

Genetic variability in salt tolerance during germination of *Stylosanthes humilis* H.B.K. and association between salt tolerance and isozymes

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ABSTRACT

Variation in salt tolerance of six natural populations of *Stylosanthes humilis* from three ecogeographic regions, Mata (wet tropical climate), Agreste and Sertão (semi-arid tropical climate) of Pernambuco State, Northeast Brazil, was evaluated on germination in 201 mM NaCl. There were significant differences among families of all populations for germination percentage and of five populations (except Tamandaré, from Mata) for germination rate. Populations from semi-arid regions presented high coefficients of genetic variation, those from Agreste being higher than those from Sertão. Populations from Mata showed low coefficients of genetic variation. The coefficients of genotypic determination were high for five populations, except Tamandaré, both for germination percentage (≥ 0.89) and for germination rate (≥ 0.79), indicating the possibility of selection for salt tolerance in these populations. An electrophoretic analysis of esterase and peroxidase isozymes was also performed in the six populations, and correlations were estimated between salt tolerance and allelic frequencies. The analysis of salt tolerant and salt sensitive families of populations from Agreste suggested an association of alleles of a peroxidase locus with salt tolerance during germination in the Caruaru population.

INTRODUCTION

Salinity is a serious problem in many parts of the world, decreasing crop productivity and it is an important edaphic factor, affecting the natural distribution of plants in natural habitats (Tal, 1985). The semi-arid regions are characterized by drought, and commonly by saline soils, thus the plants from these regions must be adapted to the adverse situations of these habitats (Epstein, 1972).

Stylosanthes humilis is an annual herbaceous legume endemic to Central and South America, pres-

enting a wide geographic and ecological distribution. In Brazil it is found in semi-arid regions, as well as in areas with an annual rainfall of up to 3000 mm (Williams *et al.*, 1984). Apart from these characteristics, which make it an interesting species for genetic/ecological studies, it is considered as an important pasture legume for the tropics.

S. humilis presents hard or water-impermeable seeds. Dormancy is broken by high surface soil temperatures as the growing season approaches, which ensures that seeds only germinate during the rainy season (Mott *et al.*, 1981), and thus avoid drought (Fisher and Ludlow, 1984). The response of *S. humilis* to saline stress is stage-specific with high tolerance during germination (Lovato *et al.*, 1994), and greater sensitivity during growth (Russell, 1976; Lovato, 1991). However, a significant variation in salt tolerance has been found

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among natural populations both during germination (Lovato *et al.*, 1994) and during the growth stage (Lovato, 1991), although it was not determined how much of this variation is due to genetic components. Intraspecific genetic variation is important to develop cultivars with a higher salt tolerance.

Salt tolerance has been reported in various species as a quantitative trait, e.g., tomato (Fooland and Jones, 1991), alfafa (Allen *et al.*, 1985) and sorghum (Igartua *et al.*, 1994). A method for understanding the inheritance of quantitative traits is the detection of linkage between molecular and biochemical markers. Associations between biochemical marker loci and quantitative traits suggest a functional significance for the extensive enzymatic polymorphism frequently found in natural populations (Price *et al.*, 1984).

MATERIAL AND METHODS

Plant materials

Six populations of *S. humilis* from Pernambuco State (Northeast Brazil), two from each of three ecogeographic regions, Mata, Agreste, and Sertão, were analyzed. Mata is the coastal region (originally forested) with small annual temperature variation and high rainfall. Agreste and Sertão are characterized by semi-arid tropical climates, with a predominance of thorny shrubs. The populations analyzed and their respective locations were: 1) Janga and 2) Tamandaré, from Mata; 3) São Caetano and 4) Caruaru, from Agreste; 5) Sertânia and 6) Flores, from Sertão (see Lovato *et al.*, 1994).

Seeds collected from each plant (18 to 24 plants for each population) were kept separately. In order to prevent the effects of different habitats during the development of seeds and to increase the number of available seeds, one seed from each collected plant was planted in an experimental field located at the Genetics Department of the Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, in Piracicaba, SP, and their seed progenies were used in this work. Seeds taken from 18 families (seed progeny from single maternal parents) of each population were used to assess the genetic variation for salt tolerance and for electrophoretic analysis.

Variation in salt tolerance

Seeds stored for eight months at room temperature were mechanically scarified and treated with fungicide, and incubated in plastic boxes Gerbox

type (50 seeds in each) with 12 ml of 201 mM NaCl. This salt concentration discriminates well between these populations (Lovato *et al.*, 1994). After 5 h of incubation at 25°C in darkness, the temperature was decreased to 16°C for 2 h, to break the embryo dormancy that could exist in some families. The seeds were then incubated at 25°C in darkness. Germinated seeds, based on the emergence of the radicle, were counted every 24 h, over a period of 14 days. The experimental design was a completely randomized one, with 18 treatments (families) for each population, and three replications of 50 seeds each. Germination rates were calculated according to Popinigs (1977) as $\Sigma(n_i/t_i)$, where n_i = number of germinated seeds on day i and t_i = time (days) to germination. Data for percentage of germination were transformed into $\arcsin \sqrt{\%}$ prior to the statistical analysis, in order to achieve variance homogeneity.

The statistics consisted of an analysis of variance for each population (Steel and Torrie, 1980). The genetic variance among families (σ_f^2) and the phenotypic variance (σ_F^2) were estimated by the following equations: $\sigma_f^2 = (Q_1 - Q_2)/r$ and $\sigma_F^2 = Q_1/r$, where Q_1 = mean square of families, Q_2 = mean square of error and r = number of replications. The coefficient of genetic variation (CV_g) and the coefficient of genotypic determination (b) were obtained from these estimates for each population, as follows: $CV_g = (\sqrt{\sigma_f^2}/m)100$ and $b = \sigma_f^2/\sigma_F^2$, where m = general mean. These parameters were estimated for both germination rate and germination percentage.

Isozyme electrophoresis and analysis of association with salt tolerance

Seedlings 2 cm in length (36 to 48 h after incubation in distilled water) were used for analyzing peroxidases (PER, EC 1.11.1.7) and seeds which were soaked for 14 h in distilled water were used for esterase analysis (EST, EC 3.1.1.1). Enzymes were extracted in a buffer composed of 0.1 M Tris, pH 7.5, 0.2 M sucrose, 0.6% polyvinylpyrrolidone, 0.1% bovine serum albumine and 20 μ l 0.6% 2-mercaptoethanol in 15 ml buffer. Electrophoresis was performed in 12% Sigma starch gel in Tris-citrate/lithium-borate buffer (Scandalios, 1969).

First, the electrophoresis was performed on one seed or seedling of each of 18 families of each population. Then, peroxidases were analyzed for five to 10 seedlings of each of the five most salt tolerant and five most sensitive families of the populations São Caetano and Caruaru. Allelic frequencies, determined by direct allele counting, were established for each

population and for each of the 10 families of populations from São Caetano and Caruaru. For those staining zones which were difficult to interpret, the presence or absence of a specific band was recorded and its frequency for each population was obtained by dividing the number of seedlings with the band by the total number of seedlings analyzed. Linear correlations were used to analyze the association of salt tolerance with frequencies of bands or alleles, both for populations and for families within São Caetano and Caruaru populations. For this statistical study the allelic or band frequencies were transformed into arcsin $\sqrt{\text{frequency}}$. For the analysis of the association of salt tolerance during germination with isozymes, linear correlations of band or allele frequencies with rate and percentage germination on 201 mM NaCl were calculated. The relationship of isozymes with salt tolerance during growth was analyzed using the number of necrotic leaves and the shoot dry weight of plants grown in 80 mM NaCl, established in another experiment (Lovato, 1991).

RESULTS

Genetic variation for salt tolerance

There was a significant variation ($P < 0.01$) within all populations in relation to the germination percentage (Tables I and III) and within five populations (except Tamandaré) for germination rate (Table II and IV) screened on 201 mM NaCl. The populations from São Caetano, one of the most sensitive (mean 44% germination), and Caruaru, one of the most tolerant (mean 70% germination), both from the Agreste region, presented the greatest variation among families, where the differences between the highest and the lowest percentage of germination were 90% and 75.4%, respectively. Populations from Mata (Janga and Tamandaré) were the least phenotypically variable, these values being 46.3 in Janga and 50.7 in Tamandaré (Table I).

The coefficients of genetic variation for germination percentage (Table III) were also higher for both populations from Agreste (Caruaru and São Caetano), followed by populations

from Sertão. The populations from Mata presented the lowest coefficients of genetic variation. Five populations, with the exception of Tamandaré, showed high coefficients of genotypic determination, indicating that a high proportion of observed phenotypic variation was genetic, and therefore allowing selection for saline tolerance, even in populations with intermediate values of variation, as in the population from Janga.

Populations from Agreste also had the highest variation in germination rate and Tamandaré the lowest phenotypic variation, although other population from Mata (Janga) had a high variation (Table II). However, considering the genetic variation (Table IV), the values of coefficients of genetic variation, although higher than those for germination percentage, maintained the same relation between populations, i.e., populations from Agreste had the highest variation, followed by populations from Sertão, and the populations from Mata presented the lowest coefficients of genetic variation among families. The coefficients of genotypic determination for germination rate were of the same magnitude as those for germination percentage, being relatively low for Tamandaré and high for all other populations.

Table I - Germination percentage of families of populations of *Stylosanthes humilis* in 201 mM NaCl.

Family	Population					
	Flores	Sertânia	Caruaru	São Caetano	Janga	Tamandaré
1	50.7 ^{bcd}	40.7 ^{bcd}	84.0 ^{ab}	42.0 ^{bc}	98.7 ^a	40.0 ^{abc}
2	75.3 ^{ab}	42.7 ^{bcd}	94.0 ^a	17.3 ^{cde}	92.0 ^{abcd}	53.3 ^{abc}
3	58.0 ^{bc}	56.7 ^b	24.0 ^c	24.7 ^{cde}	100.0 ^a	42.0 ^{abc}
4	64.7 ^{ab}	17.3 ^{de}	86.0 ^{ab}	90.7 ^a	76.7 ^{bcd}	67.3 ^{ab}
5	76.7 ^{ab}	32.0 ^{bcd}	90.7 ^{ab}	54.7 ^b	92.7 ^{abc}	38.0 ^{abc}
6	88.0 ^a	39.3 ^{bcd}	91.3 ^{ab}	14.7 ^{de}	95.3 ^{ab}	36.0 ^{abc}
7	59.3 ^{abc}	36.7 ^{bcd}	94.7 ^a	88.0 ^a	53.7 ^e	42.7 ^{abc}
8	82.0 ^{ab}	14.7 ^e	20.7 ^c	54.7 ^b	92.0 ^{abcd}	44.7 ^{abc}
9	58.7 ^{abc}	35.3 ^{bcd}	31.3 ^c	24.0 ^{cde}	97.3 ^a	26.7 ^{bc}
10	57.3 ^{bc}	30.0 ^{bcd}	94.0 ^{ab}	12.0 ^{de}	74.0 ^{cde}	35.3 ^{abc}
11	57.3 ^{bc}	38.0 ^{bcd}	82.7 ^{ab}	86.7 ^a	99.3 ^a	42.7 ^{abc}
12	29.3 ^{cd}	30.0 ^{bcd}	89.3 ^{ab}	8.0 ^e	57.3 ^e	48.7 ^{abc}
13	50.7 ^{bcd}	26.7 ^{bcd}	19.3 ^c	30.0 ^{bcd}	95.3 ^{ab}	50.7 ^{abc}
14	22.0 ^d	30.0 ^{bcd}	70.7 ^b	92.0 ^a	92.0 ^{abcd}	74.0 ^a
15	66.7 ^{ab}	27.3 ^{bcd}	26.0 ^c	16.7 ^{cde}	56.0 ^e	44.0 ^{abc}
16	79.3 ^{ab}	24.0 ^{cde}	91.3 ^{ab}	15.3 ^{de}	70.7 ^{de}	31.3 ^{abc}
17	68.7 ^{ab}	47.3 ^{bc}	92.0 ^{ab}	22.7 ^{cde}	54.7 ^e	23.3 ^c
18	29.3 ^{cd}	86.0 ^a	78.7 ^{ab}	98.0 ^a	97.3 ^a	41.3 ^{abc}
Mean	59.7	36.4	70.0	44.0	83.0	40.1
Range	22.0-88.0	14.7-86.0	19.3-94.7	8.0-98.0	53.7-100.0	23.3-74.0

The values in each column followed by the same letter do not differ from each other at the 5% level (Tukey test).

Table II - Germination rate of families of populations of *Stylosanthes humilis* in 201 mM NaCl.

Family	Population					
	Flores	Sertânia	Caruaru	São Caetano	Janga	Tamandaré
1	8.2 ^{bcdefg}	7.6 ^{abc}	23.3 ^{ab}	6.6 ^b	24.1 ^{ab}	3.8 ^a
2	11.0 ^{abcd}	3.8 ^{bc}	23.2 ^{ab}	2.5 ^{bcde}	21.2 ^{bcd}	8.0 ^a
3	8.8 ^{abcdef}	6.3 ^{bc}	3.1 ^c	3.3 ^{bcde}	25.0 ^{ab}	6.7 ^a
4	8.9 ^{abcde}	2.9 ^c	20.0 ^{ab}	19.6 ^a	15.0 ^{cde}	11.4 ^a
5	9.4 ^{abcde}	4.9 ^{bc}	21.6 ^{ab}	5.3 ^{bcd}	22.7 ^{ab}	5.7 ^a
6	12.0 ^{abc}	5.0 ^{bc}	22.2 ^{ab}	2.1 ^{cde}	24.3 ^{ab}	7.7 ^a
7	6.1 ^{defg}	5.1 ^{bc}	27.0 ^{ab}	18.4 ^a	14.1 ^{de}	6.0 ^a
8	13.6 ^{ab}	2.2 ^c	4.2 ^c	6.2 ^{bc}	21.8 ^{abc}	7.0 ^a
9	6.2 ^{cdefg}	6.8 ^{abc}	6.4 ^c	2.8 ^{bcde}	24.0 ^{ab}	3.4 ^a
10	6.3 ^{cdefg}	5.6 ^{bc}	28.1 ^a	1.6 ^{de}	12.6 ^e	4.3 ^a
11	7.1 ^{cdefg}	7.0 ^{abc}	20.0 ^{ab}	18.7 ^a	28.6 ^{ab}	5.6 ^a
12	2.9 ^{fg}	3.1 ^{bc}	23.5 ^{ab}	0.8 ^e	9.0 ^e	6.3 ^a
13	6.1 ^{defg}	3.2 ^{bc}	4.6 ^c	3.3 ^{bcde}	25.3 ^{ab}	6.9 ^a
14	2.3 ^g	4.4 ^{bc}	19.9 ^{ab}	19.3 ^a	23.1 ^{ab}	9.9 ^a
15	8.7 ^{abcdef}	6.2 ^{bc}	3.7 ^c	1.9 ^{cde}	10.6 ^e	5.7 ^a
16	14.6 ^a	4.4 ^{bc}	21.7 ^{ab}	1.4 ^{de}	11.3 ^e	2.9 ^a
17	7.5 ^{cdefg}	9.6 ^{ab}	22.0 ^{ab}	3.3 ^{bcde}	9.9 ^e	3.4 ^a
18	3.5 ^{efg}	13.0 ^a	19.1 ^b	21.7 ^a	29.2 ^a	5.3 ^a
Mean	8.0	5.6	17.4	7.7	19.5	6.1
Range	2.3-14.6	2.2-13.0	3.1-28.1	0.8-21.7	9.0-29.2	2.9-11.4

The values in each column followed by the same letter do not differ from each other at the 5% level (Tukey test).

Table III - Summary of variance analysis of arcsin $\sqrt{\%}$ germination transformed data and parameters¹ of six populations of *Stylosanthes humilis* sown in 201 mM NaCl.

Source of variation	Means squares					
	Flores	Sertânia	Caruaru	São Caetano	Janga	Tamandaré
Family	393.64*	315.72*	1176.54*	1470.43*	589.01*	181.23*
Error	40.93	34.38	36.49	29.68	33.42	70.28
Mean	51.0	36.8	58.8	41.9	69.0	41.0
CV (%)	12.5	15.9	10.3	13.0	8.4	20.4
CV _g (%)	21.3	26.3	33.2	52.3	19.7	14.6
b	0.90	0.89	0.97	0.98	0.94	0.61

*, Significant at 1% level.

¹CV, CV_g, b represent coefficients of variation, genetic variation and genotypic determination, respectively.

Table IV - Summary of variance analysis of germination rate, and respective coefficients of variation (CV), genetic variation (CV_g) and genotypic determination of seeds of six populations of *Stylosanthes humilis* sown in 201 mM NaCl.

Source of variation	Means squares					
	Flores	Sertânia	Caruaru	São Caetano	Janga	Tamandaré
Family	34.75*	20.61*	222.85*	179.38*	138.53*	15.02
Error	3.53	4.41	7.00	1.99	5.79	7.79
Mean	8.0	5.6	17.4	7.7	19.6	6.1
CV (%)	23.5	37.5	15.2	18.3	12.3	45.8
CV _g (%)	40.5	41.3	48.7	99.6	34.0	25.3
b	0.90	0.79	0.97	0.99	0.96	0.48

*, Significant at the 1% level.

Salt tolerance and isozymes

The esterase system exhibited two staining zones consistent with a monomeric subunit structure. The two zones were interpreted as two loci, each with two alleles. The peroxidases showed three staining zones, one anodic with only one band in all populations, that was interpreted as a monomorphic locus (Per1), and two cathodic zones. The fast cathodic zone presented two bands, and was interpreted as a locus (Per2) with two alleles. The slow cathodic zone was difficult to be interpret, exhibiting in all populations two bands, assigned as bands 3 and 4.

The analysis of the association of salt tolerance of populations during germination and during growth, with the allele or band frequencies of locus or staining polymorphic zones showed significant correlations ($P < 0.05$) only between the allele frequency in the Per2 locus with germination rate and with the number of leaves with necrosis (Table V). This preliminary analysis (based on electrophoresis of one seed for each family) showed that in the Caruaru population all the tolerant families presented only the allele Per2^S, and the sensitive ones only the alternative allele, Per2^F. In the São Caetano population, the sensitive families also had only the Per2^F allele, but the tolerant ones presented one or the other allele.

Considering that within both populations from Agreste region (Caruaru and São Caetano) there were families that were very different in relation to salt tolerance (Tables I and II), a more detailed study was made, analyzing the peroxidases of five to 10 seedlings within each of five tolerant and five sensitive families of each of these populations. *S. humilis* presents an intermediate mating system (Marcon, 1988), and thus segregation within families can occur. All the tolerant families of Caruaru population presented only homozygote seedlings

Table V - Linear correlations among bands or allele frequencies of populations and their germination in 201 mM NaCl and growth in 80 mM NaCl (mean of 18 families).

Alleles or bands	n	Germination percentage	Germination rate	No. of necrotic leaves	Shoot weight
Alleles					
Per2 ^S	6	0.69	0.86*	0.83*	-0.29
Est1 ^F	6	-0.64	-0.61	-0.55	0.65
Est2 ^F	6	0.57	0.29	-0.06	0.02
Bands					
P3	6	-0.05	0.22	0.41	-0.27
P4	6	-0.77	0.77	-0.39	0.18

*, Significant at the 5% level.

for the Per2^S allele, and the seedlings of sensitive families presented only the alternative allele, Per2^F (Table VI). In the São Caetano population, all the seedlings of sensitive families were also homozygotic for the Per2^F allele, however the tolerant families were homozygotic for this or for the alternative allele, or segregated for both (Table VI). The high correlation coefficients between the frequencies of allele Per2^S of the families and the rate and percentage germination of families of Caruaru population confirmed the association of this allele with salt tolerance during germination in this population (Table VII).

Table VI - Frequencies of Per2^S allele in tolerant and sensitive families of Caruaru and São Caetano populations.

Caruaru		São Caetano	
Families	Per2 ^S frequencies	Families	Per2 ^S frequencies
2 (T) ¹	1.00	4 (T)	0.00
5 (T)	1.00	7 (T)	1.00
10 (T)	1.00	11 (T)	1.00
14 (T)	1.00	14 (T)	0.00
16 (T)	1.00	18 (T)	0.11
3 (S)	0.00	2 (S)	0.00
8 (S)	0.00	3 (S)	0.00
9 (S)	0.00	6 (S)	0.00
13 (S)	0.00	9 (S)	0.00
15 (S)	0.00	15 (S)	0.00

¹T = Salt tolerant and S = salt sensitive according to Tables I and II.

Table VII - Linear correlations among Per2^S allele frequencies within families and germination in 201 mM NaCl and growth in 80 mM NaCl of these families.

Population	Number of families	Germination percentage	Germination rate	No. of necrotic leaves	Shoot weight
Caruaru	10	0.97**	0.98**	-0.27	0.28
São Caetano	10	0.46	0.49	0.64*	-0.51

*, **, Significant at 5% and 1% levels, respectively.

It appears that there was no association between alleles of the Per2 locus and salt tolerance during growth, since a significant correlation was observed only for the number of leaves with necrosis for São Caetano population, and none for the other character used to assess salt tolerance during growth, the shoot weight (Table VII).

DISCUSSION

Genetic variation for salt tolerance during seed germination has been shown in several species, for example, in alfafa the estimated broad sense heritability is 50% (Allen *et al.*, 1985), and in the cultivated tomato, 74% (Fooland and Jones, 1991).

The populations from Agreste presented the highest genetic variation, followed by those from Sertão. The populations Janga and Tamandaré from Mata region showed lower genetic variation. Marcon (1988) also found lower variation in isozymes in populations from Mata than those from Agreste and Sertão, and the variation within populations was correlated with environmental heterogeneity (climatic, topographic and edaphic conditions). The Agreste region is the most variable in relation to environmental conditions. The two populations from Agreste besides having shown great intrapopulation variation presented different levels of saline tolerance, Caruaru being one of the most tolerant and São Caetano one of the most sensitive. The spatial environmental heterogeneity in relation to soil salinity and rainfall between and within habitats of these populations could, through disruptive selection mechanisms, contribute to the maintenance of high genetic variation on salt tolerance in these populations. It is known that in many salt affected soils there is extreme variability in salinity both spatially (Richards, 1983) and temporally (Ungar, 1987). According to Hedrick (1995), when an unfavorable environment can be avoided by either delayed germination or diapause, the conditions for genetic polymorphism are greatly broadened in a temporally varying environment. *S. humilis* presents dormancy and this could maintain variation in salt tolerance. The patterns of spacial and temporal variation had different effects on the maintenance of polymorphism (Hedrick, 1986, 1995). Although in the present study the patterns of environment variation were not characterized, it is known that there are great temporal fluctuations in rainfall in Agreste and Sertão regions, which could lead to temporal variations in soil salinity.

Marcon (1988), based on levels of genetic variation, on genetic distances between populations, and on historical evidence that populations from Mata are recent and have been introduced by migrants from Sertão region, suggested that populations from Mata have a marginal distribution, and their relative genetic uniformity is due to a founder effect. The low levels of genetic variation in salt tolerance of these populations favor this hypothesis.

Our results showed an association of salt tolerance during germination with alleles of one locus of peroxidase only in the population from Caruaru. The fact that a relation between saline tolerance and allozymes of peroxidases was found only in one population suggests that even if peroxidases have some functional relation with saline stress in *S. humilis*, the two allozymes do not confer differences in salt tolerance during germination.

The association found in this study could be due to a physical association (linkage) between the peroxidase locus and the saline response loci. The putative gametic disequilibrium found only in Caruaru population could be explained by historical events, such as founder effect or bottleneck, both leading to genetic drift (Hedrick *et al.*, 1978).

The populations from Janga and Tamandaré, both from Mata region, are from an environment that was altered by man, and probably are recent (Marcon, 1988). The presence of allele Per^{2S} in Janga suggests that this population was founded by seeds from São Caetano and/or Caruaru populations, since it is common to bring goats into the Mata region (Marcon, 1988). The dispersal of *Stylosanthes humilis* seeds is facilitated by the presence of a hooked beak (hardened style which remains after anthesis) that adheres readily to fur, clothing, hay, etc (McKeon and Mott, 1984).

RESUMO

A variação na tolerância salina na germinação dentro de seis populações naturais de *Stylosanthes humilis*, provenientes de três regiões ecogeográficas do Estado de Pernambuco, Mata (clima tropical úmido), Agreste e Sertão (clima tropical semiárido), foi determinada submetendo-se sementes para germinar em NaCl 201 mM. Os resultados mostraram diferenças significativas entre famílias de todas as populações para porcentagem de germinação e de cinco populações para velocidade de germinação, com exceção da população Tamandaré (Mata). As populações das regiões semiáridas mostraram altos coeficientes de variação genética, sendo as do Agreste maiores que as do Sertão. As populações da Mata apresentaram baixos coeficientes de variação genética. Os coeficientes de determinação genotípica foram altos para todas as populações, com exceção de Tamandaré,

tanto para porcentagem de germinação ($\geq 0,89$), como para velocidade de germinação ($\geq 0,79$), indicando a possibilidade de seleção para tolerância salina na germinação nessas populações. Foram também realizadas análises de isoenzimas de esterases e peroxidases e estabelecidas correlações entre tolerância salina e frequências alélicas dessas populações. A análise de famílias sensíveis e tolerantes ao sal de populações do Agreste mostrou uma associação de alelos de um loco de peroxidase com a tolerância salina durante a germinação na população proveniente de Caruaru.

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