CYTOLOGICAL OBSERVATIONS IN *COFFEA*. IV

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CYTOLOGICAL OBSERVATIONS IN *COFFEA*. IV[1]

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(With Eighteen Text-figures)

CONTENTS

I. Introduction

In one of the previous articles on the cytology of *Coffea* (Krug, 1937) the senior author published a preliminary account on the occurrence of a triploid coffee plant obtained by hybridization between *C. arabica* L. ($2n=44$) and *C. canephora* Pierre ($2n=22$). The plant was at that time only in its seedling stage, its triploid nature having been determined at mitosis in root tips. At metaphases some of the longer *canephora* chromosomes could be easily detected. In other articles on the same subject it was suggested that some of the interspecific hybrids grown in Java were highly unproductive due to their triploid nature; a high sterility was expected to occur in these plants.

The present article confirms the hypothesis on the sterility of these triploids, giving a detailed account on the meiotic behaviour of their chromosomes. In the course of our work a few more hybrid plants of the same nature were obtained.

II. Morphological characters of two interspecific triploid hybrids (*Coffea arabica* × *C. canephora*)

The interspecific hybrids as yet studied are intermediate with respect to most of their morphological characters when compared with their parents. The germination of the hybrid seeds is somewhat retarded and the growth at early seedling stages very slow; this may be caused by the

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general lack of sufficient nutritive tissue contained in the seed; in later stages the growth rate is normal. No hybrid vigour is perceptible.

TABLE I

		Leaf dimensions			
Tree no.	Species and hybrids	Length mm.	Breadth mm.	Index	Angle of veins
45	*C. arabica* var. *bourbon* (2n = 44)	131·94	53·44	2·47	57°
45 × 37	Triploid hybrid (2n = 33)	172·00	71·88	2·39	63°
37	*C. canephora* (2n = 22)	192·50	87·92	2·19	67°
34	*C. arabica* var. *mokka* (2n = 44)	87·40	29·16	2·99	48°
36 × 34	Triploid hybrid (2n = 33)	126·34	43·92	2·88	55°
36	*C. canephora* (2n = 22)	210·62	88·52	2·38	69°

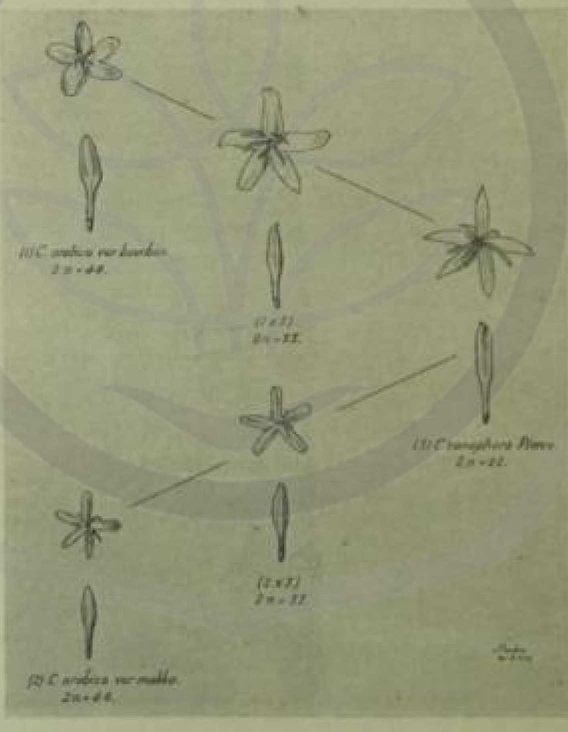

Fig. 1.

Table I contains the leaf dimensions of the parents and hybrids grown under half shade, and Fig. 1 shows differences in flower types ($\times\frac{1}{2}$).

In another instance a cross was made between *C. canephora* and *C. arabica* var. *Murta*, a small-leaved heterozygous variety (Krug, 1938); the morphological characters of the hybrid, not mentioned in the above table, are very similar to those obtained by crossing the var. *bourbon* with *C. canephora*. The results which are being obtained in these interspecific hybridizations are mainly of genetical interest and will be dealt with in future publications on the genetics of *Coffea*.

All these interspecific hybrids have been flowering abundantly, the flowers being normally developed, but practically no fruit set has occurred.

III. CYTOLOGICAL OBSERVATIONS IN TRIPLOID *COFFEA*

(*a*) *Material and methods*

For the study of microsporogenesis of these triploid plants, flower buds were fixed in a solution of three parts of alcohol and one of acetic acid; aceto carmin was used for staining the smears.

All flower buds examined originated from one single hybrid plant (45 × 37–1).

The drawings were made with camera lucida with a magnification of 3350 times; Zeiss 100 × immersion objective and 20 × ocular were used; Figs. 2–18 were reduced ½ for reproduction.

(*b*) *Microsporogenesis*

The initial prophase stages are difficult to observe in detail, the chromatic filaments not showing up very clearly. In this stage one notes that most of them condense close to the relatively large nucleolus; gradually the filament begins to expand throughout the nucleus; however, as long as the nucleolus exists, a good deal of the filaments remain more or less attached to it (Fig. 2). The chromonemata appear merely as dotted lines.

At this initial stage, the nucleus of the pollen mother cell retains its normal round shape, being less intensively stainable than the surrounding cytoplasm.

At the stage when the filaments begin to appear more clearly, the cell volume increases, its cytoplasm becomes less dense and takes up less stain than in earlier stages. Pressing the cover glass on the slide, caryoplasm and cytoplasm become completely undistinguishable from each other. Due to this procedure, the filaments become better separated and a clear doubleness of some of the chromosome strands can sometimes be noted (Fig. 3). The pairing is difficult to be observed due to the condensation of the filaments close to the nucleolus in most of the cells. It is however clearly noticeable that the pairing of the filaments becomes gradually more frequent and also that sometimes a third filament tries to attach itself to a pair of chromosomes and apparently mainly at those regions where the two strands are not intimately united.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

The chromatic filaments begin to contract themselves and the chromosomes appear shorter and thicker; they unite, the contracted filaments appearing deeper stained at certain regions, probably due to differences in the degree of contraction along the chromosomes (Fig. 4). As this contraction is terminal for some chromosomes and subterminal or median for others, certain regions of the chromosomes appear at this obtained; these were misshaped and only some of these contained a few irregular seeds with completely abnormal embryos; not a single one germinated.

Many flowers were also pollinated by *C. arabica* and *C. canephora* pollen, but no seeds were obtained.

As the triploid plants flowered abundantly, at the same time many other coffee species and varieties of varied cytological constitution flowered around them, a few fruits were formed on the triploid, yielding two apparently normal seeds. Two plants were obtained from them which revealed to have $2n = 44$ *chromosomes*. Very few of their chromosomes seem to be of the *canephora* type.

V. Discussion and conclusions

As expected, the meiosis of the interspecific triploid hybrid *C. arabica* × *C. canephora* is completely abnormal, sterile pollen grains with varying number of chromosomes being formed. It is very probable that the same irregular behaviour of the chromosomes noted in microsporogenesis also occurs at megasporogenesis. The sterility of some of the interspecific hybrids grown in Java is therefore completely cleared up.

With respect to the behaviour of the chromosomes at meiosis a few interesting conclusions may be drawn. Up to the present date nothing is known regarding the genetical relations between the varied species of the genus *Coffea* and the origin of some of the cultivated types. The triploid hybrid above examined got one chromosome set of 11 elements from *C. canephora* and another of 22 from *C. arabica*; the fact that a pairing of chromosomes occurs at early prophase up to metaphase of meiosis, in average at a rate of respectively 61·5 and 56·5%, indicates that certain homology exists between many of the chromosomes; due to the fact that some of the *canephora* chromosomes are longer than the *arabica* ones, and that *C. arabica* is already considered as a tetraploid species, it is probable that in most of the cases autosynapsis occurred between *arabica* chromosomes. No data are available, however, to deny the occurrence of alosynapsis.

If the *arabica* species should be an autotetraploid, the existence of characters determined by duplicate genes should be frequent; however genetical studies which are in course revealed that most of the main characters, analysed up to the present, are determined by single pairs of genes. If the hypothesis of an autotetraploid origin should be confirmed by later cytological and genetical analysis, it must be assumed that the duplication occurred at a very remote date.

The loose association of a third member to some of the chromosome pairs in prophase and metaphase suggests that this third element belongs to the *canephora* set of chromosomes, as the occurrence of neither an association of three *arabica* chromosomes nor the pairing of *canephora* elements *inter se* is probable. It may be that certain regions of some of the *canephora* chromosomes are analogous to some existing in certain *arabica* elements; or that the association of a third chromosome to a group of two is merely caused by the attraction of inert regions (Kostoff & Arutiunian, 1938). The data obtained are as yet too incomplete to make any considerations regarding the possible genetical relations between the two species crossed.

Regarding the cytological constitution of the two progeny plants of one of the triploid hybrids the following considerations can be made. Both have $2n=44$ chromosomes and it is apparent that some of the chromosomes derived from *C. canephora*. The plants are still in the seedling stage and therefore nothing can be said yet about their morphological characters. As stated above, they originated from seeds of open pollinated flowers. If one analyses Table V, one concludes that these plants did not originate through self pollination, as it was observed that only one nucleus out of 74 was formed at second telophase with 21 chromosomes; the association, through self pollination, of two gametes summing up 44 is therefore highly improbable. Considering cross pollination, pollen of 11, 22 and 33 chromosomes was available from neighbouring plants. 11-chromosome pollen did not fertilize the triploid, as it does not produce 33-chromosome gametes. From the other two types of pollen, the one with 22 chromosomes is more probable to have been the pollinating agent, as it was available around the triploid plant in great majority. The only hexaploid plant, which furnished a small percentage of viable 33-chromosome pollen grains, flowered very little in the neighbourhood. The future study of the meiosis of these two tetraploid progeny plants will probably clear up its cytological constitution.

VI. Summary

1. A short description is given of the main morphological characters of two interspecific triploid hybrids (*C. arabica* × *C. canephora*); their growth habit is normal and leaf and flower characters are intermediate in shape and size when compared with their parents.

2. The meiotic behaviour of the chromosomes at microsporogenesis is given in detail; the expected abnormalities were observed at the distribution of the chromosomes at first and second divisions, resulting

in the formation of sterile pollen grains which are extremely variable in size.

3. The megasporogenesis in this triploid is believed to show the same abnormalities as observed in microsporogenesis.

4. The sterility of some interspecific hybrids grown in Java, as suggested in previous articles, is therefore confirmed and cleared up.

5. The intimate pairing of some chromosomes at early prophase up to metaphase suggests the possible autotetraploid origin of *Coffea arabica*.

6. The occasional association of a third chromosome at prophase and metaphase to groups of two suggests that some of the *canephora* chromosomes may have regions analogous to ones of some *arabica* elements; nothing can be stated, however, regarding a possible genetical relationship between *C. arabica* and *C. canephora*.

7. Two progeny plants of a triploid, obtained from open pollinated flowers, revealed to have $2n=44$; it is suggested that they derived from cross pollination, 22 chromosome pollen from normal *arabica* plants in the neighbourhood having fertilized two occasional 22 chromosome egg cells of the triploid.

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