Cytogenetical Studies in *Pongamia pinnata* (L.) Pierre¹

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Pongamia pinnata (L.) Pierre (syn. *P. pinnata* (L.) Merrill = *P. glabra* Vent.), a monotypic genus (Hutchinson, 1964) occurring in tropical Asia, Malaya and Australia, is a leguminous tree of ornamental as well as of medicinal and economic value. It is believed to be native to tropical Asia and grown along the bank of streams and rivers. In India, it is found along the bank of rivers in the Konkan and Deccan not far from the sea coast. The plant is a member of tribe Dalbergieae of Papilionaceae (Fabaceae). The leaves are used as green fodder for cattle as well as for green manuring. Seeds yield an oil, popularly known as 'Karnaj Oil', is employed as a remedy in skin diseases and also for burning purposes.

A perusal of literature shows that only chromosome numbers have been reported in this plant. Patel and Narayana (1937) were the first to report 22 as diploid number of chromosomes in the species. Raghavan and Arora (1958) confirmed this number. Atchison (1951) on the other hand recorded 20 as diploid chromosome number.

The present paper deals with the detailed cytogenetical studies including mitotic and meiotic studies in this monotypic genus.

Material and methods

The tree of *Pongamia pinnata* (L.) Pierre is growing in the Botanical garden of Botany Department, Allahabad University, Allahabad. Materials for somatic and meiotic studies were collected from it.

As the trees present much difficulty in yielding well stained and well spread chromosome preparations. It was necessary to try various cytological techniques in order to standardize the most suitable method of fixation and staining for the present material.

Root tips from seeds, young anthers and leaves were used for the study of somatic chromosomes. The study of karyotype was made from temporary preparations. To facilitate the study of the morphology of chromosome and to get clear cut picture the materials were pretreated separately in either of the following chemicals recommended as pretreating agents.

1. 0.002 M aqueous solution of 8-hydroxyquinoline – 4 hours (Tjio and Levan 1950).
2. Aqueous saturated solution of p-dichlorobenzene – 4 hours at 10–14°C

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(Sharma and Mookerjee 1955).

3. Aqueous saturated solution of coumarin – 5 hours at 10–15°C (Sharma and Bal 1953).

4. Aqueous saturated solution of aesculin for young stem-tips and leaf-tips only – 4 hours at room temperature (Sharma and Sharma 1957).

However, best results were obtained with the use of p-dichlorobenzene in the case of root-tips and this was used for the pretreatment of the material throughout the investigations. After pretreatment root-tips were fixed in acetic alcohol (1:3) for 2 to 6 hours. Root-tips were then transferred to a mixture of 2% aceto-carmine and normal hydrochloric acid in the ratio of 8:2 at 60°C for 30 to 40 seconds. Deeply stained portions of the root-tips were squashed in a drop of 1% aceto-carmine by standard methods.

For meiotic studies flower buds at the division time were fixed in Carnoy's fixative (3 ethylalcohol: 1 acetic acid: 1 chloroform) for 6 to 12 hours. To improve the staining property of the chromosomes few crystals of iron-acetate were added to the fixative. Squash preparations were made following Belling's 1% aceto-carmine method.

Permanent preparations of the temporary squashes were made as detailed before (Sarbhoy 1971 and Naithani and Sarbhoy 1973).

Pollen fertility was determined on the basis of stainability in aceto-carmine. Well stained pollen grains with regular shape were considered viable and empty ones without any stain were taken as sterile.

The photomicrographs were taken with the aid of Leitz 'Panphot' microscope fitted with photographic attachment at a magnification of 1800 $\times$, using oil immersion objective (100.01 $\times$) and 10 $\times$ eye piece.

Observations

Somatic chromosomes

Times for maximum mitotic activity in the root-tip cells were found to be between 12 noon to 1 P.M. and 7 P.M. to 8 P.M.

The normal diploid chromosome number was found to be 22 (Fig. 1). The size of chromosomes ranges between 1.5 μ to 2.5 μ . The chromosome could be classified as follows:

1. Three pairs of long chromosomes (2.2 μ to 2.5 μ) with median primary constrictions and sub-median secondary constrictions.
2. Five pairs of medium size chromosomes (1.8 μ –2.1 μ) with submedian primary constrictions.
3. Three pairs of short chromosomes (1.5 μ –1.7 μ) with subterminal primary constrictions.

Total chromatin length is 40.8 μ .

Meiosis

At pachytene (Fig. 2) chromosomes get completely paired to form bivalents which appeared as greatly extended threads not clearly distinguishable individually.

One or two bivalents were seen attached to the nucleolus. Generally eleven bivalents were observed at diakinesis (Figs. 3 and 4). Both ring and rod shaped bivalents were encountered. However, ring bivalents were more in number. In

Figs. 1-6. 1, Mitosis in *Pongamia pinnata*. Metaphase showing $2n=22$ chromosomes. $\times 1800$. 2-12. Meiosis in *Pongamia pinnata*. $\times 1800$. 2, pachytene showing normal pairing of chromosomes. 3, diakinesis showing 11 bivalents. One bivalent is attached to nucleolus. 4, diakinesis showing 11 bivalents. 5, diakinesis showing one IV and nine bivalents. 6, diakinesis (late) showing 2 IV and 7 II. Nucleolus has disappeared.

10–15% cases a quadrivalent and 9 bivalents were observed at diakinesis (Fig. 5). The maximum number of such quadrivalents in a pollen mother cell (PMC) was observed to be two (Fig. 6). Table 1 gives the various types of configurations observed at diakinesis.

Table 1.

Types of configurations		Number of PMC's observed	
11 _{II}	=	42	
1 _{IV} 9 _{II}	=	6	
2 _{IV} 7 _{II}	=	2	
Total		50	

The maximum number of chiasmata per PMC was found to be 16. The chiasma frequency per bivalent was 1.4. At metaphase I eleven bivalents were generally observed (Fig. 7). In few cases one or two quadrivalents were seen (Fig. 8). Table 2 gives the various types of configurations observed at metaphase I. Usually bi-

Table 2.

Types of configurations		Number of PMC's observed	
11 _{II}	=	43	
1 _{IV} 9 _{II}	=	5	
2 _{IV} 7 _{II}	=	2	
Total		50	

valents disjoined regularly and eleven chromosomes were observed at each pole of anaphase I (Fig. 9).

Prophase II was of very short duration and chromosomes immediately entered into metaphase II. Eleven chromosomes were observed to orient themselves at each equator of metaphase II. In 5% cases an unusual and interesting phenomenon was observed at metaphase II where a chromatin bridge was encountered in between two metaphase spindles (Figs. 10 and 11). Presumably, this configuration is due to the fact that one of the quadrivalent with an inversion, encountered during diakinesis and metaphase I, remained in the centre unaccounted after the completion of first division due to non-disjunction or slow movement. At the second division this quadrivalent having two centromeres disjoined incompletely; each part with one centromere moving towards spindles and in doing so it got stretched up forming a bridge between the two spindles of metaphase II.

The further fate of the bridge could not be traced due to rare occurrence of such bridges. Anaphasic disjunction of chromosomes was regular with equal number of chromosomes at each pole at anaphase II (Fig. 12). Telophase II was normal.

Pollen sterility was found to be 15–18%.

Figs. 7-12. Meiosis in *Pongamia pinnata*. $\times 1800$. 7, metaphase I showing 11 bivalents. 8, metaphase I showing 2 IV and 7 II. 9, anaphase I showing normal 11:11 separation of chromosomes on the two poles. 10, metaphase II showing chromatin bridge inbetween two metaphase spindles. Spindle fibres can be seen (polar view). 11, metaphase II showing chromatin bridge inbetween two metaphase spindles (Equatorial view). 12, telophase II chromosomes have reached towards the poles.

Discussion

Basic chromosome number

The genus *Pongamia*, as far as the records reveal, indicates the presence of both $X=11$ (Patel and Narayana 1937 and Raghavan and Arora 1958) and $X=10$ (Atchison 1951) chromosomes in the basic set. The present investigations in *Pongamia pinnata* confirms 11 and 22 as haploid and diploid number of chromosomes. The two basic numbers $X=10$ and $X=11$ in the genus—*Pongamia* are to be considered. It seems that there exists two races of *Pongamia pinnata* with basic numbers one with $X=10$ and other with $X=11$ in nature. It is clear that basic numbers 10 and 11 are derived one from the other. The origin may involve mere non-disjunction of chromosomes. On the basis of multivalent and bivalent configurations observed at diakinesis and metaphase I of meiosis it appears that $X=10$ is the basic number for the genus *Pongamia*. Therefore, it can be emphasized that $X=11$ race in this genus is derived from $X=10$ race and consequently 10 is the original basic chromosome number and 11 is secondarily derived by non-disjunction of chromosomes.

The most interesting feature of meiotic behaviour of *Pongamia pinnata* is the occasional presence of multivalents and chromatin bridges. The normal chromosome number of the species has been found to be $2n=22$ which is the ordinary diploid chromosome number for the genus *Pongamia*. The presence of multivalents in the diploid plant shows that it is a structural heterozygote involving segmental interchanges.

The presence of quadrivalents shows that at least four chromosomes are involved in the translocations. The chromatin bridge at metaphase II indicates the presence of inversion heterozygosity in the chromosomes of *Pongamia pinnata*.

The meiotic studies in *Pongamia pinnata* clearly lead to the conclusion that it is heterozygote both for translocations and inversions. Presumably, this is the first record of translocation and inversion heterozygosity in the genus *Pongamia*.

The translocation heterozygotes may arise spontaneously or in response to irradiation or treatments with certain chemicals or by hybridization of two different species or races. Several cases are known in plants where translocation heterozygotes have been obtained by crossing different homozygous species or races, eg. *Pisum* (Sansome 1929, Håkansson 1936), *Triticum* (Smith 1935) and *Allium* (Levan 1936).

The spontaneously occurring multivalent configurations in *Pongamia pinnata* indicates a failure of chiasma formation in one pairing segment. Such failure is generally brought about when the translocation segments are not very long as in the case of maize T_{3-8} (Clarke and Anderson 1935), Barley (Burnham *et al.* 1954) and *Thelepodon elegans* (Sisodia 1970 a, b).

Venkateswarlu (1958) and Koul (1964) have reported a spontaneously occurring translocation heterozygote in *Coix aquatica*. Koul (1964) explained the formation of 4 and 6 chromosomes as due to associations between non-homologous heterochromatic segments, which also sometimes results in the interlocking of bivalents.

The first cytological demonstration of the location of an interchange between

two non-homologous chromosomes was given by McClintock (1930) in a semi-sterile line of maize. Since then extensive cytological work has been carried out in maize as well as in other plants and animals. Burnham (1956) cited 49 genera in Dicotyledoneae and 47 genera in Monocotyledoneae besides 2 genera in Gymnosperms and 3 genera in fungi in his review referring to chromosomal interchanges in plants.

The spontaneously occurring interchange has been reported in different plants. Among others, Naithani and Raghuvanshi (1958 a, b) and Raghuvanshi (1962 a, b) have reported such translocation interchange in *Citrus assamensis*, for the first time in the genus. They observed the formation of multivalents, such as tri-, quadri- and hexavalent at metaphase I in PMCs of *C. assamensis*, which is a diploid form. Rana and Jain (1965) observed a ring or chain of 4 chromosomes, their number varies from 1 to 2 in different populations of diploid annual *Chrysanthemum carinatum*. Khushoo and Mukherjee (1966) reported a ring or chain IV and 7 bivalents in diploid garden *Canna*. Sisodia (1970, a, b) reported rings and chains of VIII+1_{II}, VIII+2_{II}, VI+2_{II} and IV+3_{II} association at diakinesis and metaphase I in monotypic genus *Thelepogon elegans*. He explained these associations is due to interlocking of bivalents at meiosis.

Burnham (1956) listed seven possible ways in which reciprocal translocations may arise spontaneously:

1. Crossing over between duplicated segments which are present in non-homologous chromosomes.
2. The spontaneous association between heterochromatic regions.
3. The interlocking of bivalents at meiosis.
4. Accidental entanglement of chromosomes.
5. Chromosome breakages followed by reunion of broken ends.
6. In progeny of plants homozygous for the 'sticky chromosomes' character (in maize).
7. When plants are grown from aged seeds.

Out of these no evidence of spontaneous association between heterochromatic regions or spontaneous chromosome breakage or sticky nature of the chromosomes so far observed in the present investigations. It is also believed that the tree was not raised from aged seeds.

The multivalent formation in *Pongamia pinnata* may be explained on the basis of reciprocal translocations as evident by high pollen sterility of 15–18%. Among the other possibilities are interlocking of bivalents at meiosis or an accidental entanglement of chromosomes.

Pollen sterility in *P. pinnata* has been found to be 15–18%. The same plant is having both inversion heterozygosity and segmental interchange or translocation heterozygosity. Upcott (1937) observed that the amount of structural hybridity and pollen sterility are directly related to each other in *Tulipa*. It is well known fact that PMC showing inversion chromatin bridges at anaphase I gives rise to microspores with deficient or duplicate chromatid segments. Microspores with a deficient chromatid does not develop into viable gametophyte due to genic unbalance but gives rise to collapsed pollen grains. On the other hand multivalents due to their

unequal separation and non-disjunctional orientation add to pollen sterility. Therefore, high pollen sterility in *Pongamia pinnata* is clearly chromosomal due to inversion bridges and non-disjunction of multivalents causing deficiency and genic unbalance in microspores.

Summary

Detailed cytogenetical investigations in *Pongamia pinnata* (L.) Pierre, a monotypic genus, have been carried out. Eleven and twenty-two have been found to be as haploid and diploid chromosome numbers respectively. The plants have been reported to be an heterozygote for both inversions and translocations. Meiotic abnormalities in the form of quadrivalents at diakinesis and metaphase I and chromatin bridges at metaphase II are recorded for the first time in this plant. The basic chromosome number and high pollen sterility have been discussed. Pollen sterility is clearly chromosomal in nature and caused by deficiency and genic unbalance in the microspores.

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