

OPTIMIZATION OF TREATMENTS OF GAMMA RAYS AND EMS TO INDUCE GENETIC VARIABILITY IN SAFFLOWER

(Carthamus tinctorius L.)

J.P. Khatod¹, S.J. Gahukar^{1*}, V. J. Dhole² and P. Kadirvel³¹Oilseeds Research Centre, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola- 444104. (MS), India.²Bhabha Atomic Research Centre, Trombay, Mumbai - 400085 (MS), India.³Indian Institute of Oilseed Research, Hyderabad – 500030 (TS), Indiaj_khatod@rediffmail.com

(MS Received: 07.02.2024; MS Revised:09.03.2024; MS Accepted:10.03.2024)



MS 3193

(RESEARCH PAPER IN SERICULTURE,)

Abstract

Induced mutation is one of the quickest methods of plant breeding to induce genetic variability for various desirable traits in crop like safflower which is having less genetic variability. Study of mutagenic sensitivity is the first step in mutation breeding to optimize mutagenic dose hence, present study was carried out to estimate LD₅₀ dose in safflower varieties AKS 207 and PKV Pink on the basis of plant survival. The dry, pure seeds of both varieties were treated with different doses of physical mutagen gamma rays viz., 50, 100, 150, 200, 250 and 300 Gy and different concentrations of chemical mutagen ethyl methane sulphonate (EMS) viz., 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6%. The treated seed along with control were sown in plastic trays. The plant survival was recorded in each treatment up to 30 days after sowing. LD₅₀ value was computed based on mortality percentage and Probit value. The survival percentage was affected by mutagenic treatments of both gamma rays and EMS. The plant survival decreases with increasing dose of mutagens in both varieties treated with mutagens. Chemical mutagen EMS caused the higher decline in plant survival percentage than gamma rays in both the varieties. On the basis of LD₅₀ values, safflower variety 'AKS 207' was found to be more sensitive to both gamma rays (LD₅₀ ~288.03 Gy) and EMS (LD₅₀ ~0.46%) as compared to variety 'PKV Pink' (325.64 Gy and 0.49% of LD₅₀ values of gamma rays and EMS respectively). The optimum mutagenic doses for 'AKS 207' will be 200 to 250 Gy and 0.3 to 0.4% and for 'PKV Pink' will be 250 to 300 Gy and 0.4 to 0.45% of gamma rays and EMS respectively. The differential response of varieties to mutagenic treatments has emphasised the importance of the present study in safflower.

Key words: EMS, Gamma rays, LD₅₀, safflower, seedling survival

Introduction

Safflower (*Carthamus tinctorius* L.) is one of the important oilseed crops belongs to the Asteraceae/Compositae family and valued for its quality oil and dye from its petals. It is also known as "Kardai" in Marathi and "Kusum" in Hindi in India. Out of 25 species of genus *Carthamus*, only one species *Carthamus tinctorius* L. (2n=24) is cultivated. Owing to its deep root system, safflower is drought-tolerant crop that thrives in heavy soils with low soil moisture (Pushpavalli *et al.*, 2017). Apart from India, safflower is cultivated in various countries like China, Mexico, United States, Ethiopia, Argentina and Australia. There is urgent need to increase oilseed production as India is importing oil from other countries spending huge foreign reserve. In India, it is mainly grown in Maharashtra (58%), Karnataka (21%), Gujarat (12%) along with Telangana accounts for 94% of the total acreage and about 99% of the country's production. Safflower is seventh important oilseed crop in India where it is mainly grown in rainfed conditions thereby leading to low productivity. The area, production and productivity are not increasing in India as productivity is very less and fluctuating between 600 to 700 kg/ha. The main reasons for low productivity of safflower are susceptibility to various biotic and abiotic stresses, low oil content (24-36%) due to high proportion of hull in seed hence fetches less price in the market. Safflower is having less genetic variability which is the main obstacle in the genetic improvement and development of high yielding varieties with resistant to various biotic and abiotic stresses, high oil content with improvement in quality. Most of the existing genetic variability is exploited for genetic improvement of safflower and yield levels is plateaued. Creation of new genetic variability for yield and yield contributing traits is require breaking the yield plateau and broadening the genetic base of existing varieties. Induced mutations are one of the most important methods which can create genetic variability in crop plants there by inducing micro and macromutations which was evident from the development of more than 3400 mutant varieties of various crop plants all over the world (MVD, IAEA, 2023). Safflower has very narrow genetic variability for morphological, agronomical and biochemical traits (Rampure *et al.*, 2014). Few reports are available towards the use of mutation technique in improvement of safflower (Mallikarjunradhaya, 1978; Ramchandram and Goud, 1983; Velasco *et al.*, 2000; Kotcha *et al.*, 2007). But most of them were depicted the usage of chemical

mutagenesis. Usage of gamma rays and other physical mutagens are limited in the literature. Physical and or chemical agents can be used for induced mutagenesis. In terms of physical mutagens, gamma rays have been widely used due to their ionizing nature and high penetrating capacity (Khin, 2006). In case of chemical mutagens, EMS is a common alkylating agent which causes G/C to A/T transitions leads to point mutations whereas ionizing radiations produce chromosomal abnormalities and deletions (Bhat *et al.*, 2007). However, when these two agents (gamma rays and EMS) diverge in their potential to create irreversible abnormalities such as lethality and sterility, then they may be considered to differ in their mutagenic efficiency. Use of suitable mutagen, their dose/ concentration and handling of mutant generations are very important steps to create maximum variability with minimum mortality. As a result, determining the optimal dose range is critical for inducing macro mutants with the least amount of biological damage.

Mutagen treatments in general reduce seed germination, growth rate, vigour and fertility. There is substantial killing of plants during different stages of development, thus considerably reduces the survival of plants. The dose required for high mutagenic efficiency depends on properties of the mutagenic agent and material to be treated. Hence, a hyper-dose might kill too many treated individuals and a hypo-dose will produce fewer mutations. The optimum dose will produce the high frequency of mutations and cause minimum killing. Many workers felt that a dose near to LD₅₀ should be the optimum which varies with crop species and mutagen used (Singh, 2000). Therefore, the information on suitable dose of a particular mutagen for a specific crop, species and different varieties/ material of a same species is highly important. In Safflower crops, there is very limited information on optimum dose of physical and chemical mutagens and the reports on species wise and cultivar differences are not available. Hence present investigation was carried out to find out the lethal dose (LD₅₀ values) and optimum dose of gamma rays and Ethyl methane Sulfonate (EMS) in safflower varieties AKS 207 and PKV Pink based on plant survival rate.

Materials And Methods

The present study was conducted at Dr. Panjabrao Deshmukh Krishi Vidyapeeth (Dr. P.D.K.V.), Akola, Maharashtra (India) during 2021-22. The experimental material consisted of two well adopted and popular varieties of safflower viz., AKS 207 and PKV Pink. The pure

seeds were procured from the Senior Research Scientist (Oilseeds), Dr. PDKV, Akola. Uniform 300 pure dry, wellfilled seeds with about 8-10% moisture content were exposed each to 50, 100, 150, 200, 250 and 300 Gy doses of gamma rays (CO^{60}) at Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Trombay, Mumbai. For chemical mutagen treatment the uniform 300 pure dry seeds of both varieties were presoaked in distilled water for 3 hours and then dipped in enough mutagenic solution of different concentrations (0.1%, 0.2%, 0.3% 0.4%, 0.5% and 0.6% EMS). Chemical mutagenic treatment was carried out in a shaker at 100 rpm at 25 ± 2 °C for 12 hours. The dry but un-treated seeds soaked in distilled water served as control in case of both varieties of safflower. Hundred seeds of each genotype for each replication were first presoaked in distilled water for 3 h and then treated overnight with the chemical mutagen. The overnight treated seeds were washed under running tap water for 2 hrs to remove residual EMS from the seeds. The mutagen treated seeds were sown in the plastic tray during rabi 2021-22 at Oilseeds Research Centre, Dr. P.D.K.V., Akola by dibbling method in simple RBD design with control in three replications. All the recommended agronomical practices and fertilizer dose were given. In M_1 generation, the effect of mutagens on plant survival was studied. Seedlings survived up to 30th days after sowing were counted. Survival percentage was calculated by using the following formula:

Plant Survival (%):

$$\text{Survival (\%)} = \frac{\text{Total no. of seedling survived.}}{\text{Total no. of seeds emerged.}} \times 100$$

Probit Analysis for LD₅₀ value

The LD₅₀ (lethal dose) value of gamma rays and EMS for safflower varieties 'AKS 207' and 'PKV Pink' was calculated by following the Probit analysis (Finney, 1978). The inverse cumulative distribution function or quantile function associated with the standard normal distribution is represented by the probit function. The dose/concentration of mutagens was transformed into log₁₀ values. The mortality per cent was determined and corrected mortality percent was calculated using Abbott's formula.

Mortality (observed – control)

$$\text{Corrected mortality (\%)} = \frac{\text{Mortality (observed – control)}}{100 - \text{Control mortality}} \times 100 \quad (1)$$

The corrected mortality proportions (P) were converted to empirical probits (y) and a dose response regression curve drawn using log₁₀ doses (x) and empirical probits (y). Empirical probits (y) values <1 and >7 is ignored (Hayes, 2014).

$$(x - \mu) \text{ Empirical probits (y)} = 5 + \text{-----} (2)$$

From equation (2) the expected probits (Y_i) were derived. The LD values were derived from the curve drawn using probits and log doses. Antilog to the Log₁₀ value corresponds to respective probit value and 95% fiducial confidence limits are calculated using the formula:

$$\text{Fiducial Limits} = \text{Antilog (Log}_{10} \text{ Dose} \pm 1.96 \text{ (SE)})$$

Probit analysis used to determine the optimal lethal dose and empirical probit units and LD₅₀ were computed in Microsoft Excel 2010.

Result And Discussion

Effect of Mutagen on Plant survival percentage:

The number of seedlings surviving of varieties 'AKS 207' and 'PKV Pink' in M_1 generation was recorded and converted to percentage. The seedling survival percentage has been presented in Table 2. The seedling survival percentage of 'AKS 207' and 'PKV Pink' was reduced in all the mutagenic treatment as compared to control. Chemical mutagen EMS caused the higher decline in plant survival percentage than gamma rays in both the varieties. The variety 'AKS 207' was more sensitive to mutagenic treatments as compared to the variety 'PKV Pink'. The maximum plant survival of 89 % and 90% was recorded in 'AKS 207' and 'PKV Pink' treated with 50 Gy dose of gamma rays, respectively. The lowest plant survival per cent was recorded in 'AKS 207' (35%) and 'PKV Pink' (38%) treated with 0.6% EMS followed by the 300 Gy dose of gamma rays in 'AKS 207' (42%) and 'PKV Pink' (44%). In the present investigation 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 in safflower (Gawande *et al.*, 2022; Satpute and Kothekar 1996), Soybean (Bhoite *et al.*, 2019; Ozdinc and Yalcin, 2019), rapeseed (Yadav *et al.*, 2016) for EMS, sesame (Singh *et al.*, 2018; Diouf *et al.*, 2010) and African sesame (Boureima *et al.*, 2009) and sunflower (Kumar *et al.*, 2010).

Table 1: Effect of mutagens on plant survival in M_1 generation of safflower varieties 'AKS 207' and 'PKV Pink'

S. N.	Dose/ Concentration	Seedling Survival (%) in AKS 207	Seedling Survival in (%) in PKV Pink
Gamma rays			
1	50 Gy	89	90
2	100 Gy	83	85
3	150 Gy	77	80
4	200 Gy	62	66
5	250 Gy	53	58
6	300 Gy	42	44
Ethyl methane sulfonate			
7	0.1% EMS	86	88
8	0.2% EMS	81	82
9	0.3% EMS	73	72
10	0.4% EMS	55	59
11	0.5% EMS	46	48
12	0.6% EMS	35	38
13	Wet Control	90	92
14	Dry Control	89	91

Determination of LD₅₀ (Lethal Dose) Values

The LD₅₀ values for 'AKS 207' and 'PKV Pink' varieties of safflower were determined through Probit analysis based on plant mortality. The expected LD₅₀ values and Probit units based on mortality percentage of mutated population presented in Table 2 and 3. The

lethal dose refers to the minimum concentration that causes 50% of mortality or 50% survival of mutated seeds. The LD₅₀ differs between genotypes depending on the genetic background, nature of treatment and environmental conditions (Singh, 2005). The optimal dose for high frequency mutations has been determined to be LD₅₀ for

artificially induced mutations using physical or chemical mutagens (Anbarasan *et al.*, 2015). The LD₅₀ value for safflower variety 'AKS 207' was estimated to be at 288.03 Gy for gamma rays and 0.46% EMS (12 hr soaking) for EMS. Similarly, the LD₅₀ value for safflower variety 'PKV Pink' was 325.64 Gy dose of gamma rays and 0.49% (12 hr soaking) EMS dosage, respectively. On the basis of LD₅₀ values, safflower variety 'AKS 207' was found to be more sensitive to both gamma rays (LD₅₀ ~288.03 Gy) and EMS (LD₅₀ ~0.46%) as compared to variety 'PKV Pink' (325.64 Gy and 0.49% of LD₅₀ values of gamma rays and EMS respectively). The differential response to mutagenic treatment was also reported in various oilseed crops. Similarly, Gawande *et al.*, (2022) estimated LD₅₀ of gamma rays in safflower as 299.5 Gy and 0.25% EMS (18 hr. soaking) while Niu *et al.*, (2009) reported LD₅₀ around 300 Gy for gamma rays in safflower. Yadav *et al.* (2016) recorded the LD₅₀ doses of EMS at

0.42%, 0.73% and 0.3% for duration of 12 hr for Indian mustard varieties (RH-749, NRCR-101) and *S. alba* respectively. Ozdinc and Yalcin (2019) estimated the GR₅₀ doses for three soybean varieties (229 to 265 Gy). On the basis of LD₅₀, the optimum mutagenic doses for 'AKS 207' were estimated to be 200 to 250 Gy gamma rays and 0.3 to 0.4% EMS while for 'PKV Pink' it was estimated to be 250 to 300 Gy gamma rays and 0.4 to 0.45% EMS. The differential response of varieties to mutagenic treatments has emphasized the importance of the present study in safflower. The above estimated optimum dose of gamma rays and EMS for both varieties will be very useful for safflower breeders to create new genetic variability.

Table 2: Estimation of LD₅₀ dose based on Plant survival (Gamma rays)

Dose of gamma rays (Gy)	Log ₁₀ value of dose	Reduction in plant survival % (Mortality %)	Probit value	LD ₅₀ value	LD ₅₀ Dose
AKS 207					
50	1.70	11	3.77	2.4594	Antilog (2.4594) = 288.03 Gy
100	2.00	17	4.05		
150	2.18	23	4.26		
200	2.30	38	4.69		
250	2.40	47	4.92		
300	2.48	58	5.20		
PKV Pink					
50	1.70	10	3.72	2.5127	Antilog (2.5127) = 325.64 Gy
100	2.00	15	3.96		
150	2.18	20	4.16		
200	2.30	34	4.59		
250	2.40	42	4.80		
300	2.48	56	5.15		

Table 3: Estimation of LD₅₀ dose based on Plant survival (EMS %)

Conc. of EMS (%) for 12 hr.	Conc. of EMS (ppm)	Log ₁₀ value of conc.	Reduction in survival % (Mortality %)	Probit value	LD ₅₀ value	LD ₅₀ Dose
AKS 207						
0.1	1000	3.00	14	3.92	3.6688	Antilog (3.6688) = 4665.50 =0.4665%
0.2	2000	3.30	19	4.12		
0.3	3000	3.48	27	4.39		
0.4	4000	3.60	45	4.87		
0.5	5000	3.70	54	5.10		
0.6	6000	3.78	65	5.39		
PKV Pink						
0.1	1000	3.00	12	3.82	3.6966	Antilog (3.6966) = 4973.39 =0.4973%
0.2	2000	3.30	18	4.08		
0.3	3000	3.48	28	4.42		
0.4	4000	3.60	41	4.77		
0.5	5000	3.70	52	5.05		
0.6	6000	3.78	62	5.31		

Conclusion:

The present investigation successfully estimated LD₅₀ values of mutagenic treatments for two safflower varieties. On the basis of LD₅₀, the optimum mutagenic doses for 'AKS 207' were estimated to be 200 to 250 Gy gamma rays and 0.3 to 0.4% EMS while for 'PKV Pink' it was estimated to be 250 to 300 Gy gamma rays and 0.4 to 0.45% EMS. The differential response of varieties to mutagenic treatments has emphasized the importance of the present study in safflower. The above estimated optimum dose of gamma rays and EMS for both varieties will be very useful for safflower breeders to create new genetic variability.

Acknowledgement

Authors are thankful to Dr. B. K. Das, Scientific Officer, Nuclear Agriculture and Biotechnology Division, BARC, Mumbai for their co-operation during the present study.

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