

Research Article**Chromosome studies in the genus *Jatropha* L.**

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Abstract :

The inflorescences of ten species of the genus *Jatropha* were fixed in Cornoy's fluid (6:3:1). Acetocarmine stain (2%) was used for staining the pollen mother cells. Seven species exhibited 11 bivalents and $2n = 22$ and $x = 11$. But the two other species, *J.villosa* var. *villosa* and *J.villosa* var. *ramnadensis* showed only 10 bivalents and $2n$ number of 20 chromosomes and $x = 10$. The study concluded the occurrence of two kinds of haploid chromosome numbers of $n = 10$ and $n = 11$. Except *Jatropha tanjorensis*, cytological investigation in all species exhibited normal and complete pairing and bivalent formation in metaphase I and equal separation of chromosome in anaphase and indicated that the course of meiosis was normal. *Jatropha tanjorensis* did not exhibit normal course of meiosis and no proper count of chromosomes could be made. Present chromosomal studies in *Jatropha* revealed the existence of two basic chromosome numbers $x = 5$ and $x = 6$.

Key words:

Cornoy's fluid, pairing of chromosomes, course of meiosis, basic chromosome number, *Jatropha*.

Introduction

The genus *Jatropha* L. is a widespread and diverse genus with 175 species (Airy Shaw, 1972). It is still uncertain where the centre of origin is; but it is believed to be Mexico and Central America. *Jatropha curcas* was introduced in India and parts of Asia and Africa by the Portuguese in the 16th century (Dehgan, 1984 & Heller, 1996). In India, 12 species have been recorded (Paramathma *et al.*, 2004) of which ten species have been assembled in Tamil Nadu Agricultural University, Coimbatore (Paramathma *et al.*, 2007). The objective of the present study is to investigate and throw light on the course of meiosis and the chromosome pairing behaviour in metaphase. And to determine the $2n$ and basic chromosome numbers.

Materials and methods**Materials**

The experimental material for the study consisted of 10 species viz *Jatropha villosa* var. *villosa*, *Jatropha villosa* var. *ramnadensis*, *Jatropha multifida*, *Jatropha podagrica*, *Jatropha maheswarii*, *Jatropha glandulifera*, *Jatropha gossypifolia*, *Jatropha tanjorensis* and *Jatropha curcas* of the genus *Jatropha*, assembled from various locations of India.

Methods

For the study of meiosis, young developing inflorescence of the above listed species were fixed in Cornoy's fluid (chloroform, absolute alcohol and

glacial acetic acid at 6:3:1) between 6.00 and 8.30AM when there was bright sunlight. The material was kept in the fixative for 24 hours. After 24 hours, it is transferred to 70% ethanol. Immediately after fixing, the material was stored in refrigerator until it was taken out for use. The course of meiosis was studied in temporary smears of pollen mother cells using 2% acetocarmine stain. The fixed anthers were placed on slide. A scalpel was used to press out the microsporocytes and the anther walls and the debris were removed thoroughly. A drop of acetocarmine was then added and a cover slip was placed on the squash such that no air bubble forms. The slide was firmly held and gently heated over a flame for 2 seconds and then pressed between folds of plotting paper to have a good spread of cells. Slide was examined under the microscope. Microscopic photographs were taken by using of CETI microscope with (100X lens) JVC colour video camera attachment.

Results and discussion

All the chromosomes were paired into bivalents at first metaphase and separated in equal number in the first anaphase. Eight species and one hybrid showed 11 bivalents and the chromosome number of $2n = 22$ and haploid chromosome number of $x = 11$. Two species i.e. *Jatropha villosa* var. *villosa* (Fig.1) and *Jatropha villosa* var. *ramnadensis* (Fig.2) have the chromosome number of $2n = 20$ only and haploid chromosome number of $x = 10$. The chromosome numbers (ICPN) database indicated that among all *Jatropha* species *Jatropha tirucalli* species have the $2n = 20$ chromosomes (Kothari *et al.*, 1981).

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Jatropha multifida (Fig.3) and *Jatropha curcas* (Fig.10) have 11 bivalents comprising seven bivalents and four rod shape bivalents during first metaphase. Similar findings were reported by Soontornchainasaeng and Jenjittikul (2003). Observation of *J. podagrica* (Fig.4) pollen mother cells showed chromosome number of $2n = 22$ and metaphase I showed 11 bivalents ($2n = 22$ chromosome) and confirmed the total count of chromosome number in this species as $2n = 22$ (Sarkar, 1989 and Krishnappa and Rashme, 1980). *J. maheswarii* (Fig.5) showed the total chromosome number of $2n = 22$. At metaphase II the daughter cells had each 11 chromosomes arranged in equatorial plate. This result was similar to the findings of Kothari *et al.*, (1980). The total chromosome number of the species *J. glandulifera* (Fig.6) was $2n = 22$. Metaphase I had an equatorial arrangement of 11 bivalents. This result was in agreement with the previous result by Navaneetham *et al.*, (1983) who reported $2n$ number of chromosomes in *J. glandulifera* to be $2n = 22$.

In *J. gossypifolia*, anaphase I had the equal separation of chromosomes namely, 11/11 (Fig.7). Soontornchainasaeng and Jenjittikul (2003) reported that the species *J. curcas* and *J. multifida* and *J. gossypifolia* appeared to be closely related to each other based on their meiotic configurations because all these three species have the 7 ring + 4 rod bivalents in the microsporocytes. Sarkar (1989), Trivedi and Trivedi (1992) and Krishnappa and Rashme (1980 and 1982) also reported the $2n$ number of chromosome in this species was 22.

Cytological observation of *J. tanjorensis* species showed there is no proper count of chromosomes. After completion of first and second meiotic cycles, tetrads are formed to produce microspores. In this species, each tetrad has four unequal sporads (Fig.8) indicating disturbances in the course of meiosis. Prabakaran and Sujatha (1999) reported *J. tanjorensis* is a new species showing intermediary in phenotypic characters of *J. curcas* and *J. gossypifolia*. Meiotic studies revealed abnormal divisions with the formation of tri and univalent at metaphase I and unequal anaphase separation leading to the formation of laggards and sporads of unequal size.

Jatropha integerrima showed six ring and five rod bivalents at metaphase I (Fig.9) and *J. curcas* showed four ring and seven rod bivalents at prophase I (Fig.10). Soontornchainaksaeng and Jenjittikul (2003) obtained the same result in this species in karyology of *Jatropha*. Missouri botanical Garden, 2008 also confirmed the $2n$ number of *Jatropha integerrima* as $2n = 22$.

The course of meiosis up to tetrads stage and formation of the bivalents in the eight species such as *J. multifida*, *J. podagrica*, *J. villosa* var. *villosa*, *J. villosa* var. *ramnadensis*, *J. maheswarii*, *J. glandulifera*, *J. integerrima* and *J. curcas* were normal. The bivalent formation and regular equal separation of chromosomes indicated that the species investigated now are essentially diploids with $2n = 22$. However, distinct differences were noticed in respect of number of bivalents at metaphase I. All the species including the hybrid studied indicated 11 bivalents and $2n = 22$ and $x = 11$. But in *J. villosa* var. *villosa*, *J. villosa* var. *ramnadensis* which showed only 10 bivalents and $2n$ number of 20 chromosomes and haploid number of $x = 10$. The existence of diploid number of $2n = 22$ and $2n = 20$ in *Jatropha* is of significance bringing out the fact that two kinds of diploids, in which eight species had $2n = 22$ and two other species had $2n = 20$ chromosomes.

Previous study on cytology of *Jatropha* also indicated occurrence of different $2n$ numbers of chromosomes (*Jatropha tirucalli* $2n = 20$; Kothari *et al.*, 1981). In this context, it may be inferred that the diploid species with $2n = 20$ ($n = 10$) and $2n = 22$ ($n = 11$) have had a common origin. This was evident from morphological studies. Precise understanding can be made only when interspecific hybrids are synthesized between the diploid species with $2n = 20$ and $2n = 22$ and examined for the interrelationships between species having $2n = 20$ and $2n = 22$ chromosomes. The species *J. villosa* var. *ramnadensis* with $2n = 20$ are endemic to Tamil Nadu alone. Hybrids between species with $2n = 20$ or between species with $2n = 20$ and $2n = 22$ have not so far attempted or reported to occur naturally.

Furthermore, if amphidiploids of the two interspecific hybrids (*J. curcas* x *J. integerrima* and *J. curcas* x *J. gossypifolia*) reported between species having $2n = 22$ are synthesized by chromosome doubling, the course of meiosis and chromosome pairing in each amphidiploids with $2n = 44$ will give a critical understanding of the genome homology and the nature of chromosome association as homogenetic or heterogenetic nature. This is indicated for future studies.

A systematic and critical study on the karyotype and pachytene pairing in meiosis in species and hybrids will help in a better understanding of chromosome morphology and nature of karyotype evolution in the diploid species of *Jatropha*. Karyotype analysis will provide clues as to how the basic chromosome number has evolved. As such, this present study helped to conclude the occurrence of two kinds of haploid chromosome number of $n = 10$ and $n = 11$. It

may be postulated that $n = 10$ has arisen from basic number of $x = 5$ and the $n = 11$ in its turn evolved from dibasic chromosome numbers of $x = 5$ and $x = 6$, thereby indicating the present day diploid species with $2n = 20$ ($n = 10$) and $2n = 22$ ($n = 11$) are secondary diploid species. It is yet another cytological evolutionary step to understand as to how the basic chromosome numbers of $x = 5$ and 6 have evolved. It may be either by increase in number from $x = 5$ to $x = 6$ or by decrease of chromosome numbers and $x = 5$ resulting from $x = 6$. In the karyotype evolution, it is known that basic (x) chromosome numbers evolve as a result of addition or loss of chromosomes by structural rearrangements. The study of chromosome association in the F_1 s of $2n:20 \times 2n:22$ species alone can be the critical step to get precise understanding of the existence of genome homology, genetical similarities and probable basic chromosome number and scope to generate recombinants in the biofuel crop species.

The interspecific hybrids of *Jatropha curcas* and *Jatropha integerrima* offered adequate opportunities for genetic recombination and to generate backcross recombinants (Paramathma *et al.*, unpub). The strategies to further exploit the other interspecific hybrid *Jatropha tanjorensis* which is completely male and female sterile may be through synthesizing allotetraploid amphidiploids of *Jatropha curcas* and *Jatropha gossypifolia* and further backcrossing to *Jatropha curcas* so that genetic traits of *Jatropha gossypifolia* can be introgressed to *Jatropha curcas* background through amphidiploidy.

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The chromosomes association in meiosis in the species of jatropha

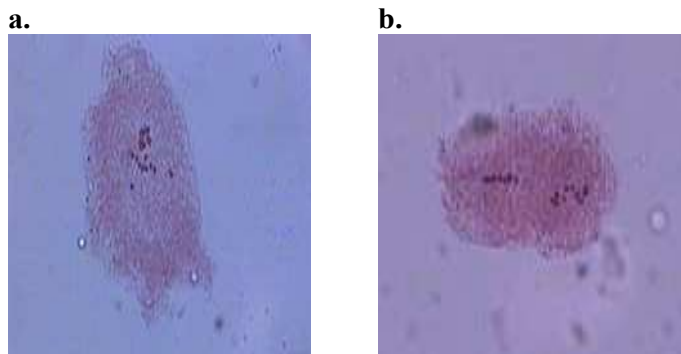


Fig.1: *Jatropha villosa* var. *villosa*, **a.** Metaphase 10_{II}, **b.** Anaphase 10: 10

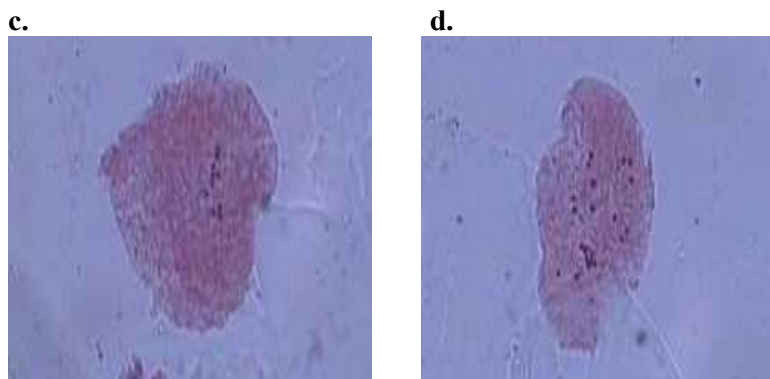


Fig 2: *Jatropha villosa* var. *ramnadensis*, **c.** Metaphase 10_{II}, **d.** Early Anaphase 10: 10

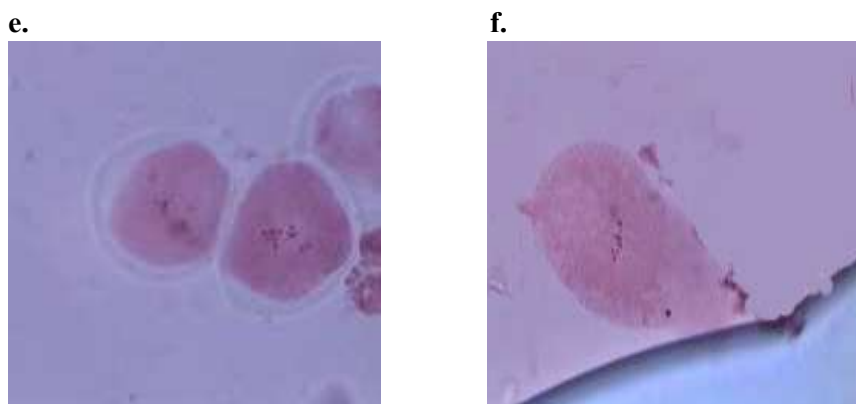


Fig 3: *Jatropha multifida*, **e** and **f.** Metaphase 11_{II}.

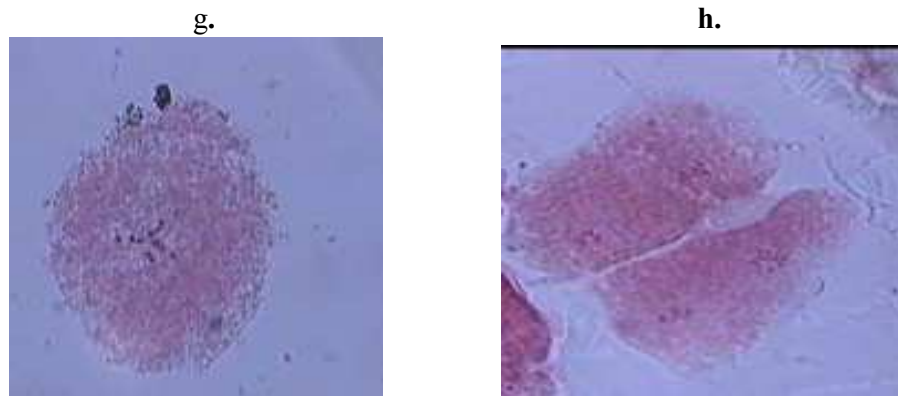


Fig 4: *Jatropha podagrica*, **g.** metaphase 11_{II}, **h.** anaphase 11: 11

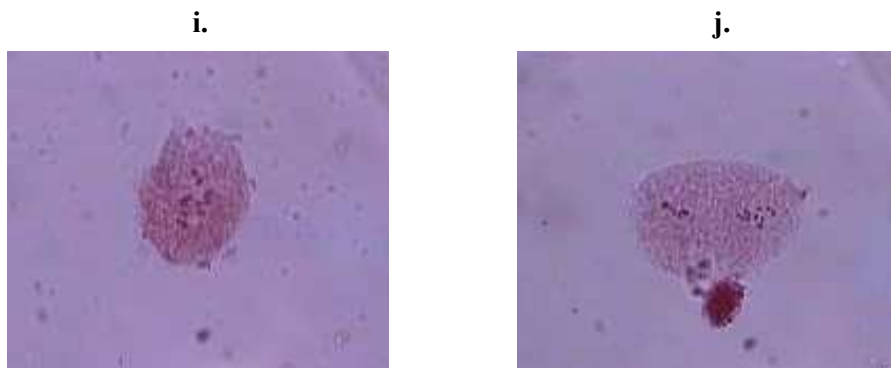


Fig 5: *Jatropha maheswarii*, **i.** anaphase 11: 11, **j.** metaphase 11_{II}.

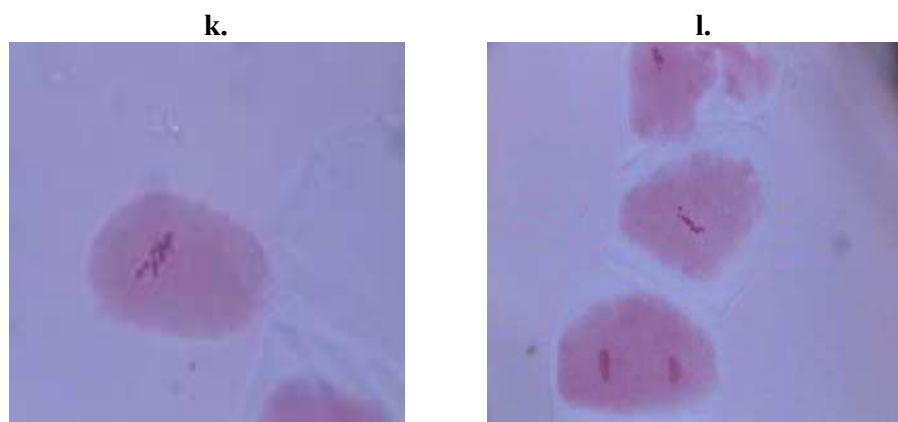


Fig 6: *Jatropha glandulifera*, **k.** metaphase 11_{II}, **l.** anaphase 11 by 11 separation

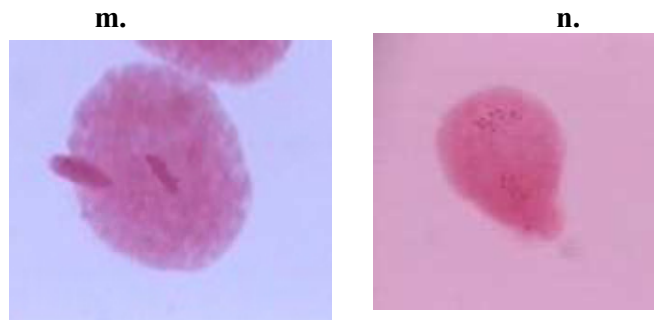


Fig 7: *Jatropha gossypifolia*, **m.** metaphase 11_{II}, **n.** anaphase 11: 11

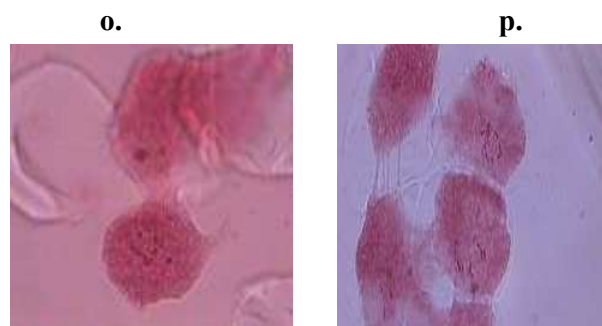


Fig 8: *Jatropha tanjorensis*, **o and p.** unequal separation of chromosomes.

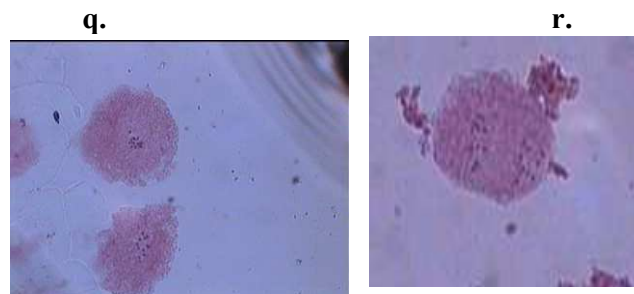


Fig 9: *Jatropha integerrima*, **q.** metaphase 11_{II}, **r.** anaphase 11: 11

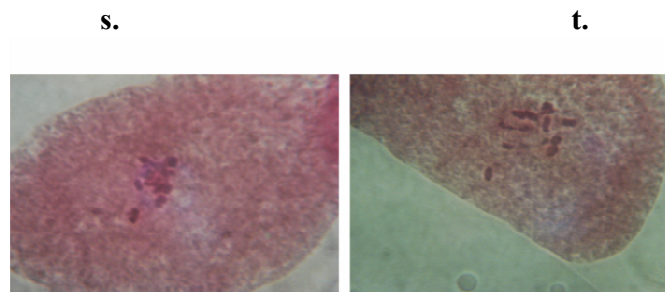


Fig 10: *Jatropha curcas*, **s and t.** prometaphase 11_{II}.