British Biotechnology Journal
6(4): 174-190, 2015, Article no.BBJ.2015.040
ISSN: 2231–2927

SCIENCEDOMAIN *international*
www.sciencedomain.org

Morphological and SSR Assessment of Putative Drought Tolerant M_3 and F_3 Families of Wheat (*Triticum aestivum* L.)

A. M. M. Al-Naggar[1*], Kh. F. Al-Azab[2], S. E. S. Sobieh[2] and M. M. M. Atta[1]

[1]Department of Agronomy, Faculty of Agriculture, Cairo University, Giza, Egypt.
[2]Department of Plant Research, Nuclear Research Center, Atomic Energy Authority, Inshas, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. Author AMMAN designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors KFAA and SESS managed the experiments and analyses of the study. Author MMMA managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BBJ/2015/15898
Editor(s):
(1) Mahalingam Govindaraj, ICRISAT, Patancheru, India.
(2) Giuliana Napolitano, Department of Biology (DB), University of Naples Federico II, Naples, Italy.
Reviewers:
(1) Klára Kosová, Department of Plant Genetics, Breeding and Crop Quality, Crop Research Institute, Prague, Czech Republic.
(2) Shilpi Srivastava, Department of Botany, D.D.U. Gorakhpur University, Gorakhpur, India.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=1028&id=11&aid=8512

Original Research Article

Received 24th December 2014
Accepted 28th January 2015
Published 18th March 2015

ABSTRACT

In an attempt to develop drought tolerant genotypes of bread wheat, two procedures, i.e., mutation and hybridization were used to induce new genetic variation. Selection for high grain yield/plant (GYPP) and other desirable traits was practiced in the M_2 populations of 7 gamma irradiated genotypes and F_2 populations of 15 diallel crosses among 6 genotypes of wheat under well watering (WW) and water stress (WS) conditions. Progenies of these selections (53 M_3 and 109 F_3 families) and their seven parents were evaluated in the field under WW and WS. Significant yield superiority of twelve families (7 M_3's and 5 F_2's) over their original and better parents, respectively under WS reached 74.71% (SF9). These putative drought tolerant families were assessed on the DNA level using SSR analysis. Fifteen SSR primers were used for PCR amplification of the genomic DNA of these 12 selections and their parents. The SSR analysis proved that the 12 families are genetically different from their 7 parents, with an average polymorphism of 86.67%. The genetic similarities (Gs) ranged from 30% to 88%. Both mutants SF3 and SF4 exhibited very

**Corresponding author: Email: ahmedmedhatalnaggar@gmail.com;*

low Gs (42 and 40%, respectively) with their common parent (Giza-168), indicating that gamma rays were very effective in changing the genetic background of Giza-168 towards high GYPP under WS conditions. SSR assay permitted the identification of seven unique bands (5 positive and 2 negative) for three drought tolerant wheat genotypes (SF3, SF4 and Aseel-5). These bands might be considered useful as markers associated with drought tolerance in bread wheat breeding programs.

Keywords: Putative mutants; transgressive segregants; bread wheat; drought tolerance; SSR markers; genetic similarity.

1. INTRODUCTION

At present, the average annual consumption of wheat grains in Egypt is about 14 million tons, while the average annual local production is about 8 million tons with an average grain yield of 18.0 ardab/feddan (6.43 t/ha) [1]. The gap between annual local production and consumption is about 6 million tons, which is imported from Russia, France and other countries. To achieve self sufficiency, the area cultivated with wheat should be increased, which is possible only in the North coast and Egyptian deserts. But the soil in these areas is sandy with low water holding capacity and thus exposes wheat plants to drought stress. Such drought stress causes great losses in wheat yield and its components [2,3]. Using drought tolerant wheat cultivars that consume less water, and can tolerate soil water deficit could solve this problem.

Wheat breeders always search for broad genetic variation to start a successful breeding program for improving the trait of interest. Unfortunately, with present distribution of improved high yielding pure line cultivars in all of the world's wheat growing areas, selection from established cultivars would rarely isolate a new genotype [4]. Gamma rays proved to be effective in broadening genetic variability of wheat cultivars for grain yield and its components, helping plant breeders to practice an efficient selection in the M_2 and next mutated generations [5-8]. In a little less than a century, mutation breeding programs resulted in developing more than 3200 crop varieties that are being grown all over the world; of which 254 mutant wheat varieties were developed by physical mutagens and mutants induced *via* gamma rays have been obtained in bread wheat for resistance to drought leading to the release of 26 varieties worldwide [9].

Hybridization procedure between diverse genotypes is used to create hybrid populations with wide genetic variation, from which new recombinations of genes may be selected [10]. Selection from segregating generations of wheat hybrid combinations succeeded to develop new genotypes that possess adaptive traits of drought tolerance, such as early maturity [12], glaucouness [7,12] and high grain yield/plant under water deficit conditions [13,14].

Molecular markers have been proven to be more powerful tools in the assessment of genetic variation and elucidation of genetic relationships within and among species than the morphological and biochemical markers, which may be affected by environmental factors and growth practices [15,16]. A wide variety of DNA-based markers has been developed in the past few years. Simple sequence repeats (SSRs) are present in the genome of all eukaryotes and consist of several repeats to over hundreds of nucleotide motif and flanked by sequence that can be used as primers, so they are more specific than RAPDs [17]. SSRs offer a potentially attractive combination of features that are useful as molecular markers. First, SSRs have been reported to be highly–polymorphic and highly informative in plants, providing many different closely related individuals [18]. Second, SSRs can be analyzed by a rapid, technically simple, and inexpensive PCR-based away that requires only small quantities of DNA. Third, SSRs are co-dominantly inherited and reveal simple Mendelian segregation has been observed. Finally, SSRs are both abundant and uniformly dispersed in plant genomes [18,19]. Many investigators concluded that SSR molecular markers are significantly associated with wheat traits related to salinity tolerance [20] and drought tolerance [21-27].

The present investigation was carried out in an attempt to develop new wheat genotypes (mutants *via* gamma rays selected from M_2 populations and transgressive segregants selected from F_2 populations of hybrid combinations) tolerant to water stress conditions.

1.1 The Objectives were to

(i) evaluate the putative mutants (M_3) and transgressive segregants (F_3) families along with their parents for drought tolerance in the field, (ii) assess the genetic diversity between the best selections (seven M_3 mutants and five F_3 segregants) and their parents on the DNA level using SSR analysis and (iii) identify unique molecular markers for drought tolerant selections and/ or parents.

2. MATERIALS AND METHODS

This investigation was carried out during four successive wheat growing seasons (2008/2009 through 2011/2012) at the Experimental Farm and Molecular Genetics Laboratory of the Plant Research Department, Nuclear Research Center, Inshas, El-Sharkyia Governorate. The latitude and longitude of the experimental farm are 30º 24` N and 31º 35` E, respectively, while the altitude is 20 m above the sea level.

Six cultivars of bread wheat (*Triticum aestivum* L.), *i.e*., Sids-4, Sakha-61, Aseel-5, Sakha-93, Giza-168 and Sahel-1 and the experimental line Maryout-5 were used in the present study. Name, pedigree, origin and important traits of these genotypes are presented in Table (1). The six genotypes, *viz.* Sids-4 (P1), Sakha-61 (P2), the line Maryout-5 (P3), Aseel-5 (P4), Sakha-93 (P5) and Giza-168 (P6) were grown in 2008/2009 season. All possible diallel crosses (excluding reciprocals) were made among the six parents, and seeds of 15 direct F_1 crosses were obtained. F_1 seeds from each of the 15 crosses were sown in the field on 20 Nov. 2009 under well watering conditions in separate plots. Each plot consisted of 6 rows, 3 m long and 30 cm wide; with hills spaced 10 cm apart (plot size = 1.8 m^2). At maturity F_2 seeds of each cross were separately harvested and kept for use in the third season (2011/2012).

Seeds of each of the seven parents irradiated with a selected dose of gamma rays (350 GY) determined by a preliminary experiment, were immediately sown on 20 Nov., 2009 in separate plots to obtain M_1 plants of each bulk. Each plot consisted of 30 rows; each row was 4 m long and 30 cm wide. Spaces between each two plants were 10 cm in each row. The plants were left for natural self pollination. At harvest, ten kernels were taken randomly from each M_1 plant (M_2 seed) and seeds from each bulk were blended to represent seed of the respective M_2 bulk. These seeds of M_2 bulks were kept for use in experiments of the third season (2010/2011). The recommended cultural practices for wheat production at Inshas were followed in M_1 and F_1 generations.

Seeds of the 15 F_2's and 7 M_2's were sown on 25 Nov., 2010 in the field under water stress (WS) and well watering (WW) in separate plots. Each plot consisted of 18 rows, 3 m long and 30 cm wide; with hills spaced 10 cm apart (plot size =5.4 m^2). Two irrigation regimes (starting 21 days after sowing) were used, viz., irrigation every 5 days (WW) and every 15 days (WS). The calculated total quantity of irrigation water for WS was 70% of that for WW and the soil at the experimental site was sandy to loamy sandy. At harvest individual plant selection, using *ca* 1% selection intensity was practiced in the same season (2010/2011), in the 15 F_2s and 7 M_2s. Selection was performed for grain yield/ plant and some other favorable traits, such as spike length, spike weight, spikes/plant, earliness, glaucousness…*etc*., under water stress and non-stress conditions. One hundred and sixty two individual plant selections were separately harvested (53 from M_2 and 109 from F_2 populations).

2.1 The Field Experiment

A field experiment was conducted in 2011/ 2012 season to compare the selected individual genotypes with their parents. The experimental design used was a split-plot in a balanced lattice (13 x 13) arrangement with three replications. Main plots were assigned to two irrigation regimes (WW and WS) and sub-plots were devoted to 169 genotypes (162 selections + 7 parents). Each plot consisted of 4 rows, 2.25 m long and 30 cm wide; with hills spaced 10 cm apart (plot size = 2.7 m^2). Rainfall in 2010/2011 and 2011/2012 seasons were very light and intermittent with a total precipitation of 10.3 and 13.9 mm, respectively, suggesting that rainfall during the stress period was of negligible influence on moisture content of the experimental soil.

Data was recorded in the field on: 1. days to 50% heading (DTH), 2. days to 50% anthesis (DTA), 3. days to 50% physiological maturity (DTM), 4. plant height (PH), 5. spike length (SL), 6. spikes / plant (SPP), 7. grains / spike (GPS), 8. spike weight (SW), 9. 100-grain weight (100GW) and 10.grain yield / plant (GYPP). Data on traits No. 1, 2 and 3 were measured on a per plot basis.

Table 1. Pedigree, origin and the most important traits of the studied wheat genotypes

Genotype	Designation	Pedigree	Origin	Important trait
Sids-4 cv.	Sd-4	Maya"S"Mon"S"/CMH74.A592/3/Sakha8 X2SD10002-140sd-3sd-1sd-0sd	ARC – Egypt	Earliness
Sakha-61 cv.	Sk-61	Lina/RL4220//7c/Yr"S"CM 15430-25-55-0S-0S	ARC – Egypt	Earliness
Maryout-5 Line	Mr-5	Giza 162 // Bch's /4/ PI-ICW 79Su511Mr-38Mr-1Mr-0Mr	DRC – Egypt	High yielding and Salt tolerant
Aseel-5 cv.	As-5	BIG INC 08 104	ICARDA - Syria	Drought tolerant
Sakha-93 cv.	Sk-93	Sakha 92/ TR 810328 S8871-1S-2S-1S-0S	ARC – Egypt	High yielding
Giza-168 cv.	Gz-168	Mrl / Buc // Seri CM 930468M-0Y-0M-2Y-0B	ARC – Egypt	High yielding
Sahel-1 cv.	Sah-1	NS 732 / PIMA // VEERY "S"	ARC – Egypt	Drought tolerant

ARC = Agricultural Research Center, DRC = Desert Research Center, ICARDA = International Center for agricultural Research in the Dry Areas, cv. = cultivar

Data on other traits (No. 4 through 10) were measured on 30 individual plants/plot. Recorded data were subjected to the normal analysis of variance for balanced lattice design, and the least significant differences (LSD) between means were estimated according to Snedecor and Cochran [28].

2.2 Molecular Analysis

SSR analysis was used in the present study to assess the genetic diversity among the best 12 drought tolerant selections (7 putative mutants and 5 transgressive segregants) and their 7 parents and to identify markers associated with drought tolerance.

Young green leaves were collected from ten days-old seedlings germinated from seeds of each genotype and quickly frozen in liquid nitrogen and then ground using mortar and pestle. Extraction of genomic DNA from these leaves was carried out according to Doyle and Doyle [29] and Sumar et al. [30].

The polymorphism among the 12 drought tolerant selections (7 putative mutants and 5 transgressive segregants) and their 7 parents was detected based on SSR analysis. A set of fifteen random primers (Table 2) chosen according to Bousba et al. [31] among the publicly available sets catalogued in the Grain Genes database (http://wheat.pw.usda.gov) as WMC (Xwmc). Roider et al. [32] described the WMS (Xgwm) as specialized for *Triticum aestivum* and used for screening drought tolerance. These primers were synthesized by BioShop® Canada Inc. and used for SSR analysis.

The PCR master mix for the simple sequence repeat (SSR) primers consisted of 2 µl of 20 ng/µl genomic DNA template, 0.40 µl of10 µM a forward and reverse primer mixture, 0.18 µl (0.9 U) of Taq polymerase, 1.20 µl of 10X buffer (10 mMTris-HCl, 50 mM KCl, 1.5 53 mM $MgCl_2$, pH 8.3), 0.96 µl of a 100 µM mixture of dNTPs and 7.26 µl of water bringing the total reaction volume to 12 µl. Reaction conditions for SSR markers were as follows: 8.33 µl ddH20, 2.4 µl 10 X reaction buffer, 0.9 µl 50 mM $MgCl_2$, 1.92 µl 2.5 mM dNTPs, 1.9 µl 1pM of 19bp M-13. The PCR master mix was carried out in a volume of 20 µl and contained 200 ng of genomic DNA, 0.2 mM of dNTPs, 10 pmol of each primer, 2.0 mM of $MgCl_2$, 50 mM of KCl, 10 mM of Tris-HCl (pH 9.0 at 25ºC), 0.1% TritonX-100 and 0.5 U of Taq DNA Polymerase. The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5 ug/ml) in 1 X TBE buffer at 95 volts. PCR products were visualized on UV light and photographed using a Polaroid camera. Amplified products were visually examined and the presence or absence of each size class was scored as 1 or 0, respectively.

The banding patterns generated by SSR-PCR marker analysis were compared to determine the genetic relatedness of the genotypes. Bands of the same mobility were scored as identical. The genetic similarity coefficient (GS) between two genotypes was estimated according to Dice coefficient [33] as follows: Dice formula: GS_{ij} = 2a/(2a+b+c), where GS_{ij} is the measure of genetic similarity between individuals i and j, a is the number of bands shared by i and j, b is the number of bands present in i and absent in j, and c is the number of bands present in j and absent in i.

The similarity matrix was used in the cluster analysis. The cluster analysis was employed to organize the observed data into meaningful structures to develop taxonomies. At the first step, when each accession represents its own cluster, the distances between these accessions are defined by the chosen distance measure (Dice coefficient). However, once several accessions have been linked together, the distance between two clusters is calculated as the average distance between all pairs of accessions in the two different clusters. This method is called unweighted pair group method using arithmetic average (UPGMA) according to Sneath and Sokal [33].

3. RESULTS AND DISCUSSION

3.1 Field Experiment

3.1.1 Analysis of variance

Analysis of variance of the split plot experiment that included two irrigation regimes in the main plots and 169 wheat genotypes in the sub-plots (53 M_3 selected families, 109 F_3 selected families and 7 parents) for studied characters is presented in Table (3).

Results indicated that mean squares due to irrigation regimes and those due to genotypes were highly significant for all studied traits, suggesting the significant effect of both irrigation regime and genotype on such traits. Mean squares due to genotypes X irrigation regimes interaction were highly significant for all studied traits, suggesting that performance of the studied genotypes in this experiment varied with water supply, confirming the results of other workers [7,8,11,34,35].

3.1.2 Morphological assessment of the best 12 selected families

The best twelve selected families (SF) included 7 M_3 families; two (SF2 from M_2 of Sakha-93 and SF3 from M_2 of Giza-168) selected under WS, and five (SF1 from M_2 of Aseel-5, SF4 and SF5 fromM_2 of Giza-168, SF6 and SF7 from M_2 of Sahel-1) selected under WW and 5 F_3 families; three (SF9, SF10 and SF11) selected under WS, from the F_2 of Sd4 X Mr5, Sk61 XAs5 and Sk61 X Sk93, respectively and two (SF8 and SF12) selected under WW, from the F_2 of Sd4 X Sk61 and Mr5 X Sk93, respectively.

Means of studied traits of the best 12 families and the 7 parental genotypes under WS and WW are presented in Table (4). On average, under WS conditions the group of the best 5 F_3families showed the highest mean grain yield (41.2g), while the group of 7 parents exhibited the lowest grain yield (26.6g). Moreover, yield reduction due to water stress in the best M_3 and best F_3 groups (12.0 and 13.3% on average, respectively) was less than that of the parents group (17.1%). This means that, in this experiment, selection practiced in both M_2 and F_2 populations was effective in producing higher yielding families under WS than the original parents and the success of the two procedures, *i.e.,* gamma-rays mutation induction and hybridization followed by transgressive segregation, in isolating new variants of higher drought tolerance. This conclusion was previously confirmed by Sobieh [6] and Al-Naggar et al. [7,8] for the success of mutation breeding. It is worth noting that the group of best F_3 families was, on average, earlier than the group of parents for DTH (by 5.3 days), DTA (by 3.9 days) and DTM (by 1.9 days) under WS Table (3). Comparing all the 12 best families (Table 5), it is interesting to mention that the best family in grain yield/plant under water stress was SF9 (45.6 g), followed by SF11 (44.2 g) and SF3 (42.8 g) with a very low reduction due to water stress (6.9, 6.2 and 11.2%, respectively). It is worth noting that the best three families under WS resulted from selection for high yield under water stress conditions.

The earliest M_3 family for DTM was SF6 as compared with the earliest parents Sids-4, Sakha-61 and Aseel-5, under water stress. The best M_3 and F_3 families for grain yield/plant were characterized by high value of one or more of yield components.

Table 2. Description of the SSR loci used in this study

No.	Primer	Sequence	
		Forward	Reverse
1	WMS 06	5 - CGT ATC ACC TCC TAG CTA AAC TAG - 3	5 - AGC CTT ATC ATG ACC CTA CCT T - 3
2	WMS 30	5 - ATC TTA GCA TAG AAG GGA GTG GG - 3	5 - TTC TGC ACC CTG GGT GAT TGC - 3
3	WMS 108	5 - ATT AAT ACC TGA GGG AGG TGC - 3	5 - GGT CTC AGG AGC AAG AAC AC - 3
4	WMS 118	5 - GAT GGT GCC ACT TGA GCA TG - 3	5 - GAT TG TCA AAT GGA ACA CCC - 3
5	WMS 149	5 - CAT TGT TTT CTG CCT CTA GCC - 3	5 - CTA GCA TCG AAC CTG AAC AAG - 3
6	WMS 169	5 - ACC ACT GCA GAG AAC ACA TAC G - 3	5 - GTG CTC TGC TCT AAG TGT GGG - 3
7	WMC 177	5 - AGGGCTCTCTTTAATTCTTGCT - 3	5 - GGTCTATCGTAATCCACCTGTA - 3
8	WMC 179	5 - CATGGTGGCCATGAGTGGAGGT - 3	5 - CATGATCTTGCGTGTGCGTAGG - 3
9	WMS 198	5 - TTG AAC CGG AAG GAG TAC AG - 3	5 - TCA GTT TAT TTT GGG CAT GTG - 3
10	WMC 235	5 - ACTGTTCCTATCCGTGCACTGG - 3	5 - GAGGCAAAGTTCTGGAGGTCTG - 3
11	WMS 304	5 - AGGAAACAGAAATATCGCGG - 3	5 - AGG ACT GTG GGG AAT GAA TG - 3
12	WMC 307	5 - GTTTGAAGACCAAGCTCCTCCT - 3	5 - ACCATAACCTCTCAAGAACCCA - 3
13	WMC 322	5 - CGCCCCACTATGCTTTG - 3	5 - CCCAGTCCAGCTAGCCTCC - 3
14	WMS 375	5 - ATTGGCGACTCTAGCATATACG - 3	5 - GGGATGTCTGTTCCATCTTAGC - 3
15	WMC 445	5 - AGAATAGGTTCTTGGGCCAGTC - 3	5 – GAGATGATCTCCTCCATCAGCA - 3

Table 3. Analysis of variance of split plot design for 169 genotypes including 162 selected families (53 from M_2 and 109 from F_2) and 7 parents under water stress and well watering conditions (Inshas, 2011/ 2012 season)

S.V.	d.f.	Mean squares				
		Days to heading	Days to anthesis	Days to maturity	Plant height	Spike length
Replication	2	14.1	9.3	2.8	1.1	0.0183
Watering (W)	1	1063.4**	1550.3**	943.7**	9040.4**	67.9**
Error [a]	2	1.6	5.2	7.5	1.0	0.01
Genotypes (G)	168	71.1**	101.8**	72.7**	336.3**	7.5**
G x W	168	4.0**	5.0**	0.6**	23.2**	0.4**
Error [b]	672	0.3	0.2	0.2	0.5	0.01
		Spike weight	Spikes/plant	Grains /spike	100-grain weight	Grain yield /plant
Replication	2	0.0002	0.0005	92.2	0.01	0.1
Watering (W)	1	44.2**	285.3**	9263.7**	96.0**	13576.2**
Error [a]	2	0.02	0.0005	5.8	0.003	1.0
Genotypes (G)	168	0.9**	14.7**	187.5**	1.0**	220.2**
G x W	168	0.2**	1.2**	8.6**	0.2**	24.7**
Error [b]	672	0.003	0.01	0.6	0.003	0.4

*** = significant at 0.01, probability level*

Table 4. Mean performance of the 12 best selected families (7 best M_3 and 5 best F_3 families) and their parents for studied wheat traits under water stress (WS) conditions (2011/ 2012 season)

Genotypes	DTH (day)	DTA (day)	DTM (day)	PH (cm)	SL (cm)	SW (g)	SPP (No)	GPS (No)	100GW (g)	GYPP (g)	Red. %
Best M_3											
SF1	95	111	141	95	13.7	3.6	11.7	75	4.4	42.1	10.0
SF2	102	112	141	96	14.1	3.3	13.3	68	4.7	42.0	12.1
SF3	91	102	135	89	14.1	3.7	11.9	74	4.3	42.8	11.2
SF4	94	103	137	87	13.7	4.0	10.1	71	4.4	39.9	10.1
SF5	93	102	137	84	13.5	3.5	11.3	65	4.6	39.3	11.9
SF6	95	105	129	101	13.4	3.7	10.9	68	4.8	40.2	13.0
SF7	98	111	139	80	13.1	3.3	11.9	64	4.8	38.2	15.3
Av. (M_3)	95.4	106.6	137.0	90.3	13.7	3.6	11.6	69.3	4.6	40.6	12.0
Best F_3											
SF8	89	98	131	103	13.5	3.6	10.9	67	5.0	38.2	11.6
SF9	82	92	131	97	14.3	4.1	11.2	71	5.0	45.6	6.9
SF10	92	100	132	90	12.0	4.0	9.7	72	5.5	38.5	29.0
SF11	88	96	133	85	13.9	3.9	11.4	64	5.6	44.2	6.2
SF12	87	99	131	85	16.3	5.0	8.0	64	5.6	39.4	12.6
Av. (F_3)	87.6	97	131.6	92	14	4.1	10.2	67.6	5.3	41.2	13.3
Parents											
Sids-4	87	95	132	96	16.2	4.3	5.3	84.0	5.0	23.1	24.6
Sakha-61	92	100	132	79	10.3	3.1	8.1	63.0	4.4	24.8	17.7
Maryout-5	95	103	138	94	14.2	3.8	6.9	76.0	4.9	26.1	13.4
Aseel-5	96	101	132	92	13.1	3.4	9.1	69.0	4.6	33.3	10.6
Sakha-93	94	101	132	81	12.2	3.2	8.7	66.0	4.4	28.2	17.0
Giza-168	95	102	136	86	12.6	3.6	7.3	65.0	4.2	26.0	15.5
Sahel-1	94	107	133	100	13.3	3.3	7.5	68.0	4.8	24.7	20.8
Av. (P)	92.9	100.9	133.5	89.9	13.1	3.5	7.6	70.1	4.6	26.6	17.1
$LSD_{0.05}$	0.67	0.58	0.56	1.08	0.13	0.08	0.13	0.90	0.07	0.80	

Red. (Reduction %) = 100(GYPP under WW - GYPP under WS)/ GYPP under WW, P = Parents, Av. = Average
F_3 = best F_3 families, M_3 = best M_3 families

Practicing selection in the F_2 generation of the studied crosses resulted in a significant superiority (selection gain) over the better parent of the corresponding cross in grain yield/plant ranging from 15.48% for SF10 to 74.71% for SF9 under water stress and from 32.76% for SF12 to 60.24% for SF9 under non-stress conditions (Table 5). The SF9 selected F_3 family showed the highest selection gain under both water stress and non-stress conditions.

The five selected F_3 families (SF8, SF9, SF10, SF11 and SF12) showed significant superiority in grain yield over their better parents under both stress and non-stress conditions. These superior families in grain yield are the result of transgressive segregation and may be considered promising lines having tolerance to drought conditions. Observations on transgressive segregation in segregating hybrid generations were previously explained by several research workers, *e.g.*, Al-Bakry et al. [36]. The results from classical genetic studies have provided fairly convincing evidence for the hypotheses that transgressive segregation can result from the complementary gene action [37].

Practicing selection for high grain yield in the M_2 populations derived from gamma radiation treatment of parent cultivars of wheat resulted in an actual progress over the corresponding original parent in GYPP ranging from 26.27 to 64.36% under WS for SF1 and SF3, respectively (Table 5). The SF3 selected M_3 family showed the highest selection gain followed by SF6 (62.62% under WS). These two M_3 families showed also superiority in SPP and in DTM, *i.e.*, earliness of maturity.

Superiority in grain yield of the 12 best families over the Egyptian cultivar Sids-4 reached 97.8% for SF9, 91.8% for SF11and 85.7% for SF3 under water stress. The twelve selected families should further be selfed for more generation to reach complete homozygosity to be tested for their stability under a variety of water stress conditions.

3.1.3 The most important traits of the best 12 selections

SFI: It is a high yielding mutant under WS (2nd highest best M_3's) with low reduction (10.0%) due to water stress, *i.e.*, drought tolerant. It recorded the highest number of grains/spike amongst the 7 best M_3 families (Fig. 1).

SF2: It is a high yielding mutant under WS conditions; with low yield reduction due to water stress (drought tolerant). It recorded the highest number of spikes (13.3) under water stress (Fig. 2).

Table 5. Actual progress (%) of the best selections over the original parent (from M_2's) and over the better parent (from F_2's) for DTM, SPP and GYPP under water stress (WS) and well watering (WW) conditions (2011/ 2012 season)

Best families	Pedigree	DTM		SPP		GYPP	
		WW	WS	WW	WS	WW	WS
Best M_3 families		**Progress (%) over the original parent**					
SF1	As-5-WW-PM5	6.77	7.22	21.78	28.57	25.44	26.27
SF2	Sk-93-WS-PM2	5.97	7.22	40.21	52.87	40.71	49.04
SF3	Gz-168-WS-PM2	-1.09	-0.74	53.01	63.01	56.34	64.36
SF4	Gz-168-WW-PM5	1.09	0.74	31.33	38.36	44.02	53.23
SF5	Gz-168-WW-PM6	1.09	0.74	50.60	54.79	44.66	50.92
SF6	Sh-1-WW-PM6	-2.60	-3.01	40.24	45.33	48.03	62.62
SF7	Sh-1-WW-PM7	4.83	4.51	50.00	58.67	44.50	54.53
Best F_3 families		**Progress (%) over better parent**					
SF8	Sd4XSk.61-WW-PS8	-0.37	-0.76	26.37	34.57	41.27	54.22
SF9	Sd4XMr5-WS-PS2	-1.12	-0.76	68.06	62.32	60.24	74.71
SF10	Sk61XAs5-WS-PS3	0.37	0.00	17.82	6.59	45.27	15.48
SF11	Sk61XSk93-WS-PS2	0.37	0.76	20.62	31.03	38.65	56.85
SF12	Mr5XSk93-WW-PS8	-0.75	-0.76	-2.06	-8.05	32.76	39.82

Fig. 1. The highest number of grains/spike for SF1 and SF3 as compared with their parents As-5 and Gz-168, respectively, and the longest spike for SF3

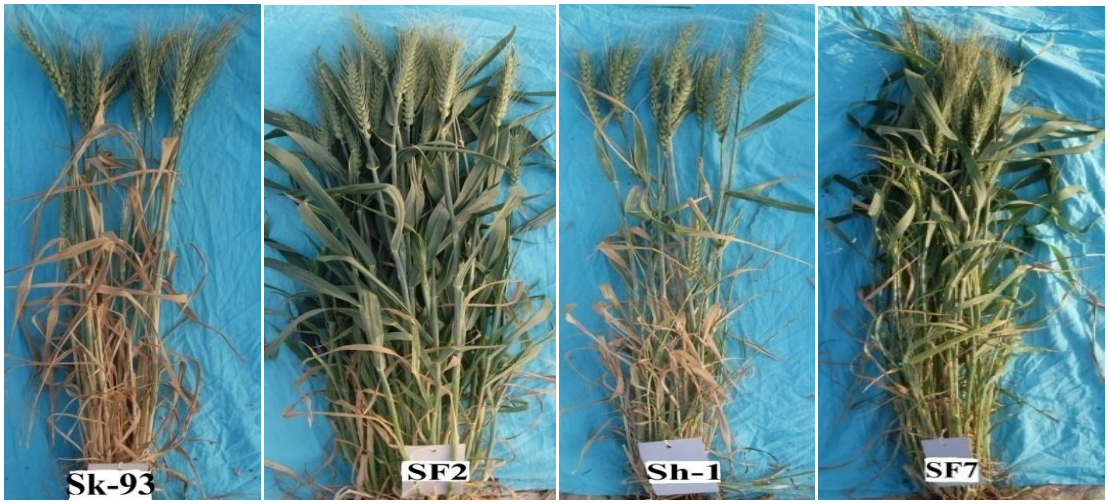

Fig. 2. The highest number of spikes for SF2 and SF7as compared with their parents Sk-93 and Sh-1, respectively

SF3: This mutant ranked first in grain yield/plant amongst the 7 best M_3 families under both WS and WW conditions; with low yield reduction due to water stress, *i.e.*, a drought tolerant family. It recorded the second largest number of grains/spike under WS and the longest spike (Fig. 1) and the earliest in DTH and DTM under WW and WS.

SF4: It is a high yielding mutant under both WW and WS; with low yield reduction due to water stress, *i.e.,* drought tolerant. It recorded the heaviest spike and grain under both irrigation regimes.

SF5: It is a high yielding mutant under WS conditions; with low reduction in GYPP due to water stress, *i.e.,* a drought-tolerant family.

SF6: It is a high yielding mutant under WS conditions, with low reduction in GYPP due to water stress, *i.e.,* a drought-tolerant family. It ranked the earliest amongst the best 12 families and the 7 parents. It recorded the heaviest grain under both irrigation regimes.

SF7: It is a high yielding M_3 family under both WW and WS conditions; with low yield reduction due to water stress. It is also characterized by the shortest plant height, the heaviest grain and

the second highest in SPP (Fig. 2) amongst the 7 best selected M_3 families.

SF8: It is a transgressive segregant in the F_3 generation. It showed high GYPP under WS; with low yield reduction due to water stress. It also recorded the tallest plant (Fig. 3) and was earlier than the earliest parent.

SF9: It is a transgressive segregant in the F_3 generation. It showed the highest GYPP under WS; with the second lowest yield reduction (6.9%) due to WS, *i.e,* the 2nd most drought tolerant F_3family. It is the earliest F_3 for DTH and DTA (Fig. 4).

SF10: It is a transgressive segregant in the F_3 generation. It recorded significantly higher yield than the best parent (Mr-5) under drought stress conditions. This family (SF10) also recorded the heaviest grain (Fig. 5) under both irrigation regimes.

SF11: It is a transgressive segregant in the F_3 generation. It is the most drought tolerant selected family; since reduction in its yield due to water stress was the lowest (6.2%). Its yield under WS ranked the second highest and amongst the 5 best F_3 families. This selected family showed the heaviest grain (Fig. 5) under both WW and WS conditions.

SF12: It is a high yielding family under WS; with low yield reduction (12.6%) due to water stress. It is characterized by the longest and heaviest spike (Fig. 5).

3.2 SSR Assessment

3.2.1 Genetic polymorphism among the 19 wheat genotypes

Fifteen SSR primers revealed discernible amplification profiles, therefore they were employed to investigate the genetic polymorphism among the 19 wheat genotypes (Table 5).

Fig. 3. The earliest maturity and tallest plant shown by SF8 as compared with the better parent Sids-4

Fig. 4. The earliest heading shown by SF9 as compared with the better parent Sids-4

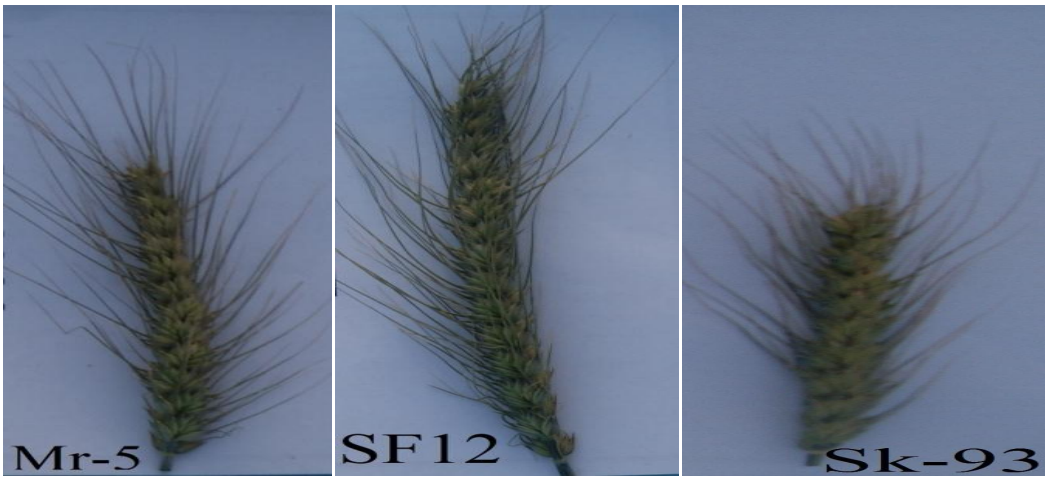

Fig. 5. The longest and heaviest spike of SF12 as compared with the better parent Maryout-5 and Sakha-93

The 15 SSR primers produced 46 amplicons, out of them 42 were polymorphic and the average percentage of polymorphism was 95.65% (Table 6). The number of amplicons per primer ranged from 1 (WMS 30, WMC 235 and WMS 304) to 10 (WMC 179) with an average of 3.07 fragments/primer across the different genotypes. However, the number of polymorphic amplicons varied from 0 (WMC 235 and WMS 304) to 10 (WMC 179) with an average number of polymorphic amplicons of 2.93 fragments/primer. Thirteen out of the 15 primers exhibited 100% polymorphism, while two primers (WMC235 and WMS 304) showed no polymorphism. The size of amplified fragments varied with the different primers, ranging from 50 to 1500 bp. In this context, Naghavi et al. [38] used RAPD and SSR analyses to estimate genetic diversity among bread wheat genotypes including nineteen Iranian cultivars and two lines (Shain and Line 518). The level of polymorphism was 88% with RAPDs compared to 100% with SSRs. Abd El-Hadi [25] investigated the genetic diversity among three durum wheat cultivars and their six selected drought tolerant lines with ISSR analysis. He reported that out of 99 amplified DNA fragments, 70 were polymorphic, representing a level of 71.42% polymorphism. Moreover, Bousba et al. [31] reported that a total of 136 fragments were obtained from the 26 SSR primers and all the bands were polymorphic across all screened genotypes. They added that polymorphism information content (PIC) values ranged from 38% to 94%, with an average of 74%. The results of the present study are in good agreement with those reported in the literature and confirm that polymorphism is a general phenomenon in wheat populations resulting after irradiation with gamma rays and hybridization followed by segregating generations, as in the case of this study.

3.2.2 Identification of unique SSR markers for drought tolerance

Unique markers are defined as bands that specifically identify an accession from the other by their presence or absence. The bands that are present in one accession but not found in the others are termed positive unique markers (PUM), in contrast with the negative unique markers (NUM), which are absent in a specific genotype. These bands could be used for genotype identification [39].

As shown in Table (7), the SSR assay permitted the identification of three out of 19 wheat genotypes by unique positive and/or negative markers. These three genotypes, namely, SF3, SF4 and Aseel-5 (all are drought tolerant) are characterized by five positive unique markers, while one of them (SF4) was characterized by two negative unique markers.

The selected drought tolerant mutant (SF3) was characterized by three unique positive markers amplified by the primers WMC 177 (100 bp) and WMC 179 (800 and 1000 bp). The selected drought tolerant mutant (SF4) was characterized by one unique positive marker amplified by the primer WMC 179 (50 bp) and two negative unique markers amplified by the primers WMC 177 (200 bp) and WMC 179 (550 bp). The drought tolerant Syrian parent (Aseel-5) was

characterized by one positive unique marker amplified by the primer WMS 198 (100 bp). The remaining 16 wheat genotypes did not exhibit any unique marker. The highest number of unique markers (four) was amplified by the primer WMC 179 (3 positive and one negative) followed by the primer WMC 177 (two unique markers; one positive and one negative). The size of these unique markers ranged from 50 to 1000 bp.

In this context, Moghaieb et al. [40] determined the genotype specific SSR markers in nine bread and pasta wheat genotypes. They reported that 13 markers can be considered as a useful marker for screening for salt tolerance in these wheat genotypes. Abd El-Hadi [25] reported that in durum wheat, ISSR analysis showed four genotype–specific markers for the drought tolerant putative line S_3 that show a significant increase in grain yield/plant over their parents under drought stress conditions. Using SSR analysis, we were able to identify seven unique bands in some drought tolerant wheat genotypes. These bands might be considered useful as markers associated with drought tolerance in bread wheat breeding programs. Further experiments need to be achieved to determine the linkage between the genotype–specific SSR markers used in the present study and gene(s) for drought tolerance in the studied bread wheat genotypes. The present results support the idea that SSR analysis can provide a fast detection of species-specific markers linked to drought stress tolerance in bread wheat.

3.2.3 Genetic similarities based on SSR analysis

The scored data from the SSR analysis in this study were used to compute the similarity matrices according to Dice coefficient [33]. As shown in Table (8) the genetic similarity ranged from 30% (between SF4 and each of Sakha-61 and Maryout-5) to 88% (between SF7 and each of SF1 and SF6). High genetic similarity between SF6 and SF7 is attributed to the fact that both of them were derived from the Sahel-1 cultivar irradiated by 350 Gy gamma rays.

The results of this investigation indicated that all the twelve selected drought tolerant families differ from their parents at the DNA level where the average of genetic similarity (GS) between selections and their parents was about 68%. The mutants SF3 and SF4 exhibited very low genetic similarity with their common parent Giza-168 (42 and 40%, respectively), indicating that gamma rays were very effective in changing the genetic background of Giza-168in a positive direction, *i.e*., towards high GYPP under WS conditions. In this context, Abd El-Hadi [25] reported that the genetic similarity between six selected putative durum wheat mutants (derived *via* gamma rays) and their parents, based on ISSR analysis, ranged from 12.7 to 87.4%. Munir et al. [20] also reported that genetic similarity coefficients for SSR markers between 18 salt tolerant wheat accessions ranged from 45 to 95%.

3.2.4 Cluster analysis as revealed by SSR

The Dice SSR-based coefficients of genetic similarity among the 19 wheat genotypes were employed to develop a dendrogram using the UPGMA method (Fig. 6). The dendrogram separated the selected F_3 family (SF4) from the other wheat genotypes, which formed a cluster in which the selected F_3 family SF3 was separated from the remaining 17 genotypes. This demonstrates the distinctiveness of the genetic background of these two genotypes (SF3 and SF4) from all the other genotypes.

The remaining 17 genotypes were divided into three main groups. The first group was divided into two sub-groups; the first sub-group separated Sakha-61 from two other genotypes (Maryout-5 and Aseel-5) and the second sub-group was divided into two classes; one of which included two genotypes (Sids-4 and SF2) and the second class separated SF6 from the other two genotypes (SF1 and SF7).

The second group separated SF8 (in one sub-group) from 4 other genotypes (in another sub-group); the latter sub-group separated SF5 from three other genotypes in a separate class; this class separated SF10 from the other two genotypes (Giza -168 and SF9) in one sub-class. The third group separated SF12 (in one sub-group) from the remaining 3 genotypes in another sub-group. The second sub-group separated SF11 in one class from the remaining two genotypes (Sakha-93 and Sahel-1) in another class.

Table 6. Number of monomorphic and polymorphic amplicons and percentage of polymorphism, as revealed by SSR primers for 19 wheat genotypes (12 selected families and their 7 parents)

Primer	Total no of amplicons	No of mono- morphic amplicons	No of poly- morphic amplicons	Polymorphism (%)
WMS 06	2	0	2	100
WMS 30	1	0	1	100
WMS 108	6	0	6	100
WMS 118	3	0	3	100
WMS 149	3	0	3	100
WMS169	2	0	2	100
WMC 177	2	0	2	100
WMC 179	10	0	10	100
WMS 198	5	0	5	100
WMC 235	1	1	0	0
WMS 304	1	1	0	0
WMC 307	2	0	2	100
WMC 322	2	0	2	100
WMS 375	2	0	2	100
WMC 445	4	0	4	100
Total	46	2	44	
Average	3.07	0.13	2.93	95.65

Table 7. Unique positive and negative SSR markers generated for 19 wheat genotypes (12 selected families and their 7 parents), marker size (bp) and total number of markers identifying each genotype

Genotype	Positive unique markers		Negative unique markers		
	Primer (band size/bp)	Total no.	Primer (band size/bp)	Total no.	Grand total
Sids-4	-		-		
Sakha-61	-		-		
Maryout-5	-		-		
Asseel-5	WMS 198 (100)	1	-		1
Sakha-93	-		-		
Giza-168	-		-		
Sahel-1	-		-		
SF1	-		-		
SF2	-		-		
SF3	WMC 177 (100), WMC 179 (800, 1000)	3	-		3
SF4	WMC 179 (50)	1	WMC 177 (200), WMC 179 (550)	2	3
SF5	-		-		
SF6	-		-		
SF7	-		-		
SF8	-		-		
SF9	-		-		
SF10	-		-		
SF11	-		-		
SF12	-		-		
Total		5		2	7

Table 8. Genetic similarity (GS) matrices among the nineteen wheat genotypes (12 selected families and 7 parents)

Genotype	Sd-4	Sk-61	Mr-5	As-5	Sk-93	Gz-168	Sah-1	SF1	SF2	SF3	SF4	SF5	SF6	SF7	SF8	SF9	SF10	SF11
Sd-4	1.00																	
Sk-61	0.68	1.00																
Mr-5	0.63	0.82	1.00															
As-5	0.69	0.80	0.84	1.00														
Sk-93	0.73	0.72	0.67	0.72	1.00													
Gz-168	0.74	0.82	0.78	0.77	0.73	1.00												
Sah-1	0.63	0.73	0.81	0.83	0.83	0.74	1.00											
SF1	0.77	0.75	0.84	0.86	0.71	0.84	0.76	1.00										
SF2	0.85	0.68	0.71	0.78	0.69	0.70	0.63	0.81	1.00									
SF3	0.57	0.50	0.51	0.45	0.36	0.42	0.43	0.48	0.51	1.00								
SF4	0.69	0.30	0.30	0.39	0.44	0.40	0.33	0.46	0.50	0.37	1.00							
SF5	0.73	0.71	0.73	0.72	0.72	0.81	0.69	0.78	0.68	0.41	0.50	1.00						
SF6	0.71	0.65	0.75	0.74	0.70	0.75	0.71	0.85	0.76	0.50	0.49	0.73	1.00					
SF7	0.80	0.74	0.75	0.81	0.78	0.75	0.71	0.88	0.84	0.45	0.54	0.82	0.88	1.00				
SF8	0.70	0.68	0.62	0.57	0.61	0.70	0.54	0.59	0.65	0.44	0.34	0.73	0.62	0.67	1.00			
SF9	0.79	0.72	0.65	0.68	0.64	0.88	0.65	0.76	0.74	0.42	0.47	0.81	0.70	0.74	0.80	1.00		
SF10	0.80	0.73	0.67	0.69	0.73	0.79	0.67	0.77	0.75	0.46	0.44	0.77	0.67	0.76	0.81	0.84	1.00	
SF11	0.70	0.77	0.70	0.73	0.80	0.78	0.81	0.75	0.61	0.39	0.42	0.76	0.71	0.75	0.70	0.77	0.78	1.00
SF12	0.60	0.67	0.65	0.64	0.71	0.64	0.72	0.67	0.64	0.43	0.31	0.71	0.69	0.69	0.73	0.71	0.68	0.79

Sd-4= Sids-4, Sk-61= Sakha-61, Mr-5= Maryout-5, As-5= Aseel-5, Sk-93= Sakha-93, Gz-168= Giza-168, Sah-1= Sahel-1, SF1 to SF12= Selected families

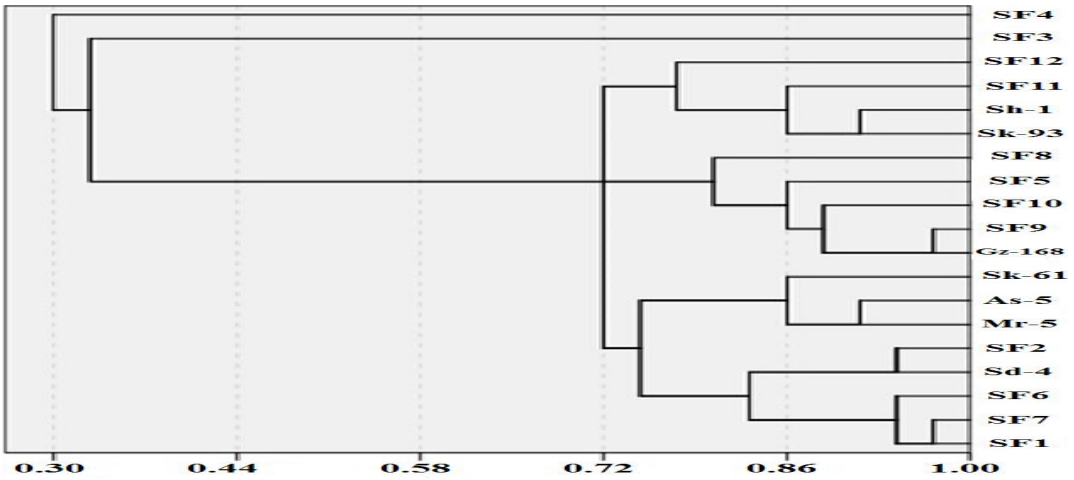

Fig. 6. Dendrogram for the nineteen wheat genotypes (12 selected families and 7 parents) constructed from SSR data using (UPGMA) according to Dice coefficients

4. CONCLUSION

The present investigation concluded that exposing some wheat cultivars and lines to gamma rays at a dose of 350 GY could induce a number (7) of putative mutants, that showed significant superiority in grain yield over the best parents reaching 64.36% for SF3 under water sress conditions. Also, some transgressive segregants (5) selected from F_2 generation of hybrids between wheat cultivars and lines showed significant superiority in grain productivity under water deficit reaching 74.71% for SF9. These new genotypes were considered drought tolerant. Molecular assessment of these mutants, transgressive segregants and their parents by SSR analysis proved the genetic dissimilarity among these new genotypes and their parents, indicating the efficiency of the two breeding methods used in this study in inducing drought tolerant genotypes.SSR assay permitted the identification of seven unique bands (5 positive and 2 negative) for three drought tolerant wheat genotypes (SF3, SF4 and Aseel-5). These bands might be considered useful as markers associated with drought tolerance in bread wheat breeding programs. Further experiments need to be achieved to determine the linkage between the genotype–specific SSR markers used in the present study and gene(s) for drought tolerance in the studied bread wheat genotypes.

ACKNOWLEDGMENTS

We would like to acknowledge the Atomic Energy Authorities in Egypt for exposing wheat seeds to gamma rays and offering the laboratory facilities for carrying out the SSR analysis. Thanks are also due to Dr. Reda Shabana and Dr. Mazhar Fawzi, Professors of Plant Breeding, Faculty of Agriculture, Cairo University for revising the manuscript of this article.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. MALR/ARE. Ministry of Agriculture and Land Reclamation, Arab Republic of Egypt. Agricultural Statistics; 2012.
2. Clarke JM, DePauw RM, Townley-Smith TF. Evaluation of methods for quantification of drought tolerance in wheat. Crop Sci. 1992;32(3):723-728.
3. Mirbahar AA, Markhand GS, Mahar AR, Abro SA, Kanhar NA. Effect of water stress on yield and yield components of wheat (*Triticum aestivum* L.) varieties. Pak. J. Bot. 2009;41(3):1303-1310.
4. Poehlman J M, Sleper DA. Breeding Field Crops. 4th ed. Iowa State University Press, Ames, USA. 1995;494.
5. Khanna VK, Bajpai GC, Hussain SM. Effect of gamma radiation on germination and mature plant characters of wheat and triticale. Haryana Agricultural University Journal of Research. 1986;16(1):42-50.
6. Sobieh ESS. Induction of short culm mutants for bread wheat by using gamma rays. Arab Journal of Nuclear Sciences and Applications. 2002;35(1):309-317.

7. Al-Naggar AMM, Ragab AEI, Youssef SS, Al-Bakry RIM. New genetic variation in drought tolerance induced via irradiation and hybridization of Egyptian cultivars of bread wheat. Egypt. J. Plant Breed. 2004; 8:353-370.
8. Al-Naggar AMM, Atta MM, Shaheen AM, Al-Azab Kh F. Gamma rays and EMS induced drought tolerant mutants in bread wheat. Egypt. J. Plant Breed. 2007;11(3): 135-165.
9. FAO / IAEA. Mutant Variety Database. Cereals and Legumes. December, 2012. FAO/IAEA, Vienna. Available:http://mvgs.iaea.org.
10. Singh BD. Breeding for resistance to abiotic stresses. I. Drought resistance. In: Plant Breeding Principles and Methods. Kalayani Publishers, New Delhi, India. 2000;381-409.
11. Al-Naggar AMM, Abdel- Raouf MS, El-Borhamy HS, Shehab-El-Deen MT. Gene effects controlling inheritance of earliness and yield traits of bread wheat under drought stress conditions. Egypt. J. Plant Breed. 2012;16(3): 41- 59.
12. Al-Bakry MRI. Glaucous wheat mutants. I. Agronomic performance and epicuticular wax content. Egypt. J. Plant Breed. 2007;11(1):1-9.
13. Al-Naggar AMM, Shehab-El- Deen MT. Predicted and actual gain from selection for early maturing and high yielding wheat genotypes under water stress conditions. Egypt. J. Plant Breed. 2012;16(3):73 -92.
14. Tharwat E, Akbar H, Jaime A, Teixeira DS. Genetic analysis and selection for bread wheat (*Triticum aestivum* L.) yield and agronomic traits under drought conditions. International Journal of Plant Breeding. 2013;7(1):61- 68.
15. Xiao J, Li J, Yuan L, Mccouch, S, Tanksley SK. Genetic diversity and its relationship to hybrid performance and heterosis in rice as revealed by PCR-based markers. Theor. Appl. Genet. 1996; 92:637-664.
16. Ovesna J, Polakova K, Lisova L. DNA analysis and their applications in plant breeding Czech. J. Genet. Plant Breed. 2002;38: 29-40.
17. Momtaz OA, Hashem MM, Moghaieb REA, Hussein MH. Genetic polymorphism among Egyptian rice genotypes as revealed by RAPD, SSR and AFLP analyses. Arab J. Biotech. 2010;13(2): 173-184.
18. Akkaya MS, Shoemaker RC, Specht JE, Bhagwat AA, Cregan PB. Integration of simple sequence repeats DNA markers into a soybean linkage map. Crop Sci. 1995;35 :1439-1445.
19. Wang Z, Weber JL, Zhong G, Tanksley SD. Survey of plant short tandem DNA repeats. Theor. Appl. Genet. 1994;88:1-6.
20. Munir A, Armghan S, Iqbal M, Asif M, Hirani AH. Morphological and molecular genetic variation in wheat for salinity tolerance at germination and early seedling stage. AJCS. 2013;7(1):66-74.
21. Ivandiç V, Hackett CA, Nevo E, Keith R, Thomas WTB, Forster BP. Analysis of simple sequence repeats (SSRs) in wild barley from the fertile crescent: Associations with ecology, geography and flowering time. Plant Mol. Biol. 2002;48: 511–527.
22. Liviero L, Maestri M, Gulli E, Nevo N, Marmiroli E. Ecogeographic adaptation and genetic variation in wild barley, application of molecular markers targeted to environmentally regulated genes. Genet. Resources and Crop Evol. 2002;49:133–144.
23. Quarrie SA, Dodig D, Pekiç S, Kirby J, Kobiljski B. Prospects for marker-assisted selection of improved drought responses in wheat. Bulg. J. Plant Physiol. 2003;83-95.
24. Ciucă M, Petcu E. SSR markers associated with membrane stability in wheat (*Triticum aestivum* L.). Romanian Agricultural Research. 2009;26:21-24.
25. Abd El-Hadi AA. Molecular characterization of some durum wheat drought tolerant mutant by RAPD and ISSR analysis. Arab J. Biotech. 2012;15 (1):77-90.
26. El-Ameen T. Molecular markers for drought tolerance in bread wheat. African Journal of Biotechnology. 2013;12(21): 3148-3152.
27. El Siddig MA, Baenziger S, Dweikat I, El Hussein AA. Preliminary screening for water stress tolerance and genetic diversity in wheat (*Triticum aestivium* L.) cultivars from Sudan. Journal of Genetic Engineering and Biotechnology. 2013;2(2): 87-94.
28. Snedecor GW, Cochran WG. Statistical Method. 8th ed. Iowa State Univ. Press, Ames, USA; 1989.
29. Doyle JJ, Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf. Phytochem. Bull. 1987;19:11-15.

30. Sumar A, Ahmet D, Gulay Y. Isolation of DNA for RAPD analysis from dry leaf materials of some *Hesperis* L. Speciments. Plant molecular Biology Reporter. 2003;21: 461-461.
31. Bousba R, Michael B, Abdelh AD, Samer L, Abdulkader D, Kadour B, Mustapha I, Gaboun F, Ykhlef N. Screening for drought tolerance using molecular markers and phenotypic diversity in durum wheat genotypes. World Applied Sciences Journal. 2012;16 (9):1219-1226.
32. Roider MS, Korzum V, Wendehake K, Plaschke J, Tixier M, Leroy P, Ganal MW. A microsatellite map of wheat. Genetics, 1998;149:2007-2023.
33. Sneath PHA, Sokal RR. Numerical Taxonomy. Freeman, San Francisco, California, USA. 1973; 513.
34. Sharma HP, Bhargava SC. Relative sensitivity of wheat genotypes under moisture stress conditions. Annals of Biology Ludhiana. 1996;12(1):39-42.
35. Ragab AI, Sobieh E-SSS. An attempt to improve bread wheat for water stress tolerance using gamma irradiation. Egypt. J. Appl. Sci. 2000;15(11):25-45.
36. Al-Bakry MRI, Al-Naggar AMM, Moustafa HAM. Improvement of grain yield of a glaucous wheat mutant line *via* backcrossing. Egypt. J. Plant Breed. 2008; 12(2):123-131.
37. Vega U, Frey KJ. Transgressive segregation in inter and intra-specific crosses of barley. Euphytica. 1980;29: 585-594.
38. Naghavi MR, Mohsen M, Ramshini HA, Bahman F. Comparative analysis of the genetic diversity among bread wheat genotypes based on RAPD and SSR markers. Iranian Journal of Biotechnology. 2004;2:195-202.
39. Hussein EHA, Abd- Alla SM, Awad Nahla A, Hussein MS. Assessment of genetic variability and genotyping of some Citrus accessions using molecular markers. Arab J. Biotech. 2003;7(1):23-36.
40. Moghaieb REA, Talaa NB, Abdel-Hadi AA, Youssef SS, El-Sharkawy AM. Genetic variation for salt tolerance in some bread and pasta wheat genotypes. Arab J. Biotech. 2010;13(1):125-142.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=1028&id=11&aid=8512