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Optimal lethal dose (LD_{50}) of gamma rays and EMS induced mutagenesis in PBNS-12 variety of safflower (*Carthamus tinctorius* L.)

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Abstract
In present investigation, the seeds of safflower variety PBNS-12 were treated with different doses of gamma rays *viz.*, 100 Gy, 200 Gy, 300 Gy, 400 Gy, 500 Gy and different concentrations of EMS *viz.*, 0.1%, 0.2%, 0.3%, 0.4% and their combination treatments. The plant survival percentage was greatly affected by mutagenic treatments of gamma rays and EMS which showed a negative dose dependent relationship in PBNS-12 variety. Based on mortality percentages and probit values, the expected LD_{50} value was computed. The LD_{50} value for PBNS-12 was fixed at 299.5 Gy for gamma rays and 0.25% for EMS. Gamma rays caused the greatest decline in plant survival percentage, followed by combination and EMS treatments. Gamma rays exhibited significant reduction in plant survival percentage (1%) than EMS (24.45%) in PBNS-12 variety of safflower. It is concluded that both the mutagens are effective to produce significant variations in safflower which can be further explored for mutation mapping.

Keywords: EMS, gamma rays, plant survival, LD_{50}

Introduction
Safflower (*Carthamus tinctorius* L.) is one of the most significant and oldest oil-producing crops, having been cultivated for centuries around the world, primarily as a source of edible oil and colors. It is also known as kardai in Marathi and kusum in Hindi in India. It belongs to the Asteraceae/Compositae family. Carthamus is a genus with 25 species, of which only *Carthamus tinctorius* L. (2n=24) is cultivated. It's a drought-tolerant crop that thrives in heavy soils with low soil moisture (Pushavalli *et al*., 2017). Safflower is cultivated in over 60 countries; especially India, China, Mexico, the United States, Ethiopia, Argentina and Australia are the top producers. In India, it is mainly grown in Maharashtra, Karnataka and parts of Andhra Pradesh, Madhya Pradesh, Orissa, and Bihar etc. Maharashtra and Karnataka are the two most important safflower growing states accounting for 72 and 23 per cent of area and 63 and 35 per cent of production, respectively (Pattar and Patil, 2020) [12].

Mutation breeding helps the geneticists and breeders to create tremendous variability that cannot be achieved through selection or hybridization process. Any agronomic trait can be improved simply by inducing mutations through the development of variability and using a selection system (Cheema *et al.* 2003) [5]. Physical or chemical factors can be used to create artificial mutations. In terms of physical mutagens, gamma rays have been widely used due to their ionizing nature and high penetrating capacity (Khin, 2006) [8], which produce free radicals (Spencer-Lopes *et al.* 2018) when they interact with water molecules present on exposed biological materials, disrupting the H-bond between complementary base pairs of double helix DNA. 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) [2]. 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.

The selection of an efficient mutagen and optimum doses for inducing variability is critical. As a result, determining the optimal dose range is critical for developing desired macro mutants with the least amount of biological damage. In the present investigation studied the lethal dose (LD_{50} values) for gamma irradiated and EMS treated safflower variety PBNS-12 on the plant survival rate.

Material and Methods

The pure seeds of a well adopted and popular variety of safflower (*Carthamus tinctorius* L.), PBNS-12 (Parbhani Kusum) were procured from the safflower Breeder, AICRP on safflower, VNMKV, Parbhani. Uniform 200 pure dry, well filled seeds with about 8-10% moisture of variety PBNS-12 were exposed to 100, 200, 300, 400 and 500 Gy dose of gamma rays (CO^{60}) at Nuclear and Agriculture Division, B.A.R.C., Trombay, Mumbai. For chemical mutagen treatment the uniform 200 pure dry seeds of variety PBNS-12 were presoaked in distilled water for 3 hours and then dipped in enough mutagenic solution of different concentrations (0.1%, 0.2%, 0.3% and 0.4% EMS) and duration. Chemical mutagenic treatment was carried out in a shaker at 200 rpm at 25+2 ^{O}C for 18 hours. Gamma irradiated seeds were also combined treated with EMS at 200 Gy + 0.1% EMS, 200 Gy + 0.2% EMS, 300 Gy + 0.1% EMS and 300 Gy + 0.2% EMS respectively. The dry but un-irradiated and seeds soaked in distilled water served as control in case of both the mutagenic treatments. A detailed account of treatments is tabulated as under.

Table 1: Mutagens

Physical (gamma rays)	Chemical (EMS)	Combined treatment
100 Gy	0.1%	200 Gy + 0.1% EMS
200 Gy	0.2%	200 Gy + 0.2% EMS
300 Gy	0.3%	300 Gy + 0.1% EMS
400 Gy	0.4%	300 Gy + 0.2% EMS
500 Gy	-	-

Field experiment

The mutagen treated seeds were sown in the field during *rabi* 2019-20 at AICRP on safflower, VNMKV, Parbhani by dibbling method with a spacing of 45 x 20 cm in simple RBD design with control in three replications. All the recommended agronomical practices and fertilizer dose was given. In M_1 generation, the effect of mutagens on plant survival was studied.

Plant Survival (%)

Seedlings survived on 30th days after sowing were counted. Survival percentage was calculated by using the following formula:

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

Probit Analysis for LD_{50} fixation

The LD_{50} (lethal dose) value of gamma rays and EMS for PBNS-12 variety of safflower was calculated according to the probit analysis (Finney, 1978) [7]. The inverse cumulative distribution function or quantile function associated with the standard normal distribution is represented by the probit function. The steps for probit analysis are as follow:

- Transformation of the dose/concentration of mutagens into log_{10} values.
- Determination of the mortality % due to treatments doses.
- Corrected mortality percentage was calculated using Abbott's formula

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

The corrected mortality proportions (P) were converted to empirical probits (y) and a dose response regression curve drawn using log_{10} doses (x) and empirical probits (y). Empirical probits (y) values <1 and >7 are ignored (Hayes, 2014).

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

From equation (2) the expected probits (Yi) were derived. The LD or LC values are derived from the curve drawn using probits and log doses. Antilog to the Log_{10} value corresponds to respective probit value and 95% fiducial confidence limits are calculated using the formula:

Fiducial Limits = Antilog (Log10 Dose ± 1.96 (SE))

Statistical Analysis

Probit analysis used to determine the optimal lethal dose and empirical probit units and LD_{50} were computed in Microsoft Excel 2010.

Result and Discussion

Effect of Mutagen on Plant survival percentage

Observations on survival percent was made on 30 days after sowing. The number of plants survived of variety PBNS-12 in M_1 generation was recorded and converted to percentage. The plant survival percentage has been presented in Table 2. The plant survival percentage of PBNS-12 was reduced in all the mutagenic treatment as compared to control. The maximum plant survival 78.15% was recorded at 100 Gy dose of gamma rays followed by 0.1% EMS (75.30%) and 200 Gy +0.1% EMS (74%) doses/ concentrations of mutagenic treatments.

The maximum reduction of plant survival percentage was recorded in gamma rays followed by combination and EMS treatments respectively. The lowest plant survival percentage (1%) was recorded at 500 Gy dose of gamma rays followed by 300 Gy+ 0.2% EMS (14.55%), 0.4% EMS (24.45%) and 200 Gy+ 0.2% EMS (30.67%) doses/ concentrations of mutagenic treatments. In the present investigation the plant survival rate decreased with an increase in doses /concentrations of both physical and chemical and their combinations mutagenic treatments over control in M_1 generation. Similar types of results have been reported by Boureima *et al.* (2009) [4] in african sesame, Satpute and kothekar (1996) [15] in safflower, Bhoite *et al.* (2019) [3] in soybean, Singh *et al.* (2018) [16] in sesame, Kumar *et al.* (2010) [9] in sunflower and Diouf *et al.* (2010) [4] in sesame.

Table 2: Effect of mutagens on plant survival in M_1 generation of safflower variety PBNS-12

Treatment	Plant survival%	Treatment	Plant survival%
100 Gy	78.15	200 Gy + 0.1% EMS	74.00
200 Gy	69.23	200 Gy + 0.2% EMS	30.67
300 Gy	65.06	300 Gy + 0.1% EMS	54.38
400 Gy	60.86	300 Gy + 0.2% EMS	14.55

500 Gy	01.00	Dry control	89.13
0.1%	75.30	Wet control	87.95
0.2%	72.41	-	-
0.3%	49.61	-	-
0.4%	24.45	-	-

Determination of LD_{50} (Lethal Dose) Values

The LD_{50} value for PBNS-12 variety of safflower determined through probit analysis based on plant survival. The expected LD_{50} values and probit units based on mortality percentage of mutated population of PBNS-12 presented in Table 3 and 4.

The lethal dose refers to the minimum concentration that cause 50% of mortality or 50% survival of mutated seeds. The LD_{50} 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 LD_{50} for artificially induced mutations using physical or chemical mutagens (Anbarasan *et al*., 2015) [1]. The LD_{50} of safflower (*Carthamus tinctorius* L.) variety PBNS-12 was found at 299.5 Gy and 0.25% of gamma rays and EMS dosage respectively. Similarly, Niu *et al*., (2009) [10] estimated the optimum dose (LD_{50}) of gamma rays in safflower to be around 300 Gy, but no data on the optimum concentration (LD_{50}) of EMS in safflower. Yadav *et al.* (2016) [18] found that the optimum concentration (LD_{50}) of EMS in *S. alba* is around 0.3% EMS concentration.

Table 3: Estimation of LD_{50} dose based on Plant survival (Gamma rays)

Dose of gamma rays (Gy)	Log_{10} value of dose	Reduction in Plant survival% (Dead %)	Probit value	LD_{50} value	LD_{50} Dose
100	2.00	21.85	4.23	2.4765	Antilog (2.4765) = 299.5 Gy
200	2.30	30.77	4.50		
300	2.48	34.94	4.61		
400	2.60	39.14	4.72		
500	2.70	99.00	6.28		

Table 4: Estimation of LD_{50} dose based on Plant survival (EMS)

Concentration of EMS (%)	Concentration of EMS (PPM)	Log_{10} value of concentration (PPM)	Reduction in Plant survival (%) (Dead %)	Probit value	LD_{50} value	LD_{50} dose
0.1	1000	3.00	24.70	4.33	3.4040	Antilog (3.4040) =2536.0 =0.2536%
0.2	2000	3.30	27.49	4.42		
0.3	3000	3.48	50.39	5.03		
0.4	4000	3.60	75.55	5.71		

Conclusion

It was concluded from the present study that the mutagenic treatment given between 250-350 Gy in the case of gamma rays and 0.2-0.3% concentration of EMS were appropriate for mutagenic treatment in the case of safflower, according to the obtained LD_{50} values. However, gamma rays found to cause higher mortality rates than EMS concentration in PBNS-12 examined. Both the mutagens are efficient to produce significant induced variations in safflower which can be further explored for developing populations and mutation mapping.

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