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Evaluation of Optimal Doses for Gamma Rays and Sodium Azide in Linseed Genotypes

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ABSTRACT

Background: Linseed is one of the most important medicinal plants grown for its various health benefits. The seeds of linseed contain a good and essential fatty acid profile that is omega-3 fatty acid/Alpha linolenic acid. It helps in the prevention of various disease including inflammation, cardiovascular problems, cancer, diabetes *etc*. Induced mutagenesis is an easy and cost effective technique to induce desired genetic variability, which either does not occur naturally or is not accessible to plant breeders. Genetic variability is enhanced by the influence of various chemical or physical mutagens. The usefulness of any mutagen relies not only on its efficiency but also on its effectiveness.

Methods: Dry and healthy seeds of linseed (var. Padmini and IC0096650) were treated with different doses of gamma rays and sodium azide. The experiment was conducted during Rabi season of November 2016-March 2017. The selection of optimum doses of mutagens through the determination of LD_{50} values has been calculated on the basis of the seed germination as well as plant survival.

Result: The present investigation reveals genotypic response of two linseed varieties towards different doses of gamma rays and sodium azide. Variety IC0096650 exhibited higher degree of sensitivity than variety Padmini with respect to the mutagens used. Results showed that 200Gy dose of gamma rays and 0.4% dose of sodium azide was the maximum non-lethal strength of the respective mutagen for the induction of the mutation in linseed genotypes.

Key words: Gamma rays, Lethal dose (LD_{50}), *Linum usitatissimum* L., Seed germination (%), Sodium azide (SA).

INTRODUCTION

Linseed (*Linum usitatissimum* L.) is a self-pollinated, annual diploid (2n=30) oilseed and fibre crop which belong to family Linaceae with relatively narrow genetic base. Linseed contains vital bio-active substances such as omega-3 fatty acid, proteins, oil, dietary fibre, phenolic compounds, minerals and vitamins (Bhatty and Cherdkiatgumchai, 1990; Bernacchia *et al*., 2014; Goyal *et al*., 2014).

Mutagenesis is a best method to improve the polygenic variability of crop plants in a short period of time. In recent years, a great deal of work has been conducted on induced mutagenesis in various crops by using physical and chemical mutagens (Khan *et al*., 2006; Wani, 2019). There are total eight varieties of linseed developed in different countries till 2019 through mutation breeding. Out of eight, six mutant varieties of linseed were released by physical mutagens and two varieties through chemical mutagens (MVD, IAEA/FAO, 2019). The availability of genetic alteration in the attributes of interest is the prerequisite for any crop improvement programme. Mutation breeding suggests an uncertain possibility of originating expected traits in a crop that cannot be found in nature or lost during the course of evolution (Novak and Brunner, 1992). Mutation breeding encourages to the plant breeders to screen and select suitable mutants with enhanced agro-economic characteristics (Khursheed *et al*., 2015; 2016; Laskar *et al*., 2018). Expression of agro-economic characteristics, especially quantitative traits is a complex process involving several genes with a small additive impact but the reactions of each gene varies towards different doses of mutagens (Laskar *et al*., 2015). Therefore the selection of mutagens and their optimum doses is very important in order to unleash the huge potential of crop improvement through mutation breeding (Raina *et al*., 2017). The LD_{50} calculation (dose at which the inhibition of germination is 50%) gives an indication of the optimum doses of various mutagens. A dose that induces higher rate of mutations with less biological damage could be considered as an optimal dose. Germination and survival percentage of the treated seeds were one of the most accurate indices for estimating LD_{50} values for the choice of the mutagenic dose.

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MATERIALS AND METHODS

Dry (moisture content 12%) and healthy seeds of the linseed varieties Padmini and IC0096650 were used for mutagenic treatments of gamma rays and Sodium azide (NaN_3). The seeds of both the varieties were directly irradiated (30 seeds each) with different doses/concentrations *viz*., 50Gy, 100Gy, 150Gy, 200Gy, 250Gy, 300Gy, 350Gy, 400Gy, 450Gy and

500Gy of gamma rays with a radioisotope Co-60 (Cobalt-60). For chemical treatments, the seeds were first pre-soaked in distilled water for 9 hours and then directly transferred (30 seeds each) to the 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%,0.9% and 1.0% doses of SA for 6 hours with intermittent shaking at room temperature of 25±2°C. To determine the lethal dose (LD50), the treated seeds along with respective controls (untreated seeds) were allowed to grown in six replications of fifty seeds each. The experiment was conducted during winter (Rabi) season of November 2016-March 2017. In control population, seed germination was observed after six days of sowing however, in mutagen treated populations the germination was delayed by five to seven days in both the varieties. The estimation on seed germination was recorded in each treatment including control (untreated seeds) from the beginning of first shoot emergence up to 25 days. From the data recorded, percentage of seed germination and lethal dose (LD_{50}) in each treatment was determined by calculating the survival rate of the plants (Laskar *et al*., 2018):

$$\text{Seed germination (\%)} = \frac{\text{No. of seeds germinated}}{\text{No. of seeds sown}} \times 100$$

$$\text{Lethal Dose } (LD_{50}) = \frac{\text{Frequency of treated seedlings}}{\text{Frequency of control seedlings}} \times 100$$

RESULTS AND DISCUSSION

The Response of chemical (SA) and physical (gamma rays) mutagens on linseed genotypes Padmini and IC0096650 in relation to seed germination was studied and represented in "Fig 1 and 2". It was observed that seed germination was gradually decreased with increasing doses of gamma rays and sodium azide in both the linseed varieties. In both varieties, the level of germination inhibitions was different. In case of var- IC0096650, effect of mutagen treatments on seed germination was more negative than var. Padmini. In general, the genotypic lethality has been demonstrated to be more in SA treatments followed by gamma rays treatments, particularly in higher treatments/doses in both the varieties. Such a dose dependent reduction in seed germination has also been recorded in various crops Ramchander *et al*. (2015) in rice, Laskar *et al*. (2017) in lentil and Raina *et al*. (2018) in cowpea.

Determination of lethal dose (LD_{50})

LD_{50} is the dose at which highest frequency of mutation occurs. The LD_{50} value of gamma ray and SA is significant for a plant breeder to create mutation as the value of the LD_{50} determines mutating effects of crops. The estimation of the lethal dose (LD_{50}) values showed that an increase in genotypic lethality to the mutagen doses with an increase in the intensity of gamma rays and SA treatments in both the linseed varieties. The lethal dose values were found to exceed 50 percent above 0.4% in SA and 200 Gy gamma rays in both the linseed varieties. (Table 1 and Table 2). Sood *et al*. (2016) determine lethal dose (LD_{50}) based on probit curve by using gamma rays and EMS in *capsicum annuum* L. Gaul (1964) proposed that biological damage caused by mutagens such as germination inhibition; pollen sterility and plant survival at maturity can be considered as an indication of mutagenic sensitivity of the genotype. It helps in regulating the biological sensitivity and mutagenic potency at the same time, which is requisite for the selection of the mutagen concentrations at which the mutation rate is high and biological damage is less. In both the linseed varieties, the seed germination generally changed according to the mutagen and their doses. The assessment of germination percent of the treated seeds represents the primary concept of mutagenic effectiveness with the germination percent below 50% being regarded as lethal or non-desirable.

Furthermore, the survival percentage of the plant produced from mutagen-treated seeds at the maturity stage is also an indicator of mutagenic lethality. While, the aim is to establish large mutagenized populations for successful mutant screening, therefore, lethal treatments have usually been discarded to ensure high mutation rate with lower

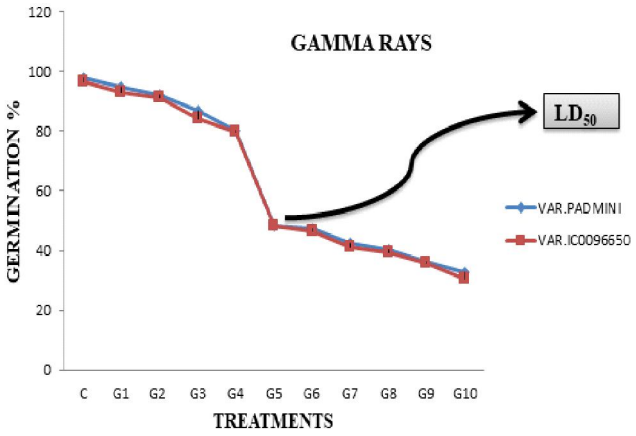


Fig 1: Comparative effect of gamma rays on seed germination in two varieties of linseed (Var. Padmini and Var. IC0096650) #C: Control, G1-G10 (Gamma rays doses)

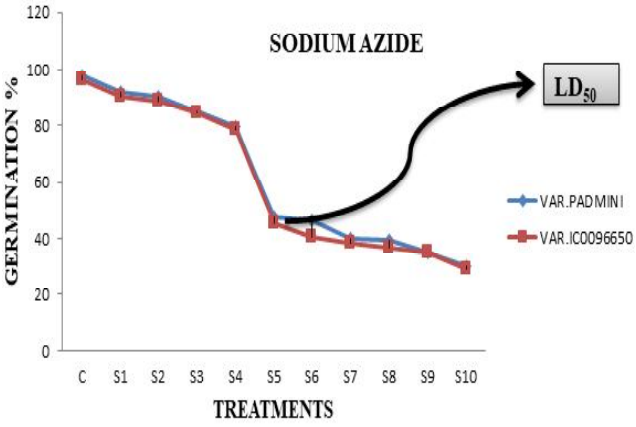


Fig 2: Comparative effect of sodium azide on seed germination in two varieties of linseed (Var. Padmini and Var.IC0096650) #C: Control, S1-S10 (Sodium azide doses).

Table 1: Comparative LD_{50}values of gamma rays in two varieties of linseed.

Code	Mutagen	M_1 treated seeds	M_1 plant survival (%)	LD_{50}	M_1 treated seeds	M_1 plant survival (%)	LD_{50}
		Var. Padmini			Var. IC0096650		-
C	Control	300	95.00	—	300	92.00	-
G1	050 Gy	300	90.33	4.92	300	87.40	5.00
G2	100 Gy	300	84.32	11.25	300	79.18	13.94
G3	150 Gy	300	85.67	9.83	300	83.67	9.06
G4	200 Gy	300	80.00	15.79	300	78.33	14.86
G5	250 Gy	300	47.38	50.13	300	47.00	48.92
G6	300 Gy	300	47.00	50.53	300	46.23	49.75
G7	350 Gy	300	41.25	56.58	300	40.25	56.25
G8	400 Gy	300	39.45	58.48	300	39.23	57.36
G9	450 Gy	300	35.62	62.51	300	34.67	63.32
G10	500 Gy	300	30.20	68.22	300	29.94	67.46

Table 2: Comparative LD_{50} values sodium azide in two varieties of linseed.

Code	Mutagen	M_1 treated seeds	M_1 plant survival (%)	LD_{50}	M_1 treated seeds	M_1 plant survival (%)	LD_{50}
		Var. Padmini			Var. IC0096650		
C	Control	300	95.00	—	300	92.00	—
S1	0.1% SA	300	91.47	3.72	300	86.40	6.09
S2	0.2% SA	300	82.33	13.34	300	78.18	15.08
S3	0.3% SA	300	78.00	17.90	300	80.00	13.05
S4	0.4% SA	300	78.32	17.56	300	80.40	12.61
S5	0.5% SA	300	46.57	50.98	300	45.20	50.87
S6	0.6% SA	300	45.67	51.93	300	39.67	56.89
S7	0.7% SA	300	38.12	59.88	300	36.23	60.62
S8	0.8% SA	300	37.45	60.58	300	35.45	61.47
S9	0.9% SA	300	32.15	66.16	300	30.69	66.65
S10	1.0% SA	300	28.21	70.31	300	28.67	68.84

germination inhibition in the treated population. The decline in germination percentage of the treated seeds could be due to the mutagen-induced disruptions in the genetically controlled bio-physiological and metabolic pathways that play a crucial role in the seed germination process, which may include altered enzyme activity (Kurobane *et al*., 1979), inhibition of mitotic process (Ananthaswamy *et al*., 1971) and hormonal imbalances (Chrispeels and Varner, 1967). It has also been documented that inhibition of DNA synthesis by mutagenic treatments due to induced mutations may be a reason for reducing seed germination (Hevesy, 1945). Chauhan and Singh (1975) suggested that due to gamma rays, the disruption and disorganization of the tunica layer contributes to inhibition of seed germination. In the enzyme synthesis interference of gamma irradiation and the degradation of the active enzymes necessary for plant auxin development (Usuf and Nair, 1974) is another reason for the inhibition of germination of seeds treated. Delay in germination over seven days at higher doses of mutagens could be due to inhibition of the mitotic proliferation in shoot and root meristems. Since, the mutagens interacts independently with the genetic material, the use of chemical and physical mutagens at sufficient doses in view of the value of LD_{50} could be useful in inducing a large number of beneficial mutations in agro-economic characteristics of crop plant.

CONCLUSION

The variable sensitivity of the varieties Padmini and IC0096650 towards similar mutagenic doses/concentrations of gamma rays and sodium azide showed that the frequency of mutations depends solely on the interaction between the individual genotypes and mutagen when other variable factors were stable. The results of the current study confirmed that the suitable doses of gamma rays (200Gy) and sodium azide (0.4%SA) were sub-optimal for mutagenesis beyond which the genotype had higher degree of lethality**.** For selecting suitable mutants from the linseed genotype, the mutagen doses used should be less than 200 Gy for gamma radiation and 0.4% SA for sodium azide in order to induce high frequency mutation with optimum survival populations.

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REFERENCES

Ananthaswamy, H.N., Vakil, U.K., Srinivasan, A. (1971). Biochemical and physiological changes in gamma irradiated wheat during germination. Radiation Botany. 11: 1-12.

Bernacchia, R., Preti, R., Vinci, G. (2014). Chemical composition and health benefits of flaxseed. Austin Journal of Nutrition and Food Sciences. 2(8): 1045.

Bhatty, R.S. and Cherdkiatgumchai, P. (1990). Compositional analysis of laboratory prepared and commercial samples of linseed meal and of hull isolated from flax. Journal of the American Oil Chemists' Society. 67(2): 79-84.

Chauhan, Y.S. and Singh, R.P. (1975). Morphological studies in safflower (*Carthamus tinctorius* Linn.) with special reference to the effect of 2,4-D and gamma rays I. Vegetative shoot apex. Radiation Botany. 15: 69-77.

Chrispeels, M.J. and Varner, J.E. (1967). Gibberellic acid induced synthesis and release of Lamylase and ribonuclease by isolated barley aleurone layers. Plant Physiology. 42: 396-406.

Gaul, H. (1964). Mutations in plant breeding. Radiation Botany. 4: 155-232.

Goyal. A., Sharma, V., Upadhyay, N., Gill, S., Sihag, M. (2014). Flax and flaxseed oil: an ancient medicine and modern functional food. Journal of food science and technology. 51(9): 1633-1653.

Hevesy, G. (1945). On the effect of roentgen rays on cellular division. Review of Modern Physics. 17: 102.

Khan, S., Wani, M.R. and Parveen, K. (2006). Sodium azide induced high yielding early mutant in lentil. Agricultural Science Digest. 26: 65-66.

Khursheed, S., Laskar, R.A., Raina, A., Amin, R., Khan, S. (2015). Comparative analysis of cytological abnormalities induced in *Vicia faba* L. genotypes using physical and chemical mutagenesis. Chromosome Science. 18(3-4): 47-51.

Kurobane, I.H., Yamaguchi, H., Sander, C., Nilan, R.A. (1979). The effects of gamma irradiation on the production and secretion of enzymes and enzymatic activities in barley. Environmental and Experimental Botany. 19: 75-84.

Laskar, R. A., Amin, R., Khan, S. (2017). Evaluation of Optimal Doses for Gamma Rays and Hydrazine Hydrates in Lentil Genotypes. Trends in Bioscience. 10(27).

Laskar, R.A., Chaudhary, C., Khan, S., Chandra, A. (2018). Induction of mutagenized tomato populations for investigation on agronomic traits and mutant phenotyping. Journal of the Saudi society of agricultural sciences. 17(1): 51-60.

Laskar, R.A., Khan, S., Khursheed, S., Raina, A., Amin, R. (2015). Quantitative analysis of induced phenotypic diversity in chickpea using physical and chemical mutagenesis. Journal of Agronomy. 14: 102.

Laskar, R.A., Laskar, A.A., Raina, A., Khan, S., Younus, H. (2018). Induced mutation analysis with biochemical and molecular characterization of high yielding lentil mutant lines. International Journal of Biological Macromolecules. 109: 167-179.

Mutant Varieties Database, IAEA/FAO, 2019.

Novak, F.J. and Brunner, H. (1992). Plant breeding induced mutation technology for crop improvement. IAEA Bull. 4: 25-33.

Raina, A., Khursheed, S., Khan, S. (2018). Optimisation of mutagen doses for gamma rays and sodium azide in Cowpea genotypes. Trends in Biosciences. 11(13): 2386-2389.

Raina, A., Laskar, R.A., Khursheed, S., Khan, S., Parveen, K., Amin, R., Khan, S. (2017). Induce physical and chemical mutagenesis for improvement of yield attributing traits and their correlation analysis in chickpea. International Letters of Natural Sciences. 61: 14-22.

Ramchander, S., Ushakumari, R., Pillai, M.A. (2015). Lethal dose fixation and sensitivity of rice varieties to gamma radiation. Indian Journal of Agricultural Research. 49(1): 24-31.

Sood, S., Jambulkar, S.J., Sood, A., Gupta, N., Kumar, R., Singh, Y. (2016). Median lethal dose estimation of gamma rays and ethyl methane sulphonate in bell Pepper (*Capsicum Annuum* L.). Sabrao Journal of Breeding and Genetics. 48 (4): 528-535.

Usuf, K.K., Nair and P.M. (1974). Effect of gamma irradiation on the indole acetic acid synthesizing system and its significance in sprout inhibition of potatoes. Radiation Botany. 14: 251- 256.

Wani, M.R. (2019). Comparative biological sensitivity and mutability of chemo-mutagens in lentil ((*Lens culinaris* Medik). Legume Research. 0976-0571 (1-6).