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GENETICAL AND CYTOLOGICAL STUDIES ON AN INTERSPECIFIC HYBRID OF HIBISCUS ESCULENTUS L. AND HIBISCUS MANIHOT L.

BY

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(With 8 plates and 12 text-figures)

Contents

Introduction

The study of the chromosome number and its behavior in interspecific and intergeneric hybrids of plants and of animals has attracted special attention of modern biologists, assuming that it may afford some vantage for the comprehension of the origin of species. It is naturally impossible to draw any distinct line across a species or genus not only from the morphological point of view but also from genetical, because the affinity between chromosomes of different.plants is variable. A study of these chromosome relations may lead to the formation of some definite ideas concerning the basis of taxonomy. Thus new ideas regarding the formation and evolution of plant species have already materialized concerning the origin of new species by hybridization.

Closely allied species are often known to possess chromosome numbers which are related to each other as common multiples. For instance, SAKA-MURA (1918), KIHARA (1919) and SAX (1922) have shown that the cereals furnish an excellent example of polyploidy. Among wheats diploid, tetraploid and hexaploid species are known. TAHARA (1915) found in the genus *chrysanthemum*, haploid number of 9, 18, 27, 36 and 45 chromosomes. *Prunus laurocerasus* L. is reported by MEURMAN (1929) as a merely 22–ploid in its chromosomal construction, the basic numer in the family being 8. In addition a number of polyploid series have been reported by TÄCKHOLM (1922) in *Rosa* species, by LONGLEY (1923) in *Rubus* and *Crataegus*, by KIHARA and ONO (1925) in *Rumex* of the group *Eulapathum* species, by JØRGENSEN (1928) in *Solanum* species etc.

For the questions which have arisen as to the differences of chromosome number in different species, three possibilities are to .be considered : that polyploid multichromosome number is due to fragmentation of chromosomes, or spontaneous doubling of chromosomes or to summing of chromosomes from two parents through intercrosses between species which possess a small number of chromosomes. WINGE (1917, 1924) has assumed that in the hybrid from the crossing of two species, a new constant species may result when two.kinds of chromosomes show no affinity and a longitudinal splitting of all the chromosomes takes place.

Cytological and genetic analyses of wide crosses involving a certain amount of incompatibility have been made by many investigators as an especially important work in regard to the origin of important domesticated plants and animals.

The species cross which will· cause the occurrence of many polymor-

phic groups of plants is now a well known and widely recognized fact.
To solve the question how and why the formation of these new forms
occured is not only of especial interest, but also it is a practically impor-
tant problem in order to breed valuable new plants.

Constant fertile hybrids between two species of plants have been
obtained by many investigators. COLLINS and MANN (1923), and COLLINS,
HOLLINGSHEAD and AVERY (1929) have noted the possibility of securing
constant fertile forms of *Crepis* possessing a chromosome complex derived
from two distinct species. *C. artificialis* (2n=24, 10 pairs of biennis and
2 pairs of setosa chrom.) has been produced from interspecific crosses of
C. biennis (n=20) × *C. setosa* (n=4). MEISTER (1928) obtained a new
type of wheat by crossing common wheat with durum wheat. This new
type outyielded its vulgare parent by *12%*. CLAUSEN (1926) obtained a
constant fertile 14 paired new species from the crossing of *Viola tricolor*
(n=13) with *V. arvensis* (n=17). SAX and SAX (1924) have noted in their
study of genus crosses of *Aegilops cylindrica* (n=14) and *Triticum vulgare*
(n=21): "Theoretically it is possible for a new species with 28 chromo-
somes to be derived from this genus cross, but the chances for such an
occurrence are extremely small". They suggested the possible origin of
the vulgare wheat from a cross of aegilops with a wheat species of the emmer
series. KARPECHENKO (1927) in intergeneric hybrid of *Raphanus sativus*
(n=9)× *Brassica oleracea* (n=9) obtained constant new forms having 18
chromosomes derived from both parents. ERLANSON (1929) has studied
cytologically the many wild roses in North America and noted; "It seems
highly probable that some of the North American diploid roses have arisen
in the manner suggested from crosses between some other diploid with a
polyploid form".

New species formation by hybridization were also reported by NINA
and TJUMJAKOFF (1928) in common wheat and durum wheat hybrid, by
JØRGENSEN (1928) in *Solanum* species hydrid, and by TSCHERMAK and BLEIER
(1926) in *Aegilops ovata* and *Triticum dicoccoides* and others.

Cytological and genetical studies of the behaviour of chromosomes in
polyploid species and their hybrids are now abundant, however, the inter-
specific hydrid of non-polyploid species, is still incompletely understood.

The hybridization of *Hibiscus* species was carried on as early as 1762
by KÖLREUTER (1764). He crossed *Hibiscus Manihot* (female) and *H.
vitifol* (male) and their reciprocal. He observed in these crosses the simi-
larity of F_1 individuals, but he could not obtain any later generation plant
because the wet and cold weather at the season when these hybrid plants

were grown so increased the longevity of vegetative growth that they were unable to reach the flowering.   He described the results of his experiments briefly as follows; "Die grosse Aehnlichkeit, die zwischen dem Hibisc. Manihot. und Hibisc. vitifol. herrscht, veranlasste mich, im Jahr 1762 eine wechselsweise vermischung unter ihnen zu bewerkstelligen.   Die befruchtung gieng in beeden Fällen glücklich von statten, und ich erzog den letzten Sommer von einem jeden Versuche vier Pflanzen.   Sie hielten in Ansehung der Blätter das Mittel zwischen ihren Eltern, und waren einander ganz ähnlich.   Die nasse und kalte Witterung, die fast den ganzen Sommer hindurch anhielt, verzögerte den Wachstum dieser Pflanzen so sehr, dass sie nimmer zur Blüte kamen; ich kann daher von den wesentlichen Eigeschaften derselben vor diessmal nichts melden; es wird aber, wie ich hoffe, mit der nächsten Gelegenheit geschehen können."

The possibility of crossing between *Hibiscus esculentus* L. and *H. Manihot* L., though the morphological characters of these species are fairly different, aroused the present author's interest as early as 1926 when examing many interspecific crosses among plants of several genera.

This study on *Hibiscus* species was begun in that year and in the following pages are given the results of some genetical and cytological investigations which were carried out during the past few years.   The data presented here are specially those on specific hydrids obtained by crossing *H. esculentus* with *H. Manihot* as well as their back-crossed plants, F_2 and other generation plants.   In this work some remarks are made on the semi-heterotypic division of pollen mother cell in F_1 hybrids, the very irregular reduction division in back-crossed plants and their later generation, and the formation of tetraploid (Tetragenomous) plants.

Part I.   Genetical studies of an interspecific hydrid between
H. esculentus L. and H. Manihot L.

1.   Material and technique

The material on which this research is based was collected from several districts, but one variety (Blue long A) of *H. esculentus* and *H. Manihot* which were used in detail in this research were pedigree-cultured several years in the experimental field of Tottori Agricultural College and were uniform in respect to morphological characters, perhaps not more variable than could be accounted for by fluctuation around mean.

In the course of this work crossings between four species were made

and the results are as follows ;

> *H. esculentus* L. × *H. Manihot* L. .... Seed obtained
>
> *H. Maninot* L. × *H. esculentus* L. .... Capsule developed but only abortive seeds were obtained.
>
> *H. esculentus* L. × *H. coccineus* WALT. ...... No seed, capsule dropped off soon after flowering.
>
> *H. coccineus* WALT. × *H. esculentus* L. ...... No seed,   ,,
>
> *H. esculentus* L. × *H. syriacus* L. .... No seed,   ,,
>
> *H. syriacus* L. × *H. esculentus* L. .... No seed,   ,,
>
> *H. Manihot* L. × *H. coccineus* WALT. ...... No seed,   ,,
>
> *H. coccineus* WALT. × *H. Manihot* L. ...... No seed,   ,,
>
> *H. Manihot* L. × *H. syriacus* L. .... No seed,   ,,
>
> *H. syriacus* L. × *H. Manihot* L. .... No seed,   ,,
>
> *H. coccineus* WALT. × *H. syriacus* L. .... No seed,   ,,
>
> *H. syriacus* L. × *coccineus* WALT. .... No seed,   ,,

By using *H. esculentus* as the female parent and pollinating it with *H. manihot* a large number of hybrid seeds were obtained and giant F_1 plants have been raised from these seeds. It is very convenient for us that we can obtain many hybrid seeds with a single crossing for the single flower of this species bear above a hundred ovules. In this way many plants of later generation have been raised. Back-cross and further crossings were made to determine, to some extent, the cytological and genetical behavior in these plants, as it was of great interest to follow the distribution of the chromosome through future generations.

The crosses were made in the summers of 1926, 1927, 1928 and 1929. Some of the capsules cross-pollinated quickly turned brown, shrivelled and dropped off, and were found to be completely empty. Other capsules developed and set well formed seeds intermediate in size between the larger *H. esculentus* and smaller *H. Manihot* seeds. The percentage of fertile seeds and the size and weight of the hybrid seeds as compared with the parents will be described later in detail.

The F_1 plants have very rarely set seeds, though they were repeatedly self-pollinated applying considerable quantity of pollen-grains, or open-pollinated, notwithstanding the fact that the capsules swell to a considersble size.

In 1926 eight flowers of *H. esculentus* were pollinated with the pollen of *H. Manihot* and the result was five capsules which contained many fertile seeds. None was obtained from the crosses of twelve flowers of

H. Manihot pollinated with the pollen of *H. esculentus*.   The details will be described later.

In 1927, crossed seeds to the number of 242 were sown in the field of Tottori Agricultural College and 29 hybrid plants (11.98%) made vigorous growth all of which flowered.   The hybrid plants possessed the diploid number of chromosomes ; their reduction division was quite abnormal ; they indicated hybrid vigor and were almost completely sterile.   At the same year the author obtained many fertile seeds by back-crosses (*H. esculentus* × F_1 hybrid) but very few seeds from the capsules of F_1 hybrid plants.   In 1928 these seeds were sown out and a cytological analysis of the plants grown from them showed that the back-crossed plants were allo-polyploid[1] i.e. that they possessed a multiple number of chromosomes in relation to F_1 hybrid.   Three plants were raised from nineteen seeds   . which were obtained from the capsules of F_1 hybrid.   One of these died at the young seedling stage while two developed to maturity.   One of these latter two exhibited morphological characters similar to those of *H. esculentus* and the other characters similar to those of the F_1 hybrid.   Unfortunately attempts to make a cytological investigation about those plants were unsuccessful.   The procedure for securing F_2 plant was altered in the following year.   This is the circumstance that induced the author to undertake a manifold investigation of this hybrid and of following generations. In 1928 and 1929 the interspecific crossing, back-crossing and further crossings were made more extensively to secure much material, enough to prove the behaviour of these plants.   The seeds of the hybrids as well as of the parental forms were usually sown in the seed bed where the plants remained until the fifth to sixth leaf had appeared, then they were transplanted into the ground.   At the same time a number of these seeds were sown in the glass house and until left the fifth or sixth leaf had appeared, and then transplanted into the ground in the open air as well as in the glass house in order to secure the material over a long period.   When . sown in mid-April, towards the middle of summer many of the plants were already beginning to bloom.   It also proved possible to prolong the life of the hybrids and to make the flower buds numerous by cutting the stem at the beginning of summer.

Either parental plants or these hybrids were capable of self-pollination

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1)   Amongst polyploids KIHARA and ONO and other have made distinction according to their gametic complements.   *"Auto-polyploidy"* is constituted by reduplication of similar series i.e. by the doubling of the chromosome number in a theoretically pure line; *"Allo-polyploidy"* is constituted by the combination of dissimilar series by doubling in a hybrid.

as well as cross-pollination by the insect visitors.

The hybridization of *H. esculentus* with *H. Manihot* presents no special technical difficulties. In every case the flowers used for crossing were emasculated one day before opening and protected with victoria lawn bags (10 cm wide, 15 cm long), covering the bud at the place where it was tied up with woolen threads. The flowering time of these species is not particularly influenced by meteorological conditions. It takes place early in the morning. It was observed that the anthers of these species dehisce in most instances at 7–8 a.m. The author could, therefore, safely perform castration and artificial pollination in the afternoon and from 8 to 10 a.m. respectively. The flowers from which the pollen was taken for pollination were also covered with insulators before they began blooming.

2. Compatibility in crossing between H. esculentus L., H. Manihot L., H. coccineus Walt., H. syriacus L. and F₁ hybrid

    (1) Compatibility between interspecific crossing

In 1926 twelve flowers of *H. manihot* were pollinated with the pollen of *H. esculentus* but none of the capsules developed. They dropped off on the day following pollination. Many hybrid seeds were obtained using *H. esculentus* as the female parent pollinated with the pollen of *H. Manihot*. Out of eight flowers of *H. esculentus* thus pollinated five flowers were developed to large capsules which contained many fertile hybrid seeds. In 1927 the field pollination notes record 22 attempts with *H. esculentus* as a female parent of which 17 proved failures after a few days and five or 22.7 per cent, were successful. Seventeen attempts in which *H. esculetus* was employed as the male parent in the same series resulted in 7 capsules developed to normal size, without a single good seed. These capsules contained nothing but numerous ungerminable empty seeds. In 1928 many attempts of crossing with *H. Manihot* and many varieties of *H. esculentus* were carried out. The varieties "White long," "Blue long A" "Blue long B", "Blue short", "Green giant", "Dwarf prolific" and "Dwarf long pod green" were used as the female parent of the crossing.

Back-crossing and crossing between *H. esculentus* and *H. coccineus*, between *H. Manihot* and *H. coccineus* and betwen back-crossed plant and *H. esculentus* and *H. Manihot* were also tried in this year. In 1929 similar crossings to those of the previous year were carried out and many seeds were obtained. The results of these crossings are shown in Tables 1 and 2. In Table 1 are shown the data of the crossing between different species summarizing the results obtained in the previous 2 to 4 years and in Table

2 are shown the data obtained in 1928 and 1929, the results of crossing with several varieties of *H. esculentus* used as the female parent.

Table 1. *Result of crossing between species.*

Combinations	Year	No. of flowers pollinated	No. of. capsules developed	Total no. of seeds obtained	Av. no. of seeds per capsule	No. of seeds sown	No. of seeds germinated
H. escu. × H. Mani.	1926 1927 1928 1929	8 22 93 46	5 5 47 28	290 270 439 354	58.0 54.0 9.3 12.6	280 212 98	19 95 58
H. Mani. × H. escu.	1926 1927 1928 1929	12 17 1ɪ 3	0 7 2 3	0 0 0 0			
H. escu. × H. cocci.	1928 1929	8 12	0 0	Capsules soon dropped off ”			
H. cocci. × H. escu.	1928 1929	8 6	0 0	Capsules soon dropped off ”			
H. Mani. × H. cocci.	1928 1929	18 7	0 0	Capsules soon dropped off ”			
H. cocci. × H. Mani.	1928 1929	12 8	0 0	Capsules soon dropped off ”			
H. escu. × H. syri.	1928 1929	13 8	0 0	Capsules soon dropped off ”			
H. syri. × H. escu.	1928 1929	12 8	0 0	Capsules soon dropped off ”			
H. Mani × H. syri.	1928 1929	12 —	0 . 0	Capsules soon dropped off ”			
H. syri. × H. Mani.	1928 1929	12 8	0 0	Capsules soon dropped off ”			

Combina-tions	Year	No. of flowers pollinate	No. of capsules developed	Total no. of seeds obtained	Av. no. of seeds per capsule	No. of seeds sown	No. of seeds germinated
H. cocci. × H. syri.	1928	12	0	Capsules soon dropped off			
	1929	8	0	,,			
H. syri. × H. cocci.	1928	18	0	Capsulee soon dropped off			
	1929	8	0	,,			

Table 2.   *Results of crossings between H. Manihot and several varieties of H. esculentus.*

Variety of H. escu. as female P.	Year	No. of crosses	No. of pods matured	Total seeds obtained	Fertile seeds	Av. no. of fertile seeds per capsule
White velvet	1928	14	7	306	186	26.6
	1929	3	1	7	0	0.0
Blue long A	1928	10	8	296	1	0.1
	1929	12	9	339	10	1.1
Blue long B	1928	7	6	224	108	18.0
White long	1928	7	4	158	42	10.5
	1929	9	7	141	128	18.3
Dwarf prolific	1928	20	6	305	25	4.2
	1929	11	4	244	124	31.0
Green giant	1928	8	4	167	72	18.0
	1929	6	2	94	58	29.0
Dwarf long pod green	1928	7	5	164	3	0.6
Blue short	1928	16	5	236	1	0.2

In these interspecific crosses, as many be seen in these tables, it was only in the case when *H. esculentus* was emasculated and pollinated with the pollen of *H. Manihot* that a number of seeds were secured which germinate well, producing vigorous seedlings. The hybrid plants showed intermediate or dominated characters of both parents and extreme hybrid vigor was observed notwithstanding the fact that decreased size and weight were shown by the cross-pollinated seeds. All the reciprocal crosses in which the *H. Manihot* was emasculated were unsuccessful. Many trials of crossings between *H. esculentus* and *H. coccineus* and other combinations were unsuccessful. The gametes of these unsuccessful species are so different in their genetic constitution that there is no harmony between them, a case of "Misozygoty" in Winge's term. Such a difference in crossability may not only be due to the genetical constitution but also to the physiological incompatibility of germ plasm.

In the case of *H. esculentus* × *H. Manihot* the capsules which developed after pollination contained many unfertile empty seeds besides the good germinable seeds. These two kinds of seeds were separated by the water treatment without difficulty. The embryo of lighter seeds was incomplete, stopped in its development at the young stage. In addition to these two kinds of seeds these capsules produced many small empty grains; these small ungerminable seeds are very rare in the case of open pollination. When the *H. Manihot* was used as the female and pollinated with the pollen of *H. esculentsu*, the developed capsule contained numerous empty abortiv seeds only. The parthenocarpic development of the fruit by the stimulation of pollen which germinated on the stigma is a well-known fact. It is known that the germination of pollen on the stigma besides being necessary for the development of the embryo resulting directly from fertilization, induces other processes, among which is a stimulation to fruit development. Two types of parthenocarpy were seen in our crop plants: the one type is vegetative parthenocarpy, in which the development starts without any pollination, the second type is stimulative parthenocarpy, where pollination is necessary to induce the development of the fruit. In the *Hibiscus* species which the present author treated, the last type exclusively is found. The shape, size and other characters of the pod are similar to the capsules which developed in natural pollination. The measurements of these characters are indicated in Table 3.

Table 3. *The shape, size and other characters of pod
obtained by cross pollination in 1928.*

Crosses	Capsule number	Length of capsule	Diameter of capsule	No. of fertile seeds	No. of sterile seeds	No. of abortive seeds	No. of longitudinal ribs	No. of embryos in a capsule	Percentage of fertilization
		cm.	cm.						
(♀) H. esculentus × (♂) H. Manihot	No. 1	18.0	2.3	55	11	28	6	94	58.5
	No. 2	19.2	2.5	43	9	44	6	96	44.8
	No. 3	17.0	2.1	73	2	32	7	107	68.2
	No. 4	20.0	1.9	36	3	55	6	94	38.3
	No. 5	18.8	2.3	65	0	28	6	93	69.9
	Av.	18.60	2.22	54.4	5.0	37.4	6.2	96.8	55.94
(♀) H. Manihot × (♂) H. esculentus	No. 1	3.7	2.4	0	32	61	5	93	0
	No. 2	3.9	2.6	0	39	61	5	100	0
	No. 3	4.1	1.9	0	30	77	5	107	0
	No. 4	4.5	2.5	0	35	56	5	91	0
	No. 5	4.4	2.5	0	61	31	5	92	0
	No. 6	4.9	2.6	0	61	25	5	87	0
	No. 7	4.5	2.4	0	58	28	5	86	0
	Av.	4.29	2.41	0	45.3	48.4	5	93.7	0

(2) Compatibility of F_1 hybrid and back-crossed plant with their parents

In spite of many attempts, the author has failed to obtain seed from
self-pollinated flowers of F_1 hybrid, nor does the F_1 hybrid set seed with
the pollen of either of the parental species or of back-crossed plant. Seed
has, however, been obtained by pollination of *H. esculentus* (mother parent)
with the pollen of the F_1 hybrid, or with that of back-crossed plant, but
none of the seed were secured from flower of *H. Manihot* (father parent)
pollinated with the pollen of F_1 hybrid or back-crossed plant. Even in
these cases of incompatible crossing, the capsule developed normally, but
contained no seeds except only small and abortive ones. Twenty flowers
of *H. esculentus* (Blue long A, mother parent) were pollinated with the
pollen of F_1 plant in 1927 and 6 flowers gave rise to capsule having many

seeds. From these 6 capsules 329 seeds were raised; an average of 54.8 seeds per capsule. In 1928 out of 23 flowers of *H. esculentus* which were pollinated with the pollen of F_1 plant, 18 capsules developed to normal size and set seeds. From these 8 capsules 868 seeds were obtained and 304 heavy seeds were separated by water treatment, an average 38.0 per capsule. In 1929, 25 flowers of *H. esculentus* were pollinated and 7.0 seeds per capsule were obatined. 4393 seeds were obtained from 56 capsules in intervarietal crosses of *H. esculentus*; it corresponds to 76.64 seeds per capsule. It is noticeable that the back-crossings were much more reduced in fertility than the intervarietal cross of *H. esculentus*.

The back-crossed plants are capable of producing seed by self-pollination and also by cross-pollination with *H. esculentus* employed either as male parent or female parent. In 1928 and 1929 many attempts were made with these crossings and there were obtained 2 and 8 fertile seeds per capsule respectively. The results of crossing between F_1 hybrid, back-crossed plant and their parents are shown in Tables 4 and 5.

Table 4. *Results of crossing between F_1 hybrid, back-crossed plant and their parents.*

Combinations	Year	No. of flowers pollinated	No. of capsules obtained	Total no. of seeds obtained	No. of fertile seeds	Av. no. of fertile seeds per capsule
H. esculentus (♀) (Blue long A) x F₁ (♂)	1927 1928 1929	20 23 25	6 8 16	361 868 230	329 304 112	54.8 38.0 7.0
(White long) x F₁	1930	14	8	54	50	6.25
(Blue long A) x F₁	1930	2	2	40	14	7.00
(Blue long B) x F₁	1930	1	1	0	0	0.00
(Dwarf prolific) x F₁	1930	2	2	57	12	6.00
(Green giant) x F₁	1930	6	3	79	36	12.00
Total or av.		93	46	1689	857	18.63

Combination	Year	No. of flowers pollinated	No. of capsules obtained	Total no. of seeds obtained	No. of fertile seeds	Av. no. of fertile seeds per capsule
H. Manihot (♀) × F₁ (♂)	1927	15	10	522	0	0.00
	1928	5	5	215	0	0.00
	1929	10	8	418	0	0.00
F₁ (♀) × H. escu. (♂)	1927	15	7	1	0	0.00
	1928	11	1	13	0	0.00
F₁ (♀) × H. Manihot (♂)	1927	16	8	2	0	0.00
	1928	12	0	0	0	0.00
H. escu. (♀) × Back-crossed plant (♂)	1927	15	2	47	4	2.00
	1928	18	4	60	32	8.00
B. C. plant (♀) × H. escu. (♂)	1928	16	6	155	56	9.33
	1929	10	2	0	0	0.00
H. Manihot (♀) × B. C. plant (♂)	1928	2	1	7	0	0.00
	1929	8	3	18	0	0.00
B. C. plant (♀) × H. Manihot (♂)	1928	16	4	29	4	1.00
	1929	8	3	16	0	0.00
F₁ (♀) × B. C. plant (♂)	1928	19	2	2	0	0.00
B. C. plant (♀) × F₁ (♂)	1928	15	12	414	147	12.25

Table 5. *The shape, size and other characters of pod of H. esculentus and H. Manihot obtained by pollination with the pollen-grains of F_1 hybrid (1928).*

Crosses	Capsule number	Length of capsule (cm.)	Diameter of capsule (cm.)	Length of fruiting branch (cm.)	Diameter of fruiting branch (cm.)	Number of fertile seeds	Number of unfertile seeds	Number of small abortive seeds	Number of longitudinal ribs of capsule	Number of embryos in a capsule	Percentage of fertilization
H. esculentus × F_1	No. 1	19.5	2.4	3.5	0.91	51	0	54	7	105	48.6
	No. 2	19.6	2.9	3.0	1.00	70	2	51	8	123	56.9
	No. 3	19.5	2.3	4.6	0.90	52	5	39	6	96	54.2
	No. 4	21.2	2.4	4.0	0.80	52	9	45	7	106	49.1
	No. 5	15.5	2.5	4.3	0.88	59	7	49	8	115	51.3
	No. 6	17.2	2.6	3.6	0.85	45	9	38	6	92	48.9
	Total					329	32	276			
	Av.	18.75	2.52	3.83	0.890	54.8	5.0	46.0	7.0	106.2	51.5
H. Manihot × F_1	No. 1	3.3	2.1	5.5	0.31	0	15	77	5	92	0
	No. 2	4.8	2.3	6.1	0.30	0	60	27	5	87	0
	No. 3	4.4	2.2	5.0	0.31	0	49	37	5	86	0
	No. 4	4.5	2.6	3.7	0.31	0	61	29	5	90	0
	No. 5	4.3	2.4	3.7	0.38	0	49	44	4	93	0
	No. 6	4.8	2.4	4.5	0.30	0	70	17	5	37	0
	No. 7	4.5	2.2	5.4	0.27	0	62	33	5	95	0
	No. 8	3.8	2.0	4.9	0.30	0	40	47	5	87	0
	No. 9	4.7	2.5	4.9	0.34	0	67	21	5	88	0
	No. 10	4.2	2.2	5.3	0.30	0	49	37	4	86	0
	Total					0	522				
	Av.	4.33	2.29	4.90	0.312	0	52.2	3.69	4.8	89.1	0

Many investigators have reported that the F_1 hybrid between different species in plants does not ordinarily produce seeds when self-fertilized or intercrossed, but can be made to produce fertile seeds by back-crossing with one or the other, or both parents. That the F_1 hybrids are more fertile with the pollen of either parent than with their own was found as early as 1849 by GÄRTNER with *Nicotiana*. BAUR (1922) has reported this kind of crossability in the hybrid of *Antirrhinum majus* and *A. siculum*. And similar results have been reported by GOODSPEED and CLAUSEN (1927), (1922) in *Nicotiana* and by STEVENSON (1922) in rye-wheat hybrid etc.

The behavior of crossability of F_1 hybrids with their parents is not always the same. Some results are quite contrary to the author's findings with his material. *Nicotiana paniculata-rustica* hybrid was described by LAMMERTS (1928). When crossed back to the parental species as a female plant the capsules developed and produced an average of approximately 40 seeds each, but when back crossed as a male plant on the parental species the number of seeds thus produced always averaged less than 40 per capsule. COLLINS and MANN (1922) have observed in the back-crosses of F_1 hybrid of *Crepis setosa* HALL × *C. capillaris* (L) WALLR. with the parent, that very little of the pollen appears to mature properly, but most of it was left in the anther locules where it disintegrated. They found none of the back-crosses in which the F_1 was used as the pollen parent to be successful. That some ovules are functional has been shown by the fact that back-crosses were obtained when F_1 was used as the pistillate parent. Similar cases have been reported by MEISTER (1921). The seed from the F_1 hybrid wheat × rye can only be obtained by repeated pollination with rye or wheat, which has also been verified by MEISTER and TJUMJAKOFF (1928). But we have seen in the table compiled by these investigators, that the results are not the same when the pollen of rye or wheat was pollinated to F_1 hybrid. Latter cases were much more effective. The results of investigations with wheat-rye hybrid were also reported by TSCHERMAK (1927), who observed that the wheat-rye hybrids are invariably cross-sterile when back crossed with wheat, but much more rarely so with rye. This inequality of crossability of F_1 hybrid to either parent is similar to the auther's findings, but in the author's material F_1 hybrid is still sterile when pollen of either parent is used for its pollination. Only when the pollen of F_1 hybrid is used for pollination to female parent (*H. esculentus*) is it effective.

As to the cause of this cross-sterility between F_1 and parental plants, various interpretations have been suggested by many investigators. THOM-

PSON (1929), in his study of species crosses in wheat states, "in back-crosses of F_1 with vulgare the great majority of seeds are plump when vulgare is female, though wrinkled ones occur; in the reciprocal nearly all are wrinkled or badly shrivelled. In back-crosses with emmer also the seeds are better when the pure parent is female". For the interpretation of the cause of a consistent difference between these reciprocal crosses he suggested the chromosome condition as follows: "If the chromosome number of the parents are different the number present in the endosperm will depend on the direction of the cross. If the parent with the larger number is female, the endosperm will have twice the larger number plus the smaller; whereas if it is male, the endosperm will have twice the smaller number of reciprocal-crosses". And from the cytological investigation of back-crossed plants he reached this conclusion on the correlation between chromosome and. endosperm condition: "The endosperm is plump and large when it cotains (a) none or few of the extra 7 vulgare chromosomes, (b) 3 sets of 7 complete or nearly so. It is usually plump and small when it contain 2 sets, complete or nearly so. It is wrinkled or shrivelled when, (a) it is haploid for all or many of the 7, (b) diploid or triploid for some only". Similar results were obtained by MICHAELIS (1925) by reciprocal crossing of *Epilobium* species. The embryo of *E. hirsutum* or *montanum* only slightly developed and degenerated when pollinated with the *E. angustiforium* or *Dodonaei*, but that of *angustifolium* or *Dodonaei* pollinated with the *hirsutum* or *montanum*, were normally developed. CHITTENDEN (1928) has obtained many more seeds by back-crossing with parental pollen to the F_1 of *Godetia amoena* × *G. whitneyi* than by selfing the F_1 hybrid. As to the cause of this compatibility of this back-crossing he wrote: "A possible explanation of this fact is that the introduction of a balanced chromosome complement by the pollen of the parental species in the back-cross families induces the development of the ovules which if met by un-balanced F_1 pollen, would hence give little chance of further growth". The results of the present author's experiments did not agree with Chittenden's and they may hardly be explained by his assumption. In the author's material the balanced parental (female) pollen was not capable of producing seed by back-crossing to F_1 hybrid while the unbalanced F_1 pollen was functional on the parental flower. NEWTON and PELLEW (1929), in their study of *Primula kewensis*, explained the phenomenon of the ste-rility of F_1 and of back-crosses as follows: "The complete sterility of the female side as compared with the partial sterility of the male may be a consequence of a difference in viability between male and female gametes

of the same nuclear constitution, but we would suggest a simple possibility, if the effective gametes are the same on the male and female sides, each hybrid ovary would nevertheless bear only a very small number, relatively to the number applied in pollination. Thus the chance of obtaining viable zygotes would be very greatly reduced when the hybrid is the female parent, and if a certain number of fertile seeds is required to bring about the development of a capsule, this number may be attained when pollen of the hybrid is used on floribunda though not in the reciprocal cross". In the author's study, the cross-sterility between parents and F_1 plants is not comprehensible merely by this interpretation. Only in case *H. esculentus* was used for male parent were seeds obtained. *H. esculentus* and *H. Manihot* are both homogeneous in their own hereditary units, but their crossability is not the same. And although the pollen of F_1 hybrid bears both of the parental chromosomes simultaneously, seed was obtained only when *H. esculentus* was used for the female parent.

3. Difference in success of reciprocal crosses between H. esculentus L. and H. Manihot L.

The reciprocal compatibility of interspecific crossing is not always the same. In the case of *H. Manihot* employed as a female parent only empty ungerminable seeds were produced. GÄRTNER (1848) succeeded in making the cross reciprocally between *Digitalis purpurea* L. and *D. ambigua* MURR., though he obtained only one plant of *D. ambigua* × *purpurea* after repeated attempts. The hybrid between *D. ambigua* and *D. purpurea* was got by BUXTON and NEWTON (1928) who obtained fifty seedlings from the seeds of *D. purpurea* (♀) × *D. ambigua* (♂), while no seed germinated from the crossing of *D. ambigua* (♀) × *D. purpurea* (♂). SALAMAN (1911–1916) made his experiments with crosses between *Solanum utile* and domesticated potato; 25 per cent of the crosses in which *S. u.* was used as a female were successful, but 48 attempts in which *S. u.* was used as a male parent in the same series failed without a single success. BALLS (1912) has observed that Hindi cotton plant, as a male plant, crosses readily with Egyptian cotton, yet Hindi × Egyptian hybrid is more frequent in the field. BABCOCK and COLLINS (1920) have indicated the equivalence of reciprocal crosses with interspecific hybrid in *Crepis capillaris* × *C. tectorum*. The reciprocal cross with *Nicotiana rustica humilis* and *N. paniculata* was also made by by EAST (1921). This hybrid was not quite so easy to obtain as when the parentage was reversed, but the only difference noticeable in the reciproal F_1 pollination was a slightly taller plant in the case of *N. rustica*

humilis (♀) x *N. paniculata* (♂).   CLAUSEN and GOODSPEED (1925) stated :
"The two species *Nicotiana glutinosa* and *N. Tabacum* have frequently
crossed, reciprocal hybrid may be obtained although hybridization is attend-
ed with some difficulty."   They (1926) also stated in regard to *N. pani-
culata-rustica* hybrid, "the hybridization is readily accomplished, particularly
when rustica is used as female parent."   JOHN PERCIVAL (1926) succeeded
in obtaining seed by crossing wheat-aegilops only when *Aegilops ovata* was
used as a male ; the reciprocal crosses were unsuccessful.

It is possible that in some cases the pollen-tubes of one species may
be injured on the stigma of the other.   DEMEREC (1928) found that rice-
pop corn was almost sterile to the pollen of several varieties of maize.
Crosses in which popcorn was a pollen parent had a normal fertility, and
he indicated the cause of cross sterility as due to a number of different
factors, such as ; inability of pollen with recessive gametophyte factor to
germinate on the silks having dominant allelomorph or inability of recessive
gametophyte factor to germinate on the silks having dominant allelomorph
or inability of recessive pollen-tubes to reach the ovules in the plant having
the dominant allelomorph.   BUCHHOLZ and BLAKESLEE (1929) reported that
the pollen-tubes of tetraploid *Datura* grow quite abnormally on the stigma
of diploid *Daturas*, so that the cross in this direction is quite unsuccessful.
But the pollen-tubes of diploids grow normally in the stigma of tetraploid
Datura, so that the reciprocal cross was successful.   In his material the
author of the present paper examined the pollen-tube development of *H.
esculentus* upon the stigma and in the style of *H. Manihot*, and found that
the pollen-tubes of *H. esculentus* grow rapidly in the stigma and style of
H. Manihot and reach the ovary within a hundred minutes in the summer
time (See Table 47).   The causes of inability of reciprocal cross as indicated
by DEMEREC, BUCHHOLZ and BLAKESLEE have not been observed in this
material.

From the fact that the capsules of *H. Manihot* secured by the polli-
nation by foreign flowers were developed and contained abundant abortive
seeds by the stimulation of germinal substance, it is doubtless noticeable
that the foreign pollen-tubes could reach the ovules of *H. Manihot* within
sufficient time without being injured by toxic or other chemical inhibitory
factors.   The growth rate of pollen-tube indicates these facts very certainly.

It is a well known fact that the crosses between wheat and rye where
the pistilated parent is rye are quite impossible although the crosses where
wheat is used as the mother plant present no special difficulties.   But quite
recently MEISTER and TJUMJAKOFF (1928) have succeeded in these reciprocal

crossings.   They obtained as many cross-fertilized seeds as over 60 per
cent in some variety of wheat pollinated with rye pollen, while from re-
ciprocal crosses they obtained only 96 grains from 3894 rye flowers emascu-
lated and pollinated with wheat pollen, i. e, 2.5 per cent of successful fertili-
zations.   LEIGHTY and SANDO (1928) have reported that they obtained
90.5 per cent of seeds by Chinense wheat emasculated and pollinated with
rye pollen and 89.4 % of seed when wheat pollen was used.   They dis-
cussed the conditions of successful crossings and said, "These results proved
that success depends largely on the time when the rye plants are ema-
sculated.   This may be explained by the fact that the pollen of wheat,
usually slow in germination on the stigma of rye, germinates more rapidly
under the moist conditions of the green house than in the field where,
with high temperature and greater dryness of the air, it is in con-
siderable danger of sericcation."   In the author's material the unsuccessful
crossings where *H. Manihot* were used as female parent were not due to
the condition described by MEISTER and TJUMJAKOFF in the case of rye-
wheat hybrid.   The pollen-tube development of *H. esculentus* was more
rapid in the style of *H. Manihot* than that of *H. Manihot* in the style of
H. esculentus, and the pollen-tubes reached the ovule within one hundred
minutes.   Abortive grains and capsule development confirm this.

As to the cause of such differences in success of reciprocal interspecific
crosses a difference between the parents with respect to the length of the
style is thought to be the important factor in crosses between *Polemonium
pauciflorum* and *P. mexicanum* made by OSTENFELD (1929).   He succeeded
in obtaining seeds only when *P. pauciflorum* was used as the male parent.
In this case the style of *pauciflorum* was about eight times as long as that
of *mexicanum*.   On the other hand in the author's material the style of
H. Manihot was rather shorter than that of *H. esculentus*.   The author
failed to obtain seed even when the short parent (*H. Manihot*) was used
as the female parent.   Consequently this explanation is out of the question
in *Hibiscus*.

The relation between chromosome number and inequality of reciprocal
cross has been studied by many investigators.   Thompson (1928, 1929)
reported that in crosses between 14- and 21 chromosome wheat, the F_1
grains are plump when the 21- chromosome species is female and wrinkled
when it is male, and he indicated that in the former case the endosperm
of the seed is diploid with respect to the extra 7 vulgare chromosomes,
and in the latter case haploid.   THOMPSON (1930) discussed the cause of
difference in success of reciprocal interspecific crosses and summarized his

opinion as follows; "Consequently when the species with the larger number is female the cross is more successful than when it is male. A review of the literature shows that as a rule the results are similar in other genera of plants: in cases in which reciprocal interspecific crosses differ in success the more successful is generally the one in which the species with the larger chromosome number is used as the female. There are also other causes of a difference in success, such as a difference in the length of the styles or possibly a faulty interaction of the cytoplasm of one parent and the genes of the other." Similar results have been obtained by many investigators in many wide crosses; in *Emmer* wheat × *Aegilops speltoides* by THOMSON, and by LEIGHTY, SANDO and TAYLOR (1926), in *Nicotiana* species crosses by EAST (1928), in genus *Brassica* by SUTTON (1909), KARPECHENKO (1922), TERASAWA and SHIMOTOMAI (1909), in *Raphanus-Brassica* crosses by FUKUSHIMA (1929) and by others in other plants.

Cross fertility and chromosome irregularity of polyploid plants do not always depend upon the differences of chromosome number of both species. The investigation of COLLINS and MANN (1923) indicated that the crosses between *Crepis setosa* (n=4) and *C. biennis* (n=20) are more easy and that division of the pollen mother cells of the hybrid are very nearly normal much more frequently than in the cross *C. setosa* (n=4) × *C. capillaris* (n=2). An apparent exception to the general rule which holds in the causes of THOMPSON's experiments is found in other wide crosses as reported by ERLANSON (1929). He has crossed *Rosa branda* (n=14, diploid) with *R. acicularis* (n=20, hexaploid) and succeeded several times in its reciprocal crosses, but the former cross yielded as good a crop of achenes. *R. woodsii* (diploid) crossed with hexaploid types gave good result. ERLANSON stated that crosses between diploid and polyploido roses are more often successful when the diploid is the female parent. LEHMANN (1918) studied the reciprocal hybrid of *Epilobium parviflorum* and *roseum* and the form obtained by crossing of these two species using *parviflorum* as a mother plant he denoted "Rigidum"; that obtained by reciprocal cross, "Curvatum".

The inequality of reciprocal hybrid was also observed by WETTSTEIN (1926) in *Funaria mediterranea* (Me) × *F. hygrometrica* (Hy), genus crosses of *Physcomitrium piriform* × *Funaria hygrometrica* and subspecies hybrid of *Physcomitrella pratens* (Pp) × *Funaria hygrometrica*. In *Me* (♀) × *Hy* (♂) hybrid were produced small, poor, upright capsules with a broad cone-shaped cover. The colour was bluish red. The reciprocal hybrid *Hy* (♀) × *Me* (♂) showed dominating characters of *Hy*-type. WETTSTEIN has written as follows; "Wenn im Vorhergehenden der beweis versucht wurde,

dass die genetische Konstitution nicht nur aus Kerngenen besteht, sondern dass auch im Plasma ein wesentliches genetisch-spezifisches Element vorhanden ist." THOMPSON (1930) said as to a cause of difference in success of reciprocal interspecific crosses: "Similarly it is conceivable that the the cytoplasm of one may lack materials which are necessary for development to occur at all when the genes are from the other parent. At any rate some peculiar interaction for failure to interact between the genes of one parent and the cytoplasm of the other may be responsible for cases of difference in success of reciprocal crosses". SCHWEMMLE (1924) observed inequality of reciprocal hybrid in *Epilobium parviflorum* and *Ep. roseum* to be due not only to morphological character, but also to sterility of the hybrid.

Ep. rigidum (parviflorum × roseum) was sterile, but *Ep. curvatum (Ep. roseum × parviflorum)* was sterile, but *Ep. curvatum (Ep. roseum × parviflorum)* was fertile. As to the cause of this sterility, SCHWEMMLE said: "Wir hätten damit eine ganze Reihe der allerverschiedensten Entwicklungshemmungen, wie sie auch schon Oehlkers am Schluss seiner Arbeit (1921) aufgestellt hat. In einer solchen Reihe liessen sich nun Bastarde, in deren Samenanlagen keine Archesporzellen mehr angelegt werden, leicht unterbringen, allerdings nun unter einer Voraussetzung, dass nämlich sämtliche Samenanlagen sich hierin gleich verhielten". LEHMANN (1928) observed inequality of reciprocal crosses in genus *Epilobium* and he said as to the cause of this inequality: "Ich selbst war von dem Gedanken ausgegangen, diese reziproken Differenzen im Anschluss an die reziproken Verschiedenheiten der heterogametisch- heterogamen Oenothera bastarde erklären können. Hier liegen die Verhältniss so, dass die beiderlei Faktoren oder Faktorenkomplexe jeweils nur in einer Geschlecht zur Wirkung kommen, im anderen Geschlecht dagegen sich nicht entfalten können: die Entfaltungshemmung aber kommt sehr früh zustande, so dass schon die Gameten, welche die in Frage kommenden Faktoren tragen, zu Grunde gehen .... Die Erklärung der grossen Verschiedenheiten der reziproken Epilobiumbastarde durch reine Plasmawirkung war zweifellos anregend".

The nucleus-plasma relation in respect to plant and animal heredity is one of the most interesting problems in modern biology and the investigation of inequality in reciprocal crossing will do something to throw light upon this problem. As stated before, in different species the cytoplasm is different in many qualities which will influence the heritable characters, hence in some species there may be special characters which inhibit the development of zygote at very young stage. The cytoplasmic influence
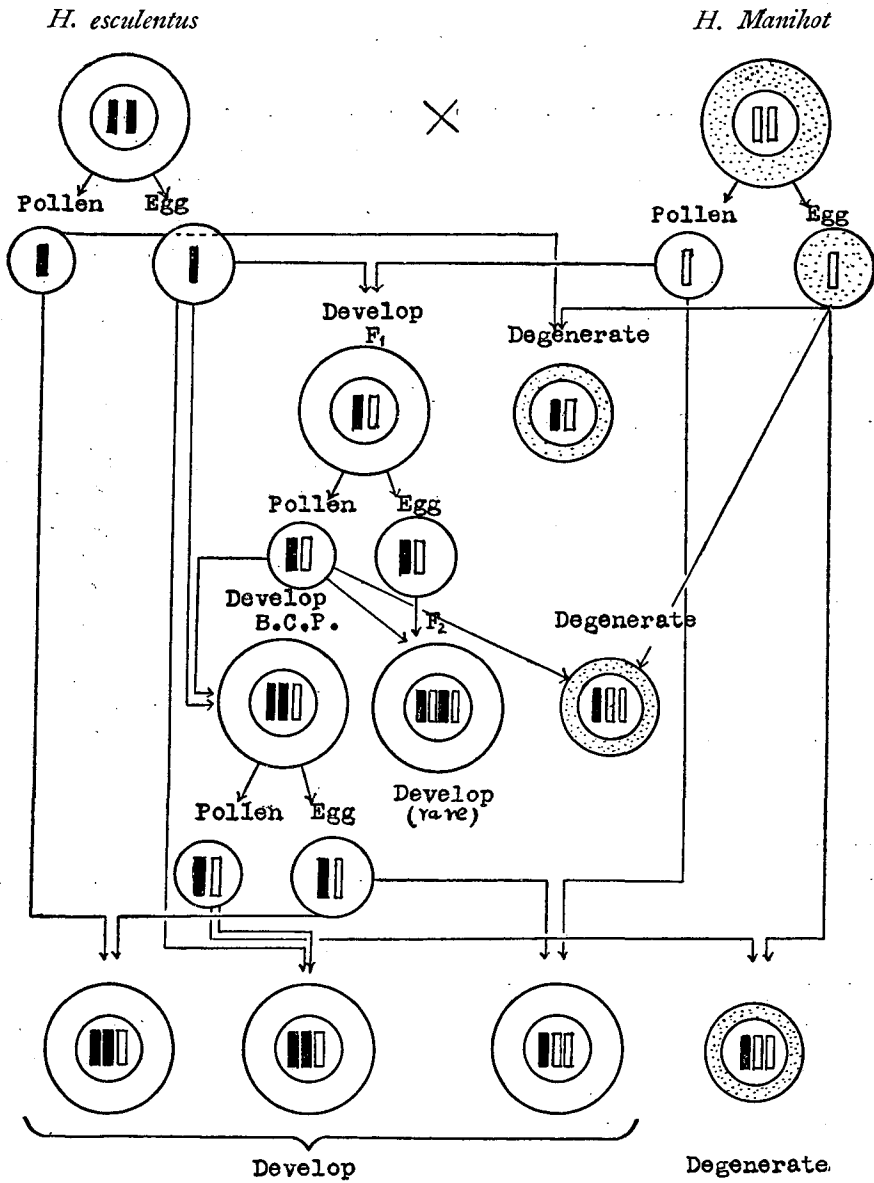
Scheme 1. Scheme of reciprocal crossings between *H. esculentus, H. Manihot*, F_1 hybrid and back-crossed plant, supposing that the cytoplasm of *H. Manihot* has a special factor which inhibits the development of zygote arised from crossing with pollen of *H. esculentus*.

will be seen in the maternal resemblance in interspecific F_1 hybrid. On this point, HILL (1929), in his study of reciprocal F_1 hybrids between *Digitalis lutea* and *D. purpurea*, made this summary statement: "Reciprocal F_1 hybrids between *D. lutea* and *D. purpurea* are unlike and matroclinous in respect to flower size. These differences appear in both corolla and calyx". Similar matroclinic inheritance was also reported by TSCHERMACK in wheat-rye hybrid, WETTSTEIN (1926) in *Funaria* species hybrid and *Physcomstella* species. CHITTENDEN (1928) in *Godetia* species, COLLINS and MANN (1923) in *Crepis*, MORINAGA (1928) and FUKUSHIMA (1929) in *Brassica-Raphanus* hybrid and others. In the author's material the F_1 hybrids are inclined to *H. esculentus* in general appearance but this was not true, when separate characters of F_1 hybrid plants were examined. Most of the characters are intermediate or dominant over. But cytoplasmic influence may be, in the author's material, the most important factor to dombnstrate the inequality of reciprocal crosses, Scheme I, derived from Schwemmle's idea, supposing that the cytoplasm of *H. manihot* has a special character which inhibits the development of hybrid zygote at the young stage, shows these relations between pure species, F_1 hybrid and back-cro ssed plant.

4. Tests for selective fertilization[1]

As has already been mentioned, when the *H. esculentus* is used as the female parent, the degree of compatibility of these two *Hibiscus* species is relatively great compared to that of *H. esculentus* which has been fertilized in open-pollination. These two species have been cultivated a short distance apart for many years in the experimental field of Tottori Agricultural College. It was supposed that the pollen of *H. Manihot* was very often carried to the stigma of *H. esculentus* by the insect visitors, but a hybrid has never thus been raised.

In 1928, an experiment was carried out to determine whether or not selective fertility exists between the pollen-grains of *H. esculentus* and *H. Manihot*, when the *H. esculentus* is the female parent.

The methods used in this experiment were as follows:

(1) A mixture of approximately equal quantities of pollen-grains taken from the flowers of both species was dusted on the flower of *H.*

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1) DONALD F. JONES (1928) employed the term "Selective fertilization" to cover broadly all forms of discrimination in reproduction. It also includes "Gametophytic selection", "Differential pollen-tube growth" and "Selective pollen-tube stimulation".

esculentus. The two kinds of pollen-grains were mixed in equal quantity as nearly as possible.

(2) The same number of pollen-grains (each 150) of each species was pollinated equally all over the stigma at the same time.

(3) The pollen-grains of *H. Manihot* were used for dusting $\frac{1}{2}$ of each stigmatic surface of *H. esculentus* and the remaining half of it was dusted with the pollen-grains of a sister plant. This way of pollination was expected to determine whether there is any selective fertilization between these two kinds of pollen-grains even when the locality of pollinations was limited to only one side of a stigma. In each case the two kinds of pollen-grains were applied in abundant quantity and both sides of a stigma were dusted many times alternately.

This experiment was performed during the summer of 1927, the variety "Blue long A" of *H. esculentus* being used. In every case the flowers to be pollinated were emasculated in the evening before flowering and were bagged to prevent accidental cross-pollination. The flowers which were to supply the pollen were also bagged at the same time.

For the counting of pollen-grains SCHNEIDERHÖHN's ore dressing microsope was used. Before the pollination the stigma were submitted to inspection to learn whether pollination had occurred accidentally or not. *Hibiscus* species are a particularly favorable material as is maize also, for this kind of experiment as a large number of seeds result from a single application of pollen. The pollen-grains are so large that we could carry them one by one from anther to the stigma. Crossed seed and self fertilized seed can be accurately distinguished at maturity because of the small size of crossed seed compared with self-fertilized seed (See Table 28), the plants which grew from these seed in 1929 proved this supposition correct.

The writer obtained in this experiment many capsules developed to normal size and calculated the number of crossed seed, number of selfed seed, the percentage of crossed seed and some characters of capsules. The results of these experiments are shown in Tables 6, 7 and 8.

Table 6. *Results of pollinations with a mixture of two kinds of pollen on the stigma of H. esculentus.*

Flower number	Length of pod	Diameter of pod	Number of ribs of pod	Total number of fertile seeds	Number of selfed seeds	Number of crossed seeds	Number of abortive seeds	% of crossed seeds
	cm.	cm.						
No. 1	19.6	2.5	7	77	76	1	0	1.30
No. 2	18.5	2.6	7	66	59	7	6	10.61

Table 7. *Results of pollination with the same number of pollen-grains of each species at the same time.*

Flower number	Length of pod	Diameter of pod	Number of ribs of pod	Total number of fertile seeds	Number of selfed seeds	Number of crossed seeds	Number of abortive seeds	% of crossed seeds	Number of pollen-grains of each species applied
	cm.	cm.							
No. 1	21.0	2.0	6	57	57	0	2	0.00	
No. 2	19.0	2.4	6	50	50	0	4	0.00	
No. 3	19.0	2.1	6	62	62	0	0	0.00	... 150
No. 4	18.0	2.2	6	68	68	0	2	0.00	
No. 5	18.7	2.0	5	49	49	0	1	0.00	 100

Table 8. *Results of pollinations with two kinds of pollen applied
to each half of the stigmatic surface separately.*

Flower number	Length of pod	Diameter of pod	Length of fruiting branch	Diameter of fruiting branch	Number of ribs of pod	Number of crossed seeds	Number of selfed seeds	Number of abortive seeds	Total number of fertile seeds	% of crossed seeds
	cm.	cm.	cm.	cm.						
No. 1	19.5	2.2	3.5	0.80	5	2	45	15	48	4.17
No. 2	17.0	2.1	2.5	0.80	6	4	37	13	41	9.76
No. 3	17 0	2.1	3.4	0.70	6	0	50	0	50	0.00
No. 4	19.0	2.6	4.2	0.92	7	16	47	0	63	25.40
No. 5	18.5	‚2.2	4.0	0 86	7	2	58	1	60	3.33
No. 6	19.7	2.1	5.0	0.77	6	1	59	0	60	1.67
No. 7	20.4	2.6	5.0	0.90	7	38	38	0	76	50.00
Total or av.						63	334	29	398	15.83

From these three tables it is evident that the occurrence of a selection of gametes in the flower of *H. esculentus* is significant. In the case of pollination in which two kinds of pollen of *H. esculentus* and *H. Manihot* were applied to the opposite sides of a stigma separately (Table 8) only 29 crossed seed out of 398 in total were obtained. The percentage of crossed seeds in this case varied from 0.00 to 50.00 with an average of 13.48. When the mixture of two kinds of pollen was used abundantly (Table 6) only a few crossed seeds were obtained, the percentage of crossed seeds in these two flowers was 1.30 and 10.61 respectively. In the case of pollination where the same number of pollen-grains from each species was applied at the same time (Table 7) strict selective fertilization was shown; all of the fertile seeds came from self-fertilization. It is interesting that in the first and third experiments a significantly higher percentage of crossed seeds was obtained than in the second experiment. The cause of this difference in the results of these experiments may be axplained by the less competition which occurred among the pollen-tubes germinated at the opposite sides of the stigma. It is supposable from the fact that in the third case (Table 8) the crossed seeds are much less than half the

entire number of fertile seeds, that there occurs also strong competition between the two kinds of pollen-grain even when pollinated separately each on one half of a stigmatic surface.

The present instances seem to show that the fertilizing power of the pollen-grain of its own flower is greater than that of another species, and also how it would be a very rare case for natural hybrids to occur even though these two species are growing near together and flowering at about the same time. However the pollen-grain of *H. Manihot*, which is less effective when in competition with the pollen-grains of *H. esculentus* on the stigma of *H. esculentus*, is able to function on the stigma of *H. esculentus* when applied alone.

In seeking an explanation of the selective fertilization, the rapidity of pollen-tube development is the first point to be considerd. The cause of the selective fertilization may be explained to some extent by the differences pollen-tube development. As indicated in Table 47 the results of the experiment, in which measurement was made of relative rates of growth of pollen-tubes at successive intervals of time after pollination, show an inferior rate of pollen-tube development of *H. Manihot* in the style of *H. esculentus* in comparison with that of *H. esculentus* in its own style. From Table 47 it will be seen that the pollen-tube of *H. esculentus* on the sister flower grew 22 mm. within 80 minutes and all of the pollen-tubes have already arrived at the micropyle of the ovary, while that of *H. Manihot* had grown down 19.20 mm. through the style of *H. esculentus* within the same time and none of the pollen-tubes arrived at the ovule even after one hundred minutes. Some of the unlike pollen-grains (*H. Manihot*) were able to accomplish fertilization, possibly because they happened to be so placed as to avoid the strong competition on the stigma and developed their pollen-tubes as well as the pollen of the same type to reach the ovule. Or some unlike pollen-tubes may be inferior in their development to that of like pollen but they reached the ovule without any competition and united with the female gamete. It would be difficult to say that this relation of pollen-tube development and fertility of like and unlike pollen occurs in every case such as when the *H. Manihot* was used for female plant and dusted with both sorts of pollen-grains, for, in general, the pollen-tube development of *H. Manihot* was somewhat more tardy than that of *H. esculentus* even in its own flower.

As to the cause of selective fertilization KEARNY and HARRISON (1924) have noted "The only hypothesis which seems to fit the observed facts is one previously advanced that the presence of like pollen in some way

prevents the germination or subsequent development of many of the unlike pollen-grains when both kinds are present on the stigma. .... It is conceivable, however, that the presence of pollen of the same type may induce a physiological reaction in the stigmas which makes them a relatively unfavorable medium for the germination or growth of pollen of a different type." . .

. . When the two kinds of pollen are applied to the flower it commonly occurs that they may be unequal in their ability to fertilize. In the case of intervarietal cross it is a well known fact that the mutual fertilization of distinct plants is effective. The prepotent action of another pollen will be noticed markedly in the plant characterized by self-sterility. Many results of experiments obtained from interspecific crosses are directly opposite to the results obtained by the present writer. KEARNEY and HARRISON (1924) have measured the relative rapidity of penetration of the ovary of Pima cotton by Pima and the Upland cotton pollen-tubes. In these two kinds of pollen-grains, they observed strict selective fertilization. Approximately three quarters of the ovules were fertilized by pollen of the same type when the mixture of two kinds of pollen was applied. They noted "It affords no evidence that the rate of growth of the tubes of the unlike pollen is inferior to that of the like pollen when the two kinds of pollen are in direct competition." They have also noted the results of double pollination in cotton flowers. They pollinated one half of the flowers first with the like pollen and then with unlike pollen and reversed the order of application on an equal number of flowers. The percentages of F_1 hybrids resulting from such pollination was, in double pollination on Egyptian; 25.8±0.61, in double pollination on Upland; 27.2±0.80. KEARNEY (1923) (From JONES;" Selective fertilization" p. 71) has made similar experiments to those carried on by the present author. He applied two kinds of pollen of Egyptian and Upland cotton to opposite sides of the stigmatic colum of Egyptian cotton and obtained 33.4± 1.13 percent of hybrid plants, while from another set of flowers to which mixture of two kinds of pollen was applied 13.6± 1.08 percent of hybrid plants were obtained. BALLS (1912) has found that mixed pollinations of Egyptian cotton by Upland gave 3 percent of hybrids and there was no significant difference between the reciprocal mixings. He observed also in the cotton plants that the different varieties or even different plants grown side by side show differences in their liability to natural crossing. As to the causes of selective fertilization in cotton species he suggests a comparison between a pollen-tube and the hypha of a parasitic fungas. He has written as follows ; "We

find in mycology that within the same strain of host-plant, different species
and varieties of the same fungus may be able to attack one strain of host-
plant with ease, while another strain may be practically immune." This
hypothesis which has been framed to account for the facts is based upon
an analogy drawn by Prof. MARSHALL WARD.

The results of the present author's experiments support JONES' con-
sideration of selective pollination in corn. He removed the husks and cut
off the silks about 1 cm. from their place of attachment to the ovules and
dusted the pollen mixture over the entire spike and compared the selec-
tive action with another which had been treated normally with the pollen
mixture. JONES observed that the selective action is four times as great
in the longer styles as in the cut styles. This indicates evidently that
the difference is due to an unequal rate of growth and not to an inhibition
of one type of pollen by the other at, or shortly after, the time of ger-
mination. JONES concluded as to the cause of the selective action : "In
maize, the differential fertilization is apparently not due to an inhibition of
the germination of the pollen but an unequal rate of growth of the pollen-
tubes."

Even between the like pollens there seems to occur strict competition.
The number of pollen-grains dusted on the stigma are much greater than
that of the total seeds of a capsule, hence we may suggest that there may
occur some competition between these pollen-tubes. MILLER (1919) has
observed pollen development of corn flowers and he stated : "If pollen is
supplied abundantly, a great number of pollen-tubes start to grow down
the bundle regions of each silk. However, as one examines the silk from
the tip downward, the number of pollen-tubes becomes smaller and smaller
so that when the cavity of the ovary is reached only one pollen-tube is
to be observed. In nearly a hundred observations no more than one pollen-
tube was seen in each ovary cavity."

JONES (1918) has observed in a study of a large number of crosses
between inbred strains of corn that the hybrid plants are vigorous and
more productive of grains, while these plants manifest a decided preference
for their own kind of pollen when the pollen mixture was used. He
concluded, "These plants manifest a definite receptiveness to their own
pollen, discriminating against foreign pollen even though it comes from
plants only slightly differentiated from them." He studied this point with
Lycopersicum esculentum MILB. also and the results agreed with those from
maize.

The style of *H. esculentus* is much longer than that of *H. Manihot*

The pollen-tubes of *H. Manihot* must travel a longer distance to reach the ovule than to reach their own when pollinated to the flower of *H. esculentus*. The selective fertilization in the author's material seems to be due partly to the slowness of pollen-tube development of *H. Manihot* and longer style of *H. esculentus*. As to the style length and fertility in cross pollination, McCLELLAND (1919) has pointed out in his study of vanilla fruit: "It seems guite reasonable to suppose from the heavy fertilization of ovules near the apex and sparse fertilization or entire absence of fertilization near the base of the ovary when the Vanillon stigma has been pollinated with *V. planfoilia* pollen that these pollen-tubes are unable to reach, or reach only limited numbers of the ovules in the far end of the ovary, which are at a considerably greater distance from the stigma than the farthest ovules of the *V. planifolia* ovary. Even in its own ovary the *V. planifolia* pollen causes a much heavier fertilization near the apex than near the base. This inability of *V. planifolia* pollen-tubes to reach the farthest ovules was particularly marked when *V. planifolia* pollen was applied to V 43, which is one of the largest flowered of the Vanillon varieties."

5. The comparative morphology and physiology in some characters of H. esculentus L., and H. Manihot L. and their F₁ hybrid

(1) General characters of H. esculentus, H. Manihot and F₁ hybrid

The general morphological characters indicated by *H. esculentus* (female parent) and *H. Manihot* (male parent) and F_1 and hybrid plant need be given shortly here.

These two species used in this research are very distinct and well-defined in their characters. *H. esculents* (Okra or Gumbo) is an especially widespread and well-known vegetable plant. The height of okra is 50–100 cm. in dwarf variety and 120–240 cm. in tall variety, whereas *H. Manihot* is 30–60 cm. The stems of both species are cylindrical and usually rough-haired especially in *H. Manihot*, the colour of hair being darker in *H. Manihot*. The leaves of *H. Manihot* are much smaller than those of *H. esculentus* and the leaf lobation is deeper than that of some varieties of *H. esculentus* which were used in this research and examined in detail. The flowers of both species arise in the leaf axils and are similar to those of cotton in size, shape and in colour. They are always single. Five large yellows petals are subtended by numerous, narrow, involucral bractlets. The stamen forms a column which is five-toothed at the apex. There are five or six style branches each tipped by a capitate stigma. The involucral

bractlets of *H. Manihot* are shorter and more closely massed together
around the stem than those of *H. esculentus.* The fruit of okra is a cap-
sule dehiscing longitudinally after maturity. The seeds of *H. Manihot* are
covered with short, gray hairs and those of okra are burried in long fine
tangled hairs. The pollen-grains of both species are large, spherical and
spiny.

The uniformity of the two parent types in all characters has already
been spoken of. The F_1 hybrid plants were also just as uniform in their
traits and no aberrant plants were raised. They differed considerably from
the parental plants in general appearance owing to the vigorous growth,
abundant branching, and enormous flower-production for which the sterility
of the plants accounts. The flowers of parents are similar so that the
differences in the flowers of F_1 hybrid are not very pronounced but the buds
are bigger than those of parents and the opened flowers are a little longer
than the parents'. The flower colour is exactly the same in all individuals,
a clear soft yellow and the center of flowers is deeply pigmented reddish
purple in the same tone as seen in both parents. The capsule and stems of
H. Manihot are more hairy than those of *H. esculentus* especially on the ca-
psules and give to the capsule a golden grayish shimmer. The capsules
of F_1 plants are more rough haired than those of either parent and the
hair is so stiff as sometimes to injure the human skin. The F_1 hybrid has
very rarely set seed, though it has repeatedly been self-fertilized and
crossed with both parental species and back-crossed (*H. esculentus* $\times F_1$
hybrid) plants. The root system is considerablly developed corresponding
to its hybrid vigor.

Many investigators of interspecific hybrids have obtained individuals
uniform in their morphological characters while in some intergeneric hybrids,
the F_1 plants were polymorphous. KARPECHENKO (1928) has obtained in
F_1 plants of *Raphanus sativus* L. $\times$ *Brassica oleracea* L. extreme polymor-
phous individuals. Only in the structure of fruit and in the shape of the
flower parts were the hybrids more or less homogeneous.

One of the significant features which usually attend the species hybrid
is the increased vigor of F_1 hybrid. The F_1 hybrids of the author's ma-
terial showed extreme vigor in their vegetative growth, about twice the
height of *H. esculentus*, the taller parent, and about 13 times that of the
H. Manihot, the smaller parent.

The hybrid vigor of species or generic hybrid has been frequently
commented upon by many investigators from the time of KÖLREUTER, for
instance, BABCOCK and COLLINS (1920) in *Crepis* hybrid, ROSENBERG (1909) in

Drosera longifolia × *D. Rotundifolia*, KARPECHENKO (1924) in Radish-Cabbage hybrid, SAX and SAX (1924), and KAGAWA (1924) in *Aegilops cylindrica* HOST × *Triticum vulgare* VILL. *var. lutescens* KORN., THOMPSON (1926) in wheat-rye hybrid and others. Sometimes but exceptionally, the species hybrids have shown weak development of vegetative growth. F_1 hybrids of *Crepis Capillaris* (L.) WALLS (n=3) × *C. tectrum* L. (n=4) were so weak that they were unable to grow beyond the seedling stage. Babcock and COLLINS (1920) have pointed out that the two haploid sets of chromosomes were unable to function together. In 1927 the present author obtained a few seeds by crossing *H. esculentus* with the pollen of Upland cotton. These seeds were sown in the spring of 1928, but all failed to germinate. CLAUSEN and GOODSPEED (1926) have reported that the F_1 hybrids of *Nicotiana glutinosa* (n=12) × *N. Tabacum* (n=24) are weak in germination and development.

Despite these differences in vigor or intermediacy in some characters the F_1 hybrid plants resemble *H. esculentus* (female parent) in general appearance rather than *H. Manihot* (male parent). Similar cases have been reported in many interspecific hybrids, for instance, by TSCHERMACK (1927) in wheat-rye hybrid, by WETTSTEIN (1926) in *Funaria* species and *Physcomstrella* species, by *Chittenden* (1928) in *Godetia* species hybrid, by COLLINS and MANN (1923) in *Crepis*; *setosa* characters predominante in F_1 plant of *C. setosa* HALL. × *C. Capillaris* (L.) LALLR,, and quite similar in *C. setosa* HALL. × *C. biennis* L.

In the interspecific hybrid the cytoplasm and gene seem to have im portant influence in its growth. As SCHWEMMLE (1924) has noted in different species the cytoplasm is different in its quality, hence the F_1 hybrid will be much more influenced by the parent which furnished both gene and cytoplasm than by the male parent. The preponderance of F_1 characters also seems to be partly due to the chromsome number of parental species. THOMPSON (1926) has reported the strong preponderance of wheat character in the hybrid between wheat and rye, when wheat is used as the female parent. Out of eighteen characters, he recognized two of these as like rye, six as like wheat and the remaining ten as intermediate of both parents. Out of these ten characters five resembled wheat more than rye. He noted "This is thus a strong preponderance of wheat characters, a fact which may be due to the large number of wheat chromosomes." MORINAGA (1928) has reported that the F_1 hybrid of *Brassica* species resembled more the larger chromosomal parent than the smaller. The *H. esculentus* used as a female parent in the author's investigation

has a larger number of chromosome than that of *H. Manihot*. The reciprocal crossings have failed in success, so that whether the preponderance of F_1 characters is due to this relation of chromosome number or not is still obscure.

(2) Duration of life and annual period of growth

H. Manihot is a biennial and *H. esculentus* is an annual plant in nature; the F_1 hybrids of these species are annual. In 1927, after harvesting the pods, four stamps of each of these species and the F_1 hybrid were left in the glass house to test their longevity. The results of these experiments were very distinct. *H. Manihot* sprouted new shoots in the Spring of the following year and reached the flowering time at the end of May, while *H. esculentus* and F_1 hybrid plants died in the winter without sprouting. Biennial plants were never obtained from later generations of these hybrid. COLLINS, HOLLINGSHEAD and AVERY (1929) have obtained a constant annual hybrid from, *Crepis artificialis*, by the crossing of *C. biennis* (biennial) × *C. setosa* (annual).

It is a general character of species hybrid that the plants increase the length of their life. JONES (1925) has said, "Plants which differ in their length of life usually give to their hybrid offspring a longevity equal to, if not greater than, that of the longer-lived parent. GÄRTNER notes especially that the crosses of annual Herbaceous species with perennial Shrubby species (As illustrated by the genera; *Hyoscyamus*, *Nicotiana*, *Calceolaria*, *Malva* and *Digitalis*) are as enduring as the perennial parent." The author got annual F_1 hybrid plants by crossing an annual plant (*H. esculentus*) with a biennial plant (*H. Manihot*), but the growth curve and flowering time clearly show the increased longevity of F_1 hybrid in one season. Table 9 shows the average length in cm. of 20 plants of each parent, F_1 and back-crossed plants in each period.

Table. 9.   *Records of the growth of the main stem of parents,*
F_1 and back-crossed plants in 1928 on the same plot.

Date / Plants	Jun. 25	July 10	July 25	Aug. 9	Aug. 24	Sept. 9
H. esculentus	cm. 9.73	17.60	34.40	81.74	137.80	187.80
H. Manihot	5.31	10.51	12.37	12.68	11.10	
F_1 hybrid	6.95	17.69	61.88	130.07	220.66	301.56
H. escu. × F_1	10.54	23.00	59.35	123.63	196.66	269.00

Date / Plants	Sept. 24	Oct. 9	Oct. 24	Nov. 8	Nov. 23.	Dec. 8
H. esculentus	cm. 223.23	230.20	232.50			
H. Manihot						
F_1 hybrid	364.08	398.18	416.88	430.10	432.17	431.85
H. escu. F_1	328.63	358.17	368.97	380.00	389.25	388.49

The data shown in Table 9 are given graphically in Text-fig. 1. In these figures curves H. e and H. M represent averages for 20 plants each of *H. esculentus* and *H. Manihot* respectively. The growth curve of F_1 hybrid and back-crossed plants is represented respectively by curves F_1 and B. C.

By a glance at Table 9 and Text-fig. 1, one is convinced that heterosis is exhibited in F_1 plants compared to parental plants and that the F_1 vigor is reduced in the back-crossed plants. There are large differences in length between the parents and between parents and F_1 plants or back-crossed plants, and each curve also shows that the plants increased their length slowly at first, rapidly afterwards, more slowly again at the end of the season, and finally ceased entirely. From these curves one can see remarkable differences in the longevity of growth; even though a noticeable increase in length is not shown in the parents, both F_1 and back-crossed plants still increase their length.

(3) **Leaf size and lobation**

A simple method of comparing the leaves of parents and their hybrid
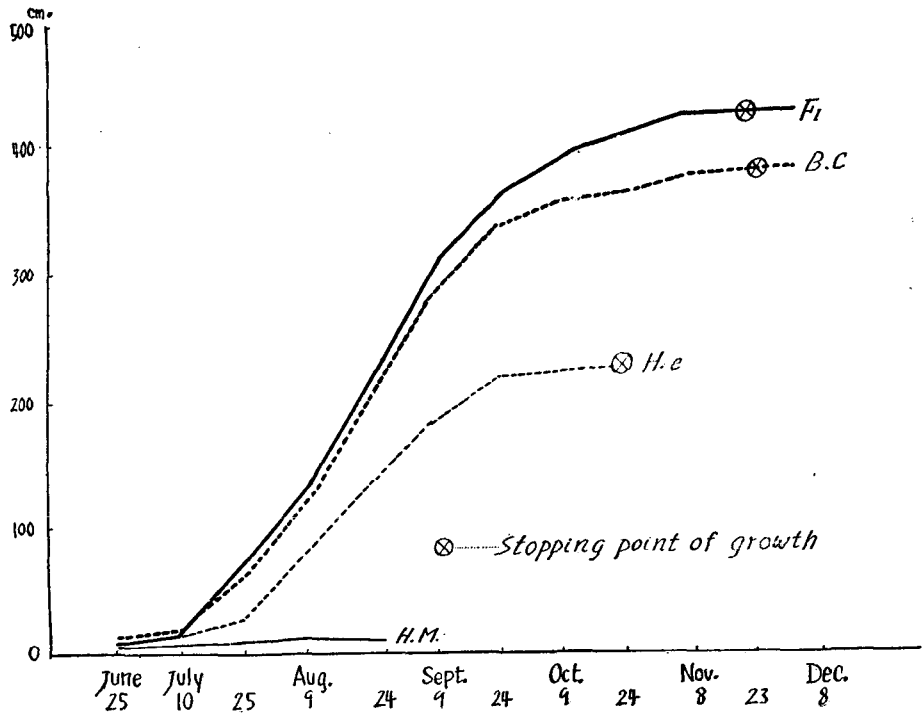
Text-fig. 1. Comparison of mean growth-curve for main stems of
parents, F_1 hybrid and back-crossed plants. in 1928
on the same plot.

was worked out by means of the leaf length and leaf-lobe index[1]. In 1928
the author measured the leaf length and calculated the leaf-lobe index of
parents and F_1 hybrid plants to compare the size and shape of leaves.
Five leaves were measured on each plant from the middle part of the stem
and the average of the five indices computed from these measurements was
taken as the leaf-lobe index of the plant.   The variation table, mean,
standard deviation and coefficient of variability for the leaf length and leaf-
lobe index of parents and F_1 hybrid plants are shown in Tables 10 and
11 (See Plate III).

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1)   The leaf-lobe index, as defined by KEARNY (1924), is "the distance from the base of the
blade to the botton of the sinus between the terminal lobe and the upper right hand
lateral lobe, expressed as a percentage of the length of the leaf. A high lobe-index in-
dicates a shallow lobed leaf and vice versa".   The vein angle of the leaf is negatively
correlated with the lobe-index.   Deeply lobed leaves, whose lobe index is low, tend to
have a wide vein-angle and vice versa.

Table 10.   *Frequency distribution of leaf length in parents and F$_1$ hybrid plants.*

Leaf length (cm.)	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	22	23	24
H. esculentus																		8
H. Manihot	2	4	7	14	20	21	15	9	5	2	1							
F$_1$ hybrid																		

Leaf length (cm.)	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	Total
H. esculentus	8	8	16	17	8	8	2	1									76
H. Manihot																	100
F$_1$ hybrid		1	1	2	2	3	4	11	15	19	13	13	10	5	3	3	105

	Mean (cm.)	STD. DEV. (cm.)	C. V. (%)
H. esculentus	27.29±0.149	±1.93 ±0.106	7.08±0.387
H. Manihot	10.66±0.134	±1.99 ±0.095	18.63±0.920
F$_1$ hybrid	34.23±0.180	±2.73 ±0.108	7.98±0.371

Table 11.   *Frequency distribution of leaf-lebe index in parents and F$_1$ hybrid plants.*

Leaf-lobe index (%)	16	18	20	22	24	26	28	30	32	34	36	38	40
H. esculentus									1	1	1	4	7
H. Manihot			3	6	12	25	15	13	9	5	4	3	2
F$_1$ hybrid	1	4	23	25	19	20	4	5	2	1	1		

Leaf-lobe index (%)	42	44	46	48	50	52	54	56	Total
H. esculentus	13	12	11	9	10	4	2	1	76
H. Manihot	2	1							
F$_1$ hybrid									

	Mean (%)	STD. DEV. (%)	C. V. (%)
H. esculentus	44.79±0.368	±4.75 ±0.260	10.60±0.587
H. Manihot	28.64±0.339	±5.03 ±0.240	17.56±1.618
F_1 hybrid	23.52±0.236	±3.58 ±0.166	15.24±0.709

Conspicuous differences between leaves of *H. esculentus* and *H. Manihot* are to be found in the size and shape of the leaves which, in *H. esculentus* are invariably larger in size and broader in lobe. The leaf-lobe index is also greater to some degree than *H. Manihot* as indicated in these Tables. The mean values of the leaf-length of *H. esculentus* and that of *H. Manihot* are 27.29±0.149 cm. and 10.66±0.134 cm. respectively, but that of F_1 plants is 34.23±0.180 cm.. The standard deviation of parent species is similar in value and smaller than that of F_1 plants. The coefficient of variability of *H. Manihot* is much larger than that of *H. esculentus* while that of F_1 hybrid is smaller than that of *H. Manihot* but slightly bigger than *H. esculentus*. The leaf-lobe index of F_1 hybrid is 23.52±0.236, which is much less than even that of the small parent *H. Manihot* (28.64±0.339), (See also Plate III). This indicates that the leaf-lobation of F_1 plants is not the intermediate of both parents but that they are more deeply lobed than either parent. The leaf lobation varied by the variety of *H. esculentus*, some varieties show a deeper lobation than that of blue long A which was examined in this experiment in detail. But similar results were obtained in the case of another variety of *H. esculentus*. Table 12 shows the results of measurements in the case of other varieties of *H. esculentus*.

Table 12. *Frequency distribution of leaf-lobe index in some varieties of H. esculentus and their F_1 hybrid.*

Leaf-lobe index (%)	16	18	20	22	24	26	28	30	32	34	36	38	40
White long								8	6	7	9	5	6
Blue long B				1	0	0	0	0	2				
White velvet													
Green giant										1	2	1	2
F_1 { White long × H. Manihot		2	8	4	3	0	1						
Blue long B × "			1	1	3	2							
White velvet × "	1	2	2	1	4	3	2						
Green giant × "							2						

Leaf-lobe index (%)	42	44	46	48	50	52	54	56	58	60	Total
White long	1	5	3								50
Blue long B											3
White velvet	2	3	1	3	3	1					13
Green giant	0	1	2	1	5	5	5	1	5	3	34
F_1 { White long × H. Manihot											18
Blue long B × ,,											7
White velvet × ,,											15
Green giant × ,,											2

The inheritance of leaf-lobation in intervarietal or interspecific hybrid in some plants has been studied by some investigators, but usually the F_1 hybrid indicated an intermediate leaf-lobation. MARTIN (1908) crossed two species of Indian cotton (*Gossypium indicum* and *G. arboreum*) one of which has a deeply and narrowly lobed leaf and the other a shallowly and broadly lobed leaf. The first generation plants were intermediate and the second generation segregated in a 1 : 2 : 1 ratio. Similar results were obtained by PEABLE and KEARNEY (1928) in crossing between "Okra-leaf" form of Scala cotton and a normal broad-leaved form of the same variety. Crosses made by SHOEMAKER and others gave results also like those of preceding investigations. ROSENBERG (1906) in his study of species hybrid of *Drosera longifolia* (long leaved form) × *D. Rotundifolia* (round leaved form) reported as follows: "Die Länge des ganzen Blätters bei dem Bastard ist gleich der bei *D. long*. und grösser als bei *D. rot*. Die Lange der Blattspreite bei dem Bastard stimmt mehr *D. long*. als mit *D. rot*. überein. Vielleicht ist die Auffassung berechtigt, dass die in dem Bastard enthaltene, von *D. rot*. geerbte Anlage der 'kurzen Blattspreite,' bei dem Bastard in Form einer deutlichen Hemmung der entsprechenden Anlage von *D. long*. hervortritt. Dagegen scheint in grossen und ganzen die 'Anlage' zu breiten Blättern von *D. rot*. her vollständig über die Schmalblattrigkeit von *D. long*. her zu domonieren." The F_1 hybrid of *H. esculentus* and *H. Manihot* indicated extreme hybrid vigor in its general characters, the leaf-lobe index also indicates a value beyond the mean value of both parents.

(4) Stem-length and stem-diameter

The F_1 hybrids of these species are very vigorous in their general appearance at flowering time, but in their young stage they are rather small and slender compared to their parents. Table 13 gives the results of measurements of stem-length in parents and F_1 hybrid plants at the

time of transplantation from glass-house to the open field in 1927. The mean value of the stem-length in *H. esculentus* is 9.67±0.095 cm. and that of *H. Manihot* is 3.59±0.071 cm., while that of F_1 plants is 7.38±0.198 cm., just the intermediate number of these two parents. The standard deviation is also intermediate between both parents. Thus there is no indication of any hybrid vigor at this stage.

Table 13. *Mean, Standard deviation and Coefficient of variability of stem-length of parents and F_1 plants, measured in young stage.*

Plants	Mean	STD. DEV.	C. V.	N
H. esculentus	cm. 9.69±0.095	cm. ±1.87 ±0.068	% 19.33±0.724	175
H. Manihot	3.59±0.071	±1.00 ±0.050	27.85±1.505	90
F_1 hybrid	7.38±0.198	±1.44 ±0.140	19.50±1.971	24

Afterward the F_1 hybrid plants increase the rate of growth rapidly and continue growth with abundance of flowers until the end of November. The results of measurements of stem-length and stem-diameter at the end of the season are presented in Tables 14 and 15.

Table 14. *Mean, Standard deviation and Coefficient of variability of stem-length in parents and F_1 plants, measured at end of season.*

Plants	Mean	STD. DEV.	C. V.	N
H. esculentus	cm. 227.72±1.357	cm. ±22.68 ±0.960	% 10.95±0.423	127
H. Manihot	30.43±0.521	±6.46 ±0.368	21.23±1.445	70
F_1 hybrid	392.80±1.630	±11.60 ±1.150	2.95±0.294	23

Table 15. *Mean, Standard deviation and Coefficient of variability*
of stem-diameter in parents and F_1 plants, measured
at full growth.

Plants	Mean	STD. DEV.	C. V.	N
H. esculentus	cm. 3.23±0.0195	cm. ±0.303 ±0.0138	% 9.37±0.426	110
H. Manihot	1.95±0.0166	±0.227 ±0.0117	11.65±0.669	85
F_1 hybrid	4.92±0.0740	±0.548 ±0.0523	11.14±1.075	25

The average length of *H. esculentus* is 227.72± 1.357 cm. and that of
H. Manihot is 30.43±0.521 cm., yet the F_1 plants have an average of
392.80± 1.630 cm. This is about twice the height of the stem of *H. escu-
lentus* and about 13 times that of the *H. Manihot*, corresponding to three
times the average length of both parents. The stem diameter of F_1 plants
measured at the base of the main stem is 4.92±0.074 cm., while that of
H. esculentus and *H. Manihot* is 3.23±0.0195 cm. and 1.95±0.0166 cm.
respectively. That is about 50 per cent greater than that of *H. esculentus*
and about 150 per cent greater than that of *H. Manihot.*

(5) Flower

a) *Size*

As already mentioned, the flowers of both parents are similar in colour
and shape, beautiful soft yellow, with a deep purple blotch at the base of
each petal. The flowers of F_1 plants appear only as a somewhat enlarged
form of all the parts of the parents, (Plate II, Fig. 4). Enough measure-
ments have not yet been made for accuracy, but the breadth of opened
flowers will be found approximately as follows;

H. esculentus .... 9 – 10 cm.
H. Manihot .... 8 – 9 cm.
F_1 plants .... 14 – 17 cm.

That is, the flower size of the F_1 plants is not the intermediate of
both parents nor the same size as the larger parent, but is markedly in-
creased in its breadth above that of the larger flowers of the species.

b) *Style*

The anthers, style and stigma of F_1 plants all resemble those of *H.*

esculentus but are greater in length and size. The only marked difference from the flowers of both parents is the behaviour of the stigma at flowering time. The style of these species and hybrid terminates in a large round stigma and there are five, more or less (usually five), distinct projections at the top of the staminal tube. In the *H. esculentus* the branches of the stigma do not spread out and bend down, so as to bring the stigmatic surface into contact with the anthers, before the evening of the day of opening, while those of *H. Manihot* spread out early in the morning. In the F_1 plants the stigma does not spread out until noon and then it opens slowly after noon. This character of the stigma is just between that of the two parents.

(6) Involucral bracts

The involucre of *H. esculentus* consists of six to twelve narrow bractlets separated at the base. In *H. Manihot*, there are four to eight broad heart-shaped bractlets. The involucral bractlets of F_1 plants are longer than those of either parent and intermediate in width and number.

a) *Number of involucral bractlets*

The data obtained from 300 flowers of each species and F_1 hybrid are given in Table 16.

Table 16. *Mean, Standard deviation and Coefficient of variability of number of involucral bractlets.*

Plants	Mean	STD. DEV.	C. V.
H. esculentus	cm. 9.477±0.0395	cm. ±1.015 ±0.0280	% 10.710±0.2982
H. Manihot	5.317±0.0282	±0.723 ±0.0199	13.602±0.3801
F_1 hybrid	6.297±0.2645	±0.680 ±0.1870	10.794±0.3008

From Table 16 it is evident that in F_1 plants the number of involucral bractlets is intermediate in mean value, but inclined towards the *H. Maninot*. The standard deviation is less than that of either parent. The coefficient of variability is nearly the same as that of *H. esculentus*.

b) *Width of involucral bractlets*

The involucral bracts of *H. esculentus* are narrow and sharp in form, while in *H. Manihot* there are heart-shaped bractlets. The data obtained from 200 bractlets of each species and F_1 plants are given in Table 17.

Table 17. *Mean, Standard deviation and Coefficient of variability of width of bractlets in parents and F_1 Plants.*

Plants	Mean	STD. DEV.	C. V.
H. esculentus	cm. 2.270±0.0281	cm. ±0.589 ±0.0199	% 25.96±1.321
H. Manihot	9.965±0.0140	±2.061 ±0.0695	20.68±0.826
F_1 hybrid	5.655±0.0518	±1.227 ±0.0414	21.66±0.766

It is seen in this table that the width of bractlets of F_1 plants is just intermediate between the two parents, not only in mean value but also in standard deviation and coefficient of variability.

c) *Length of bractlets*

The data obtained from 200 bractlets of each species and F_1 plants are given in Table 18.

Table 18. *Mean, Standard deviation and Coefficient of variability of length of bractlets in parents and F_1 plants.*

Plants	Mean	STD. DEV.	C. V.
H. esculentus	mm. 14.70±0.073	mm. ±1.52 ±0.051	% 10.37±0.353
H. Manihot	19.15±0.081	±1.70 ±0.057	8.89±0.300
F_1 hybrid	24.70±0.079	±1.66 ±0.056	6.72±0.227

It is apparent from Table 18 that the length of F_1 plants markedly increases in mean value; that the standard deviation lies between that of the two parents and that the coefficient of variability is smaller than that of the smaller parent.

.   (7) Capsule

The chief characteristics of the capsules; namely the colour, shape, length and diameter of pod, and length and diameter of fruiting branches are measured when they are full-grown.   The diameter of pods and fruiting branches are measured with callipers.   The shape of pods are determined by number of longitudinal ribs of pod.   These characters partly depend upon the environmental condition during the growing time after flowering, only the form of pod is wholly independent from such conditions.

a) *Number of longitudinal ribs*

The fruit of these plants is a several-celled capsule with longitudinal ribs.   The cross-section of pod varies in shape according to the number of longitudinal ribs.   The number of ribs in *H. esculentus* varies from five to ten, so that the shape of cross-section of pod varies from pentagon to decagon, while that of *H. Manihot* and F_1 hybrid are all pentagon (five ribs or five ovary cells) with only a small number of exceptions.   Table 19 presents the frequency in number of longitudinal ribs of pod in the parental species and F_1 plants.   The mean, standard deviation and coefficient of variability calculated from this variation table are also presented.

Table 19.   *Variation table in number of longitudinal ribs of pod and Mean, Standard deviation and Coefficient of variability.*

Plants	Number of longitudinal rbs							N
	4	5	6	7	8	9	10	
H. esculentus		39	198	201	55	6	1	500
H. Manihot	2	889	2					893
F_1 hybrid		402	2					404

Plants	Mean	STD. DEV.	C. V.
			%
H. esculentus	6 588±0.0255	±0.452 ±0.0180	12.830±0.0278
H. Manihot	5.000±0.0015	±0.067 ±0.0010	0.013±0.0214
F_1 hybrid	4.995±0.0002	±0.007 ±0.0002	0.001±0.0000

From this table it will be seen that the pentagonal character of *H. Manihot* is dominant over the larger number of ribs of *H. esculentus*. The coefficient of variability is 12.830±0.0300 in *H. esculentus* while in both *H. Manihot* and F_1 plants it is negligible.

b) *Length of capsule*

The okra fruit is 13 to 23 cm. in length.   It bends to the outside of the main stem.   The capsules of *H. Manihot* are very short compared to those of *H. esculentus* varying from 3.5 to 5.6 cm. in length.   They are straight in form.   The F_1 pods are straight as those of *H. Manihot* and vary from 7 to 16 cm. in length, just the intermediate of parents.   The data of calculated values are presented in Table 20 (See Plate II, Fig. 3). The capsules which were used in these measurements were taken from individuals which developed normally.   Five to ten capsules were taken rom each plant and measured.

Table 20.   *Mean, Standard deviation and Coefficient of variability in length of pod.*

Plants	Mean	STD. DEV.	C. V.	N
H. esculentus	cm. 18.56±0.076	cm. ±1.96 ±0.054	% 10.54±0.294	300
H. Manihot	4.55±0.107	±0.34 ±0.009	7.35±0.203	300
F_1 hybrid	11.74±0.057	±1.31 ±0.040	11.11±0.345	242

From this table it is evident that the length of F_1 capsules is intermediate of the parents in mean and standard deviation and that the coefficient of variability of F_1 hybrid is larger than either parent.   This comparatively high value of C. V. may depend upon the length of flowering time.

c) *Diameter of capsules*

Five to ten capsules, which were considered to be of normal size were selected from the middle part of a stem for measurement.   The diameter of capsules was measured at the point of maximum breadth.   Table 21 gives the mean, standard deviation and coefficient of variability.

Table 21.   *Mean, Standard deviation and Coefficient of variability in diameter of capsules of parental species and F_1 plants.*

Plants	Mean	STD. DEV.	C. V.	N
H. esculentus	cm. 2.57±0.011	cm. ±0.27 ±0.009	% 10.55±0.294	300
H. Manihot	2.46±0.068	±0.18 ±0.048	7.14±0.197	300
F_1 hybrid	2.45±0.010	±0.23 ±0.007	9.23±0.283	242

From Table 21 it is evident that the diameter of F_1 capsules is intermediate in mean and standard deviation between the two parents and that the coefficient of variability is larger than either parent.   The value of coefficient of variability may depend upon the length of the flowering time.

d) *Length of fruiting branches*

H. esculentus has long pods and short fruiting branches, on the contrary, *H. Manihot* has short pods and long fruiting branches.   The F_1 plants have intermediate capsules and intermediate fruiting branches in length between the two parents.   The result of measurements of length of fruiting branches is presented in Table 22.

Table 22.   *Mean, Standard deviation and Coefficient of variability of fruiting branches of parental species and F_1 plants.*

Plants	Mean	STD. DEV.	C. V.	N
H. esculentus	cm. 3.95±0.043	cm. ±1.09 ±0.030	% 27.69±0.819	300
H. Manihot	5.05±0.028	±0.72 ±0.020	1.419±0.224	300
F_1 hybrid	4.00±0.044	±1.01 ±0.031	25.24±0.826	242

e) *Diameter of fruiting branches*

Middle part of fruiting branches were measured with callipers.   The results of these measurements are presented in Table 23.

Table 23. *Mean, Standard deviation and Coefficient of variability in diameter of fruiting branches of parental species and F_1 hybrid plants.*

Plants	Mean	STD. EDV.	C. V.	N
H. esculentus	cm. 8 45±0.046	cm. ±1.19 ±0.033	% 14.08±0.403	300
H. Manihot	3.45±0.014	±0.36 ±0.010	10.37±0.289	300
F_1 hybrid	5.12±0.023	±0.54 ±0.016	10.49±0.325	242

Table 23 shows that the mean, standard deviation and coefficient of variability of *H. esculentus* are greater than those of *H. Manihot*; that the mean and standard deviation of F_1 plants lie between those of these two species and that the coefficient of variability is smaller than that of the smaller parent.

f) *Colour of pod*

Mature pods of *H. esculentus* turn yellowish brown in colour, while those of *H. Manihot* are darker and the capsules of F_1 plants are inter-mediate in colour.

(8) Trichomes

Abundant trichomes or plant hairs are distributed over the entire sur-face of stem, pod and leaves of these plants. The length, structure and toughness of these hairs are dissimilar in the different species and F_1 hybrid.

a) *Trichomes on the stem and leaves*

Trichomes distributed on the stem and leaf surface are similar in shape and structure; they are unicellular and sharpened at the tip, the simple extention of an epidermal cell of the stem or leaves, as indicated in Plate VI, figs. 2, 6, 8. Only the length of these trichomes is different in these species and F_1 hybrid. The measurements of these trichomes were taken under the microscope with the aid of a micrometer. The summarized results are shown in Table 24.

Table 24. *Mean, Standard deviation and Coefficient of variability in length of trichomes from the stem of parents and F_1 hybrid.*

Plants	Mean	STD. DEV.	C. V.	N
H. esculentus	Micron 86.23±0.802	Micron ±20.59 ±0.570	% 23.88±0.800	300
H. Manihot	117·94±1.600	±45.24 ±1.246	38.36±1·404	300
F_1 hybrid	101.03±1.813	±27.16 ±1.056	26.88±0.795	300

As shown in Table 24, the mean, standard deviation and coefficient of variability of *H. Manihot* are greater than those of *H. esculentus*; those of F_1 hybrid are intermediate between these two parents. The trichomes of F_1 hybrid are more stiff than those of either parent. The differences in length of this kind of hairs are not so significant as those of capusles.

b) *Trichomes of capsule*

(i) *Trichomes on the pod of H. esculentus*

The trichomes grown on the capsules of *H. esculentus* are very different from those of the stem and leaves. The hairs grown on the capsule of *H. esculentus* are uniform in structure and in shape; multicellular hairs extending from an epidermal cell of pod, tip not sharp but rather slender and shrivelling before the maturity of seeds, average diameter at base 25.77 microns (Plate VI, Fig. 4).

In addition to this kind of hairs, the capsules of *H. esculentus* have also a very small number (about 3 %) of tough unicellular hairs only close to the longitudinal ribs (Plate VI, Fig. 5).

For convenience the multicellular hairs are referred to in this paper as H. e. M. type, the tough unicellular hairs are referred to as H. e. U. type of hair.

(ii) *Trichomes on the pod of H. Manihot*

There are two kinds of trichomes on the capsule of *H. Manihot*; (1) long hairs and (2) short hairs. Both trichomes are unicellular, tender and fine, but they do not shrivel as easily as the multicellular trichomes of *H. esculentus*. The average diameter of long trichomes at the base is 32.12 microns and that of short trichomes 8.56 microns. For convenience the long

trichome is referred to in this paper as H. M. L. type, while the short trichomes are referred to as H. M. S. type. These two kinds of trichomes are mixed together over the entire surface of pod but the short trichomes are much more abundant than the long ones (Plate VI, Fig. 7). The numerical data of these two kinds of trichomes are as follow.

Total number of trichomes counted	Number of short tricomes	Number of long trichomes	Percentage of long trichomes
1581	1290	291	18.4

(iii) *Trichome on the capsules of F_1 plant*

The hairiness of F_1 capsules is somewhat different from that of parental species (Plate VI, Fig. 1, 2, 3). It is possible to classify these trichomes of F_1 capsules into the following four types;

(1) H. e. M. type. Multicellular trichomes similar to those of *H. esculentus*, on the average 22.52 microns in diameter.

(2) H. M. S. type. Unicellular trichomes resembling the short trichomes of *H. Manihot*, only somewhat enlarged in size, on the average 13.27 microns in diameter.

(3) H. e. U. type. This type of trichomes is grown only close to the longitudinal ribs as those of *H. esculentus*. Very few in number, average length 431.3 microns, average diameter 39.65 microns.

(4) F_1 type. Instead of long fine trichomes of *H. Manihot* there are many giant stiff trichomes, average diameter at the base is 64.63 microns.

These four kinds of trichomes are mixed together over the surface of F_1 capsules. Only the H. e. U. type is limited in position close to the longitudinal ribs. The number of these four kinds of trichomes were counted on a certain area under the microscope. The summary results of these counts are presented in Table 25.

Table 25. *Number of trichomes of each type grown on the capsules of F_1 hybrid.*

Types	M.e.M. type	M.e.U. type	H.M.S. type	F_1 type	Total
Number counted	1611	217	348	413	2589
Percentage	62.22	8.38	13.44	15.95	99.99

The measurements of these four kinds of trichomes were taken under microscope with the aid of a micrometer.   The results of these measurements are presented in Table 26.

Table 26.   *Mean, Standard deviation and Coefficient of variability in length of various types of trichomes grown on the capsules of H. esculentus, H. Manihot and F_1 hybrid plants.*

Plants	Type of trichome	Mean	STD. DEV.	C. V.
H. esculentus	H.e.M. type	Micron 333.0±3.834	Micron ± 98.45 ±2.711	% 29.56±1.027
H. Manihot	H.M.L. type	2726.9±23.380	±600.30 ±16.53c	22.01±0.728
	H.M.S. type	118.0+1.6c9	± 41.32 ±1.138	35.02±1.258
F_1	H.e.M. type	186.5±3.094	± 79.45 ±2.198	42.60±1.356
	H.M.S. type	138.0±1.721	± 44.20 ±1.217	32.03±0.961
	F_1 type	1590.7±17.660	±453.60 ±12.493	28.52±0.913

As described above, the F_1 plants have many kinds of trichomes, derived from both parents, mixed together over the surface of the capsules. Among these trichomes the majority are multi-cellular (H.e.M. type) derived from *H. esculentus*, but are shorter compared with the same type of trichomes of *H. esculentus*.   The H. M. S. type trichomes which are thought to be derived from *H. Manihot*, are greater in length and width.

The F_1 type trichomes, which are thought to be derived from *H. Manihot* (H. M. L. type) are very vigorous and stiff but the length of this type is less than that of H. M. L. type (about 58.3 %), and the width is more than $7\frac{1}{2}$ times as large.   On the whole, it may be concluded that in the capsule of F_1 plants there are present both unicellular and multi-cellular trichomes, which may be derived from each parent independently. The trichomes which are thought to be derived from *H. esculentus* are predominant in number but decrease in size ; on the other hand, the trichomes which are thought to be derived from *H. Manihot* develop vigorously but decrease in number.

(iv) *Trichomes on the inner-surface of capsules*

The inner surface of the capsules of *H. esculentus* is quite smooth and

has no trichomes, while that of capsules of *H. Manihot* and F_1 hybrid plants are hairy.   The trichomes of both *H. Manihot* and F_1 hybrid plants are similarly unicellular, straight and tapering gradually towards the end.   However the trichomes of F_1 hybrid are much longer and more variable in length and size compared with those of *H. Manihot* (Plate VI, Figs. 9, 10, 11).   The numerical data obtained from *H. Manihot* are as follow :

> Mean in length ....   70.40±0.478 (microns)
> STD. DEV. in length ....   ±10.04±0.339 (microns)
> C. V.   in length ....   14.26±1.134 (%   )
> Width at the base ....   about   7.93 (microns)

The trichome of F_1 hybrid vary greatly in length and there are three or more groups of trichomes each having a mean value of 79.06±1.09 microns, 133.01±0.740 microns and 184.78±0.836 microns in length.

Besides these trichomes, there are three groups of extraordinarily long trichomes ranging from 300 to 650 microns in length, which grow only on the side opposite the longitudinal ribs.

(9) Size and weight of hybrid seed

Significant differences between *H. esculentus* and *H. Manihot* are even more striking when the size and weight of seeds are considered.   The weight of a single seed of *H. esculentus* is about 63 milligrams, while that of *H. Manihot* is only about 20 milligrams.   The seeds resulting from the immediate cross (F_1 embryo) are relatively small compared with the seed of mother plants (*H. esculentus*), hence they may be distinguished easily from the self-pollinated seeds of *H. esculentus*.   The seeds obtained by crossing with the pollen of *H. Manihot* are slightly smaller than those of mother parent (*H. esculentus*) but larger than those of the father parent (*H. Manihot*).   In 1926 the length, breadth and thickness of the crossed seed and parent seeds were measured with a micrometer screw gauge.   The number of self-pollinated and cross-pollinated seeds in each capsule and the order of these capsules on the stem are indicated in Table 27.   In this table crossed capsules are indicated by sign ( ).

Table 27. *Number of self-pollinated and cross-pollinated seeds*
in each capsule according to the order of capsules
on the stem.

Plan number	Order of capsules from below							
	1	2	3	4	5	6	7	8
A	81	81	72	80	89	(48)	86	107
B	75	67	31	93	(61)	86	114	116,
C	68	80	88	(54)	70	93	106	101
D	25	71	(73)					
E	26	71	(54)	107				

It was found that the average size of seeds per capsule decreased from
bottom to top on a single stem.   As it is apparent that the decreasing
ratios comply to the logarithmic curve, it may be necessary to calculate
the theoretical value of seeds of *H. esculentus*, supposing it were grown
in the position of crossed capsules, and to compare the size of crossed seeds
with the self-fertilized seeds.   The following formula was used to calculate
the theoretical value of the size of open-pollinated seeds at the assumed
position of crossed capsule.

$$\text{Log}_e y = \log_e a + bx$$

where $\log_e a$ and bx were substituted by the following value;

$$\log_e a = \frac{\Sigma x \log_e y \Sigma x - \Sigma \log_e y \Sigma x^2}{(\Sigma x)^2 - 1^2 \Sigma x^2}$$

$$b = \frac{\Sigma \log_e y \Sigma x - \Sigma x \log_e y \Sigma 1^2}{(\Sigma x)^2 - \Sigma 1^2 x^2}$$

In these formulas; $x=$ The position of capsule from below as
1. 2. 3.........

$y=$ Average length, width and thickness of
seeds of each capsule calculated.

The results of these calculations are shown in Table 28.

Table 28. *Average size of crossed seeds in a capsule in comparison to the theoretical value of mother parent seeds.*

Plant number		Theoretical value of mother parent seeds at the position of crossed capsule	Average value of crossed seeds (F$_1$ embryo)	Percentage of crossed seeds value in comparison with the theoretical value of seeds of mother parent
	A	mm. 4.990	mm. 4.420±0.017	% 88.58
	B	5.030	4 362±0.020	86.72
Length	C	5.020	4.330±0.010	86.25
	Av.	5.013	4.371	87.18
	A	mm. 4.570	mm. 4.409±0.017	% 96.48
	B	4.590	4.344±0.015	94.64
Breadth	C	4.610	4.289±0.015	93.04
	Av.	4.590	4.347	94.72
	A	mm. 4.400	mm. 2.868±0.014	% 87.91
	B	4.360	3.792±0.021	86.97
Thickness	C	4.410	3.883±0.012	88.05
	Av.	4.390	3.848	87.64

As indicated in Table 28, the average length of seeds obtained by crossing in 1926 is less than the seed of mother parent being 87.18 per cent of the latter. In breadth the average is 94.72 per cent and in thickness 87.65 per cent of that of the open-pollinated seeds of mother parent (*H. escu.*). Crossed seeds are larger than the seeds of *H. Manihot*. Table 29 presents the comparative length, breadth and thickness of *H. Manihot* seeds compared with those of F$_1$ hybrid seeds obtained from five capsules in 1926.

Table 29.   *Comparative length, breadth and thickness of H.
Manihot seeds and the crossed seeds obtained from
five capsules A,B,C,D and E in 1926.*

		Crossed seeds av. of 260	H. Manihot seeds av. of 200	% of crossed seeds
Length	Mean	mm. 4.359 ±0.008	mm. 2.663 ±0.006	119.00
	STD. DEV.	±0.190 ±0.006	±0.129 ±0.004	
Breadth	Mean	3.895 ±0.007	3.243 ±0.007	120.10
	STD. DEV.	±0.159 ±0.005	±0.140 ±0.005	
Thick- ness	Mean	3.731 ±0.006	2.399 ±0.007	155.52
	TSD. DEV.	±0.150 ±0.004	±0.145 ±0.005	

From the data in Table 29 it may be seen that the crossed seeds
are 119 per cent in length, 120.1 per cent in breadth and 155.52 per cent
in thickness of *H. Manihot* seeds.   As a whole the crossed seeds obtained
in 1926 were just intermediate in size between the two parents, only slightly
inclined to that of *H. esculentus*.

In 1927 an experiment[1] was conducted in which two kinds of pollen-
grains of *H. esculentus* and *H. Manihot* were dusted separately upon both
sides of stigma of *H. esculentus*.   In this experiment many cross-pollinated
and self-pollinated seeds were obtained in each capsule which are convenient
for the purpose of comparing the two kinds of seeds exactly.

The results of these calculations are presented in Table 30.

Table 30.   *Comparison of length, breadth, and thicknees of cross-
pollinated and self-pollinated seeds of H. esculentus
obtained by double pollination in 1927.*

Length	Mean	STD. DEV.	C. V.	N
Selfed-seeds	mm. 4.967±0.006	mm. ±0.147 ±0.004	% 2.96±0.085	273
Crossed-seeds	4.381±0.014	±0.160 ±0.010	3.65±0.221	21

1)   This experiment was carried out to determine whether there is any selective fertilization
between these two kinds of pollen-grains even when the locality of the pollination was
limited to both sides of the stigma.   See "Selective fertilization" in this paper.

| Difference | – 0.586 | | | |
| % of crossed seeds | 8.820 | | | |

Breadth	Mean	STD. DEV.	C. V.	N
	mm.	mm.	%	
Selfed-seeds	4.581 ±0.005	±0.132 ±0.004	2.88±0.083	273
Crossed-seeds	3.879±0.011	±0.125 ±0.075	3.22±0.194	62
Difference	–0.702			
% of crossed seeds	84.68			

Thickness	Mean	STD. DEV.	C. V.	N
	mm.	mm.	%	
Selfed-seeds	4.330±0.053	±0.130 ±0.037	3.00±0.087	273
Crossed-seeds	3.808±0.002	±0.140 ±0.008	3.69±0.222	62
Difference	–0.522			
% of crossed seeds	87.94			

Weight	Average weight of single seed	Ratio	N
	mg.		
Selfed-seed	63.92	100.0	273
Crossed-seed	34.26	53.6	62

From Table 30 it is seen that the crossed seeds are as much smaller as 88.20 per cent in length, 84.68 per cent in breadth and 87.94 per cent in weight of those of self-pollinated seeds. These results obtained in 1927 are nearly identical with those of 1926.

Crossed seeds are more variable in size than the self-pollinated, hence the standard deviation and coefficient of variability of crossed are generally greater than those of self-pollinated seeds. The results of these studies seem to indicate that the unbalanced condition of the chromosome sets might give rise to the abnormal endosperm development of seeds resulting from the cross-pollination with the pollen of *H. Manihot*.

Although the F_1 hybrid plants grow very vigorously, the crossed seeds do not indicate greater vigor but they are nearly intermediate in size between the two parents and their weakness is shown by the fact that they ger-

minate very poorly. From this fact it is apparent that the size of the seeds of *H. esculentus* is determined not only by the female parent but also by the male parent, in other words, the hybrid seeds (F_1 embryo) are influenced by the combination of small character of the seed of *H. Manihot*.

It is a widely known fact that the seeds resulting directly from cross-pollination within species, which grow into vigorous F_1 plants are usually followed by an increase in the weight and size. The results of such experiments were reported by many investigators, for instance, COLLINS and KEMPTON (1912) in corn, EAST and HAYS (1917) in tomato. The author of this paper has observed (1928) in the crossed seeds of sesame (*Sesamum indicum* L.) an increase in average weight of about 23 per cent compared with that of the parental varieties. In the case of interspecific crossing, on the contrary, there are many instances of decrease in the size of the crossed seeds compared to those of the parents as observed in the author's material. For instance, BUXTON (1928) has observed in the crossing of *Digitalis* species a few seeds which are intermediate in size between the larger *ambigua* and the smaller *purpurea* seeds.

(10) Seed-hairs of H. esculentus, H. Manihot and F₁ hybrid

There are abundant hairs over the entire seed coat in *H. esculentus*, *H. Manihot* and F_1 hybrid. These hairs are similarly unicellular elongations from the epidermal layer of the ovule. The seed hair of *H. esculentus* is bent and takes on a flattened ribbon-like appearance. Twisting like cotton fiber is a characteristic of this plants seed hair, and these hairs are light yellow in colour. The seed hairs of *H. Manihot* are straight, untwisted, conical, deep yellow in colour and abundant in number, shorter than those of *H. esculentus*. The F_1 hairs resemble those of *H. esculentus* but they may be distinguished from the latter by length, shape and by colour. These characters of F_1 hairs are just intermediate between the two parents (Plate VI, Figs. 12, 13, 14). Several hundreds of fibers were taken from the seed coat of these plants and their length measured under microscope with the aid of a micrometer. The results thus obtained are indicated in Table 31. From these results it would appear that mean, standard deviation and coefficient of variability are all intermediate between the two parents.

Table 31. *Mean, Standard deviation and Coefficient of varia-
bility of length of seed hairs in H. esculentus. H.
Manihot and F_1 hybrid.*

Plants	Mean	STD. DEV.	C. V.	N
H. esculentus	microns 380.55±4.40	microns ±93.09 ±3.14	% 24.46±0.90	200
H. Manihot	80.55±0.66	±15.95 ±0.47	19.81±0.60	266
F_1 hybrid	208.20±2.37	±51.11 ±1.67	24.55±0.85	212

(11) Flowering time

On each plant of parental species and F_1 hybrid grown in 1928 the
date of appearance of the first flower was recorded to determine the flower-
ing time.   The results are shown in Table 32.   The flowering period of
each individual was continued in *H. Manihot* until the middle of September
in *H. esculentus* until the beginning of October, and in F_1 plants until the
beginning of December.

Table 32. *Frequency distribution of flowering date in parents
and F_1 hybrid.*

Plants	July	10	11	12	13	14	15	16	17	18	19	20	21	22
						Date of flowering								
H. esculentus		1	2	8	6	9	13	9	9	2	2	2	1	2
H. Manihot		1	2	1	3	12	9	7	7	1	4	2	2	1
F_1 hybrid							2	3	2	7	9	9	5	9

Plant	July	23	24	25	26	27	28	29	30	31	Aug. 1	2	Total
						Date of flowering							
H. esculentus		0	1	1									68
H. Manihot		1	1	2	2	1	1						60
F_1 hybrid		3	2	8	3	5	10	2	5	3		2	89

Table 33.' *Mean and Standard deviation of flowering date*
(July) in parents and F₁ hybrid.

Plants	Mean	STD. DEV.
H. esculentus	15.47±0.244	±2.99 ±0.173
H. Manihot	16.98±0.361	±4.15 ±0.256
F₁ hybrid	23.23±0.324	±5.54 ±0.229

It will be noticed that the flowering time of the first flower of the hybrid plants is delayed 6–7 days in mean from that of parents. The standard deviation is slightly larger than that of parents. Usually the flowering time of the interspecific hybrids departs very widely from a position intermediate between those of their parents as DARLINGTON (1928) observed in some of the hybrids of *Prunus* species.

(12) Fertility of F₁ hybrid

The fact that there is some correlation between the percentage of apparently poor pollen-grains and the sterility of the plant was indicated by GATE (1915) in *Oenothera*, by SAX (1921) in wheat hybrid, and by BLAKESLEE (1921) in *Datura* as well as by others. But of course this is only an approximate measure and for general purposes it should be considered satisfactory. F₁ hybrids of *H. esculentus* × *H. Manihot* produced an abundance of good and well developed germinable pollen-grains but they are almost entirely sterile without exception, even though these pollen-grains functioned to produce hybrid seeds when pollinated to the flower *H. esculentus* (back-cross) and to back-crossed plant (*H. esculentus* × F₁).

From the beginning of summer, all these F₁ plants had flowered abundantly and produced many fully developed pollen-grains but, as has been mentioned, some percentage of abortive pollens was included among them. The F₁ plants were not isolated either from *H. esculentus* or *H. Manihot* but grew along with both species and blossomed in the same season. At the beginning of the flowering time almost all of the capsules after flowering quickly turned brown and soon shrivelled. They were found to be completly empty. Some capsules swelled a little and produced only minute seeds. After the middle age of the plant the capsules were developed normally and some of them contained a few well formed

seeds, but only a very small quantity. Generally the capsules contained numerous empty unfertile small grains. Results of self-pollination were also examined. Thirty flowers were bagged and pollinated with their own pollen. The capsules developed by this pollination produced no seeds.

Two hundred and ninety-nine capsules secured from 27 F_1 plants were examined, in 1927 among which only 19 capsules produced one grain each in open pollination. Hence the total number of 19 seeds was secured. The average number of ovules in 20 capsules was 96.47. The percentage of fertility calculated from these data was 0.066 per cent. This is a surprisingly small value compared with that of *H. esculentus*, 80.69 per cent and that of *H. Manihot*, 85.84 per cent. Tables 34 and 35 show in summary form the results of these calculations.

In 1928, 89 individual plants of F_1 hybrid were examined and out of 946 capsules only 69 contained one or two grains each and a total of 73 seeds was obtained. This corresponds to 0.077 per capsule and 0.08 per cent of the total number of embryos (Table 37). In 1929 out of 1485 capsules which were examined from 38 F_1 plants only 83 seeds were secured, one or three seeds per capsule. The fertility of F_1 plants in this year was from 0 to 0.101 per cent (Table 36 and 37).

Table 34. *Number of fertile and abortive seeds per plant of F_1 hybrid obtained in 1927.*

Plant number	No. of capsules examined	No. of capsules producing seeds	Number of fertile seeds	Number of small abortive seeds			
				Max.	Min.	Av.	Total
A–1	13	2	2	81	0	32.9	428
A–2	10	1	1	51	0	22.3	223
A–3	7	0	0	74	0	35.9	251
A–4	8	1	1	76	0	22.5	180
B–1	8	0	0	85	0	26.6	213
B–2	8	0	0	72	0	27.1	217
B–3	10	0	0	82	0	32.2	322
B–4	10	1	1	80	0	60.4	604
B–5	9	2	2	77	0	33.0	297
B–6	11	0	0	70	0	44.0	484
B–7	24	1	1	74	0	19.3	464
B–8	12	0	0	38	0	7.5	90
B–9	16	1	1	87	0	23.8	380
B–10	11	0	0	60	0	14.4	158

Plant number	No. of cupsules examined	No. of capsules producing seeds	Number of fertile seeds	Number of small abortive seeds			
				Max.	Min.	Av.	Total
D-1	15	1	1	83	0	37.8	567
D-2	4	0	0	61	1	16.8	97
D-3	9	1	1	77	0	34.4	310
D-4	7	0	0	66	0	46.9	258
D-5	10	2	2	70	0	21.8	218
D-6	10	1	1	62	0	34.1	341
D-7	9	1	1	35	0	9.2	83
D-8	13	0	0	74	0	19.8	257
D-9	17	0	0	65	0	9.2	157
D-10	9	0	0	69	0	29.9	269
D-11	13	1	1	59	0	18.4	239
D-12	10	1	1	71	3	41.2	412
E-1	16	2	2	94	0	29.5	472
Total or av.	299	19	19	70.1	0	38.99	7961

Table 35. *Fertility of parents and F_1 hybrid (1927 test)*.

Plants	No. of ribs in capsule	No. of capsule examined	No. of ovules (Av.)	Average number of seeds		Fertility (%)
				Fertile	Unfertile	
H. esculentus	6	20	94.1	83.15	10.95	88.36
	7	20	111.3	87.65	23.65	78.75
	8	20	118.9	95.05	23.80	79.94
	9	20	134.7	102.90	32.70	75.72
	Av.					80.69
H. Manihot	5	20	90.4	77.60	12.75	85.84
F_1 hybrid	5	299	96.47	0.064	96.406	0.066

Table 36.   *Seeds per plant of F_1 hybrid (1929 test).*

Crosses	Number of plant	Number of capsules examined	Number of capsules producing seed	Number of fertile seeds	Min. No. of fertile seeds per capsule	Max. No. of fertile seeds per capsule
F_1 hybrid White long × H. Manihot	20	928	20	24	0	3
F_1 hybrid White velvet × H. Manihot	15	503	49	59	3	3
F_1 hybrid Blue long × H. Manihot	2	49	0	0	0	0
F_1 hybrid Dwarf pro. × H. Manihot	1	5	0	0	0	0

Table 37.   *Fertility of F_1 hybrid (1928 and 1929 test).*

Crosses	No. of plant	No. of capsules examined	No. of fertile seeds	Av. No. of seeds per plant	Av. No. of fertile seeds per capsule	Fertility (%)
1928 White long × H. Manihot	89	946	73	0.82	0.077	0.080
1929 White long × H. Manihot	20	928	24	1.20	0.026	0.027
White velvet × H. Manihot	15	503	69	3.93	0.117	0.101
Blue long B × H. Manihot	2	49	0	0	0	0.000
Dwarf prolific × H. Manihot	1	5	0	0	0	0.000
Total	38	1485	83			

It is a well known fact that wide species or genus crosses in plants result in partially or completely sterile F_1 plants and that there are some variations in the sterility of F_2 plants.   Some investigators have reported that the F_1 plants were completely sterile and other investigators found in the partially sterile F_1 plants all degrees of sterility ranging from plants which never pass to the rosette stage, to those quite as fertile as the parents. F_1 plants between *Aegilops* and a certain variety of *Triticum vulgare* were reported as completely sterile by SAX and SAX (1924) and by JOHN PERCIVAL (1926) and as highly sterile, though not completely so by KAGAWA (1928).   KARPECHEKO (1928) and MORINAGA (1928) observed the *Raphanus-Brassica* F_1 to be almost entirely sterile but very few of them were developed.   The hybrid between the cultivated sugarbeet and *Beta trigyna* were reported by TSCHERMACK (1927) as almost completely sterile.   The only exception has been reported by MORINAGA (1928) who obtained hybrids between ten chromosome species of Brassica which showed neither abnormal pollen-grains, nor any decrease in fertility.   NEWTON's experiments (1928) showed in the case of species hybrid *Digitalis purpurea* × *D. ambigua* that two plants among the hybrid showed a low degree of fertility, while from the others no seeds were obtained.   In he writer's material, among all the F_1 plants no plant was as fertile as the parental species and it appears probable that all of the F_1 plants should be almost entirely sterile, although a single plant produced 12 seeds in 1929.   The author did not observe any peculiar differences in fertility between them.   The degree of sterility, as indicated by the grains per plant of 1927 and 1929 are as follows in frequency distribution :

No. of seeds	0	1	2	3	4	5	6	7	8	9	10	11	12	Total
No. of plants	24	17	8	5	1	4	0	1	0	0	0	1	1	62

It appears that there is some variation in the sterility of F_1 plants, but we can not notice any definite differences among them. F_1 plants were apparently quite uniform and the variation in seed produced in each plant may not be due to the differences of gametic construction.   The irregular distribution of the fertile seed over the plant is admissible regarding the formation of fertile spore as purely a matter of chance.   Some F_1 plants produced not more than 12 grains but 1 or 2 seeds were usually obtained in a fertile capsule.   These fertile seeds were not aggregated in a mass but were separated by infertile ovules.

EAST (1921) obtaind from F_1 hybrid of *Nicotiana paniculata* × *N. rustica* only from 1 to 30 seeds per capsule when crossed *inter se* or when

self-pollinated, even though considerable quantities of pollen were applied. No greater quantities of seed were obtained when pollen-grains of F_1 plants were applied to either parent, nor when pollen-grains of either parent were applied to them. Accordingly EAST considered this sterility to be equally of ovules and of pollen. It is doubtful that the sterility of F_1 plants in *H. esculentus* × *H. Manihot* is equally due to a sterility of ovules and of pollen-grains, for a great quantity of seeds was produced when the pollen of F_1 plants was applied to the mother plant but no seed were produced when the pollen of either parent was applied to F_1 palents.

As to the causes of sterility of F_1 plants, a study in view of the chromosome relationships should be of value. THOMPSON and CAMERON (1928) concluded in their study of wheat species hybrid (14×21) "There are maked differences between male and female gametes of F_1 the latter showing less elimination of intermediate number". The sterility of F_1 plants of *H. esculentus* × *H. Manihot* can be attributed, in general, to incompatibility of parental chromosomes and to differences in parental chromosome number which give rise to certain unbalanced univalent chromosomes at the time of meiotic division. And the functional pollen-grains show the main cause of sterility of F_1 plants to exist in the female side.

In general, that sterility increases in proportion to the univalent chromosomes, is a well known fact. SAX (1923) has suggested in his studies of wheat species hybrids, that the sterility in the F_1 and later generations can be attributed to the unbalanced numerical relation of the chromosomes, due to the random distribution of the univalents and to incompatibility of certain chromosome combinations even among the bivalents. He also indicated, in wheat, that the sterility is greater when the proportion of univalents to bivalent chromosomes is $1 : 1$ than when the ratio is $1 : 2$. Similar facts were demonstrated in *Oenothera* and *Datura*. In the hybrids of wheat, it is now known that the gametes with an intermediate chromosome number are more or less sterile and the gametes which have a multiple of 7 are functional. TSCHERMAK (1914) and KIHARA (1924) have reported on the sterility of F_1 hybrid between several combinations of *Triticum*-species. They have discussed the degree of sterility and chromosome relations. KIHARA wrote as follows: "Ich habe mich schon oben dahin geäussert, dass in diesem Bastarde die Sterilität und die Fertilität hauptsächlich vhn Kombinationsweise der 7 überschüssigen Dinkelchromosomen in den Zygoten abhänging sind. Die 28- und 42- chromosomigen Nachkommen von Triticum durum × vulgare und T. polonicum × Spelta waren demnach alle fertil.

Ein Abkömmling von T. polonicum × compactum war aber fast vüllig

steril. Die Kombination der Kreuzungen bedingt daher in den pentaploiden Bastardnachkommen grosse Unterschiede des Fertilitätsgrades." GOODSPEED and CLAUSEN (1922) studied F_1 and back-crossed *Nicotiana* and made an explanation, that in the formation of the gametes of hybrid plants there is a random assortment of chromosomes derived from both parents. Among these gametes only those which had a predominance of chromosomes of one or other parental type were functional. In the case of *Nicotiana* (GOOD-SPEED, CLAUSEN and others, 1926) the supposition is that the two sets of homologous chromosomes derived from both species are conjugated at the meiotic division of the F_1 hybrid leaving other chromosomes un-paired. When the hybrid is pollinated by either parental species only those gametes may function which have none or all of the unpaired chromosomes. In some instances there is objective evidence for this assumption. In the case of F_1 *paniculata-rustica* hybrid (LAMMERTS, 1929) the variable gametes apparently represented a random sample as respects the chromosome num-ber of the entire series. In general odd multiple polyploids are relatively infertile with some exception. NISHIYAMA (1929) in his study of oat hybrid concluded that the triploid and pentaploid oat hybrids under consideration are highly sterile but not completely, and that the hexaploid hybrids show complete fertility.

(13) Comparison of pollen-grains of F_1 hybrid with those of parental species

It is a matter of first importance in a study of sterile hybrids and in-compatible matings to know how many abortive pollen-grains there are and how far the normal appearing pollen-grains produced by these plants are capable of functioning in reproduction. The morphology of pollen-grains is important in giving hints on the nature of reduction division. The correlation between sterility in a plant and poor or abortive pollen-grains should also be studied, remembering also that the apparent morphological perfection in a pollen-grain is not always to be construed as positive evi-dence of ability to function.

In order to compare the characters of pollen-grains in parental species and F_1 hybrid the author calculated in the first instance the abortive pollen-grains and lastly examined the germinability of the pollen-grains in relation to their size and pollen-tube growth in natural state. These investigations were carried out during the summer of 1927.

a) *Percentage of abortive pollen-grains in parental species and F_1 hybrid*

The pollen-grains of F_1 hybrid develop normally for a time; the large

number of small abortive pollen-grains are found later on.   At the time of
the dehiscence of the anthers the pollen was shaken out on the slide-glass
by filliping slightly with a pincette.   Random samples were taken by measu-
ring the pollen-grains in the fields taken in definite areas across the slide.
Calculations were made of the number of large round and small abortive
pollen-grains.

Abortive pollen-grains are very small in size ranging from 20 to 50
microns in diameter, deep yellow in colour and they are non-functional,
(Plate VII, Fig. 3).   In shaking out the mature pollen-grains the heavier
ones will come out more readily than the small abortive ones so that the
absolute percentage of abortive pollen cannot be determined by this method
but for the purpose of comparison it may be quite satisfactory.   The data
on which these percentages are based are brought together in Table 38.

Table 38.   *Percentage of abortive pollen-grains in parents
and F_1 hybrid.*

Plant number		Number of pollen grains counted			% of abortive pollen-grains
		Total	Normal	Abortive	
♀ P H. esculentus	No. 1	299	296	3	1.00
	No. 2	81	78	3	3.70
	No. 3	143	138	5	3.50
	No. 4	630	621	9	1.43
	No. 5	397	486	11	2.77
	No. 6	508	504	4	0.79
	No. 7	303	303	0	0.00
Total or av.		2361	2326	35	1.884±0.340
♂ P H. Manihot	No. 1	256	248	8	3.13
	No. 2	133	130	3	2.26
	No. 3	716	700	16	2.23
	No. 4	734	709	25	3.41
	No. 5	207	203	4	1.93
	No. 6	235	230	5	2.13
	No. 7	79	79	3	3.80
Total or av.		2360	2296	64	2.70±0.173

Plant number		Number of pollen-grains counted			% of abortive pollen-grains
		Total	Normal	Abortive	
F$_1$ hybrid	No. 1	313	230	83	26.52
	No. 2	200	171	29	14.50
	No. 3	340	257	83	24.41
	No. 4	487	389	98	20.12
Total or av.		1340	1047	293	21.39±1.568

It will be observed that less than 3 per cent of the pollen-grains from the parental plants are abortive, on the other hand, over 20 per cent are obviously non-functional in F$_1$ hybrid. It is probable that the formation of abortive pollen-grains may be partly caused by environmental conditions, e. g. high temperature, as HILBORN (1928) observed in Apple varieties.

The abortive pollen in F$_1$ plants of interspecific hybrid has been observed by many investigators. KIHARA (1921) has reported that about 20 per cent of the pollen-grains of F$_1$ hybrid resulting from Emmer group-Vulgare group wheat crosses are obviously imperfect as indicated by their smaller size and meager contents. NINA MEISTER and TJUMJAKOFF (1928) have stated in their studies of F$_1$ hybrid of rye × wheat that they observed out of 31 crosses, 0–1 per cent of normal pollen-grain in 18 crossed, 2–8 per cent in 6 crosses, 4–5 per cent in 6 crosses, 7.4 per cent in 1 cross. KARPECHENKO (1928) found in his study of F$_1$ hybrid between *Raphanus sativus* L. and *Brassica oleracea* L. a majority of degenerated pollen-grains and only a small number of more or less normally developed pollen-grains. HOEPPENER and RENNER (1928) in *Oenothera biennis* × *ammophila*, MATSUDA (1927) in hybrid *Petunia*, BRINK (1927) in F$_1$ hybrid between *Linaria vulgaris* L. and *L. purpurea* have similarly observed the abortive pollen-grains.

The pollen of the author's F$_1$ hybrid is non-functional with the female gamete of its own plants, but many fertile seeds were obtained by mating it to the flower of the female parent (*H. esculentus*). From this relation of compatibility of gametes we may say that there is no decided relation between the percentage of normally developed pollen and the fertility of plants nor between germinability and fertility. Different investigators have also called attention to the possibility that normal appearance may not necessarily indicate functional reproduction. EAST (1921) has stated that 20–70 per cent of the apparently normal grains formed by a partially sterile

hybrid between *Nicotiana rustica* and *N. paniculata* failed to extrude pollen-tubes under conditions which allowed about 80 per cent germination of the parental pollen. WATKINS (1925) has reported that in certain wheat hybrids only about 10 per cent of the morphologically perfect pollen-grains are capable of germinating upon the stigma. He considers that failure to set seed in these plants is largely caused by a lack of functionable pollen.

b) *Variation in pollen-grain size*

The pollen was examined in dry condition under a micrometer with the aid of an ocular micrometer giving a magnification of 120 diameters. In all cases only the apparently good pollen-grains were used. Apparently environmental conditions, especially moisture, may cause differences in pollen-grain size which is much increased after rainfall. To avoid the trouble of keeping pollens-grains in a desiccator 4–5 hours to make its form constant in size, the measurements were made on the pollen-grains which were secured on fine days after 3 or 4 consecutive fine days. Table 39 shows the relative variability in size of pollen of the parents and F_1 hybrid. Among the well-developed round pollen-grains large elliptical ones were sometimes observed. Only the longer axis was measured. The comparison of pollen-grains in mean, standard deviation and coefficient of variability is shown in Table 40.

Table 39. *Variability in size of pollen-grains of parents and F_1 hybrids in dry condition.*

	Plant number	Diameter of pollen (Class center)													Total
		11.0	11.5	12.0	12.5	13.0	13.5	14.0	14.5	15.0	15.5	16.0	16.5	17.0 17.5	
H. esculentus	No. 1	3	4	6	25	49	50	6							143
	No. 2			3	21	85	59	12							180
	No. 3		1	5	16	48	31	4	3						108
	No. 4	1	8	10	38	57	25	6							145
	Total	4	13	24	100	239	165	28	3						576
H. Manihot	No. 1			4	17	94	75	13							203
	No. 2	1	13	27	68	78	41	2							230
	No. 3			1	2	13	32	20	8						76
	Total	1	18	46	175	185	74	10							509

	Plant number	Diameter of pollen (Class center)														Total
		11.0	11.5	12.0	12.5	13.0	13.5	14.0	14.5	15.0	15.5	16.0	16.5	17.0	19.5	
F₁ hybrid	No. 1						1	5	6	34	56	72	56	21	2	253
	No. 2						7	16	17	46	53	24	7			170
	No. 3						3	15	23	43	56	28	8	1		177
	Total						11	36	46	123	165	124	71	22	2	600

(1 unit of class value corresponding to an absolute size of 6.85 microns)

Table 40.   *Summary of Mean, Standard deviation and Coefficient of variability in size of pollen-grains of parents and F₁ hybrid plants.*

Plants	Total	Mean	STD. DEV.	C. V.
		Micron	Micron	%
H. esculentus	576	89.21±0.105	±3.72 ±0.074	4.17±0.083
H. Manihot	509	87.50±0.106	±3.54 ±0.075	4.05±0.086
F₁ hybrid	600	106.02±0.146	±5.33 ±0.103	5.00±0.097

In the first place it is to be noticed that the pollen-grains of F_1 hybrid show probably significant differences not only in mean diameter but also in variability as may be seen in standard deviation and coefficient of variability.   Pollen-grains of *H. esculentus* are slightly larger and more variable than those of *H. Manihot*, but the difference is not significant. When the pollen of F_1 hybrid is compared with the mean value of pollen diameter of both parents it is seen to be about 1.2 times as large as that of parents.   So far as their genetic composition is concerned, the F_1 pollen-grains are certainly bigger and more diverse than those of the parents.   The size of pollen-grains depends mostly upon the chromosome number contained.   The cytological facts which will be described later might help to explain this situation.

c) *Increasing size of pollen-grain when mounted in white of an egg*

When the pollen-grains of these plants are embedded in water or a sugar solution, even 60 per cent, they all expand to a maximum size by the absorption of water, then immediately burst.   In the white of an egg

the pollen-grains absorb water slowly until they reach their maximum size and still remain in perfection. The ratio of swelling of pollen-grains in white of an egg is conspicuously different in these species and F_1 hybrid. Tables 41 and 42 show the relative variability in size of pollen-grains of these plants after two hours from the time of mounting in white of an egg.

Table 41. *Variation in pollen diameter of parents and F_1 hybrid when embedded in the white of an egg and increased in volume to the maximum size.*

Plants	Diameter of pollen-grains																		N
	12.0	12.5	13.0	13.5	14.0	14.5	15.0	15.5	16.0	16.5	17.0	17.5	18.0	18.5	19.0	19.5	20.0	20.5	
H. escu.						8	45	94	105	40	8								300
H. Mani.	13	26	90	86	67	14	4												300
F_1											16	24	60	69	60	35	28	8	300

(1 unit of class value=6.85 microns)

Table 42. *Mean, Standard deviation and Coefficient of variability of pollen diameter of parents and F_1 hybrid when embedded in the white of an egg and increased in their volume to maximum size.*

Plants	Total	Mean	STD. DEV.	C. V
		Micron	Micron	%
H. esculentus	300	107.87 ± 0.143	$\pm 3.66 \pm 0.101$	3.39 ± 0.093
H. Manihot	300	91.63 ± 0.164	$\pm 4.21 \pm 0.116$	4.5 ± 0.126
F_1 hybrid	300	127.78 ± 0.225	$\pm 5.78 \pm 0.226$	4.52 ± 0.124

More accurate data showing the true relations would be afforded if volume or superficial area of the pollen were used rather than diameter in expressing the size. Table 43 shows thus calculated values, in which superficial area and volumes were calculated according to the formulae $4\pi r^2$ and $\frac{4}{3}\pi r^3$ respectively.

Table 43. *Ratio of increase in diameter, superficial area and volume of the pollen-grains in parents and F_1 plants when embedded in the white of an egg.*

	Plants	Not embedded	Embedded	Increasing ratio	
				Not embedded	Embedded
Mean diameter (Micron)	H. esculentus	89.21	107.87	100	120.92
	H. Manihot	87.50	91.53	100	104.72
	F_1 hybrid	106.02	127.78	100	120.52
Mean superficial area (Square micron)	H. esculentus	25,044.8	36,555.7	100	145.96
	H. Manihot	24,052.1	26,376.9	100	109.67
	F_1 hybrid	25,312.6	51,296.0	100	145.26
Mean volume (Cubic micron)	H. esculentus	372,669.0	657,198.0	100	176.35
	H. Manihot	350,745.0	402,829.0	100	114.85
	F_1 hybrid	623,980.0	1092,401.0	100	175.07

From Table 43 it will be noticed that the ratio of increase in diameter of pollen-grain when embedded in white of an egg is no more than 4.73 per cent in *H. esculentus*, while that of *H. esculentus* and F_1 hybrid is above 20 per cent. The superficial area and volume of pollen-grains indicate still more significant differentiations. In superficial area the pollen-grains of *H. Manihot* are increased only 9.67 per cent while those of *H. esculentus* and F_1 hybrid are increased above 45 per cent. In volume of pollen-grains the ratio of increase of *H. esculentus* and F_1 hybrid is above 75 per cent while that of *H. Manihot* is no more than 14.85 per cent. From these data it is evident that increase in volume under the moist condition is a specially provided character of pollen-grains in each of these species. In this case the increase of volume above 75 per cent in moist condition is a character of *H. esculents* which is transmitted to the F_1 hpbrid as a complete dominant character. In connection with this table it may also be noticed that the volume of F_1 pollen is 623,980 cubic microns in dry condition, this is 172.2 per cent of the average corresponding measurement of the two parents. The volume of F_1 pollen-grain at maximum size in white of an egg is 1,092,401 cubic microns which is 206.1 per cent of the average maximum volume of the two parents.

The pollen-grains are especially good for testing indirectly the chromo-

some number and behavior of chromosomes in reduction division, although it is evident that the pollen-grain size is influenced by several developmental factors. There is a correlation between chromosome number and size of the pollen-grains and between irregular reduction division and the uniformity of size. A number of cases are known where larger nuclei and cells contain a larger number of chromosomes. BLAKESLEE (1922) found that polyploid *Datura* show larger nuclei and cells than the diploid original forms. SAX (1922) has concluded that the size of the pollen-grains is closely correlated with the chromosome number in the various species of wheat and oats, and the pollen-grains of fertile species hybrids are more variable than the pollen-grains of the parental species due to various degrees of compatibility of the combination of non-homologous chromosomes in the gametophytic generation. MÜNTZE (1927) has studied the chromosome number, nuclear volume and pollen-grain size in *Galeopsis* and conculuded; "The 16 chromosome species have larger nuclei, pollen mother-cell and pollen-grains than the other species (8) of the genus." This close relation between chromosome number and nuclear volume has been found also by many other investigators, CLAUSEN and GOODSPEED (1925) in *Nicotiana*, CLAUSEN J. (1926) in *Viola*, KAGAWA (1928) in *Aegilops* species and others.

d) *Germination of pollen-grains in parents and F_1 hybrid*

The pollen-grains of these plants absorb water quickly in water or in dilute (up to 60 per cent) sugar solution and within a few seconds or a few minutes they are broken. Sugar solution to which has been added 10–20 per cent of gelatin, honey or glycerine was used for germination bed in moist chamber or hanging drop cultures, but all of these methods were unsuccessful and none of the pollen-grains were germinated upon these media. The another medium of germination bed which was tried was stem juice (Mucus) of tissue extracted from these plants. This stem juice is a very viscous liquid and it is not easy to keep it in constant moisture, for in a moist chamber the mucus absorbs water quickly and furnishes it to the pollen-grains excessively; in a dry chamber it loses water immediately. Finally attempt was made to germinate the pollen-grains of these plants on a longitudinal thin section, about 0.2–0.5 mm. in thickness, of style of flower. The pollen-grains were sown on these sections arranged on the slide glass and were kept in the moist chamber. The water to supply moisture in chamber was placed in the bottom of each dish. Pollen for germination was obtained from ripe anthers which were allowed to dehisce in the open air. The germination of pollen-grains occurred within a few

minutes. It was found that this method is successful. The central pith of the style of these flowers seems to have some chemical composition which stimulates the pollen-grain to germinate. Thus the style section was generally the most favourable for the germination of pollen of *H. esculentus*, *H. Manihot* and F_1 hybrid and it is a rapid method determine merely the germinability of pollen-grains in these plants. However, in general, it was not reliable for measuring the pollen-tube length exactly, for all parts of the style are not similar in character as media for the germination test and irregular development of pollen-tubes may occur.

The germination was completed within 20 minutes in the laboratory at the temperature of $30°-31°$ C, hence germination counts were made after the laspe of 20 minutes with a microscope under a magnification of 120 diameters. The pollen-tubes were stained with 0.5 per cent solution of cotton blue in lactic acid to facilitate the counting, and the total number of pollen-grains and number of germinated pollen-grains were recorded. Table 44 shows the results of germination test of parents and F_1 hybrid on the style section of the same flower and of the other species.

Table 44. *Percentage of germinated pollen-grains of parents and F_1 hybrid on the style section of the same flower and that of another species.*

	Total number of pollen-grains	Germinated	Ungerminated or broken	% of germinated pollen
Pollen-grains of H. esculentus on the style section of the same flower	577	554	23	96.01
Pollen-grains of H. esculentus on the flower of H. Manihot	369	345	24	93.50
Pollen-grains of H. Manihot on the same flower	423	187	236	44.21
Pollen-grains of H. Manihot on the flower of H. esculentus	763	384	379	50.33
Pollon-grains of F_1 hybrid on the same flower	535	467	68	87.29

	Total number of pollen-grains	Germinated	Ungerminated or broken	% of germinated pollen
Pollen-grains F₁ hybrid on the flower of H. esculentus	808	534	274	66.09
Pollen-grains of F₁ hybrid on the flower of H. Manihot	280	160	120	57.14

From this table it will be noted that the pollen germinability of *H. esculentus* is higher in every case than that of *H. Manihot* and F_1 hybrid, even though the esculentus pollen-grains are non-functional when pollinated to the flower of *H. Manihot*. The relatively larger style of *H. esculentus* seems to afford better conditions for germination than the smaller style of *H. Manihot*. The germination percentage of *Manihot* pollen-grain is 44.21 on the section of its own style and 50.33 on the style section of *esculentus*. This is nearly half the number of *H. esculentus* on the average. The cause of increase in the percentage of germination on the *esculentus* section may be the same condition as in the former case. The germination of F_1 hybrid pollen-grains is quite remarkable notwithstanding they showed totally sterile when pollinated on the same flower. The round pollen-grains of F_1 hybrid are germinable on the section of their own style up to 87.29 per cent, on the *esculentus* and *Manihot* style section to 66.09 and 57.14 per cent respectively. As a whole the percentage of germination of F_1 hybrid pollen is intermediate between the two parents and it is apparent that there is no relation between pollen-grain germinability and the function of pollen-grains to produce seeds.

e) *Germination percentage of pollen-grains of F_1 hybrid and the relation between size and germinability*

These experiments were arranged so that the relation between size and germinability could be determined as well as the germination percentage of a random sample of normally developed pollen-grains. The pollen-grains of F_1 plants were taken at random and arranged singly in lineal series on a thin section of flower style taken from *H. esculentus*. They were arranged in order in lines each containing 6 grains distributed in such a way that each pollen-grain could be identified with certainty. The diameter of each grain was then promptly measured with the aid of an ocular micrometer giving a magnification of 120 diameters in the same way as des-

cribed before. After a period of about 20 minutes the cultures were examined. During the incubation period the cultures were closed in moist chamber.

Table 45. *Relation between size and germinability of pollen-grains in F_1 hybrid.*

	Plant number	Diameter of pollen-grain														Total
		12.0	12.5	13.0	13.5	14.0	14.5	15.0	15.5	16.0	16.5	17.0	17.5	18.0	18.5	
Variation of pollen-grains tested	No. 1		2	0	2	2	5	20	14	29	12	4	2	1	1	94
	No. 2		1	2	0	4	3	12	14	16	12	14	5	2	1	86
	No. 3			1	5	5	7	22	20	24	16	11	5	1		117
	No. 4	4	0	4	1	6	4	16	14	33	31	26	11	4		154
	No. 5			1	3	3	3	9	13	28	20	21	10	3		114
	No. 6				1	1	11	9	12	12	12	3	2	2		65
	Total	4	3	8	11	21	23	90	84	142	103	88	36	13	4	630
Variation of pollen-grains germinated	No. 1					1	2	4	8	20	8	4	2	1	1	51
	No. 2							2	5	3	6	7	3	1	1	28
	No. 3							4	3	4	7	5				23
	No. 4						1	7	5	18	18	21	5	2		77
	No. 5					1	1	1	7	16	8	14	4	3		55
	No. 6							8	6	8	11	7	2	1	2	45
	Total					2	4	26	34	69	58	58	16	8	4	279
Percentage of germinated pollen grains		0	0	0	0	0.95	1.74	28.89	40.48	48.59	56.31	65.91	44.44	61.54	100.00	

(1 unit of class value = 6.85 microns)

In such a way 630 pollen-grains were tested under observation in the laboratory at a temperature of 30°–32° C. The average percentage of germination in this total of 630 pollen-grains was 44.29 per cent. Presumably the special treatment of the pollen-grains by arranging in linear order is a cause of the decrease in the percentage of germination compared with that of data indicated in Table 44.

The relation between the size of pollen and germinability is likewise of much interest. As seen in Table 45, as a whole, the percentage of germination increases as the diameter of the pollen-grain rises. The small

pollen-grains show a low percentage of germination, namely 26 grains below class 13.5 (92.48 microns) entirely failed to germinate; only two grains out of 21 of class 14.0 (95.9 microns) were germinated. Thereafter the percentage of germination rose slowly until it reached class 17 (115.45 microns), 65.91 per cent germination being attained. In this class among the 88 grains tested 58 grains were germinated. From this point the percentage of germination decreased slightly. The decrease in germination exhibited by the largest grains is of much interest in connection with the genetical construction of germinal substance. The giant pollen-grains containing a surplus of chromosomes are elliptical in form, germinability is low and they break easily without germination. Table 46 shows the variation of germinability in relation to diameter of pollen-grains arranging the data of Table 45 in fewer classes to simplify it.

Table 46. *Simplified variation table of germinability as related to the diameter of pollen-grains.*

	Diameter of pollen-grains					
	−140.1	14.5–15.0	15.5–16.0	16.5–17.0	17.5–18.5	Total
No. of pollen-grains tested	47	113	226	191	53	630
No. of pollen-grains germinated	2	30	103	116	28	279
Percentage	42.6	20.55	45.58	67.84	52.83	44.29

It is a well known fact that the big pollen-grains which have surplus chromosome number as the result of an irregular meiotic division are apt to be non-functional, while the big pollen which contains a multiple number of chromosomes are often proved to be fertile. MATSUDA (1927) observed in *Petunia* that the percentage of germination in the giant pollen-grains are smaller than in the normal. BUCHHOLZ and BLAKESLEE (1929) have studied pollen-tube growth in crosses of *Datura stramonium* and proposed three hypotheses to explain the nature of the ungerminated portion of the pollen from haploids $(n = 12)$; (1) Some may contain new genes which are lethal in the male gametophytic stage, (2) some may be pollen-grains with 13 chromosomes, (3) some may be pollen-grains with 11 chromosomes arising from pseudo-reduction.

f) Pollen-tube growth in parents and F_1 hybrid

As already noted none of the crosses in which *H. Manihot* or F_1 hybrid were used as a female parent and pollinated with the pollen of *H. esculentus* and F_1 hybrid were successful. Moreover, selective fertilization exists when a mixture of approximately equal quantity of pollen-grains taken from the flower of *H. esculentus* and *H. Manihot* were dusted on the *esculentus* flower. Between the germinable pollen-grains produced by *H. esculentus* and F_1 hybrid marked differences may exist in ability to carry out the further process required in delivering the male gametes to the embryosac of *H. Manihot* or F_1 hybrid. If such a difference does exist, the differential fertility of these plants could be explained in several ways. We may suppose that the gametes having different genetic constitution produced by these two species and F_1 hybrid may be unequal in their ability to reach the ovule and accomplish fertilization. Hence in seeking an explanation of cross-sterility and self-sterility of these plants, the possibility of a difference in pollen-tube development and rapidity of growth of each pollen-grain on the flower of each plant is the first point to be considered expecting to find some correlation, indirectly, between the gametic difference and physiological differences in growth rate of the pollen-tube in its own and different somatic tissue.

During the summer of 1928 the pollen-tube growth was studied carefully in both compatible and incompatible matings of these two species and F_1 hybrid. Abundant pollen-grains of the parents and F_1 hybrid were sown on the stigma of each flower which was castrated and covered with a bag one day before flowering.

The pollen-grains sown on the stigma began to germinate within a few minutes and the pollen-tube developed along the central pith of the style toward the embryosac. The styles of flowers used in cultivation of of the pollen-grains were cut off at intervals of 40, 60 and 80 minutes after the pollens were deposited (sometimes after 50, 70, 100, 120 minutes), then treated with aceto-alcohol solution (Abs. alcohol 3 + glacial acetic acid 1) for a few minutes. Next the central pith of the style was reduced to a thin slice with a needle after washing it out in the water. These slices were examined after being stained with cotton blue solution (Cotton blue 0.5 + lactic acitic 100). Measurements were made only of the pollen-tubes distinguishable from each other. The results of these measurements and the air temperature at the time these experiments were carried out are summarized in Table 47.

Table 47. *Comparative growth rate of pollen-tubes.*

		Time after pollination (minutes)	Date	Temp. (C)	Weather	Number of pollen-tubes	Av. length of pollen-tubes	Style length	% of pollen-tube length to style length	Remarks
							(mm.)	(mm.)		
Pollen-grains of H. esculentus	Pollinated on the flower of H. esculentus	40	8.7	31.7	Clear	21	8.06	20.90	38.56	
		60	8.7	31.7	Clear	15	16.13	20.80	77.55	
		80	8.7	31.7	Clear	35	22.00	22.00	100.00	All reached an ovary
	Pollinated on the flower of H. Manihot	40	7.31	26.5	Cloudy	5	7.00	20.30	34.48	
		60	7.25	25.0	Cloudy	24	10.50	19.00	55.26	
		80	7.31	26.5	Cloudy	13	13.69	18.60	73.60	
		100	7.31	26.5	Cloudy	20	18.18	18.30	99.34	14 reached an ovary
	Pollinated on the flower of F_1 hybrid	40	7.29	30.0	Clear	26	7.61	22.20	34.28	
		60	7.29	30.0	Clear	15	11.66	23.30	50.04	
		80	7.29	30.0	Clear	13	16.32	22.50	72.53	
		100	7.29	30.0	Clear	13	22.50	22.50	100.00	All reached an ovary
Pollen-grains of H. Manihot	Pollinated on the flower of H. Manihot	40	7.21	25.0	Cloudy	13	5.15	18.00	28.61	
		60	7.21	25.0	Cloudy	19	12.16	19.40	62.68	
		80	7.21	25.0	Cloudy	28	17.22	17.40	98.97	24 reached an ovary
	Pollinated on the flower of H. esculentus	40	8.7	32.0	Clear	28	9.14	21.50	42.51	
		60	8.7	32.0	Clear	10	12.08	20.50	58.93	

		Time after pollinat'on (minutes)	Date	Temp. (C)	Weather	Number of pollen-tubes	Av. length of pollen-tubes	Style length	% of pollen-tube length to style length	Remarks
							(mm.)	(mm.)		
		80	8.7	32.0	Clear	8	15.88	21.60	73.52	
		100	8.7	32.0	Clear	17	21.20	22.80	92.98	
	Pollinated on the flower of F_1 hybrid	40	7.30	27.0	Cloudy	17	5.95	26.00	22.88	
		60	7.30	27.0	Cloudy	21	12.50	23.70	52.74	
		80	7.30	26.0	Cloudy	20	17.07	27.10	62.99	
		100	7.30	26.0	Cloudy	6	22.35	23.40	95.51	2 reached an ovary
	Pollinated on the flower of F_1 hybrid	40	7.30	27.0	Clear	11	6.82	24.60	27.72	
		60	7.25	33.5	Clear	21	13.32	25.30	52.65	2 reached an ovary
		80	7.26	30.0	Clear	23	20.58	25.70	80.08	
		100	7.26	30.0	Clear	17	26.26	26.90	97.62	13 reached an ovary
Pollen-grains of F_1 hybrid	Pollinated on the flower of H. esculentus	40	8.10	27.0	Clear	16	9.29	19.60	47.40	
		60	8.8	30.0	Clear	14	15.78	20.20	68.12	1 reached an ovary
		80	8.16	32.5	Clear	9	19.98	20.80	96.06	
		100	8.9	28.0	Clear	10	19.40	19.40	100.00	All reached an ovary
	Pollinated on the flower of H. Manihot	40	7.21	25.0	Cloudy	18	7.00	18.20	38.46	
		60	7.21	25.0	Cloudy	20	11.31	18.20	62.11	
		80	7.21	26.0	Cloudy	20	17.81	18.50	96.27	6 reached an ovary

From Table 47 it is seen that when pollination was made between these plants at the height of flowering season almost all of the pollen-tubes grew within only 100 minutes across the whole length of the style. The pollen-tube growth in every case was at the same rate and there was no such marked differences as observed by EAST and MANGELSDORF (1926) in *Nicotiana* species. They observed the rapidly accelerated rate of growth which permits fertilization in the compatible mating of *Nicotiana* species and a slower and more nearly uniform rate of growth which ordinarily does not allow the generative nucleus to reach the micropyle before the flower withers and falls in the incompatible matings of *Nicotiana* species. It is noticeable, however, that the rate of pollen-tube growth of *H. Manihot* is rather slower than any other ones. In the case of pollination upon the stigma of *H. esculentus* the pollen-tube of *H. Manihot* within 100 minutes grew across 92.98 per cent of the style length, nevertheless the rate of growth indicates a straight line. The pollen of *H. esculentus* indicated the same rate of growth and even within 80 minutes each of 35 pollen-tubes reached the ovule of its own flower. The same pollen-grains indicated a somewhat slower growth of pollen-tube when pollinated upon the stigma of *H. Manihot* and F_1 hybrid. But 14 pollen-tubes of *H. esculentus* reached the ovule of *H. Manihot* within 100 minutes and all of the pollen-tubes reached the ovule of F_1 hybrid, although in these cases the pollen-tubes are non-functional so as to produce hybrid seed. The pollen-tube growth of F_1 hybrid was as rapid as that of *H. esculentus*; even within 80 minutes some of the pollen-tubes arrived at the micropyle of the ovules.

6. Comparative morphology and physiology in some characters of back-crossed plants

The following pollinations were carried out in order to obtain back-crossed plants; $F_1 \times$ *H. esculentus*, $F_1 \times$ *H. Manihot*, *H. esculentus* $\times F_1$, *H. Manihot* $\times F_1$; but only when the flower of *H. esculentus* was pollinated with the F_1 pollen were fertile seeds secured. From these seeds many back-crossed plants were raised. The back-crossed plants showed general uniformity and here again segregation was not evident. They took on more or less F_1 characters, but in some characters they appeared intermediate between F_1 and *H. esculentus*. A brief description will be made here.

(1) Duration of life and annual period of growth

Back-crossed plants were all annual and as stated in Table 9, Text-fig. 1 together with those of *H. esculentus*, *H. Manihot* and F_1 hybrid

plants, they exhibited heterosis even though they were reduced in vigor to some extent compared to the F_1 hybrid.

(2) Leaf size and lobation

The leaf length and lobation of back-crossed plants were measured by the same method as used in the measurements of F_1 and parental species. Table 48 summarizes the results of measurement of leaf length and leaf-lobe index in back-crossed plants.

Table 48. *Comparison of Mean, Standard deviation and Coefficient of variability of leaf length and leaf-lobe index in back-crossed plants with those of F_1 and H. esculentus.*

	Leaf length			
	Mean	STD. DEV.	C. V.	N
H. esculentus	cm. 27.29±0.149	cm. ±1.93 ±0.087	% 7.08±0.387	76
F_1 hybrid	34.23+0.180	±2.73 ±0.108	7.98±0.371	105
Back-crossed plant	32.70±0.211	±2.50 ±0.149	7.56±0.456	64

	Leaf-lobe index			
	Mean	STD. DEV.	C. V.	N
H. esculentus	cm. 44.79±0.368	cm. ±4.75 ±0.260	% 10.58±0.586	76
F_1 hybrid	23.52±0.236	±3.58 ±0.166	15.24±0.709	105
Back-crossed plant	26.47±0.390	±4.62 ±0.275	17.45±1.072	64

As shown in this table the mean, standard deviation and coefficient of variability of leaf length are intermediate in back-crossed plants between F_1 hybrid and *H. esculentus*. However they are inclined to the F_1 hybrid to a certain degree. Here also is exhibited the hybrid vigor. The leaf-lobe index of back-crossed plants is intermediate in mean and standard deviation between F_1 hybrid and *H. esculentus*, but the coefficient of variability of back-crossed plants is greater than any other plant.

(3) Stem-length

As is apparent from the growth curves shown in Text-fig. 1 and Table

9 the stem-length of back-crossed plants is intermediate between *H. esculentus* and F_1 hybrid and they still exhibit hybrid vigor in general appearence, even though their vigor is reduced to some extent.

At their young stage the F_1 plants do not exhibit the hybrid vigor but are rather lacking in that respect compared to either parent (This finding of F_1 inferiority is identical with the results obtained in the previous year (1927), see Table 13), while the back-crossed plants exhibited very vigorous growth even at this stage. Table 49 gives the results of comparative measurements of stem length at young stage of back-crossed plants with those of *H. esculentus*, *H. Manihot* and F_1 hybrid obtained in 1928.

Table 49. *Mean, Standard deviation and Coefficient of variability in stem-length of back-crossed plants at young stage compared with those of F_1 hybrid, H. Manihot and H. esculentus obtained in 1928.*

	Mean	STD. DEV.	C. V.	N
H. esculentus	cm. 5.87±0.056	cm. ±1.17 ±0.039	% 19.86±0.696	206
H. Manihot	4.06±0.049	±0.83 ±0.034	20.40±0.621	132
F_1 hybrid	3.43±0.059	±0.91 ±0.c62	26.66±1.305	1c9
Back-crossed plants	6.44±0.104	±1.26 ±0.073	19.57±1.118	67

(4) Number of involucral bractlets

In 1928 five flowers were examined on each plant and the average of the five indices computed from these measurements was taken as the number of involucral bractlets of the plants. Thus 62 back-crossed plants were calculated. The results of these measurements were as follows: Mean = 7.10±0.046, Standard deviation = ±0.54 ±0.028, Coefficient of variability=7.62±0.311 (%), N=62.

It is apparent that the mean value is intermediate between *H. esculentus* and F_1 hybrid. (See also Table 16).

(5) Capsule

The capsule of back-crossed plants is just intermediate between F_1 hybrid and *H. esculentus* in every character. The two main characters of capsule which were measured and calculated will be described here.

a) *Number of longitudinal ribs*

In 1928, 228 capsules obtained from 45 back-crossed plants were examined. The summarized results are presented in Table 50.

Table 50. *Frequency distribution of longitudinal ribs in
capsules of back-crossed plants.*

Number of longitudinal ribs	5	7	8	Total
Number of capsules	133	79	16	228

From this table it is seen that the form of capsules of back-crossed plants is intermediate between F_1 hybrid and *H. esculentus.*

b) *Length of capsule*

In 1928 two hundred capsules secured from 26 back-crossed plants were measured. The results are presented in Table 51.

Table. 51. *Mean, Standard deviation and Coefficient of variability
of capsule length in back-crossed plants.*

Mean	STD. DEV.	C. V.	N
12.66±0.069	±1.75 ±0.059	13.82±0.475	200

The mean value of capsule length in back-crossed plants is intermediate between F_1 hybrid and *H. esculentus,* but inclined to that of F_1 hybrid; the standard deviation is also intermediate between F_1 hybrid and *H. esculentus*; the coefficient of variability of back-crossed plants is larger than that of either F_1 hybrid or *H. esculentus.*

(6) Seed hair length

The seed hairs of back-crossed plants range from 330 microns to 670 microns in length in some individuals. The mean value is 492.20 ±3.938 microns, the standard deviation is ±82.56 ±2.78 microns, and the coefficient of variability is 16.77±0.581 (%) in 200 hairs. These results indicate that the mean length value is greater than that of either F_1 hybrid

or *H. esculentus*, that the standard deviation is intermediate between F_1 hybrid and *H. esculentus*, and that the coefficient of variability is smaller than that of F_1 hybrid or *H. esculentus*. Other individuals were examined but they showed no significant differences between these individuals.

(7) Size and weight of back-crossed seed

As stated before, the seeds resulting from the immediate cross (F_1 embryo) of *H. esculentus* with the pollen of *H. Manihot* are relatively small compared with the seeds of the mother plant (*H. esculentus*). Such direct effects of pollination upon the size and weight of seed were observed in other cases of crossings. The seeds obtained from *H. esculentus* pollinated with the pollen-grains of F_1 hybrid were markedly larger in their size and weight in every case. This decrease and increase in size and weight of seeds by crossing should depend upon the number and nature of the chromosomes.

In 1927, the length, width, thickness and weight of seeds resulting from the immediate crosses of *H. esculentus* with the F_1 pollen-grains were measured. The results of these measurements have been summarized in Tables 52, 53 and 54, compared with those of *H. esculentus*, *H. Manihot*, F_1 hybrid seeds (F_1 embryo) and seeds from open-pollinated F_1 hybrid (F_2 embryo). Among these back-crossed seeds which failed to germinate, a majority lie in the lower class value. Table 52 shows the relation between seed weight and germinability of back-crossed seeds tested in 1928.

Table 52. *Relation between weight and germinability*
of back-crossed seeds.

Weight of seed	Class value in mg.															Total
	44	47	50	53	56	59	62	65	68	71	74	77	80	83	86	
Number of seed sown	3	2	5	8	13	8	15	20	26	22	21	5	3	0	1	152
Number of seed germinated								4	2	12	11	3	6			38

Among 38 plants which grew, it seems there is little or no correlation between weight of back-crossed seeds and either the fertility or height of the resulting plants.

Table 53. *Comparison of length, breadth and thickness of back-crossed seeds with those of H. esculentus, H. Manihot, open-pollinated F_1 hybrid seeds (F_2 embryo) and F_1 hybrid seeds (F_1 embryo).*

	Seed	Mean	STD. DEV.	C. V.	N
		mm.	mm.	%	
Length	H. esculentus	5.03±0.006	±0.15 ±0.004	2.92±0.081	300
	H. Manihot	3.63±0.007	±0.14 ±0.005	3.78±0.127	200
	F_2 embryo	4.94±0.033	±0.19 ±0.023	3.94±0.470	19
	F_1 embryo (H. escu. × H. Mani.)	4.29±0.008	±0.18 ±0.005	4.26±0.126	249
	Back-crossed seeds (H. escu × F₁)	5.29±0.012	±0·29 ±0.008	5.48±0.153	291
Breadth	H. esculentus	4.58±0.005	±0.12 ±0.003	2.71±0.075	300
	H. Manihot	3.24±0.006	±0.13 ±0.004	4.06±0.137	200
	F_2 embryo	4.25±0.021	±0.13 ±0.015	2.99±0.357	19
	F_1 embryo (H. escu. × H. Mani.)	3.63±0.007	±0.17 ±0.005	4.77±0.144	249
	Back-crossed seeds (H. escu. × F₁)	4.85±0.007	±0.18 ±0.005	3.59±0.142	291
Thickness	H. esculentus	4.35±0.005	±0.12 ±0.003	2.78±0.077	300
	H. Manihot	2.33±0.006	±0.12 ±0.004	5.21±0.176	200
	F_2 embryo	3.76±0.020	±0.12 ±0.014	3.17±0.379	19
	F_1 embryo (H. escu. × H. Mani.)	3.78±0.007	±0.17 ±0.005	4.45±0.135	249
	Back-crossed seeds (H. escu. × F₁)	4.59±0.007	±0.18 ±0.005	3.85±0.108	291

(F_1 embryo is for seed directly obtained by crossing, not for seeds borne on F_1 plants resulting from crossing, F_2 embryo is for seed obtained from F_1 hybrid plant by open-pollination).

Table 54.   *Comparison of seed weight of the back-crossed, F_1
embryo, F_2 embryo, H. Manihot and H. esculentus.*

	Mean	STD. DEV.	C. V.	Ratio of mean	N
H. esculentus	mg. 61.83±0.117	±2.46 ±0.083	% 3.98±0.135	100.0	200
H. Manihot	20.03±0.072	±1.50 ±0.051	7.49±0.253	32.4	200
F_2 embryo	34.50±2.392	±14.62 ±1.692	42.38±8.445	55.8	17
F_1 embryo	29.86±0.148	±3.09 ±0.104	10.35±0.353	48.3	200
Back-crossed seeds	64.48±0.390	±8.16 ±0.275	12.09±0.413	104.3	200

(Back-crossed seeds are for those directly obtained by crossing the flower of *H. escu-
lentus* with the pollen of F_1 hybrid plants).

(8) Flowering time

The date of the first flower in back-crossed plants was recorded in
1928 and the results of the calculations arc as follows:

Mean = 17.37±0.339 (July) STD. DEV. = ±3.86 ±0.239 N = 56

It is noted that these values are intermediate between those of *H.
esculentus* and F_1 hybrid.   The extent of the flowering time of all the
flowers of each individual was prolonged until the end of November.   This
length of flowering time is likewise intermediate between F_1 hybrid and
H. esculentus.

(9) Fertility

In progeny of back-crossings (*H. esculentus* × F_1 hybrid), out of eight
capsules from each plant which were self-pollinated only two produced
seeds in 1928, in total 9 and in average 1.13 per capsule, while when
freely pollinated up to 70–80 seeds could be gathered from a single capsule
·and an average of 26.84 seeds per capsule.   It is, however, interesting to
note, although meiosis is very irregular producing many abnormal pollen-
grains, that the fertility of back-crossed plants is relatively higher than the
F_1 hybrid in cases of both free-pollination and self-pollination.   It may
also be noted that they showed reduced fertility when isolated for self-
pollination.   The number of capsules and number of seeds secured from
back-crossed plants in 1928 are presented in Table 55.

Table 55.   *Number of seeds secured from back-crossed plants
             in 1928.*

	Capsule number	Total number of seeds	Heavy seeds	Empty seeds
	No. 1	6.	0	6
	No. 2	10	0	10
	No. 3	16	0	16
Secured by self-pollination	No. 4	9	0	9
	No. 5	6	0	6
	No. 6	6	6	0
	No. 7	13	3	10
	No. 8	18	0	18
Total	8	84	9	75
Average		10.5	1.13	9.38

	Total number of plants	Total number of seeds	Heavy seeds	Empty seeds
Secured by free pollination	65	4915	2308	2607
	Av. per plant	Av. per capsule (86)		
	35.508	26.84		

As a whole the fertility of back-crossed plants in free pollination is intermediate between *H. esculentus* and F$_1$ hybrid (See Tables 34, 35, 46, 47),

GOODSPEED and CLAUSEN (1917, 1922) obtained many varied plants by back-crossing the hybrid plants of *Nicotiana sylvestris* × *N. Tabacum* to either parental species. These plants were unlike either of them, and were also completely sterile as F$_1$ plants. Some of those plants produced a few seeds by self-fertilization, and plants grown from these seeds were identical with the parental species used in the back-cross. The sterility in wheat hybrids can be measured by grains set per spikelet and by the percentage of obviously poor pollen-grains as indicated by many investigators, while in the author's material the obviously good or poor pollen-grains do

not indicate the measurement of sterility.   The F_1 plants produced many apparently good pollen-grains but these plants exhibit almost complete sterility while the pollen of back-crossed plants contained many more abnormal and abortive grains but the fertility is much higher than that of F_1 plants.

(10) Trichomes

a) *Trichomes on the stem and leaves*

Trichomes distributed on the stem and leaf surface of back-crossed plants were all uniform in construction and similar to those of F_1 hybrid in length and in variability, but the difference is that they are slightly longer than those of F_1 hybrid.   The summarized results in a certain plant (B. C. No. 227) are presented in Table 56 (See also Table 24).

Table 56.   *Frequency distribution and Mean, Standard deviation and Coefficient of variability of trichomes distributed on the stem of a back-crossed plant.*

V	Length of trichomes (micron)															N
	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	
F	7	2	20	17	25	12	18	14	17	26	20	12	6	2	2	200

Mean$= 124.35 \pm 1.605$ STD. DEV.$= \pm 33.65$ ± 1.165 C. V.$= 27.01 \pm 0.968$

b) *Trichomes on the capsule*

The four kinds of trichomes, as was observed in F_1 hybrid capsules, were found on the capsules of back-crossed plants. The number of each of these four kinds of trichomes was counted under the microscope.   The summarized results of these counts are presented in Table 57.

Table 57.   *Number of trichomes of four types counted on the capsules of a back-crossed plant.*

Types	M.eM. type	M. e U. type	H.M.S. type	F_1 type	Total
Counted number	2192	90	121	391	2794
Percentage	78.45	3.22	4.33	13.99	100.00

From Table 57, it may be seen that the percentage of M. e. M. type (Multicellular trichomes) which are thought to be derived from *H. esculentus* is increased in the back-crossed plant, on the contrary, the percentage of H. M. S. type (Short unicellular trichomes) which may be derived from *H. Manihot* is decreased, and that of F_1 type (Giant trichome) and that of M. e. U. type (Short unicellular) which are thought to be derived from *H. esculentus* are slightly decreased.   As a whole the trichomes of back-crossed plants are still very prominent and very coarse in construction but the number of trichomes of each kind is somewhat intermediate between F_1 hybrid and *H. esculentus*.

(11) Pollen-grain

In 1928 and 1929, the pollen-grains of back-crossed plants were examined and found to have larger variation in size and form than those of F_1 plants, and to include more abortive, irregular and elliptical big pollen-grains than the F_1 hybrid, though on the other hand these plants have pollen-grains larger than those of F_1 hybrid and also pollen-grains smaller than those of parental plants. (Plate VII, Fig. 4 and 5).   The pollen-grains develop normally for a time, but a majority degenerate in one or the other stage of development and a larger number of small abortive pollen-grains are found later on.   Thus full majority is reached only by very few of them.   Accordingly when, in these plants, mature pollen is being examined under the microscope, only a small number of more or less normally developed pollen-grains and giant pollen-grains may be seen within the field of sight.

In 1929, at the time of dehiscence of anthers the pollen-grains were knocked off upon slide glass with a pincette by filliping the anther slightly and then the number of abortive pollen-grains and deformed ones was calculated and the diameter of the well developed pollen-grains was measured under the microscope.   The results are as indicated in Table 58.   The frequency distributions of the pollen-grain diameter are usually so diverse that it seems to be impossible to calculate a representative mean value from this variation table.

The observation of KARPECHENKO (1924) that the number of normal pollen-grains in triploid hybrid is considerably greater than in F_1 of *Raphanus sativus* L. × *Brassica oleracea* L., is quite contrary to the observations with the author's material.

The irregularity of reduction division of P. M. C. may cause increased variation in size and shape of pollen-grains in back-crossed plants, ranging

from completely abortive grains to those much larger than F_1 pollen-grains. The increased variability is due here to combinations of non-homologous chromosomes acting in the gametophytic generation and to differences in chromosome number. The size of pollen-grains depends not only on the fluctuation, but also strictly on the chromosome number in the hybrid, the greater the number the larger the grains. Similar conclusions have been reported by COLLINS, HOLLINGSHEAD and AVERY (1929). They observed in trivalent *Crepis artificialis* from examination of the anthers which showed only 30–38 per cent bad pollen, that the apparently good poollen showed variation in size. This relation may be seen in the author's material in the pollen of the balanced and unbalanced types under the same magnification. Plate VI, Fig. 1 shows a field of pollen-grains from a *H. esculentus*; Fig. 2, pollen-grains from a *H. Manihot*; Fig. 3, larger grains of pollen from an F_1 hybrid plant, while figs. 4 and 5 show pollen-grains from back-crossed plants. These pollen-grains from a back-crossed plant are not only characterized by a larger proportion of empty grains, but also a great diversity in size brought about by the differences in the number of chromosomes which they contain.

(12) Pollen-tube growth

The pollen-tube growth of back-crossed plants was measured in 1929 by the same method as used in the F_1 pollen-grains. Only a small number of good pollen-grains of back-crossed plants sown on the stigma of *H. esculentus*, of F_1 hybrid and their own flower germinated soon and the pollen-tube reached the ovule within 100 minutes.

Table 58. *Frequency distribution of pollen-grain diameter,
tion of number of cells in microsporocytes of*

Plant number	Pollen-grain-diameter																					
	10.5	11.0	11.5	12.0	12.5	13.0	13.5	14.0	14.5	15.0	15.5	16.0	16.5	17.0	17.5	18.0	18.5	19.0	19.5	20.0	20.5
No. 1-2	1	3	2	2	7	1	12	7	9	4	22	8	10	2	5	1	3	2	5		
No. 2-2			3	3	27	20	36	8	22	4	1	0	2	0	4	3	1				
No. 2-3			1	3	6	7	14	25	24	6	8										
No. 2-4		2	3	6	7	19	19	30	28	51	20	16	5	2							
No. 3-1					3	4	11	27	22	27	5	1									
No. 3-2	1	3	0	8	5	13	18	21	21	13	2	1	1	1	0	0	1	1			
No. 3-3		1	1	3	1	2	2	9	2	7	3	8	2	2	0	2	1	2			
No. 3-4			2	9	7	6	12	23	22	25	15	4									
No. 3-5		3	1	6	9	17	20	20	2												
No. 3-6		1	3	1	7	2	5	20	18	33	12	11	5	1							
No. 3-7		1	0	1	2	12	19	60	32	37	8	3	1	0	2	0	3	1			
No. 3-8	1	3	16	15	22	9	4	3	2	0	4	10	14	23	10	2					
No. 3-9		1	0	6	3	7	11	26	13	20	3	7	2	2	0	0	2	3			
No. 3-10	1	1	3	2	3	1	13	6	17	8	2	6	1	1	3	3	2	1	3		
No. 3-11		1	2	3	2	3	1	4	3	4	1	2	2	1	0	1					
No. 3-12			2	0	0	3	11	10	10	4	2	1	1								
No. 3-13								1	0	1	1	2	3								
No. 3-14					1	2	4	4	4	12	11	14	8								
No. 3-15				2	2	6	13	15	15	13	4	3	1	3	2	1	3	3	3	1	
No. 3-16				1	3	1	4	2	4	1	1	0	0	0	0	0	0	0	1		
No. 3-17			1	0	0	0	0	0	1	0	1										
No. 7-11				6	0	18	13	20	18	24	11	5	1	0	0	1	0	1			
No. 8-11				1	4	1	1	10	3	4	9	8	5	0	0	0	1	7	3	1	1
No. 9-1				3	8	5	9	32	24	24	7	7	3	2	0	1					
No. 9-33				2	7	10	13	15	5	9	1	9	1	6	6	7	0	4			
No. 16-1		1	1	1	2	7	14	14	13	24	11	12	3	2	0	0	0	1	0	1	

Class value of pollen diameter is shown by the unit of micrometer, which corresponds to an absolute length of 6.85 microns.

Pollen-grains are sometimes massive, pyramidal and often semicircular or pyriform.

percentage of abortive pollen-grains and frequency distribu-
back-crossed plauts raised in 1929.

Total	Abortive pollen	Deformed pollen	Sum total	% of abortive pollen	Number of cells in microsporocytes											Total
					2	3	4	5	6	7	8	9	10	11	12	
106	73	1	180	41.0	1	7	143	2								153
134	76	5	216	35.5	6	12	43	63	53	29	10	4				220
93	109	0	202	53.7	9	19	104	48	26	9	4	0	2			221
208	302	0	510	59.2	3	79	76	52	24	13	1					252
100	164	0	264	62.1			3	422	7							432
110	30	0	140	21.4	2	6	202	4	2	1	1					218
48	167	1	216	77.3	1	6	57	55	48	18	8	2				195
125	181	0	306	59.1	1	12	154	6	4	1						178
78	140	0	218	64.2	54	36	30	3								123
119	284	0	403	70.5	8	10	126	38	19	3	1					205
182	53	0	235	22.6	1	4	92	42	25	1						165
138	73	2	213	34.2	1	7	42	52	90	29	10	4	0	1		236
106	248	1	355	69.0			56	46	82	16	3	2				205
77	158	1	236	66.9			2	8	21	36	18	11	7	3	1	107
30	308	3	341	90.3		3	58	27	41	24	8	3	1			165
44	281	0	325	89.2	1	1	52	49	59	16	3	1				182
8					1	5	83	23	25	4						141
60	521	0	581	89.7			66	48	54	39	23	18	4	4	2	258
90	106	7	203	52.2	3	3	41	32	32	13	7	1	2			134
18	152	0	170	89.4	1	1	36	11	18	14	8	4	3	2	2	100
3							260	4	5							269
118	230	0	248	66.1		2	83	43	47	29	15	6				225
59	91	0	150	60.1	3	6	144	69	74	42	26	8	4			376
125	92	0	217	43.3		3	121	13	13	1						151
95	216	1	312	69.2			121	11	4	2						138
107	132	0	239	35.5			99	48	25	15	6	4	2			199

7. Comparative morphology in some characters of the progeny resulting from the seeds of back-crossed plants

About 200 plants from the seeds obtained from the back-crossed plants by self-pollination and cross-pollination were grown in 1929. They were so variable in many characters that every one could be classed as a different form.

Fertility A few of the plants were nearly fertile as back-crossed plants but some were completely sterile as F_1 plants, on the whole the fertility in this group of plants was apparently much higher than that of F_1 plants. The fertility of these plants is shown in detail in Table 59.

Height The stem-length in this group of plants varied from 1.30 to 4.40 meters and most of the plants were 2.20 to 4.60 meters in height. The frequency distribution of main stem-length is as follows:

Class value (meter)	1.2	-1.4	-1.6	-1.8	-2.0	-2.2	-2.4	-2.6	-2.8	-3.0	-3.2	-3.4
Frequency	1	1	4	4	5	19	16	17	21	29	30	

Class value	-3.6	-3.8	-4.0	-4.2	-4.4	Total
Frequency	19	14	4	4	1	189

Branches Some plants formed abundant lateral branches but some only a few. The hairiness and colour of the stem also varied widely.

Leaf Shape of leaves varied from shallow Okra-like leaf to deeply lobed and coarsely toothed leaf as that of F_1 plant (Plate IV, Fig. 1, 2, 3).

Flower Some plants flowerd abundantly but some of the plants bore only a few flowers, notwithstanding they had grown with many branches.

Pollen-grain The pollen-grains of these plants were examined and found to have larger variation in size and percentage of abortive pollen-grains. In some plants there were only a few abortive pollen-grains as in case of the pure species while in another plants there were only a few good pollen-grains. The percentage of abortive pollen-grains varied from 5.2 per cent to 97.1 per cent. The diameter of these pollen-grains was more variable than that of back-crossed plants. In some plants the pollen-grains varied in diameter from 60.86 to 143.85 microns while those of some plants were as uniform as those of pure species.

Capsule Usually the capsules of this group of plants had rough and stiff hairs as those of F_1 and back-crossed plants but some plants (BCN,

No. 24) had soft hairs on the pods as those of Okra.   The form of pod
varied from short and broad to long and slender as that of Okra.

Number of cells in microsporocytes Number of cells in microspo-
rocytes varied in one plant (BCN, No. 48) from 2 to 12 while in some
plants (BCN, No. 24, 25, 26, 28, 46, 54) there was only a tetrad.

As regards morphological features that the progeny of back-crossed
plants varied to such an extent that no two of them were identical was
also reported by GOODSPEED and CLAUSEN (1917, 1922), and LAMMERTS
(1929) in *Nicotiana* species and others.

Table 59.   *Fertility of progeny of back-crossed plants.*

	Plant number	Number of empty seeds	Total no. of fertile seeds	Number of capsules	Av. number of seeds per capsule	Max. no. in a capsule	Min. no. in a capsule
Progeny of back-crossed plants by open-pollination	No. 15	40	163	14	11.64	20	4
	No. 16	1	56	7	8.43	16	0
	No. 17	33	145	15	9.67	22	0
	No. 18	14	61	6	10 18	20	0
	No. 19	48	190	19	10.00	12	0
	No. 20	16	123	11	11.18	20	0
	No. 21	5	54	5	10.80	22	4
	No. 22	16	25	3	8.33	14	4
	No. 23	4	52	4	13 00	20	7
	No. 24	43	291	14	20.79	30	11
	No. 25	21	14	4	3 50	11	0
	No. 26	8	36	7	5.14	14	0
	No. 27	8	22	4	5.50	15	0
	No. 28	7	29	5	5.80	8	2
	No. 29	0	0	0	0.00	0	0
	No. 30	2	2	3	0.67	1	0
	No. 31	1	15	4	3.75	7	1
	No. 32	54	313	29	10.79	21	0
	No. 33	16	139	11	12.64	29	4

Plant number	Number of empty seeds	Total no. of fertile seeds	Number of capsules	Av. number of seeds per capsule	Max. no. in a capsule	Min. no. in a capsule
No. 34	46	49	7	7.00	10	1
No. 35	2	0	6	0.00	0	0
No. 36	0	0	2	0.co	0	0
No. 37	70	144	17	8.47	23	1
No. 38	32	35	6	5.83	17	0
No. 39	5	10	3	3.33	6	2
No. 40	16	14	2	7.00	7	7
No. 41	44	373	32	11 66	14	1
No. 42	25	91	9	10.11	19	1
No. 43	13	47	5	9.40	16	1
No. 44	4	5	4	1.25	4	0
No. 45	0	1	3	0.33	1	0
No. 46	2	4	2	2.co	4	0
No. 47	2	18	2	9.00	10	8
No. 48	0	28	4	7.00	12	2
No. 49	25	164	15	10.93	26	5
No. 50	47	134	17	7.88	13	0
No. 51	3	18	7	2.57	12	0
No. 52	20	140	12	11.67	19	11
No. 53	34	120	16	7.50	14	2
No. 54	21	52	10	5.20	11	1
No. 55	11	25	6	4.17	9	0
No. 56	14	17	12	1.42	2	0
No. 57	12	15	5	3.00	5	1
No. 58	22	63	9	7.00	15	1
No. 59	14	92	13	7.08	12	0
No. 60	18	27	6	4.50	8	1
No. 61	8	23	4	5.75	8	2
No. 62	10	50	5	10.00	20	4

Progeny of back-crossed plants by open-pollination

	Plant number	Number of empty seeds	Total no. of fertile seeds	Number of capsules	Av. number of seeds per capsule	Max. no. in a capsule	Min. no. in a capsule
	No. 63	54	352	28	12.57	20	2
	No. 64	105	219	44	4.98	12	0
	No. 65	6	24	5	4.80	14	0
	No. 66	5	1	2	0.50	1	0
	No. 67	0	0	0	0.00	0	0
	No. 68	39	106	12	8.83	19	1
	No. 69	18	116	18	6.44	13	1
	No. 70	1	3	1	3.00	3	3
	No. 71	0	0	1	0.00	0	0
	No. 72	4	49	4	1.23	19	8
	No. 73	0	0	1	0.00	0	0
	No. 74	1	7	1	7.00	7	7
Progeny of back-crossed plants by open-pollination	No. 75	17	159	11	11.36	19	0
	No. 76	12	31	14	2.21	9	0
	No. 77	0	0	0	0.00	0	0
	No. 78	25	147	30	4.90	12	1
	No. 79	0	0	0	0.00	0	0
	No. 80	13	13	10	1.30	13	0
	No. 81	7	31	5	6.20	12	1
	No. 82	62	63	22	2.86	9	0
	No. 83	5	6	4	1.50	2	1
	No. 84	0	0	0	0.00	0	0
	No. 85	0	0	0	0.00	0	0
	No. 86	7	23	5	4.60	15	0
	No. 87	35	197	14	14.07	45	1
	No. 88	16	77	12	6.42	16	0
	No. 89	15	35	10	3.50	18	0
	No. 90	160	95	13	7.31	14	0
	No. 91	31	11	9	1.22	3	0

	Plant number	Number of empty seeds	Total no. of fertile seeds	Number of capsules	Av. number of seeds per capsule	Max. no. in a capsule	Min. no. in a capsule
	No. 92	64	80	21	3.81	19	0
	No. 93	8	9	4	2.25	3	2
	No. 94	0	0	0	0.00	0	0
	No. 95	15	104	8	1.30	17	4
	No. 96	74	158	40	3.95	13	0
	No. 97	14	15	5	3.00	9	0
	No. 98	16	18	6	3.00	12	0
	No. 99	4	33	4	8.25	14	3
	No. 100	5	0	1	0.00	0	0
Progeny of back-crossed plants by self-pollination	No. 1	0	0	0	0.00	0	0
	No. 4	4	1	2	0.50	1	0
	No. 5	0	0	0	0.00	0	0
	No. 6	0	0	0	0.00	0	0
	No. 7	0	0	0	0.00	0	0

Low fertility of the progeny of back-crossed plants by self-pollination is due to the bad condition because grown in glass house.

8. Comparative morphology in some characters of F_1 plants

In 1929, 24 seeds were obtained from 20 F_1 plants (White long × *H. Manihot*) and 59 seeds from 15 plants (White velvet × *H. Maninot*) by open pollination. All of these seeds were sown in 1930 in the experimental plots of Hokkaido Imp. Univ., Coll. of Agric., Sapporo. Of these, 41 seeds germinated and the F_2 plants were raised to maturity. These F_2 plants were fairly uniform, resembling F_1 hybrid or back-crossed plants in general characters, e. g., stiff hairiness in capsule and stem, deep lobation of leaf, shape of capsule etc., but the only marked difference was their poor development of vegetative growth compared to that of F_1 and back-crossed plants.

They opened an abundance of flowers but only a few of the capsules were developed to normal size. Nearly all of the capsules stopped their

development a few days after flowering, their colour turned to brown and they shrivelled up, consequently some of the plants produced no seed. Among 41 F_2 plants 15 plants produced capsules. From 20 of these capsules out of 25 many plump seeds varying 1 to 37 per capsule were obtained. All of the capsules were five ribbed without exception as those of *H. Manihot* or F_1 hybrid.

The number of involucral bractlets per capsule varied from 5 to 8. The data obtained from 189 flowers of 41 plants are as follow:

Mean$=6.34\pm0.039$, Standard deviation$=\pm0.79\pm0.03$,

Coefficient of variability$=12.46\pm0.627$.

These data and frequency distribution are quite similar to those of F_1 hybrid. (See Table 16).

The leaf-lobe indices were calculated to compare the leaf shape of F_2 plants with that of F_1 hybrid and pure species, for which similar methods were used as employed in F_1 hybrid. The frequency distribution of leaf-lobe index of F_2 plants compared with those of pure species and F_1 plants are shown in Table 60.

Table 60. *Frequency distribution of leaf-lobe index of F_2 plants compared with those of pure species and F_1 hybrids.*

	Class value	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	Total
Frequency	White velvet (Pure species)													2	3	1	3	3	1		13
	White long (Pure species)								8	6	7	9	5	6	1	5	3				50
	F_1 (White velvet × H. Manihot)			1	2	2	1	4	3	2											15
	F_1 (White long × H. Manihot)	1	2	8	4	3	0	1													19
	F_2 (White velvet × H. Manihot)		1	6	2	2	3	5	3	2	1										25
	F_2 (White long × H. Manihot)	1	2	2	1	4	2	1													13

From these data it is seen that the leaf-lobation of F_2 plants is apparently similar to that of F_1 hybrid plants. (Pl. IV, Fig. 4).

Tetrad formation usually occurs without exception among these 41 plants. This indicates the regular meiotic division of pollen mother cells. In consequence of this regular meiotic division the pollen-grains were very uniform and only a small percent of abortive pollen-grains were contained,

but the occurrence of larger sized pollen-grains having digenomous chromo-
somes was evident.

The pollen-grains of F_2 plants were examined in dry condition under
a microscope and the diameter measured. The data obtained in these
measurements in some of the F_2 plants are shown in Table 61.

Table 61. *Frequency distribution of pollen-grain diameter of
F_2 and Mean, Standard deviation and Coefficient
of variability. (1 unit of class value corresponding
to an absolute size of 6.85 microns).*

Class value	14.0	14.5	15.0	15.5	16.0	16.5	17.0	17.5	Total	Abortive pollen
Frequency F_2 (WL, No. 11)	1	5	13	19	36	21	5		100	4
F_2 (WV, No. 5)		2	10	14	33	21	17	3	100	8

	Mean	STD. DEV.	C. V.
F_2 (WL, No. 11)	micron 108.30±0.300	micron ±4.46 ±0.213	% 4.30±0.262
F_2 (WV, No. 5)	114.56±0.312	±4.62 ±0.222	4.18±0.200

From the data shown in Table 61 it may be concluded that the Mean,
Standard deviation and Coefficient of variability of pollen-grains of F_2 plants
are similar to those of F_1 plants. (See Table 39 and Pl. VII, Fig. 10).

9. **Comparative morphology in some characters of progeny resulting from seeds
obtained from the back-crossed plants by pollination with the pollen of H.
esculentus, [(H. esculentus × F₁) × H. esculentus], and with the pollen of F₁
hybrid, [(H. esculentus × F₁) × F₁]**

In 1928, 56 plump seeds were obtained from the back-crossed plants
by crossing with the pollen of *H. esculentus* and 147 seeds by crossing
with the pollen of F_1 hybrid. From these seeds 18 and 52 plants res-
pectively were raised to maturity in 1929. They varied in every character
to such as extent that no two of them were identical. But in general,
the plants resulting from the seeds of back-crossed plants pollinated with
the pollen of *H. esculentus* rather resembled *H. esculentus* in general charac-
ters and the plants resulting from the seeds obtained from the back-crossed

plants pollinated with the pollen of F_1 plants resembled the F_1 plants rather than back-crossed plants.   Plate IV. Fig. 2, BC × F_1, 9–10 show the leaf form of a progeny resulting from the back-crossed plant with the pollen of F_1 plant; BC × HE, No. 10–2 and BC × HE, No. 15–1 show the leaves of two plants resulting from the seeds of back-crossed plants with the pollen of *H. esculentus*.   Plate V, Fig. 2, BC × F_1 shows a capsule of a plant resulting from the seeds obtained from the back-crossed plants by cross-ing with the pollen of F_1 plant; BC × HE, No. 15–1 and BC × HE, No. 6–1 show the capsules resembling the capsule of *H. esculentus* resulting from the seeds obtained from the back-crossed plants by pollination with *H. esculentus* pollen.

Stem length, number of cells in microsporocytes of P. M. C., frequency distribution of pollen-grain diameter, percentage of abortive pollen-grain and other characters vary extensively as in progeny of back-crossed plants. The fertility of those plants varies as that of progeny of back-crossed plants. Some characters of pollen-grains and number of cells in microsporocyte of these plants are shown in Table 62.

Table 62. *Frequency distribution of pollen-grain diameter and grains and percentage of tetrad in microsporocytes of H. esculentus and F₁ hybrid.*

Plant number	10.0	10.5	11.0	11.5	12.0	12.5	13.0	13.5	14.0	14.5	15.0	15.5	16.0	16.5	17.0	17.5	18.0	18.5	19.0	19.5	20.0	20.5	21.0	21.5
[(H. esculentus × F₁) × H. esculentus																								
No. 1-1	3	0	2	0	2	1	10	5	9	7	16	5	2	3	4	4	3	0	1	1				
No. 1-2						2	9	0	3	1	0	2												
No. 1-3							15	26	34	29	28	6	4											
No. 6-1								1	17	31	86	58	46	10	1									
No. 15-1	8	0	9	4	9	23	59	18	17	2	2													
No. 15-2								1	12	30	86	45	36	2	1									
No. 15-4						3	3	17	12	39	17	7	1	1										
[(H. esculentus × F₁) × F₁]																								
No. 7-1		2	0	0	0	1	0	2	0	2	0	3	0	0	0	0	0	0	0	0	1			
No. 7-2	4	1	2	3	1	1	1	3	2	8	11	52	27	37	8	5	2	2	1	2	0	3	0	2
No. 7-5		1	0	1	2	9	13	21	24	40	11	15	0	4										
No. 2-3	11	1	2	2	1	0	1	0	4	4	6	2	4	4	2	5	5	9	14	6	10			
No. 7-6						2	0	4	4	8	22	47	32	33	7	3	2							
No. 7-9						9	6	22	16	35	20	32	6	9	1	2	0	1						
No. 7-10						1	2	0	9	10	74	76	75	23	21	7	1							
No. 7-11	2	0	0	0	0	1	1	5	8	11	34	30	23	6	5	1	0	0	1	2	4	0	1	
No. 9-2			1	0	1	2	1	0	1	1	9	17	24	39	39	5	2	1						
No. 9-6			1	0	1	0	2	2	7	8	43	27	29	5	9									
No. 9-8					4	2	5	5	43	27	57	15	14											
No. 9-11								1	8	22	16	15	12	10	2									
No. 12-1	5	2	1	2	5	1	1	3	12	9	25	12	11	5	3	0	0	0	0	0	1	1	1	
No. 12-3			3	0	2	1	8	8	14	5	7	1	1	1	0	6	5	9	14	7	1			
No. 9-6						2	1	12	23	47	22	16	9	1	0	0	0	0	0	1				

Class value of pollen diameter is shown by the unit of micrometer, which corresponds to an absolute length of 6.85 microns.

number of cells in microsporocytes, percentage of abortive pollen—
in the progeny of back-crossed plants pollinated with the pollen

Total	Abortive pollen	Sum total	% of abortive pollen	Number of cells in microsporocyte															Total	% of tetrad
				2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
78	83	161	51.55	4	7	208	1	1											221	94.12
17	297	314	94.54	1	7	164	37	2	2										213	77.00
142	236	378	62.43			200													200	100.00
250	22	272	8.09	4	3	170													177	96.05
151	1	152	0.66			208													208	100.00
213	1	214	0.47			200													200	100.00
100	70	170	41.18			200													200	100.00
11	22	33	66.67			116	51	45	34	11	5	2	0	1					265	43.77
178	25	203	12.32	4	5	86	23	7											125	68.80
141	123	264	46.59				20	2											22	90.91
94	57	151	37.75			105	65	28	20	16	8	2							244	43.03
164	38	202	18.85			286	2	1											289	98.96
159	198	357	55.46				70	25	23	9	3								130	53.85
299	94	393	23.92																	
135	47	182	25.82																	
143	13	156	8.33		1	250	0	1											252	99.21
134	46	179	25.13			389	3	3	1										396	98.23
172	86	258	33.33			192	21	16	11	4	2								246	78.05
86	186	272	68.38				18	23	20	26	44	26	10	6	6				179	10.06
100	27	127	21.26			41	11	5	0	6	2	3	3	7	4	0	0	1	83	49.40
93	11	104	10.59		8	185	15	10	2										220	84.02
134	36	170	21.18			252	81	32	11	4									380	66.32

Part II. Cytological studies of an interspecific hybrid between Hibiscus esculentus L. and H. Manihot L.

1. Material and method

In 1928, 1929 and 1930 studies of chromosomes of *Hibiscus* species and their hybrids and later generations were undertaken in order to determine the chromosome relationships in these species and the chromosome behavior in these hybrids and later generation.

Since it was impossible to obtain well fixed root tips unavoidably only anthers were used for the preparations. Differentiation of the archesporium in the anthers of these plants takes place at a very early stage, pollen mother cell formation usually occuring when the flower bud is 2–3 mm. in diameter, about ten days before flowering. Anthers were fixed as a whole with petals in order to prevent their separation from each other. Flemming's weaker solution enabled us to obtain good results. The aceto-carmine method was also tried, although it does not permit counts of chromosome number to be made on account of minuteness in size and comparatively great number of chromosomes, but it did enable the author to pick out the right stage of the buds for imbedding. For the selection of buds the anthers of each bud were dissected out and broken on a slide and tried in a drop of acetocarmine. The fixed materials were imbedded in paraffin, longitudinal microtome section of buds, varying from 25 to 30 microns in thickness were cut and stained with Heidenhain's iron-alum haematoxylin. The fixation of the buds was carried on during the whole summer usually several times for the same plant.

The chromosomes of parental species are comparatively small, so that somatic counts have been found difficult. Both parental species and F_1, F_2, back-crossed plants and another generation plants, as a rule, have been counted in the metaphase of the heterotypic division of P. M. C., some additional countings have been made in diakinesis and in the metaphase of the homotypic division in the P. M. C.. Observations on the stages preceding metaphase will be omitted and only chromosome behavior after that stage will be considered.

2. Cytological investigation of parental species

As it is important to study the cytological feature of the pure species before examing the chromosome behavior of hybrids, a brief résumé of meiotic behavior in diploid parents may be made.

After diakinesis the bivalent chromosomes shorten, the nuclear mem-

brane disappears, and the chromosomes become oriented on the equatorial plate. Pl. VIII, Fig. 1 and 2.

The diploid chromosome number in *H. esculentus* L. (female parent) was counted as 72, and haploid number as 36; this can be seen in the metaphase of the first division.

In the heterotypic division the spindle fibers have a terminal attachment to the chromosomes. The division proceeds regularly and there are no lagging chromosomes. After telophase the chromosomes of both poles soon pass into the resting stage and the cell plate is formed. The second or homotypic division is normal. All the chromosomes are regularly arranged on the equatorial plate in the metaphase. In the anaphase each of the chromosomes splits, the longitudinal halves of the chromosomes pass into both spindles, and the four cells of the tetrad are formed in one plane. The chromosomes as seen in this division are of round form as in the first division and are somewhat difficult to count.

The chromosomes of *H. Manihot* (male parent) are similar to those of *H. esculentus* in their shape and behavior. There is little difference between these species in the size of individual chromosomes. The author counted the number of haploid chromosomes in *H. Manihot* as 30 in the heterotypic and in the homotypic metaphase. The second division is also normal and the four cells of the tetrad are formed in one plane.

3. Cytological investigation of F_1 hybrid between Hibiscus esculentus L. and H. Manihot L., (Heterogenomous plant)

(1) Usual way of meiotic division in F_1 hybrid

The F_1 plants receive 36 chromosomes from *H. esculentus* and 30 chromosomes from *H. Manihot*. Thus, there should be 66 chromosomes in the somatic cells of the F_1 hybrid plants. The meiosis in the pollen mother cells of F_1 hybrid plants is exceedingly abnormal and is of the type usually found in the hybrid between species with different chromosome number but something different from others may be found.

At the metaphase of the first division 66 chromosomes, more or less, make their appearance in a majority of cases and arrange regularly at the equatorial plate. This is the sum of the haploid number of the two parental species. Evidently no conjugation of chromosomes occurs in these cases and bivalents are never formed, $30_I + 36_I$ seems to be the underlying type (Text-fig. 2-1, 2, Pl. VIII, Fig. 3). There is reason to suppose that they are qualitatively so different that it is impossible for them to pair together. The threads of the spindle are markedly discernible, the uni-

valent chromosomes usually do not split at the equatorial plate.   In the anaphase of the first division about half of these chromosomes move towards the poles.   At this time the author counted about 66 of chromosomes all scattered all over the spindle for a long time (Text-fig. 2–3, 4, Pl. VIII, Fig. 4), some of the chromosomes still remain at the equatorial plate even though some have reached the poles, and fail consequently to form two nuclei in the regular way.   Then all the chromosomes are oriented at the equatorial plate again and siderophile matter[1] is formed around the whole spindle. There is some probability that the formation of restitution nucleus[2] may occasionally occur after this stage.   Here the univalents probably have been preparing to divide (Text-fig. 2–5).   Then these univalents split lengthwise and the siderophile matter soon disappears, so that at the time of this division there often may be counted above 120 chromosomes (Pl. VIII, Fig. 5.).   Text-fig. 2–6 show the above 120 small chromosomes including some which are still not divided.   These split chromosomes move towards the poles in about equal number and form two nuclei with about 66 chromosomes at each of the poles (Text-fig. 2–7, 8, 9).   In the course of meiosis in which the first division has been dropped away one very broad spindle is formed as shown in Text-fig. 2–7, 8.

Dyads occur in consequence of such a division instead of tetrads.   Text-fig. 2–10, 11, 12 and Pl. VIII, Fig. 6 show the dyads resulting from such an abnormal division, and if the distribution of chromosomes has proceeded evenly, each cell of the dyads receives 66 chromosomes.   These cells develop directly into the pollen-grain and consequently gametes arise with a diploid number or correctly two sets of different genomes[3].

---

1)   Dark coloured thick layer, MATSUDA (1927) called "Siderophile matter".

2)   "A peculiar process during the development of the pollen mother cells in the subgenus *Eu- Hieracium*, in which a semiheterotypic division was interrupted at about late prophase or metaphase and a new nucleus constituted with the diploid number of chromosomes, all split.   I proposed for such a nucleus the term "Restitution nucleus". ROSENBERG (1927).

3)   According to WINKLER (1920) the haploid set of chromosomes is termed "Genom". All the author's hybrids will be heterogenomous, as they always contain different genomes of *Hibiscus esculentus* and *H. Manihot*. But since the *H. esculentus* and *H. Manihot* genomes are not identical as to the number of their chromosomes, our digenomous hybrids with respect to number exclusively, will have a something different meaning from "Diploid". Those having three genomes also will be different from "Triploid" etc.

Text-fig. 2
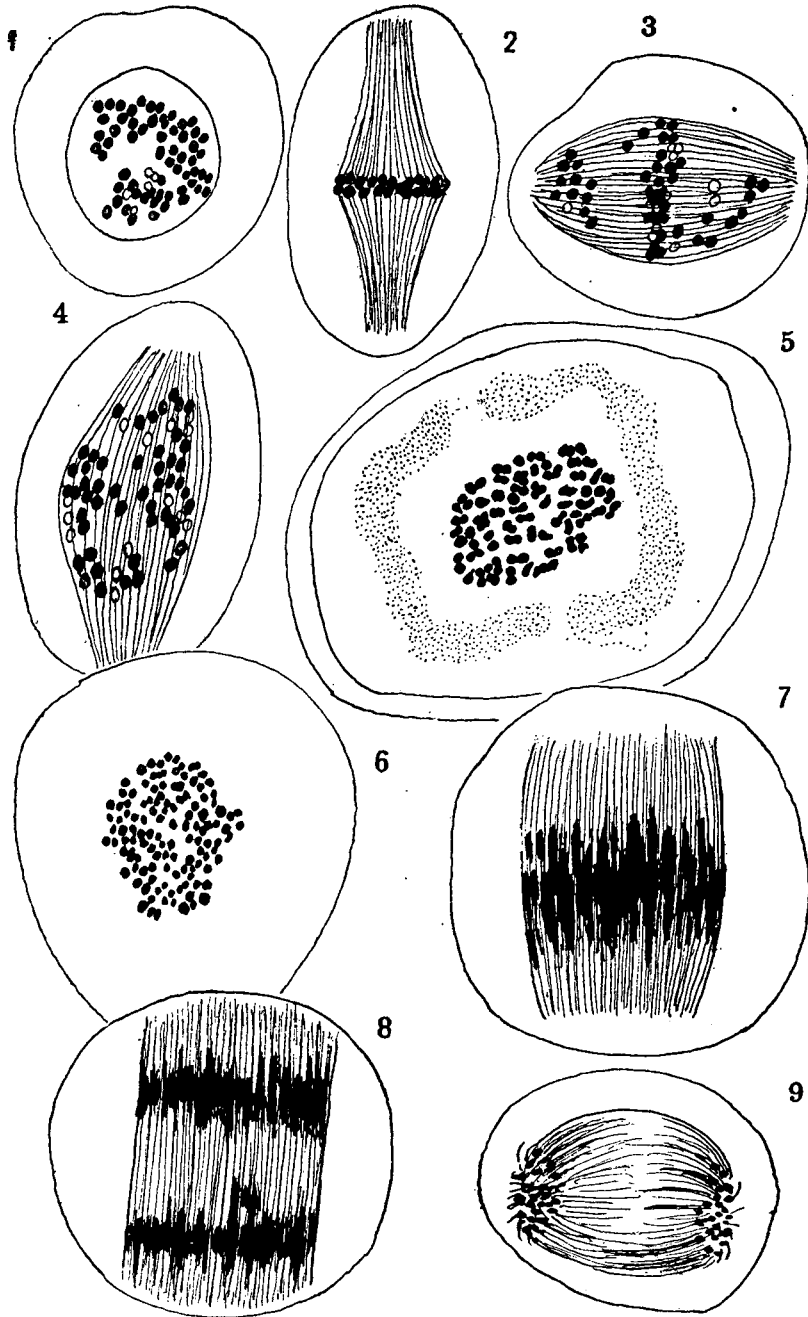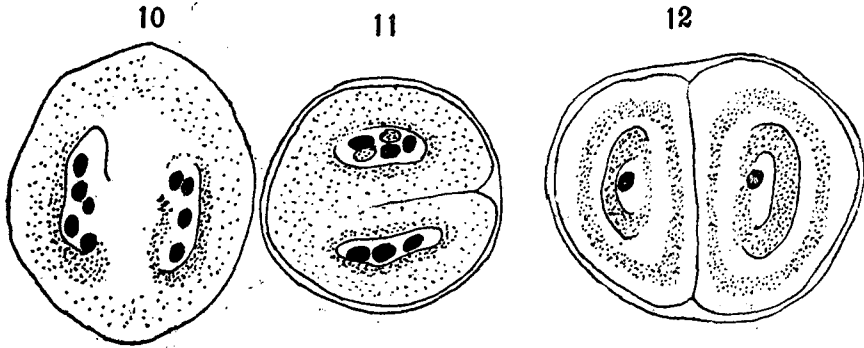
Text-fig. 2. 1-12. Dyad formation by abnormal meiotic division of P.M.C.
in F$_1$ hybrid.

1. Metaphase, polar view, 66 chromosomes are seen.
2. Side view of metaphase, all the univalent chromosomes oriented
    at the equatorial plate.
3. Late metaphase, some chromosomes going to the poles, but ma-
    jority of the chromosomes still remaining at the middle part of
    the spindle.
4. Anaphase, all the chromosomes scattering over all parts of the
    spindle.
5. 66 chromosomes are seen oriented at the equatorial plate and
    siderophile matter is formed.
6. Univalent chromosomes split lengthwise and siderophile matter
    disappeared; above 120 small chromosomes are seen including
    some chromosomes which are still not divided.
7, 8, 9. Split chromosomes moving toward the poles in about equal
    number and forming two nuclei. Broad spindles are formed and
    divided chromosomes are in obscure thread form.
10, 11, 12. Three stages of dyad formation.

Scheme 2 illustrates the usual process of abnormal meiotic division
of P.M.C. in F$_1$ hybrid.

Parents	
Parental gametes	
F_1 hybrid	
Diakinesis	
Metaphase of fiirst division	
Before splitting	
All the univalents split	
Digenomous F_1 gametes	
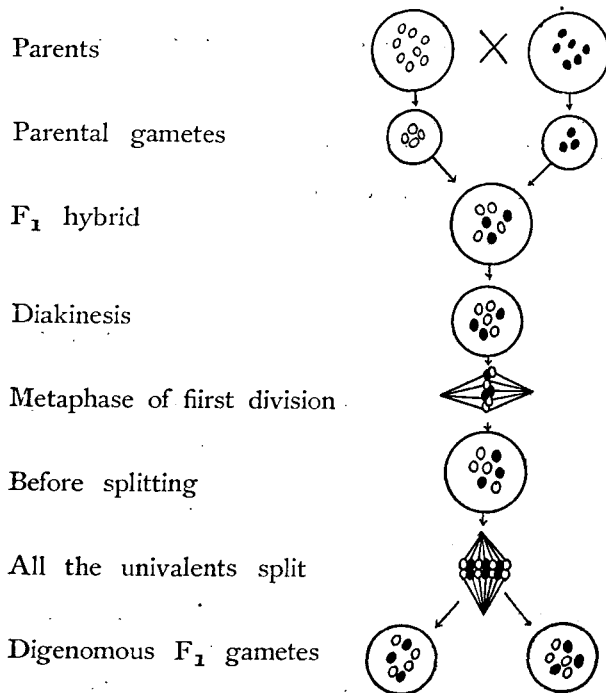
Scheme 2. Formation of digenomous gametes in the P.M.C. in F_1 hybrid of *H. esculentus* L. × *H. Manihot* L.

(2) Some irregularities observed

It is necessary to presume the occurrence of irregularites in the meiotic division of P.M.C. to be able to explain the formation of abortive and abnormal pollen-grains by means of which new species or forms with changed chromosome numbers arise. The pollen mother cells of F_1 plants sometimes show certain irregularities, which may be of some interest.

a) *Formation of tetraploid (Tetragenomous) gametes*

It has been sometimes observed that the split chromosomes do not distribute towards the poles and are inclosed in one nuclear membrane. Siderophile matter is already recognizable before splitting. In such a case a big pollen-grain will be produced (Text-fig. 3–1). It is not frequent nor is it extremely exceptional. It is also imaginable from the occurrence of big pollen-grains as described before.

b) *Formation of tetrads*

After each chromosome at the equatorial plate splits, two homo-typic spindles may be formed in parallel. Such plants are rarely observed in these hybrids, and tetrads instead of dyads will be produced in this case. Text-fig. 3–4, 5 and 6 are polar and side views of this abnormal division. In Text-fig. 3–6 two spindles enclosed completely by siderophile matter are seen.

c) *Irregular distribution of chromosomes in daughter nuclei*

Sometimes two neighboring spindle poles formed in the manner described under b) fuse together leaving the other two free. As each forms a haploid nucleus, consequently a triad will be formed (Text-fig. 3–3). Text-fig. 3–2 shows the split chromosomes distributing in unequal number in two nuclei surrounded by siderophile matter. In this case, large and small abortive pollen-grains are formed. Text-fig. 3–7, 10, 11 shows the chromosomes being divided in unequal mass by the siderophile matter. On one side only a few chromosomes are seen, where an extra dwarf pollen-grain will be formed and the number of chromosomes in one nucleus may be greatly in excess of that in the other. A giant pollen-grain then results.

Sometimes observations were made, as shown in Text-fig, 3–9, 12, although two masses of siderophile matter make their appearance in the form of two rings, the chromosomes are still remain between them (Pl. VIII, Fig. 7). In this case some of the chromosomes are inclosed in either nucleus but some chromosomes lagging between the two nuclei form another third nucleus.

Text-fig. 3–8 shows divided chromosomes lagging all over the spindle and siderophile matter constricted in three parts. Then all the chromosomes distribute in three nuclei in unequal number. In this case triads will be formed.

Text-fig. 3.
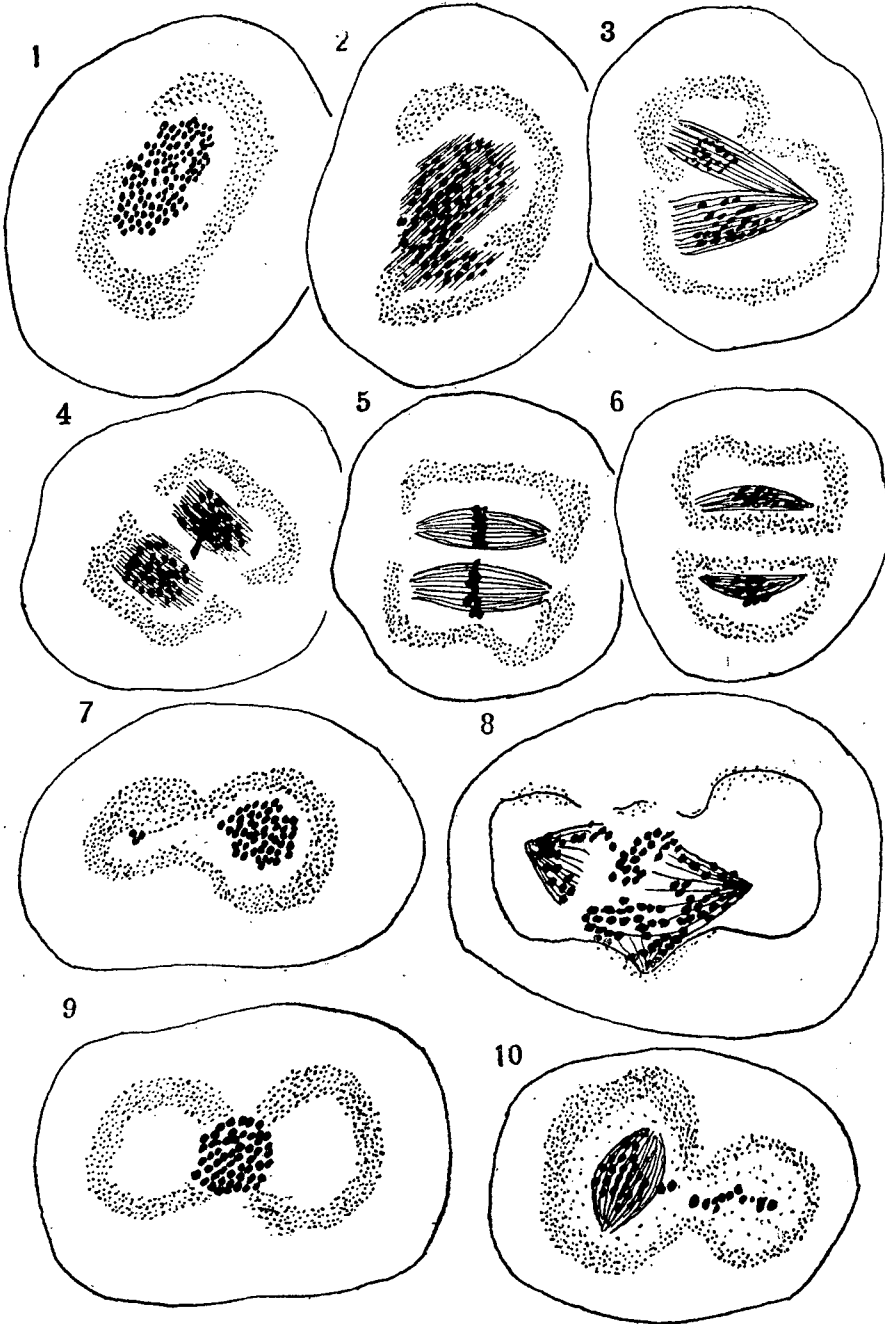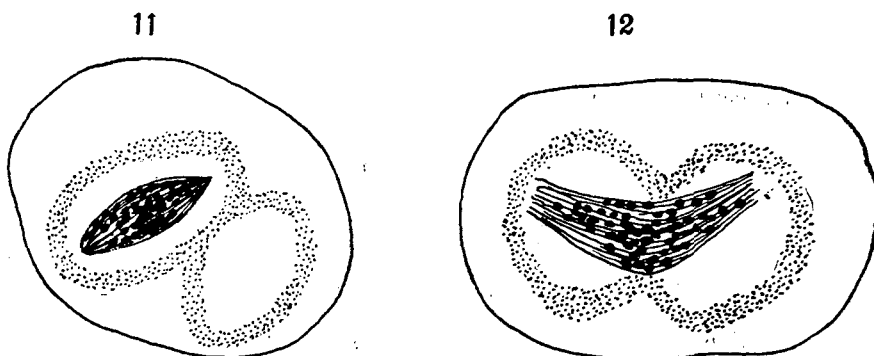
11   **12**

Text-fig. 3.   1-12. Some irregularities observed in meiotic division
of pollen mother cells in F_1 hybrid.

1. Formation of tetraploid gametes.
2. Formation of large and small pollen-grains.
3. Formation of triad.
4, 5, 6. Formation of tetrad.
7, 8, 9, 10, 11, 12. Irregular distribution of chromosomes.

(3) Conclusion

Summing up all that has been said on the formation of the sexual
cells in the F_1 hybrids, we come to the conclusion that many of them
possess two genomes of chromosomes as a result of non-conjugation
and omission of the first division in the meiotic division of P.M.C. Some
of them may have a number of chromosomes more or less approaching the
digenomous cells, i.e a small number of hypo and hyper[4] digenomous
gametes will be formed by the unequal distribution of chromosomes. There
may arise also gametes containing tetragenomes and gametes having only
a few chromosomes in consequence of an irregular distribution of chromo-
somes in the pollen mother cells.   This is coincident with the formation of
giant elliptical pollen-grains and small abortive pollen-grains as described
before.

It is not possible to ascertain with exactness how often the gametes
arise, which have greater or less number of digenomous chromosomes. The
deviation in pollen size and dyad formation indicates indirectly the occurrence
of irregular processes.

4) According to KARPECHENKO (1928), the gametes which are in their number of chromo-
somes close to tetraploid, but do not exactly correspond, is termed "Hypotetraploid"
or "Hypertetraploid" according to the number of chromosomes included in the hybrid or
gametes. Writer used here new terms combining the term "Hyper-" or "Hypo-" with
WINKLER's term "genom".

It would appear from the results shown in Table 63 that almost all of the sporocytes of F_1 hybrids are dyad and only a small number are monad, triad, tetrad or polyad.

Table 63.   *Frequency distribution of monad, dyad, triad, tetrad and polyad sporocytes formed in F_1 hybrids in 1929.*

F_1 plants	· Number of microspores in a sporocyte						
	I	2	3	· 4	5	6	Total
White long × H. Manihot	3	3141	7	20	I	2	3174
White velvet × H. Manihot (Field)	4	3305	10	I	I	2	3323
(Glass house)	I	227	80	36	12	I	357

As regards white velvet, in plants grown in the open field $(27°-31°$ C$)$ 3305 sporocytes out of 3323 calculated were dyad.   In plants grown in glass house $(36°-38°$ C$)$ out of 357 sporocytes only 227 were dyad, 80 were triad, 36 were tetrad, 12 were pentad and one each was hexad and monad respectively (Table 63).   This gives the impression that here the external condition (presumably high temperature) of the plants at the time of examination plays a certain role.   The possibility of the influence of external condition on the reduction division is already indicated by the experimental investigations of SAKAMURA (1920, 1926), SAKAMURA and STOW (1916), STOW (1927), BELLING (1923), HEILBORN (1928), TAKAGI (1928), SHULL (1930) and others.   BELLING and BLAKESLEE (1920) indicated that the formation of large dyad pollens in *Daturas* by non-reduction can be greatly intensified by transient cold.   TAKAGI observed various abnormalities in pollen formation of *Lychnis Sieboldii* when the pollen mother cells are subjected to the temperature of $38-39°$ C for $3-3\frac{1}{2}$ hours and some of the mother cells produced only dyads having diploid number of chromosomes.

Each of the chromosomes of these parental species is incapable of association with the others owing to lack of harmony in the constitution of the gametes, i. e. there is an exteme case of pathozygoty[5].   This lack of association between those chromosomes which were derived from the two parents indicates that there is no special genetical relationship between the

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5)   WINGE (1917) has employed "Pathozygoty" to indicate the fact that two gametes are only partly capable of association owing to the less marked harmony.

chromosome sets in question, or in other words, their failure to pair in these hybrids may be due to a general lack of balance in the chromosomes of both parents.

.   ROSENBERG (1917) gave to the formation of diploid gametes as a result of non-conjugation of chromosomes and non-reduction in the meiotic division, i. e. the omission of the first division, the name "Semi-heterotypic division". He first described this process in *Hieraceum loevigatum* and *lacerum*. NEWTON (1928) has pointed out in his investigation of interspecific hybrid *Digitalis purpurea* × *D. ambigua* : "Dyads arise in two ways, (1) from failure of the first division, (2) from combination of two second division plates." The latter has not been observed in the present author's material.   In the case of *Digitalis* the dyad formation was not after the usual manner but was rather a rare case ; usually there were numerous or only two spindles formed in the second division.   In the present author's material, the former case usually occurred but the latter case did not.   ROSENBERG (1927) observed in parthenogenetic *Eu-Hieracia* a graded series of degeneration of the meiosis, beginning with a type having a variable number of doubles and singles, (this type was called by him "Boreale type"), to types where the conjugation between the chromosomes at the first division is quite lacking to which he gave the name "Levigatum type" or "Semiheterotypic division".   As to the Levigatum type of *Eu-Hieracia* he wrote as follows ; "In Levigatum type all chromosomes in the first division are univalent and distributed irregularly along the spindle figure.   The chromosomes are short and thick as in the heterotypic division of sexual forms.   As a result of this division the nuclei will have a variable number of chromosomes.   The semiheterotypic meta- and anaphase very often are not completed, but are interrupted by a premature homotypic division, whereby restitution nuclei are formed.   Round the entire spindle figure a new nuclear wall is formed resulting in the production of a single large nucleus, the chromosomes of which divide quite in the same manner as in normal interkinesis, but with the diploid number of chromosome.   Such pollen mother cells divide only once and become dyads, thus producing pollen cells with the diploid number of chromosomes."   He observed these two types of division in the same species.   .

In the present author's material described in this paper there is the case corresponding to this Levigatum type, but diploid gamete formation seems to be usual, the rare occurrence of triad, tetrad, monad or polyad gametes indicating this indirectly.

The non-conjugation of all chromosomes of the F_1 hybrid was reported

by COLLINS and MANN (1923) in F_1 hybrid of *Crepis setosa* (n=4) × *C. Capillaris* (n=3), but in this case the division was irregular and usually the chromosomes passed into the poles without dividing.

J. CLAUSEN (1924) observed semi-heterotypic division in a cross of *Viola* species. KARPECHENKO (1924) reported the formation of diploid gametes as a peculiarity in the formation of 18 (2n) chromosome gametes in the hybrid of *Raphanus* × *Brassica*. KARPECHENKO (1927) and SAKAOKA (1930) observed a peculiarity of diploid gamete formation in F_1 hybrid of *Raphanus* × *Brassica*. MEURMAN (1928) found in the sterile hybrid forms of genus *Ribes* a certain number of large pollen mother cells showing 16 univalents arranged in a regular equatorial plate which divided equationally twice and gave rise to diploid gametes. The formation of diploid gametes by omission of first division has been described also by KIHARA (1924) in wheat-rye hybrid, BELLING (1925) in *Uvularia*, KAGAWA (1929) in *Aegilops* × *Triticum*, FUKUSHIMA (1929) in F_1 hybrid of *Brassica* × *Raphanus*, LAMMERTS in *Nicotiana paniculata-rustica* hybrid, GOODSPEED and CLAUSEN (1927) in F_1 hybrid of *Nicotiana Bigelovi* × *suaveolens*, JØRGENSEN (1928) in *Solanum* species, LONGLEY (1930) in wheat-rye and *Sacale cereale* × *S. montanum* hybrid, and by WOODWORTH (1929) in *Betula papyrifera* var. *condifolia* × *B. davurica*.

Restitution nuclei were also observed by KAGAWA (1929) in the meiotic division of *Aegilops cylindrica* × *Triticum dicoccum*, by KUWADA (1928) in *Balenophora japonica* MARK. and by others.

In the many above mentioned cases, the diploid gametes formation as a result of non-conjugation and the omission of the first division of pollen mother cell was observed not to be the usual manner of meiotic division but rather to be the irregular and rare case. In the *Betura Sandbergi Britton*, WOODWORTH (1929) reported that five to ten per cent of the pollen mother cells behave in the manner above mentioned. The usual course of the meiotic division leads in the hybrids of *Hibiscus esculentus* × *H. Manihot* to the production of gametes with a somewhat varying number of chromosomes, but with a number near to that of the digenomous gametes. The gametes which have a less or greater number of chromosomes arise only as results of certain modifications in the process of division.

4. Cytological investigation of the back-crossed plant (Trigenomous plant)

The cytological study of back-crossed plants in interspecific hybrid makes possible the exact determination of chromosome number of F_1 gametes which are capable of functioning.

(1) The first reduction division

In 1928 and 1929 many of the back-crossed plants (*H. esculentus* ♀ ×
F_1 hybrid ♂) were examined cytologically. It was found that in the meta-
phase of reduction division these plants are triploid or trigenomous. These
trigenomous plants had, without doubt, two sets of *H. esculentus* and one
set of *H. Manihot* chromosomes, that is $36_{II} + 30_I$ in Drosera schema[6].
Text-fig. 4–1 shows 56 chromosomes in the metaphase of the first division.
It is not easy to distinguish the bivalent and univalent chromosomes. The
calculated number of chromosomes in metaphase of meiotic division varies
between 63 and 68, but the counting is very difficult, partly due to the
relatively great number of chromosomes, partly to the minuteness in size.
Out of 12 plants 5 were 66; other cases 66+ or 66−, but very near to
66 in number.

All these chromosomes were often observed oriented at the equa-
torial plate as indicated in Text-fig. 4–2, Pl. VIII. Fig. 9. But also the
bivalent chromosomes oriented themselves on the equatorial plate and single
chromosomes were found scattered on both sides of that plate, though
some of them remained on the equatorial plate, as indicated in Text-fig. 4–
6. This is similar to that case reported by ROSENBERG as early as 1906
in the hybrid of Drosera. The number of single chromosomes on each
side varies somewhat, but the total number is approximately 30 in most
cases. In general, however, the distribution of single chromosomes to
either pole occurs in about equal number. The trivalents, such as DAR-
LINGTON (1928) observed in hyacinth, were not formed supposedly because
the two kinds of chromosomes are not functionable to conjugate.

At the anaphase of this first division the bivalent chromosomes divide
at the equatorial plate. At this stage we should ordinarily find 102 chromo-
somes (Pl. VIII, Fig. 9), but often the number cannot be stated definitely and
we can only state that it is somewhere between 100–105. In several cases
the author was able to count over 120 chromosomes because, evidently,
some of the univalents from *H. Manihot* are divided. The split chromo-
somes move towards the poles and at this stage a very broad spindle is
formed as indicated in Text-fig. 4–4.

The two groups of chromosomes having reached the poles are soon
inclosed each in one nuclear membrane. Text-fig. 4–5 shows the two
groups of chromosomes having reached the poles; the fibers are still
visible.

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6) ROSENBERG (1906) observed in hybrid of *Drosera* species hybrids ten bivalents and ten
univalents at the metaphase of meiotic division.
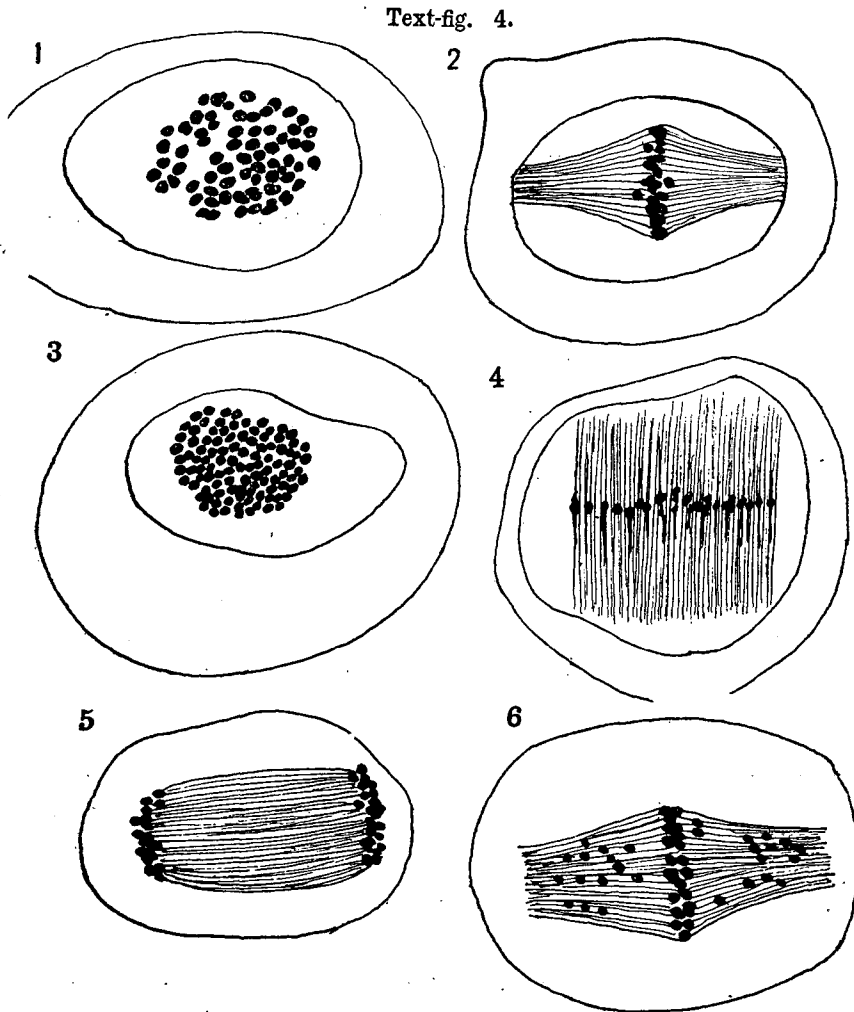
Text-fig. 4.

Text-fig. 4. 1-6. Normal first reduction division of P.M.C. in back-crossed plants.

1. Heterotypic metaphase, 66 chromosomes are seen.
2. Heterotypic metaphase, side view, all the chromosomes oriented at the equatorial plate.
3. Anaphase of first division, showing all the bivalent chromosomes divided. 102 chromosomes in total are seen.
4. Anaphase of first division, side view showing the split chromosomes and broad spindle.
5. Late anaphase, two groups of chromosomes have reached the poles, the fibers are still in sight.
6. Metaphase of first division, univalent chromosomes are scattering at both sides of equatorial plate.

(2) Some irregularities observed in the first reduction division

From the just described usual course of meiosis in back-crossed plants, deviations are met with, to which attention should now be directed. These back-crosses showed more irregularity in meiosis than in F_1 hybrid.

1) After the bivalent chromosomes split at the equatorial plate two homotypic spindles are formed as shown in Text-fig. 5-1. In this case polyspory will occur. It is not a rare case.

2) The two spindles are not parallel and two of the spindle poles may join at one side to make a single nucleus, the other two remaining free as indicated in Text-fig. 5-2. This type of irregularity was observed in F_1 hybrid occasionally. A triad, one large and two comparatively small, will be formed in this case.

3) In some cases the divided chromosomes in metaphase of the first division remained for a long time together with the undivided univalent chromosomes and divided into three parts by the constriction of nucleus membrane; the siderophile matter was also seen in constriction. In this case the second division may be very irregular and the pollens resulting from this division are highly variable in form. The mother cell may contain many young pollen-grains. (Text-fig. 5-3). In these cells the author calculated above one hundred chromosomes and often 132, this is presumably $36 + 36 + 30 + 30$.

4) In very rare cases the author observed a few like or unlike spindles, free or connecting with each other at various angles, taking very complicated forms with above one hundred of the small chromosomes scattered all over them. In this case very irregular nuclear division will occur and hexads or other polyad forms will be formed. This type of cell division is shown in Text-fig. 5-4, 5, 6, 7, 8.

5) As indicated in Text-fig. 5-9 and 10, the chromosomes first reaching the end may form a new nucleus before the later ones arrive, so that several chromosomes may lie out of the reconstructed nucleus. In this way the later chromosomes which remain outside of the new nucleus often form a third nucleus.

6) Between the two poles the membrane is constricted into two or three parts giving the appearance of the nucleus pulling apart without the formation of a spindle as shown in Text-fig. 5-11, 12. In this case one cell may have all the univalent chromosomes while one or two of them have nothing.

7) In very rare cases the membrane is constricted into three parts in

Text-fig. 5.
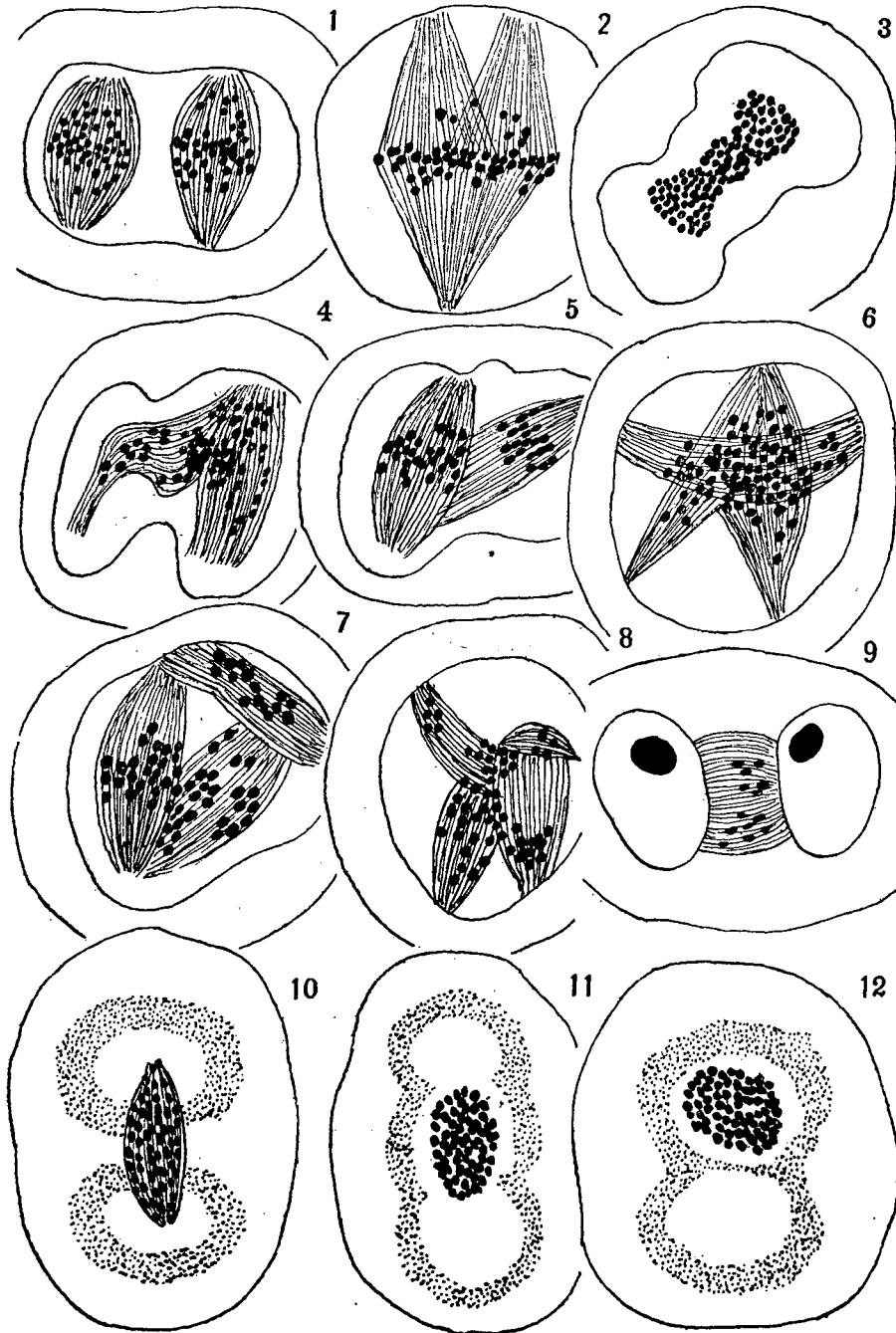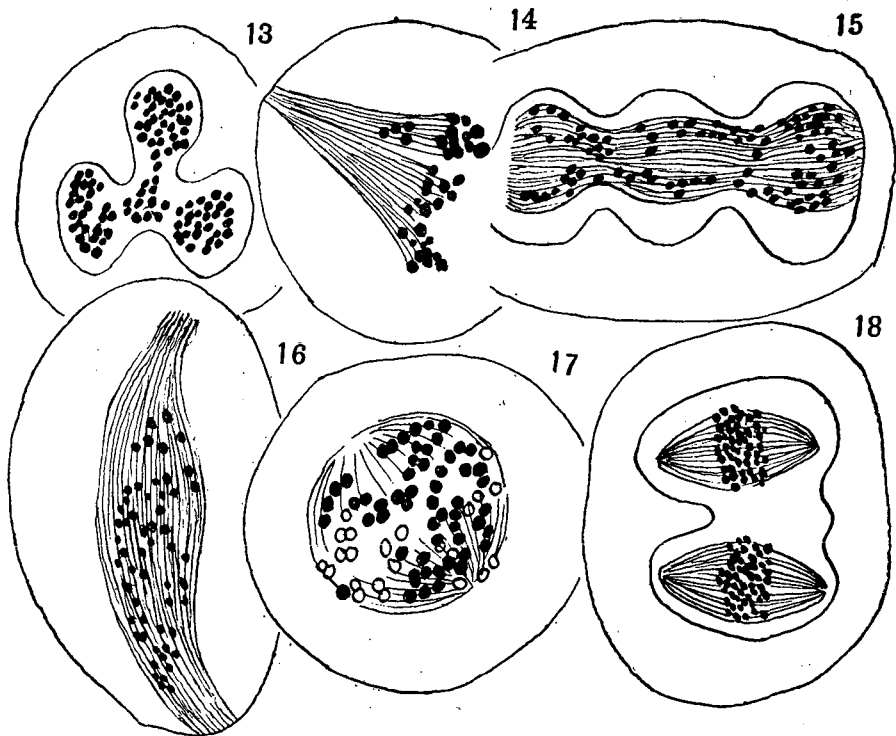
Text-fig. 5. 1-18. Irregular meiotic division of back-crossed plants.

1. After splitting, the univalent chromosomes formed two new homotypic spindles in parallel.

2. Two unparallel spindles are formed and the two spindle poles may join at one side.

3. All the univalents divided into three parts by the constriction of nucleus membrane.

4, 5, 6, 7, 8. A few like or unlike spindles free or connecting with each other at various angles and making very complicated forms.

9, 10. Two nucleus membranes are formed while some chromosomes are still lagging between the two poles. The remaining chromosomes outside of either nucleus may form the third nucleus.

11, 12. Nucleus membrane constricted into two or three parts; only one part may have all the univalent chromosomes.

13. The nucleus membrane is constricted into three parts in the formula of radiation and the chromosomes are distributed in each part approximately in equal number.

14. Monospindle formed in first division.
15. All the univalents lagging all over the spindle and the nucleus membrane constricted into three parts.
16. The curving of the spindle at anaphase of the first division.
17. Exceptional plant of back-crosses, BG No. 3-17, at metaphase of first division 79 chromosomes were counted.
18. Normal second division metaphase.

the formula of radiation and the chromosomes are distributed in each part approximately in equal number as indicated in Text-fig. 5–13.

8) The author observed in very rare cases the monospindle formed in the first division as shown in Text.fig. 5–14.

9) One may see the univalents lagging all over the spindle, the nucleus membrane constricted into three parts and the chromosomes distributed in three nuclei in inequal numbers as shown in Text-fig. 5–15.

10) The curving of the spindle is often seen at anaphase of the first division just as was found by GOODSPEED (1927) in *Nicotiana Bigovii* × *suaveolens* as shown in Text-fig. 5–16.

(3) The second reduction division

The second division of pollen mother cells in back-crossed plants is much more irregular than that of first division. The formation of spindles with varying number of chromosomes and other irregulalities as we observed in the first division also occured in this division. At the second division the univalents split; the distribution of the split chromosomes again proceeds unequally. Instead of tetrads there arise groups with a different number of cells up to 12. In the metaphase of this division the author observed in some cases a normal 51 chromosomes in both daughter nuclei as indicated in Text-fig. 5–18, that is presumably 36+15 and in some cases 66 small chromosomes, which is presumably 36+30. The first and second division of pollen mother cells in back-crossed plants are explained by Scheme 3.

1   Zygote (3 genomes)
2   Diakinesis
3   Metaphase of first division
4   Telophase of first division
5   Metaphase of second division
6   Pollen-grains

A   B   C   D
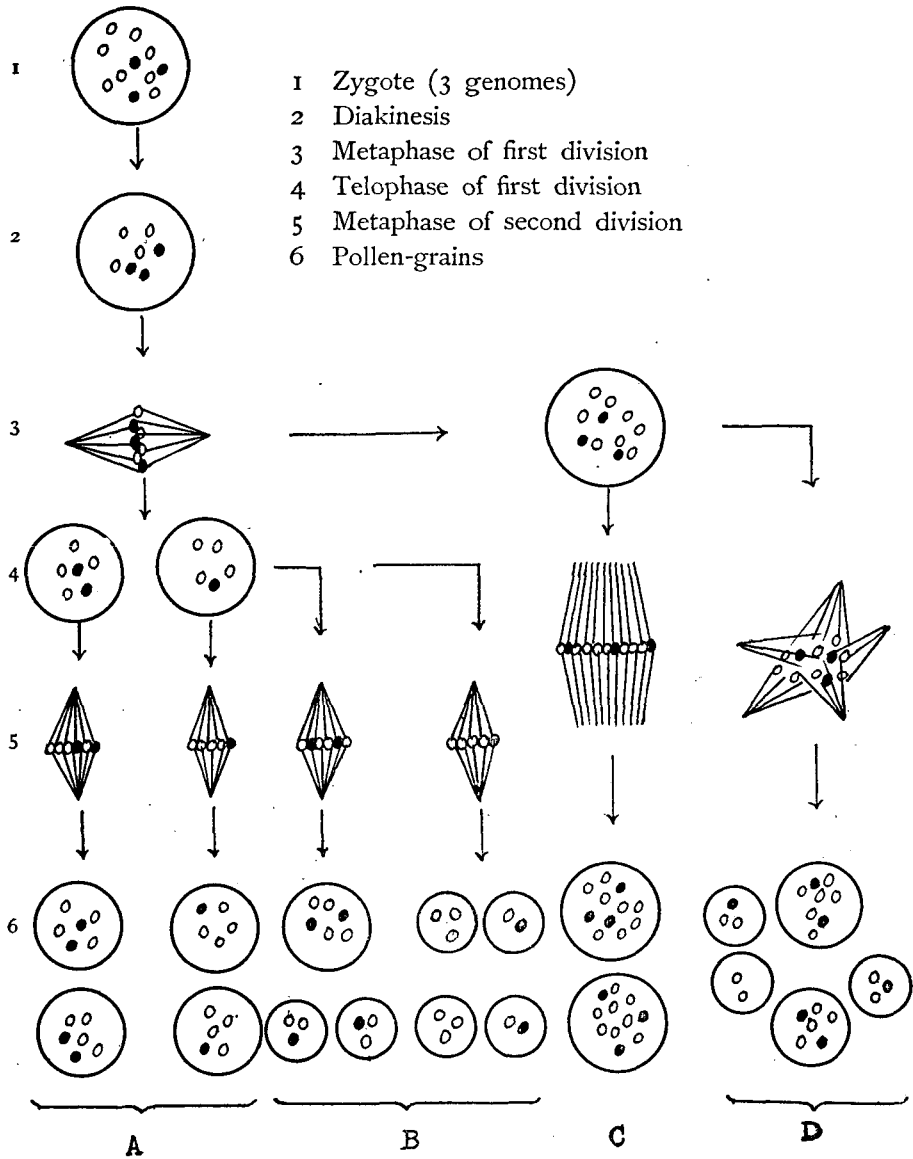
Scheme 3.   Gamete formation in back-crossed plant.

A.   Normal course of trigenomous plant.
B.   Some irregularly formed gametes.
C.   Formation of triploid gametes.
D.   Some irregularly made polyads with a different
number of chromosomes in each cell.

(4) Conclusion

In consequence of the irregularities attending division a single sporocyte can produce from one to many spores, with nuclei which contain various amounts of chromatin.   The size of the cells which contain more chromosomes is larger than those containing a smaller number.   As shown in Table 58 one to fourteen microspores were observed produced from a single sporocyte.

The back-crosses raised in 1928, 1929 and 1930, with some exceptions, were generally quite uniform without any conspicuous differences although fluctuation caused by environmental conditions may exist.   There is no doubt in this case but that the chromosome constitution of these plants represents the series of $36_{II} + 30_{I}$ or it allies.   BC No. 3–17 is one of the exceptional plants which the author examined.   This plant differed from other ordinary back-crosses not only in general appearance but also in fertility.   The general appearance was much like that of *H. esculentus* and only about 2 per cent of normal pollen-grains were produced.   The tetrads were much more numerous than dyads, triads or polyads.   The fertility of this plant was so low that only two capsules were obtained by open pollination, each having one to nine fertile seeds.   Seventy nine chromosomes altogether were calculated in the metaphase of the first division of this plant (Text-fig. 5–17).   Presumably $36_{II} + 30_{I} + 13_{I}$ is the costruction of chromosomes.   The extra 13 chromosomes may be of *H. esculentus* as this plant shows an appearance much more like *H. esculentus* than the others.

The appearance of exceptional plants among back-crosses which have a surplus number of chromosomes was often reported by several investigators.   For instance, LAMMERTS (1929) observed, among 117 back-cross progeny of F_1 *Nicotiana paniculata-rustica* × *paniculata*, 28 plants having 48 chromosomes ($12_{II} + 24_{I}$).   The remaining 89 plants belonged to the chromosome series $12 + i$, value of i ranging from 1 to 9.

From these results of a cytological investigation of back-crosses it is evident that the functional gametes of F_1 hybrid are mostly of category $36_{I} + 30_{I}$ with some exception.   This result is something like the case with *Raphanus-Brassica* hybrid, as KARPECHENKO reported (1927) only the gametes possessing the entire haploid set of both parents or even twice their number appear, among these hybrids, to be able to produce offspring.

The lack of affinity and non-conjugation of chromosomes derived from *H. esculentus* and *H. Manihot* cause somewhat different results from those described by other investigators.

The results from *Hibiscus* hybrid are different from those of GOOD-

SPEED and CLAUSEN (1922) in back-crosses of (*Nicotiana sylvestris* × *Tabacum*) × *N. sylvestris*, from which they obtained only pure *sylvestris* plants. They explained their results as due to zygotic elimination of all combinations not genetically identical with *sylvestris*.

The author's results differ from those of NEWTON and PELLEW (1929) in back-crosses of *Primula floribunda* × diploid hybrid, who found 18 (2n) chromosomes in their five back-crossed plants examined. But in this case no two *floribunda* appeared as in the case of *Nicotiana*, although 1 in 512 gametes contained a complete set of *floribunda* chromosomes. GOODSPEED, CLAUSEN and CHIPMAN (1926) reported the results of the cytological examination in the back-cross progeny of F_1 hybrid of *Nicotiana · rustica* (24^{II}) and *paniculata* (12^{II}) to the *rustica*. The functional gametes of the F_1 hybrid were mostly of the category $R+i$, in respect to chromosome number with i having an average value of approximately 6, as is to be expected from random distribution of univalents. In the *paniculata* back-cross progeny, however, they reported that there was one exceptional group of plants which have $12_{II}+12_I$ chromosomes; LAMMERTS (1929) in his studies of back-cross plants, F_1 *Nicotiana paniculata-rustica* × *paniculata*, observed two main classes in these progeny in respect to chromosome number. The plants which belong to class (1) had the series of $12_{II}+i_I$, value of i ranging from 1 to 9 and the plants of the other class (2) had approximately $12_{II}+24_I$ or 48 chromosomes. Moreover he also observed the exceptional individuals which uniformly exhibited $13_{II}+8_I$. In LAMMERTS' experiment there is no doubt but that it is to be expected that one will obtain individuals which belong to class (1) in ordinary case and the occurrence of individuals which belong to class (2) is rather exceptional. But those belonging to class (2) appear to be very interesting and coincident to the author's result which is reported in this paper as usual manner of case. THOMPSON and CAMERON (1928) reported the results of back-cross in *Triticum* species hybrid that in nearly every case the gametes with a number of extra vulgare chromosomes intermediate between 0–7 were in much smaller proportions than were to be expected, and in most cases the gametes with univalent chromosomes (14 in all) were much more numerous than those with 7 (21 in all).

5. Cytological investigation of progeny of back-crossed plants

The progeny of back-crossed plants obtained by open-pollination grown in 1929 were examined cytologically. The observed behavior in these plants was of great interest to forecast the distribution of the chromosomes

through future generations.   Over one hundred plants were examined but in only fourteen plants was the approximate number of chromosomes counted partly due to the relatively great number of chromosomes, partly due to the lagging chromosomes.

It might be expected from the irregular distribution of chromosomes in the meiotic division of pollen mother cells of back-crossed plants that the progeny of back-crossed plants have an extremely varied number of chromosomes.

In the metaphase of the first division all of the chromosomes are arranged at the equatorial plate and a varied number of chromosomes was counted. At the anaphase of this division usually small chromosomes may be observed scattering at both sides of the spindles.   At the metaphase there are some variations in size of chromosomes which it is not easy to distinguish distinctly but it is not improbable that these large chromosomes are bivalents and small ones are univalents.   The number of single chromosomes at each side varies somewhat but the total varies from 2 to 40.   Text-fiig. 6, 1 shows the metaphase of first division of P.M.C. in BCN, No. 42 where about 70 chromosomes are seen.   Some of these are constricted and small but larger chromosomes are seen; 2 shows the side view of the spindle at anaphase of first division, presumably the chromosomes oriented at the equatorial plate are the bivalents and those that are scattered at both sides of the plate are single chromosomes.   In some cases the second division is regular as shown in 3 in this Text-figure, but most cases of first and second division are usually irregular.   Many irregularities which were observed in the pollen mother cells of F_1 plants and
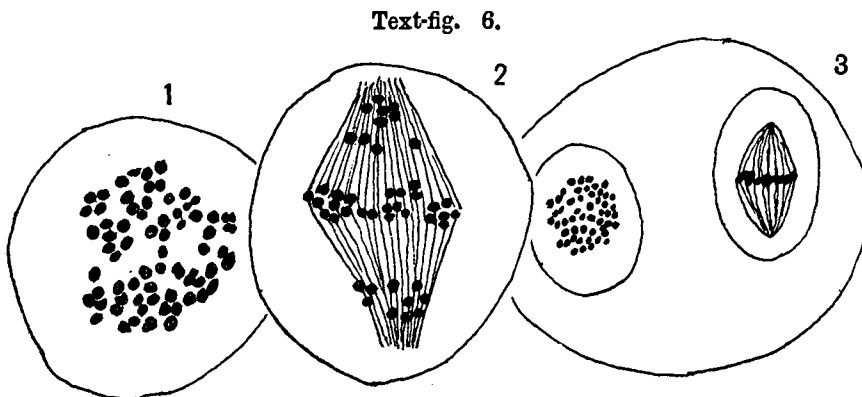
Text-fig. 6.

Text-fig. 6.   Polar and side view of first division and normal second division of P.M.C. in the progeny of back-crossed plants.

back-crossed plants were also observed in these plants, consequently poly-spory is much in evidence. There are many tetrads of pollen mother cells but usually from 3 to 12 cells resulted from one sporocyte.

The following number of chromosomes was counted at the metaphase of the first division in twelve plants; 48, 50, 50, 50, 55, 55, 56, 64, 65, 69, 70, 70. It is not improbable to suppose that the back-crossed plants produce the male and female gametes having various numbers of chromosomes. Among these gametes the functional ones are mostly of category 36+i as respects chromosome number, value of i ranging from 0 to 30; 36 are *esculentus* chromosomes and i represents *Manihot* chromosomes.

There is a definite correlation between chromosome number and fertility of the plants, the plant having the higher number of chromosomes possessing greater fertility than those having the smaller number of chromosomes. The fertility of these plants and their chromosome number is shown in Table 64.

Table 64. *Fertility and chromosome number in the progeny of back-crossed plants.*

Plants having 48 to 56 chromosomes			Plants having 64 to 70 chromosomes		
Plant number	Number of chromosomes	Total seeds obtained	Plant number	Number of chromosomes	Total seeds obtained
BCN, No. 1	50	9	BCN, No. 21	65	54
No. 9	56	0	No. 42	70	91
No. 14	50	1	No. 49	64	164
No. 26	55	36	No. 53	70	120
No. 35	50	0	No. 63	69	352
No. 48	48	28			
No. 62	55	50			
Average	52.0	17.7	Average	67.6	156.2

From the result represented in Table 64 it may be concluded that the fertility of these plants increases when the plants have a number of chromosomes aproaching to 66 (36+30) at the metaphase of the first division.

6. Reestablishment of constant types in later generations

Many seeds obtained from the progeny of back-crossed plants by open-pollination were sown in 1930 in the field of Hokkaido Imp. Univ., Coll. of Aric., Sapporo. From these seeds many varied plants were raised. Among these groups of plants it was found that No. 24 and No. 125 were quite similar to *H. esculentus* in general appearance, and the mother plants of these groups of plants which were grown in the previous year also quite nearly resembled the pure species (Plate IV, Fig. 1, BCN, No. 24, Plate V, Fig. 1, BCN, No. 24 and BCN. No. 125). Among 33 plants of BCN, No. 24, three plants still remained in the hybrid form but other plants were all uniform in every character. Among 19 plants of No. 125 only two plants were hybrid in form, while the remainder were quite similar to the pure species in general appearance. Apparently these plants my be constant ancestral types which came into appearance by reestablishment. In view of the similarity in characters to *H. esculentus* and uniformity in appearance, chromosome number and behavior similar to *H. esculentus* might be expected. It is possible that the elimination of the univalents through lagging on the spindles at the meiotic division led to the establishment of the stable diploid type resembling the diploid ancestor.

The calculated number of chromosomes at the metaphase of the first division is 36 or 37 in BCN, No. 24-4 and 36 in BCN, No. 125-5.

The chromosome behavior in heterotypic and homotypic division is very regular as in the pure species and there is no lagging chromosome, Text-fig. 7-1 shows 37 bivalent chromosomes in the metaphase of the

Text fig. 7.
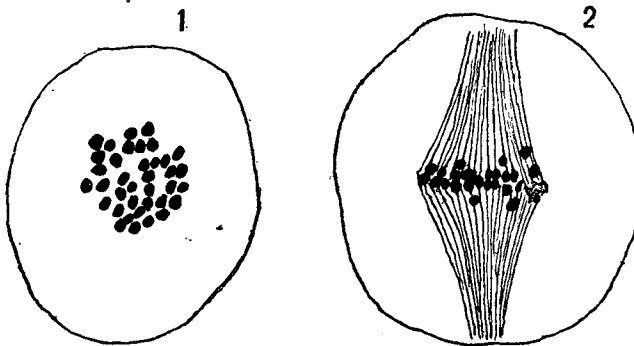
Text-fig. 7. Polar and side view of metaphase of first division in P.M.C. of constant ancestral type appearing by reestablishment.

first division of BCN, No. 24–4, 2 shows a side view of a spindle at the metaphase of the first division in BCN, No. 125–5.

Such reestablishment of constant type in later generations from crosses between individuals with different chromosome numbers has been found by many other investigators. ERLANSON (1929) has noted it on a diploid rose which he recognized in a later generation of cross between *Rosa blanda* (Diploid) and *R. acicularis* (Hexaploid), KIHARA (1921) and SAX (1922) in *Triticum*, BOEDIJN (1926) in *Oenothera*, YASUI (1921) in *Papaver*, THOMPSON and HOLLINGSHEAD (1927) in *Triticum*, and also it was suggested by HEILBORN in *Drabe*. GOODSPEED and CLAUSEN (1922) obtained only pure sylvestris plant from back-crosses of *(Nicotiana sylvestris × Tabacum) × N. sylvestris*. They explained their results as due to zygotic elimination of all combinations not genetically identical with *sylvestris*.

7. Cytological investigation of F_2 plants

It might be expected that tetraploid form would be obtained by union of unreduced F_1 gametes of *H. esculentus × H. Manihot* as suggested by FEDERLEY (1913) in *Lepidoptera* hybrid and as corroborated by CRANE and DARLINGTON (1927) in *Rubus-rusticans vinermis × R. thyrsiger* and by KARPECHENKO (1928) in *Raphanus-Brassica* hybrid. The results of cytological investigation of the F_2 plants cultivated in the trial ground of Hokkaido Imp. Univ., Coll. of Agric. in 1930 without being isolated from either *esculentus* or *Manihot* proved that these F_2 plants are tetragenomous having 132 chromosomes. These tetragenomous plants had, without doubt, two sets of *esculentus* and two sets of *Manihot* chromosomes, that is $36_{II}+30_{II}$ in Drosera schema. The chromosome behavior in heterotypic and homotypic division are very regular as in pure species, the calculated number of diploid chromosomes in the metaphase of first division being 66 or nearly 66.

At the metaphase of first division all of the bivalent chromosomes oriented at the equatorial plate and there were no lagging chromosomes as shown in Text-fig. 8–1 and 2, Pl. VIII, Fig. 10 and 11; at the anaphase the divided chromosomes are seen in smaller size than the bivalents (Text-fig. 8–3). The second division is very regular, at the anaphase it was possible to count over 120 small chromosomes (Text-fig. 8–6). In consequence of this regular division the tetrads are always formed and they developed into big pollen-grains.

Only in a few cases was it found that there occurred some irregularities. Text-fig. 8–5 shows the one of irregularities which was observed in these plants. The siderophile matter makes its appearance in

the form of two rings and three spindles are seen. Text-fig. 8–4 represents another irregularity showing two rings and remaining chromosomes between them.
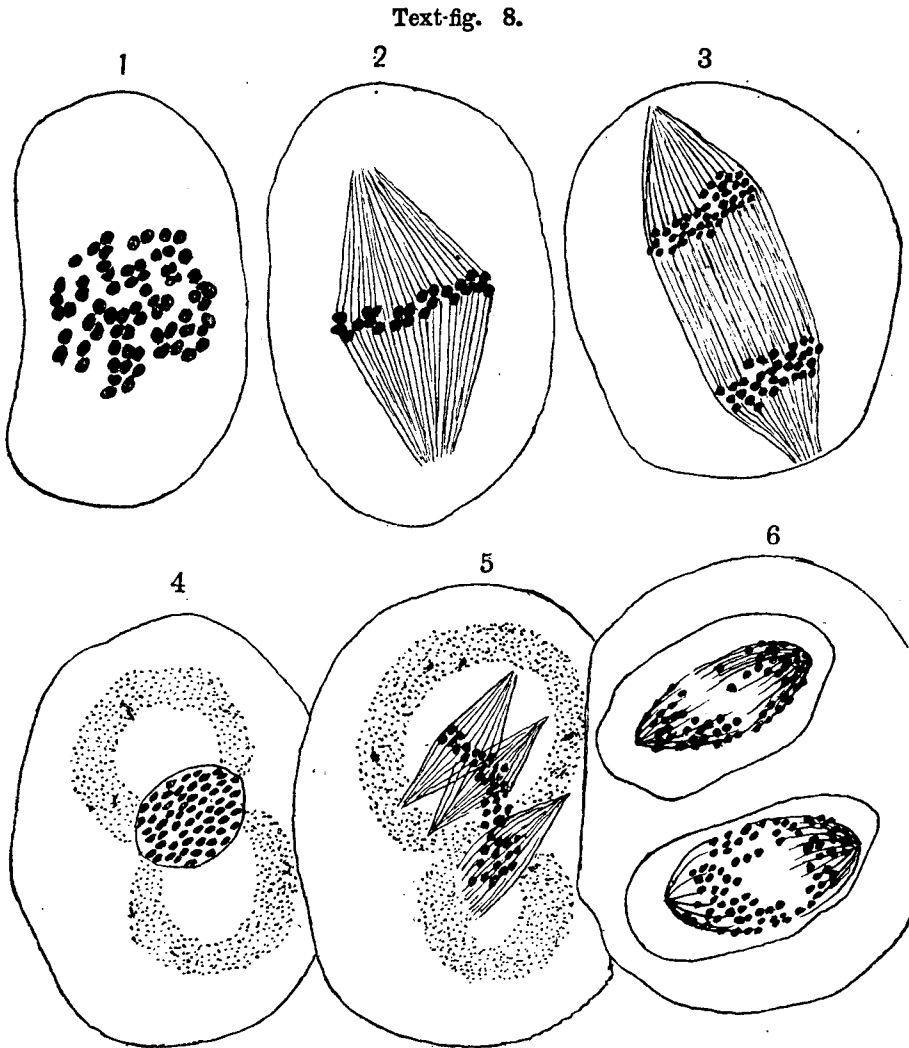
Text-fig. 8.

Text-fig. 8. Regular meiotic division of P.M.C. in F_2 plants and some irregularities observed.

1, 2. Polar and side view of metaphase of first division.
3. Anaphase of first division.
4, 5. Irregularity of meiotic division.
6. Anaphase of second division.

It is not an improbable supposition that the F_1 plants produce female gametes having more varied chromosomes than male gametes. Among these gametes functionable are those having somatic number of chromosomes of F_1 hybrid and capable of making tetragenomous F_2 plants with the male gametes having the same number of chromosomes. Such an increase in the chromosome number in F_2 over F_1 was also found by many investigators in several plants; for instance, BREMER (1923) in species hybrids of *Saccharum*, ERLANSON (1929) in *Rosa*. CLAUSEN (1924) has explained this increase in the chromosome number over F_1 by a double splitting of unpaired chromosomes during meiosis in *Viola*. In interspecific hybrid of *Digitalis*, BUXTON and NEWTON (1928) obtained tetraploid (n= 112) F_2 plants by artificial self-fertilization and triploid plants from natural pollination. In the author's material it was found impossible to obtain seed from F_1 plants by controlled pollination and only progeny of the F_2 hybrids resulting from open-pollination were examined.

8. Cytological investigation of progeny resulting from the seeds obtained from the back-crossed plants by pollination with the pollen of H. esculentus, [(H. esculentus × F₁) × H. esculentus], and with the pollen of F₁ hybrid, [(H. esculentus × F₁) × F₁]

Many of these plants were examined cytologically but the result of this investigation was obtained from only a small number of individuals.

BC × HE, No. 1–3, (*H. esculentus* × F_1) × *H. esculentus*

In the metaphase of the first meiotic division of this plant relatively large chromosomes, which are thought to be bivalents, and small chromomosomes were seen, oriented at the equatorial plate and about 5 or 7 lagging chromosomes were usually seen at both sides of the spindle. (Text-fig. 9–2). At the anaphase of this division about 87 relatively small split chromosomes are seen, Text-fig. 9–1.

If the female gametes of a back-crossed plant, which has 36 *esculentus* chromosomes and 15 *Manihot* chromosomes, mates with the pollen-grain of *H. esculentus* which has 36 chromosomes, then, there should be $36_{II}+$ 15_I chromosomes at the metaphase of the first division and after these double chromosomes split we might expect 87 chromosomes to appear. About half of these split chromosomes move toward the poles. The second division occurs evenly and as a consequence of regular division there usually occurs a tetrad. If the distribution of chromosomes has proceeded evenly, each cell of the tetrad receives 43 or 44 chromosomes. These cells develop directly into the pollen-grains. The diameter of there pollen-grains is

Text-fig. 9.
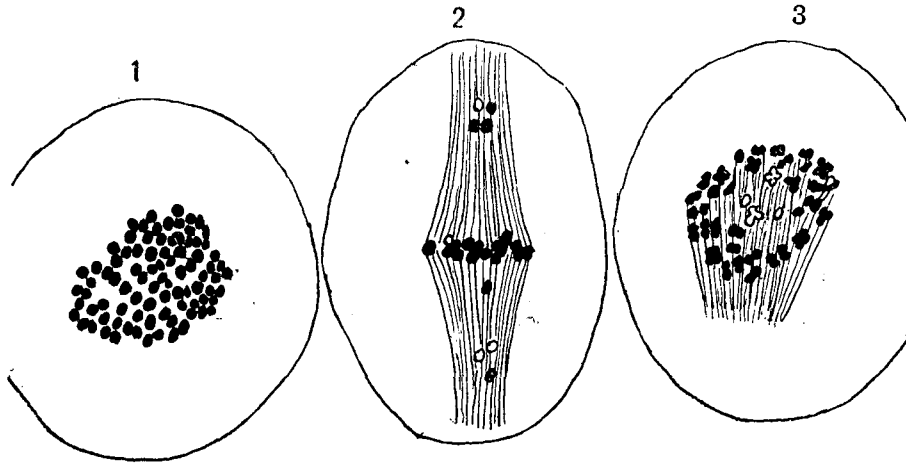
Text-fig. 9. Side and polar view of first meiotic division in BC×HE, No. 1-3 and No. 6-1.

97.41±0.289 (microns). This value is just the intermediate of *H. esculentus* and F_1 hybrid (The average value of pollen-grain diameter of *H. esculentus* and F_1 hybrid is 97.61±0.123 microns).

BC × HE, No. 6-1

At the metaphase of the first division in this plant a few of the large mass of chromosomes, which are thought to be trisome or tetrasomes, were observed among the double and single chromosomes. There is some reason to suppose that trisomes arise as a consequence of crossing F_1 hybrid twice with *H. esculentus* successively. Text-fig. 9-3 shows many chromosomes which are thought to be single, double and trivalent chromosomes ; some univalents going to the poles are not in sight. Plate V, Fig. 2, BC × HE, No. 6-1 shows the capsule of this plant.

BC × F_1, No. 7-10, (*H. esculentus* × F_1) × F_1

At the metaphase of the first reduction division of this plant about 66 chromosomes were observed in the total number of univalent and bivalent chromosomes, although there were no distinct differences in size among these chromosomes. This plant received 36 *esculentus* chromosomes and 30 *Manihot* chromosomes through the pollen-grain of F_1 plant and if it received 36 *esculentus* chromosomes and 15 *Manihot* chromosomes from back-crossed plant then it might be expected to have 36_{II} (*esculentus*

chromosomes) $+ 15_{II}$ (*Manihot* chromosomes) $+ 15_{I}$ (*Manihot* chromosomes), in total 66 chromosomes.

The bivalents split at the equatorial plate (Text-fig. 10–12) and divided univalent chromosomes move toward the poles to make two nuclei after the second division. Usually only a small number of lagging chromosomes were observed.

Text-fig. 10.
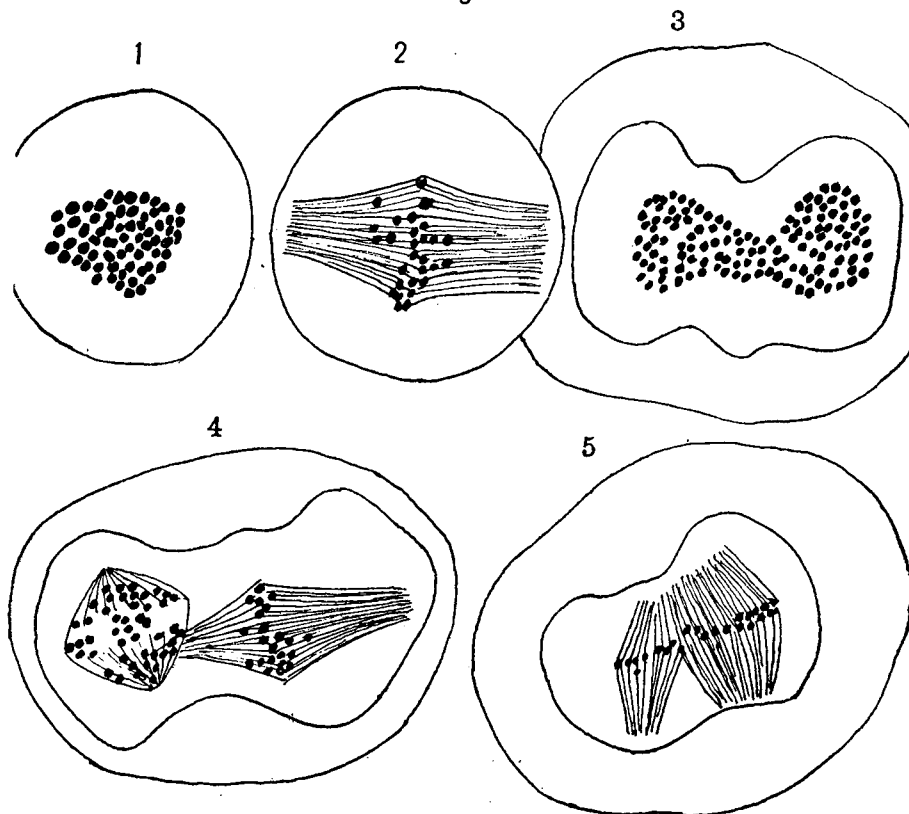
Text-fig. 10. Metaphase of first division and some irregularities in P.M.C. of $BC \times F_1$, No. 7–10.

In this plant many irregularities of meiotic division were observed. Text-fig. 10–3 shows one of the irregularities. About 120 small chromosomes are still remaining at the equatorial plate and are about to be divided into three parts by the constriction of nuclear membrane. Text-fig. 10–4 and 5 illustrates another irregularity. The two spindles are formed in unparallel position.

$BC \times F_1$, No. 7-6

At the metaphase of the first division about 65 or 66 chromosomes were found, including univalents and bivalents. Some large masses of chromosomes which were thought to be trivalent, were observed, (Text-fig. 11-1, Pl. VIII, Fig. 12). At the anaphase a small number of chromosomes varying from 5 to 10 was usually observed on both sides of the spindle (Text-fig. 11-2). The second division was regular. At this time about 66 small chromosomes moving toward the poles were observed. In Text-fig. 11-3 are shown about 65 small chromosomes in both sides, the same number of small chromosomes going to the other pole are not in sight.

Text-fig. 11.
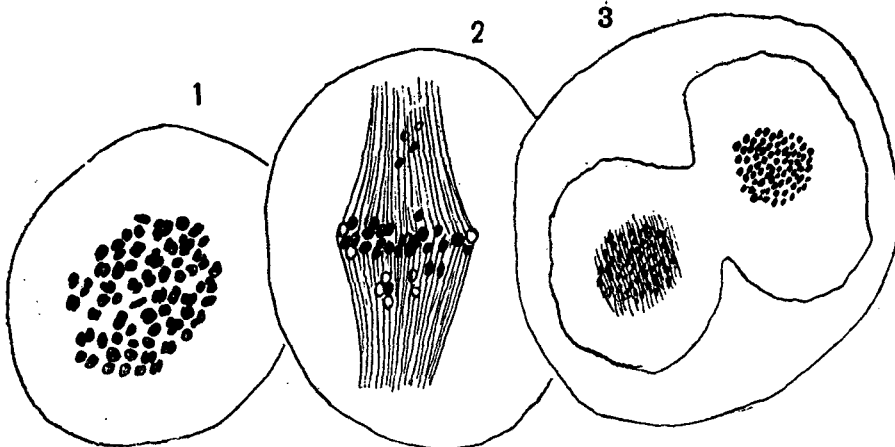
Text-fig. 11. First and second division of P.M.C. in $BC \times F_1$, No. 7-6.

$BC \times F_1$, No. 7-7

The chromosome number and chromosome behavior of this plant are just like those of $BC \times F_1$, No. 7—6. Text-fig. 12-1 and 2 show the first division metaphase.

Judging by the results of a cytological investigation of these plants the supposition is that the female gametes of back-crossed plants vary in chromosomal constitution. Among these female gametes the functional ones are those which have about 36 *esculentus* chromosomes and about 15 *Manihot* chromosomes as the result of normal meiotic division. The plant resulting from this female gamete combined with the pollen-grain of *H. esculentus* or with the pollen-grain of F_1 plant is a hyper-digenomous or hyper-trigenomous plant.

Text-fig. 12.
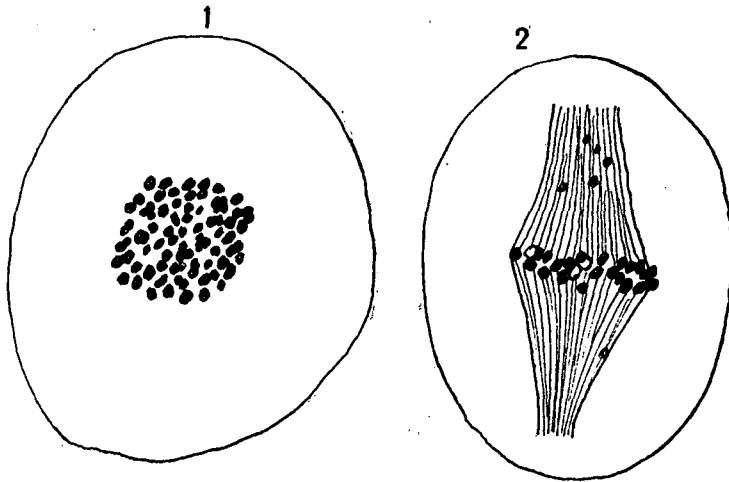
Text-fig. 12. Polar and side view of first metaphase of P.M.C. in BC × F_1, No. 7-7.

General conclusion

If it is of importance for taxonomy in determining the relationships of species and genera by studying the phylogenetic nature of the various kinds of chromosome change, a genetical study of crossings between species or genera and a knowlege of chromosome behavior in these hybrid and later generations should be of value in analysis of the origin and relationships of various species or genera.

Many trials of crossings between *H. esculentus*, *H. Manihot*, *H. cocci-neus* and *H. syriacus* have been carried on during these past five years but only when *H. esculentus* was emasculated and pollinated with the pollen of *H. Manihot* were a number of hybrid seeds secured which germinated and developed to maturity. KÖLREUTER (1764) obtained many hybrid seeds by crossing *H. Manihot* and *H. vitifol.* These differences of compatibility between species and degree of affinity between two kinds of chromosomes, without doubt, indicate the definite relationship of species or genera. De CANDOLLE (1824) classified the *Hibiscus* species in the following eleven sections:

Sect.   I   *Cremotia*   Sect.   IV   *Ketomia*
Sect.   II   *Pentaspermus*   Sect.   V   *Furgaria*
Sect.   III *Manihot*   Sect.   VI   *Abelmoschus*

Sect. VII *Bombicella*   Sect. X *Azanza*
Sect. VIII *Trionum*   Sect. XI *Lagunaria*
Sect. IX *Sabdariffa*

H. esculentus belongs to Sect. VI, *Abelmoschus* MED.; *H. Manihot* belongs to Sect. III, *Manihot*; *H. syriacus* L. belongs to Sect. IV, *Ketomia* and *H. vitifol* L. to Sect. VI, *Abermoschus* Med. ENGLER A. (1895) made a new genus "*Abermoschus* Med." combining Sect. III, *Manihot* and Sect. VI, *Abermoschus* Med. Both *H. esculentus* L. and *H. Manihot* L. are species included in this new genus. He named these species "*Abelmoschus esculentus* MAY." and "*A. Manihot* MEDIK." respectively. He classified the *Hibiscus* species in the follwing five sections, Sect. I. *Ketomia* ENGL., Sect. II *Furgaria* DC., Sect. III *Bombycella* DC. Sect. IV. *Azanza* DC., Sect. V, *Trionum* DC.. *H. syriacus* L. was placed in Sect. III, *Bombycella* and *H. vitifolius* L. in Sect. I *Ketomia*.

From the results of the author's investigation *H. esculentus* and *H. Manihot* are more closely related to each other in genetical constitution than the other two species. Thus the results of these crossing experiments point to the conclusion that ENGLER's rectified classification of *Hibiscus* species seems to be much more reasonable.

According to DE CANDOLLE the original home of *H. Manihot* and *H. vitifolius* is India oriental. According to ENGLER the *H. vitifolius* originated from tropical Africa, Asia, and Australia. According to ENGLER the original home of *H. esculentus* is east India though some writers ascribed it to tropical Africa or Old world tropics. These three species which are thought to be more closely related than *H. coccineus* or *H. syriacus* from the genetical point originated in the same part of the world, while *H. syriacus* L. which is thought not to be a closely related species to *H. esculentus* or *H. Manihot* is considered to be of Turkish or American origin by ENGLER or Syrian or Carniolian by DE CANDOLLE.

With regard to the following characters the F_1 hybrids are generally intermediate of both parents; character of stigma, number and width of involucral bractlets, length of capsule, color of pod, length and diameter of fruiting branch, cotyledon and seed hair. The following characters of F_1 hybrid are similar to those of the male parent (*H. Manihot*); number of longitudinal ribs in capsule, diameter of capsule. The duration of life is similar to that of female parent (*H. esculentus*). The following characters of F_1 hybrid are considered to dominate over either parent; annual period of growth, leaf size and leaf lobation, stem length and stem diameter, flower size, length of involucral bractlets, trichomes on the stem and cap-

sule, flowering time and diameter of pollen-grain.

The almost complete sterility and enormous vigor of F_1 hybrid are most significant features.   It is not the author's intention to discuss here the cause of hybrid vigor but it is noticeable that hybrid vigor is also exhibited in the back-crossed plants, even though they are of reduced vigor to some extent compared to the F_1 hybrid.   But the F_2 plants (tetra-genomous plant) are rather smaller than the digenomous plants.   Thus the hybrid vigor in these plants seems to correspond with the number of un-paired chromosomes.

As has been previously shown by numerous investigators the chromo-some number of a species in a plant genus is often in arithmetical pro-gression, for instance, in *Triticum, Rosa, Chrysanthemum, Betula, Crepis* and others.   Many investigators have dealt with the problem how the number of chromosomes is increased and special attention has been paid to tetraploidy as a method of increasing chromosome number.   KAGAWA (1929) has studied the comparison of somatic chromosomes in four species of *Triticum* and two species of *Aegilops* and described the phylogenetic relationships among these species.   He concluded, "Consequently, the chro-mosome sets of the above stated tetraploid and hexaploid species may also be regarded not to present the reduplication of the chromosome set of any basic diploid species other than *Triticum monococcum.*   So that, these tetrap-loid and hexaploid species were not formed, in their course of the phyloge-netic development, by any possible method which involved the reduplication of a basic chromosome set.   They may have probably been formed by the crosses among ancestral forms having different chromosome contents." There are abundant detailed evidences and a few experimental verifications to indicate that new species are derived from intercrossing two species. The behavior or chromosomes in polyploid species and their hybrid is now well known and their genetical investigations are also abundant.   How-ever, the methods of new species formation by the interspecific hybridization between non-polyploid species are still not clear.   It is the purpose of the present paper to describe the behavior of chromosomes of both species and formation of tetragenomous plant and the cause of occurrence of many poly-morphic groups of plants through interspecific hybridization.   OSTENFELD (1925) maintained that species description should be accompanied by cytolo-gical investigation and that hybridization probably formed new species in the polymorphic genera.   DIGBY (1912) and FARMER and DIGBY (1914) found that the diploid number of chromosomes, alike in the parental species and sterile hybrid in *Primula kewensis*, was 18, while in the fertile giant

form the number was 36. FARMER and DIGBY suggested that the increase in chromosome number was due to fragmentation, and not to longitudinal splitting of each chromosome. WINGE (1917), NEWTON and CAROLIN PELLEW (1929) and others have pointed out the difficulties of this theory. WINGE (1917) considered as to the cause of possible origin of fertile tetraploid form that doubling of the chromosomes might result from failure to conjugate at meiosis, followed by splitting and subsequent pairing of the identical halves.

WINGE (1917, 1924) has indicated a scheme in which the chromosome number of species is in each case added together in the offspring and new species arise which contain the double or manifold chromosome number of parental species. In this case chromosomes of both parents are in such a condition as that when the chromosome pairing entirely fails to appear after hybrid fertilization and after which a formation of gemini takes place by longitudinal fision of all the chromosomes ("Indirect chromosome binding"). He further pointed out that successive doubling of chromosome number would give rise to geometrical rather than arithmetical series. The process suggested by Winge would establish fertile tetraploid interspecific hybrids having $2 (n_1 + n_2)$ chromosomes, where n_1 and n_2 represent the haploid number of the parental species.

Confirmation of WINGE's hypothesis has been afforded by many investigators. CLAUSEN and GOODSPEED (1925) gave the instance of some F_1 hybrid plants between *Nicotiana glutinosa* and *N. Tabacum*. They found in one of the group of F_1 plants, which exhibited partial fertility, 36 bivalent chromosomes in the first metaphase figures and they were able to count both metaphase plates in the second division to determine that each contained 36 chromosomes. This number of chromosomes is undoubtedly tetraploid, since *Nicotiana glutinosa* has 12 haploid number of chromosomes and the haploid number of *N. Tabacum* is 24. They concluded, "The original fertile F_1 plant must have arisen from a doubling of the chromosome number immediately or soon after fertilization, by which a tetraploid hybrid with 36 pairs of chromosomes was produced." They made the following suggestion as to the occurrence of tetraploid hybrids; (1) by doubling of chromosome number immediately subsequent to fertilization; (2) by bud-variation in an F_1 interspecific hybrid; (3) by crossing together tetraploid representatives of two different species; and (4) by irregular distribution of chromosomes in an interspecific hybrid in which the chromosomes do not pair in meiosis, as suggested by COLLINS and MANN (1923). The confirmation of these suggestions has been afforded similarly also by DIGBY

(1912) and by PELLEW and DURHAM (1916) in *Primula*. WOODWORTH (1928) made a cytological study of the *Betula* species and found it to be a polyploid genus containing diploid, triploid, tetraploid, pentaploid, hexaploid and dysploid species. He found the semiheterotypic division to produce diploid pollen in hybrid *B. sandbergi* and apparent hybrid *B. japonica* var. *mandshurica*. He also found that in *B. sandbergi* a restitution nucleus is formed, not only about the first spindle, but also about the homotypic spindles in cells which completed the heterotypic division, which again results in the production of a dyad. WOODWORTH observed in *B. lenta - pumila* cross that the dyads make up some 5–10 per cent of the pollen while in *B. japonica* var. *mandshurica* dyads often appear in number equal to tetrads. He concluded consequently that heterozygosis is to be considered one of the methods of the origin of polyploidy and the polymorphism in *Betula* is apparently due to the readiness with which the species cross in nature.

It has usually been assumed that lack of conjugation promotes the production of diploid gametes in interspecific F_1 hybrid. J. CLAUSEN (1924) observed in some of the pollen mother cells of an F_1 plant between *Viola tricolor* L. and *V. arvensis* MURR. a large increase in chromosome number which he explained in the following way. "In F_1 of the cross 13 (tr) × 17 (ar) occasionally only 6 pairs of chromosomes from the two species have been able to conjugate, of the remaining 18 unpaired chromosomes, 17 have divided in the heterotypical metaphase and are distributed equally to the two poles, whereas 1 has remained unsplit in the original plane of the nuclear plate. In this way two homotypical plates arise. In the homotypical anaphase the chromosomes once divided must have split again." He confirmed WINGE's theory (1917) as to the manner in which a new chromosome number originated. The increase in chromosome number has arisen from the failure of conjugation of chromosomes and through a subsequent division of the unpaired chromosomes during the heterotypic division, and he suggested the view that new species can arise from crossing between already existing ones. ROSENBERG (1926, 1927) suggested that the semi-heterotypic division is instrumental in the formation of tetraploid hybrids. KARPECHENKO (1927) has explained the process of tetraploid formation in the *Raphanus-Brassica* hybrid by non-conjugation of chromosomes. LAMMERTS (1929) observed in the pollen mother cell of *Nicotiana* F_1 hybrid that 32 per cent of the viable female gametes were of somatic category notwithstanding the pollen mother cell indicated a highly regular conjugation according to the Drosera schema. SKOVSTED (1929) has studied *Aesculus carnea* WILLD. cytologically and found in heterotypic metaphase

large chromosomes obviously originated from *A. pavia* and 20 small ones like those of *A. hippocastanum*,   He concluded this form to have arisen by species crossing followed by indirect chromosome binding according to WINGE's hypothesis.

Owing to selective fertilization the crosses between *H. esculentus* and *H. Manihot* are presumably infrequent in nature even if these two species are planted close to each other in open field.   However, it is not improbable that these crosses occur very rarely and succeed in growing in nature. These two species are different in chromosome number and are supposed to be derived from a common origin.   We may feel sure that the differentiation of species in *H. esculentus* and *H. Manihot* has proceeded by way of structural changes within the individaul chromosomes as well as changes in the chromosome number.   It is not unreasonable to suppose that the difference of chromosome number in *H. esculentus* and *H. Manihot* must have resulted from a process of gradual change continued so far that all the chromosomes of each species have lost their affinity of conjugation to make gemini normally in the meiotic division of the pollen mother cell.

The F_1 hybrid plants are highly sterile but they produce some seeds occasionally.   Thus the non-conjugation of all chromosomes derived from both parents in the F_1 hybrid leads to the production of digenomous (Diploid) gametes, consequently trigenomous (Triploid) back-crossed plants and tetragenomous (Tetraploid) F_2 plants originated.   From these experiments, the heterozygosis in *Hibiscus* species is to be considered one of the methods of the origin of polygenomous plants (Polyploidy) and the formation of tetragenomous forms which are to be considered as constantly fertile types confirming WINGE's hypothesis.   Even though the F_1 plants of the author's material are highly sterile, the back-crossed plants and their progeny are fertile and the possibility of the production of strong fertile descendants of intermediate forms will occur in second or third generation plants.   In the gamete formation in some of these progeny the unpaired lagging chromosomes are eliminated during the meiotic division, so that these stable progeny of back-crossed plants do not appear intermediate between two parents but they exhibited so nearly *esculentus* appearance in every character that no one can imagine they are the later generation on a cross between *H. esculentus* and *H. Manihot*.   This would give rise to taxonomic confusion. Moreover it is noticeable that the author obtained, among the progeny of back-crossed plants by pollination of *H. esculentus* or F_1 hybrid plant, plants which are more stable than hybrid or back-crossed plants and are regular in tetrad formation and give uniform pollen-grains which have hyper-

digenomous chromosomes.

From a cytological point of view JØRGENSEN (1928) has classified the plant genera in the following three groups;

(1) Those in which all the species examined have the same chromosome number.

(2) Those in which different, but not polyploid number occur.

(3) Those in which the species have multiple chromosome number.

It is probable that increase in chromosomes (multiplication or addition) and changes in the chromosome material are necessary for the formation of new species. The process suggested by WINGE would establish tetraploid interspecific hybrids, but the results of the author's experiments indicate not only tetraploid interspecific hybrid but also that more than digenomous plants will be formed by interspecific crossing and back-crossings. The course of the author's experiments is reproduced diagramatically below;

Species   Species

H. esculentus   ×   *H. Manihot*
n (es) = 36 | n (Ma) = 30
                              ↓

F_1 zygote
36 (es) + 30 (Ma)
↓

F_1 gametes
Non-reduction
Pollen   Egg
36 (es) + 30 (Ma).   36 (es) + 30 (Ma)
↓

F_2 zygote
36_{II}(es) + 30_{II}(Ma)
↓

F_2 gametes
Regular meiosis
Pollen   Egg
36_I (es) + 30_I (Ma). 36_I (es) + 30_I (Ma)
↓

Constant

Back-crossed plant
Irregular meiosis
36_{II} (es) + 30_I (Ma)
↓
Back-crossed plant
gamete
↓
Polymorphic group
having various number
of chromosomes
↓
Later generation
Reestablishment of
original species

Double back-crossed
plants
36_{II} (es) + i_I (Ma) usually
↓
New types having new
combination of
chromosmes

Double back-crossed
Plants

36_{II} (es) + i_{II} (Ma) + i_I (Ma)
usually
↓
New types having new
combination of
chromosomes

Interspecific hybridization is not the only way to forming the tetraploid hybrid plant, another way of doubling the chromosomes will be considered. For instance, JØRGENSEN (1928) succeeded in the formation of polyploid plants in genus *Solanum* by using WINKLER's cross-grafting method with seedling of tomato and potato plants.

The behavior of chromosomes and the genetical characters in inter-specific hybrid of *H. esculentus* and *H. Manihot* are of interest in comparison with chromosome behavior in other species crosses or in a genus cross. The behavior of chromosome and genetical characters of species hybrids or genus hybrids indicates great variability in different cases, but in species hybrids the bivalents only or bivalents and univalents are usually present at the metaphase of the first division. CHITTENDEN (1928) in the F_1 hybrid between *Godetia amoena* (n=7) × *G. Whitneyi* (n=7) usually found seven bivalents at the metaphase of the heterotypic division. TÄCKHOLM (1920) found only paired chromosomes at the metaphase of *Rosa* species hybrid. The univalents and bivalent chromosomes at the metaphase of the first meiotic division were found in many interspecific hybrids; in *Drosera longi-folia* × *D. rotundifolia* by ROSENBERG (1909), in *Oenothera lata* × *O. gigas* by GATE, in *Brassica cernua* × *B. chinensis* by T. MORINAGA (1929), in *Solanum nigrum* × *S. luteum* by JØRGENSEN (1928), in *Digitalis purpurea* × *D. ambigua* by BUXTON and NEWTON (1928) in *British Rosa* by BLACK-BURN and HARRISON (1921), in *Triticum* species by KIHARA (1919) and SAX (1921), in *Oenothera Lamarckiana* × *Oe. gigas* by GEERTS (1911), in *Hi-eracium aurcicula* × *H. aurantiacum* by ROSENBERG (1917) in *Nicotiana* by GOODSPEED, CLAUSEN and CHIPMANN (1926), in *Crepis* by COLLINS, HOLLING-SHEAD and Avery (1929) and COLLINS and MANN (1926) and others. In intergeneric crosses none or only a small number of bivalent chromosomes usually appear at the metaphase of the first division in consequence of non-conjugation of all or nearly all the chromosomes. These are of much impor-tance to our conception of the process of species formation and so these phenomena should be of great interest. THOMPSON (1925) reported in his study of wheat-rye hybrid that in the heterotypic division the entire absence of an equatorial plate and one or two pairs of chromosomes occur occasio-nally and three pairs rarely. KIHARA (1924) also reported 0,1,2 or 3 biva-lents at the metaphase of the first division of rye-wheat hybrid. In *Rapha-nus-Brassica* intergeneric hybrids KARPECHENKO (1924, 1927, 1928) and FUKUSHIMA (1929) reported the non-conjugation of 9 radish and 9 cabbage chromosomes with each other. In *Aegilops* (n=14)-*Triticum* (n=21) hybrid K. SAX and J. SAX (1924) found 6 or 7, occasionally 5 or 6, bivalent chromosomes at the metaphase of the first division. The F_1 hybrid of *Crepis capillaris* (L.) WALLR. (n=3) × *C. tectorum* L. (n=4) is reported by COLLINS and MANN (1923) as an exceptional case in which the compatibility between these two species chromosomes was so low that the two haploid sets of chromosomes were unable to function together. The cytological

behavior of the F_1 hybrid between *H. esculentus* and *H. Manihot* is similar to that of *Raphanus-Brassica* hybrid in many respects. It is impossible to draw any distinct line across the innumerable gradations between different plants but if the affinity of chromosomes does show the taxonomic relationships of plant species or plant genera as MONTGOMERY pointed out as early as 1906, we may feel sure that the differentiation of chromosomes in *H. esculentus* and *H. Manihot* has proceeded beyond the species differentiation.

It is the author's pleasant duty to thank all persons that in one way or other facilitated his work, in the first place Dr. GENTARO YAMADA, the Director of the Tottori Agricultural College who afforded many facilities for this investigation and has always taken much interest in the progress of the undertaking, and special acknowledgment is due to the Prof. MASAO AKEMINE, N.H., Prof. of Genetics. for much valuable help. Further the author is indebted to KINGO MIYABE, D. Sc., N. H., TETSU SAKAMURA, N. H. and Prof. S. FUKUSHI for much valuable help and information. The author is also very much indebted to SHOICHI TANAKA, assistant Y. YOSHI-OKA, K. YORITA, S. INOUE, S. MORIMOTO, H. KAKITA, K. ARIYOSHI, K. KOYAMA and S. KOMEI for much help in the field or laboratory work.

Summary

I. Genetical studies

1. Many species crossings between *Hibiscus esculentus* L., *H. Manihot* L., *H. coccineus* WALT., and *H. syriacus* L. were carried on but hybrid seeds were obtained only when *H. esculentus* was used as the female parent and pollinated with the pollen of *H. Manihot*. The results of these crossing experiments point to the conclusion that ENGLER's rectified classification of *Hibiscus* species is much more reasonable than DE CANDOLLE's classification, and that closely related species originated in the same part of the world.

2. The rate of pollen-tube growth is not the cause of difference in success of reciprocal crosses between *H. esculentus* and *H. Manihot* but the cytoplasmic influence may be the important factor to demonstrate the inequality of reciprocal crosses.

3. F_1 plants showed a high degree of sterility, but abundant seeds were obtained by back-crossing the *H. esculentus* with the pollen of F_1 hybrid.

4. Selective fertilization in the flower of *H. esculentus* is significant when

the pollen-grains of *H. esculentus* and *H. Manihot* are applied at the same time.   Self-fertilization is much easier than cross-fertilization.

5.   The F_1 hybrid is uniform in every character and the hybrid vigor is one of the significant features.   The average length of the main stem of *H. esculentus* is 227.72 ± 1.357 cm. and that of *H. Manihot* is 30.43 ± 0.521 cm., yet the F_1 plants have an average of 392.80 ± 1.630 cm.   This is about twice the height of the mother parent and about 13 times that of the father parent.

6.   Leaf lobation, flower size, involucral bracts, capsule, fruiting branches, trichomes, seed, flowering time and some other characters of F_1 plants were measured to compare with those of parents.   Among these characters the length of capsule and 8 other characters are intermediate of both parents, annual period of growth and 9 other characters are considered to be dominant over either parent, the number of longitudinal ribs in capsule and diameter of capsule are similar to those of the male parent (*H. Manihot*) and the duration of life is similar to that of female parent (*H. esculentus*).

7.   Small abortive and giant pollen-grains are more numerous in F_1 hybrid than in pure species.

8.   The pollen-grains of *H. esculentus* are slightly larger and more variable than those of *H. Manihot* but the difference is not significant.   The diameter of pollen-grains in *H. esculentus* is 89.21 ± 0.105 and that of *H. Manihot* is 87.50 ± 0.106 microns, while that of F_1 hybrid is 106.02 ± 0.146 microns in dry condition.   The ratio of increase in diameter of pollen-grain when imbedded in white of an egg is no more than 4.72 per cent in *H. Manihot*, while the increase of both *H. esculentus* and F_1 hybrid is above 20 per cent.

9.   The pollen-grains showed as good measure of ability to germinate on the section of flower style as on the stigma.

10.   The germination of F_1 hybrid pollen is quite remarkable notwithstanding the grains proved to be totally non-functional when pollinated on the same flower.   The relation between size and germinability of pollen is, as a whole, that the percentage of germination increases to some extent as the diameter of the pollen-grain increases.

11.   When pollination was made between parental species and F_1 hybrid and between themselves at the height of the flowering season almost all of the pollen-tube traversed the whole length of the style.   The pollen-tube growth in every case was at the same rate and there was no marked difference between these pollen-tube growths no matter whether they are

functional or non-functional.

12. Segregation was not evident in the back-crossed plants and many of their morphological characters are generally intermediate between F_1 hybrid and *H. esculentus*, but the microsporocytes and the pollen-grains are more variable than those of F_1 plants or pure species.

13. The progeny of back-crossed plants resulting from controlled or open pollination were so variable in may characters that every one could be classed as a different form. From these progeny a small number of the original form were reestablished.

14. The F_2 plants resulting from F_1 plants by open-pollination were fairly uniform and their morphological characters were similar to those of F_1 hybrid plant, but the higher fertility and poor vegetative growth were significant differences. From these facts the hybrid vigor seems to be accompanied by the unpaired chromosome number.

II. Cytological studies

1. The meiotic division of pollen mother cell in parental species is very regular. The haploid chromosome number in *H. esculentus* was counted by the author as 36 and in *H. Manihot* as 30.

2. At the metaphase of the first division of F_1 hybrid plants the entire number of chromosomes make their appearance in the majority of cases. Evidently no conjugation of chromosomes occurs from lack of affinity between two kinds of chromosomes. The heterotypic division is prematurely interrupted and all the chromosomes now divide on one large spindle, forming a dyad each cell of which has the somatic number of chromosomes 66. Occasionaly when some irregularities had taken place the resulting pollen-grains were big or small abortive ones.

3. The back-crossed plants were examined cytologically and it was found in the metaphase of reduction division that these plants are triploid or trigenomous, $36_{II} + 30_I$ being the chromosomal constitution at the metaphase. The P.M.C. exhibit irregularities during meiosis; at the anaphase many lagging chromosomes were usually obtained, consequently dyads, triads, tetrads and other polyads are formed.

4. The fourteen progeny of back-crossed plants were examined cytologically and various number of chromosomes counted at the metaphase of the first meiotic division varying from 48 to 70. This number of chromosomes is correlated with the fertility of plant, the plant having the higher number of chromosomes having the greater fertility. At the anaphase a small number of lagging chromosomes were usually observed.

5. The meiotic division of P.M.C. in the reestablished plants which are similar to *H. esculentus* is very regular. It is possible in some plants, that the elimination of the univalents through lagging on the spindles at the meiotic division led to the establishment of the stable diploid type resembling the diploid ancestor. In these reestablished plants the author counted 36 or 37 bivalent chromosomes at the metaphase of the first division.

6. From the results of cytological investigation the F_2 plants were found to be tetragenomous with $36_{II} + 30_{II}$ chromosomes at the metaphase of the meiotic division. The division is very regular and consequently tetrads and pollen-grain having two genomes of chromosomes are formed.

7. Examination was made of a few plants of progeny resulting from the seeds obtained from the back-crossed plants by crossing with the pollen of *H. esculentus*, (*H. esculentus* × F_1) × *H. esculentus*. At the anaphase of the first division in one plant about 87 relatively small chromosomes were observed, it may be supposed $36 + 36$ (*esculentus*) + 15 (*Manihot*) chromosomes. At the metaphase of the meiotic division in another plant a few chromosomal masses which are thought to be trisome or tetrasome were observed.

8. At the metaphase of the meiotic division of progeny resulting from the seeds obtained from the back-crossed plants by crossing with the pollen of F_1 hybrid, (*H. esculentus* × F_1) × F_1, about 66 chromosomes were observed in the total number of univalents and bivalents. These plants received 36 *esculentus* chromosomes and 30 *Manihot* chromosomes through F_1 pollen-grain and presumably 36 *esculentus* chromosomes and 15 *Manihot* chromosomes from back-crossed plant. Then this plant might have 36_{II} *esculentus* chromosomes, 15_{II} *Manihot* chromosomes and 15_I *Manihot* chromosomes so that a total of 66 chromosomes will be seen at the metaphase. A large mass of chromosomes which are thought to be trivalent was observed in some cases.

9. Hybridization of these species leads to the production of diploid gametes by the semiheterotypic division of F_1 plant, consequently heterozygosis is to be considered one of the ways of the origin of polyploidy.

10. From the very irregular meiotic division in back-crossed plants polyspory with various numbers of chromosomes resulted. Consequently from the progeny of back-crossed plants fertilized with pollen of the original species or of F_1 plants stable new forms having chromosomes not in polyploid number originate.

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Explanation of plates

Plate I.

Fig. 1. Parents and F_1 plants, showing general appearance at the height of their blooming period. Plant at left (e) *H. esculentus* (female parent), middle, (M) *H. Manihot* (male parent); and right, (F_1) F_1 hybrid plants.

Fig. 2. Stems with pods in parents and F_1 hybrid. Left *H. esculentus* (Blue long A); middle, F_1; and right, *H. Manihot.*

Fig. 3. Stems with pods in parents and F_1 hybrid. Right, *H. esculents* (White long); middle, F_1; left, *H. Manihot.*

Fig. 4. Stems with pods in parents and F_1 hybrid. Right, *H. esculentus* (Dwarf prolific); middle, F_1 hybrid; and left, *H. Manihot.*

Plate II.

Eig. 1. Stems with pods in parents and F_1 hybrid. Right, *H. esculentus* (Green giant); middle, F_1; and left *H. Manihot.*

Fig. 2. Stems with pods in parents and F_1 hybrid. Right, *H. esculentus* (Blue long B); middle, F_1; and left, *H. Manihot.*

Fig. 3. Capsules obtaneid from a single plant; in upper row *H. esculentus* (Blue long A); in middle row, F_1 hybrid; in lower row *H. Manihot.*

Fig. 4. Flowers from left to right; *H. esculentus*, F_1 and *H. Manihot.*

Plate III. Typical leaves of parents, F_1 hybrid and back-crossed plants.

Fig. 1. Taken from lower (right), middle (middle) and upper (left) part of stem. Upper row, *H. esculentus* (Blue long A); middle row, F_1; lower row, *H. Manihot.*

Figs. 2 and 3. Showing some variation in leaves of back-crossed plants grown in 1928. Garden number BCNo, 3-17 (left end in Fig. 3) is different from others in form reembling that of *H. esculentus.*

Fig. 4. Taken from lower (right), middle (middle) and upper (left) part of stem. Upper row, *H. esculentus* (White long); middle row, F_1; lower row, *H. Manihot.*

Plate IV. Leaves from progeny of back-crossed plants.

Figs. 1. and 3. showing variation in leaf lobation of progeny of back-crossed plants.

Fig. 2. Leaves, progeny of back-crossed plants (upper row) and some leaf types of back-crossed plants × F_1 plant (BC × F_1, No. 9-10) and back-crossed plants × *H. esculentus* (BC × H. E, No. 10-2, BC × H. E, No. 15-1).

Fig. 4. Leaves from F_2 plants. Taken from middle part of stem.

Plate V.

Fig. 1. Capsules from the progeny of back-crossed plants.

Fig. 2. Capsules from the back-crossed plant × F_1, back-crossed plants × H. *esculentus* and *H. esculentus*.

Fig. 3. Showing capsules of *H. esculentus* (upper left), F_1 hybrid (upper right) and back-crossed plants (lower).

Fig. 4. Showing the capsules of five kinds of intervarietal F_1 hybrids and their parents.

    1. White long × Blue long A.

    2. White long × Blue short.

    3. Dwarf long pod green × White long.

    4. Dwarf prolific × White long.

    5. Blue short × Dwarf prolific.

Plate VI. Figs. 1-8. Line drawings of morphological details of trichomes on capsule and flowering stalk.

Fig. 1. From a capsule of F_1 plant.

Fig. 2. From the neighbourhood of longitudinal ribs in F_1 capsule.

Fig. 3. From the flowering stalk of F_1.

Fig. 4. From the capsule of *H. esculentus*.

Fig. 5. From the neighbourhood of longitudinal ribs in *H. esculentus*.

Fig. 6. From the flowering stalk of *H. esculentus*.

Fig. 7. From the capsule of *H. Manihot*.

Fig. 8. From the flowering stalk of *H. Manihot*.

Figs. 9-14. Line drawings of morphological details of trichomes on the inner epidermis of capsule and seed hairs.

Fig. 9. From inner epidermis of *H. esculentus*, showing no trichome or hair.

Fig. 10. From inner epidermis of *H. Manihot*.

Fig. 11. From inner epidermis of F_1 hybrid.

Fig. 12. From the seed of *H. esculentus*.

Fig. 13. From the seed of *H. Manihot*.

Fig. 14. From the seed of F_1.

Plate VII. Photomicrographs of pollen-grains. × 90.

Fig. 1. From a *H. esculentus*.

Fig. 2. From a *H. Manihot*.

Fig. 3. From F_1 hybrid showing small number of abortive and large diploid pollens.

Figs. 4-5. From back-crossed plants (*H. escu.* × F_1).

Fig. 6. From a back-crossed plnat, (BC. No. 2-3), containing many abortive pollen-grains.

Fig. 7. From a back-crossed plant, (BC. No. 3-7), containing many abortive pollen-grains.

Fig. 8. From a progeny of back-crossed plant, (BCN. No, 22), containing none of the abortive pollen-grains.

Fig. 9. From a progeny of back-crossed plant (BCN. No. 50).

Fig. 10. From a F_2 plant showing large diploid pollen-grains and no abortive pollen-grain.

Plate VIII.

Figs. 1-2. First division metaphase in the pollen mother cells of parents. (K. 20 x Apo. 2 mm).

Fig. 1. *H. esculentus* L. (female parent).

Fig. 2. *H. Manihot* L. (male parent).

Figs. 3-5. Pollen mother cells of F_1 hybrid of *H. esculentus* x *H. Manihot*. (K. 20 x Apo. 2 mm).

Fig. 3. Unpaired chromosomes arranged on the equatorial plate.

Fig. 4. Unpaired univalents scattering all over the spindle.

Fig. 5. All the univalents are splitting and small chromosomes are seen.

Fig. 6. Dyads formed in F_1 hybrid. (10 x 8).

Fig. 7. An abnormal meiotic division of a F_1 hybrid, split chromosomes still remaining between rings.

Figs. 8-9. Pollen mother cells of back-crossed plants.

Fig. 8, Heterotypic metaphase, bivalents are splitting.

Fig. 9. Heterotypic anaphase, broad spindle is formed.

Figs. 10-11. First division metaphase in the pollen mother cells of F_2 plants.

Fig. 10. Polar view, 66 pairs of gemini are arranged at the equatorial plate.

Fig. 11. Side view, broad spindle is formed.

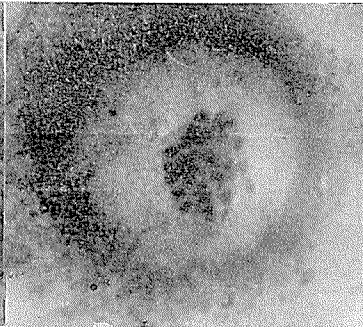Fig. 12. Metaphase of first division in BC x F_1, No. 7, variable size of chromosomes is seen.

Fig. 1

Fig. 2
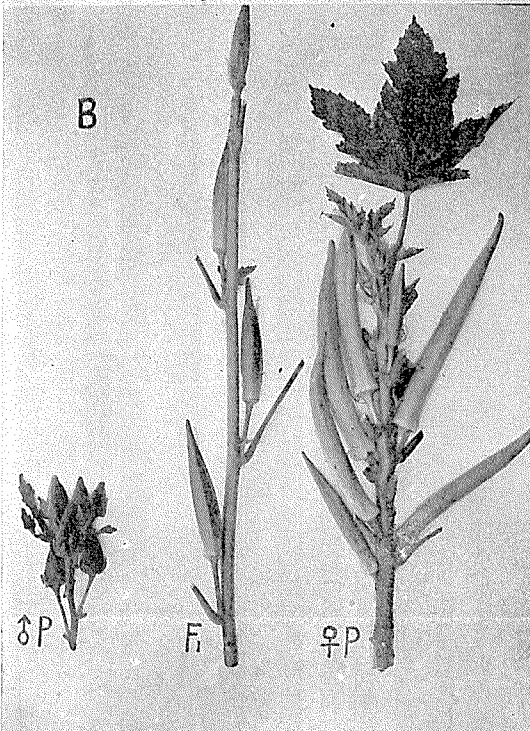
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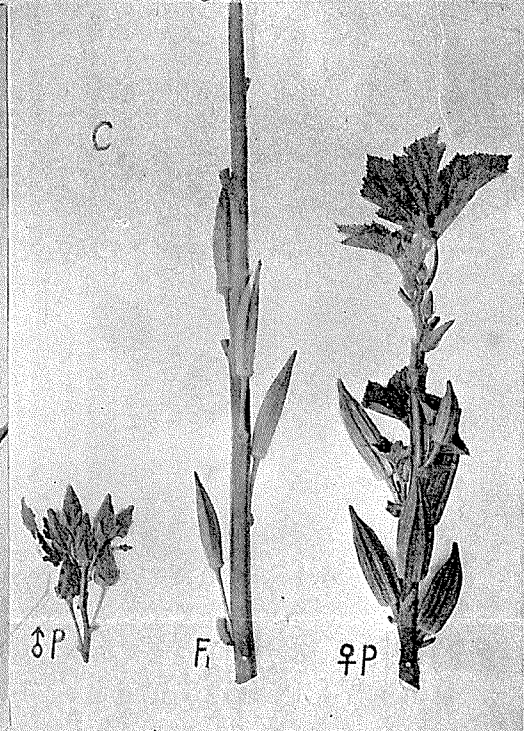
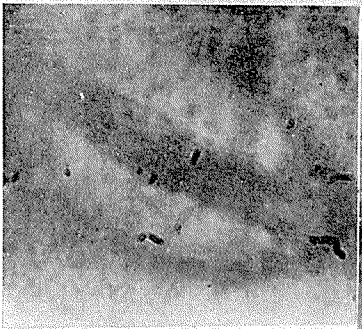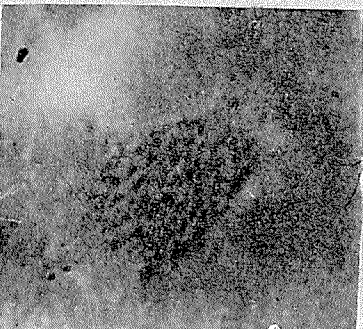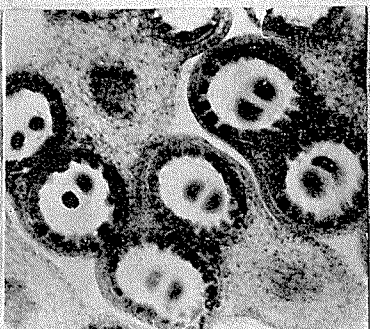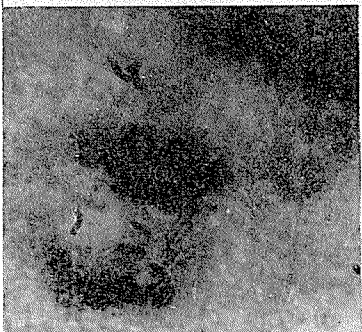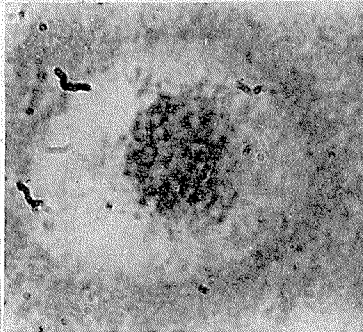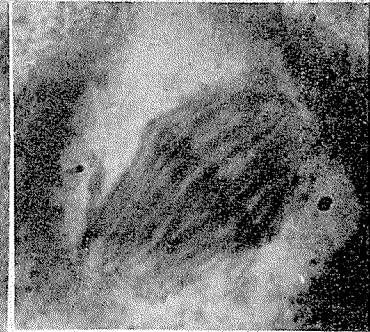Fig. 4
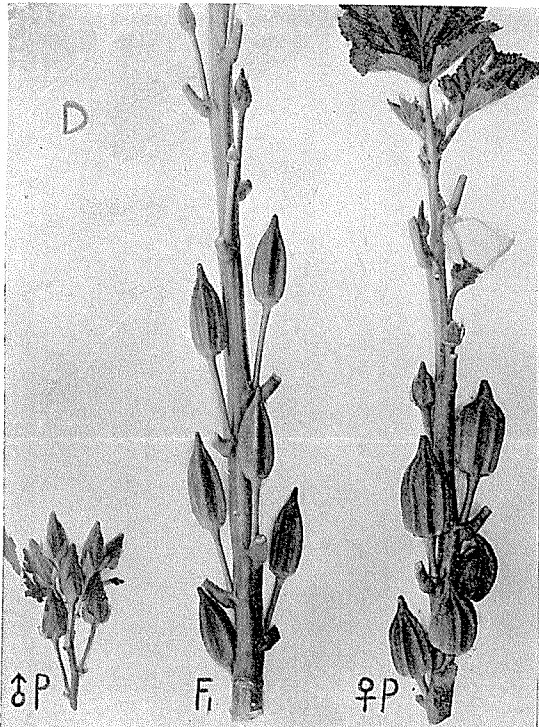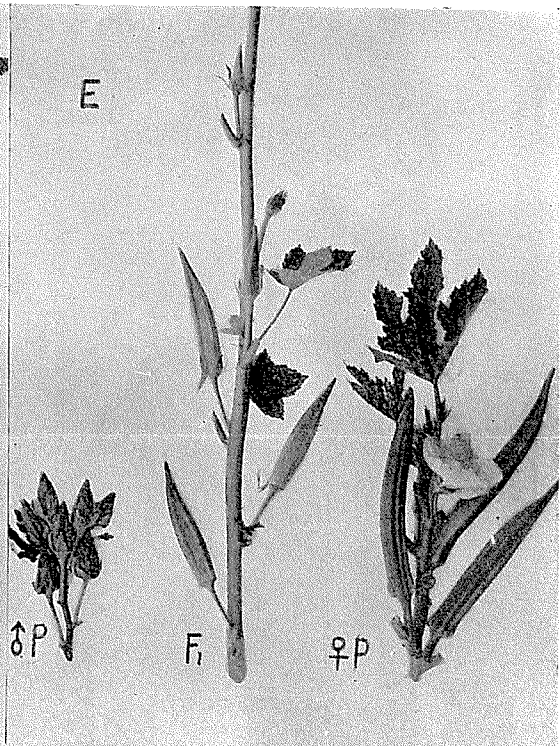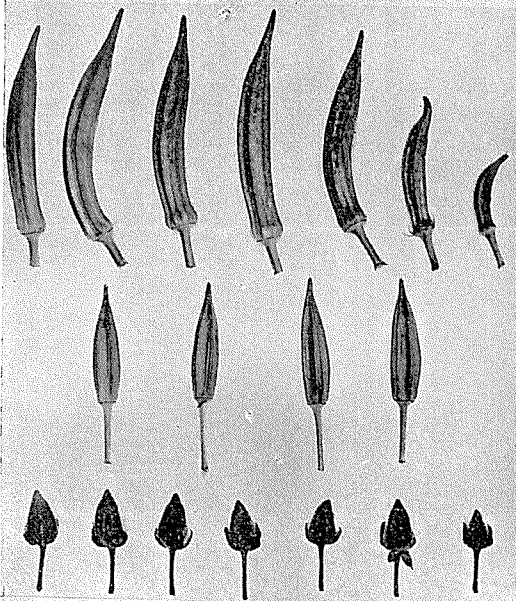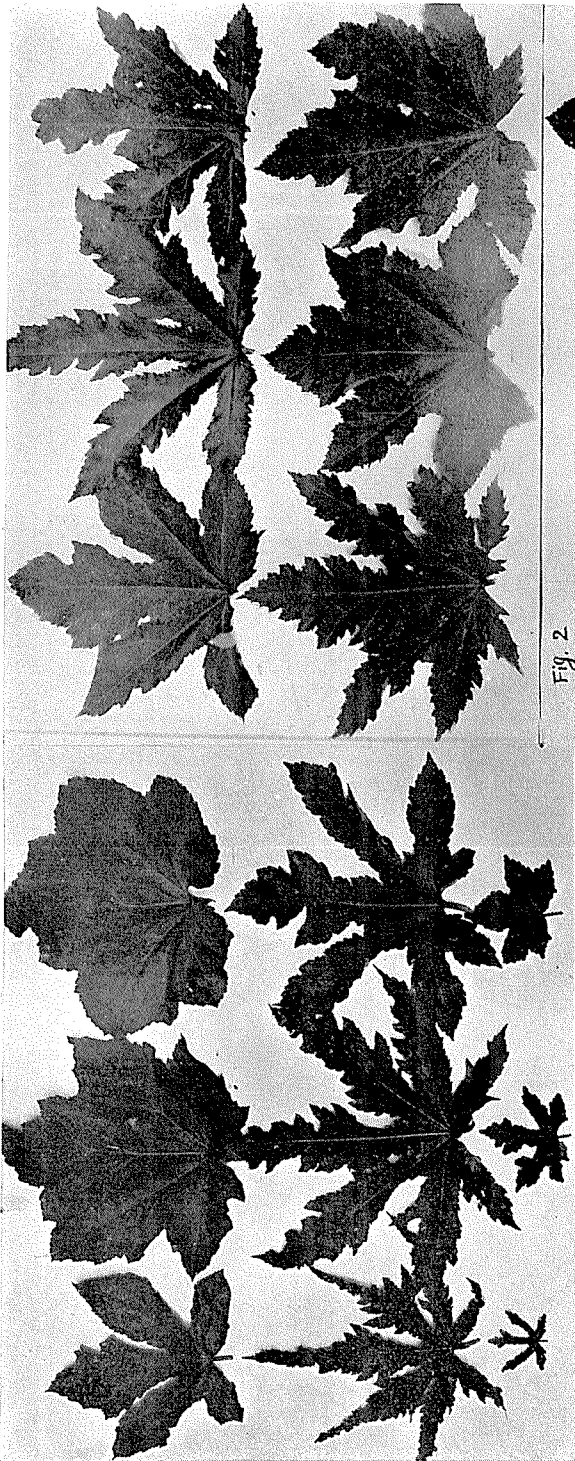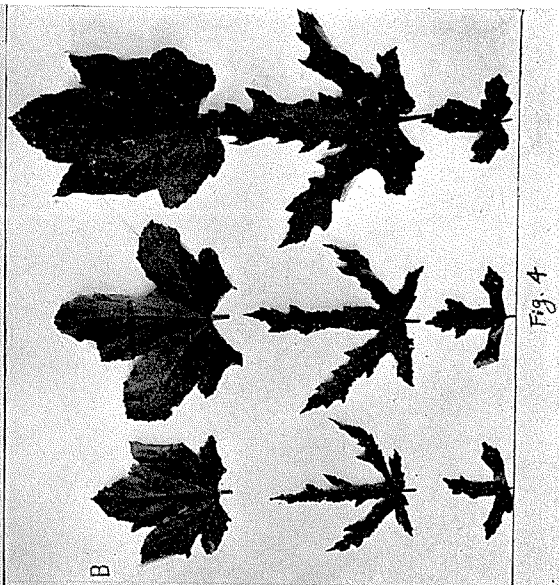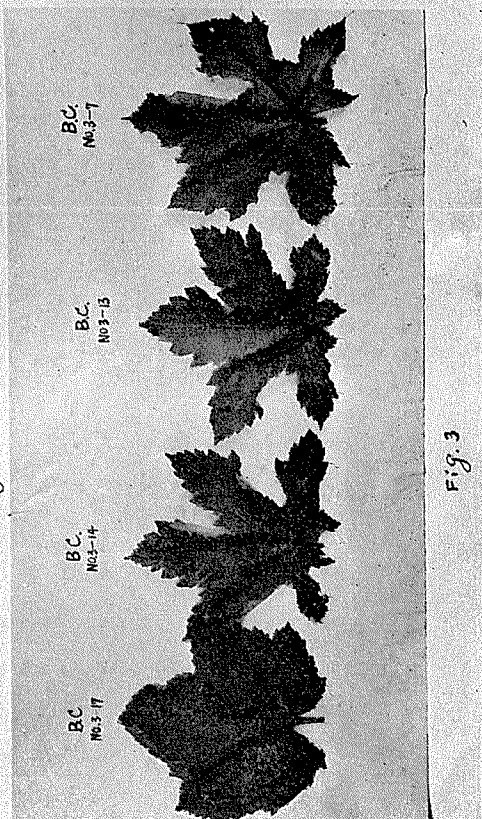
Fig. 1

Fig. 2

Fig. 3

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Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 1

Fig. 2

Fig. 3

Fig. 4

BCN No.16 BCN No.19 BCN No.24 BCN No.25 BCN No.29

BCN No.32 BCN No.48 BCN No.56 BCN No.65 BCN No.125

Fig. 1

BC×F₁ No.7-4 BC×F₁ No.7-5 BC×F₁ No.9-2 BC×F₁ No.9-3 BC×F₁ No.9-9

BC×F₁ No.9-12 BC×HE No.6-1 BC×HE No.15-1 HE H.E.

Fig. 2

Fig. 3

Fig. 4

X 10

Fig. 1
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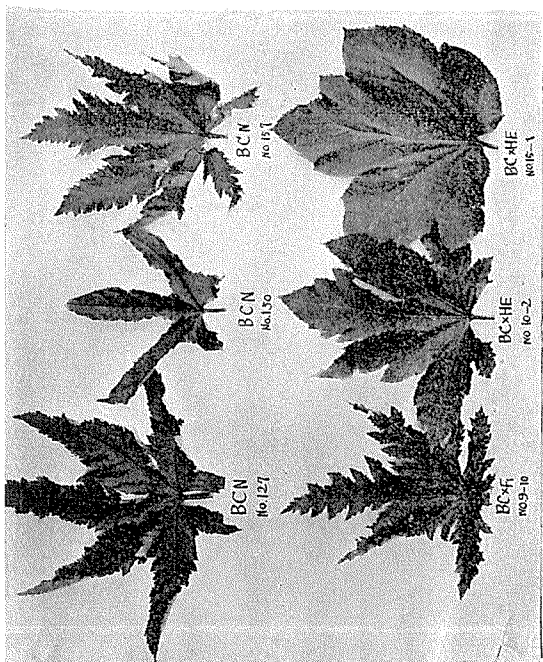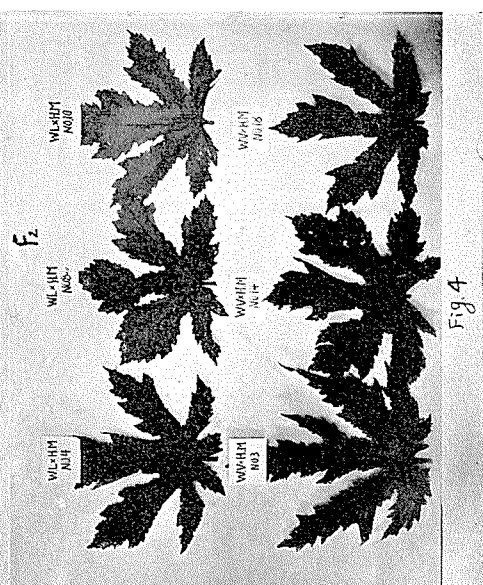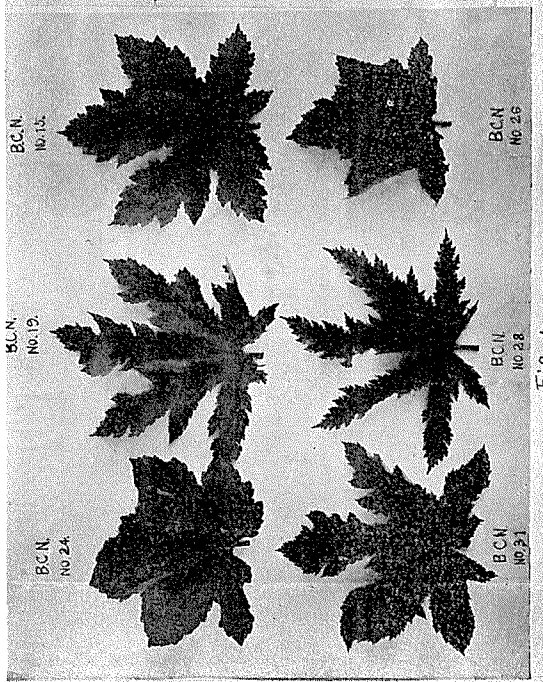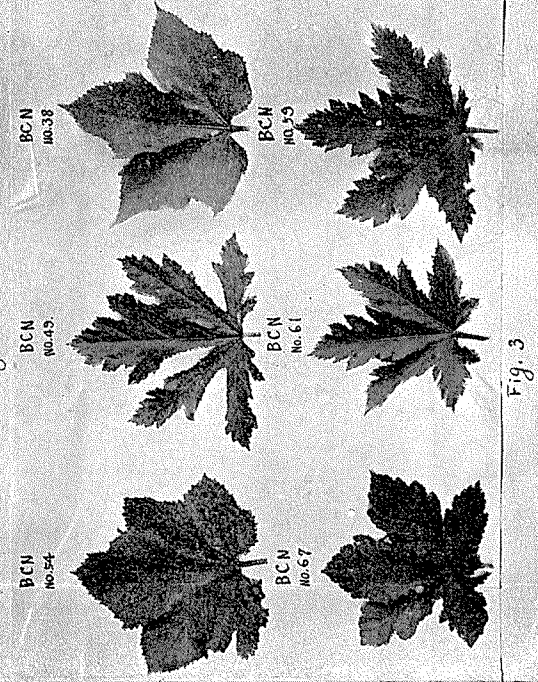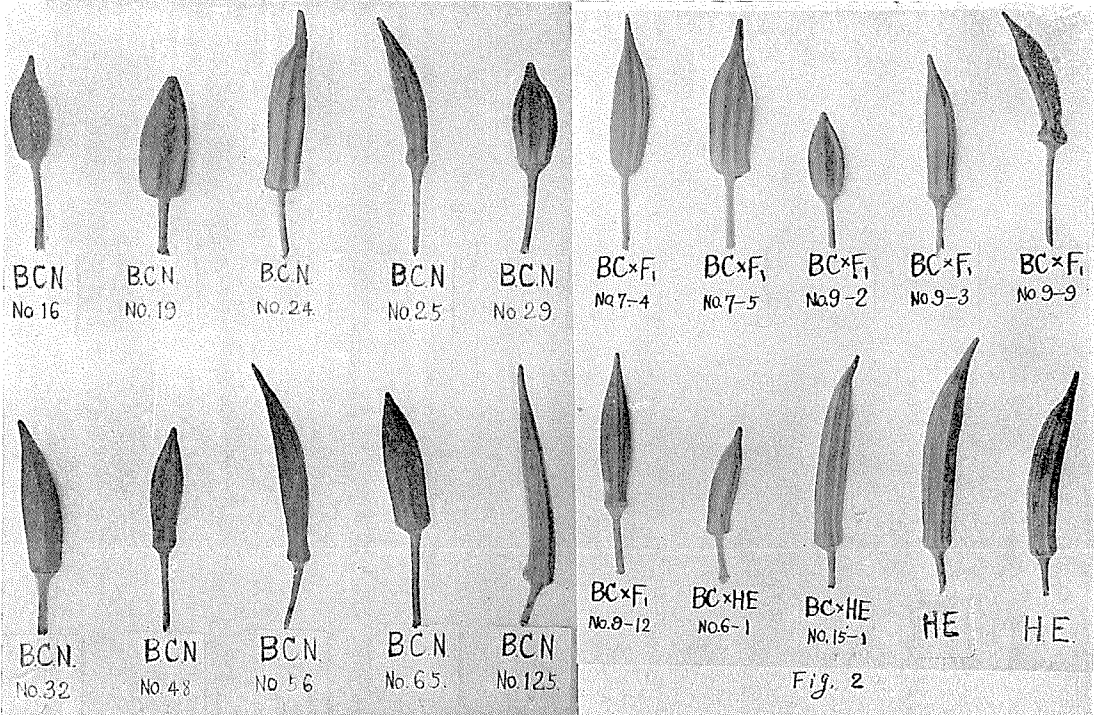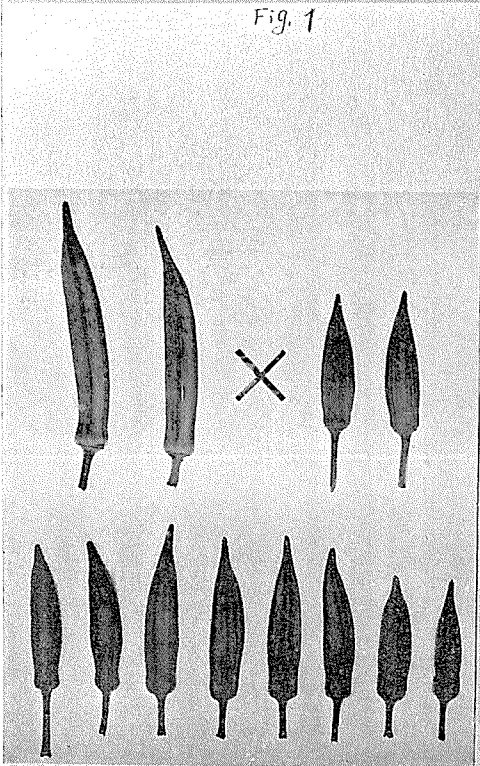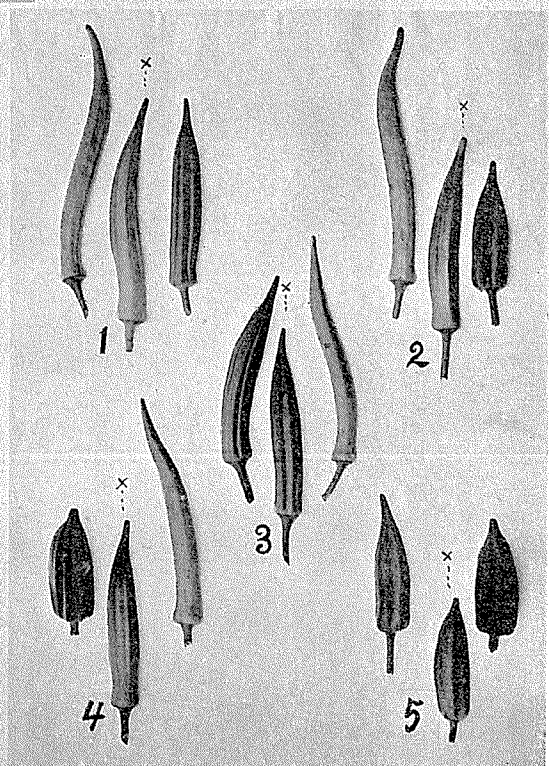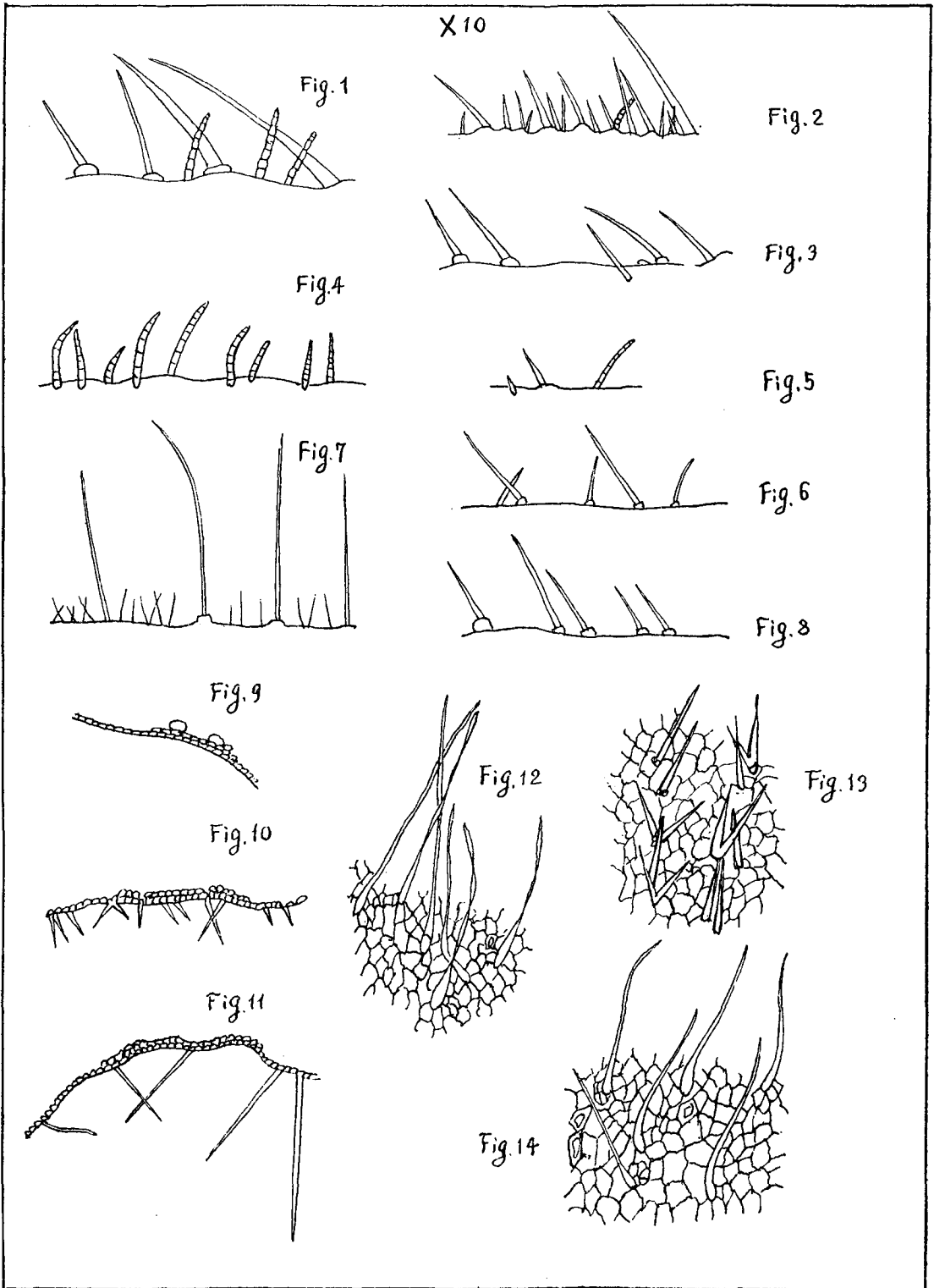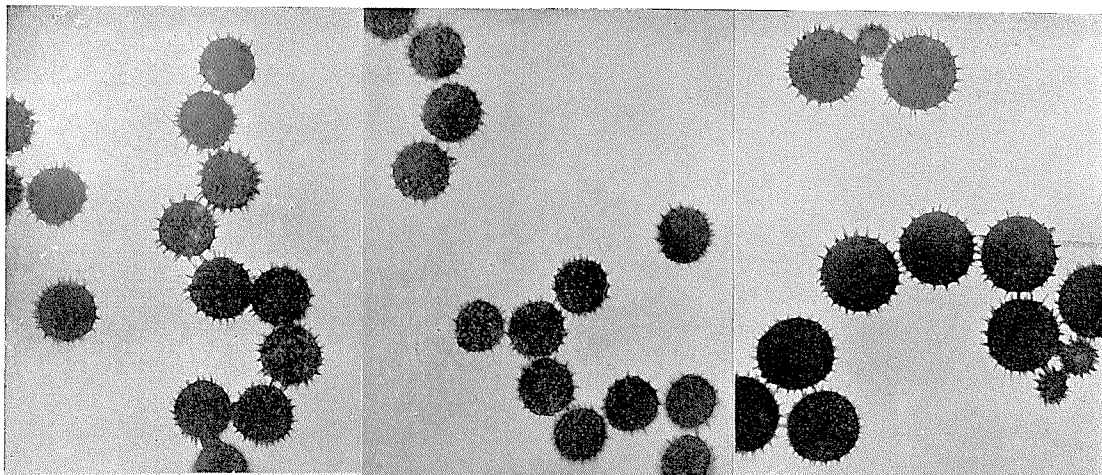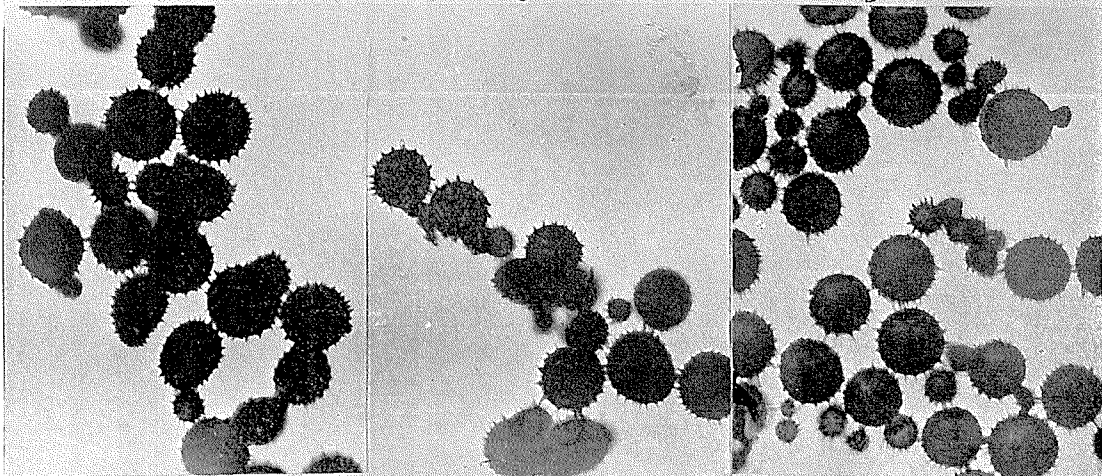
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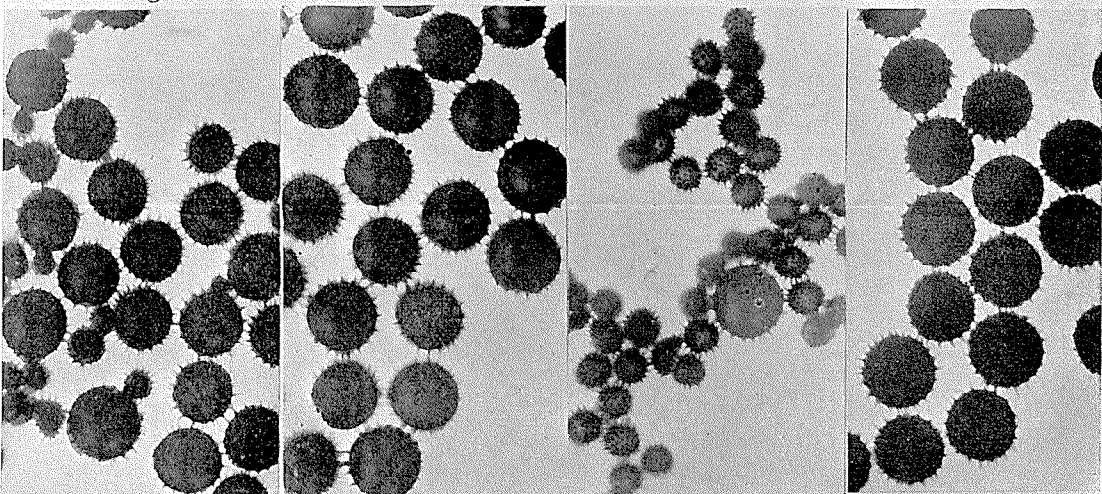
Fig. 3

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