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Impacts of EMS on Cytological and Palynological behavior in *Dolichos lablab* L.

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ABSTRACT
*To estimate the toxicity level of mutagen on the plants, it is mandatory to examine the mutagenic sensitivity of any mutagen on the palynological parameters alongwith meiotic irregularities, as these parameters are critical factors in plant's productivity.So, the present study evaluate scyto-palynological impacts of chemical mutagen on the test plant material, Dolichos lablab L.(plant family; Fabaceae, with diploid chromosome number 2n=22).Here, Ethyl Methane Sulphonate (EMS) was used for the mutagenic action.The inbred seeds of D.lablab L.were immersed in different concentrations of EMS viz; (0.1%),(0.3%) and (0.5%) alongwith control.These seeds were sown in triplicates.The young flower buds of appropriate size were fixed in Carnoy's fixative for screening of the meiocytes.Different types of meiotic anomalies such as stickiness, precocious movement, laggard, unorientation etc were encountered in the EMS treated sets.In vitro pollen germination was carried out by providing nutrition medium of sucrose solution. But, the total abnormality percentage was increased from control to EMS treated sets.The maximum effects were observed at **(0.5%)** concentration of EMS.On the basis of experimental findings it has been concluded that the dose (0.5%) of EMS has higher mutagenic potentialas compared to (0.1%) and (0.3%).*
Keywords: *Ethyl Methane Sulphonate, Meiotic abnormalities, In vitro Pollen germination, Pollen tube growth, Dolichos lablab L.*

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INTRODUCTION

In recent era, mutation breeding established a landmark position in the area of agriculture by providing us desirable traits.But, the traits are not always found in terms of benefit, it could also impart harmful effects, when the doses are higher than the critical level. The chemical mutagen such as EMS (Ethyl Methane Sulphonate) induces the meiotic abnormalities due to its involvement in transition mutation among the nitrogen bases. It brings alkylation of guanine bases and leads to mispairing-alkylated G pairs with T instead of C, resulting in primarily G/C- to-A/T transitions[1].Incorporation of alkyl group into a base may result in the formation of a gap in the DNA template [2,3]and subsequent replication defects leading to mutations. These mutations are responsible to the generations of the various harmful effects inside the plants.Numerous researches have documented the deleterious mutagenic effects of EMS on the cytological and palynological parameters.It has been reported that this mutagen firstly act on chromosomes and results in various chromosomal anomalies, which adversely effects the palynological parameters of the plants.The alteration in percentage range of these parameters depends on the optimum dose of this mutagen.

Sexual incompatibility between male and female flowers during pollination is also a kind of harmful effects of mutagens.This adverse effect is closely related to irregularities induced in palynological parameters (Pollen germination and pollen tube growth). These effects ultimately confirm a major disadvantage for seed production in the plants. Studies on *in vitro* pollen germination and pollen tube growth are very useful for explaining the lack of fertility [4].

Therefore, the present study has been focused on the mutagenic sensitivity of highly potent mutagen i.e. EMS (Ethyl Methane Sulphonate,) on Meiotic disturbances and *in vitro* Pollen biology of *Dolichos lablab*L.(Fabaceae; Total Diploid genome(2n=22). This plant is considered as a multipurpose crop since it

is used for food, forage, soil improvement, weed control and soil protection [5]. The palynological studies was assessed on the basis of three criteria (1) *In vitro* pollen germination (2) Pollen tube growth.

MATERIALS AND METHODS

Study species

The plantations established from the seeds of *Dolichos lablab* L.,which were obtained from Indian Institute of Horticulture Research(IIHR), Bengaluru. Seeds were surface sterilized, followed by pre-soaking in distilled water.

EMS treatment

These seeds were then immersed in different concentrations of EMS viz. (0.1%),(0.3%) and (0.5%) (v/v) which were prepared in phosphate buffer solution**.**Some seeds were soaked in only distilled water which served for control set. After 3 hrs, both the treated and control seeds were sown in experimental plots in triplicates, at the Roxburgh Botanical Garden, University of Allahabad.

Cytological analysis

The plants were monitored carefully and young flower buds of appropriate size were fixed in Carnoy's fixative (3.1;Alcohol:Glacial Acetic Acid) for 24 hrs. Next day, these buds were transferred in absolute alcohol and preserved at 4^{o}C. To study meiotic cell division, anthers were teased and squash was prepared in 2% acetocarmine stain. Slides were microphotographed using Nikon microscope. Simultaneously, the chiasma per bivalent were also calculated in Metaphase I in the microsporogenic cells.

Palynological studies

In vitro pollen germination and pollen tube growth

For monitoring pollen germination, flowers were randomly selected from all the 3 treatment concentrations in the morning alongwith control sets**.** Flowers were air dried and fresh pollen grains were then dusted onto cavity slides containing *in vitro* germination medium consisting of boric acid (0.01 g/l), calcium nitrate (0.03 g/l), magnesium sulphate (0.02 g/l), potassium nitrate (0.01 g/l) and sucrose (15%) in distilled water. The medium alongwith pollen grains was then incubated at 25°C.After 4 h. of inoculation, the germinated pollen grains were then fixed by putting several drops of acetic alcohol **(**1**:**3**)** solution**.** A drop of 2% acetocarmine was added to germinated pollen grains for staining and the slides were observed under microscope[6].Pollen tube length was measured using Dewinter optical microscope.

Statistical Analysis

All the data obtained from the present study were analyzed by the use of SPSS 16.0 software. There were three replicates for each treatment and one independent variable was used. A one way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT, $P < 0.05$) were performed for mean separation and the graph was plotted by using sigma plot 10.0 software. Actual mean and standard error were calculated.

RESULTS

The cytological observations of all meiotic phases are shown subsequently in Fig-1(a-i) where (1c) to (1i) are outcome of abnormal meiosis.Meiosis with normal Diakinesis and normal Anaphase I (Fig-1a and 1b respectively) was observed in plants of control sets during microsporogenesis.However in EMS treated sets, different kinds of meiotic irregularities were recorded.Among these meiotic chromosomal irregularities such as stickiness, precocious movement and unorientation of chromosomes at MetaphaseI/II, laggard and forward movement at AnaphaseI/II were found frequently. Cytological Bridge formation at AnaphaseI/II were also observed but its percentage was less. The Percentages of all meiotic irregularities in EMS treatment have been summarized in (Table-1).The frequency of meiotic chromosomal irregularities was increased (7.93 to 23.48% to 17.63±0.04%) from control to increasing concentration of EMS.

The pollen germination was also found to be normal in control sets. In EMS treatment, these facets were affected in dose dependent manner (as mentioned in Table-2). The average decreased in percentage of pollen germination (85.55±0.98% to 30.86±0.49%) from control to higher concentration of EMS. Moreover, the length of pollen tube was also greater (47.60±0.98)µm in control sets as compared to treated sets which was reached to (3.49±0.05) µm at 0.5% conc. of EMS. At the higher concentration of EMS, most of the pollen showed reduced tube growth (Fig-2f and 2g) and at few instances, pollen cytoplasm oozed out without forming pollen tube (Fig-2h). The concentration (0.3%) and (0.5%) also showed pollen with twisted tube formation (Fig-2i) instead of straight tube. Some abnormal pollens with

2 germ pores (Fig-2e) were also evidenced, which was in contrast to the control pollens that possess 3 germ pores (Fig-2a).

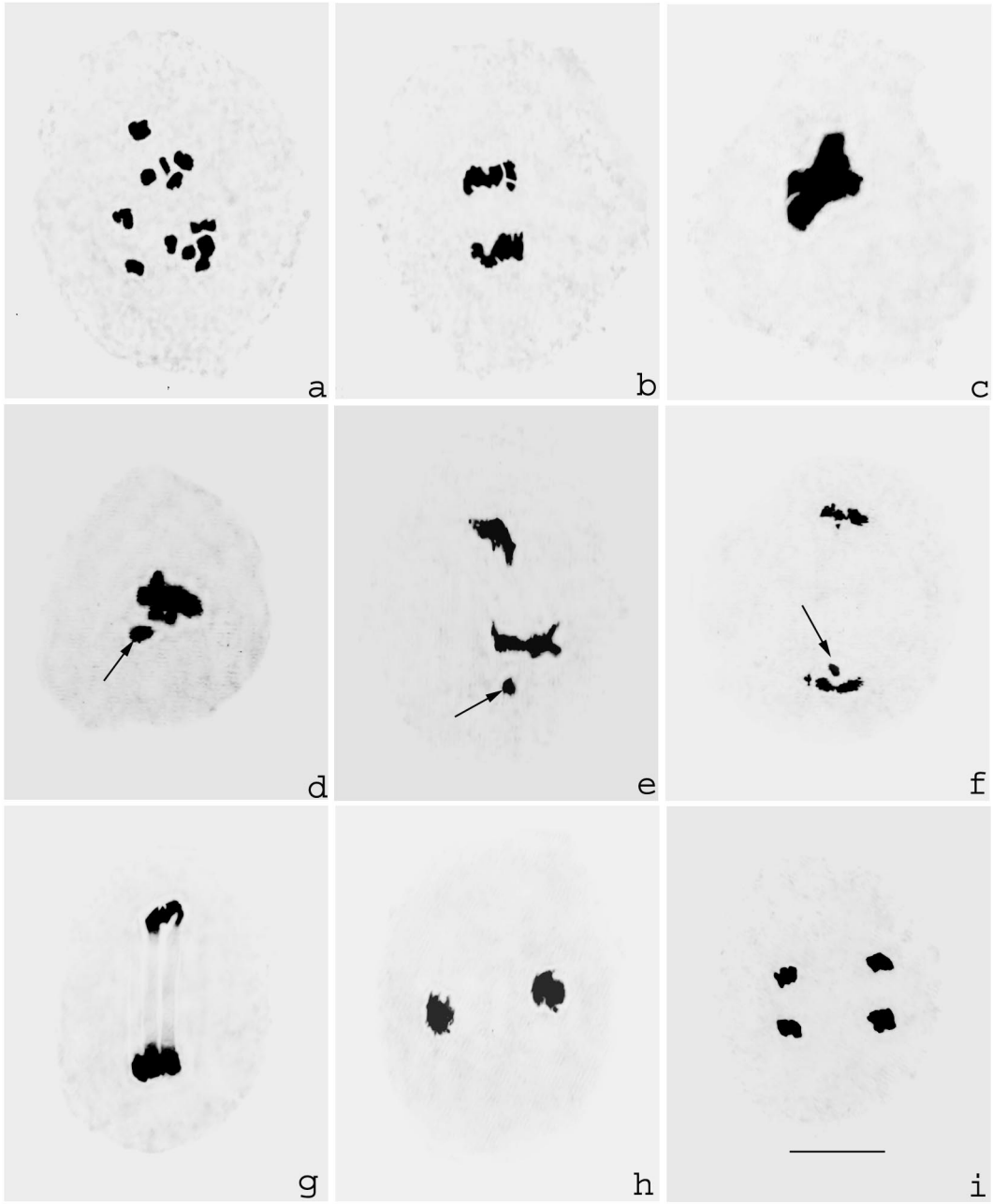

Figure1. Representative microphotograph of PMC with meiotic irregularities prompted by EMS in *D.lablab*L.**a-**Normal Diakinesis, **b** Normal AnaphaseI, **c**.Stickiness at MetaphaseI, **d**-one precocious chromospme at Metaphase I, **e**-One forward chromosomes with unoriented Anaphase I, **f**-Laggard chromosome at Anaphase I, **g**-Sticky chromosomes at Anaphase I, **h**-Stickiness at Metaphase II, **i-**Stickiness at Anaphase II,**(Scale bar-**22.654µm**).**

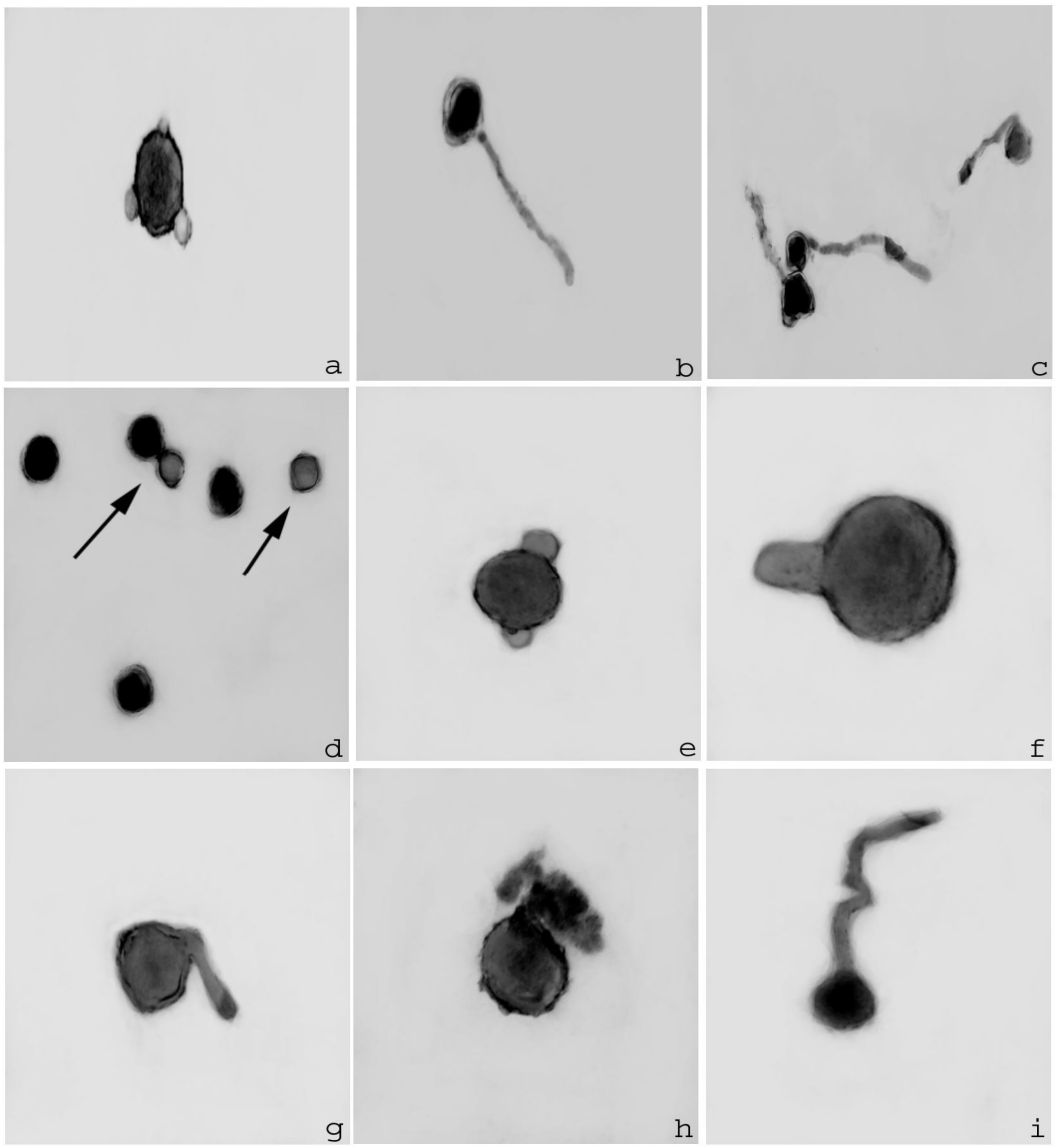

Figure 2. Representation of Pollen behavior effected by EMS in *D.lablab* L. **a-3** Pollen germ pores in the control set, **b and c-**Normal pollen tube formation, **d**- fertile pollen grains stained with acetocarmine (arrowheads represent sterile pollengrains), **e-**Abnormal pollen with only 2 germ pores, **f and g-** Abnormal pollen tube with reduced length, **h**-.Pollen protoplasm oozing out without forming pollen tube, **i**- Twisted pollen tube.

DISCUSSION

Studies focusing mutagenic sensitivity on the meiotic chromosomal irregularities and palynological parameters of *D.lablab* L.are very scarce.In present study, different types of meiotic abnormalities were induced by EMS, leading to abnormal cyto-palynological behaviors in *D.lablab* L. According to [7]the main reason behind the existence of this abnormal cyto-palynological behaviors could be as, EMS influences a very short segment of chromosome that carries one or several genes, and can affect the cytological, genetic and palynological traits of plant tissues and cells.

For the existing meiotic abnormalities, there are many views. [8]suggested that sticky chromosomes reflect highly toxic effects, usually of an irreversible type, and probably lead to cell death. Stickiness (a chromatid type aberration) has been attributed to depolymerization of chromosomal DNA[9].The observed precocious movement of chromosome arose due to the deviation of one chromosome from its native equatorial plate.[10]considered the anaphase bridges as obtained from structural changes of deficiency, some of them surviving to late telophase, indicative of their stability. A laggard was defined as a chromosome that did not overlap along axis of the spindle with any of the properly segregating

chromosomes[11].A considerable decrease in chiasma frequency showed the prominent effect of EMS on chromosomes.

Kumar and Rai (2006)[6]stated that a decreased in pollen germination can be attributed to abnormal meiosis forming abnormal or unequal gametes.They also supported that the structure and the physiology of the pollen grains is also under the genetic control and irregular or abnormal meiosis may cause significant changes in the pollen properties. Shivanna and Johri [12], Ahmed *et al*.[13] suggested that the tapetum supplies nutrients necessary for pollen development and provides the precursors of exine formation.Hence, any disturbances occurred in normal functioning of tapetum, resulted in poor pollen germination.The growth of pollen tube depends on the amount of amino acids and sugars stored in pollen grains during microsporogenesis [14,15].On treatment with mutagen, the balance between amino acids and sugars could be fluctuated and this effected the normal growth of the pollen tube.Reductions in percentage germination and inhibition of pollen tube growth caused by higher doses of mutagen might decrease the effectiveness of pollination and fertilization and may consequently change the quantity and quality of seed [16,17].

Table 1. Estimation of percentage of Meiotic Irregularities induced by EMS in Pollen mother cells of *D.lablab* L.

Concen. (%)	NO of PMCs	Metaphasic abnormalities (Mean±S.E.)							Anaphasic abnormalities (Mean±S.E.)					Telophasic abnormalities (Mean±S.E.)		Oth	Tab %
		St	Un	Pr	Sc	Mv	SA	Asyn	St	Un	Lg	Fw	Br	Dp	Mn		
		EMS (3h)															
Control	409	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1%	417	0.52±0.04	0.75±0.03	0.43±0.04	0.22±0.015	0.26±0.02	0.21±0.02	0.42±0.06	0.55±0.07	0.78±0.068	1.29±0.06	1.24±0.06	0.29±0.05	0.42±0.05	0.22±0.01	0.34±0.07	7.93±0.05
0.3%	421	0.55±0.07	1.34±0.04	0.90±0.05	0.71±0.04	0.34±0.081	0.34±0.08	0.93±0.04	0.54±0.07	1.23±0.03	2.33±0.07	2.13±0.03	0.42±0.05	0.52±0.04	0.53±0.04	0.25±0.14	13.10±0.15
0.5%	388	1.15±0.05	1.74±0.07	1.56±0.04	0.93±0.03	1.08±0.09	0.85±0.04	1.15±0.05	1.17±0.05	1.61±0.04	4.67±0.09	3.76±0.17	1.08±0.09	1.07±0.10	0.81±0.05	0.84±0.19	23.48±0.05

Where, St- Stickiness, **Pr**- Precocious movement, **Sc**-Scatterring,**Un**- Unorientation, **Mv**- multivalent, **SA**-secondary association, **Asy**- asynchronous **Br**-Bridge formation, **Lg**- Laggard, **Fw**- Forward movement, **Dp**-disturbed polarity, **MN**-micronuclei. Data are means±standard error of three replicates (n = 3**).** Different letters show significant difference at P<0**.**05 significance level according to the Duncan's multiple range test**.**

CONCLUSION

The present study concluded the mutagenic sensitivities of the EMS on the meiotic cell division, which consequence in numerous deleterious effects in Chiasma frequency and palynological parameters of *D. lablab* L.These observed findings support that mutagens not only provided desirable traits but could also adversely affect the plants.It can be estimated that dose of mutagen should be in optimum range.Here, it was also observed that the concentration(0.5%) of EMS highly harmed the plant than the (0.1%) and (0.3%) concentrations.

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