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Assessment of genetic diversity of sorghum (*Sorghum bicolor* L. Moench) genotypes under saline irrigation water based on some selection indices

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Abstract

Genetic improvement for salt tolerance is of high importance due to the extent and the constant increase in salt affected areas. Sorghum has been considered relatively more salt tolerant than maize and has the potential as a grain and fodder crop for salt affected areas. In order to study the reaction of twenty two genotypes of sorghum to saline irrigation treatments (0.33, 6.25 and 9.37 dS m^{-1}), a split-plot experiment was conducted under randomized complete block design with three replications. The results showed that sorghum genotypes differed in their response to salinity levels, and genotypes G3, G8, G9, G20, and G14 recorded reasonable forage yield under saline and non-saline irrigation water treatments. Selection indices, ie; GMP (Geometric mean productivity), MP (Mean productivity), STI (Stress tolerance index) were more informative towards classification of better or superior genotypes with respect to tolerant and susceptible groups. In addition, these indices recorded positive correlations either with fresh and dry weights of shoots, root dry weight and K^+ content or negative correlations with Na^+ and Cl^- contents and Na^+/K^+ ratio. Cluster analysis based on resistance indices discriminated the genotypes into three and four groups under 6.25 and 9.37 dS m^{-1}, respectively. The genotypes with high STI, GMP and MP values were suitable for cultivation under saline and non-saline environments. In contrast, cluster with highest TOL (Tolerance Index) and SSI (Stress Sensitivity Index) is sensitive to saline irrigation and more suitable for non-saline conditions. Such genotypes are considered new valuable sources for sorghum breeding programs under saline affected areas.

Keywords: Cluster analysis; salinity; selection indices; sorghum; fresh weight; ion content.
Abbreviations: Chla_chlorophyll a; Chlb_chlorophyll b; RDW_root dry weight; RSR_root: shoot ratio; SFW_shoot fresh weight; SDW_shoot dry weight; STI_stress tolerance index; SSI_tress susceptibility index, TOL_tolerance, YI_yield index, YSI_yield stability index, MP_mean productivity; GMP_geometric mean productivity.

Introduction

According to the FAO Land and Plant Nutrition Management Service, over 3% of the world's land is salinity affected, which cover over 397 million hectares. Much of the world's land is not cultivated, but a significant proportion of cultivated land is salt-affected. Of the current 230 million ha of irrigated land, 45 million ha are salt-affected (19.5%), and of the 1500 million ha under dryland agriculture, 32 million are salt-affected to varying degrees (2.1%). Therefore, overcoming on salt stress in these regions is a main issue to ensure agriculture sustainability and crop production. In Egypt, 33% of the cultivated lands, which comprises only 3% of total land area, is already salinized due to low rainfall and irrigation with saline water (Ghassemi et al., 1995). As salinity stress is becoming one of the major constrains in agricultural productivity, particularly in arid and semiarid areas of the world, breeding for salinity tolerance should be given a high research priority in research programs (Arzani, 2008). Over the past 15 years, bioengineering has not delivered salt-tolerant cultivars of conventional crops, such as wheat (*Triticum aestivum* L.) or rice (*Oryza sativa* L.), for release to farmers (Munns, 2005; Rozema and Flowers, 2008). The likely explanation for this difficult problem is that salt tolerance is a complex trait, determined by many different genes (Flowers, 2004) and many of the stress-related metabolic phenomena are unknown which limiting the success of improving crops for salt tolerance. Development of salinity tolerant plants using physiological, biochemical and molecular markers are recommended and may offer mechanistic understanding of tolerance (El-Baz et al., 2003). The deleterious effects of salinity on plant growth are associated with the decreased osmotic potential of the growing medium, specific ion toxicity and nutrient ion deficiency (Netondo et al., 2004). There are various reports on the different ionic contents, accumulation of amino acids and their contributions to salinity tolerance among genotypes of particular species, and, hence, high degree of diversity of plants responses to salinity (Bavei et al., 2011). For example, the control of ion accumulations under salt stress in higher plants is usually caused by either ion exclusions at the root cortex (Jeschke, 1984) or redistribution of excess ions to senescing leaves (Yeo and Flowers, 1984). Several earlier reports indicated that salt tolerance of plants is associated with Na^+ and Cl^- exclusion and K^+/Na^+ or Ca^{2+}/Na^+ discrimination in rice and wheat (Garcia et al., 1995; El-

Hendawy et al., 2005), high shoot K^+/ Na^+ ratio in sorghum (Bakht et al., 2006). Furthermore, Zhen-hua et al. (2012) found an increase in chlorophyll concentration in leaves under salt stress, and attributed salinity tolerance to the ability to accumulate higher concentrations of starch in plant tissues and chlorophyll *a* in leaves. In contrast, Chutipaijit et al. (2011) recorded a decrease in Chla, Chlb and Carotenoids contents in NaCl treated rice seedlings. They concluded that chlorophyll concentration can be used as a sensitive indicator of the cellular metabolic state, and its decrease signifies toxicity in tissues due to the accumulation of ions. Thus, Na^+ and Cl^- exclusion, K^+/Na^+ or Ca^{2+}/Na^+ discrimination, and photosynthesis could be used as tolerance indicators for screening germplasm (Netondo et al., 2004). The degree of genotypic diversity of particular species in responses to salinity differed according to ionic contents, accumulation of amino acids and their contributions to salinity tolerance.

Different selection indices are being used by many researchers to assess the genotypes sensitivity / tolerance to different environmental stresses. Salt tolerance index (STI) is defined as the observed value of a target trait under a given salinity level divided by the mean value for that trait under the control treatment (Zeng et al., 2002). It can be used to identify genotypes that produce high yield under both stressed and non-stressed conditions (Fernandez, 1992). Rosielle and Hamblin (1981) defined stress tolerance (TOL) as the differences in yield between the stress and non-stressed environments, and mean productivity (MP) as the average yield of genotypes under irrigated (Yi) and rainfed (Yr) conditions. Singh et al. (2011) used stress sensitivity index (SSI) to evaluate drought tolerance and spotted stem borer resistance in wheat and sorghum genotypes. They found variation in SSI for genotypes and could rank their pattern. In sorghum genotypes, it is suggested that SSI <1.00 or >1.00 indicate high or low tolerance to environmental stress, respectively. Geometric mean productivity (GMP) accounts for large differences in performance between stressed and unstressed conditions, and identifies genotypes with high grain yield potential and adaptation to borer infestation in sorghum (Samper and Adams, 1985; Ramirez and Kelly, 1998).

Sorghum is a C4 grass usually grown under hot and dry conditions and plays an important role as a major grain cum fodder crop. It is extensively grown as a major source of fodder as it is preferred over maize because of its high tolerance to various stresses. Although sorghum is relatively tolerant to drought, many of the areas potentially suitable for sorghum cultivation suffer from elevated soil salinities. Maas and Hoffman (1977) reported a threshold of 6.8 dS m^{-1} and 50% yield reduction at salinity level of 9.9 dS m^{-1}. In plant breeding programs, assessment of genetic relationship and genetic resources are useful for determining the uniqueness and distinctness of a genotype, genetic constitution of genotypes, selection of parents for hybridization (Bretting and Widrelechner, 1996) and generating new high yielding crop varieties with resistance to biotic and abiotic stresses.

Water-scarce countries (like Egypt) will have to rely more on the use of non-conventional water resources to partly alleviate water scarcity**.** The use of saline water and groundwater for agriculture is expected to increase, as fresh water supplies are being depleted and became insufficient to meet the food demand of an increasing population worldwide. Combined with the problem of summer forages shortage for livestock production, there is an urgent need for solving the problem of animal feeding sources with exploitation of the available water resources.

Therefore, this study aims to evaluate the efficiency and profitability of different selection indices in identifying salt tolerant sorghum genotypes, so that suitable genotypes can be recommended for cultivation in salt-prone areas.

Results

Performance of sorghum genotypes for various morpho-physiological traits and content of mineral ions

Results on the average performance of sorghum genotypes under salt stress conditions are presented in Table 4. Growth parameters differed significantly due to salinity levels, genotypes and their interactions. The increase in water salinity decreased the sorghum SFW (49.87% and 35.18%), SDW (48.61% and 30.74%), RDW (42.78% and 38.16%) and chlorophyll content (74.62% and 53.96%) at 6.25 dS m^{-1} and 9.37 dS m^{-1}, respectively. However, RSR showed 5.47% decrease when irrigated with 6.25 dS m^{-1} level, and 16.20% increase at 9.37 dS m^{-1} level.

At 6.25 dS m^{-1} level, the maximum SFW was produced by G14, G3, G9 and G8, respectively. The highest SDW was produced by the genotypes G14, G3, G7, G8 and G9. The least SFW and SDW were recorded for G15. The greatest RDW was obtained by G7, G8, G18 and G11, whereas the lowest values were represented by G22. Both genotypes G18 and G15 showed the highest RSR, whereas the lowest value was represented by the G5 and G14. Highest chlorophyll content was found in genotypes G3, G9, G17 and G22. At 9.37 dS m^{-1}, five genotypes (G14, G9, G11, G7 and G8) gave the highest shoot fresh weight. The genotypes G7, G8, G9 and G19 recorded the highest shoot dry weight, while the least fresh and dry weights were displayed by G16. The genotypes G7, G8 and G11 and G14 exhibited the highest RDW (Table 4). Among the tested genotypes, the highest RSR was observed for G2, G18, G15 and G14, while the lowest values were recorded for the local cultivar. The maximum chlorophyll content was observed for G3 followed by G9, G7, G12 and G8, whereas the least values were recorded by G2 and G16.

An immediate and primary effect of the imposition of salt stress is a perturbation in tissue cation and anion levels. Salinity levels and their interactions with genotypes demonstrated significant differences in Cl^- content. No genotypic differences for this ion were detected (Fig. 1). On the other hand, Na^+ and K^+ content and Na^+/K^+ ratio showed significant responses among salinity levels, genotypes and their interactions. Significant rise in Na^+ (59.82 and 161.03%) and Cl^- (60.90 and 177.96%) contents and Na^+/K^+ ratio (115.85 and 341.95%) of forage sorghum were detected at both salinity levels compared to control. In contrast, K^+ content showed 27.49 and 41.29% reduction at 6.25 and 9.37 dS m^{-1}, respectively.

The responses of sorghum genotypes based on ions accumulation differed according to the salinity level. Data on the average mineral contents demonstrated an increase in the uptake of Na^+ and Cl^- and high Na^+/ K^+ ratio as the salinity level increase (Fig. 1). At 6.25 dS m^{-1} salinity level, four genotypes (G22, G21, G18 and G17) maintained the least Cl^- and Na^+ contents and the lowest Na^+/K^+ ratio. The highest K^+ content was determined in G9, G10, G7 and G2. At 9.37 dS m^{-1} salinity level, G7, G8, G9 and G14 accumulated the

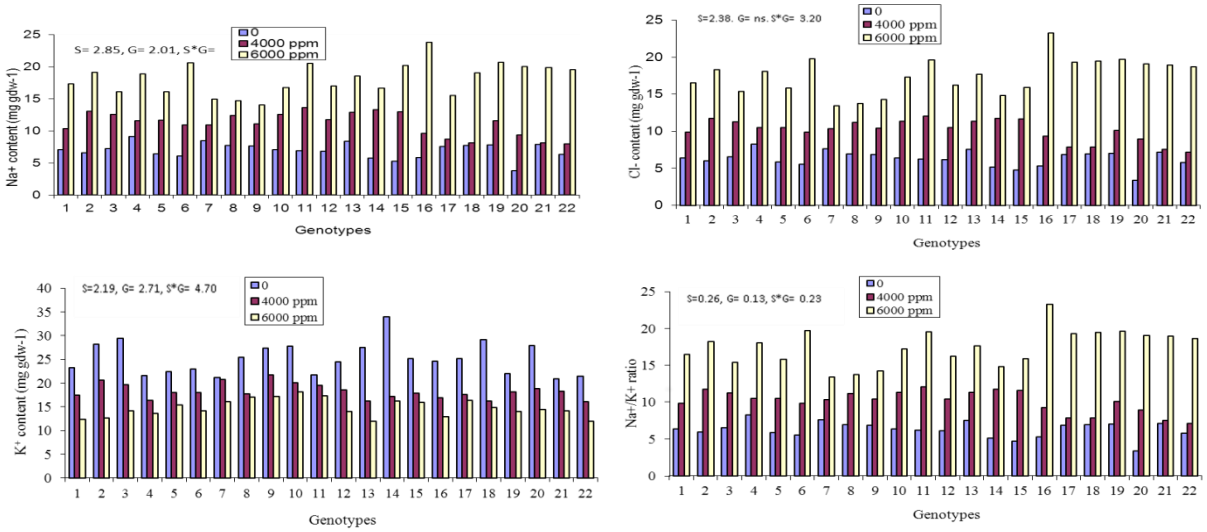

Fig 1. Effect of irrigation with 0.34, 6.25 and 9.37 dS m^{-1}saline water on Na^+, Cl^-, K^+ and Na^+/K^+ ratio of 22 forage sorghum genotypes.

Table 1. Number and origin of sorghum genotypes used for salinity tolerance evaluation.

Serial	Accession identifier	Origin	Serial	Accession identifier	Origin
G1	IS 613	USA	G12	IS 12695	South Africa
G2	IS 1255	Zaire	G13	IS 18711	USA
G3	IS 2192	India	G14	IS 24906	Zambia
G4	IS 2375	India	G15	IS 30890	Uganda
G5	IS 3323	USA	G16	IS 33903	India
G6	IS 5078	India	G17	IS 33917	India
G7	IS 5124	India	G18	IS 33921	India
G8	IS 5204	India	G19	IS 35223	Pakistan
G9	IS 6014	India	G20	PI195754	China
G10	IS 8007	Japan	G21	PI 34911	U.S. (Hegari)
G11	IS 8754	South Africa	G22	Giza 2 (Local variety)	Egypt

lowest levels of Na^+ and Cl^-, while the highest K^+ content was accumulated by G10, G11, G8, G9, G14 and G7. Low Na^+/K^+ ratios were allocated for G7, G8, G9, and G10. Most of the introduced genotypes demonstrated their potential for salinity tolerance as they exhibited greater values for yield traits, chlorophyll and K^+ content, lower Na^+ and Cl^- uptake and Na^+/K^+ ratio than the local genotype.

Selection indices

As shown in Table 5, at 6.25 dS m^{-1} level, sorghum genotypes G3, G7, G9 and G11 exhibited the least TOL and SSI values, whereas the highest values were recorded in G22 followed by G18, G16 and G17. Highest YSI and YI indices were recorded for sorghum genotypes G2, G9, G3, G11 and G14. The highest GMP, MP, STI values were recorded for the genotypes G14, G20, G9, G8, G3. At the highest salinity level, sorghum genotypes G22 and G16 showed the highest TOL and SSI values, whereas G4, G7 and G11 recorded the least values. The genotypes G14, G9, G11 and G8 registered the highest values for STI, YI, MP and GMP indices, in contrast to sorghum genotypes G16 and G15 which showed the lowest values.

Correlation coefficients between Yp, Ys and the various stress tolerance indices are presented in Table 6. The values provide interesting information reflected and determine the most effective salt-tolerant indices. In this respect, positive and significant correlations were recorded among the tolerance indices; MP, STI, TOL, SSI and GMP with Ypi at both salinity levels. The indices MP, YI, GMP, YSI and STI showed positive and significant correlations with Ysi. There was positive and non-significant correlation between Ypi and Ysi at 6.25 dS m^{-1} level, while negative correlation was recorded under the highest salinity level. While positive and significant correlations were detected between TOL and SSI at both levels, a negative correlation with Ysi was recorded. Also negative correlations were detected between YSI with TOL and SSI.

Correlation analysis between tolerance indices and measured attributes in two separate conditions are presented in Table 7. The SFW, SDW and RDW correlated positively and significantly with STI, MP, GMP, YSI and YI at both salinity levels. In case of RSR, there were positive correlations with TOL and SSI at both levels. Other indices recorded high and negative associations with RSR. Regarding chlorophyll content, the association was weak and either positive or negative in direction. At the highest salinity level, Na^+ and Cl^- contents and Na^+/K^+ ratio showed negative associations with GMP, MP, STI, YSI and YI and positive relations with TOL and SSI. However, under lower salinity levels, the previous indices (GMP, MP, STI, YSI and YI) showed positive association with the same traits, while TOL and SSI recorded negative values. The view was different in case of K^+ content, where it showed positive associations with all indices except TOL and SSI at both levels of salinity.

Cluster analysis using selection indices data

The 22 sorghum genotypes were grouped into three and four clusters under 6.25 and 9.37 dS m^{-1}levels of saline water treatments, respectively, based on the selection indices using Ward's minimum variance clustering method (Fig. 2). At 6.25 dS m^{-1} level, cluster III encompassed the maximum number of genotypes (10) followed by cluster I (8), and cluster II (4). At 9.37 dS m^{-1}, cluster I contained the maximum number of genotypes (10) followed by cluster II (6), cluster IV (4) and cluster III (2). To identify the characterization of each cluster group, cluster means of selection indices at each salinity level are summarized in Table 8. At both levels, cluster of genotypes with high GMP, MP and STI indices considered suitable for growing in saline and non-saline environments. Genotypes have the highest SSI and TOL values, are suitable for growing under non-saline environments. Genotypes have the highest YSI, YI and Y_s values, are stable under saline-affected environments.

Discussion

The significant genotypic variation for all characters in control and salinity treatments suggested that the magnitude of differences was sufficient to provide some scope for selection to improve salinity tolerance. The two saline irrigation levels used in the current study were chosen to meet threshold salinity level of sorghum crop (6.8 dS/m) and high level of salinity (9.37 dS/m). The plant growth parameters, K^+ and chlorophyll content were adversely affected by water salinity. In contrast, ions (Na^+ and Cl^-) content and Na^+/K^+ ratio were progressively increased. The responses of most genotypes differed according to the stress level. At both levels of salinity, the most tolerant genotypes (G3, G7, G8, G9 and G14) produced high SFW, SDW, RDW, maintained low Na^+, Cl^- and Na^+/K^+ ratio, high K^+ and chlorophyll contents compared to the most sensitive genotypes (G15 and G16). The local variety, Giza 2, surpassed the introduced germplasm in SFW, RDW and chlorophyll content under non-saline irrigation, while it was severely affected by 9.37 dS m^{-1} level.

The present results on reducing growth traits due to salinity are similar to those of previous investigations. Vasilakoglou et al. (2011) recorded a reduction in sorghum chlorophyll content index from 7% to 13% with increasing soil salinity from 3.2 dS/m to 6.9 dS/m. Also**,** Chutipaijit et al. (2011) found a decrease in Chl a, Chl b and carotenoids contents and a decline in growth efficiency in rice seedlings treated with NaCl. They considered that chlorophyll concentration can be used as a sensitive indicator of the cellular metabolic state. Therefore, its decrease indicates toxicity in tissues due to the accumulation of ions. In contrast, an increase in leaf chlorophyll content under saline conditions, possibly due to the reduction in the leaf area, was observed in spring wheat. However, Del Zoppo et al. (1999) found that, levels of 50-150 mM NaCl caused a relatively small decrease of the chlorophyll a and chlorophyll b content in wheat plants. Netondo et al. (2004) recorded 75% and 53% reduction in dry shoot weight; 58% and 70% reduction in Chl a and 68% and 69% reduction in Chl b for two sorghum cultivars treated with 250 mM NaCl. Root dry weight indices showed decline with increasing levels of NaCl from 50 to 200 mM (Kausar et al., 2012). Meanwhile, Akram et al. (2007) concluded that, roots are the first developing organ sensitive to increasing levels of salinity. They attributed root growth inhibition under saline conditions to the lower availability of O_2, which deprive the plants from energy source and accumulate high level of ethylene. The slight reduction in average RSR value under low salinity and its increasing under higher salinity level demonstrate that, while low salinity does not affect this trait harmfully, high salinity encourage root growth compared to shoots. While the majority of genotypes (13 genotypes) showed increasing RSR at 9.37 dS m^{-1}compared to control, other genotypes recorded values ranged from 0.67-0.46. Therefore, the response of the genotypes in terms of root/shoot ratio suggests that root growth is less inhibited by salt stress than shoot growth. Our results are in harmony with those obtained by Mannan et al.(2010) reduction in shoot

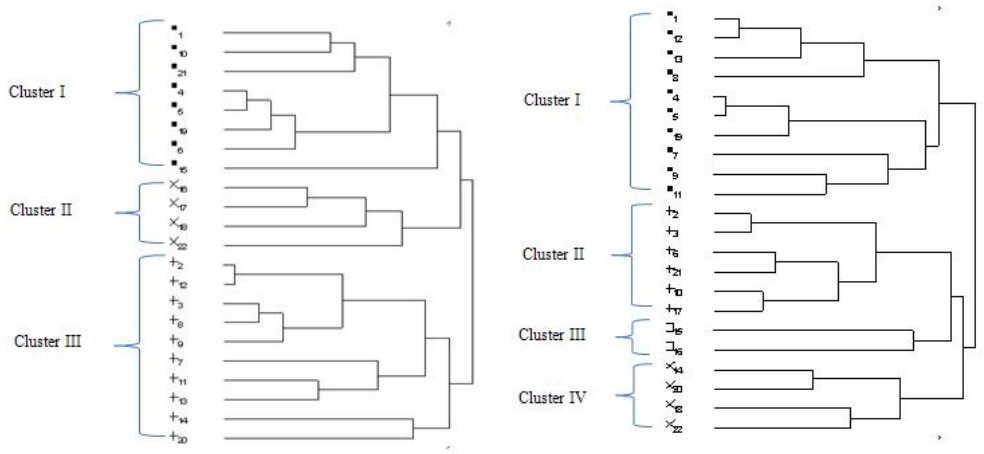

Fig 2. Diagram of 22 sorghum genotypes for 7 selection indices using hierarchical cluster analysis (ward's method and squared Euclidean distance) under 6.25 dS m^{-1} (left) and 9.37 dS m^{-1} (right).

Table 2. Chemical analysis of the irrigation water used for the experiment.

Parameter	Control (Nile water)	6.25 dS m^{-1}	9.37 dS m^{-1}	Sea water
pH	7.30	8.25	8.22	7.54
EC (dS m^{-1})	0.336	6.25	9.375	45.30
Soluble cations, (mg l^{-1})				
Ca^{2+}	0.84	13.80	21.0	58.6
Mg^{2+}	0.73	4.0	5.20	37.0
Na^{+}	1.04	43.60	65.30	347.9
K^{+}	0.61	1.31	1.97	8.46
Soluble anions, (mg l^{-1})				
CO_3^{2-}	-	-	-	-
HCO^{3-}	1.30	4.0	4.50	6.50
Cl^{-}	1.21	41.80	61.70	360.61
SO_4^{2-}	0.83	16.70	26.80	85.00
Sodium Adsorption Rate	1.17	14.63	18.04	50.33

growth than root in saline conditions, and increasing RSR in saline treated plants. Takele (2000) reported that water stress significantly increased root-shoot ratio of sorghum seedlings, which also could be an important selection parameter for selection of drought tolerant genotypes (Misra, 1994). Nour et al. (1978) correlated high RDW/SDW ratios of young plants with superior drought resistance in sorghum genotypes.

Results on ion accumulation and its relation to salinity tolerance are presented by many reports. Epstein (1985) mentioned that, tolerant species usually absorb more K^+ and exclude Na^+ to maintain the osmotic balance in the plant parts. Venkata et al. (2012) found no change in K^+/Na^+ ratio as soil salinity increase from 3.2 dS/m to 6.9 dS/m. Conversely, Netondo et al. (2004) found a significant increase in Na^+ concentration in leaves of two sorghum genotypes when treated with 250 mM NaCl. However, there was no effect of NaCl on K^+ concentration in leaf blades, whereas in leaf sheaths, K^+ concentration was declined with increasing NaCl from 0.00 mM to 100 mM. Renault et al. (2001) mentioned that, as K^+ is a macronutrient involved in turgor control, the growth stunt attributed to the inhibition of K^+ uptake as a result of the salinity effect.

Zan et al. (2011) concluded that, salinity not only causes the accumulation of Na^+, but also inhibit the uptake of essential nutrients such as K^+ through the effects of ion selectivity. They attributed salinity tolerance to the lower Na^+/K^+ ratio in leaves under salt stress conditions. Venkata et al. (2012) reported that, the tolerant genotype is characterized by having the lower values for Na^+, Na^+/K^+ of leaf or stem and more K^+ absorption to maintain the osmotic balance in the plant. In contrast, Tavakkoli et al. (2012) found the importance of Na^+ and Cl^- exclusion to salt tolerance in faba bean is varied with the severity of stress. While it was important to salinity tolerance at low levels of salinity (75 mM NaCl), its importance was diminished at 150 mM NaCl. As the concentration of salt increases, the ability to exclude salt may become less effective in protecting the plant from salt stress and other mechanisms, such as osmotic tolerance, may become increasingly important. The different responses of various sorghum genotypes to salinity treatments could be attributed to the differences in the genetic makeup and genotype x salinity interaction.

The positive correlations of MP, GMP and STI with Yp and Ys affirm their reliability for predicting yield under normal and stress conditions than TOL and SSI. The observed relations were in agreement with those reported by Talebi et al. (2009) in wheat and Kharrazi and Rad **(**2011) in sorghum. They demonstrated the consistency of these indices in identifying tolerant genotypes under both conditions. They added that the negative associations of TOL and SSI with Ys, and the positive association between both indices confirm that low values of these indices are desirable. Golabadi et al. (2006) and Mohammadi et al. (2010) mentioned that, the validity of selection index for screening genotypes for stress conditions depends on its good correlation with yield under normal and stressed durum wheat. Similarly, three indices (STI, GMP and MP) had the highest positive correlation coefficient with yields under normal and drought stress conditions and introduced as selection indices for post water stress tolerance in sorghum and wheat (Rad et al., 2009; Talebi et al., 2009).

At the highest salinity level, the negative and significant correlations of ions content (Na^+ and Cl^-) and Na^+/K^+ ratio with MP, GMP, STI, YI and YSI indices, suggested their suitability for salinity screening. Moreover, genotypes able to exclude Na^+ and Cl^- from their green parts should have high values of these indices. In contrast, TOL and SSI indices recorded positive correlations with the above mentioned variables and positive relations with high RDW and K^+ content. Under 6.25 dS m^{-1}, ion inclusion did not present an adverse effect on the growth, as correlations recorded positive values with selection tolerance indices.

The negative RSR correlations with all selection indices except TOL and SSI at both levels confirm that this trait is not reliable indicator for salinity tolerance screening, and the high reduction in SDW relative to RDW is a consequence of stress. A significant contribution of K^+ ion to fresh weight of sorghum under salt stress (Bavei et al., 2011) was detected. Similarly, Tavakkoli et al. (2012) found that yield production in faba bean is significantly associated with the exclusion of Na^+ and Cl^- in leaves, both under field conditions and hydroponics. In addition, K^+ content in plants demonstrated a great genotypic variation and was well correlated with the salt tolerance ranked using grain yield.

Based on MP, STI, GMP indices and cluster analysis, high rates of these indices indicate endurance of genotypes to salt stress. Among the 22 genotypes screened, the entries G14, G20, G8 and G9 are identified to be grown under both-saline (6.25 and 9.37 dS m^{-1}, respectively) and non-saline conditions. The genotypes G8 and G9 belong to cluster I, while G14 and G20 belong to cluster IV. The moderate values of YI, YSI and Ys which characterize genotypes in cluster I confirm their suitability for cultivation at 6.25 dS m^{-1}. While at 9.37 dS m^{-1}, cluster I showed the highest values for the previous indices with the genotypes G7, G8, G9 and G11 being the most tolerant to saline conditions. Three genotypes (G16, G18 and G22) at 6.25 dS m^{-1} and two genotypes (G15 and G16) at 9.37 dS m^{-1} combined in cluster III showed the highest TOL and SSI values and the lowest Ys yield. These genotypes are sensitive to salt stress and suitable for cultivation under non-saline environments. Results on using different selection indices for screening crop genotypes for stress conditions have been widely reported. Sio-Se et al. (2006) and Khayatnezhad et al. (2011) stated that high rates of YI, MP, STI and GMP indicate tolerance of corn genotypes to water stress and considered for selection under stress conditions. Additionally, they are reliable indices being able to identify high-yielding and drought tolerant genotypes under both environmental conditions. Also, Awaad et al. (2010) indicated that the results of relative performance and Tolerance index (ToL) are coupled with lead sensitivity index (LSI) and might be considered as reliable measurements for identifying tolerant or sensitive genotypes to stress. Talebi et al. (2009) mentioned that, the greater the TOL value, the larger the yield reduction under stress conditions and the higher the drought sensitivity of wheat cultivars. On the other hand, genotypes with low TOL index have low yield under normal conditions and selection for this index tend to favor low yielding genotypes (Rad et al., 2009). Moreover, Khayatnezhad et al. (2011) observed positive and meaningful correlation between STI and YI, MP, GMP; MP and YI, GMP; GMP with YI and TOL with SSI. Also, negative correlation was observed between YSI with SSI and TOL which by increasing of the above indices, the value of this index will be decreased. It is interesting to note that genotypes suitable for saline and non-saline soils are more preferred by the farmers (group I and IV under 6.25 dS m^{-1} and 9.37 dS m^{-1} levels, respectively).

The choice of the present research problem is most appropriate and practical utility. Because the self-pollination nature of sorghum, the genetic variation is low in this species. Moreover, in Egypt only one local genotype is culti-

Table 3. Salt tolerance/sensitivity indices and their equations.

Salt Tolerance/sensitivity indices	Equation	Reference
Stress Sensitivity Index (SSI)	$SSI= [(1-(Y_{si} - Y_{pi}) / SI$	Fischer and Maurer (1978)
Stress Tolerance Index (STI)	$STI= [Ypi \times Ysi] / (Yp)^2$	Fernandez (1992)
Tolerance Index (TOL)	$TOL = Ypi - Ysi$	Hossain et al. (1990)
Geometric Mean Productivity (GMP)	$GMP = (Ypi \times Ysi)^{0.5}$	Fernandez (1992)
Mean Productivity (MP)	$MP = (Ypi + Ysi) / 2$	Hossain et al. (1990)
Yield index (YI)	$YI = Y_{si} / Ys$	Gavuzzi et al. (1997)
Yield stability index (YSI)	$YSI = Y_{si} / Y_{pi}$	Bouslama and Schapaugh (1984)

Where, Ypi and Ysi are the fresh weight of a genotype in normal and stress conditions, respectively. SI is stress intensity, where: SI= 1– (Ys /Yp); Ys and Yp are the mean fresh weights of all genotypes under stressed and controlled conditions, respectively.

Table 4. Effect of seawater salinity on SFW, SDW, RDW, RSR and chlorophyll content of 22 forage sorghum genotypes.

Genotypes	Shoot fresh weight (SFW) (g)			Shoot dry weight (SDW) (g)			Root dry weight (RDW) (g)			Root : Shoot Ratio (RSR)			Chlorophyll (mg g^{-1} fw)		
	Cont.	6.25 dS m^{-1}	9.37 dS m^{-1}	Cont.	6.25 dS m^{-1}	9.37 dS m^{-1}	Cont.	6.25 dS m^{-1}	9.37 dS m^{-1}	Cont.	6.25 dS m^{-1}	9.37 dS m^{-1}	Cont.	6.25 dS m^{-1}	9.37 dS m^{-1}
G1	55.32	30.68	30.31	14.03	9.78	8.77	5.95	2.84	2.43	0.42	0.29	0.28	0.87	0.61	0.42
G2	55.73	48.06	19.35	15.82	11.83	5.53	3.84	3.5	3.81	0.24	0.30	0.69	0.92	0.19	0.02
G3	58.75	53.35	19.15	17.12	13.74	7.56	5.77	4.10	3.43	0.34	0.30	0.45	1.65	1.06	0.87
G4	40.18	31.17	30.69	14.85	9.74	9.93	7.19	3.09	3.47	0.48	0.32	0.35	2.70	0.64	0.17
G5	41.01	29.78	29.94	13.76	9.51	9.70	4.25	2.08	4.58	0.31	0.22	0.47	2.78	0.75	0.51
G6	44.37	29.92	23.25	17.29	9.54	6.31	5.82	3.69	2.33	0.34	0.39	0.37	1.04	0.52	0.40
G7	39.41	37.88	37.68	14.12	13.58	12.05	5.69	6.22	5.17	0.40	0.46	0.43	0.90	0.86	0.64
G8	59.18	50.49	34.68	13.19	13.48	10.09	5.02	4.66	4.52	0.38	0.35	0.44	0.93	0.54	0.55
G9	54.83	51.58	38.68	15.32	13.24	11.84	2.52	3.50	3.65	0.16	0.26	0.31	1.43	1.04	0.70
G10	53.10	35.10	21.11	11.91	11.27	7.55	3.36	2.91	2.36	0.28	0.26	0.31	0.60	0.25	0.12
G11	48.34	45.71	38.23	14.78	12.51	9.19	5.86	4.26	5.43	0.40	0.34	0.59	1.96	0.30	0.11
G12	55.41	46.30	29.04	11.67	11.78	8.25	5.74	3.81	2.93	0.49	0.32	0.36	0.76	0.66	0.62
G13	51.25	43.34	31.35	13.55	11.61	9.73	4.97	3.95	3.39	0.37	0.34	0.35	0.98	0.59	0.13
G14	79.23	62.95	30.13	25.32	17.41	8.74	6.66	3.81	5.23	0.26	0.22	0.60	0.98	0.41	0.12
G15	36.38	12.70	12.73	10.24	4.15	3.61	4.18	3.18	2.06	0.41	0.77	0.57	0.98	0.67	0.29
G16	63.52	26.73	3.32	13.97	8.46	1.07	5.69	3.59	1.26	0.41	0.42	1.18	1.14	0.16	0.06
G17	56.20	26.02	24.23	12.14	8.04	8.10	5.27	4.29	3.67	0.43	0.53	0.45	1.25	1.06	0.35
G18	70.92	32.36	19.85	14.02	4.64	6.08	10.60	4.54	4.33	0.76	0.98	0.71	1.23	0.89	0.13
G19	42.63	33.43	28.46	12.50	11.05	10.38	6.26	3.56	4.42	0.50	0.32	0.44	1.33	0.60	0.17
G20	80.23	43.69	26.41	19.32	10.40	6.70	8.18	3.00	1.90	0.42	0.29	0.28	1.67	0.37	0.21
G21	47.38	26.53	24.83	13.32	7.23	5.41	9.32	2.47	1.72	0.70	0.34	0.32	1.10	0.53	0.16
G22	85.84	26.34	16.98	25.35	8.05	7.90	5.65	1.92	1.00	0.22	0.24	0.20	1.94	1.07	0.26
LSD (0.05)															
S		8.81*			1.86*			0.75*			0.11			0.05*	
G		10.28*			3.12*			1.05*			0.15*			0.5*	
S x G		17.81*			4.41*			1.81*			0.20*			0.09*	

vated. In the present study, new germplasm of sorghum were introduced to be evaluated with the local material under local conditions. Therefore, new sources with different resistance degree would be selected for salinity prone environments of Egypt.

Materials and Methods

Plant materials, growth conditions and experimental setup

Twenty two genotypes of sorghum (*Sorghum bicolor* L. Moench) were used in this study based on their wide diversity of origins (Table 1). Eighteen genotypes were imported from ICRISAT, India; one genotype was imported from Germplasm Resources Information Network (GRIN), USA; and one Egyptian local cultivar (Giza 2) was provided by the Agricultural Research Centre, Giza, Egypt.

A greenhouse experiment was conducted during the summer season 2011 to examine the genotypes' responses to saline irrigation at the experimental farm of Suez Canal University, Ismailia, Egypt. Three different levels of saline irrigation water (0.33, 6.25 and 9.37 dS m^{-1}) were applied. Salt treatments were prepared by diluting sea water, taken from Suez Canal, with fresh water taken from Ismailia Canal (Nile water as control). The salinity levels of control, 6.25 and 9.37 dS m^{-1} were equivalent to an electrical conductivity of 0.336, 6.25 and 9.375 dS m^{-1}, respectively. The chemical analyses of sea water, Ismailia Canal as well as irrigation water treatments are presented in Table (2).

Grains were seeded in plastic pots filled with 20 kg sandy soils collected from virgin sandy soils of Ismailia Governorate. Soil was mixed with 2.16 g nitrogen fertilizer in the form of ammonium nitrate (33.5%), 5.67 g phosphorus fertilizer as calcium super phosphate (15.5% P_2O_5), and 2.06 Potassium fertilizer as potassium sulphate (48% K_2O). After one week, the seedlings were thinned to four plants per pot and irrigated with non-saline water for four weeks for plant growth establishment as outlined by Almodares et al. (2008). Thereafter, pots were irrigated with salt solution twice a week and increased with plant development to prevent additional drought stress being suffered. Split plot combination of treatments was laid out in a randomized complete block design replicated three times. Three levels of salinity (0.33, 6.25 and 9.37 dS m^{-1}) were assigned to the main plots and sorghum genotypes were assigned to the subplots. Each sub-subplot consisted of one pot with four plants.

Table 5. Tolerance indices of 22 sorghum genotypes grown under 6.25 dS m^{-1} (regular) and 9.37 dS m^{-1} (bold) seawater salinity.

Genotype	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11
Ypi	55.32	55.73	58.75	40.18	40.01	44.37	39.41	59.18	54.83	53.09	48.34
Ysi	30.68	48.06	53.34	31.16	29.78	29.92	37.88	50.48	51.58	35.10	45.71
Ysi	**30.30**	**19.35**	**19.15**	**30.69**	**29.94**	**23.24**	**37.67**	**34.68**	**38.68**	**24.10**	**38.23**
SSI	1.37	0.43	0.28	0.69	0.84	1.00	0.12	0.45	0.18	1.05	0.17
SSI	**0.85**	**1.23**	**1.27**	**0.45**	**0.51**	**0.90**	**0.08**	**1.28**	**0.56**	**1.03**	**0.39**
TOL	24.64	7.67	5.40	9.01	11.23	14.45	1.53	8.69	3.25	17.99	2.63
TOL	**25.01**	**36.38**	**39.60**	**9.49**	**11.07**	**21.13**	**1.73**	**24.50**	**16.10**	**28.99**	**10.11**
STI	0.55	0.87	1.02	0.41	0.40	0.43	0.49	0.97	0.92	0.61	0.72
STI	**0.55**	**0.35**	**0.37**	**0.40**	**0.40**	**0.34**	**0.48**	**0.67**	**0.69**	**0.42**	**0.60**
MP	43.00	51.90	56.05	35.67	35.39	37.14	38.65	54.83	53.20	44.10	47.02
MP	**42.81**	**37.54**	**38.95**	**35.43**	**35.47**	**33.81**	**38.54**	**46.93**	**46.75**	**38.60**	**43.29**
GMP	41.20	51.75	55.98	35.38	34.94	36.43	38.64	54.66	53.18	43.17	47.01
GMP	**40.94**	**32.84**	**33.54**	**35.11**	**35.04**	**32.11**	**38.53**	**45.30**	**46.05**	**35.77**	**42.99**
YSI	0.56	0.86	0.91	0.78	0.73	0.67	0.96	0.85	0.94	0.66	0.94
YSI	**0.55**	**0.35**	**0.33**	**0.76**	**0.73**	**0.52**	**0.96**	**0.59**	**0.71**	**0.45**	**0.79**
YI	0.82	1.28	1.42	0.83	0.79	0.80	1.01	1.35	1.38	0.94	1.22
YI	**1.16**	**0.74**	**0.71**	**1.18**	**1.15**	**0.89**	**1.45**	**1.33**	**1.48**	**0.92**	**1.47**
Genotype	G12	G13	G14	G15	G16	G17	G18	G19	G20	G21	G22
Ypi	55.41	51.25	79.22	36.38	63.52	56.20	70.92	42.63	80.23	47.38	85.84
Ysi	46.30	43.34	62.95	12.69	26.72	26.02	32.36	33.43	43.69	26.53	26.34
Ysi	**29.04**	**31.35**	**30.12**	**12.73**	**3.32**	**24.23**	**19.85**	**28.46**	**26.41**	**24.83**	**16.98**
SSI	0.51	0.48	0.63	2.01	1.79	1.66	1.68	0.67	1.40	1.36	2.14
SSI	**0.90**	**0.73**	**1.17**	**1.23**	**1.79**	**1.07**	**1.36**	**0.63**	**1.27**	**0.90**	**1.51**
TOL	9.11	7.91	16.27	23.68	36.80	30.18	38.56	9.20	36.54	20.85	59.50
TOL	**26.37**	**19.90**	**49.10**	**23.65**	**60.20**	**31.97**	**51.07**	**14.17**	**53.82**	**22.55**	**68.86**
STI	0.83	0.72	1.62	0.15	0.55	0.48	0.75	0.46	1.14	0.41	0.74
STI	**0.52**	**0.52**	**0.78**	**0.15**	**0.07**	**0.44**	**0.46**	**0.39**	**0.69**	**0.38**	**0.47**
MP	50.85	47.30	71.09	24.54	45.12	41.11	51.64	38.03	61.96	36.95	56.09
MP	**42.22**	**41.30**	**54.67**	**24.55**	**33.42**	**40.21**	**45.38**	**35.54**	**53.32**	**36.11**	**51.41**
GMP	50.65	47.13	70.62	21.49	41.20	38.24	47.91	37.75	59.20	35.45	47.55
GMP	**40.11**	**40.09**	**48.85**	**21.52**	**14.53**	**36.90**	**37.52**	**34.83**	**46.03**	**34.30**	**38.18**
YSI	0.84	0.85	0.79	0.35	0.42	0.46	0.46	0.78	0.54	0.56	0.31
YSI	**0.52**	**0.61**	**0.38**	**0.35**	**0.05**	**0.43**	**0.28**	**0.67**	**0.33**	**0.52**	**0.20**
YI	1.24	1.07	1.68	0.34	0.70	0.69	0.86	0.89	1.17	0.71	0.70
YI	**1.11**	**1.20**	**1.16**	**0.49**	**0.13**	**0.93**	**0.76**	**1.09**	**1.01**	**0.95**	**0.65**

Table 6. Correlation coefficients between Ypi, Ysi and salt tolerance indices at 6.25 dS m^{-1} (regular) and 9.37 dS m^{-1} (bold) seawater salinity.

Index	Ypi	Ysi	SSI	STI	TOL	MP	GMP	YSI	YI
Ypi	1.00	0.34	0.33	0.72*	0.65**	0.85**	0.73**	-0.33	0.35
Ypi	**1.00**	**-0.25**	**0.66****	**0.41**	**0.88****	**0.81****	**0.34**	**-0.66****	**-0.24**
Ysi		1.00	-0.74**	0.87**	-0.49**	0.79**	0.89**	0.74**	0.99**
Ysi		**1.00**	**-0.80****	**0.76****	**-0.67****	**0.37**	**0.80****	**0.86****	**1.00****
SSI			1.00	-0.33	0.90**	-0.20	-0.37	-1.00**	-0.74**
SSI			**1.00**	**-0.27**	**0.89****	**0.16**	**-0.34**	**-0.97***	**-0.80****
STI				1.00	-0.03	0.97**	0.98**	0.33	0.88**
STI				**1.00**	**-0.05**	**0.85**	**0.98****	**0.34**	**0.76****
TOL					1.00	0.15	-0.04	-0.33	0.35
TOL					**1.00**	**0.44***	**-0.12**	**-0.92****	**-0.67****
MP						1.00	0.98**	0.20	-0.48*
MP						**1.00**	**0.81****	**-0.11**	**0.37**
GMP							1.00	0.37	0.89**
GMP							**1.00**	**0.41**	**0.80****
YSI								1.00	0.74**
YSI								**1.00**	**0.86****
YI									1.00
YI									**1.00**

Table 7. Correlations coefficients between stress tolerance indices and measured traits under 6.25 dS m^{-1} (regular) and 9.37 dS m^{-1} (bold) seawater salinity.

Trait	SSI	STI	TOL	MP	GMP	YSI	YI
SFW	-0.74**	0.87**	-0.49*	0.79**	0.89**	0.74**	0.99**
SFW	**-0.79****	**0.76****	**-0.67****	**0.36**	**0.80****	**0.86****	**1.00****
SDW	-0.80**	0.69**	-0.60**	0.58**	0.69**	0.80**	0.88**
SDW	**-0.79****	**0.65****	**-0.68****	**0.27**	**0.70****	**0.85****	**0.93****
RDW	-0.46*	0.40	-0.36	0.33	0.40	0.46*	0.52*
RDW	**-0.59****	**0.43***	**-0.51***	**0.15**	**0.45***	**0.64****	**0.65****
RSR	0.31	-0.24	0.20	-0.22	-0.26	-0.31	-0.32
RSR	**0.42**	**-0.51***	**0.09**	**-0.31**	**-0.60***	**-0.44***	**-0.56***
Chlorophyll	0.07	0.11	0.12	-0.04	-0.09	-0.072	-0.11
Chlorophyll	**-0.15**	**-0.03**	**-0.21**	**-0.08**	**-0.02**	**0.25**	**0.15**
Na^+ content	-0.65*	0.21	-0.73**	0.03	0.16	0.65	0.47*
Na^+ content	**0.35**	**-0.55***	**0.33**	**-0.32**	**-0.59***	**-0.44***	**-0.61***
Cl^- content	-0.67**	0.22	-0.74**	0.40	0.17	0.66**	0.48*
Cl^- content	**0.37**	**-0.48***	**0.39**	**-0.20**	**-0.49***	**-0.46***	**-0.56***
K^+ content	-0.57*	0.11	-0.55*	0.04	0.14	0.34	0.40
K^+ content	**-0.26**	**0.31**	**-0.33**	**0.07**	**0.29**	**0.34**	**0.40**
Na^+/K^+ ratio	-0.57*	0.10	-0.60*	-0.07	0.04	0.51*	0.29
Na^+/K^+ ratio	**0.38**	**-0.50***	**0.41**	**-0.20**	**-0.51***	**-0.47***	**-0.60***

Table 8. Comparison profile of the sorghum genotypes groups classified by Ward's minimum variance clustering method based on selection indices.

Cluster groups	Salinity level 1 (6.25 dS m^{-1})								
	Ypi	Ysi	TOL	MP	YI	SSI	YSI	STI	GMP
Cluster I (8)	45.04	28.66	16.38	36.85	0.77	1.12	0.64	0.43	35.73
Cluster II (4)	69.12	27.86	41.26	48.49	0.74	1.82	0.41	0.63	43.72
Cluster III (10)	58.24	48.33	9.90	53.28	1.28	0.47	0.85	0.93	52.88
	Salinity level 2 (9.37 dS m^{-1})								
Cluster I (10)	48.76	32.91	15.85	40.83	1.26	0.64	0.69	0.52	39.90
Cluster II (6)	52.59	22.49	30.10	37.54	0.86	1.07	0.43	0.38	34.25
Cluster III (2)	49.95	8.03	41.93	28.99	0.31	1.51	0.20	0.11	18.02
Cluster IV (4)	79.06	23.34	55.71	51.20	0.90	1.33	0.30	0.60	42.65

Values between parentheses are number of genotypes

Sampling and measurements

Data were recorded per plant one month after salinity treatments application. This corresponded to 10% of flowered plants. Plants were pulled out and weighted to record shoot fresh weight (SFW), then shoots and roots were separated and dried at 75°C for 48 h for shoot dry weight (SDW), root dry weight (RDW) and root/shoot ratio (RSR) determination. Chlorophyll content in the leaves was determined according to the method of Arnon (1949). Inorganic ions content were measured in three replications of green forage as follow: 0.5 g of oven-dried samples was made into ash in a Muffle furnace at 580°C for 5 h, then ground into a fine powder by passing them through a 0.5-mm diameter sieve. Dried materials were digested in tertiary acid mixture ($HClO_4$+ HNO_3+H_2SO_4) according to Motsara and Roy (2008) Concentrations of Na^+ and K^+ were determined by Flame photometer (Flamephotometer Model Jenway PFP7, Spectronic Analytical Instruments, UK), Cl^- was determined by titration with $AgNO_3$ and results were expressed as mg/g dry weight of forage. Salt tolerance/sensitivity indices were calculated for each genotype based on shoot fresh weight (Table 3).

Statistical analysis

Analyses of variance and mean comparison of variables were performed by MStat-C, version, 2.10 (software, MSU, USA). Correlation analyses were performed among different selection indices; and with measured traits for each salinity level using Microsoft Excel 2007. Ward's minimum variance clustering method was used to classify genotypes into discrete clusters (Romersburg, 1988).

Conclusion

This study succeeded to assess the extent of genetic variation among 22 sorghum accessions from different origin under saline irrigation water using salinity indices. Some of the introduced genotypes (G7, G8, G9 G11 and G14) are better suited to face high saline irrigation (9.37 dS m^{-1}) than the local. These genotypes should be subjected to further research at field level to evaluate green yield production and use them as genetic resources in plant breeding programs.

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