Genetic diversity for different sorghum (*Sorghum bicolor* L. Monesh) genotypes under saline water irrigation based on RAPD markers

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Abstract: Soil salinity affects a large and increasing amount of arable land worldwide, and genetic and agronomic solutions to increasing salt tolerance are urgently needed. Experiments were conducted to measurement the genetic diversity of sorghum germplasm to provide practical information for the selection of desired parental genotypes thus assist in planning breeding strategies under salinity stress conditions. In order to study the reaction of twenty two genotypes of sorghum to saline irrigation treatments (control, 4000 ppm and 6000 ppm), split-plot experiment was conducted following a randomized complete blocks design (RCBD) with three replications. Twenty two sorghum genotypes were fingerprinted using seven random amplified polymorphic DNA (RAPD) markers. Out of 22 genotypes and 7 primers, only two primers B-01 and B-10 created clearly fragments with 5 genotypes G3, G8, G15, G18 and G22. Unweighted pair-group mean analysis grouped them into two main group clusters, group A consisted of 2 genotypes, namely G3 and G8 were clustered together while the other genotype G22 remained unclustered. While group B consisted of two genotypes, namely G15 and G18. The genotypes G3 and G18; G3 and G15 showed the lowest similarity index (20.0 %), these genotypes could be useful for hybridization. Also, positive and negative markers were produced to distinguish the sorghum genotypes.
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Abbreviation: ds m^{-1} – desiemens per meter; ICRIST – International Crop Research Institute for the Semi-Arid Tropics; TEB – Tris EDETA Buffer; UPGMA- unweight pair-group method with arithmetic average .

1. Introduction

Increased use of fertile agricultural lands for human activities other than crop production pushes crop cultivation to less productive lands, including saline area **(Sang *et al.*, 2005)**. Salinity in soil and irrigation water is a major limiting factor in crop productivity all over the world **(Fuller *et al.*, 2012).** Salinity affects around 60 to 80 million ha of the earth and saline soils are estimated about 5 – 10% of the world's arable land **((Munns *et al.*, 2002).** Salinity restricts yield on 40,000,000 hectares of irrigated land, which is approximately one third of the irrigated land on earth **(Bernstein, 1975)**. In Egypt, 33% of the cultivated land, which comprises only 3% of total land area, is already salinized due to low rainfall and irrigation with saline water **(Ghassemi *et al.*, 1995).** In Sinai where an area of about 0.6 million faddans is proposed to be cultivated with relatively low quality irrigation water through El-Salam canal and under ground water. Wadi Suder region is one of the most promising regions located on the South of Sinai where rainfall or the existing water is limited. So, the irrigation in this region is dependent upon under water contains 3000-5000 ppm as dissolved salts. Development of salt tolerant sorghum cultivars would complement salt management programs to help maximize yields in these areas.

Sorghum rank fifth in worldwide economic importance among cereal crops with an annual production of 60 million tons **(Iqbal *et al.*, 2010).** More than half of the world's sorghum is grown in semi-arid tropics of Africa and India, where it is a staple food for millions of poor people **(Mehmood *et al.*, 2008).** It is extensively grown as a major source of fodder as it is preferred over maize (*Zea mays* L.) because of it's moderately tolerance to salinity **(Reddy *et al.*, 2004; Vahid *et al.*, 2011). Shannon (1999)** reported a threshold of 6.8 dS m^{-1} and 50% yield reduction at salinity level of 9.9 dS m^{-1}. It is a predominantly self-pollinated crop with 2–20% outcrossing **(Rai *et al.*, 1999).** Because self pollination nature makes the genetic variation low in this species, collections and introduction of genetic materials have become an important component of several breeding programs **(Iqbal *et al.*, 2010).** Sorghum breeding can play a major role in narrowing the gap between production and consumption through utilizing improves varieties that can be grown under stress

conditions. Choice of parents for crossing is considered as an important step in any plant-breeding program aimed at improving yield and related attributes (**Arzani, 2008).** 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 and generating new high yielding crop varieties with resistance to biotic and abiotic stresses **(Murray *et al.,* 2008).** Morphological, biochemical and molecular procedures have been exploited for evaluating these resources.

Many studies have been devoted the assessing patterns of sorghum genetic variation based on morphology **(Dje *et al.,* 1998 ; Tulole *et al.,* 2009),** but more recent, DNA techniques have been used successfully in DNA fingerprinting of sorghum plant genome **(Iqbal *et al.,* 2010; Taryono *et al.,* 2011).** The utility of DNA markers for marked-assisted selection is the current trend in modern agriculture. Molecular markers are practically unlimited in number and are not affected by environmental factor and /or the developmental stage of plant **(Romero *et al.,* 2009).** These molecular DNA markers allow the construction of a complete genome map and can be integrated with conventional linkage maps, which play an important role in plant breeding strategies **(Ahmad *et al.,* 2010).** The discovery of polymerase chain reaction was a landmark in this effort and this effort and this has facilitated the development of DNA marker based gene tagging and mapping, biodiversity studies, marker-assisted selection of desirable genotypes **(Akkaya *et al.,* 1992; Nagaoka and Ogihara, 1997; Adnan and Katsuhiko, 2011).**

These molecular markers have been used efficiently to characterise and estimate the genetic diversity among sorghum germplasm **(Abe *et al.,* 2013).** Among the different molecular markers, random amplified polymorphism DNA (RAPD) have been widely used for genetic diversity studies in Sorghum **(Agrama and Tuinstra, 2003; Jeya *et al.,* 2006; Taryono *et al.,* 2011).** Random amplified polymorphic DNA (RAPD) is a DNA polymorphic assay, based on the amplification of random DNA segments with a single primer of arbitrary nucleotide sequences. RAPD produces DNA profiles of varying complexity, depending on the primer and template used. RAPD marker offer many advantages, than other molecular markers, such as higher frequency of polymorphism, rapidity, less costly, easier to use, faster, technical simplicity, requirement of a few nanograms of DNA sequences and feasibility of automation **(Senthil and Gurusubramanian, 2011).**

Therefore, the objectives of this study were to evaluate the efficiency and profitability of different selection indices in identifying salt tolerant sorghum genotypes using RAPD molecular markers to assess the genetic diversity among sorghum lines from local and exotic germplasm, so that suitable genotypes can be recommended for cultivation in salt-prone areas

2. Material 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 (control, 4000 and 6000 ppm) 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, 4000 and 6000 ppm 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 (50 x 70 cm) filled with sandy soils collected from virgin sandy soils of Ismailia Governorate and mixed with 2.16 g N (33.5%) 5.67 g P (15.5%) and 2.06 K (46.5%). 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 two times. Three levels of salinity (control, 4000 and 6000 ppm) were assigned to the main plots and sorghum genotypes were assigned to the subplots. Each sub-subplot consisted of one pot with four plants.

Plant genomic DNA extraction

Fresh leaf samples were collected from 5-week-old seedlings, and genomic DNA was extracted from one or more individual plants from each genotype following a hexadecyltrimethylammonium bromide (CTAB) method according to **Maniatis *et al.* (1982).** The DNA was redissolved in TE buffer and quantified by taking the Optical Density (OD) at 260 and 280 nm with a spectrophotometer (Specto. 23

RS). Reading at 260 and 280 nm was taken to obtain the L260/L280 ratio as an indicator of DNA purity **(Sambrook *et al.*, 1989).** The purified DNA was observed on 1.5% Agarose gel after staining with ethidium bromide to ascertain its integrity.

DNA amplification by PCR-RAPD

For amplification DNA, out of seven different primers (Table 3) initially tested (QIAGEN Operon) only two primer (B-01 and B10) produce clear PCR product were screened for RAPD analysis. The polymerase chain reaction (PCR) was run in a final volume of 25 µL containing 2.5 µL DNA template, 0.2 µL Taq DNA polymerase, 2.5 µL 10x buffer, 3.0 µL $MgCl_2$, 4.0 µL dNTPs, 2.0 µL RAPD primer and 10.8 µL deinonized autoclaved water. The thermal cycler (Perkin Elmer GeneAmp 9700 PCR Thermal Cycler) was operated for PCR reaction as follow: 94°C for 4 min, 45 cycles (94°C for 30 sec, 36°C for 30 sec, 72°C for 2 min) and final elongation of 8 min at 72°C. RAPD amplification products were analyzed by electrophoresis on 1.4% Agarose gels and visualized with ethidum bromide staining under a UV transilluminator, photographed using a digital camera and scanned with Bio-Rad video densitometer Model 620, at wave length of 577. The size of fragments were estimated by comparing the bands size with standard band of 50bp DNA Ladder which consists of 17 bands (1500, 1200, 1000, 900, 800, 700, 600, 500, 450, 400, 350, 300, 250, 200, 150, 100 and 50 bp). The data generated from the detection of polymorphism fragments was analyzed using SPSS package program software (Version 17). All amplifications of each RAPD marker were scored as 1 or 0 where 1; indicated the presence of specific bands and 0; indicated its absence. Using genetic similarity matrix values, a dendrogram was conducted based on unweight pair-group method with arithmetic average (UPGMA).

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).**

3. Results and Discussion

Understanding the genetic diversity of sorghum germplasm collections is important for effective and efficient exploitation of their genetic potential as well as for selection of landraces and other genotypes as breeding material, maintenance and for conservation. Random amplified polymorphic DNA (RAPD) technology was used to discriminate among the 22 sorghum genotypes. Seven randomly primers were used and only the primer B-01 and B-10 produced strong and clear bands with cultivars G3, G8, G15, G18 and G22 (Fig. 1) . Number of fragments amplified using these two different primers showed that: the number of amplified fragments different from one genotype to another indicating that all sorghum genotypes are not always identical in their DNA ability to be amplified. The B-01 primer recorded the highest percentage polymorphism (100%) as it revealed 22 polymorphic bands in 22 amplified fragments, while the B-10 primer recorded the least percentage (80%) by revealing 8 polymorphic bands in 10 amplified fragments (Table 4). This data was close to study of **(Sun *et al.*, 1998),** when detected 81.3% polymorphism among 46 genotypes of *Triticum aestivum* L.

The oligonucleotide of primer B-10 produced one amplified DNA fragment at 1857 bp in two salt tolerance genotypes G3 and G8. On the other hand primer B-01 did not produced any common bands among genotypes G3, G8 and G22. Two amplified fragment at 3173 and 911 bp was obtained in two salt tolerance G3 and G8 while , only, in salt sensitive genotypes G22 eight fragments at 4878, 3454, 2233, 1572, 1310, 1113, 551 and 417 bp were created (Fig. 1). Two bands at 656 and 363 bp were common band in these genotypes while other bands were polymorphic (Table 4).

Depends on the RAPD analysis, the exotic salt moderate genotypes G15 and G18 also showed higher (100%) while similarity between salt tolerant genotypes G3 and G8 was (62.9%). On the other hand, the lowest similarity was observed between G3 and G18; G3 and G15 (20.0 %) (Table 5). This Result indicate that the landraces were related, and this most likely results from the exchange of seeds between farmers in the collection regions, although no duplications were found in the material. This can be exploited in the selection of crossing parents for sorghum variety development program. The information about genetic similarities will be helpful to avoid any chance of elite germplasm becoming genetically uniform and endangering long term productivity gains **(Messmer *et al.*, 1992). Jeya *et al.* (2006)** reported the lowest similarity index between two different genotypes of sorghum K2 and AS376 was (0.28). They varied considerably in plant height, earhead length and dry matter production. These genotypes could be useful for hybridization, since hybrid vigor has a positive relation with genetic distance **(Xiao *et al.*, 1996).** But this results was not agreement with **Iqbal *et al.* (2010)** who observed no correlation between molecular marker and morpho-physiological traits. This an insufficient correlation may be due to the influence of different environmental conditions on the phenotypic traits, leading to

apparent differences even among identical genotypes **(Shehzad *et al.*, 2009)** and also insufficient of number of primers **(Gowhar *et al.*, 2010),** they indicated that the genetic diversity among 5 pea cultivars could be exploited further by increasing the number of random primers and validating it which other available DNA marker. While, **Osman *et al.* (2013)** indicated that PCR-RAPD of DNA provided more precise information concerning relationships between corn (*1. Zea mays* ssp. *mays*, 2. *Sorghum valgare*, 3. *Zea mays* ssp. *Mexicana,* 4. *Zea mays* spp. *Parviglumis*, 5. *Sorghum bicolor*, and 6. *Sorghum grande*.) using Five random oligonuclotide primers OPA-16, OPB-11, OPO-09, OPO-11 and OPZ-7.

Cluster analysis define two main group, group A consisted of 2 genotypes, namely G3 and G8 were clustered together while genotype G22 remained unclustered, which may due to similarity in their genetic structure and common selection history. While group B consisted of two genotypes, namely G15 and G18 (Fig. 2). **Esmail *et al.* (2008)** indicate that determining true genetic dissimilarity between individuals is an important and decisive point for clustering and analyzing diversity within and among populations. **Zhang *et al.* (2005)** found DP555BR and Dp449BR shared cv.DP5690 in their pedigree but they were grouped separately and they concluded that pedigree information or geographic origins of cultivars may not accurately reflect genetic relatedness among genotypes, whereas DNA markers could better reveal the genotypic relationships when there are sufficient markers and they are distributed across all chromosomes.

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

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

Parameter	Control (Nile water)	4000 ppm	6000 ppm	Sea water
pH	7.30	8.25	8.22	7.54
EC (dS/m)	0.336	6.25	9.375	45.30
Soluble cations, (meq/l)				
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, (meq/l)				
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

Table 3. List of seven arbitrary primers and their nucleotide sequences used to generate RAPD markers in sorghum.

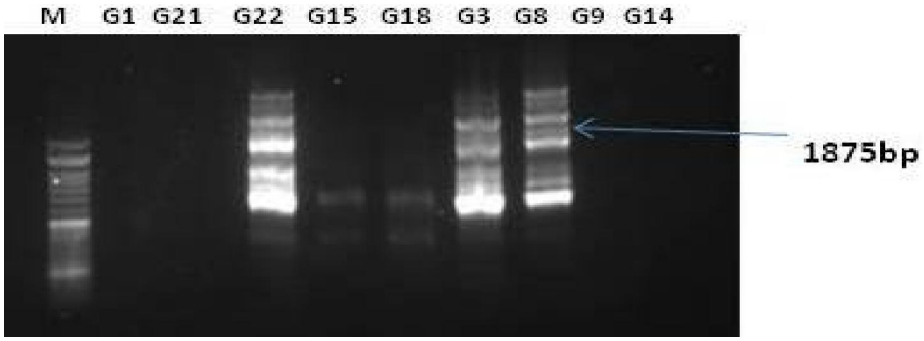
No.	Primer name	Sequence	No.	Primer name	Sequence
1	B-01	5' GTT TCG CTC C 3'	5	B-09	5' TGG GGG ACT C 3'
2	B-04	5' GGA CTG GAG T 3'	6	B-10	5' CTG CTG GGA C 3'
3	B-05	5' TGC GCC CTT C 3'	7	O-03	5' CTG TTG CTA C 3'
4	B-06	5' TGC TCT GCC C 3'			

Table 4. Total number of amplified bands and number of polymorphic bands generated by PCR using two random primers B-01 and B-10.

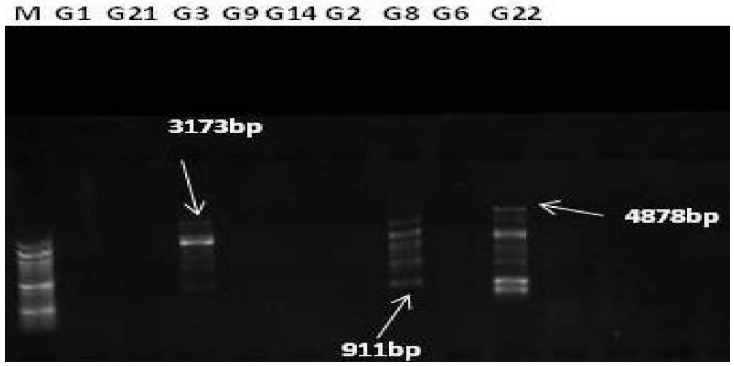
Primer name	Primer Sequence (5'⟶ 3')	Size range (bp)	Total amplified bands	No. of polymorphic bands	% polymorphic bands
B-01	GTT TCG CTC C	417 - 4878	22	22	100
B-10	CTG CTG GGA C	363- 3472	10	8	80
Average					**90**

Table 5. Dice's similarity coefficient matrix for sorghum based on RAPD data.

Genotypes	**G3**	**G8**	**G15**	**G18**	**G22**
G3	100				
G8	62.9	100			
G15	20.00	21.1	100		
G18	20.00	21.1	100	100	
G22	45.7	52.9	21.1	21.1	100



Primer B-01



Primer B-10

Figure 1. RAPD banding pattern obtained with different genotypes (G) of sorghum using primers B-1 and B-10. Lane (M) represented 50bp DNA marker.

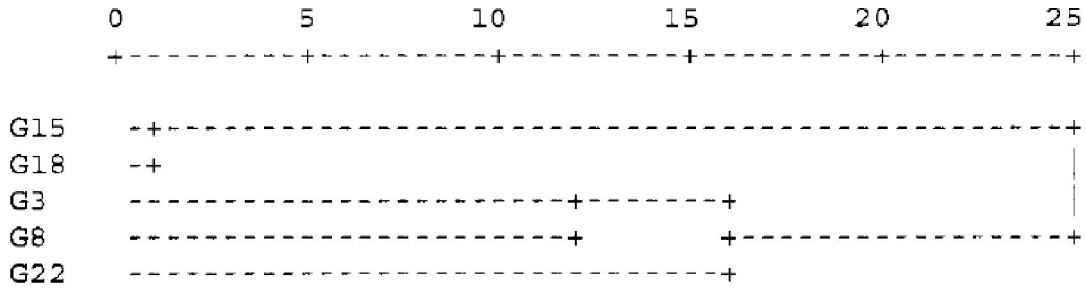


Figure 2. Dendrogram of the five sorghum genotypes based on the similarity matrix produced by RAPD-PCR.

Conclusion

The RAPD molecular marker carried out with seven random primers only revealing the genetic diversity among the 5 genotypes of sorghum. It is essential to rationalize conservation and use of genetic resources to guide in the establishment of strategies that ensure the maintenance of genetic variability essential in plant breeding. To avoid this lack in the RAPD-PCR product, in the future work, increasing the number of random primers must using.

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