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EFFECT OF EMS (ETHYL METHANE SULPHONATE) ON SEED GERMINATION, OVULE FERTILITY AND POLLEN FERTILITY IN DIFFERENT VARIETIES OF *LINUM USITATISSIMUM* L.

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ABSTRACT

In present investigation, effect of four different concentrations of EMS *i.e*, 0.2%, 0.4%, 0.6% and 0.8 % EMS concentration were studied on four different varieties of linseed *i.e*, Himalsi-1, Surbhi, Jeevan and Janki. Four different treatments of EMS i.e, 0.2, 0.4.0.6 and 0.8% were given to the presoaked seeds. Seed germination was found to be decreased with increase in dose of mutagen. 0.2 % EMS showed increase germination percentage in all the 4 varieties of linseed. In seed germination there was an increased reduction when increasing dose of chemical mutagen. While in ovule fertility, all the treatments showed decrease in percentage of ovule fertility and reduction showed an increase in all the treatments with increasing dose of mutagen. In pollen fertility, there was also decrease in the percentage of pollen fertility. The maximum per cent pollen fertility was found at 0.2% EMS concentration in all the 4 varieties of linseed. While, there was an increased reduction in all the 4 varieties of linseed when increasing dose of mutagen. The highest reduction was observed in 0.8% EMS concentration in all 4 varieties of linseed. The treatment was found effective for lower concentrations.

Key words : *Linum usitatissimum*. var. Himalsi-1, Surbhi, Jeevan and Janki, Chemical mutagen, Ethyl methane sulphonate, Seed germination, Ovule and Pollen fertility.

Introduction

Linseed (*Linum usitatissimum* L., 2n=30) is an important oilseed and fibre crop belonging to family *Linaceae*. Linseed is unique among oilseeds as its oil is one of the richest vegetarian sources of linolenic (omega-3) and linoleic (omega-6) polyunsaturated fatty acid (PUFA) which offer potential health benefits since they are not synthesized in the organism and must be ingested through food. Its seeds contain about 33 to 45% of oil, which is used in human and animal diets and for various industrial uses. Linseed oil helps in decreasing cholesterol level (Singh and Marker, 2006), preventing cancer especially breast, prostate and other hormone sensitive cancers when included in the diet chain. Linseed occupies an area of 32.23 lakh ha yielding 30.68 lakh tonnes with an average productivity of 952 kg/ha in the world, whereas in India, it occupies an area of 1.7 lakh ha with a production and productivity of about 1 lakh tonnes and 574 kg/ha, respectively. Linseed production volume in India is estimated to produce nearly 113 thousand metric tons of linseed. (FAO, 2024). For the genetic improvement of linseed, conventional breeding methods have been the basic tools for releasing new cultivars with durable resistance to diseases, agronomic fitness and greater yield stability (Green *et al*., 2008). However, the narrow genetic base used for the development of linseed cultivars (Fu *et al*., 2002; Cloutier *et al*., 2009), the scarce availability of related species to incorporate new variation, the lack of hybrid production systems (Green *et al*., 2008) and the limited genomic tools for molecular breeding (Cloutier *et al*., 2011). Development of high yielding varieties requires the knowledge of existing genetic variability, but due to selective breeding over the generation in linseed, conventional breeding has limited scope. Thus, mutation breeding is an alternative effective tool for creation of variability. The physical and chemical mutagen has been employed successfully in genetic improvement of many crops *i.e.* linseed (Green, 1986; Green and Marshall, 1984; Deka, 2016; Jahan *et al*., 2019), Sesame (Kumari *et al*., 2016), Mustard (Julia *et al*., 2018), Cowpea (Gririja and Dhanavel, 2013; Bind *et al*., 2016), peas (Dhulgande *et al*., 2011). This mutagen provides a powerful tool for creation of variation in crop plant for both qualitative and quantitative traits (Das and Misra, 2005; Baisakh *et al*., 2011). EMS is the most commonly used mutagen which are known to influence the plant growth and development by inducing cytological, genetical, biochemical and morphological changes in plants (Gunkel and Sparrow, 1961; Das and Prusti, 2020). However, after continuous work done over the generation, it was observed that only a few mutagenic treatments have been effective for inducing high frequency of mutation. A highly effective mutagen may not necessarily show high efficiency and vice versa. Therefore, selection of effective and efficient mutagen is very essential to recover high frequency of desirable mutations in any mutation breeding studies (Usharani and Kumar, 2015). Hence, the study was undertaken to assess the effect of different doses of EMS on seed germination, pollen fertility and ovule fertility.

Materials and Methods

Genetically pure seeds of *Linum usitatissimum* L. var. Himlasi-1, Surbhi, Jeevan and Janki were treated with chemical mutagen *i.e*, EMS (Ethyl methane sulphonate). Seeds were treated with EMS concentrations of 0.2,0.4,0.6 and 0.8% (v/v) periods for 18 Hrs. All the treatments were carried out at 25+0.5. After mutagen treatment seeds were rinsed for 30 min with running tap water to remove the mutagen and post soaked for 1Hr. in distilled water and then seeds were kept in seed germinator.

Seed germination percentage

100 seeds of each dose along with control were kept in petri-dishes on blotting paper. The emergence of radical was recorded as indication for germination of seeds. Seeds were considered germinated when the radicle was at least 4mm long. Germination percentage was calculated after three and seven days, by counting the germinated seeds and total number of seeds sown. Further percent over control and reduction over control was also calculated.

$$\text{Seed Germination percentage} = \frac{\text{Number of seeds germination}}{\text{Total number of seeds}} \times 100$$

Ovule fertility

As expressed in percentage of control, ovule fertility was measured in terms of seed setting per capsule. Marked variations in number of seeds per capsules were observed in treatment of EMS. With increase in dose of EMS, decrease in ovule fertility was observed in Him Alsi-1, Surbhi, Jeevan and Janki.

Pollen fertility

100 seeds were sown in field for each treatment at experimental field of School of Agriculture, Abhilashi University, Chailchowk. After first flower opening from main branch, buds form 3rd to 9th were used for pollen fertility study. At initial stage of flowering, flower buds were collected from 20 plants at random. Anthers of each flower were tested with the help of needle on the slide, stained with 1% Aceto-carmine stain and observed under compound microscope.

$$\text{Pollen fertility} = \frac{\text{Number of viable pollens}}{\text{Total number of pollens}} \times 100$$

Results

Results of present investigation indicated that 18 Hrs treatment significantly influenced seed germination, ovule fertility and pollen fertility have been tabulated in Tables 1 and 2, respectively (Plate 1).

Seed germination

A decrease in seed germination was observed with increase in mutagen dose. The percent germination and survival was recorded on 3rd and 7th DAT respectively and the results indicated that the germination and survival decreased with increase in the concentration of the mutagen i.e EMS (Thakur and Paul, 2020). The results indicated that the average percentage germination and survival were reduced with increasing concentration of mutagen (EMS) in lab condition (Kumar and Paul, 2021). Seed germination is the initial stage of a plant’s life. It is a three-phase process. The first phase is imbibition. Dormant seeds take up water, and the hydrolysis process takes place. Seed imbibition results from the interaction of proteins, carbohydrates, lipids and variations in their contents can affect the process. Protein and oil bodies are the primary reserves in oilseed crops that provide energy, carbon and nitrogen to seedlings during their establishment (A.S. *et al*., 2017). The second phase is the regulation of germination, characterized by the

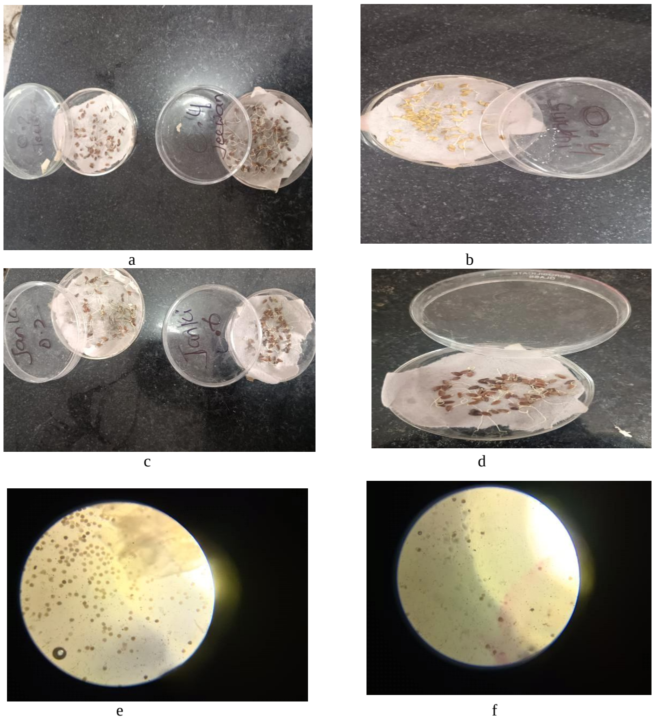

Plate 1 : Micrograph showing chemical mutagen treatment, seed germination and pollen fertility.

activation of ATP synthesis in glycolysis, the Krebs cycle, respiratory chain, and the translation of stored mRNA. However, the third phase represents the completion of germination, when the radicle protrudes from the seed coat and forms a root, and the plumules form a shoot system capable of utilizing inorganic matter, water and light energy for healthy growth (Nonogaki *et al*., 2010). Germination is a complicated process from a physiological standpoint, involving multiple signals and it is influenced by both intrinsic and extrinsic factors (Miransari and Smith, 2014). Intrinsic factors include seed dormancy and available food stores, and extrinsic factors include water, temperature, oxygen, light and relative humidity. The visible sign that germination is complete is usually the penetration of the structures surrounding the embryo by the radicle, the result is often called visible germination. The results indicated that the average percentage germination and survival were reduced with increasing concentration of mutagen (EMS) in lab condition (Kumar and Paul, 2021). Several workers reported decrease in seed germination with increasing concentrations such as Sharma *et al*. (2013) in citrus Jambhiri seeds and epicotyl, Dhulgande *et al*. (2015) in pea, Sandhiya *et al*. (2020) in white seeded sesame, Omuson *et al*. (2021) in two okra varieties, Kumar *et al*. (2023) in Mungbean, Sowmya *et al*. (2023) in field bean, Dhange *et al*. (2024) in stevia, Meng *et al*. (2024) in marigold seeds, Modi *et al*. (2024) in *Brassica campestris* stated that the decrease in seed germination was mainly due to the interference of mutagens with metabolic activities of the seeds. Disturbance in the formation of enzymes involved in the germination process may be one of the physiological effects caused by mutagenic treatment particularly chemical mutagen like EMS leading to decrease in germination (Kulkarni, 2011).

Ovule fertility

As expressed in percentage of control, ovule fertility was measured in terms of seed setting per capsule Marked variations in number of seeds per capsules were observed in treatment with EMS. With increase in EMS, decrease in ovule fertility was observed in Him Alsi-1, Surbhi, Jeevan and Janki. Data pertaining to percent reduction over control in pollen and ovule fertility showed that there was inverse relation with the dose of EMS with the ovule fertility which proves that with increase in concentration of mutagens, the ovule fertility got reduced drastically. Also it was observed that different

Table 1 : Effect of different doses of EMS on seed germination and plant survival percentage (Lab condition).

Treatments	Seed germination (%)	Percent reduction over control	Plant Survival	Percent reduction over control
Himalsi-1				
0.2	79	20.20	91.75	6.37
0.4	61	38.38	87.50	10.71
0.6	49	50.50	79	19.38
0.8	30	69.69	63	35.71
Control	99		98	
Surbhi				
0.2	80	18.36	67.52	29.66
0.4	71	27.55	65.83	31.42
0.6	11	88.77	10	89.58
0.8	5	94.89	2	97.91
Control	98		96	
Jeevan				
0.2	87	8.42	86.66	8.77
0.4	71	25.26	84.16	11.41
0.6	67	29.47	80	15.78
0.8	49	48.42	75.83	20.17
Control	95		95	
Janki				
0.2	86	10.41	91.66	0.36
0.4	82	14.58	88.33	3.98
0.6	71	26.04	85	7.60
0.8	50	47.91	74.16	19.39
Control	96		92	

Table 2 : Effect of different doses of EMS on pollen and ovule fertility.

Treatments	% Pollen fertility	% Reduction	% Ovule fertility	% Reduction
Himalsi-1				
0.2	91.30	8.7	90.71	9.29
0.4	88.69	11.31	89.28	10.72
0.6	86.08	13.92	87.85	12.15
0.8	79.13	20.87	84.28	15.72
Control	100		100	
Surbhi				
0.2	5.26-6.60	10.91	77.60	2.16
0.4	3.56-5.55	25.43	58.60	26.56
Control	97		79.80	
Jeevan				
0.2	82.60	13.64	81.42	16.18
0.4	80.86	15.46	78.57	19.11
0.6	75.65	20.90	76.42	21.33
0.8	72.17	24.54	74.28	23.53
Control	95.65		97.14	
Janki				
0.2	87.21	11.73	86.42	10.37
0.4	83.47	15.51	84.28	12.59
0.6	76.52	22.55	82.85	14.07
0.8	74.56	24.53	79.28	17.77
Control	98.80		96.42	

concentrations have their own biological effects, and effect varies with genotypes based upon the physiological and genetic properties of seeds of cultivars.

Pollen fertility

In case of pollen fertility, 18 Hrs treatment showed reduction in pollen fertility with an increase in mutagen doses. The pollen fertility was seen maximum in 0.2% EMS concentration in all the varieties of linseed and the percentage reduction was seen maximum in 0.8% EMS concentration. The increasing pollen sterility has been mainly attributed to chromosomal interchange, chromosomal aberration, gene mutation (Gautam *et al.*, 1992), cytoplasmic factors (Malinoveskii *et al.*, 1973). The negative effect of mutagens on pollen fertility percentage in mutagenic treatment plants may be due to meiotic aberrations that were induced by mutagens leading to the formation of aberrant pollen grains (Rani and Kumar, 2016). Effect of mutagens on pollen fertility in different plants were reported by different scientists- Devmani and Dwivedi (2014) in cowpea, Monica and Seetharaman (2015) in garden bean, Thakur and Paul (2020) in linseed, Akilan *et al*. (2019) in rice, Gawande *et al*. (2022) in safflower, Shukla (2017) in mustard, Sinha (2017) in Indian squill, Kumar and Paul (2021), Malik *et al*. (2021) in ajwain, Kumar *et al*. (2022), Kumar *et al*. (2023) in mungbean, Hasan *et al*. (2025) in *Capsicum annum* L. var. NS1101. Increase in pollen sterility in mutant plants may be interpreted as that the mutagenic efficiencies that have been observed in the form of reduced or increased germination and growth parameterss has been carried over through subsequent cell divisions from seed germination up to the flowering of plant. It affects normal pollen development from pollen mother cells and ultimately increases pollen sterility.

Conclusion

The treatments for lower concentrations were found to be effective while higher concentrations showed inhibitory effect on seed germination, ovule fertility and pollen fertility. Drastic reduction in all parameters were found in EMS mutant. Over control. The stimulatory effect at lower dose is due to to the fact that mutagens at lower concentrations stimulate the role of enzyme and growth hormone responsible for growth and yield while the inhibitory effect is due to the fact that biological damage increased at a faster rate in higher concentrations of mutagens (Selvaraj *et al.*, 2019). This study proves that the importance of mutation treatment in crop improvement as suggested by Malode (1995) that the ultimate source of all heritable variations to select from are mutations. Further investigation is required to determine the effects of other variables viz. cytological studies, agronomic growth parameters and biochemical parameters is in progress.

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