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### Screening of tropical maize for salt stress tolerance

Carlos Daniel Giaveno<sup>1\*</sup>, Rafael Vasconcelos Ribeiro<sup>2</sup>, Gustavo Maia Souza<sup>3</sup>, and Ricardo Ferraz de Oliveira<sup>1</sup>

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**ABSTRACT** - Since salinity is a common stress factor in agricultural areas, the objective of this study was to evaluate the feasibility of morphological and physiological traits as selection criteria of maize genotypes under salt stress. The experiments were carried out in the stages of germination and early seedling growth. Root and shoot weight, leaf area, root and leaf water potential, photochemical efficiency and growth rate were measured during salt stress and stress recovery. Our results indicated the presence of genetic variability for germination, but no association between germination and early seedling vigor, such as seedling weight and growth rate, and photochemical efficiency under stress conditions can be used as selection criteria for salt-tolerant maize in breeding programs.

Key words: maize, screening, salinity, NaCl, stress.

### INTRODUCTION

The rapid increase in the world population demands an expansion of crop areas to raise food production. In this context, a significant fraction of agricultural crops are cultivated on low quality soils, sometimes affected by salinity (Allen et al. 1983). According to Steppuhn and Wall (1999), salinity could be defined as a water property that indicates the concentration of dissolved solutes. Soil salinity refers to the state in which dissolved constituents concentrate beyond the needs of plant roots. It is well-known that salinity is a common stress factor in agricultural areas as a result of extensive irrigation with saline water and fertilizer application (McKersie and Leshem 1994). The phenomenon is closely related to low osmotic potential in the root zone. As soil salt concentration increases, the soil osmotic potential decreases, resulting in a

marked reduction in root water uptake. According to McKersie and Leshem (1994), plant roots may not only fail to absorb water but under extreme salt stress conditions they can also lose their water to the soil.

In some high-salinity areas, desalinization may be an economically possible option (Allen et al. 1983). Alternatively, the use of crops that have some degree of salt tolerance can also be a possibility to overcome the constraints caused by salinity. Among the crops of economic interest, there are large variations from highly sensitive species such as bean and citrus, over tolerant species such as wheat, maize and sunflower, to highly tolerant ones like cotton and barley (Francois and Maas 1994). The availability of genetic variation, both at intra or inter-specific level, is a prerequisite for the success of breeding programs (Ashraf et al. 1987, Maas 1986). Genetic variability for salt tolerance was reported in

<sup>&</sup>lt;sup>1</sup> Laboratório de Fisiologia de Plantas Sob Condições de Estresse, Departamento de Ciências Biológicas, Escola Superior de Agricultura Luiz de Queiroz (ESALQ), Universidade de São Paulo. C.P. 11, 13.418-900, Piracicaba, SP, Brasil. <sup>\*</sup>E-mail: cdgiaven@yahoo.com.br

 <sup>&</sup>lt;sup>2</sup> Labotarório de Fisiologia Vegetal, Centro de Pesquisa e Desenvolvimento de Ecofisiologia e Biofísica, (IAC), C.P. 28, 13.012-970, Campinas, SP, Brasil
<sup>3</sup> Laboratório de Ecoifísiologia Vegetal, Universidade do Oeste Paulista, 19.067-175, Presidente Prudente, SP, Brasil

alfalfa (McKimmie and Dobrenz 1991), *Trifolium* (Ashraf et al. 1987), sunflower (Francois 1996) and maize (Maiti et al. 1996, Jan et al. 1998, Sharif et al. 1999, Khan et al. 2003).

As suggested by Souza and Cardoso (2000), a marked increase of germination inhibition is expected at higher NaCl concentrations in the substrate. In general, salt stress is directly related with drought stress due to the capacity of the dissolved solutes to retain water. However, two different mechanisms of salt tolerance enable seeds to germinate at high salt concentrations. Seeds can tolerate the effects of a lower water potential in the substrate (Allen et al. 1986) or they may present specific tolerance to the inhibitory effect of NaCl (Rumbaugh et al. 1993). In some cases, germination inhibition of Eucaliptus grandis was higher in NaCl than in PEG 6000 solutions at equal osmotic potential (Souza and Cardoso 2000). Germination inhibition may be equivalent in species that exhibit a mechanism of Na exclusion or of Na shoot accumulation. In maize, differences in Na shoot accumulation were observed among genotypes and the lack of correlation between this trait and salt tolerance was reported (Alberico and Cramer 1993, Cramer et al. 1994, Wang et al. 2003). According to Amzallag et al. (1993), sorghum plants have the capacity to broaden salt tolerance after previous exposure to sublethal NaCl concentrations. This adaptation period induced the capacity to grow at lethal concentrations.

The objective of this work was to evaluate the feasibility of using morphological and physiological traits as selection criteria of tropical maize tolerant to salt stress.

#### MATERIAL AND METHODS

### Experiment 1 - Germination under osmotic stress: comparison of maize hybrids

This experiment was conducted to evaluate the germination of 14 commercial maize hybrids (Table 1) in a range of NaCl concentrations. Based on previous evaluations (data not shown), the NaCl concentration of 200 mmol  $L^{-1}$  was insufficient to inhibit germination by 100%. Two concentrations (250 and 300 mmol  $L^{-1}$ ) were therefore added to the treatment set in this trial.

Three replications of 50 seeds of each hybrid were used. Seeds were surface-sterilized, placed to germinate within a folded paper towel soaked with appropriate treatment solution, and covered with plastic trays. The plastic trays were maintained in a germination chamber Screening of tropical maize for salt stress tolerance

Table 1. Commercial hybrids used in experiment 1

Number	Hybrid	Company
1	A 4646	Aventis
2	A 4454	Aventis
3	A 3663	Aventis
4	A 3575	Aventis
5	A 2560	Aventis
6	A 2555	Aventis
7	A 2345	Aventis
8	A 2288	Aventis
9	Tractor	Syngenta
10	P32R1	Pioneer
11	BRS 3123	Embrapa
12	BR 3060	Embrapa
13	BR 206	Embrapa
14	AGS 5010	Monsanto

(NT 708-AT, Novatecnica, Brazil) at 25 °C for seven days. Seeds were considered germinated when radicle and shoot were longer than 15 mm. The germination inhibition index (GI) was estimated as the difference between germination in the control and each salt treatment, respectively. At the end of the germination period, all seedlings of each folded paper were individually weighed to assess seedling fresh weight (SW). Three seedlings of each treatment were randomly chosen to determine the root water potential (Yr). Yr was measured by the psychrometric method, in dewpoint mode, with a micro-voltmeter (HR-33T, Wescor, Logan, USA) and a sample chamber (model C-52, Wescor, Logan, USA). For this evaluation, 1 cm tissue excised from the root tip was used.

For the evaluation of recovery from salinity, ten seedlings of each hybrid with similar length and weight were transferred to full-strength Hoagland solution (Hoagland and Arnon 1950) for 72 h. After this period, seedlings were individually weighed and the recovery rate (R) calculated as the difference between the final and initial seedling weight, divided by the evaluation period (in days).

# Experiment 2 - Seedling growth in stress and recovery conditions

This experiment evaluated the existence of an association between plant development at seedling stage and seed germination (shown in experiment 1) in different salt treatments. Seedlings of the maize hybrids A4646 and P32R1 (with the best and worst responses in

experiment 1, respectively) were grown in a greenhouse, in 3 L plastic pots containing half-strength Hoagland nutrient solution with 0, 100, 200 and 300 mmol L<sup>-1</sup> of NaCl. The root medium was replaced weekly and the daily water loss compensated by addition of deionized water.

Moreover, the presence of adaptation mechanisms related to salt tolerance was tested in varying salt concentrations and by applying the salt treatments in different ways. Different salt concentrations were applied at different seedling ages, depending on the treatment. Control plants were supplied with Hoagland's nutrient solution (HNS) without NaCl (treatment 1), whereas the treatments 2, 3 and 4 consisted of HNS combined with 100, 200 and 300 mmol L<sup>-1</sup> of NaCl, respectively. The salt treatments were applied when the seedlings were 5 days old. In treatments 5, 6 and 7, gradual increases of NaCl concentration were applied from the fifth day onwards. In treatment 5 (total of 100 mmol L<sup>-1</sup>), 25 mmol L<sup>-1</sup> were added every day for four days, whereas in treatment 6 (total of 200 mmol  $L^{-1}$ ), the process (25 mmol L<sup>-1</sup> day<sup>-1</sup>) was repeated for six days totaling 150 mmol L<sup>-1</sup>, and 50 mmol L<sup>-1</sup> was added on the 11<sup>th</sup> day to complete the desired salt concentration. A similar approach was used in treatment 7 (total of 300 mmol L<sup>-1</sup>), where 25 mmol L<sup>-1</sup> was added daily from the 5<sup>th</sup> to the 10<sup>th</sup> day and completed with two applications of 75 mmol L<sup>-1</sup> on the 11<sup>th</sup> and 12<sup>th</sup> day. In treatments 8, 9 and 10, salt concentrations were similar to those in treatments 2, 3 and 4, respectively, but they were applied on the 8<sup>th</sup>, 11<sup>th</sup> and 12<sup>th</sup> day in a single application, coinciding with the end of the gradual salinity increase in treatments 5, 6 and 7.

Seedlings were grown under salt stress for 20 days after the first salt application in each treatment. After this period, the surviving seedlings were individually weighed and solutions were substituted for full-strength Hoagland solution without NaCl for recovery evaluation. With respect to nutrient solution, the same procedures of water reposition and solution exchange were repeated as described in experiment 1. Recovery from salt stress was evaluated after 20 days in absence of NaCl, by weighing seedling roots and shoots. Green and senescent leaves were detached from seedlings and the leaf area (LA) was measured using a planimeter (model LI-3100, LICOR, Lincoln, USA).

Before the recovery period, photochemistry activity was evaluated by measuring the potential quantum efficiency of photosystem II (Fv/Fm) with a portable modulated fluorometer (PAM-2000, Walz, Germany). In addition, the leaf water potential ( $Y_L$ ) was measured by the psychrometric method as described in experiment 1. For this evaluation, 0.2 cm<sup>2</sup> leaf discs taken from fully expanded leaves were measured.

The data presented here for both experiments were obtained in the set of experiments conducted between April and September 2002. Previous experiments, carried out between June and November 2001 (data not shown), were used to select genotypes and adjust salt concentrations and evaluation techniques. Data were analyzed in univariate comparisons using ANOVA (experiment 1) and GLM (experiment 2) procedures (SAS 1994).

#### **RESULTS AND DISCUSSION**

## Experiment 1- Germination under osmotic stress: comparison of maize hybrids

Significant differences were observed among genotypes and salt levels for all traits with exception of  $\Psi$ r (Table 2). The 14 hybrids evaluated in this trial exhibited differences in GI values (Figure 1A), mainly at salt concentrations above 50 mmol L<sup>-1</sup>. Two main GI groups could be established: a tolerant and a susceptible. In the tolerant group, hybrids BRS3060 and AGS5010 presented the lowest GI values even at a salt concentration of 200 mmol L<sup>-1</sup>. At higher concentrations, the GI in these hybrids rose sharply (Figure 1A). On the other hand, hybrid A4646 was less affected by increasing salt concentrations, presenting the lowest GI values at 300 mmol L<sup>-1</sup> (Figure 1A). At this salt concentration, the germination performance of hybrid A4646 was excellent (GI 44.4%), and good in the hybrids Tractor and AGS5010 (GI 62.3 and 69.5%, respectively). Of the susceptible group, the GI values of hybrid P32R1 were highest in all tested salt concentrations. From 50 mmol L<sup>-1</sup> upwards the GI of this hybrid increased significantly until complete inhibition (100 %) at 300 mmol L<sup>-1</sup> (Figure 1A), similarly to hybrid BR206 (GI 97.4%) at 300 mmol L<sup>-1</sup>. At this salt concentration, the hybrids A4454, A3663, A3575, A2560, A2555, A2345, A2288 and BRS3123 also presented high susceptibility to osmotic stress (GI between 80.5 and 95.2 %) (Figure 1A).

This experiment also evaluated seedling fresh weight (SW), measured at the end of the germination period. As expected, high NaCl concentrations caused

		Experin	nent 1					Experi	ment 2				
	GI	SW	Ψr	R	SWs	GRs	Fv/Fm	₽	SWr	GRr	RFW	SFW	ΓV
Replication	0.6	0.5	3.3 *	0.5	5.23 *	9.4**	2.18	7.1*	1:1	0.9	0.8	0.9	0.6
Hybrids	5.9**	3.9**	0.6	8.1**	$1338^{**}$	1379**	35.4**	14.1**	1.8	1.6	2.5	1.9	2.5*
Salt level	$169.9^{**}$	71.7**	$18.3^{**}$	85.6**	56.6**	27.4**	3.51*	76.5**	3.1*	1.9	2.7*	3.2*	3.8*
nteraction	1.4 *	0.4	0.5	0.8	31**	32.3**	0.9	1.6	0.7	0.6	0.6	0.8	0.8
JV%	11.2	22.8	14.5	18.6	9.8	5.2	16.8	-17.7	14.8	18.5	13.3	18.6	16.7

(Yr), and recovery rate (R) and in experiment 2: Seedling fresh weight (SWs), seedling growth rate (GRs), photochemical efficiency (Fv/Fm), leaf water potential

Table 2. F-values and probability levels of evaluated traits in experiment 1: germination inhibition index (GI); seedling fresh weight (SW); root water potential

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an evident suppression of early seedling growth. As consequence of the osmotic stress, SW was reduced considerably as salt concentration increased (Figure 1B). SW reductions were more evident in susceptible hybrids than in tolerant ones. For instance, SW was least reduced in hybrids A4646 and AGS5010 at 300 mmol L<sup>-1</sup>, exhibiting decreases of 47.2% (0.47 vs. 0.89 g seedling<sup>-1</sup>) and 53.2% (0.44 vs. 0.94 g seedling<sup>-1</sup>), respectively. In the susceptible group, SW decreased most in hybrids A2560 and A2288 (Figure 1B), and by 62.5% in hybrid A4454, in relation to the control treatment.

Regarding the Ψr value, non-significant differences among hybrids were observed (Table 2). The Yr values of all hybrids ranged from -0.3 to -0.5 MPa in the control treatment. The hybrids tended to differ with respect to germination at high salt concentrations (Figure 1C). The hybrid Tractor, with one of the lowest GI values (Figure 1A), presented the highest (i.e. most negative.)  $\Psi$ r value (Figure 1C). Likewise, low Yr values were observed in some hybrids of the susceptible group. For example, hybrids A2560 and A2555 with GI values of over 90% presented  $\Psi$ r values of -3.3 and -3.2 MPa, respectively. There are two possible explanations for these apparently contradictory results: First, hybrids with high germination rates may increase the  $\Psi$  value, which allows them to absorb water by a physiological mechanism such as osmotic adjustment (Hare et al. 1998), which was not studied here. Second, some hybrids presented high GI and low  $\Psi$ r values, indicating the occurrence of root water loss (McKersie and Leshem, 1994).

In the recovery period, the evaluated hybrids exhibited random performance with no consistent tendency, apart from hybrid A4646, which presented highest growth rates in all conditions (data not shown). Based on results of experiment 1, it can be inferred that there is considerable genetic variability for germination under salt stress among maize hybrids, which can be grouped in tolerant and susceptible. A highly tolerant and a highly susceptible hybrid, A4646 and P32R1, respectively, were further identified in this period.

# Experiment 2- Seedling growth under stress and recovery conditions

The highly tolerant hybrid A4646 and very susceptible hybrid P32R1 were both tested in a range of salt treatments. Significant differences among salt treatments were verified in all evaluated traits, with exception of growth rate in the recovery period (Table 2).



Figure 1. Germination inhibition (A), seedling fresh weight (B) and root water potential (C) of 14 tropical maize hybrids as affected by increasing NaCl concentrations. Symbols are mean values of three replications

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Figure 2. Seedling fresh weight (A), growth rate (B), photochemical efficiency (C) and leaf water potential (D) after 20 days of salt stress in maize hybrids A4646 (dashed bar) and P32R1 (black bar) submitted to a range of NaCl treatments. Bars represent mean values of three replications

The measurements of seedling weight at the end of the salt stress period showed the influence of the salt treatments (Figure 2A). While hybrids A4646 and P32R1 in the control condition presented SW values of 23.17 and 20.17 g seedling<sup>-1</sup>, respectively, both hybrids died after seven days at 300 mmol L<sup>-1</sup> supplied to the 5day-old seedlings (treatment 4). Important suppressive effects on SW were observed in treatments 3, 9 and 10 (application of 200 mmol L<sup>-1</sup> to 5-day-old seedlings, and 200 and 300 mmol L<sup>-1</sup> to 11and 12-day-old seedlings, respectively). Under these conditions SW of hybrid A4646 decreased by about 88.7, 83.6 and 86.8 % in treatments 3, 9 and 10, respectively, compared to the control. Similarly, SW of hybrid P32R1 decreased by 90.6, 83.0 and 77.1 % in the treatments 3, 9 and 10, respectively. The apparently good development of seedlings submitted to treatments 6 and 7 (gradual application of 200 and 300 mmol  $L^{-1}$  to 5-days-old seedlings) was result of the high growth rate of these seedlings before the imposition of salt treatments. After the 20-day stress period, seedlings of both hybrids submitted to treatments 6 and 7 exhibited symptoms of toxicity such as leaf wilting and generalized chlorosis. The different combinations of 100 mmol  $L^{-1}$  resulted in the highest SW values, without toxicity symptoms

The highest seedling growth rates (GRs) in the stress period were observed in the control treatment (without NaCl), whereas the treatments 3, 9 and 10 inhibited seedling growth (Figure 2B).

The leaf water status, as evaluated by the leaf water potential ( $\Psi_{I}$ ) and photochemical efficiency (Fv/ Fm) (Figure 2C and D) agreed with the tendency observed for SWs and GRs (Figure 2A and B). While the highest SWs and GRs values were found in seedlings that grew in the control treatment, the others under different degrees of osmotic stress exhibited significant reductions in both  $\Psi_L$  and Fv/Fm. These patterns were in agreement with the visual aspect of the seedlings. Thus, non-stressed seedlings presented the highest  $\Psi_L$  and Fv/Fm values, indicating the presence of high leaf turgor and photochemical efficiency. The lowest values  $\Psi_L$  values were induced by treatments 3, 6, 7, and 10 in both hybrids (Figure 2D). The Fv/Fm values were lowest in treatments 3 and 7 (0.13 and 0.07 in A4646, and 0.18 and 0.04 in P32R1, respectively), indicating the deleterious effect of salinity on the photochemical apparatus (Figure 2C). This effect was consistent with observations of Khodary (2004). In fact, optimum Fv/Fm values are around 0.8, whereas values below 0.725 indicate photoinhibition of photosynthesis (Critchley 1998). Important effects on the activity of antioxidative enzymes were reported by Azevedo Neto et al. (2006).

After 20 days of osmotic stress, the surviving seedlings were transferred to full-strength Hoagland solution without NaCl to measure the recovery capacity. As predicted, based on the physiological traits measured after the stress period, the seedlings of treatments 3, 6 and 7 died, indicating that the gradual as well as the unique application of 200 and 300 mmol L<sup>-1</sup> of NaCl to 5-day-old seedlings were lethal for both hybrids. Moreover, seedlings of treatment 10 died on the 10<sup>th</sup> day of the recovery period. Although the values of SW, GR and Fv/Fm for the seedlings of this treatment were similar to those in treatment 9 after the stress period (Figure 2A-C), differences in performance were verified during the recovery period.

The control treatment achieved the highest values of seedling fresh weight (SW) after the recovery period. This fact was supported by the highest growth rates (GR) of seedlings in control condition. No significant differences between hybrids were detected in relation to SW and GR (Table 2), but in a comparison of the three treatments with 100 mmol L<sup>-1</sup>, the highest SW and GR values were found in treatment 2, followed by 8 and 5. The fact that treatment 9 was the only salt treatment of over 100 mmol L<sup>-1</sup> that presented surviving seedlings after salt stress recovery period, may indicate the existence of a physiological mechanism that allows seedlings to cope with the early effects of salt stress. Interestingly, the salt concentration of 200 mmol L<sup>-1</sup> was only lethal for maize seedlings when supplied in a single application either in the beginning (5-day-old seedlings) or in the end (11- day-old seedlings) of the salt treatment. On the other hand, seedling growth was maintained when plants were submitted to a gradual salinity increase.

Salt treatments affected root (RFW) and shoot (SFW) fresh weight as well as leaf area (LA) (Table 2). As salt concentrations increased, these traits decreased in both hybrids (Figure 3). In the control treatment, hybrid A4646 tended to higher values of RFW, SFW and LA in relation to hybrid P32R1; RFW, SFW and LA were, respectively, higher in A4646 than in P32R1 by 31.0, 49.0 and 42.5%. Although not significantly different, these values suggest distinct dry matter accumulation in the tested genotypes.

When comparing the control and treatment 9 (200 mmol L<sup>-1</sup> applied to 11-day-old seedling), SFW, RFW and LA were sharply reduced, especially in hybrid P32R1. In hybrid A4646, SFM, RFM and LA values were respectively decreased by 83.8, 74.5 and 76.4%, whereas hybrid P32R1 exhibited reductions of 91.2 (in SFM), 89.3 (in RFM) and 90.6% (in LA) compared to the control (Figure 3). Similarly, effects of salt stress on leaf expansion were reported in maize (Cramer et al. 1994) and cowpea (Silveira et al. 2001). According to Larcher (1995) growth processes are particularly sensitive to salinity; biomass yield and growth rate are considered reliable criteria for evaluating the degree of salt sensitivity. As reported by Grumberg et al. (2002), these phenomena were closely related with the extensibility of the cell wall, affecting cell growth and cell division process.

Our results indicated that root fresh weight (RFW) was less affected than shoots (SFW) by salt stress in both maize hybrids. Generally, shoots are more affected than the root system, owing to an emergency mechanism that intensifies nutrient and water uptake to prevent plant death in stressful conditions. In some cases, stress can trigger an alternative process in plants, such as



Figure 3. Root (A) and shoot (B) fresh weight, and leaf area (C) after 20 days of recovery from salt stress in maize hybrids A4646 (dashed bar) and P32R1 (black bar) submitted to a range of NaCl treatments. Bars represent mean values of three replications

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osmotic adjustment, allowing the maintenance of root growth. However, this mechanism was not verified in this study. Pascale et al. (1999) found differential salt effects on shoots and roots of snap bean. According to these authors, the reduction of the shoot/root ratio was weakly related with lower turgor of salt-stressed leaves.

The results reported here are an evidence of genetic variability among tested hybrids. Furthermore, the salt tolerance observed at germination was not wellcorrelated with hybrid responses at the seedling stage. Although seedling growth of hybrid A4646 was more intense than in P32R1 at recovery from salt treatments (experiment 2), the difference was non-significant. In spite of these responses, the differences between hybrids were evident in experiment 1 (Figure 1), where A4646 exhibited lower GI, higher SW and higher Yr than P32R1. Therefore, the lack of correlation between seedling response at germination and in the salt stress period suggests that studies on plant stress responses should consider the phenological stage. The absence of correlation between germination and seedling growth under salt stress was also reported in alfalfa by Johnson et al. (1991). The uninterrupted seedling growth under lethal NaCl concentrations after previous exposure to sublethal concentrations may suggest the presence of an adaptation mechanism for coping with salt stress, as found in sorghum plants (Amzallag et al. 1993).

In conclusion, screening of maize for salt tolerance by an evaluation of seed germination under salt stress is not possible due to the lack of association with early seedling growth. Alternatively, selection could focus on seedling vigor under salt stress. Either morphological (such as SW and GR) or physiological (Fv/Fm) traits can be used as selection criteria. The presence of an adaptation mechanism could be used to select superior genotypes, though more studies on the genetic bases of this phenomenon are needed.

## Seleção para tolerância ao estresse salino em milho tropical

**RESUMO** - Desde que a salinidade é um fator de estresse muito comum em regiões agrícolas, o objetivo deste trabalho foi avaliar a possibilidade de utilização de parâmetros morfológicos e fisiológicos como critérios de seleção de genótipos de milho sob estresse salino. Os experimentos foram conduzidos durante os estágios de germinação e inicial de crescimento da plântula. A massa de raiz e folhas, área foliar, potencial da água de raiz e folhas, eficiência fotoquímica e taxas de crescimento das plântulas foram avaliadas durante o estresse e no período de recuperação. Nossos resultados demonstraram a presença de variabilidade genética na germinação e ausência de associação entre a germinação e o crescimento inicial das plântulas

sob estresse salino. Os parâmetros associados com o vigor da plântula, com peso e taxa de crescimento, e a atividade fotoquímica avaliados durante o estresse podem ser utilizados como critério em programas de melhoramento que visem a seleção de genótipos de milho tolerantes à salinidade.

Palavras chave: milho, seleção, salinidade, NaCl, estresse.

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