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Field Evaluation and Molecular Analysis of Three Early Flowering Canola Mutants under Natural Salinity-Stressed Environment

Hassan, M. S.[1] and T. Bashandy[2*]

[1] Department of Agronomy, Faculty of Agriculture, South Valley University, Qena, Egypt
[2] Department of Genetics, Faculty of Agriculture, New Valley University, The New Valley, Egypt.

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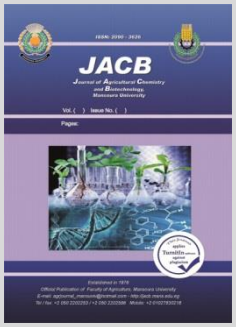

ABSTRACT

Mutagenesis is a good way to create new plant lines having desirable traits. Therefore, this study was conducted to evaluate three gamma ray-induced early flowering canola mutants, their parent (Serw4) and chick verity (Serw6). Some agronomic traits were evaluated at M6 and M7 generations, under natural saline conditions. The results revealed that all the mutants were earlier and had more seed yield production than their parent and the chick in both two generations and combined. Otherwise, the mean performance of the other studied traits is significantly decreased. Furthermore, the ability of the mutants for salt stress tolerance was evaluated *in vitro* by treating seedlings with different concentrations of NaCl. All the mutants were the most salt tolerant. On the other hand, genetic diversity and similarity relationships among all genotypes were measured by inter simple sequence repeat (ISSR) molecular marker. The percentage of polymorphism was 50%, while the highest similarity was 0.90 between Serw4 and Serw6, but the lowest similarity (0.79) was detected between Serw6 and M11-4-1 mutant. Moreover, the dendrogram classified all the genotypes into two main clusters, the mutant's parent and the chick variety were gathered in one cluster, while all the mutants were collected in the other one. These induced earlier flowering mutants having higher seed yield production and salinity tolerance, suggested that these mutants could be new great promising mutant lines.

Keywords: induced mutations, gamma ray, genetic improvement, ISSR, field trials, dendrogram, polymorphism.

INTRODUCTION

Canola (*Brassica Napus* L.) is one of the most important sources of edible oil in many world countries. In Egypt, providing of edible oil is considered as great economic problem, because the oilseed crops cultivation are not farmers priority. In recent years, the importance of canola cultivation is increased, especially in new reclaimed lands to overcome the huge demand and consumption of edible oil. These new reclaimed lands needs to grow the most adaptable genotypes for biotic and abiotic stress. Salinity is one of the major abiotic stresses which greatly affecting canola yield and yield components (Bray *et al.*, 2000; Ahmadzadeh *et al.*, 2015; Tahmasebpour *et al*., 2018).

Additionally, induction of earlier cultivars is also one of breeder aims in order to reduce water consumption during cultivation season and helping in using of effective crop intensive rotation. Creating and increasing genetic variation of useful traits is the major factor for crop genetic improvement. In fact, induction of mutations is an effective approach to generate different genetic variations in plants (parry *et al.*, 2009). Gamma radiation has been widely used as mutagenesis agents for inducing genetic variations in many plants (Khatri *et al*., 2005; reviewd by Szarejko and Forster, 2007; Badr *et al*., 2014; El-Khateeb *et al*., 2017). Many mutants having desirable traits have been identified in canola and other crops via agro-morphological characterization (Emrani *et al*., 2012; Malek *et al*., 2014a; Laskar *et al*., 2015; Dey *et al*., 2016). Recently, the DNA molecular markers have been used to assess individual genetic diversity and differentiate among different genotypes at DNA molecule levels. Moreover, they are not influenced by environmental effects. Several PCR based molecular markers have been used to determine genetic diversity. Inter simple sequence repeats (ISSR) one of many PCR based molecular markers (Zietkiewicz *et al*., 1994; Fernández *et al.*, 2002). It is highly polymorphic, a simple, low-cost and quick method that use of microsatellite sequences as primers to detect multi-locus markers throughout the genome (Godwin *et al*., 1997; Reddy *et al.*, 2002).

This study was applied to evaluate some gamma ray-induced early flowering canola mutants and their parent in M6 and M7 generations by using agro-morphological and ISSR molecular marker analysis.

MATERIALS AND METHODS

Plant material: Three promising early flowering Canola mutants (M11-2-1, M11-2-4 and M11-4-1), their parent (Serw4) and check variety (Serw6) were used in this study. These mutants previously developed among 10 promising early flowering mutants from M5 generation (Hassan 2014). They were induced through treating Serw4 variety seeds with 15 kr of gamma rays (Hassan and Abd-El-Haleem, 2014).

Field trials: The field experiment was performed during two growing seasons (2016/2017 and 2017/2018), at the Experimental Farm of Agriculture Faculty, South Valley University, Qena, Egypt. It is new reclaimed land and irrigate by underground water. Furthermore, the salinity (EC_e) value of both its soil and irrigation water was 13.99

* **Corresponding author.**
E-mail address: talat55@yahoo.com
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and 7.71 ds m^{-1}, respectively. Experimental layout was a randomized complete blocks design (RCBD) with three replications. The experimental plot included three rows each 3 m long and 50 cm apart, sowing was in hills spaced 10 cm. All farming applications were performed as recommended.

Studied traits:

Earliness was recorded in each plot as number of days from sowing to flowering of 50 % of plants (NDF). At harvest time, 15 random plants were taken from each plot to estimate, plant height (PHt), number of siliquas $plant^{-1}$ (NSP), and seed yield $plant^{-1}$ (g.) (SYP). Seed oil content (oil %) was estimated by Soxhelt apparatus according to AOAC (1980).

***In vitro* salinity tolerance evaluation:** The seeds of all genotypes were grown in pots filled with cotton and irrigated with tap water. Then, after six days of germination, the pots were divided into three groups each of them contained 15 pots, three pots for each genotype. One group was irrigated with MS liquid medium (as a control), while the second and the third groups were watered with MS liquid medium supplemented with 200 mM NaCl or 250 mM NaCl, respectively for two weeks.

Statistical analysis: Analysis of variance (ANOVA) was performed according to Gomez and Gomez (1984) for randomized complete blocks design for each preliminary trial, separately. Combined analysis over the two trials was also done after testing the homogeneity. Mean comparisons were performed using Least Significant Differences (L.S.D.) test.

Molecular characterization:

DNA extraction: Genomic DNA was extracted from fresh leaves of mutants of M7 generation, their parent and the check variety by using method described by Anna *et al*., (2001).

PCR amplification and electrophoresis: Nine primers of ISSR markers (UBC 807, UBC 808, UBC 810, UBC 811, UBC 815, UBC 826, UBC 834, UBC 840 and UBC 846) as represented in Table (3) were used in this study (EZBiolab-USA). PCR amplification was performed in 25µl reaction volume containing 1x PCR buffer, 4 mM $MgCl_2$, 0.2 mM dNTPs, 20 pmole primer, 2 units Taq DNA polymerase and 50 ng template DNA. Amplifications were performed in a Thermal Cycler (Labocon, U.K.), with initial denaturation at 94°C for 5 min followed by 40 cycles: denaturation at 94°C for 1min, annealing at (each primer has specific annealing temperature) for 1min, extension at 72°C for 2min, with final extension at 72°C for 7min. PCR products were separated on 1.5% agarose gels using 1×TBE (Tris-Borate-EDTA) buffer at 5 V/cm., then visualized by staining with ethidium bromide.

Data analysis:

The detected bands were scored as 1 (present) and 0 (absent). Genetic similarity was estimated using Nei-Li's similarity index (Nei and Li, 1979). A dendrogram was constructed on the basis of the similarity matrix data by unweighted pair group method with arithmatic average (UPGMA), cluster analysis was achieved using the software MEGA program. Resolving power (Rp) of each primer was calculated using the formula: $Rp= \Sigma I_b$ (Band informativeness) according to Prevost and Wilkinson, 1999. Whereas, I_b was calculated by the formula of $I_b= 1-(2*|0.5-p|)$, where *p* is the percentage of genotypes containing the band.

RESULTS AND DISCUSSION

A. Agronomic characterization:

Results of combined analysis of variance of M6 and M7 generations and the mean performance for earliness, plant height, siliquas/plant and seed oil content are summarized in Tables 1 and 2, respectively. All the genotypes showed highly significant differences in performance of all the evaluated traits (Table 1). According to the earliness trait, all the mutants were earlier than their parent and the check variety at M6, M7 and combined, except the M11-2-4 which lost its earliness in M7 generation. Morever, the combined data showed that the M11-4-1 mutant was the earliest with 23.17 days over their parent followed by M11-2-4 (15.34 d) and M11-2-1 (9.84d), respectively (Table 2). Similarly, several early flowering gamma ray induced mutants have been isolated by Yokoo and Okuno (1993), Tulmann and Alves (1997), Isfahani and Fotokian (2002) and Malek *et al.* (2014b). The earliness of the evaluated mutants may be due to interruption of one or more genes which controlling flowering time and biological clock. Whereas, Park *et al*. (2007) found that inhibition of COG gene expression can induce the early flowering in plants. On the other hand, the plant height of all the mutants was varied in M6 as compared with M7. In M6 the M11-2-1 and M11-4-1 mutants were taller than their parent, but M11-2-4 mutant was shorter than their parent. In contrast, all the mutants were the shortest in M7. The combined analysis showed that the plant height of only M11-2-1 (85.02) and M11-2-4 mutant (90.03) was shorter than their parent (102.17) (Table 2). Reduction in plant height as response to gamma rays irradiation treatment has been reported in several studies (Khatri *et al*., 2005, Monshi and Malek, 2013 and Gunasekaran and Pavadai, 2015). This reduction in height may be due to induction of mutation(s) which can affect different biosynthesis pathways. Additionally, the shortness has also been found to be associated with the earliness in maturity (Olejniczak and Adamska, 1999).

Concerning of siliqua number per plant trait, the recorded number was decreased in all the mutants in comparison with their parent and the check variety in M6, M7 and the combined. The combined analysis showed that the reduction was 75, 72.17 and 46.5 in M11-4-1, M11-2-4 and M11-2-1 mutant, respectively (Table 2). In agreement with our results, either decrease or increase of NPS in gamma induced mutants was detected by Monshi and Malek (2013), Yassein and Aly (2014). Alteration in siliqua number of all the mutants may be due to alteration in genetic make-up in original derived their parent. Furthermore, positive correlation between siliqua number and plant height was observed by Yassein and Aly (2014).

On the other hand, all the mutants had better performance in SYP than their parent in all the M6, M7 and the combined, except only M11-2-1 mutant was the worst in M7. The superiority of all the mutants over their parent was noted in the combined, where the differences from their parent were 1.16, 1.82 and 1.85 for M11-2-1, M11-2-4 and M11-4-1 mutant, respectively. Our results are similar to the finding of Khan *et al*. (2003), Khatri *et al.* (2005), Malek *et al.* (2012) who isolated short statured rapeseed and mustard mutants had high yield performance by using gamma rays irradiation treatment. Moreover, Khan *et al*. (2003), Khatri *et al.* (2005) found that short plants would be having higher grain yield productivity because they have good fertilization performance and more tolerant to the bad weather

conditions. Furthermore, early flowering helps for good seed filling which could produce better seed yield. Additionally, radiation makes genetic make-up changes which can increase yield production capacity (Shah and Rahman, 2009, Monshi and Malek, 2013).

According to seed oil content, at M6 and M7 the oil % value was not affected in M11-2-1 and M11-2-4 mutant, respectively, but it decreased in the other two mutants. Moreover, the combined analysis revealed that this value decreased in all the mutants that recorded 35.47, 36.07 and 36.23 for M11-2-4, M11-4-1 and M11-2-1 in comparison to their parent value (37.05) (Table 2). Similar findings have also been detected by Kumar *et al*. (2011), Yassein and Aly (2014). Superiority of the mutants over their original derived parent for M7 generations, in earliness and yield under salinity conditions indicate that, these mutants could be new great promising mutant lines. However, some traits were revisable in their performance, thus those mutants needs more evaluation trials before registration.

Table 1. Significance of mean squares due to different sources of variation for studied traits in M6, M7 generations and their combined

Generations	M6			M7			Combined				
Source of variation	Reps.	Mut./ Var.	Erorr	Reps.	Mut./ Var.	Erorr	Gen. (a)	Erorr	Mut./Var. (b)	A × b	Erorr
Degrees of freedom	2	4	8	2	4	8	1	4	4	4	16
					Mean squares						
S.O.V/ Trait	Mut./Var.		Erorr	Mut./Var.		Erorr	Gen.(a)	Erorr	Mut./Var. (b)	A × b	Erorr
NDF	836.23**		0.88	232.1**		0.55	246.53**	0.47	616.72**	451.62**	0.72
PHt	761.61**		16.79	1200.40**		14.85	5561.05**	2.81	296.84**	1665.17**	15.82
NSP	29114.99**		17.21	1924.5**		11.65	218627.45**	34.96	17468.22 **	13571.27**	14.43
SYP	103.67**		0.20	3.01**		0.26	710.83**	0.09	57.4**	49.29**	0.32
Oil %	11.74**		0.11	2.60**		0.13	1.12^{ns}	0.23	3.05**	11.29**	0.12

Note: ^{ns} = not significant, ** =significant at 1 % probability level, NDF= number of days to 50% flowering, PHt= plant height, NSP = number of siliquas plant^{-1}, NSP = seed yield plant^{-1}, oil %= Seed oil content.

Table 2. Mean performance of the mutants, their parent and the check variety for earliness and the other studied traits in M6, M7 generations and their combined

Mut./ Var.	M6					M7					Combined				
	NDF Days	PHt cm.	NSP	SYP gm.	Oil %	NDF Days	PHt cm.	NSP	SYP gm.	Oil %	NDF Days	PHt cm.	NSP	SYP gm.	Oil %
Serw 4 (parent)	90.67	96.67	303.17	9.89	36.98	89.67	107.7	131.7	4.85	37.12	90.17	102.17	217.42	7.37	37.05
Serw 6 (Control)	90.33	118.37	422.34	24.99	39.23	91	68.67	116	5.58	35.14	90.67	93.52	269.17	15.29	37.19
M11-2-1	88.67	112.04	276.84	13.66	36.81	72	58	65	3.4	35.66	80.33	85.02	170.92	8.53	36.23
M11-2- 4	61	86.6	179.5	12.9	33.7	88.67	94	111	5.48	37.25	74.83	90.30	145.25	9.19	35.47
M11- 4-1	58	125.48	190.17	12.5	36.26	76	74.67	94.67	5.94	35.87	67	100.07	142.42	9.22	36.07
$LSD_{0.05}$	1.77	7.72	7.81	0.84	0.62	1.40	7.26	6.43	0.96	0.68	1.47	6.88	6.58	0.98	0.60
$LSD_{0.01}$	2.57	11.22	11.36	1.23	0.91	9.03	10.56	9.35	1.40	0.99	2.02	9.49	9.06	1.35	0.83

Note: LSD= Least significant difference.

B- Response of the mutants for *in vitro* salinity stress:

To study tolerance ability of these mutants in comparison with their parent and the check variety for salinity stress, they were subjected to two different levels of NaCl (200 mM and 250 mM) at seedling stage. At 200 mM NaCl level, seedlings of the parent and the check variety turned to yellow colure and more dried than the mutants (Fig.1). Moreover, at 250 mM NaCl level, seedlings of the parent and the check variety dried and died, but plants of all the mutants mostly survived in this treatment, especially M11-4-1 mutant. Suggesting that the mutants are more tolerant to the salinity effect than their parent (Fig.1). This *in vitro* treatment confirm the field evaluation of the mutants through M6 and M7 generations under high saline conditions of both soil (EC_e= 13.99 ds m^{-1}) and irrigation water (7.71 ds m^{-1}). Even though these saline conditions, the mutants produced higher seed yield than their parent. Induction of mutation has been employed in several studies by Hossain *et al*. (2006) and Patade *et al*. (2008) not only for high yield improvement but also to produce more salt tolerant lines.

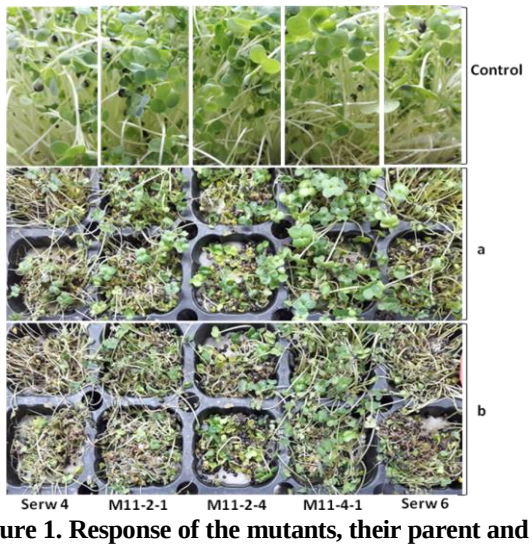

Figure 1. Response of the mutants, their parent and the check variety for salinity stress. (Control) watered with liquid free NaCl MS medium, (a) watered by MS medium supplemented with 200 mM NaCl for 14 days after 6d germination, (b) watered by MS medium supplemented with 250 mM NaCl for 14 days after 6d germination.

C- ISSR marker analysis:

To compare the evaluated mutants, their parent and the check variety genetically, nine ISSR primers were used for detection of several DNA bands from genomic DNA (Fig. 2).

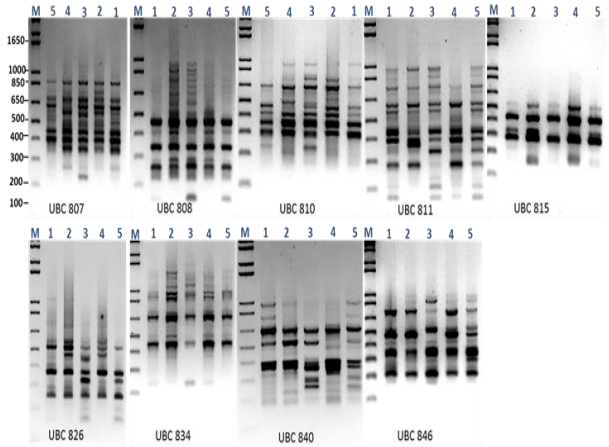

Fig. 2. ISSR pattern of M7 generation of three canola mutants, their parent and the check variety was produced by nine primers. 1, original parent; 2, M11-2-1 mutant; 3, M11-2-4 mutant; 4, M11-4-1 mutant; 5, Serw6. M, kbp DNA marker.

A total of 72 bands were detected with variable size ranged from 212 bp to 990 bp, 36 of them were polymorphic. From all, 6 bands were found to be mutants specific which only presented in all the mutants.

Moreover, some unique bands were present in one or two of mutants, and the polymorphism frequency was 50% (Table 3). To determine the best discriminative primer, the resolving power (Rp) for each primer was estimated, the UBC 840 prime had the highest value (4.8), but both UBC 815 and UBC 826 primers had the lowest value (0.8).

Furthermore, the relationships among all the genotypes were determined by a UPGMA cluster analysis of genetic similarity matrices, depending on the Nei-Li's similarity coefficient matrices cluster analysis and revealed that the similarity was high and its values ranged from 0.79 to 0.90. The highest similarity value (0.90) was between Serw 4 and Serw 6, while Serw 6 and M11-4-1 mutant showed the lowest similarity (0.79) (Table 4). On the other hand, the dendrogram of genetic distance classified all the evaluated genotypes into two main clusters (Fig. 3).

Table 3. Polymorphism obtained by nine ISSR primers in three mutants, their parent and the check variety

Primers	Primer sequence	Range of fragment size bp	Total No. of fragments	Monomorphic fragments	Polymorphic fragments	Polymorphism %	Resolving Power (RP)
UBC 807	$(AG)_8T$	342-853	9	5	4	44.44	2.4
UBC 808	$(AG)_8C$	260-495	6	4	2	33.33	1.6
UBC 810	$(GA)_8T$	280-925	11	4	7	63.64	4.4
UBC 811	$(GA)_8C$	288-965	11	4	7	63.64	3.6
UBC 815	$(CT)_8G$	260-490	4	3	1	25.00	0.8
UBC 826	$(AC)_8C$	246-556	6	5	1	16.67	0.8
UBC 834	$(AG)_8TT$	235-970	6	4	2	33.33	1.2
UBC 840	$(GA)_8TT$	244-990	12	4	8	66.67	4.8
UBC 846	$(CA)_8AT$	212-732	7	3	4	57.14	2
Total			72	36	36		

Table 4. The similarity index among the three mutants, their parent and the check variety based on ISSR

Genotypes	Serw 4	M11-2-1	M11-2-4	M11-4-1	Serw 6
Serw 4	1.00				
M11-2-1	0.87	1.00			
M11-2-4	0.82	0.85	1.00		
M11-4-1	0.87	0.88	0.85	1.00	
Serw 6	0.90	0.80	0.81	0.79	1.00

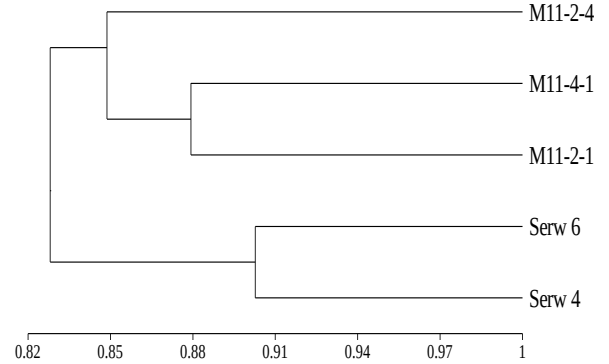

Fig. 3. The dendrogram of genetic distances among the three mutants, their parent and the check variety using UPGMA cluster analysis of Nei-Li's similarity coefficient based on ISSR markers.

The first one contained Serw 4 and Serw 6, while as all the mutants presented in the second cluster which divided into two sub-clusters, one of them contained only M11-2-4 mutant but the other one contained M11-4-1and M11-2-1 mutants. Detection of genetic variability among gamma rays induced mutants and their original parent by using ISSR marker analysis was also achieved by Kumar *et al.* (2011), Hamideldin and Hussin (2014) and El-Khateeb *et al.* (2017).

This study showed that the mutants and their parent and the check possess a moderate polymorphism as well as revealed by Xi *et al.* (2012) and Wang *et al.* (2017) who also detected a moderate polymorphism among gamma ray-induced mutants. The appearance or disappearance of bands as specific for γ-ray induced mutants is may be attributed to DNA structural rearrangements caused by different types of DNA damages (Selvi *et al.*, 2007 and Mejri *et al.*, 2012). On the other hand, the high similarity which was detected among all genotypes may be attributed to that they have the same basic genetic background. Furthermore, the constructed dendrogram could be able to differentiate between induced mutants and their parent and check variety, whereas its classification was convenient with the difference among them in some evaluated agronomic traits and salinity stress response.

CONCLUSION

Three gamma ray-induced mutants were evaluated at M6 and M7 generations for earliness, their performance in some agronomic characters, salinity tolerance response and ISSR molecular marker analysis. All the mutants were earlier and produced higher seed yield than their parent and

the check variety. Furthermore, the mutants revealed the highest ability for salinity tolerance. Moreover, ISSR molecular marker analysis classified all the mutants in one group. However, our results suggest that mutagenesis could be an applicable source for induction of genetic variability to obtain canola superior lines.

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REFERENCES

Ahmadzadeh N., Mostafavi K. and Zabet M. (2015). Effect of Salinity Stress on Yield and Yield Components in Rapeseed Cultivars. International Research Journal of Applied and Basic Sciences, 9: 1592-1595.

Anna M.P., Hirsikorpi M., Kämäräinen T., Jaakola L. and Hohrola A. (2001). DNA isolation methods for medicinal and aromatic plants. Plant Molecular Biology Reporter,19: 273-273.

AOAC (1980). Official Method of Analysis of the Association of Official Agriculture Chemists. 13th Edn., AOAC, Washington, DC., USA.

Badr A., Ahmed H.I.S., Hamouda M., Halawa M. and Elhiti M.A. (2014). Variation in growth, yield and molecular genetic diversity of m2 plants of cowpea following exposure to gamma radiation. Life Science Journal, 11: 10-19.

Bray E.A., Bailey-Serres J.and Weretilnyk E. (2000). Responses to abiotic stress. In: Biochemistry and Molecular Biology of Plants. American Society of Plant Physiology, (Eds.): B. Buchanan, W. Gruissem, R. Jones. Rockville, MD, pp. 1158-1203.

Dey S., Badri J., Prakasam V., Bhadana V.P., Eswari K.B., Laha G.S. and Ram T. (2016). Identification and agro-morphological characterization of rice genotypes resistant to sheath blight. Australasian Plant Pathology, 45: 145-153.

El-Khateeb M.A., Eid R.A., Mahfouze H.A., Ashor H.A. and Mabrouk R.M.S. (2017). Induction of Mutation with Gamma Radiation in *Helichrysum bracteatum* L. and Identification of Mutants by Molecular Markers. Middle East Journal of Agriculture Research, 6 : 282-293.

Emrani S.N., Arzani A.H., Saeidi G.O., Abtahi M., Banifatemeh M.O., Parsa M.B. and Fotokian M.H. (2012). Evaluation of induced genetic variability inagronomic traits by gamma irradiation in canola (*Brassica napus* L.). Pakistan Journal of Botany, 44: 1281-1288.

Fernández M.E., Figueiras A.M. and Benito C. (2002). The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with known origin. Theoretical and Applied Genetics, 104: 845-851.

Godwin I.D., Aitken E.A.B. and Smith L.W. (1997). Application of inter-simple sequence repeat (ISSR) markers to plant genetics. Electrophoresis, 18: 1524-1528.

Gomez K.A. and Gomez A.A. (1984). Statistical Procedures for Agriculture Research. Wiley Inter Science Publication. John Wiley and Son, Inc. New York, USA.

Gunasekaran A. and Pavadai P. (2015). Effect of gamma rays on germination, morphology, yield and biochemical studies in groundnut (*Arachis hypogaea* L.). World Scientific News, 23: 13-23.

Hamideldin N. and Hussin O. (2014). Morphological, physiological and molecular changes in *Solanum tuberosum* L. in response to pre-sowing tuber irradiation by gamma rays. American Journal of food science and technology, 2: 1-6.

Hassan M.S. (2014). Selection of earlier flowering mutants from canola (*Brassica napus* L.) cv. Serw 4. PARIPEX- Indian journal of research, 3: 1-3.

Hassan, M.S. and Abd-El-Haleem S.H.M. (2014). Effectivenss of gamma rays to induce genetic variability to improve some agronomic traits of canola (*Brassica napus* L.). Asian Journal of Crop Science., 6: 123-132.

Hossain Z., Mandal A.K.A., Datta S.K. and Biswas A.K. (2006). Isolation of a NaCl-tolerant mutant of *Chrysanthemum morifolium* by gamma radiation: in vitro mutagenesis and selection by salt stress. Functional Plant Biology, 33: 91-101.

Isfahani M. and Fotokian M.H. (2002). Induction of earliness and awn less mutants in rice (*Oryza sativa* L.) Domsiah cultivar. Iranian Journal of Crop Science, 1: 95-106.

Khan A., Dahar N.A., Khanzada M.H. and Khan R. (2003). Development of high yielding mutants of *Brassica campestris* L. cv. Toria selection through gamma rays irradiation. Asian Journal of Plant Science, 2: 192-195.

Khatri A.B., Khan I.A., Siddiqui M.A., Raza S., and Nizamani G.S. (2005). Evaluation of high yielding mutants of *Brassica juncea* cv. S-9 developed through gamma rays and EMS. Pakistan journal of botany, 37: 279.

Kumar H., Anubha M.K.V. and Lal J.P. (2011). Morphological and molecular characterization of *Brassica rapa* ssp yellow sarson mutants. Journal of Oilseed Brassica, 1: 1-6.

Laskar R.A., Khan S., Khursheed S., Raina A. and Amin R. (2015). Quantitative analysis of induced phenotypic diversity in chickpea using physical and chemical mutagenesis. Journal of Agronomy, 14: 102-109.

Malek M.A., Begum H.A., Begum M., Sattar M.A., Ismail M.R. and Rafii M.Y. (2012). Development of two high yielding mutant varieties of mustard [*Brassica juncea* (L.) Czern.] through gamma rays irradiation. Australian Journal of Crop Science, 6: 922-927.

Malek M.A., Rafii M.Y., Afroz S.S., Nath U. and Mondal M. (2014a). Morphological characterization and assessment of genetic variability, character association, and divergence in soybean mutants. The Scientific World Journal, 2014:1-12.

Malek M.A., Rafii M.Y., Samad M.A. and Khatun M.K. (2014b). M8 rapeseed (*Brassica napus* L.) mutants: Evaluation for earliness with higher seed yield. Research on Crops, 15: 797-801.

Mejri S., Mabrouk Y., Voisin M., Delavault P., Simier P., Saidi M. and Belhadj O. (2012). Variation in quantitative characters of faba bean after seed irradiation and associated molecular changes. African journal of biotechnology, 11: 8383-8390.

Monshi F.I. and Malek M.A. (2013). Evaluation of twelve motants of Rapeseed earliness, seed yield and response to changing environments in M6 generation. Bangladesh Journal, 24: 123-130.

Nei M. and Li W.H. (1979). Mathematical model studying genetic variations in terms of restriction endonucleases. Proc. Nat. Acad. Sci., 76: 5269-5273.

Olejniczak J. and Adamska E. (1999). Achievement of mutation breeding of cereal and oilseed crops in Poland. Proc. 3rd Int. Symp. New Genetical Approaches to Crop Improvement. (eds. Arain *et al*.,) Nuclear Institute of Agriculture, Tando Jam, Allied Printing Corporation, Hyderabad, Pakistan, pp. 55-63.

Park D.H., Nam H.G. and Lim P.O. (2007). Gene controlling flowering time and method for controlling flowering time in plants using the gene. U.S. Patent No. 7,217,855. Washington, DC: U.S. Patent and Trademark Office.

Parry M.A., Madgwick P.J., Bayon C., Tearall K., Hernandez-Lopez A., Baudo M. and Labhilili M. (2009). Mutation discovery for crop improvement. Journal of Experimental Botany, 60: 2817-2825.

Patade V.Y., Suprasanna P. and Bapat V.A. (2008). Gamma irradiation of embryogenic callus cultures and *in vitro* selection for salt tolerance in sugarcane (*Saccharum officinarum* L.). Agricultural Sciences in China, 7: 1147-1152

Prevost A., Wilkinson M.J. (1999). A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theoretical and Applied Genetics, 1: 107-112.

Reddy M.P., Sarla N. and Siddiq E.A. (2002). Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. Euphytica, 128: 9-17.

Selvi B.S., Ponnuswami V. and Sumathi T. (2007). Identification of DNA polymorphism induced by gamma ray irradiation in Amla (*Emblica officinalis* Gaertn.) grafts of V1 M1 and V2 M1 generation. Journal of Applied Science Research, 3:1933-1935.

Shah S.A. and Rahman K. (2009). Yield and growth response of rapeseed (*Brassica napus* L.) mutants to different seeding rates and sowing dates. Pakistan Journal of Botany, 41: 2711-2716.

Szarejko I. and Forster B.P. (2007). Doubled haploidy and induced mutation. Euphytica, 158: 359-370.

Tahmasebpour B., Nojadeh M.S., Esmaeilpour M. (2018). Salt Stress Tolerance of Spring Canola (*Brassica napus* L.) Cultivars. International Journal of Plant Biology and Research, 6: 1098.

Tulmann Neto A. and Alves M.C. (1997). Induction of mutations for earliness in the soybean cultivar Paraná. Brazilian Journal of Genetics, 20: 1-10.

Wang P., Zhang Y., Zhao L., Mo B. and Luo T. (2017). Effect of gamma rays on *Sophora davidii* and detection of DNA polymorphism through ISSR marker. BioMed research international, 2017.

Xi M., Sun L., Qiu S., Liu J., Xu J. and Shi J. (2012). *In vitro* mutagenesis and identification of mutants via ISSR in lily (*Lilium longiflorum*). Plant cell reports, 31: 1043-1051.

Yassein A.A.M. and Ali A.A. (2014). Effect of gamma irradiation on morphological, physiological and molecular traits of *Brassica napus*. Egyptian Journal of Genetics And Cytology, 43: 25-38.

Yokoo M. and Okuno K. (1993). Genetic analysis of earliness mutations induced in the rice cultivar Norin 8. Japanese Journal of Breeding, 43: 1-11.

Zietkiewicz E., Rafalski A. and Labuda D. (1994). Genome fingerprinting by simple sequence repeat (SSR) anchored polymerase chain reaction amplification. Genomics, 20: 176-183.

التقييم الحقلي والتحليل الجزيئي لثلاث طفرات مبكرة من نبات الكانولا تحت ظروف إجهاد ملحية طبيعية

محمد سيد حسن[١] و طلعت بشندي[٢]

[١]قسم المحاصيل، كلية الزراعة بقنا، جامعة جنوب الوادي، قنا، مصر.

[٢]قسم الوراثة، كلية الزراعة، جامعة الوادي الجديد، الوادي الجديد، مصر.

يعتبر استحداث الطفرات طريقة جيدة لإنتاج سلالات نباتية جديدة ذات سمات جيدة مرغوبة . لذلك ، أجريت هذه الدراسة لتقييم ثلاث طفرات كانولا ذوات إزهار مبكر مستحدثة بأشعة جاما ، مقارنة بأحد آبائهم (Serw4) و بالصنف (Serw6) ككنترول. حيث تم تقييم بعض الصفات المحصولية في الجيلين M6 و M7 تحت ظروف ملحية طبيعية. وقد أوضحت النتائج أن جميع الطفرات كانت مبكرة و ذات إنتاجية عالية للبذور عن ما هو في الأب والكنترول في كلا الجيلين معاً. ولكن علي عكس ذلك فقد انخفض متوسط الأداء للصفات المدروسة الأخرى بشكل معنوي. علاوة على ذلك، تم تقييم قدرة الطفرات لتحمل الإجهاد الملحي تحت ظروف المعمل من خلال معاملة البادرات بتركيزات مختلفة من كلوريد الصوديوم. و كانت جميع الطفرات هي الأكثر تحملاً للملوحة. وأيضا تم قياس التنوع الوراثي ومدي التشابه بين جميع التراكيب الوراثية بواسطة الواسمات الجزيئية (ISSR). حيث كانت النسبة المئوية لتعدد الأشكال المظهرية ٥٠٪ بينما كانت قيمة أعلي تشابه ٠.٩٠ بين الصنفين Serw4 وSerw6 و كانت أقل قيمة تشابه (٠.٧٩) بين الصنف Serw6 و الطفرة M7-11-4-1 . وعلاوة على ذلك ، فقد قسم التحليل العنقودي وقياس القرابة الوراثية كل التراكيب الوراثية إلى عنقودين رئيسيين، العنقود الأول شمل الأب و الصنف الكنترول، بينما جميع الطفرات تم جمعها في العنقود الآخر. هذه الطفرات المستحدثة المبكرة الإزهار ذوات إنتاجية عالية للبذور و تتمتع بقدرة عالية على تحمل الملوحة ، ومن المتوقع لهذه الطفرات أن تكون سلالات واعدة جديدة.