J. Phytol. Res. 18(1) : 77-81, 2005

DESYNAPTIC VARIATIONS IN SUNFLOWER (*HELIANTHUS ANNUUS*)

G. KUMAR and NEELAM GAUTAM
Plant Genetics Laboratory, Department of Botany, University of Allahabad, Allahabad-211 002, India.

Seeds of *Helianthus annuus* were treated with 0.5% aqueous solution of EMS for three durations. A strong type desynaptic plant was isolated from 3h treated set. Meiotic studies showed varying number of univalents at diakinesis and metaphase-I. Random distribution of univalents lead to highly irregular distribution of chromosomes resulting into unequal separation, laggards, bridges at anaphase-I. Formation of micronuclei with high frequency was also observed. Meiosis-II also exhibited varying degree of irregularities. Desynapsis was strong type with large count of univalents and few bivalents. Increased frequency of univalents causes unbalanced gametes which lead to impaired pollen fertility and seed production.

Keywords: Chromosomal abnormalities; Desynapsis; *Helianthus annuus*.

Introduction

During the normal course of meiosis at pachytene stage, the homologous chromosomes synapse to form chiasmata. But, sometimes these homologues fail to retain chiasmata and the chromosomes separate prematurely remaining as univalents. This phenomenon is recognized as desynapsis. Though desynapsis may be confused with asynapsis, the latter can be characterized as complete absence of synapsis of chromosomes even at pachytene. The genes governing the desynapsis of chromosomes are known to be recessive. Formation of univalents causes irregular and unequal distribution of chromosomes which lead to varying degree of chromosomal imbalance in the gametes, due to which the possibilities of altered chromosome progeny arise. It may also results in partial or complete sterility of pollen and ovules.

Researches on desynaptic mutants have been performed by various workers in many species including plants of agronomic value such as *Zinnia*[1], Chilli[2,3], *Chlorophytum comosum*[4], Soybean[5], *Avena sativa*[6], Rice[7], Cicer[8], Barley[9] and *Anogeissus*[10].

The present study documents the analysis of meiotic behaviour of EMS induced desynaptic plant in sunflower (*Helianthus annuus*).

Material and Method

Dry seeds of *Helianthus annuus* were presoaked in distilled water for 6h and then treated with 0.5 % aqueous solution of Ethyl - Methane Sulphonate (EMS) for 3 durations viz. 3h, 5h and 7h.

Treated seeds were thoroughly washed and sown in experimental pots in replicates to raise the plants. Corresponding control sets were also maintained separately. At maturity, the young flowering buds were fixed in Farmer's fixative (1: 3 acetic alcohol) for 24h and then preserved in 70% alcohol. Anther squashes were made in 2% acetocarmine. Meiotic chromosomes from pachytene phase onwards were cytologically analysed and observations were recorded. Plants showing variant morphology in comparison to controls were studied and cytologically analysed.

Pollen sterility in desynaptic mutant was determined by acetocarmine- glycerine stainability test. Stained pollen grains were considered as fertile whereas unstained and small sized pollen grains were recognized as sterile.

Results and Discussion

Among the population raised from 5h EMS treated seeds, one plant showing weak morphology was encountered. This plant showed reduced height, smaller leaves and late flowering. Cytological investigation revealed it as a strong desynaptic plant. This plant also exhibited high levels of pollen sterility. Only a few seeds could be obtained from this desynaptic plant. In controls, the meiosis was normal with regular occurrence of 17 bivalents at diakinesis and metaphase–I (Fig.1) and normal 17 :17 segregation at anaphase–I (Fig.2). However, only a few PMCs showed complete bivalent formation in the desynaptic plant. As a result, they showed variable number. of univalents at diakinesis and metaphase–I stage. Frequency of open bivalents and ring bivalents also varied but open bivalents were more frequent thus causing a further reduction in the chiasma frequency. The number of bivalents ranged from 0–17 (tabulated in Table-1). The most frequently occurring bivalent number was of 5 bivalent (Fig.6) with frequency 10.34 %. Following it, were 7 (9.2 %) and 9 (8.43 %) bivalents. Least frequent bivalent was that of 1 bivalent with frequency 2.68 %. Only few PMCs showed fully separated and scattered 34 univalents with frequency 1.15 %.

In all the PMCs, bivalent were more or less oriented

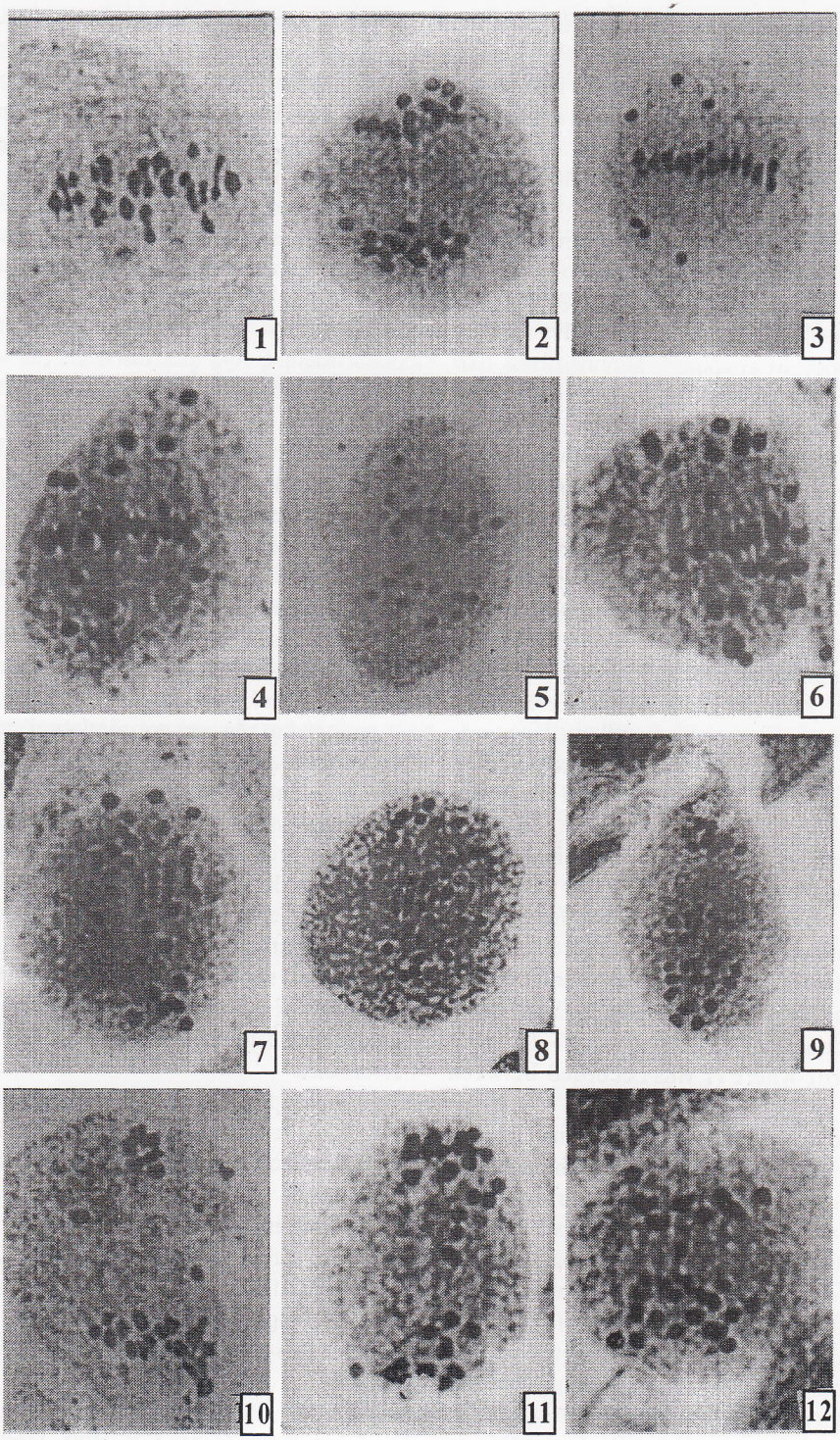
Table 1. Frequency of univalents and bivalents during metaphase - I in the desynaptic plant.

Chromosome configuration	No. of PMCs	Frequency (%)
17 II	9	3.45
16 II + 2 I	10	3.83
15 II + 4 I	14	5.36
14 II + 6 I	16	6.13
13 II + 8 I	13	4.98
12 II + 10 I	18	6.9
11 II + 2 I I	12	4.6
10 II + 14 I	15	5.75
9 II + 16 I	22	8.43
8 II + 18 I	14	5.36
7 II + 20 I	24	9.2
6 II + 22 I	17	6.51
5 II + 24 I	27	10.34
4 II + 26 I	19	7.28
3 II + 28 I	10	3.83
2 II + 30 I	11	4.21
1 II + 32 I	7	2.68
34 I	3	1.15

Table 2. Frequency of different segregations at anaphase - I in the desynaptic plant.

Segregation and lagging chromosome(s)	No. of PMCs	Frequency (%)
17 : 17	8	3.19
16 : 18	10	3.98
15 : 19	12	4.78
14 : 20	18	7.17
11 : 23	11	4.38
10 : 24	16	6.37
8 : 26	13	5.18
7 : 27	16	6.37
17 : 1L : 16	14	5.58
26 : 2L : 6	17	6.77
18 : 2L : 14	15	5.98
25 : 3L : 6	9	3.59
17 : 4L : 13	19	7.57
17 : 6L : 11	22	8.76
17 : 6L : 10	24	9.56
16 : 8L : 11	17	6.77
15 : 9L : 10	6	2.39
15 : 11L : 8	4	1.59

L = Laggard(s)



Figs. 1- 2. Meiosis in normal plants.

1. Normal metaphase - I with 17 bivalents; 2. Normal anaphase - I with 17 : 17 separation.

Figs. 3-8. Metaphase configuration in desynaptic plant.

3. 14 II + 6 I; 4. 12 II + 10 I; 5. 9 II + 16 I; 6. 5 II + 24 I; 7. 4 II + 26 I; 8. 2 II + 30 I.

Figs. 9-12. Anaphase segregations in desynaptic plant.

9. 8 : 11 L : 15; 10. 6 : 2L : 26; 11. 10 : 7L : 17; 12. Unequal separation + Bridges.

at the metaphase–I plate but univalents were scattered irregularly. Univalents seem to aggregate more towards the pole in advance from the disjoining bivalents. Chromosomes lying at the equatorial plate were mostly clumped. In most of the PMCs observed here, univalents were found to lie in pair which may be due to prophase pairing of homologues.

Due to unorientation and irregular arrangement of univalents at equatorial plate, there was no clear cut distinction of metaphase–I and anaphase–I. All the cells showed scattered univalents and few loose bivalents which increased the tendency of anaphasic abnormalities like laggards and unequal separation. Lagging chromosomes lead to the formation of micronuclei.

Anaphase – I stage was remarkable with highly unequal and irregular distribution of chromosomes (shown in Table – 2). Normal segregation (17 : 17) was recorded only in 3.19 % PMCs of the variant plant. The most frequent abnormalities found in the species analysed were irregular chromosome segregation, laggards and bridges both at anaphase–I and telophase–I. The most common unequal separation of chromosomes was 14 : 20 with 7.17 % frequency followed by 10 :.24 and 7 : 27 separation with frequency 6.37 % in each case, respectively. Laggards encountered, varied from 1 – 11. The most common configuration was 17 : 7L : 10 (Fig.11) with frequency 9.56 %.

The second meiotic division also exhibited drastic irregularities. Micronuclei ranging from 1-10 per tetrad were also observed. At metaphase–I, the mean number of bivalent was 8.67 and that of univalent was 16.65.

The desynaptic plant was highly sterile. Pollen fertility recorded in this plant was 18–20 %. Seed set in desynaptic plant drastically reduced to 10–12 % as compared to control where it was recorded between 80–85 %.

In meiosis, a series of complex mechanical and biochemical changes occur which culminate in the reduction of chromosome number. Extensive work performed on different plant and animal species suggests that each step of meiosis is genetically controlled[11,12]. In meiosis synapsis of homologous chromosomes occur to facilitate crossing over so that recombination occurs. Desynapsis furthermore leads to highly reduced recombination frequency because of inability of chromosomes to synapse during meiosis. Occurrence of desynapsis may be attributed to failure of chiasma formation and gene action[13]. According to Ehrenberg[14], change in proteolytic enzymes interfere with the normal process of crossing over. Also, mutation in the gene responsible for formation of synaptonemal complex (SC) might result in defective SC protein which would not hold homologues together for long[15]. It was suggested that the recombination modifier mutation in *rec* gene restricts recombination which lead to desynapsis. In the present text desynaptic mutant was induced by treatment of seeds with EMS. Analysis of pachytene stage showed normalcy in initial pairing hence, presence of univalents is probably due to desynapsis.

Depending on the intensity of pairing, Prakken[16] divided desynapsis into 3 categories weak, medium and strong by counting the number of univalents. Presently described desynaptic plant can be grouped under strong type because of presence of many univalents and few loose bivalent. Desynapsis can occur due to environmental factor, induced or physical stress, apomixis or genotypic abnormality[17].

Meiotic abnormalities have been observed at every stage from diakinesis onwards. At metaphase–I univalents were distributed more towards the pole or periphery of the spindle. The distribution of univalents depends on the number of bivalents per cell. Large count of bivalents leads to the equatorial position while few number of bivalents leads to the polar distribution of univalents[18].

In the PMCs studied, no clear cut distinction of metaphase–I and anaphase–I was found as univalents were not arranged at the equatorial plate. Person[19] coined the term meta – anaphase stage for this stage.

Univalent chromosomes at diakinesis or metaphase–I may result from low chiasma frequency, precocious chiasma terminalization or by the presence of asynaptic or desynaptic genes in prophase – I [20,21].

Partial or complete desynapsis generally exhibits a number of anaphase irregularities. At anaphase – I chromosome showed differential migration towards the poles which lead to the occurrence of laggards. High frequency of laggards usually results into formation of micronuclei.

Desynapsis causes high pollen sterility and reduced seed production. Desynapstic variants raise the possibilities of formation of aneuploids in subsequent generations.

Acknowledgements

One of the author (N. G.) is thankful to CSIR for financial assistance in form of JRF. Authors are highly indebted to Mr. Vivek Singh for his valuable guidance and help. Sincere thanks are also due to other laboratory members for their support.

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