Chapter III

MEIOSIS IN
Passiflora incarnata L.

MEIOSIS IN PASSIFLORA INCARNATA

INTRODUCTION

Passiflora incarnata which is a vegetatively propagated plant and shows some desirable characteristics such as disease resistance, flowering through out the year and general vigour, but it is self and cross-incompatible. Meiotic analysis is helpful in obtaining some idea about the extent and nature of homology of chromosomes of a taxon. A knowledge of the reproductive stability of a taxon is an essential prerequisite for the formulation of any rational breeding programme.

It reveals from the previous literature that Passiflora is poorly understood from the standpoint of meiotic studies (Beal 1971). Previous studies also show that there is very poor fruit formation in Passiflora. Therefore a study of meiotic analysis is helpful in obtaining some idea about the extent and nature of homology of chromosomes of a taxon. So in the present work attempts are made to study meiosis in P. incarnata.

MATERIALS AND METHODS

Flower buds of P. incarnata were fixed in Carnoy's fluid absolute alcohol:acetic acid (3:1) for 24 h. stored in 70 % alcohol at 7°C. (Darlington and La Cour 1976). Anthers were

hydrolysed in a mixture of 2 % aceto-orcein and 1 N HCl (9:1) by gently heating over a spirit flame for a few seconds. Then anthers were macerated in 2 % aceto orcein for staining. All easily visible pieces of tissue were removed and the PMCs were squashed in 45 % acetic acid.

The slides were made permanent following the freezing and butyl alcohol-acetic acid series method using Depex as mountant (Conger and Fairchild 1953).

Meiotic abnormalities were examined under light microscope. Number of dividing cells were counted under one view and possible meiotic aberrations were detected. Average abnormality percentage is determined, by drawing the camera-lucida of diplotene, diakinesis and metaphase-I, number of chiasmata per nucleus and coefficient of chiasmata terminalization were calculated.

Photomicrographs of abnormal meiotic stages were taken under light microscope. (X 1500).

RESULTS

The meiosis in P.incarnata was highly irregular, being characterized by the presence of bridges, laggards, univalents and abnormal bivalent associations (Figs. 3.1 a,b,c; 3.2 a,b; 3.3 a to h; 3.4 a,b). Among the meiotic abnormalities detected diplotene and diakinesis were highest in number than any other meiotic aberrations (Table 3.1). Abnormal metaphase-I, and anaphase-I were also observed (Fig. 3.5). Metaphase-II and anaphase-II were also highly irregular. Telophase-II was highly

Fig. 3.1 a, b, and c showing Bridges.

Fig. 3.2 a, and b showing Laggards.

Fig. 3.3 a to h showing Univalents.

Fig. 3.4, a and b showing abnormal bivalent association.

Fig. 3.5 showing abnormal Metaphose I and anaphase I.

Fig. 3.6 - Telophase II showing cytomixis and unequal distribution of chromosomes.

Fig. 3.7 showing Micronuclei formation.

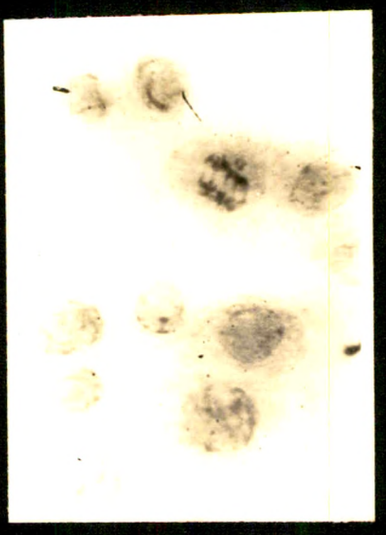


FIG. 3.1a

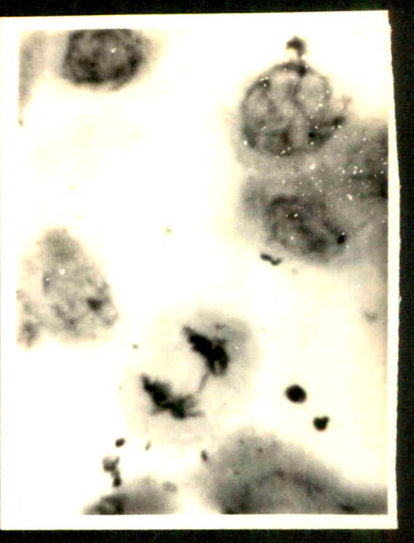


FIG. 3.1b

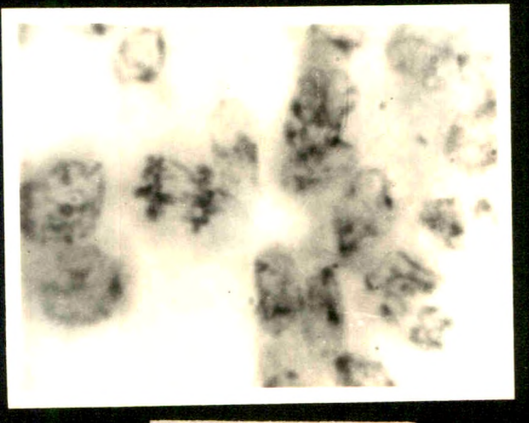


FIG. 3.1c

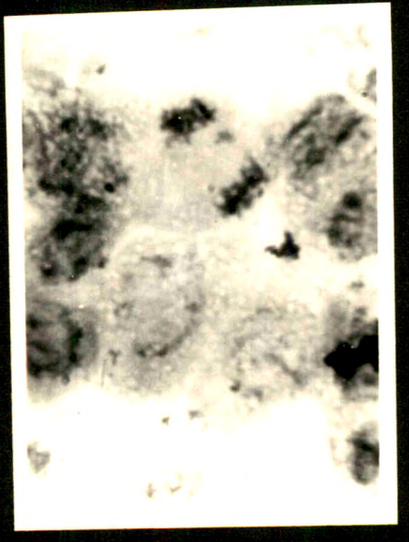


FIG. 3.2a

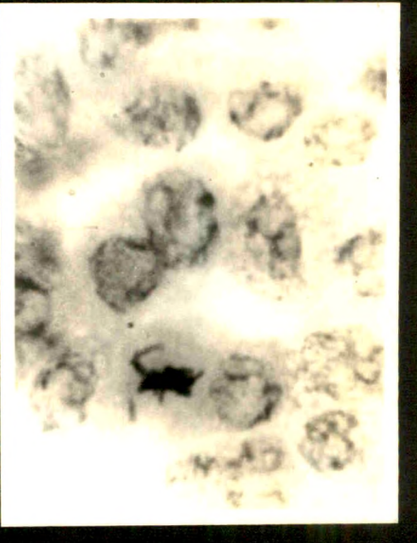


FIG. 3.2b

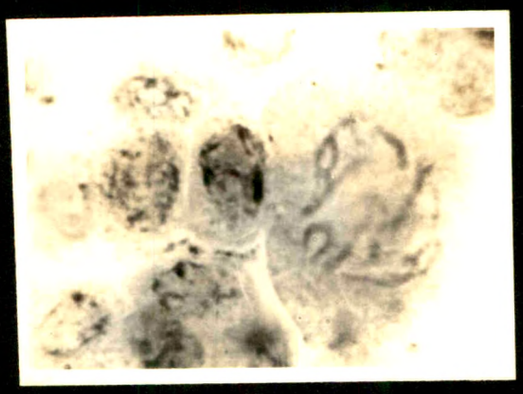


FIG. 3.3a

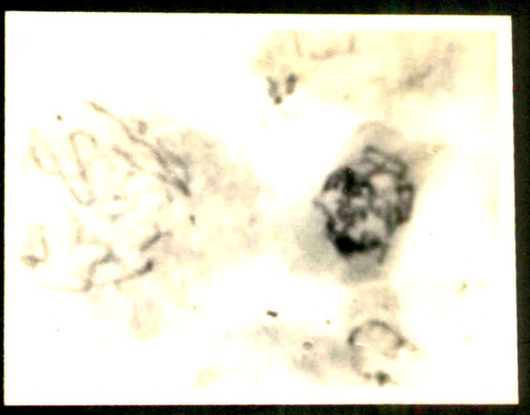


FIG. 3.3b

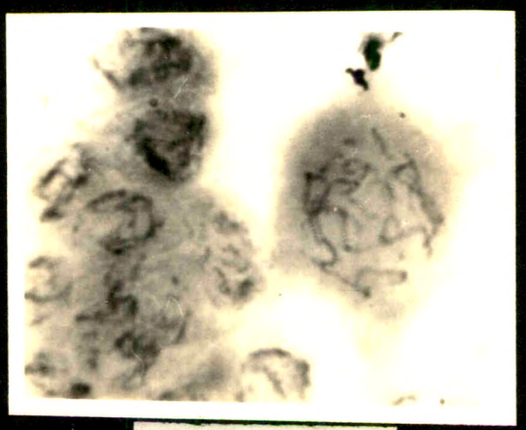


FIG. 3.3c

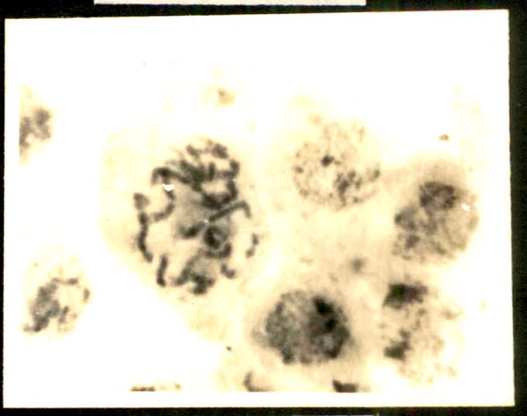


FIG. 3.3d

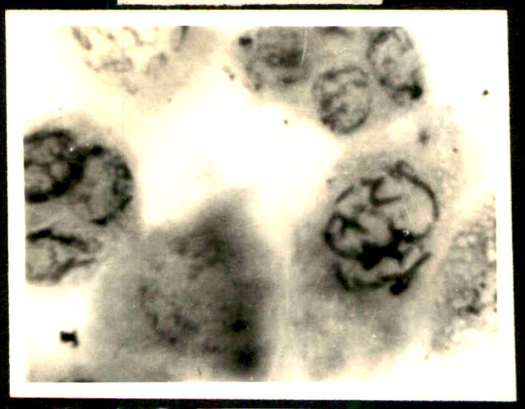
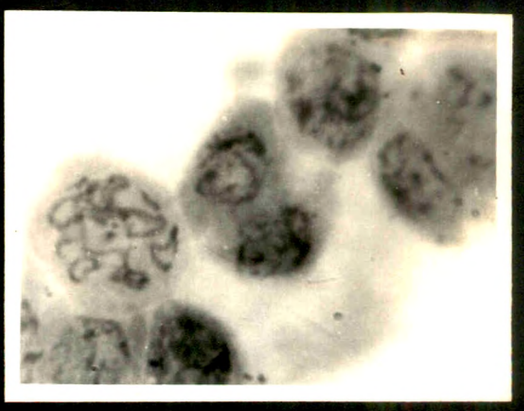


FIG. 3.3e



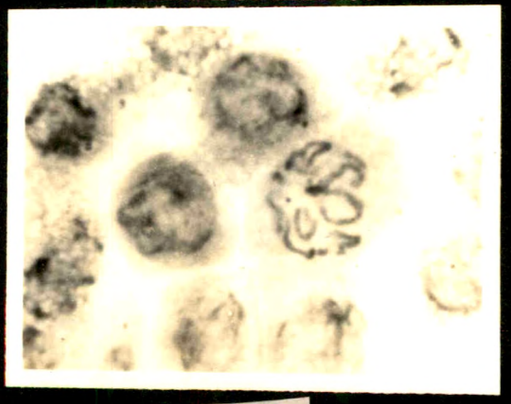


FIG. 3.3g

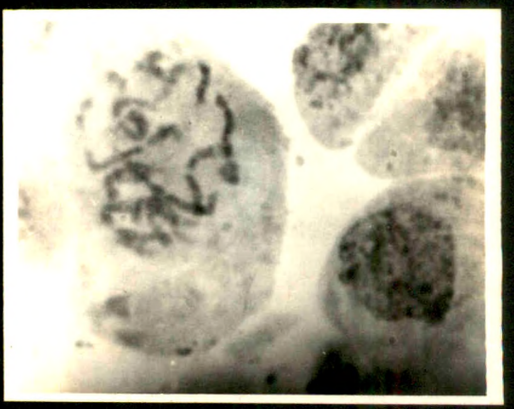


FIG. 3.3h

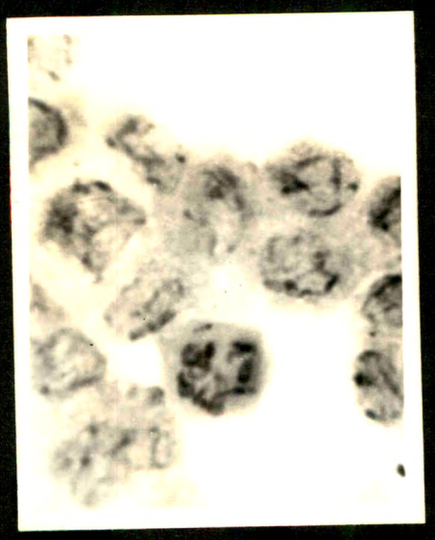


FIG. 3.4a

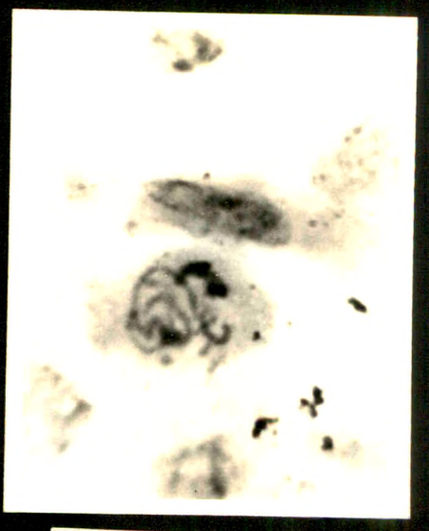


FIG. 3.4b

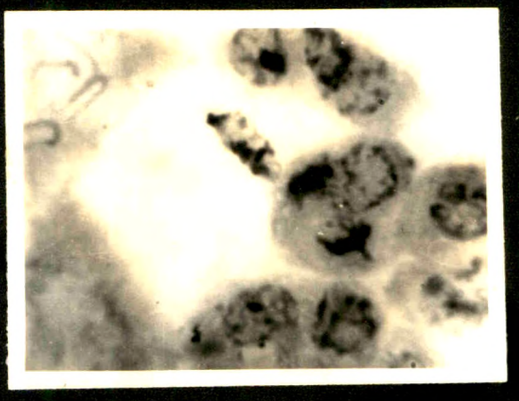


FIG. 3.5

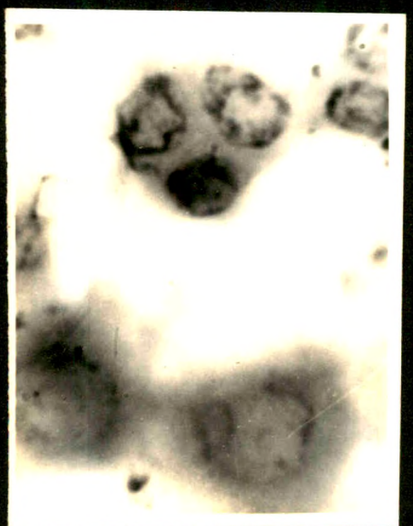


FIG. 3.6

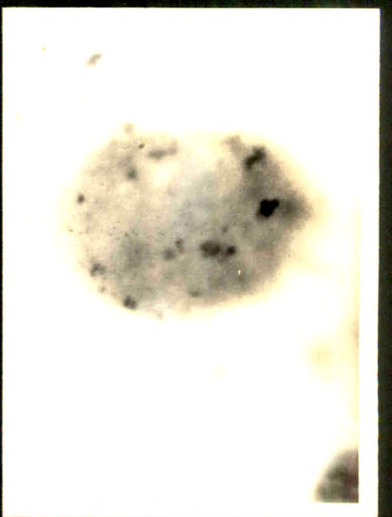


FIG. 3.7

TABLE 3.1

Percentage of meiotic abnormalities in *P.incarnata*

Abnormality at	Pachytene	Diplotene	Diakinesis	Metaphase I	Anaphase Bridge	Anaphase Bridge laggard	Telophase II	Total
%	7.00	30.00	16.23	13.74	4.60	4.50	0.23	76.30

TABLE 3.2

Chiasma frequency at Diplotene to Metaphase-I (*P. incarnata*)

State	No.of cells analysed	Total No. of		0 X'ta	1 X'ta	2 X'ta	Total No.of X'ta	Total No.of X'ta/ nucleus	Coefficient of chiasma terminalization
Diplotene	11	Univalents	43	43	--	--			
		Bivalents	74	--	56	18	92	8.36	0.413
Diakinesis	17	Univalents	59	59	--	--	124	7.77	0.594
		Bivalents	111	--	98	13			
Metaphase-I	21	Univalents	64	64	--	--	138	6.57	0.748
		Bivalents	128	--	118	10			

irregular showing cytomixis and unequal distribution of chromosomes (Fig. 3.6). Number of cells have also shown Micronuclei formation (Fig. 3.7). Average abnormality percentage calculated was 76.3 % (Table 3.1).

Average number of chiasmata per nucleus and chiasmata terminalization at diplotene, diakinesis and metaphase-I were calculated (Table 3.2). The coefficient of chaismata terminalization was more at metaphase-I, indicating complete terminalization of chiasmata at metaphase-I. Number of chiasmata per nucleus were more in diplotene than diakinesis and metaphase-I. Occurrence of single chiasmata was more.

Early disjunction of some of the bivalents resulted in increase in the number of univalents at prometaphases and metaphase-I, with a corresponding decrease in the number of chiasmata per cell. Failure of univalents to orient themselves on the metaphase plate and early disjunction of some of the bivalents resulted in an irregular separation in some of the cells.

DISCUSSION

Abnormality percentage determined was 76.3 % which indicates in P.incarnata meiosis is highly irregular. In P.incarnata 2 types of chromosome associations have been observed. First is the primary association of chromosome forming bivalents and second is the variable number of univalents occurrence during diakinesis and metaphase-I. The presence of fragments and bridges denotes an inversion heterozygote. Univalents are caused by a

hybrid constitution of a numerical nature. Other meiotic abnormalities like non-disjunction of bivalents, lagging and early separation represents lack of co-ordination between the chromosomes and the spindle. Pairing of chromosomes at meiosis indicates perfect genetic homology.

Average number of chiasmata per nucleus was quite low in P.incarnata as compared to P.edulis and P.edulis flavicarpa (Dixit 1979). According to Rees and Ahmed (1963) Lolium plants and other short lived populations which are annuals have higher chiasmata frequencies than those of perennial populations. The variation in the chiasma frequency is inferred to be adaptive. Jones and Rees (1966) have suggested, however that the variation in chiasma frequency is directly dependent on the longevity of the population. The low frequency of univalents and bivalents is the cause of genome differentiation (Celarie 1957). However a number of workers have reported that univalent and bivalent formation is dependent on chiasma frequency (Rees and Ahmed 1963).

Muntzing (1938) reported that interspecific sterility is mainly caused by gene mutations and structural differences in chromosomes. The presence of small structural differences have been recorded in some plant species reported by Magoon and Shambulingappa (1962a and b), therefore it becomes necessary to look for a sensitive tool to detect minor structural differences in the chromosomes.

As a consequence of the presence of many univalents and bivalents in the PMCs of P.incarnata, manifold irregularities

appeared during meiosis causing a high reduction in fertility. Present results indicate that the genome of P.incarnata contains several groups of different genes or one or several series of multiple alleles causing reduction in fertility. Fertility depends directly on the degree of reduction of chiasmata frequency; the small the number of chiasmata, the higher the number of univalents and bivalents, higher the meiotic irregularities, lower is the degree of fertility.

According to stebbins (1947) presence of these abnormalities at meiosis indicates that hybridization, gene mutations and chromosomal interchanges has taken place, hence the plants behave as segmental polyploids instead of true diploids. The entrance of a particular genomic set into foreign cytoplasm leads to such effects which indicate that the individual is not a true diploid (Sharma 1976). The number of chiasmata found at diakinesis is not always constant. This indicates that the most frequent number is exactly not the same as in true diploids, i.e. P.incarnata is not a true diploid. Absence of multivalents in P.incarnata at meiosis rules out the possibility of their being of autopolyploid origin, hence it is quite likely that it also originated as secondary polyploids.

The significance of chromosomal alterations in evolution and speciation had often been under-estimated in the past due to overemphasis on the role of gene mutations in evolution. But recently with the aid of improved chromosome techniques, it has been possible to work out the chromosomal basis of intervarietal and even interstrain differences (Sharma 1961).

From the above discussion it can be concluded that extensive hybridization and structural alterations of chromosomes have played an important role in the evolution of P.incarnata.

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