Cytological Studies in the Tetrasomics of Coix gigantea (Poaceae)

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Aneuploids that have an extra chromosome pair from the euploid complement in their constitution (2n+2) are known as tetrasomics and such individuals have been reported in plants and animals, including man. Tetrasomics in Datura (Blakeslee and Belling 1924), Zea (McCIntock 1929) and Nicotiana (Goodspeed and Avery 1939) have been recorded in the past. Autotriploid constitution is the most dependable source of tetrasomics (and also other aneuploids) since in them a variable number of univalent, bivalent and trivalent associations lead to segregational irregularities during meiosis giving more and/or less chromosomes than the haploid set. Tetrasomics from the selfed progeny of autotriploids have been isolated in tomato (Rick and Barton 1954, 16.4%) barley (Tsuchiya 1960, 17.5%) and rye (Kamanoi and Jenkins 1962, 20.7%). In addition, tetrasomics can also come from the selfed progeny of trisomics, tetrasomics themselves and asynaptic/desynaptic mutants in a population (Khush 1973). Non-disjunction of a bivalent during meiosis in diploids is also likely to directly produce aneuploids from nullisomic (2n–2) to octosomic (2n+6) constitutions (Sapre and Barve 1985).

Addition or deletion of chromosome(s) in a diploid or even polyploid complement is known to seriously affect the general health of the aneuploid individuals drastically reducing their survival in nature. They may, however, thrive well under domestication and have been cytogenetically exploited in several crop plants. Occurrence of higher polysomics in nature is a rare situation, although they have been reported in human abortuses (Hamerton 1969). However, viable higher polysomics among plants, a pentasomic (2n+3) and a hexasomic (2n+4) each in Coix gigantea, have been recently isolated and a detailed chromosomal behaviour in them has been reported by us (Sapre and Barve 1985a, b). From among the same series of aneuploids, tetrasomics have been isolated and all possible meiotic configurations are reported in this communication.

Materials and methods

Coix gigantea Koen. ex Roxb., an oriental member of the tribe Maydeae (Poaceae), was collected from the Purandar Fort (Maharashtra). A free-breeding population grown and maintained at the Botanical Garden of this University for the past nine years was screened cytologically and a series of aneuploids from nullisomy to hexasomy (2n=18–24, Sapre and Barve 1983, 1984) was isolated. Two tetrasomic plants were recorded one each in 1982 and 1984. Young male racemes of these plants were fixed in acetic-alcohol (1: 3) and the anthers were squashed in acetocarmine (1%). Micropreparations were made permanent using carbon dioxide freezing technique (Conger and Fairchild 1953). Desirable chromosomal configurations were photographed and the microslides have been deposited with the Cytogenetics Unit of the Botany Department.

Results

Most of the pollen mother cells showed nine bivalents and a quadrivalent at diakinesis as
expected in typical tetrasomics (Table 1). The two extra chromosomes that made a total of four homologues in the complement formed two most common types of quadrivalent configurations (Figs. 1, 2) in addition to many others. Depending on the degree of synapsis and the number and position of chiasma (ta), other associations like 2Is, II and 2I, III and I or 4Is were noted in decreasing order (Table 1). These various multivalent and univalent associations involving the four homologues presented some difficulty in orientation and segregation with the result that variable number of chromosomes were noted at two poles with some lagging at the equator by the end of meiosis-I. Quadrivalents usually orientated at metaphase plate but segregated into two bivalents rather late (Figs. 8, 10) or showed complete nondisjunction (Figs. 11, 12) getting incorporated in one of the polar complements. When the four homologues formed two bivalents (Fig. 4), they invariably identified themselves from the rest of the bivalents not only by their larger size but also by their behaviour. The two bivalents got distributed one each to the two poles, where they either merged with the polar complements (Fig. 9) giving 11-11 distribution of univalent chromosomes and ultimately n+1 gametes (Fig. 18) or both of them lagged (Figs. 14, 15) keeping away from the rest of the complement. At times, one bivalent moved to one of the poles, while the other disjointed into two univalents that lagged (Fig. 16). When the four homologues formed a bivalent and two univalents (Fig. 5), the former lagged while the latter precociously reached the opposite poles (Fig. 17). In the trivalent and univalent configurations (Fig. 3) the trivalent segregated as II-I, while the remaining univalent precociously reached a pole ultimately giving equal (11-11) distribution of chromosomes (Fig. 7). However, sometimes the trivalent and the univalent both moved to the same pole (Fig. 13) ultimately giving 13-9 distribution of chromosomes (Figs. 20, 21). Failure of pairing in the homologues resulting in four univalents is a rare situation (Table 1) and as such the behaviour of univalents through meiosis could not be traced. Even the various chromosomal segregations presented through Figs. 7-21 could be noted in a few clear PMCs, most of the configurations going sticky after diakinesis (Figs. 6-8) and even during meiosis-II. As such, it was almost impossible to analyse with clarity any of the chromosomal associations during meiosis-II in a large number of PMCs. However, some clear anaphase-I and prophase-II configurations noted are

<table>
<thead>
<tr>
<th>Total number of PMCs observed at diakinesis</th>
<th>9II+IV</th>
<th>9II+II+II</th>
<th>9II+II+2Is</th>
<th>9II+III+I</th>
<th>9II+4Is</th>
</tr>
</thead>
<tbody>
<tr>
<td>181</td>
<td>97 (54.59%)</td>
<td>49 (27.07%)</td>
<td>21 (11.60%)</td>
<td>10 (5.52%)</td>
<td>4 (2.20%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total number of PMCs observed at prophase-II</th>
<th>11-11</th>
<th>12-10</th>
<th>10+I-10+I</th>
<th>13-9</th>
<th>9+II-9+II</th>
</tr>
</thead>
<tbody>
<tr>
<td>148</td>
<td>83 (56.08%)</td>
<td>42 (28.37%)</td>
<td>11 (7.43%)</td>
<td>7 (4.72%)</td>
<td>5 (3.37%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chromosome number of the gametes</th>
<th>n=9</th>
<th>n=10</th>
<th>n=11</th>
<th>n=12</th>
<th>n=13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage</td>
<td>6.08</td>
<td>26.90</td>
<td>42.14</td>
<td>21.31</td>
<td>3.54</td>
</tr>
<tr>
<td>Decreasing order 1-5</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>5</td>
</tr>
</tbody>
</table>
Figs. 1-7. Meiotic configurations in tetrasomics showing association of the four homologues. 1-2, diakinesis showing nine bivalents and quadrivalent. 3-5, diakinesis showing III+I, 2IIIs and II+2Is configurations of the four homologues. 6-7, polar and equatorial mataphases with sticky chromosomes. Note quadrivalent in 6 and segregation of trivalent as II–I; the remaining univalent is precociously at pole in 7.
Figs. 8–16. Meiotic configurations in tetrasomics showing behaviour of four homologues. 8–10, anaphase-I showing late disjunction of quadrivalent. Note dissociation of one of the bivalents into two univalents in 9. 11–12, early and late anaphase-I showing nondisjunction of a quadrivalent. 13, anaphase-I showing nondisjunction of a trivalent. Note a univalent precociously at the pole giving 9I–9I+III+I. 14, anaphase-I showing unorientated bivalents. 15, lagging bivalent at prophase-II in one of the dyads. 16, telophase-I showing two lagging univalents giving 10I–I+I–10I.
presented in Table 2. Microspores with $n+1$ ($n=11$, Fig. 18) appeared in maximum number followed by those with $n$ ($n=10$, Fig. 19, Table 3). Male gametophytes with $n+3$ ($n=13$) chromosomes (Fig. 20) due either to total nondisjunction of quadrivalent or the trivalent and the univalent both having passed to the same pole were few in number (Table 3). The $n-1$ ($n=9$) gamates could arise through many situations (Table 2) and more so when univalent(s)/bivalent(s) having dissociated from the quadrivalent lagged and got eliminated through pycnosis.

Figs. 17-21. Meiotic configurations showing various possible gametic constitutions. 17, anaphase-I showing precocious movement of two univalents and a lagging bivalent. 18-19, prophase-II showing 11-11 and 12-10 distribution of chromosomes. 20-21 prophase-II showing 13 and 9 chromosomes at two poles. Two figures are separated for convenience in photomicrography.

Discussion

Addition and deletion of individual chromosomes in the basic complement of organisms provide interesting information on cytogenetics and evolution. However, literature on the cytology of aneuploids is relatively fragmentary, and more so in regard to the higher polysomics. There are several reasons for this; abortion of embryos with polysomic constitutions, failure of germination of polysomic seeds, weak constitution of the polysomic plants imparting them a great set back in the struggle for existence, seedling lethality/premature death, failure of
flowering/deformation of flowers etc. are some of the inhibitions in carrying out detailed cytological studies on polysomics. However, chromosomal behaviour in a pentasomic and a hexasomic plant has been recently reported by us (Sapre and Barve 1985a, b). Tetrasomics, that occur comparatively rarely among the polysomics, have likewise been poorly worked out cytologically, although breeding experiments and cytological screening of progeny involving them have been carried out in some plant species (Burnham 1962, Khush 1973). It is for the first time that detailed cytology of tetrasomics is reported here.

**Chromosomal behaviour**

Since the tetrasomics carried four homologous chromosomes in their constitution formation of quadrivalents was very common. However, other associations such as 2IIs, II and 2Is, III and I and rarely 4Is also occurred at diakinesis. In the present tetrasomics, all these various associations of the four homologues apparently did not disturb the rest of the bivalents and their behaviour up to diakinesis. However, one of the major disturbances due to tetrasomic condition was the chromosomal stickiness. A large number of PMCs showed sticky configurations soon after diakinesis and it was only in a few PMCs that stages in meiosis-II could be noted with clarity. Chromosomal stickiness obliterating meiosis in a large number of PMCs was also recorded in the pentasomic and hexasomic constitutions (Sapre and Barve 1985a, b). Although chromosomal stickiness is reported to be controlled by a recessive gene (Beadle 1932), the variable number of sticky PMCs and the degree of stickiness recorded in the present tetrasomics indicate no genetic control over this condition, and especially when chromosomes behave so well up to diakinesis. The multivalent/univalent configurations involving four homologues presented some difficulty in orientation and segregation. Nondisjunction of quadrivalent/trivalent and lagging of unorientated univalents/bivalents were common situations leading to variable number of chromosomes at poles.

A new type of tetrasomic plant in barley reported by Tsuchiya (1962, 1969) needs a special mention in this context. This barley carried an extra pair of chromosome-6 that showed two structural changes, a pericentric inversion and a deletion. This changed the morphology of the additional pair of chromosome-6 in barley so much that most of the PMCs showed 8IIIs (and a few 7IIIs+2Is) at diakinesis. That none of the PMCs with a quadrivalent or trivalent were noted by him indicates how homologous chromosomes can behave as non-homologous with the induction of major structural changes. Plants with new basic chromosome number from tetrasomics can thus arise in a wild population.

**Variable gametic constitution**

The male and the female gametic constitution in the tetrasomics is generally assessed through selfing and crossbreeding experiments. Using tetrasomics as female and male parents and crossing them with normal disomics provide some clue as regards the gametic make-up both on the male and the female sides if their progeny is screened cytologically. Although breeding is the practical and most reliable way to assess the constitution of the functional gametes, it is only through detailed study on chromosomal behaviour that the possibility as to the range of gametic composition can be understood. Whether all the various types of gametes produced are functional and if so whether they can efficiently compete with normal haploid gametes, can then be estimated through breeding experiments. Cytological study thus reveals the capacity of tetrasomics in the production of various gametic types.

Banks et al. (1982), who induced aneuploidy in maize by treating seeds with para-fluorophenylalanine (PFP), reported disomics (2n=20), trisomics (2n=21) and tetrasomics (2n=22) in the progeny of one of the tetrasomics. They are of the opinion that the female reproductive tissue of this tetrasomic probably had 21 chromosomes that gave eggs with n=10 and n=11
chromosomes and hence disomic and trisomic appeared among the descendents. While this may well be so, the present study on the chromosome behaviour, especially the segregation of the quadrivalent, indicates clearly how gametes in the range of \(n-1\) to \(n+3\) are likely to be produced in a tetrasomic constitution (Tables 2, 3). Similar chromosomal segregations in the maize tetrasomic of Banks et al. (1982), in our opinion, are also likely to give gametes with \(n=10\) and \(n=11\) chromosomes rather than changing from \(2n=22\) (in roots) to \(2n=21\) (in the female reproductive tissue) as proposed.

Data on the breeding studies of tetrasomics involving chromosome-8 of Datura (Blakeslee and Avery 1938) indirectly indicated 2–2 disjunction of the quadrivalent at anaphase-I resulting in \(n+I\) spores and producing trisomics in large number. However, a considerable number of disomics and tetrasomics appearing in the selfed progeny of tetrasomic Datura also suggested 3–1 disjunctions giving spores with \(n+2\) and \(n\) constitutions. The present cytological data is in complete agreement with the above results since majority of the PMCs showed equal (11–11) distribution of chromosomes (Fig. 18) giving 2–2 disjunction of quadrivalent, followed by 3–1 disjunction in some PMCs giving 12–10 segregations (Fig. 19, Table 2). However, nondisjunction of the quadrivalent (Figs. 11, 12) and passing of both trivalent and univalent to the same pole (Fig. 13) created situations with 13–9 chromosomal distribution (Figs. 20, 21) giving \(n+3\) (\(n=13\)) and \(n-1\) (\(n=9\)) gametes. Additional \(n-1\) gametes can also arise due to lagging bivalents (Figs. 14, 15, Table 2) and also if the nondisjoined quadrivalent (Fig. 12) becomes pycnotic and gets eliminated during tetrad formation. Thus, theoretically a range of gametes from \(n-1\) to \(n+3\) can possibly be obtained in any tetrasomic plant. Among these, \(n+1\) and \(n\) gametes would occur in large numbers compared to \(n+2\) and \(n+3\) (Table 3), and the latter may fail to function in competition with normal gametes (\(n\)). It is for this reason that higher polysomics, \(2n+3–2n+6\), have not appeared even in controlled breeding experiments conducted by Blakeslee and Avery (1938). Hamerton (1969) has recorded polysomic constitutions among human abortuses. It may be of interest to cytologically screen young aborting plant embryos through embryo-squash techniques (Mujeeb et al. 1978) since abnormal chromosomal constitutions of embryos are likely to adversely affect their growth and differentiation. There is no record where such embryo-screening-tests have been carried out, nor are there any efforts made to grow such weak and underdeveloped embryos to maturity in artificial cultures.

**Origin of tetrasomics**

Although tetrasomics generally appear in the progeny of autotriploids and trisomics, they are also likely to originate from diploids and monosomics. It has been demonstrated that normal diploids can produce gametic range between \(n-1\) to \(n+3\) through nondisjunction of a single bivalent during meiosis (Sapre and Barve 1985). Even monosomics have been noted to produce gametes with \(n-1\), \(n\) and \(n+1\) constitutions through variable behaviour of the monosome during meiosis; \(n-1\) when the monosome becomes pycnotic and lags, \(n\) when it gets included in one of the anaphase-I groups and \(n+1\) when it divides into two chromatids both of which get included in one of the four groups at the end of meiosis-II (Mashalkar 1983). Since the present tetrasomics have been isolated from a free-breeding population comprising aneuploids, they could have arisen in any of the ways discussed above.

**Summary**

Detailed cytological study in the tetrasomics of Coix gigantea Koen. ex Roxb. (Poaceae) has been carried out revealing a possibility of wider range in the gametic constitution than is generally predicted through breeding experiments. Although majority of the PMCs showed nine bivalents and a quadrivalent association at diakinesis, the four homologues gave two
bivalents, bivalent and two univalents, trivalent and a univalent and rarely four univalents. Orientation and segregation in all these associations, especially irregularities involving quadrivalent and univalent, have resulted in gametes with from \( n=9 \) to \( n=13 \) chromosomes. There is every reason to believe that gametes up to \( n+2 \) (\( n=12 \)) constitution are functional since polysomics, pentasomic (2n+3, 2n=23) and hexasomic (2n+4, 2n=24), have been isolated and cytologically studied by us in the past.

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References


