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CYTOGENETICS OF THE F<sub>1</sub> HYBRID BETWEEN CASSAVA  
AND CEARA RUBBER, AND ITS BACKCROSS

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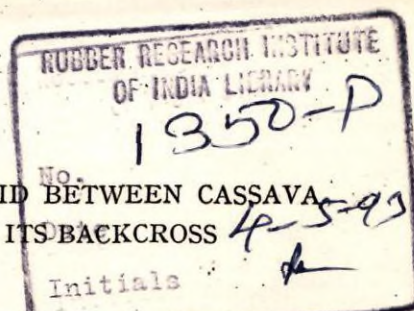
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Cytogenetical studies of the F<sub>1</sub> hybrid between the commercially cultivated tuber crop, cassava (*Manihot esculenta* CRANTZ.) and the closely related wild species *Manihot glaziovii* MUELL. (Ceara rubber) used as donor species for "Cassava mosaic" disease and drought-resistant genes and back crosses (to cassava parent) were made. The contrasting parental characters showed partial to total dominance in the F<sub>1</sub> hybrid, while the back cross plants were similar to cassava in most of their characters. Eleven of the twelve backcross plants exhibited resistance to "Cassava mosaic" under field conditions. Karyological similarities and differences as resolved on the basis of a comparative study of the karyotypes of the cassava parent and Ceara rubber were corroborated by the study of chromosomal pairing in the F<sub>1</sub> at pachytene. Major chromosomal differentiation in the two species involved three chromosomes of their haploid complement which were represented by three heteromorphic bivalent associations in F<sub>1</sub> each consisting of a probably basic chromosomal type and a derived type. Pachytene analyses of three back cross plants provided direct proof for random transmission of marker chromosomes of both the parents through male gametes of the F<sub>1</sub> hybrid. An increase in the chiasma frequency in the back cross plants over the F<sub>1</sub> hybrid at metaphase I stage was also observed. Pollen fertility of the backcross plants showed considerable variation.

### Introduction

*Manihot glaziovii* MUELL. (Ceara rubber) has been used in cassava (*Manihot esculenta* CRANTZ.) breeding programmes as donor of genes conferring resistance to 'Cassava mosaic' disease and drought. Even though successful hybridization of the two taxa has been reported by several workers, intensive cytological studies of the F<sub>1</sub> hybrid to bring out the nature of chromosomal differentiation in these two species have not been carried out (see for reviews, MAGOON et al., 1966; MAGOON, 1967 and 1968). With the recent success in the identification of all the 18 haploid chromosomes of the pachytene complement of *M. esculenta* (MAGOON et al., 1969) and *M. glaziovii* (KRISHNAN





et al., 1970), it is now possible to investigate the pairing behaviour of individual chromosomes of the parental complement in the  $F_1$  hybrid and the backcrosses. The present paper reports the results of such studies carried out on the  $F_1$  of *M. esculenta*  $\times$  *M. glaziovii* and its backcrosses to cassava.

### Materials and Methods

The  $F_1$  of *M. esculenta*  $\times$  *M. glaziovii* used in this study was produced earlier by MAGOON et al., (1966). The hybrid was backcrossed as female and as male parent to cassava. Of the 214  $F_1$  flowers pollinated with cassava pollen, 53 (24.8%) set fruit, which at maturity gave six seeds. None of these six seeds, however, did germinate. In the reciprocal crosses, 54 out of 198 cassava flowers pollinated with  $F_1$  pollen, set fruit (27.3 percent), yielding at maturity 31 seeds. Thirteen backcross plants were raised from these seeds and were vegetatively multiplied in experimental plots along with the  $F_1$  hybrid and the parents. Attempts to backcross the  $F_1$  hybrid with *M. glaziovii* were unsuccessful.

Cytological studies of the cassava parent, Acc. No. CTCRI-155, the  $F_1$  hybrid and the backcross plants were conducted adopting the technique employed for the study of the pachytene chromosomes of cassava (MAGOON et al., 1969). Pollen fertility was assayed on the basis of iodine stainability of pollen.

### Observations

#### MORPHOLOGY

The contrasting characters of the parents,  $F_1$  and backcross plants are given in Tables 1 and 2. The  $F_1$  hybrid was intermediate in some morphological features: stem shape, nodal swelling, flower and fruit size etc. The morphological characters of the cassava parent: leaflet number, phyllotaxy and the discrete nature of disc were dominant in the  $F_1$ . The backcross plants resembled the parents and the  $F_1$  in one or more characters (Table 1) and showed increased pollen fertility over the  $F_1$  (Table 4).

TABLE 1  
CONTRASTING CHARACTERS OF PARENTS, F<sub>1</sub> HYBRID AND BACK CROSS PLANTS (INDICATED AS  
NUMBER) OF THE CROSS, *M. esculenta* × *M. glaziovii*

Character	<i>M. esculenta</i> (P <sub>1</sub> )	F <sub>1</sub> hybrid	<i>M. glaziovii</i> (P <sub>2</sub> )	Back cross parental, F <sub>1</sub> or other type	Plant No.
shape of stem	ridged	slightly ridged	round	P <sub>1</sub> F <sub>1</sub> P <sub>2</sub>	2, 3, 5, 6, 11 1, 4, 7, 9 8, 10
stipule base	swollen	slightly swollen	not swollen	P <sub>1</sub> F <sub>1</sub> P <sub>2</sub>	2, 3, 8, 10, 11 1, 5, 6, 7, 9 4
node	swollen	slightly swollen	flat	P <sub>1</sub> F <sub>1</sub> P <sub>2</sub>	1, 2, 3, 5, 6, 8, 10, 11 7, 9 4
phyllotaxy: alternate +	1/5	1/5	1/3	1/5 1/4 1/3 1/8	3, 4, 5, 9, 10, 11; 1, 7 6, 8; 2
ovary disc	discrete	discrete	confluent with ovary	P <sub>1</sub> and F <sub>1</sub>	1, 5, 7, 9, 10, 11
fruit	winged	winged	not winged	P <sub>1</sub> and F <sub>1</sub>	1, 3, 5, 7, 9, 10, 11
fruit size	small	large	large	P <sub>1</sub> F <sub>1</sub> and P <sub>2</sub>	3, 7, 11 1, 5, 6, 9, 10
pollen fertility (%)		low		25.0-50.0 51.0-75.0	1, 2, 5, 6, 7, 9, 10 3
disease reaction	susceptible	resistant	resistant	resistant susceptible	1, 2, 3, 4, 5, 6, 7, 8, 9, 11 10

## CYTOLOGY

The pachytene karyology of the *M. glaziovii* parent used in the present study has been analysed earlier (KRISHNAN et al., 1970). Of *M. esculenta*, pachytene has been analysed previously only for another cultivar (Acc. No. CTCRI S-2371). It was necessary, therefore, to analyse also the pachytene karyotype of the *M. esculenta* cultivar (Acc. No. CTCRI-155) used as parent of the present hybrid. The karyotypes of the two cultivars were not found to differ. Karyotype as well as pairing behaviour at pachytene were worked out in detail for the hybrid and three backcross plants: Nos 3, 5 and 6. Metaphase I



TABLE 2

FREQUENCY OF LEAVES WITH DIFFERENT NUMBER OF LEAFLETS, AND AVERAGE NUMBER OF LEAFLETS PER LEAF. (% IN PARENTHESES)

Plant	Frequency of leaves with leaflets							No. of leaflets		Total No. of leaves examined
	1	2	3	4	5	6	7	Average	Range	
Ceara rubber	38 (9.4)	16 (4.0)	202 (50.0)	13 (3.2)	135 (33.4)			3.5	1-5	404
F <sub>1</sub>	4 (0.4)	—	108 (11.9)	10 (1.1)	759 (83.3)	—	30 (3.3)	5.0	1-7	911
Cassava	2 (0.3)	—	36 (6.0)	16 (2.7)	431 (71.8)	11 (1.8)	104 (17.3)	5.0	1-7	600
Back cross										
Plant 1	18 (14.9)	7 (5.8)	94 (78.3)	1 (0.8)				2.7	1-4	120
" 2	—	—	9 (3.5)	2 (0.8)	45 (17.5)	3 (1.2)	198 (77.0)	6.5	3-7	257
" 3	19 (4.8)	8 (2.0)	139 (35.4)	—	170 (43.3)	17 (4.3)	40 (10.2)	4.3	1-7	393
" 4	1 (0.7)	—	67 (45.6)	7 (4.8)	72 (49.0)			4.0	1-5	147
" 5	85 (6.3)	56 (4.1)	1189 (87.7)	10 (0.7)	17 (1.3)			2.9	1-5	1356
" 6	12 (8.8)	6 (4.4)	119 (86.9)					2.8	1-3	137
" 7	4 (3.8)	3 (2.8)	91 (85.8)	2 (1.9)	6 (5.7)			3.0	1-5	106
" 8	19 (18.1)	7 (6.7)	79 (75.2)					2.6	1-3	105
" 9	80 (5.4)	65 (4.4)	1272 (86.0)	17 (1.1)	45 (3.0)			2.9	1-5	1479
" 10	69 (6.4)	46 (4.3)	938 (86.8)	—	27 (2.5)			2.9	1-5	1080
" 11	—	—	85 (43.8)	15 (7.7)	94 (48.5)			4.0	3-5	194

and tetrad stages were studied in backcross plants 1, 3, 5 and 6. The length of the bivalents at pachytene of *M. esculenta* ranged from 21.2  $\mu$  to 36.5  $\mu$ . Among the seven metacentric chromosomes (II, V, VI, X, XI, XII and XVIII), chromosomes VI and XI carry a telo-

chromomere in one of the arms. In both the arms of chromosome XVIII telochromomeres are present, and one of the arms is totally heteropycnotic. The three nucleolar chromosomes of the complement, chromosomes IV, IX and XV belong to the submetacentric group (others being I, III, VII, VIII, XIII, XIV, XVI and XVII) and are found in association with the nucleolus. Chromosomes I, VIII and XIII, bear a telochromomere in their short arm. The short arm of chromosome VII is totally heteropycnotic.

A comparative study of the pachytene karyology of *M. glaziovii* and *M. esculenta* presents an overall similarity in the morphology of most of the chromosomes of the two taxa. However, the following differences are noted. The longest chromosome of the complement (chromosome I) in *M. glaziovii* is metacentric as also chromosome II, while chromosome I of *M. esculenta* is submetacentric. *M. glaziovii* has six metacentric chromosomes namely chromosomes I, II, VII, IX, X and XI in contrast to seven such chromosomes present in *M. esculenta*. Two of the metacentric chromosomes namely chromosomes II and IX, bear a telochromomere each and a chromosome corresponding to chromosome XVIII of *M. esculenta* with heteropycnotic arm is absent. The three nucleolar chromosomes in both the taxa correspond in their general morphology, but two of them differ in their relative position in the karyotype. In *M. glaziovii* they are chromosomes VI, XII and XV. The *M. glaziovii* complement has two submetacentric and telochromomere-bearing chromosomes, chromosomes IV and V and lacks the third chromosome present in *M. esculenta*. The *M. glaziovii* karyotype has an additional heteropycnotic short-armed chromosome and thus three chromosomes (III, XI and XVIII) belong to this category.

In the  $F_1$  hybrid, the pachytene bivalents range in length from 35.3  $\mu$  to 15.3  $\mu$ . The mean total chromatin length of the  $F_1$  nuclei (421.7  $\mu$ ) was lower than that of the two parents (*M. esculenta* = 505.0  $\mu$  and *M. glaziovii* = 527.1  $\mu$ ). The difference is larger than can reasonably be attributed to differences in stage of pachytene. The parental chromosomes, in general, exhibited total synapsis along their entire length and only three bivalents were heteromorphic. Except in the case of the latter and of the bivalent involving chromosomes XVIII of *M. esculenta* and *M. glaziovii*, chromosomal associations in the  $F_1$  included chromosomes belonging to similar arm ratio





Figures 1 to 6. Pachytene in the  $F_1$  hybrid, *M. esculenta*  $\times$  *M. glaziovii*.

Figure 1. Nucleus with 17 of the 18 bivalents; IV not included. Note association of three nucleolar chromosomes. Heteromorphic bivalents indicated (VI, VIII and XV), but not very clear at this magnification ( $\times 1250$ ).

Figure 2. Bivalent I with telochromomere in short arm:  $\times 2200$ .

Figure 3. Bivalent XVIII with telochromomere ( $\dagger$ ) in short arm.  $\times 2200$ .

Figure 4. Bivalent IV. Note excentric attachment of accessory nucleolus (arrow)  $\times 2200$ .

Figure 5. Heteromorphic bivalent, VI. Note the presence of telochromomere (arrow) in one of the homoeologues.  $\times 2200$ .

Figure 6. Heteromorphic bivalent, XV. One of the homoeologues with heteropycnotic short arm (arrow).  $\times 2200$ .

groups (Figure 1). The chromosomal complement consisted of six metacentric and 12 submetacentric chromosomes which are numbered in the order of decreasing length. The longest bivalent of the  $F_1$  hybrid (I), unlike that of *M. glaziovii* is submetacentric and bears a telochromomere in its short arm (Figure 2). The homoeologues in this association are chromosome I of *M. esculenta* and chromosome V of *M. glaziovii*. The shortest bivalent of the  $F_1$  complement is submetacentric and carries a telochromomere in its heteropycnotic short arm (Figure 3). The homoeologues of this bivalent are chromosomes XVIII of *M. esculenta* and XVIII of *M. glaziovii*. Chromosome XVIII of *M. esculenta* is a metacentric chromosome with telochromomeres in both its heteropycnotic and non-heteropycnotic arms. Chromosome XVIII of *M. glaziovii* is a submetacentric chromosome carrying a telochromomere only in its heteropycnotic short arm. In the longest metacentric bivalent of the  $F_1$  (IV), chromosome V of *M. esculenta* and chromosome I of *M. glaziovii* are associated. Both these chromosomes are known to organize accessory nucleoli near their centromeres. In the  $F_1$ , however, only one of the homoeologues seemed to organize an accessory nucleolus as could be deduced by the excentric attachment of the accessory nucleolus to the bivalent (Figure 4). Three heteromorphic bivalents, VI, VIII and XV were detected at pachytene (Figures 1, 5 and 6); one of the partners in each case is characterized by a heteropycnotic short arm (Figure 6). In case of heteromorphic bivalents VI and VIII, the other partner carries a telochromomere in the short arm. The two telochromomere-bearing chromosomes of these bivalents are derived each from one of the parents, as are the chromosomes carrying the heteropycnotic short arm. In case of bivalent XV, the homoeologue with heteropycnotic short arm is chromosome XI of *M. glaziovii*.

All the three nucleolar chromosomes of the parents are functional in the  $F_1$  hybrid (Table 3).

In the three backcross plants of which pachytene was studied (plants 3, 5 and 6) chromosome pairing at pachytene was complete. The total chromatin length is 463.0  $\mu$ , 505.4  $\mu$  and 474.8  $\mu$  respectively. Bivalent length and arm ratio for the bivalent corresponding to bivalent XVIII of the  $F_1$  hybrid are given in Table 4 along with the values recorded for this type in the  $F_1$  and the parents. In plant No. 6 this bivalent is similar to that of *M. esculenta* and in plants 3 and 5 it is similar to



TABLE 3  
LENGTH AND ARM RATIO OF THE PACHYTENE BIVALENTS OF THE F<sub>1</sub> HYBRID,  
*M. esculenta* × *M. glaziovii*

Bivalent	Total length (in $\mu$ )	Arm ratio	Bivalent	Total length (in $\mu$ )	Arm ratio
I	35.3 ±7.1	1:4.1 ±0.4	X	22.4 ±1.2	1:2.3 ±0.2
II	29.4 ±1.2	1:1.9 ±0.1	XI	21.2 ±0.6	1:1.2 ±0.1
III *)	27.9 ±2.4	1:5.0 ±0.3	XII *)	21.2 ±2.4	1:3.7 ±0.6
IV	27.7 ±2.9	1:1.2 ±0.1	XIII	20.6 ±2.4	1:1.1 ±0.1
V	27.1 ±1.2	1:1.3 ±0.1	XIV	20.0 ±1.2	1:1.5 ±0.2
VI **)	26.9 ±2.9	1:2.2 ±0.4	XV **)	19.4 ±1.2	1:2.1 ±0.3
VII	24.1 ±1.8	1:1.2 ±0.1	XVI	18.5 ±1.4	1:1.4 ±0.1
VIII **)	24.1 ±1.2	1:2.3 ±0.2	XVII *)	18.2 ±2.4	1:2.9 ±0.3
IX	22.4 ±0.6	1:1.2 ±0.1	XVIII	15.3 ±1.2	1:1.8 ±0.1

\*) Nucleolar chromosome.

\*\*\*) Heteromorphic chromosome; value for the longer of the homoeologue.

± Standard error.

that of the F<sub>1</sub> hybrid. The heteromorphic bivalent corresponding to bivalent XV of the F<sub>1</sub> hybrid, is present in plant No. 5 and presence of one of the heteromorphic bivalent types corresponding to VI and VIII of the F<sub>1</sub> hybrid, is recorded in plant No. 5. Both are present in plant No. 3. In plant No. 5 a submetacentric bivalent with heteropycnotic short arm (similar to chromosome VII of *M. esculenta*) is detected.

Later stages of meiosis were analysed in the backcross plants, Nos. 1, 3, 5 and 6. The chiasma frequency at metaphase I in these plants where eighteen bivalents are regularly formed, along with the values reported for the parents by MAGOON et al. (1966), is given in Table 4. The chiasma values per cell in the backcross plants are nearly equal to those of the cassava parent. In plant No. 5 six percent PMCs

TABLE 4

LENGTH AND ARM RATIO FOR BIVALENT XVIII AT PACHYTENE, AND DATA ON CHIASMA FREQUENCY AT METAPHASE I, FREQUENCY OF MICROSPORES AND POLLEN FERTILITY OF PARENTS, F<sub>1</sub> HYBRID AND BACK CROSS PLANTS OF THE CROSS BETWEEN *M. esculenta* × *M. glaziovii*

Plant	length (in $\mu$ )	arm ratio	chiasmata per cell	microspore stage (%)						pollen ferti- lity (%)
				tetrads		dyads		triads		
				normal	micro- nuclei	normal	micro- nuclei	normal	micro- nuclei	
<i>M. esculenta</i> *)	21.2	1 : 1.3	27.8							
<i>M. glaziovii</i> *)	21.2	1 : 2.0	20.5							
F <sub>1</sub> hybrid	15.3	1 : 1.8	17.3							
Backcross										
plant No. 3	20.5	1 : 1.7	27.4	—	—	—	—	—	—	68.2
" 5	17.1	1 : 1.8	23.3	58.3	37.2	—	—	3.3	1.2	32.3
" 6	18.2	1 : 1.2	25.6	76.0	11.1	2.3	1.8	7.0	1.8	32.1
" 1	—	—	24.4	76.7	19.5	—	0.8	1.5	1.5	26.4

\*) Based on data of MAGOON et al., 1966.

exhibited precocious movement of chromosomes. In the four backcross plants at the end of division, 58.3 to 76.7 percent of PMCs produced four microspores and in 11.1 to 37.2 percent of the PMCs one to four micronuclei occurred. In addition to these, dyads and triads with and without micronuclei are observed in a low percentage of PMCs (Table 4).

### Discussion

The unidirectional nature of crossability, observed in crosses between *M. esculenta* and *M. glaziovii* (see for review, MAGOON, 1968) is reflected in the crossability of the F<sub>1</sub> hybrid which could be successfully backcrossed only as male parent to *M. esculenta*. In their exomorphic characters, vigour and pollen fertility, the backcross plants showed variation and resembled the parents and F<sub>1</sub> in one or more characters (Table 1). Although the number of backcross plants is not large enough for genetical studies of contrasting parental characters, the available data show clearly the predominance of *M. esculenta* and F<sub>1</sub> hybrid characters in the backcross plants. In addition to the two



types of parental phyllotaxy ( $1/5$  in *M. esculenta* and  $1/3$  in *M. glaziovii*) two new types i.e.  $1/4$  and  $1/8$  are seen. Another important exomorphic character studied in the backcross plants relates to the differences in the leaflet number of the parents (Table 2). Even though the  $F_1$  hybrid resembles the female parent in this respect, nine of the eleven backcross plants are similar to the male parent in range and mean number of leaflets. The correlation of disease reaction and exomorphic characters, if any, is not apparent under field conditions in the backcross plants. Only one plant, plant No. 10, manifested susceptibility to "Cassava mosaic" under field conditions. The recovery of a majority of mosaic resistant plants with predominantly *M. esculenta* characteristics among the backcross plants suggests a great possibility of incorporating the disease resistance trait in *M. esculenta* through repeated backcrossing using *M. esculenta* as recurrent parent (see also JENNINGS, 1957).

Pachytene analysis of the  $F_1$  hybrid confirmed several of the karyological differences established by comparative karyology of the parents. The relative position of the bivalents in the  $F_1$  karyotype, however, often does not correspond with that of the homoeologues in their respective parental karyotypes. Chromosome I of *M. esculenta* is also one of the homoeologues of the longest bivalent in the  $F_1$ , whereas the other homoeologue of this bivalent is chromosome V of *M. glaziovii*. Similarly, the nucleolar chromosomes of the  $F_1$  hybrid are chromosomes III, XII and XVII, but IV, IX and XV in *M. esculenta* and VI, XII and XV in *M. glaziovii*. The *M. esculenta* and  $F_1$  karyotypes have seven metacentric chromosomes, while *M. glaziovii* has six metacentric chromosomes. In the  $F_1$  hybrid, the metacentric chromosome XVIII of *M. esculenta* is found to pair with the submetacentric chromosome XVIII of *M. glaziovii* resulting in the formation of a submetacentric bivalent with intermediate arm ratio. The behaviour of this chromosomal type in the backcross plants is discussed in detail elsewhere. Six of the metacentric bivalents in the  $F_1$  hybrid are a result of association of six parental metacentric chromosome pairs.

The parental karyotypes also differ in the number of chromosomes with heteropycnotic short arm. *M. esculenta* has one such chromosome (VII) and *M. glaziovii* has two (III and XI). Chromosome XVIII has a heteropycnotic arm in both parents but the pairing behaviour of this



chromosome in the  $F_1$  hybrid will be discussed separately. In the  $F_1$  hybrid the first three do not pair among themselves, but with chromosomes bearing distal light-staining regions. These heteromorphic bivalents in the  $F_1$  hybrid are VI, VIII and XV. In XV the heteromorphic arm remains frequently unpaired even though the other arm shows total synapsis. In the other two heteromorphic bivalents (VI and VIII), though one of the partners lacks the distal light-staining region in an arm and thus is shorter, the homoeologues are intimately paired and homomorphic in respect of the paired regions. The homoeologues bearing a heteropycnotic arm can hence be conceived as derived types. Since the three derived chromosomal types of the parents bear homoeology to the original chromosomal types in the  $F_1$  hybrid, the independent origin of these types as a result of terminal deletion can be adduced. This must have occurred in both taxa, but involving different chromosomes.

The pachytene analyses of the three backcross plants provide evidences for random transmission of at least some of the parental chromosomal types through the male gametes of the  $F_1$  hybrid. The bivalent type represented by chromosome XVIII in the  $F_1$  was studied in several PMCs of the three backcross plants (Table 4). In two of these (3 and 5), this type by virtue of its arm ratio is similar to the  $F_1$  type but in plant No. 6, it is similar to that of *M. esculenta*. On this basis, it can be deduced that one of the homoeologues in plant Nos. 3 and 5 belong to the *M. glaziovii* parent and was transmitted through the male gamete of the  $F_1$  hybrid. Thus, the inclusion of this chromosomal type in the viable male gamete clearly demonstrates the random segregation and transmission of this parental chromosome. Similarly, 1. the presence in plant No. 5 of a chromosome with a heteropycnotic short arm corresponding to chromosome VII of *M. esculenta*, and 2. the presence, also in No. 5 of one of the two heteromorphic bivalent types VI and VIII of the  $F_1$  hybrid, and 3. the presence of both these heteromorphic bivalents in plant No. 3 provide further evidence of the random transmission of parental chromosomes in the male gametes of the  $F_1$  hybrid.

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## RELATIONSHIPS OF JUSTICIEAE (ACANTHACEAE) BASED ON CYTOLOGY

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The cytology of some species representing the subtribes *Barlerieae*, *Andrographideae*, *Eujusticieae* and *Dicliptereae* of the *Justicieae* of BENTHAM & HOOKER, suggests that *Barlerieae* are the most primitive subtribe. Separation of *Barlerieae* in Engler's system from the tribe *Justicieae* has been supported. But separation of *Ecbolium* and *Rungia* in a different taxon, *Odontonemeae* in the classification of ENGLER does not find justification. The position of *Rungia* under *Dicliptereae* (BENTHAM & HOOKER) is supported.

### Introduction

Of the five tribes of *Acanthaceae*, the tribe *Justicieae* (BENTHAM & HOOKER, 1873) includes six subtribes, of which *Barlerieae*, *Andrographideae*, *Eujusticieae* and *Dicliptereae* are common in India. Most of the species have wide therapeutic application (vide CHOPRA et al., 1956).

ENGLER & DIELS (1936) have placed *Barlerieae* in a different taxon, wide apart from their *Justicieae* and *Andrographideae*, by which their classification differs markedly from that of BENTHAM & HOOKER. In the system of ENGLER, *Ecbolium* and *Rungia* are placed in the *Odontonemeae* (vide DE DALLA TORRE & HARMS, 1900-1907) while BENTHAM & HOOKER have placed *Rungia* under subtribe *Dicliptereae* and *Ecbolium* under subtribe *Eujusticieae*, both under *Justicieae*.

The present paper is an attempt to contribute to the solution of the taxonomic controversies by utilizing cytological principles (SHARMA, 1964).

### Materials and Methods

The following species were collected from Calcutta and its suburbs, identified by consulting the key of CLARKE (1885) and the herbarium of Calcutta University. Sheet numbers are given in parentheses.

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