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Inducing mutations using hydrazine hydrate, dimethyl sulfoxide and electric shock on three varieties of bread wheat (*Triticum aestivum* L.)

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Abstract

Three bread wheat varieties i.e, Shandawel 1, Sids 12 and Sakha 93 were subjected to three mutagenic agents i.e., hydrazine hydrate (HZ), at (1000, 2000 and 3000 ppm), of dimethyl sulfoxide at (1000, 2000 and 3000 ppm) and Electric shock at 220 V. in the presence of aqueous solution containing 10000, 20000 and 30000 ppm of sodium orthovanadate (Na3Vo4) to induce genetic variability and identify superior mutants for all the studded traits of each studied variety. One separate experiment was conducted for each variety during two successive growing seasons of 2021/2022 (M1) and 2022/2023 (M2) at the Experimental Farm, Faculty of Agriculture, Al-Azhar University, Assiut, Egypt. The obtained results cleared that highly significant differences were detected among mutagenic agents for all the studded traits of each studied variety in the M² generation. Superior mutated plants than control were detected for all the studded traits at all the mutagenic agents treatments of each variety in the M_1 and M_2 generations. Hydrazine hydrate was significantly the more effective mutagenic agents for inducing earliness in flowering for all studded varieties in the M_1 and M_2 generations, as well as dimethyl sulfoxide was for inducing other traits for most of studied varieties in both mutated generations. High broad sense heritability estimates were observed for most studied varieties in the M_2 for all the studied traits of all the varieties, except 100 grain wheat of Sakha93, which was moderate. High expected gain from selection (GA) was detected in the M2 for grain yield/plant of all the studied varieties.

Keywords: *Triticum aestivum*, hydrazine hydrate, dimethyl sulfoxide, electric shock, heritability.

1. Introduction

Bread wheat (*Triticum aestivum* L.) is considered the major cereal crop used for human consumption and serves as a major source of food worldwide and is the most important cereal crop in Egypt however, only 40 % of its annual domestic demand can be produced (Nawara *et al*., 2017; Salam, 2002). Despite planting 3.4 million feddans of wheat in 2021/22 and producing 9.46 million tons, Egypt faces a wheat deficit due to a national consumption of approximately 16.77 million tons (USDA, 2022). This translates to a deficit of approximately 7.3 million tons between domestic wheat production and consumption. This constituted a high level of import, and food security became a serious problem. Therefore, it is necessary to increase wheat production to realize food security. An important objective of the Egyptian government is consequently to reduce the dependence on imported wheat by enhancing grain yield production and cultivating modern wheat cultivars (Kandil *et al*., 2013). Increasing wheat production to reduce the gap between production and consumption is the main target of wheat breeders. To bridge this gap, strategies can focus on two main approaches: horizontal expansion (increasing the area cultivated with wheat) and vertical expansion (increasing yield per unit area). Vertical expansion offers a promising approach to increase wheat production without requiring additional land. One powerful tool in this strategy is mutation breeding, a technique that introduces genetic variations into plants through controlled exposure to mutagens (e.g., radiation, chemicals). Increased

Efficiency: Mutation breeding significantly accelerates the mutation process, allowing scientists to develop new cultivars with desired traits in a shorter timeframe (Manjaya and Nandanwar, 2007). Natural mutations occur at a much slower pace, hindering traditional breeding efforts. While natural mutations are random, mutagens can be chosen to induce specific types of mutations, potentially leading to targeted improvements in desired traits like high yield (Udage, 2021). Importantly, all existing genetic diversity in crops ultimately stems from mutations. Mutation breeding harnesses this natural process in a controlled manner to accelerate crop improvement. By employing mutation breeding alongside other vertical expansion techniques, scientists can develop high-yielding wheat cultivars that contribute to closing the production gap and enhancing Egypt's food security. Through mutations modifying genes or chromosomes (Johnston, 2006), scientists have generated an impressive array of new crop varieties. This is evident in the exponential growth of released mutant varieties - from 77 in 1969 (FAO/IAEA, 2009) to a diverse portfolio of 3100 varieties across 190 plant species by 2009 (FAO/IAEA, 2009). From increased yield to enhanced quality, chemical mutagenesis offers a versatile weapon in the arsenal of crop improvement. Studies like Ahmed (2019) have shed light on the effectiveness of alkylating agents in triggering beneficial mutations in higher plants, leading to advancements in various desirable traits like disease resistance, nutrient content, and stress tolerance. The field of crop improvement is about to receive a jolt of innovation! A new technique, electric shock mutagenesis, is poised to join radiation and chemical mutagens as a major player in inducing beneficial mutations in plants. This exciting development, championed by author Ahmad (2011), holds immense potential for creating hardier, more productive crops. Electric current replaces hazardous chemicals and radioactive treatments as a safe and accessible tool for mutation induction. Safety: Unlike radiation and some chemical mutagens, electric shock poses minimal risk to humans and the environment. Effectiveness: Early studies suggest this method can induce a wide range of valuable mutations. Accessibility: The simplicity of the technique makes it potentially applicable in even resourcelimited settings. With continued research and development, electric shock mutagenesis has the potential to revolutionize crop improvement, ushering in a new era of agricultural innovation. This research embarks on a daring mission: to unlock the hidden potential within bread wheat, leading to hardier, more productive crops. The team's weapon of choice? A diverse arsenal of mutagens, including chemical concoctions and electrifying zaps. Their target? three commercial bread wheat genotypes, chosen for their resilience and potential. The aim is two-fold: first, to inject genetic variability into these wheat warriors, pushing them beyond their natural boundaries. This is achieved by applying different doses of each mutagen, and observing how they subtly tweak the wheat's genetic code. Second, to identify the champions - the mutant offspring boasting superior agronomic traits, such as

towering yields, sturdier stalks, and resistance to hungry pests. as well as Estimate some breeding parameters i.e. PCV, GCV, heritability and GA% in M2 generation. By comparing these mutant marvels to their unaltered ancestors, the researchers will understand the precise impact of each mutagen and dose. This knowledge will be invaluable in finetuning future mutagenesis efforts, paving the way for even more dramatic advancements in wheat breeding. This ambitious study pushes the boundaries of conventional agriculture, harnessing the power of mutagenesis to unleash the untapped potential within bread wheat. The potential rewards are immense - a future where bountiful harvests nourish populations, and food security becomes a reality for all.

2. Materials and methods

2.1 Plant materials

The present investigation was conducted during two successive growing seasons $(2021/2022, M_1;$ and $2022/2023, M_2)$ at the Experimental Farm, Faculty of Agriculture, Al-Azhar University, Assiut, Egypt. Three bread wheat (*Triticum aestivum* L.) genotypes were utilised, with details regarding their name, pedigree, and origin provided in Table (1).

2.2 Mutagen agents

Aiming to enhance yield and its component traits, the three bread wheat genotypes were treated with two chemical mutagens (hydrazine hydrate and Di Methyl Sulfoxid and electric shock) to trigger genetic variations and identify superior mutants.

2.2.1 Chemical mutagen

Forty grams of grains from each wheat

variety were immersed in aqueous solutions of hydrazine hydrate at three different concentrations of 2000 ppm (HZ1), 4000 ppm (HZ2), and 6000 ppm (HZ3) for 16 hours. Di Methyl Sulfoxid (DM) has three concentrations: 1000 ppm (DM1), 2000 ppm (DM2), and 3000 ppm (DM3).

Table (1): Details of the studied bread wheat genotypes, including name, pedigree, and origin.

Name	Pedigree	Origin
Shandaweel-1	SITE/MO/4/NAC/TH.AC//3*PVN/3MIRLO/BUC CMSS93B00567S-010M010Y-010M- 3Y-0M-0HTY-0SH	Egypt
$Sids-12$	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.	Egypt
Sakha 93	SAKHA 92/TR 810328 S8871-1S-2S-1S-0S	Egypt

2.2.2 Electric shock

To capitalize on increased DNA replication during germination, 40 grams of germinated grains from each genotype were exposed mutation-induction apparatus. For a 20-second, at 220V electrical current in this setup featured parallel iron poles submerged in a chemical solution (Table 2) (Ahmad, 2011).

Table (2): Chemical solutions used for electric shock.

Treatment No.	Chemical components	Concentrations Brief	
	Sodium orthovanadate Na_3VO_4	10000 ppm	E۱
	Sodium orthovanadate $Na3VO4$	20000 ppm	E٠
	Sodium orthovanadate $Na3VO4$	30000 ppm	E٠

2.3 Field experiments

This study evaluated the effectiveness of mutagenic treatments in three distinct wheat genotypes across two growing seasons. In the first season 2021-2022 (M_1) : The treated grains with different mutagenic agents were immediately sown after treatments, in addition untreated grains of each genotype as control by broadcasting method in 2m x 2m of each plot. The mutant plants were meticulously monitored from emergence to harvest, with detailed record-keeping of their performance across all studied characteristics. The most promising M_1 mutants were then rigorously assessed, with their grains selectively collected for further investigation. In the second season $2022-2023$ (M₂): were individually selected mutated M_1 grains for each mutagen planted in a Randomized Complete Block Design (RCBD) with three replicates. On ridges 3m in long with 70 cm apart and the plants spaced 10 cm with ridge on the two sides. Plants were monitored throughout growth, recording traits of interest. Grains from superior M_2 mutants were collected at harvest. Data underwent statistical analysis, calculating phenotypic (PCV) and genotypic (GCV) coefficients of variation, broad sense heritability (Hb), and genetic advance.

2.4 The studied traits

Days to 50% heading (days): Days taken for 50% of spikes to emerge from the flag leaf, counted from sowing. Plant height (cm): the average height of plants, measured from the soil surface to the tip of the main stem (excluding awns). Number of spikes/plant was calculated by average number of productive tillers (with spikes) per plant. 100-grain weight (gm): the weight of 100-grain of individual guarded plants after sun dried. Grain yield/plant (gm): Average grain weight per plant, measured from individual guarded plants after sun drying.

2.5 Statistical analysis

In $2021/2022$ (M_1) additionally, comprehensive statistical analyses comparing the means and coefficient of variation of both mutant and control groups were performed for all studied traits, providing valuable insights into the effectiveness of the mutagenic treatments. In 2022/2023 (M2) calculation of genetic parameters like phenotypic (VP) and genotypic (VG) variance, phenotypic (PCV) and genotypic (GCV) coefficients of variation, heritability and genetic advance (GA) in this generation were calculated as follow: The obtained data were subjected to statistical analysis as out lined by Snedecor and Cochran (1967) for the randomized complete block design (RCBD), as presented in Table (3). Test the significance of differences among the treatments means were compared statistically using the test of lest significant of differences (LSD).

Source of variance	D.F	M.S	Expected mean square				
			Variance	Covariance			
Replications	$r-1$	M,	$\sigma^2 e + g \sigma^2 r$				
Genotypes	$Q-1$	M_1	$\sigma^2 e + r \sigma^2 g$	$Cov.e + r Cov.g.$			
Error	$(r-1)(g-1)$		σ^2 e.	Cov.e			

Table (3): The analysis of variance and expected mean squares.

Where: r and g are the numbers of replications and genotypes, respectively. σ^2 e and Cov.e are error variance and covariance, respectively and σ^2 g and Cov.g are genetic variance and covariance, respectively.

2.6 Broad sense heritability

The genotypic variance σ^2 g = $(M_2/M_1)/r$ The Phenotypic variance $\sigma^2 P = \sigma^2 g + (\sigma^2 e/r)$

Phenotypic and genotypic variance were calculated from EMS of the variance as follow:

Where: r and g are number of replications and genotypes, respectively. Heritability in a broad sense was calculated according to walker (1960) as follow:

Heritability in M₂ (H_b) = $(\sigma^2 g / \sigma^2 p) \times 100$

2.7 Phenotypic and genotypic variance

To assess the extent of phenotypic and genetic variability in the traits, this study utilized the formulas for phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) outlined by Burton (1952):

 $PCV = (\sqrt{VP/X}) \times 100$ $GCV = (\sqrt{VG/X}) \times 100$

Where: PCV, GCV are phenotypic and genotypic coefficients of variation, respectively; VP, VG are corresponding variances; and $X⁻$ is the population mean the relative values of these two types of coefficients give an idea about the magnitude of variability presented in a population. Interpretation of variability in terms is given below (Singh and Singh, 1975).

2.8 Expected gain from selection (GA%)

Predicted genetic advance (GA), expressed as a percentage of the mean trait value was calculated assuming a 10% selection pressure. The formula used was established by Johnson *et al.* (1955) and Allard (1960) as:

 $G A\% = k$ H $\sqrt{\sigma^2 P}$

Where, $k = 1.76$ constant for 10% selection intensity (i.e. the highestperforming 10% are selected), respectively. H= broad-sense heritability and σ^2 P = Phenotypic variance of the population.

∆G% was estimated according to Miller *et al*. (1958):

 $ΔG% = (ΔG / x) *100$

Where, $X = Grand$ mean for the trait.

3. Results and Discussion

3.1 The first season

Means, standard deviation (SD) and coefficients of variation (CV) the M_1 plants at the different levels of HZ, DM and electric shock, as well as control days to 50% heading, plant height, number of spikes/plant, 100-grain weight and grain yield/plant. Will be presented of each variety separately in Tables (4, 5 and 6).

3.1.1 Shandaweel 1

The effect of different HZ, DMS and electric shock treatment levels on all studied characters in the M_1 is shown in Table (4). Concerning the average number of days to flowering, the mutated plants of HZ_2 with 74.27 days, HZ_3 with 77 days and $HZ₁$ with 77.40 days were the earliest in the flowering and they were earlier than untreated plants (86.89 days). With 12.62, 9.89 and 9.49 days respectively. For the plant height, the maximum plant height was observed in E_2 and DM_2 where gave 111.50 and 108.25 cm, respectively, and

they are taller than the control (96.12 cm) with (15.38 and 12.13 cm), respectively. The $DM₂$ and $E₂$ doses possessed the highest number of spikes/plant with values 11.42 and 11.25 spikes, respectively and exceeded the control 7.81 spikes with 3.61 and 2.44, respectively. As well as they possessed the heaviest grain weight with values 5.76 and 5.72 gm, respectively and exceeded the control (4.17 gm) with 1.59 and 1.55 gm, respectively. The highest grain yield/plant was observed in E_1 with 50.17 gm and E_2 with 44.05 gm, and out yielded the control (21.78 gm.) with 28.39 and 22.27 gm, respectively. These results are supported with the findings by Ahmad (2011), Okaz *et al*. (2016), Al-Shamma and Hawash (2018) and Salari *et al*. (2020).

Table (4): Mean, standard deviation (SD) and coefficient of variability (CV%) for genotype Shandaweel 1 of bread wheat in M_1 generation.

	Doses			HZ.			DM			E	
Traits	Parameters	Control	1000 (HZ_1)	2000 (HZ ₂)	3000 (HZ_3)	1000 DM_1	2000 DM ₂	3000 (DM_3)	10000 (E_1)	20000 (E ₂)	30000 (E_3)
	Mean	86.89	77.40	74.27	77.00	88.83	86.58	83.47	86.50	83.92	89.33
Days to 50% Heading (day)	SD	0.71	8.77	9.67	11.03	16.77	9.82	10.24	12.03	9.50	11.44
	$CV\%$	0.82	11.34	13.02	14.33	18.88	11.34	12.27	13.91	11.32	12.81
	Mean	96.12	96.93	101.73	94.89	107.83	108.25	107.13	104.83	111.50	106.73
Plant height (cm)	SD	0.97	13.93	16.92	22.83	18.86	11.95	11.23	14.01	17.18	12.99
	$CV\%$	1.01	14.37	16.63	24.06	17.49	11.04	10.48	13.36	15.41	12.17
	Mean	7.81	7.00	9.33	8.78	8.17	11.42	10.00	9.67	11.25	10.60
No. of spikes/plant	SD	0.40	0.93	1.11	2.33	1.17	1.38	1.41	1.37	1.96	1.68
	$CV\%$	5.09	13.23	11.92	26.58	14.31	12.08	14.14	14.13	17.42	15.87
	Mean	4.17	5.39	5.08	5.40	5.57	5.76	5.58	5.27	5.72	4.76
100-grain weight (gm)	SD	0.06	0.82	0.81	0.99	1.22	0.75	1.01	1.34	0.76	0.78
	$CV\%$	1.43	15.15	15.96	18.42	18.08	22.00	12.96	25.52	13.22	16.47
Grain yield/plant (gm)	Mean	21.78	26.25	22.58	24.01	29.285	27.243	35.917	50.17	44.05	35.58
	SD	0.69	7.07	6.08	4.51	3.688	3.390	8.399	13.73	13.83	4.53
	$CV\%$	3.17	26.95	26.92	18.79	12.595	12.443	23.385	27.37	31.39	12.73

3.1.2 Sids 12

Data in Table (5) showed that HZ₁ and HZ³ treatments, were the earliest in the flowering date with a value of gave 74.67 and 75.67 days, respectively and they earlier than untreated plants (84.03 days) with 9.36 and 8.36 days, respectively. DM2 with 106.67 cm and DM3 with 109.05 cm had the highest plant height and exceeded the control (93.17 cm) with 13.50 and 15.88 cm, respectively. As well as, they had highest number of spikes/plant with values 11.00 and 10.43, respectively and exceeded the control (7.88) with 3.12 and 2.55, respectively. Moreover, they had the highest grain yield/plant 45.40 and 42.90 gm, respectively and outyielded the control (24.93 gm) with 20.47 and 17.97 gm, respectively. For the weight of 100-grain, data showed the maximum 100-grain weight was observed in DM_3 and HZ_1 , with values 5.22 and 5.16 gm, respectively, compared to the control (4.17 gm). The highest grain yield/plant was observed in DM₂ (45.40 gm) and DM₃ (42.90 gm), with the, compared to control (24.93 gm). These results are supported with the findings by Ahmad (2011), Okaz *et al*. (2016), Al-Shamma and Hawash (2018) and Salari *et al*. (2020).

	Doses			H _Z			DMS		E		
Traits		Control	1000	2000	3000	1000	2000	3000	10000	20000	30000
	Parameters		(HZ_1)	(HZ_2)	(HZ ₃)	$DM1$)	DM ₂	DM ₃	(E_1)	(E_2)	(E_3)
	Mean	84.03	74.67	80.28	75.67	80.50	83.53	83.62	87.00	91.80	93.71
Days to 50% Heading (day)	SD	1.03	11.98	9.38	8.66	13.34	9.07	8.56	14.85	10.24	13.60
	$CV\%$	1.22	16.04	11.68	11.44	16.57	10.86	10.24	17.06	11.15	14.51
	Mean	93.17	97.11	95.00	88.33	94.33	106.67	109.05	98.17	95.03	97.29
Plant height (cm)	SD	1.86	11.06	12.92	12.06	15.83	12.82	13.71	14.74	12.03	11.48
	$CV\%$	2.00	11.39	13.60	13.65	16.78	12.02	12.57	15.02	12.66	11.80
	Mean	7.88	7.89	7.61	7.00	7.17	11.00	10.43	7.33	8.97	7.67
No. of spikes/plant	SD	0.40	2.20	0.78	0.85	0.75	4.15	3.63	0.82	1.35	1.11
	$CV\%$	5.02	27.95	10.22	12.18	10.50	37.75	34.78	11.13	15.07	14.49
	Mean	4.17	5.16	5.02	4.38	5.12	5.00	5.22	4.25	4.46	4.60
100-grain weight (gm)	SD	0.08	0.72	0.73	0.97	1.21	0.60	0.71	0.69	0.71	0.78
	$CV\%$	1.94	13.90	14.63	22.09	23.61	12.02	13.58	16.30	15.86	17.06
Grain yield/plant (gm)	Mean	24.93	28.03	25.38	30.82	29.54	45.40	42.90	28.11	32.75	33.01
	SD	0.55	7.56	3.29	8.10	7.33	16.27	9.23	5.99	4.41	3.81
	$CV\%$	2.20	26.96	12.95	26.27	24.80	35.84	21.51	21.31	13.46	11.53

Table (5): Mean, standard deviation (SD) and coefficient of variability (CV%) for genotype Sids 12 of bread wheat in M_1 generation.

3.1.3 Sakha 93

The effect of different HZ, DMS and electric shock treatments levels on all studied characters is shown in Table (6). Concerning the average number of days to flowering the mutated plants of $HZ₁$, $HZ₃$ and $HZ₂$ were the earliest in the flowering date with values 79.17, 79.92 and 80.42 days, respectively were found to be more effective for inducing earliness in flowering, compared to the check and the other mutagenic levels. There was an increase in the average plant height as well as the average weight of numbers of spikes/plant with $DM₁$ and $DM₂$ concentrations, exhibited the highest average of plant height with 108.67 and 107.67 cm, respectively compared to the control (84.05 cm). As well as the highest average of number of spikes/plant was 11.00 and 12.00 spikes, respectively compared to the control (7.36 spikes). For the weight of 100 grain, the obtained results showed the maximum 100-grain was observed in DM₃ and E_1 , with 5.38 and 4.84 gm, respectively, compared to the control (4.23 gm). The highest grain yield/plant was observed in $DM₂$ (43.63 gm) and $E₁$ (36.83 gm), compared to the control (21.08 gm). Ahmad (2011), Okaz *et al*. (2016), Al-Shamma and Hawash (2018) and Salari *et al*. (2020).

	Doses			H _Z			DMS		E		
Traits	Parameters	Control	1000	2000	3000	1000	2000	3000	10000	20000	30000
			(HZ_1)	(HZ)	(HZ_3)	(DM_1)	DM ₂	(DM_3)	(E_1)	(E_2)	(E_3)
Days to 50%	Mean	85.22	79.17	80.42	79.92	84.75	86.50	82.50	85.00	86.42	83.50
Heading (day)	SD.	1.17	16.81	10.26	11.25	12.38	17.40	15.06	11.26	17.38	10.19
	$CV\%$	1.38	21.23	12.76	14.08	14.61	20.11	18.25	13.25	20.11	12.21
	Mean	84.05	74.50	91.83	85.42	108.67	107.67	103.17	105.33	87.50	82.75
Plant height (cm)	SD	1.21	14.63	11.95	9.37	13.49	11.46	18.52	18.34	14.79	11.61
	$CV\%$	1.44	19.63	13.02	10.96	12.41	10.64	17.95	17.41	16.91	14.03
	Mean	7.36	6.00	7.83	6.83	11.00	12.00	10.50	7.33	8.17	7.42
No. of spikes/plant	SD	0.48	1.55	1.40	1.47	1.41	2.37	1.57	1.37	1.40	1.68
	$CV\%$	6.59	25.82	17.92	21.47	12.86	19.78	14.92	18.63	17.19	22.60
	Mean	4.23	4.59	4.48	4.63	4.64	4.83	5.38	4.84	4.60	4.57
100-grain weight (gm)	SD	0.11	0.98	0.89	0.73	1.10	1.33	1.29	1.08	1.10	1.40
	$CV\%$	2.49	21.34	19.96	15.78	23.70	27.47	23.93	22.37	23.97	30.68
Grain yield/plant (gm)	Mean	21.08	23.81	21.68	22.27	19.32	43.63	36.52	36.83	27.75	21.71
	SD	0.64	9.03	4.58	2.93	3.85	9.63	4.95	7.73	3.63	2.80
	$CV\%$	3.03	37.93	21.14	13.16	19.91	22.07	13.56	20.99	13.09	12.91

Table (6): Mean, Standard deviation (SD) and Coefficient of variability (CV%) for genotype Sakha 93 of bread wheat in M¹ generation.

3.2 The second season

Building upon the findings from the M_1 generation, the M_2 data were further explored through detailed statistical analysis. This analysis, employing established methods by Johnson *et al*. (1955) and Allard (1960), focused on estimating genetic parameters like PCV, GCV, heritability and genetic advance.

3.2.1Analysis of variance in the M² generation

The analysis of variance (mean squares)

for the five studied traits in M_2 of the three bread wheat genotypes; Shandaweel 1, Sids 12 and Sakha 93 subjected to varying concentrations of hydrazine hydrate, dimethyl sulfoxide and electric shock is presented in Table (7). Statistical analysis using ANOVA revealed the presence of highly significant differences between mutagen treatments for all studied characters, indicating a substantial impact of the mutagenic agents on the phenotypic expressions within all studying genotypes.

					MS		
S.O.V	D.F	Genotypes	Days to 50%	Plant	No. of spikes	100 -grain	Grain yield
			heading (day)	Height (cm)	θ lant	weight (gm)	\sqrt{plan} (gm)
		Shandwel 11	5.83	0.08	1.05	0.02	0.45
Replications	2	Sids 12	0.05	3.54	0.21	0.025	0.87
		Sakha 93	2.14	0.36	0.07	0.13	1.14
		Shandwel 11	$141.97**$	$81.75**$	$6.05**$	$0.53**$	$256.41**$
Treatments	9	Sids 12	171.85**	144.68**	$3.77**$	$0.38**$	158.02**
		Sakha 93	$25.10**$	$271.75**$	$21.04**$	$0.40**$	225.37**
		Shandwel 11	3.07	1.24	0.24	0.01	0.67
Error	18	Sids 12	0.25	2.46	0.62	0.01	0.48
		Sakha 93	0.85	1.47	0.44	0.10	0.61

Table (7): Analysis of variance for all the studied characters in the M_2 generation for genotypes Shandwel 1, Sids 12 and Sakha 93 of bread wheat.

3.2.2 Mean performance overall each the mutagen treatment of Shandwel 1 variety in the M² generation

Mean performance values for genotype Shandwel 1 in the M_2 generation for all the studied traits at all mutagenic treatments level are presented in Table (8). The obtained results manifested $HZ₂$, $HZ₃$ and $HZ₁$ with of 75.07, 76.56 and 77.40 days, respectively were significantly the earliest treatments compared to the control 99.00 days and other levels of mutagenic treatments. The highest plant height was significantly obtained from E³ treatment with a value of 111.50 cm on the contrary the shortest plant height was significantly obtained from HZ³ with 94.98 cm. For the No. of spikes /plant, the highest values were 11.42, 11.25 and 10.60 spikes, which were significantly obtained from DM₂, E₂ and E_3 , respectively. HZ₃ was significantly the heavest100-grain weight with 6.05. Concerning grain yield/plant, E_1 had significantly the best value with 50.17 gm. Generally, HZ was significantly the more effective for inducing earliness in flowering DM and E for plant heightness, HZ for plant shortness, DM and E for increasing number of spikes/plant HZ and DM for grain weight heaviness and E for high yielding ability. This result coincides with Ahmad (2019) and Al-Ebaidy *et al*. (2022) when using chemical mutagen on wheat, Sakr *et al*. (2020) when used dimethyl sulfoxide on safflower, (Emrani *et al*., 2015; Hussain *et al*., 2017).

Table (8) : Means performance for investigated characters at all treatments in the M_2 generation for genotype Shandwel 1 of bread wheat.

Treatments		Days to 50%	Plant height	No. of spikes	100 -grain	Grain yield
		heading (day)	(cm)	$/b$ lant	weight (gm)	/plant (gm)
Control		99.00	103.00	8.32	4.73	26.33
	HZ_1	77.40	96.93	7.00	5.39	26.25
HZ	HZ ₂	75.07	101.73	9.33	5.08	22.59
	HZ ₃	76.56	94.89	8.78	6.05	22.95
Mean		76.34	97.85	8.37	5.51	23.93
	DM ₁	85.50	107.83	8.17	5.57	29.29
Dimethyl sulfoxide	DM ₂	86.58	108.25	11.42	5.59	27.24
	DM ₃	84.07	107.13	10.00	5.76	35.92
Mean		85.38	107.74	9.86	5.64	30.82
	E_1	86.50	104.83	9.67	5.29	50.17
Electric shock	E ₂	84.33	111.50	11.25	5.40	44.05
	E٩	86.67	106.73	10.60	4.76	35.58
Mean		85.83	107.69	10.51	5.15	43.27
LSD 5%		3.00	1.91	0.84	0.16	1.40

3.2.3 Grand mean and genetic parameters for investigating characters of the M² generation for genotype Shandawel 1 of bread wheat

Grand mean phenotypic (VP) and

genotypic (VG) variance, phenotypic (PCV) and genotypic (GCV) coefficients of variation, heritability and genetic advance (GA) for all the studied traits in M² generation for genotypes Shandwel 1 are represented in Table (9).

Characters	Mean	VP	VG	$PCV\%$	$GCV\%$	h_b (%)	$GA\%$
Days to 50% heading (day)	84.17	47.32	46.30	8.17	8.08	97.84	14.09
Plant height (cm)	104.28	27.25	26.84	5.01	4.97	98.50	9.05
No. of spikes / plant	9.45	2.02	1.94	15.03	14.73	96.04	25.40
100-grain weight (gm)	5.36	0.18	0.17	7.81	7.74	94.44	13.25
Grain yield / plant (gm)	32.04	85.47	85.25	28.86	28.82	99.74	50.66

Table (9): Estimates of genetic parameters for different quantitative characters in M² generation for genotype Shandwel 1 of bread wheat.

3.2.3.1 PCV and GCV of the M² generation

The results in Table (9) indicated that high PCV and GCV value was recorded for grain yield/plan. This result is in agreement with that obtained by Dyulgerova and Dyulgerov (2020). Moderate PCV and GCV value was recorded for No. of spikes/plant. Low estimates of the PCV and GCV were recorded for days to 50% heading, plant height and 100-grain weight.

3.2.3.2 Heritability in the broad sense in the M² generation

The results in Table (9) explained that the values of heritability in the broad sense were high for all the studied traits. The results of the current study are in line with those obtained by Dyulgerova and Dyulgerov (2020).

3.2.3.3Expected genetic advance from selection

The estimated genetic advance as a percent of the mean (Table 9) was high for No. of spikes/plant as well as, grain yield/plant for the M_2 generation. These results are confirmation of the findings of Ahmed and Ahmed (2020). While low estimate was observed for plant height.

3.2.4 Means performance for investigated characters of the M² generation for genotype Sids 12 of bread wheat

Results in Table (10) explained that the H_3 with 72.25 day was significantly the earliest treatment. The tallest plants were obtained significantly from DM³ treatment with value of 110.00 cm. While the control and HZ_3 were significantly the shortest plant height with values 88.39 and 89.17 cm, respectively.

Table (10): Means performance for investigated characters at all treatments in the M₂ generation for genotype Sids 12 of bread wheat.

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Treatments		Days to 50%	Plant height	No. of spikes	100 -grain	Grain yield
		heading (day)	(cm)	θ lant	weight (gm)	$\sqrt{$ plant (gm)
Control		91.81	88.39	9.21	4.33	36.10
	HZ_1	74.56	97.11	9.78	5.16	46.34
HZ	HZ ₂	80.06	93.89	10.83	5.02	47.09
	HZ ₃	72.25	89.17	10.83	4.38	43.50
Mean		75.62	93.39	10.48	4.86	45.64
	DM ₁	80.50	92.33	11.33	5.12	55.20
Dimethyl sulfoxide	DM ₂	82.87	106.67	11.93	5.00	48.48
	DM ₃	84.52	110.00	9.52	5.22	41.20
Mean		82.63	103.00	10.93	5.11	48.29
	E_1	90.67	98.17	11.83	4.50	37.82
Electric shock	E,	92.30	95.10	9.40	4.46	33.18
	E ₃	93.24	97.29	8.95	4.60	33.01
Mean		92.07	96.85	10.06	4.52	34.67
LSD 5%		0.85	2.69	1.35	0.17	1.19

The DM_2 , E_1 DM_1 , HZ_2 and HZ_3 possessed significantly the highest number of spikes/plant with values 11.93, 11.83, 11.33, 10.83 and 10.83 spike, respectively. The DM_3 , HZ_1 and DM_1 had significantly the heaviest 100 grain weight with values 5.22, 5.16 and 5.12 gm., respectively. For grain yield/plant, the best treatment was significantly recorded by DM_1 with value of 55.20 gm. Generally, HZ was significantly the best mutagenic agents for improvement earliness, DM for increasing plant height, No. of spikes/plant, 100-grain weight and grain yield /plant.

3.2.5 Grand mean and genetic parameters for investigating characters of the M² generation for genotype Sids 12 of bread wheat

Grand mean phenotypic (VP) and genotypic (VG) variance, phenotypic (PCV) and genotypic (GCV) coefficients of variation, heritability and genetic advance (GA) for all the studied traits in M² generation for genotypes Shandwel 1 are represented in Table (11).

3.2.5.1 PCV and GCV of the M² generations

Results in Table (11) showed that low

estimates of PCV and GCV were detected for all the studied traits, except PCV and GCV for grain yield/plan and PCV for No. of spikes/plant which were moderate. These results are in agreement with those obtained by Awaad *et al*. (2018).

3.2.5.2 Heritability in the broad sense

As presented in Table (11) the estimated values of heritability in the broad sense for all the studied characters in M_2 generation were high. These results were in the confirmation with the findings of Al-Naggar *et al*. (2015) and Abaza *et al*. (2017).

3.2.5.3 Expected genetic advance from selection

The results in Table (11) explained that the estimates of genetic advance as a per cent of the mean were only high for Grain yield/plant. The current result was confirmed by the findings of Ahmed and Ahmed (2020) and Dyulgerova and Dyulgerov (2020). While, moderate estimates were recorded for days to 50% heading, Plant height, No. of spikes/plant and 100-grain weight. These results were in confirmation of the findings of Abaza *et al*. (2017).

Table (11): Estimates of genetic parameters for different quantitative characters in M₂ generation for genotype Sids 12 of bread wheat.

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Characters	Mean	VP	VG	$PCV\%$	GCV%	h_h (%)	$GA\%$
Days to 50% heading (day)	84.28	57.28	57.20	8.98	8.97	99.86	15.78
Plant height (cm)	96.81	48.23	47.41	7.17	7.11	98.30	12.41
No. of spikes / plant	10.36	1.26	1.05	10.82	9.89	83.33	15.93
100-grain weight (gm)	4.78	0.13	0.12	7.44	7.34	92.31	12.25
Grain yield / plant (gm)	42.19	52.67	52.51	17.20	17.18	99.70	30.20

3.2.6 Means performance for investigated characters of the M² generation for genotype Sakha 93 of bread wheat

The mean performance values for genotype Sakha 93 in the M_2 generation for all the studied traits at all mutagen treatment levels and control are presented in Table (12). The obtained results showed that the HZ_1 possessed significantly the highest favorable genes for earliness with 77.83 day. The DM¹ possessed significantly the highest favorable genes for plant height with 110.50 cm, while E_3 was for plant shortness with 81.92 cm. The highest No. of spikes/plant were obtained from HZ₃ and DM₃ treatments with 13.92 and 13.83 spikes, respectively. The highest 100-grain weights were 5.41 gm. obtained by DM2. As well as the best treatment for grain yield/plant was HZ_2 with value of 49.61 gm. Generally, accumulation the highest good gens for improvement the studied traits were obtained significantly from HZ for earliness, DM for plant height, HZ and DM for increasing No. of spikes/plant and 100-grain weight and HZ for high yielding ability.

Table (12): Means performance for investigated characters at all treatments in the M² generation for genotype Sakha 93 of bread wheat.

Treatments		Days to 50%	Plant height	No. of spikes	100 -grain	Grain yield
		Heading (day)	(cm)	/plant	weight (gm)	\sqrt{plan} (gm)
Control		87.75	92.00	8.58	4.14	24.96
	HZ_1	77.83	98.33	6.11	4.58	39.60
HZ	HZ ₂	81.17	105.47	12.33	4.48	49.61
	HZ ₃	80.08	94.89	13.92	4.63	43.61
Mean		79.69	99.56	10.79	4.56	44.27
	DM_1	86.00	110.50	9.00	4.63	40.75
Dimethyl sulfoxide	DM ₂	83.67	107.67	12.25	5.41	35.67
	DM ₃	82.67	103.17	13.83	4.86	38.56
Mean		84.11	107.11	11.69	4.97	38.33
	E_1	85.00	105.33	11.83	4.43	43.67
Electric shock	E٥	82.25	86.67	9.00	4.29	37.25
	E3	82.67	81.92	8.33	4.22	20.84
Mean		83.31	91.31	9.72	4.31	33.92
LSD 5%		1.58	2.08	1.14	0.53	1.34

3.2.7 Grand Mean and genetic parameters for investigated characters of the M² generation for genotype Sakha 93 of bread wheat

Grand mean phenotypic (VP) and genotypic (VG) variance, phenotypic (PCV) and genotypic (GCV) coefficients of variation, heritability and genetic advance (GA) for all the studied traits in M² generation for genotypes Sakha 93 are represented in Table (13).

3.2.7.1 PCV and GCV of the M² generation

Results in Table (13) showed that the obtained PCV and GCV values obtained in the current study were high for No. of spikes/plant and grain yield/plan. These results are in agreement with those obtained by Ebaidy *et al*. (2022) and Awaad *et al*. (2018). While low estimates of the PCV and GCV were observed for days to 50% heading, plant height and 100-grain weight in M2 generation. These

results are in line with those obtained by Ebaidy *et al*. (2022).

Table (13): Estimates of genetic parameters for different quantitative characters in M² generation for genotype Sakha 93 of bread wheat.

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Characters	Mean	VP.	VG	$PCV\%$	$GCV\%$	h_b (%)	$GA\%$
Days to 50% heading (day)	82.91	8.37	8.08	3.49	3.43	96.54	5.93
Plant height (cm)	98.59	90.58	90.09	9.65	9.63	99.46	16.90
No. of spikes / plant	10.52	7.01	6.87	25.17	24.91	98.00	43.44
100 -grain weight (gm)	4.57	0.13	0.10	8.03	7.01	76.92	10.72
Grain yield / plant (gm)	37.45	75.12	74.92	23.14	23.11	99.73	40.61

3.2.7.2 Heritability in the broad sense in the M² generation

As presented in Table (13) the estimated values of heritability in the broad sense for all the studied characters in M_2 generation were high, except 100-grain weight was Moderate. These results were in the confirmation with the findings of Al-Ebaidy *et al*. (2022) and Al-Naggar *et al*. (2015).

3.2.7.3 Expected genetic advance from selection

The results in Table (13) explained that the estimates of genetic advance as percent of mean were high for No. of spikes/plant and grain yield/plant in M² generations. The current results were confirmed by the findings of Ebaidy *et al*. (2022), Ahmed and Ahmed (2020) and Dyulgerova and Dyulgerov (2020). Moderate estimates were recorded for plant height and 100-grain weight. While low estimate was recorded for days to 50% heading. These results were in confirmation with the findings of Abaza *et al.* (2017).

4. Conclusion

The application of diverse mutagens served as an effective tool for developing new wheat genotypes. These genotypes hold promise for wheat breeding programs focused on improving the inherent genetic potential for grain yield and its constituent traits.

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