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CYTOLOGICAL EFFECT OF ETHYL METHANE SULPHONATE AND SODIUM AZIDE IN *LINUM USITATISSIMUM* L.

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ABSTRACT : The present investigation provides a comparative account of cytological and developmental effects on EMS (Ethyl Methane Sulphonate) and SA (Sodium Azide) on meiotic features. Chemical mutagens have become important tools in crop improvement. These mutagens are being used to produce resistance in various susceptible crops to improve their yield and quality traits against harmful pathogens. There are several mutagens available for crop improvement and each mutagens has its important role as positive or negative effect on crops. Studies undertaken in M1 generation on the NDL-2002 variety of *Linum usitatissimum* L., showed that both the mutagens EMS and SA elicit various chromosomal aberrations in meiosis. Different types of meiotic abnormalities such as laggard, 3-nucleate conditions, unsynchronized movement at metaphase I and bridges at anaphase I were recorded. In general, the meiotic abnormalities increased along with the increase in concentration in mutagens. However, the induction of meiotic aberrations was observed to be higher in EMS and SA treatments, suggesting that EMS could be more effective in inducing additional variability than SA, in *Linum usitatissimum* L. var. NDL-2002.

Key Words: *Linum usitatissimum*, EMS, SA, Meiotic aberrations, M1 generation

INTRODUCTION

Mutations can be beneficially utilized for tailoring better varieties of crop plants. But in general, chemical mutagens like EMS affects a wide range of chromosomal aberrations resulting into abnormal behavior. Cytological analysis with respect to meiotic behavior is considered to be one of the most dependable index to estimate the potency of mutagens. Many researchers have mutagens on different crops. While many researchers like Rao and Rao (1983), Kumar and Dubey (1998), Dhanayanth and Reddy (2000) and Bhat *et al.,* (2005) found chemical mutagens to be more effective than physical ones, others like Tarar and Dnyansagar (1980), Zeerak (1991) and Singh (2003) found the reverse case. Induced mutagenesis has rarely been used to increase genetic variability in flax breeding (Gill, 1987). Chemical mutagens provide a good scope for selection, as a tool for alteration in the genotype to enhance the variability of characters. In the present investigation two potent mutagens EMS and SA were used to treat *Linum usitatissimum* L. var. NDL-2002.

Mutation may take place in genetic information causing a cell or living creature to be different from the other. Mutations are the tools used to study the nature and function of genes which are the building blocks and basis of plant growth and development, thereby producing raw materials for genetic improvement of economic crops (Adamee *et al*., 2007). Induced mutation have great potentials and serve as a complimentary approach in genetic improvement of crops (Mahandjiev *et al*., 2001). Chemical mutagens are the one cause of mutations in living organism. It is known that various chemicals have positive or negative effects on living organisms. Many of these chemicals have clastogenic (chromosomal damaging) effects on plants through reactive oxygen-derived radicals (Ricardo *et al*., 1998).

A number of workers have reported the role of chemical mutagens in enhancing genetic variability in higher plants (Coe *et al*., 1977; Mashenkov *et al*., 1986 and Ricardo *et al*., 1998). Sodium Azide is a mutagen and has been one of the most powerful mutagens in crop plants. It is known to be highly mutagenic in several organisms, including plants and animals (Rines *et al*., 1985; Veleminsky *et al*., 1987; Owais *et al*., 1988; Raicu *et al*., 1992 and Grant *et al*., 1994) and its mutagenic potential has been reported in several screening assays. The promutagen NaN_3 is highly used with seeds to create mutation, which must be metabolized by plant cells to the mutagenic agent, presumably azideoalanine (Owais *et al*., 1983). To effect of chemical mutagens depends on the permeability of seed coat and nature of the mutagens.the present study has been undertaken to assess the effect of EMS and SA in *Linum usitatissimum* L. var. NDL-2002.

MATERIALS AND METHODS

Healthy and dried seeds of *Linum usitatissimum* L. var. NDL-2002 was obtained from Acharya Narendradev University of Agriculture and Technology, Kumarganj, Faizabad, U.P. were subjected to 24 hrs treatment by five different concentrations (0.02, 0.04, 0.06 0.08 0.10%) of EMS and SA after presoaking of 12 hrs. The treated seeds were thoroughly washed in running tap water for half an hour to remove the residual effects of mutagen sticking to the seed coat. One set of seeds was kept untreated to act as control for comparison. All sets of seeds (including control), containing 50 seeds in each set, were sown in pots with 10 seeds in each pot to raise M1 generation. For meiotic studies young flower buds 20-3- randomly selected M1 plants were fixed in freshly prepared carnoy's fixative (Absolute alcohol, Chloroform and Acetic acid in 6:3:1 ratio) for 24 hrs, washed and preserved in 70% alcohol. Anthers were squashed in 2% aceto-carmine, dehydrated in NBA series (50% acetic acid+ 50% N-butyl alcohol then passed through 100% N-butyl alcohol), mounted in Canada balsom and dried at 45^0C. Microphotographs were taken from freshly prepared slides using X30 olympus research photomicroscope.

Table-1: Percent frequency due to different concentrations of EMS

Meiotic stages	Characters	Control	Total no. of cells	% Frequency				
				0.02	0.04	0.06	0.08	0.10
Prophase I (Diakinesis)	-	-	150	-	-	-	-	-
Metaphase I	Unsynchronized movement	-	150	8 (5.33)	-	-	-	-
Anaphase I	Bridges	-	150	-	-	11 (7.33)	15 (10)	18 (12)
Telophase I	Laggards	-	150	-	-	9 (6)	14 (9.33)	16 (10.66)
Metaphase II	-	-	156	-	-	-	-	-
Anaphase II	-	-	160	-	-	-	-	-
Telophase II	-	-	158	-	-	-	-	-

Table-2: Percent frequency due to different concentrations of SA

Meiotic stages	Characters	Control	Total no. of cells	% Frequency				
				0.02	0.04	0.06	0.08	0.10
Prophase I	-	-	150	-	-	-	-	-
Metaphase I	Unsynchronized movement	-	150	4 (2.66)	-	-	-	-
Anaphase I	Pro-anaphase	-	150	-	-	4(2.66)	8(5.33)	12(8)
Telophase I	3-nucleate	-	150	-	12(8)	18(12)	19(12.66)	19(12.66)
Metaphase II	-	-	150	-	-	-	-	-
Anaphase II	-	-	150	-	-	-	-	-
Telophase II	-	-	150	-	-	-	-	-

RESULTS

Meiosis was perfectly normal at Prophase I (Diakinesis) and Anaphase II (Fig 1 & 2) which segregated into 6 bivalents and 9 univalents (fig 1). However, a number of meiotic aberrations were recorded in plants raised from seeds treated with different concentrations of the mutagens. The most frequent aberrations were bridges and unsynchronized movement at Anaphase I (Fig 8) and Metaphase I (fig 5 & 6). A dose dependent increase in meiotic aberrations was observed with all mutagenic treatments in both the mutagens. The maximum aberrations were found at higher doses of the mutagens (Table 2). Although most of abnormalities Laggards (Fig 3) and 3-nucleate condition (Fig 4) were dominant at Telophase I. Bridges were found without fragments. Most of the cells with single bridges were observed at Anaphase I. unsynchronized movement was observed in 0.02% concentration of EMS and SA at Metaphase I with 5.33% and 2.66% frequency. Same pattern were also followed in the other concentrations of EMS and SA. EMS 0.06-0.10% concentration showed increasing value of Bridges aberration at Anaphase I with the increased frequency from 7.33-12% respectively.

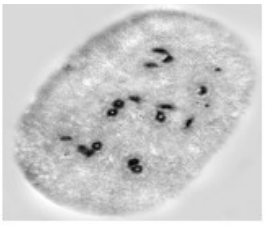
Fig: 1

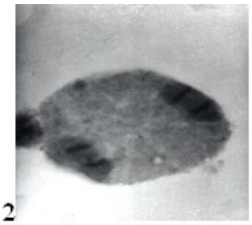
Fig: 2

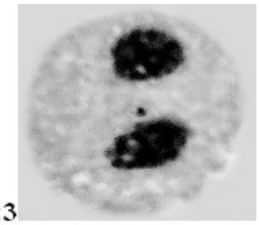
Fig: 3

Fig: 1-Shows Prophase I (diakinesis) of control, Fig: 2-Shows Anaphase II of control
Fig: 3-Shows Laggards at Telophase I

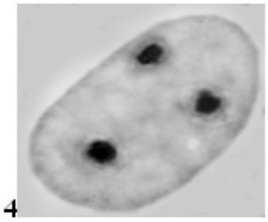
Fig: 4

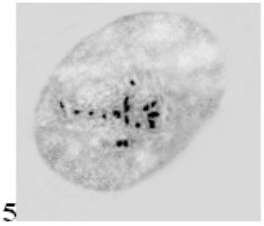
Fig: 5

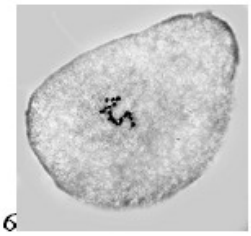
Fig: 6

Fig: 4-Shows 3-nucleate conditions at Telophase I, Fig: 5 & 6-Unsynchronized movement at Metaphase I,

At Anaphase I of SA different concentration showed the minimum abberation of Pro-Anaphase (Fig 7) 2.66-8% as compared to EMS. At the Telophase I of EMS showed Laggard aberration which increased on increasing the concentrations from 0.06-0.10% as increasing pattern of percent frequency from 6-10.66%. The Telophase I stage showed the another type of abnormality 3-nucleate condition in which the plant were treated with different concentrations from 0.04-0.10% of SA, showed the increasing order of percent frequency from 8-12.66%.

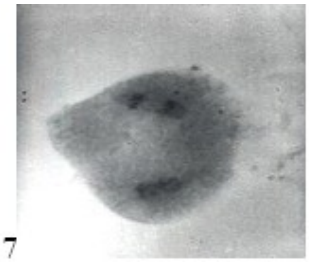

Fig: 7

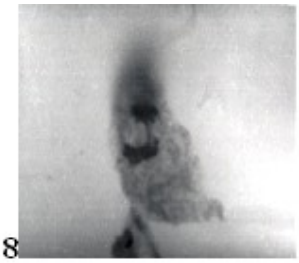

Fig:8

Fig: 7-Shows Pro-Anaphase I condition, Fig: 8-Shows Bridges at Anaphase I

DISCUSSION

In the present investigation the mutagenic treatments exhibited similar types of meiotic abnormalities but the percent abnormalities was different in different treatments. This showed that these mutagens have different mutagenic potential for *Linum usitatissimum* L. Meiotic abnormalities increased along with the increasing concentrations of EMS and SA.

The major abnormalities at Anaphase I was bridges and laggards at Telophase I of EMS. Similar results were also observed earlier by different workers Bhat *et al.* (2006b) and Anis and Wani (1997). Anaphasic bridge formation was attributed to interlocking of bivalents chromosomes (Bhattracharjee, 1953), failure of chiasmata in a bivalent to terminalize (Saylor and Smith, 1966); unequal exchange or dicentric chromosomes etc. Different types of chromosomal abnormalities observed during the present investigation as laggard, bridges, pro-anaphase, 3-nucleate condition and unsynchronised movement have been reported by various workers in different plant materials after treated with physical and chemical mutagens (Ahmed, 1993; Kumar and Dubey, 1998; Dhamayanthi and Reddy 2000; Bhat *et al*., 2005). The varietal sensitivity to mutagenic treatment have also reported by some workers (Evans and Sparrow, 1961; Akbar *et al.,* 1976; Ahmed and Godward, 1981; Bhat *et al*., 2006a). anaphasic bridges may be formed due to unequal exchange or dicentric chromosomes. Sax (1960) suggested that the formation of bridges might be due to the failure of chiasmata in a bivalent to terminalise and chromosomes get stretched between the poles. The laggards observed might be due to the delayed terminalization stickiness of chromosomal ends or because of failure of the chromosomal movement (Jayabalan Rao, 1987; Soheir et al., 1989).

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