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Rapid screening for aluminum tolerance in maize (*Zea mays* L.)

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Abstract

A significant decrease in maize grain yield due to aluminum toxicity is considered to be one of the most important agricultural problems for tropical regions. Genetic improvement is a useful approach to increase maize yield in acid soils, but this requires a rapid and reliable method to discriminate between genotypes. In our work we investigated the feasibility of using hematoxylin staining (HS) to detect Al-tolerant plants at the seedling stage. The original population along with two populations obtained after one cycle of divergent selection were evaluated by net root growth (NRG) and HS after 7 days in nutrient solution. Results showed a negative correlation between NRG and HS in all populations, in which sensitive plants, characterized by low NRG, exhibited more intense staining than tolerant plants. These results indicate that HS is a useful procedure for selecting Al-tolerant maize seedlings.

INTRODUCTION

Aluminum toxicity is the major factor limiting plant growth in the acid soils that comprise large agricultural areas, principally in tropical and subtropical regions (Kochian, 1995). Several approaches have been suggested to increase grain yield in these soil types, closely related to root growth inhibition, which is considered to be the main effect of aluminum toxicity in higher plants. The degree of Al-induced root growth inhibition can be used to screen plants at the seedling stage for their relative aluminum sensitivity (Delhaize and Ryan, 1995; Kochian, 1995). There is considerable genetic variability in sensitivity in the major crops and the evaluation of root elongation in nutrient solution has been useful in developing Al-tolerant varieties (Delhaize *et al*., 1993; Pellet *et al*., 1995).

Higher plants make use of two main mechanisms to avoid the effects of Al toxicity, one being an exclusion mechanism, by which aluminum is prevented from crossing the plasmalemma and reaching the root cytoplasm by cell wall binding (Kochian, 1995), release of organic acids (especially citrate and malate) (Miyasaka *et al*., 1991; Delhaize *et al*., 1993; Pellet *et al*., 1995), and modification of the pH of the rhizosphere (Miyasaka *et al*., 1989). If aluminum does cross the plasmalemma, it is excluded by the ATPase pump located in the plasmalemma (Kochian, 1995). The other mechanism is an internal response, characterized by the production of specific proteins capable of forming complexes with toxic aluminum (Aniol, 1984; Basu *et al*., 1994).

Selection and breeding for aluminum tolerance are important approach for increasing grain yield in acid soils. Field trials have proved to be effective in selecting resistance but are very expensive and time-consuming. A rapid and reliable screening system is needed to discriminate sensitive and resistant genotypes. The selection of seedlings in nutrient solution is a rapid screening method based on net root growth (NRG), developed to screen for aluminum tolerance in several crops. A rapid method using hematoxylin staining (HS) has been widely used for the direct visualization and localization of aluminum in root tissues (Rincón and Gonzales, 1992). It is a useful approach for macroscopically detecting aluminum accumulation in root tips by the formation of an intense blue coloration in the root tips of sensitive genotypes. This reaction occurs by the oxidation (in the presence of $NaIO_3$) of hematoxylin to hematyn, which in the presence of aluminum produces nucleic acid coloration (Polle *et al*., 1978). The reaction of hematoxylin with aluminum-stressed roots has been used by several researchers in different crop species, such as wheat (*Triticum aestivum* L.) (Polle *et al*., 1978; Carver *et al*., 1988; Rincón and Gonzales, 1992; Tice *et al*., 1992), soybean (*Glycine max* Merrill) (Fonseca Jr., 1982; Fonseca Jr. *et al*., 1982; Spehar and Makita, 1994), the common bean (*Phaseolus vulgaris* L.) (Braccini *et al*., 1996) and maize (*Zea mays* L.) (Cançado, 1997).

Information about the utilization of hematoxylin stain in maize (*Zea mays* L.) is scarce and the work reported in this paper was undertaken to determine the effectiveness of this methodology for detecting Al-tolerant and -sensitive genotypes.

MATERIAL AND METHODS

Maize populations

Two thousand seedlings of tropical maize population SIKALQ were grown in a greenhouse for seven days in nutrient solution at pH 4.2 and 5 ppm of aluminum as described by Furlani and Furlani (1988). The population SIKALQ is the result of an introgressive cross between

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the local population ESALQ-PB23A (50% ESALQ PB2 and 50% ESALQ PB3; yellow endosperm) and the exotic variety SIKUANI ICA V-110 (Giaveno *et al*., 1998). Secondary roots of seedlings with similar length to the principal root were eliminated by hand and the length of the principal root measured to obtain the initial root length (IRL). After seven days, seedlings were removed from the nutrient solution and the final length of the principal root (FRL) of each plant was measured. NRG for individual plants was calculated as NRG = FRL - IRL.

One cycle of divergent selection was completed by selecting the 10% most tolerant and 10% most sensitive seedlings. Selected seedlings of both groups were transplanted to the field and randomly crossed to obtain cycle-I sub-populations, designated as CI-AT (Al-tolerant) and CI-AS (Al-sensitive) as described by Giaveno *et al*. (1998).

Growth measurements

Treatments were the three maize populations described above, and the seedlings of each population were grown in a growth chamber for seven days at a constant temperature of 24°C and a 16-h light and 8-h dark photoperiod. Treatments were placed into four 3.5-liter buckets containing the same nutrient solution (Furlani and Furlani, 1988) in a completely randomized block design, in which the buckets were considered as blocks. At the end of this period an NRG value was calculated and the Pearson's coefficient of correlation with HS calculated. Values of NRG were analyzed by ANOVA and the means compared by the Duncan test.

Hematoxylin staining of roots

Preliminary studies using the tropical maize (*Zea mays* L.) populations CMS 36 (Al-tolerant) and BR 106 (Al-sensitive) were carried out in growth chambers to assess the best combination of time of exposure and Al concentration for HS (Giaveno and Miranda Filho, 1999).

After 24-h exposure to aluminum, seedlings were removed from the nutrient solution and stained for visual detection of aluminum using the method proposed by Polle *et al*. (1978), modified by Cançado (1997). Seedlings were placed in flasks containing deionized water and washed for 30 min in a horizontal shaker, and stained with hematoxylin solution (2 g/l of hematoxylin and 0.02 g/l of KIO_3) for 15 min. The seedlings were washed again for 20 min in deionized water to remove excess of stain.

Stained root tips were evaluated using a visual scale varying from 0 (highly tolerant) to 5 (highly sensitive). Values of HS were analyzed by ANOVA and the means compared by the Duncan test.

RESULTS AND DISCUSSION

In previous work we have shown that 24 h of Al exposure was sufficient to result in staining when the roots were subsequently treated with hematoxylin (Giaveno and Miranda Filho, 1999).

In the present work two parameters, NRG (Figure 1) and HS (Figure 2), were used to measure aluminum tolerance in maize seedlings, and both showed statistical differences among the populations (Table I). These results confirmed the effectiveness of the divergent selection for NRG.

The phenotypic correlation between NRG and HS for all populations showed a negative trend (Figure 3). It could be explained by the fact that susceptible seedlings have poor NRG as a consequence of high quantities of accumulated aluminum in the root cap and therefore, these genotypes show higher HS. On the other hand, tolerant seedlings have some mechanism to avoid aluminum toxicity, therefore they presenting higher NRG and low HS.

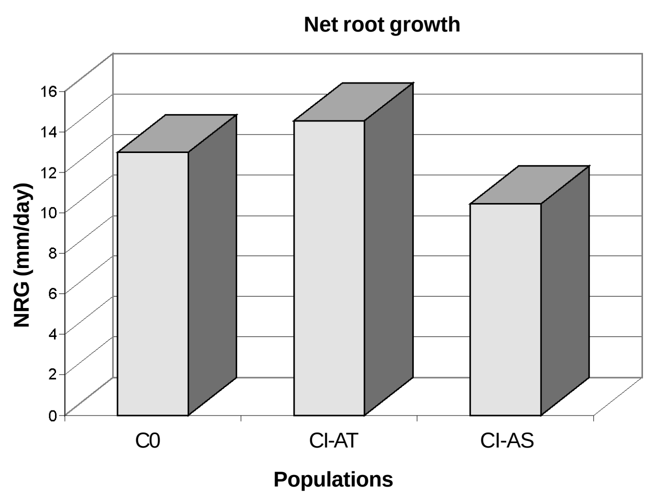

Figure 1 - Mean net root growth (NRG) values of the original (C0), Al-tolerant (CI-AT) and Al-susceptible (CI-AS) populations.

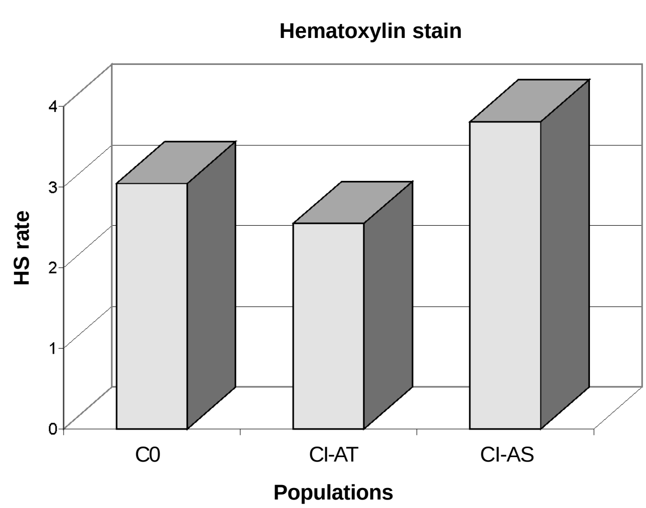

Figure 2 - Mean hematoxylin staining (HS) values of the original (C0), Al-tolerant (CI-AT) and Al-susceptible (CI-AS) populations.

Table I - Observed means for net root growth (NRG) and hematoxylin staining (HS) for the original population (C0), and divergently selected populations (Al-tolerant (CI-AT) and Al-sensitive (and CI-AS)) along with the phenotypic correlation (r_P) value between NRG and HS.

Treatment (populations)	NRG**	HS**	r_P
C0	12.99 A	3.04 B	-0.77
CI-AT	14.55 A	2.55 B	-0.79
CI-AS	10.45 B	3.81 A	-0.75
All plants	12.65	3.30	-0.82
Coefficient of variation (%)	18.57	22.55	

**Significant differences among populations (C0, CI-AT, and CI-AS) at P ≤ 0.01. Duncan's test. Means with the same letter are not significantly different.

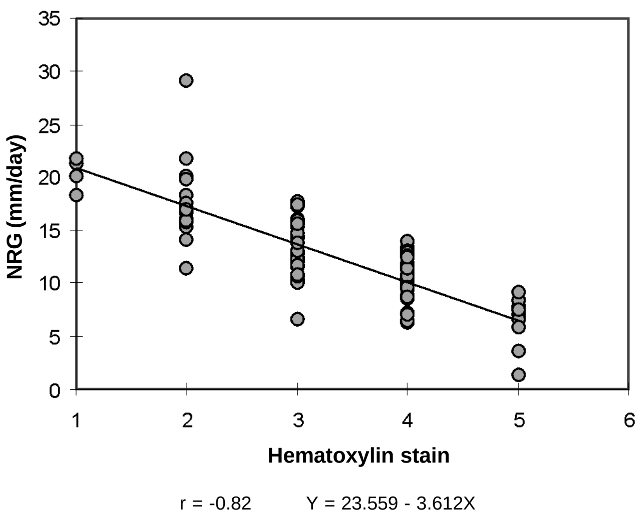

Figure 3 - Regression analysis of the data for all plants. NRG, Net root growth.

Our results show a possible selection effect on the phenotypic correlation value in both selected populations. In the tolerant population (CI-AT) there was a small increase in the phenotypic correlation coefficient (-0.792) when compared to the original population (-0.771). On the other hand a decrease in the correlation coefficient (-0.757) was observed in the sensitive population. However, such trends cannot be necessarily assured in further selection cycles, and we can say that hematoxylin staining is highly negatively correlated trait with NRG in both the original and divergently selected populations.

Overall results are in agreement with other reports, such as that of Cançado (1997) who reported a correlation between HS and NRG of -0.693 and a relative root growth of -0.816 using S_3 inbreed lines. Guevara *et al*. (1992) concluded that hematoxylin staining was a good criterion for discriminating between tolerant and sensitive maize seedlings. These results were partially confirmed by Ryan *et al*. (1993).

Our results lead us to conclude that hematoxylin staining is a suitable approach to the screening of maize seedlings because it allows the rapid evaluation of a large number of genotypes without destroying the root apical meristem.

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RESUMO

A importante diminuição nos rendimentos de milho causados pela toxidez produzida pelo alumínio é considerada um dos mais importantes problemas nas regiões tropicais. O melhoramento genético é uma metodologia útil para aumentar os rendimentos do milho em solos ácidos, requerendo um método rápido e seguro que permita diferenciar os diferentes genótipos. O objetivo deste trabalho foi avaliar a possibilidade de utilizar a técnica da coloração com hematoxilina (HS) na detecção de plântulas tolerantes ao alumínio. Duas populações obtidas de um ciclo de seleção divergente e a original, foram avaliadas depois de sete dias em solução nutritiva utilizando os parâmetros NRG (crescimento líquido da raiz principal) e HS. Os resultados apresentaram uma correlação negativa entre NRG e HS em todas as populações devido ao fato de que as plântulas suscetíveis, caracterizadas por um baixo NRG, apresentaram uma coloração mais intensa do que as tolerantes. Nossos resultados permitem concluir que a técnica de coloração com hematoxilina é um procedimento adequado para selecionar genótipos tolerantes ao alumínio em estado de plântula.

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