Decrease in Quadrivalent Frequency over a 10 Year Period in Autotetraploids in Two Species of Grain Amaranths¹

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Improvement in pollen and seed fertility has been observed in advanced generations of induced autotetraploids of a number of crop plants like maize (Gilles and Randolph 1951), *Brassica campestris* var. *toria* (Swaminathan and Sulba 1959), rye (Hilpert 1957), barley (Bender and Gaul 1966) and *Coix* (Venkateswarlu and Rao 1976). While in some cases the improvement in fertility could be correlated with reduced quadrivalent formation and increased bivalency (Hilpert 1957, Venkateswarlu and Rao 1976) in others no such correlation was evident (Muntzing 1951, McCollum 1958, Morrison and Rajathy 1960). With this in mind the situation was examined in two autotetraploid lines of C_{10} generation of two species of grain amaranths viz. *Amaranthus caudatus* Linn. and *A. edulis* Spegazzini of which the autotetraploids raised earlier (Pal and Khoshoo 1977) were available for study. Analysis of these lines was made for their morphological features as also for pollen fertility. The cytological studies were done following usual technique (Darlington and LaCour 1976).

Results

Morphologically the plants of the C_{10} generation did not reveal any significant difference as compared to those reported for C_0 and C_1 generations and maintained the general gigantism of the determinate parts like leaves, stomata, pollen and seed. A small percentage of male plants in C_1 as observed earlier by Pal and Khoshoo (1977) was also observed in C_{10} generation.

Meiosis

Both the species at the diploid level had $2n=32$ and regularly formed 16 bivalents at meiotic metaphase I followed by regular meiotic behaviour resulting in normal pollen fertility (for details see Pal and Khoshoo 1973 and 1977).

Meiosis in the C_{10} autotetraploids in the two species revealed varying number of quadrivalents, trivalents, bivalents and univalents at metaphase I (Figs. 1-4, Tables 1 and 2). The frequency of trivalents and the univalents, however, was very low (Tables 1-2). The mean frequencies of quadrivalents, trivalents, bivalents and univalents observed per cell were 2.31, 0.66, 25.93 and 0.92 in *A. caudatus* and 1.91, 0.67, 26.76 and 0.84 in *A. edulis*, respectively. On the whole, an average of less than 20 per cent chromosomes were involved in quadrivalent formation. It

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appeared that the few trivalents and univalents, observed in low frequencies were actually disjoined quadrivalents while some univalents may be the result of precocious disjunction (Fig. 2). Cases of precocious disjunction have been reported

Figs. 1-6. 1 and 2, *A. caudatus* metaphase I, 3 IV+26 II and 8 IV+1 III+14 II+1 I. 3 and 4 *A. edulis*, metaphase I, 2 IV+28 II and 5 IV+22 II. 5 and 6, anaphase I, 32:32 *A. caudatus*, *A. edulis*. All $\times 1650$.

earlier in the diploids of the two species (Khoshoo and Pal 1972 and Pal and Khoshoo 1973).

Anaphase I as a rule is regular in the autotetraploids in both the species resulting in 32:32 distribution of chromosome (Figs. 5-6). However, a few cells

Table 1. Chromosome association at metaphase I in autotetraploid of *A. caudatus*

Generation	Number of cells	Quadrivalents			Trivalents			Bivalents			Univalents		
		Range	Mean		Range	Mean		Range	Mean		Range	Mean	
*C ₀	25	4-9	6.800±0.29		0-2	0.240±0.02		13-23	17.320±0.07		0-4	1.400±0.03	
*C ₁₀	13	2-7	4.615±0.27		0-2	0.461±0.02		18-28	26.615±0.25		0-2	0.923±0.02	
	10	3-7	6.100±0.20		0-1	0.300±0.01		10-26	18.600±0.31		0-8	1.500±0.05	
	10	4-7	5.800±0.03		—	—		17-23	19.500±0.31		0-4	1.800±0.03	
	15	2-7	5.533±0.03		0-1	0.133±0.01		18-28	20.333±0.39		0-1	0.800±0.03	
	15	2-7	5.533±0.03		0-1	0.133±0.01		18-28	20.333±0.39		0-4	0.800±0.03	
*C ₁₀	30	1-4	1.800±0.03		0-1	0.167±0.03		24-30	27.533±0.49		0-4	1.233±0.03	
	30	1-4	1.867±0.05		0-1	0.433±0.03		24-30	27.167±0.31		0-3	0.901±0.02	
	30	1-3	1.600±0.03		0-1	0.600±0.05		24-30	27.467±0.30		0-3	0.866±0.02	
	30	1-4	2.433±0.06		0-2	0.300±0.007		24-30	26.000±0.30		0-4	1.368±0.03	
	30	1-4	2.233±0.06		0-2	0.567±0.03		24-30	26.233±0.27		0-4	0.901±0.03	
	30	1-6	3.266±0.05		0-2	0.433±0.001		20-30	24.433±0.20		0-3	0.771±0.02	
	30	1-5	2.833±0.03		0-1	0.700±0.003		20-28	24.567±0.20		0-3	1.434±0.05	
	30	1-4	2.534±0.05		0-2	0.766±0.02		22-28	25.300±0.20		0-4	0.966±0.03	
	30	1-4	2.134±0.03		0-1	0.770±0.03		22-30	25.966±0.19		0-2	1.322±0.05	
	30	1-4	2.434±0.02		0-2	0.932±0.02		22-28	25.234±0.31		0-2	1.000±0.03	

* Figures for C₀ and C₁ have been taken from Pal and Khoshoo (1977).

Table 2. Chromosome association at metaphase I in autotetraploid of *A. edulis*

Generation	Number of cells	Quadrivalents		Trivalents		Bivalents		Univalents	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean
*C ₀	13	4-8	6.00 ± 0.27	0-1	0.059 ± 0.02	16-24	19.529 ± 0.23	0-4	0.765 ± 0.06
*C ₁	25	2-8	4.840 ± 0.29	0-1	0.040 ± 0.01	14-28	21.720 ± 0.71	0-4	1.080 ± 0.04
C ₁₀	30	1-3	1.400 ± 0.03	0-2	0.600 ± 0.01	26-30	27.933 ± 0.69	0-3	0.734 ± 0.03
	30	1-4	2.000 ± 0.06	0-1	0.233 ± 0.009	24-30	27.200 ± 0.73	0-2	0.601 ± 0.02
	30	1-4	2.033 ± 0.06	0-2	0.866 ± 0.02	22-30	26.202 ± 0.70	0-2	0.866 ± 0.04
	30	1-4	2.333 ± 0.05	0-1	0.633 ± 0.01	22-28	25.801 ± 0.65	0-4	1.167 ± 0.03
	30	1-4	2.133 ± 0.03	0-1	0.66 ± 0.01	22-30	26.334 ± 0.51	0-2	0.802 ± 0.02
	30	1-3	21.00 ± 0.05	0-1	0.600 ± 0.01	24-28	26.400 ± 0.52	0-2	1.000 ± 0.04
	30	1-4	2.166 ± 0.05	0-1	0.833 ± 0.02	22-30	25.900 ± 0.47	0-4	1.037 ± 0.04
	30	1-3	1.266 ± 0.04	0-1	0.833 ± 0.02	26-30	27.767 ± 0.51	0-2	0.903 ± 0.04
	30	1-3	1.466 ± 0.03	0-1	0.667 ± 0.008	26-30	27.534 ± 0.50	0-4	1.067 ± 0.03
	30	1-3	1.266 ± 0.03	0-2	0.933 ± 0.01	26-30	27.602 ± 0.50	0-3	0.933 ± 0.03

* Figures for C₀ and C₁ have been taken from Pal and Khoshoo (1977).

with laggards, bridges and unequal segregation and precocious division of univalents at anaphase I were also observed. These abnormalities perhaps resulted in the formation of micropollen which was recorded in low frequencies in the autotetraploid lines of both the species. Anaphase II was apparently normal.

Discussion

In contrast to the diploids the unique feature of the induced autotetraploids in both *A. caudatus* and *A. edulis* was the occurrence of stray predominantly male plants in the C_{10} generation. The occurrence of such predominantly male plants was also reported for these species in the C_1 generation by Pal and Khoshoo (1977). Perhaps polyploidy disturbed the intricate genic balance for sex expression and brought about some sort of hormonal disturbance disrupting the normal and well set pattern of male and female flower formation. Such an abnormal development has also been reported for some of the interspecific hybrids in the genus (Pal 1972). It is therefore clear that the selection for ten generations has not resulted in the attainment of an entirely normal sex behaviour.

At metaphase I in the autotetraploid of the two species nearly 20% chromosomes were involved in quadrivalent formation. A few trivalents and univalents were also reported in these taxa for C_0 and C_1 generations (Pal and Khoshoo 1977). The notable feature was the reduction in the frequency of quadrivalents. In the ten plants of C_{10} *A. caudatus* which were studied the mean quadrivalent frequency varied from a minimum of 1.600 ± 0.03 to a maximum of 3.266 ± 0.05 (see Table 1). Similar reduction was noted in C_{10} *A. edulis* also, where the minimum and maximum frequencies were 1.400 ± 0.03 and 2.33 ± 0.05 , respectively (Table 2). The figures in C_0 generations of mean quadrivalent frequency in the *A. caudatus* and *A. edulis* were 6.800 ± 0.29 and 6.00 ± 0.27 , respectively. As is apparent these frequencies represent a considerable reduction when compared to the data for C_0 and C_1 generations in the respective species.

A similar decline in the quadrivalent frequency accompanying selection for fertility was also reported by Gilles and Randolph (1951) in tetraploid maize, Hilpert (1957) in rye, Swaminathan and Sulba (1959) in *Brassica campestris* var. *toria*, Bender and Gaul (1966) in barley and Venkateswarlu and Rao (1976) in *Coix*.

The reduction in quadrivalent frequency in the aforementioned cases appears to be correlated with inbreeding and selection of vigorous and fertile seed stock in the succeeding generations. The only possible inference which can be drawn from these observations is that the individuals with less quadrivalents and higher bivalent frequency were more fertile and vigorous. The increased bivalency perhaps resulted from increased preferential pairing through gradual differentiation and accumulation of heterozygosity over a number of generations.

It has been observed by Skirm (1942) and Sybenga (1966) that even small amount of structural heterozygosity leads to preferential pairing. Other reasons for the noted decrease in quadrivalent frequency could be the establishment of gene or gene complexes which suppressed multivalent association of chromosomes.

In contrast to these, instances of improvement in fertility associated with an

increase in quadrivalent frequency have also been reported in rye (Müntzing 1951) and *Dactylis* (McCollum 1958). Morrison and Rajhathy (1960a) who studied a number of autotetraploids of different species found that there was neither evidence for heritable variation in quadrivalent formation nor for variation in the number of quadrivalents and their affect on fertility. They (Morrison and Rajhathy 1960b) also did not find any reduction in quadrivalent frequency in advanced generations of many autotetraploids studied by them. The data in the present study did not reveal any relationship between the formation of quadrivalents and univalents and other meiotic irregularities on the one hand, and the pollen sterility on the other. In the present investigations it was observed that the quadrivalent frequency did not affect the fertility of the plants. While Hazarika and Rees (1967) found a higher fertility correlated with higher quadrivalent frequency in rye. It may however be mentioned that the pollen and seed fertilities were high even in the C_0 and C_1 generations of the present autotetraploids (see Pal and Khoshoo 1977). Moreover, in the isogenic autotetraploids as long as there is orderly disjunction of quadrivalents with no numerical chromosome differences, quadrivalent correlated sterility should not ordinarily be expected.

It is therefore obvious that whatever sterility was encountered may be more due to genetic physiological causes rather than irregular distribution of chromosomes on account of multivalent formation and other meiotic abnormalities.

However, whatever may be the reasons for improved fertility and the reduction in quadrivalent frequency, it is significant that in C_{10} generation of the two induced autotetraploid lines of grain amaranths a considerable meiotic regularity has been achieved which, if the present studies are an indication is likely to improve further in the succeeding generations as a consequence of small chromosomes, low chiasma frequency, some amount of preferential pairing, high degree of cross pollination and heterozygosity and selection for vigour and fertility.

Summary

C_{10} autotetraploid lines in two grain species of *Amaranthus* viz. *A. caudatus* and *A. edulis* were evaluated for their morphology and meiosis. Morphologically the C_{10} plants in both the species maintained the general gigantism in the determinate parts like leaves, stomata, pollen and seed as observed in C_0 and C_1 . The meiosis was typically autopolyploid with quadrivalents, trivalents, bivalents and univalents. The mean quadrivalent frequency as observed at metaphase I varied from a minimum of 1.600 to maximum of 3.266 in *A. caudatus* while the respective figures in *A. edulis* were 1.400 and 2.33. The mean quadrivalent frequencies for C_0 generation in the two species were 6.800 ± 0.29 and 6.00 ± 0.27 , respectively. These figures represented statistically significant reduction over C_0 and C_1 data. The present results have been discussed in relation to similar or contradictory observations of earlier authors on the metaphase I associations in advanced generations of autotetraploids.

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Literature cited

- Bender, K. and Gaul, H. 1966. Zur Frage der Diploidisierung autotetraploider Gerste. *Z. Pflanzenzücht.* **56**: 164–183.
- Darlington, C. D. and LaCour, L. F. 1976. *Handling of Chromosomes*. 6th Edn. George Allen & Unwin Ltd. London.
- Gilles, A. and Randolph, L. F. 1951. Reduction of quadrivalent frequency in autotetraploid maize during a period of ten years. *Amer. J. Bot.* **38**: 12–17.
- Hazarika, M. H. and Rees, H. 1967. Genotypic control of chromosome behaviour in rye. X. chromosome pairing in autotetraploids. *Heredity* **22**: 317–332.
- Hilpert, G. 1957. Effects of selection for meiotic behaviour in autotetraploid rye. *Hereditas* **43**: 318–322.
- Khoshoo, T. N. and Pal, M. 1972. Cytogenetic patterns in *Amaranthus*. *Chromosomes Today* **3**: 259–267. Hafner Publ. Co., New York.
- McCollum, G. D. 1958. Comparative studies of chromosomes pairing in natural and induced tetraploid. *Chromosoma* **9**: 571–605.
- Morrison, J. W. and Rajhathy, T. 1960a. Frequency of quadrivalents in autotetraploid plants. *Nature* **186**: 528–530.
- and — 1960b. Chromosome behaviour in autotetraploid cereals and grasses. *Chromosoma (Berlin)* **11**: 297–309.
- Müntzing, A. 1951. Cytogenetic properties and practical values of tetraploid rye. *Hereditas* **37**: 18–84.
- Pal, M. and Khoshoo, T. N. 1973. Evolution and improvement of cultivated amaranths. VI. Cytogenetic relationships in grain types. *Theor. Appl. Genet.* **43**: 242–251.
- and — 1977. Evolution and improvement of cultivated amaranths VIII. Induced autotetraploidy in grain types. *Z. Pflanzenzüchtg.* **78**: 135–148.
- Skirm, G. W. 1942. Bivalent pairing in an induced tetraploid of *Tradescantia*. *Genetics* **27**: 635–740.
- Swaminathan, M. S. and Sulba, K. 1959. Multivalent frequency and seed fertility in raw and evolved tetraploids of *Brassica campestris* var. *toria*. *Z. Vererbungslehre* **90**: 385–392.
- Sybenga, J. 1966. The role of meiotic chromosomal pairing pattern in induced structural allopolyploidization of autopolyploids. *Acta. Agric. Scand. Suppl.* **16**: 43–45.
- Venkateswarlu, J. and Rao, P. N. 1976. Effect of inbreeding and selection for vigour and fertility on meiotic behaviour in autotetraploid Job's Tears, *Coix lacryma-jobi* L. *Theor. Appl. Genet.* **47**: 165–169.
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