Contents list available at Science Letters

Science Letters

journal homepage: http://www.scienceletters.org/

Physio-Morphological Characteristics in Linseed (Linum Usitatissimum L.) Induced by Hydrazine Hydrate

Sadaf Jafri[a], Roshan Jahan[a*], Samiullah Khan[a]

[a]Mutation Breeding Laboratory, Department of Botany, Aligarh Muslim University, Aligarh 202002, Uttar Pradesh, India.

ARTICLE INFO

Article History:
Received 10 January 2020
Revised 28 April 2020
Accepted 4 May 2020
Available Online 5 May 2020

Keywords:
Linum usitatissimum L.
Hydrazine hydrate,
Bio-physiological Damages,
Quantitative traits,
Medicinal plant.

ABSTRACT

One of the proven facts of genetics is induced mutagenesis the most successful, safe and low cost approach for producing the different varieties of a crop species. In the present scenario linseed (Linum usitatissimum L.) a medicinally as well as economically important plant of the family Linaceae, was selected for the present study for the enhancement of genetic variability by using induced mutagenesis technique. Healthy seeds of two linseed varieties were treated with different doses of hydrazine hydrate (HZ) for a short duration of six hours. The findings recorded were correlated with qualitative and quantitative traits to assess mutagenic potency and to produce desirable genetic variation in selected crop plant. The bio-physiological study showed a steady reduction in the germination percentage with the increasing doses of mutagens. The total chlorophyll content showed a significant negative shift from control to all treatment in both the varieties. The doses of hydrazine hydrate (0.2% and 0.3%) were found to be the most potent for the induction of the genetic variability in both the varieties. The lower and moderate doses were found to be applicable for the inducing the mutation in the agro-economical traits of linseed. In quantitative characters, lower biological destruction was evaluated with a high percentage of variance.

1. Introduction

Linseed is an annual, diploid (2n=30), self-pollinated crop having ~373 Mb of genome size, belongs to the genus *Linum* and family *Linaceae.* At present the plant is known worldwide for its valuable benefits including oilseed and fibrous content obtained from the crop. Linseed is divided into various categories because of availability of active compounds likewise minerals, lignans, protein, fibre as well as oil which may be beneficial in health (Jahan *et al.*, 2020). The plant is having unique profile of fatty acid i.e. Omega 3-fatty acid (Alpha-linolenic acid).

* Corresponding Author: Roshan Jahan

E-mail Address: roshanjahan.amu@gmail.com

DOI: 10.46890/SL.2020.v01i03.002

© 2020 by the authors. The license of Science Letters. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons org/licenses/by/4.0/).

Linseed represents its good status among oilseed crops due to its tremendous benefits resulting in versatile uses across the globe. Alpha- linolenic acid is one of the most pivotal and essential fatty acid among polyunsaturated fatty acid that has showed its anti-thrombotic, anti-inflammatory, anti-arrhythmic properties and prevent several types of cancer (Simopoulos, 2002; Goyal *et al.*, 2014). For non-fish eaters, linseed exhibit as the best source of omega-3 fatty acid. In spite of various proven clinical evidences of linseed the people still are not acquainted with the nutritional and therapeutic values of linseed. The increased yield of any plant is the need of the growing population; however, oil being one of the essential products of linseed has immense economic potentialities and studied in great details. Hydrazine (N_2H_4/H_2NNH_2) an inorganic compound is referred as diamidogen. Hydrazine is extremely toxic and dangerously unstable when it is treated in a solution. Hydrazine is used primarily as a foaming agent in the preparation of polymer foams but it is also known for other important applications as a substrate for polymerizing

© 2020 Science Letters. All Rights Reserved.

catalysts, in pharmaceuticals and agrochemicals. Recently, hydrazine is used in mutation breeding programmme to assess genetic variability in plants.

2. Materials and Methods

2.1. Plant Material

Experimental plant material was selected for the present study is *Linum usitatissimum* L., commonly known as Linseed. The two varieties selected were IC0096650 and Padmini. Variety IC0096650 and variety Padmini were procured from National Bureau of Plant Genetic Resource (NBPGR), New Delhi, India and Governmental Seed Store Aligarh respectively.

2.2. Experimental Design

Dry and healthy seeds of linseed (*Linum usitatissimum* L.) were used to mutagenic treatment of Hydrazine hydrate (HZ). Hydrazine hydrate (HZ) stock solution was made in phosphate buffer at pH 7. Buffer tablet were used to maintain their pH. Seeds from each variety were distributed into 13 sets of 50 seeds each for this experiment. One set of seeds were taken as control while the rest of 12 sets of linseed seeds were treated with different doses (0.1%, 0.2%, 03% and 0.4% HZ) of HZ of both the varieties. Seeds of linseed were pre-soaked for 6 hrs in distilled water and after that 9 hrs for mutagenic treatments with intermittent shaking at room temperature of 25 ± 2°C. Treated seeds were washed properly with running tap water for 20-30 minutes to remove unnecessary mutagen. In the Net House, Department of Botany, A.M.U. Aligarh, treated seeds were sown along with their respective control in five replicates of ten seeds each in earthen pots during 2017-2018 Rabi season. During the growth period, both treated and control populations were examined thoroughly for morphological mutants. For quantitative traits, Statistical analysis; Mean (X), Standard error (SE), Coefficient of variation (CV %) were done by using IBM SPSS Statistics 20 software.

2.3 Seed Germination

Seed germination percentage was determined by using the formula

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

2.4. Seedling Height

After 15 days of germination, seedling height was noted by measuring the shoot and root lengths for control and each treatment.Seedling injury was assessed in respect of control seedling height reduction.

2.5. Chlorophyll and Carotenoid Contents Estimation

According to the following equation given by Arnon (1949), chlorophyll and carotenoid content was estimated

$$\text{Total chlorophyll (mg g}^{-1}\text{leaf fresh mass)} = \{20.2\ (OD_{645}) + 8.02(OD_{663})\} \times \frac{V}{1000 \times W}$$

$$\text{Carotenoid (mg g}^{-1}\text{leaf fresh mass)} = \frac{7.6\ (OD\ 480) - 1.49\ (OD510)}{d \times 1000 \times W} \times V$$

Where,

OD_{645}, OD_{663}, OD_{480}, OD_{510} = Optical densities at OD $_{645}$ OD, $_{663}$ OD, $_{480}$ and $_{510}$ respectively

V = Volume of an extract

W = Mass of leaf tissues

d = Length of light path (d = 1.4 cm)

2.6. Plant Survival

For calculating the percentage of injury, inhibition or reduction, the following formula was used.

$$\text{Percentage injury} = \frac{\text{Control} - \text{Treated}}{\text{Control}} \times 100$$

2.7. Quantitative Traits

The quantitative traits were studied thoroughly including plant height (cm), No. of fertile branches/plant, number of capsule/plant, number of seeds/capsule, 1000 seed weight (g) and yield/plant (g).

2.7.1. Mean ($\bar{X}$)

The mean was computed by taking the sum of a number of values (X_1, X_2,……..Xn) and dividing by the total number of values (N) involved, thus;

$$\bar{X} = \frac{X_1 + X_2 + \ldots\ldots\ldots\ldots\ldots Xn}{N}$$

Where,

X_1, X_2,…...Xn = Observations

N = Total number of observations involved

2.7.2. Coefficient of variation (C.V.)

$$\text{C.V. (\%)} = \frac{\text{Standard deviation}}{\bar{X}} \times 100$$

2.7.3. Standard error (S.E.)

$$\text{S.E.} = \frac{\text{S.D. of sample}}{\sqrt{N}}$$

Where,

S.D. = Standard deviation

N = Number of observations

© 2020 Science Letters. All Rights Reserved.

3. Experimental Results

Mutagenic effects of hydrazine hydrate (HZ) on seed germination, percentage inhibition, seedling height, total chlorophyll and carotenoid content, morphological variants, and various quantitative traits of linseed were studied.

3.1. Seed Germination

The outcome of seed germination, plant survival and pollen fertility are shown in **Figure 1**. In control as well as treated plants within 1-8 days, seed germination was noted. As compared to the var-padmini, seed germination percentage was higher in var- IC0096650. While increasing the doses and duration of mutagens, germination was delayed. In var. Padmini, percentage of seed germination decreased from control (98%) to 0.4% HZ (62%) while in var-IC0096650 it decreased from control (100%) to 0.4% HZ (65%). Percentage inhibition increased with the increase in duration and doses of mutagen treatments. In higher dose (0.4%HZ) of mutagens it was 36.73% in var-padmini and 35% in var-IC0096650.

3.2. Seedling Height

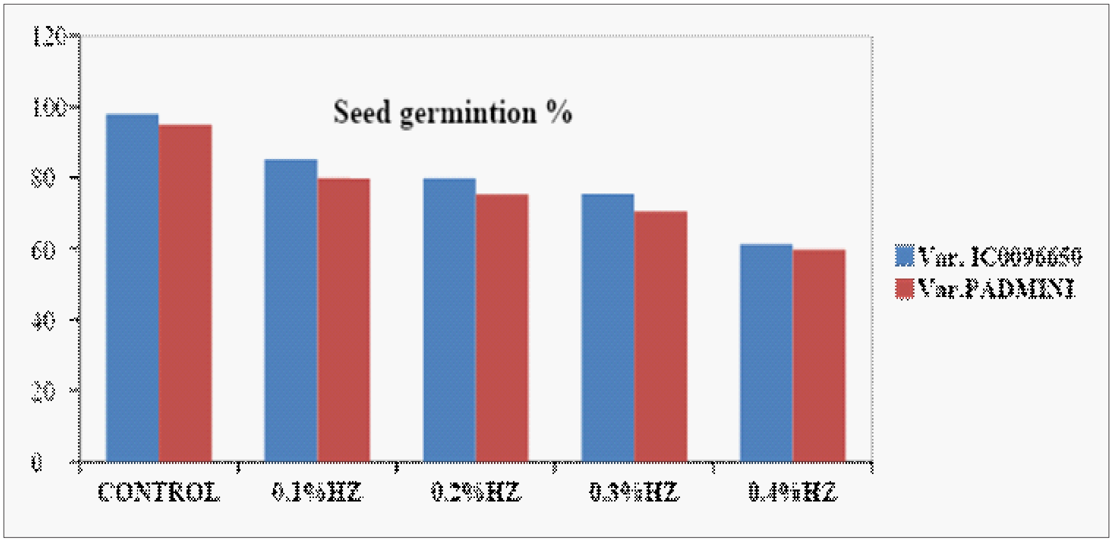
Data were recorded for 15-days-old seedlings raised in B.O.D incubator in petri plates. Increasing the concentration of mutagens, germination percentage as well as seedling height were decreased in both the varieties. The percent inhibition was more desperate at higher doses of mutagens.

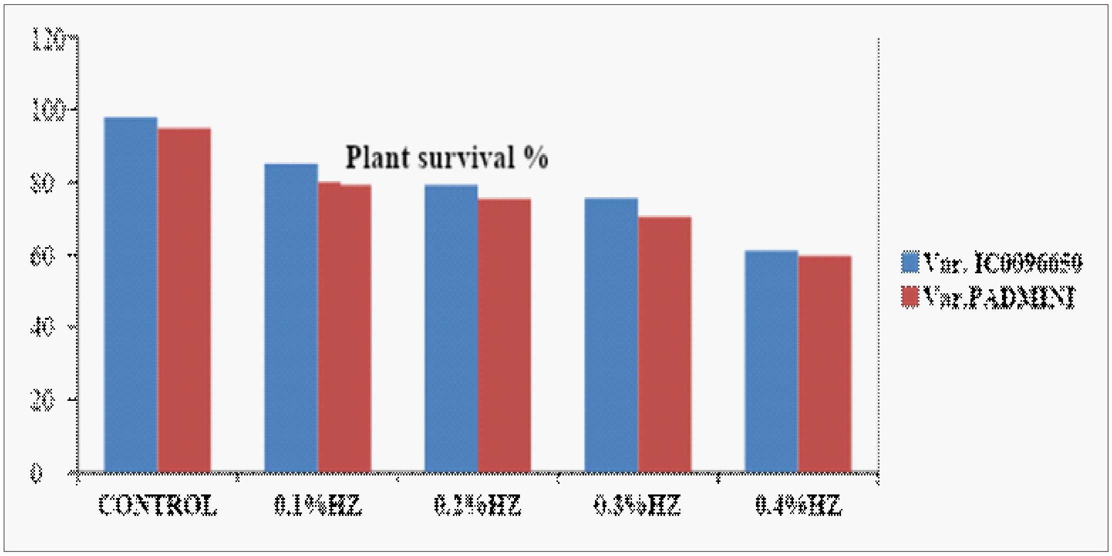
3.3. Plant Survival

Percentage of plant survival was decline with increasing the duration and doses of mutagen treatment. In controls (98% and 95%) maximum plant survival was noted of var-IC0096650 and var-Padmini, respectively, which exhibited drastic decrease in higher dose of HZ (0.4%) 61.22% and 59.73% in var-IC0096650 and Padmini respectively.

3.4. Pollen Fertility

The Present study showed a significant reduction in the degree of pollen fertility. It reduced (77.53% and 76.13%) in 0.3% of HZ treatment in both var-IC0096650 and padmini, respectively.





© 2020 Science Letters. All Rights Reserved.

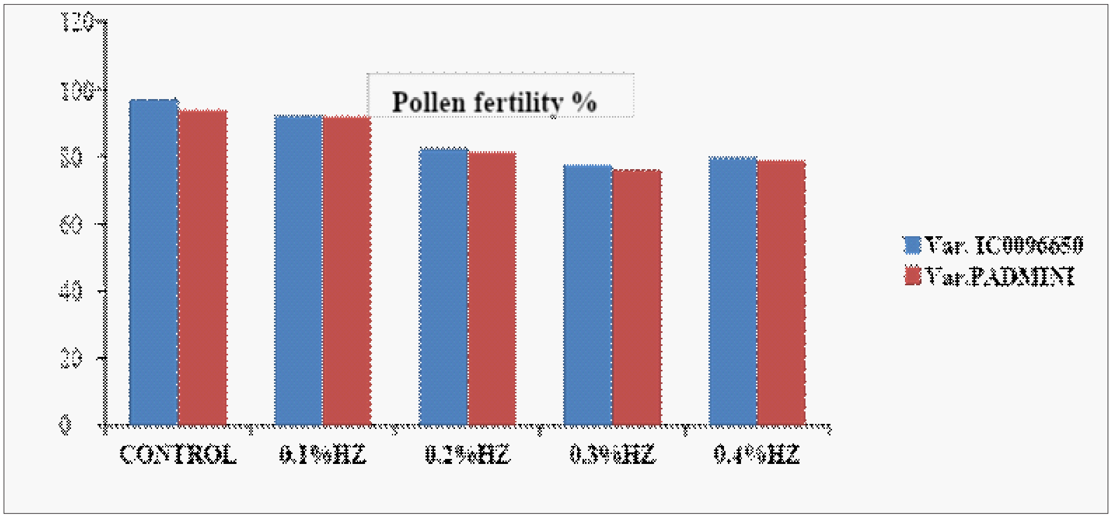


Figure 1. *Estimates of Mean ($\bar{X}$) seed germination (%), plant survival (%), pollen fertility (%) in M_1 generation of linseed (Linum usitatissimum L.) var-IC0096650 and Padmini.*

3.5. Total Chlorophyll and Carotenoids Contents

The total chlorophyll content of linseed control and treated plants are shown in the Table 1. It was found that total chlorophyll in both varieties revealed a significant negative shift from control to all the treatments. In var- IC0096650, it was 1.70 mg/g in control which decreased to 0.53 mg/g in 0.4% HZ, whereas in var-Padmini, it decreases from other treated population from 1.22 mg/g (control) to 0.14mg/g (0.4% HZ). The total carotenoid content also showed a negative shift from control to the all mutagenic treatments, in var-IC0096650, It was 0.10mg/g in control which decreased minimum to 0.03 mg/g in 0.4% HZ, whereas in Padmini, it decreases from 0.09 mg/g (control) to 0.04mg/g in 0.4% HZ.

Table 1. *Estimates of Mean, Standard Error (S.E.), and Coefficient of Variation (C.V. %) for chlorophyll content and Carotenoid content of leaves in Linseed (Linum usitatissimum L.).Var- IC0096650 and Padmini.*

Treatments/ Doses	Var-IC0096650				Var-Padmini			
	Chlorophyll Content (mg g-1 FW)		Carotenoid content (mg g-1 FW)		Chlorophyll Content (mg g-1 FW)		Carotenoid content (mg g-1 FW)	
	Mean ±SE	CV(%)	Mean ±SE	CV (%)	Mean ±SE	CV (%)	Mean ±SE	CV (%)
Control	1.70 ±0.016	2.95	0.10±0.018	50.0	1.22 ±0.009	2.31	0.09±0.015	46.0
0.1%HZ	0.70±0.013	5.83	0.06±0.001	5.11	0.18±0.001	3.03	0.08±0.003	12.1
0.2%HZ	0.63±0.009	4.72	0.09±0.018	60.0	1.19±0.004	1.20	0.09±0.013	44.0
0.3%HZ	0.55±0.002	1.49	0.04±0.001	8.81	0.18 ±0.000	1.38	0.04±0.007	55.7
0.4%HZ	0.53±0.010	6.12	0.03±0.000	5.95	0.14 ±001	4.14	0.04±0.002	16.1

3.6 Morphological Variants

Various types of morphological variants have been identified that affect almost all plant parts in the mutagens treated population which is shown in the Figure 2 & 3. Morphological variants of leaf were small, bifurcate and lance-shaped. Variation in the size of capsule and seeds were also noticed in the treated population. Variation in the flower colour (white and light blue) and shape (star-shaped, Funnel shaped, Sinuate) are presented in the Figure 4.

© 2020 Science Letters. All Rights Reserved.

Figure 2. *Plant height and growth habit mutants of linseed varieties. A. Comparison of dwarf and tall plant. B. Plant with branches emerges from base. C. Plant with single branching.D. Plant with more leaves on whole plant body. E. Plant with high yield. F. Normal plant.*

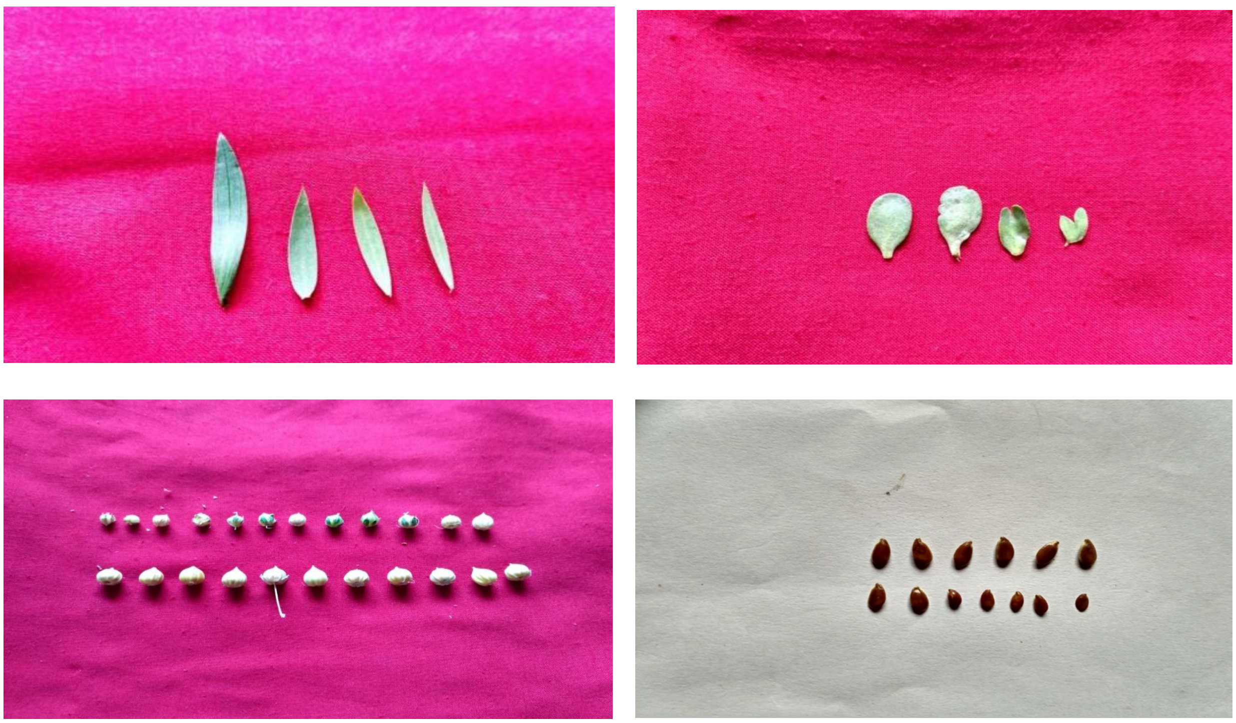


Figure 3. *Isolated morphological variations of leaf in mutagen treated populations of linseed varieties. A.Variation in the size of leaves. B. Variation in the cotyledonary leaves. D.Variation in capsule size (D-I: Capsule of var- IC0096650; D-II.Capsule of var- padmini. E. Variation in seed size (E-I: Seeds of var-padmini, E-II: seeds of var-IC0096650).*

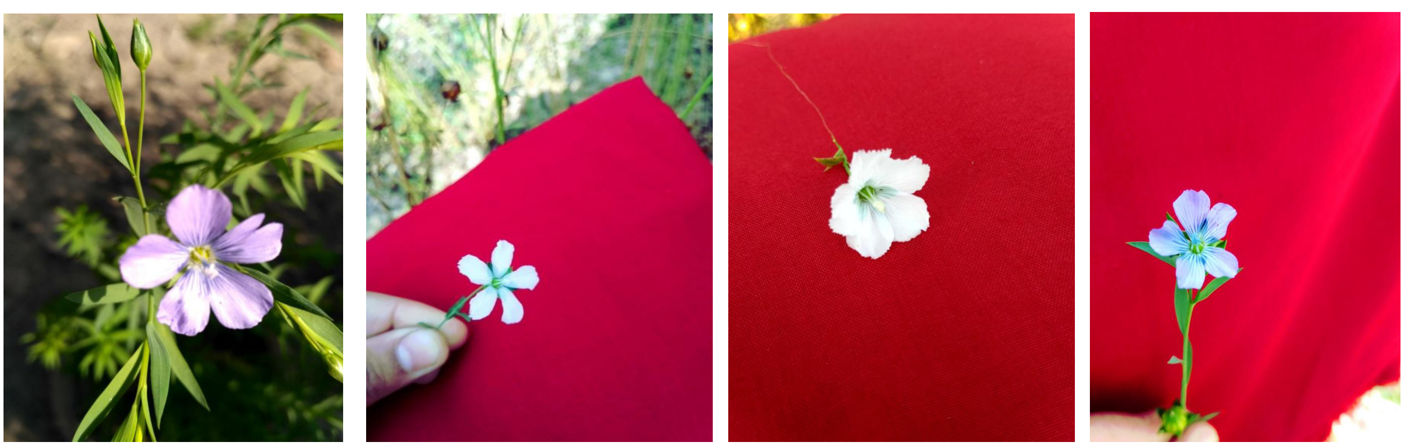


Figure 4. *Variation in flower colour and shape. A. Purple colour flower, B. Star shaped, C. Funnel shaped, D. Blue colour (Normal flower).*

© 2020 Science Letters. All Rights Reserved.

3.7. Quantitative Traits

Data on quantitative traits of both the varieties are given in the Table 2& 3. The plant height was decreased with increasing the doses of mutagen in both the varieties (IC0096650 and Padmini). Reduction in the plant height from 66.73 cm (control) to 53.93% (0.4%HZ) was noted in the var-IC0096650, while in var-Padmini, it decreased from 68.76 (control) to 61.13 (0.4%). The maximum coefficient of variation (CV %) was observed in higher doses of hydrazine hydrate in both the varieties. The minimum number of fertile branches was recorded in 0.4%HZ (3.66 and 5.66) in var- IC0096650 and Padmini respectively. The number of capsule was increased from 43.33 (control) to 46.33 (treatment 0.2% HZ) in var-IC0096650 whereas in var-Padmini it increased from 50.33 (control) to 52.33 (0.2%HZ). As compared to the control number of seeds per capsule of the treated plants was also increased in both the varieties. The average weight of 1000 seeds was 6.76 g to 7.00 g (controls) of the var- IC0096650 and Padmini. In the var- IC0096650 average weight was increased at 0.1% HZ while in var- Padmini it increased at 0.2% HZ. The maximum yield was observed at lower and moderate doses in both the varieties. The high C.V. % was found at lower doses of mutagens suggesting high genetic variability in mutagenized population of both varieties.

Table2. *Estimates of Mean, Standard Error (S.E.) and Coefficient of Variation (C.V. %) for Plant height (cm), No. of fertile branches / plant, number of capsule/ plant, number of seeds /capsule, 1000 seed weight (g), yield/plant (g) of Linseed (Linum usitatissimum L.) var. IC0096650.*

Treatments/ Doses	Plant height (cm)	No. of fertile branches/plant	No. of Capsule/ plant	No. of Seeds/capsule	1000 Seeds weight (g)	Yield/plant(g)
	Mean ±S.E.	Mean ±S.E.	Mean ±S.E.	Mean ±S.E.	Mean ±S.E.	Mean ±S.E.
Control	66.73±0.65 (1.95)	5.33±0.43 (10.85)	43.33±1.00 (7.05)	9.00±0.57 (11.11)	6.76±0.40 (7.32)	2.63±0.10 (1.33)
0.1%HZ	64.23±0.69 (2.26)	6.33±0.43 (9.11)	44.33±0.91 (5.67)	10.66±0.43 (5.41)	7.85±0.30 (3.58)	3.70±0.06 (0.31)
0.2%HZ	63.83±0.63 (1.89)	5.33±0.43 (10.82)	46.33±0.71 (3.29)	11.33±0.62 (10.18)	7.19±0.34 (5.03)	3.77±0.09 (0.70)
0.3%HZ	60.93±0.87 (3.75)	4.33±0.71 (35.25)	40.33±0.83 (5.16)	8.33±0.71 (18.33)	5.99±0.19 (1.84)	2.01±0.18 (5.03)
0.4%HZ	53.93±1.17 (7.77)	3.66±0.62 (31.49)	38.66±0.71 (3.95)	7.33±0.43 (7.87)	5.54±0.18 (1.90)	1.56±0.10 (2.21)

 # Figure in parenthesis represents Coefficient of variation (%)

Table 3. *Estimates of Mean, Standard Error (S.E.) and Coefficient of Variation (C.V. %) for Plant height (cm), No. of fertile branches / plant, capsule/ plant, seeds /capsule, 1000 seed weight (g), yield/plant (g) of Linseed (LinumusitatissimumL.) var. Padmini.*

Treatments /Doses	Plant height (cm)	No. of fertile branches/plant	No. of Capsule/plant	No. of Seeds/ capsule	1000 seeds weight (g)	Yield/plant(g)
	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
Control	68.76±0.70 (2.18)	8.00±0.75 (21.65)	50.33±1.30 (10.19)	9.66±0.43 (5.97)	7.00±0.19 (1.64)	3.40±0.49 (19.29)
0.1%HZ	67.93± 1.07 (5.14)	9.33±0.71 (16.36)	52.33±1.30 (9.80)	11.33±0.62 (10.18)	7.92±0.28 (3.11)	4.69±0.59 (21.96)
0.2%HZ	67.56±0.96 (4.14)	10.66±0.62 (10.82)	51.66±1.08 (6.79)	12.00±0.57 (8.33)	8.56±0.35 (4.40)	5.30±0.63 (20.21)
0.3%HZ	64.86±1.13 (5.94)	6.66±0.43 (8.66)	48.66±0.71 (3.13)	8.66±0.71 (17.62)	6.32±0.31 (5.06)	2.66±0.22 (4.12)
0.4%HZ	61.13±1.29 (8.16)	5.66±0.43 (10.18)	47.66±0.91 (5.27)	7.00±0.57 (14.28)	6.19±0.44 (10.49)	2.06±0.33 (11.21)

 # Figure in parenthesis represents Coefficient of variation (%)

4. Discussion

In the present study the dose and time dependent response of the hydrazine hydrate is discussed comparatively between the two varieties of linseed viz., IC0096650 and Padmini. The examination of biological damage induced by mutagen doses is a crucial tool for the mutation breeding experiments. According to Amin *et al*. (2015) biological damages caused by mutagen is considered as a clue of mutagenic sensitivity. Mick (1979) also suggested that mutagenic sensitivity analysis is the very crucial part of objective to boost the genetic variability. The authors reported reduction in the seed germination upon increasing the doses of the mutagen during the whole study of the experiments.

© 2020 Science Letters. All Rights Reserved.

The maximum inhibition 35% and 36.73% in IC0096650 and Padmini was observed respectively at 0.4% hydrazine hydrate. Similar findings of decrease in percentage of germination with increasing doses of mutagens have also been reported in *Cicer arietinum* (Barshile *et al*., 2006), *Hordeum vulgare* (Khursheed *et al.*, 2015), *Trigonella foenum graecum* (Hasan *et al*. (2018). The delay in the biological and physiological processes is required for seed germination. We observed the occurrence of reduction in seed germination in mutagen treated seeds which may possibly owing to enzyme activity (Kurobane *et al*., 1979) and inhibition of mitotic process (Ananthaswamy *et al.,* 1971). Dose dependent reduction in pollen fertility of mutagen treated population was reported which is correlated with biological damages. This is in agreement with previous studies by Amin *et al*. (2016) in *Nigella sativa* L. and Hasan *et al*. (2018) in *Trigonella foenum graecum.* Induction of morphological mutant was also observed in chickpea (Khan *et al.*, 2011), lentil (Laskar *et al*., 2018; Hasan *et al*., 2018) and Linseed (Jahan *et al*., 2020). Induced mutagenesis is considered to be the most effective technique for induction of genetic variability in crop species. The reduction in the pollen fertility is correlated with the meiotic abnormalities (Rana and Swaminathan, 1964; Sinha and Godward, 1972; Reddy and Rao, 1982; Thomas and Rajhathy, 1966 and Laskar *et al*., 2017; Amin and khan, 2018). Evaluation of chlorophyll and carotenoid pigments of treated seeds disclosed a significant increase due to mutagenic doses that also suggested an improvement in photosynthetic rate of mutagen treated population as compared to the control of both the varieties (Khursheed *et al*., 2016; Hasan *et al.,* 2018; Amin *et al*., 2019). One of the most important indicators of photosynthetic activity is the chlorophyll content (Larcher, 1995). The highest amount of chlorophyll content occurs at the beginning of flowering season and chlorophyll is considered to be the part of organogenesis (Simova *et al*., 2001). Nitrogen is a structural element of chlorophyll and protein molecules, and it thereby influence chloroplast formation and accumulation of chlorophyll in them (Daughtry, 2000; Haboudane *et al*., 2002; Cabrera, 2004; Tucker, 2004).

Increased frequency of morphological variations has been correlated with dose and time dependent effects of the mutagen. The variations in the leaf morphology were usually observed at higher doses of mutagen. The different variants with respect to growth like tall plants, bushy plants and highly branched plants were also observed. Similar finding have also been reported in mung bean (Khan and Siddiqui, 1993), faba bean (Bhat *et al*., 2007), lentil (Laskar and Khan, 2017), Black cumin (Amin and Khan, 2018), Linseed (Jahan *et al*., 2019). Induction of micromutation in polygenic methods is an essential to control and improve the quantitative and qualitative traits of various crops. Several researchers have made it quite clear that micromutations are significant inducers of genetic variability in mutagen treated population (Swaminathan, *et al*., 1962). In the current study, the quantitative traits including plant height, number of fertile branches, number of capsules/plant, number of seeds/capsule, 1000 seed weight and yield/plant, were taken to assess the extent of genetic variability in both the varieties of linseed. The mean height of the mature plants indicated a reduction with the increase of the mutagen dose. The maximum reduction in average height was recorded in var. IC0096650 than var. Padmini. The reduction in plant height was also observed by Ansari and Siddiqui (1995), Yaqoob and Rashid (2001), Wani *et al.* (2011), Amin *et al.* (2016), Jahan *et al*. (2017), Jahan *et al*., (2019) in different crops upon mutagenic treatments. The yield of linseed can also be affected by various other quantitative characters such as capsule bearing branches, number of capsule/ plant, number of seed/ capsule and 1000 seed weight. The increase in yield attributing traits as observed in this study has also been reported in *Cicer arietinum* (Shaikh *et al*., 1982: Sharma *et al*., 1990), mung bean (Tickoo and Chandra, 1999), *Hordeum vulgare* (Ramesh *et al*., 2001) *Triticum durum* (Sakin and Yildirium, 2004), *Lens culinaris* (Laskar and Khan, 2017), *Linum usitatissimum* (Jahan *et al*., 2019). The declined number of capsule at higher doses of hydrazine hydrate as observed in this study has also been documented by Amer and Farah (1976) and Vandana and Dubey (1988) in *Vicia faba,* Reddy Rao (1982)and Lakshmi *et al*.(1988) *Capsicum annum*, Hasan *et al*. (2018) in *Trigonella foenum-graecum*, Jahan *et al.* (2020) in *Linum usitatissimum* L.

5. Conclusion

The present study was done to study the mutagenic effects of hydrazine hydrate on two varieties of *Linum usitatissimum* L. var. IC0096650 and Padmini**.** Induce mutagenesis provides desired genetic variation and selection of mutants with high yield and yield attributing traits. It was noticed in this study that induction of genetic variability by using HZ in linseed varieties depends upon mutagen concentrations and duration of treatments. The treatment duration 6 hrs was found to be more accurate for breeding purposes intensive on productivity, consider low biological damages by high degree of variation in quantitative traits, while 9 hrs treatments were successful in inducing remarkable morphological variations with higher rate of qualitative traits. It was established that the careful selection of mutagen doses and treatment durations are the key to achieve maximum breeding objectives.Therefore, both the dose and treatment durations were found to be useful for accelerating the development of noble farmer friendly varieties in linseed.

Acknowledgements

The authors are thankful to the Department of Botany, Aligarh Muslim University, Aligarh and NBPGR.

© 2020 Science Letters. All Rights Reserved.

Conflict of Interest: The authors declared no conflict of Interest

6. References

[1] **Amer, S. M. and Farah, O. R. (1976**). Cytological Effects of Pesticides: VIII. Effects of the carbamate pesticides" IPC"," Rogor", and" Duphar" on *Vicia faba. Cytologia*, 41(3): 597-606.

[2] **Amin Laskar, R., Wani, M. R., Raina, A., Amin, R. and Khan, S. (2018).** Morphological characterization of gamma rays induced multipodding mutant (mp) in lentil cultivar Pant L 406. *International journal of radiation biology*. 94(11):1049-1053.

[3] **Amin, R. and Khan, S. (2018).** Studies on The Effects of Gamma Rays on *Nigella sativa* L. In M1 Generation. International Research Journal of Pharmacy. 9(4):62-66.

[4] **Amin, R., Laskar, R. A. and Khan, S. (2015).** Assessment of genetic response and character association for yield and yield components in Lentil (*Lens culinaris* L.) population developed through chemical mutagenesis. *Cogent Food and Agriculture.* 1: 1000715.

[5] **Amin, R., Laskar, R. A., Khursheed, S., Raina, A. and Khan, S. (2016)**. Genetic sensitivity towards MMS mutagenesis assessed through in vitro growth and cytological test in *Nigella sativa* L. *Life Sciences International Research Journal*. 3: 2347-8691.

[6] **Amin, R., Wani, R.M., Raina, A., Khursheed, S. and Khan, S. (2019**). Induced Morphological and Chromosomal Diversity in the Mutagenized Population of Black Cumin (Nigella sativa L.) Using Single and Combination Treatments of Gamma Rays and Ethyl Methane Sulfonate. Jordan Journal of Biological Sciences. 12(1):23-30.

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

[8] **Ansari, M.Y.K. and Siddiqui, S. A. (1995).** Mutagenic effects of EMS on *Ammi majus* L. I. Morphological variations, mutation frequency and yield. *Phytomorphoplogy*. 45:23-29.

[9] **Barshile, J. D., Auti, S. G., Dalve, S. C. and Apparao, B. J. (2006).** Mutagenic sensitivity studies in chickpea employing SA, EMS and gamma rays. *Indian Journal of Pulses Research*. 19(1): 43.

[10] **Bhat, A., Koul, A., Rai, E., Sharma, S., Dhar, M. K. and Bamezai, R. N. K. (2007).** PGC-1α Thr394Thr and Gly482Ser variants are significantly associated with T2DM in two North Indian populations: a replicate case-control study. *Human Genetics*. 121(5): 609-614.

[11] **Cabrera, R. I. (2004).** Evaluating yield and quality of roses with respect to nitrogen fertilization and leaf nitrogen status. XXV International Horticulture Congress. *Acta Horticulturae*. 511.

[12] **Daughtry, C. S. T., Walthall, C. L., Kim, M. S., De Colstoun, E. B. and McMurtrey Iii, J. E. (2000).** Estimating corn leaf chlorophyll concentration from leaf and canopy reflectance. *Remote sensing of Environment*. *74*(2): 229-239.

[13] **Goyal, A.; Sharma, V.; Upadhyay, N.; Gill, S. and Sihag, M. (2014).** Flax and flaxseed oil: an ancient medicine & modern functional food. *Journal of Food Science and Technology*. 51: 1633-1653.

[14] **Haboudane, D., Miller, J. R., Tremblay, N., Zarco-Tejada, P. J. and Dextraze, L. (2002).** Integrated narrow-band vegetation indices for prediction of crop chlorophyll content for application to precision agriculture. *Remote sensing of environment*. 81(2-3): 416-426.

[15] **Hasan, N. Laskar, A. R., Raina, A. and Khan, S. (2018).** Maleic Hydrazide Induced Variability in Fenugreek (*Trigonella Foenum-Graecum* L.) Cultivars CO1 and Rmt-1. *Research & Reviews: Journal of Botanical Sciences.* 7(1): 19-28.

[16] **Jahan, R., Amin, R., Ansari, Bi. S., Malik, S. and Khan, S. (2019).** Sodium Azide Affects The Qualitative and Quantitative Traits Of *Linum usitatissimum* L. (Var. Padmini) In M1 Generation. *International Research Journal of Pharmacy*. 10(12):1-4.

[17] **Jahan, R., Ansari, Bi. S., Malik, S. and Khan, S. (2020).** Cytological Aberrations in M2 Morphological Mutants of *Linum usitatissimum* (L.) Induced By Physical And Chemical Mutagens. *Plant Archives.* 20 (2):1343-1348.

[18] **Jahan, R., Shahwar, D., and Ansari M.Y.K. (2017**). Assessment of morphological and quantitative variability in Linseed induced by caffeine and MMS. 16-9-2017, *Gyan Vigyan Journal of Science*. 1226.

[19] **Khan, S. and Siddiqui, B. A. (1993).** Chlorophyll mutation in *Vigna radiata* (L.) Wilzcek. II. Mutagenic effectiveness and efficiency of chemical mutagens. *Pakistan Journal of Botany*. 25:161-166.

[20] **Khan, S., Parveen, K. and Goyal, S. (2011).** Induced mutations in chickpea-morphological mutants. *Frontiers of Agriculture in China*. *5*(1):35-39.

[21] **Khursheed, S., Laskar, R. A., Raina, A., Amin, R. and 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.

[22] **Khursheed, S., Raina, A. and Khan, S. (2016).** Improvement of yield and mineral content in two cultivars of Vicia faba L. through physical and chemical mutagenesis and their character association analysis. *Archives of Current Research International*. 1-7.

[23] **Kurobane, I., Vining, L. C. and Mcinnes, A. G. (1979).** Biosynthetic relationships among the secalonic acids. *The Journal of antibiotics*. 32(12): 1256-1266.

[24] **Lakshmi, N., Prakash, N. S. and Harini, I. (1988).** Genetics and breeding behaviour of male sterility mutant in *capsicum.* In *Proceedings of National Seminar on Chillies, Ginger and Turmeric*. 28-32.

[25] **Larcher, W. (1995).** Physiological plant ecology. In: Ecophysiology and Stress Physiology of Functional Groups. Springer, New York. 1-528.

[26] **Laskar, A. R. and Khan, S. (2017).** Assessment on induced genetic variability and divergence in the mutagenized lentil populations of

© 2020 Science Letters. All Rights Reserved.

microsperma and macrosperma cultivars developed using physical and chemical mutagenesis. PLOS ONE. 12(9):1-18.

[27] Laskar, R. A., Amin, R. and Khan, S. (2017). Evaluation of optimal doses for gamma rays and hydrazine hydrates in lentil genotypes. *Trends in Bioscience*, *10*, 5822-5825.

[28] Micke, A. (1979). Crop improvement by induced mutations. Use of mutation induction to alter the ontogenic pattern of crop plants. *Gamma Field Symp*. 18: 1-23.

[29] Ramesh B., Prasad, B. K. and Singh, V. P. (2001). Semi-dwarf, high yielding and high protein mutants in barley. *Mutation Breeding Newsletter.* 45: 26-27.

[30] Rana, R. and Swaminathan, M. (1964). Advances in Palynology. *Ed. PKK Nair, National Botanical Gardens, Lucknow, India*. 276-304.

[31] Reddy, S. S. and Rao, G. M. (1982). Cytogenetic Effects of Agricultural Chemicals. *Cytologia*, *47*(2), 257-267.

[32] Sakin, M. A. and Yildirim, A. (2004). Induced mutations for yield and its components in durum wheat (*Triticum durum* Desf.). journal of *Food*, *Agriculture* and *Environment*. 2:285-290.

[33] Shaikh, M. A. Q., Ahmed, Z. U., Majid, M. A. and Wadud, M. A. (1982). A high-yielding and high-protein mutant of chickpea (*Cicer arietinum* L.) derived through mutation breeding. *Environmental and Experimental Botany*. 22(4): 483-489.

[34] Sharma, H. C., Vidyasagar, P. and Leuschner, K. (1990). Components of resistance to the sorghum midge, Contariniasorghicola. *Annals of applied biology*. 116(2): 327-333.

[35] Simopoulos, A.P. (2002). The importance of the ratio of omega-6/ omega-3 essential fatty acids. *Biomedicine and Pharmacotherapy*, 56: 365-379.

[36] Simova-Stoilova, L. J., Stoyanova, Z. and Demirevska-Kepova, K. (2001). Ontogenic changes in leaf pigments, total soluble protein and Rubisco in two barley varieties in relation to yield. *Bulgarian Journal of Plant Physiology.* 27(1-2): 15-24.

[37] Sinha, S. S. N. and Godward, M. B. E. (1972). Radiation studies in *Lens culinaris*. *Indian Journal of Genetics and Plant Breeding.* 32(3):331-339.

[38] Swaminathan, M. S., Chopra, V. L. and Bhaskaran, S. (1962). Chromosome aberrations and the frequency and spectrum of mutations induced by ethyl methanesulphonate in barley and wheat. *Indian Journal of Genetics and Plant Breeding.* 22(3): 192-20

[39] Thomas, H. and Rajhathy, T. (1966). A gene for desynapsis and aneuploidy in tetraploid *Avena*. *Canadian Journal of Genetics and Cytology*. 8(3):506-515.

[40] Tickoo, J. L. and Chandra, N. (1999). Mutagen induced polygenic variability in mung bean (*Vigna radiata wilczek* L.). *The Indian Journal of Genetics and Plant Breeding*. 59(2): 193-201.

[41] Tucker, R. (2004). Primary nutrients and plant growth. In: Essential Plant Nutrients, North Carolina Department of Agriculture.

[42] Vandana, D. and Dubey, D. K. (1988). Effect of ethyl methane sulphonate (EMS) and diethyl sulphonate (DES) on germination, growth, fertility and yield of *Vicia faba* L. *FABIS Newsletter*. 20: 25-30.

[43] Wani, M. R., Khan, S. and Kozgar, M. I. (2011). Induced chlorophyll mutations. I. Mutagenic effectiveness and efficiency of EMS, HZ and SA in mung bean. *Frontiers of Agriculture in China*. 5(4):514-518.

[44] Yaqoob, M., and A. Rashid (2001). Induced mutation studies in some mung bean (*Vigna radiata* (L.) Wilczek) cultivars. *Online Journal of Biological Sciences.* 1(9): 805-808.

© 2020 Science Letters. All Rights Reserved.