Theoretical and Applied Genetics 39, 274–279 (1969)

The Pachytene Chromosomes of *Ipomoea crassicaulis*

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Summary. The detailed morphology of the pachytene chromosomes and microsporogenesis have been studied in a diploid ($2n = 30$) American species, *Ipomoea crassicaulis* (Bth) B. L. Robinson. Idiogram of the pachytene chromosomes is presented and taking advantage of the extreme precision that pachytene analysis can lend, karyological characteristics of the haploid complement have been worked out in detail and individual chromosomes are identified. The course of meiosis was normal and over ninety five percent of pollen were found stainable. The urgent need for extending similar studies to other taxa in this economically important genus for unravelling phyletic relationships has been stressed.

The genus *Ipomoea* (*Convolvulaceae*) is an exceedingly large and diversified genus consisting of species widely distributed throughout the tropical and warm temperate regions of the world. A number of species of *Ipomoea* are of considerable importance either as medicinal plants or ornamental plants. The *Ipomoea batatas* (sweet potato) extensively cultivated for its edible root tubers is one of the staple food crops of mankind and, because of its capacity to give high outturns per acre, its role in feeding the ever increasing number of humanity is likely to be even more important especially in the context of present overall shortage of staple cereals. Many wild *Ipomoea* species possess several valuable and desirable characteristics such as resistance to diseases and pests and adaptation to different phytogeographical regions etc., incorporation of which into the cultivated varieties would appear to be of prime importance in any modern sweet potato breeding programme.

However, in spite of its great economic importance, comparatively little attention has been paid to the various existing cytogenetic problems and taxonomic relationships within this genus. Though considerable data on the morphological characters and geographic distribution of wild and cultivated species of *Ipomoea* have already been collected, in contrast, cytological and genetical studies in *Ipomoea* species have been relatively few and less intensive till recently (see for review JONES, 1968). The cytological information is largely restricted to the determination of chromosome numbers and most of the cytologically analyzed taxa are diploids having 30 somatic chromosomes. However, a few tetraploid ($2n = 60$) and hexaploid ($2n = 90$) species do occur and in the latter category falls *Ipomoea batatas*. Neither the mode of origin of polyploid species nor the interrelationships amongst the diploid species are known, even though such knowledge is very essential for breeding programmes aiming at interspecific transfer of genes from wild taxa into the cultivated ones.

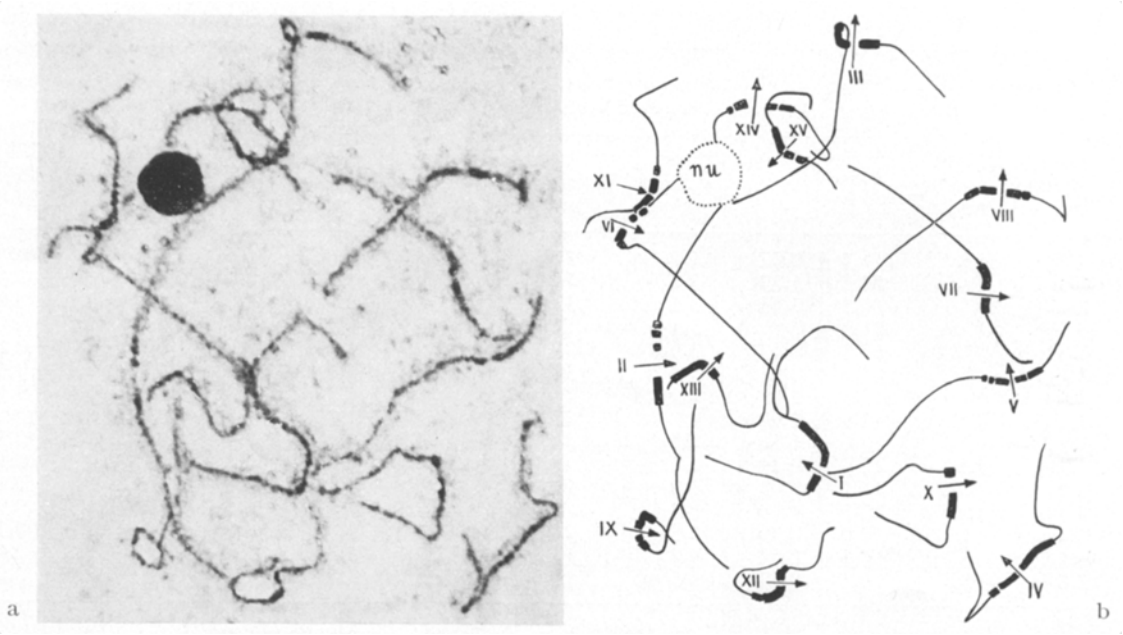
In fact, an understanding of the interrelationships between the taxa and also in the group as a whole as well as an adequate assessment of the nature of factors which may hinder or help such gene transfer is essential, if the desirable genes present in this allied, wild material are to be successfully incorporated into the cultivated varieties. The importance of such fundamental knowledge in crop improvement has, however, now been fully realized and little wonder then that considerable attention is now being paid in several parts of the world to the amelioration of the crop. The present paper which reports the pachytene karyology of *Ipomoea crassicaulis* (Bth) B. L. Robinson, a South American diploid species is a forerunner to similar studies in this genus.

Material and methods

Plants of *Ipomoea crassicaulis* were raised vegetatively from stem cuttings at the farm of the Institute. Flower buds of suitable sizes were fixed in a mixture of one part acetic acid and three parts of alcohol and smeared following the technique adopted earlier for cassava (see for review MAGOON, KRISHNAN and VIJAYA BAI, 1969). Measurements of pachytene chromosomes were made from camera lucida drawings of ten well spread cells at a table magnification of × 1450 obtained from optical combinations of 7× eyepiece and 1:100, 1.30 apochromatic oil immersion objective. The chromosomes are arranged and numbered in accordance with their decreasing lengths whereby the longest chromosome is Chromosome I and the shortest, Chromosome XV. For comparison of relative length of the different chromosomes, the methods described by HAZEGAWA (1932) and HUZIWARA (1956) are used in the present study. Various stages of meiosis were critically analyzed from temporary preparations. However, some slides were also made permanent by following the procedure described by BHADURI and GHOSH (1954). All photomicrographs presented were taken from temporary preparations.

Observations

All the fifteen pachytene bivalents of the complement (Fig. 1) were identified and the idiogram is presented in Fig. 3. In the identification of individual chromosomes, criteria such as total length,



Figs. 1 a and b. Fifteen pachytene bivalents of *I. crassicaulis*. Arrows indicate position of centromere. nu = nucleolus

relative length, position of centromere, size, shape and distribution of chromomeres, distribution and length of dark and light staining regions and nucleolar association proved useful. At pachytene, the chromosomes are differentiated into proximal dark staining segments that are localized in the immediate proximities of the apparently unstained centromere. In many chromosomes these regions can be further resolved into distinct chromomeres of varying sizes. In each arm, the dark staining regions are distally followed by light staining regions. In addition to the variation in the length of chromosomes that ranged from 56.6 μ to 27.3 μ variations in the total length of the constituent light and dark staining regions as well as their distribution in the two arms was also evident. In the pachytene nuclei, the light staining regions of the chromosomes contributed nearly four-fifth of the total chromatin length. The chromosome complement has two bivalents in constant association with the principal nucleolus and no accessory nucleoli are observed. Additional diagnostic features of each of the fifteen bivalents are as follows:

Chromosome I: This is generally the longest chromosome of the complement and measures about 56.6 μ and has an arm ratio of 1:2.1. The heteropycnotic segments of the two arms are unequal, the segments in the long arm being approximately three times longer. This feature coupled with its relative length provides criteria for its identification. Another characteristic feature of this chromosome pertains to the total length of heteropycnotic segments which are the longest in the complement.

Chromosome II: It is the longest metacentric chromosome having an arm ratio of 1:1.1. It

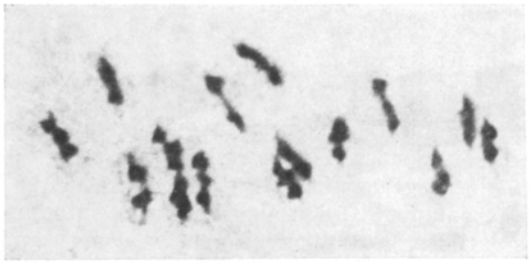


Fig. 2. Metaphase I showing 15 bivalents

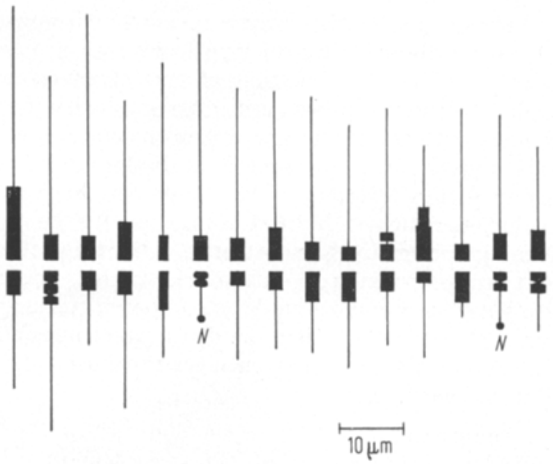


Fig. 3. Idiogram of *I. crassicaulis* pachytene chromosome complement. N = nucleolar chromosome

measures on an average 52.4 μ. The heteropycnotic segments of the two arms are more or less equal in length but is differentiated in one arm into three distinct chromomeres.

Table 1. *Cytological values for pachytene*

Chromosome No.	Short arm Length in μ of			Long arm Length in μ of			Total length in μ	Arm ratio as length of long arm/ short arm
	Dark staining regions	Light staining regions	Total	Dark staining regions	Light staining regions	Total		
I	3.2	15.0	18.2 ± 0.97	11.0	27.4	38.4 ± 2.76	56.6 ± 3.31	2.1 ± 0.14
II	4.9	19.6	24.5 ± 1.79	3.6	24.3	27.9 ± 1.38	52.4 ± 0.97	1.1 ± 0.05
III	2.6	7.9	10.5 ± 0.83	3.7	33.3	37.0 ± 3.52	47.5 ± 2.28	3.5 ± 0.64
IV	3.8	17.2	21.0 ± 0.90	5.4	17.7	23.1 ± 1.03	44.1 ± 1.93	1.1 ± 0.10
V	6.3	7.0	13.3 ± 0.83	3.6	25.7	29.3 ± 1.24	42.6 ± 1.66	2.2 ± 0.15
VI*	2.1	5.6	7.7 ± 0.97	3.6	30.6	34.2 ± 1.17	41.9 ± 1.93	4.4 ± 0.54
VII	2.4	10.9	13.3 ± 0.97	2.9	23.0	25.9 ± 1.03	39.2 ± 1.52	1.9 ± 0.17
VIII	2.8	9.1	11.9 ± 0.97	4.7	21.2	25.9 ± 1.17	37.8 ± 1.31	2.2 ± 0.18
IX	5.3	7.3	12.6 ± 1.52	2.8	21.7	24.5 ± 0.76	37.1 ± 1.38	1.9 ± 0.30
X	4.7	10.7	15.4 ± 0.34	1.6	18.7	20.3 ± 1.24	35.7 ± 0.83	1.3 ± 0.10
XI	3.2	8.0	11.2 ± 0.62	3.9	19.2	23.1 ± 1.24	34.3 ± 1.59	2.1 ± 0.13
XII	2.3	11.0	13.3 ± 0.69	8.3	9.2	17.5 ± 1.17	30.8 ± 1.72	1.3 ± 0.05
XIII	5.2	1.8	7.0 ± 0.76	2.5	21.3	23.8 ± 1.93	30.8 ± 2.34	3.4 ± 0.36
XIV*	3.1	5.3	8.4 ± 0.62	4.2	18.2	22.4 ± 3.86	30.8 ± 3.38	2.7 ± 0.44
XV	3.8	5.3	9.1 ± 0.14	4.6	13.6	18.2 ± 1.72	27.3 ± 1.03	2.0 ± 0.20

* denotes nucleolar chromosome. — L.A. = Long arm; S.A. = Short arm; ± = standard error

Chromosome III: This measures about 47.5 μ and has an arm ratio of 1:3.5. The heteropycnotic segments on either side of the centromere are almost equal in length. But the light staining segments of the long arm are about four times longer.

Chromosome IV: This is the second metacentric chromosome of the complement measuring about 44.1 μ in length with an arm ratio of 1:1.1. This chromosome can be distinguished from Chromosome II essentially on the basis of relative length and by the presence of longer dark staining segments in the long arm of this chromosome.

Chromosome V: This is an acrocentric chromosome measuring about 42.6 μ in length with an arm ratio of 1:2.2. The identification of this chromosome is rendered difficult on account of its similarity in morphology with the three acrocentric chromosomes VII, VIII and IX. Chromosome V is distinguished from Chromosomes VII and VIII chiefly on the basis of its lower ratio of light-staining regions to dark-staining regions in the short arm. The ratio of total light staining region to total dark staining region is also lower in chromosome V as compared to Chromosome VII. On the other hand, Chromosome V and IX can be distinguished only on the basis of their relative length.

Chromosome VI: This is the longest of the two nucleolar chromosomes of the complement. It is almost 41.9 μ long and has an arm ratio of 1:4.4. The nucleolar organizer is located nearly terminally.

Chromosome VII: This is 39.2 μ long and has an arm ratio of 1:1.9. The heteropycnotic regions of the two arms are short that in the longer arm being relatively longer. This chromosome can be differentiated from chromosome VIII by virtue of its higher ratio of total dark staining regions to light staining regions in which respect it is also distinct from chromosome IX. Further, it can also be distinguished from Chromosome IX by the differences in the ratio of dark staining regions of the two arms and total length of light-staining regions.

Chromosome VIII: It measures about 37.8 μ in length and has an arm ratio of 1:2.2. This chromosome differs from Chromosome IX in the ratio of light staining regions to dark-staining regions in the long arm, (higher in Chromosome IX) as well as in the distribution of dark staining regions in the two arms.

Chromosome IX: This is an acrocentric chromosome measuring about 37.1 μ in length and with an arm ratio of 1:1.9. The heteropycnotic segments in both the arms are unequal, in length. This chromosome resembles, as mentioned earlier, Chromosome V in its general morphology.

Chromosome X: This is the third metacentric chromosome of the complement which is nearly 35.7 μ long. It has an arm ratio of nearly 1:1.3. The presence of a macrochromomere in one of its arms serves as a reliable diagnostic feature. In the other arm, two chromomeres can be seen.

Chromosome XI: This is also an acrocentric chromosome, about 34.3 μ long with an arm ratio of 1:2.1. Although the heteropycnotic regions of the two arms are almost equal in length, the constituent chromomeres of the short arm are larger and this feature aids considerably in its identification. This chromosome differs from Chromosome IX in the

chromosomes of Ipomoea crassicaulis

Chromosome length as % of		Ratio of the length of dark staining regions of the arms as of		Ratio of the length of light staining regions of the arms as of		Ratio of light staining region to dark staining region of		
Total length	Longest chromosome length	S.A./L.A.	L.A./S.A.	S.A./L.A.	L.A./S.A.	Short arm	Long arm	Chromosome
9.61	100.00	0.29	3.44	0.55	1.82	4.7	2.5	3.0
8.90	92.59	1.36	0.73	0.81	1.24	4.0	6.8	5.2
8.07	83.93	0.70	1.42	0.24	4.22	3.0	9.0	6.5
7.49	77.92	0.70	1.42	0.97	1.03	4.5	3.3	3.8
7.23	75.27	1.75	0.57	0.27	3.67	1.1	7.1	3.3
7.11	74.04	0.58	1.71	0.18	5.46	2.7	8.5	6.4
6.66	69.27	0.83	1.21	0.47	2.11	4.6	7.9	6.4
6.42	66.79	0.60	1.68	0.43	2.33	3.3	4.5	4.0
6.30	65.56	1.89	0.53	0.34	2.97	1.4	7.8	3.6
6.06	63.08	2.94	0.34	0.57	1.75	2.3	11.7	4.7
5.82	60.61	0.82	1.22	0.42	2.40	2.5	4.9	3.8
5.23	54.42	0.28	3.61	1.20	0.84	4.8	1.1	1.9
5.23	54.42	2.08	0.48	0.08	11.83	0.4	8.5	3.0
5.23	54.42	0.74	1.35	0.29	3.43	1.7	4.3	3.2
4.64	48.24	0.83	1.21	0.39	2.57	1.4	3.0	2.3

pattern of distribution of dark-staining regions in the arms.

Chromosome XII: This is the fourth and the shortest of the metacentric chromosomes of the complement with an arm ratio of 1:1.3. The average length of this chromosome is 30.8 μ. The presence of a small chromomere in one of the arms is the conspicuous feature of this chromosome. The other arm has a heteropycnotic region in which chromomeric pattern cannot be readily resolved.

Chromosome XIII: This is about 30.8 μ long and has an arm ratio of 1:3.4. The diagnostic features of this acrocentric chromosome are the presence of relatively longer dark staining segments in its short arm as well as the high ratio (1:8.5) of light staining region to dark-staining region of the long arm.

Chromosome XIV: This is the shorter of the two nucleolar chromosomes and measures about 30.8 μ in length. The arm ratio of this chromosome is 1:2.7. The nucleolar organizer is almost terminally located. The dark staining regions of the two arms are more or less equal.

Chromosome XV: This is the shortest chromosome of the complement and can therefore be distinguished from the other chromosomes even on the basis of its relative length alone. The chromosome is about 27.3 μ in length and has an arm ratio of 1:2.0. The dark staining regions of the two arms are almost equal in length and the differences in the length of the light staining regions of the two arms contribute to the asymmetry of this chromosome.

Later stages of meiosis: Post-pachytene stages commencing from diakinesis were also studied. At diakinesis, most of the nuclei had two nucleolar-associated bivalents and occasionally nuclei with single bivalent in nucleolar association were also observed. The chromosomal association both at this stage and at metaphase I consisted entirely of bivalents (Fig. 2). The mean chiasma frequency per cell (average of 20 cells) was observed to be 28.4 at metaphase I. Anaphase I was usually normal and subsequent stages of meiosis were also regular resulting in normal tetrad formation followed by high percentage of pollen stainability (over 95%).

Discussion

There is a good deal of controversy regarding the present classification in the genus *Ipomoea* both from the point of taxonomic treatment and of tracing phylogenetic interrelationships amongst the various *Ipomoea* species (see JONES, 1968). The delimitations of intra- and infraspecific categories in this genus lack the sharpness that a study of evolutionary interrelationships amongst the entities would require. Thus, a mere taxonomic comparison of the various taxa belonging to this genus, which also exhibit no marked morphological differentiation among themselves, do not provide us a complete picture of the interrelationships amongst them. The importance of conducting combined study of morphological, cytological and genetical aspects in finding out a more natural classification based on ancestral relationships, cannot therefore be overemphasized.

Several workers employed somatic metaphases for karyomorphological studies in the genus *Ipomoea* (see SHARMA and DATTA, 1958, NAKAJIMA, 1963). However, for reasons of smallness of size and poor resolutions of chromosomal details at somatic meta-

phases in this genus, karyological data secured from these studies, apart from resulting in conflicting interpretations, have proved to be unrewarding in interpreting phyletic relationships of the taxa studied. Analysis of karyotype employing pachytene stages, on the other hand, can prove to be very useful in this regard and also information of far greater precision can be obtained by the study of pachytene stages of the various taxa within this complex and in hybrids between them. This is clearly evident when we consider that in the mitotic metaphase one is dealing with chromosome lengths varying only in a few micra (1.50—2.80 μ) (SHARMA and DATTA, 1958) while in the pachytene the magnitude of variation is very high as may be seen from the data presented in the present study resulting in much less error in classifying the chromosomes on the basis of their size. Thus, pachytene analysis, apart from providing information on chromosomal lengths and arm-ratios, permits the analysis of linear differentiation of the chromosomes such as chromomeric pattern, extent of eu- and hetero-chromatic segments, nucleolus-organizing regions of the chromosomes and in certain instances special land marks like knobs etc. Further, in as much as the homologous chromosomes are closely paired, the occurrence of even small structural differences in one of them will be clearly brought out at this stage. In fact, pachytene analysis in species and species hybrids have proved to be an important tool in recent years in successfully tackling of several cytogenetic and evolutionary problems in several taxa (see MAGOON, 1966; MAGOON and SHAMBULINGAPPA, 1963; MAGOON *et al.*, 1961, 1964 and 1967; MAGOON and TAYYAB, 1968 and SADASIVAIAH and MAGOON, 1966 and MAGOON *et al.*, 1969). In order, therefore, to get a better picture of the interrelationships amongst the different taxa in the genus *Ipomoea*, it has become necessary to extend this analysis to as many species as are available and in the present study hitherto unanalyzed species is considered.

The differentiated pachytene chromosomes of this species spread well and thus lend themselves amenable for critical study. The easy location of apparently unstained centromere along the chromosomal length is facilitated by the proximal deep-staining segments of the chromosomal arms that flank its sides. The well delineated proximal dark-staining segments are succeeded distally by light staining regions which generally contribute to the bulk of chromosomal length. The chromosomes that range in length from 56.6 μ to 27.3 μ maintain longest chromosome to shortest chromosome ratio of 1:2.1. However, differences in absolute chromosomal lengths did not prove a very useful criterion in identification. The bulk of the chromatin is accounted for by light staining regions of chromosomes. The ratio of these regions to the proximal dark-staining regions was computed (see Table 1) and used as one of the morphological criteria for identification. On the basis of this ratio, the chromosomes could be classified into one of the three categories, namely category I with a ratio varying from 1.9 to 3.5, category II varying from 3.6 to 5.0 and category III with ratio varying from 5.1 and above. The chromosomes belonging to these categories are I, V, XII, XIII, XIV, and XV; IV, VIII, IX, X and XI; II, III, VI and VII respectively.

The outstanding feature of the karyotype of *Ipomoea crassicaulis* is the preponderance of asymmetrical chromosomes which are eleven in number. Although the presence of 'submedian and extremely sub-median' chromosomes in the complement is reported by SHARMA and DATTA (1958) for some of the taxa of *Ipomoea* studied by them, a further categorization of these chromosomes on the basis of the differences in centromeric position proved beyond the resolution of the stage examined by them. In the present study, however, the chromosomes could be assigned to three groups on the basis of their degree of asymmetry. Thus to the first group with an arm ratio varying from 1:1.5—2.4 belong chromosomes I, V, VII, VIII, IX, XI and XV. Chromosomes XIII and XIV fall in the second group with arm ratio varying from 1:2.5—3.4 and finally to the third group belong the highly asymetrical chromosomes III and VI with arm ratio of 1:3.5 and above.

The pachytene chromosome complement of this species has two nucleolar bivalents in constant association with nucleolus which bear nearly terminal nucleolar organizers in their short arm. The two nucleolar chromosomes show significant difference in their relative-lengths which permit their easy distinction. A perusal of the number of secondary constriction-bearing chromosomes in the taxa studied by SHARMA and DATTA (1958), bear out lack of relationship between the number of such chromosomes and the ploidy level of each taxa. Thus, for example, the hexaploid *I. batatas* ($2n = 90$) has three pairs of chromosomes with secondary constrictions while the diploid species *I. leari* ($2n = 30$) has been shown to possess as many as six pairs. Here again, since the stage of analysis being mitotic metaphase does not permit verification of the exact number of chromosomes contributing to nucleolar organization, the answer can be best sought only from pachytene analyses of these taxa. Further, the operation of nucleolar organizer suppression phenomenon, if any, can also be brought to light by studies at pachytene stages as has been shown by similar analyses in *Phaseolus* (KRISHNAN and DE, 1968). This, again, suggests that the data from the study of somatic chromosomes are to be interpreted with great caution since one is dealing with lengths of relatively short magnitude subject to vagaries of fixation and further processing. The study of the nucleolar chromosomes of the other diploid *Ipomoea* species would prove useful in interpreting the mode of origin of

tetraploid and hexaploid species, where the higher chromosome number may otherwise make detailed karyological studies arduous. To this end, the study of nucleolar chromosomes of diploid species in their synthetic autopolyploids hybrids and amphiploids in addition to the naturally-occurring tetraploid and hexaploid species would greatly aid in understanding the behaviour of nucleolar chromosomes in the latter.

The successful identification of the entire pachytene chromosome complement of this diploid species indeed proves to be a good augury in extending similar studies to the other taxa of the genus. In fact, preliminary data obtained on several other *Ipomoea* species suggest that these species are ideally suitable for pachytene analysis and that valuable information on the construction of karyotypes, on the genetic differentiation between the species and on the nature of pairing in hybrids between them can be secured by this method.

Acknowledgements

The authors are thankful to Mr. C. S. ANTONISAMY, Photographer-cum-Artist, C.T.C.R.I., for his help in photomicrography.

Zusammenfassung

Bei der diploiden amerikanischen *Ipomoea crassicaulis* (Bth) B. L. ROBINSON ($2n = 30$) wird die Morphologie der Pachytänchromosomen und die Mikrosporogenese untersucht. Das Idiogramm der Pachytänchromosomen wird aufgestellt und unter Ausnutzung der großen Präzisionsmöglichkeit der Pachytänanalyse werden die charakteristischen Daten des haploiden Chromosomensatzes erarbeitet und alle Chromosomen identifiziert. Die Meiose verläuft normal und liefert zu mehr als 95% guten Pollen. Die Notwendigkeit umfassender ähnlicher Untersuchungen bei anderen taxonomischen Einheiten aus dieser wirtschaftlich wichtigen Gattung zwecks Klärung phylogenetischer Beziehungen wird nachdrücklich hervorgehoben.

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Received February 5, 1969

Communicated by F. MECHELKE

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