Theoretical and Applied Genetics 47, 165-169 (1976)

Effect of Inbreeding and Selection for Vigour and Fertility on Meiotic Behaviour in Autotetraploid Job's Tears, *Coix lacryma-jobi* L.

J. Venkateswarlu and Panuganti N. Rao

Department of Botany, Andrha University, Waltair (India)

Summary. Meiotic behaviour of the colchicine-induced "raw" autotetraploid (4n = 40) Job's tears (*Coix lacryma-jobi* L.) was compared with that of the tetraploid envolved from it through selection for vigour and fertility over a 4 year period and selfing of the selected plants for 3 generations. A significant decrease in quadrivalent frequency and an increase in bivalent frequency per cell, greater frequency of ring quadrivalents, more cells with regular separation of chromosomes and fewer cells with laggards at anaphase I, fewer irregularities in meiosis II, fewer pollen quartets with micronuclei and fewer aneuploids in the progenies were found in evolved tetraploid. The average chiasma frequency per cell, per quadrivalent and per bivalent were more or less the same in both tetraploids. All these facts indicate that inbreeding and selection for vigour and fertility have brought about a shift towards regular meiosis in the evolved tetraploid. The increase in fertility during the period of selection was not, however, significant, suggesting that its response to selection is slow, that a number of factors are probably involved and that, besides multivalents, genic factors also govern sterility in the tetraploid.

Introduction

Job's tears (*Coix lacryma-jobi* L., 2n = 20), also known as Adlay, an asiatic relative of maize, is used as an article of food, fodder, medicine and ornament (see Watt, 1904; Vallaeys, 1948; Schaaffhausen, 1952; Nirodi, 1955; Venkateswarlu and Chaganti, 1973; Arora, 1973). Autopolyploidy was induced in Job's tears by Murakami and Harada (1959), Venkateswarlu and Krishnarao (1966) and Venkateswarlu and Rao (1967), through colchicine treatment. In the present study, the effects of inbreeding and selection for vigour and fertility on chromosome behaviour during meiosis in autotetraploid Job's tears have been studied and the results are reported here.

Materials and Methods

Pollen fertility was determined by staining in acetocarmine a minimum of 1,000 grains from three anthers coming from different florets. Those stained were counted as fertile and unstained as sterile.

For determining seed set, all the fully mature false fruits of a given plant were harvested, the soft chalky-white ones being scored as sterile and the hard brownish-black or dark brown ones as fertile.

For selfing, entire plants were enclosed in large cloth bags. Job's tears also reproduces vegetatively through basal stem suckers, so that the three originally induced autotetraploids in 1966 could be perpetuated in the same pots for 5 years, by carefully nursing them; after this they died, apparently of some root disease. The original tetraploids and their vegetatively propagated plants are referred to as "raw" tetraploids, and the tetraploid derived through 3 generations of selfing with selection for vigour and fertility as the "evolved" tetraploid. The first generation open-pollinated progeny (C_1 generation) of the raw tetraploids is referred to as S_0 generation and the successive selfed generations as S_1, S_2 and S_3 generations.

Cytological studies were made on pollen mother cells after fixation of the young male spikelets in a freshly prepared solution of 1:4 acetic-alcohol for 24 hours at room temperature and storage in 70% alcohol.

The frequencies of chromosome associations in the selected vigorous plants, during the inbreeding programme, were obtained from at least 25 cells per plant.

Results and Discussion

From the open-pollinated seed of the 3 raw tetraploids, a large progeny (S_0 generation) was raised in 1967. The progeny contained both euploids and aneuploids (Rao, in press). Of these, 5 vigorous plants with euploid number were selected, and pollen fertility, quadrivalent and bivalent frequency per cell determined. Among these plants, the one showing the highest pollen fertility was selfed. At maturity, seed set was scored in all 5 plants. From the selfed seed, the S_1 progeny was raised in 1968 and again 5 vigorous euploids were chosen, and pollen fertility, seed set and the frequency of chromosome associations determined. This process of selection for vigour and fertility in selfed progenies and determination of chromosome associations in the selected plants was continued through S_3 generation in 1970. To compare chromosome behaviour at meiosis in the evolved tetraploid (the most fertile plant among the

Table 1. Bivalent and quadrivalent frequency per cell and percentage of pollen fertility and seed set in a tetraploid plant (4n = 40) selected for high pollen fertility, among a group of 5 vigorous plants, in each of 3 generations of selfing, together with mean values per generation (mean of the 5 selected vigorous plants) and in raw tetraploid (4n = 40) Job's tears

Year	Selfed generation		Bivalent frequency	Quadrivalent frequency	Pollen fertility	Seed set
1967	S_0	Selected plant	8.69 ± 0.3216	5.68 ±0.2646	66.4	27.5
		Mean value per generation	9.15 ± 0.4052	5.48 ±0.1739	55.0 ± 4.1635	23.4 ± 3.9669
		Raw tetraploid	8.60 ± 0.4688	5.69 ±0.2434	52.4	28.3
1968	S_1	Selected plant	8.88 ± 0.3001	5.56 ±0.2434	71.4	28.9
		Mean value per generation	9.52 ± 0.3800	5.25 ±0.1869	57.0 ± 4.8509	25.0 ± 3.2011
		Raw tetraploid	8.42 ± 0.4446	5.77 ±0.1948	54.1	23.1
1969	S_2	Selected plant	9.58 ± 0.2674	5.21 ±0.1469	67.4	25.9
		Mean value per generation	9.76 ± 0.2276	5.12 ±0.1162	60.1 ± 4.0014	27.1 ± 4.1803
		Raw tetraploid	8.52 ± 0.4140	5.74 ±0.1568	56.0	32.1
1970	S_3	Selected plant	10.26 ± 0.3898	4.87 ±0.2277	69.8	40.8
		Mean value per generation	10.34 ± 0.2548	4.94 ±0.0447	60.3 ± 2.7378	27.7 ± 4.2306
		Raw tetraploid	8.42 ± 0.4519	5.76 ±0.2308	54.4	28.8

group of 5 vigorous plants in S_3 generation) and the raw tetraploid, male spikelets were fixed from the vegetatively propagated raw tetraploid and from the evolved tetraploid on the same day during 1970 and detailed meiotic study was made in both tetraploids.

Pollen fertility, seed set and chromosome associations from S_0 to S_3 generation

Table 1 shows the mean pollen fertility and seed set, and the quadrivalent and bivalent frequency per cell in the selected selfed plant from each of S_0 to S_3 generations, the mean values of the 5 selected plants for each generation from S_0 to S_3, and the fertility and chromosome associations observed in the raw tetraploid from 1967 to 1970.

When the selected vigorous plants during the inbreeding programme were subjected to an analysis of coefficient of correlation, only a low positive correlation (r = +0.2795) was obtained between seed set and pollen fertility; a similar situation was also found under open-pollinated conditions in euploids as well as aneuploids in S_0 generation (Rao, in press). Quadrivalent frequency per cell gave a negative correlation with pollen fertility as well as seed set (r = - 0.2230 and - 0.4487 respectively). There was a positive correlation between bivalent frequency per cell and pollen fertility and also seed set (r = + 0.1747 and + 0.4523 respectively). In none of these cases, however, were the r values significant.

While the average pollen fertility, seed set and bivalent frequency per generation showed a steady increase, quadrivalent frequency suffered a gradual decline from the S_0 to S_3 generation (Table 1), but the differences in mean values of S_0 and S_3 generations for pollen fertility and seed set (Table 1) are not sig-

Table 2. Differences in meiotic behaviour of raw and evolved tetraploids (4n = 40) of Job's tears

S. No.	Criteria	Raw tetraploid	Evolved tetraploid
1.	Mean number of IV per cell **	5.76 ± 0.2308	4.87 ± 0.2277
2.	Mean number of III per cell	0.03	--
3.	Mean number of II per cell **	8.42 ± 0.4519	10.26 ± 0.3898
4.	Mean number of I per cell	0.03	--
5.	Mean number of ring II per cell **	7.00 ± 0.2666	8.39 ± 0.2388
6.	Mean number of rod II per cell	1.42 ± 0.2435	1.87 ± 0.2184
7.	Mean chiasma frequency per cell	37.80 ± 0.2636	37.53 ± 0.1434
8.	Mean number of chiasmata per IV	3.88	3.88
9.	Mean number of chiasmata per II	1.83	1.82
10.	% ring bivalents	83.09	81.79
11.	% rod bivalents	16.91	18.21
12.	% chromosomes going into multivalent formation	57.80	48.69
13.	% chain quadrivalents	15.0	11.8
14.	% ring quadrivalents	67.4	83.8
15.	% other types of quadrivalents	17.7	4.3
16.	% cells with 20:20 distribution at AI	59.5	75.0
17.	% cells with irregular distribution at AI	27.2	14.2
18.	% cells with laggards and/or bridges at AI	13.3	6.3
19.	% cells with irregularities in meiosis II	13.6	7.2
20.	% pollen quartets with micronuclei	33.1	14.6
21.	% aneuploids in progeny	18.8	4.0

** Significant at 1% level

nificant. This indicates that progress of improvement in fertility under selection was slow, which might be expected if the plant selected initially in S_0 was already near the upper levels of fertility possible in the tetraploid, and that a number of other factors, such as inbreeding (in a habitually outbreeding species), acting to limit the fertility gains in selection, may also have been involved. However, the mean values of quadrivalent and bivalent frequency per cell in S_0 and S_3 generations (Table 1) are significantly different ($P = 0.05 - 0.01$). A similar decline in quadrivalent frequency accompanying selection for fertility was recorded by Gilles and Randolph (1951) in maize, Hilpert (1957) in rye, Swaminathan and Sulbha (1959) in *Brassica campestris* var. *toria* and Bender and Gaul (1966) in barley. The opposite, where improvement in fertility was associated with an increase in quadrivalent frequency, was also found again in rye (Müntzing, 1951) and *Dactylis* (McCollum, 1958). However, in the study of Morrison and Rajhathy (1960), involving a number of autotetraploids of different species, there was neither evidence for heritable variation in quadrivalent formation nor indication that variation in the number of quadrivalents affects fertility. It should be pointed out here that, in the present study, selection for fertility was coincidental with selection for vigour. It is probable that the latter also contributed simultaneously to the rapid reduction in quadrivalent frequency and enhancement of bivalent frequency per cell, as was found in the case of rye (Hilpert, 1957), where selection for tillering was effective in bringing about a shift towards a more regular meiosis. However, the fact that a significant reduction in quadrivalent frequency did not bring about a concomitant improvement in fertility during the four-year period of selection for the latter, together with the low negative r values between quadrivalent frequency and fertility, suggests that sterility in the tetraploid can not be ascribed entirely to multivalent formation and irregular segregation of chromosomes, particularly when gametes with aneuploid numbers also function (Rao, in press), but genic causes may also be involved.

Meiotic behaviour in raw and derived tetraploids

A comparison of the meiotic behaviour in the evolved tetraploid with that in the raw tetraploid revealed certain differences between the two (Table 2). The evolved tetraploid, apart from registering a significant lowering of quadrivalent frequency and increase in bivalent frequency per cell, in which both ring and rod bivalents occurred in excess, showed marginal differences in the percentage of chromosomes going into the formation of multivalents, while the average chiasma frequency per cell in both tetraploids remained more or less the same (Table 2). However, the range of chiasmata per cell varied between 32 and 42 in the raw tetraploid and from 35 to 39 in the evolved tetraploid, indicating that selection and inbreeding brought stability in chiasma formation. The chiasma frequency per quadrivalent and per bivalent in both tetraploids were also identical (Table 2). This indicates that the lowering of quadrivalent frequency in the evolved tetraploid was not due to a reduction in the number of chiasmata but occurred because partner exchanges between homologous chromosomes during synapsis, necessary for quadrivalent formation, occurred less frequently than in the raw tetraploid. The two tetraploids showed the same range of 1 to 10 quadrivalents per cell. Both ring and chain quadrivalents were more numerous than the other types in both tetraploids, but in the evolved tetraploid, while the percentage of ring quadrivalents increased considerably, that of all other types decreased (Table 2); this suggests that selection for vigour and fertility invoked a response towards a shift in favour of ring quadrivalents, which are likely to have a more regular disjunction at anaphase I. This is evidenced by an increase in the percentage of cells showing 20:20 separation of chromosomes, and a decrease in the proportion of cells with irregular distribution and laggards at anaphase I in the evolved tetraploid (Table 2). Similarly, cells showing irregularities at division II were also reduced in number and the proportion of pollen quartets with one or more micronuclei was also far less than in the raw tetraploid (Table 2). When the evolved tetraploid was selfed and 25 plants of the progeny examined for chromosome number, only one of them showed 4n = 39 while the rest were 4n = 40; in comparison the open-pollinated progenies of the raw tetraploid, studied in 1967, contained 13 aneuploids with 4n = 41, or 39 out of a total of 69 plants examined (Rao, in press). Thus, in the progenies of the evolved tetraploid there was a considerable fall in the frequency of aneuploids (Table 2). From all these facts, it is conceivable that a fair degree of meiotic regularity was achieved as a consequence of selection for vigour and fertility and inbreeding for 3 generations.

Literature

Arora, R.K.: Adlay *(Coix)* - Crop in Meghalaya. J. Indian bot. Soc. 51, 95-98 (1973)

Bender, K.; Gaul, H.: Zur Frage der Diploidisierung autotetraploider Gerste. Z. Pflanzenzücht. 56, 164-183 (1966)

Gilles, A.; Randolph, L.F.: Reduction in quadrivalent frequency in autotetraploid maize during a period of ten years. Amer. J. Bot. 38, 12-17 (1951)

Hilpert, G.: Effects of selection for meiotic behaviour in autotetraploid rye. Hereditas 43, 318-322 (1957)

McCollum, G.D.: Comparative studies of chromosome pairing in natural and induced tetraploid *Dactylis*. Chromosoma 9, 571-605 (1958)

Morrison, J.W.; Rajhathy, T.: Chromosome behaviour in autotetraploid cereals and grasses. Chromosoma 11, 297-309 (1960)

Müntzing, A.: Cytogenetic properties and practical value of tetraploid rye. Hereditas 37, 18-84 (1951)

Murakami, M.; Harada, K.: Studies on the breeding of genus *Coix*. II. On the tetraploid plant of genus *Coix* induced by colchicine treatment. Sci. Rep. Kyoto Prefectural Univ. Agric. No. 11, 1-8 (1959)

Nirodi, N.: Studies on Asiatic relatives of maize. Ann. Missouri bot. Gdn. 42, 103-130 (1955)

Rao, P.N.: Studies on the occurrence, cytology, fertility and breeding behaviour of aneuploids in induced autotetraploid Job's tears. Cytologia (in press)

Schaaffhausen, R. v.: Adlay or Job's tears - A cereal of potentially greater economic importance. Econ. Bot. 6, 216-227 (1952)

Swaminathan, M.S.; Sulbha, K.: Multivalent frequency and seed fertility in raw and evolved tetraploids. Z. Vererbungslehre 90, 385-392 (1959)

Vallaeys, G.: Le "*Coix Lacryma-Jobi*". Bull. Agric. Congo Belge 39, 247-304 (1948)

Venkateswarlu, J.; Chaganti, R.S.K.: Job's tears (*Coix lacryma-jobi* L.). Tech. Bull. No. 44, New Delhi: Indian Council of Agricultural Research 1973

Venkateswarlu, J.; Krishnarao, M.: Chromosome pairing in autopolyploid *Coix lacryma-jobi*. M.G.C. News Letter 40, 165 (1966)

Venkateswarlu, J.; Rao, P.N.: Colohicine-induced autotetraploids of Job's tears (*Coix lacryma-jobi*). M.G.C. News Letter 41, 7 (1967)

Watt, G.: *Coix* spp. or Job's tears: A review of all available information. Agric. Ledger 11, 189-229 (1904)

Received September 5, 1975
Communicated by R. Hagemann

Dr. J. Venkateswarlu
Dr. Panuganti N. Rao
Department of Botany
Andrha University
Visakhapatnam - 530 003 (India)