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Full Length Research Article

Effect of Mutagens on Seed Germination, Seedling Height and Quantitative Characters of *Carthamus tinctorius* L.

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Abstract

Seeds of safflower (*Carthamus tinctorius* L.) cv. Phule Bhivra was treated with various doses / concentrations of mutagens such as gamma rays (200, 300Gy, 400Gy), ethyl methyl sulphonate (0.2%, 0.3%, 0.4%), as well as their combination (200Gy + 0.3%, 300Gy + 0.3%, 400Gy + 0.3%). Quantitative characteristics and seed germination percentage in the M_1 generation were also measured. Higher dosages of gamma rays, EMS and their combination of the two have been shown to significantly reduce seed germination percentage and seedling height. As a result of almost all mutagenesis treatments, the M_1 generation had a decrease in plant height, number of branches / plant, days to first flowering, days for the maturity of the first capitula, 100 seed weight, number of seeds/capitula, and number of seeds/plant (expect some for a few).

***Key words*:** Safflower, Mutagens, Treatments, Gamma rays, EMS

More than 4000 years ago, safflower (*Carthamus tinctorius* L.) was probably domesticated in the Fertile Crescent region [1]. *Carthamus tinctorius*, more commonly known as safflower, is a member of the daisy family (Asteraceae) that is grown extensively throughout the Mediterranean and South Asian regions, including India [18]. Seed germination and the subsequent emergence of seedlings are critical phases in a plant's life cycle, which are mostly controlled by environmental factors including temperature and moisture [22]. Safflower is an annual plant that is used for food and oil. This oil seed crop has a lot of promise since it can provide meal bird seed, oil, and significant financial returns with minimal irrigation. Due to its improved tolerance for oil content, drought as well as composition, and other oil-related features, safflower exhibits a higher response, yield, and economic outcome [12]. Polyunsaturated fatty acids in the oil are beneficial for a healthy heart. Safflower oil is one of the best vegetable oils since it contains both oleic and linoleic acids [8].

Induced mutation is a fast and effective way to enhance single or several economic features and qualitative attributes [15]. Treatments may be counterproductive by decreasing plant germination, ovule fertility, pollen output, plant vigour, and growth rate, despite the benefits of mutations for increasing population variety [16].

MATERIALS AND METHODS

The authentic seeds of safflower (*Cathamus tinctorius* L.) cv. Phule Bhivra was obtained from, The Breeder, All India Coordinated Research Project on Safflower, VNMKV, Solapur, Maharashtra, India. At Savitribai Phule Pune University's Department of Chemistry in Pune (M.S.) India, it is irradiated with gamma rays (200, 300, 400Gy) (^{60}Co 1000 curie). Seeds that were determined to be healthy after soaking in distilled water for 10 hours were given several EMS concentrations (0.2%, 0.3%, 0.4%) for 4 hours. As a baseline, we also tested untreated dry seeds. Seed that had been exposed to EMS was rinsed for one hour in regular water. Gamma irradiation and EMS treatment of seedlings for 4 hours: 200Gy + 0.3% EMS, 300Gy + 0.3% EMS, 400Gy + 0.3% EMS. Wash for an hour in running water. Both untreated and treated seeds were planted in field using a randomized block pattern with a spacing of 45 cm by 20 cm. For the next generation of M_1 plants, 225 seeds were planted in the field using either Gamma rays, EMS, or a combination of the two. This research has been carried out at the Research field of the Department of Botany at "Annasaheb Awate Arts, Commerce, and Hutatma Babu Genu Science College in Manchar, Tal. Ambegaon, District Pune" (Maharashtra) India. In the lab, we measured the germination rate of 30 seeds across all treatments to get an accurate percentage. At regular intervals, 5 randomly selected plants from each treatment of M_1 populations were examined for quantitative characteristics. The table contains the means for

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each parameter, such as the survival rate at 52 DAS and the micro mutations about the quantitative features at maturity.

Statistical analysis

The means of three replicates were used to summarize the data, and the measures of variability were the standard deviation. A one-way ANOVA test assessed the significance of changes resulting from different treatments. Statistical significance between treatments has been evaluated using a post hoc test (Fisher's LSD; "Least Significant Difference") at p=0.05level. Sigma Stat is being used for the statistical analysis (ver.25).

RESULTS AND DISCUSSION

The (Table 1) shows the proportion of seeds that germinated after being exposed to gamma radiation, EMS, or both. Based on the findings reported in (Table 1), it seems that, with the exception of 200Gy, a higher dose/concentration of Gamma rays reduces the rate at which seeds germinate in the lab. This finding was true for both individual EMS and their combination. In the current study, both physical and chemical mutagenic treatments resulted in a reduction in seed germination percentage with increasing dosages and concentrations. Similar types have been reported in African sesame [4], in sesame [13], in sesame [19], and in groundnut [11], in soybean [2], and in mustard [7]. In Phule bhivra, higher doses/concentrations inhibited seed germination whereas lower doses or concentrations stimulated it. The same tendency observed in horse gram [3]. The biological physiological and processes required for seed germination are delayed or inhibited in mutagenesis treatments, which includes enzyme activity [14]. Chromosomal damage and physiological abnormalities were responsible for the decline in seed germination percentage [21]. (Table 1) demonstrates that when the dose/concentration of gamma rays increased in the lab, the height of the seedlings became shorter. The same result was seen with both EMS and a combination of the two. Gamma rays and EMS, when applied in increasingly strong doses, stunted the growth of seedlings. In *Jatropha curcas* found similar outcomes [5].

Table 1 Effect of mutagens on percent seed germination and seedling height in M_1 generation

Treatment	Germination percentage	Seedling height
Control	96.67 ±3.333	9.72 ± 0.86
200Gy	100.00 ± .000	7.56 ± 0.21
300Gy	93.33 ± 3.333	4.08 ± 0.11
400Gy	96.67 ± 3.333	3.86 ± 0.11
0.2%EMS	96.67 ± 3.333	6.73 ± 0.19
0.3%EMS	93.33 ± 3.333	4.86 ± 0.14
0.4%EMS	73.33 ± 3.333	3.24 ± 0.09
200Gy + 0.3% EMS	43.33 ± 3.333	4.30 ± 0.08
300Gy + 0.3% EMS	43.33 ± 3.333	4.01 ± 0.05
400Gy + 0.3% EMS	36.67 ± 3.333	3.91 ± 0.09
SEM	± 4.47	± 0.42
F-Value	68.09	48.16
P-Value	<0.001	<0.001
$LSD_{0.05}$	8.76	0.82

The mean difference is significant at the 0.05 level
Data are means of three replicates ± standard deviation
Significant difference due to treatments was assessed by Fisher's LSD as a post-hoc test

Quantitative characters (Micro mutations)

Plant height, days until first blooming, number of main branches/plant, days until first capitula is mature, number of seeds/capitula, 100 seed weight, and number of seeds / plant were all recorded as quantitative traits that were affected by mutagens in M_1 generation (Table 2).

Plant height

In M_1 generation of the Phule bhivra, all the mutagens were successful in causing plant height variability (Table 2). Compared to the control (81.62cm), plant height was drastically lowered by GR treatments of 200, 300, and 400Gy, with the greatest drop occurring at 400Gy (60.66cm). The EMS treatments reduce the plant height in all treatments and the maximum reduction of plant height in 0.4% (56.50cm) as compared to the control (81.62cm). Combined treatments also show the same result reduced plant height in all treatments and maximum reduction of plant height in 400Gy + 0.3% (44.01cm) compared to control (81.62cm). The reduced plant height in the cultivar was caused by the higher concentrations/doses of treatment.

Number of primary branches per plant

In GR treatments including 200, 300 and 400Gy the number of primary branches / plants gets slightly reduced with increased doses / concentrations. Maximum reduction of number of primary branches / plant in 400Gy (1.60) as compared to control (2.60). The same trend follows in EMS except for 0.3% (1.73) concentration. In combination, treatments reduced the number of primary branches / plants with increased doses / concentrations of mutagens. Maximum reduction of number of primary branches / plant in 400Gy + 0.3% (1.40) as compared to control (2.60) (Table 2).

Days required for first flowering

Gamma radiation, EMS and combination treatments had succeeded in producing a variation in the GR treatments in Phule bhivra generating a minor delay in the first flowering relative to the control (52.06 DAS). The same pattern is seen in EMS except for 0.3% EMS over control. Also, the delay in the day for the first flowering in Combination treatment with increased doses /concentrations of mutagens.

Days required for maturity of first capitula

In gamma radiation treatment delayed the days required for the maturity of the capitula as compared to control. The same trend follows in EMS except for 0.2%, 0.3% concentration reduced days required for the maturity of the first capitula. In combination treatment also show the same result more days are required for the maturity of capitula as compared to control (Table 2).

Table 2 Micromutations in the M_1 generation of Phule Bhivra

Treatments	Plant height (cm)	No. of primary branches / plant	First flowering days	Days for the maturity of the first capitula	No. of seeds / capitula	No. of seeds / plant	100seed weight (gm)
Control	81.62 ± 4.71	2.66 ± 0.06	52.06 ± 3.00	81.60 ± 4.73	14.80 ± 0.23	25.80 ± 1.50	5.78 ± 0.13
200Gy	64.12 ± 3.68	2.06 ± 0.13	54.13 ± 2.50	82.40 ± 4.73	12.33 ± 0.58	23.00 ± 1.30	5.09 ± 0.11
300Gy	61.90 ± 3.55	1.73 ± 0.06	55.20 ± 3.11	83.06 ± 4.85	8.20 ± 1.11	13.13 ± 0.81	3.59 ± 0.08
400Gy	60.66 ± 3.48	1.53 ± 0.06	55.53 ± 3.29	84.33 ± 4.90	8.80 ± 1.52	14.53 ± 1.61	4.53 ± 0.10
0.2%EMS	63.17 ± 4.79	2.21 ± 0.23	52.80 ± 3.11	81.40 ± 4.39	12.46 ± 0.54	23.06 ± 1.26	3.84 ± 0.08
0.3%EMS	58.34 ± 3.39	1.60 ± 0.11	50.80 ± 2.88	81.00 ± 4.73	11.73 ± 1.41	13.73 ± 0.86	4.03 ± 0.09
0.4%EMS	56.50 ± 3.23	1.46 ± 0.06	54.86 ± 3.06	82.53 ± 4.67	12.13 ± 0.98	12.40 ± 0.52	3.08 ± 0.07
200Gy + 0.3%EMS	49.18 ± 2.82	1.53 ± 0.06	53.06 ± 3.11	83.06 ± 4.85	10.53 ± 1.35	20.86 ± 1.34	5.30 ± 1.31
300Gy + 0.3%EMS	41.34 ± 2.38	1.46 ± 0.06	55.20 ± 3.11	83.80 ± 4.84	6.80 ± 1.36	9.40 ± 0.57	3.71 ± 0.08
400Gy + 0.3%EMS	44.01 ± 1.38	1.26 ± 0.06	55.86 ± 3.17	85.73 ± 5.13	5.40 ± 1.51	8.20 ± 0.23	3.03 ± 0.06
SEM±	4.92	0.15	4.30	6.77	1.62	1.55	0.60
F-Value	10.98	15.44	0.30	0.09	6.52	32.18	4.92
P-Value	<0.001	<0.001	<0.001	1.000	<0.001	<0.001	0.001
$LSD_{0.05}$	9.64	0.29	8.42	-	3.17	3.03	9.64

The mean difference is significant at the 0.05 level
Data are means of three replicates ± standard deviation
Significant difference due to treatments was assessed by Fisher's LSD as a post-hoc test

Number of seeds per capitula

Phule bhivra showed variation from the control in terms of seed production per capitulum. The number of seeds per capitula is somewhat reduced through GR treatments. Also, the same effect was also shown in EMS treatments. In combination, treatments reduced the number of seeds/capitula with increased doses /concentrations of mutagens were observed (Table 2).

Number of seed per plant

For M1 generation, seed yield/plant data reported a decrease in the average number of seeds produced by each plant for all GR treatments except 200Gy. EMS shows the also positive impact of the doses / concentrations of mutagen decreases number of seeds / plant increases doses / concentrations of mutagens. The combination treatment exhibits the reduced no. of seeds/plants and the highest number of seeds/plants decreased at 400Gy + 0.4% (8.20) (Table 2).

100 seed weight

The average values for 100-seed weight were shown to decrease with increasing doses/concentrations of mutagen (Table 2). Seed weight is decreased by EMS, gamma rays, along with their combinations compared to the control. Higher dosages and concentrations of EMS and gamma rays lowered plant heights in the M_1 generation. Seeds subjected to both high levels of gamma radiation and EMS had fewer main branches. In the treated seeds, greater dosages of gamma rays and EMS resulted in fewer seeds per capitula. Increased gamma ray and EMS dosages result in fewer seeds produced per plant. The same finding observed in Niger [17]. Physical and chemical mutagens were present in higher quantities, delaying the initial blooming by a few days. The same thing found to be true with sesame [9]. In groundnut, the reduced weight of 100 seeds was caused by higher doses or concentrations of both chemical as well as physical mutagens [10]. Physical damage in the M_1 generation, resulting to modifications in quantitative traits, has been reported in almost all papers on induced mutation research in diverse agricultural plants. The number of main branches, days until first blooming, number of seeds per capitula, days until capitula maturity, number of seeds/plants, and weight of 100 seeds were all reduced by the mutagenic treatments, with a few exceptions. Senapati *et al*. [20] as well as Bolbhat and Dhumal [6] all claim that the mutagens may have all affected the genes producing diverse traits that can be spread throughout the genome.

CONCLUSION

As mutagen dosage/concentrations increased, seed germination decreased to a greater extent. With very few exceptions, almost all mutagenic treatments reduced height of plant, no. of primary branches/plant, no. of seeds/capitula, first flowering days, days required for the maturity of capitula, no. seed yield/plant, along with 100 seed weight during M_1 generation.

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