Stress Induced Desynapsis in *Hemerocallis*

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Desynapsis or dissociation of chromosomes shortly after pairing has been reported in a large number of plants of diverse phylogenetic relationships (for review see Gottschalk and Baquar 1971, Gottschalk and Kaul 1980a,b, Koduru and Rao 1981). It is caused by spontaneous or induced gene mutation, environmental stress and gene-environment interaction. In the present communication desynapsis in two cultivated varieties of *Hemerocallis* induced by high temperature and low water stress is reported.

Materials and methods

Sprouted tubers of *Hemerocallis* varieties 'Love Song' and 'Sleeping Beauty' were dug up from soil and after cutting off roots and part of leaves were stored in moss for 3 weeks. On replanting flowering spikes appeared first after 2 weeks and later 4 times during the year (Table 1). Male meiosis was studied during each of these flowering periods. Meiotic preparations were made from anthers fixed for 10 min in 1:3 acetic-alcohol and squashed in 1% aceto-orcein. Chiasma frequency was determined from 30 cells at metaphase I. Since during desynaptic meiosis chromosomes did not follow normal pattern of metaphase congression and anaphase disjunction these stages have been designated arbitrarily.

Observations

'Love Song' and 'Sleeping Beauty' were diploid with $2n=22$ chromosomes. In both the varieties meiosis during first flowering showed medium-strong type of desynapsis whereas during subsequent flowerings the same plants showed normal chromosome pairing.

Desynapsis:

Early stages were difficult to study because of extreme length and fuzziness of chromosomes. However, the few cells in which configurations could be clearly observed showed chromosomes loosely paired into bivalents. At 'metaphase I' a maximum of 3 bivalents per cell was observed while the rest of chromosomes were lying unpaired as univalents (Table 2, Figs. 1-3). Average association per cell was $0.76 \text{ II} + 20.48 \text{ I}$ in 'Love Song' and $0.80 \text{ II} + 20.39 \text{ I}$ in 'Sleeping Beauty'. Both terminal and interstitial chiasmata were observed in the bivalents; chiasma frequency was almost same in both the varieties (Table 3). Equatorial congression

Table 1. Period of flowering and atmospheric temperature

	Month				
	May	July	October	March	April
Varieties flowered	'Sleeping Beauty' 'Love Song'	'Sleeping Beauty'	'Love Song'	'Sleeping Beauty'	'Sleeping Beauty'
Average					
max. and	34.5°C	29.4°C	28.3°C	32.2°C	33.8°C
min. temp.	21.8°C	19.8°C	18.4°C	19.6°C	21.7°C

Table 2. 'Metaphase I' associations during desynaptic meiosis

S. no.	Associations		Number of cells	
	II	I	'Love Song'	'Sleeping Beauty'
1.	3	16	1	2
2.	2	18	5	7
3.	1	20	22	17
4.	—	22	18	20
Total			46	46

Table 3. Chiasma frequency during desynaptic meiosis

Variety	Chiasmata	Terminal	Interstitial	Total $\pm$ S.E.
'Love Song'	per cell	0.45	0.45	0.90 $\pm$ 0.153
	per bivalent	0.60	0.58	1.18 $\pm$ 0.068
'Sleeping Beauty'	per cell	0.74	0.30	1.04 $\pm$ 0.089
	per bivalent	0.95	0.38	1.33 $\pm$ 0.082

of chromosomes could not be observed in any cell nor was there any evidence of a bipolar anaphase spindle. Instead, at 'anaphase I' chromosomes in varying numbers clustered into 2-4 groups (Table 4, Fig. 4). Occasionally, upto 6 chromosomes remained outside these groups and got later differentiated into micronuclei. In 3 of the 34 cells studied, chromosomes remained completely dispersed in the cell volume and did not show any tendency to form groups. During 'anaphase I' chromosomes underwent decondensation. From tightly coiled typical meiotic metaphase chromosomes these acquired the shape of long somatic chromosomes with clear centromeres and two chromatids. Interestingly, while condensation upto 'metaphase' was synchronous, 'anaphase' decondensation on the other hand was non-synchronous in majority of the cells (Figs. 4, 5).

Second division of meiosis was lacking in both the varieties. As a result, telophase I nuclei differentiated directly into microspores. Blockage of meiosis after telophase I in desynaptics has earlier been recorded in *Allium cepa* (Koul 1962), *Sorghum* (Sadasivaiah and Magoon 1965) and *Pennisetum orientalis* (Jauhar and Singh 1969). The ensuing pollen grains were variable in size. Pollen stainability with 1% acetocarmine was 25.7% in 'Sleeping Beauty' and 30.8% in 'Love Song'.

Normal meiosis

Meiosis during later flowerings was regular in both the varieties with 11 bivalents at metaphase I (Fig. 6) and 11:11 segregation at anaphase I. Chiasma frequency during different seasons of flowering is given in Table 5. Chromosome condensation-decondensation cycle was quite synchronous in both divisions of meiosis. Even the two dyads of a tetrad showed exactly same stage of division indicating that

Figs. 1-6. 1-5: Desynaptic meiosis in 'Sleeping Beauty'. 1, 3 II+16 I. 2, 2 II+18 I. 3, 22 I, note 11:11 grouping. 4, 'anaphase I' showing 10:7:4:1 grouping. 5, 'anaphase I' showing non-synchronous decondensation. 6, normal meiosis in 'Sleeping Beauty' showing 11 II. Arrows show bivalents. Scale 10 μ .

Table 4. Chromosome distribution at 'anaphase I' during desynaptic meiosis in 'Sleeping Beauty'

S. no.	Number of cells	Chromosome distribution
1.	3	11: 11
2.	2	10: 12
3.	5	9: 13
4.	1	8: 14
5.	1	8: 8: 6 (scattered)
6.	1	6: 16
7.	1	11: 8: 3
8.	2	11: 7: 4
9.	1	11: 6: 5
10.	1	10: 9: 3
11.	3	10: 8: 4
12.	1	10: 7: 5
13.	1	10: 6: 6
14.	1	9: 8: 5
15.	1	7: 6: 6: 3 (scattered)
16.	1	10: 7: 4: 1
17.	1	9: 9: 2: 2
18.	2	9: 8: 3: 2
19.	1	9: 7: 3: 3
20.	1	7: 7: 6: 2

Table 5. Chiasma frequency during normal meiosis

Variety	Month of flowering	Chiasmata	Terminal	Interstitial	Total $\pm$ S.E.
'Love Song'	October	per cell	10.30	6.46	16.76 $\pm$ 0.395
		per bivalent	0.94	0.59	1.53 $\pm$ 0.049
'Sleeping Beauty'	July	per cell	8.91	8.23	17.14 $\pm$ 0.368
		per bivalent	0.81	0.75	1.56 $\pm$ 0.035
	March	per cell	8.53	8.30	16.83 $\pm$ 0.322
		per bivalent	0.78	0.75	1.53 $\pm$ 0.014
	April	per cell	8.73	8.10	16.83 $\pm$ 0.387
		per bivalent	0.79	0.74	1.53 $\pm$ 0.036

synchrony was maintained even after the cells within a microspore mother cell were effectively separated from one another.

Discussion

Occurrence of desynapsis during first flowering followed by normal meiosis during subsequent flowerings suggests that some factors other than gene mutation alone are responsible for desynapsis in the two *Hemerocallis* varieties. Among the several environmental stresses which lead to irregularities in meiosis the role of temperature is most well known. Reduction in chiasma frequency and some cases of desynapsis under extreme temperature stress have been reported by Darlington (1940), Barber (1942), Elliot (1955, 1958), Dowrick (1957), Wilson (1959),

Hossain (1978) and Singh and Gupta (1981). Jain (1957) reported inactivation of nucleolar organizing activity in *Lolium* resulting in appearance of several small nucleoli instead of a single large one. Environmental effect on expression of genotypically controlled desynapsis has been reported by Goodspeed and Avery (1939), Prakken (1943), Li *et al.* (1945), Soost (1951), Ahloowalia (1969) and Rao (1975). In *Coix lacryma-jobi* mutant Rao (1975) reported desynapsis in summer and normal meiosis from suckers produced in monsoon.

In the present material, meiosis during seasons subsequent to first flowering reveals no significant variation in chiasma frequency (Table 5) though there is a marked change in atmospheric temperature (Table 1). The distribution of chiasmata, whether terminal or interstitial, also shows no appreciable change from season to season. Obviously, within the given temperature range the pairing behaviour of chromosomes is not adversely affected. Further, while desynapsis was observed during high temperature month of May, in April of next year, when temperature was almost the same, meiosis was perfectly normal. Desynapsis in the first season of flowering, therefore, cannot be attributed to high temperature alone. As mentioned earlier, flowering in these plants started just two weeks after planting of tubers. In this period these were unable to produce a good root system. Most likely, stress caused by high temperature and inability of plants to draw adequate water resulted in desynapsis. Goodspeed and Avery (1939) in a mutant of *Nicotiana* reported normal meiosis during high temperature and high humidity and desynapsis during high temperature but low humidity. Similar relation between humidity and desynapsis has been reported by Prakken (1943) in rye.

As compared to normal meiosis desynaptic meiosis shows reduced per cell as also per bivalent chiasma frequency. These results suggest that desynapsis causes a general reduction in chiasma affecting all chromosomes randomly.

While lack of both equatorial congression and ordered polar movement of chromosomes would suggest that spindle is not organised during desynaptic meiosis, two other observations indicate that this may not be entirely so. One, half bivalents at 'metaphase' are stretched towards opposite poles; and two, in majority of cells at 'anaphase' chromosomes show congression into groups. These observations lend support to the contention of Martini and Bozzini (1966) that at least some spindle activity is retained despite irregular metaphase and anaphase.

An interesting feature of the presently reported desynapsis is the non-synchrony in chromosome decondensation following 'metaphase I'. Rees (1958) in *Scilla siberica* and Narain (1980) in *Gloriosa* reported non-synchronous condensation during prophase. Jain (1957) in *Lolium* and Rana (1965) in wheat induced differential condensation of bivalents by high temperature treatment. Jain (1957) attributed non-synchrony to anomalies in DNA synthesis.

Because of variation in chromosome numbers of ensuing gametes, desynapsis has been used to raise aneuploids (Gohil and Koul 1983). *Hemerocallis* being vegetatively propagated, all chromosome variations can be fixed through clonal propagation. Earlier, aneuploids have been raised in the genus through reciprocal crosses of diploid and triploid taxa (Stout 1932, Matsuoka 1972). Provided the method for induced desynapsis through environmental manipulation is standard-

ised, it can provide an alternative means of producing aneuploids.

Summary

Desynapsis, induced by water and temperature stress, was recorded in two varieties of *Hemerocallis*. Average 'metaphase I' association per cell during desynapsis was 0.76 II+20.48 I in 'Love Song' and 0.80 II+20.39 I in 'Sleeping Beauty' whereas during normal meiosis 11 II were invariably observed in the same plants. Equatorial congression in desynaptic meiosis was lacking, but majority of cells at 'anaphase I' showed clustering of chromosomes into 2-4 groups. Chromosome decondensation following 'metaphase I' was non-synchronous. Second division of meiosis was completely blocked so that the microspores were produced directly from telophase I products.

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