Genetic analysis of aluminum tolerance in maize

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ABSTRACT

Soluble aluminum in acid soils can induce severe phytotoxic effects, leading to a decrease in both the mitotic activity of roots and the yield of cultivated plants. Intraspecific variability for aluminum tolerance has been described in many species. However, results from studies on transmission of this trait are conflicting because they indicate monogenic and quantitative inheritance, prevalently with the expression of more than one locus. The aim of this research was to evaluate the inheritance of tolerance in a family of maize (*Zea mays* L.) consisting of aluminum-tolerant and aluminum-sensitive parental inbred lines, and their progenies. Net Seminal Root Length (NSRL) was analyzed in seedlings grown in nutrient solution containing 4.5 μg/mL aluminum. NSRL distributions in F_2 and in backcrosses with the aluminum-sensitive parent were bimodal and indicated ratios 3:1 and 1:1, respectively. It is concluded that, in this family, tolerance is inherited as a single trait with a main locus effect.

KEY WORDS: *Zea mays* L., aluminum toxicity, genetics of tolerance.

INTRODUCTION

Acid soils have high concentrations of soluble aluminum, which can induce severe phytotoxic effects, leading to a decrease in potential yield of almost all cultivated species. In Brazil, the 1.8 million km^2 region named 'cerrado' is a savanna-like area consisting of acid soils. In this area, aluminum toxicity is a relevant limiting factor for yield of crops that are not adapted to acid soils (Foy et al., 1978; Silva, 1976).

The mechanisms of phytotoxicity and tolerance of aluminum in diverse genotypes have not been wholly explained, although various hypothesis based on results with different species have been formulated (Kochian, 1995; Kolmeier et al., 2001; Pineros and Kochian, 2001). In sensitive plants, the most evident effect of aluminum is a decrease of mitotic activity in root meristems (Sivaguru and Horst, 1998). Deficient development of root system impairs the use of soil nutrients and the plant becomes more susceptible to droughts (Foy, 1974; Eckert et al., 1996).

Besides severe aluminum effects, in acid soils complex interactions at a low pH block available essential nutrients and favor available toxic elements, such as manganese (Foy et al., 1978). Lime application has become an alternative to raise pH of acid soils, so that aluminum becomes insoluble and precipitates (Marion et al., 1976). On the other hand, there are economical and technical difficulties to solve the aluminum problem solely by lime application. The best strategy to improve acid soils is the combination of pH neutralization by liming and the simultaneous use of aluminum-tolerant plants (Pandey et al., 1994; Spehar and Souza, 1999).

Maize is a crop with limitations for agricultural practice on acid soils. As a general rule, high yield elite germoplasms are sensitive to aluminum; However, certain cultivars of maize derived from an authocthonous race of the Atlantic coast of South America are aluminum-tolerant, albeit with low yields (Prioli, 1987). Since translocation of aluminum to the aerial part does not occur in maize (Magnavaca, 1982; Rasmussen, 1968), the most effective traits in tolerance assessment of nutrient solution are related to the root system, mainly to the root length (Garcia et al., 1979; Magnavaca et al., 1987; Prioli, 1987; Rhue et al., 1978). In spite of a consensus on their trustworthiness in the identification of tolerant genotypes, some results on root length are contradictory with regard to the inheritance of aluminum tolerance in maize. Previous studies indicate single locus inheritance of aluminum tolerance in maize, with the effect of a clearly defined main locus (Garcia and Silva, 1979; Jorge and Arruda, 1997; Rhue et al., 1978). On the other hand, in maize there are also persisting evidences of quantitative inheritance of aluminum tolerance, with continuous unimodal distribution and effect in only a few loci (Magnavaca et al., 1987; Prioli, 1987; Sawazaki and Furlani, 1987).

Because results from genetic studies have been conflicting and non-conclusive, the inheritance of aluminum tolerance is still considered an open question. The aim of the present research was to obtain additional information on the inheritance of aluminum tolerance in maize.

MATERIAL AND METHODS

Inbred lines of maize, F_1, F_2 and backcrosses

Inheritance of aluminum tolerance was investigated by using a maize family consisting of two parental inbred lines and their progenies F_1, F_2 and the backcrosses for the two parents. The two parental lines diverge in aluminum tolerance (Prioli, 1987), and they have been self-pollinated for more than 40 generations. Both inbred lines were developed at the Department of Genetics, IB, State University of Campinas, SP, Brazil. Line L922, derived from *Cateto* race with orange-color flint endosperm, is highly tolerant to aluminum. Line Ast214, derived from *Tuxpeño* germosperm, with light-yellow dent endosperm, is aluminum-resistant. Progenies F_1, F_2, and backcrosses BC(F_1 x L922) and BC(F_1 x Ast214) were obtained by controlled pollination. Seeds were used in assays within 5 to 8 months after harvesting, so that results would not be affected by loss of vigor due to aging.

Nutrient solution and aluminum level

The nutrient solution described by Clark (1975) was used with few modifications. Solution was prepared with distilled water and saline composition: 3.43 mM $Ca(NO_3)_2.4H_2O$, 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 $Na_2MoO_4.2H_2O$. Aluminum ion concentration was 4.5 μg/mL, added as double salt $KAl(SO_4)_2.12H_2O$. With such ionic strength in the nutrient solution, this aluminum dose is sufficient to discriminate aluminum-tolerant and aluminum-resistant maize genotypes (Prioli, 1987). The pH was adjusted to 4.0 and remained stable during the entire experiment without any need for acid or base corrections.

Seed germination

Seeds were germinated on filter paper rolls wetted with distilled water. Paper rolls were placed in plastic containers with a 3 cm water layer at basal level. Sealed containers were kept in a dark room at 26 ±1°C. After 2 to 3 days, germinated seeds with seminal roots of approximately 2 cm were transferred to the aluminum nutrient solution. Seedlings were placed in floating polystyrene (Styrofoam) plates with their roots immersed in the nutrient solution. Solution was constantly aired for oxygenation and its volume was maintained constant by a daily addition of distilled de-ionized water. Temperature in the growth room was kept at 26 ±1°C with a light/dark period adjusted to 14/10 hours. Light intensity was approximately 350 $\mu E.m^{-2}.s^{-1}$ at plant level.

Measurements and variables

In maize, length of seminal root is a biological trait that represents the aluminum-tolerance rate with great assurance. In our study, the Initial Seminal Root Length (ISRL) was measured individually prior to the transference of the plants to the solution. After 10 days of growth, at the end of assay, seminal roots were measured for the Final Seminal Root Length (FSRL). Aluminum-tolerance was evaluated by the Net Seminal Root Length (NSRL), which is the difference between FSRL and ISRL.

Genetic analysis and statistics

A total of 93, 95, and 101 seedlings, respectively, of the genetically uniform populations L922, Ast214, and F_1, were analyzed. In each segregating population, the number of seedlings was increased to 186 plants from backcross BC(F_1 x L922), and 311 plants of F_2 generation. Segregation in F_2 progeny was tested for the Mendelian proportion 3:1 by χ^2 test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Table 1 shows the NSRL averages of the six populations within the maize family under analysis. Contrast between lines L922 and Ast214 was obvious in the differences between the two averages for the responses to aluminum treatment. In Figure 1 is shown the effect of aluminum on the root growth of both inbred lines. There was no overlapping of frequency distributions, demonstrating a differential tolerance to aluminum. Whereas root development of Ast214 was almost paralyzed, L922 was not affected by the aluminum concentration used. In several previous studies of these inbred lines, the absence of toxic aluminum in the nutrient solution apparently benefited Ast214, which registered higher root development than that of L922. Contrastingly, aluminum at 4.5 µg/mL concentration almost completely impaired the growth of seminal roots of Ast214 seedlings. In L922 seedlings, however, seminal roots developed without any significant restrictions (Prioli, unpublished data). Because of the consistent pronounced differences of their response to aluminum treatment, these two inbred lines can be considered suitable genotypes for genetic analysis of aluminum tolerance in maize.

Table 1. Number of maize seedlings, averages (cm), variances and variation coefficient of variation (cv) of net length of root of parental inbred lines L922 and Ast214, which are aluminum-tolerant and aluminum-sensitive, respectively, and their progenies F_1, F_2 and backcrosses. Seedlings were grown for ten days in a nutrient solution supplemented with aluminum to a final concentration of 4.5 µg/mL.

Population	Number of Plants	Mean	Variance	CV (%)
L922	93	18.19	6.68	14.2
Ast 214	95	4.91	3.89	40.2
F_1(L922 x Ast214)	101	20.09	6.99	13.2
F_2(L922 x Ast214)	311	15.44	45.74	43.8
BC(F_1 x L922)	186	20.47	6.97	12.9
BC(F_1 x Ast214)	185	11.98	36.31	50.3

Mean of F_1 population was slightly higher than that of aluminum-tolerant inbred line L922 (Table 1 and Figure 1), clearly demonstrating that aluminum-tolerance in these maize genotypes is dominant over aluminum-sensitivity. This result corroborates conclusion of Garcia and Silva (1979), Jorge and Arruda (1997), Prioli (1987) and Rhue et al. (1978). On the other hand, Magnavaca (1982) and Magnavaca et al. (1987) indicated dominance of the aluminum-sensitive trait in other maize genotypes.

Frequency distributions of the populations studied (inbred lines, F_1, F_2, and backcrosses) were represented in graphs (Figure 1). In all populations, NSRL registered a continuous distribution. Another important aspect was the clear bimodality in generation F_2. Modality with the highest value, which corresponds to the tolerant phenotype, encompasses about three times as many individuals as does the sensitive phenotype. This bimodal pattern of F_2 distribution is known to be indicative of the inheritance model with effect of a main single locus,

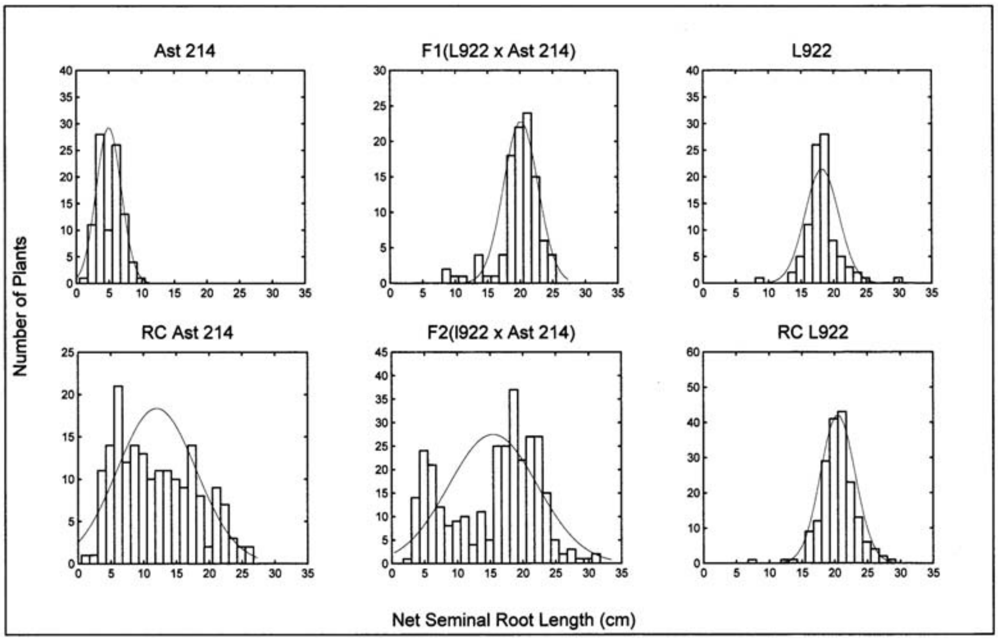

Figure 1. Frequency distribution of Net Seminal Root Length (NSRL), in cm, of maize seedlings from the parental inbred lines L922 and Ast214, aluminum-tolerant and aluminum-sensitive, respectively, and their progenies F_1, F_2 and backcrosses, after ten days of growth in a nutrient solution containing 4.5 µg/mL aluminum.

with segregation of two alleles, and complete dominance. A dominant allele would determine aluminum tolerance, while a recessive allele would determine sensitivity. The F_2 distribution continuity could be coherently interpreted as a result of the action of modifier genes and environmental factors.

Proportion of aluminum-tolerant and aluminum-sensitive F_2 seedlings was tested by χ^2 according to the 3:1 ratio, expected in the inheritance of a single locus with two alleles and complete dominance. Assuming the average of the lowest NSRL rate of aluminum-tolerant L922 and of lowest NSRL rate of aluminum-sensitive Ast214 as the limit between segregating classes, the $\chi^2 = 3.98$ was significant only at the level of 5% probability. On the other hand, adopting the highest NSRL rate of the aluminum-sensitive Ast214 as the limit between classes, the $\chi^2 = 0.31$ was nonsignificant. It may thus be admitted that these results of population F_2 are coherent with the model of inheritance of a single locus. The slight discrepancy may be attributed to participation of genes with small effects (modifiers) in the determination of aluminum tolerance.

Nevertheless, according to the model of single locus inheritance, the bimodal profile distribution would also be found in a backcross with an aluminum-sensitive father BC(F_1 x Ast214). In such a population, the two groups should be almost equal, with segregation 1:1, as foreseen by inheritance model of an allele pair and complete dominance. Although not entirely defined, the distribution in backcross with aluminum-sensitive Ast214 parent was similar to a bimodal one. A higher overlapping of the two segregating classes in this population could be a plausible interpretation for this result. It may be that the partial overlapping observed is a consequence of interaction of a principal gene with genes functioning as modifiers. This would posit greater difficulties in detecting bi-modality if a ratio of 1:1 is expected, compared to the 3:1 ratio.

When Prioli et al. (2000) employed analyses of averages and variances for these same populations (parental lines, F_1, F_2, and backcrosses) using the L922 and Ast214 parental lines, they estimated the existence of two loci determining the inheritance of aluminum-tolerance. On the other hand, The F_2 bimodal distribution of NSRL, with proportion close to 3:1, strongly suggests a single locus inheritance model for aluminum tolerance in maize, even though the interaction between the main gene and modifier genes should be admitted. This fact agrees with reports in the literature on single locus inheritance for aluminum tolerance (Jorge and Arruda, 1997; Rhue et al., 1978; Garcia and Silva, 1979).

Quantitative inheritance for aluminum-tolerance is frequently reported in the literature (Magnavaca et al., 1987; Prioli, 1987; Sawazaki and Furlani, 1987), indicating that the existence of more than one locus with enhanced effect for aluminum-tolerance in maize is possible. Certain crosses would produce genotype combinations with a single segregating locus, while in other loci the recessive alleles would be in homozygosis. In other situations, several other loci may be heterozygous in F_1 and segregation would produce unimodal distribution in F_2. The unimodal quantitative trait for aluminum tolerance heredity may be consistently explained by the activity of a major locus or a few loci coupled to moderate influences of environmental factors. Therefore, diverse combinations of inbred lines of maize could result in either unimodal or bimodal distributions due to various possible interactions of the major locus with different allelic combinations in other loci involved in the tolerance to aluminum toxicity.

RESUMO

Análise genética da tolerância ao alumínio em milho

O alumínio solúvel dos solos ácidos pode ter efeitos fitotóxicos severos, causando a redução da atividade mitótica das raízes e diminuição do potencial produtivo de plantas cultivadas. Já foi constatada variabilidade intraespecífica para a tolerância em muitas espécies. No entanto, os resultados dos estudos da herança da tolerância são conflitantes, com indicações de herança monogênica e de herança quantitativa, com manifestação pronunciada de mais de um loco. O objetivo deste trabalho foi determinar a herança da tolerância em uma família constituída por uma linhagem tolerante e uma sensível e suas gerações derivadas F_1, F_2 e retrocruzamentos. Foi analisado o Comprimento Líquido da Radícula (NSRL) de plântulas desenvolvidas em solução nutritiva com alumínio na concentração 4.5 μg/mL. As distribuições do NSRL na F_2 e no retrocruzamento com parental recessivo foram bimodais, indicando as proporções 3:1 e 1:1, respectivamente. Portanto, pode-se concluir que a tolerância é herdada de modo simples, com o envolvimento de um loco principal.

ACKNOWLEDGEMENTS

The authors would like to thank CAPES for the scholarships, and Dr. Deonisio Destro and Dra. Laudenir Maria Prioli for their valuable suggestions.

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Received: August 06, 2001;

Accepted: March 20, 2002.