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MUTAGEN INDUCED CYTOTOXICITY IN TRIGONELL FOENUM-GRAECUM L.

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Abstract:

Present experiment was design to investigate the effect of methyl methane sulphonate and sodium azide on Trigonella foenum-graecum L. treated with different concentrations (0.01, 0.02, 0.03, 0.04, and 0.05%). The results indicate that there was a direct correlation between increasing concentrations of mutagens and parameters studied. Although meiotic abnormalities, pollen fertility and chiasma frequency showed continuous decreasing trend with the increasing concentrations of both the mutagens but the maximum decline was observed at the higher concentration (0.05%) of MMS and lest was at lowest concentration (0.01%) of SA. However, MMS was found to be more effective in inducing meiotic abnormalities, reduction in chiasma frequency and decrease in pollen fertility as compared to SA. Further, a negative relationship between increasing concentrations of mutagen and reduction in chiasma frequency in treated population was also observed.

KEY WORDS:

Cytotoxicity, chromosomal aberrations, chiasma frequency, pollen fertility, mutagens and Trigonella,

INTRODUCTION:

Mutation are known to enhance the genetic variability of crop plants as the variability at species level has reached the ceiling due to high breeding intensity and rapid erosion of genetic resources. Among the different breeding method used, mutation induction has been used as an important tool to supplement existing variability and to create additional variability for quantitative as well as qualitative inherited traits in Trigonella. The possibility to induce mutations with the use of chemical compounds was discovered simultaneously and independently by Rapoport (1946) and (Auerbakh and Robson, 1946). Induced mutations have been used to generate genetic variability and have been successfully utilized to improve yield components of various crops like *Oryza sativa* (Awan et al., 1980; Singh et al., 1998), *Hordium vulgare* (Ramesh et al., 2001), *Cicer arietinum* (Wani and Anis, 2001), *Vigna mungo* (Misra et al., 2001), *Sesam indicum* (Mensah et al., 2007), *Helianthus annuus* (Elangovan, 2001). These reports show that mutagenesis is a potential tool to be employed for crop improvement. The utilization of new mutagenic agents in several plants species has played an important role in mutation breeding. Like methyl methane sulphonate and sodium azide is one of the most commonly applied mutagens in plants. SA was used for the first time as a mutagen in barley (Nilan et al., 1973). SA has been reported to induce high frequency of point mutations (base substitutions) and no detectable chromosomal aberrations (Nilan et al., 1973). The

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successful utilization of sodium azide to generate genetic variability in plant breeding has been reported in groundnut (Mensah and Obadoni, 2007), barley (Kleinhofs and Sander, 1975) and other crops (Avita and Murty, 1983; Rroutaray et al., 1995). Rascio et al. (2001) used sodium azide as a mutagenic treatment for durum wheat and screened high yield component in M4 generation. MMS is a monofunctional alkylating agent it is commonly used in plant breeding because it cause frame shift mutations.

Trigonella foenum-graecum L. (Family-Fabaceae, $2n=16$) is an important, economic and medicinal crop. As a medicinal plant, it is much used in herbal medicine (Rathee et al., 1980, and Som and Maity, 1993). It is also used in the treatment of late onset diabetes and poor digestion (Raghuram et al., 1994). The alkaloid trigonellin used in cancer therapy. Since the genotype of *Trigonella foenum-graecum* L. is homozygous with limited genetic variability, the variations were induced with the help of MMS and SA. The suitability of sodium azide and methyl methane sulphonate, as a mutagen for *Trigonella* was investigated by assessing the type of cytological anomalies. Cytological analysis with respect to their meiotic behaviour is considered to one of the most dependable indices to estimate the potency of mutagen (Siddique et al., 1982) and the dose response relationships were determined from the correlation and regression coefficients. In most of the cases, the meiotic chromosomal aberrations are closely related to pollen fertility. In this study various types of cytological aberrations induced by MMS and SA, were scored to assess its relation to M1 pollen fertility.

MATERIALS AND METHODS:

The seeds of *Trigonella* were obtained from cytogenetics and mutation breeding laboratory, Department of Botany Aligarh Muslim University Aligarh. Fresh healthy and uniform seeds of *Trigonella* were presoaked in distilled water for 12 h and then treated with the 5 different concentrations (0.01, 0.02, 0.03, 0.04, and 0.05%) of MMS and SA (pH 4) prepared in phosphate buffer for 24h. One set of seeds were soaked in distilled water to act as control. The treated sets of seeds were washed in running tap water to remove the residual effect of mutagens. Each set of seeds were sown in pot along with the control to raise M1 generation. For meiotic studies young flower buds from randomly selected plant from every dose of mutagen as well as from control, were fixed in freshly prepared Carnoy's fixative (absolute alcohol, chloroform and acetic acid in 6:3 % ratio) for 24 hours, and preserved in 70% alcohol. Anthers from the collected flower buds were squashed in 1.5% propionocarmine and made permanent through NBA-GAA series followed by mounting on Canada balsam (Bhaduri and Ghosh, 1954) for the PMCs and pollen grain analysis. The pollen fertility determined by staining the pollen grain with acetocarmine and glycerin solution at the ratio of 1:1. Microphotographs were taken from freshly prepared slides using X30 Olympus Research Photomicroscope.

RESULTS:

Meiotic study of PMCs of control plants revealed a diploid status with chromosome number $2n=16$ (fig. 2A). However, the plants in both treatments sets displayed varying degrees of chromosomal aberrations distributed in all phases of division (Table-1). A dose based increase in meiotic abnormalities was observed in both MMS and SA treatment sets (Figure-1A). Although a number of abnormalities were present in both treatment sets, stickiness (fig. 2B), stray bivalent (fig. 2C), laggards (fig. 2D) and unorientation were more common in MMS treated sets on the other hand, stickiness, stray bivalent, unequal separation, precocious movements (fig. 2E), bridges (fig. 2F) were more common in SA treated sets. In addition, other abnormalities like fragments, disturb polarity (fig. 2G), micronuclei (fig. 2H) and multinucleate (fig. 2I) condition were also found in both the mutagens. All the meiotic abnormalities observed in present investigation of MMS and SA treated plants have also been observed by workers in different plants materials after treatment with physical or chemical mutagen (Reddy and Annadanai, 1992; Mitra and Bhowmik, 1996 and Kumar and Srivastava, 2001). The most important abnormalities induced by both mutagens were stickiness at metaphase (I/II) and at anaphase (I/II).

The phenomenon ranged from slight stickiness to an indistinct compact chromatin mass involving the entire complement. It was recorded to be highest (2.38%) at 0.05% MMS treatment, while it was 2.08% at metaphase (I/II) with same treatment of SA. Stray bivalents were also common in the SA treated set but the percentage was low (1.38% at 0.05%) as compared to MMS treatment. Laggards and un-orientation were also common in MMS (1.78% and 1.48% at 0.05%, respectively) while in SA (1.17% and 1.18% at 0.05%, respectively). Bridges and unequal separation were found to be almost equal in both MMS and SA treatment set i.e. bridges 1.19% and 1.18% in both chemical at 0.05% and unequal separation i.e. 1.48% and 1.48% respectively at 0.05%. Precocious separation and disturb polarity is 1.78% and 1.48% respectively, at 0.05% of MMS versus 1.48% and 1.18% respectively, at 0.05% respectively of SA. As a consequence of

precocious separation of univalent, laggards some fragments, micronuclei and multinucleate were also observed. In the MMS treatment set, the percentage of micronuclei, fragments and multinucleate were reached as high as 0.87%, 2.08% and 1.74% respectively, at 0.05% of MMS versus 0.59%, 1.78% and 1.58% respectively, at 0.05% of SA.

The test for pollen fertility showed a very low percentage of sterile pollen grains in control sets. Pollen fertility found to be significantly correlated with meiotic irregularities as the meiotic abnormalities increased along with dose of chemical treatment, the percentage of fertile pollen grains decrease (Table-1, Figure-1B). The MMS treated set recorded a greater decrease in pollen fertility compared to the SA treated set. A dose dependent decrease in chiasma frequency per cell and per bivalent was also recorded in treated plants (Table-2, Figure-1C,1D)

DISCUSSION:

Induced mutation has been benefited the plant breeders to explore genetic variations resulting from mutagens. Though the mutagens have remarkable possibilities of causing variations in plants with regard to their qualitative and quantitative characters by altering the genetic architecture, the chemical mutagen have been reported to be more potent in inducing mutations than the physical ones Sharma (1969). During the present investigation, both chemical mutagens, i.e. MMS and SA, elicited similar type of chromosomal abnormalities, but the percentage of these abnormalities and the total abnormalities induced differed between the two treatments. This provides a case for comparison of deleterious effects of these mutagens on the concerned plant. The induction of cytological disturbances in the meiotic cells is of great value, as it results in genetic damage that is handed over to the next generation. The results also showed that close co linearity existed between the concentrations of mutagen treatment and percentage of chromosomal aberrations. The spectrum of chromosomal abnormalities, induced by both chemicals was broad, and induced a comparatively higher proportion of stickiness. Several agents have been reported to cause chromosomal stickiness, including X- rays Steffensen et al. (1969), gamma rays Al Achkar et al. (1989), temperature Eriksson et al. (1968), herbicides (Badr and Ibrahim, 1987) and some chemical present in soil Caetano-Pereira et al. (1995). Stickiness has been reported to be a result of partial dissociation of nucleoproteins and alteration in the pattern of cyto-chemically balanced reactions Jayabalan and Rao (1987). Depending on the tendency of stickiness, pollen fertility may be partially or completely affected. The sticky chromosome may result from defective functioning of one or two type of specific non-histone proteins, involve in the chromosome organization which are needed for chromatid separation and migration to poles can result from univalent chromosomes at the end of prophase 1 or precocious chiasma terminalization in diakinesis or metaphase 1. Precocious movements are possibly due to the effect of chemicals in breaking the protein moiety of the nucleoprotein backbone (Kumar and Rai, 2007). Bridges observed seem to be due to non- separation of chiasma due to stickiness. The formation of bridges may be due to the failure of terminalization of chiasmata in a bivalent and the chromosomes get stretched between the poles (Saylor and Smith, 1966). Irregular chromosome segregation in meiosis 1 and 2 could be the result of non-oriented bivalents formed due to spindle dysfunctioning or they could be due to the formation of univalents at diakinesis or metaphase 1. Un-orientation of chromosome at metaphase 1 sometimes lead to unequal separation at anaphase 1 (Khan, 1996). Fragments of chromosomes were also found in the present investigation, it occurred due to damaged mechanism of DNA repair caused by the mutagen (Cremer et al., 1981). The loss of chromosomal fragments as a result of paracentric inversion lead to deficient gametes and high sterility as observed in the present case. Laggard at anaphase can be attributed to the delayed terminalization or perhaps to stickiness of chromosomal ends (Minija et al., 1999). Laggards may arise by breakage or faulty spindle resulting into imbalanced daughter nuclei and micronuclei (Singh and Chaudhary, 2005). Micronuclei are true mutagenic aspects, which may lead to a loss of genetic material and have been regarded as an indication of the mutagenicity and their inducers Raun et al. (1992). Formation of micronuclei may be due to adhesion of fragments or due to lagging chromosomes (Anis et al., 1999). Raina et al. (1994) reported that multipolar condition is mainly due to spindle disfunction. Such conditions resulted in the unequal distribution of chromatin material in the gametes. Most of these gametes were deficient which either does not take part in fertilization or the produce aneuploids. Disturbed polarity was also reported by (Ganai et al., 2005 and Kumar and Rai, 2007) it occurred at anaphase and telophase stages could be due to spindle disturbance. As more and more abnormalities accumulate, the process of gamete formation is affected and it leads to non-viable gametes, which considerably reduce the plant fertility. The actual reason of sterility caused by these chemical mutagens may be a gene mutation or more probably invisible deficiencies.

Chiasma frequency was variable in the populations treated with different concentration of mutagen; however, reduction in chiasma frequency was dose dependent. A considerable decrease in

chiasma frequency showed the prominent effect of MMS and SA on chromosomes crossing over and chiasma formation are under genetic control Rees (1955). It is reported in wheat, sorghum and in capsicum (Goud, 1967; Sadanandam et al., 1984 and Sree Ramulu, 1973) that the reduction in chiasma frequency is the consequence of the mutagen induced structural changes. In the present investigation reduction in chiasma frequency may be attributed to the nature and potency of mutagen and to the underlying factors such as complex structural changes or to the nature of the genes responsible for chiasma formation.

The present observations have shown varying responses of these two mutagens in Trigonella. The significant karyological and cytoplasmic abnormalities induced through the mutagen provide enough scope for further improvement of this economic crop through mutagenesis and mutagenic data from plant assay are thus very important for genetic research and may serve as the basis of means for maintaining a stable ecosystem throughout the entire biological kingdom.

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Table-1: Frequency of Chromosomal Abnormalities Induced by MMS and SA in*Trigonella foenum graecum*L. (M₁ generation)

Conc .	Total No. of PMCs Observed.	Metaphase-I/I (%)				Anaphase- I/II (%)				Telophase- I/II (%)			Total % Of Abn. PMCs Observed.	Pollen Fertility %
		Stic.	Stray	Preco.	Unori.	Lag.	Bri.	Uneq.	Frag.	Micr.	Mult.	Dist.		
Control	360	-	-	-	-	-	-	-	-	-	-	-	-	94.82
	347								0.55					
	343													
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0.01 %	347	1.15	0.58	-	-	-	-	-	-	0.58	-	0.86	3.72	89.49
0.02%	345	1.15	0.86	0.86	0.86	0.86	-	1.15	-	-	0.55	1.15	7.44	85.47
0.03 %	360	1.11	0.83	1.38	0.83	1.38	0.55	1.38	0.55	0.87	0.87	1.11	10.54	78.64
0.04 %	344	1.74	1.74	1.45	1.45	1.74	1.16	1.45	0.58	0.87	1.38	1.45	15.01	73.60

0.05 %	336	2.38	1.79	1.78	1.48	1.78	1.19	1.48	2.08	1.38	1.74	1.48	20.34	67.37
SA														
0.01 %	342	0.58	0.87	0.58	-	-	0.58	-	-	-	-	-	2.61	90.65
0.02 %	350	1.14	-	-	0.57	0.57	-	1.14	0.85	0.28	-	0.85	5.40	87.60
0.03 %	342	1.16	1.16	0.87	-	0.58	0.87	1.16	-	0.58	0.58	0.87	7.83	80.16
0.04 %	340	1.47	1.17	1.17	0.88	1.17	0.88	1.20	0.88	0.58	1.17	1.17	11.68	76.75
0.05 %	337	2.08	1.38	1.48	1.18	1.17	1.18	1.48	1.78	0.59	1.58	1.38	13.20	68.28

Conc. = Concentration, Stic. = Stickiness, Stray. = Stray bivalents, Preco. = Precocious, Unosi. = Univalent, Unid. = Unidirectional, Lag. = Lagg, Uneq. =

Unequal separation, Frag. = Fragments, Mic. = Micronucleate, Dist. = Disturb polarity.

Table-2: Effect of MMS and SA on chiasma frequency at Metain *Trigonella foenum-graecum*L.

Treatment	No. of chiasma/cell	No. of chiasma/bivalent
Control	20.25±0.26	1.50±0.08
MMS		
0.01%	16.19±0.19	1.40±0.06
0.02%	14.38±0.25	1.32±0.04
0.03%	12.86±0.21	1.25±0.02
0.04%	12.16±0.18	1.20±0.07
0.05%	10.74±0.16	1.15±0.09
SA		
0.01%	18.94±0.25	1.46±0.08
0.02%	16.55±0.23	1.40±0.08
0.03%	14.78±0.10	1.32±0.04
0.04%	14.28±0.17	1.28±0.06
0.05%	12.46±0.18	1.21±0.09

Figure-1

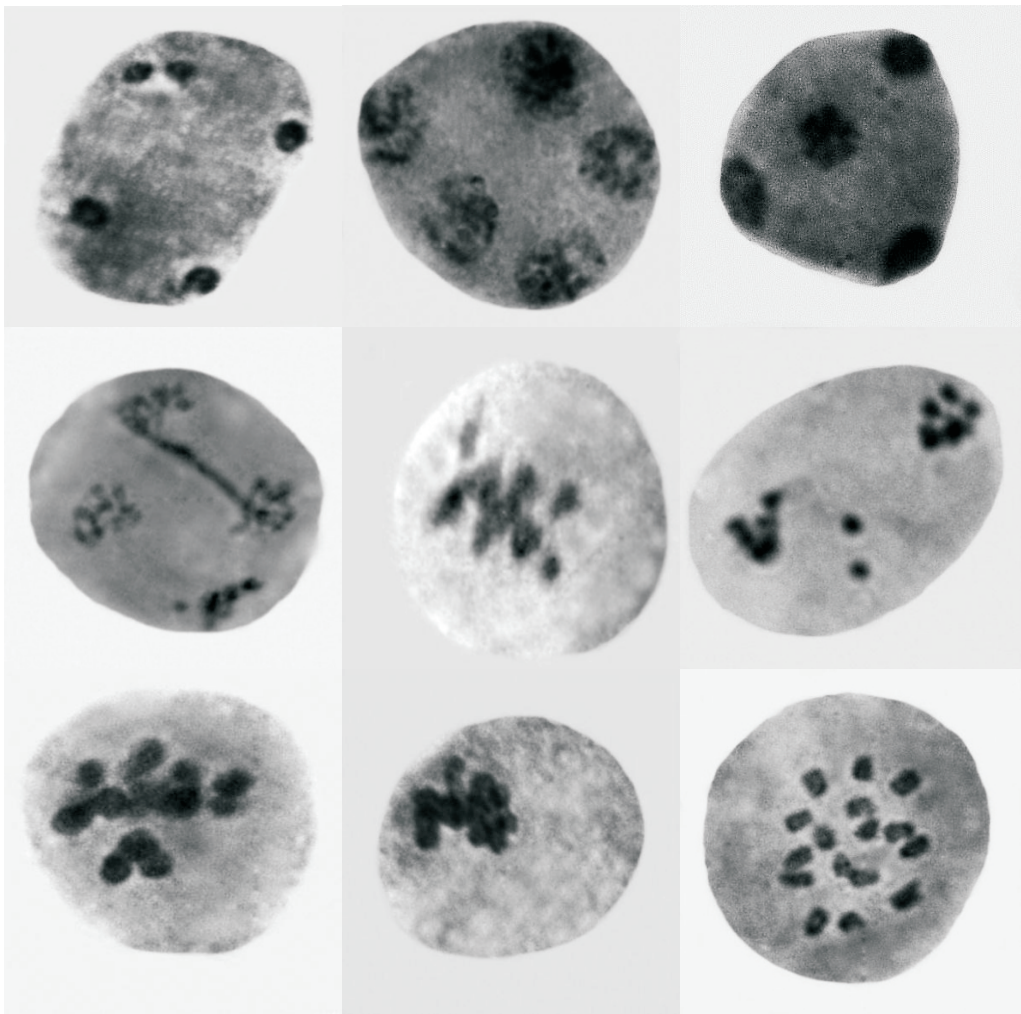


Figure-2

EXPLANATION OF TABLES AND FIGURES

Table-1: Frequency of Chromosomal Abnormalities Induced by MMS and SA in Trigonella foenum graecum L. (M1 generation).

Table-2: Effect of MMS and SA on chiasma frequency at Meta-1 in Trigonella foenum-graecum L.

Figure-1: Different co-relation graphs

- (A). Frequency of chromosomal abnormalities induced by MMS and SA in Ttigonella foenum-graecum L. (M1 generation).
- (B). Frequency of pollen fertility by MMS and SA in Trigonella foenum-graecum L. (M1 generation).
- (C). Effect of MMS and SA on Chiasma frequency per cell at Meta-1 in Trigonella foenum-graecum L.
- (D). Effect of MMS and SA on Chiasma frequency per bivalent at Meta-1 in Trigonella foenum-graecum L.

Figure-2: Different chromosomal abnormalities induced by MMS and SA in *Trigonella foenum-graecum* L.

- (A) 16 univalents at metaphase-I.
- (B) Sticky metaphase –I.
- (C) Two straybivalents at metaphase–I.
- (D) Laggards at anaphase–I.
- (E) Precocious separation of chromosome at metaphase–I.
- (F) Bridge with laggard at anaphaseII.
- (G) Disturb polarity at telophaseII.
- (H) Multinucleate condition at telophaseII.
- (I) Micronuclei at telophaseII.

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