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Ethyl Methane Sulphonate Induced Desynaptic Variants in Ajwain (*Trachyspermum ammi* (L.) Sprague)

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Abstract

Synaptic mutants are the subject of a special concern to cytologists and geneticists as they hold the variability in their recombination pattern. Seeds of *Trachyspermum ammi* (L.) Sprague (ajwain) were treated with Ethyl Methane Sulphonate (EMS) solution at 3 different concentrations *viz*. 0.1%, 0.3% and 0.5% for 5 h, prepared in potassium phosphate buffer with 7.0 pH. During meiotic observation of 0.3% concentration of EMS treated population of ajwain, the Pollen Mother Cells (PMCs) of the two plants showed a desynaptic behavior with a high frequency of a univalent formation along with a few bivalents at diakinesis/metaphase I. Apparently, EMS might be involved in the formation of substandard synaptic proteins which reduced the chiasma formation, suggesting that EMS has the ability to generate the male sterile lines. Along with the univalent formation, other various chromosomal aberrations were also analyzed in the subsequent stages of meiotic division *viz*. laggards, bridges, unequal separation, and disturbed polarity with abnormal tetrads. Thus, both the desynaptic plants displayed a high incidence of pollen sterility. The present investigation made an attempt to understand the meiotic behavior of desynapsis and its consequences on the pollen fertility of the ajwain crop.

Keywords: Synapsis, Ajwain, Desynaptic plants, Ethyl methane sulphonate, Recombination, Chiasma.

1. Introduction

Meiosis is a highly organized and conserved process, which facilitates the reduction of the chromosome number of all sexually reproducing organisms and ensures the accuracy of their genetic balance. It constitutes an array of different coordinated events. Although all of them play an essential role but synapsis during the zygotene stage of prophase I is one of the most significant events in meiosis. During this process, the homologous chromosomes recognize each other by their telomeres and synapse intimately along their length. After pairing, recombination is initiated by meiosis specific Double Strand Breaks (DSB) (Kleckner, 1996). DSB and the subsequent genetic exchange precede the formation of the Synaptonemal Complex (SC) (Hawley and Arbel, 1993). The pairing and SC formation in homologous chromosomes are essential for their consequent orderly segregation during the anaphase of the first meiotic division. However, any deviation from this normal incident results into two types of mutations (for example, asynaptic and desynaptic mutation), which affects the pairing of homologous chromosomes and chiasma formation. Mutations that partially or completely prevent homologous chromosome pairing are classified as asynaptic mutants, while those that cause the premature separation of homologous chromosomes are classified as desynaptic (Cai and Makaroff, 2001).

Ajwain (*Trachyspermum ammi* (L.) Sprague) is an annual herb of a Mediterranean origin and is used as a traditional ayurvedic medicine in India. Thymol is the main ajwain essential oil constituent and may be yielded from 35% - 60% (Ishikawa *et al.*, 2001; Zershinas *et al*., 2014). The non-thymol fraction (thymene) contains Para-cymene, γ-terpinene, α-pinene, β-pinene, α-terpinene, Styrene, δ-3-carene, β-phyllanderene, terpinene-4-ol and Carvacrol (Mohagheghzadeh *et al*., 2007; Ranjan *et al*., 2012). The phenols, thymol and carvacrol are responsible for the antiseptic, antitussive, and expectorant properties. Although a huge amount of work has been done related to the antimicrobial activity of ajwain (Khan *et al.*, 2010; Singh *et al*., 2004; Mood *et al.*, 2014), but there is much to explore in the area of cytogenetics.

The factors influencing the nuclear male sterile mutants (desynaptic mutants) having a reduced amount of chiasma formation are valuable resources in the field of cytogenetics, hold a variability in their recombination pattern resulted aneuploid formation. The objective of this study was to describe the meiotic behavior of induced desynapsis, and to evaluate the induced pollen sterility,

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and to investigate the unexplored aspect, especially in the case of ajwain.

2. Materials and Method

2.1. Procurement of seeds

Healthy and fresh seeds of *T. ammi* var.AA-1 were collected from National Research Centre for Seed Spices, Ajmer, Rajasthan, India.

2.2. Seed treatment

Seeds were treated with EMS solution at 3 different concentrations *viz.* 0.1%, 0.3% and 0.5% for 5 h, prepared in potassium phosphate buffer with 7.0 pH with a constant shaking and a thorough washing with tap water. Three replicates were given for each treatment along with their respective controls. After that, seeds were sown to raise the M_1 generation.

2.3. Meiotic analysis

At the time of flowering, young floral buds were fixed in ethanol and glacial acetic acid (3:1) solution for 24 h, after which they were transferred to 70% ethyl alcohol and stored at 40ºC. The meiotic slides were prepared using anther squash technique with 2% acetocarmine (Fürste, 1962). Slides were analyzed and suitable cells were photographed under a Nikon research photomicroscope. The pollen fertility was estimated using a glyceroacetocarmine (Marks, 1954). Fertile pollen grains were recorded with stained cytoplasm with whereas undersized and unstained pollen grains without nuclei were considered sterile.

2.4. Statistical analysis

For all measurements, data collected were subjected to analysis of variance (ANOVA) performed with SPSS 16.0. A pair wise comparison of means was made using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$ significance level.

3. Results

Five plants were cytologically analyzed out of which the Pollen Mother Cells (PMCs) of two plants showed a desynaptic chromosomal behavior. The two plants from 0.3% concentration of EMS treated population were morphologically normal and showed normal flowering. The seed formation in these plants was negligible. The cytological studies of these plants revealed aberrant meiotic behavior. In control plants, 9 bivalents were observed frequently, at diakinesis and metaphase I (Figures 1A and 1B, respectively) followed by normal anaphase I (9:9 separation). Due to some environmental factors, the PMCs of control also showed univalents, but this occurred in a very low frequency (0.55 % in diakinesis and 0.36 % at metaphase I). While in sterile plants, only few bivalents and a high frequency of univalents were recorded. In the desynaptic mutants, univalents were predominantly present at diakinesis and metaphase I stages, although most of the univalents were observed in the stages of diakinesis. The maximum chromosomal configuration *i.e.* 0-2(II)+14-18(I) in E-1 plant, was 26.09 % at diakinesis while 5.81% at metaphase I. However, in E-2 it was 12.85 % at diakinesis while 4.83 % at metaphase I (as mentioned in Table 1). In E-2, chromosome configuration of 6-8(II)+2-6(I) were found to be predominant (28.25 % at diakinesis and 5.05 % at metaphase I) as compared to E-1 (15.33 % at diakinesis and 4.15 % at metaphase I).

In the desynaptics, the univalents were not found independently but they belonged to the same pair and were preferably found in close proximity to each other. In plant E-1, a high frequency of univalent was reported (64.69 %) as compared to E-2 (56.32 %) at diakinesis/ metaphase I (Table 2). Hence, E-1 showed a high frequency of pollen sterility than E-2 (Table 2). The univalents and bivalents varied considerably from PMC to PMC in both the desynaptics. Consequently, both the desynaptic plants (E-1 and E-2) fitted into the category of medium-strong type.

Figure 2 shows the comparative frequencies of univalents, bivalents and pollen fertility of control and two desynaptic plants. At metaphase I, bivalents and univalents were arranged at the equatorial plate but the chromosome segregation was not perfect. As a consequence of univalent formation, various other chromosomal aberrations were observed in the succeeding stages of meiotic division (Table 2). At anaphase I, unequal separation of chromosomes (Figure 1I) was observed frequently in E-1 while in E-2 it was scarcely reported. A part from causing unequal separation, laggards, bridges (Figure 1J), late separation, disturbed polarity were also observed. The bivalents had 1 or 2 chiasmata, delay in chiasma terminalisation which promoted the occurrence of laggards at anaphase I. Simultaneously, the second meiotic division in desynaptic mutants also showed abnormal meiotic divisions as in the anaphase I. More commonly dyads (Figure 1L), triads were also observed. Laggards were also reported in anaphase II (Figure 1K).

A variable number of microspore formations at tetrad stage were observed from 3-6 microspores. In normal plants, quartet of cells were observed. In plant E-1 very few PMCs showed a tetrad of homosized spores, which is a characteristic of normal plants. These types of various aberrations lead to pollen sterility in both the plants (88.38% in E-1 whereas 83.79% in E-2, Table 2).

Table 1. Chromosomal configurations at diakinesis/metaphase I in two induced desynaptic plants of ajwain (*T. ammi* (L.) Sprague) var. AA-1

Plant No.	Stages	Total no. of PMCs observed	Chromosomal Configurations (Mean±S.E.*)			
			9II	6-8(II)+2-6(I)	3-5(II)+8-12(I)	0-2(II)+14-18(I)
Control	Diakinesis	379	68.65±2.35	0.55±0.01	-	-
	Metaphase I	120	21.49±3.40	0.36±0.18	-	-
E-1	Diakinesis	413	2.25±0.14	15.33±0.66	14.97±0.34	26.09±0.29
	Metaphase I	162	0.86±0.16	4.15±0.43	6.95±0.18	5.81±0.32
E-2	Diakinesis	402	1.96±0.12	28.25±0.33	15.04±0.37	12.85±0.71
	Metaphase I	157	0.71±0.17	5.05±0.52	6.60±0.48	4.83±0.22

*S.E. - Standard Error, II- Bivalents, I- Univalents.

Table 2. Frequencies of univalents and bivalents along with anaphasic abnormalities and pollen sterility in two induced desynaptic plants in ajwain (*T. ammi* (L.) Sprague) var. AA-1

Plant no.	Frequencies (%Mean±S.E.)		Anaphasic Abnormalities (Mean±S.E.)				Pollen Sterility (Mean±S.E.)
	Bivalents	Univalents	Unequal seperations (8:10/7:11)	Laggards	Bridges	Others	
Control	98.18±0.79	1.67±0.92	-	-	-	-	2.43±0.45
E-1	35.30±1.20	64.69±1.20	9.93±0.40	5.21±0.19	4.69±0.20	3.99±0.07	88.38±0.16
E-2	43.52±1.69	56.32±1.54	7.49±0.33	5.80±0.29	5.75±0.38	5.26±0.21	83.79±1.08

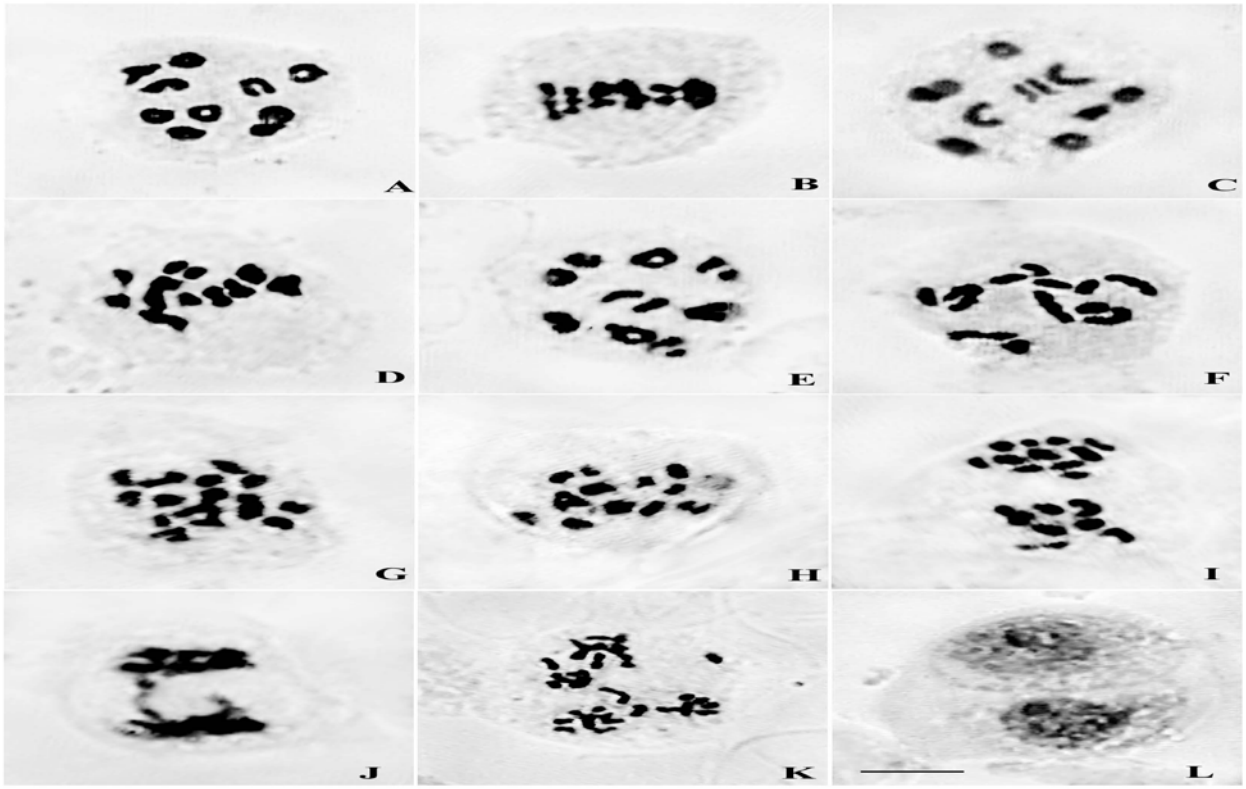

Figure 1. The normal Ajwain chromosome complement; 2n=18. **A**.Normal Diakinesis (9 II), **B.** Normal metaphase I , **C.** Diakinesis (8II+ 2I), **D.** Metaphase I (7II+ 4I), **E.** Diakinesis (6II+ 6I), **F.** Diakinesis (5II+ 8I), **G.** Diakinesis (3II+ 12I), **H.** Diakinesis (3II+ 12I), **I.** Anaphase I (7:11 separation), **J.** Bridge at anaphase I, **K.** Laggards at anaphase II, **L.** Dyad. Scale bar: 1cm.=4.28µm

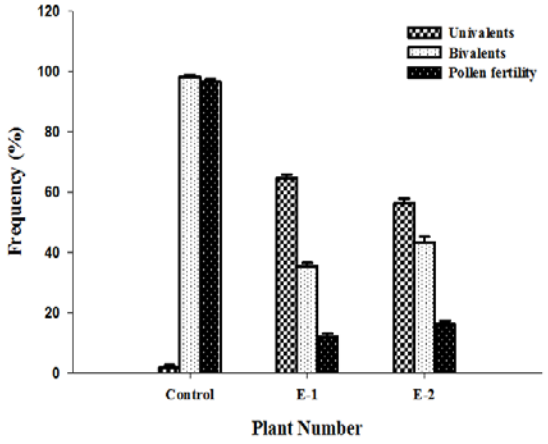

Figure 2. Comparative account of univalents, bivalents and pollen fertility of control and tow desynaptic plants of ajwain (*T. ammi* (L.) Sprague)

4. Discussion

The cytogenetical study based on M_1 generation of ajwain introduces more or less sterile individuals indicating that the mutation affects the process of microsporogenesis. Peirson *et al*. (1996) suggested that the mutation may disrupt the early stage of meiosis and results in the formation of aberrant microsporocytes and megasporocytes. Research on desynaptic mutants continues in many economically important plants but primarily used as a source of trisomics for genetic analysis (Jackson *et al*., 2002).

Desynaptic mutants are classified into three types according to chromosome dissociation into weak, medium-strong and complete (Prakken, 1943). In the present case, both the desynaptic plants (*i.e.*, E-1 and E-2) obtained are of a medium-strong type, as a number of cells possess one or two or rarely up to four bivalents and more than five bivalents, respectively, at stages of diakinesis and metaphase I (Table 1). According to Pierson *et al.* (1997), in desynapsis, the bivalents as well as univalents congregate at metaphase plate. The two plants (*i.e*., E-1 and E-2) obtained during the present investigation are in accordance with the findings.

In the present case, anomalous pairing of homologous chromosomes (Figure 1C to 1H) was induced by EMS, which is a potent mutagen creating the base pairing mistakes. However, the exact mechanism of EMS in the formation of univalents has not been known yet, but according to Naseem and Kumar (2013), EMS that might have acted on some genes was responsible for synapsis and chiasma formation, and resulted in early chiasmata dissociation.

The PMCs of both the mutant plants showed few bivalents with a high incidence of univalents. Various researchers gave many explanations regarding desynapsis stating that the univalent formation is linked with mutation at the gene level. The recombination modifier genes (*rec* genes) play an important role in the formation of viable cross over product. According to Simchen and Stamberg (1969), these genes are also defined as coarse control. The coarse control system has numerous genes that rigidly control the progression of meiotic events (Ji *et al*., 1999). A mutation in this gene system might lead to a failure of chiasma formation and recombination (Simchen and Stamberg, 1969). Mutations that alter the chiasma development and formation are more numerous than the other types of meiotic mutants, and they provide a source of variability in the otherwise conserved *rec* alteration system (Kaul and Murthy, 1985). The commencement of the mutation in these genes may lead to the non-disjunction and can generate aneuploids. Earlier workers, like Sharma and Reinbergs (1974) and Gottschalk and Klein (1976), also proposed that the recessive homozygous condition of *ds* gene might cause chiasma to dissociate early leading to desynapsis, *i.e.*, the formation of univalents.

Thus, from all these statements, it can be concluded that there is a large number of genes ingeniously involved in the formation of univalents which reflect the complexity of the genetic control system of chiasma formation and chiasma maintenance (Kitada and Omura, 1983). Cohesin is the protein which is able to hold the homologous chromosomes together. Some sort of mutations in these protein encoding genes might also be the reason of univalent formation (Figure 1C-1H and Table 2) which resulted in the premature separation of sister chromatids. Defects in synapsis not only result in recombination and chiasmata deficiency but might also hinder the establishment of sister chromatid cohesion necessary for proper chromosome segregation (Bickel and Orr-Weaver, 1996).

Consequently, due to the formation of univalents, the following meiotic stages were also asymmetrical like laggards (Figure 1K), unequal separation (Figure 1I) and bridges (Figure 1J) at anaphase I (Table 2). Klein (1970) stated 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. The unequal distribution of chromosomes at anaphase attributed to different sized microsporocyte formation and ploidy levels that do not generally develop into viable pollen grains. Alternatively, a laggard develops into the micronuclei at telophase I/II.

5. Conclusion

From the present study, it can be concluded that desynapsis emerges due to the mutated genes. Such individuals are favourable material for the cytogenetic analysis based on chiasma formation and crossing over and provide the genetic information about the male sterility and aneuploid lines and to generate the anueploids in higher plants.

Although all the control and treated plants were grown in same environmental conditions, only E-1 and E-2 showed a high incidence of univalent formation and pollen sterility. Due to the formation of univalents, some other meiotic abnormalities, *viz*. laggards, bridges, unequal separation, triads, dyads, etc., were also reported. Decisively, the present study clearly elucidates that both plants are of medium-strong type of desynaptic mutants.

The study also suggests that EMS has the ability to generate the male sterile lines and can be used for the development of aneuploids in ajwain. Further related studies are required to support these results and to explore our knowledge related to the univalent formation and its consequences for their resourceful utilization in breeding programs of higher plants.

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