Cytological studies in *Coix aquatica* Roxb. (2n =10): A possible origin of aneuploids

Shrimant D. Raut¹ and Chamile D. R.²

¹Pratibha Niketan Mahavidyalaya, Nanded - 431601,(M.S.), India
²Sharda Mahavidyalaya, Parbhani - 431401, (M.S.), India

Author for correspondence: (rshrimant@gmail.com)
Received July 15, 2017; accepted August 10, 2017

ABSTRACT: *Coix aquatica* (2n = 10) is one of the three species of *Coix* L. occurring in Maharashtra. The detailed meiotic behavior, chromosome number, and pollen fertility were studied in three distantly located populations of *Coix aquatica* Roxb. The progeny has been studied as single plant cytology for three consecutive generations. All the three populations of the species had the diploid chromosome number of 2n = 10, and morphologically they were all alike except some minor differences like height of the plant, number of tillers, shape and size of seed. The plants of the population from Tuljapur (Dist. Oosmanabad) had narrow leaves and comparatively smaller conchate seeds. The behavior of their chromosomes during meiosis was normal in first two generations. While screening the third open pollinated generation, three plants from the Aundha-nagnath shown different chromosomal behavior though the chromosome number was same i.e. 2n = 10. It results in to various abnormalities like, univalents at diakinesis, precocious movement at metaphase, laggards and early segregation in to chromatids during anaphase I etc. Therefore, the plants has been deeply investigated for results at the end of meiosis-II, and it is presented in the present communication.

KEYWORDS: Chromosomal abnormalities, Chromosome number, *Coix aquatic*, Maharashtra, Nullisomics, Poaceae

*Coix* L. is the most widely distributed oriental genus of a monococious tribe Maydeae, family Poaceae, is a wild relative of cultivated maize (Koul and Paliwal 1964). It is of wide occurrence in most of south-east Asian countries and of great economic importance as food, forage and medicinal plant, especially *Coix lacryma-jobi* L. The genus *Coix* consists of four species (Bor 1960) whose chromosome numbers range from 2n = 10 to 40 and has the basic number x = 5 (Darlington and Wylie 1955; Fedorov 1974; Goldblatt 1984). *C. lacryma-jobi* Linn. commonly known as “Job’s tears” consists of three cytotypes, diploid, tetraploid and the octoploid with the chromosome numbers 2n = 10, 20 and 40, respectively. The chromosome number of *C. aquatica* is (2n = 10) (Mangelsdorf and Reeves 1939; Nirodi 1955; Nirmala 2003; Barve and Sangeetha 2008). *C. lacryma-jobi* (2n = 20) while for *C. gigantea* it has been reported to be 2n = 12, 20, and 40 (Nirodi 1955; Venketshvarlu and Rao 1956; Koul and Paliwal 1964; Christopher and Jacob 1991).

*Coix aquatica* (2n = 10) has larger chromosomes than *C. gigantea* and *C. lacryma-jobi*. It is considered to be quiet stable and widely distributed in the various parts of India as well as Mharashtra.

MATERIALS AND METHODS
The seeds of *Coix aquatica* collected from Aundha-Nagnath (Dist. Hingoli), Tuljapur (Dist. Oosmanabad) and Nanakwadi (Dist. Nanded). Their plants were cultivated in the Botanical Garden of Pratibha Niketan Mahavidyalaya Nanded. Before the onset of flowering, all the plants in the field were serially tagged with numbers. During the flowering season; male racemes in appropriate size were fixed in the acetic:alcohol (1:3), material stored in the refrigerator till the further use for meiotic analysis. The plants were studied as single plant cytology. For meiotic studies 1% aceto-carmine was used as nuclear/chromosomal stain to squash the anther and prepare the slides. From each collected plant material a number of freshly prepared slides were examined for meiotic analysis and chromosome counts at different stages in each population. Pollen fertility was estimated through stainability test for which mature anthers were squashed. Well-filled pollen grains with uniformly stained cytoplasm were considered fertile and unstained or partially stained shriveled pollen grains were counted as sterile. Photomicrographs were taken from the temporary slides using a Nikon Coolpix s2700 under Olympus research Microscope.

RESULTS AND DISCUSSION
The detailed meiotic analyses of the three populations of *Coix aquatica* showed the presence of five regular bivalents at diakinesis in most of the PMCs (Fig. 4). At metaphase I they were arranged on equator or metaphase plate (Fig. 5). They segregate in to 5I-5I on respective poles during anaphase I (Fig. 9). Asserting that all the three populations showed the same meiotic chromosome number (n = 5). This number is similar to the previous chromosome number reports by various workers for the species. The data obtained from three populations (Number of plants scored), meiotic chromosome number, the ploidy level and place of collection of seeds of *Coix aquatica* was mentioned in Table 1. Cytological investigation has been performed by single plant cytology.
Table 1. Locality of populations, number of plants screened, chromosome number and ploidy level of *Coix aquatica* Roxb.

| Sr. No. | Locality               | No. of plants in 1st generation | No. of plants in 2nd generation | No. of plants in 3rd generation | 2n chromosome number | Ploidy level (x) |
|---------|------------------------|---------------------------------|---------------------------------|---------------------------------|----------------------|------------------|
| 1       | Nanakwadi              | 25                              | 67                              | 47                              | 2n =10               | 2x               |
| 2       | Aundha-nagnath         | 21                              | 56                              | 51                              | 2n =10               | 2x               |
| 3       | Tuljapur               | 17                              | 43                              | 49                              | 2n =10               | 2x               |

Figs. Meiosis in *Coix aquatica* having 2n = 10.
Figs. 1 and 2. Diakinesis showing cross bivalent and end to end pairing of bivalent. Fig. 3. Diakinesis showing univalent. Fig. 4. Perfect 5II at diakinesis. Fig. 5. Metaphase I with normally oriented bivalents. Fig. 6. Metaphase I. Precociously moving univalents at same pole. Fig.7. Precocious univalents towards opposite pole. Fig. 8. Anaphase I showing precocious univalent. Fig. 9. Anaphase showing 5I – 5I segregation. Fig. 10. Telophase I lagging univalents at equator. Fig. 11. Anaphase I precocious splitting in to chromatids. Fig. 12. Chromatids moving at respective poles.
The plants of all three populations showed normal meiotic behavior for first two generations, without any numerical and behavioral variation during both the meiosis I and II. However, while screening the third generation of open pollinated population from Aundha-Nagnath (District Hingoli), the meiotic irregularities occurred. Their plants studied had commonly the same chromosome number of $2n = 10$. Most frequent abnormalities found at diakinesis in this population were univalents (Fig. 3) (17%) chains, rings (Fig. 1) and end to end paring of chromosomes (Fig. 2) in PMCs (12%). It may be because of desynapsis or asynapsis desynapsis is associated with appearance of univalents (formed as a result of precocious separation of bivalents) at the post pachytene stage. This probably occurs due to failure of the crossing over. Asynapsis, on the other hand, has been linked with total prevention of chromosome pairing. Practically, in the absence of detailed study of Prophase I it is difficult to show whether asynapsis has occurred or desynapsis, and hence it is hard to use either of these terms with accuracy.

The presence of un-oriented univalents (Fig. 6) (7.33%), precocious movement of univalents (Fig. 7) (30.67%) is a very common condition in the PMCs at
metaphase I. The most interesting condition at anaphase I was the division of univalents into chromatids (Fig. 11) instead of anaphase II in (35.73%) PMCs. At telophase I and prophase II the early seplitted chromatids or univalents remain separate from the rest of the chromosome complement as micronuclei (Fig. 10, 14, 15, 16 and 17) in about 37.12% of PMCs latter on some of them might be get included and behave normally in metaphase and anaphase II. The miss behavior of the univalents continues during meiosis II. At prophase I they remain separate and condensed either as chromosome or chromatids (Fig. 18). During metaphase II they were either unorinted (Fig. 18) (10.33%) or precociously moving (25.66%) to the opposite poles and rarely orient at equator as two univalents with other chromosomes (Fig. 19). While in anaphase and telophase-II they showed variety of configurations, randomly placed in the cell, late segregating towards the poles, (6.77%) or remain on the equator. The behavior of the chromosome pair made it highly difficult to predict about the fate of them in the newly formed tetrads (Fig. 30). In 36.53% tetrads variable number of micronuclei have been reported in one, two, three or all the four cells. The behavior results in to the hypoloid $n = 4$ and hyperploid ($n = 7$) gametes, and affected the fertility of pollen grains which falls down up to 79.4% against the normal which is almost 100% in the plants. This misbehavior of single pair of chromosome may lead to aneuploid plants with chromosome number $2n = 9$ to $2n = 14$ at least theoretically. The behavior and the results of the chromosome pair may be suggesting that this plant is nullisomic ($2n = 2n-2$) in chromosome constituent and the genes controlling the meiotic behavior get disturbed due to inter population breeding. The through investigation at genetic level may help in knowing the basic chromosome number of the genus *Coix* L.

**LITERATURE CITED**

Barve, S. S. and Sangeetha, J. S. 2008. Cytological studies in 2n=17 *Coix gigantea* (Maydeae). Journ. Cytol. Genet. 9: 79–83.

Bor, N. L. 1960. Grasses of Burma, Ceylon, India and Pakistan. New York: Pergamon Press.

Christopher, J. and Jacob, B. 1991. Cytological studies of *Coix gigantea* Koen ex Roxb from South India. Cytologia 56: 265–268.

Darlington, C. D. and Wylie, A. P. 1955. Chromosome atlas of flowering plants. G. Allen and Unwin, London.

Fedorov, A. 1974. Chromosome numbers of flowering plants Rept. Koenigstein.

Goldblatt, P. 1984. Index to plant chromosome numbers. Missouri Bot. Gard.

Koul, A. K. and Paliwal, R. L. 1964. Morphology and cytology of a new species of *Coix* with 32 chromosomes. Cytologia 29: 378-386.

Mangelsdorf, P. C. and Reeves, R. G. 1939. The origin of Indian corn and its relatives. Texas Agri. Exp. Sta. Bull. 574: 1-315.

Nirodi, N. 1955. Studies in Asiatic relatives of maize. Ann. Missouri Bot. Gard. 42: 103–130.

Nirmala, A. 2003. Cytomorphological study of *Coix gigantea* Koen (2n=8x=40) and its interracial and interspecific hybrids. Nucleus 40: 115–119.

Venkateswarlu, J. and Rao, Rajeswara G. 1956. Progress Report on the 1.C.A.R. Scheme on Cytological Studies in Maize and Its Relatives.
Table 2. Chromosomal abnormalities during meiosis I and II

| Population       | Meiotic Abnormalities |
|------------------|-----------------------|
|                  | Unoriented univalents at metaphase I (%) | Precocious moving univalent at metaphase II (%) | Lagging univalents at anaphase I (%) | Dividing in to chromatids anaphase I (%) | Segregation in to 5I-5I at anaphase I (%) | Prophase II 4I+cc-4I+cc (%) | Unoriented univalents at metaphase II (%) | Precocious moving univalents at anaphase II (%) | Laggrds univalent s at A-II (%) | Tetrads with micronuclei | Pollen fertility |
| Nanakwadi        | 00                     | 0.10                                 | 00                                     | 00                                      | 100                                    | 00                                      | 00                                     | 00                                      | 00                                      | 00                                      | 100                                        |
| Aundha- Nagnath  | 7.33                   | 30.67                                | 4.43                                   | 35.73                                   | 65                                     | 37.12                                   | 10.33                                  | 25.66                                   | 6.77                                   | 36.53                                   | 79.36                                      |
| Tuljapur         | 00                     | 00                                   | 00                                     | 00                                      | 100                                    | 00                                      | 00                                     | 00                                      | 00                                      | 00                                      | 99                                         |