A Cytogenetic Study of Triploids and Aneuploids in Job's Tears, *Coix lacryma-jobi* L.

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Job's tears (*Coix lacryma-jobi* L., $2n=20$), an asiatic member of the monoecious tribe Maydeae, is cultivated since ancient times for food, forage, ornamental and medicinal purposes (see Watt 1904, Vallaey 1948, Schaaffhausen 1952, Nirodi 1955, Venkateswarlu and Chaganti 1973, Arora 1973). Cytogenetically it is regarded as a stable species but the occurrence of cytological races with and without chromosomes differentiated into eu and heteropycnotic regions has been reported (see Venkateswarlu and Chaganti 1973, Venkateswarlu, Chaganti and Rao 1976). Studies on chromosome behaviour in induced autotetraploids (Venkateswarlu, Rao and Krishnarao 1976) and their progenies (Rao 1976) have been made earlier. This paper deals with a study of the cytogenetic behaviour of triploids and aneuploids in Job's tears.

Materials and methods

Autotetraploids ($4n=40$) of *C. lacryma-jobi* var. *typica* Watt were produced through colchicine treatment (Venkateswarlu and Rao 1967). By crossing diploids and tetraploids reciprocally, autotriploids ($3n=30$), hypo-triploids ($3n=29$) and hyper-triploids ($3n=31$) were obtained. In the progenies of these, trisomics ($2n=21$) double trisomics ($2n=22$) and multiple trisomics ($2n=27$) were found. In controlled crosses using diploids and triploids, and diploids and aneuploids and in open pollinated progenies of double and multiple trisomics, further aneuploids, though of the same types, were isolated.

Meiotic behaviour of the triploids and aneuploids was studied from one of the plants for each chromosome class from pollen mother cells. Fertility was scored from 5 or more plants in each chromosome class. Pollen fertility was scored as percentage of grains stained in acetocarmine for each plant and the range in percentage fertility is given for each chromosome class. Similarly the range in number of seeds produced per plant for each chromosome class, rather than percent of seed set, is given as the seeds formed in triploids and many of the aneuploids were fewer in number.

Results

Morphology of triploids and aneuploids

The triploids and aneuploids in their general morphological features like plant

height, number of culms, length of leaf sheath and blade, leaf width, length and diameter of internode, and number of inflorescences at each node, are not different from either tetraploids or diploids. However, in length of male spikes and of lower glume of male spikelets, diameter of pollen, shape and size of capsular spathes and false fruits, and colour of false fruits, the triploids and hypo- and hyper-triploids resembled the tetraploids more closely. The other aneuploids—trisomics, double trisomics and multiple trisomics—resembled in all these characters the corresponding diploid, except that the colour of the false fruit in these is more often brownish-black or dark brown as in tetraploids and triploids, in contrast to bluish-white in diploids. The only way by which the triploids (and hypo- and hyper-triploids) could be distinguished from the tetraploids in the field was by means of their greater sterility. Similarly the other aneuploids could be tentatively identified from the corresponding diploids by their lower seed set and in part by the colour of the false fruit. The aneuploids and triploids could be maintained for several years through vegetative propagation.

Cytology of triploids, hypo-triploids and hyper-triploids

The autotriploids originated by the union of a 20-chromosome gamete ($2n$) of the tetraploid and a haploid gamete ($n=10$) of the diploid parent. The hypo- and hyper-triploids obviously originated respectively by the fusion of a 19-chromosome gamete ($2n-1$), and a 21-chromosome gamete ($2n+1$) from the tetraploid with the normal haploid gamete from the diploid.

Chromosome pairing was studied from diakinesis and metaphase I cells (Table 1).

In triploids, number of trivalents varied between 10 and 5, bivalents between 5 and 1, and univalents from 4 to 1, with an average frequency of 8.59 trivalents, 1.41 bivalents and 1.41 univalents per cell (Figs. 1–3). The types of trivalents formed were rod-and-pan (44.7%), chain (41.1%) and Y-shaped (14.2%). Chiasma frequency per cell varied between 20 and 27 with an average of 23.34 ± 0.2009 , which is significantly less than one-and-half times that in the corresponding diploid (19.28 ± 0.0990). The average chiasma frequency per chromosome in trivalents was 0.82 and that per chromosome in bivalents 0.83.

In hypo-triploids, trivalents varied between 9 and 5 (Figs. 10–11), bivalents between 5 and 1, and univalents between 4 and 1 with an average frequency of 7.16 trivalents, 2.84 bivalents and 1.84 univalents per cell.

In hyper-triploids, only one quadrivalent per cell (Fig. 12) occurred in 12.9% of the cells with an average frequency of 0.13. The number of trivalents per cell was between 9 and 5, bivalents between 6 and 1, and univalents between 4 and 1, with an average frequency of 7.23 trivalents, 3.74 bivalents and 2.00 univalents.

Anaphase I and telophase I separation of chromosomes was highly irregular, with frequent occurrence of laggards (Table 2; Figs. 4–6, 13). Bridges due to delayed disjunction of bivalents or trivalents occurred in 7.7% of the cells in triploid, 10.4% of the cells in hypo-triploid and 8.8% of the cells in hyper-triploid. Persistent laggards ranging from 1–8, and often dividing, were found in 75.0% of the 150 cells in triploid (Fig. 6), 68.4% of the 86 cells in hypo-triploid and 78.5% of the 120 cells in hyper-triploid. Second meiotic division was also highly irregular

Table 1. Chromosome associations at diakinesis and metaphase I in diploids, aneuploids and triploids of Job's tears

Chromosomal class	Chromosome associations				No. of cells	%
	IV	III	II	I		
2n=20	—	—	10	—	50	100.0
2n=21	—	1	9	—	45	61.6
	—	—	10	1	28	38.4
2n=22	—	2	8	—	28	50.9
	—	1	9	1	16	29.1
	—	—	10	2	11	20.0
2n=27	—	7	3	—	18	54.5
	—	6	4	1	12	36.4
	—	5	5	2	3	9.1
3n=29	—	9	1	—	5	11.1
	—	8	2	1	11	24.5
	—	7	3	2	17	37.8
	—	6	4	3	10	22.2
	—	5	5	4	2	4.4
3n=30	—	10	—	—	13	19.1
	—	9	1	1	24	35.3
	—	8	2	2	24	35.3
	—	7	3	3	5	7.3
	—	6	4	4	1	1.5
	—	5	5	5	1	1.5
3n=31	1	9	—	—	2	6.5
	1	8	1	1	1	3.2
	1	7	2	2	1	3.2
	—	9	2	—	2	6.5
	—	8	3	1	4	12.9
	—	7	4	2	11	35.4
	—	6	5	3	7	22.6
	—	5	6	4	3	9.7

(Figs. 7–9) and some of the persistent laggards organizing themselves into micro-nuclei were found from dyad to tetrad stage (Table 2). The fertility was comparatively less in both hypo- and hyper-triploids than in triploids (Table 3).

Chromosome behaviour and fertility were studied in the corresponding diploid also (Tables 1–3).

Cytology of trisomics, double trisomics and multiple trisomics

In all the trisomics ($2n+1$), double trisomics ($2n+2$) or multiple trisomics ($2n+7$), the chromosomes involved in trisomy might not be the same. It was, however, not possible to recognise any one aneuploid from the other within the same or between different chromosome classes.

Chromosome associations were studied from diakinesis and metaphase I cells (Table 1).

In trisomics, although several were obtained, none were found to be trisomic

Figs. 1-9. Meiosis in autotriploid ($3n=30$) Job's tears. $\times 700$. 1, diakinesis: 7 III, 3 II(b) and 3 I(u). 2, metaphase I: 9 III, 1 II(b) and 1 I(u). 3, metaphase I: 10 III. 4, anaphase I: 10:11 + 9 laggards. 5, anaphase I: 11:9 + 10 laggards. 6, telophase I: 4 persistent laggards (3 of them divided). 7, metaphase II: precocious movement of chromosomes. 8, anaphase II: precocious separation of chromosomes. 9, telophase II: 4 persistent laggards.

Table 2. Chromosome distribution at anaphase I and telophase I and micronuclei in pollen quartets in diploids, aneuploids and triploids of Job's tears

Chromosomal class	Chromosome distribution		Total cells studied	Micronuclei		Total quartets studied
	Range	%		Range	%	
2n=20	10:10	100.0	50	0	00.0	50
2n=21	10:11	66.7	75	1	41.7	60
	1 laggard	33.3				
2n=22	11:11 to 10:12	43.8	39	1 to 2	58.3	48
	1 to 2 laggards	56.6				
2n=27	17:10 to 14:13	18.8	48	1 to 4	82.8	58
	1 to 7 laggards	81.2				
3n=29	9:20 to 15:14	22.2	90	1 to 6	80.0	140
	1 to 9 laggards	77.8				
3n=30	10:20 to 15:15	24.2	104	1 to 6	90.8	240
	1 to 10 laggards	75.8				
3n=31	11:20 to 15:16	21.8	110	1 to 8	83.8	148
	1 to 11 laggards	78.2				

Table 3. Range of fertility in diploids, aneuploids and triploids of Job's tears

Chromosome class	No. of plants	Male	Female
		% stained pollen	No. of seeds/plant
2n=20	5	80.5-94.8	168-390
2n=21	15	38.4-72.6	15- 68
2n=22	9	42.0-70.2	12- 32
2n=27	5	18.4-30.1	4- 9
3n=29	6	21.8-30.2	5- 10
3n=30	10	20.8-41.4	3- 38
3n=31	6	16.8-28.4	3- 5

for the nucleolar chromosome. Although the pairing behaviour in a $2n+1$ plant was studied earlier (Venkateswarlu and Rao, 1975), one other plant was studied again as this might represent trisomy for a different chromosome. Only one trivalent per cell was found (Fig. 14) with an average frequency of 0.62. The mean frequency of bivalents and univalents per cell was 9.38 and 0.38 respectively.

In double trisomics, 1-2 trivalents occurred in 80.0% of the cells (Figs. 15-16) with an average frequency of 1.31 per cell and in the rest of the cells the extra chromosomes remained as univalents (Fig. 17). The mean frequency of bivalents and univalents per cell was 8.69 and 0.69 respectively. The nucleolar chromosome was not involved in trisomy in any of the double trisomics.

In multiple trisomics, number of trivalents varied between 5 and 7 with an average of 6.45 per cell (Fig. 18). The mean frequency of bivalents and univalents respectively was 3.55 and 0.58. In two of the multiple trisomics, nucleolar chromosome was involved in trisomic condition.

At anaphase I and telophase I, the three kinds of aneuploids showed laggards in more than 50.0% of the cells; the maximum number of laggards observed in each

Figs. 10-18. 10-11. Meiosis in hypo-triploid ($3n=29$) Job's tears. $\times 1200$. 10, metaphase I: 8 III, 2 II(b) and 1 I(u). 11, metaphase I: 9 III and 1 II(b). 12-13. Meiosis in hyper-triploid ($3n=31$) Job's tears. $\times 1000$. 12, diakinesis: 1 IV(q) and 9 III. 13, anaphase I: Suggesting 14:17 distribution. 14, meiosis in trisomic ($2n=21$) Job's tears. $\times 1000$. Metaphase I: 1 III(t) and 9 II. 15-17. Meiosis in double trisomic ($2n=22$) Job's tears. $\times 1000$. 15, diakinesis: 2

kind being the same as the number of chromosomes involved in trisomy (Table 2). Bridges were found in 3 cells each in double and multiple trisomics. Persistent laggards dividing occasionally or undivided formed micronuclei in dyads and tetrads (Table 2). Pollen and seed sterility was proportionately higher with increase in chromosome number in the aneuploids (Table 3).

From the observations presented above, it is found that the average frequency of trivalents formed for a set of 3 chromosomes varied, in different aneuploids and triploid, between 0.62 and 0.92. Chromosome associations were studied earlier in $4n$ (Venkateswarlu, Rao and Krishnarao 1976, Venkateswarlu and Rao 1976), and $4n-1$ and $4n+1$ Job's tears (Rao 1976). The average frequency of quadrivalents for a set of 4 chromosomes varied in these between 0.49 and 0.58. $4n-1$ plants, where one of the chromosomes is represented only thrice, showed an average frequency of 0.57 trivalents per that chromosome, and $4n+1$ plants, where the chromosome involved in excess is represented 5 times, showed a mean frequency of 0.45 pentavalents per that chromosome. Thus it is apparent from the data, in general that the chances for trivalents to be formed are much higher when a chromosome in the complement is represented thrice, than the formation of quadrivalents, pentavalents or multivalents of a higher order respectively when it occurs 4, 5 or more times in autopolyploids or aneuploids.

The average chiasma frequency per cell in $4n$ is about double that in the corresponding diploid (Venkateswarlu, Rao and Krishnarao 1976) but the general depression in chiasmata in $3n$ is difficult to explain. The maximum number of chiasmata found at diakinesis and metaphase I in a bivalent in diploid is two. In tetraploid, the quadrivalent types requiring 4 or more chiasmata occurred more frequently (82.4%) than those requiring less than 4. In triploid, trivalent types requiring 3 chiasmata occurred less frequently (44.7%) than those requiring only 2 chiasmata. Further the average number of chiasmata per bivalent in triploid was 0.83 while the same in corresponding diploid was 0.96. In view of all these and the formation of univalents, it is probable that the chiasma frequency in triploid is not as high as expected on the basis of the observed chromosome associations in diploid and tetraploid. The types of trivalents formed in the aneuploids were also the same as those in the triploid.

Transmission of extra chromosomes to the progeny (Table 4)

When triploids, hypo-triploids and hyper-triploids were taken as female parents, either in controlled crosses or open pollination, the overall progeny contained 47.1% diploids, 28.7% trisomics, 9.2% double trisomics, 5.8% multiple trisomics, 6.9% hypo-triploids and 1.1% each of triploids and tetraploids, out of a total of 87 individuals studied. The $4n$ plant resulted from the functioning of an unreduced gamete in the triploid female parent (Venkateswarlu and Rao 1975). In open pollination, the male gametes represent a random sample from all the plants growing around. But when triploid was used as male in crosses with the diploid, only 4.0% of the progeny were trisomic and the rest were diploids. When trisomic plants were

III(t) and 8 II. 16, diakinesis: 1 III(t), 9 II and 1 I(u). 17, diakinesis: 10 II and 2 I(u). 18, meiosis in multiple trisomic ($2n=27$) of Job's tears. $\times 1000$. Diakinesis: 7 III and 3 II(b).

either selfed or crossed as female with the diploid, 83.3% of the progeny were diploid and 16.7% were trisomic, out of a total of 47 individuals examined. But when trisomic was used as male in crosses with the diploid, 95.2% were diploids and only 4.8% were trisomic in the progeny. The above data and that given in Table 4

Table 4. Distribution of chromosome numbers in the progenies of triploids and aneuploids of Job's tears under self pollination, open pollination and controlled cross pollination

Pollinations	Progeny							Total no. of plants
	% 2n=20	% 2n=21	% 2n=22	% 2n=27	% 3n=29	% 3n=30	% 4n=40	
3n=30* O.P.	42.3	38.5	15.4	3.8	—	—	—	26
3n=30 ⊗	58.4	33.3	8.3	—	—	—	—	12
3n=29 O.P.	71.4	28.6	—	—	—	—	—	7
3n=31 O.P.	75.0	25.0	—	—	—	—	—	4
3n=30** × 2n=20	39.5	21.1	7.9	10.5	15.8	2.6	2.6	38
2n=20 × 3n=30	96.0	4.0	—	—	—	—	—	25
2n=21 ⊗	83.3	16.7	—	—	—	—	—	18
2n=21 × 2n=20	83.3	16.7	—	—	—	—	—	24
2n=20 × 2n=21	95.2	4.8	—	—	—	—	—	21
2n=22 O.P.	75.0	25.0	—	—	—	—	—	20
2n=27***O.P.	77.8	16.7	5.5	—	—	—	—	18

O.P.=Open pollination; ⊗=self pollination; *=O.P. seed collected from several plants;

** =3 triploid plants were used as female; ***O.P. seed from 5 plants were sown.

show clearly that the transmission frequency of extra chromosomes to the progeny was much higher through the female parent than male. Apparently, male gametes carrying extra chromosome(s) are frequently sterile and/or less successful in competition with normal gametes in fertilization. Aneuploids with $2n+3$ to $2n+6$ and $2n+8$ chromosomes were not found in the progenies. This may be due to the small size of the population observed in each case.

Summary

In Job's tears (*Coix lacryma-jobi* L.) autotriploids ($3n=30$), hypo-triploids ($3n=29$) and hyper-triploids ($3n=31$) were produced by crossing diploids and colchicine induced autotetraploids. Trisomics ($2n=21$), double trisomics ($2n=22$) and multiple trisomics ($2n=27$) were obtained in the progenies of triploids. In none of the trisomics or double trisomics, the nucleolar chromosome was involved in trisomy. In each of the chromosome classes, meiotic behaviour, fertility and transmission of extra chromosomes to the progenies were studied. The average frequency of trivalents for a set of 3 chromosomes in triploids and aneuploids varied between 0.62 and 0.92, which is much higher compared to the average frequency of quadrivalents for a set of 4 chromosomes in the tetraploids (including hypo- and hyper-tetraploids) which varied between 0.49 and 0.58. These apparently indicate that the frequency of multivalents tends to decrease as the number of homo-

logues of a chromosome in the complement increases. The average chiasma frequency per cell in triploid was significantly less than one-and-half times that in diploid. Although this depression in chiasmata in triploid is difficult to explain, some probable causes were mentioned. In general, aneuploids were lower in fertility compared to diploids, and both hypo- and hyper-triploids were less fertile than triploids. Transmission of extra chromosomes to the progenies occurred more frequently through the female gametes than male gametes.

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