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GENETIC IMPROVEMENT ASSESSMENT OF MORPHO-PHYSIOLOGICAL AND YIELD CHARACTERS IN M3 MUTANTS OF BREAD WHEAT

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ABSTRACT: ***The present investigation was carried out at the Experimental Farm of Plant Research Department, Nuclear Research Center, Atomic Energy Authority, Inchas, Egypt. The aim of this study was to induce genetic variability in Sids 12, Sakha 94 and Gemmeiza 9 wheat cultivars using Gamma ray, LASER beams and Ethyl Methan Sulphonate. Field studies were conducted on M1, M2 and M3 generations. Bulked M3 mutants were evaluated during growing 2013/2014 season for performance and genetic variability of morpho-physiological and yield characters. The most promising M3 mutants derived from mother cultivar Sids 12 were Sd-12 0.3EMS and Sd-12 0.4EMS for high grain yield, no. of spikes/plant, flag leaf chlorophyll content, flag leaf area and spike length with moderate plant height. Whereas, the most promising populations derived from mother cultivar Sakha 94 were Sk-94 350 Gy, Sk-94 400 Gy and Sk-94 2hr LASER for grain yield and its components, flag leaf chlorophyll content, spike length. Furthermore, those obtained from Gemmieza 9 were Gm-9 0.3EMS for grain yield and its components, flag leaf area, flag leaf chlorophyll content, plant height and spike length. Maximum estimates of phenotypic (PCV), genotypic (GCV) and environmental (ECV) coefficients of variability were detected for No. of spikes/ plant followed by grain yield/plant and then No. of grains/spike in both Sids 12 and Sakha 94 as well as No. of grains/spike followed by grain yield/plant and flag leaf area.***

Heritability estimates in the broad sense in M3 generation varied from moderate to high for grain yield/plant and its contributing characters. The genetic advance was high for No. of grains/ spike and ranged from low to moderate for the remaining yield contributing characters in the three mutant cultivars. The cluster analysis based on the mean performance of grain yield/plant grouped wheat genotypes into three main clusters at 95% similarity. Interestingly, the derived mutants were clustered in separate groups than their mother cultivars (Sids 12, Sakha 94 and Gemmeiza 9). These results confirm the efficiency of the mutagens to induce genetic variations.

Key words: ***Bread wheat, Mutagens, Genetic variability, Heritability, Genetic advance and cluster analysis***

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is usually known as the king of cereals and is considered the foremost source of food products worldwide. Rising wheat production through improving the traditional local cultivars and application of mutation breeding methods is the greatest national target to minimize the gap between the production and consumption in Egypt. The area allocated to wheat crop in Egypt is 3 million faddan and total production is 8.68 million tons of grains with an average yield 2.89 tons/faddan (19.30 ardab/faddan) (Anonymous, 2017). Nevertheless, the

total production is far away than that required for local consumption. Excessive efforts have been made to increase food and agricultural production, mainly through evolving new cultivars categorized by high yielding potentiality and better quality to fill the gap between wheat production and consumption. This is the main aim of wheat breeders where they apply biotechnological techniques beside the traditional breeding methodology.

The use of physical and chemical mutagens like gamma ray, LASER beams and Ethyl Methan Sulphonate (EMS) for inducing variation and developing of many agronomical important traits is well proven. Recently the application of physical methods for plant growth promoting is receiving more interest due to its less harmful effect on the environment. Govindaraj *et al.* (2017) indicated that physical factors can be employed to get positive biological changes in crop plants without affecting the ecosystem. It causes physiological and biochemical changes, which reflect the plant growth and development processes and finally improve the yield and quality of produce.

The fundamental purpose of using mutagens has been to induce genetic variation, which is considered the first step in a breeding programme. Since wheat is a polyploid plant, duplication of genes permits great changes to be preserved and transferred to the next generation. The segregating populations have been used to improve wheat genotypes, which were competitive in yielding ability with the best wheat varieties. For accurate expression of genetic yield potential, any new wheat genotypes should also have a suitable combination of disease resistance, plant height, fertile tiller, spike length and grain weight. These characteristics must also be genetically modified to permit maximum yield stability under adverse conditions. Mutation induction and detection, has been an essential tool for plant breeders for more than 70 years to increase the genetic diversity of plants and originate new mutant lines with improved characteristics (IAEA, 2017).

The increase in variation of quantitative characters for a given generation can depend on the genotype, the trait investigated and the mutagen used. The success of induced variation will depends on the precision in selection techniques. So, selection of mutants can only begin in the M_2 generation. In the respective M_3 populations, many new mutants were selected which were not observed in the parental M_2 families. The expected response to selection can be measured by determining the parameter like mean, variation, standard deviation, heritability and genetic advance (El-Degwy and Hathout, 2014; El-Naggar *et al.* 2015; Nazarenko *et al.* 2018).

LASER irradiation is considered as a new branch in agriculture. The LASER is one of the physical sources for inducing a biostimulation effect and genetic changes in higher plants. Lower doses of LASER activate plants, resulting in increasing bioenergetic potential of the cell and higher activation of their biochemical and physiological processes. Higher doses influence genetical material of the cell leading to genetic changes of plant traits, besides its use in the cell and molecular biology. It is successfully used for other purposes in plant genetics and breeding. He-Ne LASER applied under field conditions to improve morphological parameters and the yield of wheat crop (Chen and Han, 2014).

Ethyl methane sulphonate (EMS) is the most useful chemical mutagen to induce genetic variation in plant breeding programs. Key factors in inducing mutations with EMS include dose,

genotype, temperature and duration of treatment (Ndou *et al.,* 2013). Meanwhile, Elsevier (2015) stated that production of mutants with altered phenotypes through EMS is a powerful approach for determining the biological functions of genes in an organism. A high-grain-weight mutant line could be obtained of the common wheat cultivars treated with EMS. So, inducing mutations are considered to be an effective way to create novel genetic variations and hence novel agronomical traits in wheat. The objectives of the present study were to 1) detect the most promising mutants derived from different doses of gamma ray, LASER beams and ethyl methane sulfonate concentrations for some agronomic traits in Sids 12, Sakha 94 and Gemmeiza 9 wheat cultivars, 2) identify genetic variability, heritability and genetic advance in M3 generation plants.

MATERIALS AND METHODS

The present investigation was carried out to induce genetic variability in bread wheat using gamma ray, LASER ray and EMS mutagens for obtaining mutants with increases in yield. The experiments of this study were conducted at the Experimental Farm of the Plant Research Department, Nuclear Research Center, Atomic Energy Authority, Inchas, Egypt during three seasons i.e., 2011/2012, 2012/2013 and 2013/2014.

Mutagenesis and obtaining mutant seeds

Seeds of three bread wheat cultivars (Sids-12, Sakha-94 and Gemmeiza-9) were obtained from the Wheat Research Section, Field Crops Research Institute, Agricultural Research Center, Egypt. The name and pedigree of the studied cultivars are presented in Table 1. Two hundred seeds of each cultivar were treated separately by three mutagens; two physical (Gamma ray and laser beams) and one chemical (Ethyl Methane Sulfonate, EMS) at Nuclear Research Center, Atomic Energy Authority, Inchas, Egypt. The three cultivars were irradiated by laser ray with 1, 1.5 and 2 hr exposure. The helium-neon laser (He-Ne) at the wave length 632.8 nm and the power density of 1Mw-2 was applied as the source of radiation. On the other hand, Gamma irradiation at a cobalt 60 (Co 60), was used to irradiate the seeds by Gamma Unit 7.5 k Gy (7500 Gy) per hour. Basically, to define the proper dose for each cultivar preliminary experiment (sensitivity test) was conducted using Petri-dishes with 0, 150, 200, 250, 300, 350, 400, 450 and 500 Gray. Based on mean values of epicotyl length (cm) and reduction % of seedling, three gamma ray doses were selected for each cultivar. Doses 250, 300 and 350 Gy were used for both Sids-12 and Gemmiza-9 cultivars, whereas, Sakha-94 was irradiated with 350, 400 and 450 Gy. Furthermore, EMS was used with concentrations 0.2, 0.3 and 0.4% (v/v). The seeds were soaked in distilled water for 6 h and then were moved to the mutagen for 2 hours at 24°C and were washed in tap water after treatment at ambient temperature then the seeds were air-dried.

Field trails and generation advance

In the first season, (2011-2012) seeds of M1 generation were immediately sown after treatment in three separate experiments for each mutagen. The experimental design was laid out in spilt-plot in which cultivars were in the main plots and treatments were randomized in the sub-plots, in three replications. Individual seeds were sown in plots consisted of five rows 3-m long with 30 cm apart, and seeds were spaced with 10 cm on the row. Harvested seeds were sown in the second season to obtain M2 generation with the same experimental design as M1 generation. In 2012/2013, the selection was performed on M2 plants in the three experiments at harvesting by 1% selection intensity based on agronomic traits *i.e.* plant height, spike length, grain yield/plant and its components. Seeds of the selected plants from each treatment were bulked and sown on 26 November 2013/2014 season in plots, each plot contain 5 rows with 3 m length, spaced 30 cm. apart, and the plants within row were 10 cm. the experimental design was randomized complete block design with three replicates for evaluation. The number of promising mutants in M_3 were 5, 3, and 2 were derived from Sids 12, Sakha 94 and Gemmeiza 9 cultivers, respectively. The recommended cultural practices for wheat production were applied under this condition. Physical analysis of the soil at the experimental site is given in Table (2).

Data recorded:

Data were recorded on 15 individual guarded plants from M3 generation in each replicate for morpho-physiological characters *i.e.,* flag leaf area (cm^2), flag leaf chlorophyll content (SPAD reading) and plant height, spike length, as well as grain yield and its components *i.e.,* number of spikes/plant, number of grains/spike and 1000-grain weight.

Statistical analysis:

Data were statistically analyzed using randomized complete block design in M_3 generation with three replicates, and mean values were compared by using the least significant difference test (L.S.D) at 5% level (Steel *et al.,* 1997). The genotypic coefficient of variation (GCV), the phenotypic coefficient of variation (PCV) and environmental coefficient of variation (ECV) was estimated according to Burton (1952). Heritability in a broad sense ($H_{b\ \%}$)) and genetic advance as the % of population mean (G.s %) were computed according to Johanson *et al.,* (1956) following to Singh and Choudhary (1985). Cluster analysis based on mean performance of grain yield/plant for studied wheat mutants was done using statistical software SPSS version 16.0 (SPSS Inc., 2007).

Table (1): Name and pedigree of the three used wheat cultivars.

Name	Pedigree
Sids 12	BUC//7C/ALD/5/MAYA74/ON//1160-147/3/BB/GLL/4/CHAT"S"/6/ MAYA/VUL//CMH74A.6304/*Sx.SD70964-SD-1SD-1SD-0SD.
Sakha 94	Opata/Rayon//KauZ.CMBW90Y3180-0T0PM-3Y-010M-010Y-10M-15Y-0Y-0AP-0S.
Gemmeiza 9	Ald"S"/Huac "S"//CMH 74A.630/5xCGM4583-5GM-IGM-0GM.

Table (2): Physical analysis of the soil at the experimental site.

Depth of soil sample	Sand	Silt	Clay	Soil texture
1-25 cm	77.0	17.8	5.2	Sandy loam
25-50 cm	97.2	1.80	1.0	Sandy

RESULTS and DISCUSSION

A. Mean performance and variability for the selected wheat mutants:

1. Flag leaf area, plant height and spike length:

Mean performance for studied wheat mutants of morpho physiological characters in M_3 bulk generation for Sids 12, Sakha 94 and Gemmeiza 9 cultivars are presented in Table (3). Mutagenesis caused a significant variation for all traits studied, reflecting the direct effect of the applied mutagen on gene expression.

In general, flag leaf area was increased by different mutagen treatments in the three tested wheat cultivars (Table 3). The highest mean of flag leaf area was obtained from the mutants induced by 0.4 EMS and 2hr LASER in Sids 12 valued 29.237 and 29.833 cm^2, respectively rather than control 20.333 cm^2. Moreover, 2hr LASER in Sakha 94 gave 19.170cm^2 compared to the control 17.823 cm^2 as well as 250 Gy and 0.3 EMS in Gemmeiza 9 valued 20.653 and 19.810 cm^2, respectively rather than the control 17.143 cm^2. The previous studies recorded the response of wheat cultivars to mutagen treatments and found great differences between the DH lines for blade area (Rybiñski, 2000; Rybiñski and Garczyñski, 2004). LASER light was particularly efficient for increasing flag leaf area. Furthermore, Singh and Datta (2010) found that a low dose of gamma radiation 0.01–0.10 kGy reduced plant height, improved plant vigor and flag leaf area.

For flag leaf chlorophyll content (SPAD reading), as presented in Table (3) was increased with various mutagen treatments in all wheat cultivars, except in Sids 12 with 0.4 EMS which was similar to the control and Sakka 94 in all treatments. Flag leaf chlorophyll content (SPAD reading) exhibited maximum SPAD reading in Sd-12 2hr LASER (56.16), Sd-12 250 Gy (56.05), Sd-12 350 Gy (55.96) and Sd-12 0.3 EMS (55.34) rather than the control (51.74). Furthermore, Gm-9 0.3 EMS followed by Gm-9 250 Gy population produced the highest mean 53.19 and 52.74, respectively compared to the control 47.31. The highest effects of the previous treatments could be due to stimulation of physiological and biochemical processes in plant. Whereas, a slight decrease in flag leaf chlorophyll content has been derived from Sk-94 2hr LASER, Sk-94 350 Gy and Sk-94 400 Gy populations which attained 54.63, 54.61 and 53.54, respectively compared to the control (55.46). This result could be due to the inhibition effect of Gamma and LASER rays on gene expression controlling flag leaf chlorophyll content. Sids 12-2hr LASER and Gm 9-250 Gy populations had the maximum effect for both flag leaf area and flag leaf chlorophyll content.

It is interest to note that, Sids 12 produced the shortest plant height (80.24 and 81.24 cm) and spike length (15.24 and 16.24 cm) mutants derived from gamma ray dose 250 and 350 Gy, respectively, and mean performance showed a significant increase with the other mutagen treatments rather than the control. The tallest plant height (86.41 cm) and longest spikes (18.37) variants were induced from 2hr LASER. A decrease in both characters was derived from Sk-94 350 Gy, 400 Gy and 2hr LASER rather than the control. Meanwhile, Gemmeiza 9 progenies showed a slight increase in plant height and spike length due to mutagen effects. The decrease in plant height and spike length due to mutagen effect could be discussed on the basis that the effect of LASER on the activity of some phytohormones, mainly indole acetic acid in the treated seeds. It is worthy to mention that selected mutants with short straw or semi-dwarf stature, has become a highly favored trait because of a lower susceptibility to lodging, higher tillering and better harvest index. These results are in agreement with the finding of Rybinski (2001) who recorded high levels of reduction and stimulation of the M_1 plants after application of LASER light on Rudzik and Ramp cvs. for plant height, spike length and chlorophyll content. Lifen *et al.* (2014) found that plant height, spike length and flag leaf length were lower in the mutant than in the wild type. However, flag leaf width of the mutant had no significant difference with those of the wild type.

Table (3): Mean performance for the studied wheat mutants of morpho- physiological characters in M_3 generation for Sids 12, Sakha 94 and Gemmeiza 9 cultivars.

Morph-physiological characters				
Characters / M3 mutants	Flag leaf area $(cm)^2$	Flag leaf chlorophyll content (SPAD reading)	Plant height (cm)	Spike length (cm)
Sids 12				
Sd-12 Control	20.333	51.74	83.42	16.13
Sd-12 250 Gy	28.420	56.05	81.24	15.24
Sd-12 350 Gy	26.493	55.96	80.24	16.24
Sd-12 0.3 EMS	28.520	55.34	84.50	17.49
Sd-12 0.4 EMS	29.237	51.74	83.69	18.19
Sd-12 2 hr LASER	29.833	56.16	86.41	18.37
F. test	*	*	*	*
L.S.D $_{0.05}$	0.542	0.554	0.508	0.594
Sakha 94				
Sk-94 Control	17.823	55.46	83.61	10.40
Sk-94 350 Gy	18.660	54.61	78.69	12.28
Sk-94 400 Gy	18.550	53.54	77.78	11.72
Sk-94 2hr LASER	19.170	54.63	81.44	11.44
F. test	*	*	*	*
L.S.D $_{0.05}$	0.368	0.528	0.568	0.419
Gemmeiza 9				
Gm-9 Control	17.143	47.31	89.25	13.41
Gm-9 250 Gy	20.653	52.74	87.58	14.30
Gm-9 0.3 EMS	19.810	53.19	88.76	14.70
F. test	*	*	*	*
L.S.D $_{0.05}$	0.441	0.687	0.886	0.778

* , Significant at 0.05 level of probability

2. Grain yield and its components

Data presented in Table (4) show mean performance for studied wheat mutants of some yield characters in M_3 generation for Sids 12, Sakha 94 and Gemmeiza 9 cultivars. The comparison with the use of mutagen doses with the control showed significant differences between various treatments for grain yield and its components in respect to all wheat cultivars.

Sids 12 produced mutants more pronounced in No. of spikes/plant with various mutagen treatments, except Sd-12 2 hr LASER which was (2.47) less than the control. The highest mean in the M_3 population was derived from Sd-12 0.3 EMS (5.41), followed by Sd-12 0.4 EMS (5.00), Sd-12 350 Gy (4.85) and Sd-12 250 Gy (4.22) compared to the control (3.14). Moreover, Sakha 94 showed a slight significant increase in No. of spikes/plant rather than the control, whereas Gemmeiza 9 was highly affected by 0.3 EMS (6.77) rather than the control (5.91). This result could be due to the differential response of wheat cultivars to the studied treatments according to their genetic constitutions. In this respect, Sakin (2002) recorded significant variation in M_3 progenies after EMS and gamma ray treatments for tiller number in durum wheat.

Regarding No. of grains/spike (Table 4), the greatest and smallest mutants for No. of grains/ spike were Sd-12 2 hr LASER (88.79) and Sd-12 0.4 EMS (75.38) in Sids 12; Sk-94 400 Gy (74.10) and Sk-94 350 Gy (68.87) in Sakha 94 as well as Gm-9 0.3 EMS (82.28) and Gm-9 250 Gy (74.63) in Gemmeiza 9. The heaviest and lightest 1000-grain weight populations were obtained from Sd-12 2hr LASER (61.660 gm) and Sd-12 0.3 EMS (42.600 gm) in Sids 12; 350 Gy (44.330 gm) and 2hr LASER (42.730 gm) in Sakha 94 as well as Gm-9 250 Gy (49.230 gm) and Gm-9 0.3 EMS (45.260 gm) in Gemmeiza 9 (Table 4). In this connection, Elsevier (2015) treated wheat cultivar YN15 with ethyl methane sulfonate (EMS). He found that M_2 and M_3 progenies registered significant differences between M8008 and YN15 in plant height, spike length, fertile spikelets number per spike and thousand-grain weight. Various mutagen treatments caused significant differences in grain yield/plant in all studied wheat cultivars (Table 4). Grain yield/ plant affected significantly with different mutagen treatments compared to the control, and the highest populations were derived from Sd-12 0.3 EMS (15.520gm) followed by Sd-12 0.4 EMS (14.787gm), Sd-12 250 Gy (14.307gm), Sd-12 350 Gy (14.260gm) and then Sd-12 2 hr LASER (13.297 gm) compared to the control (8.177gm), with insignificant difference between 250 Gy and 350 Gy on grain yield/plant.

The slight effect has been registered under various treatments valued 16.207 gm, 16.150 gm, 16.070 gm and 10.340gm for Sk-94 400 Gy, Sk-94 2hr LASER, Sk-94 350 Gy and Sk-94 control, respectively, with insignificant influence between the studied mutagen treatments. Hereby, these mutants has similar trend respecting grain yield.

It is interest to note that, Gemmeiza 9 cultivar took similar trend; since it produced higher variants with 250 Gy and 0.3 EMS valued 19.573 and 19.280 gm, respectively rather than control 16.347gm. These results could be due to stimulation effect of both mutagen species on Gemmeiza 9.

Generally, some mutants such as Sd-12 0.3 EMS, Sk-94 350 Gy, Sk-94 400 Gy and Sk-94 2hr LASER as well as Gm-9 250 Gy and Gm-9 0.3 EMS were identified as the best progeny for grain yield/ plant and most of its components among all the progeny. Singh *et al.,* (2013) recorded significant differences among gamma

rays doses (20, 25 and 30 kR) on wheat genotype HD 2867 in M_2 and M_3 generations. They showed improvement in most of the mutagenic treatments in M_3 as compared to the corresponding treatments in M_2 generation over untreated check. The impact of this dose was promising in the number of tillers/plant, plant height, test weight and yield/plant. However, a high reduction in the mean value for all the characters was obtained in response to the higher dose of gamma rays (30 kR). Al-Naggar *et al.* (2015) selected high grain yield/plant and other desirable traits in the M_2 populations of 7 gamma irradiated genotypes under well watering and water stress conditions. Significant yield superiority of 7 M_3's variants over their original and better parents were identified under WS reached 74.71%.

Table (4): Mean performance for the studied wheat mutants of yield and its components traits in M_3 generation for Sids 12, Sakha 94 and Gemmeiza 9 cultivars.

Yield and its components				
Characters / M3 mutants	No. of spikes/plant.	No. of grains/spike	1000-grain weight (gm)	Grain yield/plant (gm)
Sids 12				
Sd-12 Control	3.14	59.03	52.260	8.177
Sd-12 250 Gy	4.22	81.29	56.630	14.307
Sd-12 350 Gy	4.85	80.90	56.130	14.260
Sd-12 0.3 EMS	5.41	76.11	42.600	15.520
Sd-12 0.4 EMS	5.00	75.38	51.930	14.787
Sd-12 2hr LASER	2.47	88.79	61.660	13.297
F. test	*	*	*	*
L.S.D $_{0.05}$	0.032	1.169	2.354	0.340
Sakha 94				
Sk-94 Control	7.00	56.86	39.930	10.340
Sk-94 350 Gy	7.66	68.87	44.330	16.070
Sk-94 400 Gy	7.64	74.10	44.260	16.207
Sk-94 2hr LASER	7.930	73.27	42.730	16.150
F. test	*	*	*	*
L.S.D $_{0.05}$	0.282	2.535	3.883	0.659
Gemmeiza 9				
Gm-9 Control	5.91	63.30	44.120	16.347
Gm-9 250 Gy	5.83	74.63	49.230	19.573
Gm-9 0.3 EMS	6.77	82.28	45.260	19.280
F. test	*	*	*	*
L.S.D $_{0.05}$	0.248	0.286	4.692	0.716

***, Significant at 0.05 level of probability**

Finally, it could be concluded that the most promising mutants derived from bulk Sids 12 was Sd-12 250 Gy for high productivity, 1000- grain weight, number of grains/ spike, flag leaf area, flag leaf chlorophyll content, but slightly moderate number of spikes/ plant; Sd-12 350 Gy for high grain yield, number of spikes/ plant, number of grains/ spike, 1000- grain weight, flag leaf chlorophyll content, but slightly moderate in both flag leaf area and plant height ; Sd-12 0.3 EMS for high productivity, number of spikes/plant, flag leaf area, flag leaf chlorophyll content, plant height and slightly moderate number of grains/spike as well as Sd-12 0.4 EMS was the same as it high productivity, higher number of spikes/plant, flag leaf area, flag leaf chlorophyll content, plant height, but slightly moderate 1000-grain weight.

Whereas, both Sk-94 350 Gy and Sk-94 400 Gy are considered as the most promising mutants characterized by high grain yield and its components, flag leaf chlorophyll content, long spike, with moderate flag leaf area and short plant stature as well as Sk-94 2hr LASER had high grain yield and its components, flag leaf area, flag leaf chlorophyll content with long plant height. Moreover, Gm-9 250 Gy and Gm-9 350 Gy are classified as the most promising mutants for grain yield and its components, flag leaf area, flag leaf chlorophyll content and plant height.

B. Reduction (-) or stimulation (+) of analyzed traits for M_3 generation

Morph-physiological characters presented in Table (5), indicate that the maximum stimulation influence of the mutagen treatments for flag leaf area was derived from 2hr LASER (43.79%) folowed by 0.4 EMS (40.26%),250Gy (39.29%), in Sids 12, as well as 7.55% (2hr LASER) in Sakha 94 cv. and Gm-9 250 Gy (20.47%) in Gemmeiza 9 cultivar. Flag leaf chlorophyll content exhibited maximum biostimulate by Sd-12 2hr LASER (8.52%) followed by Sd-12 250Gy (8.31%), then Sd-12 350 Gy (8.15%). Also, the highest stimulation was registered by Sk-94 350 Gy (6.12%), otherwise, the highest reduction -3.46% was recorded by Sk-94 400 Gy. Moreover, maximum stimulation of 12.42% was derived from Gm-9 0.3 EMS followed by 11.47% Gm-9 250 Gy.

Table (5): Reduction (-) or stimulation (+) of analyzed traits calculated as a percentage of control value for wheat mutants in M_3 generation.

Sids 12								
M3 mutants	F.L.A	F.L.C.	P.H	S.L.	N.S/P.	N.G/S	1000-G.W	G.Y/P
Sd-12 250 Gy	+39.77	+8.31	-2.60	-5.49	+34.09	+37.71	+8. 36	+74.96
Sd-12 350 Gy	+30.29	+8.15	-3.80	+0.70	+54.21	+37.05	+7.40	+74.39
Sd-12 0.4 EMS	+40.26	+6.94	+1.29	+8.43	+71.90	+28.93	-18.48	+89.80
Sd-12 2hr LASER	+43.79	-	+0.32	+12.78	+58.88	+27.69	-0.63	+80.83
Sakha 94								
SK-94 350 Gy	+4.69	+6.12	-5.88	+18.07	+9.42	+21.11	+11.01	+55.41
SK-94 400 Gy	+4.07	-3.46	-6.97	+12.75	+9.13	+30.31	+10.84	+56.74
SK-94 2 hr LASER	+7.55	-1.50	-2.59	+10.02	+13.23	+28.86	+7.01	+56.18
Gemmeiza 9								
Gm-9 250 Gy	+20.47	+11.47	-1.86	+6.66	-1.35	+17.89	+11.58	+19.73
Gm-9 0.3 EMS	+15.55	+12.42	-0.54	+9.64	+14.41	+29.96	+2.58	+17.94

FLA: Flag leaf area, FLC: Flag leaf chlorophyll content, PH: Plant height, SL: Spike length, NS/P: No. of spikes/plant, NG/S: No. of grains/spike, 1000-GW: 1000-grain weight, GY/P: Grain yield/plant.

The highest reduction in plant height was -3.80% in Sd-12 350 Gy, as well as -6.97% in Sk-94 400 Gy followed by -5.88% in Sk-94 350 Gy and negligible -1.86% in Gm-9 250 Gy. Moreover, maximum stimulation for spike length was derived from 1.5 LA × 0.3 EMS (13.93%) followed 2hr LASER (12.78%), but there was reduction at 250 Gy (-5.49%) in Sids 12. Maximum stimulation was 18.07 (350 Gy) in Sakha 94, while it was 9.64% (0.3 EMS) and 6.66% (250 Gy) in Gemmeiza 9. Rybinski (2001) recorded high levels of reduction and stimulation of the M1 plants after application of LASER light on Rudzik and Ramp cvs. for plant height, spike length, number of fertile spikes/plant and chlorophyll content.

Regarding yield characters, the greatest stimulation in number of spikes/plant was observed by Sids 12 population 0.4 EMS (71.90%) followed by 2hr LASER (58.88%), 350 Gy (54.21%) and 250 Gy (34.09%). Whereas, high stimulation 13.23% was registered by Sk-94 2hr LASER and 14.41% by Gm-9 0.3 EMS. While, the greatest biostimulate 37.71% for number of grains/spike was recorded by (250 Gy), 37.05% (350 Gy) in Sids 12; 30.31% (400 Gy) followed by 28.86% (2hr LASER) and 21.11% (350 Gy) in Sakha 94 as well as 29.96% (0.3 EMS) in Gemmeiza 9. Moreover,1000-grain weight exhibited maximum biostimulate 8.36% in Sd-12 250 Gy, while maximum reduction was 18.48% in Sd-12 0.4 EMS. Furthermore, the highest stimulation was 11.01% in Sk-94 350 Gy followed by 10.84% in Sk-94 400 Gy, however it was 11.58% in Gm-9 250 Gy. In continues, grain yield/plant exhibited the highest stimulation valued 89.80% by (Sd-12 0.4 EMS) followed by 80.83% (Sd-12 2hr LASER), 74.96% (Sd-12 250 Gy), 74.39% (Sd-12 350 Gy); while it was 56.74% in Sk-94 400 Gy followed by 56.18% (Sk-94 2hr LASER) and then 55.41% (Sk-94 350 Gy), as well as 19.73% in Gm-9 250 Gy followed by 17.94% (Gm-9 0.3 EMS).

It could be concluded that Sd-12 0.4EMS and Sd-12 2hr LASER exhibited maximum biostimulate in grain yield/plant, number of spikes/ plant, number of grains/ spike in addition to flag leaf area. Also, Sk-94 350 Gy and Sk-94 400 Gy exhibited the maximum biostimulate in grain yield/plant, 1000-grain weight, number of grains/spike with moderate value in flag leaf area and short plant height. Furthermore, Gm-9 250 Gy exhibited higher biostimulate in grain yield/plant, 1000-grain weight with moderate number of grains/spike, broader flag leaf area and flag leaf chlorophyll content. In this respect, Rybiñski (2001) studied the influence of LASER beams combined with chemo mutagen (MNU) on the variability of plant height, spike length and grain yield traits in spring barley. Irrespective of the cultivar, a short exposure to LASER light (30 min) induced a biostimulation effect on the yield contributing traits in the M_1 plants. Prolongation of irradiation to 120 min caused a reduction of the analyzed traits. Whereas, AL-Azab (2013) showed that the gamma ray irradiated genotypes Maryout-5 and Sakha-93 found to be the most responsive ones to induction of more variability via irradiation especially for grain yield and spikes/plant under water stress and non-stress conditions.

C. Genetic parameters of studied wheat mutant traits in M_3 generation for wheat cultivars.

Estimates of some genetic parameters for studied mutants in M_3 generation for Sids 12 cultivar are given in Table (6). The estimates of phenotypic (PCV), genotypic (GCV) and environmental (ECV) coefficients of variability were high for No. of spikes/ plant and grain yield / plant, while they were moderate for spike length, flag leaf area, No. of grains/spike and 1000-grain weight, except the latest character which was low in environmental (ECV) coefficient of

variability. Whereas, the three parameters were low for both plant height and flag leaf chlorophyll content, in addition to 1000-grain weight for (ECV) coefficient only. The PCV was higher than its respective GCV for No. of spikes/plant, No. of grains/spike and grain yield/plant, reflect their influenced by the environmental fluctuations. While the narrow difference between PCV and GCV were observed for the remaining yield attributes, implanted its relative resistance to the environmental condition. Singh *et al.*, (2013) induced mutation through gamma irradiation at different doses (20, 25 and 30 kR) on genotype HD 2867 in M_2 and M_3 generations. They registered genetic variability through all the three mutagenic treatments in yield and yield contributing traits. These results agreed with heritability in the broad sense. Hence the estimate of heritability was essential to get the best face of amount of gain to be expected from the selection. Broad sense heritability estimates (Tables 6) were high (≥75%) for flag leaf area, flag leaf chlorophyll content, plant height, No. of spikes/ plant, No. of grains/spike and 1000-grain weight and it was moderate (50 - < 75%) for spike length and grain yield/plant. The genetic advance as the percentage of the mean (Table 6) was high for No. of grains/ spike, followed by 1000-grain weight and flag leaf area. While, it was low for the remaining characters. High values of heritability and genetic advance were observed for most yield attributes which might be due to effective gene action regulating their expression, and phenotypic selection for their improving could be brought about by a simple method like mass and / or bulk method. In this regard, Sakin and Yildirim (2004) recorded high heritability estimates for mutant populations demonstrated that induced variation was maintained in the M_2 and M_3 generations. AL-Azab (2013) registered the highest estimates of PCV, GCV, ȣ^2 P and ȣ^2 g by grain yield/plant and spikes/plant. Also, highest GCV and ȣ^2 g were recorded for five out of seven traits, including grain yield and the most important yield component. In M_2 generation the heritability estimates in the broad sense were, on average higher for five traits *i.e.* plant height, spikes/plant, grains/spike, 100-grain weight and grain yield/plant.

Table (6): Estimates of some genetic parameters for studied wheat mutant traits in M_3 generation of Sids 12 cultivar.

Parameters / Characters	PCV	GCV	ECV	$H_{b\ (\%)}$	G.s (%)
Flag leaf area $(cm)^2$	13.510	12.950	3.84	91.880	14.640
Flag leaf chlorophyll content (SPAD reading)	3.200	3.360	1.01	90.030	6.924
Plant height (cm)	2.90	2.618	1.24	81.52	8.10
Spike length(cm)	8.874	7.638	4.37	73.70	4.210
No. of spikes/plant	34.410	30.510	15.88	78.640	4.720
No. of grains/spike	14.120	12.900	5.72	83.570	38.120
1000-grain weight(gm)	7.114	6.715	2.34	89.13	15.38
Grain yield/plant.(gm)	22.65	19.722	11.14	74.79	9.108

PCV: Phenotypic coefficient of variation; GCV: Genotypic coefficient of variation; ECV: Environmental coefficient of variation; $H_{b\ (\%)}$: Heritability in broad sense G.s (%): Genetic advance as % of population mean

For Sakha 94 cultivar, estimates of some genetic parameters for the studied mutants in M_3 generation of the bulk method are given in Table (7). The estimates of phenotypic (PCV), genotypic (GCV) and environmental (ECV) coefficients of variability were high for both No. of spikes/plant and grain yield/ plant, while the estimates were moderate for No. of grains/spike and 1000-grain weight , whereas, they were low for plant height, spike length, flag leaf area and flag leaf chlorophyll content. The PCV was higher than its respective GCV for both No. of spikes/plant and 1000-grain weight, reinforce they are influenced by the environmental fluctuations. Broad sense heritability estimates (Tables 7) were high (≥ 75%) for flag leaf area, flag leaf chlorophyll content, plant height, spike length, No. of grains/spike, and grain yield/plant. Otherwise, it was moderate (50 - < 75%) for No. of spikes/plant and low for 1000-grain weight, which was highly affected by the environmental changes and selection based on phenotypic performance was ineffective.

The genetic advance as a percentage of mean (Table 7) was high for No. of grains/spike and moderate for plant height and grain yield/plant, while it was low for the rest characters. The higher magnitude of heritability and genetic advance for plant height and No. of grains/spike indicated that these characters could be improved through phenotypic selection. It indicates that inducing genetic variability and improvement in such traits would be possible through mutagens. These results are confirmed with the previous findings of El-Degwy and Hathout (2014) shown that all genetic parameters σ^2g, GCV, PCV, $h^2_{b.s}$ and genetic advance from selection were higher and folded several times in case of irradiated plants compared to the control for plant height and yield contributing characters.

Table (7): Estimates of some genetic parameters for studied wheat mutant traits in M_3 generation of Sakha 94 cultivar.

Parameters / Characters	PCV	GCV	ECV	$H_{b\ (\%)}$	G.s (%)
Flag leaf area (cm^2)	3.099	2.932	0.99	89.72	2.259
Flag leaf chlorophyll content (SPAD reading)	2.496	2.448	0.48	96.23	3.452
Plant height (cm)	3.490	3.300	1.16	88.980	10.694
Spike length (cm)	7.356	6.790	2.81	85.370	3.066
No. of spikes/plant.	21.24	16.68	13.16	61.62	1.096
No. of grains/spike	12.447	11.591	4.53	86.720	31.25
1000-grain weight (gm)	6.043	4.054	4.48	45.010	4.237
Grain yield/plant (gm)	20.96	19.705	7.17	88.300	8.670

PCV: Phenotypic coefficient of variation; GCV: Genotypic coefficient of variation; ECV: Environmental coefficient of variation; $H_{b\ (\%)}$: Heritability in broad sense; G.s (%): Genetic advance as % of population mean

For Gemmeiza 9 cultivar, values of genetic parameters for studied mutants in M_3 generation of the bulk method are given in Table (8). The estimates of genotypic (GCV) and phenotypic (PCV) coefficients of variability were high for No. of grains/spike, while they were moderate for spike length, flag leaf area, flag leaf chlorophyll content, No. of spikes/plant, 1000-grain weight and grain yield/plant, whereas, they were low for plant height. Broad sense heritability estimates (Table 8) varied from moderate for 1000-grain weight to relatively high for remaining yield contributing characters

The genetic advance as a percentage of mean coupled with broad sense heritability estimates (Table 8) was high for flag leaf chlorophyll content, No. of grains/spike and moderate for flag leaf area, 1000-grain weight and grain yield/plant and low for the remaining yield contributing characters. Static parameters in M3 generation of bulk method for the tested wheat cultivars for the traits found to be differ from cultivar to another, this could be attributed to the different genetic background of genotypes and their interaction with mutagens treatments. Kusaksz and Dere (2010) subjected three spring durum wheat cultivars namely Salihli-92, Ege-88 and Gediz-75 to various doses of gamma irradiation *i.e.,* (0, 150 and 300 Gy). In certain mutant populations, heritability and phenotypic standard deviation values, genetic gains were higher than those of control population. Al-Azab (2013) recorded the highest h^2b estimates (61.70%) by plant height followed for grain yield/plant (25.21%). The maximum predicted genetic gain from selection in M_2 bulks was achieved from No. of spikes/plant (17.07%) followed by spike length (15.63%) and grain yield/plant (14.69%). Maximum gain from selection in the M_2 for high grain yield would be expected to be (18.09%) from Sids-4(1) followed by (17.82%) from Sakha-61(1).

Table (8): Estimates of some genetic parameters for studied wheat mutant traits in M_3 generation of Gemmeiza 9 cultivar.

Parameters / Characters	PCV	GCV	ECV	$H_{b\,(\%)}$	$G.s_{(\%)}$
Flag leaf area (cm^2)	10.196	6.676	3.64	87.310	7.288
Flag leaf chlorophyll content (SPAD reading)	6.714	6.395	2.04	90.710	13.463
Plant height(cm)	1.03	0.933	0.44	81.680	3.172
Spike length (cm)	5.084	4.466	2.42	77.170	2.331
No. of spikes/plant.	9.304	8.315	4.16	79.870	1.868
No. of grains/spike	18.123	16.394	1.93	82.92	11.310
1000-grain weight(gm)	6.771	5.258	4.26	60.29	7.361
Grain yield/plant (gm)	10.880	9.641	5.04	78.550	6.360

PCV: Phenotypic coefficient of variation; GCV: Genotypic coefficient of variation; ECV: Environmental coefficient of variation; $H_{b\,(\%)}$: Heritability in broad sense G.s (%): Genetic advance as % of population mea

D. Cluster analysis of wheat mutants.

Cluster analysis based on themean performance of grain yield/plant for studied wheat mutants was done as shown in Figure 1 using statistical software SPSS version 16.0 (SPSS Inc., 2007). At 95% similarity, the generated dendrogram divided the genotypes into three main clusters, the first cluster included two subgroups (the first subgroup was composed of Sd-12 250 Gy, Sd-12 350 Gy, Sd-12 0.4 EMS and Sd-12 2hr LASER genotypes; while the second subgroup included the genotypes Sk-94 400 Gy, Sk-94 350 Gy, Sk-94 2hr LASER, Gm- Cont and Sd-12 0.3 EMS. In addition, the second cluster contained the genotypes Gm-250 Gy and Gm-0.3 EMS, while the third cluster grouped Sk-94 and Sd-12 controls together in the same group. Interestingly, the derived mutants were clustered in separate groups than their controls (Sids 12, Sakha 94 and Gemmeiza 9). These results confirm the efficiency of the mutagens to induce genetic variations. Previous studies showed that the cluster analysis based on morphological traits of soybean gave remarkable genetic divergence and was employed to study the genetic relationships among mutants (Cui *et al.,* 2001; Iqbal *et al.,* 2008; Malek *et al.*, 2014 and Yu *et al.,* 2005).

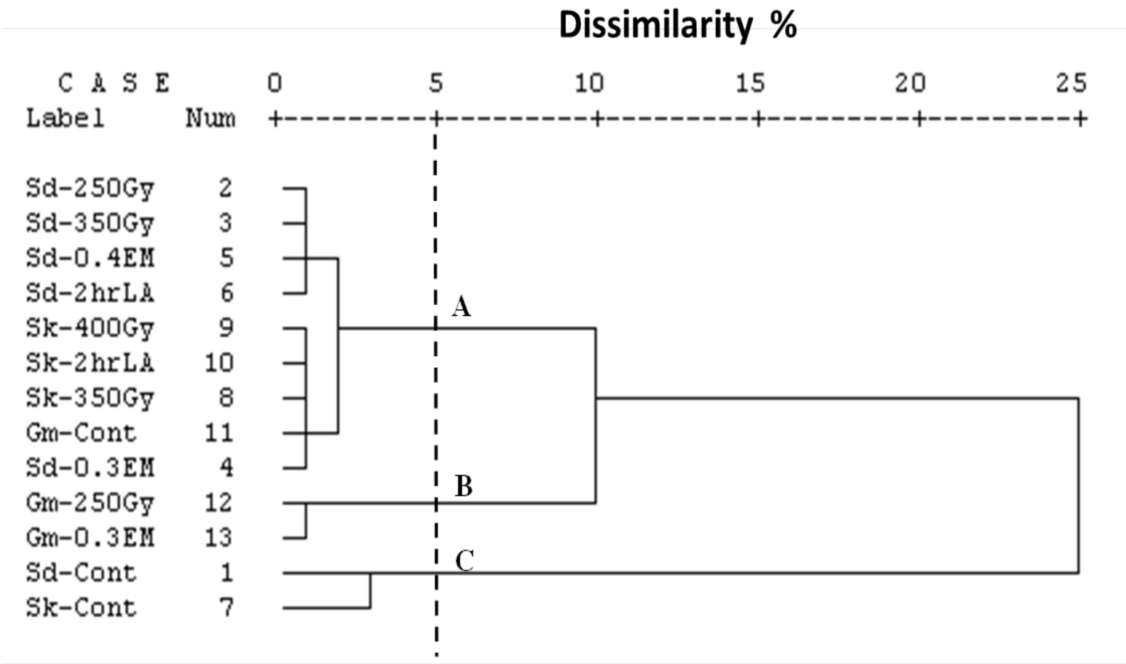

Figure (1): Cluster analysis based on mean performance of grain yield/plant for studied wheat mutants using squared Euclidian distance as revealed by the statistical software SPSS version 16.0 (SPSS Inc., 2007)

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تقييم التحسين الوراثي للصفات المورفوفسيولوجية والمحصولية في الجيل الطفري الثالث من قمح الخبز

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الملخص العربى

أجريت هذه الدراسة بالمزرعة التجريبية بقسم البحوث النباتية، مركز البحوث النوويه، هيئة الطاقه الذرية، انشاص - مصر بهدف إستحداث طفرات فى ثلاثة أصناف من قمح الخبز (سدس ١٢، سخا٩٤ ، جميزة ٩) بإستخدام أشعة جاما والليزر والمطفر الكيماوى إيثيل ميثان سلفونات. تم إجراء هذه التجارب الحقلية على الاجيال الطفرية M1 و M2 و M3. استهدفت الدراسة تقييم الصفات المورفوفسيولوجية والمحصولية للجيل الطفرى التجميعي الثالث للموسم الشتوي ٢٠١٣/٢٠١٤. وقد وجد ان الطفرات سدس ١٢ EMS 0.3 و سدس ١٢ EMS 0.4 الناتجة من الصنف سدس ١٢ كانت اكثر الطفرات المبشرة للجيل الثالث من حيث الانتاج العالى، عدد سنابل/النبات، محتوى كلوروفيل ورقة العلم، مساحة ورقة العلم وطول السنبلة ومتوسطة في صفة ارتفاع النبات، بينما كانت الطفرات سخا٩٤- ٣٥٠ جراي، سخا ٩٤- ٤٠٠ جراي وسخا٩٤ -٢ ساعة ليزر الاعلى في محصول الحبوب ومكوناتة، محتوى الكلوروفيل لورقة العلم وطول السنبلة. علاوة على ذلك كانت الطفرة جميزة ٩- EMS ٠,٣ مبشرة فى محصول الحبوب ومكوناتة، مساحة ورقة العلم، محتوى كلوروفيل ورقة العلم وطول السنبلة وارتفاع النبات. وسجلت أعلي تقديرات لمعامل الاختلاف المظهري والوراثي والبيئي لصفة عدد سنابل/ النبات يليها محصول حبوب/النبات ثم عدد حبوب/السنبلة في الصنفين سدس ١٢ وسخا ٩٤، ولصفة عدد حبوب/السنبلة يليها محصول حبوب/ النبات ثم مساحة ورقة العلم في الصنف جميزة ٩. هذا وقد تباينت تقديرات معامل التوريث بالمعنى الواسع من متوسطة الى عالية بالنسبة لمحصول حبوب/ النبات والصفات المرتبطة بة. كانت تقديرات التحسين الوراثي عالية لصفة عدد الحبوب/ السنبلة وتراوحت من منخفضة الى متوسطة لباقى صفات المحصول الاخرى للتراكيب الطفرية المختلفة. التحليل العنقودى باستخدام متوسط الاداء لمحصول الحبوب/ النبات قسم تراكيب القمح الوراثية الى ثلاث مجاميع رئيسية عند درجة تشابة ٩٥ %. ومن المثير للاهتمام ان طفرات القمح الناتجة ذهبت فى مجاميع منفصلة عن الاباء غير المعاملة (سدس ١٢، سخا ٩٤ وجميزة ٩) وتؤكد تلك النتائج كفاءة المطفرات فى استحداث تغيرات وراثية.

الكلمات الافتتاحية:

قمح الخبز، المطفرات، التباين الوراثى، معامل التوريث، التحسين الوراثى والنحليل العنقودى

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