Scientific J. Flowers & Ornamental Plants
www.ssfop.com/journal
ISSN: 2356-7864 **doi: 10.21608/sjfop.2021.198630**

RESPONSE OF EVENING PRIMROSE (*OENOTHERA* GENUS) TO INDUCED MUTATIONS BY GAMMA IRRADIATION

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ABSTRACT: *Oenothera* was documented by its pharmaceutically active ingredients that require prolonged time to upgrade. Selection of enhanced plants needs high variability. Subsequently, gamma radiation mutagenesis was applied for induction and flaring the genetic variations to get hopeful traits. To examine gamma radio-sensitivity of *Oenothera* species and the extent of induced genetic variability on earliness and morph-agronomic traits in M_1, M_2 and M_3 generations, field experiments were performed through the sequential seasons 2017/2018, 2018/2019 and 2019/2020. Seeds of both species viz. *Oenothera biennis* L. and *Oenothera albicaulis* Pursh were exposed to gamma doses (100, 200 and 300 Gy). The results displayed that both species responded to gamma radiation causing genetic variability reflected on markedly elevation of yield of some selected mutants and earliness in others. Morphological and genetic behaviors altered in both species at all mutagen doses among generations. A steady decline in the survival M_1 plants percent after 25 days of sowing was observed with doses upsurge for both species, *O. biennis* recorded lower response to mutagen doses than *O. albicaulis*. In M_2 generation, coefficient of variation was higher at all doses than the control for both species. Selected mutants significantly enhanced in most studied traits compared to the parallel controls predominantly at 100 and 200 Gy that were more effective on upgrading morpho-agronomic characteristics in M_2 generation for both species. Some mutants exhibited formerly earliness than control in M_2 generation. Moreover, the superior selected mutants were checked in M_3 as 12 mutant lines could be utilized as genetic resources for getting novel varieties with high seed yield, oil and protein percentages. Therefore, exploit gamma mutagens at low and moderate doses; 100 and 200 Gy as effective *Oenothera* breeding strategy to enrich the seed yield and constituents. Besides, extra selection and assess mutant lines under diverse environments at large-scale to attain the uppermost *Oenothera* varieties.

Key words: *Oenothera*, gamma mutagen, genetic variability, morpho-agronomic traits.

Scientific J. Flowers & Ornamental Plants, 8(3):335-352 (2021).

Received:
10/8/2021
Accepted:
25/8/2021

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INTRODUCTION

Oenothera genus belongs to Onagraceae (Oenotheraceae, Order Myrtales) includes about 145 species with 18 sections of herbaceous flowering plants (Agrawal *et al*., 2012). The flowers of many species bloom in the evening therefore it was called evening primrose. Evening primrose species originated in North America are disseminated throughout the world and currently, commonly found in many temperate zones, they thrive on waste ground, especially in dunes and sandy soil, they have a medicinal importance to treat premenstrual problems, inflammations, multiple sclerosis, tumors, diabetes and microbial infections (Munir *et al*., 2017; Bota *et al*., 2020).

Oenothera biennis L. (common evening primrose) is known as medicinal plants as all parts have plenty of pharmaceutically active components like alkaloids, phenolic acids, flavonoids and tocopherols, meantime the seeds have protein, minerals, carbohydrates and vitamins (Timoszuk *et al*., 2018). Seed oil characterized by high gamma-linoleic acid (GLA) and other important secondary compounds, especially ellagitannins and hydrolyzable tannins (Greiner and Köhl, 2014; Lasko´s *et al*., 2021). Evening primrose has yellow lance-shaped leaves in addition to elongated capsule fruits and often purple-tinged hairy stem. It contains four petals and blooms in the evening. It is known as agricultural crop instead of as wild flower and cultivated in gardens as ornamental plants (Abd El-Khalek., 2017). Evening primrose (EP) extract has a protective effect on human skin against oxidative stress (Lee *et al*., 2020). Additionally, seed extract involves polyphenols that inhibit α-glucosidase activity thus EP controls levels of blood glucose, also it has various fatty acids including; linoleic, oleic, γ-stearic and linolenic acid (Asghari, 2019). Furthermore, evening primrose oil (EPO) is used to soften the skin, facial scrubs, combined with vitamin E to prevent oxidation and the oil is applied to cosmetic preparations as EPO is an important source of sterols, polyphenol as well as fatty acids; cis-linoliec (about 70%) and cis-gamma linolenic acids (about 9%) as described by Liu *et al.* (2003) and Sany *et al*. (2021). Unsaturated fatty acid; γ-linolenic acid (GLA) is required for its nutritional and pharmaceutical application (Ghatas and Mohamed, 2020). Additionally, GLA acts as an important mediator in human metabolism and supports in the synthesis of prostaglandins as GLA metabolite (dihomo-γ-linolenic acid; DGLA is oxidized to 15-hydroxyeicosatrienoic acid under the effect of lipoxygenase or, is metabolized to prostaglandins by cyclooxygenase, these compounds act as anti-inflammatory. Consequently, EPO is effective and the plant has a valuable effect in the treatment many diseases including, atopic dermatitis, multiple sclerosis, coronary heart, some types of cancer, autoimmune conditions, premenstrual syndrome and gastrointestinal symptoms as reported by ACS (2009), Mahmoud and Soliman (2017), Timoszuk *et al*.(2018), Asghari (2019) and Bota *et al*. (2020).

Oenothera albicaulis Pursh (whitest evening primrose) is a pubescent annual or biennial herb, self-fertile growing at lower elevations, white petals. The basal leaves can be either entire and oblanceolate or pinnatifid even on the same plant. The plant has an edible seedpod. Moreover, leaves and young shoots could be cooked (Weiner, 1980) and used as a lotion to treat swellings on muscle strains (Moerman, 1998).

Plant breeding is a way to improve horticultural crop by domestication of better phenotypes plus formation of innovative genetic diversity. Morpho-agronomy description of germplasm and new varieties via descriptors plays a major role in breeding programs. Hence, modern breeding tools like mutation were applied that lead to horticultural crop improvement which is mainly constrained by the genetic basis of cultivated materials. Genetic variation for desirable characters can be stimulated through mutation breeding method and obtain mutants with preferred traits then selection in consecutive generations (Dewanjee and Sarkar, 2017). Nearly 90% (64% with gamma rays and 22% with X-rays) of the resulted mutant cultivars were obtained with radiation application (Jain, 2010).

Gamma rays are high energy electromagnetic ionizing radiation emitted in the excitation of the atomic nucleus (Koornneef, 2002), ionized radiation approach has a major role in breeding practices affected plants development with supreme features (Kharkwal, 2012). Gamma rays are the most broadly used as physical mutagens utilized to produce the desired genetic variability (Mba and Shu, 2012; Kolakar *et al*., 2018). Gamma irradiation is widely used to artificially induce mutations that may produce a new genetic variability represent a perquisite for selection in breeding (Anne and Lim, 2020). Dose rate effects are species-dependent, thus influencing on the possibility of inducing desirable parameters. Mutagenesis may alter the number of biochemical processes leading to beneficial genotype changes, thus obtaining plant varieties with high-quality.

The main goal of this work is to examine gamma radio-sensitivity of *Oenothera* species, get improved *Oenothera* by customizing specific features to increase the preferred attributes through induction of new genetic variability for yield, oil and protein content in the parental lines by subjecting *Oenothera* seeds to the optimal dose of gamma irradiation and from better mutants, new lines is further selected to get new cultivar adjusted to environmental conditions of Egypt.

MATERIALS AND METHODS

Field experiments were performed at a private farm at Mit Khamis, Dakahlia Governorate, Egypt and laboratories of Medicinal and Aromatic Plants Res. Depart., Horticulture Res. Inst., Agric. Res. Center, Giza, Egypt. Seeds of evening primrose (*O. biennis* L. and *O. albicaulis* Pursh) acquired from Vegetable Crop Seed Production and Technology Department, Horticulture Research institute, ARC, Giza, Egypt were irradiated by different gamma doses; 100, 200 and 300 Gy through ^{60}Co source at Cyclotron Department, Nuclear Research Center, Atomic Energy Authority, Anchas, Sharkia Governorate, Egypt at dose rate of 1.74 Gy/min.

The study was implemented during the three consecutive seasons of 2017/2018, 2018/2019 and 2019/2020 including two phases; 1st phase initiated from October 2017 to check the induced mutation by gamma irradiation and the 2nd one from October 2018 to 2020 to distinguish the obtained mutants and select preferred plants in the subsequent generations. The trial included 4 treatments for each *Oenothera* species which were non treated seeds (control) and three gamma irradiated treatments (410 seeds each). On 19th October, irradiated seeds were sown along with the parental controls in well prepared germination trays with a mixture of vermiculite, peat moss and sand (1:1:1) afterwards, the seedlings were transplanted into the permanent field at 50 cm×50 cm plant spacing, the percentage of the survived M_1 plants was estimated after 25 days of sowing as;

The survived M_1 plants % = (number of survived plants/number of sown seeds) ×100.

The experimental soil was clayey-loam texture with EC of 0.79 dSm^{-1}; pH of 7.83 and organic matter content of 1.76% analyzed according to Jackson (1973) and Black *et al.* (1982). Cultural practices were performed as recommended.

The experimental design was a complete randomized block and a descriptive statistical analysis of the studied traits was carried out for each treatment by recording the mean, minimum and maximum values (Range of variability) as well as the coefficient of variance (CV) percentage using appropriate statistical programs (SPSS, Excel).

The seeds of each selected M_1 plants were separately collected to create M_2 generation which grown in growing trays then the seedlings were planted in the open field. Seeds of M_2 obtained from individual M_1 were sown on October 2018 as plants consistent with vigorous growth, number of days till flowering, seed yield were individually compared along with the parents and during the growth period, mutations were characterized and mutant lines were selected and the results were statistically analyzed at each irradiation dose for both species. For further selections M_3 seeds were collected from mutant lines from selfpollinated M_2 plants and sown on October 2019 and the superiority traits viz. plant height (cm), number of branches/plant, number of capsules/plant, number of seeds/capsule, weight of 1000 seeds and duration to 1st flower appearance were estimated.

The oil was extracted by N-hexane solvent at 50–60 °C for 6 h from the crushed and ground seeds of *Oenothera* species in Soxhlet apparatus following the AOCS method (1994). The percentage of total protein in dry seed was evaluated using Kjeldal method according to A.O.A.C. (1995). Data were statistically analyzed in M_1 and M_2 using SPSS and Excel programs calculating the maximum and minimum values (Range of variability), mean, and the percentage of coefficient of variation (% CV). Duncan's multiple rang test was used for the comparison between treatment means (Leclarg *et al.,* 1962).

RESULTS AND DISCUSSION

Sensitivity of *Oenothera* sp. to gamma radiation doses:

1. The percentage of survival of M_1 plants:

As shown in Fig (1) the percentage of survival M_1 plants of both *Oenothera* species recorded the drastic decrease due to the effect of all the mutagenic treatments. The plants of *O. albicaulis* species was more sensitive to gamma radiation as under higher doses of gamma irradiation (300 Gy) showed undesirable effect as the survival percentages were reduced to 55.61 and 50.24% for *O. biennis* L. and *O. albicaulis* Pursh, respectively in relation to the maximum survival percentage observed at the corresponding controls treatments. On the other hand, the lowest dose 100 Gy had the lowest effect gave the survival percentage 91.41 and 89.99% in respective to *O. biennis* and *O. albicaulis* along with control treatments. Mutagens inhibitory effect may be attributed to adversative result on enzymatic activity decrease, vigor decline and losses of membrane integrity (Khan and Goyal, 2009), interference in the synthesis of enzyme and acceleration in enzyme degradation (Yusuf and Nair, 1974). Additionally, mitotic cycles and metabolic pathways aberrations influence on emergence of seedlings (Micco *et al*., 2011). From the recorded data, it was noted that higher doses of gamma radiation reduced the survival *Oenothera* plants in the first mutagenic generation; these are in covenant with those of Bashir *et al*. (2013) who correlated the percent survival decrease with an increase in the mutagens doses and their effect on biological damage. Verma *et al* (2017) found that the percentage of survived seedlings decreased with the increase of gamma doses and at the doses of 400 and 500 Gy, there were no survived seedlings. Pramanik *et al* (2018) stated that reduction in seed germination rate, seedling growth and other physiological and cytological disturbances were induced by gamma irradiation.

2. Duration to 1st flower appearance:

The 1st mutagenic generation (M_1):

Results presented in Table (1) indicated that all radiation doses provided variations on earliness of both *Oenothera* species in M_1. Selected plants through the first mutagenic generation showed differences in number of days till first flower appearance as some of them gave earliness nearly two weeks in *O biennis* and 4 days in *O. albicaulis*, other mutants exhibited an increase in number of days till flowering about ten days and one week in respective to *O. biennis* and *O. albicaulis* reliable to controls. Mostly, all doses adversely affected the earliness in the first mutagenic generation as the shortest period to 1st flower appearance was recorded at the control treatments of both species, while all radiated treatments increase the duration through the first mutagenic generation particularly the doses 200 and 100 Gy for *O. biennis* and *O. albicaulis*, respectively to extent 80.6 and 64.9 days as the maximum mean values of number of days till flowering.

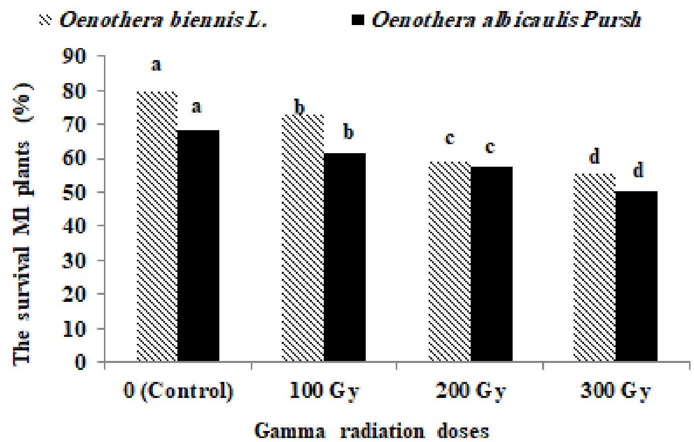

Fig. 1. Gamma radiation doses effect on the percentage of the survival M_1 plants.

Table 1. Gamma radiation doses effect on duration to 1st flower appearance of *Oenothera* sp. through M_1 and M_2 generations.

Species	Gamma doses	M_1 Max.	M_1 Min.	M_1 Mean	M_1 CV %	M_2 Max.	M_2 Min.	M_2 Mean	M_2 CV %
		Duration to 1st flower appearance (days)							
O. biennis	**Control**	75.0	67.0	69.9 c	1.17	77.0	69.0	73.7 a	1.75
	100 Gy	77.0	70.0	75.4 b	1.45	71.0	60.0	66.2 b	10.35
	200 Gy	84.0	78.0	80.6 a	3.92	80.0	59.0	64.1 c	9.32
	300 Gy	85.0	52.0	76.5 b	9.88	82.0	69.0	75.3 a	9.04
O. albicaulis	**Control**	59.0	54.0	56.2 d	1.28	61.0	54.0	59.9 a	1.13
	100 Gy	66.0	59.0	64.9 a	2.70	61.0	40.0	49.7 c	12.22
	200 Gy	59.0	55.0	57.4 c	14.38	62.0	39.0	51.3 c	7.52
	300 Gy	62.0	50.0	59.4 b	12.72	63.0	47.0	55.4 b	9.16

Means with the same letter are not significantly different at the 5% level.
Max: maximum value, Min: minimum value, CV: coefficient of variation

Table (1) showed that the coefficient of variation percentage (CV %) under all radiation doses was higher over those under control treatments in both *Oenothera* species particularly at radiation doses 300 and 200 Gy that induced the relative variability about 8.44 and 11.23 % related to the CV percentage of the control. The variability on number of days till flowering in the first mutagenic generation may be due to the high doses of gamma radiation.

The 2nd mutagenic generation (M_2):

The same table showed that gamma radiation doses presented affirmative effect on the duration to first flower through the second mutagenic generation plants that differ in their genetic variability from the first mutagenic generation plants for both *Oentohera* species. It is clear that mean values of number of days till first flower were decreased to 64.1 days at 200 Gy dose for *O. biennis* compared to the control treatment recorded 73.7 days while, 100 Gy dose displayed the positive result reached to 49.7 days in relation to the parallel control 59.9 days for *O. albicaulis* without significant differences between 100 and 200 Gy doses. All doses exhibited genetic variability through M_2 generation, some selected mutants in M_2 generation flowered early about 10 and 15 days before the control predominantly at 200 Gy dose for both *Oentohera* species while, other mutants performed longer duration till flowering at the same dose and 300 Gy dose compared to the control. These results coincide with those of Jagajanantham *et al*. (2012) on *Abelmoschus esculentus* (Bhendi) who showed that increasing gamma levels affected number of days required to first flower; Moualla *et al*. (2012) who reported that some mutants were entirely matured nearly 3 weeks before control at 150 Gy dose through M_2 generation on *Glycine max* L. The lowermost CV percentage was shown through the second mutagenic generation at the control treatments and those of all examined doses caused the genetic variability thus CV of them were greater several times particularly at 100 Gy dose than CV percentage of the control for both *Oenothera* sp. Thus, doses of gamma mutagen created and widened the variability of number of days till first flower so it will allow selecting early mutants in the consequent generations.

3. Morpho-agronomic characteristics:

a. Morphometric characteristics:

The 1st mutagenic generation (M_1):

The significances of variance obtained from the analysis of variance (ANOVA) for some morphological traits in terms of plant height and number of branches per plant in M_1 and M_2 generations were displayed in Table (2). Results revealed that both *Oenothera* species markedly varied in their response to gamma doses treatments that gave highly significant variations and malformations on the height of M_1 plants and number of branches/plant. The plant height at the highest dose 300 Gy was decreased by 19.61 and 35.55% in respective to *O. biennis* and *O. albicaulis* related to parallel controls. Many alterations appeared during M_1 generation, some mutants recorded tallest plants and others gave dwarf ones. Thus, for both species high dose 300 Gy of gamma mutagen gave destructive results on the morphological characteristics; these effects were similar to those recognized by Wi *et al*. (2006), Norfadzrin *et al.* (2007), Warghat *et al.* (2011), Fayad *et al.* (2020). Regarding CV percentages, all radiated treatments have high variability, so CV % was greater than those at control treatments for both species. These results might be owing to that low and moderate doses of gamma mutagen enhanced growth traits due to the cell division stimulus and metabolic modifications causing the synthesis of growth phytohormones (Latif *et al*., 2011; Tshilenge-Lukanda *et al*., 2013) while, high doses of gamma mutagen causes apical meristem destruction, also inactivation of growth regulators (Patel and Saha, 1974), increase of active radicals (Selim *et al*., 1974), temporary delay of cell division (Reddy and Vidyavathi, 1985; Wi *et al*., 2005) and the structurally DNA and enzyme alterations (Danylchenko and Sorochinsky, 2005; Alikamanoglu *et al*. 2007). Gamma irradiation influences the plant development by inducing cytological, physiological and morphological modifications in plant cells (Beyaz and Yildiz, 2017). As a result, the plant height in M_1 generation decreased because of such above alterations resulted from the higher doses of gamma mutagen.

Table (2) exhibited that all gamma doses influenced the formation of branches number of both *Oenothera* species and gave highly variations with no significant differences between doses 100 and 200 GY of gamma mutagen in *O. biennis*. Number of branches per plant was declined by increasing gamma doses as the extreme number was obtained from the control treatment in *O. biennis* and the significant reduction in the number of branches/plant through M_1 generation was noted at 300 Gy, the reduction percent reached 56.77%. The high mutagen doses had deleterious effect on vegetative characters maybe due to enzymatic disorders affecting the growth (Abd El-Rahman, 2000). Moreover, the difficulties in creation of RNA and protein resulted from the negative effect of gamma radiation, the number of branches might be decreased (Bajaj, 1970). Also, the harmful effect of mutagen on cellular homeostasis and highly reactive oxygen species (ROS) were produced causing cell death (Sharma *et*

Table 2. Gamma radiation doses effect on morphometric characteristics of *Oenothera* sp. through M_1 and M_2 generations.

Species	Gamma doses	M1				M2			
		Max.	Min.	Mean	CV %	Max.	Min.	Mean	CV %
					Plant height (cm)				
O. biennis	**Control**	155.00	138.00	147.78 a	18.87	167.00	150.00	153.44 c	7.81
	100 Gy	146.00	138.00	141.00 b	31.99	167.00	156.00	159.11 b	26.70
	200 Gy	138.00	127.00	138.95 c	22.39	170.00	161.00	168.20 a	27.12
	300 Gy	124.00	96.00	118.81 d	31.02	174.00	169.00	171.08 a	31.03
O. albicaulis	**Control**	37.00	23.00	30.69 b	12.30	37.00	28.00	31.05 c	6.22
	100 Gy	40.00	16.00	21.99 c	19.79	46.00	36.00	39.76 b	16.49
	200 Gy	51.00	25.00	34.04 a	18.76	50.00	35.00	43.18 a	16.05
	300 Gy	33.00	18.00	19.78 c	31.53	48.00	30.00	43.27 a	30.83
					Number of branches/plant				
O. biennis	**Control**	7.00	4.00	6.80 a	9.18	9.00	3.00	7.08 c	8.12
	100 Gy	5.00	3.00	4.16 b	9.98	13.00	5.00	8.13 b	14.66
	200 Gy	6.00	2.00	4.03 bc	12.36	14.00	9.00	12.05 a	12.17
	300 Gy	5.00	2.00	2.94 c	17.9	16.00	8.00	12.99 a	13.24
O. albicaulis	**Control**	15.00	9.00	11.71 b	7.28	14.00	8.00	10.67 c	7.19
	100 Gy	14.00	9.00	11.71 b	30.13	12.00	7.00	8.71 c	21.41
	200 Gy	20.00	12.00	18.90 a	15.44	18.00	15.00	17.66 a	14.62
	300 Gy	19.00	14.00	17.52 a	21.05	18.00	12.00	15.43 b	7.79

Means with the same letter are not significantly different at the 5% level.
Max: maximum value, Min: minimum value, CV: coefficient of variation

al., 2012). On the other side, many variations were observed in *O. albicaulis* and the treatment of 200 Gy dose exhibited the greater effect causing the highest branches number. All treatments had coefficient of variations higher than those in the control treatments, thus genetic variability in the plant height and branches number were created by gamma radiations.

The 2nd mutagenic generation (M_2):

As evident in Table (2), gamma radiation doses significantly induced genetic variability over the control as the range limits (maximum - minimum) of variability, mean values and coefficient of variation in the radiated plants were greater than those of the control. All radiation doses resulted higher genetic segregations through M_2 than those in M_1 particularly the selected plants at 200 and 300 Gy with no significant differences in-between as the mutants reaches the height in respective 168.20 and 171.08 cm for *O. biennis*, 43.18 and 43.27 cm for *O. albicaulis*. Hussein *et al.* (1974) showed that the plant height was influenced by gamma radiation and variability stimulation in M_2 generation may be as a result of the genetic variations.

Table (2) indicated that genetic variations in M_2 generation are differed from those in M_1 generation in branches number per plant in response to doses of gamma. All radiation doses exhibited optimistic effect on number of branches for both *Oenothera* species as a coefficient of variation (CV %) folded several times in the radiated plants related to the control one. The radiated treatments widened the range of variability and CV percentages in the plant height and branches number compared to those in control treatments that could permit to select the desirable mutants. The mean number of branches at radiation dose 300 Gy was significantly higher compared with the other treatments, while, the differences between 200 and 300 Gy was not significant and the increasing percentage extents to 70.20 and 83.48%, respectively compared to the corresponding control in *O. biennis*, while gamma radiation dose at 200 Gy had stimulation effect with increasing percentage 65.51% over the control in *O. albicaulis*.

It is clear that mutagenic treatments were able to generate and extent an extra variations on plant height and number of branches/plant in the M_2 generation of *Oenothera* sp. Variations and the mean values in most morphological traits were increased by gamma radiation, the range of variability and the CV percentage of treated plants particularly in M_2 generation were greater several times than those in the control. The same results were attained with Mullainathan and Aruldoss (2015) on *Capsicum annuum* L who obtained some distinctive mutants involving tallest plants. Gamma irradiation at high doses have inhibitory effect, while lower doses stimulate germination, plant gridgeth, crop yields and stress resistance (Ali *et al*., 2017). Morphological characters of *Pisum sativum* L were conspicuously affected in M_2 generation by levels of gamma mutagen (Khan *et al*., 2018). Additionally, Masry *et al*. (2019) who concluded that 20 and 30 Kr doses of gamma radiation induced a considerable variability over control treatment regarding the plant height and number of branches. Fauziah *et al*. (2020) concluded that 1000 Gy dose successfully improved morpho-agronomy of black soybean. While, Labrada *et al*. (2001) conveyed that the increase of radiation dose causes a gradual reduction for all growth traits such as plant height and length of panicle.

b. Agronomic characteristics:

The 1st mutagenic generation (M_1):

Yield mainly reflects all biological progressions in the course of plant growth and development. Table (3) showed some agronomic traits viz. number of capsules per plant, number of seeds per capsule and weight of 1000 seeds of both *Oenothera* species subjected to different doses of gamma mutagen through the first and second mutagenic generations. Concerning the number of capsules per plant in response to gamma treatments, results showed that all doses confidently influenced this trait and the genetic variability through the first mutagenic generation was induced under all gamma doses over the control mainly at 200 Gy dose as some selected plants had extreme capsule number (267 capsules per plant) while others gave 143 capsules per plant with mean value 248.2 capsules per plant compared to the parallel control for *O. biennis*. On the other hand, both 100 and 200 Gy doses created genetic variability for *O. albicaulis* with mean value without significant differences in between recording 297.3 capsules per plant at 100 Gy dose greater than those at control treatment 250.9 capsules per plant. All doses of gamma mutagen caused genetic variability by providing highly CV percentage through M_1 in order 300> 200 >100 Gy than those of control treatments for *O. biennis* and the gamma dose of 300 Gy followed by100 and 200 Gy for *O. albicaulis*.

Regarding the number of seeds per capsule as shown in Table (3), both *Oenothera* species were sensitive to all gamma doses that exhibited constructive effect on number of seeds per capsule through M_1 showing mutants related to this trait, number of seeds per capsule as in some selected mutants extremely increased to reach 362 seeds per capsule at 100 Gy dose while others provided 209 seeds per capsule. Mean values of gamma mutagen at 100 and 200 Gy doses were higher in respective of 285.4 and 296.4 seeds per capsule than those at the control treatment recording mean value 228.3 seeds per capsule for *O. biennis* while, the maximum mean value was 253.9 seeds per capsule under the effect of 200 Gy dose against the control of 198.5 seeds per capsule for *O. albicaulis*. High percentage of CV at all examined gamma doses was greater than the moderate CV percentage of the control reflecting the genetic variability for both species. The same results were stated by Mullainathan and Aruldoss (2015) on *Capsicum annuum* L and Masry *et al*. (2019) on *Pisum sativum* L., they reported that many characteristic mutants were obtained involving mutants with high number of pods under levels of gamma radiation.

Table (3) showed that the mean values were shifted at gamma mutagen doses to negative trend for weight of 1000 seeds trait except 200 Gy dose. Gamma doses providing many genetic variations through the first mutagenic generation mainly at 200 Gy dose that realized positively effect on weight of 1000 seeds for both species as some selected plants provided the heaviest weight of 1000 seeds reached to 0.779 and 0.783 g compared to the resultant control 0.717 and 0.742 g while, other mutants gave 0.438 and 0.511 g for *O. biennis* and *O. albicaulis*, respectively. On the other hand, the treatments of 100 and 300 Gy doses negatively affected this trait providing mean values of 0.486 and 0.535 g lower than that on the control (0.578 g) for *O. biennis*, while they gave 0.666 and 0.653 g in relation to the control of 0.707 g for *O. albicaulis*. Radiation doses convinced genetic variability on weight of 1000 seeds trait and exhibited moderate and high coefficient of variability for *O. biennis* and *O. albicaulis*. The CV percentage under doses of gamma mutagen was greater than that of the control. Hence, gamma radiation wide-ranging morphology and physiology of the plant through formation of radicals consistent with the gamma radiation doses (Kiong *et al.,* 2008). Majeed *et al.* (2010) on *Lepidium sativum* L. stated that gamma irradiated seeds at 20-80 Kr doses provided significantly increases in growth parameters and vice versa at high doses. Also, Moussa and Abdul Jaleel (2011) on *Trigonella foenum-graecum* L. reported that the effective dose of gamma radiation was 150 Gy recording the superiority results on morphological parameters.

Table 3. Gamma radiation doses effect on some agronomic traits of *Oenothera* sp. through M_1 and M_2 generations.

Species	Gamma doses	M_1 Max.	M_1 Min.	M_1 Mean	M_1 CV %	M_2 Max.	M_2 Min.	M_2 Mean	M_2 CV %
				Number of capsules/plant					
O. biennis	**Control**	256	208	215.6 d	11.64	259	201	223.2 c	8.04
	100 Gy	261	194	240.9 b	28.71	499	80	248.9 b	40.15
	200 Gy	267	143	248.2 a	36.53	383	77	261.2 a	36.98
	300 Gy	252	130	226.7 c	46.45	504	43	269.5 a	34.25
O. albicaulis	**Control**	288	155	250.9 c	14.26	299	204	285.1 c	7.48
	100 Gy	378	207	297.3 a	25.25	494	92	280.7 c	47.57
	200 Gy	354	189	296.7 a	24.07	602	95	341.9 a	53.08
	300 Gy	300	103	253.4 b	38.17	643	114	299.4 b	39.90
				Number of seeds/capsule					
O. biennis	**Control**	269	212	228.3 b	9.43	261	217	235.2 c	7.02
	100 Gy	362	209	285.4 a	23.19	485	104	253.4 b	39.1
	200 Gy	345	208	296.4 a	31.21	450	98	271.5 a	49.7
	300 Gy	355	100	233.8 b	36.40	451	69	251.4 b	40.5
O. albicaulis	**Control**	263	142	198.5 b	10.45	272	178	221.3 c	6.22
	100 Gy	314	160	241.4 a	43.37	325	103	238.8 b	43.91
	200 Gy	405	160	253.9 a	25.41	418	67	261.7 a	45.18
	300 Gy	338	102	206.8 b	34.52	360	113	239.5 d	36.75
				Weight of 1000 seeds (g)					
O. biennis	**Control**	0.717	0.419	0.578 b	13.78	0.679	0.564	0.647 b	5.85
	100 Gy	0.710	0.395	0.486 d	18.07	0.791	0.575	0.609 c	6.95
	200 Gy	0.779	0.438	0.699 a	18.98	0.981	0.599	0.754 a	19.82
	300 Gy	0.690	0.409	0.535 c	12.54	0.767	0.489	0.603 c	7.51
O. albicaulis	**Control**	0.742	0.685	0.707 a	8.12	0.794	0.680	0.757 b	11.36
	100 Gy	0.761	0.549	0.666 b	31.60	0.869	0.575	0.686 c	19.18
	200 Gy	0.783	0.511	0.698 a	40.65	0.921	0.641	0.846 a	24.33
	300 Gy	0.765	0.495	0.653 c	37.51	0.873	0.399	0.599 d	37.01

Means with the same letter are not significantly different at the 5% level.
Max: maximum value, Min: minimum value, CV: coefficient of variation

The 2nd mutagenic generation (M_2):

Data presented in Table (3) indicated that various segregations were recorded in both the first and second mutagenic generations due to the sensitivity of *Oenothera* species to gamma mutagen. It is clear that during the second mutagenic generation, some mutants were selected that providing the highest number of capsules per plant; 504 and 643 capsules per plant under the effect of 300 Gy dose in respective for *O. biennis* and *O. albicaulis*, and other selected plants recorded 43 and 114 capsules per plant in that order. Gamma doses recognized the extreme effect through M_2 generation on number of capsules per plant over the control. The mean values were greater under the gamma radiation doses and the treatments may be ordered as 300 or 200 > 100 Gy dose greater than the control for *O. biennis* and 200 > 300 > control or 100 Gy for *O. albicaulis*. The mean values; 261.2 and 269.5 capsules per plant at 200 and 300 Gy dose showed positive increase without significant differences in between while all examined doses exhibited significant increase compared to the control that extent to 223.2 capsules per plant for *O. biennis* and the mean value at 200 Gy dose providing highly promotion reached to 341.9 capsules per plant in relation to the control recording 285.1 capsules per plant for *O. albicaulis*.

All gamma radiation doses recorded high levels of coefficient of variation in both *Oenothera* species compared to low coefficient of variation for the control treatment. As shown in Table (3) the percentage CV of all the examined doses was greater than CV percentage of the analogous controls. Similar results have been conveyed by Tshilenge-Lukanda *et al*. (2013) on *Arachis hypogaea*. Hanafy and Akladious (2018) who reported that 100 Gy dose gave the greatest result on *Trigonella foenum-graecum*.

From the same Table, it can be found that at all studied radiation doses during M_2, much genetic segregation reflecting on number of seeds per capsule were found that lead to high variability in terms high range and coefficient of variation consequently an increase in desirable traits selection. The most effective dose on this agronomic character was 200 Gy dose for both *Oenothera* species recording a significant increase in the mean values that prolonged 271.5 and 261.7 seeds per capsule in relation to the control that donated mean values extended to 235.2 and 221.3 seeds per capsule for *O. biennis* and *O. albicaulis*. Some selected plants possessed the highest number of seeds 485 seeds per capsule and others gave 104 seeds with the same treatment (100 Gy) dose related to the comparable controls; 261 and 217 seeds per capsule of *O. biennis*. Furthermore at 300 Gy dose some mutants were obtained providing greater number of seeds per capsule (451 seeds) than control (261 seeds per capsule) for *O. biennis*. However, at the doses 200 and 300 Gy, some mutants produced the uppermost number of seeds per capsule gotten 418 and 360 seeds against 272 seeds in the control treatment of *O. albicaulis.* As general there were highly variations between both species at all levels of gamma mutagen on agronomic traits as the coefficient of variation was larger many times than those at control for both species. Hanafy and Akladious (2018) concluded that low doses ranged from 25 to 200 Gy gave the stimulatory effect concerning the number of pods/plant in M_1 generation while, the high dose of gamma mutagen at 400 Gy in M_1 and M_2 showed inhibitory effect. However, regarding the number of seeds per pod the obtained results revealed no obvious upsurges.

The averages of 1000 seeds weight (g) of *Oenothera* during M_2 generation were evaluated under gamma radiation doses. Results in Table (3) showed that through progenies of radiated treatments, the supreme yielding mutants were recognized. Much genetic segregations on this important agronomic trait at all studied doses for both *Oenothera* species were resulted.

For *O. biennis* some selected plants produced superlative weight reached 0.981g related to the control (0.679 g) and other mutants gave 0.599 g greater than the parallel control 0.564 g at the dose 200 Gy. Likewise, other mutants were selected at 100 or 300 Gy doses realized heavier seeds in respective 0.791 and 0.767 g than the control. For *O. albicaulis,* the selected mutants concerning the weight of 1000 seeds produced 0.921 g at 200 Gy dose compared the control that reached 0.794 g and other mutants were obtained at 300 and 100 Gy doses recording in that order 0.873 and 0.869 g compared the control that produced 0.794 g. It is clear that throughout M_2 the mean values were shifted at radiation doses to negative direction for this trait except 200 Gy that performed affirmative result on the trait of 1000 seeds weight for both *Oenothera* species so the plants recorded the highest mean values of this trait recording 0.754 and 0.846 g in respective for *O. biennis* and *O. albicaulis* compared to the control providing 0.647 and 0.757 g. As regards the average of 1000 seeds weight in most plants, the radiation doses at 100 and 300 Gy displayed adverse results and the mean values decreased in both *Oenothera* species. The percentage of CV at all the examined doses was higher than those at the control, thus all doses create genetic variability and the highest CV percentage was 19.82% at the dose 200 Gy compared to the control 5.85% in *O. biennis*. While the highest CV percentage was observed at 300 Gy dose producing 37.01% followed by 24.33% at 200 Gy dose then 19.18% compared to the control 11.36% at 100 Gy dose in *O. albicaulis* consequently, these doses are crucial to induce the genetic variability vital to selection of the desirable mutants of these traits.

Gamma radiation enhanced agronomic characteristics through the second mutagenic generation contingent to the used doses of radiation. Similar results were found by Khan *et al*. (2005) who stated that 600 Gy gave the highest yield of *Pisum sativum* L. whereas, Mudibu *et al*., 2010 showed that gamma mutagen dose at 200 Gy provide a significant increase of *Glycine max* L. yield. While, El-Degwy (2013) stated that M_1 and M_2 generations were influenced by gamma radiation in most traits except the weight of 1000-grain. Furthermore, yield attributes of *Pisum sativum* L. were prominently influenced in M_2 generation by gamma mutagen (Khan *et al*., 2018).

The 3rd mutagenic generation (M_3) for induced mutant lines:

According to the superiority of morpho – agronomic traits, 12 mutant lines were selected. Some morpho-agronomic traits and percentages of oil and protein content of the selected mutant lines compared to the original parent lines through the third mutagenic generation for both *Oenothera* species were significantly varied as shown in Table (4). Mostly, selected lines compared to the parental controls exhibited significant differences between the mean values for all studied parameters. On the topic of *O. biennis* the shortest duration till first flower appearance was provided from mutant lines 6 and 2 obtained from the radiated treatments at 200 and 100 Gy doses associated with significant increases on number of capsules per plant and high seed yield and oil %, while those promotion results were accompanied with a decrease of protein percent from mutant line number 6 resulted from the treatment at 200 Gy dose. The elevated plants were observed in selected mutant line number 5 induced as a result of 200 Gy dose that presented the superlative effect on seed yield and number of capsules per plant. The superiority in branches number per plant was recognized in mutant lines 6 and 2 as the increments extended to 92.49 and 87.95%, respectively compared to the original line. High seed oil percent was obtained from the mutant lines 3 and 6 resulted from the treatment at 200 Gy dose. While, the significant differences among mutant lines in relation to the parental control regarding the percentage of protein were showed in mutant lines 4 and 1 created from the treatments at 200 and 100 Gy

Table 4. Oil and protein percentages of *Oenothera* seeds and some morpho-agronomic traits of induced mutants (12 selected mutant lines) compared to the parental lines in M3.

Species	Gamma doses	Mutant line number	Duration to 1st flower appearance (days)	Plant height (cm)	No. of branches/ plant	No. of capsules/ plant	Seed yield (g/plant)	Oil (%)	Protein (%)
O. biennis	Control		67.11 b	154.97 de	7.72 c	216.30 d	19.39 d	15.73 f	12.99 ef
	100 Gy	1	68.90 a	160.50 c	13.20 a	229.70 c	23.08 c	15.88 ef	17.25 b
		2	59.01 e	148.32 f	14.51 a	234.75 b	23.71 c	16.05 e	14.01 c
	200 Gy	3	67.62 b	159.52 cd	6.08 c	219.40 d	19.73 d	24.18 a	14.02 c
		4	68.33 a	151.44 ef	10.36 b	229.61 c	22.24 cd	15.94 e	17.54 a
		5	65.20 c	176.12 a	10.52 b	243.52 a	31.77 a	16.25 d	13.09 e
		6	58.17 f	168.03 b	14.86 a	240.25 a	28.19 b	23.41 b	12.97 f
	300 Gy	7	60.42 d	157.12 cd	6.74 c	216.40 d	20.06 d	16.66 c	13.20 d
O. albicaulis	Control		55.61 a	37.57 bc	10.18 b	269.42 d	21.03 d	16.33 d	12.08 de
	100 Gy	1	56.90 a	36.16 c	11.34 b	275.33 cd	24.93 c	19.45 b	15.16 b
		2	48.33 c	39.40 b	8.75 c	269.91 d	20.72 d	22.18 a	18.16 a
	200 Gy	3	52.42 b	48.12 a	10.63 b	290.30 b	34.54 a	17.91 c	11.97 e
		4	50.17 bc	37.91 bc	18.98 a	309.40 a	28.61 b	16.05 d	12.59 c
		5	57.95 a	39.22 b	11.34 b	281.24 c	27.27 b	17.63 c	12.25 d

Means with the same letter are not significantly different at the 5% level.

doses, respectively. Instead, for *O. albicaulis*, the dominance in the plant height and branches number were noted in mutant lines 3 and 4 induced at 200Gy dose, respectively with a significant decrease in number of days till the first flower appearance that distinguished in mutant lines 2 and 4 induced at 100 and 200 Gy doses. The significant increase in number of capsules per plant was obtained from mutant line number 4 at 200 Gy dose compared to the original line and this enhancement correlated to the significant increase in seed yield and the supreme yield was prominent in mutant line 3 induced at 200 Gy dose. The selected mutants with significant enhancement in seed oil and protein percentages were gained from mutant line 2 due to the effect of 100 Gy dose compared to those from the parental line. Youssef and Moussa (1998) on *Chamomilla recutita* L; Rahimi and Bahrani (2011) on *Brassica napus* L, they showed that oil % and composition were affected by gamma radiation. Hussein (2010) stated that gamma radiated seeds ranged from 50 to 250 Gy improved protein contents of mungbean. Moussa and Abdul Jaleel (2011) on *Trigonella foenum-graecum* L. resulted that the total protein was increased at gamma dose of 150 Gy. Moemen (2012) on *Ambrosia maritima* L stated that gamma mutagen at 10 and 20 Gy doses created higher concentration of protein than the control. Accordingly, the radiated treatments generate mutations allowing for selecting the promising traits.

These results are in line with those reported by Malek (2012) on mustard variety BARI sarisha-11 who stated that the selected plants from M2 generation and 10 breeding mutants with higher seed yield desirable traits were confirmed in M4 generation. Furthermore, selected mutant lines might be appreciated platform to improve genetic and breeding programs of *Abelmoschus esculentus* L. in Egypt as reported by Jadhav *et al*. (2013) and Fayad *et al*. (2020).

CONCLUSION

Both *O. biennis* and *O. albicaulis* responded to mutagenesis involving phenotypic evaluation from M1 to M3 generations thus permitted to select hopeful mutant lines. All studied doses (100, 200 and 300 Gy) in M_1 generation produced malformations such as dwarf plants, No. of lateral shoots decline, duration till flowering upturn and many variations and vice versa in M_2. During the consecutive generations (M_1 and M_2), all mutagen doses induced the extreme variability particularly in M_2 in which the most promising traits were selected. Depending on the mutagen doses; for obtaining the tallest plants 200 Gy or 300 Gy doses can be applied in both *Oenothera* species in M_2 while, the most effective doses regarding branches number were 200 or 300 Gy dose in M_2 for *O. biennis* while, 200 Gy dose in both generations (M_1 and M_2)for *O. albicaulis*. However, 100 and 200Gy doses for earliness in M_2 and enhancement of yield in both generations. In consequence, gamma mutagen induced much genetic variability contingent on doses and useful lines were selected reflecting on enrichment some morpho-agronomy traits and earliness. It could be concluded that using gamma mutagen at low and moderate doses (100 and 200 Gy) for augmenting the percentage of oil, protein and yield in both species. Also, more selection and further improvement were required to examine mutant lines under diverse environments across-the-board to obtain new improved *Oenothera* varieties. Thus the genetic variability and selected mutant lines are appreciated platforms for upgrading the genetic and breeding approaches of *Oenothera*.

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استجابة نبات زهرة الربيع المسائية (جنس الاونثرا) للطفرات المستحدثة بالاشعاع الجامى

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تكمن أهمية نبات زهرة الربيع المسائية (الاونثرا) في احتوائه للمكونات الفعالة والهامة دوائيا، ونظراً لأن زيادة مثل هذه المواد يستغرق وقتا طويلاً كما يلزم أن يكون التباين بدرجة عالية عند الإنتخاب لإقتناء نباتات بالمواصفات الجيدة لذا دعت الحاجة إلى احداث التباين الوراثي عن طريق الطفرات بالاشعاع الجامي الفعال لزيادة واتساع قاعدة التنوع الوراثي الهام والوصول للصفات المأمولة لذا أعدت تجارب حقلية خلال الاعوام المتعاقبة ٢٠١٧/٢٠١٨، ٢٠١٨/٢٠١٩ و٢٠١٩/ ٢٠٢٠وتم تعريض البذورللاشعاع الجامي عند المستويات ١٠٠،٢٠٠ و٣٠٠ جراى لدراسة مدى الحساسية للجرعات الاشعاعية لنوعين من الاونثرا *O. biennis* and *O. albicaulis* واحداث التباين الوراثى واتساع القاعدة الوراثية لانتخاب النباتات الطافرة ذات الصفات الجيدة على النمو والتبكير وبعض الصفات الانتاجية في الاجيال الطافرة الثلاثة ج١،ج٢ ،ج٣. بينت النتائج أن كلا النوعين من نباتات الأونثرا قد استجاب للاشعاع الجامى الذى أدى الى حدوث التباين الوراثي وانعكس بدوره على تحسين الانتاجية في بعض النباتات الطافرة والبعض الأخر فى صفة التبكير. كما حدثت تغيرات مورفولوجية واختلافات في السلوك الوراثى بين الاجيال لكلا النوعين عند كل الجرعات المطفرة. وقد لوحظ إنخفاضاً ملحوظاً في نسبة عدد النباتات الحية في نباتات الجيل الطافر الأول بعد ٢٥ يوم من الزراعة وذلك بزيادة الجرعات الاشعاعية في كلا النوعين كما أشارت النتائج أن *O. biennis* أقل استجابة للجرعات الاشعاعية عن *O. albicaulis* كما وجد أن معامل الإختلاف أعلى عند كل الجرعات الاشعاعية في كلا النوعين خلال الجيل الطافر الثاني مقارنة بالكنترول وسجلت النباتات الطافرة المنتخبة زيادة معنوية عن معاملات الكنترول في معظم الصفات تحت الدراسة

وعلى الأخص جرعتي (١٠٠- ٢٠٠ جراي) والتى تفوقت لكلا النوعين في تحسين معظم الصفات مورفولوجياً وإنتاجياً في الجيل الطافر الثاني. كما بينت بعض النباتات الطافرة تبكيرعن الكنترول خلال الجيل الثاني. واضافة الى ذلك تم انتخاب النباتات الطافرة ذات الصفات الاكثر تفوقاً ودراستها في الجيل الطافر الثالث حيث تم تقييم ١٢ سلالة طافرة في هذا الجيل والتي يمكن استخدام هذه السلالات كمصادر وراثية للحصول منها على اصناف بمواصفات جديدة فائقة في انتاجية البذور ومحتواها من الزيت والبروتين العالية. لذا يمكن التوصية باستخدام الاشعاع الجامي كمطفرات عند الجرعات المنخفضة والمتوسطة (١٠٠- ٢٠٠ جراى) كوسيلة فعالة في طرق تربية الأونثرا لتحسين انتاجية البذور ومحتواها من المواد الفعالة. كما نوصى باجراء المزيد من الانتخاب وتقييم السلالات الطافرة على نطاق واسع تحت ظروف بيئية مختلفة والحصول على أفضل الاصناف من الاونثرا.