Studies on the Occurrence, Cytology, Fertility and Breeding Behaviour of Aneuploids in Induced Autotetraploid Job's Tears

Panuganti N. Rao

Department of Botany, Andhra University, Waltair, India

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The occurrence and frequency of aneuploids in relation to their effects on fertility and stability of autotetraploids have been studied in many plant species (see Ellerström and Sjödin 1966, Ahloowalia 1971). In the present investigation, the occurrence and meiotic behaviour of aneuploids, their fertility and breeding behaviour in induced autotetraploid ($4n=40$) Job's tears (*Coix lacryma-jobi* L.), were studied and the results obtained are presented below.

Materials and methods

Cytological investigations were restricted to studies of pollen mother cells from acetocarmine squash preparations.

Pollen sterility was determined by staining with acetocarmine. Those unstained or imperfectly stained were scored as sterile. In each case, a minimum of 1,000 grains coming from three different male spikelets were counted.

For female fertility, all the mature false fruits in a plant on a given day, towards the end of the crop season, were harvested; hard and brownish-black false fruits were scored as fertile, the soft and chalky-white ones as sterile.

Results and discussion

Occurrence of aneuploids

A large progeny was raised from an open pollinated seed lot collected from three colchicine induced autotetraploid plants. Of these, 70 plants were scored cytologically for distribution of chromosome numbers. One of the plants showed $2n=21$ which obviously was resultant of apomictic development of an unfertilized egg or some other nucleus of the embryo sac containing an extra chromosome ($2n+1$) due to irregular separation during anaphase I in the parent (Venkateswarlu and Rao 1974). The cytological findings of the rest of the 69 plants, together with the theoretically possible gametic unions and zygotic chromosome numbers, and their expected frequencies are given in Table 1. Theoretical expectations of the gametic combinations were arrived at on the basis of an analysis of the frequencies of cells with various anaphase I distributions of chromosomes in the parental plant (Rao, unpublished). In this calculation, cells containing laggards were ignored. Of the 134 cells, free from laggards, observed at anaphase I, 92 (about 69%) showed 20: 20, 32 (about 24%) and 10 (about 7%) cells respectively showed 19: 21 and

Table 1. Theoretically possible gametic combinations and zygotic chromosome numbers with their expected and observed frequencies in the progenies of autotetraploid ($4n=40$) Job's tears

Gametic combinations*	Chromosome numbers ($4n$) in the progeny	Expected		Observed	
		Frequency	%	Frequency	%
18 × 18	36**	1	0.15	—	—
18 × 19	37**	6	0.89	—	—
19 × 19	38**	9	1.33	—	—
20 × 18	38**	36	5.33	—	—
19 × 20	39	108	15.98	5	7.25
18 × 21	39**	6	0.89	—	—
20 × 20	40	324	47.93	56	81.16
19 × 21	40**	18	2.66	—	—
18 × 22	40**	2	0.30	—	—
21 × 20	41	108	15.98	8	11.59
19 × 22	41**	6	0.89	—	—
20 × 22	42**	36	5.33	—	—
21 × 21	42**	9	1.33	—	—
21 × 22	43**	6	0.89	—	—
22 × 22	44**	1	0.15	—	—

* Calculated on the basis of anaphase I separation of chromosomes in the autotetraploid (Rao, unpublished).

** Zygotes formed by gametes having more than one chromosome difference.

Table 2. Chromosome associations at diakinesis and metaphase I in the 39-chromosome plant of Job's tears

Chromosome associations				No. of cells	%
IV	III	II	I		
9	1	—	—	1	1.32
9	—	1	1	1	1.32
8	1	2	—	2	2.63
8	—	3	1	2	2.63
7	1	4	—	5	6.58
7	—	5	1	3	3.95
6	1	6	—	7	9.21
6	—	7	1	6	7.89
5	1	8	—	10	13.16
5	—	9	1	7	9.21
4	1	10	—	9	11.84
4	—	11	1	8	10.53
3	1	12	—	4	5.26
3	—	13	1	4	5.26
2	1	14	—	4	5.26
2	—	15	1	2	2.63
1	1	16	—	1	1.32
Total				76	

18:22 separation of chromosomes. Thus the different distributions of 20:20, 19:21 and 18:22 were approximately in the proportion of 9:3:1 respectively. Assuming i) occurrence of similar distributions with the same relative frequencies in female gametogenesis, ii) all gametes, euploid and aneuploid, derived from such distributions are functional and iii) random mating of gametes, the expected zygotic contents of chromosomes and their frequencies were calculated. However, as is evident from Table 1, none of the zygotic chromosomal possibilities except 39, 40 and 41 were actually realized. This might possibly suggest that gametes having 18 or 22 chromosomes were not functional. This does not, however, seem to be the case because in both natural and artificial interspecific crosses between *C. aquatica*

Figs. 1-4. 1 and 2. Diakinesis in 39-chromosome plant. $\times 1050$. 1, 7 IV, 1 III (middle of the cell) and 4 II. 2, 2 IV, 15 II and 1 I. 3 and 4. Diakinesis in 41-chromosome plant. 3, 5 IV, 1 III and 9 II. 4, 1 V, 4 IV and 10 II.

Roxb. ($2n=10$) and tetraploid *C. lacryma-jobi* ($4n=40$), made reciprocally, F_1 hybrids with chromosome numbers of $2n=24, 25, 26$ and 27 (with 19, 20, 21 and 22 chromosomes of *lacryma-jobi* respectively and 5 of *aquatica*) occurred indicating that aneuploid gametes even with 22 chromosomes, in the tetraploid parent, functioned successfully in fertilization even on the male side (Rao unpublished). This being so, then it might be argued that the progeny examined is too small to enable recovery of the remaining zygotic classes. But a subsequent cytological study of 108 tetraploid plants, made on different occasions for screening interspecific hybrids, also did not reveal the presence of plants with less than 39 and more than 41 chromo-

somes. Among these 108 plants, 88 (81.48%) showed $4n=40$, 13 (12.04%) had $4n=41$ and 7 (6.48%) showed $4n=39$ chromosomes. These were not included in the data given in Table 1, because the exact chromosome numbers of the parents from which these 108 plants came were not known, and the observed progenies in Table 1 have all come from plants having $4n=40$. Eighty six plants of a selfed progeny of the tetraploid, examined cytologically, also did not show chromosome numbers beyond the 39–41 range. Furthermore, none of the progenies of aneuploid plants ($4n=39$ and 41) studied contained any plant outside the 39–41 chromosome range (Table 5). It is, therefore, quite possible that the remaining zygotic classes were not recovered not because of the small population examined or non-

Table 3. Chromosome associations at diakinesis and metaphase I in the 41-chromosome plant of Job's tears

Chromosome associations					No. of cells	%
V	IV	III	II	I		
1	9	—	—	—	1	0.91
1	8	—	2	—	4	3.64
1	7	—	4	—	8	7.27
1	6	—	6	—	8	7.27
1	5	—	8	—	14	12.73
1	4	—	10	—	8	7.27
1	3	—	12	—	3	2.73
1	2	—	14	—	3	2.73
—	10	—	—	1	1	0.91
—	9	1	1	—	1	0.91
—	9	—	2	1	2	1.82
—	8	—	4	1	6	5.45
—	7	—	6	1	8	7.27
—	6	—	8	1	12	10.91
—	5	1	9	—	2	1.82
—	5	—	10	1	12	10.91
—	4	—	12	1	9	8.18
—	3	—	14	1	5	4.55
—	2	—	16	1	2	1.82
—	1	—	18	1	1	0.91
Total					110	

functional nature of the 18- or 22-chromosome aneuploid gametes, but that zygotes outside the 39–41 chromosome range are inviable. Thus the limits of aneuploidy in the autotetraploid were set most probably by the embryo abortion.

There were no morphological differences between aneuploids and euploids and the two can be distinguished only by a count of chromosome numbers.

Cytology and origin of the 39- and 41-chromosome plants

Chromosome pairing was studied at diakinesis and metaphase I in aneuploids. Although the data on chromosome associations were recorded from only one of the plants of each of the aneuploid classes in C_1 generation, all the aneuploid plants obtained in C_1 and C_2 generations were, nevertheless, critically examined at these

Table 4. Average per cent of pollen and ovule fertility and coefficients of correlation between pollen and ovule fertility of individual plants of the various chromosome number (39, 40 and 41) classes of Job's tears

Chromosome no. (4n)	No. of plants	Average pollen fertility	t-tests for pollen fertility	Average ovule fertility	t-tests for ovule fertility	Coefficients of correlation (r)
39	14	39.54	39 vs. 40 $t=4.3927^{**}$	15.52	39 vs. 40 $t=6.1489^{**}$	Within 39-chromosome class: $r=+0.174$
40	22	55.09	39 vs. 41 $t=0.3577$	31.63	39 vs. 41 $t=3.1377^{**}$	Within 40-chromosome class: $r=+0.279$
41	16	38.54	40 vs. 41 $t=5.0767^{**}$	19.85	40 vs. 41 $t=4.2993^{**}$	Within 41-chromosome class: $r=+0.215$

** Significant at 1% level.

stages for chromosomal associations for an understanding of the origin of these plants.

In the 39-chromosome plants, a trivalent or univalent was invariably found in every cell (Table 2, Figs. 1 and 2). Trivalents were present in 43 (56.58%) of the 76 cells studied. Such a high frequency of trivalents or univalents was not observed in the autotetraploid (Rao, unpublished). The average frequencies of quadrivalents, trivalents, bivalents and univalents per cell were 4.87, 0.57, 8.59 and 0.43 respectively. From the cytological studies it is apparent that the 39-chromosome aneuploids arose by the union of gametes having 19 and 20 chromosomes.

In the 41-chromosome plants, as expected, a pentavalent was present in 49 (44.55%) out of 110 cells studied and in the rest of them upto quadrivalent associations (Table 3, Figs. 3 and 4) were found. The mean frequencies of pentavalents, quadrivalents, trivalents, bivalents and univalents per cell were 0.54, 5.55, 0.03, 8.16 and 0.53 respectively. It is thus apparent that the 41-chromosome plants originated by mating of gametes with 20 and 21 chromosomes.

All the 40-chromosome progenies showed chromosome associations to the extent of quadrivalents only. These plants have probably arisen by fusion of gametes with 20 chromosomes.

Table 5. Chromosome numbers in the progenies of 39- and 41-chromosome plants of Job's tears

Chromosome nos. ($4n$)	Progeny of $4n=39$ plant		Progeny of $4n=41$ plant	
	Frequency	%	Frequency	%
39	5	16.13	4	10.26
40	25	80.65	28	71.79
41	1	3.22	7	17.95

Although the possibility that the 39- and 41-chromosome plants could have arisen by the union of two aneuploid gametes (21 and 18, and 19 and 22 chromosomes respectively) does exist but such would be rare as the frequency of occurrence of aneuploid gametes itself was quite low that the chance of two such gametes coming together in mating would be further reduced. If such matings did occur, however, then some of the 39- and 41-chromosome plants could have shown multivalents of a much higher order than were actually recorded in this study. Similarly the 40-chromosome plants could also have come about by fusion of gametes with 21 and 19, or 18 and 22 chromosomes. Nevertheless, since all the 40-chromosome plants in the progeny contained multivalents of the order of only quadrivalents, it is obvious that such matings did not occur. Thus none of the 40-chromosome progenies examined were compensating aneuploids. The 40-chromosome plants, with typical autotetraploid behaviour, could, however, arise by mating of gametes with 19 and 21 chromosomes which are mutually compensating (deficient and excess for the same chromosome) but the probability of such matings would be extremely low.

Alternatively, it may be explained that zygotes resulting from mating between gametes having more than a single chromosome difference (Table 1) are inviable.

Such zygotes might well be within the tolerable 39–41 chromosome range, but still are inviable, due to quantitative reasons, if the same chromosome is in excess or deficient more than once. Similar situations were found also in maize (Shaver 1962), barley (Rommel 1961), red clover (Ellerström and Sjödin 1966) and ryegrass (Ahloowalia 1971).

Fertility of aneuploids

Both ovule and pollen fertility were determined from 14 plants in 39-chromosome class, 16 plants in 41-chromosome class and 22 plants in 40-chromosome class. It is likely that the different aneuploids could be deficient or excess for a different chromosome and that each one might exert its influence on fertility in a different way. Since it was not possible to identify one aneuploid from the other within the 39- or 41-chromosome class, the fertility data were collected together from plants, occurring in both C_1 and C_2 generations, in each of 39-, 40 and 41-chromosome classes, and the mean fertility of these was shown in Table 4.

In pollen fertility, the aneuploids among themselves did not differ significantly, but in ovule fertility 41-chromosome plants were significantly higher than 39-chromosome plants. Both pollen and ovule fertility in 40-chromosome plants were significantly higher than in aneuploids. Compared to pollen fertility, female fertility in all chromosome classes was low. This low female fertility was probably due to the method employed for determining the same, which includes both gametic inviability and embryo abortion. This probably also accounts for the low but positive coefficients of correlation between male and female fertility in individual plants of all chromosome classes (Table 4). The fact that aneuploids were significantly lower in fertility than euploids indicates that the state of their being aneuploid—with an imbalance in the chromosome number of the sporophyte—itself is probably responsible for it. Even though the aneuploid gametes were functional, seed set and pollen fertility were quite low indicating that all sterility in aneuploids and euploids might not be chromosomal but that much of it is genetic, as shown in many autopolyploids (Randolph 1935, Müntzing 1936, 1961, Rommel 1961, Shaver 1962, Ellerström and Sjödin 1966, Ahloowalia 1971).

Breeding behaviour of aneuploids

Open pollinated progenies of 39- and 41-chromosome plants were raised and the chromosome numbers in the progenies determined (Table 5). None of the progenies had chromosome numbers beyond the 39–41 range and most of the progenies (more than 70%) showed $4n=40$, the occurrence of which was as frequent as that in progenies of 40-chromosome plants (Table 1). The average chromosome number of the progenies of 39-chromosome plants was 39.87, and that of 41-chromosome plants 40.08. The corresponding figure for the progenies of 40-chromosome plants was 40.04. This is suggestive that there is a rapid return to euploidy in the progenies of aneuploids.

Summary

In the progenies of autotetraploid ($4n=40$) Job's tears, aneuploids with 39 and 41 chromosomes occurred in some proportion. Non-recovery of aneuploids

having less than 39 or more than 41 chromosomes was attributed to embryo abortion. The 39-chromosome plants regularly formed a trivalent or univalent in a cell and the 41-chromosome plants showed a pentavalent in a proportion of cells. Both aneuploid classes were less fertile than 40-chromosome plants. Much of the sterility in both aneuploids and euploids seemed to be genetic. Open pollinated progenies of 39 and 41-chromosome plants did not show chromosome numbers beyond the 39–41 range and a great majority of these exhibited an euploid number of 40.

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