Pachytene Karyology of *Ipomoea biloba*

M. L. Magoon, R. Krishnan and K. Vijaya Bai

Central Tuber Crops Research Institute,
Trivandrum 10, India

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In resolving the existing confusion on the taxonomic treatment and phylogenetic relationships of the genus *Ipomoea* (Jones 1968), karyological studies at somatic metaphase due to small size of chromosomes (*vide* Sharma and Datta 1958 for details) have proved to be inadequate. With the realization of the utility in both karyological and chromosomal homology studies, pachytene analysis was successfully carried out in the diploid ($2n=30$) species, *I. crassicaulis* (Krishnan *et al.* 1969) leading to identification of entire haploid complement. Similar studies were later extended to a tetraploid ($2n=60$) form of *I. biloba* (Vijaya Bai *et al.* 1969) and the hexaploid ($2n=90$) sweet potato, *Ipomoea batatas* (Magoon *et al.* 1970) which have thrown considerable light on their mode of origin and interrelationships of their constituent genomes. The present investigation on the pachytene karyology of diploid, pantropical *I. biloba* Forsk (Syn. *I. pescaprae* L.) was initiated in the course of cytogenetical and evolutionary studies of the genus *Ipomoea*. It is also hoped that this study would provide the necessary basic informations for carrying out further investigations on the earlier reported tetraploid form.

Material and methods

Diploid plants of *I. biloba* Forsk were collected from the western coast of Kerala and grown in the experimental nursery of the Institute. The plants were raised vegetatively from vine cuttings. For the study of microsporogenesis, flower buds of appropriate sizes were fixed in a mixture of three parts of alcohol and one part of glacial acetic acid and anthers smeared in two percent propiono-carmines adopting the technique described by Krishnan *et al.* (1969). Measurements of the pachytene bivalents were made from camera lucida drawings of eight well spread cells at a table magnification of $\times 1450$ obtained from optical combinations of $7\times$ eyepiece and 1:100, 1.30 apochromatic oil immersion objective. The bivalents were arranged and numbered in order of their decreasing length—the longest chromosome being chromosome I and the shortest chromosome XV. Photomicrographs were taken from temporary preparations.

Observations

Pachytene and post-pachytene stages of meiosis were critically studied. At pachytene, the bivalents showed complete pairing in their proximal dark-staining

and distal light-staining segments (Fig. 1). The primary constriction appeared as unstained area amidst dark-staining segments. The distribution of differentially stained segments in the fifteen bivalents which ranged in their total length from 25.5 to 60.0 μ was varied (Table 1). Two of the bivalents were readily recognized by their nucleolar associations. In the identification of bivalents, criteria such as i) total length, ii) relative length, iii) position of centromere, iv) distribution and length of dark- and light-staining segments in the arms v) size and distribution pattern of chromomeres and vi) nucleolar association were used. The pachytene idiogram was constructed incorporating all these criteria (Fig. 2). The diagnostic characters of the fifteen bivalents are as follows:

Fig. 1. Fifteen pachytene bivalents of *I. biloba*. Arrows indicate centromere. nu: nucleolus. $\times 1100$.

Chromosome I: This is the longest chromosome of the complement. It measures about 60.0 μ and has an arm ratio of 1:2.0. The heteropycnotic segments of the arms are of equal length and in the long arm is characterized by two distal chromomeres. Its relative length coupled with submedian position of centromere aid in its identification.

Chromosome II: This is readily recognized as the longest metacentric chromosome of the complement (59.3 μ long) and has an arm ratio of 1:1.2. The dark-staining segments in the arms are equal and also help in its identification. In some cells the dark-staining regions of both arms can further be resolved into three macrochromomeres each.

Chromosome III: This chromosome is 53.0 μ long. It is a metacentric chromosome with an arm ratio of 1:1.1. This chromosome is distinguished from chromosome II on account of the unequal lengths of heteropycnotic segments in the arms that of one arm being nearly one and half times longer. The ratio of

Table 1. Cytological values for the haploid complement of *I. biloba* at pachytene stage

Chromosome no.	Short arm length in μ of			Long arm length in μ of			Total length in μ	Arm ratio as length of LA/SA	Ratio of the length of dark-staining regions of the arms as of LA/SA	Ratio of the length of light-staining regions of the arms as of LA/SA	Ratio of the length of the total LSR/DSR
	LSR	DSR	Total	DSR	LSR	Total					
I	13.8	6.2	20.0 $\pm$ 2.8	6.9	33.1	40.0 $\pm$ 4.1	60.0 $\pm$ 6.9	2.0 $\pm$ 0.1	1.11	2.39	3.6
II	20.7	6.2	26.9 $\pm$ 3.4	6.2	26.2	32.4 $\pm$ 3.4	59.3 $\pm$ 7.6	1.2 $\pm$ 0.07	1.00	1.26	3.8
III	19.3	6.2	25.5 $\pm$ 2.1	4.1	23.4	27.5 $\pm$ 4.8	53.0 $\pm$ 6.9	1.1 $\pm$ 0.1	0.66	1.21	4.1
IV	15.8	9.7	25.5 $\pm$ 2.8	4.1	22.1	26.2 $\pm$ 4.1	51.7 $\pm$ 6.2	1.0 $\pm$ 0.2	0.42	1.39	2.7
V*	6.2	3.4	9.6 $\pm$ 1.8	5.5	35.2	40.7 $\pm$ 4.5	50.3 $\pm$ 3.4	4.2 $\pm$ 0.5	1.61	5.67	4.7
VI	9.6	7.6	17.2 $\pm$ 0.7	4.1	27.6	31.7 $\pm$ 2.1	48.9 $\pm$ 5.5	1.8 $\pm$ 0.3	0.54	2.87	3.2
VII	7.6	6.9	14.5 $\pm$ 0.7	4.1	26.9	31.0 $\pm$ 2.8	45.5 $\pm$ 4.1	2.1 $\pm$ 0.2	0.59	3.53	3.1
VIII	17.9	4.1	22.0 $\pm$ 2.1	7.6	15.2	22.8 $\pm$ 3.4	44.8 $\pm$ 1.4	1.0 $\pm$ 0.1	1.85	0.84	2.8
IX*	4.8	4.8	9.6 $\pm$ 1.8	3.5	26.2	29.7 $\pm$ 3.4	39.3 $\pm$ 2.8	3.1 $\pm$ 0.4	0.73	5.45	3.7
X	8.3	4.8	13.1 $\pm$ 2.8	5.5	20.7	26.2 $\pm$ 2.8	39.3 $\pm$ 4.8	2.0 $\pm$ 0.3	1.14	2.49	2.8
XI	10.4	3.4	13.8 $\pm$ 2.1	6.9	18.6	25.5 $\pm$ 2.8	39.3 $\pm$ 1.4	1.8 $\pm$ 0.3	2.03	1.78	2.8
XII	8.3	6.2	14.5 $\pm$ 2.8	2.8	22.0	24.8 $\pm$ 2.1	39.3 $\pm$ 3.4	1.7 $\pm$ 0.1	0.45	2.65	3.4
XIII	6.2	6.9	13.1 $\pm$ 1.4	3.4	21.4	24.8 $\pm$ 2.8	37.9 $\pm$ 4.8	1.9 $\pm$ 0.1	0.49	3.45	2.7
XIV	7.6	9.0	16.6 $\pm$ 0.7	2.0	17.2	19.2 $\pm$ 2.8	35.8 $\pm$ 2.8	1.2 $\pm$ 0.1	0.22	2.26	2.3
XV	8.3	4.1	12.4 $\pm$ 0.7	4.1	9.0	13.1 $\pm$ 0.7	25.5 $\pm$ 1.4	1.1 $\pm$ 0.1	1.00	1.08	2.1

* denotes nucleolar chromosome; LA = long arm; SA = short arm; LSR = light-staining regions;

DSR = Dark staining-regions.

 $\pm$ = standard error.

total dark-staining to light-staining regions (1:4.1) of this chromosome is highest among non-nucleolar chromosomes.

Chromosome IV: This is also a metacentric chromosome having an average length of 51.7μ . It has an arm ratio of 1:1.0. This chromosome, like chromosome III, is characterized by unequal dark-staining segments in the arms but differ in respect of their length relationships. In one arm, the dark-staining regions are nearly two and half times longer and in the other the dark-staining region can be resolved into four micro-chromomeres.

Fig. 2. Idiogram of *I. biloba* pachytene chromosome complement.
N: nucleolar chromosome.

Chromosome V: This is the longest of the two nucleolar chromosomes. It measures on an average 50.3μ and has an arm ratio of 1:4.2. The nucleolar organizer is located terminally. This chromosome is distinguished from the other nucleolar chromosome, chromosome IX, by its relative length and also by the unequal distribution of dark-staining regions in the arms. The short arm has two macrochromomeres.

Chromosome VI: This submetacentric chromosome has a mean length of 48.9μ and an arm ratio of 1:1.8. The dark-staining regions of the short arm are nearly two times longer than that of the long arm. In the latter, it is represented by two macrochromomeres. This chromosome is similar to chromosome VII

from which it is distinguished based on length ratio of light-staining regions of the arms.

Chromosome VII: This chromosome is also a submetacentric chromosome (arm ratio 1:2.1), measuring on an average $45.5\ \mu$ in length. As in chromosome VI, the dark-staining regions of the short arm are longer and are more than one and half times longer than that of the long arm. In suitable preparations a macro- and two micro-chromomeres can be distinguished in the long arm.

Chromosome VIII: This metacentric chromosome exhibits unequal distribution of dark-staining regions in the arms. It is $44.8\ \mu$ long and has an arm ratio of 1:1.0. In the arm carrying shorter dark-staining regions, a macrochromomere and a microchromomere are observed. In the other arm, two macro- and three micro-chromomeres are present.

Chromosome IX: This is the shorter of the two nucleolar chromosomes, measuring about $39.3\ \mu$. It has an arm ratio of 1:3.1. The nucleolar organizer is located terminally in the short arm which has the longer dark-staining segments.

Chromosome X: This chromosome measures on an average $39.3\ \mu$ in length and has an arm ratio of 1:2.0. The dark-staining regions of the arms are of equal length. The short arm has a macrochromomere and a medium sized chromomere and the long arm, two macrochromomeres. This chromosome can be distinguished from chromosomes XI and XII by the symmetrical distribution of dark-staining regions in the arms.

Chromosome XI: This is also a submetacentric chromosome having an arm ratio of 1:1.8 and is $39.3\ \mu$ long. The dark-staining regions in the arms are of unequal length, that in the short arm being shorter and represented by a macro- and two medium sized chromomeres and in the long arm by a medium sized chromomere and a segment consisting of 4 or 5 micro-chromomeres.

Chromosome XII: Like chromosome X and XI, this is a submetacentric chromosome with an arm ratio of 1:1.7. It measures $39.3\ \mu$ in length. The dark-staining segments of the arms are unequal but unlike chromosome XI, the longer being present in the short arm. In this arm the dark-staining regions consist of one macro- and two micro-chromomeres. In the long arm one medium sized chromomere is present.

Chromosome XIII: This is readily recognized as the shortest sub-metacentric chromosome of the complement. It measures on an average $37.9\ \mu$ and has an arm ratio of 1:1.9. In this chromosome also, like chromosome XII, the dark-staining regions of the long arm are shorter. This chromosome can be distinguished from chromosome XII essentially on the basis of its relative length and longer ratio of total light-staining to dark-staining regions.

Chromosome XIV: It is a metacentric chromosome with an arm ratio of 1:1.2 and mean length of $35.8\ \mu$. The distribution of dark-staining segments of the arms are highly asymmetrical and nearly four and half-times longer in one arm. In the other arm it is represented by a single medium sized chromomere.

Chromosome XV: This is the shortest chromosome ($21.5\ \mu$) in the complement and is a metacentric chromosome. It has an arm ratio of 1:1.1. This chromosome is further characterized by the symmetrical distribution of differentially

stained segments in the arms. However, the dark-staining regions of one of the arms are more deeply stained than the other.

Later stages of meiosis

Fifteen bivalents were regularly present both at diakinesis and metaphase I stages and in the former stage, two bivalents were in nucleolar association. At anaphase I chromosomal disjunction was normal leading to equal distribution of chromosomes to poles. No abnormalities were recorded in the later stages of meiosis and at the end of division in all the cells four microspores were formed. Pollen fertility as determined by carmine stainability of pollen was over 95%.

Discussion

As reported by Sharma and Datta (1958), the somatic chromosomes of *I. biloba* ranging from 1.5 μ to 2.8 μ could be resolved into seven types, which include the three long chromosomal types, A, C and F with one pair in each of which A and C bear satellites, types J and K of 'medium' length with three and six pairs, respectively and 'short' types L and M having two and one pairs, respectively. The position of centromere is median in types J and L, median to submedian in type A and submedian to extremely submedian in the rest.

There is a fairly close agreement between the karyotypes at somatic metaphase as reported by Sharma and Datta (1958) and at pachytene. In both stages two nucleolar chromosomes have been detected though the relative position in respect of length are variable (1 and 2 in root-tip metaphase and V and IX at pachytene). The ratio between the lengths of shortest and longest chromosomes at root-tip metaphase (1:1.87) and at pachytene (1:2.35) are also comparable. The number of metacentric chromosomes are also more or less in agreement being five at somatic metaphase and six at pachytene.

Notwithstanding these similarities, the advantages of karyological studies at pachytene over that at somatic metaphase in this species is, indeed, striking. This is particularly evident in the accuracy with which arm ratios could be determined. Thus, at pachytene among the nine submetacentric chromosomes seven have arm ratios ranging from 1.5–2.4, one each in the arm ratio groups of 2.5 to 3.4 and above. This criterion along with such additional criteria as chromomere number, size and pattern of distribution, differential distribution of dark- and light-staining regions in the chromosomes and in their arms and relative lengths of bivalents have rendered further classification of chromosomal types leading to identification of the entire haploid complement.

It is now possible to draw a comparison of pachytene karyotypes of *I. biloba* and the earlier investigated *I. crassicaulis* (Krishnan *et al.* 1969) and evaluate the extent of chromosomal differentiation in these two diploid species. Such a comparison is of further interest on account of the recent success in the hybridization between these two distantly related taxa (Jones and Kobayashi 1969). Their gross karyological data are compared in Table 2. As may be seen, the two karyotypes are comparable in respect of relative lengths and ratio of longest and shortest chromosomes, the number, relative position and arm ratio of nucleolar chromosomes,

number of submetacentric chromosomes in arm ratio group of 1.4 to 2.4, mean length and ratio of total light- and dark-staining regions of the pachytene nuclei, mean chromosomal length and general morphology of differentiated chromosomes. The major karyological differences relate to number and position of both metacentric and submetacentric (especially in the arm ratio groups of 2.5 to 3.4 and above) chromosomes.

Table 2. Comparative study of gross karyological details of *Ipomoea biloba* and *crassicaulis*

	<i>I. biloba</i>	<i>I. crassicaulis</i> **
<i>Chromosome length (in μ)</i>		
Longest	60.0	56.6
Shortest	25.5	27.3
Longest/Shortest	2.4	2.1
Mean	44.7	39.3
<i>Chromosomes in arm ratio group</i>		
1:1.3 and less	II, III, IV, VIII, XIV and XV	II, IV, X and XII
1:1.4 to 2.4	I, VI, VII, X, XI, XII and XIII	I, V, VII, VIII, IX, XI and XV
1:2.5 to 3.4	IX*	XIII and XIV*
1:3.5 and above	V*	III and VI*
<i>Mean length (in μ) of total nuclear</i>		
Light-staining regions	509.6	466.8
Dark-staining regions	160.3	122.1
Light-staining regions/ dark-staining regions	3.18	3.82

* denotes nucleolar chromosome.

** based on data from Krishnan *et al.* 1969.

From a detailed comparison of the pachytene complement of the two species, homoeology in respect of eight chromosomes of the complement could be adduced. Their cytological values are given in Table 3. The extent of chromosomal homoeology as deduced from comparative karyology is also borne out by the data on chromosomal pairing at metaphase I stage in the F_1 hybrid as reported by Jones and Kobayashi (1969). These authors have recorded a maximum of nine bivalent chromosomal association at metaphase I in this F_1 hybrid. However, it must be mentioned that the data obtained from study of metaphase I stage may not truly reflect the actual extent of chromosomal pairing that had occurred at early prophase stages, since many intrinsic and extrinsic causes are known to affect chromosomal associations at late prophase and metaphase I stages (for detailed discussion *vide* Krishnan *et al.* 1970, Malik and Thomas 1967). This, in fact, is well supported even in this interspecific cross where variation in the extent of mean chromosomal association was recorded in different hybrid plants secured from the same set of parental plants. A thorough investigation of the chromosome-wise pachytene pairing in this hybrid would indeed be very rewarding in estimating more precisely

Table 3. Cytological values of eight homoeologous chromosomes of the haploid complement of *I. biloba* and *I. crassicaulis***

Chromosome number	Total length (in μ)	Arm ratio LA/SA	LSA LA/SA	DSR LA/SA	Total LSR/total DSR
II b	59.3	1.2	1.3	1.0	3.8
II c	52.4	1.1	1.2	0.7	5.2
III b	53.0	1.1	1.2	0.7	4.1
IV c	44.1	1.1	1.0	1.4	3.8
V * b	50.3	4.2	5.7	1.6	4.7
VI * c	41.9	4.4	5.5	1.7	6.4
VI b	48.9	1.8	2.9	0.5	3.2
V c	42.6	2.2	3.7	0.6	3.3
VII b	45.5	2.1	3.5	0.6	3.1
XI c	37.1	1.9	3.0	0.5	3.6
IX * b	39.3	3.1	5.5	0.7	3.7
XIV * c	30.8	2.7	3.4	1.4	3.2
X b	39.3	2.0	2.5	1.1	2.8
XI c	34.3	2.1	2.4	1.2	3.8
XI b	39.3	1.8	1.8	2.0	2.8
VIII c	37.8	2.2	2.3	1.7	4.0

LA = long arm; SA = short arm; LSR = light-staining region; DSR = dark-staining region;

* denotes nucleolar chromosome; ** based on data from Krishnan *et al.*, 1969; b = *I. biloba*; c = *I. crassicaulis*.

the extent of genomic relationship between these two species.

There is also an urgent need for pursuing similar studies in the various diploid taxa of the genus *Ipomoea* which constitute practically an unexplored wealth of breeding material. In the utilization of the diploid species in the hexaploid sweet potato improvement programme, tetraploid bridging species would be useful. A number of tetraploid forms have been already recorded in several species (Jones 1968, Gustafsson and Gadd 1965, Vijaya Bai *et al.* 1969) but detailed cytogenetical investigations on the origin and interrelationships with diploid form have only been carried out in *I. biloba* (Vijaya Bai *et al.* 1969). In this species, pachytene analyses in conjunction with morphological, geographical and other cytological data have provided considerable insight into the genomic homology between these two forms.

Summary

The entire haploid complement of the pantropical diploid ($2n=30$) *Ipomoea* species, *I. biloba* Forsk has been identified at pachytene stage employing criteria such as relative length, centromere position, nucleolar association, distribution and length of dark- and light-staining segments in the arms and chromomere number, size and pattern of distribution. The diagnostic features of the differentiated pachytene bivalents and the idiogram at this stage are presented. The advantages

of pursuing karyological studies at pachytene over that at somatic metaphase in this species is brought out. From a comparative study of pachytene karyotypes of *I. biloba* and the earlier investigated *I. crassicaulis* with which it has been recently hybridized, homoeology of eight chromosomes could be adduced. The need for pachytene analyses in this inter-specific hybrid, other related species and their hybrids is emphasized. Later stages of meiosis were found to be normal leading to formation of over 95% stainable pollen.

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