Effect of some mutagens for induced mutation and detected variation by SSR marker in bread wheat (*Triticum aestivum* L.)

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**Abstract**

Two chemical mutagens sodium azid (SA) and hydrazine hydrate (HZ) were used to induce genetic variability for improving some morphological and agronomical traits in three bread wheat genotypes namely, Sids1, Sids12 and Giza168. Some mutations were selected in M1 generation plants *i.e.*, three for Sids1 variety (S1 29, S1 49, and S1 75), four for Sids12 variety *i.e.*, (S12 116, S12 161, S12 168 and Sids12 177) and one for Giza168 (G168 202). Selected genotypes were grown in field experiment to obtain M2 generation. Results showed that most agronomical traits were significantly increased in M2 than M1 generation plants. The highest values in selected mutated plants for 100 grain weight were 6.31 gm for S12 161 plant, 5.79 gm for G168 202 plant and 5.55 gm for S1 29 plant. Genetic variation in M2 plants were used of evaluated by SSR molecular markers. The results showed representable the variation between treated genotypes and untreated (control) for all studied traits.

**Keywords**: bread wheat, sodium azid, hydrazine hydrate, SSR marker, mutation.
1. Introduction

Wheat (*Triticum aestivum* L.) belongs to the grass family Poaceae and considered one of the most important food crops in the world, with genome size (~17.6 GB) (Hussain *et al.*, 2018; Mohamed *et al.*, 2018; Nielsen *et al.*, 2014). Bread wheat is an allohexaploid species (2n=6x=42) (Babben *et al.*, 2018). In Egypt, wheat is one of the oldest and most important cereal crops (Al-Naggar *et al.*, 2015). Physical and chemical mutagens have been induced for various plant characters in variety of crops including wheat (Singh and Balyan, 2009). Induction of mutation in crop plants contribute by increasing genetic variability and enrich plant germplasm for direct selection and cross-breeding. Induction of mutation has been applied to produce mutant plants vary by changing the plant characteristics for a significant increase in production and improve quality (Nazarenko, 2016). Sodium azide (SA) used with seeds to create mutation. Sodium azide is a very potent mutagen in barley and induced chlorophyll deficiency as well as a wide range of morphological and physiological variation (Pande and Khetmalas, 2012). Also, hydrazine hydrate (HZ) was used to induce mutations (Laskar and Khan, 2017). Chlorophyll mutations induced by ethylmethane sulphonate (EMS) - an alkylating agent, hydrazine hydrate (HZ) - a base analogue and sodium azide (SA) (Wani, 2017). Molecular markers have been proved valuable tools in the characterization and evaluation of genetic diversity within and between species and populations (Sadigova *et al.*, 2014). Assessment of genetic diversity, increasing the efficiency of selection for both qualitative and quantitative traits can be achieved with DNA marker (El-Sherbeny *et al.*, 2020; Farhan *et al.*, 2019). Sequence Tagged Sites (STS), and Random Amplified Microsatellite Polymorphism (RAMP) (Altintas *et al.*, 2008; Ercan *et al.*, 2010). Several PCR based molecular markers are available for investigation of genetic diversity. SSR, RAPD, AFLP and ISSR (Singh and Singh, 2018). This study aims to use chemical mutagens to induced morphological and agronomical variations in three widely different varieties of bread wheat and to use simple sequence repeats (SSR) molecular markers to detect the variation between treated varieties and their untreated varieties (control) for all studied traits.

2. Materials and methods

2.1 Field materials

Three genotypes of bread wheat were used in this study namely Sids1, Sids12 and Giza168. The pedigree and origin of these genotypes are shown in Table (1). In season 2018/2019, dry seeds (~200 grain/treatment) were soaked into distilled water for 16 h then soaked in three concentrations of both sodium azid (SA) and hydrazine hydrate (HZ) i.e., (0.0, 0.01, 0.02 and 0.03 %) for 16 h. Treated seeds were washed in distilled water for 2 h. Grains from each of the three genotypes and treated seeds were sown in soil. The experimental plot consisted of five rows 2.5 m long and 20 cm apart. A field trial to obtain M1 generation. In season 2019/2020, selected eight mutant plants...
genotypes were and sown in the Faculty, Al-Azhar University, Assuit, Experimental Farm of Agriculture Egypt to obtain M₂ generation.

| Name   | Pedigree                        | Origin   |
|--------|--------------------------------|----------|
| Sids₁  | MRL/BUC/SER1                    | Egypt    |
| Sids₁₂ | BUC/7C/ALD/S/MAYA74/0N//1160    | Egypt    |
|        | Giza//CM 930 46-8M-OM-2Y-OB-OGZ | Egypt    |

2.2 Genomic DNA isolation and SSR analysis

Genomic DNA of wheat was extracted from the young leaves by CTAB methods (Doyle and Doyle, 1987). Seven SSR primer combinations were used (Table 2). For each primer combinations, 25 μL PCR reaction contained 5 μL buffer (5x), 1.5 μL of genomic DNA (30 ng), 2 μL of 25 mM of MgCl₂, 0.5 μL of 10 mM dNTPs and 0.15 μL of Taq DNA polymerase. PCR amplifications for SSR analysis were performed in Applied Biosystems 2720 thermal cycler system, with initial denaturation at 94°C for 5 minutes followed by 40 cycles, each consisted of denaturation at 94°C for 50 seconds, annealing at 52°C for 1 minute, extension at 72°C for 1 minute, with final extension at 72°C for 7 minutes. PCR products were separated on 1.5% agarose gels, stained with ethidium bromide, and visualized on UV transilluminator. The gel was photographed using bio-print camera.

| Code   | Sequences (5-3)                      | References       |
|--------|-------------------------------------|------------------|
| Xgwm 99| F- AAAGATGAGCTATGCACTACAGA          | Ateş Sönmezőoğlu and Terzi (2018) |
|        | R- GCATATGGATGACGACATA              |                  |
| Xgwm 186| F- GCAGGCCTGGTTCAAAAAAG            |                  |
|        | R- CCCTCTCTAGCGAGAGCTATG            |                  |
| Xgwm 337| F- CCTCTTCTCCCTCACCTAGC           |                  |
|        | R- TGCTAATGGGCTTTTGGCC             |                  |
| Xgwm 357| F- TATGCTCAAAGTTTGACCTCG          |                  |
|        | R- AGGCTGCAGCTCTCTCTCAG             |                  |
| Xgwm 484| F- ACATCGCTCTCACAACCC              |                  |
|        | R- AGTCTGCCGCTGAGCAGTGGCTAGG       |                  |
| Xgwm 626| F- GATCTAAATGTTATTTTCTTC           |                  |
|        | R- TGACATATAGCTGACGTTGG            |                  |
| Xp45 3200| F- GTTCTGAGACATCTACGGATG          |                  |
|        | R- GAGAATAGCTGTTGTTG               |                  |

F= forward, R= reverse.
2.1 Statistical analysis

The experimental design was Randomized Complete Blocks Design (RCBD). The analysis of variance (ANOVA) and Duncan multiple range testes at 5% level of probability were used to test the significant of differences between the treatments. Statistical analyses of data were performed using Costat software (Steel and Torrie, 1986). Gel images detected via PCR-based methods were analyzed using the free software Gel Analyzer 3 which is available at http://www.geocities.com/egygene (Gel Analyzer Version 3, 2007).

3. Results and Discussion

3.1 Mean performance of the three genotypes in first mutagenicity (M₁) and second mutagenicity (M₂) generations for the studied traits

3.1.1 Sids₁

The presented results in Table (3) showed that means of the studied traits i.e., plant height, number of spikes/plant, spike length, number of spikelets /spike, number of grains / spikelets and 100 grain weight in M₁ and M₂ generations of Sids₁ genotype and its mutant selected plants i.e., S₁ 29, S₁ 42 and S₁ 75 plants. Results showed that some mutations in Sids₁ genotype increased in M₂ than M₁ generations. The highest value in plant height was 121.6 cm which obtained with 0.03% HZ for mutated plant S₁ 75 as compared to untreated plants (control) with 104.6 cm. While the highest value in number of spikes /plant was 58.7 obtained with 0.02% SA for mutated plant S₁ 29 as compared to original plants (control) with 12.9 spike. The highest value of spike length was 19.1 cm obtained at 0.02% SA for mutated plant S₁ 29 as compared to untreated plants (control) with 13.5 cm. The highest value of number of spikelets /spike was 26 obtained at both 0.02% SA and 0.03% HZ for mutated plants S₁ 29 and S₁ 75 plants as compared to untreated plants (control) with 21.6 spikelet. The results (Table 3) showed that means of both number of grains /spikelet and 100-grain weight were lower in M₂ than M₁ generation. The best reduced values in M₂ generation were 4.9 and 5.55 obtained with 0.02% SA for mutant plant S₁ 29 as compared to 6.0 and 5.0 gm. in M₁ generation for number of grains/spikelet and 100-grain weight, respectively. Finally, the data in Table (3) showed that significant differences among the different concentrations of two chemical mutagens (SA and HZ) in Sids₁ genotype in both M₁ and M₂ generations for all studied traits. These results agree with those reported by Saad et al. (2010), Beche et al. (2013) and Haridy and Abd El-Zaher (2015). They found that the average of 100 grain weight of treated plants increased significantly. Khursheed et al. (2015) found that mean values of 100 grain weight recorded higher in M₂ generations than M₁ generation, and the average number of spikes per plant increased from 2.66 to 4.33 as a result to treat grains with 0.02% HZ.
Table (3): Mean performance of Sids₁ genotype and the mutated selected plants at M₁ and M₂ generations for all studied traits in 2018/19 and 2019/20 seasons.

| Genotypes | Treatments | Plant height | Number of spikes/plant | Spike length | Number of spikelet/spike | Number of grains/spikelet | 100-grain weight |
|-----------|------------|--------------|------------------------|-------------|--------------------------|--------------------------|------------------|
| Sids₁     | Control    | 103.0        | 13.0                   | 14.0        | 22.0                     | 3.0                      | 5.3              |
|           | M₁         | 104.6±10.11  | 12.9±1.6 c             | 13.5±1.40 c | 21.6±1.26 c              | 3.9±0.74 b              | 5.0±0.13 c       |
| S₁ (29)   | 0.02 SA    | 105.0        | 59.0                   | 18.0        | 24.0                     | 6.0                      | 6.0              |
|           | M₁         | 113.8±6.81 b | 58.7±17.30 a           | 19.1±1.39 a | 26.0±1.33 a              | 4.9±0.99 a              | 5.5±0.15 a       |
| S₁ (42)   | 0.03 SA    | 105.0        | 103.0                  | 17.0        | 26.0                     | 5.0                      | 6.2              |
|           | M₁         | 113.3±6.14 b | 38.2±16.61 b           | 16.7±1.06 b | 24.4±1.26 b              | 4.7±0.48 a              | 5.2±0.14 b       |
| S₁ (75)   | 0.03 HZ    | 91.0         | 38.0                   | 14.0        | 24.0                     | 6.0                      | 6.1              |
|           | M₁         | 121.6±4.55 b | 44.7±11.29 b           | 17.85±1.73 ab | 26.0±1.89 a     | 4.7±0.82 a          | 5.46±0.13 a      |

Significant * * * * * * * LSD 0.05 6.52 12.04 1.28 1.32 0.71 0.13

M₁= first mutagenicity value of one mutant plant, M₂= second mutagenicity mean value of ten mutant plants generations, * means significant at 0.05 levels of probability.

3.1.2 Sids₁₂

The data in Table (4) showed that the means of all studied traits in M₁ and M₂ generations of Sids₁₂ genotype and its mutant selected plants. Uniformly results showed that some mutations in Sids₁₂ genotype increased in M₂ than M₁ generations. The highest value in plant height was 107.1 cm which obtained with 0.03% HZ for mutated plant S₁₂ 168 as compared to untreated plants (control) with 102.0 cm. While the highest value in number of spikes /plant was 27.6 obtained with 0.02% HZ for mutated plant S₁₂ 161 as compared to original plants (control) with 8.4 spike. The highest value of spike length was 19.8 cm obtained at 0.03% HZ for mutated plant S₁₂ 177 compared to untreated plants (control) with 13.4 cm. The highest value of number of spikelets /spike was 26 obtained at both 0.03% HZ for mutated plants S₁₂ 177 as compared to control with 20.4 spikelet. The highest value of number of grains /spikelets was 6.9 obtained at both 0.03% SA for mutated plants S₁₂ 116 as compared to untreated plants (control) with 3.4 grain. The highest value of 100-grain weight was 6.31 g obtained at both 0.02% HZ for mutated plants S₁₂ 161 as compared to untreated plants (control) with 4.18 gm. These results showed that means of M₂ generation of Sids₁₂ genotype in the four mutant selected plants were higher than those obtained from M₁ generation for most studied traits. Also, data in Table (4) showed that significant differences among the different concentrations of two chemical mutagens (SA and HZ) in Sids₁₂ genotype in both M₁ and M₂ generations for all studied traits. Mensah and Obadoni (2007) reported that increasing shoots number per plant in M₂ than M₁ generation. Khursheed et al. (2015) and Khah and Verma (2015) reported that mutagen treatment increased spike length positively. Mensah and Obadoni (2007), Khah and Verma (2015), and Khursheed et al. (2015) recorded positive shifts in mean values of plant height because of the mutagen treatment.
Table (4): Mean performance of Sids12 genotype and the mutated selected plants at M1 and M2 generations for all studied traits in 2018/19 and 2019/20 seasons.

| Genotypes | Treatments | Plant height (cm) | Number of spikes/plant | Spike length (cm) | Number of grains/spike | Number of grains/spikelet | 100- grain weight |
|------------|------------|-------------------|------------------------|------------------|------------------------|--------------------------|-----------------|
| Sids12     | Control    | 98.0              | 9.0                    | 12.0             | 22.0                   | 4.0                      | 5.2             |
|            | 102.0±2.66 | 8.4±2.07 d        | 13.4±0.39 b            | 20.4±1.58 b      | 3.4±0.32 c             | 4.18±0.18 c             |
| M1         | 100.4±5.27 | 19.0              | 30.0                   | 7.0              | 5.9                    |                          |
| M2         | 102.9±3.03 | 19.0              | 30.0                   | 7.0              | 5.9                    |                          |
| S12 (116)  | 0.02 SA    | 81.0              | 17.0                   | 24.0             | 10.0                   | 5.8                      |
| M1         | 107.1±1.91 | 24.4±3.21 ab      | 27.0±1.70 a            | 2.01±0.32 b      | 6.01±0.32 b             |
| M2         | 105.0±2.14 | 21.0              | 26.0                   | 7.0              | 5.9                    |                          |
| S12 (168)  | 0.03 HZ    | 81.0              | 17.0                   | 24.0             | 10.0                   | 5.8                      |
| M1         | 107.1±1.91 | 24.4±3.21 ab      | 27.0±1.70 a            | 2.01±0.32 b      | 6.01±0.32 b             |
| M2         | 105.0±2.14 | 21.0              | 26.0                   | 7.0              | 5.9                    |                          |
| Significant | *          | *                 | *                      | *                | *                      |                          |
| LSD 0.05   | 2.99       | 4.47              | 1.15                   | 1.77             | 0.82                   | 4.17                     |

M1= first mutagenicity value of one mutant plant, M2= second mutagenicity mean value of ten mutant plants generations, * means significant at 0.05 levels of probability.

### 3.1.3 Giza168

The results in Table (5) showed that means of the studied traits in M1 and M2 generations of Giza168 genotype and one mutant selected plant Giza168 202. Means of most studied traits increased in M2 than M1 generation in mutant selected plant Giza168 202, which obtained with 0.02% SA. The increased values were 106 cm for plant height, 25.1 for number of spikes/plant, 19.9 for spike length, 27 cm for number of spikelet/spike, 5.77 for 100-grain weight as compared to untreated plants (control) with 94.1, 12.1, 13.35, 21.6, 4.0 and 4.09, respectively. However, number of grains/spikelet reduced from 7 in M1 to 5.7 in M2 generation. The data in Table (5) showed that significant differences between treated with 0.02% SA and untreated plants (control) in Giza168 genotype in both M1 and M2 generations for all studied traits. These results were agreement with those obtained by Fikre et al. (2015) showed significant variation for all the traits studied i.e., number of spikelet/spike, spike length and plant height after mutagen treatment. Ahmed et al. (2016) reported increasing of spike length and hundred-grain weight after mutagen treatment. Al-Nuaimi and Al-Shamma (2015) reported that all mutations showed significant increase in plant height and number of tillers per plant compared with control plants. However, the results of present study disagree with those obtained by Khah and Verma (2015) which found decrease in number of spikelets/spike after mutagen treatment.

Table (4): Mean performance of Giza168 genotype and one mutated selected plant at M1 and M2 generations for all studied traits in 2018/19 and 2019/20 seasons.

| Genotypes | Treatments | Plant height (cm) | Number of spikes/plant | Spike length (cm) | Number of grains/spike | Number of grains/spikelet | 100- grain weight |
|------------|------------|-------------------|------------------------|------------------|------------------------|--------------------------|-----------------|
| Giza168    | Control    | 95.0              | 10.0                   | 13.0             | 23.0                   | 4.0                      | 4.2             |
|            | 94.1±3.25 b| 12.0±4.80 b       | 13.35±0.85 b           | 21.6±1.58 b      | 4.0±0.67 b             | 4.09±0.15 b             |
| M1         | 105.0      | 21.0              | 26.0                   | 7.0              | 5.3                    |                          |
| M2         | 106.0±5.31 | 25.1±4.04 a       | 19.9±0.84 a            | 27.0±1.70 a      | 5.7±1.06 a             | 5.79±0.39 a             |
| Significant | *          | *                 | *                      | *                | *                      |                          |
| LSD 0.05   | 4.14       | 4.17              | 0.80                   | 1.54             | 0.83                   | 0.27                     |

M1= first mutagenicity value of one mutant plant, M2= second mutagenicity mean value of ten mutant plants generations, * means significant at 0.05 levels of probability.
3.2 SSR molecular marker

Seven SSR primers were used to amplify fragments in all selected M2 generation for the three varieties and its original varieties (control). Primer xgwm99 (Figure 1 A) generated five bands in Sids1 and their three selected mutant plants with DNA size ranged from 110 bp to 720 bp, lane 1 to 4 respectively. Three bands out of them were monomorphic and two bands were polymorphic and showed 40% polymorphism. In the same figure lane 5 to 9 presented molecular fragments amplified by Sids12 variety and their four selected mutant plants respectively with DNA size ranged from 110 bp to 720 bp. Three bands were produced, two bands were monomorphic, and one band was polymorphic and showed 33.3% polymorphism. In the same figure lane 10 to 11 presented molecular fragments amplified by Giza168 variety and its selected mutant plants respectively with DNA size ranged from 110 bp to 720 bp. Four bands were produced; two bands were monomorphic, and two bands were polymorphic showed 50% polymorphism.

Figure (1): SSR banding patterns among 11 genotypes, (1) Sids1, (2) S1 29, (3) S1 42, (4) S1 75, (5) Sids12, (6) S12 116, (7) S12 161, (8) S12 168, (9) S12 177, (10) Giza168, (11) Giza168 202 for xgwm99 primer (A), xgwm186 primer (B), Xgwm337 primer (C), Xgwm357 primer (D), xgwm484 primer (E), xgwm626 primer (F), xpsp3200 primer (G), and marker is 1000 bp.
Primer xgwm186 (Figure 1B) generated four bands in Sids1 and their three selected mutant plants, lane 1 to 4 respectively with DNA size ranged from 75 bp to 490 bp. One band was monomorphic, and three bands were polymorphic and showed 75% polymorphism. In the same figure, lane 5 to 9 presented molecular fragments amplified by using Sids12 and their four selected mutant plants respectively with DNA size ranged from 75 bp to 490 bp. Four bands were produced, one band was monomorphic, and three bands were polymorphic and showed 75% polymorphism. In the same figure lane 10 to 11 presented molecular fragments amplified by using Giza168 and its selected mutant plant with DNA size ranged from 75 bp to 500 bp. Five bands were produced, four bands were monomorphic, and one band was polymorphic and showed 20% polymorphism. Primer xgwm357 (Figure 1D) generated four bands in Sids1 and their three selected mutant plants, lane 1 to 4 respectively with DNA size ranged from 75 bp to 500 bp. Four bands were produced, one band was monomorphic, and three bands were polymorphic and showed 75% polymorphism. In the same figure lane 5 to 9 presented molecular fragments amplified by using Sids12 and their four selected mutant plants respectively with DNA size ranged from 210 bp to 600 bp. Four bands were produced, one band was monomorphic, and three bands were polymorphic and showed 50% polymorphism. In the same figure lane 10 to 11 presented molecular fragments amplified by using Giza168 and its selected mutant plant with DNA size ranged from 210 bp to 600 bp. Five bands were produced, four bands were monomorphic, and one band was polymorphic and showed 20% polymorphism. Primer xgwm484 (Figure 1E) generated three bands in Sids1 and their three selected mutant plants, lane 1 to 4 respectively with DNA size ranged from 210 bp to 600 bp. Two bands were produced; two bands were monomorphic, and two bands were polymorphic and showed 50% polymorphism. In the same figure, lane 10 to 11 presented molecular fragments amplified by using Giza168 and its selected mutant plant with DNA size ranged from 75 bp to 500 bp. Five bands were produced, four bands were monomorphic, and one band was polymorphic and showed 20% polymorphism. In the same figure lane 10 to 11 presented molecular fragments amplified by using Giza168 and its selected mutant plant respectively with DNA size ranged from 210 bp to 600 bp. Four bands were produced, one band was monomorphic, and three bands were polymorphic and showed 50% polymorphism. In the same figure lane 10 to 11 presented molecular fragments amplified by using Giza168 and its selected mutant plant respectively with DNA size ranged from 210 bp to 600 bp. Four bands were produced, one band was monomorphic, and three bands were polymorphic and showed 50% polymorphism. In the same figure lane 10 to 11 presented molecular fragments amplified by using Giza168 and its selected mutant plant respectively with DNA size ranged from 210 bp to 600 bp. Four bands were produced, one band was monomorphic, and three bands were polymorphic and showed 50% polymorphism.
DNA size ranged from 210 bp to 600 bp. Five bands were produced; one band was monomorphic, and four bands were polymorphic and showed 80% polymorphism. In the same figure lane 10 to 11 presented molecular fragments amplified by Giza\textsubscript{168} variety and its selected mutant plant respectively with DNA size ranged from 210 bp to 600 bp. Four bands were produced; two bands were monomorphic, and two bands were polymorphic and showed 50% polymorphism. Primer xgwm626 (Figure 1F) generated three bands in Sids\textsubscript{1} and their three selected mutant plants, lane 1 to 4 respectively with DNA size ranged from 450 bp to 720 bp. Two bands were monomorphic, and one band was polymorphic and showed 33.3% polymorphism. In the same figure lane 5 to 9 presented molecular fragments amplified by Sids\textsubscript{12} variety and their four selected mutant plants respectively with DNA size ranged from 450 bp to 900 bp. Four bands were produced, one band was monomorphic, and three bands were polymorphic and showed 75% polymorphism. In the same figure lane 10 to 11 presented molecular fragments amplified by Giza\textsubscript{168} and its selected mutant plant with DNA size ranged from 450 bp to 700 bp. Three bands were produced; two bands were monomorphic, and one band was polymorphic and showed 40% polymorphism. In the same figure lane 5 to 9 presented molecular fragments amplified by Sids\textsubscript{12} and their four selected mutant plants respectively with DNA size ranged from 110 bp to 700 bp. Three bands were produced; two bands were monomorphic, and one band was polymorphic and showed 33.3% polymorphism. In the same figure lane 5 to 11 presented molecular fragments amplified by Giza\textsubscript{168} and its selected mutant plant with DNA size ranged from 110 bp to 700 bp. Three bands were produced; two bands were monomorphic, and no polymorphic band and showed 0% polymorphism. The highest levels of polymorphism for SSRs system compared to other systems also reported in previous studies by Brbaklic \textit{et al.} (2015), Faheem \textit{et al.} (2015), Hao \textit{et al.} (2011), Nagy \textit{et al.} (2012), Ramadugu \textit{et al.} (2015), Ramya \textit{et al.} (2015), Ateş Sönmezoğlu and Terzi (2018), and Tomar \textit{et al.} (2016). This high level of polymorphism, associated with SSR markers, is to be expected because of the unique mechanism responsible for generating SSR allelic diversity (Abbasov \textit{et al.}, 2019). The codominant nature of SSR markers also permits the detection of a high number of alleles per locus and contributes to higher levels of expected heterozygosity being reached than would be possible with RAPD markers (Hao \textit{et al.}, 2006).
4. Conclusion

In conclusion, the results of this study provided most morphological and agronomical traits increased in the selected mutant plants as a result of mutagen treatment as compared to untreated plants (control). Also, most morphological and agronomical traits showed more increase in M₂ generation than M₁ generation for most studied traits. Moreover, results showed that significant differences among the different concentrations of two chemical mutagens (SA and HZ) in the treated and untreated of the three varieties of bread wheat i.e., Sids₁, Sids₁₂ and Giza₁₆₈ for both M₁ and M₂ generations for most studied traits. And in molecular studied were showed represetable the variation between treated genotypes and untreated (control) for all studied traits. in all selected M₂ plants were used to evaluate variation by SSR molecular markers. Finally, the SSR marker identify developed from this study can be used to identify mutation genotypes in wheat with chemical mutagen.

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