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Effect of recurrent mutagenesis on some induced genotypes in safflower (*Carthamus tinctorious* L.)

Sakr H. G.[a*], Okaz A. M. A.[b], Abd el-zaher I. N.[a], Haridy M. H.[a]

[a]*Department of Agronomy, Faculty of Agriculture, Al-Azhar University (Assiut Branch), Assiut, Egypt*
[b]*Department of Agronomy, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt*

Abstract

Mutation breeding is a tool to induce new genetic variation for improving agronomical important traits. Thus, an investigation was carried out during two successive growing winter seasons 2017/2018 and 2018/2019 on safflower plants, at the Experimental and Research Farm, Faculty of Agriculture, Al-Azhar University, Assiut, Egypt. Three mutagen treatments *i.e.*, dimethyl sulfoxide, electric shock and gamma rays, were used to isolate the desirable mutants in M_4 and M_5 generations of thirty three safflower genotypes. Three mutagen treatments were used, such as; the chemical mutation mutagen was more effective than the two other mutagens to induce mutant genotypes. On the other hand, some mutant progenies were derived from gamma rays treatment with dose 20 kr which was more effective mutagen for induction of stable promising mutants in safflower, according to final results of M_5 generation, particularly high yielding ability trait, softness and earliness as compared to untreated plants. Concerning the important traits, the results showed that the earliest progenies for flowering date were 11 gave 119 days while, 9 gave 120 days with the h1 treatment and rad. in M_5 generation. Also, the results showed that two mutated progenies 9 and 12 gave the highest seed yield/plant, 355.64 and 317.67 gm respectively from h3 treatment, one progeny 13 gave 382.90 gm from t1 treatment. The seed oil content trait, showed that the highest progenies no.11 and 12, gave 44.80 and 44.76 % respectively, on using h_1 treatment, while, the mutated no. 13 gave 45.32% from t1 treatment. Finally, these results supported that the recurrent mutagenic treatments can be used to induce new mutant safflower genotypes which are characterized by spineless, earliness high seed yield and seed oil content. Thus, they can be involved in breeding programs to get new suitable varieties with high seed and oil yield.

Keywords: safflower, mutation breeding, recurrent mutagenesis, gamma rays, heritability.

*Corresponding author: Sakr H. G.,
E-mail address: hemsakr87@yahoo.com

1. Introduction

Safflower seeds have been found 4,000 year-old in Egyptian tombs and were used by Chinese approximately 2,200 years ago. Safflower (*Carthamus tinctorius* L.) is one of the important oil seed crops and has been traditionally grown for its flowers as a source of dye for coloring food and fibers. Subsequently, it is grown for edible oil, animal meal, bird feed, medicinal uses, as a potential candidate crop for production of plant used in pharmaceuticals and biofuel. Oil of safflower is the richest source of linoleic acid, with average linoleic acid content around 78% of the total seed oil fatty acids (Velasco *et al.*, 2005). Safflower oil is thought to be one of the highest quality vegetable oils, it contains polysaturated fatty acids as oleic and linoleic acids, which are good for cooking and healthy heart, safflower is considered to have a good taste, cook and health (Fernandez-Martinez *et al*., 1993; Singh and Nimbkar, 2006). However, the safflower plant is neglected among oil crops i.e, soybean, rapeseed and sunflower, due to the domination of these three oil seed crops, many other crops are either underutilized or neglected (Khan *et al*., 2009; Murphy, 1999). Also, some limitations are facing this crop as spineness and decreasing both seed yield and oil percentage (Dajue and Mundel, 1996). These limitations make safflower a weak competitor with the other oil crops. The estimated world production is about 0.622 million tons of seed per year from about 0.736 million hectares (FAO, 2009). At recent statistics according to FAO (2014) statistics, safflower production in the world was realized on an area of 1,010,180 ha with a total world production reaching about 867, 659 tons (Yilmaz *et al*., 2016). Despite its vast potential and growth adaptability to a wide range of agro-ecological conditions, safflower remained as a neglected crop due to low seed oil content (28-36%), spines, fiber rich seed meal and vulnerability to a number of diseases and pests. Safflower species are known to possess several desirable genes such as, drought hardiness, shattering tolerance, non-dormancy of seeds, resistance to safflower fly, rust, and powdery mildew (Sujatha, 2007). Mutagenesis technique has been used successfully in several crops to induce genetic variation for improving of both qualitative and quantitative traits. Over the years, the usage of mutations have increased to create novel variability's, mutations are classified into natural and induced ones. In nature, the natural mutation occurs slightly. Recently, mutagenic agents such as physical, chemical, electric shock mutagen, etc., are considered effective and sufficient for induction of genetically and morphological changes in many plant species, especially the self-fertilized plants (Fahmy *et al.*, 1997; Geetha and Vaidyanathan, 1998; Hajduch *et al.*, 1999; Hassan *et al.*, 2001; Kharkwal, 2000; Mihov *et al.*, 2001; Rakesh and Pratibha, 2014; Solanki and Sharma, 1999; Soliman *et al.*, 2003; Wani and Anis, 2001). More than 1800 cultivars obtained either as direct mutants or derived from their crosses have been released worldwide in 50 countries (Ahloowalia and Maluszynski, 2001). In Egypt, safflower area decreased year after

year at Upper Egypt, because the suffering of genotypes from many problems as lateness (185 days at maturity), full thorns on leaf and heads, low seed yield and low seed oil content. Therefore, the present study aimed to induce recurrent mutations for earliness, spineless and high seed yield with high oil content as a promising mutant that could be used in breeding program to get new varieties.

2. Materials and methods

The present investigation was carried out during two successive winter seasons 2017/18 and 2018/19 at the Experimental Farm, Faculty of Agriculture, Al-Azhar University, Assuit, Egypt. The basic materials (thirty mutated progenies and their three parental lines) were extension of the previous study, master thesis Okaz *et al.* (2016 b). In the beginning of the previous study, during season 2013/14, seeds from three parental lines of safflower plants were subjected to three mutagen agents *i.e*; gamma radiation (20 kr), dimethyl sulfoxide at 1000, 2000 and 3000 ppm and the third mutagen agent was electrical shock treatments to the plantlets (seedlings) in the presence of water solution containing 30000 and 50000 ppm from monosodium phosphate and sodium nitrate 50000 ppm. In the M_1 generation (season 2013/14), the mutated plants were morphologically different in spineless heads, red and orange petals, flowering date, seed yield/ plant and seed oil content. Selection was made on these mutants who showed high yield/plant and high seed oil content with early flowering. Self-pollinated seeds from those mutants were harvested individually to represent M_2 generation seeds. In the M_2 generation (season 2014/15), an experiment was carried out to evaluate the mutant progenies. The means, variances between and within the studied genotypes were calculated, in the same time all segregated plants which didn't show the initial changes were excluded. While, the self-pollinated seeds from the plants maintained changes were harvested individually and taken as the M_3 generation seeds. In the M_3 generation (season 2015/16), seeds of those mutant progenies were planted, the means and the variances between and within of the mutant progenies were calculated as in M_2 generation. In the end of the previous study Okaz *et al.* (2016 b), during season 2015/16, thirty promising mutants with high yielding ability, increase in seed oil content and with early in flowering date as compared to the parental lines, were isolated to produce M_4 generation. In the season 2017/18, M_4 generation (R_1M_1), seeds of the thirty promising mutants which were isolated from the M_3 generation safflower plants were subjected to the same previous mutagenic treatments, during season 2013/14), with the same dose or method to induce the recurrent mutagenesis (RM) and demonstrate its effect on the subsequent mutated generations. Three mutagenic agents i.e. chemical mutagen (dimethyl sulfoxide),

electrical shock mutagen and physical mutagen (gamma rays) were used. The concentrations of dimethyl sulfoxide were 1000 ppm (h_1 treatment) on six mutated safflower genotypes, 2000 ppm. (h_2 treatment) on three mutated safflower genotypes and 3000ppm (h_3 treatment) on six mutated safflower genotypes. The electrical shock mutagen treatments, were prepared in the presence of the chemical solutions as follow; monosodium phosphate 30000 ppm (t_1 treatment) on four mutated safflower genotypes, monosodium phosphate 50000 ppm (t_2 treatment) on four mutated safflower genotypes and sodium nitrate 50000 ppm (t_3 treatment) on three mutated safflower genotypes. Four mutated safflower genotypes were isolated from gamma rays treatment (20 kr). Table (1) showed that the list of the isolated plants from each mutagenic treatment for each mutated progeny of this investigation. An experiment was carried out in the field, which involved thirty three genotypes i.e.; the three parental lines (untreated plants or control) and thirty mutated progenies. The seed of each mutated plant in M_4 generation were planted in three ridges plot, each ridge was 3 meter long in hills 0.3 m apart. Also, the three parental lines were sown, each parental line was represented by three ridges plot, using randomized completely block design with three replicates.

Table (1): Mean and coefficient of variation (c.v) of days to flowering date, seed yield/plant and seed oil content of mutagenized safflower genotypes under different mutagenic treatments for M_4 and M_5 generations.

Character	Days to flowering (day)				Seed yield/ plant (gm)				Seed oil content (%)			
	Mean		C.V (%)		Mean		C.V(%)		Mean		C.V (%)	
Generation / Treatment	R_1M_1	R_1M_2	R_1M_1	R_1M_2	R_1M_1	R_1M_2	R_1M_1	R_1M_2	R_1M_1	R_1M_2	R_1M_1	R_1M_2
Control	129.22	130.00	1.27	0.57	94.47	92.51	3.28	1.34	38.77	38.73	2.20	1.39
h1	125.29	125.61	3.27	3.45	125.45	132.35	10.52	12.65	42.40	42.97	5.90	4.60
h2	127.25	127.76	2.57	1.88	102.00	111.19	9.49	12.55	43.00	43.26	5.93	3.81
h3	124.23	124.51	3.30	3.32	138.00	139.76	10.32	11.07	43.22	43.51	5.38	3.27
t1	125.47	125.88	3.01	3.52	128.22	128.96	13.23	11.95	43.71	43.75	5.38	3.29
t2	127.76	128.15	3.35	3.28	141.98	143.50	8.11	11.00	43.59	43.60	5.51	2.76
t3	125.45	124.97	2.86	2.90	127.42	129.04	12.03	11.34	43.52	43.28	5.14	3.25
R	127.68	127.83	4.22	4.14	101.92	110.82	6.78	10.49	43.12	43.16	5.34	2.96

C.V= coefficient of variation. R1M1= M4 generation. R1M2= M5 generation.

The agriculture practices of irrigation, fertilization, weeds and pests control were used as normal recommended for safflower production. The data of the present investigation were recorded on ten guarded plants per plot for each of genotypes. At the harvest, selection was made on the best mutants who showed high yield/plant and high seed oil content with early flowering. Self-pollinated seeds from those mutants were harvested individually to represent M_5 generation

seeds. In the season 2018/19, M_5 generation (R_1M_2), the seed of each selected mutated progeny of the previous M_4 generation which maintained the changes was taken, sown and it represented M_5 generation and considered as a progeny of heritable mutant plant. From the Tables (2-4), it is clear that all mutagenic treatments induced the different types of changes. The total number of selected mutant plants were 24, in which four mutations due to h_1 treatment, two mutations due to h_2 treatment, seven mutations due to h_3 treatment, three mutations due to t_1 treatment, three mutations due to t_2 treatment , three mutations due to t_3 treatment and two mutations due to rad. treatment. Mutated plants are high in both seed yield and seed oil content per plant. An experiment was carried out in the field, which involved twenty seven genotypes *i.e.*; the three parental lines (untreated plants or control) and twenty four mutated genotypes. All the agricultural practices in the previous M_4 generation were carried out on M_5 generation. Measurements and statistical analysis for both seasons (M_4 and M_5 generations) were as done.

Table (2): The morphological description and parent-offspring regression in mutated plants derived from chemical treatments.

Character		Days to flowering date (day)			Seed yield/plant (gm)			Seed oil content (%)			Color flower-Texture plant
Treatment	Genotype	M_3	R_1M_1	R_1M_2	M_3	R_1M_1	R_1M_2	M_3	R_1M_1	R_1M_2	
Control		130	129.22	130.00	75.25	94.47	92.51	37.98	38.77	38.73	thorns - yellow
h_1	1	127	127	128	139.51	131.56	164.33	40.46	41.20	43.67	sleek - yellow
	11	130	120	119	203.31	136.3	255.36	40.34	40.60	44.80	sleek - yellow
	12	127	125	124	209.33	136.38	278.68	38.38	45.50	44.76	sleek - yellow
	27	130	123	126	149.35	147.64	170.31	45.67	39.70	43.47	sleek - yellow
h_2	4	127	126	126	187.87	105.58	181.74	39.57	41.80	44.55	thorns - orange
	6	128	127	126	166.23	100.55	162.57	38.08	39.30	44.48	sleek –orange
h_3	7	128	126	127	125.33	151.54	196.47	42.39	42.00	44.48	sleek –orange
	8	126	127	126	167.64	149.30	268.79	43.09	39.8	44.19	sleek - yellow
	9	126	127	126	127.23	159.16	355.64	44.13	44.20	44.24	thorns – red
	10	129	128	129	120.46	145.06	172.95	43.55	39.70	44.51	sleek - yellow
	11	125	128	128	103.88	157.36	175.98	40.60	41.30	44.43	sleek - yellow
	12	128	126	125	93.04	145.36	317.67	40.58	42.40	44.52	thorns - yellow
	24	128	123	127	120	152.70	180.65	40.11	42.80	43.63	thorns – yellow
Regression coefficient			-0.62	-0.26		-0.07	0.45		-0.06	0.17	

R_1M_1= M_4 generation. R_1M_2= M_5 generation.

2.1 Measurements

Days to flowering date (day), was measured as number of days from sowing to the first flower on the plant, seed yield/plant (gm) and seed oil content (%), was estimated by Soxhelt apparatus according to AOAC (1980).

2.1 Statistical analysis

The mean and coefficient of variation (c.v) of the mutants cached from each mutagenic treatment were calculated and compared with that of the same number of plants representing control treatment was made for RCBD, estimation of mean, the

coefficient of variation (c.v), were calculated according to Gomez and Gomez (1984), the analysis of regression coefficient for the parents and its offspring which represent heritability in narrow sense was estimated according to Mather (1949).

3. Results and Discussion

At the first season of the investigation all mutagenic treatments induced recurrent mutagenized plants for different desired traits such as smooth leaves, red and orange petals, earlier flowering and high yielding ability plants. Tables (2-4) showed that the chosen mutants in R_1M_1 (M_4 generation) after applying the mutagen treatments. It is clear from results, that mutants differed from the original plants of different safflower parental genotypes in three main characters *i.e.* days to flowering date (D.F), seed yield/plant (S.Y/P) and seed oil content /plant (%). In addition to thorns and sleek and petal color (Kotcha *et al.*, 2007). Results showed that all mutagens (chemical, electric shock and radiation) have led to induce of mutations in all safflower genotypes. The obtained plants in M_4 generation which shown in Tables (2-4) were planted to produce the M_5 generation. The means and the variances of the mutagenized plants under different mutagenic treatments were calculated and compared with the same number of untreated plants (control) for the three main traits *i.e*. number of days from sowing to flowering, seed yield/ plant and oil content percentage /plant (Table 1).

Table (3): The morphological description and parent-offspring regression in mutated plants derived from electric shock treatments.

Character		Days to flowering date (day)			Seed yield/plant (gm)			Seed oil content (%)			Color flower-Texture plan
Treatment	Genotype	M_3	R_1M_1	R_1M_2	M_3	R_1M_1	R_1M_2	M_3	R_1M_1	R_1M_2	
Control		130	129	130	75.25	94.47	92.51	37.98	38.77	38.73	thorns - yellow
t1	12	127	127	126	145.86	148.63	190.37	42.45	45.3	44.29	thorns - red
	13	129	123	124	215.72	144.19	382.9	40.45	44.8	45.32	sleek – red
	19	125	125	129	128.4	154.36	173.56	39.41	39.2	44.23	sleek – red
t2	5	126	128	129	125.29	148.08	195.44	38.68	43.00	43.13	sleek - yellow
	6	116	128	127	188.44	133.09	217.18	40.07	46.50	44.53	thorns - orange
	8	121	127	125	90.14	134.98	199.07	39.58	45.80	43.37	thorns - yellow
t3	1	127	121	128	133.6	158.18	157.84	37.79	42.60	43.59	sleek - yellow
	2	129	120	121	202.13	144.52	313.62	39.62	43.80	43.61	thorns - yellow
	9	126	128	122	94.26	124.86	175.83	42.76	39.50	44.5	sleek -orange
Regression coefficient			-0.28	-0.07		0.18	0.99		0.28	0.60	

R_1M_1= M_4 generation. R_1M_2= M_5 generation.

Table (4): The morphological description and parent-offspring regression in mutated plants derived from gamma rays treatment.

Character		Days to flowering date (day)			Seed yield/plant (gm)			Seed oil content (%)			Color flower-Texture plan
Treatment	Genotype	M_3	R_1M_1	R_1M_2	M_3	R_1M_1	R_1M_2	M_3	R_1M_1	R_1M_2	
Control		130	129	130	75.25	94.47	92.51	37.98	38.77	38.73	thorns – yellow
rad	7	126	125	126	166.56	112.29	174.12	42.38	39.80	43.67	thorns – yellow
	9	130	119	120	74.99	111.21	154.54	39.35	43.57	43.67	sleek – yellow
Regression coefficient			-0.22	0.97		0.10	0.55		-0.01	0.94	

R_1M_1= M_4 generation. R_1M_2= M_5 generation.

3.1 Effect of chemical mutagen treatments on the studied traits

3.1.1 Days to flowering (day)

Results in Tables (1-2) and Figure (1), illustrated that the average number of days to flowering for mutated plants of h_1 treatment ranged from 119 for mutant no. 11 to 128 days for mutant no. 1, with an average 125.61 days as against 130 days for the untreated plants, The genotypes 11 and 12 were the earliest in the flowering date, gave 119 and 124 days, respectively. The average of h_2 treatment was 127.76 days, two genotypes 4 and 6 gave 126 days, and they are the earlier than the untreated plants with 130 days. While, the average number of days to flowering for mutated plants of h_3 treatment in R_1 M_2 (M_5 generation) ranged from 125 for mutant no. 12 to 129 days for mutant no. 10, with an average of 124.51 days. The genotype 12 with 125 days and 9 with 126 days, were more responsive to chemical mutagen (h_3 treatment) as compared to the untreated plants with 130 days. These results are in agreement with those of Dhole *et al*. (2003), Sheeba *et al*. (2005), Mensah and Obadoni (2007), Nura*et al*. (2013), Gopinath and Pavadai (2015), Ravichandran and Jayakumar (2015), Okaz *et al*. (2016a) and Ahmad (2019). The coefficient of variation (c.v) is one of variation parameter, its values were categorized as low (<10%), moderate (10 to 20%) and high (>20%) as indicated by Subramaniam and Menon (1973). The results showed that all treatments *i.e*. h_1, h_2 and h_3 gave low estimates of variation coefficient (c.v), Table (1) indicated that these three treatments induced low amount of genetic variation in the studied genotypes for days to flowering date.

3.1.2 Seed yield /plant (gm)

For the chemical mutagen treatments, the mean seed yield/plant of all mutated plants is presented in Tables (1-2) and Figure (2). The results showed that all plants which maintained of the mutation until R_1M_2 (M_5 generation) surpassed the untreated plants in seed yield /plant trait. The mean seed yield / plant of h_1 treatment ranged between 164.33 for mutant no.1 to 278.68 gm. for mutant no.12 with an average of 132.35 gm. The genotypes 12 and 11 were the highest in seed yield /plant; they gave 278.68 and 255.36 gm. respectively, as compared to untreated plants with 92.51 gm. For the h_2 treatment, the mean seed yield / plant ranged from 162.57 for mutant no.6 to 181.74 gm. for mutant no.4, with an average 111.19 gm. These genotypes 6 and 4 were the heaviest in seed yield /plant with percentage 96.45 and 75.73 %, respectively as compared to the untreated plants with 92.51 gm. Meanwhile, the average of seed yield/plant for h_3 treatment ranged between 172.95 for mutant no.10 to 355.64 gm for mutant no.9 with an average 139.76 gm .The genotype 9 with 355.64 gm and 12 with 317.67 gm were out yielded the others and with percentage 284.43 and 243.39 %, respectively as against 92.51 gm in the untreated plants. These results confirm the findings of Ahmed (2012), Nura*et al*. (2013), Ravichandran and Jayakumar (2015), Okaz *et al*. (2016a) and Ahmad (2019). The results of variation

coefficient showed that all h1, h2 and h3 treatments.

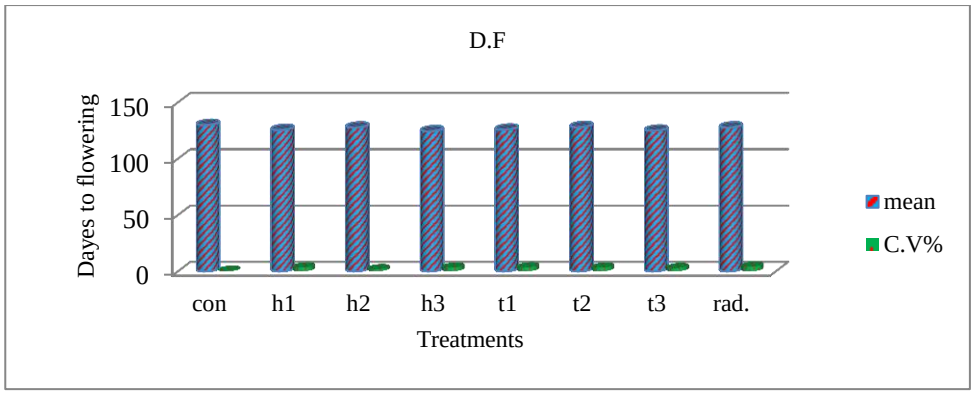

Figure (1): Number of days to flowering of safflower genotypes under different mutagenic treatments in 2018/2019 season.

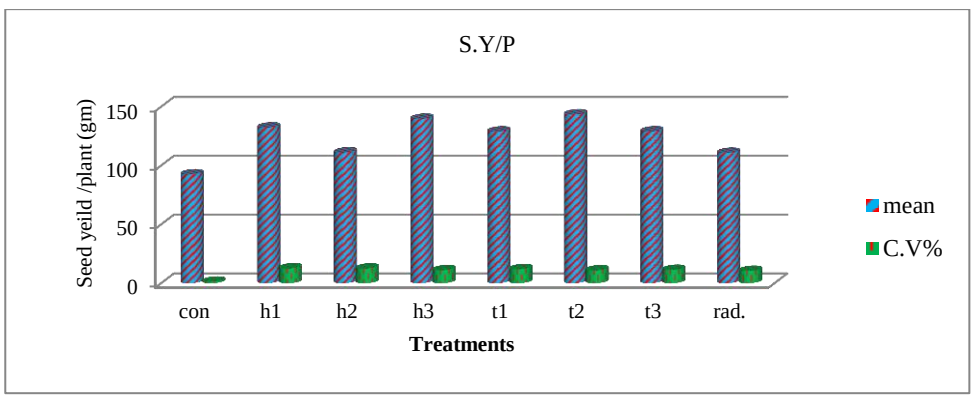

Figure (2): Seed yield/ plant of safflower genotypes under different mutagenic treatments in 2018/2019 season.

Table (1) gave moderate estimates of c.v pointing out that these three treatments induced moderate amount of genetic variation in the studied genotypes for seed yield/plant. This amount can lead to enhance this trait.

3.1.3 Seed oil content (%)

The chemical mutagen treatments, for mean of seed oil content of all mutated plants are presented in Tables (1-2) and Figure (3). The results showed that all plants which maintained of the mutation until R_1M_2 exceeded untreated plants in seed oil content. For the h_1 treatment, the average seed oil content ranged from 43.47 for mutant no.27 to 44.8 % for mutant no.11 with an average 42.97 %. The genotype 11 with 44.80 % and 12 with 44.76 % were the higher of seed oil content than the other mutants and as compared to the untreated plants with 38.73%, this result meaning that these genotypes exceeded the untreated plants with percentage 15.67 and 15.57 %,

respectively. For h_2 treatment, the average of seed oil content ranged between 44.48 for mutant no. 6 to 44.55 % for mutant no.4, with an average 43.26%. These genotypes 6 and 4 surpassed the untreated plants 38.73 %, with percentage 44.55 and 44.48 %, respectively. Also, for the h_3 treatment, the average of seed oil content ranged from 43.63 for mutant no. 24 to 44.52 % for mutant no.12 with an average 43.51 %. The genotypes 12 with 44.52 and 6 with 44.48 % were higher of seed oil content than the other mutated plants and as compared to the untreated with 38.73%. The genotypes 12 and 6 exceeded the untreated plants with 38.73% with percentage (14.94 and 14.85 %), respectively. Our results were in agreement with those obtained by Dhole *et al*. (2003), Sheeba *et al*. (2005), Mensah and Obadoni (2007), Nura*et al*. (2013), Gopinath and Pavadai (2015), Ravichandran and Jayakumar (2015), Okaz *et al*. (2016 a) and Ahmad (2019).

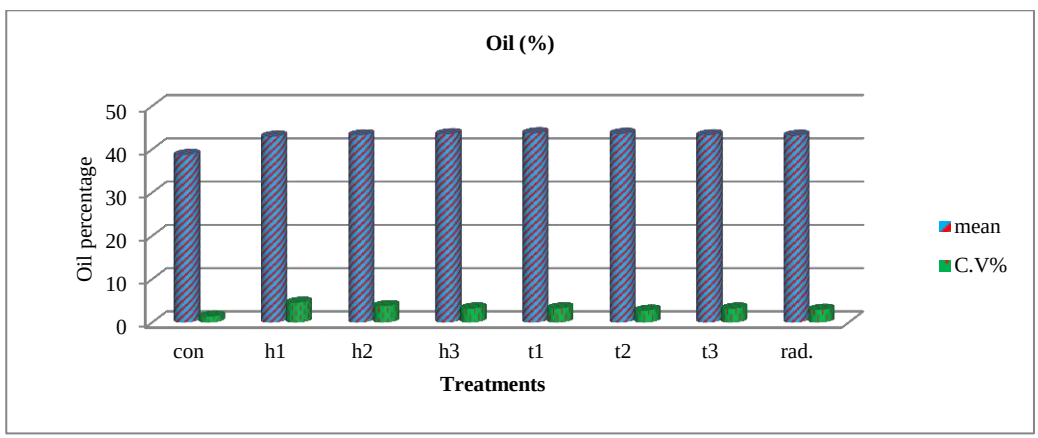

Figure (3): Oil percentage of safflower genotypes under different mutagenic treatments in 2018/2019 season.

The results showed that all treatments of chemical mutagen (h_1, h_2 and h_3) gave low estimates of variation coefficient (c.v), Table (1) indicated that these three treatments induced low amount of genetic variability in the studied genotypes for seed oil content. The parent-offspring regression coefficients values (Table 2) represent heritability in narrow sense were estimated as follow; -0.62 and -0.26 for days to flowering, -0.07 and 0.45 for seed yield /plant and 0.06 and 0.17 for seed oil content % for M_4 and M_5 generation, respectively. The large proportion of the total genetic variation due to the additive genetic effects for days to flowering with -0.62 and seed yield /plant with 0.45 in M_4 and M_5 generations, respectively, indicating that efficiency of selection for these two traits. These results were in line with those from variation coefficient values.

3.2 Effect of electric shock mutagen treatments on the studied traits

3.2.1 Days to flowering (day)

The results in Tables (1 and 3) and Figure (1), showed that the average no. of days

to flowering for mutated plants of t_1 treatment in R_1M_2 (M_5 generation), ranged from 124 for mutant no.13 to 129 days for mutant No.19, with an average of 125.88 days. The genotypes 13 with 124 and 12 with 126 days were more responsive to electric shock mutagen (t_1 treatment) as compared to the untreated plants with 130 days. While, the average number of days to flowering date for mutated plants of t_2 treatment ranged between 125 for mutant No.8 to 129 days for mutant no. 5 with an average 128.15 days. The genotype 8 with 125 days and 6 with 127 days were decreased in days to flowering date as compared to the untreated plants with 130 days. For t_3 treatment, the mean of days to flowering ranged from 121 for mutant no.2 to 128 for mutant no.1with an average 124.97 days. The genotype 2 with 121 days and 9 with 122 days were the earlier than the untreated plants with 130 days. These results coincides with Okaz *et al.* (2016a) when used electric shock on safflower, (Ahmad, 2011) when used electric shock on wheat. The results of variation coefficient showed that the electric shock treatments i.e. t_1,t_2 and t_3 had low estimates of variation coefficient (c.v), Table 1, indicating that these three treatments induced low amount of genetic variability in the studied genotypes for days to flowering.

3.2.2 Seed yield /plant (gm)

For the electric shock mutagen treatments, the mean seed yield/plant of all mutated plants is presented in Tables (1 and 3) and Figure (2). The results showed that all plants which maintained of the mutation until R_1M_2 (M_5 generation) were surpassed untreated plants in seed yield /plant trait. The mean seed yield / plant of t_1 treatment ranged between 173.56 for mutant no.19 to 382.9 gm. for mutant no.13 with an average of 128.96 gm. The genotype 13 with 382.9 and 12 with 190.37 gm. were most responsive to electric shock and gave the highest seed yield /plant with percentage 313.90% and 105.78 as compared to untreated plants with 92.51 gm. Meanwhile, for the t_2 treatment, the average seed yield/plant ranged from 195.44 for mutant no.5 to 217.18 gm. for mutant no. 6 with an average 143.5 gm. The genotype 6 with 217.18 and 8 with 199.07 gm. were the heaviest with percentage 134.76 and 115.19 gm in seed yield/plant as against 92.51 gm for untreated plants. Also, the mean seed yield /plant for t_3 treatment ranged from 157.84 for mutant no.1 to 313.62 for mutant no.2 with an average 129.04 gm. Some genotypes *i.e* 2 with 313.62 and 9 with 175.83 were the highest yield /plant with percentage 239.01 and 90.1% as compared to untreated plants with 92.51 gm. This result coincides with Okaz *et al.* (2016 a) when used electric shock on safflower (Ahmad, 2011) when used electric shock on wheat. The results of variation coefficient (c.v) showed that all t_1, t_2 and t_3 treatments. Table (1) had moderate estimates of c.v, indicating that these treatments induced moderate amount of genetic variability in the studied genotypes for seed yield plant. This amount can lead to improve this trait.

3.2.3 Seed oil content (%)

The electric shock mutagen treatments,

for mean of seed oil content of all mutated plants are presented in Tables (1 and 3) and Figure (3). The results showed that all plants which maintained of the mutation until M_5 generation exceeded untreated plants in seed oil content. For the t_1 treatment, the average seed oil content ranged from 44.23 for mutant no.19 to 45.32 % for mutant no.13 with an average 43.75 % .The genotype 13 with 45.32% and 12 with 44.29 % were most responsive to electric shock with percentage 17.02 and 14.36%, respectively, as compared to the untreated plants with 38.73% .While, for t_2 treatment, the average of seed oil content ranged between 43.13 for mutant no.5 to 44.53 % for mutant no.6 with an average 43.60%. The genotype 6 with 44.53 and 8 with 43.37 % were the highest of seed oil content, with percentage 14.98 and 11.98 %, respectively, as against 38.73 % for the untreated plants. In electric shock mutagen, t_3 treatment, results showed that mean seed oil content ranged from 43.59 (4-2-1) to 44.5% (1-1-9) with an average 43.28%. The genotypes 9 with 44.5 % and 2 with 43.61% were the highest of seed oil content with percentage 14.90 and 12.60% as compared to 38.73% for untreated plants. This result coincides with Okaz *et al.* (2016a) and (Ahmad, 2011) when they used electric shock on safflower and wheat respectively. The results of variation coefficient (c.v) showed that all t_1, t_2 and t_3 treatments, Table 1(), had low estimates of (c.v), indicating that these three treatments induced low amount of genetic variability in the studied genotypes for seed oil content. The analysis of regression coefficient for the parents and its offspring which represent heritability in narrow sense, Table 3, showed that the estimated values for studied traits, were -0.28 and -0.07 for days to flowering date, 0.18 and 0.99 for seed yield /plant and 0.28 and 0.60 for seed oil content for M_4 and M_5 generations, respectively. The large proportion of the total genetic variation due to the additive genetic effects for seed yield /plant with 0.99 and seed oil content with 0.60 in M_5 generation, indicating that efficiency of selection for these two traits.

3.3 Effect of physical mutagen treatment (gamma rays) on the studied traits

The results of the physical mutagen treatment (gamma rays, dose 20 kr), Tables (1 and 4) and Figure (1), showed that all mutated plants which maintained of the mutations until R_1M_2 (M_5 generation) exceeded untreated plants for all studied traits.

3.3.1 Days to flowering (day)

The mean of days to flowering date of (gamma rays, dose 20 kr), Tables (1 and 4), ranged between 120 for mutant no.9 to 126 days for mutant no.7 with an average of 127.83 days. The genotype 9 with 120 days and 7 with 126 days were earlier than the untreated plants with 130 days. This result coincides with those of Mia and Shaikh (1997), Veena and Ravikumar (2003), Sheeba *et al*. (2005), Gopinath and Pavadai (2015) and Okaz *et al*. (2016a).

3.3.2 Seed yield /plant (gm)

Results of mean seed yield/plant, Tables

(1 and 4), showed that the average ranged from 154.54 for mutant no.9 to 174.12 gm. for mutant no.7 with an average 110.82 gm. The genotype 7 and 9 were the higher in seed yield /plant with percentage 88.22 and 67.10% as compared to the untreated plants with 92.51 gm. This result coincides with those of Mia and Shaikh (1997), Veena and Ravikumar (2003), Sheeba *et al*. (2005), Cvejic *et al.* (2011), Gopinath and Pavadai (2015) and Okaz *et al*. (2016a).

3.3.3 Seed oil content (%)

For seed oil content, results in Tables (1 and 4), showed that the genotypes 7 and 9 gave the same performance with 43.67 %, and were higher in seed oil content, with percentage 12.75 as against 38.73% for the untreated plants. This result coincides with those of Mia and Shaikh (1997), Veena and Ravikumar (2003), Sheeba *et al*. (2005), Cvejic *et al.* (2011), Gopinath and Pavadai (2015) and Okaz *et al*.(2016 a), but opposite with that obtained by Siddiqui et al (2009), who reported that no treatment could produce oil (%) higher than control. The variation coefficient (c.v) estimates, Table (1), showed low values of days to flowering date and seed oil content. Meanwhile, the c.v value was moderate for seed yield/plant. The parent-offspring regression coefficients values (Table 4), representing heritability in narrow sense, were estimated as follow; -0.22 and 0.97 for days to flowering date, 0.10 and 0.55 for seed yield/plant and -0.01 and 0.94 for seed oil content in M_4 and M_5 generations, respectively. The large proportion of the total genetic variation due to the additive genetic effects for days to flowering with 0.97, seed yield / plant with 0.55 and seed oil content with 0.94 in M_5 generation, indicating that efficiency of selection for these studied traits.

4. Conclusions

Using the different mutagen treatments were effective tool to obtain new safflower genotypes with spineless, earliness and high seed yield. We can use these new genotypes in breeding programs to obtain new suitable varieties for cultivation at reclaimed desert lands as a new oil crop in Egyptian agriculture. In general, the chemical mutagen (h_3 treatment) was more effective than the two other treatments to induce mutagenized plants for days to flowering date with an average (124.51 days), seed yield /plant with an average (139.76 gm.) and seed oil content with an average (43.51%) as compared (130 days, 92.51 gm. and 38.73%) for untreated plants in the three studied traits, respectively. For electric shock mutagen (t_2 treatment) was more effective than the two other treatments to induce mutations and gave mutant plants for seed yield/ plant with an average (143.50 gm.). While, t_1 treatment was efficient to induce mutations and gave mutant plants with high oil percentage (45.32%).

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