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Inheritance of aluminum tolerance in maize

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ABSTRACT – *The purpose of this study was to investigate aluminum tolerance in maize. For this purpose, a nutrient solution was used with the tolerant (L922) and sensitive (Ast214) homozygous parental lines, with the segregating generation F_2 and with the $F_{2,3}$ lines derived thereof. Seeds were germinated on paper and seedlings transferred to a nutrient solution containing 4.5 mg L^{-1} aluminum. In the experiments involving $F_{2,3}$ derived lines groups of rows with $F_{2,3}$ plants were intercropped with rows of eight seedlings of the parental lines in a completely randomized block design. Results of the F_2 generation demonstrated a bimodal distribution of relative frequencies, with approximately three times more seedlings in the tolerant group. This suggests the participation of only one locus in tolerance inheritance. The narrow-sense heritability (F_2 , $F_{2,3}$) of seminal root length of F_2 plants and means of $F_{2,3}$ lines was 0.49, which partially explained the bimodal frequency distribution of means of $F_{2,3}$ lines, but was not accurate enough to differentiate tolerant from sensitive groups.*

Key words: *Zea mays* L, seedlings, nutrient solution, heritability.

INTRODUCTION

Approximately one third of the Brazilian territory is part of the Cerrado ecosystem, where a great part of the agricultural production is grown on highly acid soil. The low pH contributes to solubilize aluminum and make it available in the soil for plant assimilation, causing severe damage to non-adapted genotypes. The main effect is a slow growth of the root system resulting in the development of surface roots. This hampers the use of soil nutrients and makes plants more drought-susceptible (Foy et al. 1978, Piñeros et al. 2005, Hartwig et al. 2007).

Liming is an alternative to reduce aluminum toxicity, because it increases soil pH. However, this

process is economically unfeasible in deeper layers. Therefore, pH standardization occurs only in a restricted top soil layer, while the sub-surface layers with toxic aluminum remain acid (Custódio et al. 2002). A possible alternative would be to combine liming with the use of more aluminum tolerant genotypes.

The fact that the tolerance of maize (*Zea mays* L.) to aluminum toxicity is genetically determined and that tolerance inheritance studies must be based on characteristics of the root system are acknowledged (Rhue et al. 1978, Foy et al. 1978). The aluminum tolerance inheritance in nutrient solution was characterized as monogenic in some studies (Jorge and Arruda 1997, Prioli et al. 2002). Rhue et al. (1978) and

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Garcia and Silva (1979) also concluded that tolerance is determined by one dominant locus for sensitivity. On the other hand, the data of Miranda et al. (cited by Miranda et al. 1984), using nutrient solution, indicated that tolerance to aluminum toxicity is inherited by two dominant genes.

In contrast to the simple inheritance model, other studies indicated that aluminum tolerance is a quantitatively inherited trait, suggesting that tolerance is dominant over susceptibility (Magnavaca et al. 1987, Kochian et al. 2004). In a study using RFLP in line L922, Sibov et al. (1999) also found indications of the involvement of two loci (or two groups) located on chromosomes 6 and 10. The use of RFLP allowed Brondani and Paiva (1996) to associate aluminum tolerance with a gene or a block of genes on chromosome 2, and Torres et al. (1997) associated chromosome 8 to aluminum tolerance.

Moreover, Ninamango-Cárdenas et al. (2003) identified five genomic regions presumably related to maize aluminum tolerance, confirming that this trait is quantitatively inherited and controlled by few genes. According to the data of this study, four Quantitative Trait loci (QTLs) were identified for aluminum resistance in maize on chromosomes 2, 6 and 8.

Dissimilar results in inheritance of aluminum tolerance may be due to the germplasm used. The genotypic constitutions of germplasm can generate a differentiated and, apparently, conflicting phenotypic expression. Therefore, it is necessary to increase knowledge on the inheritance mechanisms of aluminum tolerance in maize, since the results reported in the literature are conflicting and inconclusive. The goal of this study was to evaluate aluminum tolerance inheritance in maize in nutrient solution, with homozygous parental generations, segregating F_2 generations and their $F_{2:3}$ lines.

MATERIAL AND METHODS

Two inbred lines, chosen with regard to their susceptibility to aluminum toxicity, were grown at the Universidade Federal de Campinas, Campinas, Brazil. Among the tolerant strains, L922 from the race Cateto achieved the highest tolerance degree. Inbred line Ast214, derived from germplasm Tuxpeño, was used as aluminum-sensitive and divergent genotype.

Seeds were obtained from the lines L922 and

Ast214 and from hybrid F_1 (L922 x Ast214), by controlled pollination, in the growing season 2004/05 on an experimental area of the Universidade Estadual de Maringá. New seed strains of the lines L922 and Ast214, produced in the 2005/06 growing season, were evaluated for the aluminum response in nutrient solution in 291 F_2 seedlings.

Seeds were germinated on filter paper soaked with distilled water in a dark chamber, at a controlled temperature of $26 \pm 1^\circ\text{C}$, for about three days, until the seminal root length reached approximately 2.5 cm. Seedlings were transferred to nutrient solution with aluminum for development in growth chambers. All experiments of aluminum response evaluation were carried out in chambers with controlled photoperiod (14/10 hours of dark/light) and temperature ($26 \pm 1^\circ\text{C}$). Artificial illumination was provided by fluorescent light bulbs at a light intensity of $350 \mu\text{E s}^{-1}$.

Seedlings were grown for 10 days in nutrient solution, according to Clark (1977) with some modifications (Prioli et al. 2002), with 4.5 mg L^{-1} aluminum and forced aeration. This dose is appropriate for discrimination of tolerant and non-tolerant maize genotypes. Aluminum was supplied in the form of double salt $\text{KAl}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$, with pH around 4.0.

After this period of exposure, each seedling was individually identified and seminal roots were measured to determine the final length and calculate the net length. Immediately after the evaluation, all F_2 seedlings were transferred to separate plant holes. These plants were self-pollinated and produced 158 $F_{2:3}$ lines. These lines were evaluated in the nutrient solution described above, with the same Al concentration. Between the rows of $F_{2:3}$ plants, parental lines were intercropped using a random experimental design (Cruz and Carneiro 2003).

The $F_{2:3}$ lines were disposed on floating dishes in rows composed of eight seedlings. Ten rows of seedlings of the parent lines L922 and Ast214 were randomly interspersed between them. A total of 1251 $F_{2:3}$ seedlings, 70 L922 and 80 Ast214 were evaluated.

Data analysis was performed using software Genes (Cruz 2001). The additive variance, the variance caused by dominance, the narrow-sense heritability between and within lines and the total narrow-sense heritability were estimated. The genetic progress was predicted for selection between and within $F_{2:3}$ lines. Moreover, the

expected gains with mass selection and combined selection were estimated.

RESULTS AND DISCUSSION

The means of seminal root length of the parental inbred lines L922 and Ast214, growing in plots of F_{2:3} lines, were 19.14 and 7.18 cm, respectively. This indicates a differential response to aluminum toxicity, as well as a substantial difference to the performance in absence of aluminum, where Ast214 develops roots approximately 30% longer than L922. Consequently, in the experimental conditions, aluminum-tolerant and aluminum-sensitive maize genotypes could be discriminated.

The mean seminal root length of F₂ seedlings was 18.06 cm (37% greater) while the mean length of parental lines was 13.16 cm. Therefore there is an expression of heterosis at high Al levels for seminal root length in the hybrid. However, this heterosis should not be associated to aluminum tolerance only.

It is possible to determine a bimodal frequency distribution based on the F₂ results (Figure 1a), with approximately three times more seedlings in the tolerant group than in the sensitive group. This result confirms the distribution frequency of seminal root length observed by Prioli et al. (2002), who also used L922 and Ast214 inbred lines. The proportion 3:1, which suggests that only one locus is involved in inheritance and dominance of aluminum tolerance, was also reported in previous studies analyzing segregation of the F₂ generation (Rhue et al. 1978, Garcia and Silva 1979, Jorge and Arruda 1997).

Compared to the study of Prioli et al. (2002), further information on the lines L922 and Ast214 was obtained

in the present study using F_{2:3} lines. It is noticeable that the asymmetry of the frequency distribution of F_{2:3} lines (Figure 1b) was similar to bimodal distribution. However, the relationship between the expression of aluminum tolerance of F₂ plants and the F_{2:3} lines was not strong. In fact, there is an association between root length values of the F₂ generation and mean root length values of F_{2:3} lines (Figure 2). In some F_{2:3} lines, generated by the 20% more tolerant F₂ plant groups, the performance was worse than in F_{2:3} lines derived from the 20% more aluminum-sensitive F₂ plant groups.

Once the regression value between two generations is known, the heritability in the narrow sense can be estimated as a result of the ratio $h^2_{F_1, F_{1+1}} = b / (2r_{F_1, F_{1+1}})$ (Cruz 2001, Hallauer and Miranda Filho 1982). If the F₁ generation was obtained by hybridization, as in this case, then the estimated heritability for F₂ and F_{2:3} generations is $h^2_{F_2, F_3} = b / (2r_{F_2, F_3}) = b$. Therefore, for F₂ and F_{2:3} generations from the crossing of L922 and Ast214 inbred lines, the narrow-sense heritability for root length must be equal to the regression coefficient between values of heritability of both lines, or $h^2_{F_2, F_3} = b = 0.49$.

The dispersion of F_{2:3} lines for tolerance and susceptibility selection (Figure 2) is reflected by the magnitude of the narrow-sense heritability of 0.49. This value for the heritability of root length of maize seedlings developed in nutrient solution with aluminum can be considered low compared to those reported in literature obtained by generation means and variance analysis (Magnavaca et al. 1987, Sawazaki and Furlani 1987, Prioli et. 2000).

Broad and narrow-sense heritability estimates can also be established by other genetic and statistical

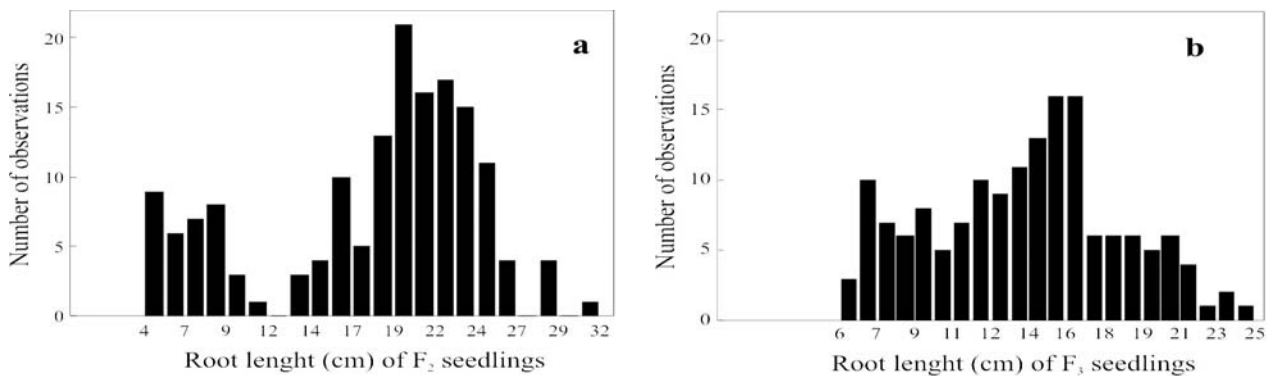


Figure 1. Frequency distribution of root length in F₂ and F_{2:3} (or F₃) seedlings grown for 10 days in nutrient solution with 4.5 mg L⁻¹ aluminum

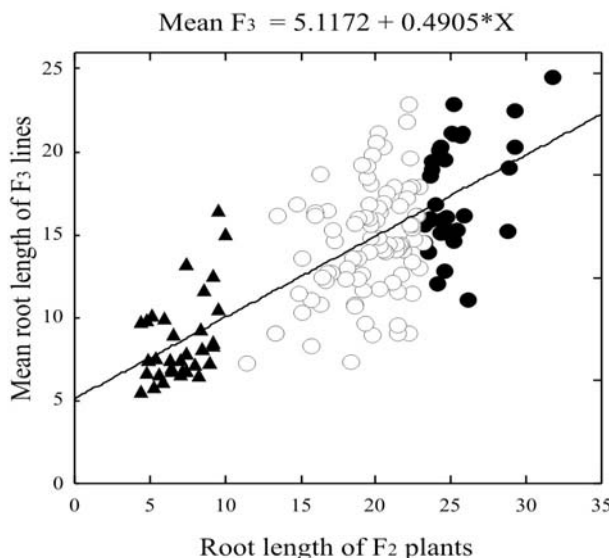


Figure 2. Scatter graph and regression equation of the relationship between root length (cm) of F_2 plants and the mean root length (cm) of $F_{2:3}$ lines of maize seedlings, grown for 10 days in nutrient solution with 4.5 mg L⁻¹ aluminum. The graphic representation shows $F_{2:3}$ lines from F_2 seedlings with: 20% more susceptibility to aluminum (▲), 20% more tolerance to aluminum (●) and non-selected seedlings (○)

procedures for the analysis model with F_2 and $F_{2:3}$ generations. Analysis of variance was performed between and within the rows, or plots, with $F_{2:3}$ lines and the homozygotic inbred lines (Table 1).

It was expected that the variation coefficient (CV) would be high in the $F_{2:3}$ generation in terms of segregation. The value of 28.49% is within the range usually found in evaluations of the F_2 generation. The CV of 8.06% in the tolerant L922 inbred line can be considered low. In experiments with aluminum-sensitive germplasm, a CV of around 20% was found for seminal root length (Magnavaca 1987, Prioli et al. 2000).

Heritability estimates for aluminum tolerance are

shown in Table 2. The broad-sense heritability values between and within lines (92.13% and 86.03 %, respectively) were similar to those obtained by other authors by generation means and variance analysis. The total narrow-sense heritability (61.29%) is also in agreement with values found by generation means and variance analysis. However, higher values of narrow-sense heritability have been found in other studies (Prioli et al. 2000).

With estimates of narrow-sense heritability, the genetic progress of selection among lines, selection within lines and mass selection was predicted (Table 3). Besides, the predicted gain was calculated in combined selection. A gain with selection of 20% was estimated for root length increase, i.e., for Al tolerance, as well as for root length decrease, i.e., for Al susceptibility. The combined selection, as shown, is probably the procedure that obtains the highest gains, far larger than other selection methods.

The results of expected gain were nearly symmetrical when the two senses of divergent selection were compared. There was great similarity in the magnitude of the absolute gain values (expressed in percentage) for increase and decrease of root length. When two selection directions among lines are considered, the gain was approximately 33%. The gains with selection within lines were lower, ranging from 7 to 14%. Gains with combined selection ranged from 102 to 125%, values that are extremely high when compared to other methods. It is possible that these forecasts are not realistic, given the value of 0.49 of tolerance heritability, obtained by regression. The mass selection achieved slightly higher gains than selection among lines. The gain values of mass selection ranged from 41 to 54%. These values indicate a fast achievement of tolerant germplasm.

Maize is an allogamous species and the cultivation

Table 1. Analysis of variance of the root length (cm) of seedlings of parental lines L922 and Ast214 interspersed with $F_{2:3}$ lines grown in nutrient solution with 4.5 mg L⁻¹ aluminum

FV	L922		Ast214		$F_{2:3}$	
	df	MS	df	MS	df	MS
Among plots	9	5.5667	9	17.5392	157	150.4345
Within plot	60	2.3817	70	2.0755	1093	15.8624
Total	69	2.7972	79	3.8371	1250	32.7647
Environmental Component of Variance		0.4558		1.9330		16.9966
Variation coefficient (CV) (%)		8.06%		20.06%		28.49%

Table 2. Estimates of genetic and environmental parameters in $F_{2,3}$, of the root length (cm) of maize seedlings grown for 10 days in nutrient solution with 4.5 mg L⁻¹ aluminum

Genetic parameters	Estimation in $F_{2,3}$		
	Between	Within	Total
Phenotypic variance	19.0000	15.862	34.8624
Environmental variance	1.4946	2.2168	3.3523
Genotypic variance	17.5054	13.6456	31.1511
Additive variance	-	-	14.2435
Variance due to dominance	-	-	13.0477
Inbreeding coefficient	-	-	0.5
Broad heritability (%)	92.13	86.03	-
Narrow- sense heritability (%)	74.97	44.90	61.29

Table 3. Prediction of gains by selection, based on selection differential, for the increase (↑) and decrease (↓) of root length (cm) of maize seedlings of $F_{2,3}$ lines, grown for 10 days in nutrient solution with 4.5 mg L⁻¹ aluminum

Parameters	20% among-line selection		20% within-line selection		20% mass selection		20% comb. selection	
	↑	↓	↑	↓	↑	↓	↑	↓
Family means / selected plants	20.10	7.66			26.38	4.54		
Selection differential	6.13	-6.35	4.31	-2.14	12.40	-9.47		
Narrow- sense heritability	0.75	0.75	0.45	0.45	0.61	0.61		
Gain by selection	4.59	-4.76	1.93	-0.96	7.60	-5.80	17.51	-14.38
Gain by selection (%)	32.86	-33.99	13.83	-6.87	54.38	-41.43	125.30	-102.63
Combined sel. efficiency/ between and within lines							2.68	2.51
Combined sel. efficiency/ mass selection							2.30	2.47

of inbred lines for large-scale production results in a heavy workload. If the goal is to follow successive self-fecundations to obtain tolerant homozygous lines, gains by combined selection are higher and therefore preferable. However, if a highly tolerant population is desired, mass selection also results in significant gains. On the other hand, in studies of QTL identification methodologies should always be more precise. In the case of aluminum tolerance in maize, the evaluation of $F_{2,3}$ lines is recommended. In literature, QTL analyses for this trait tend involve few loci, but there are divergences. The number of identified QTLs oscillates from two to five (Brondani and Paiva 1996, Sibov et al. 1999, Ninamango-Cárdenas et al. 2003).

It was possible to identify Al tolerant and sensitive segregating lines based on the frequency distribution of $F_{2,3}$ lines. Therefore, the phenotyping of F_2 plants

by the analysis of frequency distribution of $F_{2,3}$ lines in this family would be almost qualitative (discriminating seedlings in terms of susceptibility to aluminum) and more reliable. In other families using other lines, this procedure may not reduce the distance between genotype and phenotype significantly.

If the goal is to simply obtain plant material with increased aluminum tolerance, there is no reason for the increase in labor input by evaluating the tolerance in $F_{2,3}$ lines. However, with suitable genotypes such as the inbred lines L922 and Ast214, a more detailed tolerance analysis with $F_{2,3}$ lines can help identify a gene with a strong effect on tolerance determination. This would be the first step towards the elucidation, on a secure foundation, of the mechanism of aluminum tolerance in maize.

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Herança da tolerância ao alumínio em milho

RESUMO – O objetivo deste trabalho foi estudar a herança da tolerância ao alumínio em milho, em solução nutritiva, com linhagens parentais homocigóticas tolerante (L922) e sensível (Ast214), a geração segregante F_2 e as linhas $F_{2,3}$ derivadas. As plântulas, após germinação, foram transferidas para solução nutritiva contendo $4,5 \text{ mg L}^{-1}$ de alumínio. Nos experimentos envolvendo as linhas $F_{2,3}$, foi utilizado o delineamento inteiramente casualizado com pais intercalados e com cerca de oito plântulas na parcela. Os resultados da geração F_2 mostraram uma distribuição bimodal de frequências relativas, com aproximadamente três vezes mais plântulas no grupo tolerante, sugerindo o envolvimento de apenas um loco na herança da tolerância. A herdabilidade restrita (F_2 , $F_{2,3}$) entre os comprimentos da radícula dos indivíduos F_2 e das médias das linhas $F_{2,3}$ foi 0,49. Isto explica parcialmente a distribuição de frequências das médias das linhas $F_{2,3}$ bimodal, mas com baixa precisão na separação dos grupos tolerante e sensível.

Palavras-chave: *Zea mays* L, plântulas, solução nutritiva, herdabilidade.

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