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The Optimal LD50 Gamma Ray and Sodium Azide-Induced Mutagenesis in the PBNS-86 Variety Safflower

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ABSTRACT

Safflower has been grown for millennia all over the world, making it one of the most important and ancient oil-producing crops. In this work, we use gamma rays and sodium azide at LD_{50} levels to target high-yielding and desirable characters. The safflower variety PBNS-86 seeds were subjected to varying quantities of sodium azide (0.005%, 0.010%,0.015%,0.020% and 0.025%) and gamma-ray treatments at levels of 100Gy, 200Gy, 300Gy, 400Gy and 500Gy. The carcinogenic administrations of sodium azide and gamma rays had a detrimental dose-dependent association with the plant survival percentage in the PBNS-86 variety. The anticipated LD_{50} value was calculated using probit values and fatality percentages. For gamma rays and sodium azide, the LD50 value of PBNS-86 was set at 296.2 Gy and 0.1513%, respectively. The greatest reduction in the proportion of plants that survived was induced by gamma rays and sodium azide treatments. It is determined that both mutagens are capable of causing notable changes in safflower, which may be investigated further for the purpose of mutation mapping.

Keywords- Safflower, Gamma ray, sodium azide, and LD_{50}.

I. INTRODUCTION

Centuries of cultivation have yielded edible oil and colors for people all over the world, making safflower (*Carthamus tinctorius L.*) one of the most important and ancient oil-producing crops. In the nation of India, it is also referred to as kusum in Hindi and kardai in Marathi. It is a member of the Compositae/Asteraceae family. Out of the 25 species in the genus Carthamus, only *Carthamus tinctorius L.* (2n=24) is grown in cultivation. This crop can withstand drought and grows well in thick soils with low soil moisture (**Pushavalli *et al.*, 2017**) [1].

More than 60 countries farm safflower ower; the countries with the highest yields are Ethiopia, Argentina, Australia, China, India, and the United States. It is mainly produced in Maharashtra, Karnataka, and certain regions of Andhra Pradesh, Madhya Pradesh, Orissa, Bihar, and other states in India. The two states in India that grow the greatest amount of safflower are Maharashtra and Karnataka, which account for 72% of the state's acreage and 35% of its production, respectively (**Pattar and Patil, 2020**)[2].

Geneticists and breeders can create enormous variety via mutation breeding that is not possible through selection or hybridization. By utilising a selection method and creating variability, any agronomic trait can be enhanced by only introducing mutations (**Cheema *et al.* 2003**)[3]. Artificial mutations can be produced by either physical or chemical processes. Gamma rays are a common physical mutagen because of their high penetrating power and ionising character (**Khin, 2006**) [4], which leads to in the production of free radicals (**Spencer-Lopes *et al.* 2018**)[5]. when the complementary base pairs of double helix DNA's H-bond are broken by the free radicals' interaction with water particles on exposed biological substances. Regarding chemical mutagens, sodium azide is a mutagenic substance that has been shown to cause mutations in a variety of agricultural

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plants, such as rice, wheat, and winter barley. **Broyana (2020)** [6] has been observed to considerably reduce yield-related features, shoot and root length, and germination in many plant species (**Vinitashira *et al.*, 2020**)[7]. nevertheless, it might be argued that the mutagenesis efficiency of these two agent's gamma rays and sodium azide differs when it comes to their capacity to produce irreversible defects including mortality and sterility. It's crucial to choose an effective mutagen and the right dosages to induce variability. Determining the ideal dose range is therefore essential for producing the intended macro mutants with the least degree of potential biological damages. The current study examined the effects of gamma-irradiation and sodium azide treatment on the plant survival rate of the safflower variety PBNS-86.

II. MATERIALS AND METHODS

The safflower breeder, AICRP on safflower, VNMKV, Parbhani, provided the pure seeds of a well-liked and widely used variety of safflower (*Carthamus tinctorius L.*), PBNS-86 (Parbhani Kusum). The PBNS-86 variety's uniform 200 wet, well-filled seeds, containing 8–10% moisture, were subjected to doses of 100, 200, 300,400 and 500 gamma rays (CO^{60}) at the Nuclear and Agriculture Division, B.A.R.C., Trombay, Mumbai.

A sufficient amount of mutagenic solution with varying concentrations (0.005%, 0.010%,0.015,0.020 and 0.025% Sodium azide) and time was applied to 200 pure uniformly clean and wet seeds of variety PBNS-86 for a three-hour presoaking in distilled water. An 18-hour chemical mutagenic treatment was conducted in a shaker at 200 rpm and 25±2 ^{0}C. The dried yet un-irradiated and seeds soaked in distilled water served as control in case of both the mutagenic treatments. A thorough summary of the therapies is shown in the table below.

Table 1: Mutagens

Physical (gamma rays)	Chemical (Sodium Azide)
100 Gy	0.005%
200 Gy	0.010%
300 Gy	0.015%
400 Gy	0.020%
500 Gy	0.025%

Field trial During Rabi 2021–2022, the mutagen-treated seeds were planted in the field on safflower at the Govt. Vidarbha Institute of Science and Humanities, Amravati, using the dibbling method with a 45 x 20 cm spacing in a basic RBD design with control in three replications. The appropriate dosage of fertilizer and all other agronomic techniques were provided. The impact of mutagens on plant survival in the M_1 generation was investigated.

Plant Survival Rate (%) The number of seedlings that survived 30 days following seeding was counted. The following formula was utilized to determine the survival percentage:

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

Using Probit Analysis to Fix LD_{50} the PBNS-86 variety of safflower's LD_{50} (lethal dosage) value for gamma rays and sodium azide was determined using the probit analysis method (**Finney, 1978**) [8]. The probit function is a representation of the inverse cumulative distribution function, also known as the quantile function, that is linked to the conventional normal distribution. The following are the procedures for probit analysis:

- 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

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

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).

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

The anticipated probits (Yi) were obtained using equation (2). The curve created using log dosages and probits yields the LD or LC values. The corresponding probit value is antilog to the Log_{10} value, and the following formula is used to obtain the 95% fiducial confidence limits:

$$\text{Fiducial Limits} = \text{Antilog (Log10 Dose} \pm 1.96 \text{ (SE))}$$

Statistical Examining LD_{50} and empirical probit units were calculated using Microsoft Excel 2010, and probit analysis was utilized to find the ideal fatal dosage.

III. RESULT AND DISCUSSION

Impact of Mutagen on the Percentage of Plant Survival Thirty days following seeding was when the survival percentage was observed. In the M_1 generation, the number of plants that survived of variety PBNS-86 was counted and translated to a percentage. Table 2 has the plant survival % displayed. In every mutagenic treatment, the plant survival percentage of PBNS-86 was lower than that of the control. The highest plant survival rate of 64.10% was seen at a gamma ray dosage of 100 Gy. This was followed by doses/concentrations of

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mutagenic treatments of 0.010% sodium azide (61.53%). Gamma rays caused the greatest drop in plant survival percentage, which was then followed by Sodium azide treatments, in that order. The lowest plant survival percentage (34.35%) was found at a 500 Gy gamma ray dosage, which was followed by mutagenic treatment doses. In the current study, the rate of plant survival dropped as physical, chemical dosages and concentrations increased, mutagenic treatments above control in M_1 generation. Similar kinds of outcomes in African sesame, **Satpute, and Kothekar** (1996) [9] have been reported by **Boureima *et al.*** (2009)[10] in soybean, **Singh *et al.*** (2018)[11];**Bhoite *et al*.** (2019)[12] in safflower **Diouf *et al.*** (2010)[13] in sesame, in sunflower, and in sesame **Gawande *et al.*** (2022)[14] in Safflower.

Table 2: Effect of mutagens on plant survival in M_1 generation of safflower variety PBNS-86

Treatment by gamma rays	Plant survival %	Treatment by Sodium Azide	Plant survival %
100 Gy	64.10	0.005	57.94
200 Gy	60.51	0.010	61.53
300 Gy	53.84	0.015	37.94
400 Gy	48.20	0.020	13.33
500 Gy	34.35	0.025	6.15
Wet control	31.28		

Determination of LD_{50} (Lethal Dose) Values

Table 3: LD_{50} dosage calculation based on gamma-ray plant survival

Dose of gamma rays (Gy)	Log_{10} value of dose	Reduction in plant survival % (Dead %)	Probit value	LD_{50} value	LD_{50} Dose
100 Gy	2.00	35.9	4.64	2.472	Antilog (2.472) =296.4
200 Gy	2.30	39.49	4.72		
300 Gy	2.47	46.16	4.90		
400 Gy	2.60	51.8	5.05		
500 Gy	2.70	65.65	5.41		

Table 4: LD_{50} dosage calculation based on Sodium Azide plant survival

Concentration of Sodium azide` %	Conc. Of Sodium azide (PPM)	Log_{10} value of conc. (PPM)	Reduction in plant survival % (Dead %)	Probit value	LD_{50} value	LD_{50} Dose
0.005	50	1.69	42.06	4.80	3.187	Antilog (3.187) =1538.15 =0.1538%
0.010	100	2.00	38.47	4.69		
0.015	150	2.17	62.06	5.25		
0.020	200	2.30	86.67	6.13		
0.025	250	2.04	93.85	6.55		

Probit analysis was used to calculate the LD_{50} value for the PBNS-86 type of safflower based on plant survival. Tables 3 and 4 show the predicted LD_{50} values and probit units based on the mortality % of the PBNS-86 mutant population. The minimal concentration required to induce 50% of mutant seeds to die or survive is referred to as the fatal dosage. Genetic background, kind of treatment, and environmental factors all affect the LD_{50}, which varies throughout genotypes (**Singh, 2005)[10]. Anbarasan *et al***. (2015) [15] found that the LD_{50} for experimentally produced mutations utilising physical or chemical mutagens is the ideal dosage for high frequency mutations. Safflower (*Carthamus tinctorius* L.) variety PBNS-86 was reported to have an LD_{50} of 299.5 Gy and a dose of sodium azide and 0.25% of gamma rays, respectively. Comparably, **Niu *et al.*** (2009) [16] calculated that safflower's optimal dosage (LD_{50}) of gamma rays was around 300 Gy; however, they could not find any information on safflower's optimum concentration (LD_{50}) of EMS. The optimal concentration (LD_{50}) of sodium azide in S. alba was determined by **Yadav *et al.*** (2016) [17] to be around 0.3%.

IV. CONCLUSION

The results of this investigation indicated that, based on the acquired LD50 values, mutagenic treatment with gamma rays should be administered between 296.2Gy, and safflower should be treated with sodium azide at a concentration of 0.1513%. Gamma radiation, on the other hand, was discovered to have a greater death rate in PBNS-86 study than sodium azide amount. It may be further investigated for creating populations and mutation

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mapping as both mutagens are effective in producing notable induced variants in safflower.

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Conflicts of interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

REFERENCES

[1] **Pushpavalli, S. N. C. V. L., & Kumar, G. (2017).** Study of genetic variability, correlation and path analysis of safflower genotypes. *Research Journal of Agricultural Sciences*, *8*(3), 706-709

[2] **Pattar, V. K., & Patil, R. (2020).** Correlation and path analysis in safflower (Carthamus tinctorius L.) genotypes. *Journal of Pharmacognosy and Phytochemistry*, *9*(4), 1717-1719.

[3] **Cheema, A. A., & Atta, B. M. (2003).** Radiosensitivity studies in basmati rice. *Pak. J. Bot*, *35*(2), 197-207.

[4] **Khin, T. N. (2006).** Rice mutation breeding for varietal improvement in Myanmar.

[5] **Spencer-Lopes, M. M., Forster, B. P., & Jankuloski, L. (2018).** *Manual on mutation breeding* (No. Ed. 3). Food and agriculture Organization of the United Nations (FAO).

[6] **Broyana, Dyulgerova., Nykolay, Hristov, Dyulgerov. (2022).** Mutagenic effect of sodium azide on winter barley cultivars. Agricultural Science and Technology, 14(2):27-33

[7] **Vinithashri, G., Manonmani, S., Anand, G., Meena, S., Bhuvaneswari, K., & JohnJoel, A. (2020).** Mutagenic effectiveness and efficiency of sodium azide in rice varieties. *Electronic Journal of Plant Breeding*, *11*(01), 197-203.

[8] **Finney, D. J. (1978).** Statistical method in biological assay. *Statistical method in biological assay.*, (3rd edn).

[9] **Satpute, R. A., & Kothekar, V. S. (1996).** Mutagenic efficiency and effectiveness in safflower. *Journal of Nuclear Agriculture and Biology*, *25*, 230-234.

[10] **Boureima, S., Diouf, M., Silme, R. S., Diop, T., Van Damme, P., & Çağırgan, M. İ. (2009).** Radiosensitivity of African sesame cultivars to gamma-rays. *Turkish Journal of Field Crops*, *14*(2), 181-190.

[11] **Singh, L., Singh, P. P., & Mishra, M. N. (2018).** 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*, *7*(2S), 293-296.

[12] **Bhoite, B. S., Kamble, M. S., Aher, A. R., & Chavan, M. V. (2019).** Mutagenic sensitivity in M1 generation of three verities of soybean (Glycine max L.). *Journal of Pharmacognosy and Phytochemistry*, *8*(5), 1817-1820.

[13] **Diouf, M., Boureima, S., Tahir, D. I. O. P., & Ilhan Çağirgan, M. (2010).** Gamma rays-induced mutant spectrum and frequency in sesame. *Turkish Journal of Field Crops*, *15*(1), 99-105.

[14] **Gawande, S. M., Ghuge, S. B., Kalpande, H. V., & Wankhade, M. P. (2022).** Effect of mutagens on emergence, plant survival and pollen sterility in safflower (Carthamus tinctorius L.).

[15] **Anbarasan, K., Rajendran, R., & Sivalingam, D. (2015).** Studies on the mutagenic effectiveness and efficiency of gamma rays, EMS and combined treatment in sesame (Sesamum indicum L.) var. TMV3. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, *6*(4), 589-595.

[16] **Niu, S., Yan, W., Wang, X., & Li, X. (2009).** Effects of seeds irradiation with 60Cogamma-ray on shoot growth and physiological status of Carthamus tinctorius. *Zhongguo Zhong yao za zhi Zhongguo Zhongyao Zazhi China Journal of Chinese Materia Medica*, *34*(23), 3004-3007.

[17] Prashant Yadav, P. Y., Meena, H. S., Meena, P. D., Arun Kumar, A. K., Riteka Gupta, R. G., Jambhulkar, S., ... & Dhiraj Singh, D. S. (2016). **Determination of LD50 of ethyl methanesulfonate (EMS) for induction of mutations in rapeseed-mustard.**