

Evaluation of Salinity Tolerance Indices in seedling of maize (*Zea mays* L.)

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The aims of this research were: to study the application of different tolerance Indices in traits measured in maize seedlings and to assess their possible use in the identification of genotypes tolerant to salinity. Sixty eight accessions were tested in two environments (0 and 100mM NaCl). We recorded length for radicle, shoot and third leaf and dry weight for root and shoot. Six stress tolerance indices were included: stress susceptibility (SSI), stress tolerance index (STI), stress tolerance (TOL), geometric mean productivity (GMP), mean productivity (MP) and yield stability index (YSI). Saline environments show a great spatial variation in relation to the salt concentration, for this reason it would be important to identify genotypes with stable behavior in a variety of saline soils. The biplot method allowed clustering accessions, traits measured in stress and non stress environment and salt tolerance Indexes in a same graphic, and showed that GMP, MP and STI indexes were the ones who helped identify the high yielding (group A genotype) and stable accessions, characterized by a high expression of these characters in both environments. Principal Component method showed that shoot dry weight and root length had the highest contribution and both were associated with these above indices in salinity. Therefore, in this study the accessions: 1, 7, 30, 33, 43 and 45 had stable values for the traits root length and shoot dry weight. Within this group the 30 and 33 entries were superior (bellowing to Group A genotypes) because they had the highest PC1 scores but its PC2 scores were rather small for the most of the variables.

Key words: *Zea mays*, seedling growth, selection, salinity tolerance, stability

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Los objetivos de este trabajo fueron: estudiar la aplicación de diferentes índices de tolerancia en caracteres de plántulas de maíz y evaluar su posible utilidad en la identificación de genotipos tolerantes a la salinidad. Se probaron 68 accesiones en dos ambientes (0 y 100m MCINa). Se midieron: largo de raíz, vástago y 3ª hoja y peso seco de raíz y de parte aérea. Se incluyeron 6 índices de tolerancia: índice de susceptibilidad al estrés (SSI), índice de tolerancia al estrés (STI), tolerancia al estrés (TOL), media geométrica de la productividad (GMP), productividad media (MP) e índice de estabilidad del rendimiento (YSI). Debido a la variación espacial relacionada con la concentración de sal en ambientes salinos, sería importante identificar genotipos estables frente a una gama amplia de suelos salinos. El biplot agrupó las accesiones, caracteres medidos en ambientes con y sin estrés e índices de tolerancia a sal, y demostró que los índices GMP, MP y STI fueron los que permitieron identificar las accesiones estables que se caracterizan por tener una alta expresión de estos caracteres en ambos ambientes. La aplicación del método de Componentes Principales (CP) identificó a los caracteres peso seco aéreo y largo de raíz como los de mayor contribución y ambos estuvieron asociados con dichos índices de tolerancia a salinidad. De esta manera, en este estudio las accesiones 1, 7, 30, 33, 43 y 45 fueron los más estables para los caracteres peso seco aéreo y largo de raíz. Entre ellos las accesiones 30 y 33 fueron superiores (pertenecientes a genotipos del Grupo A) porque mostraron los escores más altos sobre el eje CP1 pero sus aportes al CP2 fueron bastantes pequeños, para la mayoría de las variables.

Palabras clave: Maíz, plántula, selección, tolerancia a salinidad, estabilidad

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INTRODUCCION

Salinity is one of the major obstacles to increasing crop production worldwide. Therefore, the need for genetic improvement of salt tolerance is great and is expected to increase dramatically in the future.

Different methods are used to screen for salt tolerance, and several physiological and morphological traits have been reported as being reliable indicators for the selection of germplasm possessing high degree of salinity tolerance. These characteristics include seed germination and seedling growth under saline conditions (Ashraf et al., 1992; 2002), the degree of electrolyte leakage (cell membrane stability, CMS) from salinity-damaged leaf cells and the water relations of plants (Ashraf et al., 2005; Collado et al., 2010). These traits measured in field and laboratory showed significant correlations (Ashraf et al., 1996; 1999; 2005). Then, the salt tolerance tested under laboratory and greenhouse conditions should reflect this character under field conditions (Sammons et al., 1978). Furthermore, it has been shown in several crops that the tolerance evidenced in the seedling stage is also manifested at the adult plant level. This property has been successfully used in: maize (Ashraf & McNeilly, 1990; Maiti et al. 1996), pearl millet (Kebebew & Mc Neilly, 1994), lucerne (Al-Khalib et al., 1993) and several forage grass species (Ashraf et al., 1994) since early stages of a breeding program. For improving salt tolerance, the number of genotypes or accessions to be evaluated is usually high the screening at seedling stage could be used for a preliminary selection in these species.

Selection of different entries under a wide range of environments is important in a breeding program. When the genotypes are tested in contrasting conditions (non-stress *versus* stress), these can be classified into four groups: (A) Genotypes producing high yield under both stress and non-stress environments, (B) genotypes with high yield under non-stress environments, (C) genotypes with high yield under stress environments and (D) genotypes with poor performance under both stress and non-stress environments (Fernandez, 1992). Three breeding approaches for abiotic stress tolerance have been evolved. The first one is to breed for high yield under optimum condition. As the maximum genetic potential of yield is expected to be realized under optimum condition and a high positive correlation exists between performance in optimum and stress conditions, a genotype superior under optimum level will also yield relatively well under stress condition. However, the presence of genotype x environment interaction may restrict the high-yielding genotype to perform well under stress condition. Thus, as a second approach, the breeding under stress condition has been suggested. The problem in this approach is that the intensity of stress is highly variable from year to year and as a consequence the efficiency of selection for stress tolerance is reduced. The simultaneous selection in non-stress environment for yield and in stress condition for stability may be done an alternative approach to achieve the desired goal of evolving stress-resistant genotype with high yield. Due to the spatial and temporal variability in salinity within most agricultural systems, a stable cultivar must possess stress tolerance

mechanisms that prevent excessive yield reductions in stress environments. The grain yield and yield stability under environmental stress remain a major selection criteria for stress tolerance in many breeding programs (Mitra, 2001). Yield stability is a measure of variation between potential and actual yield of a genotype across changing environments (Blum, 1980). This relative performance in salt stress compared with a non-saline soil has been used for screening salinity-resistant genotype (Igartua, 1995).

There are several tolerance indices for screen genotypes to stress tolerance such as geometric mean productivity (GMP) (Fernández, 1992), mean productivity (MP) (Rosielle & Hamblin, 1981), stress susceptibility index (SSI) (Fischer & Maurer, 1978), yield stability index (YSI) (Bousslama & Schapaugh, 1984), stress tolerance index (STI) (Fernández, 1992) and tolerance index (TOL) (Rosielle & Hamblin, 1981) that identify susceptible and resistance genotypes based on their yields in stress and non-stress environments. A suitable index must be able to distinguish group A genotypes from the other groups (Fernandez, 1992).

Even if these selection indices were used to identify drought-tolerance; they could be used for another stress like the one induced by salinity soils. Besides although seed yield is the most frequently traits, is not the only one that could be used for screening. In this view, Ashraf et al. (2006) used these indices to explore the salinity tolerance in a grass species in several traits measured in seedlings like plant height and dry matter. For estimating tolerance indices to drought stress, Moayedi et al. (2009) employed several traits of wheat seedlings like seed germination, root length, root dry weight and seedling dry weight.

The best selection index must be able to distinguish genotypes that have uniform superiority in both stress and no-stress environment (Fernandez, 1992). A larger value of TOL and SSI show relatively more sensitivity to stress, thus a smaller values of TOL and SSI are favored. Several authors noticed that selection based on these two indexes favors genotypes with low yield under non-stress conditions and high yield under stress conditions (Fernandez, 1992; Golabadi et al., 2006). STI and GMP tend to select genotypes with high yield under stress and non-stress conditions, while SSI only identifies high-yielding genotypes under stress conditions (Khalili et al., 2004; Souri et al., 2005; Karami et al., 2006). Genotypes are more stables if STI and GMP show higher values, then the selection based on these indices would result in genotypes with higher stress tolerance and good yield potential (Fernandez, 1992).

In a breeding program to identify salt tolerance, it would be very useful the early selection of tolerant accessions. In this sense the characters evaluated during germination and seedling stage would help to a early identification of the best entries. The objectives of present study were: to study the application of different tolerance Indices of traits measured in maize seedlings and assess their possible use in the identification of genotypes of maize tolerant to salinity.

MATERIALS AND METHODS

Plant material

Seeds of 68 different accessions (8 inbred lines and 60 landraces) were evaluated. The accessions represent a wide range of racial origins, maturity and grain type (Table 1). Landraces seeds were ceded by Germplasm Bank of INTA Pergamino and the inbred lines supplied by Instituto Fitotécnico Santa Catalina (La Plata University, FCAyF).

Experiment design and traits

Seeds were sterilized in the surface with 1% sodium

hypochlorite solution for 5 minutes before experimentation, then rinsed with distilled water. Pre-germinated caryopses were transferred to pots containing perlite. These pots were put in trays with half strength Hoagland's solution. The solutions were renewed every three days. The experiment was carried out in a controlled environment room at 25 °C, with 16h day length. A completely randomized design with three replicates was adopted. Each replicate was a plot with three plants (experimental unit). Three plots per accession were assigned to each treatment. Two treatments were used: 0 (as control) and 100mM NaCl (Fortmeier & Schubert, 1995; Azevedo Neto et al., 2004

Table 1. Name of accessions, kernel type, genetic structure and place of collections. *: these entries correspond to inbreds lines and the remaining genotypes are landraces belong to INTA Pergamino. N/D: no data

N°	Entry	Kernel Type	Province	N°	Entry	Kernel Type	Province
1	ARZM01001	Red flint	Buenos Aires	35	ARZM17001	Red flint	San Luis
2	ARZM01005	Red flint	Buenos Aires	36	ARZM17026	Red flint	San Luis
3	ARZM01006	Red flint	Buenos Aires	37	ARZM17035	Red flint	San Luis
4	ARZM01009	Red flint	Buenos Aires	38	ARZM17040	Red flint	San Luis
5	ARZM01013	Red flint	Buenos Aires	39	ARZM17056	Red flint	San Luis
6	ARZM01015	Red flint	Buenos Aires	40	ARZM18001	Red flint	La Pampa
7	ARZM01029	Red flint	Buenos Aires	41	ARZM18005	Red flint	La Pampa
8	ARZM01039	Red flint	Buenos Aires	42	ARZM18006	Red flint	La Pampa
9	ARZM01043	Red flint	Buenos Aires	43	ARZM18010	Red flint	La Pampa
10	ARZM01052	Red flint	Buenos Aires	44	ARZM18012	Red flint	La Pampa
11	ARZM01080	Red flint	Buenos Aires	45	ARZM18017	Red flint	La Pampa
12	ARZM01102	Red flint	Buenos Aires	46	ARZM18037	Red flint	La Pampa
13	ARZM02005	Red flint	Santa Fe	47	ARZM18043	Red flint	La Pampa
14	ARZM02006	Red flint	Santa Fe	48	ARZM19010	Yellow flint	Neuquén
15	ARZM02016	Red flint	Santa Fe	49	ARZM19068	Red flint	Neuquén
16	ARZM03003	Camelia	Entre Ríos	50	ARZM20010	White flint	Río Negro
17	ARZM03013	Camelia	Entre Ríos	51	ARZM21011	Yellow flint	Chubut
18	ARZM03020	Camelia	Entre Ríos	52	B73 *	Yellow dent	N/D
19	ARZM03023	Camelia	Entre Ríos	53	AD3 *	Red flint	N/D
20	ARZM03034	Camelia	Entre Ríos	54	SC75 *	Yellow flint	N/D
21	ARZM04012	Red flint	Corrientes	55	F564 *	Yellow flint	N/D
22	ARZM04018	Camelia	Corrientes	56	LP3*	Red flint	N/D
23	ARZM06020	Red flint	Chaco	57	P21 *	Red flint	N/D
24	ARZM07140	Red flint	Formosa	58	WXEB *	Red flint	N/D
25	ARZM14004	Red flint	Córdoba	59	A26*	Yellow flint	N/D
26	ARZM14044	Red flint	Córdoba	60	F10*	Yellow flint	N/D
27	ARZM14049	Red flint	Córdoba	61	P1338*	Red flint	N/D
28	ARZM14056	Red flint	Córdoba	62	arzm12209	Red flint	N/D
29	ARZM14103	Red flint	Córdoba	63	arzm18054	Yellow flint	N/D
30	ARZM14110	Red flint	Córdoba	64	arzm18029	White flint	N/D
31	ARZM16021	Red flint	Mendoza	65	arzm01151	Red flint	N/D
32	ARZM16026	Red flint	Mendoza	66	arzm06070	Red flint	N/D
33	ARZM16035	Red flint	Mendoza	67	arzm18052	Red flint	N/D
34	ARZM16050	Red flint	Mendoza	68	SC66 *	Yellow flint	N/D

2005 y 2006; De Costa et al., 2007). The final concentration was reached by a gradual increment of 25mM NaCl every two days (Rao & McNelly, 1999; Cicek & Cakirlar, 2002; Khan & McNeilly, 2003). After 14 days of salt treatment, the seedlings were harvested. The length for shoot, radicle and third leaf (SL, RL and LL, respectively) were recorded. Shoot and radicle were separated and the samples were dried for two days until constant weight, for dry mass determination (SDW and RDW respectively). These traits were measured in both treatments and were identified by the subscript c and s (no stress and stress conditions, respectively). The salinity tolerance indices were calculated as follows:

1. Stress Susceptibility Index (SSI): $SSI = (1 - (Y_{si}/Y_{pi}))/SI$ (Fischer & Maurer, 1978);
2. Stress Tolerance Index (STI): $STI = (Y_{pi} \times Y_{si})/Y_p^2$ (Fernandez, 1992);
3. Tolerance Index (TOL): $TOL = Y_{pi} - Y_{si}$ (Rosielles & Hamblin, 1981);
4. Geometric Mean Productivity (GMP): $GMP = \sqrt{Y_{pi} \times Y_{si}}$ (Fernandez, 1992);
5. Mean Productivity (MP): $MP = (Y_{pi} + Y_{si})/2$ (Rosielles & Hamblin, 1981);
6. Yield Stability Index (YSI): $YSI = Y_{si}/Y_{pi}$ (Bouslama & Schapaugh, 1984; Gavuzzi et al., 1997)

Where:

Y_{si} = yield of cultivar in stress condition,
 Y_{pi} = yield of cultivar in normal condition
 And SI that is stress intensity, where: $SI = 1 - (Y_s/Y_p)$
 Y_s = total yield mean in stress condition,
 Y_p = total yield mean in normal condition.

Statistical analysis

Means and standard deviation were calculated for the traits in non-stressed and stressed traits, separately. The analysis of variance for the traits and the indices were estimated in both treatments. The ANOVA allowed partitioning the total observed variance into two components, between and within accessions. As a random model was assumed, the phenotypic variance (σ_f^2) genotype variance (σ_g^2), and the residual variance (σ_s^2) were calculated as:

$$\sigma_f^2 = MST / k$$

$$\sigma_g^2 = (MST - MSR) / k$$

$$\sigma_s^2 = MSR$$

Where:

MST: Between accessions Means Square
 MSR: Within accessions Means Square

$$k: \sigma_g^2 = \sum_{i=1}^g r_i^2 \quad (r_i = \text{replicate})$$

The Intraclass correlation coefficient (t) was calculated as a relative proportion of genetic variance of the variance total, and it is considered as a broad heritability (Falconer & Mackay, 1996). It was used the GENES Software for Windows (Cruz, 2001).
 Intraclass correlation coefficient (t):

$$t = \frac{\hat{\sigma}_g^2}{\hat{\sigma}_g^2 + \hat{\sigma}^2}$$

The Pearson correlation coefficients (r) among the different indices were estimated in both treatments (Sokal & Rolf, 1995).

Principal component (PC) analysis (PCA) was calculated by each trait. The first (PC1) and second (PC2) principal component were used to graphic traits measured on both tolerance Indices and accessions (Sneath & Sokal, 1973). Cumulative Variance Percentage and eigenvectors coefficients (scores) of the PC1 and PC2 were estimated per each trait to identify the relative importance of the traits and indices and their inter-relationships.

RESULTS AND DISCUSSION

The results of analyses of variance for SL, LL, RL, SDM and RDM measured in absolute value in both environments (stress and non-stress) and the indices of tolerance are given in Table 2. All the traits showed significant differences in both environments. These results indicate the presence of genetic diversity in the material under testing, as it was found by various authors (Darvishzadeh et al., 2010; Talebi et al., 2009). Furthermore, also the indices showed significant differences, which could be indicating that genotypes were differing in each tolerance index. This is in agreement with the results of Gholipouri et al. (2009) and Anwar et al. (2011).

Characters associated with leaf length and seedling length presented the highest means and phenotypic variances growing without stress. Conversely, in a salty environment, the biomass traits (root and shoot dry weight) and root length reached the highest values (Table 3). The intraclass coefficient (t) estimated for all the traits were greater in non-stress environment than in stress environment; with the exception of the traits in root (RL). The non-stress environment favors a better expression of genotypic potential and in consequence, a higher heritability was estimated. These are in agreement with the results of Fernandez (1992). However, the RL measured in salinity conditions showed most genetic variability than in controls, which could be due to a survival strategy associated to tolerance.

The principal component multivariate method was applied to the results to identify and select the variables that best explained the observed variability (Table 4 and Fig. 1). The total variability explained retaining two axes was 0.77. SL showed the highest association with PC1 (0.51), while RL and SDW were those that presented greater association with PC2 (-0.53 and 0.56 respectively). According to the results obtained only

Table 2: Analysis of variance for different traits of maize seedling tested under non salt and salt stress conditions and tolerance indexes. Means Squares between accessions and within (Error). Where: trait in non-stressed (X_c), trait in stressed (X_s); tolerance index (TOL); yield stability index (YSI); stress susceptibility index (SSI); geometric mean productivity (GMP); mean productivity (MP) and stress tolerance index (STI). **, * , indicates differences significant at $p < 0.01$; 0.05 respectively, while ns, denotes not significantly differences. Leaf length in non-stress (LLc); Leaf length in stress (LLs); Root length in non-stress (RLc); Root length in stress (RLs); Shoot length in non-stress (SLc); Shoot length in stress (SLs); Shoot dry weight in non-stress (SDWc); Shoot dry weight in stress (SDWs); Root dry weight in non-stress (RDWc); Root dry weight in stress (RDWs)

Leaf Length (LL)									
Source of variation	df	LLc (cm)	LLs (cm)	TOL	YSI	SSI	GMP	MP	STI
Accessions	67	39.9**	31.7**	50.7**	0.13**	526**	24.6**	23.3**	0.23**
Error	134	9.04	10.55	10.4	0.03	110	7.6	5.9	0.06

Root Length (RL)									
Source of variation	df	RLc (cm)	RLs (cm)	TOL	YSI	SSI	GMP	MP	STI
Accessions	67	27.2**	32.4**	55.7**	0.22**	31.7**	16.16**	15.9**	0.22**
Error	134	5	3.39	3.37	0.01	1.9	2.2	2.3	0.03

Shoot Length (SL)									
Source of variation	df	SLc (cm)	SLs (cm)	TOL	YSI	SSI	GMP	MP	STI
Accessions	67	71.2**	55.0**	86.7**	0.13**	75.9**	42.2**	41.5**	0.19**
Error	134	12.4	19.5	19.5	0.02	15	8.05	7.9	0.04

Shoot Dry Weigth (SDW)									
Source of variation	df	SDWc (mg)	SDWs (mg)	TOL	YSI	SSI	GMP	MP	STI
Accessions	67	226**	317**	434**	0.46**	28.3**	156**	164**	0.63**
Error	134	46	74	73	0.07	4.2	39	34	0.18

Root Dry Weigth (RDW)									
Source of variation	df	RDWc (mg)	RDWs (mg)	TOL	YSI	SSI	GMP	MP	STI
Accessions	67	141**	702**	696**	2.27**	1.1**	191**	249**	2.9**
Error	134	31	236	234	0.47	0.23	43	69	0.88

these traits and their respective indices of tolerance are to be analyzed.

To determine the most desirable salinity tolerance criteria, the phenotypic correlation coefficient (r) between each morphological trait (RL, SL and SDW) and other quantitative indices of salinity tolerance were calculated (Table 5, a, b and c).

There were a peak correlation for SL in both environments ($r=0.32$) or no correlation for RL and SDW ($r= 0.07$; 0.21 respectively). Thus, indirect selection for a salinity tolerance based on the results of optimum conditions will not be efficient. These results are in agreement with those of Sio-Se Mardeh. et al. (2006), Souri, J. et al. (2005) and Anwar et al. (2011) who found that the grain yield under irrigated condition was adversely correlated with that in rain-fed condition suggesting that a high potential yield under optimum condition does not necessarily result in improved yield under stress.

There were positive and significant correlations between traits (SL, RL, and SDW) and the indices: MP, GMP, TOL and STI in non-stress and salinity stress. In consequence, GMP, MP and STI appeared as better predictors of these traits than TOL and SSI. Our results were consistent with those reported by Fernández (1992) in mungbean (*Phaseolus aureus* Roxb.), Farshadfar Sutka (2002) and Jafari et al. (2009) in maize (*Zea mays* L.), Anwar et al. (2011) in wheat and Kristin et al. (1997) in common bean (*Phaseolus vulgaris* L.).

Stress tolerance (TOL) was positive and significantly correlated with all the traits in non-stress conditions while these correlations were negative under stress. Larger TOL values represent relatively more sensitivity to stress, thus smaller TOL values is preferable. In consequence the selection based on TOL index tends to favor genotypes with low expression of the trait under non-stressed conditions and high expression under stressed conditions (Fernandez, 1992; Paunescu & Boghici, 2008; Golabadi et al., 2006).

Table 3: Means, phenotypic variance (σ_f^2) and intraclass correlation coefficient (t). Where: trait in non-stressed (X_c), trait in stressed (X_s), tolerance index (TOL), yield stability index (YSI), stress susceptibility index (SSI), geometric mean productivity (GMP), mean productivity (MP) and stress tolerance index (STI). Leaf length in non-stress (LLc); Leaf length in stress (LLs); Root length in non-stress (RLc); Root length in stress (RLs); Shoot length in non-stress (SLc); Shoot length in stress (SLs); Shoot dry weight in non-stress (SDWc); Shoot dry weight in stress (SDWs); Root dry weight in non-stress (RDWc); Root dry weight in stress (RDWs).

traits		LLc (cm)	LLs (cm)	TOL	YSI	SSI	GMP	MP	STI
Leaf Length (LL)	Means	20.26	19.82	0.42	1.00	-0.18	19.74	19.98	0.99
	σ_f^2	13.32	10.57	16.90	0.04	175.62	8.19	7.77	0.08
	t	0.53	0.4	0.56	0.55	0.55	0.43	0.49	0.50
Root Length (RL)		RLc (cm)	RLs (cm)	TOL	YSI	SSI	GMP	MP	STI
	Means	16.90	18.38	-1.47	1.12	1.44	17.46	17.64	1.08
	σ_f^2	9.07	10.81	18.57	0.07	10.57	5.36	5.30	0.08
	t	0.6	0.74	0.83	0.83	0.84	0.67	0.66	0.66
Shoot Length (SL)		SLc (cm)	SLs (cm)	TOL	YSI	SSI	GMP	MP	STI
	Means	28.82	30.00	-1.18	1.06	1.58	29.18	29.41	1.05
	σ_f^2	23.75	18.34	28.89	0.04	25.32	14.07	13.84	0.06
	t	0.61	0.38	0.53	0.58	0.57	0.58	0.59	0.56
Shoot Dry Weigh (SDW)		SDWc (mg)	SDWs (mg)	TOL	YSI	SSI	GMP	MP	STI
	Means	1.05	1.18	-0.14	1.19	1.46	1.09	1.12	1.15
	σ_f^2	75.59	105.84	144.93	0.16	9.44	52.33	54.75	0.21
	t	0.56	0.52	0.62	0.66	0.66	0.5	0.56	0.45
Root Dry Weigh (RDW)		RDWc (mg)	RDWs (mg)	TOL	YSI	SSI	GMP	MP	STI
	Means	0.77	1.87	-1.10	2.59	1.11	1.17	1.32	2.49
	σ_f^2	47.00	234.31	232.05	0.76	0.37	63.81	83.20	0.98
	t	0.53	0.4	0.39	0.56	0.56	0.53	0.46	0.44

The stress susceptibility index (SSI) had significant positive correlation with yield under stress and a lower negative correlation with the traits in normal condition. Thus, a small value of SSI is desirable and selection for this parameter would also tend to favor low yielding genotypes, but to a much smaller extent than selection for TOL index. These results were consistent with those reported by Clark et al. (1992) and Golabadi et al. (2006). The YSI index showed the same behavior than the SSI index.

No significant correlations were observed between TOL with GMP, MP and STI in all the traits. This could be indicating that each index may be a potential indicator of different biological responses to salinity and that the combination of high GM and STI with a low to moderate TOL is biologically attainable in maize, thereby combining different traits that associate with each index. The same results were reported by Golabadi et al. (2006).

The application of a combination of indices to select genotypes of maize for salt tolerance in seedling stage may provide a more useful criterion of improvement.

However the study of correlation coefficients is useful in finding the degree of overall linear association between any two attributes. Thus, a better approach than a correlation analysis such as biplot is needed to identify the superior genotypes for both stress and non-stress environments (Fig 2, a, b and c). Principal component analysis (PCA) revealed similar outcomes for each trait since the first PCAs explained about 50% of the variation in RL, SL and SDW. This component positively correlated with MP, STI and GMP. However, these traits measured in both environments showed different behavior. The RLs exhibit greater correlation with the first component than RLc and identical results were obtained for SDW; while SLc had a greater association with the first component than SLs. In this way, we could infer that GMP, MP and STI would be defined mainly by RL and SDW measured in salinity. Thus, the first dimension can be named as the yield potential and stress tolerance (table 6). Considering the high and positive value of this component, genotypes that have higher values of these indices will be high yielding under stress and non-stress environments. The second

Table 4. Phenotypic correlation estimated using Pearson's coefficient (r) for different traits of maize seedling tested under non salt and salt stress conditions and stress tolerance indices: tolerance index (TOL), yield stability index (YSI), stress susceptibility index (SSI), mean productivity (MP), geometric mean productivity (GMP) and stress tolerance index (STI). **,*, indicates differences significant at $p < 0.01$; 0.05 respectively, while ns, denotes not significantly differences. Shoot length in non-stress (SLc); Shoot length in stress (SLs). **,*, indicates differences significant at $p < 0.01$; 0.05 respectively, while ns, denotes not significantly differences Root length in non-stress (RLc); Root length in stress (RLs). **,*, indicates differences significant at $p < 0.01$; 0.05 respectively, while ns, denotes not significantly differences. Shoot dry weight in non-stress (SDWc); Shoot dry weight in stress (SDWs).

a) Shoot length (SL)							
	SLc	SLs	TOL	YSI	SSI	GMP	MP
SLs	0.32 *						
TOL	0.65**	-0.51**					
YSI	-0.70**	0.43**	-0.98**				
SSI	-0.70**	0.43**	-0.98**	1.00**			
GMP	0.84**	0.77**	0.15ns	-0.23ns	-0.23ns		
MP	0.84**	0.78**	0.14ns	-0.21ns	-0.21ns	1.00**	
STI	0.84**	0.77**	0.15ns	-0.22ns	-0.23ns	1.00**	0.99**

b) Root length (RL)							
	RLc	RLs	TOL	YSI	SSI	GMP	MP
RLs	0.07ns						
TOL	0.65**	-0.72**					
YSI	-0.70**	0.64**	-0.98**				
SSI	-0.70**	0.64**	-0.98**	1.00**			
GMP	0.71**	0.74**	-0.07ns	-0.02ns	-0.02ns		
MP	0.70**	0.76**	-0.09ns	0.00ns	0.00ns	1.00**	
STI	0.71**	0.74**	-0.07ns	-0.02ns	-0.02ns	1.00**	0.99**

c) Shoot Dry Weight (SDW)							
	SDWc	SDWs	TOL	YSI	SSI	GMP	MP
SDWc	0.21ns						
TOL	0.55**	-0.70**					
YSI	-0.57**	0.63**	-0.96**				
SSI	-0.57**	0.63**	-0.96**	1.00**			
GMP	0.76**	0.78**	-0.12ns	0.04ns	0.04ns		
MP	0.73**	0.82**	-0.17ns	0.10ns	0.10ns	0.99**	
STI	0.74**	0.79**	-0.14ns	0.06ns	0.06ns	0.98**	0.98**

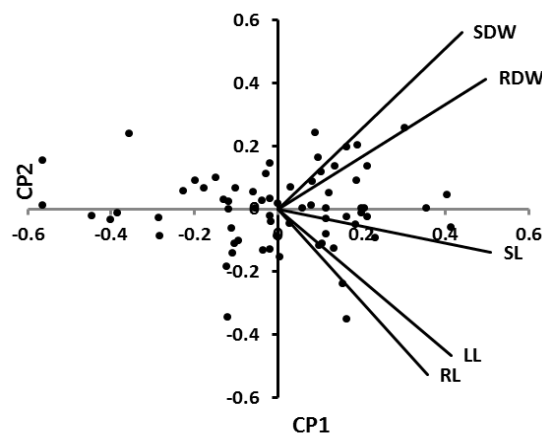


Figure 1. Biplot for accessions (dots) and traits for maize seedling growing in salinity. Where: leaf length (LL, cm), shoot length (SL, cm), root length (RL, cm), shoot dry weight (SDW, mg) and root dry weight (RDW, mg).

Table 5: Principal component analysis of maize seedling in salinity. Cumulative variance percentage and eigenvectors coefficient for first and second principal component (PC1 and PC2, respectively). Where: root length (RL, cm), shoot length (SL, cm), leaf length (LL, cm), root dry weight (RDW, mg) and shoot dry weight (SDW, mg).

Component	Cumulative variance (%)	Variables				
		RL	SL	LL	RDW	SDW
PC1	0.58	0.36	0.51	0.41	0.49	0.41
PC2	0.77	-0.53	-0.14	-0.46	0.41	0.56

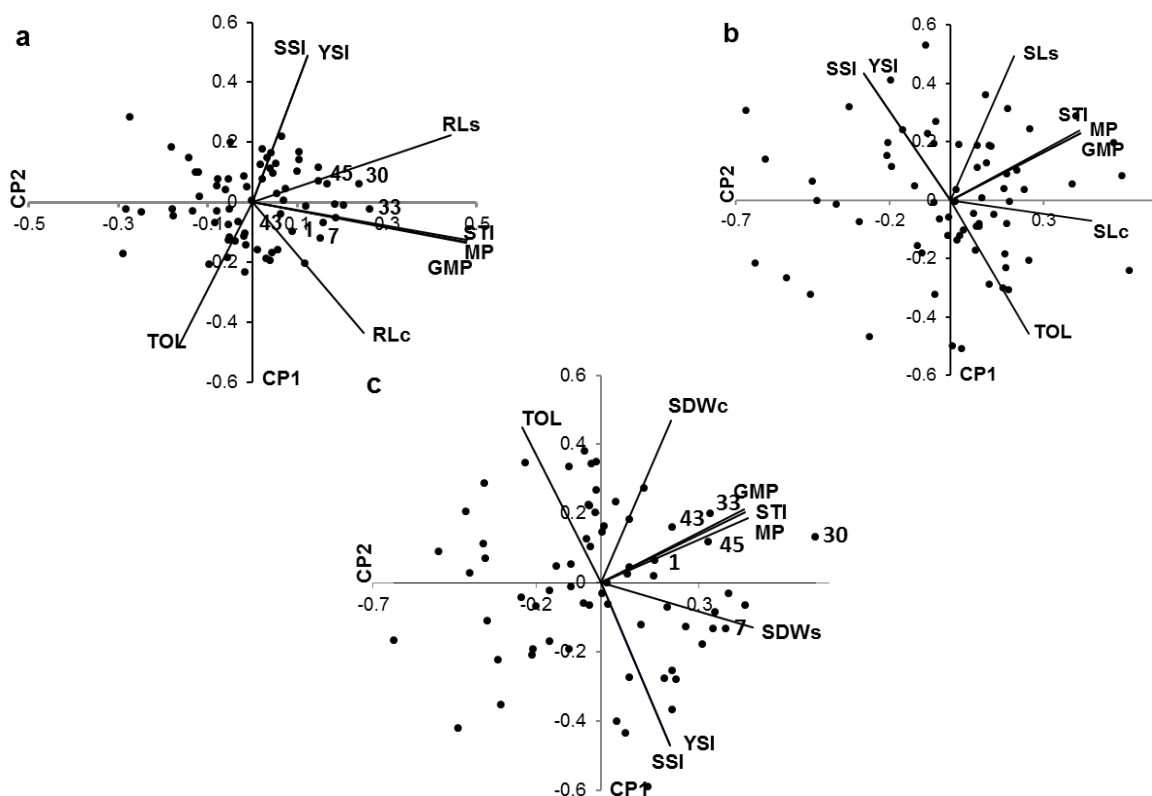


Figure 2. Biplot of accessions (dots) and e traits: a) Root Length (RL) in maize seedling; b) Shoot Length (SL) and c) Shoot Dry Weight (SDW). Where: trait in non-stressed (X_c), trait in stressed (X_s), tolerance index (TOL), yield stability index (YSI), stress susceptibility index (SSI), mean productivity (MP), geometric mean productivity (GMP), stress tolerance index (STI).

component explained about 40% of the total variation in the traits evaluated, correlated with SSI, YSI and TOL. Therefore, the second component can be named as a stress-tolerant dimension and it separates the stress-tolerant genotypes from non-stress tolerant. Thus, selection of genotypes that have high PCA1 and low PCA2 are suitable for both stress and non-stress environments. These results were in agreement with findings by Nazari & Pakniyat (2010) in barley, Siahisar et al. (2010) in lentil lines, Golabadi et al. (2006) in durum wheat and Nouri et al. (2011) in wheat genotypes (table 5).

The correlation coefficient among any two indices is approximately by the cosine of the angle between their vectors. Thus, $r = \cos 180^\circ = -1$, $\cos 0^\circ = 1$, and \cos

$90^\circ = 0$ (Yan & Rajcan, 2002). The most prominent relations revealed by these biplot are: (i) a strong negative association between SSI and YSI with TOL, as indicated by the large obtuse angles between their vectors, (ii) a near zero correlation between SSI and TOL with GMP and MP, as indicated by the near perpendicular vectors and (iii) a positive association between Yp and Ys with MP, GMP, and STI, as indicated by the acute angles. The results obtained from biplot graph confirmed the correlation analysis. These results are in agreement with the findings of Fernandez (1992), Farshadfar & Sutka (2002) and Golabadi et al. (2006) (table 4).

Table 6: Principal component analysis of maize in salinity for Root Length (RL), Shoot Length (SL) and Shoot Dry Weigh (SDW). Cumulative variance percentage and eigenvectors coefficient for first and second principal component (PC1 and PC2, respectively). Where: trait in non-stressed (c), trait in stressed (s), tolerance index (TOL), yield stability index (YSI), stress susceptibility index (SSI), geometric mean productivity (GM), mean productivity (MP) and stress tolerance index (STI).

Component	Cumulative variance (%)	RL		Tolerance Index					
		c	s	TOL	YSI	SSI	GM	MP	STI
PC1	0.51	0.25	0.44	-0.16	0.12	0.12	0.48	0.48	0.48
PC2	0.99	-0.44	0.22	-0.48	0.49	0.49	-0.14	-0.12	-0.13

	Cumulative variance (%)	SL		Tolerance Index					
		c	s	TOL	YSI	SSI	GM	MP	STI
PC1	0.58	0.46	0.20	0.25	-0.28	-0.28	0.42	0.41	0.42
PC2	0.99	-0.07	0.49	-0.46	0.43	0.43	0.23	0.24	0.23

	Cumulative variance (%)	SDW		Tolerance Index					
		c	s	TOL	YSI	SSI	GM	MP	STI
PC1	0.54	0.21	0.46	-0.24	0.21	0.21	0.44	0.45	0.44
PC2	0.98	0.47	-0.13	0.45	-0.47	-0.47	0.21	0.19	0.20

CONCLUSSION

In the world, saline environments show a great spatial variation in relation to the soil salt concentration. For this reason it is important to identify genotypes with stable behavior in a variety of saline environments during a breeding program to salinity tolerance. In this study, SDW and RL traits showed the greater contribution to indices GMP, MP and STI in salinity. Biplot graphics showed relationships among accessions, traits and salinity tolerance indices. GMP, MP and STI indices helped to identify the better (group A genotype) and stable accessions, characterized by a high expression of these characters in both environments. The application of Principal Component method showed that shoot dry weight and root length had the highest contribution and both were associated with these above indices in salinity. Therefore, the genotypes: 1, 7, 30, 33, 43 and 45 had stable values of the traits RL and SDW. Within this group the 30 and 33 entries were superiors because they had the highest PC1 scores in most of the variables but its PC2 scores were rather small for the most of the variables. As consequence both could be identified as group A genotypes determined by Fernandez (1992).

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