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Physical and chemical mutagenesis in *Linum usitatissimum* L. to induce variability in seed germination, survival, and growth rate traits

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ABSTRACT

The studies of induced mutation in *Linum usitatissimum* L. were performed by exposing the fully matured and healthy dry seeds to gamma rays at 10 and 15 Krad (Kr), Xrays at 10 and 15 Kr, hydroxylamine (HA) at 0.1% and 0.2%, and 5amino acridine (AA) at 0.1% and 0.2% doses. The observations were made for seed germination percentage, survival percentage, and growth characters such as shoot length, root length, and dry weight. Seeds treated with low dose of mutagens showed negligible effect while that with the high dose exhibited significant effects on studied parameters as compared to control. Data obtained in this study were statistically significant at 5% level. The results conclude that treatments of gamma rays and X-rays were less effective as compared to those of HA and AA treatments.

KEY WORDS: Acridine, hydroxylamine, *Linum usitatissimum* L., mutation

INTRODUCTION

The genus *Linum* belongs to family Linaceae and comprises over 200 species which exhibit great diversity in karyotype, biochemical, and morphological attributes. The species of the genus are distributed worldwide in habitats ranging from sea level to high altitudes (9000 m above mean sea level). *Linum usitatissimum* L. plant has short tap root system with fibrous branches extending up to 90120 cm in light soil. Stem of *L. usitatissimum* L. grows to a height of 30120 cm and 34 branches or tillers arise from a node near the surface of the ground. Seed types are bushy and profusely branched.

L. usitatissimum L. being a dicotyledonous plant, the first two leaves are cotyledonary and are thick in texture.

Induced mutations pave a path to induce genetic variability in some economically important selfpollinated crops where crossing/hybridization is quite difficult, *viz.*, wheat (Srivastava *et al.,* 2011) and fenugreek (Chaudhary and Singh, 2001; Basu *et al*., 2008). Hence, utilization of mutagenesis undoubtedly is capable of increasing genetic variability in a number of crops as reported by many workers (Mahandjiev *et al*., 2001; Tai *et al.,* 2007; Khan and Goyal, 2009; Kozgar *et al.,* 2011; Srivastava *et al.,* 2011).

Effects of physical mutagenic studies on this plant were studied by Abidi *et al*. (1978) while studying the effect of gamma irradiation on seed germination of *L. usitatissimum* L. variety Neelum. The X-rays have wavelength from 0.001 Å and can penetrate deep to cause ionization which results in the formation of free radicals (extreme reactive and containing unpaired electrons). Chemical mutagens such as hydroxylamine (HA) react with cytosine at NH group and form hydroxyl cytosine which pairs with adenine instead of guanine. 5amino acridine (AA) is planar (flat molecule)-like purine bases and at low concentration can be inserted or intercalated between bases of DNA helix, which stretches distance between adjacent base pairs and distorts the DNA strand.

We have taken the present investigation on induced mutagenesis in *L. usitatissimum* L. using gamma rays, X-rays, HA, and AA to study mutagen sensitivity of *L. usitatissimum* L. against varying dose rate in immediate generation.

MATERIALS AND METHODS

The physical mutagens (gamma rays and X-rays) and chemical mutagens (HA and AA) were employed in the present study. Gamma ray treatment was given from BHEL Bhopal with 200 curie Co gamma cell at a dose rate of 0.465 Kr/h at a distance of 100 cm. X-ray treatment was given by X-ray machine at Safia P.G. Science College, Bhopal. HA and 5-AA for the present study were obtained from Merck India.

The seeds of *L. usitatissimum* L. variety IP-I6 were collected from Jawaharlal Nehru Krishi Vishva Vidyalaya, Jabalpur, Madhya Pradesh, India. The variety IP-I6 is branched flowering after 30 days and maturity in 96 days. Capsules in this variety are an important variety of Madhya Pradesh, India.

The seeds presoaked in distilled water for 17 h were subjected to doses of gamma rays 10 and 15 Kr, X-rays 10 and 15 Kr, HA 0.1% and 0.2%, and AA 0.1% and 0.2% along a set of untreated seeds with some moisture content used as a control group. Hundred seeds of each treatment sown at 1 cm depth in plastic trays (23 cm × 27 cm in height) filled with river sand, red soil, and farm yard manure in the ratio 3:2:1 along with control (containing 100 seeds). Water was applied manually on alternative days, and germination was observed daily for 15 days after sowing. Emergence of coleoptiles was taken as the indication of seed germination. Finally, the overall germination percentage was calculated. After germination, plants from each treatment and control transplanted to experimental field which was ploughed well to ensure good growth of plants in 3 replications. The plant to plant and line to line distance was 12" × 12".

The survival percentage was computed in percentage from those plants surviving till maturity out of total number of plants produced through seed germination. Observations were recorded for growth parameters, *viz.,* shoot length (cm), root length (cm), and dry weight (mg) of 20 days old seedling. For this purpose, mean of the data collected for 50 seedlings was taken. Data were analyzed using one-way ANOVA in SPSS software package (17.0).

RESULT AND DISCUSSION

Table 1 shows seed germination and survival percentage in the control as well as in the treated plants of *L. usitatissimum* L. variety IP-I6.

Table 1: Effect of various doses of physical and chemical mutagens on seed germination and survival percentages in variety IPI6 of *Linum usitatissimum* L.

Mutagens	Germination %	Survival %
Control	98±3.1798	95±5.36449
X-rays (10 Kr)	71±3.84419	69±3.05505
X-rays (15 Kr)	69±2.08167	64±4.25572
Gamma rays (10 Kr)	78±1	76±5.17472
Gamma rays (15 Kr)	76±4.70225	72±7.2111
HA (0.1%)	64±4.8074	60±7.50555
HA (0.2%)	62±5.48736	58±6.06447
AA (0.1%)	70±5.54777	68±7.26483
AA (0.2%)	67±6.69162	63±5.56776
CD	7.4	11.2

HA: Hydroxylamine, AA: 5-amino acridine

A minimum number of seeds of this variety germinated with 0.2% HA. This minimum was 62% against 98% of control. Seed germination percentage of physical (gamma rays, X-rays) and chemical (HA and AA) mutagens treated seeds was gradually declined linearly with every increase of dose level. Percentage reduction in seed germination might have been due to effect of mutagens on meristematic tissue of the seed. The initiation of seed germination process was also considerably delayed with HA doses.

Statistical analysis of critical difference and standard error (Table 1) indicates that all the treatments are significantly effective on germination in comparison to control, except low doses of physical and chemical mutagens which exercised negligible effect.

It is evident from Table 1 that all the treatments of physical and chemical mutagens given to variety IP-I6 of *L. usitatissimum* L. inhibited the germination process as compared to their respective controls with higher doses of physical and chemical mutagens, and germination was significantly delayed. As the concentration increased, inhibitions of germination were also increased. Hence, all the physical and chemical mutagens found to be as germination inhibitors with varying potential.

Several workers (Akgun and Tosum, 2004; Alcantara *et al.,* 1996; Karthika and Lakshmi, 2006; Khan *et al.,* 2005; Kumar and Mishra, 2004; Thapa, 2004; Toker *et al.*, 2005) reported such behavior of physical and chemical mutagens in different crop plants. Taking into the account of the findings documented by various workers (Chaghtai and Prasad, 1979; Gichner and Veleminsky, 1974; Jain and Agarwal, 1987; Mensah *et al.,* 2007; Nazir *et al.,* 2005; Santos, 1961; Sareen and Koul, 1999) and it is inferred that presoaking renders the seed vulnerable to the effect of physical and chemical mutagens and the germination is inhibited due to biochemical interference of physical and chemical mutagens

with the physiological activities involved in the process of seed germination. On this parameter, chemical mutagens have more effect in comparison of physical mutagens.

The highest plant survival percentage was 95% observed in control with 5% mortality. Minimum number of plants, i.e., 58% survived with 0.2% HA treatment showing 42% mortality. In this variety (IP-I6), survival percentage and dose rate followed an inverse linear relationship as with higher doses survival percentage was noticeably reduced. The reduction in survival percentage is probably due to toxic effect of mutagens. In this parameter, chemical mutagens have more effect in comparison of physical mutagens. Critical difference and standard error (Table 1) analysis indicate that all treatments employed significantly affected survival of plants as compared to control.

Plant survival percentage decreased in variety IP-I6 of *L. usitatissimum* L. with increasing doses of physical and chemical mutagens which prove that clear correlation exist in between dose rate and survival of plants. Results of the present work reveal that chemical mutagens, particularly HA, is comparatively more operative to cause the lethality than physical mutagens and acridine. Here, gamma rays seem to be more effective than X-rays.

The reduction in plant survival percentage following with physical and chemical mutagens has been reported by many workers, and results of present investigation regarding survival are in agreement with earlier findings in different plants (Bari, 1971; Cheema and Atta, 2003; Davies *et al.*, 1975; Hussein and Disouki 1975; Kar *et al.*, 1995; Siddiq and Swaminathan, 1968; Singh *et al.*, 2006).

Table 2 shows shoot length mean in cm, root length mean in cm, and dry weight mean in mg of gamma rays, X-rays, HA, and AA treated plants. In variety IP-I6, highest shoot length mean was observed in control which was 5.3 cm while minimum shoot length was observed with 15 Kr gamma rays treatment which was 4.6 cm. In this parameter also, mean and dose rate followed inverse trend, i.e., with higher doses of physical and chemical mutagens, shoot length mean decreased as compared to lower doses. Statistical analysis of critical difference and standard error (Table 2) revealed that all the treatments are effective as compared to control. Among various treatments of physical and chemical mutagens, employed lower doses were found to be comparatively insignificant while higher doses of all the physical and chemical mutagens were found to have significant effect on shoot length.

Table 2: Effect of various doses of physical and chemical mutagens on growth rate in variety IPI6 of *Linum usitatissimum* L.

Mutagens	Length (cm)		Mean dry weight (mg)
	Mean shoot	Mean root	
Control	5.3±0.550757	4.9±1.12398	$1.0\times10^{-4}\pm1.53\times10^{-5}$
X-rays (10 Kr)	5.1±1.12694	4.7±1.26623	$1.0\times10^{-4}\pm2.65\times10^{-5}$
X-rays (15 Kr)	4.9±0.793725	4.5±1.08167	$9.0\times10^{-5}\pm2.08\times10^{-5}$
Gamma rays (10 Kr)	5±0.776745	4.6±1.34288	$8.0\times10^{-5}\pm2.03\times10^{-5}$
Gamma rays (15 Kr)	4.6±0.608276	4.2±0.680686	$7.0\times10^{-5}\pm2.08\times10^{-5}$
HA (0.1%)	5.1±1.02632	4.7±0.680686	$9.0\times10^{-5}\pm2.65\times10^{-5}$
HA (0.2%)	4.9±0.793725	4.3±0.43589	$8.0\times10^{-5}\pm1.53\times10^{-5}$
AA (0.1%)	5.2±1.09697	4.9±1.25033	$9.0\times10^{-5}\pm3.06\times10^{-5}$
AA (0.2%)	5±1.01489	4.5±0.953939	$8.0\times10^{-5}\pm2.52\times10^{-5}$
CD	1.32	1.16	0.000011

HA: Hydroxylamine, AA: 5-amino acridine

Highest mean root length was observed in control which was 4.9 cm while minimum mean root length observed under 15 Kr gamma rays treatment which was 4.2 cm. This parameter also follows the same trend as with higher doses mean value decreases. Critical difference in the standard error analysis (Table 2). Revealed that all the treatments except lower doses of mutagens were effective on root length in comparison to control.

Mean dry weight was found highest in control which was 0.001 mg and lowest mean dry weight was observed in 0.2% HA treatment which was 0.00007 mg. Dry weight mean and dose rate of mutagens followed similar trend with increase in dose rate, dry weight mean decreased as compared to lower doses. Analysis of critical difference and standard error (Table 2) indicated that all the treatments except lower doses significantly affect the dry weight of plant in comparison to control.

Gamma rays, X-rays, HA, and AA reduce the shoot length, root length, and dry weight in variety IP-I6 of *L. usitatissimum* L. at higher doses. Similar observations were made by several workers in different plants (Chopra, 1972; Davies, 1968; Nerkar, 1970; Pathak and Patel, 1988; Rai, 1971; Singh and Singh, 2007). The results of the present work are in agreement with these early findings. The growth rate showed a gradual recovery from the inhibitory effect of mutagens as it increased with the advance of time period after giving periods. In variety IPI6, dwarf plants were produced due to stunted growth. Different workers hold independent opinion regarding phenomenon on stunted growth (Conger and Stevenson, 1969; Gordan, 1954; Gunckel and Sparrow, 1961).

CONCLUSION

Among the mutagens used, chemical mutagens (HA and acridine) were found to be more effective in showing

inhibitory effects as compared to physical mutagens (gamma rays and X-rays). HA treatment shows the highest inhibitory effect on germination and survival of plants. In physical mutagens, gamma rays show more inhibitory effect than X-rays. In growth rate parameter, both physical and chemical mutagens show similar inhibitory effects. However, low doses show negligible effect as compared to control in this variety (IP-I6) of *L. usitatissimum* L. for growth parameter. The inhibitory effect of mutagens is due to the fact that biological damage increased at a faster rate in higher doses/concentration of mutagens. This study was one step toward exploring the most desirable treatment condition for enhancing mutagen efficiency. Further research is required to determine the effect of other variables such as temperature, pH, and post-treatment on mutagen action through PCRR-APD marker in treated population.

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