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Methyl methanesulphonate induced chromosomal variations in a medicinal plant *Cichorium intybus* L. during microsporogenesis

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Abstract

Genotoxic effect of MMS on gametic cells of chicory has been investigated in the present study. Progenies were raised from seeds treated with four different concentrations that is 0.04, 0.06, 0.08, 0.10 % aqueous solutions of MMS. Anther-smear studies revealed a wide range of chromosomal anomalies such as stickiness, univalents, multivalents and precocious separation of chromosomes at metaphase; bridges, laggards and polyads at anaphase and telophase stages. Such abnormalities were dose dependent and increased along with the increasing concentrations of mutagen; therefore MMS can be used as a potential mutagen in induction of genetic variability in this plant.

Keywords: Methyl methanesulphonate; Chromosomal anomalies.

Introduction

Induced mutagenesis has been recognized as the most efficient method for induction of morphological and genetical variabilities in plants especially in those with limited genetic variabilities. Its importance continues even in the present era of biotechnology, because in plants the gene replacement experiments through homologous recombination with introduced DNA sequences have met with limited success (Hohn and Puchta, 2003). Cytological analysis with respect to their mitotic and meiotic behaviour is considered to be one of the most dependable indices to estimate the potency of mutagen (Siddiqui et.al, 1982). Cytological studies provide information regarding the response of various genotypes to a particular mutagen and provide greater chances for the selection of desired characters. *Cichorium intybus* L. (fam. Asteraceae, $2n=18$), commonly called as chicory, is an important economic and medicinal crop. It is an important antihepatotoxic and antiulcerogenic herb. Since the genotype of *Cichorium intybus* is homozygous with limited genetic variability, the variations were induced with the help of a monofunctional alkylating agent MMS, which causes frame shift mutations.

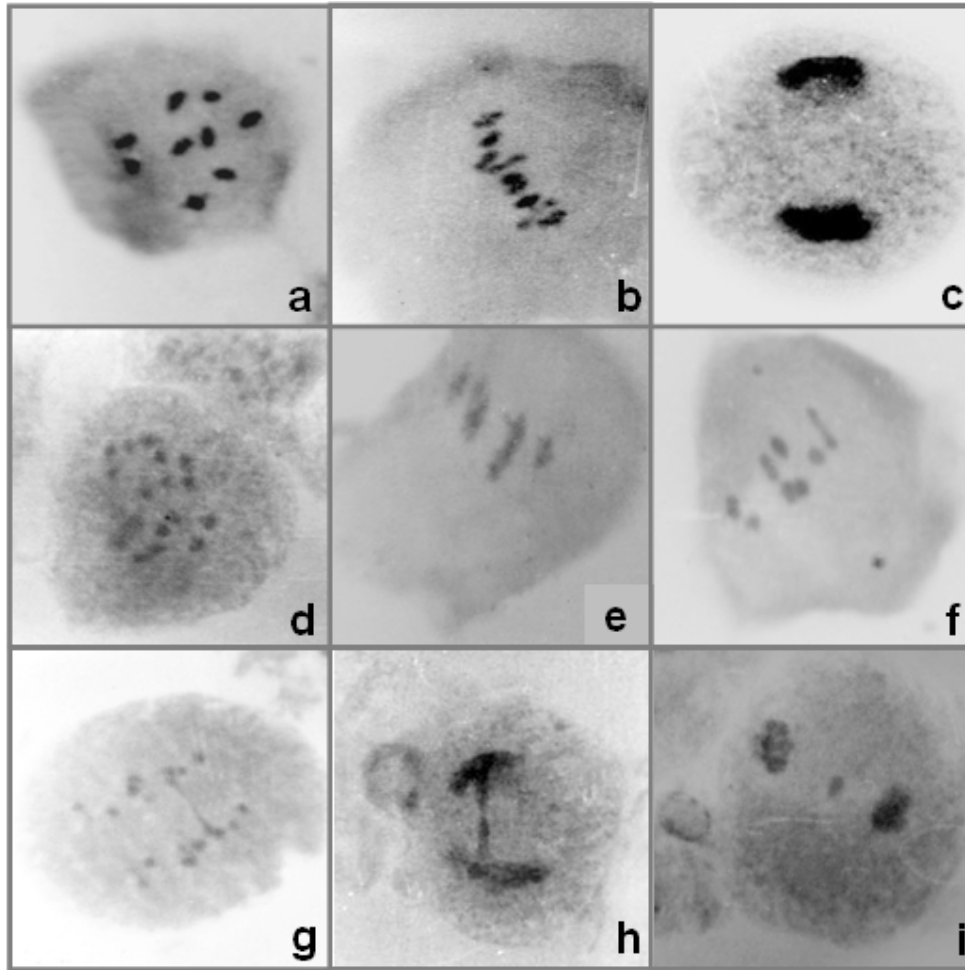
Materials and Methods

Certified healthy seeds of Chicory were treated with four different aqueous solutions (0.04, 0.06, 0.08 and 0.10 %) of Methyl Methane Sulphonate for 24 hrs. One set of seeds was soaked in distilled water as control. The seeds

were then sown in pots to raise M_1 generation. The young flower buds were fixed in Carnoy's fluid (absolute alcohol: chloroform: acetic acid in 6:3:1 ratio) for 40 min. And then transferred to propionic acid saturated with Ferric Acetate for 24 hrs. Material was then stored in 70 % alcohol. Anthers were squashed in Propionocarmine.

Results

Meiotic cells of the control plants revealed 9 perfect bivalents at diakinesis and at metaphase, separating normally at anaphase and telophase stages. Maximum irregularities were found at higher doses of MMS. Overall percentage of abnormal pollen mother cells increased from 8.10% to 19.35% in 0.04% to 0.10% MMS. At metaphase-I the pollen mother cells with univalents, multivalent (trivalent and tetravalent), stray chromosomes, stickiness and precocious separation were observed in varying frequencies (Table-1). However precocious separation appeared only at the higher dose (0.10%). PMCs at anaphase exhibited stickiness, laggards and bridges. The most prominent abnormality was the formation of bridges at anaphase and telophase stages. Various groups of chromosomes formed at metaphase due to spindle disturbances lead to the formation of polyads at telophase. The overall impact of chromosomal anomalies as a result of MMS treatment was observed on pollen fertility as it decreased from 98.50% to 63.11% in control population to 0.10% MMS treatments respectively (Table-1).



- a: Diakinesis-9 ring bivalents.
- b: Metaphase-I ; 7 ring bivalents + 2 rod bivalents at equator.
- c: Telophase-I; two groups of chromosomes at poles.
- d: Diakinesis; 18 univalents.
- e: Metaphase-I; four groups of sticky chromosomes.
- f: Metaphase-I; Precocious separation of 2¹ towards pole.
- g: Anaphase-I; Chromatin bridge formation.
- h: Telophase-I; Chromatin bridge.
- i: Telophase-I; showing one laggard.

Table 1: Frequency of chromosomal abnormalities induced by MMS in Chicory (M₁ generation)

Treatment	Total no. of PMCs	Metaphase I/II				Anaphase I/II			Telophase I/II			Abnormal PMCs	Pollen Fertility	
		Uni.	Multi.	Stray	Pr.	St.	Lag.	Bri.	Lag.	Br.	Muln.			
Control	215	-	-	-	-	-	-	-	-	-	-	-	-	98.50
0.04	222	0.45	0.45	0.4	-	0.90	-	1.80	0.90	1.80	1.35	08.10	90.21	
0.06	226	0.44	0.88	-	-	1.76	-	3.09	0.44	2.21	1.32	10.14	84.68	
0.08	230	0.86	0.43	1.30	-	1.30	1.73	2.60	2.17	2.60	2.17	16.89	75.62	
0.10	232	1.29	1.72	1.72	0.86	1.72	2.58	2.58	2.15	3.01	2.15	19.39	63.11	

Uni- Univalents, Multi.-Multivalents, Pr.-Precocious separation, St.-Stückiness, Lag- Laggards, Bri.-Bridges, Muln.-Multinucleate condition, PMCs-Pollen /mother Cells.

Discussion

Cytological studies revealed the increase in meiotic abnormalities along with increasing concentrations of MMS, confirming the observations of earlier workers (Ahmad, 1993; Dhamyanthi and Reddy, 2003; Bhat *et al.*, 2007). The frequency of abnormalities was higher in meiosis I than meiosis II (Anis and Wani, 1997; Dryanova and Dimitrov, 2000; Donbak, 2002). Kumar and Tripathi (2004) believed that the chemical mutagen induce univalent formation through cryptic structural changes in chromosomes, which restrict the pairing and in turn reduce the chiasma frequency. Multivalents may be attributed to pairing due to translocation and inversion (Dixit and Dubey, 1986; Kumar and Sinha, 1991). Mc Gill *et al.*, 1974 and Klasterska *et al.*, 1976 suggested that stickiness arises due to improper folding of chromosome fibres. It could also be due to the result of partial dissociation and altered pattern of organization of nucleoproteins (Evans, 1962; Myer *et al.*, 1992) or due to polymerisation of nucleic acid caused by mutagenic treatment. Precocious separation of chromosomes at metaphase was observed at higher concentration of MMS only. It might have resulted due to disturbed homology for chromosome pairing or disturbed spindle mechanism. Besides the precocious separation of univalents, the bivalents were also observed to move ahead and seemed as stray chromosome, this may move to one pole resulting into unequal distribution of chromosome or loss of a complete bivalent at metaphase stage.

Bridges were commonly observed at anaphase and telophase stages in present course of study. Sinha and Godward, 1972 suggested that paracentric-inversion may lead to formation of chromatin bridges at anaphase I/II and telophase I/II. According to Saylor and Smith (1966), the formation of bridges can be due to failure of chiasmata in a bivalent to terminalize and the chromosomes get stretched between the poles. The occurrence of lagging chromosomes may be due to abnormal spindle formation and as a result spindle fibres failed to carry the respective chromosomes to the polar regions and resultantly lagging chromosome appeared (Tarar and Dnyansagar; 1980). Its fate is same as in the case of precocious chromosome, that is it may be included to any of the poles or lost. Multinucleate condition may lead to the arrangement of chromosomes in various groups at metaphase due to abnormal spindle formation. The tri- and multipolar segregation

at anaphase may result into formation of polyads.

Pollen fertility decreased in higher doses of MMS. The negative effect of mutagens on pollen fertility may be due to cumulative effects of various meiotic aberrations as mentioned above. Ramanna (1974) have reported that any deviation in karyokinesis or cytokinesis could produce non-viable microspores. This is because of the fact that meiosis is more prone to any conceivable type of disturbances (Darlington, 1937; Swanson, 1957). It may therefore be assumed that cytological disturbances caused as a result of chemical mutagenesis were responsible for pollen sterility. Moreover due to frame shift mutations caused by MMS the changed protein product as a result of changes in amino acid sequences might have affected morphology and fertility of pollen grains

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