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Research Article
Determination of Genetic Markers in Some Egyptian Varieties of Wheat and Barley under Salt and Drought Stresses

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Abstract

Background and Objective: Wheat and barley considered very important cereals for 100 millions of Egyptian population. Therefore, yield improving of some Egyptian varieties via genetic markers cereal crops under abiotic stresses drought and salinity is a crucial objective of this research. **Materials and Methods:** Five varieties of quadruple wheat were evaluated under salt stress and 14 varieties of barley were evaluated under drought stress to determine the genetic mechanisms related with molecular markers responsible for salinity tolerance in wheat and water deficit in barley. The techniques used were RAPD, ISSR and SSR-PCR, the obtained data of items studied were analyzed by molecular methods. **Results:** The results obtained from SSR revealed the presence of five molecular markers related to water stress tolerance in barley, three of which were positive for endurance and durability compared with control. While, RAPD-PCR revealed 3 markers which have 2 positive and one negative with primers OPE-26, E-10 and A-12, respectively. Furthermore, molecular studies of quadruple wheat for salt tolerance revealed the presence of 15 molecular markers from RAPD-PCR and ISSR techniques, six of which were positive with Beni-Sweif4, while Beni-Sweif1, 3 and Beni-Sweif5 had two positive markers for each of them. **Conclusion:** It could be concluded that RAPD, ISSR and SSR markers played vital and successful role to identify between all the genotypes used concerning salt stress in wheat and drought stress in barley, which could be helpful in the enhancement of cereals production in Egypt. This technology can be used as an indicator of molecular breeding in barley and wheat. This stage is the strategic bit for increasing the ability of abiotic stress tolerance of the studied lines and using it in local breeding programs.

Key words: Wheat, barley, genetic markers, environment conditions, abiotic stress

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

INTRODUCTION

Drought, marginal temperatures, chemical poisonous, oxidative stress, toxicity of heavy metals and salinity are considered serious abiotic threats to husbandry, yielding the environmental deterioration, decreasing the yield of plants and damage of human health. Abiotic stress is the main reason of crop defeat across the world, this lead to average reduction in harvests for most main crop plants near 50% or more[1]. Water deficit conditions is considering one of the most significant abiotic elements preventive plant germination as well as early seedling which brought within by salinity and drought[2], in addition, these factors are considered as common problems worldwide[3].

Drought is the most important ecological stress in husbandry around the world, consequently yield improving of cereal crops under drought is a crucial objective of plant breeding. Plant breeding improvement review refers to that selection for high harvest in stress free environments has to a definite range, improved yield indirectly in several water deficiency conditions. Drought as other abiotic stresses can affect the physiological status of plants and have opposing effects on growth, development and metabolism[4]. Drought affects plant life at many stages and levels, through decreasing water content and stomatal closure and so, affect gas exchange, decreases transpiration and finally interrupts photosynthesis[5]. Water shortage has many negative effects on metabolism and mineral nutrition which lead to decrease the area of the leaves and this alter assimilate partitioning among the plant organs[6]. Both drought and salinity interrupt the plants in a parallel ways[7].

In Egypt and all over the world, salinity is a main abiotic stress affecting crops. Globally about 800 million ha of terrestrial land are salt affected, this means that more than 6% of the entire land area[8]. Egypt suffers from the severe salinity problems, only 3% of total land area in the country is cultivated where the 3% of that land is already saline[9]. Water stress implemented by reducing the percentage and germination rate, as well as plantlet growth[10], besides seeds germination which is considered as the most critical stages of plant life, is seriously influenced by salinity[11].

Barley (*Hordeum vulgare* L.) is considered the fourth most cultivated crop worldwide. Water deficiency, mineral toxicity and salinity as ecological stresses, often influence plants lives in agricultural approaches representing main restrictions to the yield and superiority of barley as well as other harvests. Due to barley used as malt in human and animals feed, therefore, it is an essential crop. In extreme environments, which are frequently described by drought, salinity and low temperature, the essential goal for barley producers is the capacity to grow and production[12]. Reduction of possible water is a common result of both drought and salinity[13].

Simple Sequence Repeat (SSR) or micro-satellite markers are valuable for the plant breeders and genetic diversity investigations for many reasons. Small amounts of DNA sample are required, simple to magnify by polymerase chain reaction (PCR) and are mostly co-dominantly inherited, multi-allelic and plentiful in genomes of the plants[14,15].

In barley, more than 775 microsatellites have been published[16] and genetic maps established on these micro-satellites for all 7 chromosomes of barley are accessible for researchers[17]. Genetic diversity analysis various studies for both wild and cultivated barley have been completed using SSRs makers[18-22].

Ramsay *et al.*[17] established a novel SSR molecular markers for 16 genotypes of barley, whereas, Ellis *et al.*[23] have been examined the SSR variation on two loci and they concluded that the SSR markers have a wide-ranging of alleles and offers an exciting model of the effects of barley domestication. Furthermore, traits of barley related to salt tolerance were diagrammed by SSR markers[24], high level of allelic variations between barley landraces were described by Naeem *et al.*[25], they estimated genetic diversity between land races of barley were found in different geographical areas using SSR molecular markers.

Wheat (*Triticum durum* L.) is the most important cereal crop in the world and because of its nutritive value and different uses it is the main food for about one third of the world's residents or more. Wheat production can be improved via the enlargement of better-quality cultivars with extensive genetic base able to producing improved yield under numerous agro-climatic conditions and stresses[26]. Wheat is found in various habitats, it is considered as the most important meal for worldwide population[27]. About one sixth of the arable land in the world is cultivated with wheat and it is giving nearly 20% of the food calories supplies for the world's rising population[28].

Salinity is one from the major problems to wheat production, developing and growing salt tolerant wheat varieties can be better approach for salty soils. Therefore, genetic diversity is a requirement for developing salt tolerant wheat varieties[29].

Genotypic markers and the agro-morphological characters of traits possibly a valuable tool for the breeders to select genotypes with suitable diversity[30]. The RAPD markers are considered as heritable markers related to salt tolerance in three wheat genotypes and hence, help in marker-assisted breeding programs[31].

Plants exposure to abiotic stresses lead to produce reactive oxygen species (ROSs), which destruct cellular constituents[32]. Consequently, plants have established a sequences of enzymatic and non-enzymatic detoxification systems to reverse ROS, which protect cells from oxidative damage[33]. Antioxidant enzymes such as catalase (CAT), peroxidase (POX), glutathione reductase (GR) and superoxide dismutase (SOD) act in detoxification of superoxide[34,35] and H_2O_2. Genotypes selection capable of tolerate water scarcity can be assisted with molecular markers, progress in genetic maps developing for cereals, containing barley[36].

The present study aimed to develop marker (s) associated with drought tolerance in barley using ISSR and SSR markers, to detect possible specific markers to be utilized in the future breeding for drought tolerance in barley. As well as, try to find molecular genetics parameters of wheat lines tolerant to salinity and water deficit.

MATERIALS AND METHODS

Plant material

Barley: Fourteen varieties of barley; 7 drought tolerant (T) namely; Giza123, Giza124, Giza126, Giza127, Giza128, Giza130 and Giza 2000 (T1, T2⋯T7) and another 7 sensitive (S) varieties for drought namely; (Giza121, Giza122, Giza125, Giza129, Giza132 Giza133 and Giza134, (S1, S2⋯.S7) were used under drought stress conditions.

Wheat: Five genotypes of durum wheat namely; Beni-Sweif1, 3, 4, 5 and Sohag3 were used under salinity stress conditions.

All the varieties were kindly provided by the Agriculture Research Centre, Giza, Egypt. Pot experiments were carried out in a greenhouse for two seasons 2014/2015 and 2015/2016 at National Research Centre, Dokki, Giza, Egypt.

Greenhouse experiment: Well-washed sandy soil with distilled water was used. Ten plastic pots were used for each replicate, (30 cm diameter×27cm length) pots were filled with the previously washed sand soil.

Drought experiment for barley: There were three treatments and control; control pots were supplemented with 1 L from tap water and three treatments supplemented with 700, 500 and 300 mL of tap water every 5 days respectively, three replications for each were used.

Salinity experiment for quadruple wheat: Three concentrations (treatments) 4000, 8000 and 12000 ppm from Na^+Cl^- solution were used, while only tap water was used for the control pots, three replications for each were used. Wheat varieties to be evaluated were grown in a temperature-controlled greenhouse under 24/16°C, day/night cycle and mean RH was (80%) and complete light hours to 12 h by artificial lamp. The pots were arranged in a factorial randomized complete block design. Five grains were sowed in each pot. The experiment was irrigated by tap water with three NaCl concentrations treatment. All planted pots were supplemented with Hoagland stock solution[37] which was used as the base nutrient medium.

Data recording: Data were scored for the following agronomic and developmental traits: plant height (PH), spike length (SL), number of sterile spikelet's/spike (SSS), number of kernels/spike (KS), total above ground biomass/plant (TBP), harvest index (HI), ag leaf area (LA) and grain yield (YLD). The stem height, spike length and the leaf area (leaf width×leaf length×0.75) were measured on 20 randomly selected plants. Harvest index and grain yield were measured after harvesting plots at maturity. As a measure of drought tolerance, four indices were calculated using the following relationship[38]:

$$SSI = \frac{(1)\ Ys/Yp}{(1)\ Xs/Xp}$$

The differences among means (mean±SD) were compared using Duncan's new multiple ranges tests[39].

DNA extraction: DNA of the barley and wheat genotypes was extracted using EZ-10 Spin column Genome DNA Minipreps kit method. RAPD, ISSR and SSR reaction conditions: RAPD analysis was performed using 5 RAPD primers (Metabion, Martinsried, Germany) (Table 1) and 5 ISSR primers (Table 2)

Table 1: Names and sequences of 5 RAPD primers used for PCR molecular analysis for barley and durum wheat

Primer type	Number	Code name	Sequences
RAPD	a	A-12	5' TCGGCGATAG 3'
	b	E-10	5' CACCAGGTGA 3
	c	OPC-19	5 'GTTGCCAGCC 3'
	d	OPE-26	5' AACGGTGACC 3'
	e	OPT-08	5' AACGGCGACA 3'

Table 2: Names and sequences of five ISSR primers used for PCR molecular analysis for durum wheat

Primer type	Code name	Sequences
ISSR	M-1	5' (AC)8 CG 3'
	UBC-811	5' (GA) 8 C 3'
	UBC-817	5' (CA)8 A 3'
	UBC 814-32	5' (CT)7CCTA 3'
	UBC 876-32	5' (GATA)2 (GACA)2 3'

Table 3: Names and sequences of 5 SSR primers used for PCR molecular analysis for barley

Primer type	Primer name	Code name	Sequences
SSR		Forward	Reverse
	WMS 06	5-CGT ATC ACC TCC TAG CTA AAC TAG-3	5-AGC CTT ATC ATG ACC CTA CCT T-3
	WMS 30	5-ATC TTA GCA TAG AAG GGA GTG GG-3	5-TTC TGC ACC CTG GGT GAT TGC-3
	WMS 108	5-ATT AAT ACC TGA GGG AGG TGC-3	5-GGT CTC AGG AGC AAG AAC AC-3
	WMS 118	5-GAT GGT GCC ACT TGA GCA TG-3	5-GAT TG TCA AAT GGA ACA CCC-3
	WMS 149	5-CAT TGT TTT CTG CCT CTA GCC-3	5-CTA GCA TCG AAC CTG AAC AAG-3

Table 4: Alleles number, fragment size range and polymorphism detected by five RAPD-PCR primers of 14 barley (*Hordeum vulgare* L.) genotypes

Primers	No.	Fragment size (bp)	Number of alleles	Monomorphic alleles	Polymorphic alleles	Polymorphism (%)
A-12	a	100-1050	11	5	6	54.55
E-10	b	300-950	22	7	15	68.18
OPC-19	c	180-790	18	8	10	55.56
OPE-26	d	200-850	13	6	7	53.85
OPT-08	e	100-1650	17	5	12	70.59
Total	-		81	31	50	
Mean						61.73

were produced from Integrated DNA Technologies Inc., (San Diego, CA, USA) based on core repeats anchored at the 5 or 3 end as shown in Table 1. Regarding to RAPD reaction, the mixture was standardized to 20 µL (PCR buffer 1X, $MgCl_2$ 2.5 mM, dNTPs 1 mM, Primer 50 ng, *Taq* Polymerase 1 unit, genomic DNA 25 ng). The PCR program was set as 45 cycles of 36°C: 1 min annealing, 2 min extension at 72°C and 7 min final extension at 72°C. The products of RAPD-PCR were analyzed on 1.5% (w/v) agarose gel.

Regarding to ISSR reaction, the mixture was standardized to 20 µL (PCR buffer 1X, $MgCl_2$ 2.5 mM, dNTPs 1 mM, 10 pmol of each primer, *Taq* Polymerase 1 unit, genomic DNA 50 ng). PCR program was set as 40 cycles of 56°C: 1 min annealing, 2 min extension and 10 min final extension at 72°C. The products of ISSR-PCR were analyzed on 1.4% (w/v) agarose gel. Gels were photographed under gel documentation system (Syngene™) and size of amplicons was detected using 1 Kb DNA ladder (Ferments Life Sciences). Likewise, 5 SSR primers were used in PCR reactions listed in Table 3.

RESULTS

Drought stress in barley: In this study,14 varieties of barley were studied under the influence of three treatments of irrigation amount with 300, 500 and 700 mL of water, where control pots supplemented with 1 L of water as well as developing plants supply lotion with nourishing Hoagland. Six traits were studied: grain yield, number of tillers, spike length, number of leaves, flag leaf area and plant height. Data revealed the following: two characters were highly significant (grain yield and flag leaf area) and three characters were significant (number of tillers, spike length and plant height), while, number of leaves character was non-significant in all varieties using statistical analysis.

Molecular studies for drought stress in barley

RAPD-PCR assay: Fourteen varieties from barley (*Hordeum vulgare* L.) had been tested using RAPD-PCR analysis. All barley varieties were found to be associated with drought stress, 7 of them were drought tolerant and the other 7 genotypes were sensitive. For this purpose, five oligonucleotide decamer RAPD primers OPE-26, A-12, E10, OPC-19 and OPT-08 were used (Table 4, Fig. 1a-b) electrophoretic profiles of PCR products obtained with used primers.

All primers revealed 81 bands in 14 genotypes where, 31 were monomorphic bands and 50 were polymorphic bands with 61.73% polymorphism as shown in Table 4. For instance, primer E-10 revealed 22 bands, 15 of them were polymorphic with ratio 68.18%, while primer OPC-19 were showed 18 bands, 10 of them were polymorphic, 8 monomorphic and polymorphism was 55.56%.

SSR-PCR assay: In this technique five SSR primers were used to detect genetic markers for drought stress in barley, primers revealed 5 markers with molecular sizes 640, 610, 590, 570 and 560 bp with primers WMS 06, WMS 30, WMS 108, WMS 118 and WMS 149 respectively, (Fig. 2, Table 5).

Amplified microsatellite loci were analyzed for polymorphism using polyacrylamide gel electrophoresis. The results of polymorphism for five SSR markers are shown in Fig. 2, number of alleles, monomorphic and polymorphic alleles found in 14 barley varieties using five SSR primers are shown in Table 5. Among 32 alleles that were detected in 14 barley varieties which they were had an average number of 6.4 allele's per-microsatellite/genotypes locus. While, these primers revealed 71.88% of polymorphism in similar experiments. Drine *et al.*[40] and Gupta *et al.*[41] revealed higher polymorphism percentage in *H. vulgar* for drought stress 77.78% when they used 12 SSR primers.

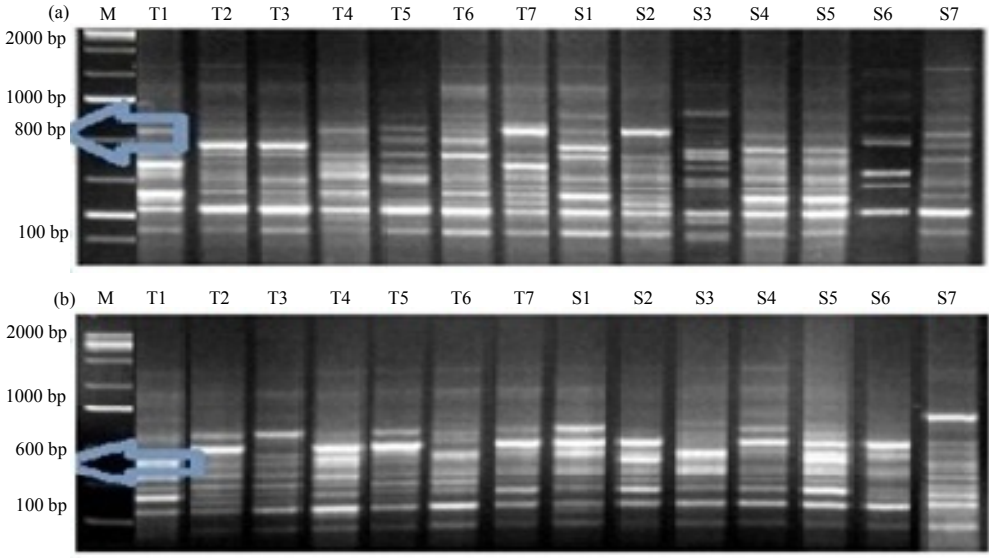

Fig. 1(a-b): Electrophoretic profiles of barley (*Hordeum vulgare* L.) of 14 varieties plants induced with RAPD d, (a) OPE-26 and (b) Primer b. E-10

M: DNA ladder, T (1-7): Tolerant variety and S (1-7): Sensitive variety

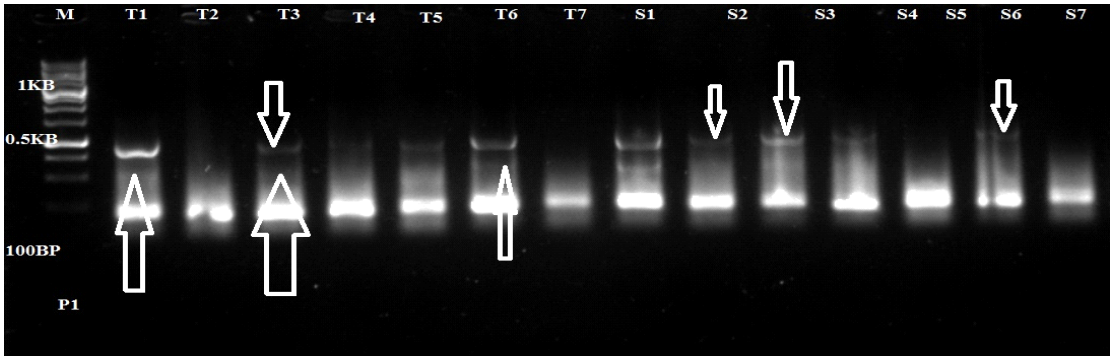

Fig. 2: SSR amplicons for drought stress in 14 varieties from barley (*H. vulgare*) genotypes using primer WMS 06 as an example

M: DNA ladder, T (1-7): Tolerant variety and S (1-7): Sensitive variety

Table 5: Allele's number, fragment size range and polymorphism detected by SSR loci in the 14 varieties of barley (*Hordeum vulgare* L.)

Primers	Fragment size (bp)	Number of alleles	Monomorphic alleles	Polymorphic alleles	Polymorphism (%)
WMS 06	250-1600	6	1	5	83.33
WMS 30	100-610	5	2	3	60.00
WMS 108	100-970	7	2	5	71.43
WMS 118	80-710	6	2	4	66.67
WMS 149	200-1500	8	2	6	75.00
Total	-	32	9	23	
Mean					71.88

Salt stress in tetraploid wheat: The analysis of variance for all the studied traits was presented in Table 6. The differences among genotypes were highly significant for all studied traits except "number of leaves per plant" which was not significant.

Mean performance of all five wheat varieties revealed that Beni-Swaif4 variety recorded the highest values for all the studied traits, while Sohag3 variety recorded the lowest values under salt stress. These results indicated that Beni Swaif4 variety was the

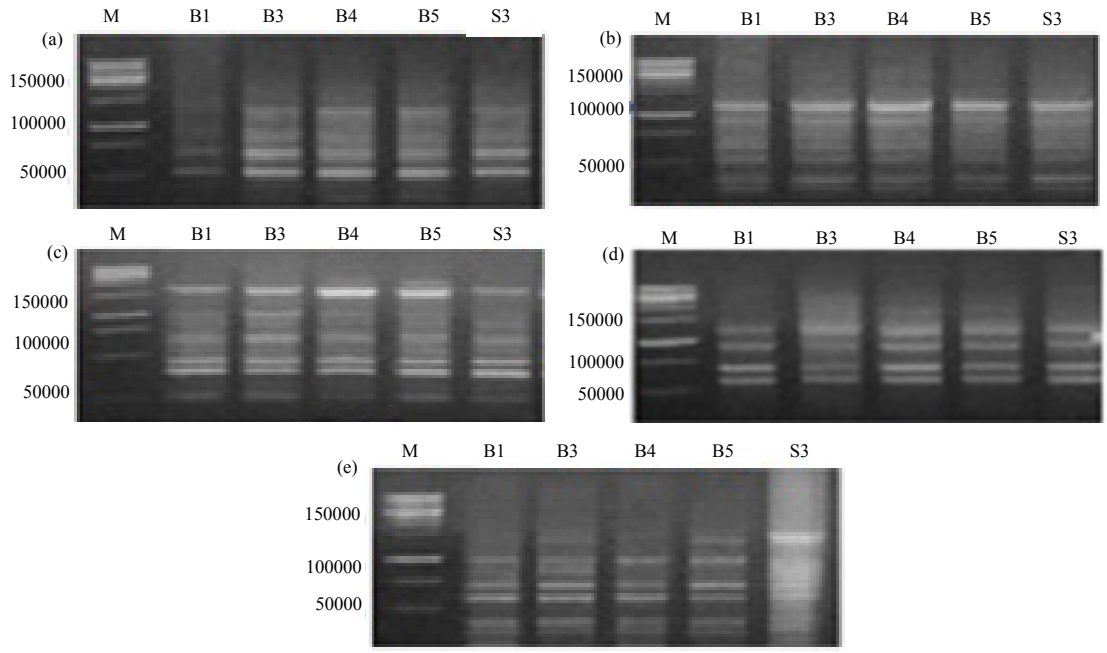

Fig. 3(a-e): (a-e) RAPD-PCR fragments of with five durum wheat (Beni-Sweif1, 3, 4, 5 and Sohag 3) varieties using five primers
M: DNA ladder

Table 6: Analysis of variance of RCBD for studied traits of 5 wheat genotypes

Sources	DF	PH	FLA	NO. L/P	SL	GY	NO. K/P
REP	2	10.87	14.47	0.47	0.61	0.00	1.07
GEN	4	1862.00**	185.23**	28.10^{ns}	29.45**	0.08**	384.90**
Error	8	70.78	3.38	0.30	0.34	0.01	0.40
CV		8.41	8.46	8.93	7.05	12.51	4.43

**indicate significance at 0.05 level and ns: Non-significance

Table 7: Mean performance of five wheat varieties

Gen	PH	FLA	NO. L/P	SL	GY	NO. K/P
Beni-Sweif1	106.00	16.67	7.00	6.40	0.60	10.00
Beni-Sweif3	100.33	26.33	3.33	7.70	0.67	12.67
Beni-Sweif5	95.67	21.67	5.33	10.07	0.56	15.67
Sohag 3	64.33	12.00	4.00	4.60	0.43	1.00
Beni-Sweif4	134.00	32.00	11.00	12.60	0.86	32.00
$LSD_{0.05}$	15.84	3.46	1.03	1.10	0.15	1.19

Table 8: Summary of statistics of high and lowest variety

	Simple statistics			
Variable	Mean	Std dev.	Minimum	Maximum
PH	100.07	23.96	56.00	144.00
FLA	21.73	7.54	11.00	34.00
NO.L/P	6.13	2.88	3.00	11.00
SL	8.27	2.95	4.50	12.80
GY	0.62	0.16	0.40	0.88
NO. K/P	14.27	10.50	1.00	33.00

most tolerant, while Sohag3 variety was the most sensitive variety (Table 7, 8).

Consistent significant highly positive correlation coefficients among all the studied traits were found (Table 9).

These results indicated that the higher of any of these traits, the higher of other traits. Therefore, any of these traits may be considered as a good selection criteria for selecting for any of the other traits.

RAPD and ISSR-PCR assays: Using RAPD and ISSR-PCR techniques, three replicates from five durum wheat varieties; Sohag 3, Beni-Sweif 1, 3, 4 and 5 were tested for salt tolerance. Fifteen molecular markers for salt tolerance were found; six of them were positive with Beni-Sweif4 and three were negative with Sohag3 at 12000 ppm concentration. While, two molecular markers were revealed for salt stress from each of Beni-Sweif1, 3 and 5, respectively, these results shown in ISSR profile listed in Table 10, Fig. 3 and 4.

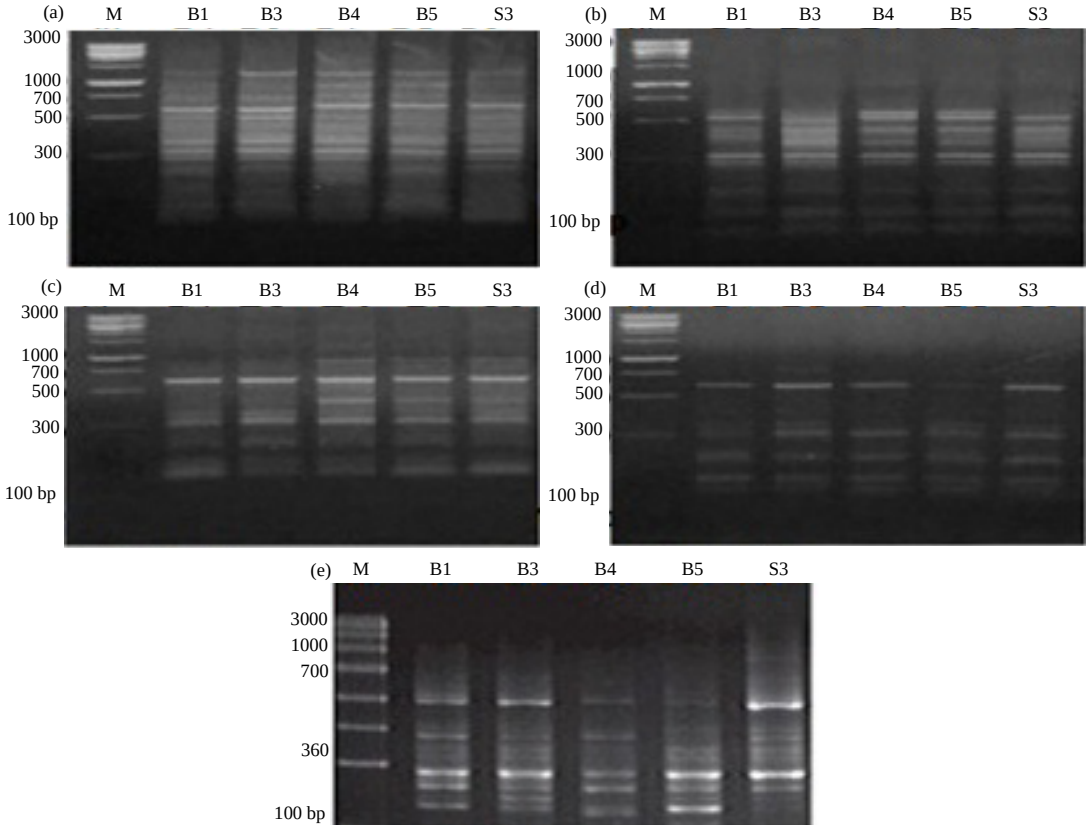

Fig. 4(a-e): (a-e) ISSR-PCR fragments for with five durum wheat Beni-Sweif1, 3, 4, 5 and Sohag3 varieties using five ISSR primers
M: 1 KB ladder

Table 9: Rank correlation coefficients between traits

Sources	FLA	NO.L/P	SL	GY	NO. K/P
PH	0.77**	0.78**	0.82**	0.81**	0.88**
FLA		0.55*	0.81**	0.81**	0.88**
NO.L/P			0.69**	0.68**	0.81**
SL				0.71**	0.94**
GY					0.86**

*,**Significant at 0.05 and 0.01 probability levels, respectively

Table 10: Total markers for salt stress with three concentrations of $Na^{+}Cl^{-}$ using five (RAPD and ISSR) primers, with five durum wheat

Primer type	Number	Primer	Band size (bp)	Beni-Sweif1	Beni-Sweif3	Beni-Sweif4	Beni-Sweif5	Sohag3
RAPD	a	A-12	1412	+	-	-	-	-
	b	E-10	1105	-	-	-	+	-
	c	OPC-19	870	-	+	-	-	-
	d	OPE-26	1500	-	-	+	-	-
	e	OPT-08	950	-	-	+	-	-
ISSR	A	M-1	765	+	-	-	-	-
			557	-	-	+	-	-
			385	-	-	+	-	-
	B	UBC-811	690	-	-	+	-	-
	C	UBC-817	668	-	-	-	-	+
			480	-	-	-	+	-
			415	-	-	-	-	+
			270	-	-	-	-	+
	D	UBC 814-32	630	-	-	+	-	-
	E	UBC 876-32	610	-	+	-	-	-
Total	-	$p^{+}2$	$p^{+}2$	$p^{+}6$	$p^{+}2$	$3N^{-}$		

The final results revealed that, the cultivar (Beni-Sweif4) recorded the highest number of positive markers (6 markers), where two of them were found with the primers (OPE-26 and OPT-08) with molecular sizes of 1500 and 950 bp in RAPD-PCR analysis, while the other four markers with molecular sizes of 557 and 385 bp with M-1 primer, 690 bp in UBC-811 primer and the molecular size (630 bp) in UBC-814-32 primer were found in ISSR analysis. On the other hand, the genotypes (Beni-Sweif 1, 3 and 5) were recorded the lowest rank of positive markers (two markers) for each one of them.

Where, the molecular size 1412 bp for primer A-12 in RAPD-PCR analysis and 765 bp for M-1 primer in ISSR analysis were found in Beni-Sweif1, the molecular size 870 bp for OPC-19 primer in RAPD-PCR analysis and 610 bp for UBC-876-32 primer in ISSR analysis were observed in Beni-Sweif3 and the molecular size 1105 bp for E-10 primer in RAPD-PCR analysis and 840 bp for UBC-817 primer in ISSR analysis were generated in Beni-Sweif5, respectively. On the same track, the three positive markers with molecular sizes 668, 415, 270 bp were generated using primer UBC-817 only in the profile of ISSR for Sohag3 cultivar.

In this research, five genotypes of wheat were studied via ISSR and RAPD techniques. Meanwhile the PCR procedures have been developed; prosperity of novel DNA marker technologies has arisen, permitting the creation of high-density molecular maps for all the main crop species. Similarly, molecular markers have been widely used in the genetic diversity analysis of plant crops. Based on the data achieved by RAPD analysis, it was possible to differentiate between the five wheat genotypes used.

DISCUSSION

The advantages of DNA-based markers have overcome disadvantages of others as isozyme markers and have been practical effectively to differentiate between individual genotypes in a wide range of plant species[42], this is frequently referred to as "DNA fingerprinting" for example random amplified polymorphic DNA (RAPD), ISSR and SSR-PCR techniques. Which is based on the use of short primers of arbitrary nucleotide sequence, have many of advantages above other DNA-based marker systems thereof, have been revealed to be functional for many applications. These studies demonstrate that it is possible to obtain RAPD, ISSR and SSR-PCR profiles that are reproducible and unique to different genotypes.

From the previous results (Table 4 and Fig. 1d-b), it could be concluded that, the primers (E-10, OPC-19 and OPT-O8) recorded the highest numbers of amplicons (22,18 and17 respectively), while the other primers coming in the second rank. In addition the highest polymorphism (%) were generated with the primers (E-10, OPT-O8) where the values were 68.18 and 70.59%, respectively. The five primers succeeded to improve that, the methodology responsible for identification and classification of the bands related with water stress tolerance in the used lines of barley and these bands also, could be used to differentiate within the previous genotypes. RAPD revealed two positive markers with 750 and 600 bp in OPE-26 and E-10 primers, respectively. On the other hand, primer A-12 showed a negative marker for drought stress with 340 bp.

Using RAPD has been proven to have several advantages over other techniques of DNA fingerprinting[42], it is very simple to perform and it does not necessitate previous knowledge of the genome in this study. These results aid to detect tolerant genotypes for drought stress in *H. vulgar*.

It was observed that the highest number of alleles per locus/genotype using SSR primers set WMS 149 showing 8 alleles, while the lowest allele number per locus among the homologous chromosomes was observed using SSR primer set WMS 30 revealed 5 alleles. However, Ivandic *et al.*[43] also, found similar findings 5.5 alleles per locus from wild barley (Fertile Crescent). These results may be aid the breeders to improve barley for drought stress tolerance under Egyptian conditions.

The results obtained from the five SSR primers (WMS06, WMS30, WMS108, WMS118 and WMS149) detected that the five markers with molecular sizes of 640, 610, 590, 570 and 560 bp, respectively succeeded to identify tolerance indices responsible for increasing, improving and enhancing the ability of water deficit tolerance in the previous barley genotypes. These markers help barley's breeders for increasing water stress resistance through hybridization between these tolerant genotypes among sensitive cultivars to produce F1 generation, then reaching to genetic stability lines and using it in the Egyptian barley breeding programs to solve water stress problem for increasing yield and quality of local varieties.

In barley, there is a little variation at allelic level and high genetic relationship among verities; hence these markers were not very effective in case of barley. Furthermore, evaluation and characterization of genetic diversity between and within species, as well as populations consequently find marker correlated to particular characters have been demonstrated to be valued tools in molecular markers[44]. Hence, different markers could be reveal different types of variations, it is related to the genome segment measured by each kind of marker, their spreading through the genome and the extent of the DNA target which is analyzed by specific assay[45-47].

In addition, DNA procedures permit the evaluation of a hypothetically indefinite polymorphic marker loci number[48]. Varieties of molecular markers were used to assess the genetic variations level. Microsatellite or Simple Sequence Repeat (SSR) is the choice marker for numerous genetic studies in barley. The SSR markers have several advantages, for example locus specificity, codominance, high level of polymorphisms, suitability when using PCR, randomize spreading through the genome and reproducibility[14,15], for barley, SSR is technically effective and are available with low cost[17]. Inter simple sequence repeat (ISSR) marker, using PCR amplifications of DNA, which consists of a microsatellite arrangement by 2-4 arbitrary, could be used to evaluate variability and genetic marker[49].

These results confirmed that, these 15 markers obtained from (RAPD and ISSR) analysis are responsible for salinity tolerance in the 5 genotypes of wheat and revealed also the vital role of (RAPD, SSR and ISSR analysis) for classification and identifying the most tolerance and sensitive genotypes for salinity.

The wheat genotypes showed different responses to Na+Cl- stress, Sohag3 was sensitive while Beni-Sweif4 was tolerant to increasing salt concentration. Previous results showed that, with increasing salinity in the environment, increased salt concentration affected wheat genotypes, these results are in agreement with those results by Sairam *et al*.[50] and Kafi *et al*.[51].

The determination of genotype specific ISSR markers was completed, 15 markers can be considered as a valuable marker for salt tolerance screening in the five genotypes of wheat. Determination of RAPD, SSR and ISSR markers were completed the big role to identify the genetically mechanisms responsible for salinity tolerance in the strategic crops such as wheat and barley through generating 15 markers can be considered as a valuable markers for the resistance of high concentration of soil salinity. Salinity influences the plant growth by affecting both of osmotic stress of the salts adjacent to the roots and by toxicity caused by extreme accumulation of salts in plant leaves[52].

Incomparable studies by Moghaieb *et al*.[52] suggested that, Giza 160 and Sids-1 are salt sensitive, while Sohag, Beni-Sweif, Gemmiza10, are salt tolerant. It could be their ability to preserve higher osmotic potential comes from the accumulation of high concentration of osmotic solutes. They concluded that, according to the determination of genotype specific molecular markers, these molecular markers can be considered as a practical tool for salt tolerance in wheat breeding programs.

DNA polymorphism using 148 RAPD primers reported by Mehboob-ur-Rahman *et al*.[53]. Whereas, Moghaieb *et al*.[31] reported the effect of genetic composition on salt tolerance in seven wheat genotypes. They determined specific RAPD markers for each cultivar genotype. likewise, they determined unique RAPD markers for salt tolerant genotypes.

In other study for Shahzad *et al*.[54], they evaluated 58 exotic and 129 Pakistani wheat cultivars/landraces which grown in Hoagland's solution, under control (where tap water equivalent to 10 mM salt) and salt stress (200 mM NaCl) conditions. They found 12 SSR markers linked to salt tolerance due to their amplification in tolerant genotypes only. Five markers were recognized as most suitable to estimate salt tolerance since these markers were associated with 4 or more salt tolerance traits in the study. Cultivars Sakha-92 from Egypt and 4098805, 10823, Pasban90, 10828 and accessions 10790 from Pakistan performed finest at both salt stress levels. SSR markers revealed high genetic variation in the wheat genotypes.

Ahmad *et al*.[29] found that Egyptian variety (11466) and Pakistani variety (11299) were found to be the most salt tolerant wheat genotypes and three unique DNA amplicons were formed from the RAPD primers OPF13 and OPA2 in some tolerant wheat genotypes only. Finally they concluded that, these fragments should be have more studies to prove their relationship with genes for salinity tolerance

Vaja *et al*.[27] examined eight RAPD primers (OPM-07, OPM-05, OPM-14, OPB-19, OPB-07, OPA-17, OPA-1 and OPR-14) which amplifying unique genotype and specific bands to classify salt tolerant and susceptible wheat genotypes. They found that the OPB-19 primer generate three unique bands to identify tolerant genotype KRL-213. They considered OPB-19 primer as a useful one to distinguish wheat genotypes against salt tolerance trait for crop improvement program.

Many mechanisms for plants to tolerate the salinity problems and most of these mechanisms are genetically controlled. New methods as biotechnological procedures to develop salinity tolerance in crops are essential to make a successful adaptation to saline environment. To enable the selection of wheat genotypes for salt tolerance DNA markers can help in this situation[55].

The SSR considered as the most variable constituent of the genome in different eukaryotes with high rate of molecular development, consequently the sequence and distribution of SSR markers may be providing approaching into phylogenetic relationships within varieties and species[52].

Wheat is the main human edible product in the majority areas of the world and it is a moderately salt tolerant crop and its harvest is significantly reduced when the salinity level of the soil rises to 100 mM NaCl[56]. Therefore, the discovery of new sources of salt tolerance genotypes is primarily essential to develop crop varieties appropriate for salty soils[54].

CONCLUSION

In this study, 14 barley varieties were evaluated under water shortage; molecular studies have been analyzed using the RAPD-PCR and SSR techniques. Results found that, 7 varieties were sensitive and 7 varieties were tolerant genotypes. RAPD-PCR data revealed the presence of two positive and one negative marker for water deficit. Furthermore, SSR technique showed 5 markers. In wheat experiment, the highest number of RAPD specific markers was scored for Beni-Sweif 4 (6 positive markers), while, Sohag3 scored three markers.

SIGNIFICANCE STATEMENT

"This study discovered the presence of many genetic molecular markers associated with drought in barley via RAPD-PCR technique; three markers and SSR-PCR technique showed 5 markers. Furthermore, results of wheat confirmed that the 15 markers obtained from RAPD and ISSR were responsible for salinity tolerance in the five genotypes. In Egypt insufficient studies have examined the genetic diversity by SSR or ISSR molecular markers within Egyptian barley and wheat genotypes. This study is a trial to fill a part of this gap. For this reasons this study will be an important in this direction.

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