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CYTOLOGICAL ABERRATIONS IN M_2 MORPHOLOGICAL MUTANTS OF *LINUM USITATISSIMUM* (L.) INDUCED BY PHYSICAL AND CHEMICAL MUTAGENS

Roshan Jahan*, Shazia Bi Ansari, Saima Malik and Samiullah Khan
Mutation Breeding Lab, Department of Botany, Aligarh Muslim University, Aligarh, Uttar Pradesh, India, 202002
*Author for correspondence email : roshanjahan.amu@gmail.com

Abstract

At present, the significant worry of the plant breeding researchers is to enhance the yield of various crops including linseed worldwide. By exploring the potential of chemical mutagens, ionizing radiation and hybridization technique, scientists are more concerned to improve quality and quantity both. The seeds of linseed plant were treated with different doses of gamma-rays (50Gy, 100Gy, 150Gy, 200Gy) and sodium azide (0.1%SA, 0.2%SA, 0.3%SA 0.4%SA) which induces various morphological putative mutants in M_2 generation such as bushy mutant (0.2% SA), narrow leaf mutant (100 Gy), and broad stem mutant (150Gy). The doses given were found appropriate to achieve the objective for increasing the productivity of linseed, focusing less biological damage with more variation in quantitative traits. In moderate and higher doses of chemical and physical mutagens, various morphological variants affecting almost all parts of the plant were observed. The cytological analysis with the manifestation of chromosomal abnormalities includes precocious movement of chromosome, unequal separation of the chromosome, laggard and chromosome bridge. Induction of morphological alterations and chromosomal aberrations were found to be higher in gamma rays treated populations than sodium azide comparatively. However the molecular mechanism responsible for the genetic modifications in these putative mutants needs to also be explored through other supporting experiments. Accelerating the rate of desirable high-yield mutants was shown to be economically important. From future perspective it has been correlated that the segregation of selected mutants will definitely contribute to the improved linseed genotype and these mutants may also be used as valuable breeding stock.
Keywords: Linseed, Physical mutagen, Chemical mutagen, Cyto-morphological mutants, Adaptive response to mutagens.

Introduction

Linseed (LS) is a self-pollinated annual, diploid (2n=30) crop belonging to the genus Linum of the family Linaceae (Jahan *et al*., 2019). It was the first crop to be domesticated by the human. As previous data indicates that the average and overall linseed production for the period of 2008-2016 were around 1988492 tons to 2925282 tons and yield was between 948 kg/ha to 1058.2 kg/ha (FAO, 2016). The crop is cultivated to obtain oil as well as fibre (Sankari, 2000; Kurt and Bozkurt, 2006) and is known as a rich source of natural antioxidant Omega-3 fatty acid which belongs to the polyunsaturated fatty acids (Oomah, 2001). It is mostly divided into several groups due to the availability of physiologically active food composition such as protein, lignans, and minerals in addition to the fiber and oil content which could be useful in extending health benefits. Linseed usually contains 4% ash, 6% moisture, 20% protein, 30% fiber and 40 % oil (Wang *et al*., 2008). Linseed helps in preventing disease by removal of molecules known as free radicals from the body. The plant has been proven for its various beneficial roles including the prevention of cardiovascular disease, inflammatory disorders, rheumatoid arthritis, diabetes, asthmatic problems, autoimmune disorders, hormonal imbalance and several types of cancer (Simopoulos, 2002; Goyal *et al*., 2014). Around one third of linseed oil produced globally, is used in pharmaceutical industries and rest is used for commercial purposes (Nykter *et al*., 2006). Apart from this its demand has been shown to rise worldwide regularly as it is being exploited for several purposes; hence the main concern of the researchers is to enhance linseed production. In this direction efforts are being made to develop the improved crop varieties through hybridization technique and induced mutagenesis. Since decades the gamma radiation and various chemical mutagens are often used to exacerbate genetic variation thus accomplishing crop improvement. As of now, through induced mutagenesis several improved varieties of linseed have been developed including a recent variety, DLV 20. The role of induced mutagenesis in the development of putative mutants has been envisaged which also help to increase yields and enhance qualitative and quantitative traits. However cytological investigations have been demonstrated at very large scale in higher plants and there are a few examinations directing with the result of chemical and physical mutagens on meiotic behaviour of *Linum usitatissimum* (Alka *et al*., 2012; Deshpande and Malode, 2018) and in *Vigna mungo* (Goyal and Khan, 2010). The analysis of meiotic activity of mutagenized population is a good indicator for the estimation of particular mutagens potency (Khan *et al*., 2015). In present study, cytological execution of morphological mutants of linseed was examined. The gamma rays and sodium azide treatments were given for induction of genetic variations.

Materials and Methods

Plant material

In linseed genetic variations were induced through various doses of physical mutagen (gamma-rays) as well as chemical mutagen (Sodium azide). The accessions were obtained from National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India.

Methodology

A field experiment was conducted during Rabi (winter) season from November-2017 to April-2018 at the agricultural farm, Aligarh Muslim University, Aligarh. Healthy and dry seeds (with 9-12% moisture) of linseed were straight forwardly irradiated with different doses of gamma rays likewise, 50Gy, 100Gy, 150Gy and 200Gy in Gamma chamber-5000 with a radioisotope Co-60 (Cobalt-60), at Indian Agricultural Research Institute (IARI), New Delhi, provider: BRIT (Leading body of Radiation and Isotope Innovation) Mumbai. For chemical treatments, pre-soaked (9hrs) seeds were treated with different dosages of Sodium Azide (SA) viz, 0.1%, 0.2%, 0.3% and 0.4% SA at room temperature of 25±2°C for 6 hours at pH-7. Soaked in distilled water for 9 hours were utilized as controls. Thereafter, the treated and control seeds were sown in the field (3 replicates) in a Complete Randomized Block Design (CRBD) in order to raise the M_1 generation. Each treatment/dose consisted of 300 seeds, including the control. Seeds harvested from the plants of M_1 generation were sown as M_2 families in the field on the basis of the plant progeny. For meiotic investigations, young unopened flower buds of the mutant plants were fixed in freshly prepared Carnoy's fixatives (absolute alcohol, chloroform and glacial acetic acid in 6:3:1 v/v) for 24 hours. After 24 hours of fixation, materials were transferred to 70% alcohol, and then anthers were squashed in 1% acetocarmine solution, at various stages of microsporogenesis, pollen mother cells (PMCs) were examined under the microscope. By using the following formula, frequencies of meiotic abnormalities were calculated.

Frequency of meiotic abnormalities (%) =

$$\frac{\text{Number of Abnormal PMCS}}{\text{Total Pollen Mother Cells observed}} \times 100$$

Results

In the present study mutagenic effects of gamma rays and SA have great potential to induce several morphological alterations (growth habit, leaf morphology, and floral morphology) and broad spectrum of cytological abnormalities in linseed. The observed and analyzed morphological alterations were found in M_2 generation which is correlated with cytological abnormalities. Various sorts of morphological putative mutants observed in M_2 generation shown (Table 1, Figs 1. A-D). These M_2 putative mutants were considered as evident rearing or true bred in the M_3 generation.

Table 1 : Morphological parameters of the M_2 mutants.

Quantitative Traits									
	Treatments	**No. of M2 plants**	**No. of mutants**	**Frequency (%)**	**Plant height(cm)**	**Capsules/ plant**	**Seeds/ capsule**	**1000 seed weight(g)**	**% age improvement/ reduction**
Control					75.71±0.91	73.06±0.42	10.03±0.25	7.26 ±0.05	-
Bushy mutant	0.2%SA	787	6	0.76	65.40±0.31	78.83±0.40	11.23±0.17	7.62±0.52	4.95
Narrow leaves mutant	100 Gy γ-rays	698	11	1.57	74.10±0.31	77.53±0.45	10.36±0.21	7.00±0.10	3.58
Broad stem mutant	150 Gy γ-rays	756	9	1.19	73.10±0.31	77.03±0.43	10.06±0.16	6.60±0.13	9.09

1. Mutants with altered growth habit

(a) Bushy Mutant (Compact)

Bushy mutant is distinguished by its vigorous growth, with higher number of branches being compactly arranged which give it a busy appearance. This mutant were noticed in moderate dose of sodium azide (0.2%SA), indicating increased yield attributing traits such as excessive branches and enlarged inflorescence number, number of capsule/plant and seeds/capsule were also increased in comparison with control. Mutant plant matured 8 days earlier than control. The average height reduced from 75.71 cm (control) to 65.40 cm in. (Table 1, Fig. 1, B.). Highest yield was noticed in Bushy mutant compared with control.

(b) Broad Stem Mutant

This type of mutant were observed in higher dose of gamma rays (150Gy), possessing a cluster of small and thick leaves spreading over the whole plant demonstrating rosette kind of morphology (Fig.1, C). Few fertile branches were notice in this mutant. Flowering and maturity period were also late with poor yield in comparison with control.

2. Mutants with altered leaf morphology

Narrow Leaves Mutants

The mutant plant was characterised by narrow leaves with pointed tips and stem were thin as compared to control plant. Capsule and seeds were smaller compared with control. Such type of mutant was noticed in moderate doses (100Gy) of gamma treated populations (Table-1 and Fig. D).

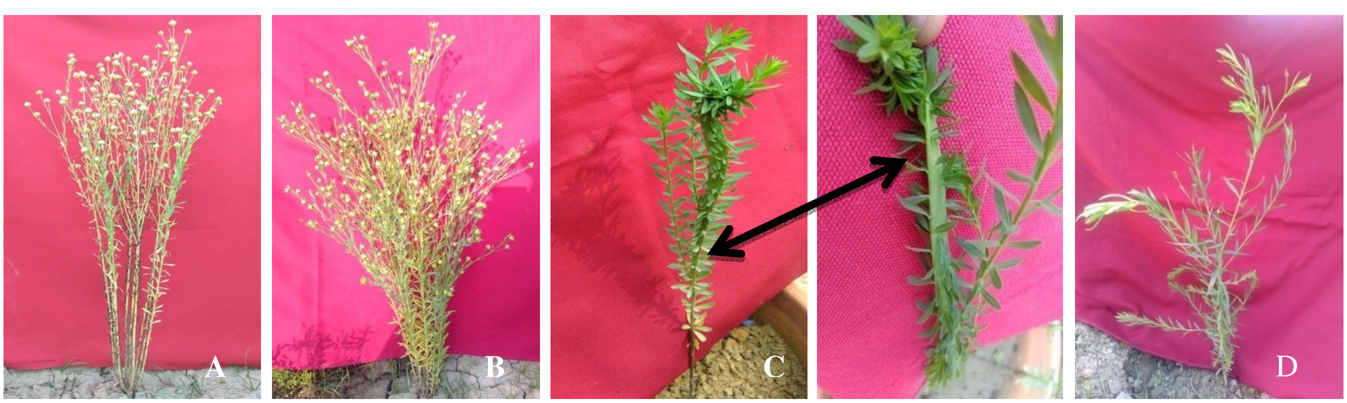

Fig. 1 : Plant height and growth habit variants: (A) Control, (B) Bushy mutant, (C) Broad stem mutant, (D) Narrow leaf mutant.

Cotyledonary leaves abnormalities

The broad spectrum of cotyledonary leaves abnormalities such as damage in shape (curled), size and numbers (Uni-seedling with one cotyledonary leaf instead of a pair as in control, tri– seedlings with an extra cotyledonary leaf) were recorded in both lower and higher dose of gamma rays and SA in M_2 generation. (Figs. 2.A-D).

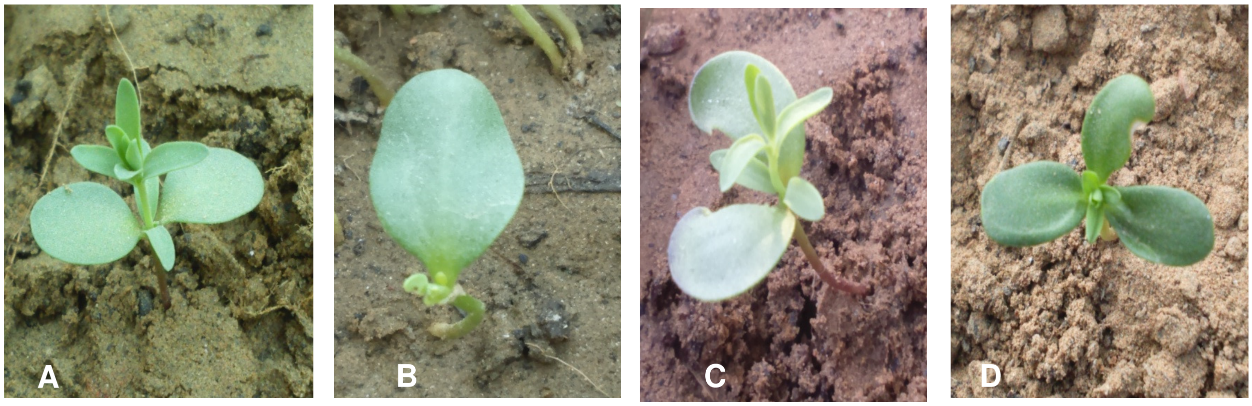

Fig. 2. (A) Normal dicotyledon; (B) Monocotyledon; (C) Dicotyledons (Curled), (F) Tricotyledon.

Leaf morphology: Mutants

Different types of leaf anomalies were noted in different doses of gamma rays and SA treated populations, such as damage in shape, size and colour. Generally, leaves were linear lanceolate in control but in mutant plants, number of notch (Fig 3. A) Acicular leaves (needle-like), Falcate (Sickle-shaped) (Fig 3. B) and Obcordate leaf (Heart shaped) (Fig 3. C) were observed.

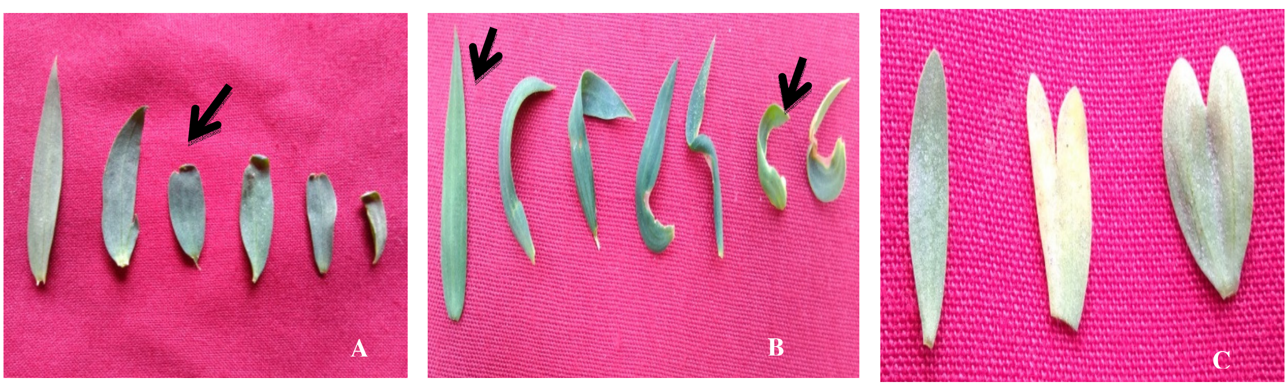

Fig. 3. Isolated morphological variations of leaf in mutagens treated populations of Linseed: (A) Control and Leaf with Notches (B) Sickle-shaped and Needle-like leaves, (C) Heart shaped leaf

Flower mutants:

The findings as shown in the figure (Fig. 4, A-I) were reported in linseed populations treated with both gamma rays and sodium azide. The variation in the form of shape, colour and size of the flower can be correlated with the mutagenic efficacy.

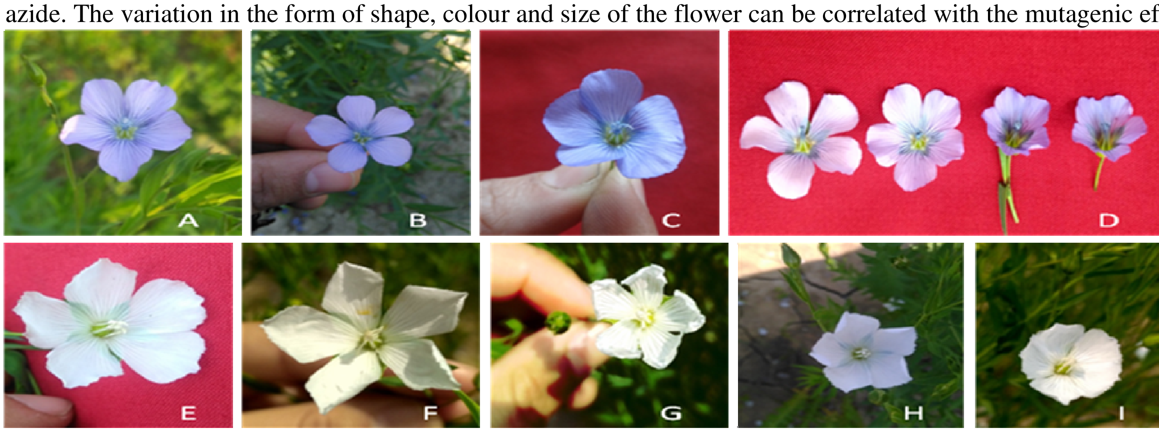

Fig. 4 : Shape and colour of flower. (A) Control, (B) flowers with free petals, (C) Disc shape (D) Bluish purple colour, Dark purple, (E) Whilte flower with broad petals, (F) Star-shaped, (G) Star shaped with curled petals, (H&I), Funnel shaped.

Meiotic analysis

By using physical (gamma rays) and chemical mutagen (sodium azide), a wide spectrum of meiotic abnormalities was induced in linseed populations. In various mutagenic treatments, spectrum of meiotic abnormalities was observed included precocious movement at Metaphase-I, unequal separation of chromosome at Anaphase-I and Laggard and chromosome bridge formation at Anaphase-I. Meiosis in control plants was found to be normal, forming 15 bivalents at metaphase-I and normal separation (15:15) in anaphase-I cells. Bushy mutants showed precocious movement at Metaphase-I, narrow-leaves mutant showed unequal separation of chromosome and laggard at Anaphase-I and broad Stem mutant showed bridges formation at Anaphase-I (Table 2, Figs.5,A-E).

Table 2: Cytological Aberrations of M_2 Morphological Mutants.

	Treatments	**No. of PMC's observed**	**No. of PMC's showing abnormalities**	**% age of abnormal PMC's**	**Chromosomal aberrations**		
Control	-	305	-	-	-	-	-
Bushy mutant	0.2%SA	286	6	2.09	Precocious movement at Metaphase-I	-	-
Narrow leaves mutant	100 Gy γ-rays	221	4	1.80	-	Unequal separation at Anaphase-I	Laggard in Anaphase
Broad stem mutant	150 Gy γ-rays	195	2	1.02	-	-	Bridge at Anaphase-I

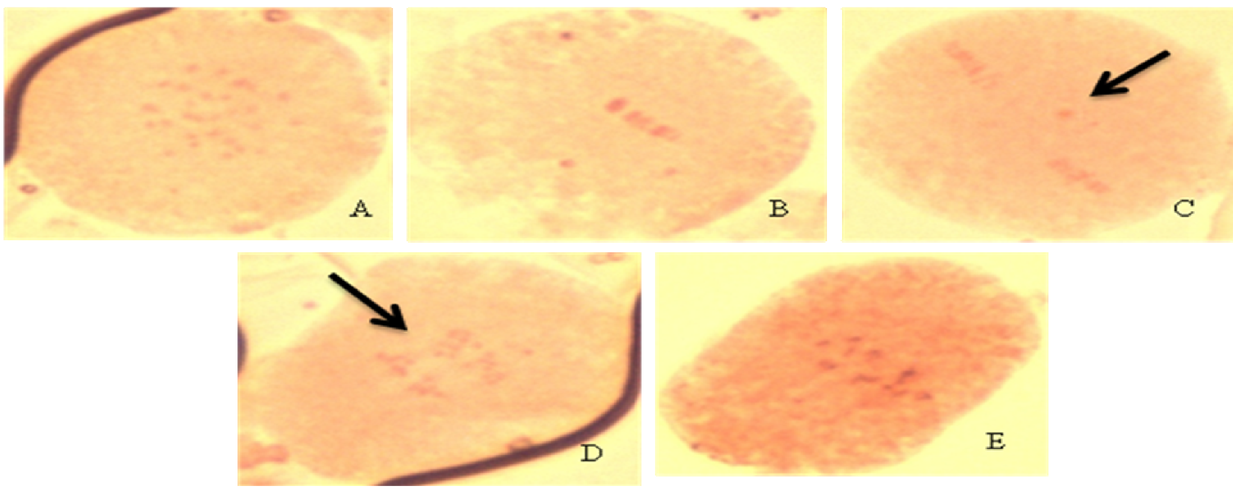

Fig. 5: (A) Prophase (Control), (B) Precocious movement at Metaphase-I, (C) Laggard at Anaphase (D) Bridge at Anaphase-I, (E) unequal separation of the chromosome at anaphase-I.

Discussion

Herein, M_2 generation plants have been screened in terms of their growth habitat, leaf and flower morphology, followed by the identification of various putative mutants and some of them contributing to increased yield. These selected putative mutants revealed the negative selection value and could be valuable for plant breeders as they are accountable for many significant genetic variations in hybridization programmes. The induced mutants were often associated with cytological abnormalities. Variations in chromosome structure are usually distinguished using traditional cytogenetic techniques, but they may fail with respect to minor chromosomal aberrations. Comparatively, gamma radiation was found to be the most effective and efficient for the induction of meiotic abnormalities than sodium azide. The experimental outcomes clearly support the general hypothesis that more cytological abnormalities are induced by the physical mutagens than the chemical mutagens (Kozgar *et al.*, 2014). Meiosis is one of the most important genetic events occurring in meiocytes of the organism consisting of highly balanced biochemical, cytogenetic, physiological and phenotypic events leading to chromosome reduction, gene rearrangements and gamete formation (Goyal *et al.*, 2019). Several studies previously done for Safflower (Kumar and Srivastava, 2010), Soyabean (Pavadai *et al.*, 2013), Chilli (Gandhi *et al.*, 2014), Faba bean (Khursheed *et al.*, 2015), Mustard (More, 2016), Black cumin (Amin and Khan, 2018), Black gram (Goyal *et al.*, 2019) had been shown well documented phenotypic variations and chromosomal abnormalities induced by both physical as well as chemical mutagens. Bushy mutant with precocious separation of chromosome exhibited increased yield attributes which was observed with the moderate dose of sodium azide. Precocious movements may be due to disturbed homology for chromosome pairing or disturbed spindle mechanism (Umar and Singh, 2003; Kumar and Gupta, 2007). Such type of precocious separation of chromosome has also been reported in different plants by several researchers, in *Cicer arientum* (Ganai *et al.*, 2005), *Trigonella foenum-graecum* (Srivastava and Kapoor, 2008), *Cichorium intybus* (Khan *et al.*, 2009; Jafri *et al.*, 2011); *Vicia faba* (Shahwar *et al.*, 2016), *Vigna mungo* (Goyal *et al.*, 2019). Narrow leaf mutant was also observed with moderate dose of gamma rays resulting in meiotic abnormalities, unequal separation and laggard chromosome at anaphase I. One of the research articles suggests that the unequal separation of chromosome was due to early or delayed separation of bivalents and multivalent in the mutated forms of chromosomes and it may result in the formation of aneuploid gametes (Zeerak, 1992). While another study has suggested that the unequal separation of chromosome occurs due to random movement of univalents to any of the pole of anaphase I (Kumar and Singh, 2003). Unequal separations of chromosome had been also reported in *Hordium vulgare* (Kumar and Singh, 2002), *Capsicum annum* (Gulfishan *et al*., 2012), *Vigna mungo* (Goyal and Khan, 2010), *Catharanthus roseus* (Verma *et al*., 2012), *Papaver somniferum* (Naseem and Kumar, 2013) and *Triticum aestivum* (Khan and Verma, 2017) by many researchers. The observed laggard formation in mutant with narrow leaf possibly may be due to the failure of chromosome separation or a delayed terminalisation and chromosome stickiness (Jayabalan and Rao, 1987) or improper spindle assembly too. Broad stem mutant confirmed maximum reduction in the yield in comparison to control plant. Other meiotic abnormality like chromosome bridges was also observed at anaphase-I that may result from breakage of chromosome and in addition to this, reunion of broken ends of chromosomes may also accomplish bridge formation (Ignacimuthu and Babu, 1989; Shreekrishna, 2006). Previously, it had been reported that the dicentric chromosome and unequal exchange can lead to bridge formation (Bhat *et al*., 2007). Many researchers have documented anaphase bridges in different crops such as *Lycopersicon esculentum* L. (Zeerak, 1992); *Tridax procumneans* (Cequea *et al*., 2003), *Capsicum annuum* (Singh and Chaudhary, 2005), *Glycine max* L. (Khan and Tyagi, 2009); *Nigella sativa* L. (Amin *et al*., 2016) and *Linum usitatissimum L.* (Alka and Ansari, 2017).

Conclusion

Herein, it can be concluded that gamma rays are most effective mutagen than sodium azide, inducing cytological aberration which could be correlated with the putative mutants as well with higher meiotic frequency in bushy followed by narrow leaf and broad stem mutants. Selection of high yield putative mutants of linseed may be beneficial for plant breeders from the future prospective.

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