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Research Article
Elicitation of Novel Sunflower Lines Tolerant to Water Deficit Conditions Through Mutation Breeding

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Abstract
Background and Objective: Water stress is one of the most important environmental reasons for the shrinkage of agricultural areas and the deterioration of the final output of various crops, especially sunflowers. On this basis, genetic improvement in sunflower for tolerance the serious environmental obstacle besides, improve its yield and oil characters observed in this study, especially after the widening gap between the production and consumption of Egyptian edible oil. **Materials and Methods:** The local sunflower cultivar Sakha 53 was a fertile material for genetic improvement and development of new mutant lines superior in all traits, especially yield and oil (%) under drought stress conditions using different doses of gamma rays. Analysis of variance and genetic parameters were the most important measurements calculated for the 6 sunflower genotypes under normal and stress conditions besides, drought tolerance indices for seed yield/plant trait in this regard. Further, 11 ISSR primers were used for comparing the local sunflower cultivar and its 5 mutant lines at the molecular level. **Results:** The final results confirmed that the 5 promising sunflower mutant lines were recorded highly rank of genetic stability and water stress tolerance in all studied traits under the stress treatment compared to the standard experiment during the 2 years. **Conclusion:** The 5 sunflower mutant lines are considered as the nucleus for producing sunflower varieties tolerated to water stress and superior in all yield and its components attributes, especially the percentage and quality of the oil.

Key words: Sunflower, drought tolerance indices, genetic parameters, ISSR primers, expected genetic advance

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

INTRODUCTION

Sunflower is considered one of the most important oilseed crops both locally and globally because its seed contains a high percentage of oil and protein compared to other oil crops. Further, sunflower oil is used in cooking and the confectionery industry where its oil and protein contents ranging from 36-52 and 28-32%, respectively[1]. Given the importance of this crop, the annual global production of it is about 35.6 million t out of the total cultivated area of 25.1 M ha[2]. But in Egypt, the agricultural area of sunflower has decreased significantly as the agricultural area was 73,000 acres in 1993 and shrunk until it reached less than 16,000 acres in the 2020 season, forcing the Egyptian state to import approximately 75,000 t annually to compensate for the shortfall in sunflower oil production. The reason for this significant decline in the area unit is due to a rise in production requirements, low yield and the competition of other crops, especially rice, cotton and maize as well as biotic and abiotic stresses especially water deficit conditions. Therefore, the attention of scientists and researchers was directed to an attempt to genetic improvement of all qualitative sunflower traits, especially the quantity and quality of oil and increase in the final seed yield in the same unit area and increasing the degree of water stress tolerance in this important crop. To establish a successful plant breeding program, it was necessary to find genetic classifications and differences tolerant to drought stress, which would enrich the genetic improvement and breeding program for sunflowers. Quantitative changes in protein expression of water-stressed versus sunflower control genotypes[3]. Also, they observed that sunflower plants exposed to water stress have already succeeded in regulating 6 proteins closely related to the physiological and molecular role of drought tolerance in sunflowers through contributing to the basic carbon metabolism. Increasing the genetic variation between sunflower entries is of great importance in selecting the most sunflower plants tolerant to water stress through some physiological characteristics that are closely related to the mechanisms of tolerance[4]. Encheva *et al.*[5] revealed the importance and fruitful role of mutagenesis for improving hybrid vigour and heterosis breeding in new sunflower entries. Iqbal *et al.*[6] detected the genetic behaviour of quantitative traits related to the oil content in sunflower during exposure to drought stress and explained ways to reduce the risk of water stress, especially in the reproductive stage. Also, they confirmed that the sunflower entries recorded positive results for the traits, the number of leaves, total leaf area, stem diameter and achene yield able to tolerate water stress while maintaining good oil content. New induced mutations have been conducted for the past 40 years to produce mutagenesis in sunflower through modifying plant attributes that reflected significantly on increasing yield and quality in sunflower[7]. Haddadi *et al.*[8] discovered genomic regions belonged to leaf related traits and yield components in recombinant inbred sunflower genotypes drought conditions. Mostafa and Alfrmawy[9] succeed in determining the new ten sunflower mutants derived from the two cultivars Giza 1 and Giza 102 by sodium azide through using 10 RAPD primers. The results revealed that eight primers only succeed in comparing the new sunflower mutants and show genetic differences between them at the molecular level through generating 98 amplicons, 83 of them were polymorphic with 84.6% polymorphism. Also, they revealed that the polymorphism percentage in all sunflower mutants confirmed the fruitful role of sodium azide as an effective mutagenic agent in this context. Ebrahimi and Sarrafi[10] studied the genetic variability under normal and drought conditions induced through using gamma irradiation between M_8 sunflower mutants and determining molecular markers related to some seed germination traits using some AFLP markers. They confirmed that the characterization of specific and non-specific markers associated with germination traits may be played a fruitful role to identify marker assistant selection responsible for enhancing some germination, yield and quality traits under both conditions. Further, the newly induced mutations have succeeded in making a great leap in improving the qualities of sunflower, especially the quality of the oil in the past 40 years such as high oleic ($\ngtr$80%), mid-oleic (50-80%) and high palmitic and so on[11]. Shehata *et al.*[12] studied salinity stress tolerance in some sunflower entries by using 7 ISSR primers and revealed that all primers tested recorded 62.5% polymorphism for all sunflower genetic materials under normal conditions, while, primer (UBC-40) showed (80%) for normal with treated genotypes with different concentrations of sodium chloride (100 and 200 mM). Water stress tolerance was enhanced in F_2 sunflower plants through using PEG 6000 solution[13] where they treated the emasculated inflorescences with 10 and 20% of PEG 6000 solution in 2 experiments besides, the control treatment using distilled water. The final results confirmed that the pollen treated with 20% PEG 6000 produced an F_2 population with a high rank of drought tolerance, unlike untreated pollen. The root system was selected as excellent experimental material to explain the physiological and molecular responsive mechanisms for water stress tolerance in plants[14]. Proline content is considered one of the most physiological indicators of water stress tolerance in sunflowers but there was a great discrepancy among parents and their hybrids as its content was more in hybrids than in parents[15]. In addition, they revealed that the proline content was highly positively correlated with osmotic adjustment and this greatly increases the tolerance of sunflowers to water stress. Further, the fruitful role of proline content was observed through increasing water stress tolerance in sunflowers entries[16] which asserted that sunflower genotypes tolerant to water stress can increase the limit of proline content in its leaves, which was estimated to be equivalent to three times what can be formed in the same genotypes under irrigated conditions. Darbani *et al.*[17] revealed the impact of water deficit stress conditions on some phenological and morphological attributes in sunflower accessions besides, determining the important indices used for screening tolerant genotypes from the other sensitive through using ISSR markers. They confirmed that Sil-96 entry recorded the highest rank of yield under the 2 experiments and was characterized as highly tolerant to water stress. In addition, 32 ISSR markers were missionary for morpho-physiological attributes where 24 of them were established using the MLM. Keipp *et al.*[18] discovered that water stress leads to a significant reduction in weight seeds of sunflower plants and thus reduces the oil yield and ultimately affects the final seed yield. γ-Aminobutyric Acid (GABA) deceasing the impact of water and heat stresses in sunflower through arranging its physiological, biochemical and molecular pathways where it enhances the mechanisms of water stress tolerance by increasing the activity of antioxidant enzymes and the total content of chlorophyll and sugars[19]. Also, they confirmed the significant association between antioxidant enzyme activities and the relative expression of genes related to heat shock proteins, dehydrin, osmotin, aquaporin, leaf embryogenesis protein, under water deficit conditions. Further, plant breeding by mutation had a major role in bringing about a revolution in genetic improvement in a large number of crops for example, for drought tolerance in soybean using a low dose of gamma irradiation[20], for water deficit tolerance in rice using gamma rays and *in vitro* pathway[21] and enhancing water stress tolerance in sorghum through using gamma rays[22]. After all that has been presented, it is possible to briefly define the aim of this investigation which is the use of breeding by mutation specifically through gamma rays as a fruitful attempt for genetic improvement in sunflower by deriving new mutants that are tolerant to water stress and superior in all yield components and oil (%) to eventually become highly tolerant sunflower cultivars to water stress in addition, it's high yielding, whether in seeds or oil (%) under Egyptian conditions.

MATERIALS AND METHODS

This study used (Sakha 53 sunflower cultivar) which has excellent morphological and physiological traits that qualify it to be high yielding and distinguished in other agro-morphological characters. As well as, the physiological traits that make it resistant to biotic and abiotic stresses. Therefore, this variety is an excellent experimental material that can be used in this investigation.

Field evaluation: The seeds used for the recent investigation were originally performed from the oil crops research department, Agriculture Research Centre. Five hundred pure seeds of the Sakha 53 sunflower cultivar were subjected for gamma irradiation treatments dosages of 100, 200, 300, 400 and 500 Gy using the Co source at the National Center for Radiation Research and Technology, Nasr City and Cairo, Egypt in the 2010 season (M_0). The irradiated materials of all doses were grown and series of selections among the mutant population under normal soil conditions in new valley farm and this process was carried out during the 2011-2018 seasons (M_1-M_8) to produce the mutant lines and all plants have reached full genetic stability at the 8th generation (M_8).

Sowing and treatments: Two experiments were conducted in new valley farm, New Valley Governorate, Egypt during the 2019 and 2020 seasons using the original sunflower cultivar (Sakha 53) and 5 mutant lines derived from it and selected from M_8 generation as follows:

- **Experiment I (normal conditions):** The 6 sunflower entries were grown under normal irrigated conditions in the new valley farm, New Valley Governorate, Egypt and the irrigation system was as follows: The 1st irrigate was conducted at sowing, the 2nd irrigate was done after 21 days from the first one, the 3rd irrigate was conducted after 21 days from the 2nd irrigate, the 4th irrigate was conducted after 21 fay from the 3rd irrigate and the 5th or last irrigate was done after 21 days from the 4th irrigate until harvest
- **Experiment II (water stress treatment):** The 6 sunflower entries were planted in the same new valley location with the same normal irrigation system but the number of irrigates was only 4 until the harvest, meaning that the 5th irrigate was excluded

The 6 sunflower entries genotypes were planted in randomized complete black design, with 3 replications. The proceeding crop was the Egyptian clover in both seasons. Seeds of each sunflower entry were sown on 15th August in the 2019 and 2020 seasons, respectively. Plot size was 12 m^2 (3×4 m) in 6 ridges each 4 m long and 60 cm apart, 3-4 seeds per hill were placed with 20 cm between hills for each experiment. One plant per hill was maintained by thinning 21 days after sowing. The conventional cultural practices of growing sunflower were conducted as recommended in the new valley region.

Each experiment was a completely independent experiment and completely isolated from the other experience. As the isolation distance was 200 m^2 and this buffer distance was covered with linoleum on both sides to prevent water infiltration from the standard experiment to drought experiment. The length of each replicate of each experiment was 20 m and the space among every 2 plants was 20 cm into each replicate.

Studied traits: Sixty plants were taken from each entry of each experiment for each season (2019 and 2020) to calculate and estimate the following traits.

Yield and its components: Plant height (cm), head diameter (cm), stem diameter (cm), seed yield (g/plant), shoot length (cm), dry weight (g) and oil (%) was determined according to the modified method[1].

Root and physiological traits related to water stress tolerance: Maximum root length (cm), number of roots/ plant, root xylem vessel number, root volume and proline content was determined from a standard curve and calculated on a fresh basis is as follows:

$$\frac{\left(\dfrac{\mu\text{g proline}}{\text{mL}\times\text{mL toluene}}\right)\Big/\left(\dfrac{115.5\ \mu\text{g}}{\mu\text{ mole}}\right)}{\left(\dfrac{\text{g sample}}{5}\right)} = \frac{\mu\text{ moles proline}}{\text{g of fresh weight material}}$$

The results related to proline content are average values of at least 3-4 samples for each entry under both experiments[23,24].

Statistical analysis: All calculated data of all traits under evaluation in 2 seasons for both treatments were analysis[25].

Estimation of drought stress tolerance indices: All drought stress tolerance indices were estimated in the 6 sunflower genotypes for seed yield/plant trait[26-28] as follows:

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

$$\text{GYD} = \frac{\text{Mean the grain yield}}{\text{Plant for the drought stress experiment}}$$

$$\text{Yield stability index (YSI)} = \frac{\text{YS}}{\text{YP}}$$

Where:

YS = Average yield under stress

YP = Average of yield under the control experiment

$$\text{Yield index (YI)} = \frac{\text{YS for each genotype}}{\text{YS for all genotypes}}$$

$$\text{Average yield for both trials (MP)} = \frac{\text{YS+YP}}{2}$$

$$\text{Drought stress tolerance index (DTI)} = \frac{\text{YP}\times\text{YS}}{(\text{Mean of YP})^2\text{, GMP: }(\text{YP}\times\text{YS})^{0.5}}$$

$$\text{Yield reduction (YR)} = \frac{\text{1-YS}}{\text{YP}}$$

$$\text{Drought susceptibility index (DSI)} = \frac{\dfrac{\text{1-YS}}{\text{YW}}}{\text{D}}$$

Where:

YS = Mean yield under salt stress

Yw = Mean yield under control condition

$$\text{Environmental stress intensity (D)} = 1 - \frac{\text{Mean of all genotypes under stress}}{\text{Mean yield of all genotypes under irrigated conditions}}$$

Genetic parameters: Variance components, heritability in the broad sense, Genetic Coefficient of Variability (GCV %), Phenotypic Coefficient of Variability (PCV %), D^z or the difference between the Phenotypic Coefficient of Variation (PCV %) and Genotypic Coefficient of Variation (GCV %), expected genetic advance, in addition, genetic advance as percentage of mean were the most important measurements calculated through the 2 seasons for both treatments in this investigation as follows:

- The Genetic Coefficient of Variability (GCV %) and Phenotypic Coefficient of Variability (PCV %) was estimates[29] as follows:

$$\text{Environmental variance } (\sigma^2 e) = MS_e$$

$$\text{Genotypic Variance (GV) or } (\sigma^2 g) = \frac{MS_g - MS_e}{r}$$

$$\text{Phenotypic Variance (Ph v) or } (\sigma^2 ph) = (\sigma^2 e) + (\sigma^2 g) \text{ or } MS_e + MS_g$$

Where:

MS_e = Mean square of error
MS_g = Mean square of genotypes
r = Number of replicates
X = Mean of trait

$$\text{Genetic coefficient of variability (GCV \%)} = \frac{\sqrt{Gv}}{x} \times 100$$

$$\text{Phenotypic coefficient of variability (PCV \%)} = \frac{\sqrt{Ph\ v}}{x} \times 100$$

Estimation of heritability in the broad sense: Broad sense heritability (h^2) is expressed as the percentage of the ratio of the Genotypic Variance (GV) to the Phenotypic Variance (Ph V) and was estimated on a genotype mean basis[29] as:

$$H^2B = \frac{\sigma^2 g}{\sigma^2 ph} \times 100$$

- **D^z:** The difference between the Phenotypic Coefficient of Variation (PCV %) and Genotypic Coefficient of Variation (GCV %), PCV (%), GCV (%)

Estimation of genetic advance: The expected Genetic Advance (GA) and percentage of the mean (GAM) assuming selection of superior 5% of the genotypes was estimated[29]:

$$GA = K\ X\ (\sigma^2 g) \times \frac{\sqrt{Ph\ v}}{Ph\ v}$$

Where:

K = Standardized selection differential at 5% selection intensity (K = 2.068)

The genetic advance as percentage of mean (GAM) was computed as:

$$GAM\ (\%) = \frac{GA}{X} \times 100$$

Molecular characterization

DNA extraction and purification: Total DNA was extracted from fresh leaves of the 6 sunflower genotypes by DNeasy Plant Kit (QIAGEN, Germany). The extracted DNA concentration and quality were estimated by NanoDrop.

ISSR-PCR reactions: Eleven ISSR primers were used in the detection of polymorphism. The amplification reaction was carried out in 25 µL reaction volume containing 12.5 L Master Mix (Sigma), 2.5 µL primer (10 pcmol), 3 µL template DNA (10 ng) and 7 µL dH_2O[30].

Thermo cycling 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 1 min, an annealing step at 45°C for 1 min and an elongation step at 72°C for 1.5 min. The primer extension segment was extended to 7 min at 72°C in the final cycle.

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. PCR products were visualized on UV light and photographed using a gel documentation system (BIO-RAD 2000).

Data handling and cluster analysis (phylogenetic tree): Data was scored for computer analysis based on the presence or absence of the amplified products for each primer. Pairwise components of the 6 sunflower genotypes based on the presence or absence of unique and shared polymorphic products, were used to determine similarity coefficients[31]. The similarity coefficients were then used to construct dendrograms, using the Unweighted Pair Group Method with Arithmetic averages (UPGMA) employing the Sequential, Agglomerative, Hierarchical and Nested clustering (SAHN) from the NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System), version 1.80 (applied biostatistics program).

RESULTS

Analysis of variance: Data viewed in Table 1 and related to the analysis of variance test showed highly significant differences among all sunflower entries (the original cultivar

Sakha 53 and their 5 mutant lines) for all studied characters under normal and water stress conditions during the 2 growing seasons (2019 and 2020). The coefficient of variance percentages was low for all studied traits under both conditions for the 2 seasons except the traits, head diameter, stem diameter, seed yield/plant and shoot length. Where the values were 57.90 and 83.52 cm under both conditions for the 1st season and 56.93 and 69.55 cm for both experiments of the 2nd season for stem diameter trait followed by 14.70 and 14.26 cm) for both treatments of the 1st season and 13.74 and 13.98 cm for the 2nd season under normal and drought conditions for shoot length trait, respectively and so on.

Mean performance: Results of mean values presented in Table 2 confirmed that the 5 sunflower mutant lines had outperformed the original cultivar (Sakha 53) in all studied traits under water stress conditions compared to the standard experiment over the 2 growing seasons and were exhibited highly trend of drought stress tolerance in this context. But the 5 sunflower mutant lines were not equal in superiority level, where the lines number (3, 4 and 5) came in the 1st level, while the rest lines (1 and 2) came in the 2nd rank in this regard under both experiments during the 2 growing seasons. For example not limited, the mean values ranged from 164.15-185.04 and 143.47-174.33 cm for the 1st season and from 165.03-183.15 and 142.05-173.08 cm for the 2nd season under both conditions for plant height trait and from 15.32-17.53 and 10.06-14.38 cm for the 1st season and 15.65-17.31 and 11.12-13.89 cm for the 2nd season under normal and drought conditions for head diameter trait. Also, the values ranged from 2.05-2.41 and 1.32-1.96 cm for the 1st season and from 2.09-2.51 and 1.55-1.93 cm for the 2nd season of both experiments for stem diameter trait and from 37.21-51.32 and 28.15-42.33 g for the 1st year and 35.42-50.09 and 23.76-40.14 g for the 2nd year under both conditions for seed yield/plant trait. In the same track, the mean values were ranged from 9.76-12.98 and 6.11-9.04 cm for the 1st season and from 9.45-12.43 and 5.98-9.28 cm for the 2nd season under both treatments for shoot length trait and ranged from 65.19-77.42 and 57.04-69.03 g for the 1st season and 66.03-76.31 and 55.12-67.18 g for the 2nd season under normal and water stress conditions for dry weight trait. While the values were ranged from 36.72-41.77 and 29.04-34.25% for the 1st season and 35.98-41.75 and 27.55-35.16% for the 2nd season of both conditions for oil (%) trait, from 92.37-117.43 and 74.55-108.23 cm for the 1st year and 90.14-115.35 and 76.12-104.21 cm for the 2nd year under normal and drought conditions for maximum root length trait. Further, the mean values were ranged from 634.11-1008.23 and 587.32-894.63 for the 1st season and from 636.53-1011.15 and 577.15-886.55 for the 2nd season of both experiments for several roots/plant traits and ranged from 28.48-61.31 and 21.17-52.49 for the 1st year and 31.05-63.02 and 24.07-54.78 for the 2nd year under normal and water stress conditions for root xylem vessel number trait. For root volume trait, the mean values ranged from 44.15-71.28 and 39.56-65.14 for the 1st season and 45.03-69.38 and 41.07-62.45 for the 2nd season under both conditions. While, the mean values of the proline content trait were ranged from 28.23-60.47 and 34.05-74.18 for the 1st season and ranged from 26.55-62.03 and 31.09-77.58 for the 2nd season under normal and water stress conditions, respectively.

Drought tolerance indices: Data of drought stress tolerance indices shown in Table 3 detected that the new sunflower mutant lines, 1, 2, 3 and 5 for YSI parameter and 2, 3 and 5 for MP and GMP parameters in 2019 season besides, the mutant lines number 1, 3, 4 and 5 for YSI and 3, 4 and 5 for MP and GMP parameters in 2020 season exhibited the highest mean values for water stress tolerance indices for grain yield trait. These results indicated that these new sunflower entries were considered highly tolerant under drought experiment compared to the control treatment. Also, the 3 sunflower mutant lines, 1, 3 and 5 for (YI) parameter besides, the mutant line 5 only for DTI in the 2019 season and the mutant lines, 3, 4 and 5 for YI and mutant line 5 only for DTI in 2020 season were recorded mean values higher than one. These results confirmed that these new sunflower entries were recorded highly drought tolerance under stress treatment compared to the normal conditions and this result was not achieved in the rest of the sunflower entries, respectively. While, all sunflower entries for the parameter (YR) in both growing seasons and the new mutant lines, 1 and 3 for the parameter DSI in 2019 season and the rest mutant lines, 1, 3, 4 and 5 for DSI in the 2020 season were showed mean values lower than one which confirmed that these superior sunflower genotypes were exhibited high tolerance for water stress in this context.

Genetic components: Results viewed in Table 4 revealed the data of phenotypic variance were higher than its counterparts in genotypic variance for the 2 experiments during the 2 growing seasons. Also, it was noticed that the environmental variance values decreased compared to the genetic variance in all the studied traits for both treatments during the 2 growing seasons in this investigation. This confirms that the great part of the phenotypic variance was in

Table 1: ANOVA analysis of all studied attributes for the 6 sunflower genotypes under normal and water stress conditions during (2019 and 2020) seasons

			Plant height (cm)		Head diameter (cm)		Stem diameter (cm)		Seed yield (g/plant)		Shoot length (cm)		Dry weight (g)	
S.O.V.	DF	Seasons	N	S	N	S	N	S	N	S	N	S	N	S
Genotypes	5	2019	33.18**	26.24**	107.11**	88.14**	25.06**	21.08**	48.05**	29.07**	15.03**	26.13**	19.62**	25.38**
		2020	23.71**	21.05**	92.23**	78.33**	21.48**	24.36**	55.24**	13.57**	18.23**	24.29**	28.07**	36.17**
Replicates	2	2019	11.32**	8.14**	6.29**	13.02**	9.28**	12.17**	32.19**	25.28**	7.13**	5.48**	23.16**	20.09**
		2020	9.36**	17.04**	7.21**	15.43**	12.07**	18.02**	38.08**	18.45**	11.04**	6.38**	21.28**	14.55**
Error	10	2019	1.37	1.28	2.17	1.33	1.82	2.04	1.56	1.27	2.74	1.26	1.79	1.65
		2020	2.05	1.57	1.96	1.58	1.73	1.55	1.42	1.54	2.39	1.19	1.59	1.48
CV (%)		2019	0.65	0.68	8.81	8.74	57.90	83.52	2.80	3.10	14.70	14.26	1.84	1.95
		2020	0.80	0.75	8.43	9.74	56.93	69.55	2.71	3.69	13.74	13.98	1.75	1.93
			Oil (%)		Maximum root length (cm)		Number of roots/plant		Root xylem vessel number		Root volume		Proline content	
			N	S	N	S	N	S	N	S	N	S	N	S
Genotypes	5	2019	16.23**	20.03**	27.11**	22.15**	13.92**	15.44**	45.19**	36.34**	12.97**	17.55**	38.79**	51.14**
		2020	19.82**	27.22**	24.33**	19.82**	15.06**	11.03**	37.83**	40.08**	23.09**	24.05**	32.14**	44.07**
Replicates	2	2019	15.32**	8.37**	12.37**	14.15**	20.04**	16.32**	8.42**	10.84**	19.06**	17.33**	6.23**	8.04**
		2020	11.28**	13.07**	9.74**	13.67**	13.69**	11.03**	13.52**	12.86**	22.17**	12.45**	10.04**	7.55**
Error	10	2019	1.42	1.67	3.24	1.95	2.13	1.79	1.86	1.67	2.43	2.15	1.02	1.07
		2020	1.28	1.08	2.96	2.03	2.27	1.81	2.05	1.73	2.08	2.66	1.17	1.05
CV (%)		2019	3.03	3.98	1.67	1.47	0.171	0.174	2.83	3.22	2.58	2.65	2.25	1.93
		2020	2.86	3.21	1.60	1.55	0.176	0.176	2.93	3.31	2.40	2.96	2.36	1.82

Table 2: Mean performance of all studied traits for the 6 sunflower genotypes under normal and water stress conditions during (2019 and 2020) seasons

	Plant height (cm)				Head diameter (cm)				Stem diameter (cm)				Seed yield (g/plant)			
	2019		2020		2019		2020		2019		2020		2019		2020	
Genotypes	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S
Local cultivar (Sakha 53)	164.15	143.47	165.03	142.05	15.32	10.06	15.65	11.12	2.05	1.32	2.09	1.55	37.21	28.15	35.42	23.76
Mutant line (1)	173.28	162.03	175.48	164.22	16.42	13.52	16.35	12.98	2.32	1.78	2.17	1.82	42.13	37.29	40.06	32.56
Mutant line (2)	179.57	168.25	180.03	170.41	16.86	12.97	16.48	12.78	2.41	1.96	2.36	1.74	44.15	36.04	46.27	29.23
Mutant line (3)	183.10	169.05	181.52	168.43	17.14	14.22	16.95	13.89	2.55	1.74	2.51	1.93	47.47	39.78	48.29	39.12
Mutant line (4)	185.04	174.33	183.15	173.08	17.06	14.38	16.82	12.79	2.28	1.65	2.34	1.88	45.19	34.19	43.68	36.55
Mutant line (5)	182.34	170.04	181.12	171.18	17.53	14.02	17.31	13.87	2.39	1.85	2.43	1.84	51.32	42.33	50.09	40.14
Mean	177.91	164.52	177.72	164.89	16.72	13.19	16.59	12.90	2.33	1.71	2.31	1.79	44.57	36.29	43.96	33.56
LSD at 5%	1.73	1.67	2.11	1.85	2.17	1.70	2.07	1.85	1.99	2.11	1.94	1.84	1.84	1.66	1.76	1.83
LSD at 1%	2.64	2.55	3.23	2.82	3.32	2.60	3.15	2.83	3.04	3.22	2.96	2.80	2.81	2.54	2.68	2.80
	Shoot length (cm)				Dry weight (mg)				Oil (%)				Maximum root length (cm)			
	2019		2020		2019		2020		2019		2020		2019		2020	
	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S
Local cultivar (Sakha 53)	9.76	6.11	9.45	5.98	65.19	57.04	66.03	55.12	36.72	29.04	35.98	27.55	92.37	74.55	90.14	76.12
Mutant line (1)	10.18	8.38	11.14	7.82	71.18	66.05	70.83	62.33	38.32	33.12	39.14	34.62	103.74	88.36	102.25	85.02
Mutant line (2)	12.07	7.83	11.95	8.03	73.56	68.13	72.09	64.76	39.07	32.75	39.15	31.23	107.05	94.23	109.33	89.48
Mutant line (3)	11.75	7.58	11.32	8.23	72.29	64.11	71.87	63.55	40.11	34.05	41.05	35.16	110.39	97.36	112.52	95.24
Mutant line (4)	10.84	9.04	11.26	9.28	75.46	69.03	73.21	67.18	39.84	31.19	40.25	32.37	117.43	103.55	115.35	101.04
Mutant line (5)	12.98	8.32	12.43	7.51	77.42	68.93	76.31	65.24	41.77	34.25	41.75	33.15	114.45	108.23	115.09	104.21
Mean	11.26	7.87	11.25	7.80	72.51	65.54	71.72	63.03	39.30	32.40	39.55	32.34	107.57	94.38	107.44	91.85
LSD at 5%	2.44	1.66	2.28	1.61	1.97	1.90	1.86	1.79	1.76	1.91	1.67	1.53	2.66	2.06	2.54	2.10
LSD at 1%	1.35	2.53	3.48	2.46	3.01	2.89	2.84	2.74	2.68	2.91	2.55	2.34	4.06	3.15	3.88	3.21

Table 2: Continue

	Number of roots/plant				Root xylem vessel number				Root volume				Proline content			
	2019		2020		2019		2020		2019		2020		2019		2020	
	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S
Local cultivar (Sakha 53)	634.11	587.32	636.53	577.15	28.48	21.17	31.05	24.07	44.15	39.56	45.03	41.07	28.23	34.05	26.55	31.09
Mutant line (1)	789.57	739.02	785.16	742.75	39.48	33.52	40.01	31.29	57.32	55.44	55.04	51.78	36.32	42.15	39.16	44.45
Mutant line (2)	866.13	812.04	872.35	796.74	49.57	41.77	52.96	44.15	63.49	58.22	61.88	57.04	41.35	49.52	42.02	53.00
Mutant line (3)	853.17	732.19	859.44	741.06	56.83	49.37	54.27	43.54	61.05	54.32	65.16	60.22	48.63	55.17	50.03	61.14
Mutant line (4)	942.17	835.47	956.03	827.49	61.31	52.49	63.02	54.78	65.22	59.34	63.48	57.04	53.77	65.92	55.04	70.14
Mutant line (5)	1008.23	894.63	1011.15	886.55	53.22	42.49	51.07	40.39	71.28	65.14	69.38	62.45	60.47	74.18	62.03	77.58
Mean	848.89	766.77	853.44	761.95	48.14	40.13	48.73	39.70	60.41	55.33	59.99	54.93	44.79	53.49	45.80	56.23
LSD at 5%	2.15	1.97	2.22	1.99	2.01	1.91	2.11	1.94	2.30	2.16	2.13	2.41	1.49	1.53	1.60	1.51
LSD at 1%	3.29	3.01	3.40	3.03	3.07	2.91	3.23	2.96	3.51	3.30	3.25	3.68	2.27	2.33	2.44	2.31

N: Normal conditions, S: Water stress conditions

Table 3: Estimates the drought tolerance indices parameters for the 6 sunflower genotypes especially for seed yield/plant trait under both conditions in 2 seasons

	Seed yield/plant (g)																	
	Season 2019									Season 2020								
Genotypes	GYP	GYD	YSI	YI	MP	DTI	GMP	YR	DSI	GYP	GYD	YSI	YI	MP	DTI	GMP	YR	DSI
Local cultivar (Sakha 53)	37.21	28.15	0.75	0.77	32.68	0.52	32.36	0.25	1.38	35.42	23.76	0.67	0.70	29.59	0.43	29.0	0.33	1.43
Mutant line (1)	42.13	37.29	0.88	1.02	39.71	0.79	39.63	0.12	0.66	40.06	32.56	0.81	0.97	36.31	0.67	36.11	0.19	0.82
Mutant line (2)	44.15	36.04	0.81	0.99	40.09	0.80	39.88	0.19	1.05	46.27	29.23	0.63	0.87	37.75	0.69	36.77	0.37	1.60
Mutant line (3)	47.47	39.78	0.83	1.09	43.62	0.95	43.45	0.17	0.94	48.29	39.12	0.81	1.16	43.70	0.97	43.46	0.19	0.82
Mutant line (4)	45.19	34.19	0.75	0.94	39.69	0.77	39.30	0.25	1.38	43.68	36.55	0.83	1.08	40.11	0.82	39.95	0.17	0.73
Mutant line (5)	51.32	42.33	0.82	1.16	46.82	1.09	46.60	0.18	1.00	50.09	40.14	0.80	1.19	45.11	1.04	44.83	0.20	0.86

YI: Meaning yield index (YS for each genotype/mean of YS for all genotypes), DTI: Means drought stress tolerance index (YP X YS/(mean of YP)^{2}, GMP: (YP X YS)$^{0.5}$, YR: Meaning yield reduction (1-YS/YP), GYP: Meaning the grain yield/plant for the control experiment and GYD: Meaning the grain yield/plant for the drought stress experiment

Table 4: Estimates of genetic parameters for all studied traits under both conditions during (2019 and 2020) seasons

		Plant height (cm)		Head diameter (cm)		Stem diameter (cm)		Seed yield (g/plant)		Shoot length (cm)		Dry weight (g)	
Genetic parameters	Seasons	N	S	N	S	N	S	N	S	N	S	N	S
Mean	2019	177.91	164.52	16.72	13.19	2.33	1.71	44.57	36.29	11.26	7.87	72.51	65.54
	2020	177.72	164.89	16.59	12.90	2.31	1.79	43.96	33.56	11.25	7.80	71.72	63.03
Genotypic variance	2019	10.60	8.32	34.98	28.93	7.74	6.34	15.49	9.26	4.09	8.29	5.94	7.91
	2020	7.22	6.49	30.09	25.58	6.58	7.60	17.94	4.01	5.28	7.70	8.82	11.56
Environmental variance	2019	1.37	1.28	2.17	1.33	1.82	2.04	1.56	1.27	2.74	1.26	1.79	1.65
	2020	2.05	1.57	1.96	1.58	1.73	1.55	1.42	1.54	2.39	1.19	1.59	1.48
Phenotypic variance	2019	11.97	9.60	37.15	30.26	9.56	8.38	17.05	10.53	6.83	9.55	7.73	9.56
	2020	9.27	8.06	32.05	27.16	8.31	9.15	19.36	5.55	7.67	8.89	10.41	13.04
Heritability in broad sense	2019	88.55	86.66	94.15	95.60	80.96	75.65	90.85	87.93	59.88	86.80	76.84	82.74
	2020	77.88	80.52	93.88	94.18	79.18	83.06	92.66	72.25	68.83	86.61	84.72	88.65
GCV (%)	2019	1.83	1.75	35.37	40.77	119.40	147.24	8.83	8.38	17.96	36.58	3.36	4.29
	2020	1.51	1.54	33.06	39.20	111.04	154.01	9.63	5.96	20.42	35.57	4.14	5.39
PCV (%)	2019	1.94	1.88	36.45	41.70	132.70	169.28	9.26	8.94	23.20	39.26	3.83	4.71
	2020	1.71	1.72	34.12	40.39	124.79	168.98	10.00	7.01	24.61	38.22	4.49	5.72
D^z	2019	0.11	0.13	1.08	0.93	13.30	22.04	0.43	0.56	5.24	2.68	0.47	0.42
	2020	0.20	0.18	1.06	1.19	13.75	14.97	0.37	1.05	4.19	2.65	0.35	0.33
Expected genetic advance (GA)	2019	6.33	5.55	11.86	10.87	5.17	4.52	7.75	5.90	3.23	5.54	4.41	5.29
	2020	4.90	4.72	10.99	10.15	4.72	5.19	8.43	3.52	3.94	5.34	5.65	6.62
Genetic advance as percentage of mean (GAM %)	2019	3.55	3.37	70.93	82.41	221.88	264.32	17.38	16.25	28.68	70.49	6.08	8.07
	2020	2.75	2.86	66.24	78.68	204.32	289.94	19.17	10.48	35.02	68.46	7.87	10.50

		Oil (%)		Maximum root length (cm)		Number of roots/plant		Root xylem vessel number		Root volume		Proline content	
		N	S	N	S	N	S	N	S	N	S	N	S
Mean	2019	39.30	32.40	107.57	94.38	848.89	766.77	48.14	40.13	60.41	55.33	44.79	53.49
	2020	39.55	32.34	107.44	91.85	853.44	761.95	48.73	39.70	59.99	54.93	45.80	56.23
Genotypic variance	2019	4.93	6.12	7.95	6.73	3.93	4.55	14.44	11.55	3.51	5.13	12.59	16.69
	2020	6.18	8.71	7.12	5.93	4.26	3.07	11.92	12.78	7.0	7.13	10.32	14.34
Environmental variance	2019	1.42	1.67	3.24	1.95	2.13	1.79	1.86	1.67	2.43	2.15	1.02	1.07
	2020	1.28	1.08	2.96	2.03	2.27	1.81	2.05	1.73	2.08	2.66	1.17	1.05
Phenotypic variance	2019	6.35	7.79	11.19	8.68	6.06	6.34	16.30	13.22	5.94	7.28	13.61	17.76
	2020	7.46	9.79	10.08	7.96	6.53	4.88	13.97	14.51	9.08	9.79	11.49	15.39
Heritability in broad sense	2019	77.63	78.56	71.04	77.53	64.85	71.76	88.58	87.36	59.09	70.46	92.50	93.97
	2020	82.84	88.96	70.63	74.49	65.23	62.90	85.32	88.07	77.09	72.82	89.91	93.17
GCV (%)	2019	5.64	7.63	2.62	2.74	0.23	0.27	7.89	8.46	3.10	4.09	7.92	7.63
	2020	6.28	9.12	2.48	2.65	0.24	0.22	7.08	9.0	4.41	4.86	7.01	6.73
PCV (%)	2019	6.41	8.61	3.10	3.12	0.28	0.32	8.38	9.06	4.03	4.87	8.23	7.87
	2020	6.90	9.67	2.95	3.07	0.29	0.28	7.67	9.59	5.02	5.69	7.40	6.97
D^z	2019	0.77	0.98	0.48	0.38	0.05	0.05	0.49	0.60	0.93	0.78	0.31	0.24
	2020	0.62	0.55	0.47	0.42	0.05	0.06	0.59	0.59	0.61	0.83	0.39	0.24
Expected genetic advance (GA)	2019	4.04	4.53	4.91	4.72	3.30	3.73	7.39	6.56	2.97	3.93	7.05	8.19
	2020	4.67	5.75	4.63	4.34	3.44	2.87	6.59	6.93	4.80	4.71	6.29	7.55
Genetic advance as percentage of mean (GAM %)	2019	10.27	13.98	4.56	5.0	0.38	0.48	15.35	16.34	4.91	7.10	15.74	15.31
	2020	11.80	17.77	4.30	4.72	0.40	0.37	13.52	17.45	8.0	8.57	13.73	13.42

favour of the genetic variance, while the environmental variance was very little and almost non-existent. Further, the values of heritability in the broad sense were observed high in all studied attributes under normal and drought conditions in both seasons except the traits, shoot length under normal conditions for the 2 years where the values appeared medium and were 59.88 and 68.83% and the number of roots/plant under both conditions for the 2 growing seasons 64.85, 71.76, 65.23 and 62.90%, respectively. In the same regard, the results of PCV (%) were higher than GCV (%) for all studied traits under normal and water stress conditions and the differences between PCV and GCV (%) (D^z) were very low in all traits under study except the trait stem diameter where it was recorded high values 13.30, 22.04 and 13.75, 14.97% for both experiments of the 2 growing seasons. On the same track, the values of expected genetic advance and genetic advance as percentage of mean (%) were appeared low in the number of roots/plant trait, medium in the traits, plant height, dry weight, maximum root length and root volume under both conditions during the 2 growing seasons. While, the rest studied traits namely, head diameter, stem diameter, seed yield/plant, shoot length, oil (%), root xylem vessel number and proline content were recorded highly rank in these two genetic parameters for the 2 years under normal and water stress conditions.

Molecular characterization

Profile of ISSR analysis: The 11 ISSR primers used for comparing among the 6 sunflower genotypes (The local cultivar Sakha 53 and their 5 mutant lines) namely, ISSR-08, 10, 14, 16, 18, 19, 20, UBC-829, UBC-835, UBC-836 and UBC-839 recorded a total of 155 bands, 77 of them were monomorphic. While that, 78 fragments were polymorphic with 50.32% (polymorphism) included 10 unique bands or positive specific markers as viewed in Table 5, Fig.1a-k. The average numbers of polymorphic ISSR markers were 7.09 fragments for each primer. Polymorphic bands number ranged from 2-13 and a molecular size ranging from 160-1300 bp, respectively. The 1st primer ISSR-08 recorded 16 fragments (9 monomorphic and 7 polymorphic) with 43.75% polymorphism including one unique or positive specific marker with sizes from 160-950 bp in Fig. 1a. While primer ISSR-10 exhibited 15 amplicons, 6 of them were monomorphic and 9 polymorphic with 60.0% polymorphism with sizes from 180-1100 bp in Fig. 1b. For primer ISSR-14, there were 14 bands (7 monomorphic and 7 polymorphic including one unique or positive specific marker) with 50% polymorphism and the sizes ranged from 240-1250 bp in Fig. 1c. Further, the primer ISSR-16 generated10 fragments where 5 of them were monomorphic and 5 polymorphic with 50% polymorphism with sizes ranging from 200-920 bp in Fig. 1d. While that, the primer ISSR-18 exhibited 16 fragments (7 of them were monomorphic and 9 polymorphic included one unique band) with 56.25% polymorphism with sizes ranging from 180-830 bp in Fig. 1e. Concerning primer ISSR-19, there were 12 bands (9 of them were monomorphic and 3 polymorphic) with 25.0% polymorphism and the molecular sizes ranged from 200-580 bp in Fig. 1f. Primer ISSR-20 exhibited 16 fragments where 10 of them were monomorphic and 6 polymorphic included one unique or positive marker with 37.50% polymorphism with sizes ranging from 170-940 bp in Fig. 1g. Also, the primer UBC-829 produced 8 fragments where 6 of them were monomorphic and 2 polymorphic with 25.0% polymorphism and the sizes ranged from 270-1000 bp in Fig. 1h. For the primer UBC-835, there were 15 amplicons where 7 of them were monomorphic and 8 were polymorphic included 2 unique or positive markers with 53.33% polymorphism and the sizes ranged from 200-1300 bp in Fig. 1i. Fifteen bands were generated by the primer UBC-836 where 6 fragments of them were monomorphic and 9 polymorphic included 4 unique or positive markers with 60.0% polymorphism and the sizes ranged from 300-1200 bp in Fig. 1j. While that, the primer UBC-839 exhibited 18 bands

Table 5: Band variation and polymorphism percentage in the 6 sunflower genotypes using 12 ISSR primers

No.	ISSR primers	TB	MB	PB	UB or PSM	P (%)	RS (bp)	Sequence
1	ISSR-08	16	9	7	1	43.75	160-950	5'-AGACAGACAGACAGACGC-3'
2	ISSR-10	15	6	9	0	60.0	180-1100	5'-GACAGACAGACAGACAAT-3'
3	ISSR-14	14	7	7	1	50.0	240-1250	5'-CTCCTCCTCCTCCTCTT-3'
4	ISSR-16	10	5	5	0	50.0	200-920	5'-TCTCTCTCTCTCTCTCA-3'
5	ISSR-18	16	7	9	1	56.25	180-830	5'-HVHCACACACACACACAT-3'
6	ISSR-19	12	9	3	0	25.0	200-580	5'-HVHTCCTCCTCCTCCTCC-3'
7	ISSR-20	16	10	6	1	37.5	170-940	5'-HVHTGTGTGTGTGTGTGT-3'
8	UBC-829	8	6	2	0	25.0	270-1000	5'-TGTGTGTGTGTGTGTGC-3'
9	UBC-835	15	7	8	2	53.33	200-1300	5'-AGAGAGAGAGAGAGAGCC-3'
10	UBC-836	15	6	9	4	60.0	300-1200	5'-AGAGAGAGAGAGAGAGCA-3'
11	UBC-839	18	5	13	0	72.22	180-1050	5'-TATATATATATATATARG-3'
Total		155	77	78	10	50.32	160-1300	

TB: Total bands, MB: Monomorphic bands, PB: Polymorphic bands, UB or PSM: Unique bands or positive specific marker, P (%): Polymorphism percentage and RS (bp): Range size

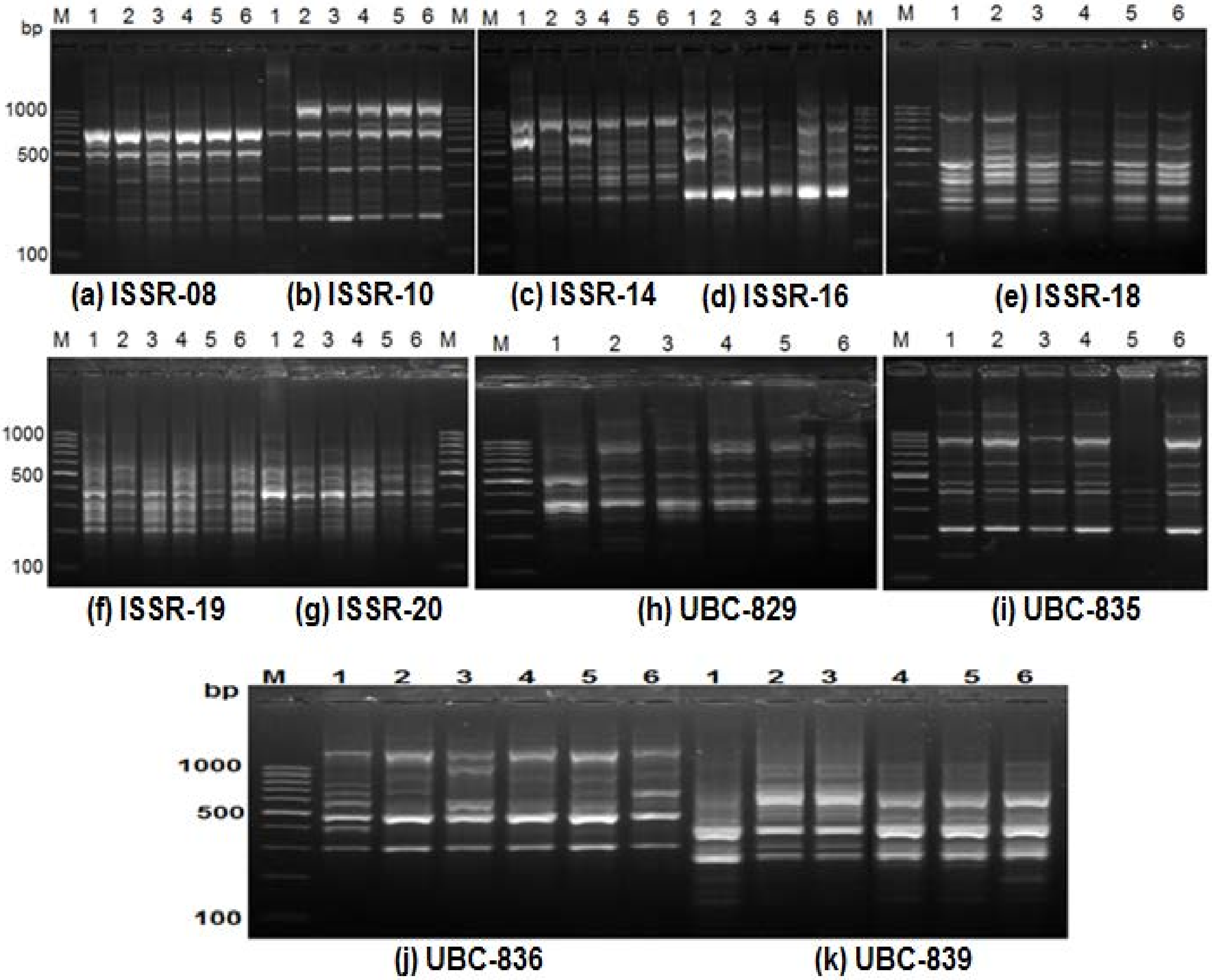

Fig. 1(a-k): PCR fragments using 11 ISSR primers, (a) ISSR-08, (b) ISSR-10, (c) ISSR-14, (d) ISSR-16, (e) ISSR-18, (f) ISSR-19, (g) ISSR-20, (h) UBC-829, (i) UBC-835, (j) UBC-836 and (k) UBC-839 in the six sunflower genotypes

1: Sakha 53, 2: Mutant line 1, 3: Mutant line 2, 4: Mutant line 3, 5: Mutant line 4 and 6: Mutant line 5 and M: DNA ladder (100 bp) as a marker

where 5 of them were monomorphic and 13 polymorphic with 72.22% polymorphism and the sizes were ranged from 180-1050 bp in Fig. 1k, respectively. Results obtained in the Table 5 confirmed that the highest numbers of total bands were observed in the primer UBC-839 (18) while the lowest numbers of fragments were observed in primer UBC-829 (8). In the same context, primer UBC-839 produced the highest number of polymorphic fragments (13) and UBC-829 primer was recorded the lowest number of bands (2), respectively. Also, the primer UBC-836 exhibited the highest number of the unique band or positive specific markers (4), followed by UBC-835 primer (2) and then followed by the primers, ISSR-08, 14, 18 and 20 where they produced one unique positive marker for each one of them and the rest primers namely, ISSR-10, 16, 19, UBC-829 and UBC-839 were recorded no unique bands in this regard, respectively. Further, the highest polymorphism (%) was observed in the primer UBC-839 (72.22%) while the primers ISSR-19 and UBC-829 were recorded the lowest rank (25.0%) in this regard. Data presented in Table 6 detected that the sunflower genotypes, mutant lines 1, 2 and 3 recorded the highest number of fragments and were coming in the 1st rank in this regard and their values were 123, 121 and123 while, the rest of sunflower accessions Sakha 53 and mutant lines 4 and 5 coming in the second rank and their values were 114, 113 and 117, respectively. Further, primers ISSR-08, 10 and 20 produced the highest number of amplified fragments 74, 74 and 79 for each one of them in all sunflower genotypes. While the primers ISSR-16 and UBC-829 generated the lowest number of bands 54 and 44 for both of them and the rest primers were exhibited a various number of amplified fragments.

Data viewed in Table 7 revealed 10 positives and 18 negative specific markers produced by 11 ISSR primers. These primers namely, ISSR-08, 10, 14, 16, 18, 19, 20, UBC-829,

Table 6: Total bands obtained from the 11 ISSR primers of the 6 sunflower entries and all amplified fragments for each entry

	Primers										
Entries	ISSR-08	ISSR-10	ISSR-14	ISSR-16	ISSR-18	ISSR-19	ISSR-20	UBC-829	UBC-835	UBC-836	UBC-839	Total
Local cultivar (Sakha 53)	13	6	9	8	11	11	15	8	13	11	9	114
Mutant line (1)	11	14	11	10	13	10	13	7	11	11	12	123
Mutant line (2)	14	14	10	8	13	11	15	7	10	7	12	121
Mutant line (3)	13	15	12	8	8	12	14	8	13	9	11	123
Mutant line (4)	12	12	12	10	10	10	10	7	8	11	11	113
Mutant line (5)	11	13	12	10	10	11	12	7	11	9	11	117
Total bands	74	74	66	54	65	65	79	44	66	58	66	711

Table 7: Mapping of positive and negative specific markers for the 6 sunflower genotypes using 11 ISSR primers

ISSR primers	MS (bp)	G_1	G_2	G_3	G_4	G_5	G_6	Specific marker
ISSR-08	400	-	-	+	-	-	-	P (G_3)
	370	+	-	+	+	+	+	N (G_2)
	290	+	-	+	+	+	+	N (G_2)
ISSR-10	1100	-	+	+	+	+	+	N (G_1)
	850	-	+	+	+	+	+	N (G_1)
	610	-	+	+	+	+	+	N (G_1)
	430	-	+	+	+	+	+	N (G_1)
	280	-	+	+	+	+	+	N (G_1)
ISSR-14	600	+	+	-	+	+	+	N (G_3)
	490	-	-	+	-	-	-	P (G_3)
ISSR-16	920	+	+	+	-	+	+	N (G_4)
	450	+	+	+	-	+	+	N (G_4)
	380	-	+	+	+	+	+	N (G_1)
	330	+	+	-	+	+	+	N (G_3)
ISSR-18	250	-	-	-	+	-	-	P (G_4)
	200	+	+	+	-	+	+	N (G_4)
ISSR-19	310	+	+	+	+	-	+	N (G_5)
ISSR-20	940	+	-	-	-	-	-	P (G_1)
	490	+	+	+	+	-	+	N (G_5)
	210	+	+	+	+	-	+	N (G_5)
UBC-829	750	+	+	-	+	+	+	N (G_3)
UBC-835	250	-	-	-	-	+	-	P (G_5)
	150	+	-	-	-	-	-	P (G_1)
UBC-836	760	+	-	-	-	-	-	P (G_1)
	520	-	+	-	-	-	-	P (G_2)
	410	+	-	-	-	-	-	P (G_1)
	370	-	-	-	-	+	-	P (G_5)
UBC-839	690	-	+	+	+	+	+	N (G_1)
Range	150-1100							
Total		15	17	17	16	17	18	10 positive+18 negative markers

G_1: Sakha 53, G_2: Mutant line 1, G_3: Mutant line 2, G_4: Mutant line 3, G_5: Mutant line 4 and G_6: Mutant line 5

UBC-835, UBC-836 and UBC-839 used in this study which succeeded in determining the molecular genetic differences among the different sunflower genotypes. Also, these molecular genetic differences were very important in this regard and considered t he taxonomic basic between the 7 sunflower genetic materials (the local variety Sakha 53 and its 5 mutant lines derived from it by mutation). The following is a detailed explanation of ISSR primers that gave positive and negative markers in this track. ISSR-08 primer produced 1 positive specific marker in genotype 3 with a molecular size of 400 bp and 2 negative markers in genotype 2 with sizes of (290 and 370 bp). While that, ISSR-10 primer generated 5 negative specific markers in genotype 1 with sizes of (280, 430, 610, 850 and 1100 bp), respectively. Also, the ISSR-14 primer generated 2 specific markers in genotype 3, the first one was positive at the size of 490 bp and the second marker was negative at the size of 600 bp. Further, ISSR -16 primer exhibited 4 negative markers where the first two markers with sizes of (450 and 920 bp) were observed in genotype 4 besides, the 2 negative markers in the genotypes (1 and 3) with molecular sizes of (380 and 330 bp), respectively. For the ISSR-18 primer, there were 2 markers were obtained in genotype 4 where the first one was positive at a size of 250 bp and the 2nd marker was negative

Table 8: Genetic similarity (%) in the 6 sunflower genotypes using 11 ISSR Primers

Genetic similarity	G_1	G_2	G_3	G_4	G_5	G_6
G_1	1.0					
G_2	0.692	1.0				
G_3	0.702	0.781	1.0			
G_4	0.692	0.757	0.706	1.0		
G_5	0.644	0.722	0.636	0.801	1.0	
G_6	0.723	0.764	0.676	0.832	0.854	1.0

G_1: Sakha 53, G_2: Mutant line 1, G_3: Mutant line 2, G_4: Mutant line 3, G_5: Mutant line 4 and G_6: Mutant line 5

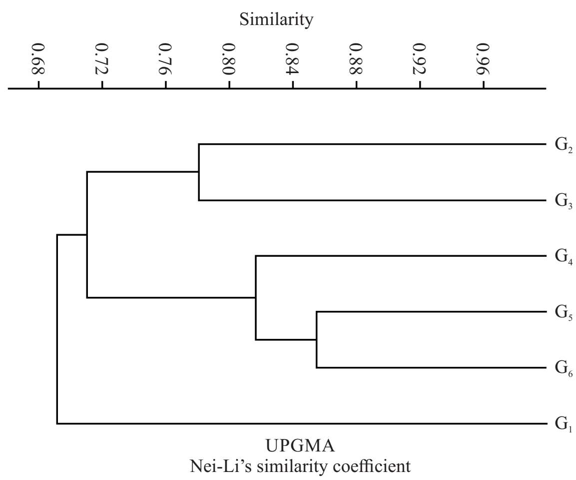

Fig. 2: Dendrogram representing the genetic relationship between the 6 sunflower genotypes using UPGMA cluster analysis of Nei-Li's similarity coefficient generated from the 11 ISSR markers

at a size of 200 bp. One negative specific marker at a molecular size of 310 bp was generated by ISSR-19 primer and showed in genotype 5. In the same context, 3 specific markers were produced by ISSR-20 primer where the first one was positive with the size of 940 bp and observed in genotype 1 and the rest 2 markers were negative with sizes of 210 and 490 bp) were shown in the sunflower genotype number 5. Also, the UBC-829 primer gave only one negative marker in genotype 3 with the size of 750 bp. Two positive specific markers were observed in the sunflower genotypes number 1 and 5 with sizes of 150 and 250 bp by UBC-835 primer. For UBC-836 primer, there were 4 positive markers with molecular sizes of 410 and 760 bp for genotype 1, 520 bp for genotype 2 and 370 bp for genotype number 5, respectively. While the primer UBC-839 produced only one negative specific marker for the sunflower genotype number one with the molecular size of 690 bp.

Proximity matrix analysis (genetic similarity): Data presented in Table 8 exhibited 15 pair wise comparisons to debate the genetic relationships among the 6 sunflower genotypes detected in terms of genetic similarity. The genetic similarity values ranged from 0.636-0.854 with an average of 0.745. Where the biggest level of genetic similarity was 0.854 between (genotype 5 and 6). While that, the lowest level of similarity was 0.636 within genotype 3 and 5, respectively. Also, some genetic similarity values were shown high such as the genetic relationships obtained among (genotype 4 and 6) (0.832), (genotype 4 and 5) (0.801), (genotype 2 and 3) (0.781) and (genotype 2 and 6) (0.764), respectively. Further, the rest genetic similarity values were gradually from higher than the average to high in this regard.

Cluster analysis (phylogenetic tree): Results of cluster analysis or phylogenetic tree which presented in Fig. 2 divided all sunflower genotypes into 2 main clusters. Where the first one included genotype number one only. While that, cluster 2 contained 2 sub-clusters. Where the sub-cluster 1 included genotypes 2 and 3. Whatever, sub-cluster number 2 included genotype 4 and 1 group (genotype 5 and 6), respectively.

DISCUSSION

Results presented in Table 1 detected that the 6 sunflower genotypes (Sakha 53 and its 5 mutant lines) were different genetically from each other especially the 5 sunflower mutant lines that descended from one species. Also, these new genetic materials were all genetically different from each other and from the original variety that descended from them. In addition, the cultivation of these new mutant lines over 2 years also confirmed that they are of a high degree of genetic stability and that the differences between them, if any, will be environmental only. Therefore, this heralds the emergence of new sunflower mutant lines that are high yielding and genetically stable as well, tolerant to water stress. Thus, this fact confirms 2 things that the first one is succeeding in mutagenic events by various degrees of gamma rays which would make positive changes in yield and its components, root and physiological traits in the original variety (Sakha 53) especially high yielding and water stress tolerance. The 2nd result is reaching to highly rank of genetic stability for the new sunflower mutant lines by 100% after nine segregation generations[10,11,32,33]. Mutation breeding is considered one of the most important scientific techniques for genetic improvement of yield and its components and resistance to biotic and abiotic stresses for various strategic crops, especially sunflower. This was evident through the optimum use of different doses of gamma rays, which was credited with causing a genetic change for the Sakha 53 sunflower variety and which eventually succeeded in producing 5 new mutant lines derived from it. These 5 mutant lines have proven unparalleled success and great superiority in their high yielding and water stress tolerance than the original variety which they descended from it over the 2 growing seasons. When presenting the data of mean performance for all studied traits of the 6 sunflower entries in both growing seasons, it was noted that the sunflower mutant lines number 3, 4 and 5 had achieved the 1st rank of high yielding and the other studied traits under both normal and water stress conditions. This confirms that these new genetic materials came in the 1st place in terms of water stress tolerance and then followed by the mutant lines 1 and 2 for the 2 growing seasons in Table 2 in this regard, respectively. As both sunflower groups were able to give ideal values for all studied traits especially yield and its components and oil (%) trait under water stress conditions compared to the standard experiment over 2 years and this proves and confirms their high genetic stability as new sunflower mutant lines tolerant to water stress and these results are consistent with the previous results[34,35]. One of the most important mechanisms that led to an increase in the degree of water stress tolerance in these 5 sunflower mutant lines is the physiological and genetic development that occurred as a result of exposure to different doses of gamma rays. This development was clearly and positively reflected in improving the efficiency of the root system represented by deepening the final root length to reach the water stored in deeper layers in the soil at the time of water stress. In addition, increasing the total number of roots/plant, root volume and root xylem vessel number which will store a large amount of water and keep it for use during the time of water shortage and these traits combined have already succeeded in making an integrated system of water stress tolerance of the 5 sunflower mutant lines compared to the original variety[36-39]. On the same track, it was noted that the sunflower mutant lines that are more tolerant for water stress have already succeeded in producing proline content in large quantities under stress treatment compared to the normal conditions. This result confirmed that organic compounds, especially proline content have a strong relationship with raising the level of plants' tolerance to environmental stresses, especially water stress conditions. Sunflower entries that are only tolerant to water stress are enjoying this mechanism, while sensitive plants lack it as they cannot produce these compounds in large quantities. Accordingly, a very large section of researchers has shed light on the importance of proline content and its main role in increasing and raising the degree of plant tolerance to water stress, for example in pea[40], in plants[41], in maize[42,43], in sunflower [44,45] and wheat[46].Thus, it can be said that the genetic improvement in sunflower plants using gamma rays has yielded fruit and shown great success in deriving 5 sunflower mutant lines with high genetic stability and yielding besides, their high tolerance to water stress under normal experiments compared to water stress treatment and this is the real goal in this study. It is worth mentioning to shed light on the physiological development resulting from breeding with mutations, especially the use of gamma rays. This trend in breeding was credited with deriving improved mutation lines from the local sunflower cultivar (Sakha 53), which recorded the highest level of water stress tolerance as well as their high yielding over 2 growing seasons. Simply, these 5 new mutant lines were able to reduce the loss rate (YR) in the final output compared to the original variety under drought stress conditions compared to the standard experiment in both seasons. Moreover, most of these new mutant lines have already outperformed the original cultivar (Sakha 53) which they descended from it by giving the lowest values of (DSI) in the 2 growing seasons. These results confirmed that these new sunflower genetic materials were able to adapt to water stress by improving the root system represented through deepening root length and increasing the number of roots/plants. As well, producing a high level of proline content responsible for water stress tolerance under drought stress compared to the standard experiment. All these factors led to genetic change and evolution at the physiological level, which eventually led to ultimately increasing their tolerance to water stress while maintaining an acceptable level of the final output, Table 3[47,48]. Results presented in Table 4 revealed that heritability in a broad sense was high in all studied traits under both conditions during the 2 growing seasons which confirms that the genetic variation was a great part of phenotypic variance and played a fruitful role in inheriting and controlling these traits for water stress tolerance. While the impact of environmental variance was very small and almost non-existent. Also, this fact indicated the impact of additive variance and its interactions for the improvement of drought tolerance in sunflowers. On the other hand, in the case of the values of heritability in the broad sense were medium in shoot length under normal conditions and the number of roots/plants under both conditions for the 2 growing seasons indicated that the environmental variance was the medium influence and its effect cannot be ignored in any case[49,50]. Also, it was noted that the PCV (%) values of all traits under study were greater than their counterparts in GCV (%) during both growing seasons under normal and drought conditions. This explains that the genetic improvement of these traits for water stress tolerance was not dependent on the genotype only but the environment and the interaction between environmental X genotype. Further, the selection processes for enhancing these studied traits mentioned above through phenotype could be the biggest goal in this regard. This confirms beyond a reasonable doubt the magnitude of the genetic stability of the new sunflower mutant lines and its high efficiency for drought tolerance as well, it's high yielding under these conditions[24].Further, the differences between PCV and GCV (%) (D^Z) were very low in all studied traits except stem diameter trait which indicated that the environmental impact on controlling and inheriting these traits was very small and did not affect the process of genetic improvement for water stress tolerance in sunflower plants. Results of expected Genetic Advance (GA) based on 5% selection and GAM (%) (Genetic advance as percentage of mean (%) for all studied traits for the two treatments in both growing seasons were appeared low to medium confirmed that additive and non-additive types of gene action were played a fruitful role for controlling and inheriting all studied traits and reflect the positive trend of increasing genetic improvement for water stress tolerance in sunflower genotypes. Also, the simple selection process for these traits would be effective through individual plants[24,51-55]. There is no doubt that molecular genetics using ISSR markers had a great role in determining genetic differences at the molecular level between the different sunflower genotypes (The local check variety Sakha 53 and its 5 mutant lines derived from it). Where ISSR primers succeed in producing 155 fragments, 78 of them were polymorphic with 50.32% polymorphism which confirms that the five sunflower mutant lines were different from the original parent generated from it. Further, this is reflected in the most positive way about the success of plant breeding through mutations in sunflower crop Table 5, (Fig. 1a-k). Also, ISSR primers can be considered as a taxonomic basis among the new sunflower lines and prove that they are indeed completely different from the local variety descended from it depended on its morphological shape as well, all yield and its components and physiological attributes associated with water stress tolerance. The sunflower genotypes, (lines 1, 2 and 3) exhibited the highest rank of amplicons and were coming in the 1st and the rest of sunflower accessions, Sakha 53 and the 2 lines (4 and 5) were coming in the 2nd rank. This fact indicated that the new genetic materials of sunflower were not only different from each other and the original variety descended from it but also superior in all the traits under study and this was confirmed by the profile of ISSR analysis in Table 6. In addition, primers ISSR-08, 10 and 20 produced the highest number of amplified fragments in all sunflower genotypes indicated that these primers were the most accurate criterion in the genetic comparison between the new sunflower genotypes in this investigation[12,17,24,56]. In the same context, the 11 ISSR markers exhibited 28 unique or specific markers (10 positive and 18 negatives) where they were the basis for dividing, classifying and differentiating the 5 sunflower mutant lines from each other and the local variety (Sakha 53) derived from it. Further, these specific markers were one of the most important reasons for distinguishing among tolerated lines to water stress from the moderately and sensitive one in Table 7. Accordingly, in the future, the sunflower mutant lines that have been identified as tolerant to water deficit conditions will become cultivars tolerant to this serious environmental obstacle as well, their high yielding. On this basis, the 2 types of unique bands are of great strategic importance in determining the important quantitative traits related to high productivity, salt and water stress tolerance and resistance to various diseases not only in sunflower but in other crops[57] few authors compared the relative water content and chlorophyll concentration traits in 70 sunflower genotypes under normal and water deficit conditions through using 210 (SSRs) where 11 genes of them were located in 17 linkage groups. In addition, a total of 10 and 8 QTLs were identified for chlorophyll levels and relative water content, respectively. El-Mouhamady *et al.*[52] observed 13 unique bands for genetic diversity among some wheat accessions. Liang *et al.*[58] revealed 876 and 269 DEGs by DGE sequencing in leaves and roots of sunflower as the index for drought tolerance. El-Mouhamady *et al.*[24] discovered 50 unique markers for salinity stress tolerance in canola included (32 positive and 18 negatives) and Khatab *et al.*[56] showed 4 alleles for the specific marker with the molecular size of 260 bp in all tolerance barley genotypes. Based on all that has been mentioned, it can be considered that the unique or specific markers are considered the most important genetic evidence for the genetic improvement of water stress tolerance in sunflower plants. Further, they have a high priority in tracking useful quantitative traits in plant breeding programs during segregation generations. Also, these unique bands help plant breeders to select and improve yield and its components, oil and protein qualities, besides, improving their quality. In the same track, unique or specific markers are widely used to transfer tolerance and resistance genes during hybridization between sensitive and tolerant cultivars for biotic and abiotic stresses after identifying those tolerant based on locating of the genes responsible for the above-mentioned quantitative traits. The analysis of the UPGMA ISSR dendrogram is a very important and fruitful biochemical marker for the determination at the species level[59] and revealed the results mentioned above observed in Table 8 and Fig. 2. Further, cluster analysis is one of the most common genetic tests and evidence at the molecular level to determine the degrees of genetic similarity between the different genotypes of any crop. Data of genetic similarity obtained in Table 8 detected that the highest similarity was (0.854) among the genotypes, (5 and 6) and followed by (0.832) among the genotypes (4 and 6) which refers to the close relation and the strong genetic compatibility between them. In contrast, the similarity percentage among the genotypes, (3 and 5) was (0.636) and although it was not a bad result, it is indicated that these were distantly related genotypes in Table 8 and Fig. 2 in this regard. From this point of view, the new sunflower mutant lines which are highly compatible with each other and proved to be highly tolerant to water stress besides gave a high yielding was the nucleus for producing new drought-tolerant sunflower cultivars, which would be a great leap in the future in the path of genetic improvement in the sunflower crop in this regard[24,50,56,60,61].

CONCLUSION

Genetic improvement for drought tolerance in sunflower is considered one of the most important priorities of this investigation due to its economic and nutritional importance not only locally but also globally where its seeds contain high percentages of oil and protein with high-quality rank compared to other oil crops. Mutation breeding using gamma rays was the most effective method to achieve this goal and to derive 5 new and beneficial sunflower mutants from the Egyptian cultivar Sakha 53. Where the 5 mutant lines outperformed on the original cultivar derived from it for water stress tolerance measurements with all studied traits and all genetic parameters under drought stress conditions compared to the standard experiment. Also, the 5 sunflower mutant lines were recorded a high level of water stress tolerance indices compared to the local cultivar (Sakha 53) for seed yield/plant trait. Further, the profile of ISSR primers analysis succeed in comparing among the 6 sunflower genotypes and proved that the 5 mutant lines were different from each other and the original cultivar descended from it during generating 78 polymorphic fragments with 50.32% polymorphism.

SIGNIFICANCE STATEMENT

This study succeeds in developing some new mutant lines of sunflower with a higher rank of yield and its component especially the percentage and quality of oil trait as well, highly stable and tolerating for drought stress under Egyptian conditions. So, it can be considered that these new promising lines as a nucleus for producing new sunflower varieties high-yielding and tolerant for water stress in this regard. Also, the profile analysis of ISSR primers recorded 28 specific markers (10 positive and 18 negatives) as molecular genetic markers that characterize the promising previous sunflower mutant lines. Finally, elicitation five promising sunflower lines with high-yielding, genetically stable and tolerant to drought stress will lead to a great deal to bridge the gap in the Egyptian edible oil industry through increasing its production and reducing the import rate from abroad and this is the great goal in this study.

REFERENCES

1. Rosa, P.M., R. Antoniassi, S.C. Freitas, H.R. Bizzo, D.L. Zanotto, M.F. Oliveira and V.B.R. Castiglioni, 2009. Chemical composition of Brazilian sunflower varieties. Helia, 32: 145-156.
2. Canavar, Ö., F. Ellmer and F.M. Chmielewski, 2010. Investigation of yield and yield components of sunflower (*Helianthus annuus* L.) cultivars in the ecological conditions of Berlin (Germany). Helia, 33: 117-129.
3. Fulda, S., S. Mikkat, H. Stegmann and R. Horn, 2011. Physiology and proteomics of drought stress acclimation in sunflower (*Helianthus annuus* L.). Plant Biol., 13: 632-642.
4. Kiani, S.P., P. Grieu, P. Maury, T. Hewezi, L. Gentzbittel and A. Sarrafi, 2007. Genetic variability for physiological traits under drought conditions and differential expression of water stress-associated genes in sunflower (*Helianthus annuus* L.). Theor. Appl. Genet., 114: 193-207.
5. Encheva, J., P. Shindrova and E. Penchev, 2008. Developing mutant sunflower lines (*Helianthus annuus* L.) through induced mutagenesis. Helia, 31: 61-72.
6. Iqbal, M., M.A. Ali, A. Abbas, M. Zulkiffal, M. Zeeshan and H.A. Sadaqat, 2009. Genetic behavior and impact of various quantitative traits on oil contents in sunflower under waters stress conditions at productive phase. Plant Omics J., 2: 70-77.
7. Cvejic, S., R. Afza, S. Jocic, S. Prodanovic, V. Miklic, D. Skoric and S. Dragin, 2011. Radiosensitivity of sunflower inbred lines to mutagenesis. Helia, 34: 99-105.
8. Haddadi, P., B. Yazdi-Samadi, M.R. Naghavi, A. Kalantari, P. Maury and A. Sarrafi, 2011. QTL analysis of agronomic traits in recombinant inbred lines of sunflower under partial irrigation. Plant Biotechnol. Rep., 5: 135-146.

9. Mostafa, G.G. and A.M. Alfrmawy, 2011. RAPD analysis for detection and fingerprinting of sunflower mutants induced by sodium azide. Int. J. Plant Breed. Genet., 5: 277-285.
10. Ebrahimi, A. and A. Sarrafi, 2012. Genetic variability and identification of markers associated with germination parameters in gamma-irradiation induced mutants of sunflower under water stress condition. Iran. J. Genet. Plant Breed., 1: 1-8.
11. Cvejić, S., D. Miladinović and S. Jocić, 2014. Mutation Breeding for Changed Oil Quality in Sunflower. In: Mutagenesis: Exploring Genetic Diversity of Crops, Tomlekova, N.B., M.I. Kozgar and M.R. Wani (Eds.)., Wageningen Academic Publishers, Wageningen, Netherlands, pp: 77-96.
12. Shehata, A.I., Z.H. Messaitfa, F.E. Quraini, H. Rizwana, A.A.A. Hazzani and M.S.E. Wahabi, 2014. Genotypic of salt stressed sunflower (*Helianthus annuus*). Int. J. Pure Appl. Biosci., 2: 40-61.
13. Totsky, I.V. and V.A. Lyakh, 2015. Pollen selection for drought tolerance in sunflower. Helia, 38: 211-220.
14. Yang, Y., Y. Guo, J. Zhong, T. Zhang and D. Li *et al*., 2020. Root physiological traits and transcriptome analyses reveal that root zone water retention confers drought tolerance to *Opisthopappus taihangensis*. Sci. Rep., Vol. 10. 10.1038/s41598-020-59399-0.
15. Khalil, F., S. Rauf, P. Monneveux, S. Anwar and Z. Iqbal, 2016. Genetic analysis of proline concentration under osmotic stress in sunflower (*Helianthus annuus* L.). Breed. Sci., 66: 463-470.
16. Tyagi, V., S.K. Dhillon, P. Kaushik and G. Kaur, 2018. Characterization for drought tolerance and physiological efficiency in novel cytoplasmic male sterile sources of sunflower (*Helianthus annuus* L.). Agronomy, Vol. 8. 10.3390/agronomy8100232.
17. Darbani, S.P., A.A. Mehrabi, S.S. Pordad, A. Maleki and M. Farshadfar, 2020. Effect of drought stress on agro-morphological traits in sunflower (*Helianthus annuus* L.) genotypes and identification of informative ISSR markers. Plant Genet. Resour.: Charact. Util., 18: 49-62.
18. Keipp, K., B.W. Hütsch, K. Ehlers and S. Schubert, 2020. Drought stress in sunflower causes inhibition of seed filling due to reduced cell extension growth. J. Agron. Crop Sci., 206: 517-528.
19. Razik, E.S.A., B.M. Alharbi, T.B. Pirzadah, G.S.H. Alnusairi, M.H. Soliman and K.R. Hakeem, 2021. γ-Aminobutyric acid (GABA) mitigates drought and heat stress in sunflower (*Helianthus annuus* L.) by regulating its physiological, biochemical and molecular pathways. Physiol. Plant., 172: 505-527.
20. Moussa, H.R., 2010. Low dose of gamma irradiation enhanced drought tolerance in soybean. Acta Agronomica Hungarica, 59: 1-12.
21. Zain, C.R.C.M., A.A. Kadhimi, A.N. Alhasnawi, A. Isahak, A. Mohamad, F. Doni and W.M.W. Yusoff, 2016. Enhancing of drought-tolerant rice (*Oryza sativa*) variety MRQ74 through gamma radiation and *in vitro* pathway. Biotechnology, 15: 125-134.
22. Nikièma, M.P., N. Ouédraogo, H. Traoré, M. Sawadogo, L. Jankuloski, M.A.G. Abdelbagi and D. Yonli, 2020. Sorghum mutation breeding for tolerance to water deficit under climate change. J. Plant Breed. Crop Sci., 12: 192-199.
23. Ábrahám, E., C. Hourton-Cabassa, L. Erdei and L. Szabados, 2010. Methods for determination of proline in plants. Methods Mol. Biol., 639: 317-331.
24. El-Mouhamady, A.B.A., A.A.M. Gad and M.A.F. Habouh, 2021. Determination of genetic parameters associated with salt stress tolerance in canola based on SCoT markers and protein pattern analysis. Asian J. Plant Sci., 20: 534-554.
25. Abu-Ellail, F.F.B., E.M.A. Hussein and A. El-Bakry, 2020. Integrated selection criteria in sugarcane breeding programs using discriminant function analysis. Bull. Nat. Res. Centre, Vol. 44. 10.1186/s42269-020-00417-6.
26. Aghaie, P., S.A.H. Tafreshi, M.A. Ebrahimi and M. Haerinasab, 2018. Tolerance evaluation and clustering of fourteen tomato cultivars grown under mild and severe drought conditions. Sci. Hortic., 232: 1-12.
27. Wasae, A., 2021. Evaluation of drought stress tolerance based on selection indices in haricot bean varieties exposed to stress at different growth stages. Int. J. Agron., Vol. 2021. 10.1155/2021/6617874.
28. Sun, F.L., Q. Chen, Q.J. Chen, M. Jiang, W. Gao and Y.Y. Qu, 2021. Screening of key drought tolerance indices for cotton at the flowering and boll setting stage using the dimension reduction method. Front. Plant Sci., Vol. 12. 10.3389/fpls.2021.619926.
29. Debebe, A., H. Singh and H. Tefera, 2012. Genetic variability and heritability studies in F4 progenies of Tef (*Eragrostis tef*). Asian J. Agric. Sci., 4: 225-228.
30. Ibrahim, S.D., A.F.A. El-Hakim, H.E. Ali and R.M.A. El-Maksoud, 2019. Genetic differentiation using ISSR, SCoT and DNA barcoding for quinoa genotypes. Arab. J. Biotech., 22: 103-118.
31. Alzahib, R.H., H.M. Migdadi, A.A.A. Ghamdi, M.S. Alwahibi, M. Afzal, E.H. Elharty and S.S. Alghamdi, 2021. Exploring genetic variability among and within hail tomato landraces based on sequence-related amplified polymorphism markers. Diversity, Vol. 13. 10.3390/d13030135.
32. Al-Khaishany, M.Y., F.H. Al-Qurainy, I.A. Alaraidh, M.N. Barakat and A.A. Elshafei *et al*., 2018. Genetic variation of wheat for salt tolerance based on physiological and agronomic traits. Int. J. Agric. Biol., 20: 2853-2861.
33. Hussain, S., M. Ahmad, S. Ahmad, J. Iqbal, M.N. Subhani *et al*., 2013. Improvement of drought tolerance in sunflower (*Helianthus annuus* L.) By foliar application of abscisic acid and potassium chloride. Pak. J. Nutr., 12: 345-352.

34. García-López, J., I.J. Lorite, R. García-Ruiz, R. Ordoñez and J. Dominguez, 2016. Yield response of sunflower to irrigation and fertilization under semi-arid conditions. Agric. Water Manage., 176: 151-162.
35. Gul, R.M.S., M. Sajid, S. Rauf, H. Munir, M. Shehzad and W. Haider, 2021. Evaluation of drought-tolerant sunflower (*Helianthus annuus* L.) hybrids in autumn and spring planting under semi-arid rainfed conditions. OCL, Vol. 28. 10.1051/ocl/2021012.
36. Rauf, S., H.A. Sadaqat, R. Ahmad and I.A. Khan, 2009. Genetics of root characteristics in sunflower (*Helianthus annuus* L.) under contrasting water regimes. Indian J. Plant Physiol., 14: 319-327.
37. Comas, L.H., S.R. Becker, V.M.V Cruz, P.F. Byrne and D.A. Dierig, 2013. Root traits contributing to plant productivity under drought. Front. Plant Sci., Vol. 4. 10.3389/fpls.2013.00442.
38. Ghaffari, M., M. Toorchi, M. Valizadeh and S. Komatsu, 2013. Differential response of root proteome to drought stress in drought sensitive and tolerant sunflower inbred lines. Funct. Plant Biol., 40: 609-617.
39. Pekcan, V., G. Evci, M.I. Yilmaz, A.S.B. Nalcaiyi, S.Ç. Erdal *et al*., 2016. Effects of drought stress on sunflower stems and roots. Int. Con. Chem. Agric. Life Sci., 3: 96-103.
40. Osman, H.S., 2015. Enhancing antioxidant-yield relationship of pea plant under drought at different growth stages by exogenously applied glycine betaine and proline. Ann. Agric. Sci., 60: 389-402.
41. Per, T.S., N.A. Khan, P.S. Reddy, A. Masood, M. Hasanuzzaman, M.I.R. Khan and N.A. Anjum, 2017. Approaches in modulating proline metabolism in plants for salt and drought stress tolerance: Phytohormones, mineral nutrients and transgenics. Plant Physiol. Biochem., 115: 126-140.
42. Naeem, M., M.S. Naeem, R. Ahmad, M.Z. Ihsan, M.Y. Ashraf, Y. Hussain and S. Fahad, 2018. Foliar calcium spray confers drought stress tolerance in maize via modulation of plant growth, water relations, proline content and hydrogen peroxide activity. Arch. Agron. Soil Sci., 64: 116-131.
43. Shemi, R., R. Wang, E.S.M.S. Gheith, H.A. Hussain and S. Hussain *et al*., 2021. Effects of salicylic acid, zinc and glycine betaine on morpho-physiological growth and yield of maize under drought stress. Sci. Rep., Vol. 11. 10.1038/s41598-021-82264-7.
44. Unyayar, S., Y. Keles and E. Unal, 2004. Proline and ABA levels in two sunflower genotypes subjected to water stress. Bulg. J. Plant Physiol., 30: 34-47.
45. Ocvirk, D., M. Špoljarević, M. Kristić, J.T. Hancock, T. Teklić and M. Lisjak, 2021. The effects of seed priming with sodium hydrosulphide on drought tolerance of sunflower (*Helianthus annuus* L.) in germination and early growth. Ann. Appl. Biol., 178: 400-413.
46. Gyugos, M., M. Ahres, Z. Gulyás, G. Szalai and É. Darkó *et al*., 2021. Light spectrum modifies the drought-induced changes of glutathione and free amino acid levels in wheat. Acta Physiol. Plant., Vol. 43. 10.1007/s11738-021-03253-x.
47. Darvishzadeh, R., A. Pirzad, I. Bernousi, A.B. Mandoulakani and H. Azizi *et al*., 2011. Genetic properties of drought tolerance indices in sunflower. Acta Agric. Scand. Sec. B: Soil Plant Sci., 10.1080/09064710.2010.530292.
48. Gholinezhad, E., R. Darvishzadeh and I. Bernousi, 2014. Evaluation of drought tolerance indices for selection of confectionery sunflower (*Helianthus anuus* L.) landraces under various environmental conditions. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, Vol. 42. 10.15835/nbha4219394.
49. Singh, D., A.K. Mall, V. Misra, M. Kumar and A.D. Pathak, 2018. Assessment of coefficient of variation, correlations between yield and yield attributes in sugar beet (*Beta vulgaris* L.). Plant Arch., 18: 15-18.
50. El-Mouhamady, A.B.A. and M.A. El-Metwally, 2020. Appreciation of genetic parameters and molecular characterization in some promising accessions of soybean (*Glycine max* L.). Pak. J. Biol. Sci., 23: 425-438.
51. Hamawaki, O.T., L.B. de Sousa, F.N. Romanato, A.P.O. Nogueira, C.D.S. Júnior and A.C. Polizel, 2012. Genetic parameters and variability in soybean genotypes. Comunicata Scientiae, 3: 76-83.
52. El-Mouhamady, A.B.A., H.M. Abdel-Rahman, A.A. Rizkalla and M.A. El-Metwally, 2019. Assessment of water stress tolerance in wheat genotypes based on half diallel analysis and DNA fingerprinting. Pak. J. Biol. Sci., 22: 103-116.
53. Chandrawat, K.S., K.S. Baig, S. Hashmi, D.H. Sarang, A. Kumar and P.K. Dumai, 2017. Study on genetic variability, heritability and genetic advance in soybean. Int. J. Pure Appl. Biosci., 5: 57-63.
54. Ejaz-Ul, H., K.A. Farooq, H. Sajida, S.H. Ahmad and B.S.M. Ahmad, 2020. Genetic diversity of sunflower genotypes under drought stress by principle component analysis. Genetika, 52: 29-41.
55. Ali, S. and M. Khodadad, 2020. Study of genetic and phenotypic diversity of sunflower (*Helianthus annus* L.) genotypes for agro-morphological traits under normal and drought stress conditions. Plant Prod. Sci. J. Agric., 43: 227-240.
56. Khatab, I.A., A.B.A. El-Mouhamady, S.A. Mariey, M.M. El-Hawary and M.A.F. Habouh, 2021. Molecular evaluation and identification of some barley hybrids tolerant to salt stress. Pak. J. Biol. Sci., 24: 997-1014.
57. Abdi, N., R. Darvishzadeh, H.H. Maleki, P. Haddadi and A. Sarrafi, 2013. Identification of quantitative trait loci for relative water content and chlorophyll concentration traits in recombinant inbred lines of sunflower (*Helianthus annuus* L.) under well-watered and water-stressed conditions. Zemdirbyste Agric., 100: 156-166.

58. Liang, C., W. Wang, J. Wang, J. Ma and C. Li*et al*., 2017. Identification of differentially expressed genes in sunflower (*Helianthus annuus*) leaves and roots under drought stress by RNA sequencing. Bot. Stud., Vol. 58. 10.1186/s40529-017-0197-3.
59. Dangi, R.S., M.D. Lagu, L.B. Choudhary, P.K. Ranjekar and V.S. Gupta, 2004. Assessment of genetic diversity in *Trigonella foenum-graecum* and *Trigonella caerulea* using ISSR and RAPD markers. BMC Plant Biol., Vol. 4. 10.1186/1471-2229-4-13.
60. Chaabane, R., M.E. Felah, H.B. Salah, M.B. Naceur and C. Abdelly*et al*., 2009. Molecular characterization of tunisian barley (*Hordeum vulgare* L.) genotypes using microsatellites (SSRS) markers. Eur. J. Sci. Res., 36: 6-15.
61. Mohamed, E.A.A.M. and E.T.A.E. Adel, 2012. Genetic diversity of some Saudi barley (*Hordeum vulgare* L.) land races based on microsatellite markers. Afr. J. Biotechnol., Vol. 11. 10.5897/ ajb11.2908.