Morphology and Cytology of a New Species of *Coix* With 32 Chromosomes¹

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Of the oriental Maydeae, *Coix* is the most widely distributed genus. There is quite disparity of opinion among morphologists about the nomenclature of different species of *Coix*, the same species being called by different names by various workers (see Nirodi 1955). Celarier (1957) reviewing the cytological work done on some of the members of Maydeae including *Coix*, remarks that this genus seems to consist of complexes of types in which only a few species are distinct. Five or six species are generally recognized in the genus *Coix*, which are more or less restricted to south east Asia, except the most variable *C. lacryma-jobi* which has been introduced throughout the tropics of the world (Nirodi 1955, Celarier 1957).

Mangelsdorf and Reeves (1939) have described the cytology of *C. aquatica* ($2n=10$) and *C. lacryma* ($2n=20$). Janaki Ammal (unpubl., see Darlington and Wylie 1956) has reported the highest number of $2n=40$ of this series in *C. gigantea*. Recently Nirodi (Nirodi 1955) has described the cytology of several Asiatic relatives of maize. She has confirmed the previous reports of $2n=10$, 20 and 40 in three different species of *Coix*. The species with the lowest chromosome number ($n=5$) studied by her is *C. poilaeni* Mimeur (?) instead of *C. aquatica* studied by Mangelsdorf and Reeves (1939).

A detailed cytomorphological study of different accessions of *Coix* was undertaken at this Laboratory. During this study one accession of *Coix* collected from Madhya Pradesh was found to show a different karyotype with $2n=32$, a number which has not been reported in any member of Maydeae so far. In the present paper morphology and cytology of this new form of *Coix* has been described.

Materials and methods

The seeds of above mentioned accession of *Coix* were sown in pots and later the seedlings were transplanted to plots. Morphological comparisons of these plants of *Coix* were made with other species of *Coix* from the literature and with *C. lacryma-jobi* grown in our garden.

For cytological studies the male spikelets were fixed in Carnoy's fluid (6 abs. alcohol +3 chloroform+1 acetic acid saturated with ferric acetate) and stored in refrigerator.

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Meiotic chromosomes were studied from temporary smear preparations of microspore mother cells at various stages of development. The tapetal cells forming the innermost layer of the wall of the anther, were found to be very suitable for the study of somatic chromosomes. For this purpose very young anthers were squashed under the coverslip in acetocarmine and then the slides were handled in the same way as for spreading the chromosomes in the microspore mother cells. Several well spread figures of somatic chromosomes of the tapetal cells could be easily obtained. Niródi (1955) has also studied the somatic chromosomes from the tapetal cells in different species of *Coix*. One of us (Paliwal, unpubl.) has used the tapetal chromosomes in *Pennisetum typhoides* to correlate the structural changes seen in the meiotic chromosomes with the somatic chromosomes. The advantages of the study of the somatic chromosomes from the tapetal cells, wherever easily and accurately possible are many fold: (a) The tapetal cells are easily separated from each other and the anther wall, and in this respect they are as good as microspore mother cells for squash preparations. (b) Because of the fairly large size of the cells and the nucleus there is no difficulty in spreading the chromosomes well, which is experienced in the cells of the root tips. (c) The somatic chromosomes of the tapetal cells can be studied at the same time and from the same material whose meiosis is being studied. (d) And lastly the most important advantage in case of *Coix* is that the chromosomes undergo a sort of incomplete endomitosis. Therefore, a very large number of nuclei can be found in division stages whose duration is considerably lengthened. Secondly at the end of metaphase the chromosomes are seen clearly as double structures with the chromatids separated throughout their length except at the centromere where they remain attached and present a X or V shaped appearance. This facilitates the identification of the centromere position on each chromosome with fair certainty.

Observations

Morphology. Fig. 1 shows a plant of the present form of *Coix* in the field. Fig. 2 shows a photograph of the specimen from the herbarium sheet made for inclusive herbaria. Plants annual, 2-3 m tall; with 2-5 tillers; vigorously branching with one branch at each node. Internodes 7-18 cm long, 1.5-3.5 cm thick. Leaf blades linear; leaf length 65 cm-1.04 m; leaf width 2.5-4 cm; length of the leaf blade/length of the leaf sheath, 7:1 to 9:1. Surface of the leaf sheath smooth; leaf blade hairy on the upper surface; on the lower surface only upper 1/3 of the midrib shows ascending hairs, while the rest of the surface is smooth. Leaf margin spinously serrate. Lower part of the leaf sheath and the blade pigmented pink. Plant monoecious. Usually 4 spikes produced at each node, but sometimes as many as 6; each spike borne on a long triangular and glabrous stalk. Usually one or occasionally two basal spikelets female, rest male; male portion of the spike 2-2.7 cm long, drooping. Male spikelets arising in three's below and in pairs above. When in three's the central spikelet is pedicelled while laterals are sessile and when in two's one is pedicelled while the other is sessile. The spike is terminated by a sterile spikelet. False fruits are enclosed at maturity within a hard shining shell. Diameter of the fruit 1-1.25 cm. The nature of the inflorescence, the floral structure and the development of the false fruit definitely show that these plants belong to the genus *Coix*. The detailed morphological description, however, does not fit exactly with

any established species of *Coix*. The morphological comparison of this species of *Coix* with *C. lacryma* grown in our garden and *C. gigantea*'s

Figs. 1-4. A plant, pressed twig and somatic chromosomes of aneuploid *Coix*. 1, plant showing the habit. 2, pressed twig. Note the axillary inflorescences. 3 and 4, tapetal cell squashes showing 32 somatic chromosomes. Note the variation in chromosome size. $\times 3360$.

description given by Nirodi (1955) has been tabulated in the Appendix 1. This comparison clearly shows that these *Coix* plants resemble *C. lacryma*

in some characters and *C. gigantea* in others, while it differs from both in certain vegetative as well as floral characters.

Cytology. Eight plants of the present form of *Coix* that reached flowering were cytologically examined and all were found to have $2n=32$ chromosomes in the somatic cells. We shall first describe the somatic chromosomes from tapetal cells followed by meiosis.

Somatic chromosomes. The tapetal chromosomes could be spread well enough for study, when they become contracted and condensed taking up a metaphase appearance (Fig. 3). Most of the cells at this stage had one nucleolus though cells with two nucleoli, one major and one minor, were also seen. The feebly stained nucleolus could always be seen in all the cells when the chromosomes were in metaphase condition or were even showing a separation at their ends (Fig. 4). Invariably all the cells where the chromosomes could be counted showed the presence of 32 chromosomes (Figs. 3, 4 and 5). The chromosomes exhibited marked variation in their length, twelve chromosomes being conspicuously larger than the remaining twenty chromosomes. The 12 chromosomes belonging to the large size group could be further subdivided into the following three categories:

- a) Two very large chromosomes.
- b) Eight large chromosomes.
- c) Two intermediate size chromosomes.

The twenty chromosomes belonging to small size group showed only slight variation in length. Two of the chromosomes of the larger group were satellited. Two pairs from the larger size group of the chromosomes and one pair from the smaller size group of chromosomes had a subterminal centromere while all the rest showed either median or slightly submedian centromeric position (Fig. 5).

Meiosis. The pachytene and diplotene preparations were far from satisfactory, the chromosomes remaining overlapped and clumped together. One thing was, however, always very clear that the pachytene chromosomes are not uniformly stained throughout their length. Instead they exhibited distinct densely stained segments mostly on the proximal parts. These differentially stained segments may be regarded as heterochromatic. In *C. poilaeni* Nirodi (1955) has used this character in the identification of individual chromosomes. Her figure of pachytene chromosomes in *C. gigantea* also shows heteropycknotic segments of variable sizes on different chromosomes.

The meiotic chromosomes could best be studied at late diplotene, diakinesis and metaphase I. Invariably at all these stages $16n$ could always be easily counted (Figs. 6–9). Further, the perfect pairing of the homologous chromosomes through all these stages of meiosis points towards the very balanced nature of the karyotype. Though most of the cells at diakinesis showed the presence of one nucleolus and one bivalent associated with it

(Fig. 6), cells with one major and one minor nucleolus were also met with (Fig. 7). Out of 230 cells scored, 50 cells showed the presence of two nucleoli. Nirodi (1955) has reported the occurrence of two nucleoli in *C. gigantea*. In the present form of *Coix* the minor nucleolus was, however, always seen appressed against the major nucleolus.

At metaphase I 16II (Fig. 8) always assembled at the equatorial plate. Both at diakinesis as well as metaphase I the significant difference in the size of the chromosomes as described for the somatic chromosomes was quite conspicuous. In Figs. 8 and 9 one largest bivalent at metaphase I can be easily spotted out. The next four large pairs of chromosomes are also easily discernible.

One pair classified as intermediate pre-

sents some difficulty but in favourable preparations it can also be identified from ten pairs of small chromosomes (see the bivalent marked by an arrow in Fig. 9). The five bivalents apparently belonging to the larger size group, are always present as closed rings at metaphase I. The intermediate size bivalent may either form a ring or a rod. The small bivalents are usually present as rods though occasionally some one of them may also form a ring

Figs. 5-8. Somatic and meiotic chromosomes of aneuploid *Coix*. All figures. $\times 2900$. 5, tapetal cell squash showing 32 somatic chromosomes. 6-7, diakinesis showing 16II . 7, shows binucleolate condition. 8, metaphase I showing 16II .

bivalent. The number of ring bivalents at metaphase I thus varies from 5-7. In majority of cells the configuration is 5 rings+11 rods. The data on the number of ring and rod bivalents at metaphase I is presented in Table 1.

Table 1.

S. N	No. of cells	Configuration at M I
1	160	5 rings+11 rods
2	30	6 rings+10 rods
3	20	7 rings+ 9 rods

Some of the rod bivalents, particularly the most small ones, show a precocious separation at metaphase I whereas the larger bivalents forming rings are the last to separate (Figs. 9 and 10). The

distribution of chromosomes at anaphase I is very regular with clear 16 chromosomes moving to each pole (Fig. 11). Out of several cells scored at this stage only one showed 17-15 distribution. At anaphase I too the difference in size of the large and small chromosomes is clearly discernible and the count of larger and smaller chromosomes at each pole confirms the classification of the chromosomes into various categories as has already been described.

At telophase I the two daughter nuclei are separated through wall formation which is of the successive type. Prophase II is very short and the chromosomes immediately acquire metaphase appearance (Fig. 12). Generally the division in both cells of a dyad is synchronous, but sometimes one of the cells may be seen one step ahead in division. Anaphase II is quite regular. At telophase II a wall is layed at right angles to the previous one and this results in the formation of isobilateral tetrads of microspores. These soon separate and develop into healthy pollen grains (Fig. 13). Pollen grains are tricelled at maturity.

On the female side too the plants were fully female fertile and large number of healthy fruits could be obtained through self pollination as well as sibbing.

Discussion

Karyotypes of Coix. The most commonly found haploid number in Maydeae is 10, but *C. aquatica* and *C. poilaeni* (?) show the lowest chromosome number of the series with $n=5$. Taking this as the basic chromosome number the genus *Coix* forms an euploid complex; *C. aquatica* and *C. foilaeni* (?) representing the diploids with $2n=10$; *C. lacryma* a tetraploid with $2n=20$ and *C. gigantea* an octaploid with $2n=40$. The present form of *Coix* with $2n=32$ is the only case of a natural aneuploid so far known in the genus *Coix*. Nirodi (1955) who has studied the cytomorphology of the diploid, tetraploid as well as octoploid species of *Coix*, suggests that the tetraploid species arose by a natural crossing of two similar diploids and a doubling of the chromosome number of the hybrid, while the octoploid

species probably arose as an autopolyploid from such amphidiploid. The evidences sought for the autopolyploid nature of the octoploid species are (Nirodi 1955);

(a) the occasional occurrence of two nucleoli per cell; (b) some cases of cells with two nucleolus or organizing chromosomes; and (c) the occasional occurrence of one or two quadrivalents in a very few cells at diakinesis in the octoploid species, *C. gigantea*. She further adds that the genome of the octoploid species has undergone such changes that it has come to function as diploid. It should be remembered, however, that *C. gigantea* has only one pair of satellite chromosome, the characteristic nucleolus

Figs. 9-13. Meiotic chromosomes and pollen grains of aneuploid *Coix*. 9, metaphase I showing 16II. Note the difference in chromosome size. $\times 2900$. 10, metaphase I. The small bivalents have separated while the large ones are still intact. $\times 2900$. 11, anaphase I showing 16 chromosomes at each pole. $\times 2900$. 12, metaphase II. In the upper dyad cell 16 chromosomes are countable. Also mark the difference in their size. $\times 2200$. 13, mature pollen grains. $\times 550$.

organizer of all the species of *Coix*, and not two, which should be expected if *C. gigantea* is an autopolyploid. In the present form of *Coix* also only one pair of satellite chromosome is found. Regarding the karyotype of *C. gigantea* Nirodi (1955) writes, "...many of the chromosomes of *C. gigantea* (?) are intermediate in size between those of *C. poilaeni* (?) and of the 20 chromosome *Coixes*". Evidently therefore, the autopolyploid nature of the octoploid should be accepted with caution. It may also be added that occasional presence of quadrivalent is not a sure sign of the autotetraploid nature of the plant since if the plant is a structural heterozygote for a very small reciprocal translocation, quadrivalents may be formed in some cells. As regards the presence of two nucleoli per cell it may be pointed out that *C. lacryma* (Koul unpubl.) also shows the occasional presence of two nucleoli per cell at diplotene and diakinesis.

The karyotype of the present species of *Coix* is essentially different from other species of *Coix* in some important features, particularly the symmetry of the karyotype. The karyotypes of all the species of *Coix* studied by previous workers appear to be quite symmetrical. There is no mention of a grouping of the chromosomes into distinct size groups and no acrocentric chromosomes have been reported. We have calculated the ratio of the longest to the shortest chromosome in different species of *Coix* from our preparations. These are presented in Table 2 along with the proportion

Table 2

Name of the species	Stage at which compared	$\frac{\text{Longest}}{\text{Shortest}}$	Proportion of meta to acrocentric chromosomes
<i>C. poilaeni</i>	Mid prophase (Nirodi 1955)	1.66	10:0
<i>C. poilaeni</i>	Metaphase "	1.60	10:0
<i>C. lacryma</i> var. typica	Mid prophase "	1.82	20:0
<i>C. lacryma</i> var. Ma-yuen	Mid prophase "	1.83	20:0
<i>C. gigantea</i>	Early prophase "	2.71	40:0
<i>Coix</i> sps	Metaphase Present work (Average of 10 cells)	3.95	26:6

of the metacentric to acrocentric chromosomes in these species of *Coix* to determine the symmetry of the karyotype (after Stebbins 1958).

It is evident from the above comparisons that there is slightly increased assymetry in the octoploid species of *Coix* as compared to the tetraploid and the diploid species. It may be pointed out, however, that for *C. gigantea* only one figure of early prophase was available to calculate the ratio of the longest to the shortest chromosome. The present species of *Coix* shows greater assymetry in two important features (see Stebbins 1958), that is, the

ratio between the longest and the shortest chromosome as well as the proportions of the acrocentric chromosomes, both being higher in the 32 chromosome aneuploid form of *Coix* than the 40 chromosome species, *C. gigantea*.

Mode of origin of 32 chromosome Coix. There are at least two basic chromosome numbers in Maydeae i.e. 5 and 9. The species of *Zea*, *Euchlaena*, *Polytoca* as well as *Coix*, except the present form, have a chromosome number multiple of 5. The species of the allied genus *Tripsacum* have chromosome numbers multiple of 9. In the related tribes Ischaeminae and Rottboellinae three basic numbers, 5, 7 and 9 are found (see Celarier 1957). None of the species of the above mentioned tribes show a chromosome number multiple of 8. It will therefore, appear naive to conceive of another basic number of 8 in Maydeae from which this form of *Coix* with $2n=32$ might have originated.

There is also nothing to indicate that this aneuploid form might have originated from an hexaploid with $2n=30$ through a duplication of one pair of chromosomes. It seems more likely that the 32 chromosome *Coix* might have originated from a polyploid species (with $2n=35$ or 40) through a reduction in the number of chromosomes. Unless an hexaploid or an heptaploid with $2n=30$ or 35 respectively is found, we will have to assume that the 32 chromosome *Coix* has originated from an octoploid species with $2n=40$ chromosomes.

Repatterning of karyotype through a reduction in the number of chromosomes by unequal reciprocal translocations followed by elimination of the centromere of the small mutant chromosomes carrying only a very small amount of genetically important chromatin, was originally proposed by Navashin (1932) and elaborated by Darlington (1937). The validity of this hypothesis has been proved from factual evidence in several cases (see Stebbins 1950, 1958, Swanson 1957 for reviews of earlier work, Hair and Beuzenberg 1958, Randolph and Mitra 1959 for recent work). Another mechanism responsible for the repatterning of the karyotype through a change in the position of the centromere is the pericentric inversions. Both these mechanisms appear to have played a role in the evolution of the karyotype of the 32 chromosome *Coix*. Small acrocentric chromosomes with subterminal centromere in 32 chromosome *Coix* could have arisen most probably through inversions in some metacentric chromosomes rather than deficiency and deletions of the terminal segments. The presence of a very large pair of metacentric chromosomes and two other large pairs of acrocentric chromosomes, not seen in any other species indicates that unequal reciprocal translocations involving a large segment from an acrocentric chromosome to another chromosome with a submedian or subterminal centromere have also occurred. Subsequently a reduction in the number of chromosomes may have been brought about by the elimination of the small chromosomes left

with very little or no genetically important (essential) chromatin material.

A very important point, however, is that in the evolution of the present 32 chromosome *Coix* by the above mechanism at least four pairs of chromosomes should have undergone unequal reciprocal translocations followed by an elimination of the four pairs of mutant small chromosomes. It could not have been brought about just in one step, instead it should have taken quite a long time for such a drastic reduction in the number of chromosomes by the slow process of translocations and eliminations. In such a case it should be possible to find out forms of *Coix* with chromosome numbers ranging from $2n=32$ to $2n=40$. However, since the 32 chromosome *Coix* is quite fertile and is capable of maintaining itself, the extra chromosomes may be present as supernumerary chromosomes. A more extensive work on a large number of accessions of *Coix* may reveal the presence of such a series. We should also keep in view that along with the above mentioned mechanism some other mechanism also may have been involved in the reduction of the number of chromosomes from $2n=40$ to $2n=32$. In this connection it is pertinent to cite some recent findings of Smith (1958) on *Nicotiana*. He has shown that when a multiple allopolyploid—*Nicotiana-bigelovii-tabacum* with $n=72$ was subjected to continued self pollination and selection through S_{10} generations three different races with different aneuploid numbers of chromosomes, but all less than the haploid number of $n=72$ of the multiple allopolyploid, were established. Thus it has been shown that higher allopolyploids may give rise to populations with low novel aneuploid numbers having higher fertility and distinctive morphologies in sexually reproducing forms. It is possible that a similar mechanism operating under a natural selection on an octoploid species of *Coix* may have been involved along with amphiplasty in producing a new aneuploid race of *Coix* with $2n=32$. Whatever might have been the mechanisms involved in the evolution of this aneuploid, the present observations point out the necessity of a more detailed study of several accessions of Oriental Maydeae to explore the existing karyotypic variability.

Status of the 32 chromosome Coix. The 32 chromosome *Coix* has quite a distinctive morphology supported by its different karyotype, warranting it to be raised to a new species level. But it is essential to find out the homology of the chromosomes of the 32 chromosome *Coix* with *C. lacryma* and *C. gigantea* through hybridization experiments and the fertility of the hybrids between the 32 chromosome and the 40 chromosome *Coixes*, before the specific status of the aneuploid *Coix* is decided.

Summary

The morphology and cytology of an accession of *Coix* collected from Madhya Pradesh has been studied in detail. Morphologically the plants are distinct from all the species so far on record. The plants invariably possess

32 chromosomes, a number new to the tribe, Maydeae. Karyotypic analysis shows a pronounced assymetry with regard to the ratio between the longest and the shortest chromosomes and the proportion of acrocentric chromosomes.

Appendix 1. Morphological comparisons of three species of *Coix*

Character	<i>C. lacryma</i> Jobi $2n=20$	<i>C. gigantea</i> $2n=40$	<i>Coix</i> sps. $2n=32$
Root system	Annual or perennial	Perennial	Annual or perennial
Stem	Erect, annual	Erect, perennial	Erect, annual
Height	1.3 m-3.3 m	2.5-5 m	2-5 m
Tillering	Vigorous tillering	—	2-5 tillers
Internodes	5.15 cm thick	—	18-7 cm long and 1.5-3.5 cm thick
Branching	Vigorous branching	—	Vigorous
Leaf blades	Lanceolate, 5.7-8.2 cm broad; 55-107 cm long	Linear-lanceolate, 2-2.5 cm broad; 30 cm long	Linear lanceolate, 2.5-4 cm broad; 65 cm-1.04 m long
Leaf surface	Glabrous, backwardly hispid due to ascending hairs along each veinlet of the upper surface. Lower surface quite smooth.	Both surfaces hairy.	Upper surface hairy. Lower surface smooth except 1/3 portion of the mid rib which is hispid
Leaf sheath	—	—	Blade / Sheath ratio, 7:1 to 9:1
Margin	Serrate	Serrate	Spinously serrate
Sheath surface	Smooth	Hairy	Smooth
Sheath colour	Usually green, occasionally pigmented pink	—	Lower part of sheath internodes pink
Ligule	Membranous	Membranous, Just above the ligule a tuft of hairs	Membranous
No. of spikes at each node	Usually 3, sometimes 5	2 or more	Usually 4, sometimes 5 or 6
Peduncle	Grooved on one side, glabrous	Flattened on one side, glabrous	Triangular, glabrous
Female spikelet	One per spike, rarely two	One, basal most	One, rarely two
Male portion of the spike	3.5 cm long, erect	4 cm long, drooping	2-2.7 cm long drooping
Male spikel	In 2's or 3's	In 3's. Central pedicelled, laterals sessile	In 3's below and 2's above
Terminal spikelet	Sterile	—	Sterile
False fruit	Ovoid, or oblong. 1.25-1.5 cm in diameter, bluish white	Elongate, pyri-form 1 cm long 5-6 mm in diameter. Flattened on one side	Ovoid, 1-1.25 cm in diameter

The distinctive morphology supported by the different karyotype warrants a specific rank for this form of *Coix*. The possible mode of origin of this new form has been discussed in detail.

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