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Elicitation of salt stress-tolerant mutants in bread wheat (*Triticum aestivum* L.) by using gamma radiation

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Abstract

Background: Given the strategic importance of wheat being the staple food for the vast majority of people, it was necessary to know reasons for the contraction and decline of its area globally and consequently its lower yield. Among the most important of these problems is the problem of a high level of salinity in both soil and irrigation water coming at the forefront of environmental challenges that hinder its production process at the local and global levels. Therefore, the genetic improvement of wheat for salinity tolerance was one of the most important priorities of this investigation.

Results: The seven wheat accessions (Sakha 8 and its six M5 derived mutants) succeeded in drawing unique cases of salinity tolerance and were excellent especially mutants 1, 2, 3, and 5. The rest genotypes were coming in the second rank for this purpose and were very good in this regard. The promising wheat genotypes which recorded high salinity tolerance in the recent investigation exhibited also high genetic stability. This fact was proved after estimating some agro-morphological and physiological traits related to salinity tolerance based on evaluating some important genetic parameters besides salinity tolerance indices under stress experiment compared to the control treatment within two seasons. Data evaluated of expected genetic advance (GA) based on 5% selection proved that all values calculated during the two seasons under both treatments appeared low for all studied traits in this regard. However, it reflects the success of breeding for salinity tolerance in wheat using mutations but in relative terms. Molecular marker analysis profile using the six ISSR primers exhibited a total of 173 markers, 12 of them were monomorphic, while that 161 bands appeared polymorphic included 56 unique bands or positive markers and 7 negative markers with 93.06 % (polymorphism).

Conclusion: The original wheat variety (Sakha 8) and its six M5 derived mutants exhibited high tolerance of salinity stress in all studied traits based on all genetic parameters and salinity tolerance indices calculated for both seasons under salinity treatment compared to the normal conditions. DNA fingerprinting analysis as well for the six wheat mutants besides the local variety proved that these genotypes were recorded highly genetic differences among them.

Keywords: Wheat, Salinity tolerance, Gamma rays, Mutation, Genetic variation, Heritability, Expected genetic advance, ISSR markers, DNA fingerprinting

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Background

Wheat is one of the most important food crops over the centuries not only for humans, but also for animals and birds with the increasing of the global wheat consumption due to the growing population. At the present time, it is noted that the area and productivity of wheat in Egypt are subjected to many environmental challenges and constraints such as high level of soil salinity and irrigation water, water poverty, and high toxicity of heavy metals. In addition, various diseases and all these factors would reduce productivity. Particularly noteworthy in this context, the imminent danger resulting from increasing salinity level in soil and irrigation water because high salinity directly impedes on all vital processes necessary for growth and germination that cause a reduction in wheat productivity by 40 to 50%.When we talk about the problem of water poverty and the decline in the share of water needed for agriculture in Egypt, it is noted that this will be in line with the rising of salinity level in soil especially in coastal land nearing from the seawater. Therefore, scientists have joined forces to solve this problem and reduce the risk of high salinity. This will do through traditional methods of plant breeding including a breeding program by mutations besides modern methods such as biotechnology for improving and developing new wheat accessions tolerant for salinity as well as keeping high yielding under this stress. Singh and Balyan (2009) studied induced mutations in bread wheat for improving plant height, grain quality, and some yield traits in Kharchia 65 cultivar using four levels of gamma rays (10, 20, 30, and 40 KR) in addition to the control. This investigation succeeds in generating 3 mutant progenies that may be fruitful and excellent for development and increasing yield traits. High limit of salinity or water stresses decreasing from 35 to 57% of the final yield of crops when drought stress is taken as 40–45% of soil normal water content (NWC: 100%) (Balla et al. 2011). The improvement and depiction of anew TiLLING population in selected mutant wheat accessions were conducted by Chen et al. (2012). Results confirmed that this mutant population representatives are considering a fruitful and important new material for the wheat explore society and the use of this invert genetic differences will supply version allelic divergence for wheat amelioration and practical genomics. Heiba et al. (2016b) showed the impact of 0.3% of ethylmethane sulphonate (EMS) for the detection of mutation chances of DNA in 3 bread wheat entries using RAPD and ISSR primers. Results revealed that RAPD primers given a total of 57 fragments under the normal conditions where 27 of them were polymorphic besides 17 new amplicons observed after treating with (EMS), while the ratio of mutation induction by ISSR markers was 0.08% which generated 4 various new bands, respectively. Genetic diversity in sodium azide induced wheat mutants through analyzing SSR primers was determined by Sen and Sarsu (2018). Results detected that SSR marker profiles generated total mean values of polymorphism rate (29.44%), polymorphic information content (PIC; 0.82), marker index (MI; 1.95), and resolving power (Rp; 1.31) in 44 generation advanced wheat mutant lines which indicated that SSRs succeed to screen genetic diversity in sodium azide induced of the previous wheat mutant accessions. Sahoo et al. (2018) detected salinity stress tolerance in some wheat accessions which emphasized that it is difficult to rely solely on the genetic aspects to reduce risks of salinity on wheat crops. But, in addition, management in reclamation land damaged with salinity as well as selecting for cultivars' high tolerance for this stress. Genetic variability, physiological, and agronomical traits related to salinity stress tolerance were estimated by Al-Khaishany et al. (2018). Results detected that salinity stress is important and highly impacted all wheat accessions and diminished physio-biochemical indices and agronomic traits, whilst significantly increased free proline and Na^+ contents in leaves. Also, agro-morphological traits under investigation may be fruitful and very important to screen, discover, and enhance salinity tolerance in some wheat lines by Yassin et al. (2019) depending on the data of tolerance indices. Shoot length, shoot dry weight, and catalase content traits besides multivariate analysis, clustering, path analysis, and MANOVA were very important indices for genotype characterization to salinity tolerance as well as setting limits for this tolerance (Al-Ashkar et al. (2019)). Saddiq et al. (2019) revealed the methods of decreasing salinity stress in wheat by improving the physiological state of seedlings. The final results detected that using KCL, NaCL, and H_2O effectively prevents and relieves the effects of salt stress and ensures good, fast, and equal germination of all seedlings as well as modification of its physiological state in a way that does not affect all future vital processes. The main objective of the present investigation is producing of some wheat genotypes tolerant to salinity stress using mutations within the framework of the process of genetic improvement of wheat to face abiotic stresses with the aim of developing the Egyptian wheat crop.

Materials and methods

Background of materials under investigation

This study used Sakha 8 wheat variety, 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 tolerant to salinity and resistance for many diseases. Therefore, this cultivar is an excellent experimental material that can be used in this study.

Field evaluation

The seeds used for the present investigation were originally performed from the wheat research department, Agriculture Research Centre. Two hundred pure seeds of Sakha 8 wheat cultivar with a moisture content of 12% and highly significant traits such as high yielding and salinity stress tolerance were subjected for gamma irradiation treatment dosages of 200, 300, and 400 Gy using the Co source at the National Center for Radiation Research and Technology, Nasr City and Cairo, Egypt in 2013 year or M0 generation. The irradiated materials of all doses were grown and series of selections among the mutant population under normal soil conditions in the farm of Sakha city—Kafr Al Sheikh Governorate, and this process carried out four growing seasons from (2013/2014) season or M1 generation to (2016/2017) season or M4 generation, respectively.

Sowing and Treatments

Two experiments were done during 2017/2018 and 2018/2019 seasons using the original wheat cultivar (Sakha 8) and six *M5* mutants derived from it under two treatments as follows:

1) Treatment 1 (normal conditions): The seven wheat genotypes were grown under normal conditions of the soil in the farm of Sakha city in Kafr El-sheikh governorate, Egypt.
2) Treatment 2 (salinity conditions): The seven wheat genotypes were grown under salinity conditions of the soil in the farm of Sirw city in Damietta Governorate, Egypt.

Note: The agriculture was carried out from the second half of November and the harvesting process was done in the first of May in each growing season.

Studied traits

Fifty plants were taken from each genotype of each treatment for each season (2017/2018 and 2018/2019) to calculate and estimate some agro-morphological and chemical traits related to salinity tolerance as follows:

1) Plant height (cm): Length of the main culm was measured from the soil surface to the tip of the main panicle at maturity.
2) Number of filled grains/panicle: Filled grains of the main panicle with separated and counted.
3) 1000-grain weight (gm): it was recorded as the weight of 1000 random filled grains per plant.
4) Grain yield/plant (gm): it was recorded as the weight of grain yield of each individual plant, and adjusted to 14% moisture content.
5) Osmotic pressure (MP): values of the total soluble solids of the cell sap were obtained for the pressed sap of the (fourth upper leaf) tested plants using the Abbe Reflectometer and the osmotic pressure values (in the atmosphere) were calculated by using special tables according to the methods described by Goseve (1960).
6) Proline content: it was quantified by following the method of Bates et al. (1973).
7) Glycine betaine: it was carried out according to the method of Grieve and Grattan (1983).
8) Trehalose contents: it was carried out according to the method of Grieve and Grattan (1983).

Experiment design

All materials were grown with four replicates of each treatment for each growing season in a randomized complete block design. Fertilizer was added at a recommended rate, and hand weeding was done when needed. During the growth period, all the data for all studied traits were estimated and calculated.

Classification of replicate

Length of row: 15 m, width of replicate: 6.30 m, space within plants in the same row: 20 cm, space within rows: 30 cm; and each genotype were sowing in three rows.

Experimental replicate area (m^2): $15 \times 6.30 = 94.5\ m^2$

Statistical analysis

All calculated data of all traits under evaluation in two seasons for both treatments were analyzed using the formula by Gomez and Gomez (1984).

Estimation of salinity tolerance indices

All salinity tolerance indices were estimated according to Fischer and Maurer (1978), Bouslama and Schapaugh (1984), Lin et al. (1986), Hossain et al. (1990), Fernandez (1992), Gavuzzi et al. (1997), and Golestani and Assad (1998) as follows:

$$\begin{aligned}\mathrm{MS} &= \mathrm{YS} + \mathrm{YP}/2, \mathrm{STI} = \mathrm{YP} + \mathrm{YS}/\text{mean of } \mathrm{YP}^2, \mathrm{GMP} \\ &= (\mathrm{YP} \times \mathrm{YS})^{0.5}, \mathrm{YI} = \mathrm{YS}/\text{mean of YS}, \mathrm{YSI} \\ &= \mathrm{YS}/\mathrm{YP}, (\mathrm{YR}) = 1 - \mathrm{YS}/\mathrm{YP}, \mathrm{SSI} = (1 - \mathrm{YS}/\mathrm{YW})/\mathrm{D}.\end{aligned}$$

Genetic parameters

Variance components, heritability in a 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, and genetic advance as a percentage of mean were the most important measurements calculated through the

two seasons for both treatments in this investigation as follows:

The genetic coefficient of variability (GCV%) and phenotypic coefficient of variability (PCV%) were estimated according to the method suggested by Burton and Devane (1953) as follows:

Environmental variance (σ^2e) = MS_e

Genotypic variance (G v) or (σ^2g) = $MS_g - MS_e/r$

Phenotypic variance (Ph v) or (σ^2ph) = (σ^2e) + (σ^2g) or $MS_e + MS_g$

where MSe is the mean square of error, MSg is the mean square of genotypes, is the r = number of replicates, and X is the mean of trait.

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

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

Estimation of heritability in a broad sense

Broad sense heritability (h^2) expressed as the percentage of the ratio of the genotypic variance (g v) to the phenotypic variance (ph v) and was estimated on genotype mean basis as described by Burton and Devane (1953) and Johnson et al. (1955) as:

$$H^2B = (\sigma^2g)/(\sigma^2ph) \times 100$$

D^z: The difference between the phenotypic coefficient of variation (PCV%) and genotypic coefficient of variation (GCV%) or (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 were estimated in accordance with the methods illustrated by Johnson et al. (1955) as follows:

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

where K is the standardized selection differential at 5% selection intensity (K = 2.068).

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

$$(GAM\%) = (GA)/\text{mean} \times 100$$

Molecular depiction

Molecular genetics contributed to a positive, large, and an effective role in finding the genetic differences between any genotypes, which may be the taxonomic basis for these new lines and the local wheat cultivar at the molecular level. This has helped in determining the genetic parameters (alleles or genes) responsible for resisting all stresses of various kinds especially salinity tolerance. Molecular markers also have played a functional role in drawing the phylogenetic tree through cluster analysis to indicate the genetic relationship between different accessions besides the extent of environmental and genetic compatibility among them (each of them can compatibility and grow together).

Molecular marker ISSR profiles

Total DNA extraction: the extraction method was applied according to the manfacturer of Wizard® Genomic DNA Purification Kit Promega using Inter Simple Sequence Repeats (ISSR) analysis was applied according to Zietkiewicz et al. (1994) and procured from UBC (the University of British Columbia, Biotechnology Laboratory, Vancouver, Canada) based on core repeats anchored at the 5 or 3 end as shown in Table 6. DNA of seven wheat varieties was amplified using Taq-DNA polymerase chain reaction (PCR) according to the manufacturer's instructions (Promega # TM048) for ISSR primers. The PCR consisted of a 5-min incubation period at 94 °C followed by 40 cycles of 94 °C/30 s (38, 40, 41, and 45 °C)/1 min and 72 °C/2 min, with a final extension step of 72 °C/7 min. The PCR product was separated by 1.5% agarose gel electrophoresis using a TAE buffer and 0.04% red safe dye.

Data handling and cluster analysis (phylogenetic tree)

Data was scored for computer analysis on the basis of the presence or absence of the amplified products for each primer. Pairwise components of the seven wheat genotypes based on the presence or absence of unique and shared polymorphic products were used to determine similarity coefficients according to Jaccard (1908). The similarity coefficients according to Jaccard (1908) also were then used to construct dendrograms or cluster analysis, using the unweighted pair group method with arithmetic averages (UPGMA) employing the SAHN (sequential, agglomerative, hierarchical, and nested clustering) from the NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System), version 1.80 (Applied Biostatistics Program).

Results

Analysis of variance

Data shown in Table 1 related to the chemical analysis among the two treatments besides results obtained in Table 2 associated with the analysis of variance test (ANOVA) detected that significant and highly significant differences between all wheat genotypes (the original cultivar Sakha 8 and its six M5-derived mutants) for all studied traits under normal and saline conditions during the two seasons (2017/2018 and 2018/2019). The coefficient of variance percentage was low for all calculated

Table 1 Chemical classification of normal and salinity soils

Characteristics	Normal soil (Sakha city)	Saline soil (Sirw city)
EC (dS/m)	2.34	9.68–10.41
pH (1:2.5)	8.20	8.57
TDS mg/l (ppm)	377.18	5704–5809
Ca^{++}	1.98	14.58
Mg^{++}	1.22	12.55
Na^{+}	9.67	51.04–57.34
K^{+}	0.53	0.28
CO_3--	0.07	0.18
HCO_3-	1.97	1.26
Cl-	12.55	45.66
SO_4-	1.57	15.62
Texture	Clay	Clay

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

traits under both conditions for the two seasons except osmotic pressure trait where it was very high under normal and salinity treatments in both seasons, where it was recorded (144.03% and 152.05%) for season 2017/2018 and (116.93% and 121.19%) for season 2018/2019 under normal and salinity conditions, respectively.

Mean performance

Results obtained in Table 3 showed that the entries (Sakha 8; mutants 3, 5, and 6) for plant height and the number of filled grains/panicle traits (Sakha 8; mutants 2, 3, and 5) for 1000-grain weight trait (Sakha 8; mutant 1, 2, and 5) for grain yield/plant trait (mutants 1, 4, 5, and 6) for osmotic pressure trait (mutants 1, 2, 5, and 6) for proline content trait (mutants 2, 3, 4, and 5) for glycine betaine trait (Sakha 8; mutants 1, 2, and 5), and for trehalose content trait were exhibited the highest mean values under normal and salinity conditions in both seasons. For example but not limited to, the mean values were ranged from 108.24 to 123.43 cm and from 106.14 to 117.80 cm in season 2017/2018 and ranged from 107.13 to 121.18 cm and 104.0 to 116.23 cm in season 2018/2019 for plant height trait under both treatments. For the 1000-grain weight trait, data was ranged from 45.12 to 67.03 g and from 34.05 to 52.96 g in season 2017/2018 and ranged from 43.07 to 64.02 g and from 29.11 to 55.13 g in season 2018/2019 under normal and salinity treatments. Results were ranged from 42.35 to 75.44 g and from 29.33 to 63.17 g in the first season and ranged from 45.0 to 77.26 g and from 25.71 to 58.22 g in the second season for grain yield/plant trait under both treatments. With respect to proline content, it was ranged from 48.60 to 80.07 and from 56.01 to 89.65 in season 2017/2018 and ranged from 51.17 to 78.33 and ranged from 73.12 to 95.14 in season 2018/2019 under normal and salinity conditions, respectively.

Salinity tolerance indices

Results presented in Table 4 showed that the entries (Sakha 8, mutant 2 and 6) for YSI in season 2017/2018 and (Sakha 8; mutants 1 and 2) for the same parameter in season 2018/2019 in addition, the genotypes (Sakha 8; mutants 1, 2, 3, and 5) for MP and (Sakha 8; mutants 1 and 2) for GMP in both years exhibited the highest mean values in this investigation. This fact means that these previous accessions were recorded highly limit of salinity tolerance. On the same regard, the entries (Sakha 8; mutants 1 and 2) for YI in both growing seasons and (Sakha 8 and mutant 2) for STI in season 2017/2018 and (Sakha 8; mutants 1 and 2) for the same parameter in season2018/2019 were detected with mean values higher than the unity. This confirmed that these materials revealed high tolerance under salinity stress compared with the control, respectively. In the opposite direction, all genotypes understudying for YR in the first year and (mutants 3, 4, 5, and 6) for the same parameter in the second season besides the accessions (Sakha 8; mutants 2 and 6) in season 2017/2018 and (Sakha 8; mutants 1 and 2) in season 2018/2019 for SSI were recorded mean values lower than one which indicated that these entries were detected highly tolerance for salinity stress compared with the normal conditions.

Genetic parameters

Data showed in Table 5 detected that heritability in a broad sense was high in the studied traits; plant height under normal conditions only for the two seasons (80.93% and 83.60%), grain yield/plant under salinity conditions only for both years (63.01% and 90.33%), osmotic pressure under stress treatment only for the two seasons (67.22% and 70.25%), proline content under normal and salinity conditions and the values were 66.66% and 68.97% for the first season and 74.19% and 86.79% for the other one, glycine betaine content for the two treatments for both years (86.23% and 75.11%, and 68.82% and 63.87%), and trehalose content for the same treatments in both seasons (76.93% and 73.18%, and 80.96% and 93.29%), respectively, while values of heritability in a broad sense were ranged from low to medium for the rest calculated traits under the same conditions in both seasons. Results of GCV% and PCV% were low under normal and salinity conditions in both years for all traits understudying except osmotic pressure trait where it exhibited the highest values of this parameter under all conditions as 106.06% and 217.78% under both treatments for the first season and recorded 102.36% and 186.24% under normal and stress conditions in the second season for GCV% and recorded

Table 2 ANOVA analysis of all studied traits for the 7 wheat materials under normal and salinity conditions during the two seasons

S.O.V	D.F	Seasons	P.H		No. of. F.G/P		1000-G.W		G.Y/P		O.P		P.C		G.B		T.C	
			N	***S***	***N***	***S***	***N***	***S***	***N***	***S***	***N***	***S***	***N***	***S***	***N***	***S***	***N***	***S***
Genotypes	6	2017/2018	**66.18****	**29.23****	**13.57****	**11.06****	**8.34****	**12.57****	**37.02****	**24.0****	**9.03****	**16.11****	**7.56****	**12.0****	**45.34****	**27.23****	**17.08****	**10.15****
		2018/2019	54.35**	12.80**	16.05**	14.26**	9.56**	10.05**	15.40**	70.3**	7.49**	9.84**	13.0**	21.03**	18.02**	13.57**	18.55**	26.07**
Replicates	3	2017/2018	8.23**	10.86**	18.54**	7.05**	5.08*	9.14**	63.02**	25.36**	8.47**	14.0**	11.3**	15.48**	18.56**	14.05**	5.89*	4.80*
		2018/2019	6.15**	9.04**	6.23**	9.33**	8.60**	12.0**	54.32**	21.08**	6.04**	10.79**	9.46**	17.0**	15.97**	12.77**	7.11**	12.05**
Error	18	2017/2018	**3.68**	**7.18**	**2.14**	**6.55**	**1.39**	**1.84**	**5.73**	**3.07**	**2.84**	**1.75**	**0.84**	**1.21**	**1.74**	**2.08**	**1.19**	**0.85**
		2018/2019	2.54	5.03	3.89	4.23	2.33	1.16	4.12	1.83	1.84	0.94	1.04	0.77	1.83	1.68	1.03	0.46
C.V.%		2017/2018	1.65	2.39	1.18	2.17	2.11	3.17	3.93	3.94	144.03	152.05	1.39	1.41	1.97	1.85	2.13	1.48
		2018/2019	1.37	2.01	1.61	1.79	2.72	2.60	3.29	3.18	116.93	121.19	1.50	1.03	1.97	1.63	1.87	1.83

*Significant at 0.05
**Significant at 0.01

Table 3 Mean performance of all studied traits for the 7 wheat materials under normal and salinity conditions during the two seasons

Genotypes	P.H				No. of. F.G/P				1000-G.W				G.Y/P			
	Season 2017/2018		Season 2018/2019		Season 2017/2018		Season 2018/2019		Season 2017/2018		Season 2018/2019		Season 2017/2018		Season 2018/2019	
	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S
Sakha 8	123.43	117.80	121.18	115.03	127.18	123.0	125.24	119.36	63.84	52.96	64.0	55.13	69.36	58.02	70.15	55.03
Mutant 1	114.33	109.60	115.07	110.12	117.55	112.09	115.33	107.44	47.72	39.20	50.04	37.16	75.44	49.11	77.26	58.22
Mutant 2	110.54	106.14	111.89	105.83	123.03	116.50	121.80	113.21	55.38	44.08	57.16	39.44	71.29	63.17	69.93	56.08
Mutant 3	118.03	112.05	116.57	113.78	125.98	119.34	123.77	115.60	61.43	48.06	63.04	44.03	58.62	39.67	60.05	42.0
Mutant 4	108.24	106.59	107.13	104.0	119.37	114.56	120.03	112.66	49.55	38.77	51.03	35.64	45.86	29.33	48.26	25.71
Mutant 5	120.06	115.34	117.98	112.63	130.22	121.08	128.06	118.77	67.03	42.03	64.02	48.94	63.28	38.66	60.05	32.16
Mutant 6	117.02	115.33	119.0	116.23	124.09	117.63	122.16	114.28	45.12	34.05	43.07	29.11	42.35	33.07	45.0	28.36
Mean	115.95	111.83	115.54	111.08	123.91	117.74	122.34	114.47	**55.72**	**42.73**	**56.05**	**41.35**	60.88	44.43	61.52	42.50
LSD at 5%	3.19	3.27	1.94	2.73	1.78	3.12	2.41	2.51	1.43	1.64	1.85	1.31	2.93	2.13	2.47	1.64
LSD at 1%	4.69	4.82	2.85	4.03	2.62	4.59	3.54	3.70	2.11	2.42	2.73	1.93	4.31	3.13	3.64	2.42
Genotypes	**O.P**				**P.C**				**G.B**				**T.C**			
	Season 2017/2018		Season 2018/2019		Season 2017/2018		Season 2018/2019		Season 2017/2018		Season 2018/2019		Season 2017/2018		Season 2018/2019	
	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S
Sakha 8	1.22	1.05	1.17	0.89	57.04	77.18	60.30	80.0	48.13	62.06	50.019	57.81	48.29	53.0	49.77	57.12
Mutant 1	1.15	1.04	1.13	0.79	**66.18**	**81.90**	**68.12**	**93.73**	55.30	78.16	53.22	68.0	34.66	39.78	40.05	44.16
Mutant 2	1.26	1.07	1.20	1.03	**72.45**	**84.03**	**75.80**	**86.78**	79.44	88.78	80.06	92.21	60.05	69.34	71.26	80.04
Mutant 3	1.44	1.08	1.39	0.67	48.60	56.01	51.17	73.80	73.22	79.50	74.67	83.64	56.33	78.30	60.0	75.24
Mutant 4	1.05	0.57	1.11	0.91	55.84	68.93	62.11	73.12	63.45	74.53	67.0	77.13	39.40	51.07	40.13	55.03
Mutant 5	1.16	0.69	1.12	0.55	**79.56**	**86.22**	**78.33**	**93.70**	88.49	94.06	91.53	97.23	68.77	89.45	70.0	88.37
Mutant 6	0.94	0.61	1.02	0.82	**80.07**	**89.65**	**78.0**	**95.14**	59.97	68.55	64.01	80.32	49.77	53.12	47.56	63.33
Mean	1.17	0.87	1.16	0.80	65.67	77.70	67.69	85.18	66.85	77.94	68.64	79.47	51.03	62.0	54.11	66.18
LSD at 5%	2.06	1.61	1.64	1.17	1.11	1.33	1.43	1.07	1.61	1.75	1.64	1.57	1.33	1.12	1.23	0.83
LSD at 1%	3.03	2.37	2.42	1.73	1.63	1.96	2.11	1.58	2.37	2.57	2.42	2.32	1.96	1.65	1.81	1.22

Table 4 Estimates the tolerance indices parameters for the 7 wheat entries especially for grain yield trait under both conditions in two seasons

Genotypes	Grain yield/plant (gm)																	
	Season 2017/2018									Season 2018/2019								
	GYP	GYS	YSI	YI	MP	STI	GMP	YR	SSI	GYP	GYS	YSI	YI	MP	STI	GMP	YR	SSI
Sakha 8	69.36	58.02	0.83	1.30	63.69	1.08	63.43	0.17	**0.62**	70.15	55.03	0.78	1.29	62.59	1.01	62.13	0.22	**0.73**
Mutant 1	75.44	49.11	0.65	1.10	62.27	0.99	60.86	0.35	1.29	77.26	58.22	0.75	1.36	67.74	1.18	67.06	0.25	**0.83**
Mutant 2	71.29	63.17	0.88	1.42	67.23	1.21	67.10	0.12	**0.44**	69.93	56.08	0.80	1.31	63.0	1.03	62.62	0.20	**0.66**
Mutant 3	58.62	39.67	0.67	0.89	49.14	0.62	48.22	0.33	1.22	60.05	42.0	0.69	0.98	51.02	0.66	50.22	0.02	1.03
Mutant 4	45.86	29.33	0.63	0.66	37.59	0.36	36.67	0.37	1.37	48.26	25.71	0.53	0.60	36.98	0.32	35.22	0.47	1.56
Mutant 5	63.28	38.66	0.61	0.87	50.97	0.66	49.46	0.39	1.44	60.05	32.16	0.53	0.75	46.10	0.51	43.94	0.47	1.56
Mutant 6	42.35	33.07	0.78	0.74	37.71	0.37	37.42	0.22	**0.81**	45.0	28.36	0.63	0.66	36.68	0.33	35.72	0.37	1.23

Table 5 Estimates of genetic parameters for all studied traits under both conditions during (2017/2018 and 2018/2019) growing seasons

Genetic parameters	Seasons	P.H		No. of. F.G/P		1000-G.W		G.Y/P		O.P		P.C		G.B		T.C	
		N	***S***	***N***	***S***	***N***	***S***	***N***	***S***	***N***	***S***	***N***	***S***	***N***	***S***	***N***	***S***
Mean	2017/2018	115.95	111.83	123.91	117.74	55.72	42.73	60.88	44.43	1.17	0.87	65.67	77.70	66.85	77.94	51.03	62.0
	2018/2019	115.54	111.08	122.34	114.47	56.05	41.35	61.52	42.50	1.16	0.80	67.69	85.18	68.64	79.47	54.11	66.18
Genotypic variance	2017/2018	15.62	5.51	2.85	1.12	1.73	2.68	7.82	5.23	1.54	3.59	1.68	2.69	10.90	6.28	3.97	2.32
	2018/2019	12.95	1.94	3.04	2.50	1.80	2.22	2.82	17.11	1.41	2.22	2.99	5.06	4.04	2.97	4.38	6.40
Environmental variance	2017/2018	3.68	7.18	2.14	6.55	1.39	1.84	5.73	3.07	2.84	1.75	0.84	1.21	1.74	2.08	1.19	0.85
	2018/2019	2.54	5.03	3.89	4.23	2.33	1.16	4.12	1.83	1.84	0.94	1.04	0.77	1.83	1.68	1.03	0.46
Phenotypic variance	2017/2018	19.30	12.69	4.99	7.67	3.12	4.52	13.55	8.30	4.38	5.34	2.52	3.90	12.64	8.36	5.16	3.17
	2018/2019	15.49	6.97	6.93	6.73	4.13	3.38	6.94	18.94	3.25	3.16	4.03	5.83	5.87	4.65	5.41	6.86
Heritability in broad sense	2017/2018	80.93	43.42	57.11	14.60	55.44	59.29	57.71	63.01	35.15	67.22	66.66	68.97	86.23	75.11	76.93	73.18
	2018/2019	83.60	27.83	43.86	37.14	43.58	65.68	40.63	90.33	43.38	70.25	74.19	86.79	68.82	63.87	80.96	93.29
(GCV%)	2017/2018	3.40	2.09	1.36	0.89	2.36	3.83	4.59	5.14	106.06	217.78	1.97	2.11	4.93	3.21	3.90	2.45
	2018/2019	3.11	1.25	1.42	1.38	2.39	3.60	2.72	9.73	102.36	186.24	2.55	2.64	2.92	2.16	3.86	3.82
(PCV%)	2017/2018	3.78	3.18	1.80	2.35	3.17	4.97	6.04	6.48	178.87	256.61	2.41	2.54	5.31	3.70	4.45	2.87
	2018/2019	3.40	2.37	2.15	2.26	3.62	4.44	4.28	10.24	155.41	222.20	2.96	2.83	3.52	2.71	4.29	3.95
D^z	2017/2018	0.38	1.09	0.44	1.46	0.81	1.14	1.45	1.34	**72.81**	**38.83**	0.44	0.43	0.38	0.49	0.55	0.42
	2018/2019	0.29	1.12	0.73	0.88	1.23	0.84	1.56	0.51	**53.05**	**35.96**	0.41	0.19	0.60	0.55	0.43	0.13
GA or (expected genetic advance)	2017/2018	7.35	3.19	2.63	0.83	2.02	2.44	4.39	3.75	1.52	3.21	2.18	2.81	6.34	4.49	3.61	2.69
	2018/2019	6.80	1.51	2.38	1.99	1.83	2.49	2.21	8.13	1.61	2.58	3.08	4.33	3.44	2.84	3.89	5.05
GAM or (genetic advance as percentage of mean) %	2017/2018	6.33	2.85	2.12	0.70	3.62	5.71	**7.21**	**8.44**	**129.91**	**368.96**	3.31	3.61	**9.48**	**5.76**	**7.07**	**4.33**
	2018/2019	5.88	1.35	1.94	1.73	3.26	6.02	**3.59**	**19.12**	**138.79**	**322.50**	4.55	5.08	**5.01**	**3.57**	**7.18**	**7.63**

178.87 and 265.61 under both treatments in season 2017/2018 and 155.41 and 222.20 for the same treatments in the second year 2018/2019 for PCV%, respectively. The differences between the phenotypic and genotypic coefficient of variation (D^Z) were low for all studied traits in both growing seasons under both treatments except osmotic pressure trait where it recorded 72.81 and 38.83 for 2017/2018 season and 53.05 and 35.96 for 2018/2019 season under normal and salinity conditions, respectively. Data assessment of expected genetic advance (GA) based on 5% selection confirmed that all values evaluated during the two seasons under both treatments were low for all studied traits in this regard. Genetic advance as a percentage of the mean GAM% was recorded as the highest limit for this parameter especially in osmotic pressure trait where it exhibited 129.91 and 368.96 under both treatments in season 2017/2018 and recorded 138.79 and 322.50 under both treatments for the second season. In addition, some traits showed well results under both treatments during the two seasons such as glycine betaine, trehalose contents, and grain yield/plant, respectively.

Molecular depiction

Molecular description using ISSR primers

ISSR analysis profile

The six ISSR primers; 17898-A, 17899-A, 17898-B, 17899-B, 844-B, and HB-14 produced a total of 173 markers, 12 of them were monomorphic, while that 161 bands appeared polymorphic with 93% (polymorphism) included 63 unique bands (56 of them were positive markers besides 7 negative markers) as shown in Fig. 1 and Table 6. The average numbers of polymorphic ISSR markers were 26.83 fragments for each primer. Polymorphic fragments number ranged from 11 to 22 and molecular size ranging from 2540 to 148 bp, respectively. The highest number of polymorphic bands (22) were observed in 17899-B primer, followed by 17898-A and 17899-A (20 bands) for each of them and then followed by the two primers 844-B and 17898-B where they recorded (17 and 15) fragments for each of them, respectively. The lowest number of polymorphic bands (11) was showed in HB-14 primer, respectively. The results in Table 6 and Fig. 2 revealed that the highest polymorphism percentage was observed in 17898-A primer (100%), followed by 844-B primer (97.29%), followed by 17899-A primer (92.308%), followed by 17898-B primer (91.667%), followed by HB-14 primer (90.0%), and then followed by 17899-B primer (86.486%), respectively. The highest number of unique bands or positive specific markers (19) appeared in primer 844-B. The highest number of both polymorphic bands (22) and monomorphic band (5) were generated in 17899-B primer. In the same regard, the highest total bands (37) appeared in 17899-B and 844-B primers.

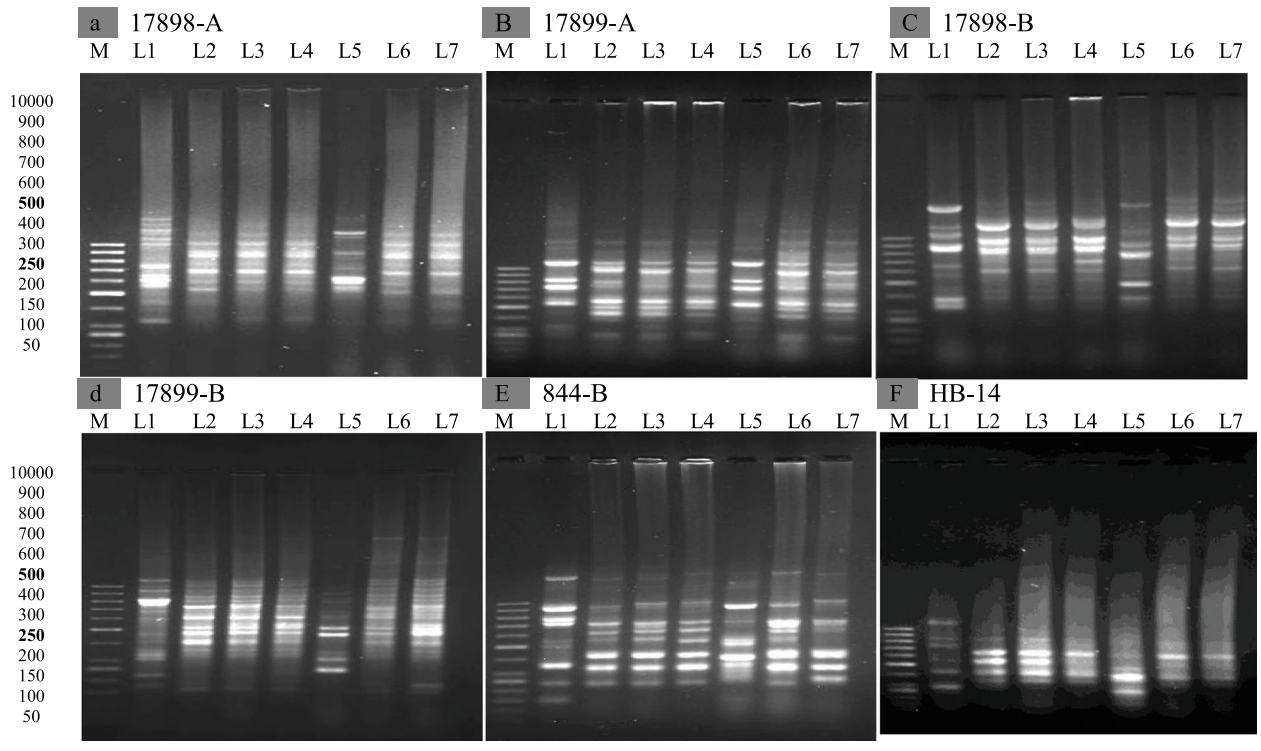

Fig. 1 The inter-simple sequence repeats (ISSR) amplification pattern obtained for seven wheat lines **a** primer 17898-A, **b** primer 17899-A, **c** primer 17898-B, **d** primer 17899-B, **e** primer 844-B, and **f** primer HB-14

Table 6 Band variation and polymorphism percentage in seven wheat lines using the six ISSR primers

Primers	Total bands	Molecular size (bp)	Number of monomorphic	Number of unique band (positive marker)	Number of polymorphic	Polymorphism %	Sequence	Annealing
17898-A	29	1525–214	0	9	20	100%	5′-(CA)6 AC-3′	38 °C
17899-A	26	2540–176	2	4	20	92.308%	5′-(CA)6 AG-3′	38 °C
17898-B	24	2173–148	2	7	15	91.667%	5′-(CA)6 GT-3′	40 °C
17899-B	37	2381–161	5	10	22	86.486%	5′-(CA)6 GG-3′	41 °C
844-B	37	1915–164	1	19	17	97.297%	5′-(CT)8 GC-3′	45 °C
HB-14	20	1262–190	2	7	11	90%	5′-(CTC)3 GC-3′	38 °C
Total	173	2540–148	12	56	105	93%		

In the same context, 17899-A primer exhibited the lowest number of unique bands or positive specific markers (4) while the lowest polymorphism percentage (86.486%) appeared in 17899-B primer.

Results obtained in Table 7 revealed that line 1 and line 3 recorded the highest number of amplified fragments (73 and 76) for each of them, while line 5 showed the lowest number of bands (56) and the rest lines were exhibited different numbers of amplified fragments. In the same regard, it is noted that primers 17899-B and 17899-A recorded the highest number of bands (115 and 90) together of all studied genotypes. But HB-14 primer gave the lowest number of amplified fragments (55) for the same materials.

Data viewed in Table 8 detected 63 markers (56 of them were positive and 7 negative specific markers) generated from 6 ISSR primers using to identify among 7 wheat accessions. Results showed that 17898-A primer exhibited 10 specific markers (9 positive and one negative) as follows: six positive markers were generated in line 1 with sizes 1290.09, 1127.28, 636.04, 544.53, 380.01, and 314.87 bp; two positive markers for line 5 with sizes 1150.56 and 707.37 bp; one positive marker for line 7 with size 404.04 bp; and one negative marker only for line 5 with size 752.10 bp, respectively. For 17899-A primer, four positive markers were observed in this regard where two positive markers with sizes 1586.04 and 512.02 bp for line 1 and the other two positive markers with sizes 703.7 and 222.75 bp were generated in line 7, respectively, while 17898-B primer was showed 10 specific markers where (7 of them were positive and 3 negative) as follows: four positive marker for line 1 with molecular sizes 1194.57, 930.92, 637.24, and 281.24 bp, one positive marker with size 499.08 bp for line 4, one positive marker with size 785.73 bp for line 5, and one positive marker with size 421.24 bp for line 6. The three negative markers were observed in lines 1 and 5 with sizes 1059.81 and 944.96 bp for line 1 and 1373.6 bp for line 5, respectively. In the same context, 17899-B primer recorded 11 specific markers (10 positive and one negative). Three positive markers were generated in line one with sizes (1053.44, 1040.88, and 220.6 bp), one positive marker with size 2353.19 bp for line 4, two positive markers with sizes 326.43 and 246.74 bp for line 5, four positive markers with sizes (689.5, 544.6, 492.79, and 381.52 bp) for line 7, and one negative marker only was observed in line 5 with size 393.93 bp, respectively. 844-B primer exhibited 20 markers where 19 of them were positive besides one negative marker only. Positive markers were as follows: six positive markers for line

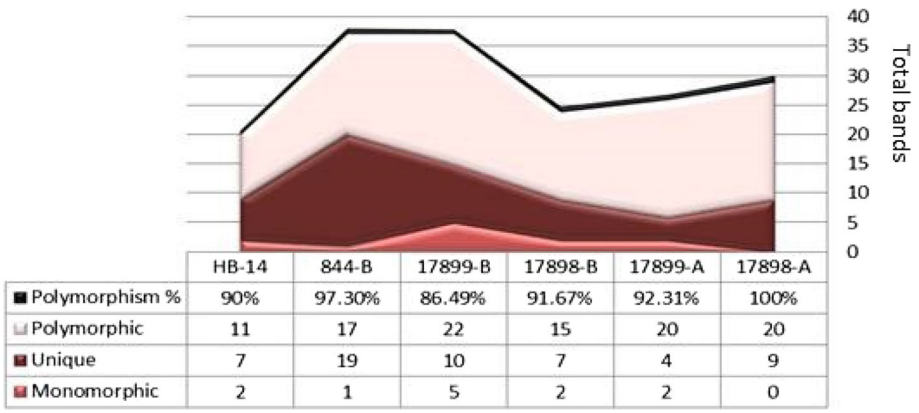

Fig. 2 The relationship between total bands and monomorphic, unique, polymorphic, and polymorphism percentage of six ISSR primers used for the detection of seven wheat lines

Table 7 Total bands produced from each primer for 7 wheat lines and all amplified fragments in each genotype

Genotypes	Primers						
	17898-A	17899-A	17898-B	17899-B	844-B	HB-14	Total
Line 1	**14**	**11**	**12**	**17**	**11**	**8**	**73**
Line 2	**9**	**14**	**12**	**15**	**11**	**8**	**69**
Line 3	**9**	**16**	**13**	**15**	**13**	**10**	**76**
Line 4	**9**	**13**	**11**	**16**	**12**	**7**	**68**
Line 5	**9**	**9**	**8**	**11**	**11**	**8**	**56**
Line 6	**10**	**13**	**12**	**18**	**11**	**6**	**70**
Line 7	**9**	**14**	**12**	**23**	**8**	**8**	**74**
Total Bands	**69**	**90**	**80**	**115**	**77**	**55**	**486**

one with sizes (813.27, 513.51, 478.95, 423.98, 376.63, and 164.39 bp), two positive markers for line 2 with sizes 1434.87 and 796.45 bp, one positive marker for line 3 with size 692.86 bp, two positive markers for line 4 with sizes 1863.24 and 653.02 bp, five positive markers for line 5 with sizes 1570.89, 827.56, 492.49, 296.16, and 265.85 bp, one positive marker for line 6 with size 700.14 bp, and two positive markers were observed in line 7 with molecular sizes of 779.97 and 252.32 bp, respectively. While the only negative marker was shown in line 1 with size 403.80 bp. Eight specific markers were generated by HB-14 primer where 7 of them were positive and one only negative. The seven positive markers were observed in line 1 with sizes 1262.81 and 271.49 bp, one positive marker with size 1017.16 bp for line 3, two positive markers with sizes 411.06 and 236.62 bp for line 5, and two positive markers were showed in line 7 with sizes 659.92 and 427.06 bp, respectively. While one negative marker was observed in line 1 only with size 311.48 bp.

Proximity matrix analysis (genetic similarity)

Data viewed in Table 9 exhibited 21 pairwise comparisons to debate the genetic relationships among 7 wheat accessions detected in terms of similarity. The genetic similarity ranged from 0.191 to 0.746 with an average of 0.468, where the biggest value of genetic similarity was 0.746 between L2 and L3 and the lowest value of similarity was 0.191 among L1 and L6, respectively. Also, highly genetic similarity values were observed for example within L3 and L4, L2 and L4, and L6 and L7 and their values were 0.714, 0.650, and 0.636, respectively. The rest data of genetic similarity exhibited values appeared low.

Cluster analysis (phylogenetic tree)

Results obtained from cluster analysis and presented in Fig. 3 divided all wheat lines into two main clusters. The cluster I included L5 while cluster II contained two subclusters. The subcluster 1 included L6 and L7 while subcluster 2 divided into L1 only and one sub-sub cluster. The sub-sub cluster included one group (L2 and L3) besides (L4), respectively.

Discussion

Results obtained in Table 2 confirmed that the seven wheat materials (Sakha 8 and its six M5-derived mutants) were different genetically from each other especially the six wheat mutants which descended from one species. These new materials were all genetically different from each other and from the original variety that descended from it. On the other counterpart, cultivating these genotypes over two growing seasons (2017/2018 and 2018/2019) also proved highly genetic stability and the differences within the seasons that emerged were only environmental. This fact confirms two things that the first one is succeeding in mutagenic events by different levels of gamma rays which would make positive changes in all agro-morphological traits in the original variety (Sakha 8) especially high yielding. The second result is reaching to a high limit of genetic stability for the six wheat mutants by 100% after five segregation generations. These results were in agreement with those reported by El-Keredy et al. (2003), El-Mouhamady et al. (2010), El-Mouhamady et al. (2011), El-Seidy et al. (2013), El-Mouhamady et al. (2016), Khatab et al. (2017), Al-Khaishany et al. (2018), El-Mouhamady et al. (2019), and Yassin et al. (2019).

Using different levels of gamma rays to irradiate the Egyptian wheat variety (Sakha 8) has proved more remarkable and also flawed results in the discovery of six excellent mutations descended from this variety and characterized it by reaching to the highest limit of genetic stability after cultivating it for five segregation generations (Table 3). These new genotypes gave great tolerance for salinity stress over two growing seasons (2017/2018 and 2018/2019) compared to the original parent that descended from it and were exhibited very promising results for all traits understudying for the salinity treatment compared to the control. This tolerance

Table 8 Mapping of positive (P) and negative specific markers for the 7 wheat lines using six ISSR primers

ISSR primers	MS (bp)	L1	L2	L3	L4	L5	L6	L7	(P or N) marker
17898-A	**1290.09**	+	−	−	−	−	−	−	**P(L1)**
	1150.56	−	−	−	−	+	−	−	**P(L5)**
	1127.28	+	−	−	−	−	−	−	**P(L1)**
	752.10	+	+	+	+	−	+	+	**N(L5)**
	707.37	−	−	−	−	+	−	−	**P(L5)**
	636.04	+	−	−	−	−	−	−	**P(L1)**
	544.53	+	−	−	−	−	−	−	**P(L1)**
	404.04	−	−	−	−	−	−	+	**P(L7)**
	380.01	+	−	−	−	−	−	−	**P(L1)**
	314.87	+	−	−	−	−	−	−	**P(L1)**
17899-A	**1586.04**	+	−	−	−	−	−	−	**P(L1)**
	703.7	−	−	−	−	−	−	+	**P(L7)**
	512.02	+	−	−	−	−	−	−	**P(L1)**
	222.75	−	−	−	−	−	−	+	**P(L7)**
17898-B	**1373.6**	+	+	+	+	−	+	+	**N(L5)**
	1194.57	+	−	−	−	−	−	−	**P(L1)**
	1059.81	−	+	+	+	+	+	+	**N(L1)**
	944.96	−	+	+	+	+	+	+	**N(L1)**
	930.92	+	−	−	−	−	−	−	**P(L1)**
	785.73	-	−	−	−	+	−	−	**P(L5)**
	637.24	+	−	−	−	−	−	−	**P(L1)**
	499.08	−	−	−	+	−	−	−	**P(L4)**
	421.24	−	−	−	−	−	+	−	**P(L6)**
	281.24	+	−	−	−	−	−	−	**P(L1)**
17899-B	**2353.19**	-	−	−	+	−	−	−	**P(L4)**
	1053.44	+	−	−	−	−	−	−	**P(L1)**
	1040.88	+	−	−	−	−	−	−	**P(L1)**
	689.50	−	−	−	−	−	−	+	**P(L7)**
	544.60	−	−	−	−	−	−	+	**P(L7)**
	492.79	−	−	-	−	−	−	+	**P(L7)**
	393.93	+	+	+	+	−	+	+	**N(L5)**
	381.52	−	−	−	−	−	−	+	**P(L7)**
	326.43	−	−	−	−	+	−	−	**P(L5)**
	246.74	−	−	−	−	+	−	−	**P(L5)**
	220.60	+	−	−	−	−	−	−	**P(L1)**
844-B	**1863.24**	−	−	−	+	−	−	−	**P(L4)**
	1570.89	−	−	−	−	+	−	−	**P(L5)**
	1434.87	−	+	−	−	-	−	−	**P(L2)**
	827.56	−	−	−	−	+	−	−	**P(L5)**
	813.27	+	−	−	−	−	−	−	**P(L1)**
	796.45	−	+	−	−	−	−	−	**P(L2)**
	779.97	−	−	−	−	−	−	+	**P(L7)**
	700.14	−	−	−	−	−	+	−	**P(L6)**
	692.86	−	−	+	−	−	−	−	**P(L3)**

Table 8 Mapping of positive (P) and negative specific markers for the 7 wheat lines using six ISSR primers *(Continued)*

ISSR primers	MS (bp)	L1	L2	L3	L4	L5	L6	L7	(P or N) marker
	653.02	–	–	–	+	–	–	–	**P(L4)**
	513.51	+	–	–	–	–	–	–	**P(L1)**
	492.49	–	–	–	–	+	–	–	**P(L5)**
	478.95	+	–	–	–	–	–	–	**P(L1)**
	423.98	+	–	–	–	–	–	–	**P(L1)**
	403.80	–	+	+	+	+	+	+	**N(L1)**
	376.63	+	–	–	–	–	–	–	**P(L1)**
	296.16	–	–	–	–	+	–	–	**P(L5)**
	265.85	–	–	–	–	+	–	–	**P(L5)**
	252.32	–	–	–	–	–	–	+	**P(L7)**
	164.39	+	–	–	–	–	–	–	**P(L1)**
HB-14	**1262.81**	+	–	–	–	–	–	–	**P(L1)**
	1017.16	–	–	+	–	–	–	–	**P(L3)**
	659.92	–	–	–	–	–	–	+	**P(L7)**
	427.06	–	–	–	–	–	–	+	**P(L7)**
	411.06	–	–	–	–	+	–	-	**P(L5)**
	311.48	–	+	+	+	+	+	+	**N(L1)**
	271.49	+	–	–	–	–	–	–	**P(L1)**
	236.62	–	–	–	–	+	–	–	**P(L5)**
Range	**164.39–2353.19**								
Total		**26**	**9**	**9**	**11**	**16**	**9**	**18**	**63 = 56(P) + 7(N)**

P positive, *N* negative, MS molecular size

is due to many physiological reasons, including the ability of new genetic genotypes to control the osmotic pressure to reach the lowest levels to maintain the water content within the cell necessary for all vital processes of growth and life under salinity stress. Thus, secreting and composition some chemical compounds that give the characteristic of tolerance and resistance to salinity stress. Controlling the sodium element and reducing its proportion as well besides increasing the level of potassium and all through a precise mechanism have been controlled by the root system. Ultimately, all these reasons are reflected to reduce the rate of loss in yield and its components under salinity stress compared to the normal conditions (El-Mouhamady (2003); El-Mouhamady (2009); El-Seidy et al. (2013); El-Mouhamady et al. (2014a); El-Mouhamady et al. (2014b); El-Mouhamady et al. (2014c); El-Mouhamady et al. (2014d); Al-Naggar et al. (2015); Heiba et al. (2016a); Khatab et al. (2017); Gadallah et al. (2017); Darwish et al. (2017); Al-Khaishany et al. (2018); Khatab et al. (2019); El-Mouhamady et al. (2019); Tawfik and El-Mouhamady (2019); Yassin et al. (2019); and Al-Ashkar et al. (2019))

Table 9 Genetic similarity percentages for the seven wheat genotypes using 6 ISSR primers

0	L1	L2	L3	L4	L5	L6	L7
L1	1.0						
L2	0.405	1.0					
L3	0.354	**0.746**	1.0				
L4	0.305	**0.650**	**0.714**	1.0			
L5	0.172	0.237	0.294	0.291	1.0		
L6	**0.191**	0.299	0.315	0.314	0.326	1.0	
L7	0.204	0.276	0.282	0.267	0.203	**0.636**	1.0

The seven superior wheat genotypes including the original cultivar and its six M5-derived mutants have succeeded in demonstrating their high efficiency of salinity tolerance. In addition, minimizing the adverse effect on their different stages of life especially germination, seedling, and other physiological aspects of flowering containing grain fullness and final yield under salinity treatment conditions compared to standard experiment. It is evident by estimating the different parameters of salinity tolerance indices for grain yield per plant in both growing seasons (2017/2018 and 2018/2019) (Table 4). This superiority is due to the high ability of these accessions to reduce the amount of loss in the final yield under salinity stress to a level that plant can accept it and continue in living well without affecting its life or the expected final

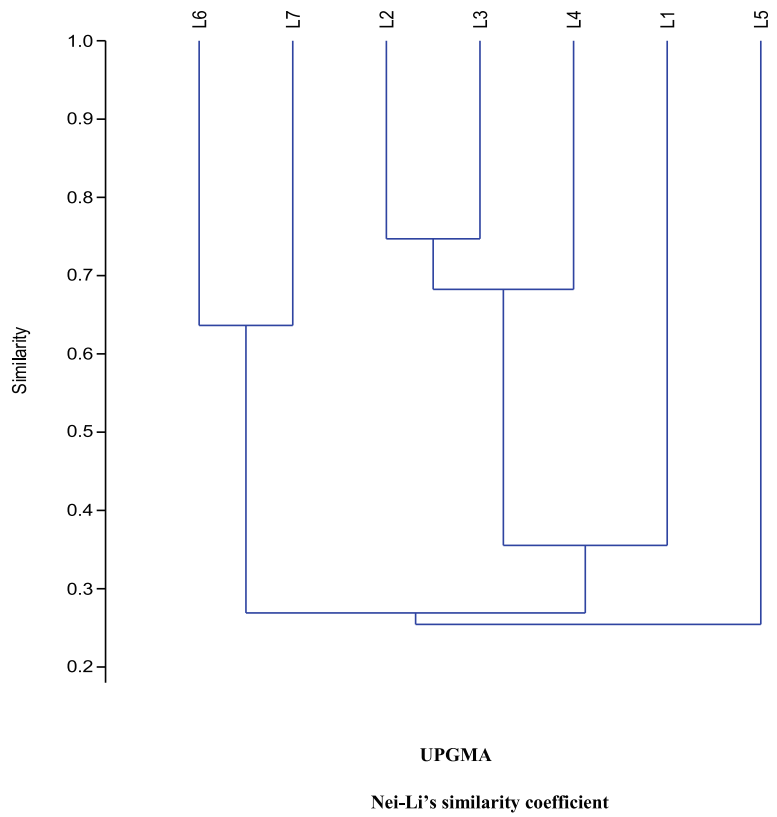

Fig. 3 Dendrogram representing the genetic relationship among the seven wheat genotypes using UPGMA cluster analysis of Nei-Li's similarity coefficient generated from the six ISSR markers

output and it is very close to natural conditions. One of the mechanisms used by the previous tolerant genetic materials to reduce the bad effect of salinity stress is reducing osmotic pressure to maintain the amount of water needed for all vital processes and also keeping the internal water in the cell from going out during high salinity and prevent the plant to reach the stage of the bollard. In addition, the high limit of the proportion of organic acids and compatible solutes would reduce this bad effect of salinity stress such as proline, trehalose, and glycine betaine contents under salinity conditions compared to the control. All these factors would have formed a degree of tolerance and resistance to salinity stress in this context of this study. These results are in agreement with those reported by Esmail et al. (2016), El-Mouhamady et al. (2016), Ramadan et al. (2016), Darwish et al. (2017), Khatab et al. (2017), El-Mouhamady and Habouh (2019), El-Mouhamady et al. (2019), Khatab et al. (2019), Tawfik and El-Mouhamady (2019), and Yassin et al. (2019).

Heritability in a broad sense was viewed high in all studied traits in both seasons under normal and salinity treatments except the number of filled grains/panicle and 1000-grain weight traits where they appeared medium for the 2 years under both treatments (Table 5). This means that low-environment effect in the first case of increasing heritability for these traits besides the genetic variation was the greatest part of phenotypic variation. At the same time, the fruitful and affective role of additive gene action was strongly visible for inheriting and improving these traits for salinity tolerance in the recent wheat accessions through a simple selection process, while that in the second case of medium limit of heritability in a broad sense for the number of filled grains/panicle and 1000-grain weight trait during two seasons under both treatments showed the medium effect for each environment and genetic variation. It confirms that these traits might be governed by non-additive gene action besides the important role of the interaction among the environment and genotype. This of course will be reflected in the genetic stimulation of increased salt tolerance in wheat lines. PCV% was always higher than GCV% in all studied traits for the two seasons under both treatments. This result confirming that all changes in all materials understudying were not only due to genetic variation but also depend on the significant effect of environmental factors and the selection process for these traits under salinity conditions besides the control treatment. Values of genetic advance (GA) in most traits in both years under normal and salinity conditions appeared low might be controlled by non-

additive gene action. The interaction among genotype and environment on the expression of these traits confirmed the weak affective of individual plant selection for enhancing and increasing salinity tolerance in these traits in the recent genotypes (the Egyptian cultivar and the six mutants) under salinity stress compared to the normal conditions. This does not mean of course that the decline in these values does not only represent a genetic advance, but also attributed to the genetic progress with a significant form in discovering and development of these excellent mutations to tolerate salinity stress in wheat crops. Moreover, the continuation of sowing these new accessions with careful follow-up during the evaluation of yield and its component and tolerance index parameters may eventually lead to raising the mechanism of tolerance and resistance for salinity stress in these lines with maintaining a good proportion of yield. With respect to GAM or (genetic advance as a percentage of mean%), high results were observed in osmotic pressure trait only under both treatments in both seasons could be indicated the effective role of additive gene action for increasing salinity tolerance. On the other hand, little values observed in the rest traits under both conditions for the two seasons may be due to non-additive gene action for controlling increasing and enhancing salinity stress in these traits. These results were in agreement with those reported by Hamawaki et al. (2012), Abou El-Nasr et al. (2013), Al-Naggar et al. (2015), Shoaib et al. (2016), Chandrawat et al. (2017), El-Mouhamady et al. (2017), El-Demardash et al. (2017), Tawfik and El-Mouhamady (2019), El-Mouhamady and Habouh (2019), El-Mouhamady et al. (2019), Al-Kordy et al. (2019), Khatab et al. (2019), Yassin et al. (2019), and Al-Ashkar et al. (2019).

Molecular genetics and especially molecular markers using 6 ISSR primers have succeeded in drawing a clear, highly accurate picture that includes all the genetic differences at the molecular level for the six salinity-tolerant wheat mutants. A clear genetic distinction was made between these new lines compared to the local wheat variety (Sakha 8) through generating a total of 173 amplified fragments by the previous ISSR primers (Table 6 and Fig. 1). Thus, this careful analysis of the molecular markers (ISSR profile analysis) turns out that the primers 17899-B, 17899-A, and 17898-B showed great success in discovering and achieving the largest number of fragments (115, 90, and 80) that were credited with confirming these molecular genetic differences. This result confirms that the six salinity-tolerant wheat lines were very different among them especially lines 1 and 3 (Fig. 2 and Table 7. These results were in agreement with those obtained by Abdel Sattar and El-Mouhamady (2012), El-Mouhamady et al. (2012a), El-Mouhamady et al. (2012b), El-Mouhamady et al. (2012c), Zian et al. (2013), Eldessouky et al. (2016), El-Mouhamady et al. (2016), El-Mouhamady et al. (2017), Khatab et al. (2017), Al-Kordy et al. (2019), Khatab et al. (2019), El-Mouhamady et al. (2019), Tawfik and El-Mouhamady (2019), and El-Mouhamady and El-Metwally (2020).

Results shown in Table 8 has already succeeded in producing 63 specific markers. These markers consisted 56 positive and 7 negative markers which were considered as a taxonomic and determinant tool for the seven wheat genotypes. Thus, it also have confirmed the saying fact that they differ from each other, an excellent indication and a new dimension to the success for the genetic improvement to salinity tolerance in wheat accessions using mutations. These results were in agreement with those reported by Eldessouky et al. (2016), Al-Kordy et al. (2019), Khatab et al. (2019), El-Mouhamady et al. (2019), Tawfik and El-Mouhamady (2019), and El-Mouhamady and El-Metwally (2020).

There is no doubt that the success achieved from determining the molecular genetic differences between the six salinity-tolerant wheat mutants compared to the local variety (Sakha 8) was the main light to determine the next most important step in this investigation. In a nutshell, this step determines the degrees of genetic similarity and (cluster analysis) or phylogenetic tree (genetic convergence or genetic affinity) among these new genotypes. This strategy will have the greatest impact on determining which of them is genetically and phenotypically compatible with the other. Thus, the purpose of reusing it in the breeding and genetic improvement program for wheat tolerance of biotic and abiotic stresses after these lines reach to a high genetic stability (Table 9 and Fig. 3). These results confirmed that the relationships L2 and L3, L3 and L4, L2 and L4, and L6 and L7 were the most compatible together and gave the highest values of genetic similarity. These results were in agreement with those obtained by Al-Kordy et al. (2019), Khatab et al. (2019), El-Mouhamady et al. (2019), Tawfik and El-Mouhamady (2019), and El-Mouhamady and El-Metwally (2020).

Conclusion

This study succeeded in dealing with the problem of salinity tolerance decreasing in wheat crops with great and radical forms through the optimum use of gamma rays with various doses for improving this purpose in Sakha 8 cultivar wheat. This variety is well known for its tolerance to salinity in a significant way, and this is what made it to be at the forefront of the genetic sources to be used in the study for the improvement and development of salinity tolerance in wheat crops not only locally, but internationally. Also, this investigation devised six M5 mutants derived from the original cultivar (Sakha 8) which have been confirmed with high genetic stability through its cultivation over 2 years under

normal and salinity conditions. All results of mean values and genetic parameters for all studied traits in Sakha 8 wheat cultivar and its six M5-derived mutants under both treatments in the two seasons (2017/2018 and 2018/2019) were proved highly significant and were very distinctive for salinity tolerance. Molecular characterization as well using six ISSR primers confirmed that the six wheat mutants were significantly different from each other and from the original cultivar descending from it through generating 63 specific markers.

Abbreviations

1000-G.W: 1000-grain weight; D^2: The difference between the phenotypic coefficient of variation (PCV %) and genotypic coefficient of variation (GCV %); G.B: Glycine betaine; G.Y/P: Grain yield/plant; GCV%: Genotypic coefficient of variance percentage; GMP: Geometrical mean productivity; GYP: Mean yield under normal conditions; GYS: Mean yield under salinity conditions; L1: Local wheat cultivar (Sakha 8); L2: Mutant 1; L3: Mutant 2; L4: Mutant 3; L5: Mutant 4; L6: Mutant 5; L7: Mutant 6; MP: Mean productivity; N: Normal conditions; No. of F.G/P: Number of filled grains/panicle; O.P: Osmotic pressure, osmotic adjustment; P.C: Proline content; P.H: Plant height; PCV%: Phenotypic coefficient of variance percentage; S: Salinity treatment; SSI: Salinity susceptibility index; STI: Salinity tolerance index; T.C: Trehalose content; YI: Yield index; YI: Yield index; YR: Yield reduction ratio; YSI: Yield stability index

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Authors' contributions

ABAEM: Done the part on plant breeding which included agriculture and statistical analysis and reviewed the full paper (50% contribution). HFI: Done the part of molecular markers and reviewed the full paper (50% contribution). Both authors read, written, and approved the final manuscript.

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Availability of data and materials

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Not applicable.

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Competing interests

The authors declare that they have no competing interests.

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