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MULTIVALENT FREQUENCY AND SEED FERTILITY IN RAW AND EVOLVED TETRAPLOIDS OF BRASSICA CAMPESTRIS VAR. TORIA

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With 9 Figures in the Text

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The extent of multivalent formation has been studied in induced autopolyploids of various plant species by many authors primarily to find out whether in such plants the frequency of occurrence of multivalents is in any way correlated with the extent of seed fertility. The formation of multivalents resulting in the production of gametes with aneuploid chromosome numbers was considered by DARLINGTON (1937) to be the primary cause for the reduced fertility of autopolyploids. However, the data obtained by several workers during the last 20 years have failed to reveal any consistent relationship between multivalent frequency and seed sterility and it is now clear that fertility in auto- as well as allo-polyploids is influenced not only by the presence or absence of multivalents but also by other kinds of genic control (MÜNTZING 1956). Thus, plants with a high frequency of quadrivalents may be perfectly fertile and plants with a regular bivalent formation highly sterile. In many of the induced polyploids selection for fertility has been possible and by providing an artificial evolution to the raw polyploids, several strains of commercial value have been produced in crops like rye and clovers. Such evolved autopolyploids provide good material for studying the effect of selection for agronomically valuable characters on meiotic behaviour. In maize, GILLES and RANDOLPH (1951) have studied meiosis in a tetraploid strain at the beginning and end of a ten year period. They found that there were fewer quadrivalents and more bivalents at the end of the period than at the beginning. Similar data concerning the frequency of aneuploid aberrants have been obtained by HAGBERG (1953) in rye. HILPERT (1957) observed that selection for seed fertility as well as for tillering during three generations in autotetraploid rye resulted in a significant increase in meiotic regularity.

Polyploidy was induced in *Brassica campestris* L. var. *toria* DUTH. & FULL. ($2n = 20$) in 1941 at the Indian Agricultural Research Institute, New Delhi, by RAMANUJAM and DESHMUKH (1945). The average seed fertility in the raw autotetraploids was found to be 2.7 seeds per siliqua, the corresponding average figure for the diploids being 13.8 seeds. Straight selection for fertility led to some improvement, but a static condition was reached as regards improvement in seed fertility after seven generations of selection and controlled cross-pollinations among plants with good fertility. At this stage, a revised selection procedure similar to the "Mass Pedigree System" of HARLAND (1949) was applied by PARTHASARATHY and RAJAN (1953). The effectiveness of this method which essentially consisted in the isolation of the elite population and bulking of the seeds of the selected plants before sowing, was seen after two generations when the frequency of plants in the higher fertility classes showed a rise and the modal classes of the tetraploids shifted to the same class as in the diploid population. The trend of improvement in fertility in the autotetraploid *Toria* was maintained during subsequent generations (RAJAN 1955). PARTHASARATHY and RAJAN (1953) concluded that the highly cross-fertilized nature of *Toria* and the operation of self incompatibility alleles govern to a large extent the fertility of the tetraploid population and that the revised breeding method adopted by them has brought about favourable gene combinations resulting in increased fertility.

In view of the strikingly high seed fertility of the tetraploid *Toria* strains developed by PARTHASARATHY and RAJAN (1953), we studied the types of chromosome associations found during meiosis in microsporocytes in plants belonging to the C_{17}, C_{18} and C_{19} generations grown during 1956, 1957 and 1958 respectively. We produced in 1956 by colchicine treatment tetraploids of strain 22, which had been used originally by RAMANUJAM and DESHMUKH (1945) for chromosome doubling and studied the frequency of occurrence of multivalents in the raw tetraploid plants also. The data are presented and discussed in this paper.

Material and Methods

Autotetraploids of strain 22 of *Toria* belonging to the C_{17}, C_{18} and C_{19} generations were used in this study. To produce again tetraploids of strain 22, seedlings were treated in 1956 with colchicine by the following method. A small quantity of cotton was dipped in a mixture of equal parts of 0.4% aqueous solution of colchicine and glycerine and placed on the growing points of 2 to 3 inch high seedlings. Drops of the colchicine-glycerine solution were applied on the cotton plug at frequent intervals and the treatment lasted for 36 hours. At the end of the treatment, the growing points of the seedlings were washed in water. Three tetraploid seedlings were obtained in this way and plants belonging to both C_1 and C_2 generations were cytologically examined.

Meiosis was studied in PMC squashes. Anthers were fixed in CARNOY's mixture (6 parts absolute alcohol, 3 parts chloroform and 1 part glacial acetic acid) for 24 hours. They were then transferred to propionic-alcohol (1:3). The propionic acid component of the fixative contained a saturated solution of ferric acetate (SWAMINATHAN et al. 1954). The anthers were squashed 24 hours later in a drop of propiono-carmine. Since the cytoplasm also tended to get stained, a drop of 45% acetic acid was added to a drop of the stain while squashing. This led to a clear differentiation and as can be seen from the microphotographs, the preparations were suitable for critical analysis. Fixations were done from C_1 and C_{17} plants during 1956, from C_2 and C_{18} plants during 1957 and from C_{19} plants during 1958. In every case, the material was fixed on the same date and at the same time and only clear diakinesis and early metaphase plates were analysed. Since no significant inter-plant differences in the frequency of multivalent associations were observed in the different generations, the detailed study was confined to one plant of each generation.

Seed fertility was calculated in the tetraploid plants used in cytological studies by taking the average of 10 siliquae from the middle of the main stem.

Experimental Results

Multivalent Frequency. The different types of chromosome associations found at diakinesis and metaphase I were scored and the data are given in Table 1. The number of cells with different frequencies of multivalents (quadrivalents + trivalents) found in the different generations is given in Table 2. Associations higher than quadrivalents were not observed and the maximum number of quadrivalents observed in a cell was 8 (Figs. 4 and 5). Two such cells were found in a plant belonging to the C_2 generation. The types of quadrivalents observed are given in Table 3. It can be seen from the data that symmetrical arrangements like simple chains and rings occurred most frequently. The "frying-pan" type of quadrivalent was seen rarely (Fig. 2). The mean number of chiasmata per cell in the different tetraploids is given in Table 1. There is apparently no difference in the chiasma frequency of plants belonging to the different generations. The mean frequency of chiasmata per cell in diploid *Toria* was 18.3. There is thus no prominent difference between the diploids and autotetraploids in the number of chiasmata per bivalent. From the data in Tables 1 and 2 it is clear that there is a decrease in the frequency of quadrivalents and an increase in the frequency of bivalents in the C_{17}, C_{18}, C_{19} generations in comparison with C_1 and C_2 generations (see also Figs. 1 to 8). The data were subjected to a 't' test and it was found that the differences in the mean frequency of quadrivalents and bivalents between the early and late generations were highly significant. Thus, there is a definite tendency for an increase of normal bivalent synapsis in the later generations. In comparison with C_1 plants, there is an increase in the frequency of trivalents and univalents in the subsequent generations (Fig. 9). Ramanujam and Deshmukh (1945) reported a quadrivalent frequency of only

Table 1. *Chromosome associations and chiasma frequency in tetraploid Toria*

Generation	No. of cells studied	Frequency per cell								Mean No. of Xta per cell
		quadrivalents		trivalents		bivalents		univalents		
		range	mean	range	mean	range	mean	range	mean	
C_1	40	2—7	3.95	0—1	0.15	6—16	11.675	0—2	0.40	36.2
C_2	41	1—8	3.41	0—3	0.73	5—16	11.50	0—3	1.17	36.8
C_{17}	80	0—5	2.08	0—3	0.44	8—20	14.86	0—5	0.64	36.6
C_{18}	40	0—6	2.00	0—2	0.55	7—20	14.70	0—3	0.95	36.7
C_{19}	50	0—5	1.68	0—4	0.74	9—20	14.98	0—4	1.10	36.4

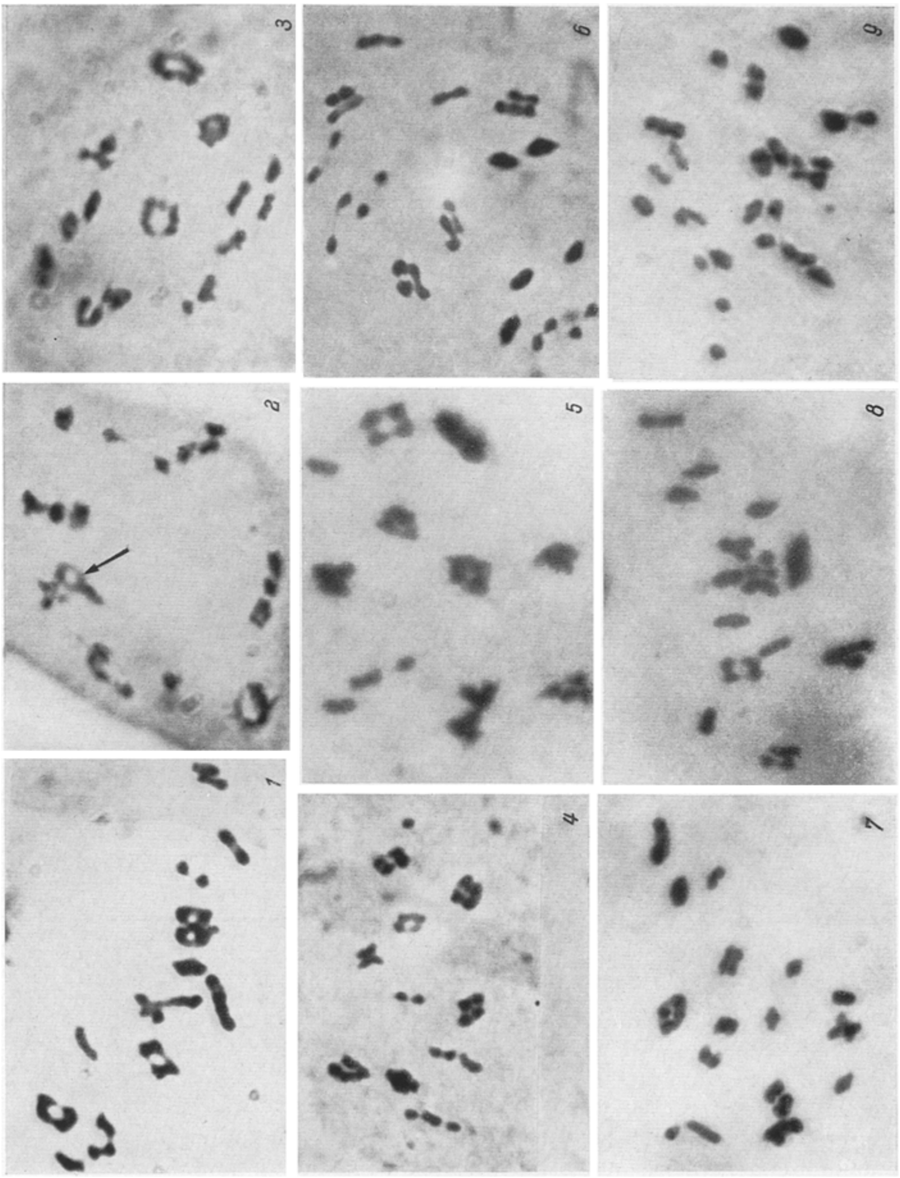
Table 2. *Frequency of multivalents in Toria tetraploids*

Generation	No. of cells with a multivalent frequency of									Mean No. of multivalent per cell ± S. E.	Coefficient of realization
	0	1	2	3	4	5	6	7	8		
C_1	0	0	4	5	18	10	2	1	0	4.10 ± 0.17	0.41
C_2	0	0	2	9	18	9	1	1	2	4.14 ± 0.19	0.414
C_{17}	5	18	18	21	8	8	2	0	0	2.52 ± 0.22	0.252
C_{18}	3	5	14	9	4	4	1	0	0	2.55 ± 0.23	0.255
C_{19}	7	11	8	9	9	5	1	0	0	2.42 ± 0.22	0.242

Table 3. *Types of quadrivalents observed*

Generation	No. of quadrivalents of type					
	— — — —	Y	X	□	∞	⚲
C_1	37	6	12	101	0	1
C_2	17	5	10	106	1	1
C_{17}	11	8	4	143	0	0
C_{18}	16	0	5	59	0	0
C_{19}	3	4	9	68	0	0

1.94 per cell in C_1 plants of autotetraploid *Toria*. The maximum number of quadrivalents in a cell observed by them was 5. The mean frequency of 4.1 multivalents per cell found by us in C_1 plants is hence much higher than that recorded by them. HOWARD (1939) observed an average of 4 quadrivalents per cell in autotetraploid *Brassica oleracea* ($2n = 36$) and the frequency observed by us in *Toria* is of a similar order. Even in auto-octoploid *Toria*, RAMANUJAM and



Figs. 1—9. Metaphase I of meiosis in autotetraploid *B. campestris* var. *toria*
Figs. 1—3. C_1 generation. Figs. 4 and 5. C_2 generation. Fig. 6. C_{18} generation. Figs. 7—9. C_{19} generation. Fig. 1. $7_{IV} + 6_{II}$. Fig. 2. $5_{IV} + 9_{II} + 2_{I}$. Note the "frying-pan" type of quadrivalent (arrow). Fig. 3. $4_{IV} + 1_{III} + 10_{II} + 1_{I}$. Fig. 4. $8_{IV} + 3_{II} + 2_{I}$. Fig. 5. $8_{IV} + 3_{II} + 2_{I}$. Fig. 6. 20_{II}. Fig. 7. $3_{IV} + 1_{III} + 12_{II} + 1_{I}$. Fig. 8. $1_{IV} + 1_{III} + 16_{II} + 1_{I}$. Fig. 9. $1 + 16_{II} + 5_{I}$.
(Magnification: ×1500)

Deshmukh (1945) observed only one quadrivalent per cell and it seems probable that the cytological preparations obtained by them were not suitable for a critical study.

Pollen and Seed Fertility. The pollen and seed fertility data collected from the plants subjected to cytological examination are summarised in Table 4. It will be noticed that while pollen fertility is more or less the same in all the plants, seed setting is very poor in the early generations. There has been a striking improvement in fertility in the C_{17}, C_{18} and C_{19} generations. As already mentioned, the plants of the later generations are derivatives of the mass pedigree system of selection adopted by Parthasarathy and Rajan (1953). The tetraploids of the C_{17}, C_{18} and C_{19} generations have nearly the same seed fertility as the diploids.

Table 4. *Pollen and seed fertility in tetraploid Toria*

Generation	Mean percentage of stainable pollen	No. of seeds per siliqua	
		range	mean
C_1	92.6	0—3	1.5
C_2	91.1	1—5	3.0
C_{17}	95.0	7—20	14.5
C_{18}	90.1	8—20	14.9
C_{19}	90.2	10—21	16.8

Discussion

Gilles and Randolph (1951) were the first to demonstrate the occurrence of a gradual shift from a multivalent to bivalent type of synapsis in an induced autopolyploid. From their observations on chromosome pairing in a strain of autotetraploid maize at the beginning and end of a ten year period, they concluded that multivalent associations of chromosomes which are characteristic of most autopolyploids of recent origin may not persist indefinitely in succeeding generations. If this happens, the possibility exists that many natural polyploids with regular bivalent configurations may have originated as autopolyploids. Müntzing and Prakken (1940) had earlier suggested the same possibility since they found a genotypically controlled tendency to form only bivalents in an autotriploid *Phleum pratense.* Of still greater interest is the recent finding of Riley and Chapman (1958) and Sears and Okomoto (1958) that the regular bivalent formation normally found in *Triticum aestivum* is under the control of a single gene or a block of genes situated in one chromosome pair. In the absence of this chromosome pair, pairing occurs among the homologous chromosomes belonging to the different genomes, while in its presence, pairing is restricted to the wholly homologous chromosome pairs within each genome. There is thus abundant evidence now to suggest that there is a tendency for autopolyploids — both naturally occurring and induced — to acquire either gradually or suddenly the cytological characteristics of a diploid.

The results of the present study indicate that the tendency for reduced multivalent formation following selection for some years found by Gilles and Randolph (1951) in maize, also exists in *Toria.* Our data show even a more striking drop in multivalent formation than that recorded in maize. This may be due to the fact that *Toria* being a plant with small chromosomes with not more than one chiasma per chromosome arm in a bivalent, the initial coefficient of realization of multivalents is itself low. Also, we have studied tetraploid plants which are nearly twice as old as the maize tetraploids investigated by

GILLES and RANDOLPH (1951), and which have been subjected to an intense breeding pressure. BREMER and BREMER-REINDERS (1954) found a positive effect on regularity of meiosis by recurrent selection of fertile plants during 7 generations in autotetraploid winter rye. HILPERT (1957) also observed that selection for high seed setting and good tillering has a pronounced effect in increasing the frequency of bivalent associations in autotetraploid rye. While the data concerning the direct correlation between multivalent frequency and seed setting in autopolyploid plants reported by different authors are in many cases contradictory and confusing, it is clear that in several autotetraploids there is a decrease in multivalent associations and a rise in bivalent frequency coincident with an increase in seed fertility. Our data as well as those of BREMER and BREMER-REINDERS (1954) and HILPERT (1957) are quite in accord on this point though the plant species studied are different. Thus, while there may be no positive correlation between multivalent frequency and seed sterility within plants of an autopolyploid population in any particular season, the increase in seed setting brought about by practising selection and breeding among them seems to be invariably accompanied by a significant increase in the number of chromosomes forming bivalents. The relationship between these two events, hence, may not be wholly casual and DARLINGTON's (1937) original surmise concerning the role of multivalents in causing seed sterility may to a great extent be correct. It is not clear whether increase in seed fertility and decrease in multivalent frequency are the only coincident effects of the evolution given by the plant breeder to an induced autopolyploid population. In autotetraploid rye, HILPERT (1957) found that even selection for tillering was effective in bringing about a shift towards more regular meiosis. On the other hand, where the stress of selection has been on factors like fodder yield in addition to seed fertility, there is apparently no increase in meiotic regularity with increasing fertility (ARMSTRONG and ROBERTSON 1956). In cross-pollinated plants like maize, rye and *Toria* which are grown solely for seed there is a common trend with regard to this relationship. Only the extent of the shift witnessed varies, probably depending on the initial coefficient of realization of multivalents and the breeding system adopted.

How is this gradual diploidization as regards meiotic behaviour brought about in an autopolyploid plant grown primarily for its seed? There are two major possibilities. First, the initially homologous chromosomes of an induced autopolyploid may undergo sufficient divergence by the accumulation of structural changes, both large and cryptic, so as to bring about a significant shift from a multivalent to a bivalent type of synapsis in the "evolved" as compared to the "raw" polyploids. STEBBINS (1956) has shown that it will be possible to experimentally induce this shift by resorting to irradiation techniques. Secondly, the changes tending to convert an autopolyploid into a functional diploid may be genically controlled. The effectiveness of selection and hybridization in increasing the seed fertility of autopolyploids is generally attributed to the evolution of favourable gene combinations. The same process is apparently effective in reducing the multivalent frequency since a genotypically controlled tendency for bivalent formation in some autopolyploids seems to be now well established. These two mechanisms, however, may not be mutually exclusive. On the other hand they may operate simultaneously and their cumulative effect may condition the pace with which the diploidization process progresses. The genetic mechanism controlling the cytologically diploid behaviour of hexaploid wheat, as postulated by Riley and Chapman (1958), however, seems to be of a single major gene type and these authors believe that the development of this "multivalent suppressor" gene (or gene system) could have arisen by mutation. No such mutation has been detected so far in induced autopolyploid plants and all the available evidence suggests that the shift from a multivalent to a bivalent type of pairing happens only slowly and progressively. The fact that sudden variation with regard to this character has not so far been observed in induced autopolyploid plants does not, however, invalidate in any way the wheat findings. Bread wheat is more a segmental allopolyploid and the possibilities for such changes to occur are hence greater than in a pure autopolyploid. In the *Toria* tetraploids studied by us, the only evidence in favour of a slight accumulation of structural changes was provided by the observed increase in the frequency of trivalents and univalents in the later generations. The small size of the chromosomes rendered critical karyotypic studies difficult and we have no direct data to suggest that structural changes have occurred in the evolved tetraploids. It is also questionable whether spontaneous structural changes would occur at such a rapid rate as to account for the observed difference in multivalent frequency between the early and late generations. It seems more likely that both genic control as well as some degree of structural differentiation are involved. Whatever may be the precise mechanisms responsible, it is of considerable interest that a gradual process of cytological diploidization occurs in autotetraploid *Toria* simultaneously with a reduction in seed sterility and as a result, it is difficult to refrain from inferring a relationship between the two both in cause and consequence. That factors other than multivalent formation are also concerned with seed fertility in the autotetraploid *Toria* population is, however, obvious since some degree of multivalent formation persists in the evolved tetraploids though the seed fertility in such plants has reached the diploid level.

Summary

1. The types of chromosome associations found during diakinesis and metaphase I of meiosis in microsporocytes was studied in plants belonging to the C_1, C_2, C_{17}, C_{18} and C_{19} generations of induced tetraploids of strain 22 of *Brassica campestris* var. *toria.* It was found that there was a considerable decrease in the frequency of multivalents and increase in the frequency of bivalents in tetraploid plants belonging to the later generations in comparison with those belonging to the earlier generations.

2. The mean number of seeds per siliqua in the C_1 and C_2 tetraploids was 1.5 and 3.0 respectively, while this number increased to 14.5, 14.9 and 16.8 in C_{17}, C_{18} and C_{19} generations respectively. The increase in seed fertility had been brought about by adopting the mass pedigree system of selection.

3. From the observation that coincident with an increase in seed fertility there is a tendency towards a decrease in multivalent frequency in autotetraploid *Toria*, it is suggested that the relationship between these two processes may not be wholly casual.

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