Induced Interchange Heterozygosity in *Sesbania aegyptiaca* (Poir.) Pers.

S. N. Zadoo

Plant Improvement Division, Indian Grassland and Fodder
Research Institute, Jhansi 284003, India

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Chromosomal interchanges can be effectively used to locate genes on a particular chromosome and establishing various linkage groups (Burnham 1962, 1966). They also form an important source of raising aneuploids (Khush 1973, Minocha *et al.* 1979). Physical mutagens have proved to be the most potent tool for induction of chromosomal aberrations including reciprocal interchanges. Irradiation induced interchanges have been reported in many species viz. *Triticum* (Yamashita 1951), *Tradescantia* (Watanabe 1962, 1973). Besides this multiple interchanges and/or those involving whole chromosomal complement have been successfully raised by recurrent irradiation, intercrossing and selection by Rana (1965) in *Chrysanthemum* and Brar *et al.* (1973), Brar and Minocha (1982) in pearl millet. Survival of interchange heterozygotes, however, depends chiefly on the mode of orientation of the multiple at metaphase plate and its subsequent disjunction, as it directly effects the viability of gametes. So an interchange heterozygote has to be accompanied by high frequency of disjunctional orientation for an adaptive advantage. The present studies are based on three interchange lines of *Sesbania aegyptiaca* (Zadoo 1984) with special reference to mode of orientation of interchange multiple and its consequences on fertility.

Material and methods

Dry seeds of *Sesbania aegyptiaca* were exposed to 10, 20, 30, 35, 40 and 45 Krad doses of gamma-rays (Co^{60} source) at Bhabha Atomic Research Centre, Trombay, India. The M_1 plants raised from the treated seeds were scored for pollen stainability by dusting freshly dehiscent anthers in 1:1 Glycerol aceto-carmin solution. The slides were left overnight and stainability of pollen was recorded from 25 microscopic fields. The plants with significantly lower proportion of stainable pollen grains were subjected to meiotic studies from pollen mother cells by staining the fresh material with 1 per cent aceto-orcin.

Observations

The plant 35-1 consistently showed an association of four chromosomes and four bivalents, as against six bivalents of the control (Figs. 1–4). In 81.25 per cent cells, a ring multiple of four chromosomes was observed, whereas, chains of four chromosomes were observed in 18.75 per cent cells. Fifty per cent of chain multiples exhibited disjunctional orientation, whereas 20.07 per cent of ring multiples showed disjunctional orientation at metaphase-I (Table 1). In the plant 40-4, 70 per cent of the cells showed a multiple of four chromosomes (Fig. 5) out of which 56 per cent depicted a ring of four and 12 per cent showed a chain of four chromosomes. Rest of the cells were having a regular formation of 6 bivalents. All the chain multiples were observed to orientate non-disjunctionally. About half the ring multiples had disjunctional orientation, whereas rest of them showed non-disjunctional/discordant orientation at metaphase-I (Table 1).

The plant 45-8 had multiples in 88 per cent cells, whereas, in 12 per cent cells a regular feature of six bivalents was observed (Figs. 6-9). An association of four chromosomes and four bivalents was observed only in 54 per cent cells, whereas, 34 per cent cells showed an association of three chromosomes, four bivalents and a univalent (Table 1).

The chiasma frequency per cell varied from 9 to 13 in 35-1 and 40-4, and 7 to 13 in 45-8, as compared to 8 to 12 of the homozygote. The corresponding averages being 11.06, 10.6 and 10.2 in 35-1, 40-4 and 45-8, respectively as compared to 10.18 of the homozygote. The terminalization coefficient works out to be 0.81, 0.78 and 0.85 in 35-1, 40-4 and 45-8, respectively as compared to 0.78 of the homozygote (Table 2). In 56.66 per cent cells of 35-1 one of the chi-

Figs. 1-6. Meiotic chromosomes of *Sesbania aegyptiaca*. 1, metaphase-I of control 6II. 2-4, Interchange multiples of 35-1. 2, disjunctional chain of 4 chromosomes. 3, non-disjunctional ring of 4 chromosomes. 4, disjunctional chain of 4 chromosomes. 5, non-disjunctional ring of four and four bivalents in 40-4. 6-10, chromosomal associations in 45-8. 6, normal 6II.

Table 1. Frequency and orientation of interchange multiple at Metaphase-I

Association	No. of cells	Orientation of multiple			Expected percentage	
		Disjunctional	Non-disjunctional	Discordant	Balanced gametes	Unbalanced gametes
45-1						
R ₄ +4II	52	12	23	17	18.75	65.21
C ₄ +4II	12	6	6	—	9.37	9.37
Total	64	18	29	17	28.12	71.88
40-4						
6II	15	15	—	—	15.00	15.00
R ₄ +4II	29	15	7	7	30.00	28.00
C ₄ +4II	6	—	6	—	—	12.00
Total	50	30	13	7	45.00	55.00
45-8						
6II	6	6	—	—	12.00	—
C ₄ +4II	11	2	9	—	4.00	18.00
A ₄ +4II	16	—	—	—	—	—
C ₃ +4II+1I	5	—	—	—	—	—
A ₃ +4II+1I	12	—	—	—	—	—
Total	50	—	—	—	—	—

Table 2. Chiasma frequency of homozygote and translocation heterozygotes

Taxon	Number of chiasma per cell		Number of terminalized chiasma per cell		Termination coefficient
	Range	Mean	Range	Mean	
Homozygote (Control)	8-11	10.18	8-10	7.96	0.781
35-1	9-13	11.06	6-11	9.06	0.819
40-4	7-13	10.6	5-11	8.32	0.784
45-8	7-13	10.2	6-11	8.76	0.858

Figs. 7-9. Chromosomal associations in 45-8, cont. 7, 1III+4II+1I. 8 and 9, A₄+4II, resulting from chiasma formation in interstitial segments.

asmata in the interchange multiple was found to be unterminalized at metaphase-I. The chiasmata in the interchange multiple of 40-4 were mostly terminalized by metaphase-I except in 14.28 per cent multiples only. The nature of metaphase-I association in 45-8, indicated that in most of the multiples the chiasmata were found in interstitial segments (Figs. 8-9).

At anaphase-I, 14 per cent cells in 35-1 show unequal disjunction of 7: 5 followed by regular anaphase-II, no chromosomes or parts thereof were found to be lagging at anaphase-I and II. In 40-4, 84 per cent of anaphase-I cells exhibited a normal numerical disjunction of 6: 6 while 16 per cent cells had a disjunction of 7: 5 chromosomes. The percentage of normal disjunction (6: 6) was however, lowest in 45-8, where it was observed in 66 per cent cells, 20 per cent cells showed an unequal disjunction of 7: 5 chromosome, while lagging chromosomes were observed in 14 per cent cells (Table 3). The pollen stainability percentage was found to be 31.35, 60.6 and 59.2 in 35-1, 40-4 and 45-8, respectively as compared to 97.12 per cent in the homozygote. The range of pollen grain size was found to be higher in 35-1 and 45-8 and lower in 40-4 as compared to that of homozygote (Table 4).

Table 3. Anaphase I disjunction of interchange heterozygotes
(Figures within brackets indicate %)

Taxon	Number of cells	Normal 6 : 6 disjunction	Abnormal 7 : 5 disjunction	With lagging chromosomes
35-1	50	43 (86)	7 (14)	—
40-4	50	42 (84)	8 (16)	—
45-8	50	33 (66)	10 (20)	7 (14)

Table 4. Pollen stainability and size in translocation heterozygotes and normal plants

Taxon	Pollen stainability %	Pollen size (μ)			
		Stainable		Unstainable	
		Range	Mean	Range	Mean
Control	97.12	27-33	29.76	18-24	21.22
35-1	31.35	24-36	30.66	18-27	21.9
40-4	60.6	30-33	30.6	21-24	21.6
45-8	59.2	30-36	31.2	18-24	21.6

Discussion

An association of more than two chromosomes in an otherwise diploid taxon, indicates the existence of a reciprocal interchange involving two or more non-homologous pairs of chromosomes. Multiples of four chromosomes found in the three cases, observed as above, show that reciprocal interchange involving two pairs of non-homologous chromosomes has taken place after subjecting the seeds of *Sesbania aegyptiaca* to various doses of gamma rays.

Realization of a multiple association, however, depends upon the length of interchanged segments. The larger the segment, more are the chances of cross-over between them, which in turn results in a multiple association. As is evident from Table 1, the occurrence of multiple of four is constant in 35-1 (i.e. realized in all cells observed), whereas it was realized in only 70 per cent and 54 per cent cells in 40-4 and 45-8 respectively. This would indicate that length of interchanged segments is largest in 35-1, followed by 40-4 and 45-8. In the latter two cases there is an occasional failure of crossing over between all the four pairing segments which in turn

results into formation of trivalents, bivalents and univalents. Furthermore, the absence of ring multiples in 45-8 also suggests that interchanged segment is very small in this case. The small interchange segment in turn results into a large interstitial segment. The presence of the large interstitial segments leads to formation of associations having chiasmata in interstitial segments.

The fertility and eventual survival of an interchange depends chiefly on the mode of orientation of the multiple at metaphase plate. Symmetrical shape of chromosomes together with terminalized chiasmata, increase the probability of disjunctional orientation, because rings with equal intercentromeric distance and terminal chiasmata tend to be more pliable and can easily arrange disjunctonally (Burnham 1956, Darlington 1965). Furthermore alternate/disjunctional orientation in ring multiples is greater if centromeres are effectively equidistant, so that each centromere is in equal communication with those adjacent to it and becomes co-orientated relative to both of them. The effective distance depends not only on morphology of chromosomes, but also on position of chiasmata at metaphase-I (Lewis and John 1963).

It appears that disjunctional orientation necessitates a greater flexibility of multiple. Keeping this in view, chain multiples should orientate in this manner more often than rings. This presumption holds good in 35-1, where 50 per cent of chain multiples observed orientate disjunctonally as compared to 18.75 per cent of rings with disjunctional orientation (Table 1), while it does not hold good in case of 40-4, where 51.7 per cent of ring multiples orientated disjunctonally and all chain multiples showed non-disjunctional orientation.

Table 5. Relationship between chiasma terminalization and orientation of multiple at metaphase I

Nature of multiple and orientation		% of multiples with all terminalized chiasma	% of multiples with one unterminalized chiasma
35-1			
Rings	Disjunctional	66.66	33.33
	Non-disjunctional	8.33	91.66
Chains	Disjunctional	66.66	33.33
	Non-disjunctional	33.33	66.66
40-4			
Rings	Disjunctional	86.66	13.33
	Non-disjunctional	85.71	14.28
Chains	Disjunctional	—	—
	Non-disjunctional	83.33	16.66
45-8			
Chains	Disjunctional	44.44	55.55
	Non-disjunctional	50.00	50.00

The observations on terminalization of chiasmata in the interchange multiples at metaphase-I may have some relationship with their orientation (Table 5). As is evident, the complete terminalization in multiple of 35-1 had some bearing on its orientation due to change in effective intercentromeric distance brought about by terminalized/non-terminalized chiasmata. However, this assumption does not hold true again, in case of interchange multiples of 40-4 where 86.6 per cent disjunctional ring multiples had terminalized chiasmata, while 85.71 per cent of non-disjunctional ring multiples also had fully terminalized chiasmata. Similar was the case with chain multiples of 40-4 where 83.3 per cent of non-disjunctional chains had complete terminalization of chiasmata.

As is apparent from the foregoing account, the orientation of multiple, which is of primary importance for fertility/survival of an interchange complex, can hardly be explained on physical characteristics of chromosomes and position of chiasmata alone. The mode of orientation

has been reported to be under genetic control (Thompson 1956; Lawrence 1958 and Rees 1961).

Besides the above factors the fertility can also be effected by discordant orientation of ring multivalents on the metaphase plate i.e. two centromeres on opposite sides of a ring are directed to different spindle poles, and the two intervening centromeres are not co-oriented. This type of orientation may lead to numerical and/or genetically unbalanced gametes depending on the fact whether the ring disjoins to give unequal (3:1) or equal (2:2) segregation. Percentage of such rings being 26.58 in 35-1, which in turn accounts for 14 per cent cells with unequal disjunction (7:5) at anaphase-I (Table 3). It implies that roughly about 50 per cent of discordant rings disjoin to give 3:1 segregation and others disjoin to give numerically equal (2:2) segregation. In 40-4, 14 per cent of cells show discordant rings, whereas 12 per cent of cells show unequal disjunction of 7:5 at anaphase-I (Table 3). In either case, however, the resultant gametes are bound to be unviable, carrying as they are deficient/duplicate combinations.

The chiasma frequency in all the interchange heterozygotes was found to be higher as compared to homozygotes (Table 2), which could be due to delayed terminalization/non terminalization of chiasmata and/or increase in crossing over following mutagenic treatment (Lawrence 1961). In the present heterozygotes, however, latter appears to be the case, as coefficient of terminalization was found to be either equal or more than that of homozygote (Table 2).

If pollen stainability is taken as an index for viability of gametes it is evident that viability is 31.35, 60.6 and 59.2 per cent in 35-1, 40-4 and 45-8 respectively as compared to 97.12 per cent of the homozygote (Table 4). This is closely corroborated by the expected percentage of viable gametes (Table 1).

Pollen grain size of translocation heterozygotes showed a higher range of variability as compared to homozygotes. This could be accounted on the assumption that at meta-anaphase-I, most of cells in heterozygotes show a non-disjunctional orientation resulting into duplication/deficiencies, that is turn manifest in terms of variability in size and stainability of pollen grains.

It is expected that studies on the progenies of these interchange heterozygotes in *Sesbania aegyptiaca* would result in establishment of hetero and homozygous translocation stocks which could be further utilized in genetic analysis.

Summary

Three plants with reciprocal translocation of chromosomes were isolated from M_1 progeny of *Sesbania aegyptiaca* (Poir.) Pers., after treating the dry seeds with 10, 20, 30, 40 and 45 Krad doses of gamma rays. The cytological observation of these plants revealed that in each case a reciprocal interchange involving two non-homologous chromosomes has taken place. The plants have been studied for their meiotic behaviour, with particular reference to type and frequency of multiple associations, mode of orientation of interchange multiple at metaphase-I, disjunction at anaphase-I, and chiasma frequency. The consequences of these factors *vis-à-vis* the fertility has been discussed.

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