots of maize seedlings of a tolerant and a nontolerant hybrid, grown for seven days in a nutrient solution containing different aluminum concentrations

Aluminum	Protein		Carbohydrate	
(μg/mL)	Tolerant	Nontolerant	Tolerant	Nontolerant
Zero	53.61	59.16	15.58	17.33
2.25	45.31	43.47	26.56	35.30
4.50	45.30	43.76	25.07	35.15
6.75	48.32	46.72	23.98	31.45

Table 2. Analysis of variance of total protein and carbohydrate contents (mg/g tissue) in the apex of seminal roots of seedlings of a tolerant and of a nontolerant hybrid of maize grown for seven days in nutrient solution containing aluminum concentrations of zero, 2.25, 4.5 and 6.75 μg/mL

Source of Variation	Degree of Freedon	Mean Square	
		Protein	Carbohydrate
Aluminum (A)	3	190.98**	269.87**
Genotype (G)	1	0.12n.s.	294.61*
A x G	3	19.52n.s.	20.12n.s.
Error	16	24.06	41.57
CV (%)		10.2	24.5

n.s. Non-significant at 5% probability level by *F*-test; * Significant at 5% probability level by *F*-test; ** Significant at 1% probability level by *F*-test

Table 3. Analysis of variance of total carbohydrate contents (mg/g tissue) in the apex of seminal roots of seedlings of a tolerant and of a nontolerant hybrid of maize grown for seven days in nutrient solution containing aluminum concentrations of 2.25, 4.5 and 6.75 μg/mL.

Source of Variation	Degree of Freedon	Mean Square
Aluminum (A)	2	16.76n.s.
Genotype (G)	1	345.26*
A x G	2	2.56n.s.
Error	12	54.26
CV (%)		24.9

n.s. Nonsignificant at 5% probability level by *F*-test; * Significant at 5% probability level by *F*-test

Results of analysis of variance (Table 2) revealed no interaction between genotypes and aluminum, in variations of protein and carbohydrate, at all aluminum levels. Absence of interaction suggests that the tolerant and nontolerant genotypes differed only in the magnitude of the response to aluminum toxicity. The same response was found for both genotypes, without any significant changes in the degree of difference within total protein content, as well as total carbohydrate content. Lack of interaction between genotypes and aluminum levels can be observed in Figure 1 (A and B).

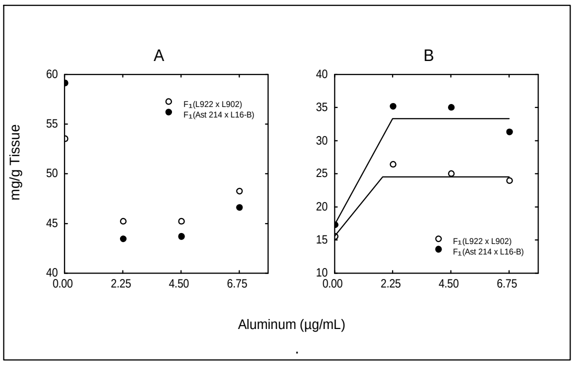

Figure 1. Comparison of a tolerant hybrid F_1(L922 x L902) and a nontolerant F_1(Ast 214 x L16-B) of maize with regard to total protein **(A)** and total carbohydrate **(B)** contents, in the apex of seminal roots of seedlings developed for seven days in a nutrient solution with different aluminum concentrations. In **B,** graphics of equations of linear response plateau of tolerant ($Y = 15.58 + 4.88X$/ Plateau = 24.52; R^2 = 90.32%) and nontolerant hybrid ($Y = 17.33 + 7.99X$/ Plateau = 33.30; R^2 = 83.92%) are shown.

As previously remarked, protein contents of both genotypes are reduced in the presence of aluminum (Table 1). Contrary to expectations, variations in protein concentrations in the two genotypes along the aluminum concentration range were almost equal (Table 1). Results shown in Figure 1A reveal this similarity between hybrids, with a pronounced decrease in protein contents caused by aluminum in all concentrations. The same response of hybrids along the range of aluminum concentrations indicates the nonsignificance of genotypes (Table 2). However, according to Prioli (1987), in aluminum concentrations up to 4.5 µg/mL no decrease occurs in the length of the seminal roots of highly tolerant genotypes, such as hybrid F_1(L922 x L902). Consequently, total protein content may not be directly related to the growth of seminal roots in the presence of aluminum, the usual characteristic for indication of tolerance.

In the absence of aluminum, tolerant and nontolerant genotypes had approximately equal total carbohydrate contents. However, the carbohydrate contents in root tips of these two genotypes differed significantly when the seedlings were grown in the presence of aluminum. There was an increase in carbohydrates in the two hybrids when aluminum was present; however, their accumulation was clearly higher in the nontolerant genotype. Discrepancy between the hybrids is demonstrated by the significance of the mean square of genotypes evaluated by carbohydrate contents (Table 2). At least part of the increase of carbohydrate rate in the presence of aluminum may be attributed to a differential production of callose, as suggested by Horst *et al.* (1997).

Increase in carbohydrate contents was detected in seminal root apex of seedlings grown in nutrient solution containing the lowest aluminum concentration (2.25 µg/mL). However, changes in aluminum concentrations, ranging from 2.25 to 6.75 µg/mL, did not influence total carbohydrate contents in the apex of seminal roots (Tables 1and 2; Figure 1B). This relatively stable response, independent of aluminum levels, suggests that genotypes are more efficiently discriminated by levels of carbohydrate concentrations, in the presence of aluminum, than by adjustment of regression equations of degree higher than one. Usually, in the analysis of variance a nonsignificant genotype x aluminum interaction would lead to the determination of a single regression equation, which results from pooling the observations of both genotypes. However, if this procedure were adopted here, it would result in losing the major interest in the objective of this study, which is the magnitude of response of each contrasting genotype. Therefore, for comparison between tolerant and nontolerant genotypes one regression equation was determined for each one of the hybrids studied. In this sense, analysis of discontinuous regression, or linear response plateau (Euclides, 1993), was applied to each genotype (Figure 1B). Significant difference in plateau values of genotypes indicates that carbohydrate contents in the apex of maize seminal roots could be used as a discriminating characteristic for tolerant and nontolerant genotypes. Inbred lines, hybrids or individual maize plants in segregant populations may be identified by means of a single aluminum concentration.

The similarity of response in different aluminum concentrations demonstrates that the genetic and physiological mechanisms of tolerance are not related to the synthesis or metabolism of carbohydrates. Accumulation of compounds may probably be the result of structural or metabolic disorders, that could be indirectly caused by aluminum. Both genotypes were not contrasting and increased differentially the carbohydrate contents in the presence of aluminum. These results suggest that the presence of aluminum causes effects that are qualitatively similar, but much less pronounced, in the tolerant genotype as compared with the nontolerant one.

It seems that the protein and carbohydrate contents are not involved in the expression of tolerance. Mechanisms of tolerance would not protect the synthesis and/or accumulation of proteins in the apex of seminal roots. There is no evidence that the metabolism of carbohydrates is one of the components of mechanisms of aluminum tolerance in maize. It seems that these mechanisms of tolerance prevent that the disarrangements in the synthesis and/or accumulation of carbohydrates in tolerant genotypes would reach the higher levels observed in nontolerant genotypes. Thus, carbohydrate accumulation in the apex of seminal roots of seedlings exposed to aluminum may be interpreted as a sign of nontolerance to aluminum and not of tolerance.

The results obtained in this work suggest that the increase of total carbohydrate contents in the apex of maize seminal roots in the presence of aluminum is useful as an indicator to discriminate tolerant and nontolerant maize genotypes.

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