CHROMOSOME NUMBERS, KARYOTYPE AND CROSSABILITY OF SOME *MANIHOT* SPECIES

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INTRODUCTION

The genus Manihot is important because of the species Manihot esculenta Crantz. This species is believed to have originated from Brazil and Central America (Pierre Martyr, 1494; Barnes, 1954; Rogers, 1964; and Schwerin, 1970). Purseglove (1968) indicates that it was grown as a crop in Peru some 4000 years ago and in Mexico 2000 years ago and that it is not known in a wild state. It is a widely cultivated tropical and subtropical root crop known by such names as cassava, manioc or tapioca in different parts of the world. It is used as a vegetable in the areas of its cultivation and as a source of high quality starch for industrial processes of various types.

Although some breeding work for improved yield and quality of the tubers has been done, no study has been made of the cytogenetics of the species in relation to other species of the genus and hence interspecific relationships of the genus for purposes of interspecific hybridisation and incorporation of desirable genes for specific economic characters and disease resistance. When chromosome morphology is compared in the different taxa, especially at the species and lower levels, an estimate of the relationship of these taxa may be obtained. Thus an evaluation of the comparative karyomorphology among the various taxa is desirable. This necessitates the precise identification of each of the pairs of chromosomes.

This type of study will indicate to the plant breeder the relationships to M. esculenta of other species of the genus and the probability of obtaining some desirable results from interspecific crosses which are a prerequisite in incorporating desirable genes in the commercial varieties. Furthermore, this type of study may open the way to locating marker genes on the different linkage groups of this species and also the genetics of inheritance of economic characters in addition to assisting in a phylogenetic classification of the taxa.

REVIEW OF LITERATURE

Taxonomy of Manihot

The genus Manihot of the family Euphorbiaceae was described by Pax (1910) to contain 128 species. Rogers and Appan (1970) made extensive explorations and collections of the various species in their native habitat which extends from southern Arizona in the United States of America through Mexico and South America to northern Argentina. They based their classification on the modern species concept of a closed gene pool, ecological and geographical distribution. Consequently they recognize 75 species and a number of sub-specific taxa in the genus. They point out that several of Pax's species were synonyms of one or another of the species, since outcrossing is the breeding system of the genus and the plants are, therefore, very heterozygous and heterogeneous. They found at least 10 different names given at one time or another to Manihot esculenta.

Chromosome numbers of Manihot

Perry (1943) determined the chromosome numbers of 90 species and varieties of 19 genera of the family Euphorbiaceae using a combination of various methods and materials. He showed that the chromosome number in the family ranged from 2n = 12 in Euphorbia dulcis to 2n = ca. 224 in Acalypha wilksiana and suggested that the primary basic number for the family is 8 with secondary basic numbers of 6, 7, 9, 10, and

11. For the genus Manihot he reported a basic number of 9 with 2n = 36 in all the five species and three varieties examined. Graner (1935, 1941) and Boiteau (1938, 1941) made chromosome counts of one species and three species respectively of Manihot and arrived at different numbers. Whereas Graner obtained a somatic number of 2n = 36 for all the varieties of one species examined, Boiteau obtained somatic numbers of 2n = 4, 8, 16, 24, and 32 for the three species examined. Perry thus seems to confirm Graner's results while Boiteau's results seem erroneous. However chromosome karyology of the genus Manihot has not been reported in the literature.

Interspecific hybridisation in the genus Manihot

Attempts at incorporating genes for mosaic disease resistance in Manihot esculenta and higher protein contents of the tubers necessitated interspecific hybridisation in the genus Manihot. The fact that Brandes and Sartoris (1936) succeeded in transferring genes for mosaic virus resistance from Saccharum spontaneum to S. officinarum by interspecific hybridisation of S. officinarum x S. spontaneum stimulated the interest of cassava breeders in attempting to carry out a similar hybridisation program. Both crops are vegetatively propagated.

Koch (1934) attempted interspecific hybridisation of Manihot esculenta and M. glasiovii. His M. glaziovii x

M. esculenta cross set no seeds but the reciprocal cross set 3.2 percent seeds. The F1 plants were much like M. glaziovii. Nichols (1947) carried out both intra- and inter- specific crosses. In M. esculenta x M. esculenta varietal crosses, seed set ranged from 0 - 56 percent. In M. esculenta x M. glaziovii he reported 1.8 percent seed set and only 0.4 percent in the reciprocal cross. In M. dichotoma x M. glaziovii, 10.7 percent seed set was obtained whereas in the reciprocal cross only 1.8 percent was obtained. Koch was however not successful in obtaining any seed set in M. dichotoma x M. esculenta cross. Nichols (1947) also carried out M. saxicola x M. esculenta cross and obtained very low seed set. He was attempting to breed for higher protein content in the tubers.

Bolhuis (1953) surveyed attempts to breed cassava cultivars with a higher protein content in the roots. He found that M. saxicola freely crossed with M. esculenta. Protein content was higher in F1 seedling roots, 0.67 - 1.32 percent dry matter, but this was not maintained in the clones when propagated vegetatively. However high HCN content was maintained in the clones. Jennings (1957) carried crosses of M. glaziovii x M. esculenta to advanced generations. The backcross to M. esculenta gave better yields in the 3rd backcross than the F1 plants but resistance to cassava mosaic virus was low. At best field resistance was obtained.

M. dichotoma x M. esculenta gave very low seed set and even after 3 backcrosses the roots were still fibrous and woody. He obtained best results when the F4 were crossed with the 3rd backcross of M. glaziovii x M. esculenta in both yield and resistance to virus mosaic.

M. catingea x M. esculenta gave tree-like F1 plants similar to the M. glaziovii x M. esculenta F1 hybrids with woody non-tuberous roots and mild transient mosaic symptoms. The F1 hybrids were crossed to resistant clones of M. glaziovii x M. melanobasis so that plants with improved tuber yields were obtained. In general no truly resistant selections were obtained from these series of interspecific hybridisations.

Abraham (1957) carried out a series of interspecific crosses between M. esculenta and M. glaziovii. His M. glaziovii x M. esculenta cross set no seeds but seed set of about 1.0 percent was obtained in the reciprocal cross. By the 4th backcross to M. esculenta individuals with improved tuber yields were obtained. Jennings (1959) showed that M. melanobasis and M. esculenta hybridised easily and the hybrids were fertile. In view of the readiness with which the two species hybridised, he doubted whether their separation as distinct species was justified. Bolhuis (1953) reached the same conclusion for M. saxicola and M. esculenta where the intercrossing was also easy.

However in most of the above crosses the percentage seed set was low. A similar situation was observed by Stout and Clark (1924) in potato. They attributed the reduction in fertility to the complete replacement of seed propagation by vegetative means and hence no selection pressure for fertility in the course of evolution. They concluded, "since potato is clonally propagated, selection and evolution have tended to be against seed set as a result of internal regulation of development and the influence of vegetative vigor may result in a systematic or plethoric sterility which in the case of potato seems to affect maleness more than femaleness and with time this has become hereditary".

MATERIALS AND METHODS

Cuttings of the cassava varieties (*Manihot esculenta* Crantz) and ceara rubber (*Manihot glaziovii* Muell. Arg.) used in these studies were collected from various parts of Hawaii between December 1970 and March 1971, (Table 1).

Some of these cuttings were rooted in vermiculite, the rest were rooted in a 2 : 1 : 1 mixture of vermiculite, sand and peat moss. Where necessary, slow release fertilizer (Osmocote) was added to promote growth. All rooting was done in plastic pots.

For somatic chromosome studies, large root tips with slight yellow tinge indicating vigorous growth were selected and cut about 2 mm in length. Some were pretreated with 0.002 molar 8-oxyquinoline solution according to the method of Tjio and Levan (1950) for 3 hours at 18° C. After this treatment these root tips and others which were not pretreated were fixed in Carnoy solution (three parts absolute alcohol, one part chloroform and one part glacial acetic acid) for 1 hour at 18° C. They were then washed in distilled water and hydrolysed with 1 N hydrochloric acid at 60° C for 10 minutes. Each root tip was macerated on a microscope slide after removing the root cap, stained with 1 percent aceto-orcein in 45 percent acetic acid and a cover slip applied. Excess stain was absorbed using bibulous paper. Application of

Table 1. *Manihot* species and varieties used in cytological studies

Species/Varieties	Source
M. glaziovii (ceara rubber)	Waimanalo Experiment Station
M. esculenta	
Itu	Waimanalo Experiment Station
?	" ex El Salvadore
Amarillo	Kauai Experiment Station
Ceiba (Cebia)	"
Mameya	"
Nina	"
Pata de Palona	"
Seda	"
Puna cassava	Hawaii (Big Island)
?	Honolulu
f. variegated	Honolulu

pressure using the wooden end of the needle spread the cells. The slide was passed through alcohol lamp flame for a few seconds to destain the cytoplasm and further spread the cells. The slide was ringed with sticky-wax around the cover slip to prevent drying out during examination.

For observation of meiotic behavior of pollen mother cells (PMC) of the species, flower buds were collected at noon and fixed in Carnoy solution (three parts absolute alcohol, one part glacial acetic acid and one part chloroform) for 24 hours at room temperature. Each anther from the fixed buds was placed in a drop of 1 percent aceto-orcein in 45 percent acetic acid on a microscope slide, cut into two halves and the pollen mother cells squeezed out of the anthers using needles. Another debris was removed before coverslip was applied and excess stain absorbed using bibulous paper. The slide was then rapidly passed through alcohol lamp flame to destain the cytoplasm and spread the cells. The coverslip was sealed with Kerr sticky wax.

Root tips and flower buds which were not used immediately after fixation were stored in 70 percent alcohol at 4^{o} C in the refrigerator and used to prepare slides as required. All examinations and analysis of the chromosomes were carried out on temporary preparations. With the aid of optical combinations of Zeiss ocular 10x, plan objective 100x and 1.30 oil immersion the chromosomes were counted and studied. Using

Zeiss photomicrographic camera attached to the above microscope, photographs were taken of cells with well spread chromosomes at 550x on Kodak High Contrast Copy film.

To make karyotypes the photomicrographic negatives were enlarged to a magnification of 5500x using a Leitz Valoy II enlarger and line tracing of whole image lengths of the chromosomes with sharp pointed pencil on standard white paper was made and the position of the centromere was indicated (Stewart, 1947). Each chromosome from the tracing was then measured with great care using a metric ruler and flexible wires. The standard deviation was calculated on the basis of the mean chromosome length of each chromosome type in each cell. The chromosomes were then arranged in order of decreasing mean length, the longest being the first and the shortest, the last. The Form Percent (F%) was used to represent the percentage of the short arm length to the entire chromosome length (Huziwara, 1956). In order to compare the relative length of the various chromosomes in the complement the ratio in percentage of the length of individual chromosome to that of the longest chromosome in the complement was used (Huziwara, 1956). In this method the length of the longest chromosome was taken as 100 and the relative lengths of all the remaining chromosomes were calculated in relation to this value. All karyotype analysis was done on unpretreated chromosomes only.

Bud pollination was carried out both in the field in the Arboretum at the Experiment Station, Waimanalo (about 22 miles from Manoa campus) and at Manoa campus on potted plants. Only three of the plants (M. glaziovii and M. esculenta varieties Itu and ? ex El Salvadore) under study were pollinated in the field at Waimanalo. These three together with the other 9 varieties of M. esculenta were grown in pots and pollinations were carried out at Manoa campus. The plants bloomed between May and September.

Seed set obtained from the crosses and selfings was determined about 3 months after pollinations when the capsules were collected before seeds dehisced. Dehiscence of the capsules occurred in the laboratory and seeds were counted. Percentage success in capsule and seed set was determined by comparing the number of fruits and seeds actually collected with the number of pollinations made. Each pollination was potentially capable of yielding three seeds from the trilocular ovaries. Seed viability was tested by germinability.

Pollen fertility was assessed in vitro by measuring stainability using tetrazolium chloride, benzidine dihydrochloride, and cotton blue in lactophenol. Attempts were also made at in vitro germination of the pollen, using Brewbaker and Kwack (1964) Ca-supplemented medium (see Appendix).

RESULTS

Chromosome numbers and karyotypes

The somatic chromosome complement of all the species and varieties studied is 2n = 36 (Fig. 1 - 12). The chromosomes on the average are small, ranging in Manihot esculenta from 1.6 to 4.0 microns (Table 3) and in M. glaziovii from 1.4 to 3.8 microns (Table 4). Based on the length of the chromosomes, centromere position, presence of secondary constriction and satellites, the chromosomes of each species were divided into types. The centromere position was used to determine the Form Percent (F%); 46 -50 percent being classified as metacentric, 26 - 45 percent as submetacentric, 11 - 25 percent as subterminal or acrocentric, and less than 10 percent as telocentric, (Huziwara, 1956). Following McClintock (1929) the chromosomes were numbered starting from the longest to the shortest.

1. Karyotype of M. esculenta

The somatic chromosome complement of this species based on average measurements of chromosomes of one cell each of 11 varieties may be divided into 13 types as follows:

Type I: A pair of longest chromosomes (1,2) 4.0 microns with submetacentric centromere.

Type II: A pair of chromosomes (3,4) of average length 3.3 microns with acrocentric (subterminal) centromere.

Type III: A pair of chromosomes (5,6) of length 3.1 microns with acrocentric centromere.

Type IV: A pair of satellited chromosomes (7,8) with secondary constriction and submetacentric centromere.

Type V: Three pairs of chromosomes (9,10; 11,12; 13,14) with submetacentric centromeres.

Type VI: Three pairs of chromosomes (15,16; 17,18; 19,20) with submetacentric centromeres.

Type VII: A pair of chromosomes (21,22) with acrocentric centromere.

Type VIII: A pair of satellited chromosomes (23,24) with submetacentric centromere and secondary constriction.

Type IX: Two pairs of chromosomes (25,26; 29,30) with submetacentric centromeres.

Type X: A pair of medium-sized chromosomes (27,28) with metacentric centromeres.

Type XI: A pair of small chromosomes (31,32) with submetacentric centromere.

Type XII: A pair of short chromosomes (33,34) with submetacentric centromere.

Type XIII: A pair of shortest chromosomes (35,36) 1.6 microns in length with metacentric centromere.

No chromosome with a telocentric centromere was observed.

2. Karyotype of M. glaziovii

The somatic chromosome complement of this species based on average measurements of chromosomes of two well spread cells is divided into 13 types as follows:

Type I: A pair of longest chromosomes (1,2) with secondary constriction and satellites. The centromere position is submetacentric.

Type II: A pair of chromosomes (3,4) with acrocentric centromere.

Type III: A pair of chromosomes (5,6) with metacentric centromere.

Type IV: A pair of satellited chromosomes (7,8) with submetacentric centromere and secondary constriction.

Type V: A pair of chromosomes (9,10) with acrocentric centromere.

Type VI: Two pairs of chromosomes (11,12; 13,14) with acrocentric centromere.

Type VII: Two pairs of chromosomes (15,16; 17,18) with submetacentric centromere.

Type VIII: Two pairs of chromosomes (19,20; 21,22) with metacentric centromeres.

Type IX: Two pairs of chromosomes (23,24; 25,26) with submetacentric centromeres.

Type X: Two pairs of chromosomes (27,28; 29,30) with submetacentric centromeres.

Type XI: A pair of medium-sized chromosomes (31,32) with submetacentric centromeres.

Type XII: A pair of short chromosomes (33,34) with submetacentric centromeres.

Type XIII: A pair of shortest chromosomes (35,36) with submetacentric centromeres.

No chromosomes with telocentric centromeres were observed.

The chromosomes of the two species M. esculenta and M. glaziovii are similar in gross morphology. Of the 36 chromosomes of M. esculenta 4 have metacentric centromeres, 26 have submetacentric centromeres, and 6 have acrocentric centromeres. In M. glaziovii 6 chromosomes have metacentric centromeres, 22 have submetacentric centromeres and 8 have acrocentric centromeres (Table 5). The mean length of the

M. esculenta chromosome complement is 2.5 microns with a range of 4.0 to 1.6 whereas in M. glaziovii the mean length is 2.6 microns with a range of 3.8 to 1.4 microns. Two pairs of chromosomes in each species possess satellites with secondary constrictions. (Fig. 13-14).

Microsporogenesis in the two species was normal (Fig. 17-20). Eighteen bivalents were regularly observed at MI and regular disjunction at AI. Metaphase II and the rest of the meiotic phases were normal ending up in the formation of tetrads. Cell wall formation of the microspore tetrads was simultaneous. On the basis of these comparative data of the two species, they must differ in gene composition which should then account for their morphological plant character differences (Fig. 21-24).

Pollination experiments

Results of pollinations which were carried out in both the field at Waimanalo and in potted plants at Manoa campus are shown in Table 6, together with germination of the seeds. The highest percentage capsule set was 42.8 in M. esculenta intervarietal cross of Seda x Itu and variegated x M. glaziovii and zero percent where M. glaziovii was used as a female parent in a cross with varieties of M. esculenta. Reciprocal cross of M. glaziovii as male parent with varieties of M. esculenta as female parents gave comparative percentage capsule set of 14.2, 24.1, and 42.8.

Varieties of M. esculenta freely intercrossed among themselves reciprocally. These results seem to suggest that restriction of reciprocal cross between M. glaziovii and varieties of M. esculenta was probably due to maternal properties of M. glaziovii when M. esculenta was used as pollen parent. This unilateral incompatibility probably results from interaction of pollen with maternal tissues. In all cases where this incompatibility was exhibited, it was observed that the female flowers dropped within 5 days after pollination. In this study no attempt was made to investigate the causes of the unilateral incompatibility except that pollen viability and size for the species were compared (Table 7).

Observation

In general it was observed that there appeared to be periodicity of flowering in Manihot species. Flowering started in May and continued through August. By late September, all young flower buds dropped prematurely without reaching anthesis. The dropping of the flowers may be closely correlated with the drop in temperatures and daylength, during the Fall through Winter months. Maximum temperatures during May to August ranged from 81.7 to 84.6° C in Waimanalo and 83.7 to 88.0° F in Manoa campus whereas daylength for the same period was 12 to 13.5 hours as compared to highest Winter temperatures of 76.5° to 79.4° F in Waimanalo and 81.7 to 83.0° F in Manoa and daylength of 10.5 to 12 hours.

Pollen physiology

Results of pollen stainability and germinability are recorded in Table 7. Although the percentage of pollen stained was high in all the stains used, showing different activities of the pollen, their germinability was zero percent. In fact, most pollen grains burst in the germination medium without germination.

Table 2. Chromosome numbers in *Manihot* spp.

Species/Varieties	Source	2n	n
Manihot esculenta Crantz			
Itu	Waimanalo Expt. Station	36	18
?	Waimanalo Expt. Station ex El Salvador	36	18
Amarillo	Kauai Expt. Station	36	18
Ceiba (Cebia)	"	36	18
Mameya	"	36	18
Nina	"	36	18
Pata de Palona	"	36	18
Seda	"	36	18
Puna cassava	Hawaii (Big Island)	36	18
?	Honolulu	36	18
f. variegated	Honolulu	36	18
M. glaziovii Muell-Arg. (ceara rubber)	Waimanalo Expt. Station	36	18

Table 3. Measurements of somatic chromosomes in M. esculenta

Chromosomes	Mean length in microns			Centro-mere	F%	Relative length percent
	Short arm	Long arm	Total			
1,2	1.3	2.7	4.0 ± 0.22	sm	32.5	100.0
3,4	0.7	2.6	3.3 ± 0.20	st	21.2	82.5
5,6	0.5	2.6	3.1 ± 0.14	st	16.1	77.5
7,8 sat.	1.3	1.6	2.9 ± 0.14	sm	44.8	72.5
9 - 14	1.2	1.5	2.7 ± 0.12	sm	44.4	67.5
15 - 20	0.9	1.7	2.6 ± 0.08	sm	34.6	65.0
21,22	0.5	2.0	2.5 ± 0.15	st	20.0	62.5
23,24 sat.	0.7	1.5	2.2 ± 0.13	sm	31.8	55.0
27,28	1.1	1.1	2.2 ± 0.13	m	50.0	55.0
25,26;29,30	0.9	1.3	2.2 ± 0.13	sm	40.9	55.0
31,32	0.9	1.1	2.0 ± 0.06	sm	45.0	50.0
33,34	0.7	1.1	1.8 ± 0.04	sm	38.9	45.0
35,36	0.8	0.8	1.6 ± 0.02	m	50.0	40.0

Table 4. Measurements of somatic chromosomes in M. glaziovii

Chromosomes	Mean length in microns			Centro-mere	F%	Relative length percent
	Short arm	Long arm	Total			
1,2 sat.	1.5	2.3	3.8 ± 0.15	sm	39.5	100.0
3,4	0.6	2.7	3.3 ± 0.13	st	18.2	86.8
5,6	1.5	1.6	3.1 ± 0.10	m	48.4	81.6
7,8 sat.	1.3	1.8	3.1 ± 0.10	sm	41.9	81.6
9,10	0.7	2.4	3.1 ± 0.10	st	22.6	81.6
11 - 14	0.5	2.2	2.7 ± 0.08	st	18.5	71.1
15 - 18	0.9	1.8	2.7 ± 0.08	sm	33.3	71.1
19 - 22	1.2	1.3	2.5 ± 0.09	m	48.0	68.4
23 - 26	0.9	1.6	2.5 ± 0.05	sm	36.0	65.8
27 - 30	1.1	1.3	2.4 ± 0.06	sm	45.8	63.2
31,32	0.6	1.6	2.2 ± 0.04	sm	27.3	57.9
33,34	0.7	1.1	1.8 ± 0.02	sm	38.9	47.4
35,36	0.6	0.8	1.4 ± 0.03	sm	42.9	36.8

Table 5. Comparison of chromosome data of *Manihot* spp

Species	2n	Number of chromosomes with different centromere positions			Average length in microns
		metacentric	submeta-centric	sub-terminal	
M. esculenta	36	4	26	6	2.5
M. glaziovii	36	6	22	8	2.6

Table 6. Fruit set, seed set and germination of seeds from reciprocal interspecific and intraspecific crosses and selfs.

Species/Variety	Number of flowers pollinated	Number of capsules set	Percentage capsules set	Number of seeds set	Mean No. of seeds per capsule	Number of seeds germinated
M. glaziovii ♀	55	9	16.3	21	2.3	19
Itu ♀	42	5	11.9	13	2.6	13
Itu x M. glaziovii	29	7	24.1	15	2.1	15
M. glaziovii x Itu	12	0	0	0	0	0
Seda ♀	31	9	29.3	23	2.6	20
M. glaziovii x Seda	15	0	0	0	0	0
Seda x M. glaziovii	21	3	14.2	8	2.7	8
Variegated ♀	5	2	40.0	5	2.5	5
M. glaziovii x Variegated	8	0	0	0	0	0
Variegated x M. glaziovii	7	3	42.8	7	2.3	7
? ex El Salvadore ♀	60	15	25.0	37	2.4	33
Itu x Seda	11	4	36.3	10	2.5	8
Seda x Itu	14	6	42.8	15	2.5	13

Table 7. Stainability, germinability and size of Manihot spp. pollen

Species/Variety	Color reaction/ Stainability percent			Germinability	Mean pollen size in microns		
	tetrazolium chloride	benzidine dihydro-chloride	cotton blue in lacto-phenol			t	P=0.05
M. glaziovii	pink/98	blue/96	blue/98	0	153.9		
M. esculenta:						0.74	2.37
Itu	pink/97	blue/95	blue/98	0	147.5		
Seda	pink/98	blue/96	blue/97	0	146.8		
Variegated	pink/97	blue/98	blue/96	0	146.4		

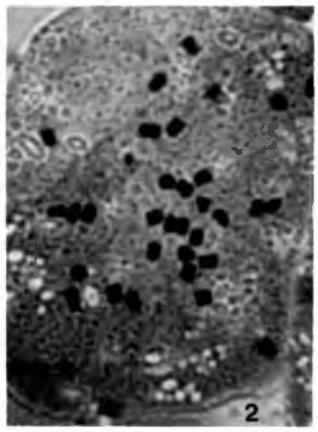
S.E. = ± 10.12

Plate 1. Somatic chromosomes of *Manihot* species pretreated with 8-oxyquinoline (1650X)

Figure:

1. *M. glaziovii* (ceara rubber)
2. *M. esculenta* var. Itu
3. *M. esculenta* f. variegated
4. *M. esculenta* var. Amarillo
5. *M. esculenta* var. ? ex El Salvadore
6. *M. esculenta* var. Ceiba
7. *M. esculenta* var. Mameya
8. *M. esculenta* var. Nina
9. *M. esculenta* var. Pata de Palona

1



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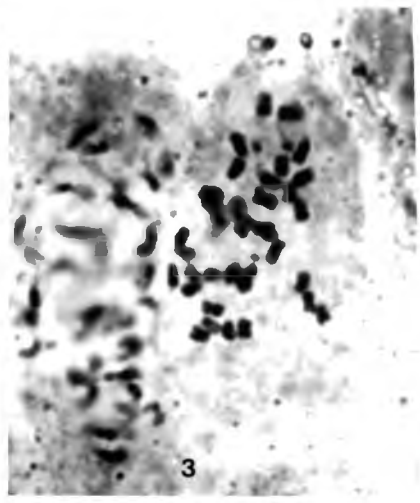

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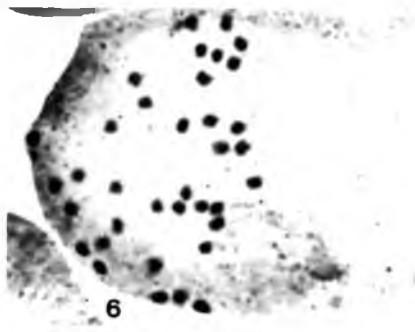

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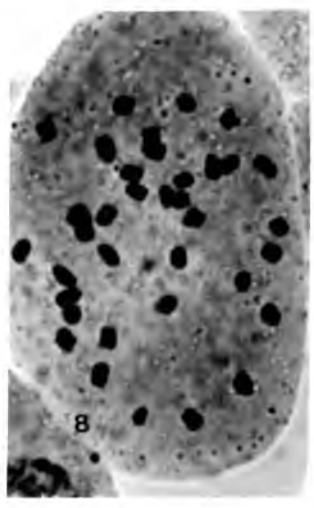


8

9

Plate 2. Somatic chromosomes of Manihot species with pretreatment and without pretreatment with 8-oxyquinoline and some meiotic configurations in microsporogenesis of Manihot spp. (1650x)

Figure:

10. M. esculenta var. Seda (pretreated with 8-oxyquinoline)

11. M. esculenta var. Puna cassava (pretreated with 8-oxyquinoline)

12. M. esculenta var. ? (pretreated with 8-oxyquinoline)

13. M. glaziovii (without pretreatment with 8-oxyquinoline)

14. M. esculenta (without pretreatment with 8-oxyquinoline)

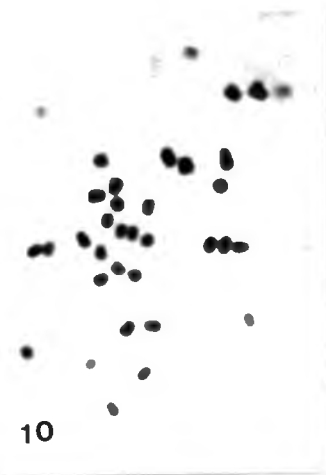
17. Early Anaphase I in M. esculenta

18. Metaphase II (polar view) in M. esculenta (n = 18)

19. Metaphase I in M. glaziovii 18 II

20. Metaphase II in M. glaziovii (polar view) n = 18

Arrows indicate satellites.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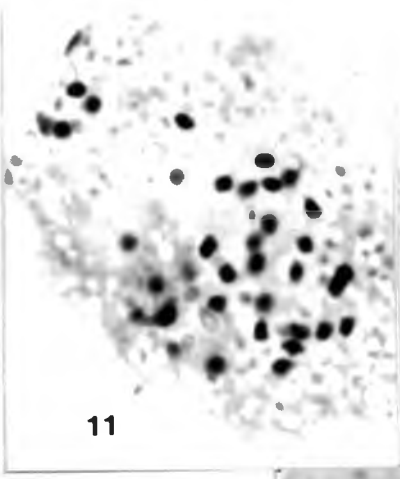
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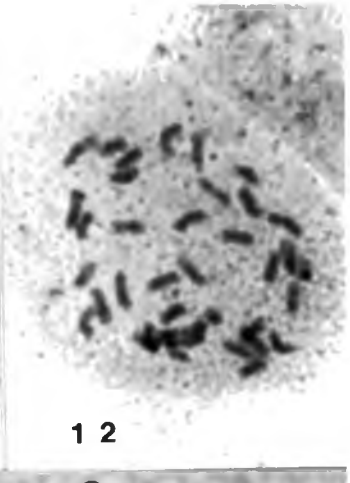
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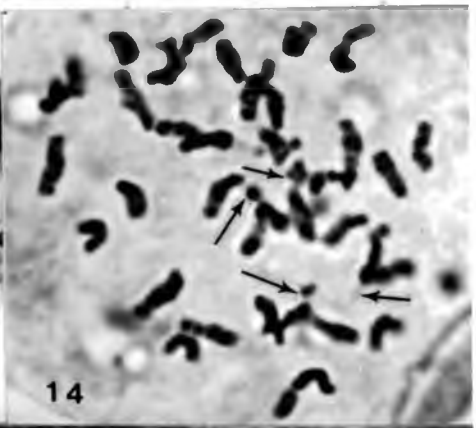
13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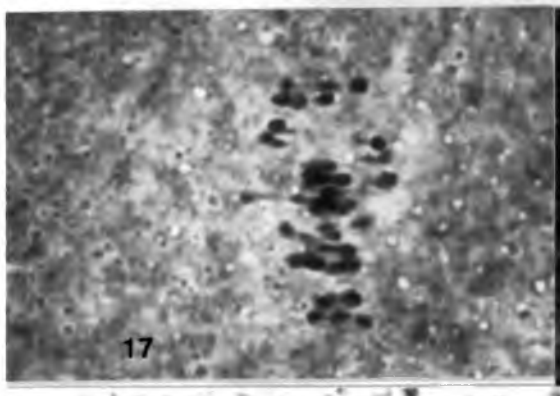
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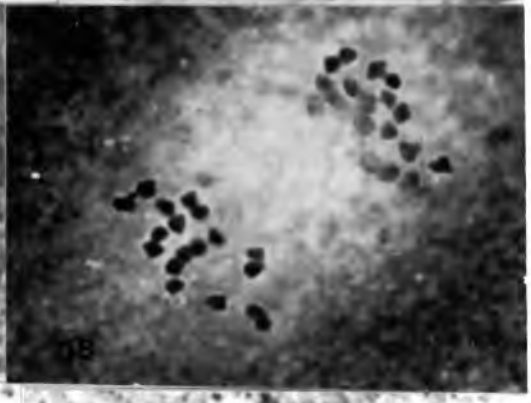
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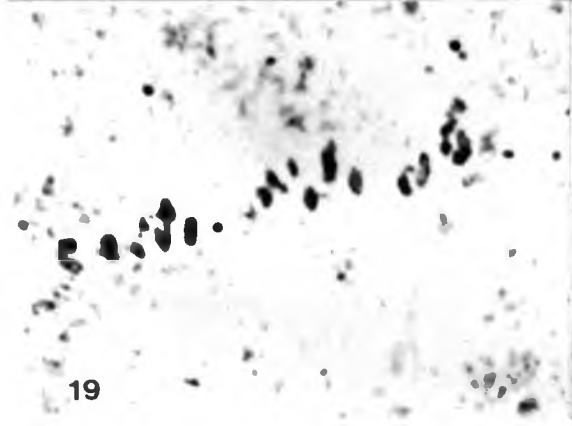
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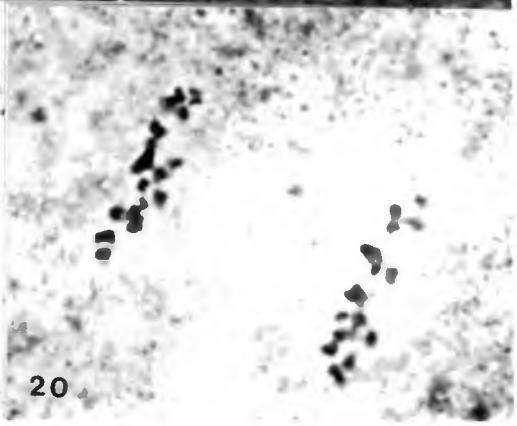
20

Plate 3. Karyotypes of *Manihot* species (5500X)

Figure:

15. *M. esculenta*

16. *M. glaziovii*

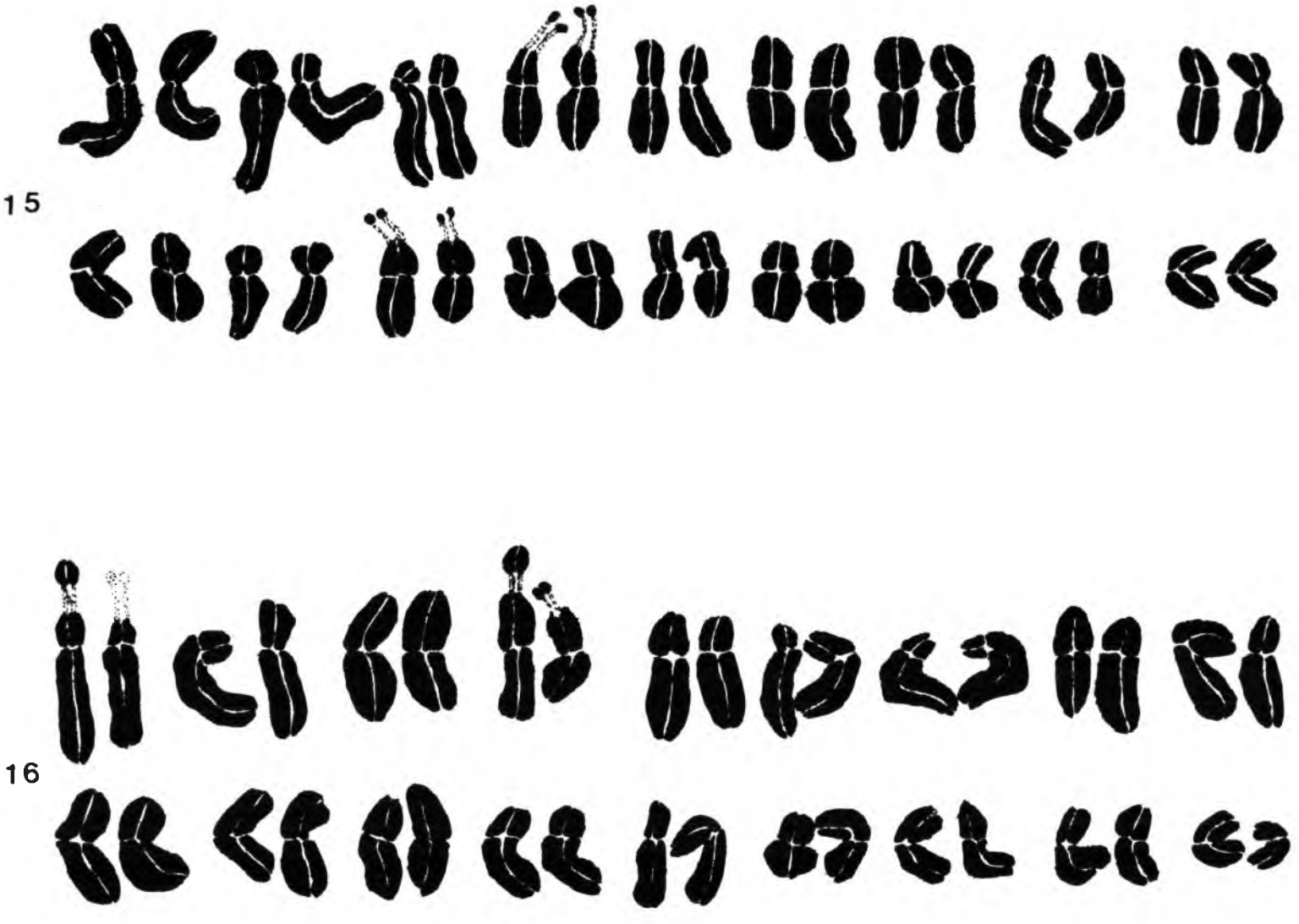
15
16

Plate 4. Vegetative morphology of *Manihot* spp

Figure:

21. Mature plant of *M. glaziovii* with flowers and fruits (0.1x).

22. Young potted plant of *M. glaziovii* (0.1x).

23. *M. esculenta* f. variegated (0.1x).

24. *M. esculenta* variety Itu with flowers and fruits (0.1x).


21


22


23


24

DISCUSSION

The number of somatic chromosomes in all the varieties and form of Manihot esculenta and Manihot glaziovii determined is 2n = 36. The gametic number is n = 18. These findings agree with those of Perry (1943) and Graner (1935, 1941). However Boiteau (1938, 1941) in Madagascar found somatic numbers of 2n = 4, 8, 16, 24, and 32 for the three species of Manihot studied. These findings seem to be erroneous for if such wide variation occurred in the genus, it should be found in the original habitat of the genus, in this case, Brazil and Central America, rather than in Madagascar where some species of the genus were only introduced in comparatively recent times.

Chromosomes of the species of the genus determined in this study are rather small. For the varieties and form of Manihot esculenta the mean chromosome length is 2.5 microns with a range of longest to shortest of 4.0 to 1.6 microns. For Manihot glaziovii the mean length is 1.6 microns and a range of 3.8 to 1.4 microns. Thus the ratio of longest to shortest chromosome in M. esculenta is 2.5 : 1 and in M. glaziovii it is 2.7 : 1. Except for 2 and 3 pairs of chromosomes respectively in each of the species which have metacentric centromeres, all the rest have either submetacentric or acrocentric centromeres. In M. esculenta the

Form Percent (F%) ranges from 15.3 to 50.0 whereas in M. glaziovii the range is from 13.3 to 50.0. No chromosomes with telocentric centromeres were observed.

These facts would seem to place these karyotypes in asymmetry class 2b according to Stebbins (1958) classification of karyotypes. In an extensive review of evolution of katyotypes in different taxa of the plant kingdom, Stebbins (1971) suggests that symmetrical karyotypes are more primitive than asymmetrical ones such that the evolutionary trend is from symmetry to greater asymmetry, although reversals of this trend may occur periodically. Karyotypes of Manihot species may therefore be considered as advanced.

Polyploid nature of Manihot species

Karyotypes of Manihot species studied show that each species possesses 2 pairs of satellited chromosomes. The two pairs are different from each other in gross morphology. Since the discovery of satellites by Navashin (1912) in Galtonia candicans this chromosomal structure has been used as one of the features to determine the level of ploidy of plants. Numerous cases of the presence of satellited chromosomes have been reported in plants. A few cases have also been reported in which no satellites have been observed. Heitz (1931) stated that all plants must have satellites, haploids with one, diploids with two, tripolids with three, and tetraploids with four, etc. According to Nandi (1936)

the absence of satellite chromosomes as has been reported in a few species is not therefore due to their real absence but due to the difficulty of obtaining good materials for their detection. And it may be added, the technique in preparing the plant materials for karyological studies, as is evident in the present study (Figs. 1 - 16). Root tips pretreated with 8-oxyquinoline before fixation shortened the chromosomes to the extent that satellites were not visible (Figs. 1 - 12), whereas direct fixation without pretreatment permitted most morphological structures of the chromosomes to be seen at metaphase (Figs. 13 - 14). The present study would therefore seem to agree with the statement of Heitz (1931) that all plants should have satellites and that their number probably coincides with the number of genomes present in the species.

Berger (1941) pointed out that in Spinacia oleracia root tips of which 2n, 4n, and 8n cells occur, the satellites in resting nuclei could be used as a method of determining the ploidy of the cells. Wilkinson (1941) found that in Salix alba and in S. fragilis both of which are allotetraploids with 2n = 76, two pairs of satellited chromosomes were present. Levan (1931) working with various species of Allium showed that triploid nutans forms each had 3-satellited chromosomes, in A. validum a tetraploid, 2-satellited chromosomes were present in pollen. However some hexaploid

A. nutans types had only 4 satellites while some diploid A. narcissiflorum had no satellites..

Satellites may be described as minute portions of chromosomes attached to the chromosomes by a tenuous thread. It is usually attached to the distal end of the chromosome. However, satellites may sometimes appear at places other than the distal end of the chromosome. Thus Darlington (1937) showed intercalary satellite (trabant) in Solanum lycopersicon and in Rhoeo species.

Satellites have been shown to play a vital role in the organization of the cell. Heitz (1931) discovered that each nucleolus arose on a satellite chromosome in telophase at the point where the satellite thread emerged. This point was later called the nucleolar organizing body. By using a combination of genetic, cytological and biochemical investigations, Brown and Gurdon (1964) showed that in a mutant of clawed frog Xenopus laevis which lacks a nucleolus, its lethality in the homozygous condition was related to a deficiency in the ribosomes which control protein synthesis through messenger RNA. Ritossa et al (1966) showed by mapping techniques that bobbed locus in Drosophila is at or near the nucleolus organizing region and that bobbed flies have less ribosomal RNA than normal flies. These flies were also shown to be deficient in DNA which codes for ribosomal RNA. This evidence would seem to indicate that bobbed mutations are deficiencies for part of the nucleolar organizer region

and that the DNA of this region codes for ribosomal RNA. Perry (1962) showed that ribosomal RNA exists in high concentrations in the nucleoli and is released into the cytoplasm, from which evidence it may be concluded that nucleoli are sites for synthesis of ribonuclear proteins and ribosomes. Lance (1957) demonstrated that in higher plants nuclei of the apical meristem during transition from the vegetative to the reproductive state contain larger nucleoli at a time when large amounts of protein are being synthesized. These pieces of evidence seem to support Heitz (1931) hypothesis that all plants, at least eucaryotes, must contain satellites.

Meiosis in the species and varieties of Manihot studied was regular in so far as there were 18 bivalents observed at metaphase I and disjunction at anaphase I. There were no quadrivalents throughout the meiotic process.

Perry (1943) suggested a basic number of 9 for the genus Manihot and basic numbers of 6, 7, 8, 10, and 11 for other genera of the family Euphorbiaceae. Considering the 2 pairs of dissimilar satellited chromosomes and regular formation of bivalents and disjunction, it is suggested that Manihot species under investigation are allotetraploid, with a basic chromosome number of x = 9. Whether these allotetraploids are true or segmental (Stebbins 1950) will have to await further investigations. Further evidence for the polyploid

nature of Manihot species studied include small chromosome size, vegetative vigor, and perennial growth habit, which according to Stebbins (1971) favour polyploid formation in many groups of plants.

Fertility of selfs and crosses

Fertility in the genus Manihot is rather low. When the different varieties and species were selfed and/or crossed, a generally low percentage seed set was obtained (Table 6). Manihot esculenta crossed only unilaterally as female parent with M. glaziovii producing low percentage seed set. Such unilateral compatibility has been observed in numerous other species of vegetable and other crops. Ricks (1960) showed that Lycopersicon esculentum crossed unilaterally as female parent with Solanum pennellii. The F1 hybrids crossed as female parents to S. pennellii but only as male parents to L. esculentum. Both parents and the F1 hybrids each had 12 pairs of chromosomes. It was observed that failure to effect a cross was due either to failure of pollen grains to germinate or very slow growth of the pollen tubes. When the meiotic chromosome cytology of the two parents and the F1 hybrids were compared (Kush and Rick, 1963) it was observed that pachytene chromosomes were very similar in gross morphology. Several of the S. pennellii chromosomes were found to have somewhat larger chromatic regions with discrete chromomeres, however. The chromomere pattern was different

in most cases. Meiosis in the hybrids was strictly regular but only size inequalities occurred in certain bivalents. On the basis of chromosome pairing, hybridisation compatibility, hybrid fertility and plant morphology, they suggested a revision of the taxonomy so as to place both S. pennellii and L. esculentum in the same genus. L. esculentum crosses unilaterally when used as female parent with L. peruvianum and embryo culture is necessary to produce F1 hybrids (Smith, 1944). Lindqvist (1960) showed that Lactuca sativa and L. serriola separately crossed with L. saligna only when the latter species was used as female parent. He observed that only slight differences exist between L. sativa, L. saligna, and L. serriola in chromosome morphology each species of which has a somatic number of 2n = 18. However L. sativa crossed reciprocally with L. serriola. In the genus Solanum section Tuberarium, Lamm (1945) showed that Solanum rhybinii (diploid) crossed easily with related species reciprocally but S. ajanhuiri was only successful when used as a female parent.

The unilateral compatibility between M. esculenta and M. glaziovii is rather similar to the above cases since their chromosome numbers and morphology are essentially the same. In a review of the phenomenon of hybrid inviability and weakness, Stebbins (1958) divided the causes into three categories of: lack of hormony between genes or chromosomes of the parent species; cytoplasmic or plastid differences between

the parent species; and disharmony between the hybrid embryo and the surrounding tissues. The first category is recognized by the fact that the crosses are equally unsuccessful in both directions whereas there is a difference between reciprocal crosses if the cause of inviability is of the kinds included in the last two categories. The cross between M. esculenta and M. glaziovii comes under the latter categories. Causes of the unilateral incompatibility are not known and will have to await future investigations. However the low seed set observed in crosses and selfs may be due to reduction in fertility in the species as a result of replacement of seed propagation by vegetative propagation and hence selection against fertility in the course of evolution. Stout and Clark (1924) suggested this reason as a probable explanation of low fertility in Solanum tuberosum and its wild relatives.

Pollen physiology of Manihot species

Pollen grains of Manihot species studied are rather large, of the order of 147 microns for M. esculenta and 154 microns for M. glaziovii, (Table 7). There was no significant difference in size between pollen of the two species. They are spherical and smooth. Stainability of newly sampled pollen at the time of anthesis was high but germinability in vitro was nil. The cytology of Manihot pollen has been reported by Schnarf (1939) who showed that the pollen grains of this genus are trinucleate. In a consideration of pollen

cytology and viability *in vitro*, Brewbaker and Majumber (1961) concluded that most pollen grains which are exceedingly difficult to germinate *in vitro* are trinucleate. Brewbaker (1967) summarized major characteristics of trinucleate pollen as absence of viability in vitro, absence of storage longevity, site of self incompatibility inhibition the stigma, and type of self incompatibility control sporophytic. He concluded that trinucleate pollen grains demand an unusual environment for germination - one which has not been duplicated effectively *in vitro* - and these more highly evolved trinucleate plants may have a more specialized breeding habit in this respect. *Manihot* species under investigation certainly have some of the above characteristics which may account for the failure of their pollen to germinate *in vitro* and perhaps also partly for the low seed set observed in controlled pollinations.

It has been shown above that the chromosomes of the species studied are equal in number and similar in karyotype. From cytological considerations, therefore, it seems clear that these species are closely related and for breeding purposes might be expected to cross reciprocally. However results of interspecific crosses reported show that reciprocal crossing between them is not practicable. The species only cross unilaterally when *M. glaziovii* was used as pollen parent and *M. esculenta* as seed parent. Although no investigation was carried out to determine the cause of

failure of reciprocal crosses, pollen analysis of the species showed no significant differences in size between them. These results would seem to indicate that similarity of karyotypes between species cannot necessarily give indication of their reciprocal crossability.

Since the subject of interspecific hybridization is rather important in vegatable crops breeding, particularly, for purposes of transferring economic characters and disease resistance from the wild to cultivated crops species, several techniques have been devised to enhance it. These techniques include: altering chromosome numbers of parental species, as in Solanum tuberosum breeding (Livermore and Johnstone, 1940; Hougas and Peloquin, 1960; in Lycopersicon esculentum, Bohn, 1948; and Soost, 1958);
use of grafts or chimeras, (Gunther, 1964, in Lycopersicon esculentum; Lamm, 1941, in Solanum tuberosum);
use of gametic diversity, (Wall and York, 1960, in Cucurbita spp; Honma and Heeckt, 1959, in Phaseolus spp);
abatement of compatibility barriers by radiation, (Pandey, 1960, in Solanum simplicifolium X S. chacoense cross);
use of bridging species, (Rhodes, 1959, in Cucurbita spp; Dionne, 1963, in Solanum tuberosum and its wild relatives);
embryo culture, (Smith, 1944, in Lycopersicon esculentum crosses with its wild relatives; Yamane, 1953, and Wall, 1954, in Cucurbita).

Some of these techniques might be useful in interspecific hybridization in Manihot species where incompatibility is demonstrated.

SUMMARY

The chromosome numbers, karyotype and crossability of some *Manihot* species were investigated.

Of the two species and 11 varieties studied, chromosome number of 2n = 36 and n = 18 were recorded. Two pairs of satellited chromosomes are reported for the first time for each of the two species and karyotypes for both species were similar. Meiosis was normal in pollen mother cells showing 18 bivalents at MI and regular disjunction at AI. On the basis of these findings, it is suggested that the *Manihot* species under investigation are allopolyploids with a basic chromosome number of x = 9.

In spite of the similarity in karyotypes of the two species, they cross only unilaterally. *M. glaziovii* crossed as pollen parent with varieties of *M. esculenta* as seed parent. *M. esculenta* varieties freely intercrossed reciprocally. The unilateral interspecific crossability of these species is discussed in relation to other such unilateral compatibility in the literature.

Pollen of neither species germinated *in vitro* in calcium-supplemented medium.

APPENDIX

Calcium-supplemented medium for germination of pollen in vitro

Sucrose 10 percent

Boric acid 100 ppm

Calcium nitrate ($Ca(NO_3)_2 \cdot 4H_2O$) 300 ppm

Magnesium sulfate 200 ppm

Potassium nitrate 100 ppm

Distilled water

pH commonly 5 - 7. But some species are best at pH 8-9.

Best growth obtained in standing aqueous drop in petri dish lined with moist filter paper at room temperature.

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