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

Roshan Jahan, Saima Malik, Shazia Bi Ansari, Samiullah Khan

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 polyclenic 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.

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\textsubscript{3}). The seeds of both the varieties were directly irradiated (30 seeds each) with different doses/concentrations viz., 50 Gy, 100Gy, 150Gy, 200Gy, 250Gy, 300Gy, 350Gy, 400Gy, 450Gy and
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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 grow 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 (LD50) in each treatment was determined by calculating the survival rate of the plants (Laskar et al., 2018):

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\text{Seed germination (\%)} = \frac{\text{No. of seeds germinated}}{\text{No. of seeds sown}} \times 100
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\text{Lethal Dose (LD}_{50}\text{)} = \frac{\text{Frequency of treated seedlings}}{\text{Frequency of control seedlings}} \times 100
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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 (LD50)

LD50 is the dose at which highest frequency of mutation occurs. The LD50 value of gamma ray and SA is significant for a plant breeder to create mutation as the value of the LD50 determines mutating effects of crops. The estimation of the lethal dose (LD50) 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 (LD50) based on probit curve by using gamma rays and EMS in capiscum 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

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\text{GAMMA RAYS}
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\[
\text{SODIUM AZIDE}
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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).
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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 interact 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 (200 Gy) 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.

### 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                 | 74.38                    | 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                 | 36.22                    | 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  |
ACKNOWLEDGEMENT
The authors are thankful to the Chairman, Department of Botany, Aligarh Muslim University, Aligarh, for providing necessary facilities. Authors are also thankful to National Bureau of Plant Genetic Resources (NBPGR) and Indian Agricultural Research Institute (IARI), New Delhi for providing accessions and gamma irradiation facility.

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