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MEAN PERFORMANCE, GENETIC DIVERSITY AND DNA ANALYSIS IN M_3 GENERATION OF SOME WHEAT MUTANTS UNDER WATER STRESS

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ABSTRACT

The field experiments was conducted during the growing season of 2019/2020, to study the mean performance and genetic diversity using RAPD Marker in M_3 generation of some wheat mutants with influenced by gamma ray and sodium azaide under water stress conditions, at the Experimental Farm, Faculty of Technology and Development, Zagazig University, Egypt. The all recommended cultural practices for wheat production except irrigation. It was irrigated immediately after sowing the first irrigation were applied after 45 days from sowing and the second irrigation up to the flowering stage. The experimental design was complete blocks design with three replicates. The analysis of variance showed a significant differences among Gimmieza-11 and there M_3 mutants for days to heading, spike length, number of spikelets/spike, number of spikes/plant, number of grains/spike, spike grain weight/g, 1000-grain weight/g, grain yield/plant, biological yield/plant and harvest index. The all mutants from Gemmeiza-11 were give the highest values of mean performance for grain yield plant, The Sids-12 0.08 sodium azide (SA) had the highest value (45.39) followed by Sids-12 350Gy3 (40.74) and Sids-12 350 Gy1 (39.87) thy were more than control (38.99) for harvest index %. RAPD data analysis showed that, primers applied on wheat genotypes produced 18 bands; 16 of these were polymorphic. Primer OPB-5 gave the highest number of bands (5) and primers O-7, A-20 and OPB-7 had the lowest number (3).The highest percentage of polymorphic bands were produced by primers O-7, A-6 and OPB-5 (100%) and the lowest percentage of polymorphic bands were produced by A-20 and OPB-7 (67%).

***Conclusively,** this study established the importance of gamma rays and sodium azide mutagenesis increating genetic variation within and among wheat breeding populations. wide phenotypic variation in*

mutants under each breeding population were identified for improving drought tolerance yield, and yield-related traits.

Key words: Mutants, mean performance, genetic diversity, DNA analysis, wheat.

INTRODUCTION

In Egypt, wheat (*Triticum aestivum* L.) is one of the most important crops and can be considered as the main source of carbohydrate. Besides being a high carbohydrate food. Wheat contains valuable protein, minerals, and vitamins. In Egypt, there is a big gap between needs and production of wheat. To fill up this gap, the imported amount reached about 49.80% of the total amount of wheat consumption (**FAO, 2018**). Although, wheat is the most cultivation area occupies more than 44.41% of cereals cultivation area (**FAO, 2018**). In the last two decades, Egypt population increased by about 84% (**FAO, 2018**), while the cultivated land and water resources remains the same (**Nassar, 2019)**. Wheat breeding program for improving drought tolerance, the source populations should possess a great amount of genetic variability amenable for efficient selection, Drought tolerant wheat mutants are the ultimate means of safeguarding the crop against adverse effects of drought.

Mutation can be defined as the change in genetic material of an organism which is heritable. Mutations are the tools used to study the nature and functions of genes which are the building blocks and the basis of plant development, thereby producing raw materials for the genetic improvement of economic crops (**Adamu and Aliyu, 2007)**. Mutation methodology has been used to produce many cultivars with improved value study of genetics and plant development phenomenal (**Van *et al*., 1990** and **Bretagne-Sagnard *et al*., 1996**). Sodium azide, a chemical mutagen has become important tool to enhance agronomic traits of crop plants. It is being used to produce resistance in various susceptible crops improve their yield and quality traits against harmful pathogens (**Khan *et al*., 2009).**

Srivastava *et al*., (2011) illustrated that yield attributing characters in both positive and negative shift in mean than those of control. They added that some of the mutant lines (eight progeny for earliness, one for plant height, three for spike length and grain yield each, two for tillering and four for test weight) were found desirable. These lines were either comparable to or better than control for yield and its components. Moreover it is concluded that sodium azide with concentration 0.02% appear to be the most effective mutagenic treatment for induction of micro-mutation in yield component traits and selection in M_2 populations of these treatment would be effective in

rectification of simply inherited morphological deficiencies and bringing out lines with yield improvement. **Sari *et al.*, (2016)** identified the selection criteria to obtain a superior mutant derived from the wheat plants of such varieties as Dewata, Selayar and Alibey, adaptive in medium land. They concluded that analysis of agronomic growth characters showed a significantly effect on growth percentage of the initial growth (8 mutants), flowering time (1 mutant), spike length (15 mutants), number of spikes/plant (7 mutants), number of grains/spike (8 mutants), grain weight/spike (8 mutants), grain weight/plant (6 mutants). Moreover, they added that the effects on the characters of ripe time, harvest, panicle length and plant height were non-significant and the mutants of Dewata, Selayar and Alibey could be selected based on the characters of spike length, number of grains/spike and grain weight/plant observation because these characters generated more mutants than the other characters.

Qadir (2016) found that the RAPD (Random Amplified Polymorphic DNA) marker a considerable potential for estimating genetic diversity among wheat cultivars with rapidly detecting a great number of drought tolerance genomic markers, it is possible and can be used for characterizing bread wheat genotypes for drought tolerant in molecular breeding, as well as for early discovery of drought tolerant genotypes for cultivation in areas with lower water. **Dubey *et al*., (2017)** reported that the mutant plants produced by the treatment of sodium azide are capable to survive under various adverse conditions and have improved yield, increased stress tolerance, longer shelf life than control treatment and reduced agronomic input in comparison to normal plants. **Ayşe and Sarsu (2018)** studied Simple Sequence Repeats (SSRs) markers were used to screen genetic diversity in sodium azide (NaN_3) induced fourteen fourth-generation advanced wheat mutant lines. The mean values of polymorphism rate (29.44%), polymorphic information content (PIC; 0.82), marker index (MI; 1.95) and resolving power (Rp; 1.31) were calculated according to SSR marker profiles. Two SSRs, Xwmc170 and Xcfd6, were detected as the most polymorphic markers, Xgwm626 proved the highest PIC and MI values, and Xcfd6 gave the highest Rp value. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram classified 15 plants into four groups. The Principle Component Analysis (PCA) showed 88.9% of the total genetic variation. **Laskar *et al.*, (2018)** used the molecular characterization of six high yielding lentil mutant lines, developed from gamma rays mutagenesis was carried out with sodium dodecyl Random Amplified Polymorphic DNA (RAPD) markers SDS-PAGE profile of seed storage proteins showed 35 unique bands with 97.14% polymorphism.

Genetic divergence analysis generated total 41 reproducible RAPD bands with average calculated polymorphic percentage of 63.06%. Among the primers, OPA-10 showed the highest polymorphism with significant PIC value.

Genetic divergent analysis revealed that genome of cultivar DPL 62 mutated relatively more than the cultivar Pant L 406 may be due to the mutagen treatments, while DPL 62-B and Pant L406-A were the most divergent mutants induced in the present study. Twenty wheat genotypes were assayed to study the genetic diversity using molecular markers. The seventy-five alleles were identified with a mean of 2.34 alleles per locus using 32 SSR markers. Majority of SSR markers showed a high level of polymorphism. Results indicated that wheat cultivars had high genetic diversity that can be used in wheat breeding programs. **(Sharma *et al.*, 2018).**

El-Mouhamady *et. al.*, (2020) conceded that traditional breeding and DNA fingerprinting could be used to clarify and sort all genotypes to generate the best of them for water stress resistance which will be in the future as a nucleus for producing resistance wheat varieties for drought stress under Egyptian conditions. **Mansour *et al.*, (2020),** showed that there were several differences in both the size and number of bands between the varieties. Based on these markers, genetic similarity coefficients were calculated and a dendrogram was constructed. The dendrogram analysis delineated three major clusters. The current study showed that RAPD markers are useful in the assessment of the genetic diversity among the wheat genotypes.

Therefore, this study aimed to determine the mean performance, genetic diversity and DNA analysis in M_3 generation of some wheat mutants under water stress.

MATERIALS AND METHODS

The field experiments were conducted during the growing season of 2019/2020, for mean performance, genetic diversity and RAPD analysis in M_3 generation of some wheat mutants influenced by gamma ray and sodium azaide under water stress, at the Experimental Farm, Faculty of Technology and Development, Zagazig University, Egypt. Four genotypes of bread wheat (*Triticum aestivum* L.), Gemmeiza-11, Sids-12, Shandweel-1 and Sahel-1were obtained from Agricultural Research Center, Giza, Egypt, for used in this study.

M_3 generation in third season (2019/2020):

The selected plants from M_2 generation sown in 19 November 2019/2020 in pedigree. These selected plants were sown individually, each plant was sown in a row, the plot contains 5 rows, the row was 3m length , space between rows

was 30 cm; and spaced between plants in row was 10 cm. The recommended cultural practices for wheat production were applied except irrigation which was irrigated immediately after sowing but first irrigation was done after 45 days from sowing and the second irrigation late up to flowering stage. The experimental design was complete block design with three replicates. Number of selected plants from M_2 population for height yield induced by mutagens; 18, 11, 8 and 24 and those promising in M_3 4, 7, 4 and 10 for Gemmeiza-11, Sids-12, Shandaweel-1 and Sahel-1 cultivars, respectively.

***The following data*:**

Number of days to 50% heading, spike length (cm.), number of spikelets/spike, spike grain weight (g), number of spikes/plant, number of grains/spike, 1000-grain weight (g), grain yield/plant (g), biological yield/plant and harvest index were recorded.

Statistical analysis:-

Data were statically analyzed using spilt plot design in M_2 and complete block design with three replication in M_3 generation. used Data were statically analyzed, and mean values were compared by using the analysis of variance (ANOVA) and least significant test (L. S. D) at 5% level probability **(Steel *et al.*, 1997)**. The least significant deference ($P<0.05$) was calculated for the parameters exhibiting significant effect and to treatments mean.

***Plant material and DNA extraction*:**

Grains of wheat were kindly provided by the wheat Research Section, Agricultural Research Center (ARC). DNA was isolated as described at *https ://primer digital.com/dna.html.*

PCR analysis:-

RAPD analysis using five primers listed in Table (2) The PCRs were performed in 20-μL reaction mixtures containing 100 ng genomic DNA, 1 × DreamTaq buffer, 200 mM deoxyribonucleotide triphosphate (dNTP), 400 nM primer and 5 U DreamTaq DNA Polymerase (Thermo Fisher Scientific). The amplifications were performed in the eppendorf thermocycler. The PCR reaction program consisted of 1 cycle at 95 °C, 5 min; 30 cycles of 95 °C for 30 s, 37 °C for 45 s, 72 °C for 90; s and a final elongation at 72 °C for 5 min. The PCR products were separated by electrophoresis at 80 V for 2 h in a 1.2% agarose gel with 1 × THE (Tris HEPES EDTA) electrophoresis buffer. The Thermo Scientific GeneRuler DNA Ladder Mix, 100–10,000 base pairs (bp), #SM0332, was used as a standard. The gels were stained with ethidium bromide (EtBr) and Scanned.

Table 1. RAPD- primers code and sequences were used for genetic diversity between wheat genotypes.

Number	Primer name	Sequences
1	OPB 5*	TGCGCCCTTC
2	OPB 7*	GGTGACGCAG
3	A-20*	GTTGCGATCC
4	A-6*	GGTCCCTGAC
5	O-7*	CCCAGTCACT

Data analysis.

The presence (1) or absence (0) of RAPD banding patterns were treated as binary character and were processed for similarity matrix and cluster analysis performed using NTSYs-pc version 2.11 software as described by **Rohlf (1993).**

RESULTS AND DISCUSSION

Mean performance of wheat mutants' traits in M_3 generation of pedigree method for Gimmieza-11.

Data presented in Table (2) revealed that the analysis of variance significant difference among Gimmieza-11 and there M_3 mutants for days to heading, spike length, number of spikelets/spike, number of spikes/plant, number of grains/spike, spike grain weight (g), 1000-grain weight (g) grain yield/plant, biological yield/plant and harvest index. These results are agreement with these reported by **Nazarenko, *et. al.*, (2018)**.

The mean performance for days to heading in Gemmeiza-11 and their M_3 mutants was more than control (93 days) for days to heading. Mean performance for spike length in Gemmeiza-11 and their M_3 mutants, were (Gemmieza-11 350Gy1 16.8 cm), (Gemmiza-11 0.08% sodium azide 17.16 cm) and (Gemmiza-11 0.06% sodium azide 17.53 cm) and higher than control (14.93 cm), but the Gemmieza-11 350 Gy2 (8.7 cm) had the lowest for spike length. Meanwhile the Gem-0.08 SA (24.93) was more than control (24.4), but Gem-350Gy2 (20.66), Gem-350Gy1 (24) and Gem-0.06 SA (24.26) for number of spikelets/spike. Mean performance for number of spikes\plant exhibited that the Gem-0.08 SA (10.7), Gem-0.06 SA (8.93), Gem-350Gy2 (8.4) and Gem-350Gy1 (8.13) were more than control (4.13) for number of spikes/plant. Meanwhile, the control (72.83) was more than Gem-

0.08 SA (72.56), Gem-350Gy2 (58.2), Gem-0.06SA (54.13) and Gem-350Gy1 (41.73) for number of grains/spike. The Gem-0.06 SA, Gem-0.08

Table 2. Mean performance for wheat mutants of some agronomic traits in M_3 generation of pedigree method for Gemmeiza-11 under water stress.

Mutants	Treatments	days to 50% heading	Spike length (cm).	No. of spikelits /spike	No. of spikes/ plant	No. of grains/ pike	Spike grain weight (g).	1000-grain weight (g)	Grain yield/plant (g)	Biological yield / plant (g.)	Harvest index %
	Control	93	14.93	24.4	4.13	72.83	2.65	37.13	7.81	31.72	24.95
Mutant-4	Gem-0.06 SA	94.33	17.53	24.26	8.93	54.13	3.41	63.63	12.55	52.68	23.79
Mutant-5	Gem-0.08 SA	98.33	17.16	24.93	10.7	72.56	3.38	48.76	27.71	93.97	29.52
Mutant-6	Gem-350Gy1	97	16.8	24	8.13	41.73	1.40	33.66	8.7	49.31	17.63
Mutant-9	Gem-350Gy2	100	8.7	20.66	8.4	58.2	3.29	56.83	17.41	49.7	35.06
	F. test	**	**	**	**	**	**	**	**	**	**
	L.S.D $_{0.05}$	1.97	1.52	1.42	1.38	12.24	0.55	3.54	1.91	6.44	4.41

*, **Significant at 0.05 and 0.01, respectively

SA and Gem-350Gy2 had the highest values (3.41, 3.38 and 3.29 respectively) were more than Gemmeiza-11 (2.65) for spike grain weight (g). The mutants (Gem-0.06 SA and Gem-0.08 SA) were recorded the highest values for 1000-grain weight (g) (63.63, 56.83 and 48.76 respectively), they were more than Gemmeiza-11 had lowest value (37.13) for 1000-grain weight. The all mutants had the highest values of mean performance for grain yield plant, Gem-0.08 SA (27.71 g), Gem-350Gy2 (17.41 g), Gem-0.06 SA (12.55 g) and Gem-350Gy1 (8.7 g) while control had the lowest value for grain yield plant (7.81 g). Meanwhile Gem-0.08 SA showed the highest value for biological yield/plant (93.97), Gem-0.06 SA (52.68), Gem-350Gy2 (49.7) and Gem-350Gy1 (49.31) they were more than control (31.72). Gem-350Gy2 had the highest value (35.06) and more than control (24.95) for harvest index, %.

Mean performance of wheat mutants traits in M_3 generation by pedigree method of Shandawel-1.

The analysis of variance in Table (3) showed significant difference among Shandawel-1 and there M_3 mutants for days to heading, spike length, number of grains/spike, 1000-grain weight(g), grain yield/plant, biological yield/plant and harvest index. **Ghada *et al*., (2020)** indicated that the used mutagens had direct impact and significantly improved agronomic traits in derivative mutants compared to their parent cultivars. Moreover, the maximum increment in yield related traits were obtained by 0.4% EMS, 1 and 2 hour-laser, 350-Gy, 1.5 hour × 0.3% EMS and 250-Gy. The obtained results highlighted the importance of these doses of applied mutagens to induce useful genetic variability in bread wheat for improving grain yield and contributing traits. The mean performance for days to heading in Shandawel-1 and their M_3 mutants, exhibited that the Sh-350Gy (104.33) was more than control (102.33 days), but the mutants (Sh-0.06SA, Sh-0.04SA and Sh-250 Gy) were (90.66, 94.33 and 98.33 days) and they were lasses than control under water stress. Mean performance for spike length in Sh-250 Gy (16.6 cm), and Sh-350Gy (16.46 cm) were more than control (15.8 cm). Mean performance for number of spikes/plant in Shandawel-1 and their M_3 mutants, the Sh-250 Gy (8.93), Sh-0.06SA (8.86), Sh-350Gy (6.4) and Sh-0.04SA (6.33) were more than control (6.13). Meanwhile the Sh-0.04SA (88.8) were more than control (79.46) for number of grains\spike. The Sh-0.04SA and Sh-250 Gy had the highest values (3.62 and 3.003) respectively were more than control (2.84) for spike grain

weight (g). The mutants (Sh-250 Gy, Sh-350Gy and Sh-0.04SA) were recorded the highest values of 1000-grain weight (g) (45.94, 42.28 and 40.98 g)

Table.3. Mean performance for wheat mutants of some agronomic traits in M_3 generation of pedigree method for shandaweel-1, under water stress.

Mutants	Treatments	days to 50% heading	Spike length (cm).	No. of spikelits/ Spike	No. of spikes/ plant	No. of grains/ pike	Spike grain weight (g).	1000-grain weight (g)	Grain yield/plant (g)	Straw yield/plant (g)	Biological yield / plant (g)	Harvest index %
	Control	102.33	15.8	24.4	6.13	79.46	2.84	36.36	13.51	37.2	50.71	26.66
Mutant-2	Sh-0.04SA	94.33	13.2	23.73	6.33	88.8	3.62	40.98	14.72	28.9	43.62	33.71
Mutant-5	Sh-0.06SA	90.66	15.5	24.93	8.86	72.52	2.50	34.51	14.63	46.25	60.88	23.02
Mutant-7	Sh-250 Gy	98.33	16.6	23.86	8.93	65.26	3.003	45.94	18.04	51.01	69.05	26.11
Mutant-8	Sh-350Gy	104.33	16.46	26.53	6.4	60.4	2.56	42.28	11.75	35.57	47.32	24.79
	F. test	**	*	Ns	Ns	*	Ns	**	*	**	**	*
	$LSD_{.05}$	2.98	1.67	2.17	3.91	11.65	0.66	3.85	2.81	4.81	5.78	4.76

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

respectively, they were more than control (36.36). Sh-250 Gy (18.04 g), Sh-0.06SA (14.7 g) and Sh-0.04SA (14.72 g) had the highest values of grain yield plant but the control (13.51 g). Meanwhile the Sh-250 Gy had the highest value of biological yield/plant (69.05 g) and Sh-0.06SA (60.88 g), were more than control (50.71). The Sh-0.04SA had the highest value (33.71) was more than control (26.66) for harvest index %.

Mean performance of wheat mutants traits in M_3 generation by pedigree method for Sids-12.

The analysis of variance in Table (4) showed significant difference among Sids-12 and there M_3 mutants for days to heading, spike length, number of spikelets/spike, number of spikes/plant, number of grains/spike, spike grain weight (g), 1000-grain weight (g), grain yield/plant, biological yield/plant and harvest index the results with agreement reported by **El-Naggar *et. al*., (2015) and Balkan (2018)**

The mean performance for days to heading in Sids-12 and their M_3 mutants, showed that the Sid-0.06 SA (101), Sid-0.04SA (95.66) and Sid-350Gy2 (94.33) were more than control (93.33days), but the mutants (Sid-250 Gy and 10) (91.66 and 92.66 days) were lasses than control under water stress. Mean performance for spike length of Sids-12 and their M_3 mutants, the Sid-250 Gy (16.2 cm), Sid-0.08 SA (15.16 cm), Sid-350 Gy1 (14.73 cm), Sid-0.04SA (14.1 cm) and Sid-0.06 SA (13.73 cm) were more than control (12.4 cm). Meanwhile the Sid-0.08 SA (24.4), Sid-250 Gy (23.46), Sid-0.04SA (23.06), Sid-0.08 SA (22.93) and Sid-350 Gy1 (22.13), were more than control (21.6) for spikelets/spike. Mean performance for number of spikes/plant in Sids-12 and their M_3 mutants, the Sid-250 Gy (9.6), Sid-0.06 SA (7.8), Sid-0.08 SA (6.6), Sid-0.04SA (5.66) and Sid-350 Gy1 (5.26) were more than control (5). Meanwhile the Sid-350 Gy1 (128.2), Sid-0.08 SA (120.64), Sid-0.04SA (107.3), Sid-350Gy3 (102.73), Sid-250 Gy (91.53) and Sid-0.06 SA (82), they were more than control (75.66) for number of grains\spike. The all mutants had the highest values and were more than control (2.98) for spike grain weight. The Sid-0.06 SA (54.37), Sid-0.08 SA (51.59), Sid-350Gy2 (48.24), 10 (47.75), Sid-350 Gy1 (45.95) and Sid-250 Gy (41.90), were more than control (39.54) for 1000-grain weight. The Sid-0.08 SA (34.08 g), Sid-0.06 SA (28.15 g) and Sid-350 Gy1 (25.64 g), Sid-250 Gy (21.21 g), Sid-0.04SA (20.62 g), Sid-350Gy3 (18.17 g) and Sid-350Gy2 (12.86 g) had the highest values of grain yield plant

but the control (11.69 g). Mean performance Sids-12 and their M_3 mutants, the all mutants had the highest of biological yield plant and they were more than control (18.33 g) and (30.02 g) biological yield/plant(g). The Sid-0.08 SA had the highest values (45.39) and Sid-350Gy3 (40.74) and Sid-350 Gy1 (39.87)

Table 4. Mean performance for wheat mutants of some agronomic traits in M_3 generation of pedigree method for Sids-12 under water stress.

Mutants	Treatments	days to 50% heading	Spike length (cm).	No. of spikelits/spike	No. of spikes/plant	No. of grains/pike	Spike grain weight (g).	1000-grain weight (g)	Grain yield/plant (g)	Biological yield / plant (g)	Harvest index %
	Control	93.33	12.4	21.6	5	75.66	2.98	39.54	11.69	30.02	38.99
Mutant – 2	Sid-0.04SA	95.66	14.1	23.06	5.66	107.3	4.26	39.55	20.62	48.32	42.68
Mutant-3	Sid-0.06 SA	101	13.73	24.4	7.8	82	4.43	54.37	28.15	79.5	35.44
Mutant-4	Sid-0.08 SA	92.33	15.16	22.93	6.6	120.46	6.20	51.59	34.08	75.04	45.39
Mutant-5	Sid-250 Gy	91.66	16.2	23.46	9.6	91.53	3.83	41.90	21.21	75.94	27.49
Mutant-6	Sid-350 Gy1	93.33	14.73	22.13	5.26	128.2	5.93	45.95	25.64	64.32	39.87
Mutant-9	Sid-350Gy2	94.33	8.66	21.2	4.73	75.06	3.6	48.24	12.86	35.34	36.54
Mutant-10	Sid-350Gy3	92.66	10.13	21.6	4.86	102.73	4.91	47.75	18.17	44.55	40.74
	F. test	**	**	*	*	**	**	**	**	**	**
	L.S.D $_{0.05}$	1.4862	1.3254	1.4938	2.394	15.781	1.234	3.7818	2.3896	4.6433	3.529

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

were more than control (38.99) for harvest index %. **Al-naggar *et. al.*, (2015)** found that the twenty-three mutant lines were generated based on spike length in M_3 generation. Five out 23 mutant lines were reevaluated based on grain yield components.

Mutant lines showed significant variation in the studied traits that were established through phenotypic and genotype coefficients of variation.

Mean performance of wheat mutants traits in M_3 generation by pedigree method for Sahel-1.

The analysis of variance in Table (5) showed significant difference among Sahel-1 and there M_3 mutants for days to heading, spike length, number of spikelets/spike, number of spikes/plant, number of grains/spike, spike grain weight(g), 1000-grain weight(g), grain yield/plant, biological yield/plant and harvest index the results with agreement reported by **Muqaddasi *et. al.*, (2019).**

The mean performance for days to heading in Sahel-1 and their M_3 mutants, showed that the Sah-0.04SA (96.66) was more than control (96 days), but all mutants it were lasses than control. Mean performance for spike length in Sahel-1 and their M_3 mutants, it ranged from 9.33 (Sah-250 Gy2) to 15 (Sah-0.06SA), the Sah-0.06SA (15 cm), Sah-0.04SA (14.4 cm), Sah-250 Gy1 (14.13 cm), Sah-250 Gy2 (14.53 cm) and Sah-0.08 SA1 (13.33 cm) were more than control (11 cm).

Meanwhile the Sah-0.08 SA2 (24.4), Sah-250 Gy1 (23.46), Sah-0.06SA (23.06), Sah-0.08 SA1 (22.93) and Sah-250 Gy2 (22.13), were more than control (21.6) for spikelets/spike. Mean performance for number of spikes\plant recorded that the Sah-250 Gy2 (9.7), Sah-350Gy3 (7.8), Sah-0.06SA (6), Sah-350 Gy1 (5.8) and Sah-350 Gy2 (4.8) were it more than control (4.46). Meanwhile the Sah-0.06SA (117.91), Sah-350 Gy2 (104.2), Sah-250 Gy1 (96.55), Sah-0.08 SA2 (89.83), Sah-0.08 SA1 (83.41) and Sah-0.04SA (80.2) were more than control (54.46) for number of grains/spike.

The all mutants had the highest values for spike grain weight and 1000-grain weight, were more than control (2.10 and38.56 g.) for spike grain weight and 1000-grain weight respectively.

The all mutants were produced the highest values of grain yield/plant, but the control (7.76 g). The all mutants had the highest of biological yield /plant (g), were more than control (15.3 g) and (23.06 g) for biological yield/plant(g) respectively. The Sah-0.08 SA2gives the highest values (40.20)

and Sah-0.08 SA1 (40.07) and Sah-350 Gy2 (38.46) it were more than control (33.65) for harvest index %.

Table 5. Mean performance for wheat mutants of some agronomic traits in M_3 generation of pedigree method for Sahel -1, under water stress.

Mutants	Treatments	days to 50% heading	Spike length (cm).	No. of spikelits/ spike	No. of spikes/ plant	No. of grains/ pike	Spike grain weight (g).	1000-grain weight (g)	Grain yield/ plant (g)	Biological yield / plant (g)	Harvest index %
	Control	96	11	22.66	4.46	54.46	2.10	38.56	7.76	23.06	33.65
Mutant-1	Sah-0.04SA	96.66	14.4	24	6	80.2	3.81	47.50	20.52	53.43	38.40
Mutant-2	Sah-0.06SA	95.33	15	24.66	5.46	117.91	5.15	43.67	16.41	54.36	30.18
Mutant-3	Sah-0.08 SA1	92.66	13.33	24.26	4.66	83.41	4.75	56.94	17.44	43.52	40.07
Mutant-4	Sah-0.08 SA2	94.33	12.86	22.26	4.76	89.83	4.18	46.53	13.58	33.78	40.20
Mutant-5	Sah-250 Gy1	90.33	14.13	23.2	3.53	96.55	5.40	55.92	16.44	42.94	38.28
Mutant-6	Sah-250 Gy2	92.33	9.33	22	9.7	61.6	2.90	47.07	19.33	53.16	36.36
Mutant-7	Sah-350 Gy1	94.33	11.53	24.4	5.8	54	2.32	42.96	8.17	30.67	26.63
Mutant-9	Sah-350 Gy2	89.33	14.53	23.6	4.8	104.2	5.45	52.30	21.43	55.72	38.46
Mutant-12	Sah-350Gy3	92.33	12.93	22.13	7.8	61.83	2.98	48.19	18.52	57.99	31.93
Mutant-14	Sah-350Gy4	93.33	11	22.93	4.26	57.6	3	52.08	10.28	28.76	35.74
	F. test	**	**	**	**	**	**	**	**	**	**
	L.S.D $_{0.05}$	1.72	1.03	1.15	1.50	8.00	0.55	6.39	3.46	8.40	5.79

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

Random amplified polymorphic DNA analysis

Detection of polymorphism based on the RAPD marker:-

In the present study, RAPD assay was used to study genetic diversity among wheat genotypes. Five RAPD primers from twenty gave results (Figure 1). RAPD primers were employed to investigate the genetic polymorphism among the 4 wheat genotypes under water stress treated with NaN_3 and Gamma irradiation. The data in Table (6) showed that RAPD primers applied on wheat genotypes produced 18 bands; 16 of these were polymorphic. Primer OPB-5 gave the highest number of bands (5) and primer O-7, A-20 and OPB-7 passed the lowest number (3).The highest percentage of polymorphic bands was produced by primer O-7, A-6 and OPB-5 (100%) and the lowest percentage of polymorphic bands were produced by A-20 and OPB-7 (67%).

The number of polymorphic amplicons per primer ranged from 3 to 5 amplicons. **Heiba *et al.* (2016b)** showed the impact of 0.3% of ethyl methane sulphonate (EMS) for detect the mutation chances of DNA in 3 bread wheat entries using RAPD and ISSR (inter simple sequence repeats) primers and the results revealed that RAPD primers displayed 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. In the present study we used RAPD markers to detect tolerant wheat genotypes under water stress using the effect of NaN_3 and gamma irradiation. The results indicated that 100% polymorphism with primer O-7, A-6 and OPB-5.

Table 6. Total number of bands, monomorphic, polymorphic bands and percentage of polymorphism detected in wheat genotypes

Primer name	Total number of allel	Polymorphic Allel	Polymorphism %	Unique bands	Positive	Negative
O-7	3	3	100	0	0	0
A-6	4	4	100	0	0	0
OPB-5	5	5	100	1	0	1
A-20	3	2	67	0	0	0
OPB7	3	2	67	0	0	0
Total	**18**	**16**	**88.8**	**1**	**0**	**1**

RAPD-Primers molecular marker traits.

The present data in Table (7), accorded that the RAPD primer OPB-5 produced 5 bands that matched between 40-300 (bP) and had a 100% polymorphism. For primer OPB-5 ratio of (H) to the amount of (0.30). The same

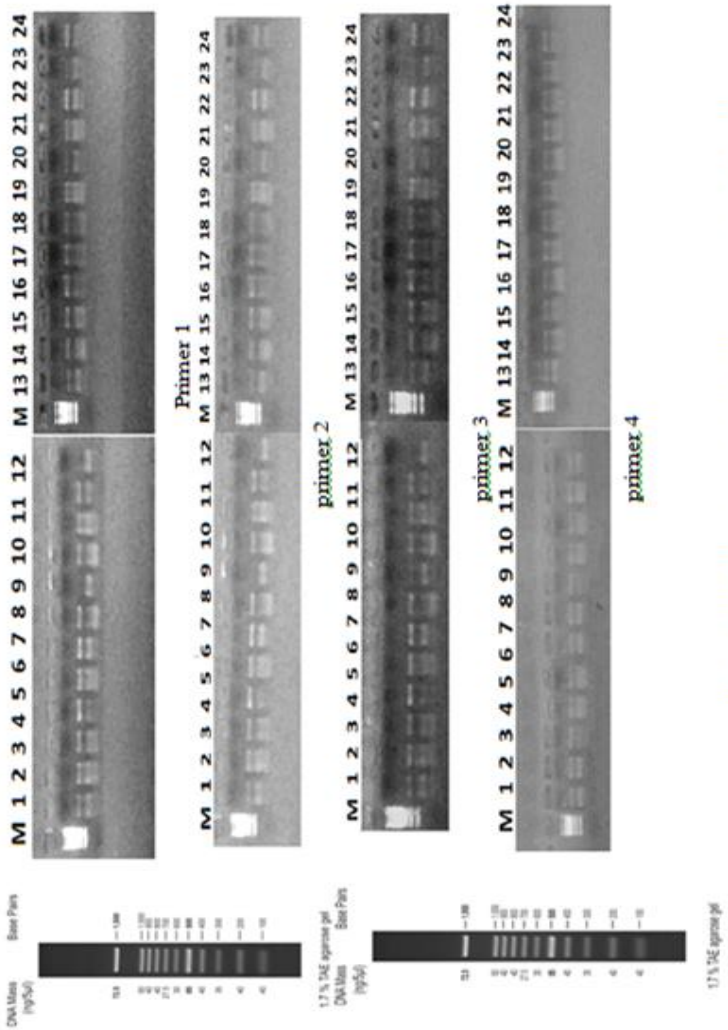

Figure 1. RAPD pattern obtained by five primers for DNA marker

1:sahel-1 control, 2:Sahel-1 250Gy, 3:Sahel-1 350Gy1, 4: Sahel-1 350Gy2 5: Sahel-1 0.04 % SA, 6:Sahel-1 0.06 % SA, 7:Sahel-1 0.08 % SA, 8: Sids-12 control, 9:Sids- 12 0.04 %SA, 10:Sids-12 0.06 %SA, 11:Sids- 12 0.08 % SA, 12:Sids-12 250Gy, 13: Sids-12 350Gy1, 14:Sids-12 350 Gy2, 15:Shandaweel-1 control, 16:Shandaweel-1 0.06 % SA, 17:Shandaweel-1 0.04 %SA, 18:Shandaweel-1 250Gy, 19:Shandweel-1 350Gy, 20: Gemmieza-11control, 21: Gemmieza-11 0.06% SA, 22: Gemmieza-110.08 %SA, 23:Gemmieza-11 350 Gy1, 24:Gemmiza-11 350Gy2.

Table 7. Heterozygocity index (H), polymorphism information content (PIC), effective multiplex ratio (E), Arithmatic mean of H (H.av) Discriminating power (D) marker index (MI), as revealed by RAPD marker in 4 wheat genotypes.

Primer	H	PIC	E	H.av	MI	D
O-7	0.23	0.22	1.00	0.23	0.23	0.06
A-6	0.26	0.25	1.00	0.26	0.26	0.07
OPB-5	0.30	0.29	1.00	0.30	0.30	0.07
A-20	0.21	0.20	1.00	0.21	0.21	0.05
OPB7	0.21	0.20	1.00	0.21	0.21	0.05

PIC value was 0.29, E value was (1.00), and the value of marker index (MI) 0.30 was highest. ratio of (D) the highest value 0.07. The second molecular primer, A-6 produced 4 bands that matched between 50-300 (bP) and had a 100% polymorphism. For primer A-6 the ratio of (H) to the amount of (0.26). The same PIC value was 0.25, E value was (1.00), and the value of marker index (MI) 0.26. Ratio of (D) the highest value 0.07.For primer O-7 ratio of (H) to the amount of (0.23). PIC value was 0.22, E value was (1.00), and the value of marker index (MI) 0.23. ratio of (D) the highest value 0.06. For primer A-20 ratio of (H) to the amount of (0.20). PIC value was 0.20, E value was (1.00), and the value of marker index (MI) 0.21.

Ratio of (D) 0.05. RAPD primer OPB7 have ratio of (H) (0.21). PIC value was 0.20, E value was (1.00), and the value of marker index (MI) 0.21. ratio of (D) 0.05.

Genetic diversity between wheat mutant treated with sodium azide using SSR was determined by **Sen and Sarsu (2018)** and 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.

Genetic relationships among the wheat genotypes:-

The presence or absence of each band was treated as a binary character in a data matrix to examine the genetic similarity among the four wheat verities and their mutants based on RAPD results (Table 8). To examine the genetic distance

Table (8): The similarity values between the wheat genotypes computed according to RAPD data.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	1																						
2	0.3	1																					
3	0.3	0.3	1																				
4	0.4	0.7	0.5	1																			
5	0.3	0.2	0.3	0.6	1																		
6	0.3	0.3	0.5	0.4	0.1	1																	
7	0.4	0.5	0.3	0.4	0.3	0.4	1																
8	0.3	0.6	0.6	0.7	0.5	0.3	0.9	1															
9	0.3	0.3	0.4	0.5	0.1	0.2	0.2	0.6	1														
10	0.3	0.1	0.4	0.7	0.1	0.2	0.3	0.6	0.1	1													
11	0.2	0.4	0.6	0.3	0.2	0.1	0.5	0.3	0.3	0.3	1												
12	0.5	0.8	0.8	0.7	0.5	0.3	0.9	0.1	0.6	0.6	0.3	1											
13	0.2	0.3	0.4	0.5	0.2	0.1	0.5	0.3	0.3	0.3	0.1	0.4	1										
14	0.3	0.3	0.2	0.4	0.1	0.3	0.1	0.7	0.2	0.2	0.3	0.7	0.3	1									
15	0.3	0.3	0.2	0.6	0.1	0.3	0.3	0.5	0.2	0.2	0.3	0.5	0.3	0.1	1								
16	0.3	0.5	0.5	0.3	0.3	0.3	0.3	0.7	0.2	0.3	0.3	0.7	0.3	0.3	0.3	1							
17	0.3	0.6	0.4	0.3	0.3	0.3	0.3	0.6	0.3	0.4	0.4	0.6	0.4	0.3	0.2	0.1	1						
18	0.6	0.7	0.5	0.4	0.6	0.6	0.4	0.9	0.5	0.7	0.7	0.9	0.7	0.6	0.4	0.3	0.2	1					
19	0.3	0.3	0.3	0.7	0.2	0.3	0.3	0.6	0.3	0.1	0.4	0.6	0.4	0.2	0.1	0.3	0.3	0.5	1				
20	0.3	0.5	0.7	0.4	0.3	0.1	0.6	0.2	0.3	0.3	0.1	0.2	0.2	0.4	0.4	0.4	0.5	0.8	0.5	1			
21	0.4	0.2	0.3	0.8	0.1	0.3	0.4	0.5	0.2	0.1	0.3	0.5	0.3	0.3	0.1	0.4	0.3	0.6	0.1	0.4	1		
22	0.3	0.4	0.4	0.5	0.2	0.1	0.5	0.3	0.3	0.3	0.1	0.3	0.1	0.3	0.2	0.3	0.3	0.5	0.3	0.2	0.2	1	
23	0.4	0.7	0.7	0.6	0.4	0.3	0.8	0.2	0.5	0.5	0.2	0.1	0.3	0.6	0.4	0.6	0.5	0.8	0.5	0.1	0.4	0.2	1
24	0.3	0.5	0.5	0.4	0.3	0.1	0.6	0.2	0.3	0.3	0.1	0.2	0.2	0.4	0.3	0.4	0.3	0.6	0.3	0.1	0.3	0.1	0.1

1:sahel-1control, 2:Sahel-1250Gy, 3:Sahel-1350Gy1, 4: Sahel-1 350Gy2 5: Sahel-1 0.04 % SA, 6:Sahel-1 0.06 % SA, 7:Sahel-10.08 % SA, 8: Sids-12 control, 9:Sids- 12 0.04 %SA, 10:Sids-12 0.06 %SA, 11:Sids- 12 0.08 % SA, 12:Sids-12 250Gy, 13: Sids-12 350Gy1, 14:Sids-12 350 Gy2, 15:Shandaweel-1 control, 16:Shandaweel-1 0.06 % SA, 17:Shandaweel-1 0.04 %SA, 18:Shandaweel-1 250Gy, 19:Shandweel-1 350Gy, 20: Gemmieza-11control, 21: Gemmieza-11 0.06% SA 22: Gemmieza-110.08 %SA, 23:Gemmieza-11 350 Gy1, 24:Gemmiza-11 350Gy2.

between the wheat mutants, the scored data were analyzed using NTSYs-pc version 2.11. In general the range of pair distance among the wheat mutants was from 0.1 to 0.9.

The highest similarity value (0.9) was observed between (Sids-12 250Gy and Sahel-10.08 % SA), (Sids-12 control and Shandaweel-1 250Gy), (Sids-12 250Gy and Shandaweel-1 250Gy), while the lowest distance value (0.1) was observed between mutants (Sahel-12 250Gy and Sids-12 0.06 %SA), (Sahel-1 0.04 % SA and Sahel-1 0.06 % SA), (Sahel-1 0.04 % SA and Sids- 12 0.04 %SA), (Sahel-1 0.04 % SA and Sids-12 0.06 %SA), (Sahel-1 0.04 % SA and Sids-12 350 Gy2), (Sahel-1 0.04 % SA and Shandaweel-1 control), (Sahel-1 0.06 % SA and Sids-12 0.08 % SA), (Sahel-1 0.06 % SA and Sids-12 350Gy1), (Sahel-1 0.06 % SA and Gemmieza-11control), (Sahel-1 0.06 % SA and Gemmiza-11 350Gy2), (Sahel-10.08 % SA and Sids-12 350 Gy2), (Sids-12 control and Sids-12 250Gy), (Sids-12 0.04 %SA, and Sids-12 0.06 %SA), (Sids-12 0.06 %SA and Shandweel-1 350Gy), (Sids-12 0.06 %SA and Gemmieza-11 0.06% SA), (Sids- 12 0.08 % SA and Sids-12 350Gy1), (Sids- 12 0.08 % SA and Gemmieza-11control), (Sids-12 0.08 % SA and Gemmieza-110.08 %SA), (Sids- 12 0.08 % SA and Gemmiza-11 350Gy2), (Sids-12 250Gy and Gemmieza-11 350 Gy1), (Sids-12 350Gy1 and Gemmieza-11 0.08 %SA), (Sids-12 350 Gy2, and Shandaweel-1 control), (Shandaweel-1 control and Shandweel-1 350Gy), (Shandaweel-1 control and Gemmieza-11 0.0 6% SA), (Shandaweel-1 0.06 % SA, and Shandaweel-1 0.04 %SA), (Shandweel-1 350Gy and Gemmieza-11 0.06% SA), (Gemmieza-11control and Gemmieza-11 350 Gy1), (Gemmieza-11control and Gemmiza-11 350Gy2), (Gemmieza-11 0.08 %SA and Gemmiza-11 350Gy2) and (Gemmieza-11 350Gy1 and Gemmiza-11 350Gy2). Appearance of differential difference between parents and their mutants derivatives to the effects of different mutagens on the DNA. **Ayşe Şen and Fatma Sarsu (2018)** used Simple Sequence Repeats (SSRs) markers to screen genetic diversity in sodium azide (NaN_3) induced fourteen fourth-generation advanced wheat mutant lines.

Cluster analyses:-

Cluster illustrating the genetic distance, based on the analysis of RAPD primers for 24 wheat mutant using the UPGMA algorithm in the MEGA 5 software given in Figure (2).

The genetic relatives in the dendrogram showed that the 24 (mutant) distributed in two clusters The first cluster contains 20 mutant divided to two sub-cluster the first have 10 divided to three groups the first have genotype sahel-

1control and second have Gemmieza-11 350 Gy1, Sids-12 250Gy and Sids-12 control, the third group have 6 mutant (Sids- 12 0.08 % SA, Gemmieza-11control,

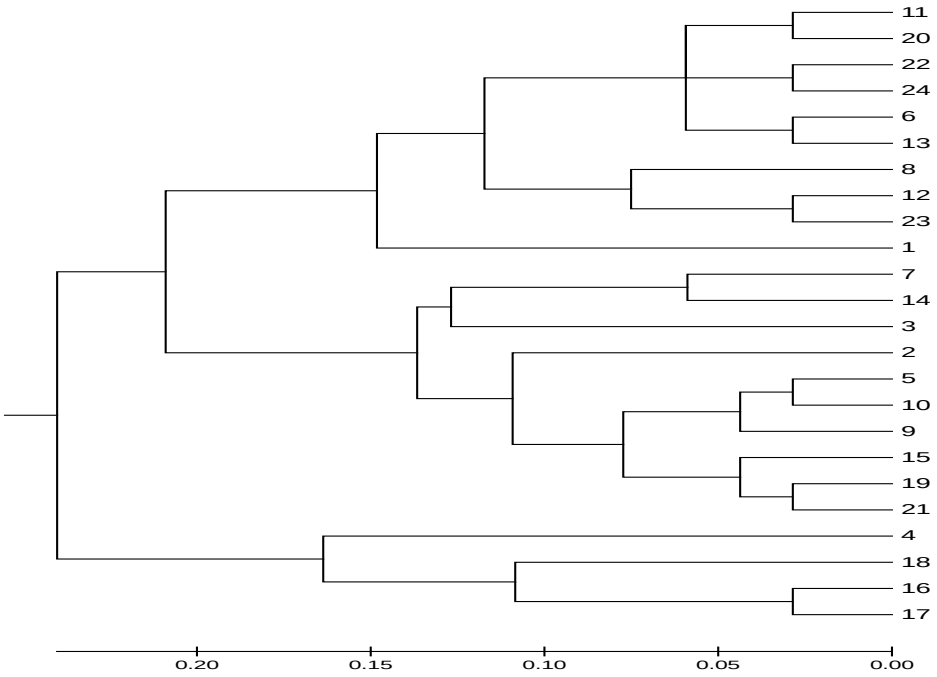

Figure (2): Cluster tree illustrating the genetic distance, based on the analysis of RAPD primers for 24 wheat mutant using the Euclidean distance and the UPGMA algorithm in the MEGA 5 software.

1:sahel-1control, 2:Sahel-1250Gy, 3:Sahel-1350Gy1, 4: Sahel-1 350Gy2 5: Sahel-1 0.04 % SA, 6:Sahel-1 0.06 % SA, 7:Sahel-10.08 % SA, 8: Sids-12 control, 9:Sids- 12 0.04 %SA, 10:Sids-12 0.06 %SA, 11:Sids- 12 0.08 % SA, 12:Sids-12 250Gy, 13: Sids-12 350Gy1, 14:Sids-12 350 Gy2, 15:Shandaweel-1 control, 16:Shandaweel-1 0.06 % SA, 17:Shandaweel-1 0.04 %SA, 18:Shandaweel-1 250Gy, 19:Shandweel-1 350Gy, 20: Gemmieza-11control, 21: Gemmieza-11 0.06% SA 22: Gemmieza-110.08 %SA, 23:Gemmieza-11 350 Gy1, 24:Gemmiza-11 350Gy2.

Gemmieza-11 0.08 %SA, Gemmiza-11 350Gy, Sahel-1 0.06 % SA and Sids-12 350Gy1) mutant and the second have 10 mutants have two groups the first have Sahel-10.08 % SA, Sids-12 350 Gy2 and Sahel-1350Gy1 and the second have Sahel-1 0.04 % SA, Sids-12 0.06 %SA, Sids- 12 0.04 %SA, Shandaweel-1 control, Shandweel-1 350Gy, Gemmieza-11 0.06% SA. The second cluster includes four mutant, mutant Sahel-1 350Gy2 and Shandaweel-1 250Gy in the separated sub-cluster and Shandaweel-1 0.04 %SA and Shandaweel-1 0.06 % SA. **Muqaddasi *et al.*, (2019)** showed that Exploiting significant, heritable genetic variation of TSN as well as a positive correlation with other traits can help to improve the grain yield in wheat. Grain yield (GY) improvement is considered as the top focus of virtually every wheat breeding program.

Conclusively, *this study established the importance of gamma rays and sodium azide mutagenesis increating genetic variation within and among wheat breeding populations. wide phenotypic variation in mutants under each breeding population were identified for improving drought tolerance yield, and yield-related traits.*

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متوسط الأداء والتنوع الجيني وتحليل DNA في جيل الثالث الطفري لبعض طفرات القمح تحت الإجهاد المائي

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أجريت التجربة الحقلية خلال موسم النمو ٢٠٢٠/٢٠١٩ لمتوسط الأداء والتنوع الوراثي وتحليل DNA باستخدام المعلم الوراثي RAPD لبعض طفرات القمح المستحدث باستخدام بأشعة جاما وأزايد الصوديوم تحت تأثير الاجهاد المائي. بالمزرعة التجريبية بكلية التكنولوجيا والتنمية. جامعة الزقازيق مصر. حيث تم اجراء جميع التوصيات الزراعية الموصى بها لإنتاج القمح ما عدا الري حيث كان كالتالي (تم الري مباشرةً بعد الزراعة، وأجريت الرية الأولى بعد ٤٥ يوم من الزراعة بينما أجريت الرية الثانية أثناء مرحلة الطرد والتزهير). حيث تم استخدام تصميم القطاعات كاملة العشوائية في ثلاث مكررات.

أظهر تحليل التباين وجود فرق معنوي بين الصنف جميزة-١١ و الطفرات المنتخبة منه لعدد الايام حتى الطرد ، طول السنبلة ، عدد السنيبلات/السنبلة ، عدد السنابل/النبات ، عدد الحبوب/السنبلة ، وزن حبوب السنبلة/جم، وزن ١٠٠٠ حبة/جم ، محصول الحبوب/النبات، المحصول البيولوجي/جم، دليل الحصاد% . حيث اعطت الطفرات المنتخبة من الصنف جميزة-١١ أعلى قيم لمتوسط الأداء محصول الحبوب/نبات ، أعطت الطفرة المنتخبة من الصنف سدس-١٢ باستخدام ٠.٠٨% صوديوم آزيد (Sid- 0.08 SA)أعلى القيم لدليل الحصاد٪.

أظهرت بيانات تحليل RAPD أن بادئات RAPD المطبقة على الطرز الوراثية للقمح أنتجت ١٨ حزمة وراثيه ؛ ١٦ من هؤلاء كانت متعددة الأشكال. أعطى البادئ OPB-5 أكبر عدد من الحزم الوراثيه (٥) بينما أعطى البادئ O-7 و A-20 و OPB-7 أقل عدد (٣). تم إنتاج أعلى نسبة من الحزم الوراثيه متعددة الأشكال بواسطة البادئ O-7 ، A- 6 و100) OPB-5 ٪(وتم إنتاج أقل نسبة من الحزم الوراثيه متعددة الأشكال بواسطة A-20 و OPB-7 (٦٧%).

التوصية: أظهرت هذه الدراسة أهمية أشعة جاما و أزيد الصوديوم في احداث التباين الجيني في القمح. تم تحديد السلوك الوراثي و التنوع الجيني باستخدام تحليل DNA في الطفرات لتحسين غلة تحمل الجفاف ، والسمات المتعلقة بالإنتاجية.