Note / Nota

SELECTION METHODS FOR MAIZE SEEDLINGS IN GREENHOUSE AS RELATED TO ALUMINUM TOLERANCE

Carlos Daniel Giaveno; José Branco de Miranda Filho*

Depto. de Genética - USP/ESALQ, C.P. 83 - CEP:13418-900 - Piracicaba, SP.
**Autor correspondente <jbmirand@esalq.usp.br>*

ABSTRACT: Genetic improvement is a useful approach to increase aluminum tolerance in maize. This experiment was carried out to compare two screening techniques under greenhouse conditions and estimate the association between the results obtained for both bioassays with grain yield. Nutrient solution (NS) and pots with acid soil (AS) were utilized as screening methodologies to perform one cycle of divergent selection for aluminum tolerance in the tropical maize population SIKALQ. The four sub-populations obtained by both approaches were compared with the original and checks in a greenhouse and in multilocation field trials. Evaluation under the conditions of NS and AS showed that the populations with the best performances were those selected for aluminum tolerance under the same condition of evaluation. The variables measured in greenhouse showed good correlation and the most closely related were fresh root weight (FRW) and dry rot weight (DRW) (0.79). All of these variables showed low correlation with yield in non acid conditions (< 0.30). The correlation was more important (≈ 0.50) as soil aluminum saturation increased. The best correlation was observed for visual scoring (VS) (0.68), FRW (0.47) and net root growth (NRG) (0.52).
Key words*: Zea mays*, acid soils, stress, divergent selection

MÉTODOS DE SELEÇÃO DE PLÂNTULAS DE MILHO EM CASA-DE-VEGETAÇÃO EM RELAÇÃO À TOLERÂNCIA AO ALUMÍNIO

RESUMO: O melhoramento genético tem se mostrado como estratégia eficiente para melhorar a tolerância ao alumínio em milho. O objetivo deste trabalho foi comparar dois metodos de seleção em casa de vegetação e estimar a associação entre os resultados obtidos em cada um desses ensaios com a produção de grãos no campo. Solução nutritiva (NS) e vasos com solo ácido (AS) foram utilizadas nas metodologias de seleção para realizar um ciclo de seleção divergente para tolerância ao alumínio na população de milho tropical SIKALQ. As quatro subpopulações obtidas foram comparadas com a original e as testemunhas em experimentos de casa de vegetação e de campo. A avaliação sob as condições de NS e AS mostraram que as populações com melhor comportamento foram aquelas selecionadas para tolerância ao alumínio nas mesmas condições da avaliação. As variáveis medidas na casa de vegetação apresentaram boa correlação e as mais fortemente relacionadas foram peso fresco da raiz (FRW) e peso seco da raiz (DRW) (0.79). Todas as variáveis apresentaram baixa correlação com a produção de grãos em solos sem acidez (<0.30). A correlação foi mais importante (≈ 0.50) na medida que a saturação com alumínio do solo foi aumentando. Os melhores valores de correlação foram observados para avaliação visual (VS) (0.68), FRW (0.47) e crescimento líquido da raiz (NRG) (0.52).
Palavras-chave: *Zea mays*, solos ácidos, estresse, seleção divergente

INTRODUCTION

Acid soils comprise large agricultural areas mainly in tropical and subtropical regions (Kochian, 1995) and aluminum toxicity is one of the major factor limiting plant growth. The main effect of aluminum in higher plants is the inhibition of root growth (Black, 1967) and the degree of Al-induced root growth inhibition is therefore used to screen plants at seedling stage for their relative sensitivity to aluminum (Delhaize & Ryan, 1995; Kochian, 1995).

Traditionally, soil acidity is corrected by adding lime and fertilizers, but this approach is useful only for the superficial layer and the subsoil may remain with high acidity and limit yield. The development of resistant genotypes may be more economical than changing soil characteristics, specially in developing countries. Pandey et al.(1994) showed the importance of the development of maize genotypes with aluminum tolerance pointing to the fact that eight million hectares of maize are cultivated under acid soil conditions.

The identification of tolerant genotypes under the condition of field trials can lead to an increase in grain yield in acid soil. However, acid soil sometimes exhibit an expressive variation and the results are not consistent. Additionally, field trials are very expensive and time consuming, mainly when a large number of genotypes are under evaluation (García et al., 1979). An alternative approach is the evaluation of genotypes under controlled environment and two main screening methodologies have been used, which are based on pots with acidic soils

(Dall' Agnol et al., 1996; Urrea Gomez et al., 1996) or in nutrient solutions (Campbell et al.,1988; Lima et al., 1992; Giaveno et al., 1998). Screening in acid soils seems to reproduce more realistically the conditions in field, but since the inhibition of root growth is the main effect of aluminum toxicity, the evaluation of this effect turns to be difficult directly in the soil. Therefore in the last years nutrient solutions have been extensively utilized in greenhouse bioassays.

MATERIAL AND METHODS

The base population (P_0) used in this study is named as SIKALQ and resulted of introgressive mating between the exotic variety SIKUANI ICA V-110 and the local population ESALQPB2-3A (50% ESALQ PB2 and 50% ESALQ PB3; yellow endosperm) as a means of introducing high aluminum tolerance from the former into the adapted germoplasm.

Two screening methodologies were utilized to complete one cycle of divergent selection: (a) nutrient solution, and (b) pots with acid soils; the 10% most tolerant and 10% most sensitive seedlings out of 2000 seedlings were selected in each instance. Selected seedlings of four groups were transplanted to the field and randomly crossed to obtain cycle-I sub-populations, named as P_I-ATNS (Al-tolerant-nutrient solution) and P_I-ASNS (Al sensitive-nutrient solution) and P_I-ATAS (Al-tolerant-acid soil) and P_I-ASAS (Al sensitive-acid soil).

Selection of the tolerant and sensitive seedlings in nutrient solution was based on NRG (net root growth) for individual plants, obtained by difference between final root length (FRL) and the initial root length (IRL), after grown in greenhouse for seven days in nutrient solution as described by Furlani & Furlani (1988). Secondary roots were eliminated by hand and the length of the main root measured to obtain the IRL value. At the end of the period, seedlings were removed from the nutrient solution and the FRL value of each plant was measured.

For the acid soil bioassay, a set of twenty germinating seedlings with the main root, 3 to 5 mm long, was placed in a greenhouse into 6.75 l plastic pots filled with acid soil (≈50% aluminum saturation) and irrigated with deionized water. After 15 days, the seedlings were pulled from the pots by washing with tap water. Visual scoring of whole plant was used as selection criterion using a visual scale based on the size of the root and the shoots, varying from 1 (very poor) to 5 (excellent).

To compare grain yield among selected sub-populations and with the original and local populations, a multilocation field trial was conducted. Locations were stratified into four groups varying from low to high aluminum saturation (Table 1). Grain yield was standardized to 14% of moisture.

The trials with nutrient solution and pots with acid soil were conducted in a greenhouse as described in the selection process. The variables measured were: net root growth (NRG) for nutrient solution; and leaf area (LA), fresh root weight (FRW), dry root weight (DRW) and visual scoring (VS) for acid soil bioassay.

Variables measured in greenhouse bioassays were associated with grain yield expressed as t ha^{-1} in the four productivity levels by using Pearson's correlation coefficient as measure of association between variables. All the estimates were made using replication means.

RESULTS AND DISCUSSION

Field trials were extensively used for evaluating tolerance in acid soils, therefore they are expensive and the results are highly variable due to the natural variability in these soils and to the climatic conditions. However, in breeding programs for nutrient stress tolerance, the comparison among selected genotypes under field stress is necessary. In this work, the total grain yield was measured with the objective of evaluating the performance of populations per se (Table 2) and the degree of association with the variables measured in the greenhouse (Table 4).

For the high productivity environment (Table 2), all the populations showed an excellent yield varying between 7.1 t ha^{-1} to 8.8 t ha^{-1}. No differences were observed among genotypes suggesting that aluminum tolerance in maize was not closely related with low productivity in non acid soils. The effect of both, the introgressive mating and the divergent selection, was more evident as nutrient stress increased. In the low productivity environment, the yield of SIKALQ was 38% higher than ESALQPB2-3A. Under these conditions, CI-ATAS showed the best yield, overcoming CI-ATNS and SIKALQ by 8% and 19.5%, respectively. This superiority of CI-ATAS could be explained by the capacity of the acid soil bioassay to detect genotypes that release organic

Table 1 - Soils used in the field trials were stratified into four groups varying in the level of productivity.

Productivity	Soil Characteristics	Location
High	no-Al soil, N,P,K fertilized and irrigated	Piracicaba (22°42'30"S; 47°38'30W)
Intermediate high	limed and N,P,K fertilized (plow layer only).	Anhumas (22°04'S; 48°07'W)
Intermediate low	35% Al saturation and 12 mg kg^{-1} of phosphorous	Anhumas (22°04'S; 48°07'W)
Low	45% Al saturation and 8 mg kg^{-1} of phosphorous	Pindamonhangaba (22°55'50"S; 45°27'22"W)
Low	53% Al saturation and 9 mg kg^{-1} of phosphorous	Sete Lagoas (19°28'00"S; 44°15'08"W)

Piracicaba, Anhumas and Pindamonhangaba are in the State of São Paulo and Sete Lagoas is in the State of Minas Gerais.

Table 2 - Grain yield means expressed in t ha^{-1} in the four productivity levels.

Genotype	Productivity level			
	H^{ns}	IH*	IL**	L**
SIKALQ	7.55	4.85	3.31	3.02
ESALQ PB2-3A	7.38	4.41	3.01	2.18
C-1 ATNS	7.62	5.28	3.82	3.34
C-1 ASNS	7.11	4.72	3.27	2.50
C-1 ATAS	7.55	5.29	4.13	3.61
C-1 ASAS	8.12	4.52	2.94	2.61
TC	8.81	5.53	3.62	3.45
SC	7.96	5.19	3.16	2.52

H, high; IH, intermediate high; IL, intermediate low and low productivity, respectively.
*, **Significant at the $P \leq 0.05$ or ≤ 0.01 probability level, respectively and ns non significant*, for Snedecor's F test.

Table 3 - Behavior of the genotypes in the greenhouse bioassays.

Genotype	NRG**	LA**	FRW**	DRW**	VS**
SIKALQ	9.75	432.54	37.40	1.51	3.51
ESALQ PB2-3A	8.45	398.97	36.46	1.36	2.50
C-1 ATNS	12.08	494.79	41.80	1.76	4.30
C-1 ASNS	7.24	409.10	35.81	1.50	2.82
C-1 ATAS	10.00	560.66	44.74	1.80	4.50
C-1 ASAS	8.39	430.22	36.29	1.65	2.70
TC	10.61	456.61	39.40	2.00	4.53
SC	5.38	383.05	32.96	1.47	3.80

NRG (net root growth) expressed in mm day^{-1}, LA (leaf area) in cm^2, FRW (fresh root weight), DRW (dry root weight) in g. and VS (visual scoring).
*, **Significant at the $P \leq 0.05$ or ≤ 0.01 probability level, respectively and ns non significant for Snedecor's F test.

Table 4 - Association among variables measured in greenhouse bioassays and grain yield.

Trait	Greenhouse					Field			
	NRG	LA	FRW	DRW	VS	H	IH	IL	L
NRG						0,02^{NS}	0,25^{NS}	0,39*	0,52**
LA	0,52**					- 0,05^{NS}	0,29^{NS}	0,43**	0,44**
FRW	0,57**	0,62**				0,30^{NS}	0,43**	0,40*	0,47**
DRW	0,56**	0,78**	0,79**			-0,03^{NS}	0,24^{NS}	0,41**	0,42**
VS	0,47**	0,53**	0,60**	0,51**		0,20^{NS}	0,46**	0,50**	0,68**

NRG = net root growth, LA = leaf area, FRW = fresh root weight, DRW = dry root weight, VS = visual scoring, H = High, IH = Intermediate high, IL = Intermediate low and L = Low productivity environments, respectively
*, **Significant at the $P \leq 0.05$ or ≤ 0.01 probability level, respectively and ns non significant for Snedecor's F test.

acid by the roots. This release, triggered by aluminum as tolerance mechanism was reported in maize by Pellet *et al*., 1995.

In each greenhouse bioassay (Table 3), the sub-population selected as Al-tolerant showed the best value. Thus, when the genotypes were availed by NRG in nutrient solution, CI-ATNS show the best value (12.08 mm day^{-1}) followed by tolerant check (10.61 mm day^{-1}) and CI-ATAS (10.00 mm day^{-1}). In the acid soil bioassay, CI-ATAS showed the best values for LA (560.66 cm^2) and FRW (44.74 g). The tolerant check showed the high value of DRW (2.00 g) and the VS value was equal to the observed for CI-ATAS (4.5). For all variables measured, the tolerant genotypes showed better performance than the sensitive ones. The most evident differences among genotypes a were exhibited by the VS variable.

The fact that each Al-tolerant sub-population showed the best performance when evaluated under the same conditions of selection suggests that tolerance evaluated by different approaches could involve different mechanisms. The same conclusion was reported for alfalfa by Campbell et al. (1989).

All of the variables measured in the greenhouse showed a good correlation varying among 0.51 (minimum) between VS and DRW and 0.79 (maximum) for DRW and FRW.

The degree of association between each variable with grain yield was not consistent (Table 4). However, all of these show a tendency to increase Pearson's correlation coefficient as aluminum saturation and nutrient stress increase. The low correlation was observed for the high productivity environment, varying between -0.05 (minimum) for LA and 0.30 (maximum) for VS.

Both, in the IH and IL environment, VS showed the best correlation 0.46 and 0.50 respectively, followed by DRW and LA (0.43) in each environment. In the L environment, again VS showed the best correlation (0.68) followed by NRG (0.52) and FRW (0.47). This increase in the correlation coefficient when increasing as aluminum stress could be explained based on the fact that the greenhouse variables were measured under conditions of high stress, similar as those found in the low productivity environment.

ACKNOWLEDGMENTS

To Dr. Akihiko Ando (Departamento de Genética ESALQ-USP) and Dr. Ricardo Ferraz de Oliveira (Departamento de Ciências Biologicas ESALQ-USP) for technical assistance. Research and publication supported by FAPESP.

REFERENCES

BLACK, C.A. **Relaciones suelo-planta**. Ames: Iowa Press, 1967. 444p.

CAMPBELL, T.A.; ELGIN, J.H. ; FOY, C.D.; MURTREY, J.E. Selection in alfalfa for tolerance to toxic levels of Aluminum. **Canadian Journal of Plant Science**, v.68, p.743-753. 1988.

DALL' AGNOL, M.; BOUTON, J.H.; PARROT, W.A. Screening methods to develop alfalfa germoplasms tolerant of acid, aluminum toxic soils. **Crop Science**, v.36, p.64-70. 1996.

DELHAIZE, E.; RYAN, P.R. Aluminum toxicity and tolerance in plants. **Plant Physiology**, v.107, p.315-321, 1995.

FURLANI, A.M.C.; FURLANI, P.R. **Composição e pH de soluções nutritivas para estudos fisiológicos e seleção de plantas em condições nutricionais adversas**. Campinas: IAC, 1988. 34p. (Boletim Técnico, 121)

GARCIA, O.; SILVA, W.J.; MASSEI, M.A.S. An efficient method for screening maize inbreeds for aluminum tolerance. **Maydica**, v.24, p.75-82. 1979.

GIAVENO C.D.; MIRANDA FILHO, J.B.; FURLANI, P.R. Tolerância ao alumínio em milho (*Zea mays* L.): seleção divergente em solução nutritiva. **Genetic and Molecular Biology**, v.21, p.242, 1998. Supplement

KOCHIAN, L.V. Cellular mechanisms of aluminum toxicity and resistance in plants. **Annual Review of Plant Physiology**, v.46, p.237-260, 1995.

LIMA, M.; FURLANI, P.R.; MIRANDA FILHO, J.B. Divergent selection for aluminum tolerance in a maize (*Zea mays* L.) population. **Maydica**, v.37, p.123-132, 1992.

PANDEY, S.; CEBALLOS, H.; GRANADOS, G.; KNAPP, E. Developing maize that tolerant aluminum toxic soils. In: C.I.M.M.Y.T. **Stress tolerance breeding:** Maize that resist insects, drought, low nitrogen and acids soils. México, 1994. p.85-93.

PELLET, D.M.; GRUNES, D.L.; KOCHIAN, L.V. Organic acid exudation as an aluminum-tolerance mechanism in maize (*Zea mays* L.) **Planta**, v.196, p.788-795, 1995.

URREA GÓMEZ, R.; CEBALLOS. E.; PANDEY, S.; BAHÍA FILHO, A.F.C.; LEÓN, L.A. A greenhouse screening technique for acid soil tolerance in maize. **Agronomy Journal**, v.88, p.806-812, 1996.

Received June 05, 2001