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Studies on Ethyl Methane Sulphonate induced Desynapsis in *Viciafaba*L.

BhatTariq A[1], WaniAijaz A[2]

[1]Department of Education, Govt. of Jammu and Kashmir, India
bhattariq110@gmail.com

[2]Cytogenetics and Reproductive Biology Laboratory, Department of Botany, University of Kashmir 190006,Srinagar, J&K, India,
aijazbotku@gmail.com

Abstract: Cytological and genetical behaviors of two desynaptic plants induced in *Viciafaba* L. var. minor by Ethyl methane sulphonate treatment have been studied. Analysis of chromosomal behavior at different stages of meiosis has been thoroughly accomplished in the desynaptic plants. At diakinesis in one plant univalents and bivalents were present while in other multivalents along with univalents and bivalents were also noticed. Bivalents were randomly distributed in one plant while non-random in the other. Abnormalities like univalents, irregular distribution of chromosomes, laggards, stikiness, bridges and micronuclei were also observed at metaphase, anaphase and telophase respectively. Meiotic studies revealed that sterility was due to irregular chromosome pairing but the genetic data indicated that sterility was because of single recessive gene in homozygous condition. The desynapsis obtained during the present investigation fit into the medium strong type.

Keyword: *Viciafaba* L., Chromosomal aberrations, Ethyl Methane Sulphonate, Micronuclei, Pollen sterility.

1. Introduction

During the meiotic cycle homologous chromosome pairing is one of the most important events that start at the early stages of prophase I and continues until the homologous chromosomes separate each one moving to its pole at anaphase. Two groups of genes namely A's and D's genes, control the homologous chromosome pairing and, if present in recessive state results in failure of pairing [1]. The A's genes inhibit the synepsis during zygotene and the condition is known as asynepsis. The D's genes act on paired chromosomes at diplotene-diakinesis stages, causing a reduction or total absence of chiasma formation leading to desynapsis i.e., formation of univalents. The distribution of univalents at metaphase I in the desynaptic plants may be either polar or equatorial and may be influenced by the number of bivalents per cell. If few bivalents were present, the univalents tend to be polar but if many bivalents occur, the univalents tend to be located at equatorial plate [2]. In either case, the major consequence is the production of gametes with varying degrees of chromosomal imbalance. The mutations that affect these genes produce immediate alterations of the meiotic procedure with deleterious effects on fertility [3]. Desynapsis during meiotic division has been reported in many plants such as pearl millet [4], chilli[5], rice[6], *Cicerarietinum* L. [7], Barley [8],*Vignamungo* [9], *Corchorusfascicularis*Lamk. [10], Solanum species [11]. During the present investigation, the desynaptic mutant has been isolated in *Viciafaba* L. var. minor, which is one of major spice crop of India and the world over. The present paper dealt with the cytogenetic characteristics of 2 induced desynaptic mutants in broad bean.

2. Materials and Methods

Seeds of *Viciafaba* L. var. minor were obtained from Indian Agriculture Research Institute (IARI), New Delhi. With a view to induce genetic variability, 250 seeds of this variety were presoaked in distilled water for 24 hr and then, four sets of 50 seeds each were treated with 0.4% freshly prepared Ethyl methane sulphonate (EMS) for 4, 6,8 and 10 h, respectively. Remaining 50 seeds were kept untreated to act as control. The treated seeds after a through washing were sown in the experimental pots. In the M_1 generation, two desynaptic plants were isolated, one each from 8 and 10h treatment and were designated as D8 and D10, respectively. At maturity, the young flower buds of appropriate sizes were fixed in freshly prepared Carnoy's solution (absolute alcohol: chloroform: glacial acetic acid= 6:3:1) for 24 hr and stored in 70% alcohol. Anthers were smeared in 2% acetocarmine [12]. The slides were made permanent using n-butyl alcohol schedule [13]. Studies on pollen fertility were also conducted by staining pollen grains

Corresponding Author: BhatTariq A

with 2% acetocarmine. Undersized and empty pollen grains were considered as sterile. Inheritance pattern of the desynaptic genes was studied by reciprocal crossing with their mother varieties. X^2 test was applied for inheritance of desynapsis using F_2 data. The F_2 segregating families were scored as normal or desynaptic and the latter were classified following Prakken [14].

3. Results and discussion

Two plants from the treated population were morphologically weak and showed late flowering (20-25 days) compared to the control. When cytological investigations were carried out of these two plants, in most of the pollen mother cells (PMCs) at pachytene, chromosome synapsis was normal while in some PMCs a few unpaired regions along the paired bivalents were present. In controls 12 bivalents were regularly formed at diakinesis and metaphase I (Fig.1a, b) followed by normal segregation at anaphase I. Diakinesis in a mutant plant was marked by irregular separation of paired chromosome. In the plant D8 only univalent (11.00±0.64) and bivalent (9.40±0.28) were recorded (Fig. 1c), while in the plant D10 associations of three (0.88±0.20) and four chromosomes (0.22±0.16) in addition to bivalent (10.24±0.26) and univalents (6.78±0.45) were observed (Table 1). In these plants regular orientation of bivalents at metaphase I could not be recognized in any of the cell examined. No spindle apparatus was observed due to the randomly presence of univalents, bivalents and higher chromosomal associations throughout the cytoplasm (Fig.1e and f).The different types of chromosomal aberrations such as precocious separation, bridges, lagards, stickiness and cytomixis were also observed. The representative Cytological figures are given in plate-1(Fig.1-9). The different types of chromosomal aberrations were also observed by different workers after treatment with several mutagens[15],[16] Occurrence of univalents at diakinesis and metaphase I in the mutants could be attributed to failure of chiasma formation[17]. The univalents and bivalents varied considerably from PMC to PMC in both plants (Table 1) and the desynapsis fitted into the medium strong category of[14]. Inter PMC frequency variation of bivalents in the mutant plant suggested that the response of different chromosomes of a genome to the influence of mutated gene might be variable and independent [18]. The distribution of bivalents in plant D10 had a good fit for the binomial distribution while in D8 the deviation from the frequencies expected on the basis of binomial distribution were significant. [19]Reported a similar situation in partial and complete asynaptic mutants of *Nicotiana* and also in several species showing desynapsis. [20],[21] postulated that differential behavior of desynaptic genes in different region of rye anther was casually related to the division sequence. Therefore, variation in metabolic status of cells undergoing meiosis at times affects the expressivity of genes controlling meiotic behavior of chromosomes. As a consequence of univalent formation at metaphase I, the following meiotic stages were also highly irregular. At anaphase I, normal 12:12 segregation was observed only in 5.35% and 3.57% in plant D8 and D10, respectively (Table 2), irregular distribution of chromosomes (Fig.1g), laggards (Fig.1h) and bridge were also observed at anaphase I.[22] pointed out that pairing and chromosome breakage are essential for crossing over to occur in the synaptic mutant during which "U" type reunions occur between sister chromatids leading to bridge and fragments instead of "X" type reunions leading to crossing over. Irrespective of the distribution pattern of chromosome at anaphase that reach the poles organized dyed nuclei and the lagging chromosome develops into micronuclei at telophase I/II (Fig. 1i). The mutants showed high degree of sterility. Genetic studies indicate that the heterozygous plant were fertile and homozygous were sterile. It can be concluded that sterility encountered in the desynaptics is associated with the homozygous condition of a single recessive gene. The pleiotropic effects of the mutant gene(s) like breakage, stickiness and spindle abnormalities, will also contribute to the pollen sterility [23]. To study the inheritance pattern, two desynaptic plant were crossed reciprocally with the normal plants, the F_1 were normal but in F_2 normal and desynaptic plant segregated in a ratio of 3:1 (Table 3). Progenies of the test cross of heterozygous normal with desynaptic segregated in a 1:1 ratio while in the back cross with normal only fertile plants were obtained in F_2 (Table 3). This is in favour of [24], [25] that desynapsis in *Capsicum annuum*is inherited as a single recessive gene. The univalent classes "weak" and "medium strong" desynaptic have been recovered from each of the segregating generation (Table 3). Such deviations in pairing frequency in F_2 are attributed to modifying genes [26], [27] and environmental factor [14].

In the present study environmental factors ruled out since both the normal and desynaptic plants were grown under similar environmental conditions. Desynapsis appear to have been brought about due to gene mutation because meiosis of untreated plants did not show such a phenomenon. This is in favour of [19]. Such desynaptic plants might be useful for physiological studies on chiasma formation and crossing over and in establishing aneuploid lines for basic studies.

4. Conclusion

The microsporogenesis and inheritance in two desynaptic mutants of *Capsicumannuum* were investigated. Due to the presence of both univalents and bivalents, desynapsis fits into medium strong type. As a consequence of univalent formation, meiosis was frequently disturbed by irregularities such as unequal separation, laggards and

micronuclei leading to high pollen sterility. The mutant condition followed a monogenic pattern of inheritance.

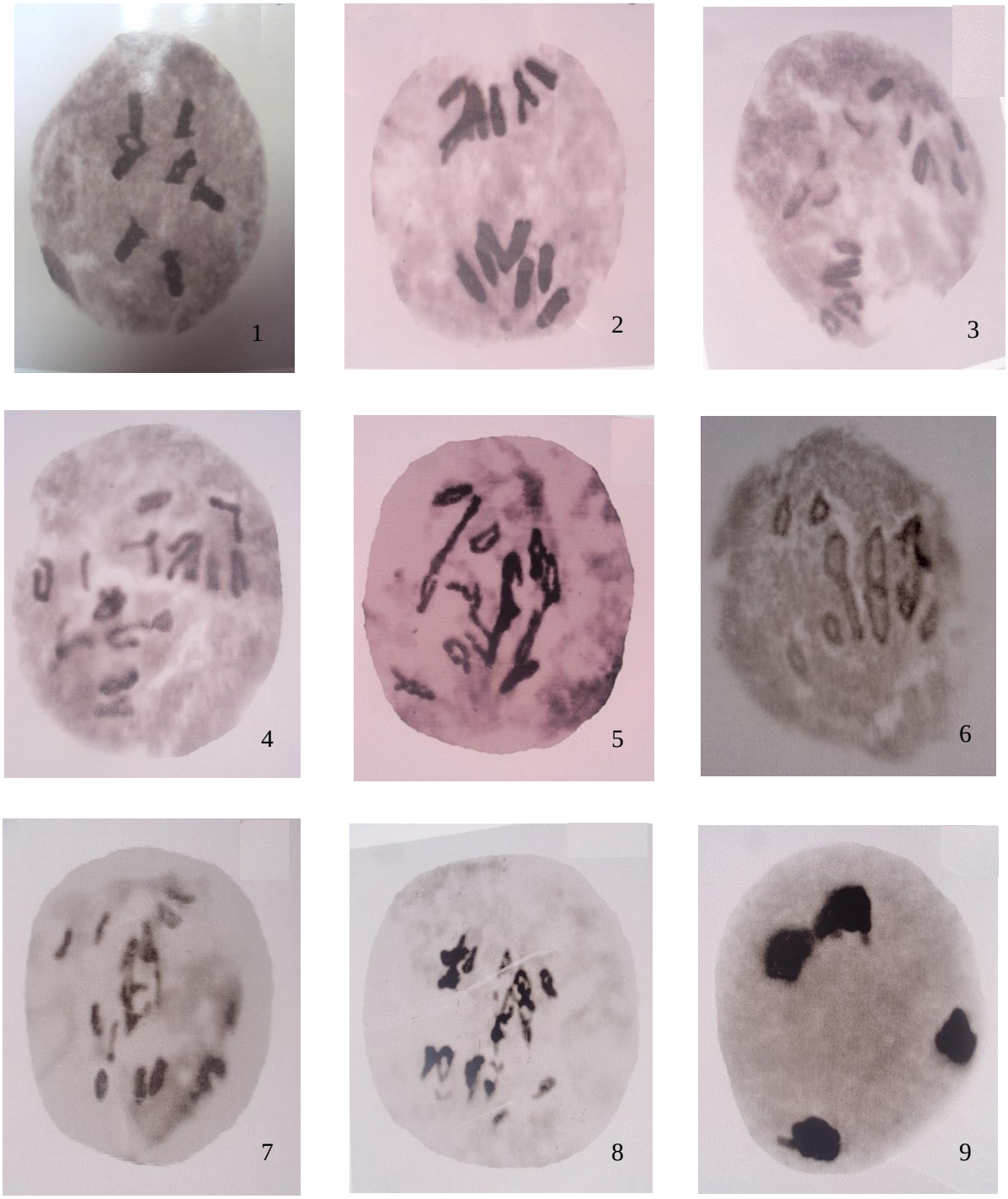
Table 1: Mean and range of chromosomal associations at diakinesis and metaphase-1 and pollen fertility in the desynaptic mutants of *Viciafaba* var. minor

Plant	*Stage*	*No. of Cells Analyzed*	*Chromosomal Association*				*Pollen Fertility (%)*
			I	*II*	*III*	*IV*	
D8	Diakinesis	250	11.00±0.64 (3-24)	9.40±0.28 (2-14)	----------	----------	16
	Metaphase-1	300	12.90±0.78 (2-20)	8.53±0.18 (1-12)	----------	----------	
D10	Diakinesis	250	6.78±0.45 (0-12)	10.24±0.26 (4-12)	0.88±0.20 (1-6)	0.22±0.16 (0-4)	26
	Metaphase-1	300	8.90±0.40 (2-16)	8.44±0.32 (3-12)	0.36±0.10 (1-5)	0.24±0.33 (0-3)	

Table 2: Chromosome distribution at anaphase-1 in two desynaptic mutants of *Viciafaba* var. minor

Chromosomal distribution	*D8*		*D10*	
	No. of PMC's	*%*	*No. of PMC's*	*%*
6:6	3	5.35	2	3.57
7:5	8	14.28	9	16.07
8:4	11	19.64	10	17.85
10:2	6	10.71	7	12.5
9:3	3	3.35	2	3.57
5:2:5	7	12.5	6	10.71
6:2:4	5	8.92	6	10.71
4:4:4	4	7.14	3	5.35
7:1:4	2	3.57	3	5.35
8:1:3	2	3.57	2	3.57
7:3:2	1	1.78	2	3.57

PLATE - I



Discription of Plate-1(Fig. 1-9)

Fig 1. 6 bivalents at diakinesis
Fig 2.6:6 bivalents at anaphase-I
Fig 3.12 univalents at diakinesis
Fig 4.12 univalents at metaphase-I
Fig 5. 4 univalents, 1 bivalent and 2 trivalents at metaphase-I
Fig 6.5 univalents, 1trivalents and 2 bivalents at metaphase-I
Fig 7.7:4 ratio and 2 bridges at anaphase-I
Fig 8. 5:5 ratio, 2 fragments and 2 bivalents yet to separate at anaphase-1
Fig 9. Disturbed polarity and non-synchronization at telophase-2

Table 3: Segregation of normal plants and desynaptic mutants in F_2 from crosses with normal and back crosses

Crosses	*Normal plant*	*Desynapsis*			*Total no. of plants*	*ratio*	X^2	*p*
		Medium strong	*weak*	*total*				
1a.D8 ×normal	220	45	30	75	295	3:1	0.30	0.9-0.8
B.normal×D8	200	35	16	51	251	3:1	0.40	0.8-0.7
C.F1(D8=normal)×normal	80	34	40	74	154	1:1	0.58	0.7-0.6
D.F1(D8=normal)×normal	150	-	-	-	150	-	-	-
2a.D8×normal	180	48	18	66	246	3:1	0.88	0.6-0.5
b.normal×D8	170	32	16	48	218	3:1	0.76	0.5-0.4
c.F1(D8×normal)×D8	60	25	28	53	113	1:1	0.54	0.4-0.3
d.F1(D8×normal)×normal	130	-	-	-	130	-	-	-

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References

1. Gottschalk. W, Klein. HD, "The influence of mutated genes on sporogenesis," TheorAppl Genet, 48, pp 23-24, 1976.
2. Ostergren. G, Vigfusson. E, "On the position correlation of univalents and quasi bivalents formed by univalents," Hereditas, 39, pp. 33-50, 1953.
3. Kaul. MLH, Murthy. TGK, "Mutant genes affecting higher plant meiosis," TheorAppl Genet, 70, pp. 449-466, 1985.
4. Singh. RB, Singh. BD, Vijayalakshmi. RM," Meiotic behaviour of spontaneous and mutagen induced partial desynaptic plants in pearl millet," Cytologia, 42, pp. 41- 48, 1977.
5. Katiyar. RB, "Radiocytogenetical studies in *Capsicum*: Induced desynapsis. Caryologia," 30, pp 347-350, 1977.
6. Reddi. TVVS, Rao. DRM, "Cytology of induced desynaptic mutants in rice," Cytologia, 65, pp. 35-41, 2000.
7. Kumar. G, Sharma. V, "Induced desynapsis in *Cicerarietinum* L." JournCytol Genet, 2, pp. 123-127, 2001.
8. Singh. MR, "Cytogenetic studies of induced desynaptic mutants in Barley (*Hordeumvulgare* L.)," Cytologia, 67, pp. 129-133, 2002.
9. Kumar. G, Kesarwani. S," Genetic analysis of induced desynaptic mutant in *Vignamungo* L. var. PDU.1," The Nucleus, 46, pp. 107-109, 2003.
10. Maity. S, Datta. AK, "Spontaneous desynapsis in *Corchorusfascicularis*Lamk. (Family: Tiliaceae)," Indian JournSciTechnol, 2: 34-36. 2009.
11. Datta. AK, Mukherjee. S, Saha. A, Das. A, "Seasonal influence on the chromosome behaviour of diploid (*Solanumnigrum* L.) andhexaploid (*S.americanum*Mill.) species of *Solanum*," Asian JournExpBiolSci, 1, pp. 193- 196, 2010.
12. Swaminathan. MS, Magoon. ML, Mehra. KL, "A simple propionocarmine PMCs smear for plant with small chromosomes," Indian Journal Genet, 14, pp. 87-88. 1954.

13. Bhaduri. PN, Ghosh. PN, "Chromosome squashes in cereals," Biotech Histochem, 29, pp. 269-276, 1954.
14. Prakken. R, "Studies of asynapsis in rye," Hereditas, 29, pp. 475-495, 1943.
15. Bhat. TA, Parveen. S, Khan. AH, "Meiotic studies in two varieties of *Viciafaba L.* (fabaceae) after MMS treatment," Asian journal of Plant Science, 61(1), pp.51-55,2007.
16. Bhat. TA, Parveen. S, Khan. AH, "MMS induced Cytomixis in pollen mother cells of Broad Bean (*Viciafaba L*)," Turk J Bot, 30, pp. 273-279, 2006c.
17. Bhat. TA, Sharma. M, Anis. M, "Comperative analysis of mitotic aberrations induced by Diethyl sulphide (DES) and sodium azide (SA) in *Viciafaba L.(*Fabaceae)," Pakistan journal of Biological sciences, 10(5), pp. 783-787, 2007.
18. Koduru. PRK, Rao. MK, "Cytogenetics of synaptic mutants in higher plants," TheorAppl Genet, 59, pp. 197-214, 1981.
19. Swaminathan. MS, Murthy. BR, "Aspects of asynapsis in plants. I. Random and non random chromosome association," Genetics, 44, pp. 1271-1280, 1959.
20. Rees. H, "Developmental variation in the expressivity of genes causing chromosome breakage in rye," Heredity, 17, pp. 427-437, 1962.
21. Rees. H, Naylor. R," Developmental variation in chromosome behaviour.," Heredity, 15, pp. 17-27, 1960.
22. Klien. HD, "Asynapsis and extensive chromosome braekage in *Pisum*," Caryologia, 23, pp. 251-257, 1970.
23. Thomas. H, Rajhathy. TA, "gene for desynapsis and aneuploidy in tetraploidAvena," Can JournCytol, 8, pp. 506- 51, 1966.
24. Rao. KGR, Kumar. OA, "Cytogenetics of a spontaneous desynaptic mutant in chilli (*Capsicum annuum* L)," Cytologia, 48, pp. 195-199, 1983.
25. Sadanadam. A, Subhash. K, "Induced desynapsis in *Capsicum*," The Nucleus, 26, pp. 7-8, 1983.
26. Koller. PC, "Asynapsis in *Pisumsativum*," Journ Genet, 36, pp. 275-306, 1983.
27. Subbarao. MV, "Genetics of desynaptic mutants in pearl millet," TheorAppl Genet, 56, pp. 85-89, 1980

1.Dr. Tariq Ahmad Bhat has received his M.Sc and Ph.D from Aligarh Muslim University, Aligarh UP India. He is engaged in active research of mutation breeding and chromosome analysis of legumes. He is actively involved in genetic improvement of legumes and medicinal plants. He is working as lecturer Botany in Department of Education, Govt. of Jammu and Kashmir, India from last 10 years.

2. Dr. Aijaz Ahmad Wani has done his Ph.D. in the field of Mutation Breeding. He is working as a senior Assistant Professor in the Department of Botany, University of Kashmir India. His current research interests are Reproductive Biology of Aquatic Angiosperms, Genetic Improvement of Rosaceous Fruits with special reference to apple (Malus x domesticaBorkh.) and Genetic Improvement of some important medicinal plants with special reference to saffron (*Crocus sativus* L.). For last five years, he has been actively engaged in Department of Biotechnology (DBT), Govt. of India sponsored projects entitled “Creating a genomics platform for apple research in India” and “Genetic improvement of Kashmir saffron”.