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Effect of Mutagenesis on Germination, Survival and Pollen Sterility in M_1 Generation of Safflower (*Carthamus tinctorius* L.)

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Abstract

Safflower is an important oilseed crops where genetic variability can be induced through mutagenesis. For deciding the appropriate doses of mutagens, the study on effect of mutagens in M_1 generation on various physiological traits are important and prerequisite. Therefore, the present investigation was undertaken to study the effect of mutagens on emergence, plant survival and pollen sterility in two varieties of safflower (*Carthamus tinctorius* L.) in M_1 generation. The seeds of safflower varieties AKS 207 and PKV Pink were treated with various doses of gamma rays viz., 250 Gy, 300 Gy, 350 Gy and concentration of EMS viz., 0.4%, 0.5% and 0.6%. Effect of these mutagens on seed germination, plant survival and pollen sterility were studied in M_1 generation. Results revealed the significant effects of various mutagenic dosages/treatments periods on emergence, plant survival and pollen sterility as compared to control in both the varieties. In this investigation, the seed emergence and plant survival decreased with increased in doses/concentrations of both gamma rays and EMS. The pollen sterility was found to be increased with an increasing doses /concentration of both gamma rays and EMS in M_1 generation. This information is very useful in deciding the doses/treatments of both mutagens in both safflower varieties.

Key words : *gamma rays, EMS, emergence, plant survival, pollen sterility.*

Introduction

Safflower (*Carthamus tinctorius* L.) is one of the important and oldest oil yielding crops cultivated around the world for centuries, mainly as a source of edible oil and dyes. In India it is most commonly known as *Kardai* in Marathi and *Kusum* in Hindi. It is a member of the compositae/ Asteraceae family. Among the 25 species of Genus *Carthamus*, *Carthamus tinctorius L.* is only cultivated species with 12 pairs of chromosomes (2n=24). Safflower is predominantly a self-pollinated crop. This deep root system allows the plant to extract water and nutrients from deeper layers of soil than many other crop plants which impart resistance to drought conditions (1, 2). Safflower is being grown mainly in India, China, Mexico, USA, Ethiopia, Argentina and Australia. In India, it is mainly grown in Maharashtra, Karnataka and parts of Andhra Pradesh, Madhya Pradesh, Orissa, Bihar, etc. At present, safflower occupies a seventh place in the acreage dedicated to oilseeds in India. Maharashtra (58%), Karnataka (21%), Gujarat (12%) along with Telangana, account for 94% of the total acreage and about 99% of the country's production. For crop breeding, the variability in the breeding material is the basic requirement for genetic improvement.

Both physical and chemical mutagens can be used to induce mutations in crop plants and subsequent improvement can be done through selection. Gamma rays were successfully employed for creating the genetic variability in safflower (3,4,5). Among physical mutagens, gamma rays is the most potent mutagen due to its better penetration and ionizing capacity in plant tissues. In case of chemical mutagens, EMS (Ethyl Methane Sulphonate) is reported as the highly effective and powerful mutagen compared to other mutagenic agents and typically produces only point mutations (6, 7). Seed germination, seedling growth, pollen sterility and chromosomal aberration are some of the commonly used criteria for studying mutagenic sensitivity in plants (8). Creation of genetic variability by induced mutagenesis proved best for strengthening crop improvement programme and represents a more efficient source of genetic variability than the gene pool conserve by nature (9). The present study was conducted to understand the immediate effects of mutagenesis on safflower in terms of emergence, plant survival and pollen sterility in order to select most appropriate doses/concentrations of mutagens. Considering the above facts the research programme was therefore, undertaken to induce genetic variability and to screen useful mutants or their use in improvement in Safflower. However, in early and late generation the germination, survival, pollen sterility are more important as initial indicators.

Materials and Methods

The experiment was conducted on field of Oilseeds Research Centre, Dr. Panjabrao Deshmukh Krishi

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Vidyapeeth, Akola (Dr. PDKV, Akola) on M_1 generation. During rabi 2021-22 Two popular varieties of safflower AKS 207 and PKV pink formed the materials for the present investigation. These varieties were recommended for cultivation in Maharashtra. The seeds of safflower were treated with gamma rays (Co^{60}) doses of 250 Gy, 300 Gy and 350 Gy at Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Trombay, Mumbai. The chemical mutagen, ethyl methane sulphonates ($CH_3SO_2OC_2H_5$) from the Himedia chemical company, was used for treating the seeds. The uniform well filled and pure 1000 dry seeds of these varieties were pre-soaked in distilled water for 3 hours and then dipped in enough mutagenic solution of different concentrations (0.4, 0.5 and 0.6% EMS) in 0.1 M Sodium Phosphate buffer (pH 7.0) for 12 hours duration in a shaker at 200 rpm at 25+2°C in 0.1 M Sodium Phosphate buffer (pH 7.0) to prevent rapid hydrolysis and incubated for 12 hr. at room temperature in a shaking incubator (200 rpm) in a dark. Control seeds were also treated with 0.1 M phosphate buffer (pH 7.0) and then processed like the EMS-treated seeds (Table 1). The mutagenic treatment was terminated by decanting the mutagen solution and washing the seeds thoroughly in running tap water for 2h. The details of treatments are given in Table-1.

Raising the M_1 generation : The M_1 generation was grown during rabi 2021-22 at Oilseeds Research Centre, Dr. PDKV, Akola by dibbling method with a spacing of 45 x 20 cm. Six treatments, dry and wet control of both varieties were sown without replication. All the recommended agronomical practices and fertilizer doses were given.

Observation of M_1 generation in the field

Emergence (%) : Emergence (%) was determined by the appearance of cotyledonary leaves above the ground surface. The observations were recorded on the 15th day after sowing and reported in percent. Germination percentage was calculated by using the following formula:

$$\text{Emergence (\%)} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Plant Survival (%) : The number of seedlings that survived 30 days after sowing was counted. The following formula was used to compute the survival percentage:

$$\text{Survival (\%)} = \frac{\text{Total number of seeds survived}}{\text{Total number of seeds sown}} \times 100$$

Pollen sterility (%) : Pollen sterility was determined in ten randomly selected plants in each treatment (in each plant five flowers were randomly selected by straining the pollen with 0.1% percent acetocarmine solution. Pollen grains stained fully were regarded as viable (fertile) while unstained, partially stained and shrivelled ones as sterile. The count was converted to percentage and calculated by using the following formula :

$$\text{Pollen sterility (\%)} = \frac{\text{No. of unstained / partially stained / shrivelled pollen grains}}{\text{Total number of pollen grains}} \times 100$$

Results and Discussion

Emergence (%) : The effect of different dosages of gamma rays and ethyl methane sulphonate (EMS) on seed emergence in AKS 207 and PKV Pink varieties of safflower were studied during M_1 generation and results are presented in Table-2. The results revealed the significant effects of various treatments of mutagen on seed emergence. The seed emergence percentage in both the varieties was reduced in all the mutagenic treatments as compared to control.

AKS 207 : The highest emergence (69.00%) was observed in 250 Gy followed by 300 Gy (64.10%) dose of gamma rays. The maximum reduction of seed emergence was observed in 0.6% EMS (35.70%) of followed by 0.5% EMS (48.60%) as compared to dry control (90.80%) (Table-2).

PKV Pink : The highest emergence (72.60%) was observed in 250 Gy followed by 300 Gy (65.70%) dose of gamma rays. The maximum reduction of seed emergence was observed in 0.6% EMS (38.20%) followed by 0.5% EMS (52.70%) as compared to dry control (88.90%) (Table-2). The seedling emergence was found to be decreased significantly with increasing doses/ concentration of physical and chemical mutagen in both the varieties of safflower. Inhibition of emergence is taken as an indication of degree of mutagen sensitivity and the extent of genetic as well as physiological damage caused by the mutagen. Similar types of results have been reported by (10) in safflower, (11) in groundnut, (12), (13) in soybean, (6, 14) in sesame in sesame.

Plant survival (%) : The observations on survival (%) were recorded on 30th days after sowing. The plant survival has been presented in Table 2. The plant survival was found to be reduced in all the mutagenic treatment and both the varieties as compared to their respective control.

AKS 207 : The highest plant survival was recorded at 250 Gy dose of gamma rays (67.70%) followed by 300 Gy (62.80%) and 0.4% EMS (54.40%) doses/ concentrations of mutagenic treatments. The maximum reduction in plant survival was recorded in EMS treatments as compared to gamma rays treatments. The lowest plant survival was recorded at in 0.6% EMS (32.30%) followed by 0.5% EMS (46.10%) and 350 Gy gamma rays (49.20%).

Table-1 : Physical and chemical mutagenic treatment details for safflower varieties AKS 207 and PKV Pink.

Mutagens	Dose/ concentration	Duration of pre-soaking (hrs.)	Duration of treatment (hrs.)	PH buffer solution	Number of seeds treated
AKS 207					
Gamma rays	250 Gy	-	-	-	1000
	300 Gy	-	-	-	1000
	350 Gy	-	-	-	1000
EMS %	0.4%	3	12	7.0	1000
	0.5%	3	12	7.0	1000
	0.6%	3	12	7.0	1000
Dry Control	-				1000
Wet Control	Phosphate buffer	3	12	7.0	1000
PKV Pink					
Gamma rays	250 Gy	-	-	-	1000
	300 Gy	-	-	-	1000
	350 Gy	-	-	-	1000
EMS %	0.4%	3	12	7.0	1000
	0.5%	3	12	7.0	1000
	0.6%	3	12	7.0	1000
Dry Control	-	-	-	-	1000
Wet Control	Phosphate buffer	3	12	7.0	1000

Table-2 : Effect of various mutagens and their treatments on emergence, plant survival and pollen sterility in M1 generation of safflower varieties AKS 207 and PKV Pink.

Sr. No.	Treatment	Emergence (%)		Plant survival at flowering (%)		Pollen sterility (%)	
		AKS 207	PKV Pink	AKS 207	PKV Pink	AKS 207	PKV Pink
1.	250 Gy	69.00	72.60	67.70	69.70	19.05	17.54
2.	300 Gy	64.10	65.70	62.80	63.30	22.78	20.32
3.	350 Gy	52.50	54.80	49.20	52.10	34.00	31.84
4.	0.4 % EMS	56.70	59.30	54.40	57.90	43.75	39.29
5.	0.5 % EMS	48.60	52.70	46.10	50.70	47.44	43.50
6.	0.6 % EMS	35.70	38.20	32.30	34.60	52.83	49.94
7.	Dry Control	90.80	88.90	89.20	87.50	7.14	5.26
8.	Wet Control	88.40	87.20	86.60	84.70	8.53	4.76

PKV Pink : The same trend as AKS 207 was noticed in PKV Pink for plant survival was observed following the mutagenic treatments. The highest plant survival was recorded in 250 Gy dose of gamma rays (69.70%) followed by 300 Gy (63.30%) and 0.4% EMS (57.90%). The maximum reduction for plant survival percentage was recorded by EMS over gamma rays treatments. The lowest plant survival percentage was recorded in 0.6% EMS (34.60%) followed by 0.5% EMS (50.70%) and 350 Gy gamma rays (52.10%). In these studies, the plant survival rate decreased with an increase in doses /concentrations of both physical and chemical mutagenic treatments over control in M_1 generation. Similar types of results have been reported by (10) in safflower, (13) in soybean.

Pollen sterility : The effect of different dosages of gamma rays and ethyl methane sulphonate (EMS) on pollen sterility in two varieties of Safflower were studied during M_1 generations and results are presented in Table-2.

AKS 207 : In the present study, the pollen sterility (%) of AKS 207 was increased in all the mutagenic treatment as compared to control. The highest pollen sterility was recorded in 0.6% EMS (52.83%) treatment followed by 0.5% (43.50%) and 0.4% EMS (39.29%). Among the gamma ray treatments, the lowest reduction in fertility was recorded in 250 Gy (17.54%) and increased with increasing doses of gamma rays.

PKV Pink : In PKV Pink, pollen sterility was also increased in all the mutagenic treatment as compared to control. The maximum pollen sterility was recorded in 0.6% EMS (49.94%) followed by 0.5% (47.44%) and 0.4% EMS (43.75%). The lowest reduction in fertility was recorded in 250 Gy (17.54%) than wet control (4.76%).

In the present investigation, it was observed that the pollen sterility rate increased with an increase in dose /concentrations of both physical and chemical mutagenic treatments in M_1 generation. Gawande *et al*. (2022) reported that the pollen sterility percent was increased with the increasing dose of gamma rays and EMS dose/ concentration. Also, the results are in agreement with the work done by (15) in safflower, (16) in sesame and in soybean.

Conclusion

It was concluded that the seed emergence, plant survival and pollen fertility of both the varieties of safflower was decreased with an increase in dose/concentrations of both physical and chemical mutagenic treatments over their respective control in M_1 generation. however, the effect of EMS was more significant as compared to gamma rays on germination, plant survival and pollen fertility in both varieties which indicating the importance of these traits for deciding the optimum doses/ concentrations of mutagen for inducing greater frequencies of desirable mutants following mutagenic treatments.

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