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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, $Sids_1$, $Sids_{12}$ and $Giza_{168}$. Some mutations were selected in M_1 generation plants *i.e.*, three for $Sids_1$ variety (S_1 29, S_1 49, and S_1 75), four for $Sids_{12}$ variety *i.e.*, (S_{12} 116, S_{12} 161, S_{12} 168 and $Sids_{12}$ 177) and one for $Giza_{168}$ (G_{168} 202). Selected genotypes were grown in field experiment to obtain M_2 generation. Results showed that most agronomical traits were significantly increased in M_2 than M_1 generation plants. The highest values in selected mutated plants for 100 grain weight were 6.31 gm for S_{12} 161 plant, 5.79 gm for G_{168} 202 plant and 5.55 gm for S_1 29 plant. Genetic variation in M_2 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.

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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) wase 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 achived 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 $Sids_1$, $Sids_{12}$ and $Giza_{168}$. 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 concentraions 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 trail to obtain M_1 generation. In season 2019/2020, selected eight mutant plants

genotypes were and sown in the Experimental Farm of Agriculture Faculty, Al-Azhar University, Assuit, Egypt to obtain M_2 generation.

Table (1): The pedigree and origin of the three bread wheat genotypes used in this study.

Name	Pedigree	Origin
$Sids_1$	MRL/BUC/SER1	Egypt
$Sids_{12}$	BUC//7C/ALD/5/MAYA74/0N//1160 Egypt/47/3/BB/GLL/4/CHAT"S"/6/MAYA/VUL-//CMH74A.63014*SX.SD7096-4SD-1SD-1SD-0SD	Egypt
$Giza_{168}$	MRL / BUC // SERI – CM 930 46- 8M-OY-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 $MgCl_2$, 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.

Table (2): SSR Primer sequences used to amplify molecular markers.

Code	Sequences (5-3)	References
Xgwm 99	F- AAGATGGACGTATGCATCACA R- GCCATATTTGATGACGCATA	Ateş Sönmezoğlu and Terzi (2018)
Xgwm 186	F- GCAGAGCCTGGTTCAAAAAG R- CGCCTCTAGCGAGAGCTATG	
Xgwm 337	F- CCTCTTCCTCCCTCACTTAGC R- TGCTAACTGGCCTTTGCC	
Xgwm 357	F- TATGGTCAAAGTTGGACCTCG R- AGGCTGCAGCTCTTCTTCAG	
Xgwm 484	F- ACATCGCTCTTCACAAACCC R- AGTTCCGGTCATGGCTAGG	
Xgwm 626	F- GATCTAAAATGTTATTTTCTCTC R- TGACTATCAGCTAAACGTGT	
Xpsp 3200	F- GTTCTGAAGACATTACGGATG R- GAGAATAGCTGGTTTTGTGG	

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 (M_1) and second mutagenicity (M_2) generations for the studied traits

3.1.1 $Sids_1$

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 M_1 and M_2 generations of Sids1 genotype and its mutant selected plants *i.e*., S_1 29, S_1 42 and S_1 75 plants. Results showed that some mutations in Sids1 genotype increased in M_2 than M_1 generations. The highest value in plant height was 121.6 cm which obtained with 0.03% HZ for mutated plant S_1 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 S_1 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 S_1 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 S_1 29 and S_1 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 M_2 than M_1 generation. The best reduced values in M_2 generation were 4.9 and 5.55 obtained with 0.02% SA for mutant plant S_1 29 as compared to 6.0 and 5.0 gm. in M_1 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 $Sids_1$ genotype in both $M_1$and M_2 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 M_2 generations than M_1 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 $Sids_1$ genotype and the mutated selected plants at M_1 and M_2 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
$Sids_1$	Control	M_1	103.0	13.0	14.0	22.0	3.0	5.3
		M_2	104.6±10.11 c	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
S_1 (29)	0.02 SA	M_1	105.0	59.0	18.0	24.0	6.0	6.0
		M_2	113.85±6.81 b	58.7±17.30 a	19.1±1.39 a	26.0±1.33 a	4.9±0.99 a	5.55±0.15 a
S_1 (42)	0.03 SA	M_1	105.0	103.0	17.0	26.0	5.0	6.2
		M_2	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	5.26±0.14 b
S_1 (75)	0.03 HZ	M_1	91.0	38.0	14.0	24.0	6.0	6.1
		M_2	121.6±4.55 a	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

M_1= first mutagenicity value of one mutant plant, M_2= second mutagenicity mean value of ten mutant plants generations, * means significant at 0.05 levels of probability.

3.1.2 $Sids_{12}$

The data in Table (4) showed that the means of all studied traits in M_1 and M_2 generations of $Sids_{12}$ genotype and its mutant selected plants. Uniformly results showed that some mutations in $Sids_{12}$ genotype increased in M_2 than M_1 generations. The highest value in plant height was 107.1 cm which obtained with 0.03% HZ for mutated plant S_{12} 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 S_{12} 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 S_{12} 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 S_{12} 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 S_{12} 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 S_{12} 161 as compared to untreated plants (control) with 4.18 gm. These results showed that means of M_2 generation of $Sids_{12}$ genotype in the four mutant selected plants were higher than those obtained from M_1 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 $Sids_{12}$ genotype in both M_1 and M_2 generations for all studied traits. Mensah and Obadoni (2007) reported that increasing shoots number per plant in M_2 than M_1 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 $Sids_{12}$ genotype and the mutated selected plants at M_1 and M_2 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
$Sids_{12}$	Control	M_1	98.0	9.0	12.0	22.0	4.0	5.2
		M_2	102.0±2.66 bc	8.4±2.07 d	13.4±0.39 b	20.4±1.58 b	3.4±0.52 c	4.18±0.18 c
S_{12} (116)	0.02 SA	M_1	98.0	18.0	19.0	30.0	7.0	5.9
		M_2	100.4±5.27 c	14.3±5.89 c	19.25±2.28 a	25.6±2.27 a	6.9±1.97 a	5.95±0.43 b
S_{12} (161)	0.03 SA	M_1	103.0	14.0	15.5	24.0	7.0	6.1
		M_2	102.9±3.03 bc	27.6±6.04 a	19.0±0.82 a	25.2±2.86 a	5.3±1.06 b	6.31±0.29 a
S_{12} (168)	0.03 HZ	M_1	81.0	17.0	18.0	24.0	10.0	5.8
		M_2	107.1±1.91 a	24.4±3.21 ab	19.75±1.21 a	22.0±1.63 b	4.8±0.82 b	6.01±0.32 b
Significant			*	*	*	*	*	*
LSD 0.05			2.99	4.47	1.15	1.77	0.82	4.17

M_1= first mutagenicity value of one mutant plant, M_2= second mutagenicity mean value of ten mutant plants generations, * means significant at 0.05 levels of probability.

3.1.3 Giza$_{168}$

The results in Table (5) showed that means of the studied traits in M_1 and M_2 generations of $Giza_{168}$ genotype and one mutant selected plant $Giza_{168}$ 202. Means of most studied traits increased in M_2 than M_1 generation in mutant selected plant $Giza_{168}$ 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 M_1 to 5.7 in M_2 generation. The data in Table (5) showed that significant differences between treated with 0.02% SA and untreated plants (control) in $Giza_{168}$ genotype in both $M_1$and M_2 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 $Giza_{168}$ genotype and one mutated selected plant at M_1 and M_2 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
Giza 168	Control	M_1	95.0	10.0	13.0	24.0	4.0	4.2
		M_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±0.13 b
G_{168} (202)	0.02 SA	M_1	105.0	21.0	18.0	26.0	7.0	5.3
		M_2	106.0±5.31 a	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

M_1= first mutagenicity value of one mutant plant, M_2= 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 M_2 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 $Sids_{12}$ 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 $Giza_{168}$ 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.

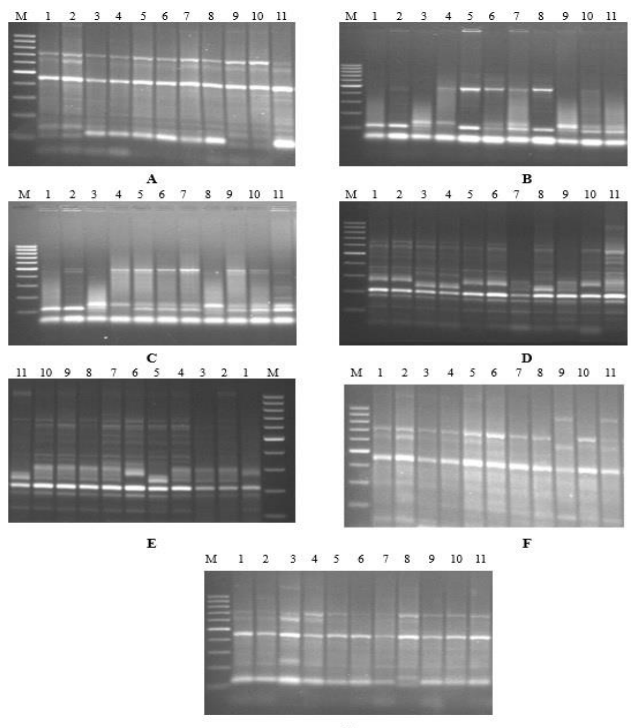

Figure (1): SSR banding patterns among 11 genotypes, (1) $Sids_1$, (2) S_1 29, (3)S_1 42, (4) S_1 75, (5) $Sids_{12}$, (6) S_{12} 116, (7) S_{12} 161, (8) S_{12} 168, (9) S_{12} 177, (10) $Giza_{168}$, (11) $Giza_{168}$ 202 for xgwm99 primer (A), xgwm186 primer (B), Xgwm337 primer (C), Xgwm357 primer (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 $Sids_{12}$ 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 Giza168 and its selected mutant plants respectively with DNA size ranged from 75 bp to 120 bp. three bands were produced, two bands were monomorphic, and one band was polymorphic and showed 33.3% polymorphism. Primer xgwm337 (Figure 1C) generated four bands in $Sids_1$ and their three selected mutant plants, lane 1 to 4 respectively with DNA size ranged from 75 bp to 500 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 $Sids_{12}$ and their four selected mutant plants with DNA size ranged from 75 bp to 500 bp. four 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 $Giza_{168}$ 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 $Sids_1$ and their three selected mutant plants, lane 1 to 4 respectively with DNA size ranged from 210 bp to 600 bp. two bands were monomorphic, and two bands were polymorphic and showed 50% polymorphism. In the same figure lane 5 to 9 presented molecular fragments amplified by using $Sids_{12}$ 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 75% polymorphism. In the same figure lane 10 to 11 presented molecular fragments amplified by using $Giza_{168}$ and its selected mutant plant respectively with DNA size ranged from 210 bp to 600 bp. Three bands were produced, three bands were monomorphic and no polymorphic bands and showed 0% polymorphism. Primer xgwm484 (Figure 1E) generated three bands in $Sids_1$ and their three selected mutant plants, lane1 to 4 respectively with DNA size ranged from 210 bp to 600 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 using $Sids_{12}$ and their four selected mutant plants respectively with

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 $Giza_{168}$ 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 $Sids_1$ 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 $Sids_{12}$ 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 $Giza_{168}$ and its selected mutant plant with DNA size ranged from 450 bp to 900 bp. Four bands were produced, two bands were monomorphic, and two bands were polymorphic and showed 50% polymorphism. Primer xpsp3200 (Figure 1G) generated five bands in $Sids_1$ and their three selected mutant plants, lane 1 to 4 respectively with DNA size ranged from 110 bp to 700 bp. Three bands were monomorphic, and two bands were polymorphic and showed 40% polymorphism. In the same figure lane 5 to 9 presented molecular fragments amplified by $Sids_{12}$ 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 10 to 11 presented molecular fragments amplified by $Giza_{168}$ and its selected mutant plant respectively with DNA size ranged from 110 bp to 700 bp. three bands were produced; three 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 *et al.* (2015), Faheem *et al.* (2015), Hao *et al.* (2011), Nagy *et al*. (2012), Ramadugu *et al*. (2015), Ramya *et al.* (2015), Ateş Sönmezoğlu and Terzi (2018), and Tomar *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 *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 *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 M_2 generation than M_1 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*., $Sids_1$, $Sids_{12}$ and $Giza_{168}$ for both M_1 and M_2 generations for most studied traits. And in molecular studied were showed represpectable the variation between treated genotyes and untreated (control) for all studied traits. in all selected M_2 plants were used to evaluate variation by SSR molecular markers. Finaly, the SSR marker identify developed from this study can be used to identify mutation genotypes in wheat with chemical mutagen.

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