www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(2): 607-610

www.thepharmajournal.com
Received: 12-12-2021
Accepted: 18-01-2022

Gawande SM
All India Coordinated Research Project on Safflower, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

Ghuge SB
All India Coordinated Research Project on Safflower, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

Kalpande HV
All India Coordinated Research Project on Safflower, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

Wankhade MP
All India Coordinated Research Project on Safflower, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

Corresponding Author:
Gawande SM
All India Coordinated Research Project on Safflower, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

Effect of mutagens on emergence, plant survival and pollen sterility in safflower (*Carthamus tinctorius* L.)

Gawande SM, Ghuge SB, Kalpande HV and Wankhade MP

Abstract
The present investigation was undertaken to study the effect of mutagens on emergence, plant survival and pollen sterility in PBNS-12 variety of safflower (*Carthamus tinctorius* L.) in M_1 generation. 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 concentration of EMS *viz.*, 0.1%, 0.2%, 0.3%, 0.4% and their combination treatments. The observations were recorded on emergence percentage, plant survival percentage and pollen sterility percentage in M_1 generation. Results revealed the significant effects of mutagen dosages and treatments periods on emergence, plant survival and pollen sterility for all the mutagens tested. In the present study, the seed emergence and plant survival rate decreased with increased in doses/ concentrations both in gamma rays and EMS and their combination treatments. The pollen sterility rate increased with an increase in dose /concentrations of both gamma rays and EMS and their combinations treatments in M_1 generation.

Keywords: EMS, gamma rays, plant survival, emergence, pollen sterility

Introduction
Among the oilseeds, 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. There are 25 species in genus *Carthamus* out of which only *Carthamus tinctorius* L (2n=24) is cultivated. It is a drought tolerant crop grown in heavy soils under receeding soil moisture conditions (Pushavalli *et. al*., 2017) [13]. Safflower is being grown in over 60 countries, among which India, China, Mexico, USA, Ethiopia, Argentina and Australia are the major growing countries. In India, it is mainly grown in Maharashtra, Karnataka and parts of Andhra Pradesh, Madhya Pradesh, Orissa, 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]. Genetic variability is the basic requirement for genetic improvement of any crop. Induced mutagenesis is an effective and potential method to create variability in a crop (Kumari *et. al.*, 2016) [10]. Both physical and chemical mutagens can be used to induce mutations in crop plants and subsequent improvement can be done through selection. Among physical mutagens, gamma rays directly penetrate the plant tissue and are partially ionizing. 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 (Hajara, 1979 and Kumari *et. al.*, 2016) [6, 10]. Seed germination, seedling growth, pollen sterility and chromosomal aberration are some of the commonly used criteria for studying mutagenic sensitivity in plants (Lal *et.al.*, 2009 and Sangle *et.al.*, 2011) [11, 17]. 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 beneficial dose / concentration of mutagen.

Material and Methods
The field experiment was conducted on experimental field at AICRP on Safflower, VNMKV, Parbhani during *rabi* 2019-20 in M_1 generation. The experimental material consisted of PBNS-12 variety of safflower, which was procured from the Safflower Breeder, AICRP on safflower, VNMKV, Parbhani. This variety is recommended for cultivation in India. The seeds of safflower were got treated with gamma rays (Co^{60}) doses of 100 Gy, 200 Gy, 300 Gy, 400 Gy and 500 Gy 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+2ºC 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.

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	-	-

Raising the M_1 generation

To raise the M_1 generation the above treated seeds of each of the treatments were sown during *rabi* 2019-20 at AICRP on Safflower, VNMKV, Parbhani by dibbling method with a spacing of 45x 20 cm^2 in simple RBD design with control in three replications. All the recommended agronomical practices and fertilizer dose was given.

The treated seed was space planted in M_1 generation and individual M_1 surviving plants were selfed by nylon nets bags before initiation of flowering. From each plant, first form three capsule was selfed and selfed capsule of an individual was threshed and seeds of each plant were kept separately for raising M_2 generation.

Observation of M_1 generation in the field

1. **Emergence (%):** The emergence of cotyledonary leaves above the ground surface was taken as indication of germination. The observations were recorded after 10^{th}, 12^{th} and 15^{th} days of sowing and were reported in percentage. Emergence percentage was calculated by using the following formula:

$$\text{Emergence (\%)} = \frac{\text{Total no. of seeds emerged}}{\text{Total no. of seeds sown}} \text{ x } 100$$

2. **Plant Survival (%):** Seedlings survived on 30th days after sowing were counted. Survival percentage was calculated by using the following formula:

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

3. **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 2 percent acetocarmine solution. Pollen grains stained fully were regarded as viable (fertile) while unstained, partially stained and shriveled ones as sterile. The count were converted to percentage and averaged out. The pollen sterility percentage was calculated by using the following formula:

$$\text{Pollen sterility \%} = \frac{\text{No. of unstained/ partially stained/ Shriveled ones pollen grains observed}}{\text{Total no. of pollen grains}} \text{ X } 100$$

Result and Discussion

Emergence percentage

The effect of different dosages of gamma rays, ethyl methane sulphonate (EMS) and their combinations on seed emergence in PBNS-12 variety of Safflower were studied during M_1 generation and obtained results are presented in Table 1.

The results revealed the significant effects of different dosages of mutagen on seed emergence for all the mutagens tested. The seed emergence percentage of PBNS-12 was reduced in all the mutagenic treatments as compared to control. The highest emergence percentage (83.4%) was observed in 0.1% EMS concentration followed by 100 Gy (80.4%) dose of gamma rays treatment. The maximum reduction of seed emergence per cent was observed in 500 Gy dose of gamma rays followed by 300Gy +0.2% EMS dose of combination treatment. The lowest emergence percentage (17.6%) was recorded at 500 Gy dose of gamma rays followed by 300 Gy + 0.2% EMS (29.6%) dose of combination treatment.

Inhibition of emergence is taken as an indication of degree of radio sensitivity and the extent of genetic as well as physiological damage caused by the mutagen. In the present investigation the seed emergence rate decreased with increased in doses/ concentrations both in physical and chemical mutagenic treatments. Similar types of results have been reported by Boureima *et. al.* (2009) [2] in African sesame, Kharade *et. al.* (2016) [8] in groundnut, Kumari *et. al.* (2016) [10] in sesame, Bhoite *et. al.* (2019) [1] in soybean, Sandhiya *et. al.* (2020) [16] in sesame and Emrani *et. al.* (2011) [5] in *B. napus*.

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 1. 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) [2] in African sesame, Satpute and kothekar (1996) [18] in safflower, Bhoite *et. al.* (2019) [1] in soybean, Singh *et. al.* (2018) [19] in sesame, Kumar *et. al.* (2010) [9] in sunflower and Diouf *et. al.* (2010) [4] in sesame.

Pollen Sterility percentage

The effect of different dosages of gamma rays, ethyl methane sulphonate (EMS) and their combinations on pollen fertility in PBNS-12 variety of Safflower were studied during M_1 generations and obtained pollen sterility percentage results are presented in Table 1.

In the present study, the pollen sterility percentage of PBNS-12 was increased in all the mutagenic treatment as compared to control. Similaraly, Deshpande and Malode (2015) reported that the pollen fertility percentage was decreased in all the mutagenic treatments over control in Linseed. The maximum pollen sterility percentage (4.97%) was recorded at 300 Gy + 0.2% EMS dose of combination treatment followed by 400 Gy (4.72%), 300 Gy + 0.1% EMS (4.64%) and 0.4% EMS (4.49%) doses/concentrations of mutagenic treatments.

In the present investigation, it was observed that the pollen sterility rate increased with an increase in dose /concentrations of both physical and chemical and their combinations mutagenic treatments in M_1 generation. Singh *et. al.* (2018) [19] reported that the pollen fertility percent was decreased with the increasing dose of gamma rays singally and in combination treatments in sesame (*Sesamum indicum* L.). Also the results are in agreement with the work done by kumari *et. al.* (2016) [10] in sesame, Saha and Paul (2017) in sesame, Kumar *et.al.* (2010) [9] in sunflower, Rampure *et. al.* (2017) [14] in safflower and Kavithamani *et. al*. (2008) [7] in soybean.

Table 1: Effect of different mutagens on emergence, plant survival and pollen sterility in M_1 generation of safflower variety PBNS-12

Sr. No.	Treatments	Emergence %	Plant Survival%	Pollen sterility %
1	100 Gy	80.40	78.15	2.45
2	200 Gy	70.40	69.23	2.70
3	300 Gy	68.20	65.06	3.71
4	400 Gy	62.80	60.86	4.72
5	500 Gy	17.60	01.00	-
6	0.1% EMS	83.40	75.30	2.54
7	0.2% EMS	73.60	72.41	3.45
8	0.3% EMS	65.20	49.61	3.92
9	0.4% EMS	61.60	24.45	4.49
10	200 Gy + 0.1% EMS	78.80	74.00	3.39
11	200 Gy + 0.2% EMS	59.60	30.67	3.65
12	300 Gy + 0.1% EMS	63.30	54.38	4.64
13	300 Gy + 0.2% EMS	29.60	14.55	4.97
14	Dry Control	90.40	89.13	0.35
15	Wet Control	88.40	87.95	0.29

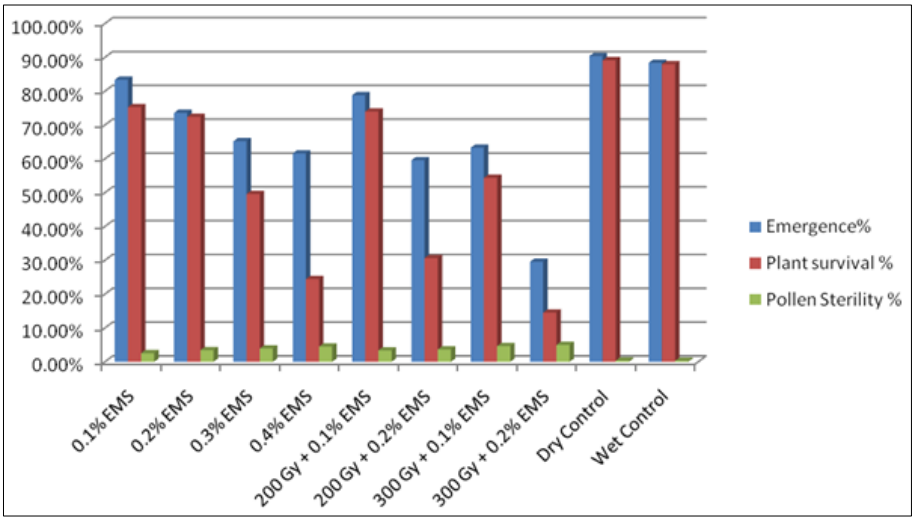

Fig 1: Effect of different mutagens on emergence, plant survival and pollen sterility in M_1 generation of safflower variety PBNS-12

Conclusion

It was concluded from the present study that the seed emergence percent, plant survival percent and pollen fertility was decreased with an increase in dose/concentrations of both physical, chemical and their combinations mutagenic treatments over control in M_1 generation.

Acknowledgement

I Shailesh Murlidhar Gawande thankful to Chhatrapati Shahu Maharaj Research, Training and Human Development Institute (SARTHI), Pune for the financial support to carry out the research work entitled on "Genetic improvement of safflower (*Carthamus tinctorius* L.) through induced mutation". Author also thanks to Dr. Ashok Badigannavar, Scientific officer, Nuclear Agriculture and Biotechnology Division, BARC, Mumbai for his suggestions during the present study.

References

1. Bhoite BS, Kamble MS, Aher AR, Chavan MV. Mutagenic sensitivity in M_1 generation of three varieties of soybean *(Glycine max* L.). Journal of Pharmacognosy

and Phytochemistry. 2019;8(5):1817-1820.
2. Boureima S, Diouf M, Silme RS, Diop T, Damme PV, Cagirgan MI. Radiosensitivity of African sesame cultivars to gamma rays. Turkish J of Field Crops. 2009;14(2):181-190.
3. Deshpande AS, Malode SN. Effect of sodium azide and ethyl methane sulphonate on seed germination, seedling height and pollen fertility in *Linum usitatissimum* var. Pkv NI 260. International E- Research Journal. 2019. ISSN: 2348-7143.
4. Diouf M, Boureima S, Diop T, Cagorgan MI. Gamma rays- Induced mutant spectrum and frequency in sesame. Turkish Journal of Field Crops. 2010;15(1):99-105.
5. Emrani SN, Arzani A, Saeidi G. Seed viability, germination and seedling growth of canola (*Brassica napus* L.) as influenced by chemical mutagens. African Journal of Biotechnology. 2011;10(59):12602-12613.
6. Hajara NG. Induced of mutations by chemical mutagens in tall *indica* rice. Indian Agriculture. 1979;23:67-72.
7. Kavithamani D, Kalamani A, Vanniarajan C, Uma D. Mutagenic effectiveness and efficiency of gamma rays and EMS in soybean (*Glycine max* L. Merrill). Madras Agricultural Journal. 2008;95(7/12):448-451.
8. Kharade MR, Ujjainkar VV, Bankar PB. Effect of mutagens on seed quality characters in groundnut (*Arachis hypogaea* L.). Trends in Biosciences. 2016, 9(7). ISSN 0974-8431, 430-435.
9. Kumar PR, Ratnam SV. Mutagenic effectiveness and efficiency in varieties of sunflower (*Helianthus annus* L.) by separate and combined treatment with gamma rays and sodium azide. African Journal of Biotechnology. 2010;9(39):6517-6521.
10. Kumari V, Chaudhary HK, Prasad R, Kumar A, Singh A, Jambhulkar S, *et al*. Effect of mutagenesis on germination, growth and fertility in sesame (*Sesamum indicum L.*). Annual Research and Review in Biology. 2016;10(6):1-9, Article no. ARRB. 26983.
11. Lal GM, Torns B, Lal SS. Mutagenic sensitivity in early generation in black gram. Asian Journal of Agricultural Sciences. 2009;1:9-11.
12. Pattar VK, Patil R. Correlation and path analysis in safflower (*Carthamus tinctorius* L.) genotypes. Journal of Pharmacognosy and Phytochemistry. 2020;9(4):1717-1719.
13. Pushpavalli SNCVL, Kumar G. Study of genetic variability, correlation and path analysis of safflower genotypes. Research Journal of Agricultural Sciences. 2017;8(3):706-709.
14. Rampure NH, Choudhary AD, Jambhulkar SJ, Badere RS. Isolation of desirable mutants in safflower for crop improvement. Indian J Genet. 2017;77(1):134-144.
15. Saha S, Paul A. Effectiveness and efficiency of gamma rays on sesame (*Sesamum indicum* L.) Genotypes. The Bioscan. 2018;13(1):197-201.
16. Sandhiya V, Kumar M, Parameswari C, Vanniarajan C, Kumaravadivel N, Dakthivel N, *et al*. Determination of optimum dose of chemical mutagen for large scale seed treatment of white seeded sesame (*Sesamum indicum* L.) varieties. Electronic Journal of Plant Breeding 2020;11(1):238-242.
17. Sangale SM, Mahamune SE, Kharat SN, Kothekar VS. Effect of mutagenesis on germination and pollen sterility in pigeonpea. Bioscience Discovery. 2011;2(1):127-130.
18. Satpute RA, Kothekar VS. Mutagenic efficiency and effectiveness in safflower. Journal of Nuclear Agriculture and Biology. 1996;25:230-234.
19. Singh L, Singh PP, Mishra MN. Effect of gamma rays, hydroxylamine and maleic hydrazide on germination, plant survival and pollen viability in sesame (*Sesamum indicum* L.). Journal of Pharmacognosy and Phytochemistry. 2018, 293-296.