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Research Article
Stability Analysis and Molecular Description of Some Promising Sorghum Lines Tolerant to Salt Stress

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Abstract

Background and Objective: Salt stress is considering the biggest environmental obstacle to crop productivity, especially sorghum. So, it was necessary to develop new sorghum lines tolerant to salt stress and high yielding to participate in bridging the large gap in the Egyptian bread industry and also as an important feed for animals. This is the biggest goalie this investigation. **Materials and Methods:** Some promising sorghum genotypes were evaluated under the control experiment and two salinity stress locations to test their stability and its salinity stress tolerance during two years. Some agro-morphological and physiological traits were the most important parameters tested under all conditions besides, 11 SCoT primers for comparing among the seven sorghum genotypes and Identification of molecular genetic markers responsible for salt stress tolerance. **Results:** The final results revealed that the five promising sorghum lines were recorded highly rank of salinity stress tolerance in all studied traits and a higher level of genetic stability during the two years. **Conclusion:** Results of agro-physiological traits, salinity tolerance indices and SCoT primers succeed in determining salt stress tolerance mechanisms in sorghum and which an important taxonomic tool is for plant breeder that helps him in sorting the tolerant genotypes from the sensitive ones.

Key words: Sorghum, salinity tolerance indices, stability analysis, SCoT primers, fodder, ionic imbalance, plant physiology

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sorghum is considering one of the important summer grain crops after rice and maize and the Arab Republic of Egypt is the first in the production of sorghum per unit area overall producing countries in the world. Most of its cultivation is concentrated in Upper Egypt, where it is grown in large areas annually, up to 400 thousand feddan. The importance of sorghum as grains is due to being a food crop for humans, especially in rural communities. Recently, it mainly contributes to the manufacture of animal and poultry feed. White grains are now supplied to the Ministry of Supply to produce good-quality municipal bread by mixing 20% sorghum flour with wheat flour, which helps reduce the import of wheat and flour from abroad. In addition, the use of some types of red-coloured sorghum in the beer and pigment industries was done. Also, green plants are used after harvesting for the short-stemmed varieties and hybrids for dual purposes as animal fodder, while the stems of the tall varieties are used for fuel and building fences and windbreaks. The current trend is expanding in cultivating high-yielding short-stemmed varieties and hybrids (green grains and fodder) that tolerance for difficult conditions such as drought, extreme heat, poor soil fertility and increased salinity. Salt stress is one of the most serious environmental constraints, as it limits the growth of plants and greatly damages the final yield[1,2]. As it has a very large role in raising the osmotic and ionic imbalance in the plant causes an imbalance of the osmotic pressure. This in turn tends to rise, resulting in a large amount of water being lost during the transpiration process. Also, the photosynthesis process is damaging and all aspects of plant physiology are negatively affected, such as the activities of antioxidant enzymes and enzymes responsible for photosynthesis that ultimately causes the final output to be destroyed[3]. This risk is most prevalent in arid and semi-arid lands, where the temperature raises parallel to the decrease in rainfall. As well as, the limited share of water allocated to agriculture and this, in turn, leads to the intensification of the devastating effects of salt stress[4,5]. But concerning the Egyptian conditions, it is noticed that the problem of high salinity is confined to the coastal lands near the seawater and the delta region. These areas do not have fresh water for sowing and salt washing and this leads to increase soil salinity. On the other hand, Egypt's share of water is limited, which began to decrease after the construction of the Ethiopian Renaissance Dam. All these factors exacerbate the problem of salt stress in the Egyptian lands and then this will be reflected in agricultural production in general. Although sorghum is tolerant to salinity stress under moderate levels, it cannot tolerate high salinity orbits. In addition, the directions of the Egyptian state for sustainable agriculture with rationalization and the use of all types of water. Therefore, breeding studies and the introduction of fine sorghum lines that are tolerant of high salinity levels have become an inevitable priority in the future[6]. The following is a quick review of the results for the most important studies and research that specifically discussed this topic. Salt-stress due to highly decreasing in germination stage[7], growth[8] and final output in sorghum[9]. Besides, it also leads to physiological and biochemical changes in the activity of the energy metabolism in plant life[10]. The salt stress using sodium chloride salt led to a significant increase in the activity of the peroxidase enzyme in a large number of sorghum accessions, as this salt stress had a major role in increasing the activity of roots compared to the activity of leaves[11]. Despite the good physiological properties of sorghum, which gives it a high tolerance to salt stress, studies at the molecular level to determine those mechanisms responsible for tolerance are still in their infancy. Therefore, it is necessary to study and devise the molecular genetics mechanisms responsible for their tolerance to this dangerous environmental factor, considering that sorghum is a good form of an African crop that gives an excellent picture of the grains' bearing of environmental stresses employing endurance, avoidance and flight[12]. Accreting of inorganic ions levels results in controlling in osmotic adjustment besides, the higher limit of K^+/Na^+ and Ca_2^+/Na^+ ratios in sorghum plants are responsible for increasing the ability of salinity tolerance under saline conditions[13]. The 181 sorghum accessions were studied to determine (QTLs) under normal and salinity conditions by Wang *et al.*[14]. They discovered a total of 53 QTLs for the studied traits, plant height, stem diameter, total biomass, stem fresh weight, juice weight and Brix under both conditions and STI ranged from 4.16-20.24%. That is, through previous studies, it is clear that the two saline and drought stresses have a great negative impact on yield and its components traits on crops and a large number of scientists have studied these effects through molecular markers such as on cucumber[15], on barley[16,17], on sorghum[18,19], oncanola[20] and maize[21]. After all that, it is noted that the aim of this study was analyzing the genetic stability for some promising sorghum hybrids that have reached a high degree of genetic stability under different environmental conditions included both natural and saline soils. As well as the genetic comparison among them using some molecular markers to determine the genes responsible for salt-stress tolerance and ultimately reaching the production of high-yielding sorghum lines that are tolerant to salt stress under Egyptian conditions. Thus, it is possible to cultivate large areas of land affected by salinity with these new lines after adopting them as varieties and

benefit from them in participating in reducing the gap in the Egyptian bread industry by mixing each of its flour with wheat flour together. Also, take advantage of its green leaves at the age of 45 days as an important feed for Egyptian livestock and this is the winning goal in this work.

MATERIALS AND METHODS

Study area: The study was carried out in the genetics and cytology department, genetic engineering and biotechnology research division, National research centre and three agriculture locations in agriculture research centre, age location in Mansoura city of the governorate of Dakahlia, Sirw location in Damietta city, Damietta governorate and El-Hosinia in the Sharqiyah governorate, Egypt from 2010-2020 (This period included choice the parents for hybridization, crossing, sowing of all genotypes; parents and the first hybrid generation, selected the best crosses, continuous of agriculture and the simple selection reaching to high genetic stability).

Plant materials: Seven sorghum genotypes were used in this investigation namely; two local check varieties and five sorghum promising lines, Table 1. The local check varieties were Giza 3 and Giza 54. While the promising lines were produced from diallel crosses (Half diallel analysis) in the 2010 season and continued sowing with simple selection processes from 2011 season until 2018 season to choose the highest five sorghum hybrids for yield and its components traits which has reached the highest levels of genetic stability under Egyptian conditions. Half diallel crossing program included the local cultivars, Giza 3 and Giza 54 and the four imported lines characterizing for salt stress tolerance from ICRISAT (International Crops Research Institute for The Semi-Arid Tropics). The four imported lines were, ICSA 34×ICSR14, ICSA 34×98 MW 6001, ICSA 34×P894108 and ICSA 21×98 MW 6100 which were conducted in season 2010 to produce 15 F1 hybrids and continuing to cultivate these crosses with simple selection to screen them and obtain the best hybrids in terms of yield and its components besides, tolerance to salt stress under both natural soil conditions in Sakha, Kafr El-Sheikh Governorate and saline conditions in the farm of Sirw city in Damietta governorate until the 2018 season. The six parents were planted in three planting dates on the basis that the interval among each two planting dates was 5 days until there is a big chance for the success of the hybridization process on June, 1, 2010 and to obtain a large number of hybrid seeds.

The process of cultivating the first hybrid generation (15 F1 crosses) continued with simple selection starting from the 2011 season until the 2018 season under normal and salinity conditions so that the hybrids had reached a high degree of genetic stability.

Sowing: The seven sorghum accessions (The two local check varieties and the best five crosses which reached highly genetic stability) were evaluated under normal and saline conditions in the first of June for the two growing seasons (2019 and 2020) with three replicates in a randomized complete block design for each experiment in each season to test the degree of genetic stability and assess the extent of tolerant to salt stress under both conditions. Where, the normal experiment was conducted in the village of Aga, Dakahlia Governorate. While salinity treatment was done in two locations where the first one was on the farm of Sirw city in Damietta governorate and the second location was conducted at the El-Hosinia village, Sharkia Governorate, Agricultural Research Station and both types of salinity soils were characterized as (salinity affected soils).

Methods

Soil analysis: Before conducting the experiments, soil samples were taken from different sites of the experimental area. Each sample was taken from a depth of 0-30 cm from both soil samples (normal and both saline treatments). The chemical analysis was carried out for each soil extract 1:5 to estimate the soluble anions, cations and Total Dissolved Salts (TDS). The Electrical Conductivity (EC) was estimated in the extract of the soil saturate paste. The procedure for preparation and measurements of the soil extract was taken[22]. The methods[23] of soil chemical analysis was followed. The description of the normal and two types of saline soils used in this investigation are shown in Table 2. Average relative humidity% and temperature were taken at the summer weather especially (June, July, August and September, months) at the three locations for the two growing seasons (2019 and 2020) and were collected from the meteorological station, the Climate Research Department of the Agricultural Research Center, Egypt for the three sites understudying in Table 3.

Studied traits: Fifty plants were taken from each replicate of each genotype for the seven sorghum accessions of each experiment or (Location) for each season to evaluate some yield and its components and attributes related to salt stress tolerance as follows:

- **Grain yield per plant (g):** It was recorded as the weight of grain yield of each plant and adjusted to 14% moisture content

Table 1: Name and pedigree of the studied sorghum genotypes

Numbers	Genotype	Origin	Reaction for salt stress
1	Parent 1: Giza 3	Egypt	Susceptible
2	Parent 2: Giza 54	Egypt	Moderately
3	Parent 3: Line 1 (ICSA 34×ICSR14)	ICRISAT	Tolerate
4	Parent 4: Line 2 (ICSA 34×98 MW 6001)	ICRISAT	Tolerate
5	Parent 5: Line 3 (ICSA 34×P894108)	ICRISAT	Tolerate
6	Parent 6: Line 4 (ICSA 21×98 MW 6100)	ICRISAT	Tolerate
Numbers of crosses	The selected 5 crosses reached highly genetic stability		
1	Line 1 or cross 1: Giza 3×Line 1 (Parent 3)		
2	Line 2 or cross 2: Giza 3×Line 4 (Parent 6)		
3	Line 3 or cross 3: Giza 54×Line 1 (Parent 3)		
4	Line 4 or cross 4: Giza 54×Line 2 (Parent 4)		
5	Line 5 or cross 5: Giza 54×Line 3 (Parent 5)		

Table 2: Some mechanical and chemical analysis before sowing at 0-30 cm depth for the three locations in both growing seasons (2019 and 2020)

	Normal soil (Aga)		Saline soil (Sirw location)		Saline soil (El-Hosinia location)	
Soil properties	Season 2019	Season 2020	Season 2019	Season 2020	Season 2019	Season 2020
Sand	12.98	13.05	11.23	10.19	12.06	11.38
Silt	33.78	34.15	35.64	38.91	37.29	35.04
Clay	53.24	52.80	53.13	50.90	50.65	53.58
Chemical analysis						
PH	7.80	8.13	9.11	9.24	9.28	8.70
EC (ds m^{-1})	2.35	2.41	9.68	9.83	15.52	14.79
ESP	7.23	7.45	12.33	12.49	13.18	13.57
TDS (mg L^{-1}) (ppm)	345.12	362.81	5711.08	5894.55	6112	6096.37
Ca^{++}	2.04	1.97	13.65	13.88	15.07	14.83
Mg^{++}	1.17	1.25	12.76	12.42	13.58	13.62
Na^{+}	9.54	9.38	53.18	55.83	61.04	59.37
K^{+}	0.62	0.57	0.26	0.24	0.22	0.17
CO_3^{-}	0.05	0.07	0.16	0.19	0.21	0.18
HCO_3^{-}	1.94	1.98	1.23	1.25	1.31	1.36
Cl^{-}	11.48	12.15	42.03	43.72	45.76	47.81
SO_4^{-}	1.64	1.72	13.61	13.58	14.09	14.28
Texture	Clay	Clay	Clay	Clay	Clay	Clay

EC: Electrical conductivity, TDS: Total dissolved salts, *Measure of soil saturation, **Measure of soil water extract 1:5

Table 3: Classification of average temperature and relative humidity (%) for all locations during the two growing seasons (2019 and 2020)

	Location (1) normal soil in (Aga) city		Location (2) saline soil (Sirw city)		Location (3) saline soil (El-Hosinia location)	
Classification of weather	Season 2019	Season 2020	Season 2019	Season 2020	Season 2019	Season 2020
Relative humidity (%)						
June	63.08	65.56	61.15	66.79	59.03	63.73
July	68.32	67.08	66.34	69.17	67.30	71.05
August	72.92	74.79	71.12	75.55	70.08	72.33
September	76.12	78.95	69.18	75.03	73.19	77.25
Mean	70.11	71.59	66.94	71.63	76.40	71.09
Average temperature (°C)						
June	36.0	37.0	37.0	36.0	35.0	34.0
July	32.0	31.0	30.0	32.0	31.0	32.0
August	34.0	36.0	35.0	33.0	33.0	34.0
September	31.0	33.0	34.0	32.0	31.0	33.0
Mean	33.25	34.25	34.0	33.25	32.5	33.25

- **Number of panicles per plant:** it was calculated by counting the total number of panicles for each plant
- **Panicle weight (g):** It was recorded by the weight of each panicle for each plant at maturity
- **1000-grain weight (g):** It was recorded by the weight of 1000 fertile grains for each plant at maturity
- **Osmotic adjustment:** It was determined by the formula[24] as follows:

$$\frac{OP \times RWC}{100}(normal) - \frac{OP \times RWC}{100}(drought)^{100}$$

Where:
OP : Osmotic pressure
RWC : Relative water content

- **Proline content:** Was determined from a standard curve and calculated on a fresh basis is as follows:

Proline (μ moles)/Fresh weight material (g) = [(μg proline/mL C mL toluene)/115.5 μg/μ mole]/[(g sample/5)]

The results related to proline content are average values of at least 3-4 samples for each species, according to previous study[25] and modified method by another study[26]:

- **Glycine betaine and trehalose contents:** It was carried out according to the method of Senthil *et al*.[27]

Planting date: All plant materials were grown on 1 June, and harvested on 21 September, in both growing seasons (2019 and 2020) for the three experiments including the control treatment in Aga location and both salinity treatments in Sirw and El-Hosinia locations.

Statistical analysis: Stability analysis was carried out[28]. This analysis included three genetic parameters bi (regression coefficient), S^2d (mean squares of deviation from the regression) and R^2 (The percentage of stability) which were used to indicate the performance on environmental indices. Yield stability statistic was calculated using the program STABLE (a basic program for calculating stability and yield stability statistics)[29].

Salinity tolerance indices: All salinity tolerance indices were estimated using grain yield/plant trait for the seven sorghum genotypes (the new five lines besides, the two local cultivars Giza 3 and Giza 54)of both salinity locations[30-32] as follows:

$$GYP = \frac{\text{Meaning the grain yield}}{\text{Plant for the control experiment}}$$

$$GYS = \frac{\text{Meaning the grain yield}}{\text{Plant for the salinity experiment}}$$

$$YSI = \text{Meaning yield stability}$$

$$\text{Index} = \frac{YS}{YP}$$

Where:
YS : Average of yield under stress
YP : Average of yield under the control experiment
YI : Meaning yield index (YS for each genotype/ mean of YS for all genotypes)
MP : Means (Average yield for both trials): YS+YP/2
STI : Meaning salinity tolerance index (YP × YS/(mean of YP)^2
GMP : $(YP \times YS)^{0.5}$
YR : Meaning yield reduction (1-YS/YP)
SSI : Meaning salinity susceptibility index

$$DSI = \frac{1\text{-}YS/YW}{D}$$

Where:
YS : Mean yield under salt stress
Yw : Mean yield under control condition
D : Environmental stress intensity = 1-(mean yield of all genotypes under stress/mean yield of all genotypes under irrigated conditions)

Molecular depiction: Molecular genetic markers played a pivotal and important role in differentiating among the seven sorghum genotypes. Also, it pointed to the most important genetic differences between them at the molecular level, especially identifying the genetic evidence responsible for salt stress tolerance in the five promising hybrids (lines) compared to the local varieties and this is what we will review in detail.

DNA Isolation and SCoT analysis: Genomic DNA was extracted from fresh leaves of 7 sorghum genotypes (the new lines or crosses reached highly genetic stability) as follows,1: Line one (Giza 3 × Line 1), 2: Line two (Giza 3 × Line 4), 3: Line three (Giza 54 × Line 1), 4: Line four (Giza 54 × Line 2), 5: Line five (Giza 54 × Line 3) and the two local check varieties namely, 6: (Giza 3) and 7: (Giza 54), respectively. Eleven (SCoT) primers namely, SCoT 1, 2, 3, 4, 5, 6, 7, 9, 10, 11 and 12 were used in this study for comparing among the seven sorghum accessions.

Start codon target (SCoT) analysis

SCoT-PCR reactions: The amplification reaction was carried out in 25 μL reaction volume containing 1 × PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 25 pmol primer, 1 U Taq DNA polymerase and 30 ng template DNA[33].

- 5×buffer 5 µL
- $MgCl_2$ (25 mM) 2 µL
- dNTPs (10 mM) 0.5 µL
- Primer (10 pmol) (ScoT) 2.5
- DNA (10 ng µL) 3 µL
- Taq DNA polymerase (5 u µL) 0.2 µL
- dH_2O Up to 25 µL

Thermocycling profile PCR: PCR amplification was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems) programmed to fulfil 40 cycles after an initial denaturation cycle for 5 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 45 sec, an annealing step at 50°C for the 50 sec and an elongation step at 72°C for 1min. The primer extension segment was extended to 7 min at 72°C in the final cycle.

DNA ladder: The marker used for primers, SCoT 1, 11 and 12 was 1kb with molecular weights of (250, 500, 750, 1000, 1500, 2000, 3000, 4000, 5000, 6000, 8000 and 10000 bp) while, the marker used for the rest SCoT primers, (2, 3, 4, 5, 6, 7, 9 and 10) was 100 bp with weights of (100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 bp), respectively.

Detection of the PCR products: The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5 µg mL^{-1}) in 1×TBE buffer at 95 volts.

Gel documentation: Gels were photographed scanned, analyzed using Gel Doc Vilber Lourmat system (Vilber Company, France) to capture the image and to calculate band intensities.

Data handling and cluster analysis (phylogenetic tree): Data were scored for computer analysis based on the presence or absence of the amplified products for each primer. Pairwise components of the 7 sorghum entries based on the presence or absence of unique and shared polymorphic products were used to determine similarity coefficients[34]. The similarity coefficients were then used to construct dendrograms, using the unweighted pair group method with arithmetic averages (UPGMA) employing the SAHN (Sequential, Agglomerative, Hierarchical and Nested clustering) from the NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System), version 1.80 (Applied Biostatistics Program).

RESULTS

Variation and interaction: Highly significant differences were observed among all studied attributes confirming that the important role of a genetic variation for controlling the seven sorghum accessions in Table 4. Data of mean squares variations of environmental were highly significant indicating that environmental factors were contributed for intension the fruitful role responsible for the recognized genotypic performance. Also, the results observed in the same table exhibited that highly significant variances were generated from the interactions among genotypes and environments for all studied attributes in all sorghum genotypes. For example not limited, the values of the traits, grain yield/plant, number of panicles/plant and 1000-grain weight were (54.18**, 7.21** and 54.23**), (17.92**, 2.15** and 12.75**) and (61.05**, 37.04** and 1.98**) for environments, genotypes×environments and Environments+(Genotypes×Environments) in Table 4, respectively. Data of Table 5 associated with (F-Ratio) were showed significant and highly significant variances of all studied traits for the most source of variance components calculated within the test of (ANOVA) for stability analysis design. The values of (F-Ratio) for the traits, grain yield/plant, number of panicles/plant and 1000-grain weight were (19.41**, 2.58** and 19.43**), (12.98**, 1.55** and 9.23**) and (48.84**, 29.63** and 1.58**) for environments, genotypes×environments and Environments+(Genotypes×Environments), respectively.

Mean performance: All results viewed in Table 6-8 indicated that the seven sorghum entries achieved great and noticeable superiority in all traits understudy for the various evaluation sites compared to the local varieties (Giza 3 and Giza 54). Where it was confirmed to a large extent that it is tolerant to salt stress in the lands affected by high salinity (Sirw and El-Hosinia locations) after comparing the data of all estimated attributes compared to the normal experiment (Aga location) during the two growing seasons. Where these five promising sorghum lines exhibited the highest mean values in grain yield/plant, the number of panicles/plant, panicle weight and 1000 grain weight and some physiological traits related to salinity tolerance like proline, glycine betaine and trehalose contents for the first location in (Aga city), followed by the location of (Sirw city) and then followed by the third location in

Table 4: Mean squares of stability analysis for all studied traits in sorghum accessions

		MS							
SOV	DF	Grain yield/ plant (g)	Number of panicles/plant	Panicle weight (g)	1000-grain weight (g)	Osmotic adjustment	Proline content	Glycine betaine	Trehalose content
Genotypes	6	83.35**	125.28**	114.21**	227.19**	163.17**	46.82**	15.31**	118.69**
Environments	5	54.18**	17.92**	8.72**	61.05**	13.91**	26.03**	32.84**	41.03**
Genotypes×environments	30	7.21**	2.15**	11.79**	37.04**	1.68**	28.19**	47.50**	13.92**
Environments+(genotypes×environments)	35	54.23**	12.75**	6.48**	1.98**	33.45**	15.09**	18.27**	20.07**
Environmental (linear)	1	24.82**	15.78**	20.07**	19.03**	10.85**	5.83**	3.46**	8.37**
(Genotypes×environments) linear	6	3.59**	25.41**	65.84**	17.93**	9.45**	11.08**	55.07**	32.48**
Pooled deviation	28	32.59**	18.62**	14.09**	9.56**	12.46**	21.15**	7.32**	16.08**
Pooled error	72	2.79	1.38	0.83	1.25	1.41	0.65	1.35	0.71

*Significant at 5%, **Significant at 1%

Table 5: F-ratio values for the components of stability analysis

	MS							
SOV	Grain yield/ plant (g)	Number of panicles/plant	Panicle weight (g)	1000 grain weight (g)	Osmotic adjustment	Proline content	Glycine betaine	Trehalose content
Genotypes	29.87**	90.78**	137.60**	181.75**	115.72**	72.03**	11.34**	167.16**
Environments	19.41**	12.98**	10.50**	48.84**	9.86**	40.04**	24.32**	57.78**
Genotypes×environments	2.58**	1.55**	14.20**	29.63**	1.19**	43.36**	35.18**	19.60**
Environments+(genotypes×environments)	19.43**	9.23**	7.80**	1.58**	23.72**	23.21**	13.53**	28.26**
Environmental (linear)	8.89**	11.43**	24.18**	15.22**	7.69**	8.96**	2.56**	11.78**
(Genotypes×environments) linear	1.28**	18.41**	79.32**	14.34**	6.70	17.04	40.79	45.74**
Pooled deviation	11.68**	13.49**	16.97**	7.64**	8.83**	32.53**	5.42**	2.64**

*Significant at 5%, **Significant at 1%, Probability>F =<0.0001

Table 6: Mean performance for the seven sorghum accessions of the six environments for grain yield/plant, number of panicles/plant and panicle weight traits, respectively

All environments	L1	L2	L3	L4	L5	Local check variety one	Local check variety two	Mean
Grain yield/plant (g)								
R1 Y1	56.18	53.6	58.13	60.03	55.73	38.19	42.13	51.99
R2 Y1	45.17	38.45	47.23	48.12	36.43	33.12	37.48	40.85
R3 Y1	37.72	32.18	34.18	39.18	29.15	27.95	28.13	32.64
R1 Y2	55.98	53.78	57.65	58.14	52.08	39.84	40.07	51.07
R2 Y2	43.62	36.89	45.57	46.05	35.92	31.64	35.97	39.38
R3 Y2	36.15	33.58	37.22	40.02	27.88	26.76	27.47	32.72
Mean	45.8	41.41	46.66	48.59	39.53	32.91	35.2	41.44
Number of panicles/plant								
R1 Y1	29.06	31.15	28.76	32.04	30.55	23.18	21.54	28.04
R2 Y1	26.44	28.46	25.29	27.93	25.12	19.22	17.68	24.3
R3 Y1	21.36	22.07	20.39	23.08	18.23	12.64	14.22	18.85
R1 Y2	30.02	28.15	32.17	31.05	29.74	22.97	20.87	27.85
R2 Y2	27.31	25.19	29.72	26.22	24.06	18.49	16.55	23.93
R3 Y2	24.2	20.15	23.12	19.47	17.96	13.38	12.74	18.71
Mean	26.39	25.86	26.57	26.63	24.27	18.31	17.26	23.61
Panicle weight								
R1 Y1	39.42	41.33	40.02	38.19	40.17	32.18	35.97	38.18
R2 Y1	36.18	38.45	37.17	32.05	34.06	28.14	31.05	33.87
R3 Y1	31.02	28.19	30.95	26.46	29.8	23.19	28.93	28.36
R1 Y2	40.25	40.11	38.48	36.67	41.55	31.07	33.32	37.35
R2 Y2	35.83	37.92	35.09	31.32	33.16	26.55	30.43	32.9
R3 Y2	30.69	26.14	29.78	25.92	27.84	24.43	27.28	27.44
Mean	35.56	35.35	35.24	31.76	34.43	27.59	31.16	33.01

R: Region, Y: Year and L: Line

(El-Hosinia city) compared to the two local varieties in both growing seasons. Further, these genetic materials recorded the lowest values of osmotic adjustment trait where the values were very little in El-Hosinia location and started for the increase in Sirw location during the two growing seasons. This result was noted in the combined data for the seven sorghum entries of all studied straits for the three experiments during the two

Table 7: Mean performance for the seven sorghum accessions of the six environments for 1000 grain weight, osmotic adjustment and proline content traits respectively

All environments	L1	L2	L3	L4	L5	Local check variety one	Local check variety two	Mean
1000 grain weight (g)								
R1 Y1	33.46	32.11	30.08	29.74	31.19	27.19	28.05	30.26
R2 Y1	30.12	28.37	26.68	27.02	25.43	24.13	25.09	26.69
R3 Y1	26.81	24.06	22.57	21.45	20.52	18.33	17.59	21.61
R1 Y2	31.75	32.04	31.04	30.17	30.85	27.03	26.89	29.96
R2 Y2	29.04	27.86	26.41	25.93	24.86	22.06	23.07	25.60
R3 Y2	27.03	24.15	23.78	22.66	20.55	15.12	16.69	21.42
Mean	29.70	28.09	26.76	26.16	25.56	22.31	22.89	25.92
Osmotic adjustment								
R2 Y1	0.58	0.61	0.75	0.49	0.58	0.97	0.89	0.69
R3 Y1	0.42	0.34	0.64	0.22	0.39	0.73	0.69	0.49
R2 Y2	0.55	0.58	0.58	0.41	0.49	0.86	0.77	0.60
R3 Y2	0.39	0.29	0.58	0.17	0.28	0.71	0.66	0.44
Mean	0.48	0.45	0.63	0.32	0.43	0.81	0.75	0.55
Proline content								
R1 Y1	45.78	39.88	41.56	43.09	38.07	24.12	28.04	37.22
R2 Y1	49.06	53.83	56.34	48.15	43.38	31.19	35.78	45.39
R3 Y1	58.54	63.97	68.09	55.93	61.75	38.98	42.08	55.62
R1 Y2	44.18	42.05	40.11	42.96	39.16	26.55	29.54	37.79
R2 Y2	52.14	54.02	57.01	50.04	46.16	33.20	37.29	47.12
R3 Y2	60.04	65.03	71.23	61.03	59.07	40.23	44.19	57.26
Mean	51.62	53.13	55.72	50.20	47.93	32.37	36.15	46.73

R: Region, Y: Year and L: Line

Table 8: Mean performance for the seven sorghum accessions of the six environments for glycine betaine and trehalose contents traits respectively

All environments	L1	L2	L3	L4	L5	Local check variety one	Local check variety two	Mean
Glycine betaine								
R1 Y1	33.18	35.81	41.38	52.28	44.07	29.38	31.16	38.18
R2 Y1	55.74	52.6	67.82	62.12	58.14	47.77	49.03	56.17
R3 Y1	76.49	81.04	75.26	82.04	69.11	68.39	72.14	74.92
R1 Y2	31.54	37.24	45.37	50.09	47.23	32.43	35.07	39.85
R2 Y2	59.03	54.22	65.32	60.13	59.86	51.06	55.96	57.94
R3 Y2	79.56	83.74	77.30	80.94	74.62	75.09	77.18	78.34
Mean	55.92	57.44	62.07	64.60	58.83	50.68	53.42	57.56
Trehalose content								
R1 Y1	29.75	32.17	38.96	30.05	35.82	24.55	28.31	31.37
R2 Y1	45.28	52.13	47.23	38.35	43.71	29.68	34.32	41.52
R3 Y1	63.03	60.33	55.94	49.85	69.18	38.58	40.17	53.86
R1 Y2	33.25	29.74	36.92	28.69	37.41	25.82	30.09	31.70
R2 Y2	47.60	49.83	45.02	40.26	51.09	31.22	37.64	43.23
R3 Y2	65.11	71.45	61.80	53.14	73.64	42.19	44.37	58.81
Mean	47.33	49.27	47.64	40.05	51.80	32.00	35.81	43.41

R: Region, Y: Year and L: Line

growing seasons in Table 9 and 10 as well and this has a logical explanation that we will deal with in some detail in the discussion part. In the same context, it was noted that the best environments that achieved a great tolerance for salt stress were (R2 Y1 and R2 Y2) in all sorghum accessions for all studied traits were the values in Table 6 were (45.17, 38.45, 47.23, 48.12, 36.43, 33.12 and 37.48 g) of (R2Y1) and (43.62, 36.89, 45.57, 46.05, 35.92, 31.64 and 35.97g) of (R2 Y1) for the seven sorghum entries in grain yield/plant, (26.44, 28.46, 25.29, 27.93, 25.12, 19.22 and 17.68) of (R2 Y1) and (27.31, 25.19, 29.72, 26.22, 24.06, 18.49 and 16.55) of (R2 Y2) for the seven sorghum entries in the number of panicles/plant and (36.18, 38.45, 37.17, 32.05, 34.06, 28.14 and 31.05 g) of (R2Y1) and (35.83, 37.92, 35.09, 31.32, 33.16, 26.55 and 30.43 g) of (R2 Y2) for the seven sorghum entries in panicle weight, respectively. While that, the mean values for the best environments in Table 7 were (30.12, 28.37, 26.68, 27.02, 25.43, 24.13 and 25.09 g) of (R2Y1) and (29.04, 27.86, 26.41, 25.93, 24.86, 22.06 and 23.07 g) of

Table 9: Mean values obtained from the seven sorghum accessions for all studied traits of all environments

All environments	Grain yield/ plant	Number of panicles/plant	Panicle weight	1000 grain weight	Osmotic adjustment	Proline content	Glycine betaine content	Trehalose content
L1	45.80	26.39	35.56	29.70	0.48	51.62	55.92	47.33
L2	41.41	25.86	35.35	28.09	0.45	53.13	57.44	49.27
L3	46.66	26.57	35.24	26.76	0.63	55.72	62.07	47.64
L4	48.59	26.63	31.76	26.16	0.32	50.20	64.60	40.05
L5	39.53	24.27	34.43	25.56	0.43	47.93	58.83	51.80
Local check variety one	32.91	18.31	27.59	22.31	0.81	50.68	50.68	32.00
Local check variety two	35.20	17.26	31.16	22.89	0.75	53.42	53.42	35.81

Table 10: Mean performances of all studied Traits for the seven sorghum accessions of all environments

All environments	Grain yield/ plant	Number of panicles/plant	Panicle weight	1000 grain weight	Osmotic adjustment	Proline content	Glycine betaine content	Trehalose content
R1 Y1	51.99	28.04	38.18	30.26	-	37.22	38.18	31.37
R2 Y1	40.85	24.30	33.87	26.69	0.69	45.39	56.17	41.52
R3 Y1	32.64	18.85	28.36	21.61	0.49	55.62	74.92	53.86
R1 Y2	51.07	27.85	37.35	29.96	-	37.79	39.85	31.70
R2 Y2	39.38	23.93	32.90	25.60	0.60	47.12	57.94	43.23
R3 Y2	32.72	18.71	27.44	21.42	0.44	57.26	78.34	58.81
Mean	41.44	23.61	33.01	25.92	0.55	46.73	57.56	43.41

R: Region, Y: Year and L: Line

(R2Y2) for the seven sorghum entries in 1000-grain weight, (0.42, 0.34, 0.64, 0.22, 0.39, 0.73 and 0.69) of (R3 Y1) and (0.39, 0.29, 0.58, 0.17, 0.28, 0.71 and 0.66) of (R3 Y1) for the seven sorghum entries in osmotic adjustment and the values were (49.06, 53.83, 56.34, 48.15, 43.38, 31.19 and 35.78) of (R2 Y1) and (52.14, 54.02, 57.01, 50.04, 46.16, 33.20 and 37.29) of (R2 Y2) for the seven sorghum entries in proline content, respectively. Further, the values in (Table 8) were (55.74, 52.60, 67.82, 62.12, 58.14, 47.77 and 49.03) of (R2 Y1) and (59.03, 54.22, 65.32, 60.13, 59.86, 51.06 and55.96) of (R2 Y2) for the seven sorghum entries in glycine betaine content and (45.28, 52.13, 47.23, 38.35, 43.71, 29.68 and 34.32) of (R2 Y1) and (47.60, 49.83, 45.02, 40.26, 51.09, 31.22 and 37.64) of (R2 Y2) for the seven sorghum entries in trehalose content, respectively. However, the tolerance degrees were not equal for the seven sorghum varieties and naturally, they were not equal in every experiment during the two years. Where the five sorghum lines surpassed the two local cultivars in their tolerance of salt stress. This superiority in Sirw experiment was higher than the El-Hosinia experiment in all traits under study after comparing it with the control location in Aga city for 2 years. This is because the level of salinity at the El-Hosinia site was greater than that at Sirw site. When ranking the superiority of the five sorghum lines compared to the two local varieties, it was observed that the lines number (1, 3 and 4) are coming in the first rank for the traits, grain yield/plant and number of panicles/plant for all experiments in both years where the values were (45.80, 46.66 and 48.59 g) for grain yield/plant and (26.39, 26.57 and 26.63) for the number of panicles/plant. While, the lines number (1, 2 and 3) are coming in the first rank in the three traits namely, panicle weight (35.56, 35.35 and 35.24 g), 1000 grain weight (29.70, 28.09 and 26.76 g) and proline content (51.62, 53.13 and 55.72) for all experiments during the two growing seasons. Also, lines numbers (2, 4 and 5) are coming at the first rank for osmotic adjustment (0.45, 0.32 and 0.43) and the lines (2, 3 and 5) for trehalose content (49.27, 47.64 and 51.80) under all conditions. Finally, the lines (3, 4 and 5) are coming at the first rank for the glycine betaine trait of the three experiments for the two growing seasons and the values were (62.07, 64.60 and 58.83) in Table 9, respectively. Also, data viewed in Table 10 showed the mean values of all studied traits under all environments where the mean values were (41.44 g) for grain yield/plant, (23.61) for the number of panicles/plant, (33.01) for panicle weight, (25.92 g) for 1000-grain weight, (0.55) for osmotic adjustment, (46.73) for proline content, (57.56) for glycine betaine content and (43.41) for trehalose content, respectively.

Stability analysis parameters: Results viewed in Table 11, confirmed that the five sorghum lines (L1, L2, L3, L4 and L5) were the most desirable materials for (bi) (Regression coefficient) parameter of all studied traits. Because these new entries exhibited values equal to one, close or exceed it very little which confirmed highly

Table 11: Estimation of stability parameters for all studies traits of the seven sorghum accessions under the 6 environments conditions, respectively

	Grain yield/plant			Number of panicles/plant			Panicle weight			1000 grain weight			Osmotic adjustment			Proline content			glycine betaine content			Trehalose content		
Lines	bi	S²di	R²	bi	S²di	R²	bi	S²di	R²	bi	S²di	R²	bi	S²di	R²	bi	S²di	R²	bi	S²di	R²	bi	S²di	R²
L1	1.02	-0.04	99.67	0.97	0.07	98.36	1.08	0.08	91.17	0.96	0.06	99.15	1.05	-0.09	96.38	0.97	0.05	92.28	1.02	-0.02	95.8	1.01	-0.04	97.14
L2	0.98	0.00	96.84	1.06	-0.03	94.82	1.02	-0.01	90.07	1.05	0.01	94.23	1.02	0.01	91.05	1.04	-0.02	88.23	1.07	0.04	98.74	1.00	-0.02	99.78
L3	1.03	-0.02	99.58	1.02	0.04	99.03	0.99	-0.02	96.12	1.03	0.03	89.15	1.00	0.08	93.66	1.07	-0.07	99.42	1.04	0.07	95.63	0.99	0.01	95.37
L4	1.01	-0.05	98.23	1.00	0.00	93.72	1.04	0.04	99.81	1.02	-0.01	97.83	0.96	0.03	88.34	1.03	-0.03	91.52	1.06	0.09	99.04	0.98	0.05	97.32
L5	0.99	-0.01	97.03	0.99	-0.07	97.13	1.02	-0.07	94.37	1.00	0.05	99.83	0.99	0.04	98.73	1.01	-0.01	86.21	1.03	0.01	92.17	1.05	-0.02	91.42
L6	1.06	0.08	97.13	1.04	0.32	98.23	1.05	0.17	92.15	0.98	0.42	95.16	1.04	0.55	96.23	1.02	0.22	90.07	1.15	0.48	99.45	0.96	0.62	93.55
L7	1.03	0.23	99.11	1.08	0.14	92.14	1.01	0.25	96.33	1.04	0.18	93.83	1.06	0.72	99.20	1.05	0.15	97.36	1.17	0.13	95.67	1.03	0.42	98.57
R1 Y1	1.02	0.01	99.87	1.00	0.02	97.34	0.99	0.07	99.05	1.01	-0.01	98.67	1.00	-0.02	96.14	0.98	0.03	94.28	1.02	-0.04	99.13	1.04	0.01	97.45
R2 Y1	1.08	0.12	91.06	1.19	0.23	93.24	1.05	0.15	96.12	1.13	0.41	91.18	1.08	0.28	94.55	1.21	0.22	90.58	1.14	0.38	94.17	1.07	0.14	91.06
R3 Y1	1.32	0.63	88.61	1.47	0.40	79.36	1.25	0.22	90.51	1.35	0.83	83.04	1.19	0.45	90.07	1.33	0.58	84.24	1.28	0.44	89.33	1.42	0.29	87.13
R1 Y2	0.99	0.03	98.16	1.01	0.04	99.18	1.02	0.05	97.45	1.03	-0.05	99.04	0.98	0.08	95.83	1.03	0.05	96.51	1.00	0.02	98.27	0.99	0.03	96.12
R2 Y2	1.06	0.15	90.17	1.11	0.18	94.86	1.07	0.12	93.22	1.09	0.62	90.38	1.05	0.19	92.48	1.18	0.19	93.57	1.15	0.21	92.88	1.12	0.17	93.70
R3 Y2	1.18	0.48	86.74	1.52	0.39	85.14	1.46	0.38	87.14	1.27	0.71	87.73	1.16	0.37	85.26	1.39	0.62	88.74	1.31	0.93	90.05	1.36	0.34	88.34

*P ≤ 0.05, **p ≤ 0, R: Region, Y: Year and L: Line, L6: Giza 3 and L7: Giza 54

genetic stability in these promising lines compared to the two local varieties. For the (S^2di) parameter, results revealed that the same lines number (1, 2, 3, 4 and 5) were recorded the optimum rank for all traits under study in this regard where these exhibited values equal (0.0) or approaches to it in both positive and negative directions. While the two local sorghum cultivars were coming in the second rank in this context. Also, these five promising sorghum lines exhibited highly data of R^2 parameter in all attributes under study where they gave results ranged from 86.21% in line 5 for proline content trait to 99.83% in line 5 for 1000 grain weight trait, respectively. Likewise, all the studied traits showed remarkable superiority for the three genetic stability analysis measures (bi, S^2 di and R^2) in both growing seasons for the first location or the standard experiment (Aga city), followed by the second location (Sirw city) and then followed by the third location (El-Hosinia city) in Table 11, respectively.

In the same context, the optimum environment or experiment of the three genetic parameters mentioned above (bi, S^2 di and R^2) for grain yield/plant was RI Y1 (1.02, 0.01 and 99.87), RI Y2 (1.01, 0.04 and 99.18) for the number of panicles/plant, RI Y1 (0.99, 0.07 and 99.05) for panicle weight, R1 Y1 (1.01, -0.01 and 98.67) for 1000-grain weight, R1 Y1 (1.0, -0.02 and 96.14) for osmotic adjustment, R1 Y2 (1.03, 0.05 and 96.51) for proline content, R1 Y2 (1.0, 0.02 and 98.27) for glycine betaine content and R1 Y1 (1.04, 0.01 and 97.45) for trehalose content in Table 11, respectively.

Salinity tolerance indices parameters: Results presented in Table 12 showed that all sorghum entries except line 5 for both salinity stress locations (Sirw and El-Hosinia) in both growing seasons were recorded the highest mean values for the YSI parameter. In the same context, the five sorghum lines, (L1, L2, L3, L4 and L5) were exhibited the highest data for both salinity stress locations in the two growing seasons for MP and GMP parameters. The genotypes number (2, 3 and 7) and (1, 3 and 4) for both locations in the first season besides, the genotypes number (1, 3 and 4) and (1, 2, 3 and 4) for the two salinity stress locations in the second year were exhibited mean values higher than one for YI parameter, respectively. While line number three only under the two salt stress locations conditions for the first grown season was recorded data higher than the unity for STI parameter in this regard. On the other hand, the seven sorghum genotypes were recorded mean values

Table 12: Estimation of salinity tolerance indices for the seven sorghum entries especially in grain yield/plant trait for the normal treatment and both salinity stress experiments during the two growing season

	Season 2019									Season 2020								
Entries	GYP	GYS	YSI	YI	MP	STI	GMP	YR	SSI	GYP	GYS	YSI	YI	MP	STI	GMP	GMP	SSI
Salinity tolerance indices (Sirw location)																		
L1	56.18	38.45	0.68	0.95	47.31	0.79	46.47	0.32	1.45	55.98	43.62	0.77	1.10	49.80	0.93	49.41	0.27	1.22
L2	53.60	47.23	0.88	1.17	50.41	0.93	50.31	0.12	0.54	53.78	36.89	0.68	0.93	45.33	0.76	44.54	0.32	1.45
L3	58.13	48.12	0.82	1.19	53.12	1.03	52.88	0.18	0.81	57.65	45.57	0.79	1.15	51.61	1.00	51.25	0.21	0.95
L4	60.03	36.43	0.60	0.90	48.23	0.80	46.76	0.40	1.81	58.14	46.05	0.79	1.16	52.09	1.02	51.74	0.21	0.95
L5	55.73	33.12	0.59	0.82	44.42	0.68	42.96	0.41	1.86	52.08	35.92	0.68	0.91	44.00	0.71	43.25	0.32	1.45
L6	38.19	37.48	0.98	0.93	37.83	0.52	37.83	0.02	0.09	39.84	31.64	0.79	0.80	35.74	0.48	35.50	0.21	0.95
L7	42.13	40.85	0.96	1.01	41.49	0.63	41.48	0.04	0.18	40.07	35.97	0.89	0.91	38.02	0.55	37.96	0.11	0.50
Salinity tolerance indices (El-Hosinia location)																		
L1	56.18	37.72	0.67	1.15	46.95	0.78	46.03	0.33	0.89	55.98	36.15	0.64	1.10	46.06	0.77	44.98	0.36	1.02
L2	53.60	32.18	0.60	0.98	42.89	0.63	41.53	0.40	1.08	53.78	33.58	0.62	1.02	43.68	0.69	42.49	0.38	1.08
L3	58.13	34.18	0.58	1.04	46.15	0.73	44.57	0.42	1.13	57.65	37.22	0.64	1.13	47.43	0.82	46.32	0.36	1.02
L4	60.03	39.18	0.65	1.20	49.60	0.87	48.49	0.35	0.94	58.14	40.02	0.68	1.22	49.08	0.89	48.23	0.32	0.91
L5	55.73	29.15	0.52	0.89	42.44	0.60	40.30	0.48	1.29	52.08	27.88	0.53	0.85	39.98	0.55	38.10	0.47	1.34
L6	38.19	27.95	0.73	0.85	33.07	0.39	32.67	0.27	0.72	39.84	26.76	0.67	0.81	33.30	0.40	32.65	0.33	0.94
L7	42.13	28.13	0.66	0.86	35.13	0.43	34.42	0.34	0.91	40.07	27.47	0.68	0.83	33.77	0.42	33.17	0.32	0.91

L: Line, L6: Giza 3 and L7: Giza 54

Table 13: Band variation and polymorphism percentage in the seven sorghum entries using 11 SCoT primers

SCoT primers	Total bands	Range size (bp)	Monomorphic band	Polymorphic band	U or P	Polymorphism (%)	Sequences
SCoT 1	11	159-1140	8	3	1	27.27	5'-ACGACATGGCGACCACGC-3'
SCoT 2	19	132-1512	9	10	3	52.63	5'-ACCATGGCTACCACCGGC-3'
SCoT 3	22	114-1652	4	18	2	81.81	5'-ACGACATGGCGACCCACA-3'
SCoT 4	17	146-1295	5	12	1	70.58	5'-ACCATGGCTACCACCGCA-3'
SCoT 5	12	167-2313	2	10	1	83.33	5'-CAATGGCTACCACTAGCG-3'
SCoT 6	13	169-1519	5	8	0	61.53	5'-CAATGGCTACCACTACAG-3'
SCoT 7	13	294-1640	2	11	1	84.61	5'-ACAATGGCTACCACTGAC-3'
SCoT 9	14	221-1315	5	9	1	64.28	5'-ACAATGGCTACCACTGCC-3'
SCoT 10	20	165-1546	3	17	0	85.0	5'-ACAATGGCTACCACCAGC-3'
SCoT 11	11	178-1154	7	4	0	36.36	5'-ACAATGGCTACCACTACC-3'
SCoT 12	10	216-748	7	3	0	30.0	5'-CAACAATGGCTACCACCG-3'
Total	162	114-2313	57	105	10	64.81	

U or P: Unique or positive specific markers

lower than one in the two salinity stress locations for the two growing seasons of the YR parameter where the values were (0.32, 0.12, 0.18, 0.40, 0.41, 0.02 and 0.04) for the first season and (0.27, 0.32, 0.21, 0.21, 0.32, 0.21 and 0.11) for the second season of Sirw location. While, the values were 0.33, 0.40, 0.42, 0.35, 0.48, 0.27 and 0.34 for the first season and 0.36, 0.38, 0.36, 0.32, 0.47, 0.33 and 0.32 for the second season for El-Hosinia location, respectively. For the SSI parameter, the lines number (2 & 3) besides, Giza 3 and Giza 54 with values (0.54, 0.81, 0.09 & 0.18) for the first season and the genotypes number (3, 4, 6 and 7) with values (0.95, 0.95, 0.95 & 0.50) for the second season of Sirw location in addition, the genotypes number (1, 4, 6 and 7) with values (0.89, 0.94, 0.72 and 0.91) for the first season and the genotypes number (4, 6 and 7) with values (0.91, 0.94 and 0.91) for the second season for El-Hosinia location were recorded mean values lower than one in Table 12, respectively.

Molecular characterization

Profile analysis of SCoT primers: The profile analysis of SCoT primers was presented in Table 13 and Fig. 1. Data viewed detected that 162 fragments were generated through using 11 SCoT primers namely, (1, 2, 3, 4, 5, 6, 7, 9, 10, 11 and 12) where (57 of them were monomorphic and 105 polymorphic bands with 64.81% polymorphism) including 16 unique bands (10 positive and 6 negatives) with range size from 114-2313 bp, e.g. The first primer SCoT 1 in Fig.1a showed 11 bands (8 monomorphic and 3 polymorphic) with 27.27% polymorphism including one unique or positive specific marker with sizes from 159-1140 bp. While SCoT 2 primer generated 19 amplicons, 9 of them were monomorphic and 10 polymorphic including 3 positive markers with 52.63% polymorphism with sizes from 132-1512 bp in Fig. 1b. Also, primer SCoT 3 produced 22 fragments (4 monomorphic and 18 polymorphic bands) with 81.81% polymorphism and 2 amplicons were unique or positive markers besides, the

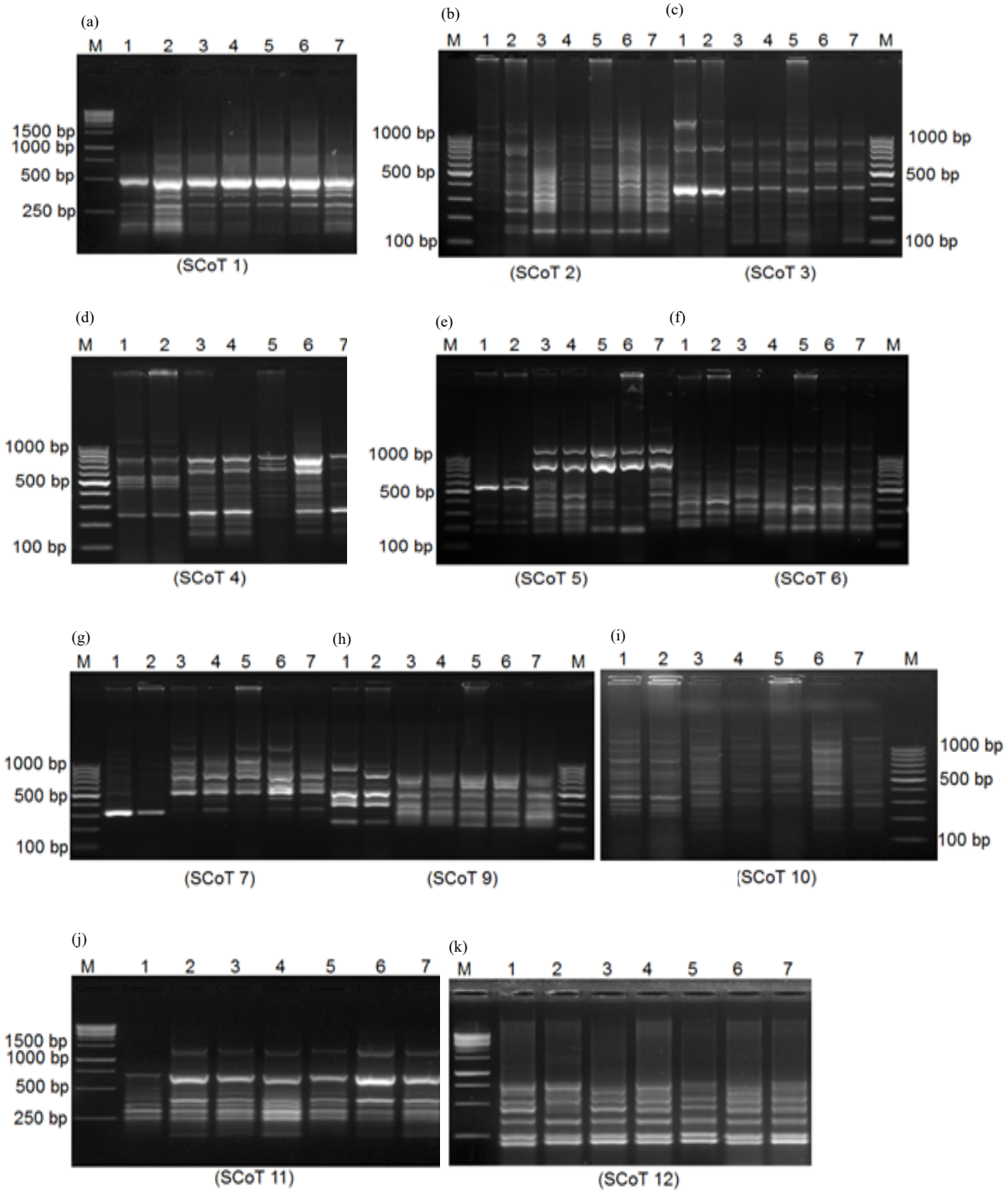

Fig. 1(a-k): SCoT profiles produced with different primers as follow; primers; a (SCoT 1), j (SCoT 11) and k (SCoT 12) by 1kb ladder marker, the rest primers; b (SCoT 2), c (SCoT 3), d (SCoT 4), e (SCoT 5), f (SCoT 6), g (SCoT 7), h (SCoT 9) and i (SCoT 10) by 100 bp ladder marker

The sorghum accessions were 1: Line 1, 2: Line 2, 3: Line 3, 4: Line 4, 5: Line 5, 6: Giza 3 and 7: Giza 54, respectively

range size ranged from 114-1652 bp in Fig. 1c. For SCoT 4 primer, there were 17 fragments (5 of them were monomorphic and 12 polymorphic) with 70.58% polymorphism including one unique marker with sizes ranging from 146-1295 bp in Fig. 1d. Further, SCoT 5 primer exhibited 12 bands divided into 2 monomorphic and 10 polymorphic fragments including one positive marker with 83.33% polymorphism and the sizes ranged

Table 14: Total bands obtained from the eleven SCoT primers of the seven sorghum entries and all amplified fragments for each entry

	Primers											
Entries	ScoT 1	ScoT 2	ScoT 3	ScoT 4	ScoT 5	ScoT 6	ScoT 7	ScoT 9	ScoT 10	ScoT 11	ScoT 12	Total
L1	8	13	11	10	4	10	6	10	11	8	9	100
L2	11	14	12	10	5	9	3	10	10	11	8	103
L3	10	14	13	11	10	8	8	9	14	11	8	116
L4	10	13	13	11	11	9	9	9	10	9	9	113
L5	10	14	15	9	9	9	10	8	9	10	8	111
Giza 3	10	13	11	12	5	10	11	8	15	8	9	112
Giza 54	9	13	11	11	11	10	7	8	11	9	9	109
Total bands	68	94	86	74	55	65	54	62	80	66	60	764

from 167-2313 in Fig. 1e. Concerning SCoT 6 primer, there were 13 fragments (5 of them were monomorphic and 8 polymorphic) with 61.53% polymorphism and the sizes ranged from 169-1519 bp in Fig. 1f. While that, ScoT 7 primer produced 13 amplicons, two of them were monomorphic and 11 polymorphic bands with 84.61% polymorphism including one unique marker with sizes from 294-1640 bp in Fig. 1g. In the same track, SCoT 9 primer generated 14 fragments (5 of them were monomorphic and 9 polymorphic included one unique band or positive specific marker) with 64.28% polymorphism and the sizes ranged from 221-1315 bp in Fig. 1h. Twenty fragments were produced by SCoT 10 primer, 3 of them were monomorphic and 17 polymorphic with 85.0% polymorphism and the sizes ranged from 165-1546 bp in Fig. 1I. SCoT 11 primers viewed in Fig. 1J recorded 11 fragments, 7 of them were monomorphic and 4 polymorphic bands with 36.36% polymorphism and the range size were ranged from 178-1154 bp. Also, SCoT 12 primer produced 10 amplicons, 7 monomorphic and 3 polymorphic with 30.0% polymorphism with sizes ranged from 216-748 bp in Fig. 1k. Results presented in Table 13 confirmed that the highest number of total bands (22) and polymorphic fragments (18) were observed in SCoT 3 primer. While, the lowest number of total bands (10) were obtained in SCoT 12 primer and the lowest number of polymorphic fragments (3) were observed in primers, SCoT 1 and 12, respectively. Also, the highest polymorphism % (85.0%) was obtained in SCoT 10 primer. But, the lowest rank of polymorphism % (27.27%) was shown in SCoT 1 primer. Further, ScoT 2 primer recorded the highest number of unique or positive specific markers (3), while primers SCoT 1, 4, 5, 7 and 9 were exhibited the lowest number of the unique band (1) for each one of them in this regard. Results presented in Table 14 revealed that the sorghum entries (L3, L4, L5 and Giza 3) exhibited the highest number of bands and were coming in the first rank in this regard and their values were (116, 113, 111 and 112), respectively. While, the rest sorghum genotypes, (L1, L2 and Giza 54) coming in the second rank and their values were (100, 103 and 109). Further, primers SCoT 2, 3, 4 and 10 recorded the highest number of amplified fragments (94, 86, 74 and 80) for each one of them in all sorghum entries. While, SCoT 5 and 7 primers generated the lowest number of bands (55 and 54) for both of them, respectively. In addition, the rest SCoT primers were exhibited a various number of amplified fragments.

Data presented in Table 15 viewed 10 positives and 6 negative specific markers generated by eleven SCoT primers. These primers namely, SCoT (1, 2, 3, 4, 5, 6, 7, 9, 10, 11 and 12) used in this investigation which succeeded in determining the molecular genetic differences between the various sorghum entries. Further, these molecular genetic differences were very fruitful in this context and considered the taxonomic basic among the seven sorghum materials. The following is a detailed explanation of SCoT primers that gave positive and negative markers in this track. Scot 1 primer showed 2 specific markers where the first one was positive in line 2 with a molecular weight of 603 bp and the second marker was negative with the size of 406 bp for line one, respectively. Three positive specific markers were generated by SCoT 2 primer as follows, two markers for line 2 with sizes of 1512bp and 148 bp besides, one marker with the size of 170 bp for Giza 54, respectively. Also, SCoT 3 primer showed two positive specific markers with sizes of 721bp and 867bp for Giza 3 and line 5. Further, only one positive marker was observed for Giza 3 with the size of 289 bp by SCoT 4 primer. Concerning primer SCoT 5, one positive and one negative specific marker were obtained in sorghum genotypes, Giza 54 and Giza 3 with sizes of 2313 bp and 518 bp, respectively. In the same track, two negative specific markers with sizes of 760 and 513 bp for line 2 and one positive marker

Table 15: Mapping of positive (P) and negative specific markers for the seven sorghum entries using 11 SCoT primers

SCoT primers	MS (bp)	L1	L2	L3	L4	L5	Giza 3	Giza 54	(P or N) marker
SCoT 1	603	-	+	-	-	-	-	-	P (L2)
	406	-	+	+	+	+	+	+	N (L1)
SCoT 2	1512	-	+	-	-	-	-	-	P (L2)
	170	-	-	-	-	-	-	+	P (Giza 54)
	148	-	+	-	-	-	-	-	P (L2)
SCoT 3	721	-	-	-	-	-	+	-	P (Giza 3)
	867	-	-	-	-	+	-	-	P (L5)
SCoT 4	289	-	-	-	-	-	+	-	P (Giza 3)
SCoT 5	2313	-	-	-	-	-	-	+	P (Giza 54)
	518	+	+	+	+	+	-	+	N (Giza 3)
SCoT 7	760	+	-	+	+	+	+	+	N (L2)
	606	-	-	-	-	-	+	-	P (Giza 3)
	513	+	-	+	+	+	+	+	N (L2)
SCoT 9	804	-	+	-	-	-	-	-	P (L2)
SCoT 10	494	+	+	+	+	+	+	-	N (Giza 54)
SCoT 11	1154	-	+	+	+	+	+	+	N (L1)
Range	2313-148								
Total		4	8	6	6	7	8	7	10 P+6 N

P: Positive, N: Negative, MS: Molecular Size, L1: Line one, L2: Line two, L3: Line three, L4: Line four and L5: Line five

Table 16: Genetic similarity (%) in the seven sorghum entries using 11SCoT primers

Similarity	LI	L2	L3	L4	L5	Giza 3	Giza 54
L1	1.0						
L2	0.812	1.0					
L3	0.489	0.469	1.0				
L4	0.468	0.449	0.846	1.0			
L5	0.485	0.486	0.816	0.806	1.0		
Giza 3	0.492	0.433	0.781	0.771	0.742	1.0	
Giza 54	0.471	0.442	0.814	0.819	0.774	0.753	1.0

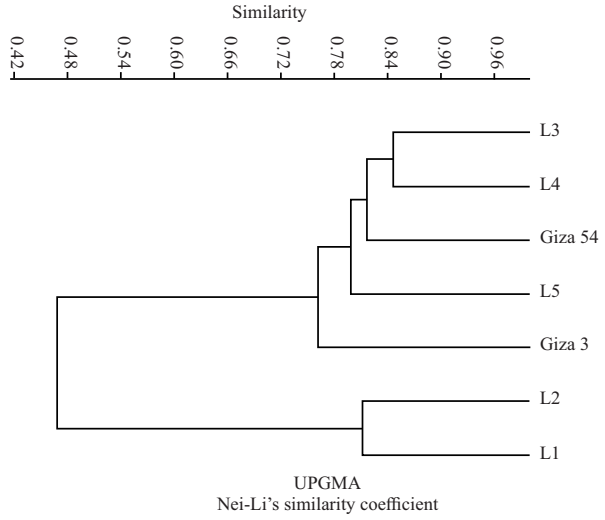

Fig. 2: Dendrogram representing the genetic relationship among the seven sorghum entries using UPGMA cluster analysis of Nei-Li's similarity coefficient generated from the 11 SCoT markers
L1: Line one, L2: Line two, L3: Line three, L4: Line four and L5: Line five

with the size of 606 bp for Giza 3 were generated by SCoT 7 primer. Also, SCoT 9 primer exhibited one positive marker for line 2 with the size of 804 bp. While, the two SCoT primers 10 and 11 recorded one negative specific marker for both of them where the first one was observed in Giza 54 with the size of 494 bp and the second marker was shown in line 1 with the size of 1154 bp, respectively.

Proximity matrix analysis (genetic similarity): Results showed in Table 16 recorded (21) pairwise comparisons to debate the genetic relationships among the seven sorghum entries detected in terms of genetic similarity. The genetic similarity values ranged from (0.846-0.433) with an average of (0.639). Where the highest rank of genetic similarity was 0.846 between Line 3 and Line 4. While that, the lowest level of similarity was 0.433 within Line 2 and Giza 3, respectively. Also, some genetic similarity values were showed high such as the genetic relationships observed between Line 1 and Line 2 (0.812), Line 3 and Line 5 (0.816), Line 3 and Giza 54 (0.814), Line 4 and Line 5 (0.806) and Line 4 and Giza 54 (0.819), respectively. The rest genetic similarity values were gradually from high to below average in this context.

Cluster analysis (phylogenetic tree): Data of cluster analysis or phylogenetic tree which viewed in Fig. 2 divided all sorghum entries into two main clusters. Where the first one included lines (1 and 2). While cluster two contained two sub-cluster. Where sub-cluster one included Giza 3. Whatever sub-cluster number two included two sup-sup clusters. The sub-sub cluster one included line 5 only. While, the two sub-sub clusters contained Giza 54 and one group (Line 3 and line 4), respectively.

DISCUSSION

This study discussed the extent of the genetic stability of seven sorghum genotypes in five Egyptian environments, which are three different agricultural regions in the degree of salinity within two years. Indeed, the degrees of genetic stability for these new genetic materials were divided into two main ranks. Also, the variation degree for the performance of these genotypes from region to region and from season to season was not significant and this confirms the validity of their high genetic stability. Data obtained in Table 3 and related with the mean squares of all sources of variance components detected that all studied traits were showed highly significant variances especially for environments, genotypes (linear), environments × genotypes (linear) and Environments+(Genotypes × Environments). This reinforces the fact that the differences were not only between genotypes but also among the three agricultural sites that included the control experiment and the two saline stress locations during the two growing seasons. But in any case, most of the differences obtained from the stability analysis confirm that the five promising lines of sorghum were different from each other compared to the local cultivars. While all differences between the two growing seasons were environmental only and in small proportions indicated highly genetic stability for the previous sorghum accessions in this context in Table 4 and 5[13,14,35-38].

Results obtained in Table 6-10 confirmed that the five promising sorghum lines namely, L1, L2, L3, L4 and L5 were exhibited highly genetic stability compared to the two local cultivars (Giza 3 and Giza 54) for the six experiments (the three locations during two growing seasons). Where these five genotypes were able to give impressive and promising results for all the studied traits, as they gave the highest results for the first location (Aga), followed by the second location (Sirw) and then followed by the third location (El-Hosinia city) during the two growing seasons. Also, they showed great tolerance to salt stress under the conditions of Sirw experiment compared to the El-Hosinia experiment and this of course compared to the standard experience at the Aga location. The reason for this is that the salinity rates at Sirw location were less than El-Hosinia location. In the same context, noted that the salt stress of the new sorghum lines is one of the biological and physiological developments. Among the most prominent of these physiological changes in the osmotic adjustment by reducing the rate of osmotic pressure during exposure to salt stress to maintain the water level necessary for metabolism and completion of growth processes and the production of dry matter and is called modified pressure or osmotic adjustment. As well as, producing a large number of organic acids closely related to salt stress tolerance such as proline, glycine betaine and trehalose contents under salinity conditions compared to the control experiment. Where the five sorghum lines that tolerate salt stress were able to produce these organic compounds in a large percentage under the conditions of El-Hosinia location, followed by Sirw location compared to the standard experiment. All these aforementioned results confirm the increase in genetic stability and the salt stress tolerance that these promising five lines reached because, in the end, they gave a good yield with an acceptable loss rate under salt stress in sirw and El-Hosinia locations compared to standard experiment (Aga location) during the two growing seasons. These results were in agreement with the previous study[11-14,17,35-45].

Results of the genetic stability parameters (bi and S^2di) showed the extent of the great genetic stability that these new sorghum genotypes (L1, L2, L3, L4 and L5) gained in the three locations during the two growing seasons. As they gave optimum values for the previous genetic parameters or were hovering around the optimal region. Moreover, the level of genetic stability was different from one line to another, but a total of the five lines was superior in terms of high genetic stability. This proves that these new lines mentioned above have indeed succeeded in environmental adaptation under different weather conditions because they simply gave perfect yield under the control treatment and its yield values were also acceptable in the two salt stress locations and damage and the final loss of yield and its components was not significant Table 11. But of course, the final yield loss rates at Sirw location were lower than at the El-Hosinia location compared to the control experiment (Aga) in both growing seasons. In the same context, the results of the genetic stability ratio (R^2) were very high and approaching 100 % for the five new sorghum lines compared to the old local varieties under different environmental conditions. This also confirms increasing and integration of the genetic stability of these new lines, which could be fine sorghum varieties that are tolerant to salt stress and have a high yield in the future[13,14,35,37,38].

Data of salinity tolerance indices obtained in the Table 12 confirmed that the new five promising sorghum lines were exhibited highly rank of salinity tolerance indices in the two saline stress locations (Sirw and El-Hosinia) compared to the two local sorghum cultivars in both growing seasons. The reason for this is that these new lines have succeeded in reducing the final yield loss rate (YR) under saline stress conditions in both locations compared to the natural experiment and gave a highly acceptable final output. Further, the SSI values of these lines were lower than one indicated highly saline stress tolerance in this regard. It reflects the extent of the change and the physiological evolution of salt stress tolerance through increasing the production of some organic compounds responsible for salinity tolerance such as proline, glycine betaine and trehalose contents. As well as, adjusting the osmosis which had a direct cause in preserving the water necessary to complete the vital processes in these promising tolerant lines sorghum[19,20,42,43,45].

SCoT markers have successfully elucidated and quantified the molecular genetic differences among the seven sorghum genotypes through producing 162 fragments (57 of them were monomorphic and 105 polymorphic) besides, 16 positive and negative specific markers which were the taxonomic basic for comparing between the new five sorghum lines and the two local check verities Giza 3 and Giza 54, in Table 13 and Fig. 1. This investigation also aimed to shed light on the genetic causes responsible for salt stress tolerance in the new five sorghum lines which reached the maximum stages of genetic stability through using 11 SCoT primers. Where, primers SCoT 2, 3, 4 and 10 are considering the highest primers which exhibited highly rank of amplified fragments (19, 22, 17 and 20) and this fact makes it so important for the molecular genetics comparison between the aforementioned genetic materials. Further, identifying the markers responsible for salt stress tolerance especially in the five sorghum lines. Also, 11 SCoT primers produced 16 specific markers of the unique band (10 positives and 6 negatives) and this was a good indication that the five sorghum lines were indeed different from their original parents and were also tolerant to salt stress. Therefore, these markers are the real evidence, not only that the seven sorghum genotypes are different from each other, but also they are tolerant to salt stress and represent the nucleus in producing high-yielding sorghum cultivars that are tolerant to abiotic stresses in the future in Table 15. In the same track, results presented in Table 14 confirmed that the four SCoT primers 2, 3, 4 and 10 considered the best primers for exhibited highly limit of fragments (94, 86, 74 and 80). Also, lines number (3, 4, 5) and Giza 3 were recorded highly rank of the total number of bands (116, 113, 111 and 112) and the rest of sorghum genotypes (line 1, line 2 and Giza 54) were coming in the second rank in this regard[17,19,20,21,46-49].

In the same context, it can be considered that the positive and negative specific markers obtained from 11 SCoT primers are remarkable scientific progress in this study. Simply, because it is considered the taxonomic basis at the molecular level to differentiate between the five new sorghum lines and the two local check cultivars namely, Giza 3 and Giza 54. Therefore, the use of these five new genotypes highly genetic stability and tolerance to salt stress in the program of breeding and improving sorghum crop for abiotic stresses tolerance will be extremely important in this regard, in Table 15[19,20,47-49].

Also, data of genetic similarity and cluster analysis shown in Table 16 and Fig. 2 revealed the extent of genetic similarity between the seven sorghum genotypes

showing the highest and lowest percentage in this regard. Where the highest limit of genetic similarity percentage was obtained between line 3 and line 4 (0.846), followed by line 4 and Giza 54 (0.819), followed by line 3 and line 5 (0.816), line 3 and Giza 54 (0.814) and followed by line 1 and line 2 (0.812). While the lowest genetic similarity was observed among line 2 and Giza 3 (0.433). The same results were confirmed by cluster analysis or phylogenetic tree which consisted of the seven sorghum accessions into two main clusters where cluster one included lines (1 and 2). But, the rest sorghum materials were in cluster two in Fig. 2. These results confirmed that the promising five sorghum lines were recorded highly rank of genetic similarity compared to the local check varieties. Further, indicates the extent of genetic and environmental compatibility that these new genotypes enjoy and that their cultivation together will be an important and fruitful step in the event of introducing it in a breeding program, improving and promoting the sorghum crop by transferring salt stress resistance genes from these new genetic materials to sensitive varieties through traditional plant breeding programs and genetic engineering methods. In addition, the process of planting them in many agricultural sites, while assessing their tolerance to water stress and disease resistance, may eventually lead to the production of new high-yielding sorghum varieties that are resistant to biotic and abiotic stresses[19-21,46-49].

In the end, the production of new sorghum lines with genetic stability and tolerance to salt stress besides, giving high yielding also may significantly contribute to the effective contribution for producing Egyptian bread, after mixing its flour with wheat flour. As well as, bridging the large food gap between the production and consumption of Egyptian bread. On the other hand, it may also help to provide high nutritional value fodder for animal production, after harvesting the fresh leaves at the age of 45 days and exposing them to the sun to get rid of the harmful HCN and this is the desired goal of this work.

CONCLUSION

One of the most prominent results of this investigation is evaluating the genetic stability, performance and ability of salinity stress tolerance for several new promising sorghum lines obtained by traditional plant breeding methods. Agro-morphological and physiological attributes associated with salt stress tolerance were the most important traits calculated under all conditions besides, molecular markers analysis using 11 SCoT primers for comparing among these five sorghum lines and the two local cultivars. Results confirmed that the five new promising sorghum lines exhibited highly rank of yielding, stable, adapting and salinity stress tolerance in this regard.

SIGNIFICANCE STATEMENT

This investigation succeeds in eliciting some new sorghum lines with highly stable and tolerated salt stress under Egyptian conditions. So, it can be considered that these new promising lines as a nucleus for producing new sorghum cultivars high-yielding and tolerant for salinity stress in this regard. Also, the profile analysis of SCoT primers recorded 16 unique bands as molecular genetic markers that characterize the promising previous sorghum genotypes. Finally, deriving high-yielding, genetically stable and tolerant sorghum lines to salt stress will lead to a great deal to bridge the gap in the Egyptian bread industry and this is the great goal in this study.

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