A STUDY ON MORPHOLOGY, FLORAL BIOLOGY AND FERTILITY OF INTERSPECIFIC HYBRIDS OF *COFFEA ARABICA* L. AND *COFFEA CANEPHORA* Pierre ex Froehner. INCLUDING BACKCROSSES OF THE HYBRID TO *C. ARABICA*

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A THESIS SUBMITTED IN PART FULFILMENT FOR THE DEGREE OF M.SC. PLANT BREEDING IN THE UNIVERSITY OF NAIROBI

- 1979 -

D E C L A R A T I O N

This thesis is my original work and has not been presented for a degree in any other University.

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This thesis has been submitted for examination with our approval as University Supervisors.

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A C K N O W L E D G E M E N T S

I am greatly indebted to my Supervisors, Dr Ir. H A M van der Vossen and to Ir. E M W van Breukelen for constant guidance, suggestions and for critically reading all the drafts of this thesis. In the same connection, I am thankful to Mr D J Walyaro, Breeder, Coffee Breeding Project for encouragement and for the many fruitful discussions on analysis of the data.

The assistance given to me by the field staff of the Coffee Breeding Unit is highly appreciated.

I am most grateful to Dr J P Moss for giving his time outside the time table teaching hours to afford me extra cytology practicals, to Professor V K Gupta and to Dr E Rowlands for resourceful discussions on the cytogenetics data, and to Dr Widdowson for allowing me the use of the phase contrast microscope.

I am thankful for the scholarship offered me by the Netherlands Government to enable me to take this course at the University of Nairobi, and to the Director, Coffee Research Foundation for granting me a fully paid study leave during the first year of the course. I am very grateful for the assistance with the dark room work offered me by Mr Kit Karanja, and to Mrs P M W Kahura for carefully typing the manuscript.

Lastly, thanks are due to members of my family for encouraging me.

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SUMMARY

A breeding programme is in progress at the Coffee Research Station, Ruiru with a main objective being to develop Coffea arabica cultivars resistant to coffee berry disease caused by Colletotrichum coffeanum Noack and coffee rust Hemileia vastatrix B. & Br. A number of C. arabica varieties have been used as progenitors of disease resistance. At the same time interspecific hybrids of C. arabica and tetraploid C. canephora called arabusta were also made to enable introgression of disease resistance and vigour of C. canephora, into cultivars of C. arabica. This report summarises the results of a comparative study on the morphology, floral biology and fertility of arabusta its parent species and backcrosses of the hybrid to C. arabica.

Arabusta plants could be classified into two phenotypic classes distinctive for leaf size and canopy shape. Notwithstanding, all arabusta plants were found to resemble other tetraploid plants in stomata density, length of the guard cells, pollen diameter and leaf length to width ratio. From observations on mitotic chromosomes in root tips and meiotic chromosomes in pollen mother cells, the indication from morphological characters that the two arabusta phenotypes were not the result of chromosome number differences was confirmed.

The results from a number of growth components showed arabusta of phenotype FI R* to have remarkable vigour.

Fertility was estimated from pollen stainability, in vitro pollen germination, % used ovules and fruit set from crosses as well as open pollination. The results showed in each case that arabusta had a very low fertility; 6.0 ± 0.9% on pollen germination compared to 72.0 ± 2.6% in C. arabica cv. SL 28 both tested under the same conditions. A large range in fertility was observed within arabusta families indicating effectiveness of selection for fertility within this material. An important result with regard to introgression of disease resistance and vigour of C. canephora into C. arabica cultivars is that fertility is restored to almost normal levels, already in the second backcross of the arabusta to C. arabica.

It was observed that arabusta produced normal flowers with very few star flowers unlike other coffee interspecific hybrids. The results also showed that artificial doubling of the number of chromosomes in C. canephora was of no consequence on self incompatibility in this species. It is suggested that the genetic system for self incompatibility in C. canephora may probably not be monofactorial gametophytic as previously reported.

It was found that at Ruiru, meiosis in pollen mother cells could be studied in flower bud samples drawn between 42 - 48 hours after breaking bud dormancy.

From an analysis of a number of meiotic components which included metaphase I chromosome associations, distribution of the chromosomes over the anaphase I poles and the frequency of microspores formed per tetrad, it was shown that the reduced fertility

of the arabusta is mostly an expression of their subnormal meiosis. More precisely, the results indicated that a poorly regulated meiosis could be responsible.

Some practical implications of these results with regard to the use of arabusta in introgression of disease resistance and vigour, as well as its possible role in robusta coffee improvement are discussed.

CHAPTER 1

BACKGROUND AND OBJECTIVES

Cultivars of Coffea arabica L. grown in Kenya have a high yield and excellent quality, but all of them are highly susceptible to coffee berry disease caused by Colletotrichum coffeanum Noack.(sensu Hindorf.) and coffee rust (Hemileia vastatrix B. & Br.). These two diseases, in particular coffee berry disease, are responsible for crop losses and in fact severe crop losses are often only averted through chemical protection using fungicides at a considerable cost to the farmer. A breeding programme with the main objective being to develop Coffea arabica cultivars resistant to coffee berry disease and leaf rust has been in progress at the Coffee Research Station, Ruiru, since 1972.

In this programme, introduced cultivars and varieties of C. arabica have been used as progenitors of disease resistance (Van der Vossen, 1973). It was however recognized in the initial stages of the programme that interspecific hybrids of C. arabica and C. canephora P. ex Fr. could also in future be important as alternative sources of disease resistance. Monaco, cited by Van der Vossen (1972), claimed that such interspecific hybrids were also likely to be superior to some varieties of C. arabica as sources of disease resistance because of vigour and yield. At Ruiru, a number of induced tetraploid C. canephora (var. robusta) clones have been found

resistant to coffee berry disease. These clones have been used in interspecific crosses with several locally adapted cultivars of C. arabica and viable arabica-robusta hybrids, coined "arabusta" by Capot et al (1968), have been obtained. These arabusta hybrids are now being used in backcross programmes for introgression of disease resistance into locally adapted cultivars of C. arabica which are already selected for their high yield and excellent quality.

In connection with this plan, the present study is an effort to provide information required about the level of fertility of the arabusta hybrid so that insight may be gained on the potential performance of materials derived from crosses involving these hybrids. Since progenies of backcross generations to C. arabica of a similar (robusta x arabica) interspecific hybrids are also available, it will be possible to obtain information about the trends of fertility with successive generations of backcrossing of the interspecific hybrid to C. arabica. This could indicate in advance the rate of restoration to normal fertility to be expected from progressive backcrossings. Such information will be important because hybrid plants used in backcrosses can then also be selected for fertility in addition to disease resistance and so adverse effects of reduced fertility on the yield of advanced generations would in this way be minimized. Prediction of the rate of restoration to normal fertility from observations of backcross generations would indicate how many additional

breeding cycles should be planned to restore the fertility to normal levels.

In addition to investigations on fertility, comparative studies on floral biology, morphology of the plants including recordings on general growth and yield components will be made on parent species and hybrids. Information from such characters would indicate whether there is hybrid vigour and how much of this vigour is lost in successive backcross generations to C. arabica. Furthermore the arabusta hybrids are of two plant types. In one group, plants have a rather open canopy, with long drooping primaries and large broad leaves. In fact these plants strongly resemble their tetraploid C. canephora parent. On the other hand, there are arabusta plants with small narrow leaves, a canopy rather tapering to the apex and the plants somewhat resembling C. arabica in general outlook. It could well be that these plant types also differ considerably in their fertility and cytogenetic aspects (e.g. chromosome number). Results from such a comparative study would facilitate a decision to be reached concerning which one of the FI plant types should be selected for backcrosses to C. arabica cultivars.

Observations on how regular the meiosis is in the arabusta hybrid and the relative trends in meiotic regularity in backcrosses and parent species could provide information about the cytogenetic basis for the level of fertility observed

in the arabusta hybrid in relation to the other generations. Meiotic components known to correlate with fertility in coffee (Louarn, 1976), such as association of chromosomes in first metaphase, distribution of the chromosomes to the anaphase poles and the frequency and number of microspores formed per tetrad will provide suitable measurements.

The objectives of the present study are therefore to examine the variation in morphological characters and fertility between the parent species, their interspecific hybrids and backcross generations. To a limited extent some cytogenetic observations will be made to check whether this variation has a cytogenetic background. The information thus collected is considered of much relevance to the breeding programme with interspecific hybrids between C. arabica and C. canephora which is in progress at the Coffee Research Station, Ruiru, Kenya.

PLATE 1

Arabusta plant type F_1A characterised by small leaves, erect branches and a canopy tapering at the apex.

PLATE 2

Arabusta Plant type F_1R characterised by large leaves, drooping branches and open canopy at the apex.

CHAPTER 2

LITERATURE REVIEW ON INTERSPECIFIC HYBRIDIZATION IN THE GENUS *COFFEA*

The genus *Coffea* has several diploid species but only *C. arabica* occurs naturally as a tetraploid. Heyn (1938), Fergelind cited by Sybenga (1960) and Mendes (1938) have stated that the basic chromosome number for the genus *Coffea* is x = 11. Diploid species therefore have 22 chromosomes and tetraploids have 44 chromosomes.

Sybenga (1960) has given an account of the early work on interspecific hybridization in the genus *Coffea*. Mainly with the purpose to create plants superior to existing *Coffea* species, a number of interspecific crosses were made in many countries. Among diploid species several interspecific hybrids, some natural and others artificially induced, have been described, but most of them turned out to be of little commercial value and so remained at the experimental stage. However, spontaneous hybrids of *C. canephora* var. robusta and *C. congensis* called "Congusta" hybrids found in Indonesia had good fertility, yield and adaptability in higher altitudes where robusta coffee does not grow well any more (Ferwerda, 1969). In the recent past interspecific hybridization among diploid coffees has been pursued more enthusiastically in a programme of robusta coffee improvement. Initially research in this field was directed at genome

relationships and fertility of the hybrids. Charrier (1976) made a detailed study of the genetic structure of the family Mascarocoffea (wild diploid coffees of the Malagasy region), and their relation with Eucoffea (Coffea originating from Africa). He made crosses between 26 Mascarocoffea species with 4 species of Eucoffea. From this study, the author concluded that Mascarocoffea and Eucoffea share the same basic genome but that slight differentiation has accompanied their evolution. Exploitation of the caffeine free attribute of Mascarocoffea through interspecific hybridization with C. canephora was found to be difficult because of the low fertility of the hybrids. Louarn (1976) crossed C. canephora and C. eugenioides and noted that the FI had a reasonable fertility, while Lanaud (1977) noted that the major problem in gene transfer from C. kianjavatensis (a Mascarocoffea) to C. canephora (an Eucoffea) was the consequence of the non-homology for about 3 chromosomes among the 11 of the complete genome of both species.

Triploid hybrids made from crosses involving diploid species and C. arabica have variously been made and occasionally turned out to have desirable attributes. Although Krug and Mendes (1940) had already obtained a C. arabica x C. canephora triploid hybrid, crosses of this nature were difficult, the embryos formed were in most cases defective and died. The few hybrids obtained flowered but with practically no seed set in accordance with their triploid

condition. However, in India, triploid hybrids of C. arabica and C. canephora were found to have resistance to H. vastatrix and this resistance was maintained in all backcross progenies of the hybrid to C. arabica (India Coffee Board Ann. Report, 1974/75). Medina et al (1975) found resistance to Leucoptera spp. in a C. arabica x C. racemosa triploid hybrid backcrossed to C. arabica. Orozco-Castano (1976) has preferred the use of triploid hybrids over duplication of chromosomes of C. canephora for introgression of simple characters from C. canephora to improve C. arabica. He contemplates that the fewer number of C. canephora genes in triploid hybrids makes it possible to use only a few number of backcrosses to obtain desired objectives.

Spontaneous interspecific hybrids of coffee at the tetraploid level have been described. The Arla hybrids referred to by Leliveld (1940) and Bouharmont (1959) arose spontaneously from natural crosses between C. arabica and C. canephora and had 44 chromosomes. Their seed production was good although they turned out to be unproductive in the long run. A spontaneous self sterile 44 chromosome plant was found in Java. Krug, Mendes and Carvalho (1950) suggested that this plant named hybrid "386" was probably an interspecific hybrid. The Kalimas and the Kawisari A and B hybrids however attained commercial prominence in Java. These were interspecific hybrids between C. arabica and C. liberica with most of the plants having C. liberica as mother. These hybrids were resistant to H. vastatrix although neither parent

had this attribute. They were however abandoned once rust resistant robusta type coffees were introduced because they had a high proportion of empty seeds.

The variety Hibrido de Timor of C. arabica is presumed to have arisen from a spontaneous hybridization between C. arabica and C. canephora (Monaco, 1977). It was found to have genotypes resistant to all known races of Hemileia vastatrix (Rodrigues, Bettencourt and Rijo, 1975) and other diseases particularly coffee berry disease (Van der Vossen, Cook and Murakaru, 1976). However, it is a low yielder probably because it arose from unselected material of inherent low yield potential.

A technique for doubling chromosome numbers in grown plants of coffee using colchine was described by Mendes, (1939). The first interspecific hybrids between induced tetraploid C. canephora and C. arabica were made in 1950 at Campinas, Brazil. Advanced generations of this cross including backcrosses to C. arabica named 'Icatu' by Monaco (1977) are currently under observation. All crosses were made with only one genotype of C. canephora (Co 254) as one parent and C. arabica variety Bourbon as the other parent and therefore materials derived from this cross are of limited variability. However, advanced backcross generations of this hybrid to C. arabica varieties Mundo novo and Caturra are of interest to the breeding programmes at Campinas and Coffee Research Station, Ruiru, because of

their resistance to coffee rust and CBD (Carvalho, Monaco and Van der Vossen, 1976).

Capot, Depautex and Durandeau (1968) used colchicine to double the chromosome number of C. canephora and made several crosses between C. arabica and induced tetraploid C. canephora obtaining on a large scale, hybrids which were subsequently named arabusta by Capot (1972). This inter-specific hybridization programme was undertaken with the objectives to improve liquor quality and reduce caffeine content of C. canephora in the Ivory Coast. Through selection, arabusta with high fertility have been obtained with a high yield potential close to the very best of robusta clones. A special arabusta development project aimed at establishing some 15,000 ha of arabusta coffee in the Ivory Coast between 1977 and 1985 with an estimated production of 20,000 tonnes clean coffee per year by 1990 was planned (IFCC, 1977).

Similar arabica x robusta interspecific hybrids utilizing arabusta is in progress at the Coffee Research Station, Ruiru with the objectives to transfer coffee berry disease and coffee rust resistance as well as vigour of these hybrids to locally adapted cultivars of C. arabica (Van der Vossen, 1974).

An interspecific hybridization programme using hexaploids produced through doubling chromosome numbers of triploid hybrids of C. arabica x C. canephora (diploid) is

in progress at the ORSTOM Research Station at-Man, Ivory Coast. These hybrids have $^{2}/_{3}$ of their chromosomes from C. arabica and resemble this species more closely than arabusta (Berthaud, 1978). They are reputed to have a lower caffeine content than arabusta. A programme of selection to improve on quality and yield is in progress but this has not reached a stage of commercial prominance as the arabusta programme.

CHAPTER 3

DESCRIPTION OF THE PLANT MATERIALS USED IN THE PRESENT STUDY

The study was made on coffee trees planted in the field in June 1976 at the field station of the Coffee Breeding Unit, Coffee Research Station, Ruiru. This Station is situated at 1600m above sea level and receives a mean annual rainfall of 1050mm. The field was planted at a spacing of 1.5m x 2.0m. Tetraploid C. canephora plants established earlier in 1972 were also available in an adjacent field. A detailed description of each group of the plant materials is as follows:

1. Parent species:

(a) Coffea arabica L. (2n = 44) cv. SL 28; widely grown in Kenya as a commercial seed cultivar. 157 plants.

(b) Coffea canephora Pierre ex. Froehner (2n = 22) var. robusta; six plants in a Museum Plot at the main Station of the Coffee Research Station, Ruiru.

(c) Coffea canephora: colchicine induced tetraploids (2n = 4x = 44) var. robusta. 15 plants representing 6 clones which had been named the Uganda Tetraploids

(UT) clones UT3, UT6, UT7, UT8, UT10, and UT12. They had been developed in 1969 by F.C. Millot at Kawanda Research Station, Uganda, by treating with colchine, open pollinated seed or seed from crosses between clones of high productivity. Cuttings from these tetraploid clones were brought to Ruiru in March 1972.

2. Interspecific hybrids.

The F_1 of C. arabica x (tetraploid) C. canephora also called arabusta. There were 271 plants in two fields (Table 1A), adjacent to each other but only 113 plants in one of the two fields were used in this study because in this field, other generations were also represented. The 113 plants were progenies of 8 crosses made with the local C. arabica cultivars as female and tetraploid C. canephora clones as male parents. Selection of the male parent clones had been based on the performance of its arabusta progeny in a preselection test for resistance to coffee berry disease (Van der Vossen, Cook and

Murakaru, (1976). Only tetraploid C. canephora clones that had been found to have disease resistant progenies in this test were used in the crosses. Details of these crosses and the number and description of plants in each group are given in Tables 1A and 1B.

3. First Backcross Generation (BC_1)

C. arabica x (C. canephora (tetraploid) x C. arabica). The plants studied were open pollinated progenies of a first backcross generation as outlined These were therefore (BC_1F_2).

These plants were available as a result of an exchange programme between the Instituto Agronomico at Campinas, and the Coffee Research Station, Ruiru. At Campinas, the interspecific hybrid of tetraploid C. canephora and C. arabica was made about 30 years ago, with one clone of tetraploid C. canephora (Co 254) as female and C. arabica cv. Bourbon as the male parent. Backcrosses of this hybrid to C. arabica varieties Bourbon, Mundo novo or Caturra were made. There were 224

plants of this generation available for observation. These progenies differed in certain characters (See Table 2).

4. Second backcross generation (BC_2)

C. arabica^2 x (*C. canephora* (tetraploid) x *C. arabica*)

There were 307 plants from ten BC_2F_2 progenies of a second backcross generation, resulting from the above type of cross. Only varieties Mundo novo or Bourbon were used as female parents in this backcross generation. The progenies were also selected for certain attributes (Table 3).

TABLE 1A Description of plants of the F_1 (arabusta) generation (in Field B6a Oaklands Breeding Station). Details of crosses and number of plants per progeny falling into group 'A' and 'R'.

PROGENY NO.	PARENTS	NO. OF PLANTS TYPE'A'	NO. OF PLANTS TYPE'R'	TOTAL
18	SL28 x UT6	5	4	9
19	SL34 x UT6	0	14	14
20	N39 x UT6	0	17	17
49	SL28 x UT3	0	14	14
50	N39 x UT3	5	10	15
51	SL28 x UT8	0	14	14
52	SL34 x UT8	0	5	5
53	SL34 x UT10	12	13	25

Notes: Type 'A' plants resemble C. arabica in general outlook.

Type 'R' plants resemble tetraploid C. canephora in general outlook.

TABLE 1B Description of plants of the F_1 (arabusta) generation. Details of crosses and no. of plants falling into groups "A" and "R" from Fields (B6a and B6b)

PROGENY NUMBER	PARENTS	NO. OF PLANTS TYPE "A"	NO. OF PLANTS TYPE "R"	TOTAL
18	SL28 x UT6	37	18	55
19	SL34 x UT6	64	56	120
20	N39 x UT6	3	20	23
49	SL28 x UT3	0	14	14
50	N38 x UT3	5	10	15
51	SL28 x UT8	0	14	14
52	SL34 x UT8	0	5	5
53	SL34 x UT10	12	13	25
	TOTAL	121	150	271

TABLE 2 Description of plants of the BC_1 (1st backcross) generation in Field B6a (Oaklands Breeding Station). Details of crosses and number of plants in each progeny falling into groups with special characteristics.

PROGENY NO.	PARENTS	NO. OF PLANTS	SPECIAL ATTRIBUTES OF PROGENIES
21	MN x (RB x B)	58	A - RAM
22	MN x (RB x B)	49	A - RAT
23	"	6	E - RAM
24	"	7	A - RAM
25	"	23	A - RAT
26	"	61	E - RAT
27	"	7	A - RAM
28	CT x (RB x B)	13	A - RAT

Notes: RB = Tetraploid Robusta (C. canephora)

B = C. arabica variety Bourbon

MN = " " " Mundo Novo

CT = " " " Cattura

A = High yielding

RA = Leaf Rust Resistant

M = Medium late ripening

T = Late ripening

E = Specially selected tree

TABLE 3 Description of plants of the BC_2 (second back cross) generation in Field B6a (Oaklands Breeding Station); number of plants in each progeny with special attributes.

PROGENY NO.	PARENTS	TOTAL NO. OF PLANTS	SPECIAL ATTRIBUTES OF PROGENIES
29	$(MN^2 x (RBxB)$	18	E - RAT
30	"	69	E - RAM
31	"	31	"
32	"	32	A - RAM
33	"	30	"
34	"	22	A - RAT
35	"	11	"
36	"	16	E - RAT
37	"	42	"
38	"	36	"

See footnotes table 2

CHAPTER 4

LEAF SIZE AND SHAPE, STOMATA DENSITY, LENGTH OF THE GUARD CELLS AND POLLEN DIAMETER

4.1 INTRODUCTION

The morphology and growth habit of plants are modified in polyploids and interspecific hybrids, so that polyploids are often more vigorous than diploids. Stebbins (1947) has listed thicker leaves, larger flowers and larger fruits as the characters most consistently present in tetraploids.

The use of morphological characters in indirect selection for polyploidy following colchicine treatment of subject plants in breeding programmes has become routine. Bradgo (1955), Savistsky (1952) and Eenink & Alvarez (1975) referred to the length of the stoma guard cell as a criterion to eliminate diploid plants in the first generation after colchicine treatment. Najcevska and Speckmann (1968) observed that doubling of the chromosome number in several Trifolium species led to an increase in the number of plastids, the increase being about the same in all the species examined. Sohoo and Gill (1976) however described reduced leaf length, leaf width and plant height following colchicine treatment in cluster bean, Cyamopsis tetragonoloba.

The relation between a morphological character and

ploidy may be inverse or direct. In pyrethrum for instance, Ottaro (1977) found that plant height, weight of flowers and stomata density could be reliably used to discriminate between ploidy levels. Triploids had heavier flowers, were taller and had lower stomata densities than diploids. Franco (1939) similarly described the relationship between ploidy and some morphological characters in coffee including stomata density and length of the guard cells. He noted that stomata density on the leaf surface decreased as the ploidy level rises whereas length of the guard cell increased with increasing ploidy level. Capot et al (1968), obtained 75 tetraploid seedlings of Coffea canephora and described larger leaves and lower stomatal density as characteristics of the tetraploid plants. Millot (1972) used leaf length to width ratio to select tetraploid seedlings of C. canephora after colchicine treatment of seeds. In the same species Berthou (1975) obtained tetraploid sprouts after treatment of twigs of mature plants. In this work, leaf length to width ratio, stomata density and length of the guard cell were used to select for tetraploid sprouts. The highest leaf length to width ratio of 4.7 has been observed in monohaploid ($n=x=11$) C. canephora (Dublin & Parvais, 1975) while the lowest ratio of 1.74 reported was obtained in a hexaploid interspecific hybrid of C. arabica and C. canephora (Berthaud, 1978).

Concerning changes in morphology in response to

interspecific hybridization, hardly any predictions can be made as to trends. Allard (1960) stated that the morphology of allopolyploids and interspecific hybrids combine in a more or less blending fashion the characters of the two parent species. The species C. canephora was described by Pierre ex Froehner cited by Haarar (1962), as a glabrous shrub or tree, with large broad leaves which are often corrugated or undulating. The leaves are 15 - 30 cm long and 5 - 15 cm wide. The species C. arabica was originally described by Linneaus cited by Haarar (1962). It is a glabrous glossy leaved shrub or small tree. The leaves are relatively small (12 - 15)cm long, about 5 cm wide.

Krug and Mendes (1940) described the morphology and growth habit of triploid hybrid plants derived from a cross between C. arabica and C. canephora and noted that their growth habit was normal and rather similar to that of parent C. arabica. The flower shape and leaf size were however intermediate between those of the parent species. Similarly Louarn (1976) observed that the stomata density on the leaf surface of hybrid plants from C. canephora x C. eugenioides crosses were intermediate between those of parent species.

4.2 METHODS

Leaf length and width were measured as follows. Mature leaves on the third and fourth node from the tip of the primaries were used. Four leaves per primary and

eight primaries per tree were observed. Primaries were chosen at midcrown of the tree. Length of the leaf was measured from the tip to the base of the leaf along its midrib, width at the centre of the leaf. To determine the mean leaf length for C. arabica Srinivasan (1972) found that a minimum of 30 or 40 leaves respectively were required, while in C. canephora, a minimum of 70 leaves was required for each of these characters. Louarn (1976) found that he could accurately estimate mean length and breadth of leaves of C. canephora as well as of interspecific hybrids of C. canephora and C. eugenioides by taking measurements on 30 leaves. In the present study, Stein's two stage sampling procedure described by Steel and Torrie (1960) was used according to the formula:

$$n = \frac{t_1^2 s^2}{d^2} \quad \text{---------(1)}$$

where t_1 = the tabulated t value for desired confidence level at the degrees of freedom of the initial sample.

s = Is the standard deviation for the initial sample.

d = Is the desired confidence interval which gives the total number of observations required.

Using an initial sample of size 10 and imposing a 95% confi-

dence interval of not more than 1 cm. for leaf breadth of C. canephora the estimated sample size according to this formula was n = 27.5 which agrees with the sample size found by Louarn (1976). In the present study, four leaves per primary on 8 primaries taken at midcrown of the tree were collected giving a sample of 32 leaves per tree.

Stomata density on the lower surface of the leaf was determined by the method described by Franco (1939) which is similar to that used by Louarn (1976). Franco demonstrated that there is little difference for stomatal density in coffee leaves sampled either from the top or bottom of the canopy, provided the leaves are of the same age and are roughly equally exposed to the sun. He nontheless recommended the use of leaves taken from mid canopy,

In the present study fully exposed leaves on the fourth node from the tip of the primaries at around mid-crown of the tree were sampled, their petioles immersed in water to avoid dehydration. A print of the lower epidermis was obtained by applying a film of nail varnish to the lower surface of the leaf. Stomata counts were made under a light microscope at a magnification of 320 x. A net micrometer fitted to the ocular gave each field of observation. Calibration with a stage micrometer gave 0.1024 mm^2 surface area at 320 x magnification.

For sample size, the following consideration was made. Franco (1939) calculated mean stomata densities for several

species of coffee from a sample of 30 leaves per tree and 30 fields of observations per leaf. Louarn (1976) determined stomata density in hybrid plants of C. canephora and C. eugenioides from a sample of 16 leaves per tree and 16 fields of observation per leaf. In the present study, an initial population of 500 counts was made from 25 leaves per tree and 20 fields of observations per leaf using leaves of diploid C. canephora. An initial sample of 20 random fields drawn from this population was used to determine the number of random fields to be observed according to formula 1 on page 22, at P = 0.05 and 4 stomata per field of view. This indicated that 55.1 such random fields would be a sufficient number of observations. Therefore in this study, 5 random fields per leaf were observed on 12 leaves taken from branches at mid-crown of the tree.

For measurements of the length of the stomata guard cell, a leaf from the fourth node (from the tip of the branch) was picked and a small piece of the epidermis pealed off from the under surface of the leaf. This piece was mounted in a drop of water on a slide and 15 stomata were observed. Measurements were made with the aid of a micrometer scale fitted to the eyepiece of a microscope. The magnification was 800 times (8 x ocular by 100 x objective). The eyepiece micrometer scale was calibrated with the stage micrometer. One scale division of the eyepiece micrometer was found to be 1.75 microns.

Pollen diameter measurements were made on fresh pollen.

A fine dust of pollen grains was applied to a drop of 1% acetocarmine solution on a slide using a camel hair brush. An eyepiece micrometer scale was used to take measurements at a magnification of 320 x. Fifty pollen grains per individual tree were observed. The eyepiece micrometer was calibrated as described above and the measurements converted to microns by multiplying the scale readings by 3.8

4.3 RESULTS

The data of dimensions of leaves are given in Table 4. Wide differences for leaf length and width of the leaves were observed between the two species. C. canephora had in general longer and broader leaves than C. arabica. Leaves of diploid C. canephora were long and narrow whereas in the induced tetraploid of this species the leaves were relatively shorter but broader. In addition, the leaves of the induced tetraploid plants were thicker and had a corrugated surface. F_1R was intermediate for leaf dimensions between its parents C. arabica and tetraploid C. canephora. F_1A on the other hand had relatively shorter, narrower leaves than C. arabica, the low parent for this character. The back crosses were generally close to C. arabica.

Unlike leaf length and width, the derived character leaf length-to-width ratio varied only with ploidy levels. The diploid species C. canephora had a higher ratio relative to tetraploid plants. Whereas C. arabica had a smaller leaf

length to width ratio than diploid C. canephora, tetraploid C. canephora and C. arabica were remarkably close for this character. It was similarly notable that while F_1R and F_1A differed widely for leaf size (Plate 3), their leaf length to width ratios were fairly similar.

Stomata density on the lower surface of the leaf was related to the ploidy level of the plants (Table 5). Diploid C. canephora had relatively higher stomata density than all tetraploid generations, which were in turn remarkably similar for this character. In F_1A however, one of the plants examined had disproportionately high stomata counts. This was the reason for the high variation for stomata density in this group.

The data of guard cell lengths showed that all tetraploid plants had longer guard cells than diploid C. canephora (Table 5). A similar trend was observed with the diameter of pollen grains. It was also noted that pollen grains of F_1 plants had thick, fuzzy outer surfaces in contrast to smooth pollen surfaces of other generations.

4.4 DISCUSSION

C. arabica and C. canephora are known to differ widely in many respects including leaf forms, C. canephora having longer and broader leaves than C. arabica. Observations on leaf dimensions confirmed these differences in the materials studied. The leaves of induced tetraploid C. canephora were in comparison

to diploids broader but shorter. In addition, the effect of increasing ploidy levels in these plants was seen in relatively thicker leaves which had corrugated surfaces. The arabusta interspecific hybrid showed a blending fashion of inheritance for leaf dimensions, but with a variation into two plant types, one group F_1A resembling C. arabica while F_1R was close to tetraploid C. canephora.

Leaf length to width ratio, stomata density on the lower surface of the leaf, length of the guard cells and pollen diameter were all found to be related to chromosome number of the plants. In interspecific breeding programmes which utilize induced tetraploids, it is a routine practice to identify morphological characters that can easily provide rough guides to ploidy levels. In coffee, leaf length to width ratio has been found to decrease as the number of chromosomes increase. The highest ratio of 4.70 has been observed in a monohaploid (n = x = 11) C. canephora (Dublin and Parvais, 1975), whereas the lowest ratio of 1.74 reported, was obtained in a hexaploid interspecific hybrid of C. arabica and C. canephora (Berthaud, 1978). Results obtained presently were in agreement with these observations. Millot (1972) has also used the ratio to select for induced tetraploids in first generation plants of C. canephora following colchicine treatment obtaining a fairly large proportion of tetraploid seedlings.

Stomata density in coffee decreases as the level of

euploidy rises (Franco 1939) and this has subsequently been applied to differentiate induced and spontaneous tetraploid as well as haploid coffee plants (Dublin and Parvais, 1975; Berthou, 1975 and Berthaud, 1978). This relation was confirmed with the materials presently studied. At the same time length of guard cells and diameter of pollen grains were also found to be effective indicators of the level of euploidy. From the range and standard errors for mean stomata density, length of guard cells, pollen diameter and leaf length to width ratio, it is clear that each of these characters can be used in indirect selection for induced tetraploids from diploids. Relative efficiency of the characters in selection is not assessed in this study. However, it is obvious that measurements of leaf length to width ratio can be made more easily relative to the others especially as the use of the microscope is not necessary. This character should therefore provide a quick check on ploidy variation in coffee.

Table 4 Leaf length, leaf width and leaf length to width ratio ($^{LL}/LW$) in *C. arabica* cv SL 28 and *C. canephora* var. robusta; their interspecific 'arabusta' hybrid and in backcrosses to *C. arabica* Dimensions in cms. Means and S.E. of the mean.

	NO OF TREES	LEAF LENGTH	LEAF WIDTH	$^{LL}/LW$ RATIO
C. arabica	10	12.6 ± 0.2	6.3 ± 0.1	1.96 ± 0.02
DIPLOID *C. canephora*	7	19.2 ± 0.5	8.0 ± 0.4	2.40 ± 0.02
TETRAPLOID *C. canephora*	14	17.8 ± 0.6	10.0 ± 0.4	1.76 ± 0.03
ARABUSTA (F_1A)	15	9.8 ± 0.3	5.5 ± 0.1	1.78 ± 0.02
ARABUSTA (F_2R)	15	14.9 ± 0.4	8.2 ± 0.2	1.82 ± 0.02
BC_1 F_2	15	10.6 ± 0.4	5.4 ± 0.2	1.97 ± 0.04
BC_2 F_2	15	9.8 ± 0.3	4.8 ± 0.2	2.06 ± 0.03

Notes: 32 leaves were observed per tree.

Table 5: Stomata density (per mm^2), length of guard cells (μ) and pollen diameter (μ) in *C. arabica* cv SL 28 and *C. canephora* var. robusta; their "arabusta" interspecific hybrid and in backcrosses to *C. arabica*. Means, s.e. of mean, ranges.

	STOMATA DENSITY			LENGTH OF GUARD CELLS			POLLEN DIAMETER		
	MEAN	RANGE	n	MEAN	RANGE	n	MEAN	RANGE	n
C. arabica	218.3±11.5	191.4-240.2	4	26.8±0.7	25.0-28.3	5	34.8±0.9	32.6-37.0	4
C. canephora (Diploid)	368.5±7.8	344.7-388.7	5	20.1±0.8	17.1-21.9	6	27.6±0.5	27.1-28.6	3
(Tetraploid) *C. canephora*	216.1±13.9	188.5-232.4	3	27.7±0.4	26.6-28.6	4	35.3±2.3	31.2-37.1	3
ARABUSTA (F_1A)	273.9±27.3	225.9-327.2	4	27.6±0.5	25.8-30.1	8	35.4±0.2	35.3-35.8	3
ARABUSTA (F_1R)	220.4±4.2	212.9-231.4	4	28.1±0.6	26.9-30.5	7	36.7±0.8	35.4-38.1	3
BC_1F_2	201.3±7.6	186.3-211.6	3	29.0±0.9	28.0-30.8	3	36.2±1.2	34.1-38.4	3
BC_2F_2	216.2±13.4	189.4-231.7	3	28.2±0.4	27.5-28.9	3	36.5±0.4	36.4-36.6	2

Notes: For stomata density: 12 leaves observed, 5 fields of view per leaf.

For guard cell lengths: 1 leaf observed, 15 stomata per leaf.

For pollen diameter: 50 pollen grains observed.

n = number of trees.

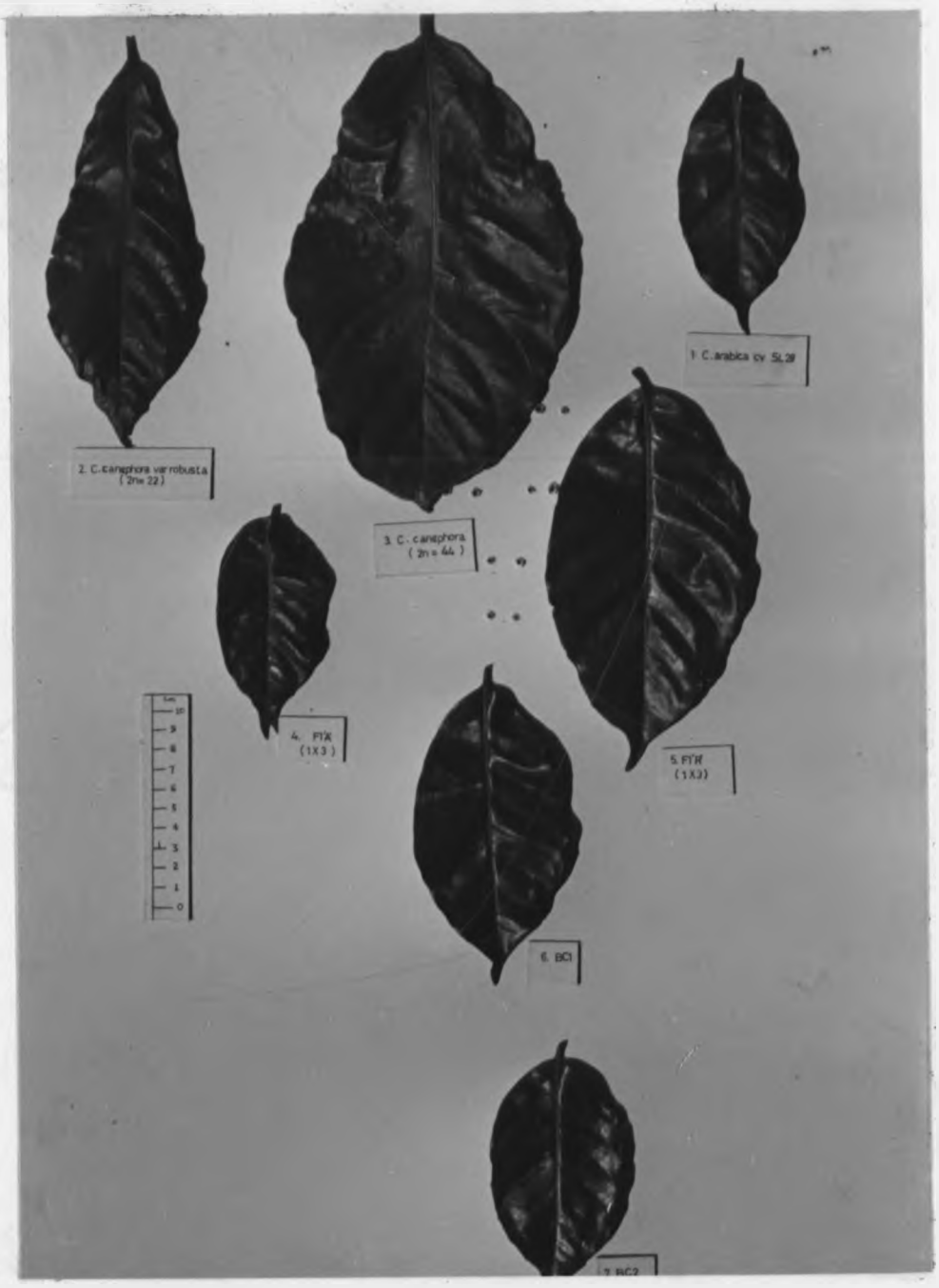

PLATE 3

Leaf forms in the two coffee species C. arabica and C. canephora (diploid and tetraploid), their arabusta hybrid and backcrosses to C. arabica.

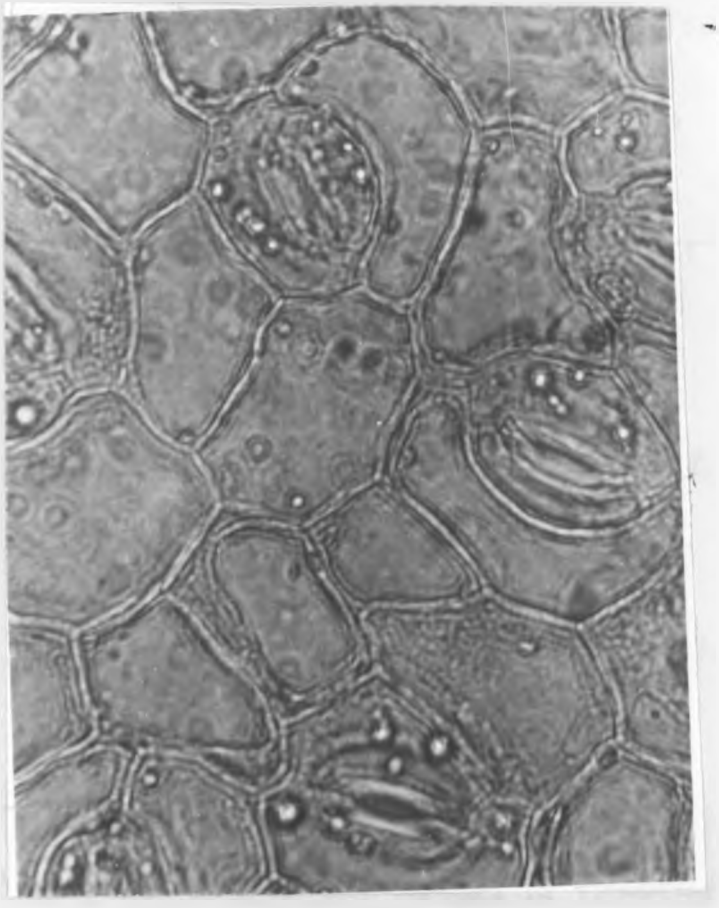

PLATE 4(a)

Stomata density on the lower leaf surface diploid C. canephora var. robusta (Magnification 400X)

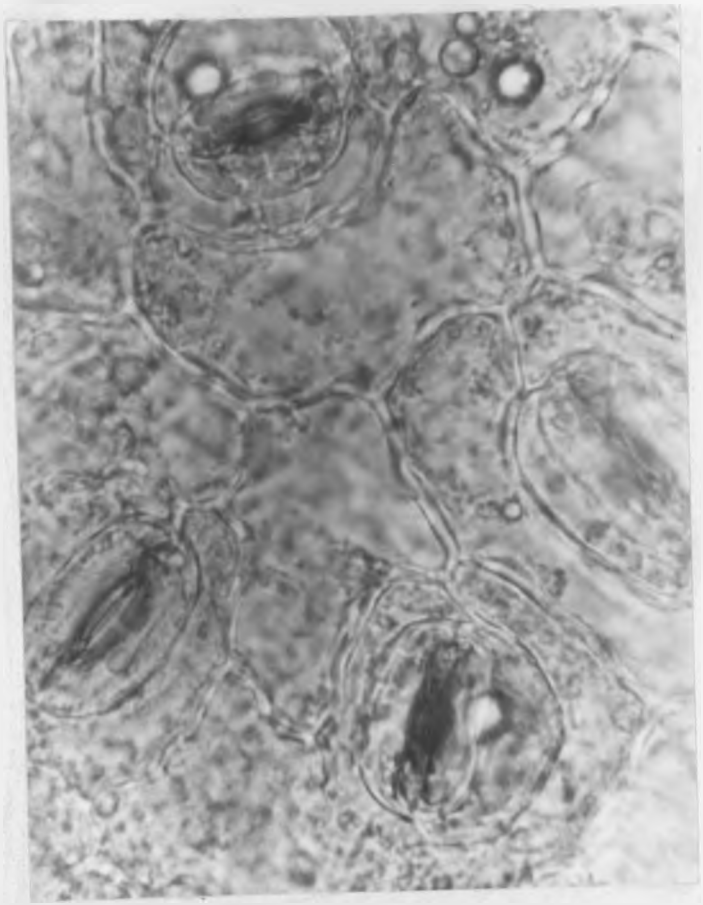

PLATE 4(b)

Stomata density on the lower leaf surface of tetraploid *C. canephora* var. robusta (Magnification 400 X)

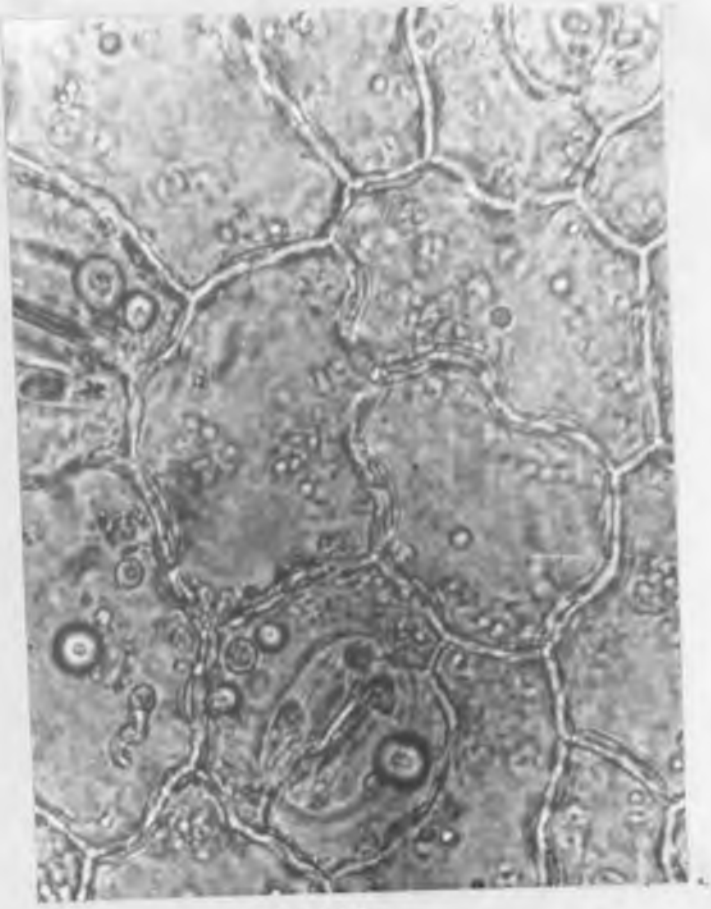

PLATE 4(c)

Stomata density on the lower leaf surface in C. arabica cv. SL 28 (Magnification 400X)

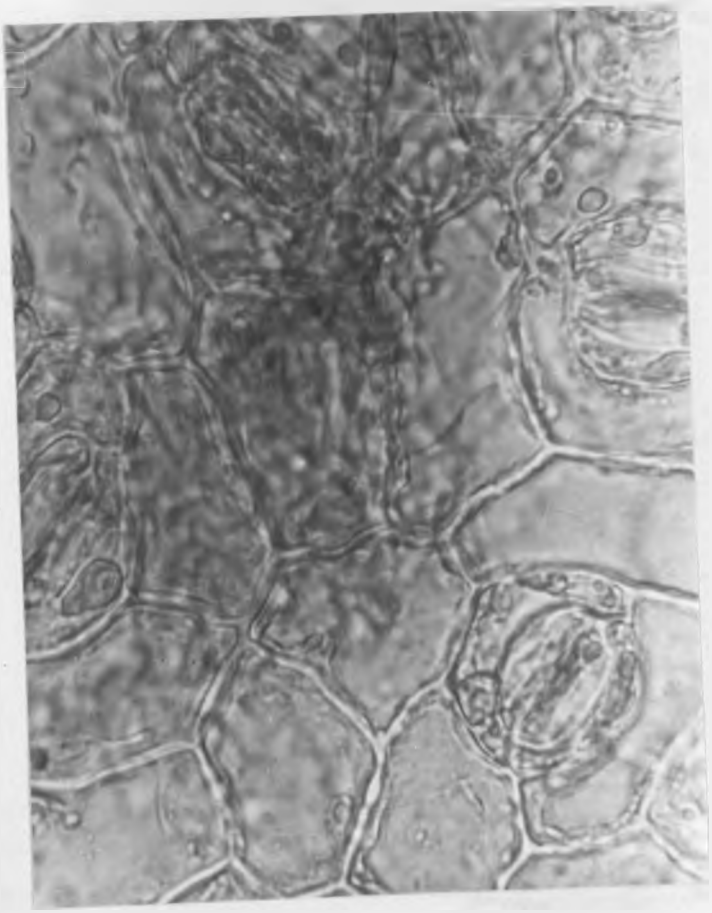

PLATE 4(d)

Stomata density on the lower leaf surface of arabusta plant type F_1A. Magnification(400X)

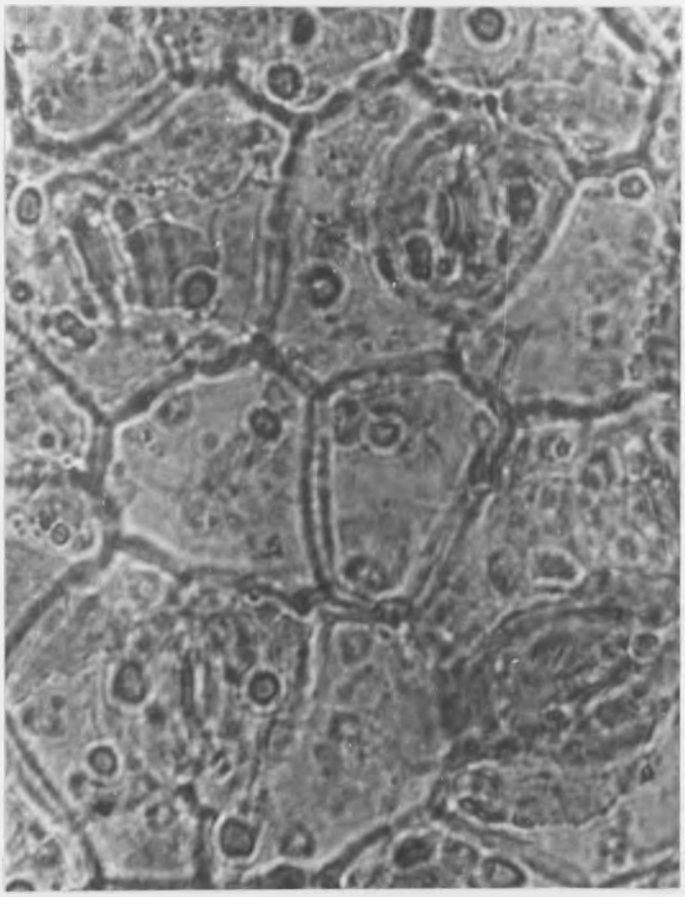

PLATE 4 (e)

Stomata density on the lower leaf surface of arabusta F_1R.
(Magnification 400X)

CHAPTER 5

GROWTH AND YIELD COMPONENTS

5.1 INTRODUCTION

Following on induction of polyploidy, alterations may not only be observed in cell size with its consequences on the morphology, but the plant's physiology may also be altered at the same time giving rise to a change in growth rate, growth habit or earliness. Although Capot et.al. (1968) noted little effect on plant height in induced tetraploids of C. canephora, very vigorous growth, probably indicating hybrid vigour was reported (Capot, 1972), in the interspecific hybrid of C. arabica and tetraploid C. canephora. This interspecific hybrid vigour apparently may not be confined to crosses between tetraploid coffee species because Louarn (1976) has also described vigorous mature plant in F_1 hybrids between diploid species C. canephora and C. eugenioides. Observations similarly made on mature plants of advanced generations of tetraploid C. canephora x C. arabica hybrids (Monaco, 1977) showed that plants of these generations were fast growing, variable for plant height and early in production.

When genotypic correlations exist between growth and yield components with yield potential, indirect selection for yield may be possible through such secondary characters especially when they also have a high heritability.

In arabica coffee, information about the relation between some growth and yield characters with yield potential is available (Walyaro & Van der Vossen, 1979) and it is now routine to include these characters for recording in any field evaluation of coffee genotypes. In C. canephora such information is not yet available and the characters chosen for recording are mostly guided by a breeder's intuition. Cramer (1952) noted that C. canephora had flowers in thick clusters on the nodes and a large number of berries per node may set so that upto 70 or 80 berries per node could be counted. Thomas (1935) based his selection of robusta coffee in Uganda on the criteria of vigour which he associated with formation of secondary branches on primaries. Vishweshwara (1978) has studied flower number per inflorescence in two coffee species C. arabica and C. canephora as well as in advanced generations of an interspecific cross between tetraploid C. canephora and C. arabica. He observed that C. canephora had a higher mean and variability for this character than C. arabica but in backcrosses of the hybrid to C. arabica the plants strongly resembled C. arabica for flowers per node.

5.2 METHODS

Growth and yield components were recorded in only four generations, one parent species C. arabica cv SL 28, the arabusta hybrids and in backcross generations. Plants of these generations had been established in one field at the same time and so could be compared for these characters.

The measurements had been made on coffee trees 2 years after planting in the field. In the F_1 generation comparisons were made between F_1A and F_1R for these characters. The growth and yield components observed were those described below:

1) Plant height was measured from ground level to the tip of the tree using wooden stakes calibrated in centimetres. Plant height measurements were made twice, once in October 1977, (H1) and at the end of the long rains season in June 1978, (H2). The difference (H2 - H1) gave height increment in the 9 months period.

2) Girth on the main stem was measured at 10 cm from the ground level with the aid of a pliable wire and a centimetre scale. Two measurements (G1) in October 1977 (beginning of the short rains) and at the end of the rainy season in June 1978 (G2) were made. Girth increment (G2-G1) was calculated.

3) Mean internode length on the main stem was calculated from plant height and total number of nodes on the main stem.

4) Number of primaries and proportion of bearing primaries. Primaries on each tree were counted

and on these, primaries that were bearing flowers and/or berries also noted. Bearing primaries were expressed as a percentage of the total number of primaries per tree.

5) Length of primaries were measured in centimetres on 8 primaries taken at mid-crown of the tree.

6) Mean Internode length of primaries was calculated from length of primary and total number of nodes on that primary.

7) Number of nodes and % bearing nodes on primaries. Nodes on each of the 8 primaries at mid-crown were counted and of these, nodes that were bearing flowers and/or berries noted. Bearing nodes were expressed as a percentage of the total number of nodes.

8) Number of lateral branches on primaries (= secondaries). Laterals on the 8 primaries of each tree were counted.

9) Extension growth and node production on primaries were observed on the 8 primaries per tree. At beginning of each season a string was tied below the tip node of each primary (November 1977 and in March 1978 respectively). Extension was measured as the length from the position of the string to the tip of the primary for each season i.e. in

January and June respectively. For overall extension growth (November-June) the length from position of string in November to tip of primary in June was measured. Node production for each period was the number of nodes counted on the extended portion.

10) Number of flowers per node and berries per node. On four primaries per tree several nodes were marked with a loop of string at the time of a main flowering for all plants of the different generations. The flowers within the loop were counted as well as the nodes in which they were borne. Flowers per node was calculated from total number of flowers and total number of nodes. Berries per node was similarly determined after 10 weeks.

11) First year cherry yield. Ripe berries (= cherries) were picked and the yield recorded in grammes at each picking on individual tree basis. Cherry yield was expressed as kg/tree per year.

5.22 Analysis of the data

The data is summarised in means and standard error of the mean for each progeny and for each generation. Variances were calculated with observations from individual trees as entries for unreplicated measurements. For replicated

measurements, on individual trees, means per tree were used as entries. The standard formula for variance calculations:

$$S_i^2 = \frac{\sum_{j=1}^{n} X_{ij}^2 - \left(\sum X_{ij}\right)^2 / n}{n - 1}$$

was used and from this the standard error (S.E.) of the sample mean:

$$\sqrt{\frac{S^2}{n}}$$

was calculated.

Individual trees of each generation had been planted unreplicated, each progeny in a single row; the rows being in the direction of a slight gradient in the field. In making comparisons of the means of generations using their standard errors, it was assumed that any inherent differences in the field (fertility, moisture) across the gradient were not as important as the genotypic differences among the generations. Some evidence of the validity of this assumption was obtained from a row (across gradient) and column (along gradient) analysis of variance performed on the height and girth data as suggested by Dr. A.C. van Eijnsbergen (personal communication). The data used in this analysis were deviations of each individual tree measurement from the mean of the genotype occupying a column and took the form:-

$$X_i' = (X_i - \bar{X}_f)$$

Where X_i = deviation from the mean

X_i = measurement on the ith individual.

and $\bar{X}_f$ = mean for population f.

Similar calculations made on the true breeding C. arabica cv SL 28 also gave some more information on the uniformity of the field.

5.3 RESULTS

Concerning soil trends in field B6, the analysis of variance did not reveal statistically significant column effects ($P \simeq 0.05$) and only at ($P \simeq 0.25$) was the row effect significant. However, partitioning of the row sums of squares through an orthogonal contrast into a linear and a residual part showed that the linear component was not statistically significant ($P \lesssim 0.05$) (Tables 6A and 6B). The same results were also obtained with the height data from the true breeding C. arabica cv SL 28 in this field (Table 6C). Thus any inherent soil trends in this field were probably not varying linearly across the field. It was concluded that the assumption that genotypic differences between the generations had more important influence on plant growth than the differences in the soil trends due to the gradient was reasonable. Comparison of the means with their standard errors was therefore considered justified.

The data on general growth is given in Table 7. Both F_1R and F_1A were taller trees than those of C. arabica cv SL 28 and trees of backcross generations, with which they had been

planted at the same time. F_1R had the widest girth on the main stem, but F_1A had a narrower girth than that of C. arabica. Increases in height or girth on the main stem gave results similar to those of absolute measurements on these characters. The longest primaries were measured in F_1R but in F_1A plants the primaries were almost as long as in those of C. arabica cv SL 28. F_1R had longer internodes than F_1A, backcrosses and C. arabica cv SL 28. The second backcross generation had the shortest internodes while the first backcross had internodes almost as long as those of C. arabica.

Table 8 is a summary of the data on general growth components for the F_1 progenies. Plant height and increase in plant height showed considerable variation among progenies but it was clear that progeny of tetraploid clone UT6 (progenies 18, 19, 20) were taller plants than those of clones UT3, UT8 and UT10. A similar, but less obvious variation was evident with girth, length of primaries, internode length of the main stem and on the primaries.

Extension growth measurements made towards the end of the short rainy season (January) and also at the end of the long rains (June) indicated in each case that F_1R had more extension growth than the local C. arabica cv SL 28 and backcrosses (Table 9), while F_1A was shown to be rather like the backcrosses in this respect. In the two seasons, and in the eight months which the extension growth measurements covered, the highest extension growth was consistently found

in F_1R. There were however hardly any differences in node production in the short rainy season, but during the long rains, C. arabica cv SL 28 and backcrosses tended to have more nodes than F_1's.

In Table 10, the data for extension growth for the F_1 progenies is given. The variation among families for extension growth was mainly between the two plant types. Almost in each family with both plant types, F_1R plants were found to have more extension growth than F_1A plants.

The data on yield components are given in Tables 11 and 12. The backcrosses and C. arabica cv SL 28 had relatively more primaries, nodes per primary and lateral branches per primary. F_1A was for these characters rather closer to C. arabica than to F_1R. There was little variation for % bearing primaries among the generations while % bearing nodes on primaries, flowers per node, berries per node and first year cherry yield showed a large variation among generations. F_1R had the highest % bearing nodes on the primaries and flowers per node while the local C. arabica cv. SL 28 had the highest number of berries per node and first year cherry yield. First year cherry yield followed roughly the trend of berries per node.

The data of yield components for the F_1 progenies is given in Tables 13 and 14. The variation for almost all components was mostly according to plant types F_1A and F_1R

especially for laterals per primary in which F_1A types tended to produce more laterals per primary than F_1R types.

5.4 DISCUSSION

Observations on general growth and extension growth on the primaries indicated that the arabusta F_1R grew more vigorously than F_1A, the backcrosses and the locally adapted cultivar SL 28. These arabusta hybrids were taller plants, with a wide girth on the main stem. They had longer primaries and gave relatively more extension growth. This vigorous growth habit of the arabusta has also been reported by Capot (1972), though the same author Capot (1968) has observed no difference in vigour between induced tetraploids of C. canephora and the diploid plants from which they were derived. In the present study growth characters could not be studied on tetraploid C. canephora clones because the plants of this generation available differed in age from plants from other generations. But it would seem that the vigorous growth habit of the F_1R arabusta may be due to their hybrid condition rather than to a resemblance to either parent species.

Arabusta hybrids of type F_1R have long internodes on the main stem and consequently they tended to produce only a few nodes on the primaries as these extended in growth. F_1R also produced significantly fewer laterals than F_1A. Long primaries with long internodes may imply that such coffee trees should be planted at wider spacings than conventional,

but this agronomic requirement imposed by the arabusta growth habit may not be a serious drawback when consideration is to be given to commercial planting of selected arabusta. In this connection, the higher drought tolerance of arabusta in comparison to diploid robusta coffee (Capot, 1972) is one of the strong points in their favour because in this country, some of the areas which were scheduled for robusta coffee growing sometimes experience extensive periods of drought. Obviously in such areas, coffee trees would have to be planted at wide spacings.

The backcrosses had the shortest internodes on the main stem and on the primaries but at the same time they were less vigorous plants. It seems that in backcross generations to C. arabica, there is generally a loss in vigour which may however be expected if the vigour is mostly due to heterosis. Monaco (1977) has observed that it is possible to retain some of the arabusta vigour in backcrosses by practising selection in early generations of backcrossing. In the present study a considerable variation has been observed for vigour in the arabusta which was mainly according to plant type i.e. whether F_1A or F_1R but even among arabusta families, there were large differences for growth. This may indicate the possibility of starting selection for vigour already in the arabusta generation.

The data on yield components did not show the situation in which F_1R arabusta were for all components superior to other

generations unlike growth components. However F_1R had significantly more flowers per node berries per node and first year cherry yield than F_1A. Vishweshwara (1978) has compared flowers per node in parent species, (arabica x robusta) hybrids, and backcrosses of the hybrid to C. arabica. He noted that C. canephora has more flowers per node than C. arabica but that backcrosses of the hybrid to C. arabica, tended to be similar to C. arabica. The arabusta studied presently which showed high flowers per node count may resemble their C. canephora parent for this character. For number of primaries, % bearing primaries and nodes per primary, trends already noted with growth components could be observed with the backcrosses being similar to C. arabica.

Table 6A Analysis of variance on the residuals of the height data for testing uniformity of Field B6 where arabusta, the backcrosses to C. arabica and C. arabica cv. SL28 were planted.

Source	d.f.	ss	ms	F	P 0.05	P 0.25
Rows	27	2584.06	95.71	1.37	n.s.	*
Columns	19	641.67	33.77	0.48	n.s.	n.s.
RowsxCols.	513	35845.18	69.87			
Total	559	39070.91				
Linear	1	1.83	1.83	0.03	n.s.	n.s.
Residual	26	2582.23	99.32	1.42	n.s.	*
Total	27	2584.06				

* Significant at P = 0.25

n.s. Not significant

Table 6B Analysis of variance on the residuals of girth data for testing uniformity of field $B6_a$ where the arabusta, the backcrosses to C. arabica as well as C. arabica cv SL28 were planted.

Source	d.f.	s.s.	m.s.	F	P 0.05	P 0.25
Rows	27	124.78	4.62	1.09	n.s.	*
Columns	19	4.76	0.25	0.06	n.s.	n.s.
RowsxCols.	513	2172.54	4.23			
Total	559	2302.08				
Linear	1	1.72	1.72	0.40	n.s.	n.s.
Residual	26	123.06	4.73	1.12	n.s.	n.s.
Total	27	124.78				

* Significant at P = 0.25

n.s. Not significant

Table 6C Analysis of variance on the height data of true breeding C. arabica cv. SL28 planted in Field B6a where the arabusta hybrids and backcrosses were also planted.

Source	d.f.	s.s.	m.s.	F	P 0.05	P 0.25
Blocks	7	289.0	41.3	0.57	n.s.	n.s.
Position of tree in column	10	969.0	96.9	1.33	n.s.	n.s.
(B1.xReps.)	70	5093.4	72.76			
Total	87	6351.4				
Linear	1	11.89	11.89	0.16	n.s.	n.s.
Residual	6	277.19	46.20	0.63	n.s.	n.s.
Total	7	289.0				

* Significant at P = 0.25

n.s. Not significant

Table 7: Growth components in 2 year old coffee trees of C. arabica x C. canephora (tetraploid) interspecific hybrids and in backcrosses of the hybrid to C arabica as well as in the local cultivar SL 28 of C. arabica. Measurements in cm. Means, s.e. of the mean.

	NO.OF TREES	HEIGHT (H_2)	(H_2-H_1)	GIRTH (G_2)	(G_2-G_1)	INT.L.ON M.ST.	LENGTH OF PRIMARIES	INT.L.ON PRI.
C. arabica	157	162.6±0.9	43.5±0.6	15.7±0.1	4.3±0.1	3.3±0.02	53.9±0.6	3.5±0.02
Arabusta F_1A	22	175.1±3.2	46.5±2.1	15.0±0.9	3.5±0.2	3.8±0.1	58.3±1.8	4.3±0.1
Arabusta F_1R	91	196.4±1.8	48.7±0.8	17.7±0.2	4.6±0.1	4.7±0.04	73.3±1.3	5.1±0.2
BC_1F_1	224	156.2±1.3	35.9±0.6	13.5±0.2	3.6±0.1	3.3±0.05	60.0±0.9	3.6±0.05
BC_2F_2	307	166.6±1.0	39.5±0.5	14.0±0.1	3.7±0.1	3.0±0.1	57.6±0.6	3.2±0.1

Notes: H_1H_2 - Plant height before and after 9 months respectively

G_1G_2 - Girth on the main stem before and after 9 months respectively

Int.L.M.ST. - Internode length on the Main Stem

Int.L.Pri. - Internode length on the primaries.

Table 8: Growth components in arabusta families measured in trees of age 2 years since planting in the field. Measurements are in cm. s.e. of the mean.

FAMILY NO AND PLANT TYPE		NO. OF TREES	HEIGHT (H_2)	(H_2-H_1)	GIRTH	(G_2-G_1)	INT. L. M.ST.	LENGTH OF PRI.	INT. L. PRI.
18	A	5	187.0±5.5	44.2±3.8	13.9±0.2	3.7±0.2	3.2±0.08	50.8±2.2	3.3±1.0
50	A	5	183.0±0.5	50.2±2.7	13.3±0.6	3.7±0.4	3.7±0.2	54.9±0.7	4.0±0.2
53	A	12	166.9±5.2	45.8±3.0	16.1±0.9	3.4±0.4	4.2±0.2	62.9±2.6	4.4±0.2
18	R	4	203.7±8.6	44.2±1.3	18.7±0.7	4.6±0.2	5.0±0.1	83.1±4.0	5.3±0.2
19	R	14	197.2±2.7	52.1±1.5	18.4±0.2	5.6±0.3	4.9±0.05	76.1±2.2	5.2±0.2
20	R	17	192.0±3.0	51.6±1.8	18.4±0.5	5.2±0.4	5.0±0.05	80.7±1.6	5.3±0.1
49	R	14	172.6±6.6	45.4±2.2	16.4±0.7	4.2±0.3	4.5±0.2	66.7±3.8	4.9±0.2
50	R	10	159.8±8.3	40.2±4.1	15.2±0.9	2.8±0.5	4.5±0.1	66.3±4.5	4.8±0.2
51	R	14	176.4±3.2	46.2±2.0	19.1±0.4	4.9±0.4	4.7±0.06	73.6±1.8	5.0±0.1
52	R	5	178.0±2.5	51.2±2.2	17.6±0.9	3.5±0.1	4.7±1.1	72.1±3.9	5.0±0.2
53	R	13	180 ± 2.8	51.1±2.0	17.4±0.4	4.3±0.2	4.6±0.1	70.7±1.7	4.9±0.1

See footnotes Tables 1 and 7.

Table 9: Extension growth and node production on primaries of arabusta hybrids, backcrosses and in the local C. arabica cultivar SL 28. Measurements in cm; means, s.e. of the mean

	NO. OF TREES	SEASON - 1 EXT.	SEASON - 1 NODES	SEASON - 2 EXT.	SEASON - 2 NODES	FULL SEASON EXT.	FULL SEASON NODES
C. arabica	157	4.9 ± 0.1	2.0 ± 0.04	7.0 ± 0.1	2.7 ± 0.05	18.0 ± 0.3	7.3 ± 0.1
Arabusta F_1A	22	4.7 ± 0.9	2.0 ± 0.1	8.1 ± 0.3	2.5 ± 0.1	20.9 ± 1.1	6.7 ± 0.3
Arabusta F_1R	91	7.8 ± 0.6	2.2 ± 0.1	11.3 ± 0.6	2.8 ± 0.5	27.8 ± 0.5	7.0 ± 0.1
BC_1F_2	224	4.5 ± 0.3	2.0 ± 0.1	7.9 ± 0.4	3.1 ± 0.1	17.1 ± 0.4	7.3 ± 0.1
BC_2F_2	307	4.5 ± 0.2	2.0 ± 0.1	6.1 ± 0.3	2.6 ± 0.1	16.2 ± 0.2	7.5 ± 0.1

Notes: Season 1 - November 1977 - January 1978 (Short rains)

Season 2 - March 1978 - June 1978 (Long rains)

Full Season - November 1977 - June 1978

Table 10: Extension growth and node production on primaries of arabusta families . Measurements in cm. Means, s.e. of the mean.

FAMILY NO. AND PLANT TYPE		NO. OF TREES	SHORT RAINS SEASON (SEASON 1)		LONG RAINS SEASON (SEASON 2)		F U L L SEASON	
-		-	Ext.	No. of Nodes	Ext.	No. of Nodes	Ext.	No. of Nodes
18	A	5	4.2 ± 0.1	1.9 ± 0.07	6.3 ± 0.8	2.4 ± 0.2	16.7 ± 1.1	6.7 ± 0.2
50	A	5	4.6 ± 0.8	1.5 ± 0.1	6.8 ± 0.9	2.3 ± 0.2	19.5 ± 1.6	6.4 ± 0.2
53	A	12	5.0 ± 0.8	1.8 ± 0.2	9.4 ± 1.1	2.6 ± 0.2	23.3 ± 1.8	6.9 ± 0.4
18	R	4	8.1 ± 1.6	2.1 ± 0.3	13.3 ± 1.7	6.5 ± 1.6	32.4 ± 1.7	7.3 ± 0.4
19	R	14	9.4 ± 0.6	2.3 ± 0.1	11.5 ± 0.7	2.5 ± 0.2	29.9 ± 1.2	6.9 ± 0.2
20	R	17	9.6 ± 0.6	2.3 ± 0.1	12.1 ± 0.5	2.7 ± 0.1	30.9 ± 0.9	7.3 ± 0.2
49	R	14	6.7 ± 0.8	1.9 ± 0.2	7.8 ± 0.8	2.0 ± 0.1	22.8 ± 1.1	6.3 ± 0.1
50	R	10	5.0 ± 0.9	1.9 ± 0.2	11.3 ± 1.0	2.5 ± 0.2	24.6 ± 1.8	6.7 ± 0.3
51	R	14	7.7 ± 1.1	2.3 ± 0.2	10.8 ± 0.8	2.7 ± 0.2	27.1 ± 1.2	7.2 ± 0.2
52	R	5	9.4 ± 0.8	2.8 ± 0.1	9.7 ± 1.4	2.3 ± 0.2	28.5 ± 0.8	7.4 ± 0.2
53	R	13	6.4 ± 1.0	2.0 ± 0.2	13.9 ± 1.2	3.1 ± 0.1	28.2 ± 1.8	7.2 ± 0.2

Table 11 Yield components in arabusta hybrids, *C. arabica* cv SL28 and in backcrosses. First year cherry yield expressed as kg/tree/year. Mean, S.E. of the mean.

	NO.OF TREES	NO. OF PRIMARIES	NO. OF LATS. PER PRIM.	% BEARING PRIM.	NO. OF NODES/PRIM.	% B.NODES ON PRIM.	FIRST YEAR CHERRY YIELD
C. arabica	157	54.3 ± 0.4	12.7 ± 0.2	81.5 ± 0.4	22.6 ± 0.1	63.5 ± 0.7	1.63 ± 0.07
Arabusta F_1A	22	50.7 ± 1.4	10.8 ± 0.8	81.8 ± 0.9	20.8 ± 0.4	60.9 ± 4.9	0.67 ± 0.13
Arabusta F_1R	91	48.1 ± 0.6	5.5 ± 0.2	80.6 ± 0.7	21.9 ± 0.2	68.9 ± 1.2	1.08 ± 0.05
BC_1 F_2	224	55.9 ± 0.5	12.1 ± 0.3	81.4 ± 0.6	23.9 ± 0.1	53.1 ± 1.3	0.73 ± 0.03
BC_2 F_2	307	63.9 ± 0.4	13.3 ± 0.2	82.6 ± 0.4	23.2 ± 0.2	52.7 ± 1.0	0.95 ± 0.04

Notes: Lats/prim. = No of laterals per primary

B nodes on prim. = Bearing nodes on primaries

Table 12 Flowers per node and berries per node in arabusta its parent species and backcrosses. Means and s.e. of the mean

	NO.OF TREES	NO. OF FLOWERS PER NODE	NO. OF BERRIES PER NODE
C. arabica	11	14.1 ± 0.6	9.1 ± 0.5
Arabusta F_1A	14	18.9 ± 2.5	2.0 ± 0.4
Arabusta F_1R	25	23.0 ± 0.5	4.5 ± 0.4
BC_1F_2	16	13.0 ± 0.8	2.8 ± 0.5
BC_2F_2	26	12.4 ± 0.8	2.1 ± 0.1

Table 13: Yield components in arabusta families and first year cherry yield expressed as kg/tree/year Means, s.e. of means

FAMILY NO. AND PLANT TYPE	NO.OF TREES	NO.OF PRIMARIES	NO.OF LATS.	% B. PRIM.	NO.OF NODES	% B.NODES ON PRIM.	FIRST YEAR YIELD
18 A	5	59.4 ± 2.6	9.2 ± 1.5	88.3 ± 2.5	22.3 ± 0.4	70.5 ± 17.5	0.12 ± 0.09
50 A	5	52.2 ± 2.1	4.4 ± 2.0	76.0 ± 2.4	20.3 ± 1.4	60.5 ± 11.2	0.13 ± 0.06
53 A	12	46.5 ± 2.1	10.4 ± 1.1	81.5 ± 3.3	20.3 ± 0.5	56.8 ± 2.3	1.12 ± 0.27
18 R	4	53.5 ± 1.7	6.4 ± 1.4	78.7 ± 3.6	22.6 ± 0.4	72.7 ± 5.7	1.25 ± 0.37
19 R	14	50.1 ± 1.1	5.8 ± 0.5	80.4 ± 2.8	21.3 ± 0.3	78.8 ± 1.6	0.91 ± 0.12
20 R	17	52.1 ± 1.1	6.1 ± 0.3	78.7 ± 0.4	22.2 ± 0.2	64.4 ± 2.6	0.69 ± 0.09
49 R	14	43.9 ± 2.8	5.3 ± 0.8	84.6 ± 1.9	19.9 ± 0.4	77.4 ± 2.2	0.98 ± 0.16
50 R	10	41.6 ± 1.7	4.8 ± 0.8	75.9 ± 3.5	20.4 ± 0.6	64.6 ± 3.7	1.32 ± 0.26
51 R	14	48.1 ± 0.9	6.3 ± 0.4	82.3 ± 1.9	21.4 ± 0.3	61.0 ± 4.1	1.26 ± 0.34
52 R	5	47.6 ± 0.7	5.7 ± 0.9	78.2 ± 1.5	21.8 ± 0.8	75.2 ± 6.9	1.50 ± 0.23
53 R	13	48.4 ± 1.3	8.6 ± 0.6	82.2 ± 1.4	21.4 ± 0.3	63.4 ± 2.4	0.72 ± 0.1

Notes: Also see footnotes Table 11.

Table 14 Flowers per node and berries per node in arabusta families. Means, S.E. of Mean

FAMILY NO.		NO. OF TREES	FLOWERS/NODE	BERRIES/NODE
18	A	5	24.6 ± 6.2	1.7 ± 0.4
50	A	4	14.2 ± 3.5	2.1 ± 1.1
53	A	5	19.3 ± 4.3	2.2 ± 0.4
18	R	4	15.6 ± 5.8	1.5 ± 0.9
19	R	10	24.2 ± 1.3	3.8 ± 0.5
20	R	4	21.0 ± 5.3	3.2 ± 0.6
50	R	4	34.5 ± 2.3	9.9 ± 1.8
51	R	3	26.6 ± 3.1	6.9 ± 2.6
53	R	-	-	-

CHAPTER 6

FLOWER DIMENSIONS, STAR FLOWERS AND SELF INCOMPATIBILITY

6.1 INTRODUCTION

The morphology of the flower in arabica and robusta coffees has been described in detail by Haarer (1962). The flowers of C. arabica are fragrant, and white to creamy in colour. The corolla is five lobed, the anthers are shorter than the corolla lobes and are wholly exserted. The style about equals the unopened flower in size, is bifid with linear lobes narrowing towards the tip. The flowers of C. canephora are white in colour, sometimes diffused with pink, are in axillary clusters, sessile with or without leafy bracts. The corolla is five lobed, the corolla tube much or a little shorter than the lobes. The stamen and the style are well exserted. Louarn (1976) described the morphology of the flower in an interspecific hybrid plant of C. canephora and C. eugenioides. He took several measurements on the various parts of the flower but found hardly any difference between the hybrid and either parent species in any of these characters. In a triploid hybrid plant resulting from an interspecific hybridization of C. arabica and C. canephora, flower size and shape were intermediate to those in parent species (Krug and Mendes, 1940).

Abnormalities in flower bud development can lead to incompletely developed, mostly sterile flowers, that open prematurely, called star flowers (Sybenga, 1960). Plate 5b depicts star and normal flowers. Porteres cited by Sybenga (1960) observed that this phenomenon occurs frequently in insufficiently adapted cultivars of C. arabica. Huxley and Ismail (1969) also noted a high star flower frequency in water stressed C. arabica plants.

The genetic background of the self-incompatibility system which is widespread among diploid coffee species is not fully understood but observational data have been interpreted as gametophytic incompatibility controlled by an oppositional- S- allele system (Devreux, et al (1959) Experience gained in Java with C. canephora showed that monoclonal planting of this species should be avoided otherwise self incompatibility will exclude any fruit set (Ferwerda, 1969). Berthaud (1978) has noted that arabusta and tetraploid C. canephora have very low or no self compatibility i.e. very little and no fruit set respectively, is obtained on selfing.

6.2 METHODS

Observations on flower parts, star flowers and self compatibility were made on the two parent species, C. arabica and C. canephora, their hybrid as well as on the backcross generations to C. arabica.

Measurements were made on several parts of the

flower which included corolla length, length and width of petals, length of styles, stigmata and anthers. Flowers were sampled on the day of anthesis for recording. The parts were removed from the flower and placed against a millimetre scale for measurement; 25 flowers were sampled from each tree. The optimum sample was determined on diploid C. canephora according to formular (1) on page 22, using an initial sample of 20 flowers and at P = 0.05 of not more than 1 mm. Several trees of each generation were observed.

During two periods of flowering, when occurrence of star flowers was generally high even on the local C. arabica cv SL 28 (in November 1977 and February 1978), star flowers were recorded. The number of star flowers was counted from a random sample of 150 flowers per tree. Several trees of each of the generations studied were screened in this way for star flowers.

Self compatibility was estimated from fruit set. About 150 flowers or more were counted at the "candle stage" which is two or three days from the day of anthesis. The flowers were bagged after removal of younger or already open flowers. On the same trees, a similar number of flowers were counted but left unbagged for natural open pollination. For the F_1 generation, another treatment of artificial controlled cross pollination between a number of trees was performed. Flowers were emasculated and bagged at the candle stage. On the day of anthesis artificial cross pollination was performed

according to methods described by Walyaro and van der Vossen (1976). Fruit set was counted after 10 weeks.

6.3 R E S U L T S

A summary of the data on flower parts is given in Table 15. Plate 5a shows representative flowers from the two species, their interspecific hybrid and backcrosses to C. arabica. The species C. canephora had larger flowers than C. arabica as indicated by the length of corolla and dimensions of the petals. Flowers of the F_1R plant type were similar in size to tetraploid C. canephora flowers, whereas those of plant type F_1A were rather like flowers of C. arabica for this character. For the reproductive parts of the flower (styles, stigmata and anthers), diploid C. canephora had relatively longer styles and stigmata but shorter anthers than C. arabica. In induced tetraploid C. canephora however, there was observed shorter styles and stigmata than in diploids while anthers were longer.

In F_1R flowers, the styles were about as long as in tetraploid C. canephora which was the parent with the longer styles. The stigmata of both F_1R and F_1A were longer than those of either parent species. It was also noted that tetraploid C. canephora resembled C. arabica more closely for length of stigmata and anthers than diploid C. canephora.

The frequency of star flowers was recorded and results expressed in percentages are given in Table 16. Induced tetraploid C. canephora and F_1 hybrids had low frequencies of star flowers. Values were close to that observed in the locally adapted C. arabica cultivar SL 28. On the other hand, the backcrosses showed a considerably much higher % star flowering.

Self compatibility was measured as fruit set under isolation by bagging and the results obtained are given in Table 17. Natural open pollination was included in each case as a control. The highest % fruit set on selfing was observed in C. arabica cultivar SL 28, though in fact, higher % fruit set values would normally be obtained on selfing C. arabica, than those obtained in this experiment. The rather wet conditions during anthesis should be partly responsible for the low fruit set. The data of self fertility indicated that tetraploid C. canephora was completely self-incompatible as virtually no fruits were set on selfing. The F_1's were close to their tetraploid C. canephora parent in this respect. Comparing first and second backcrosses, it was clear that there was an increase in self compatibility from first backcross to second backcross generation. From artificially controlled pollination between different trees of the F_1 generation, a relatively higher fruit set (6.0%) compared to about 2.0% on selfing was obtained. In all generations, there was more fruit set from open pollinations than from selfing.

6.4 DISCUSSION

Observations on flower size indicated that both diploid and tetraploid C. canephora had larger flowers than C. arabica. Doubling of the chromosome number in C. canephora seemed to have no consequence on corrola length but styles and stigmata were shorter while anthers of induced tetraploids were longer than those of diploids. Comparing C. canephora (diploid) with induced tetraploid C. canephora on the one hand, and C. arabica (natural tetraploid) with tetraploid C. canephora on the other for these characters, it was obvious that tetraploid C. canephora tended more towards C. arabica than to diploids for length of stigmata and anthers. Neither tetraploid C. canephora nor the arabusta hybrids had any abnormality in the morphology of their flowers.

The arabusta hybrids produced normal flowers with very few star flowers. Capot (1972) also reported a low star flower frequency in arabusta hybrids in the Ivory Coast. A high frequency of star flowers has been observed in interspecific hybrids of coffee (Sybenga 1960). Porteres cited by the same author also noted a high star flower frequency in relatively unadapted coffee cultivars while Huxley and Ismail (1969) have observed the same condition in water stressed coffee plants. The high frequency of star flowers observed in backcrosses in the present study could therefore well be due to their insufficient adaptation locally, although their cytogenetic condition may partly be responsible. Since the arabusta

hybrid had on the other hand only a relatively low star flower frequency, it would appear that the backcrosses which were introduced materials from Brazil suffered a high star flower frequency on account of their being insufficiently adapted to local conditions. One cause may possibly be increased water stress due to a more superficial root system. The backcrosses (ex-Brazil) were noted to start wilting earlier than cultivar SL 28 and other locally selected materials planted in the same field.

Tetraploid relatives or induced tetraploids from diploid dicotyledons with gametophytic monofactorial self incompatibility usually display a self-compatible phenotype (de Nettancourt, 1977). In the genus Coffea the widespread self-incompatibility in diploid species has tentatively been interpreted as gametophytic (Devreux et al (1959). However the self-incompatibility of diploids of C. canephora is apparently not affected by artificial doubling of chromosome numbers in this species, as it was observed in the present study, that there was no fruit set on selfing tetraploid C. canephora. It was also noted that arabusta had a higher fruit set from controlled cross pollination than from selfing although for arabusta, the high pollen sterility was the major cause of the low fruit set on selfing. These observations very much agree with the findings of Berthaud (1978), who noted that arabusta were closer to their tetraploid C. canephora parent than to C. arabica for self-compatibility. These facts also

seem to indicate that the genetic system of incompatibility in C. canephora may probably be more complex than merely of a monofactorial gametophytic incompatibility. A stimulative speculation is therefore put forward that other systems of genetic control, such as the bifactorial gametophytic, sporophytic or even the dual gametophytic/sporophytic system operating in Theobroma cacao (Cope, 1962) may be the basis of self incompatibility in C. canephora.

Table 15: Flower size in the two species C. arabica and C. canephora and in their F_1 hybrid as well as in backcrosses to C. arabica. Means, s.e. of the mean. Measurements are in mm

	C. arabica	DIPLOID C.canephora	TETRAPLOID C.canephora	ARABUSTA (F_1A)	ARABUSTA (F_1R)	B C_1F_2	B C_2F_2
LENGTH OF COROLLA	8.3 ± 0.3	9.7 ± 0.7	11.9 ± 1,0	7.1 ± 0.7	8.4 ± 0.4	7.9 ± 0.3	6.7 ± 0.3
LENGTH OF PETALS	12.8 ± 0.5	14.6 ± 0.4	14.3 ± 1.1	11.7 ± 0.9	14.5 ± 0.5	13.7 ± 0.5	11.0 ± 0.3
WIDTH OF PETALS	4.9 ± 0.1	5.0 ± 0.7	6.0 ± 0.4	5.2 ± 0.2	6.0 ± 0.2	5.2 ± 0.1	4.3 ± 0.1
LENGTH OF STYLES	13.5 ± 0.3	15.9 ± 0.7	14.7 ± 1.9	13.2 ± 1.4	16.0 ± 0.8	13.3 ± 0.6	11.4 ± 0.5
LENGTH OF STIGMATA	6.2 ± 0.2	10.9 ± 0.4	6.0 ± 0.4	7.3 ± 1.0	6.4 ± 0.2	6.4 ± 0.2	5.3 ± 0.1
LENGTH OF ANTHERS	8.3 ± 0.1	6.8 ± 0.7	7.4 ± 0.6	7.6 ± 0.3	9.7 ± 0.3	9.6 ± 0.3	8.4 ± 0.4
NO. OF TREES OBSERVED	9	4	4	7	10	17	20

Note: 25 flowers observed for each character per tree.

Table 16: Frequency of star flower in C. arabica cv SL 28 and tetraploid C. canephora var. robusta; their arabusta hybrid and backcrosses

	C. arabica	TETRAPLOID C. canephora	ARABUSTA (F_1 A)	ARABUSTA (F_1R)	B C_1F_2	B C_2F_2
% STAR FLOWERS	25.3	15.4	33.7	28.3	60.9	56.9
NO. OF TREES	18	4	6	9	15	14

Notes: Percentages are means of two main flowerings.

Table 17: Self compatibility in C. arabica, tetraploid C. canephora, their interspecific arabusta hybrid and in backcrosses, as estimated from % fruit set.

	NATURAL POLLINATION		SELF POLLINATION IN BAGS	
	% FRUIT SET	NO. OF FLOWERS OBSERVED	% FRUIT SET	NO. OF FLOWERS OBSERVED
C. arabica	73.1	288	59.5	256
TETRAPLOID C. canephora	55.5	412	0.0	400
ARABUSTA (F_1 A)	24.1	867	1.8	946
ARABUSTA (F_1 R)	13.0	876	1.6	816
B C_1 F_2	32.3	1206	11.5	1095
B C_2 F_2	56.2	915	35.5	1058

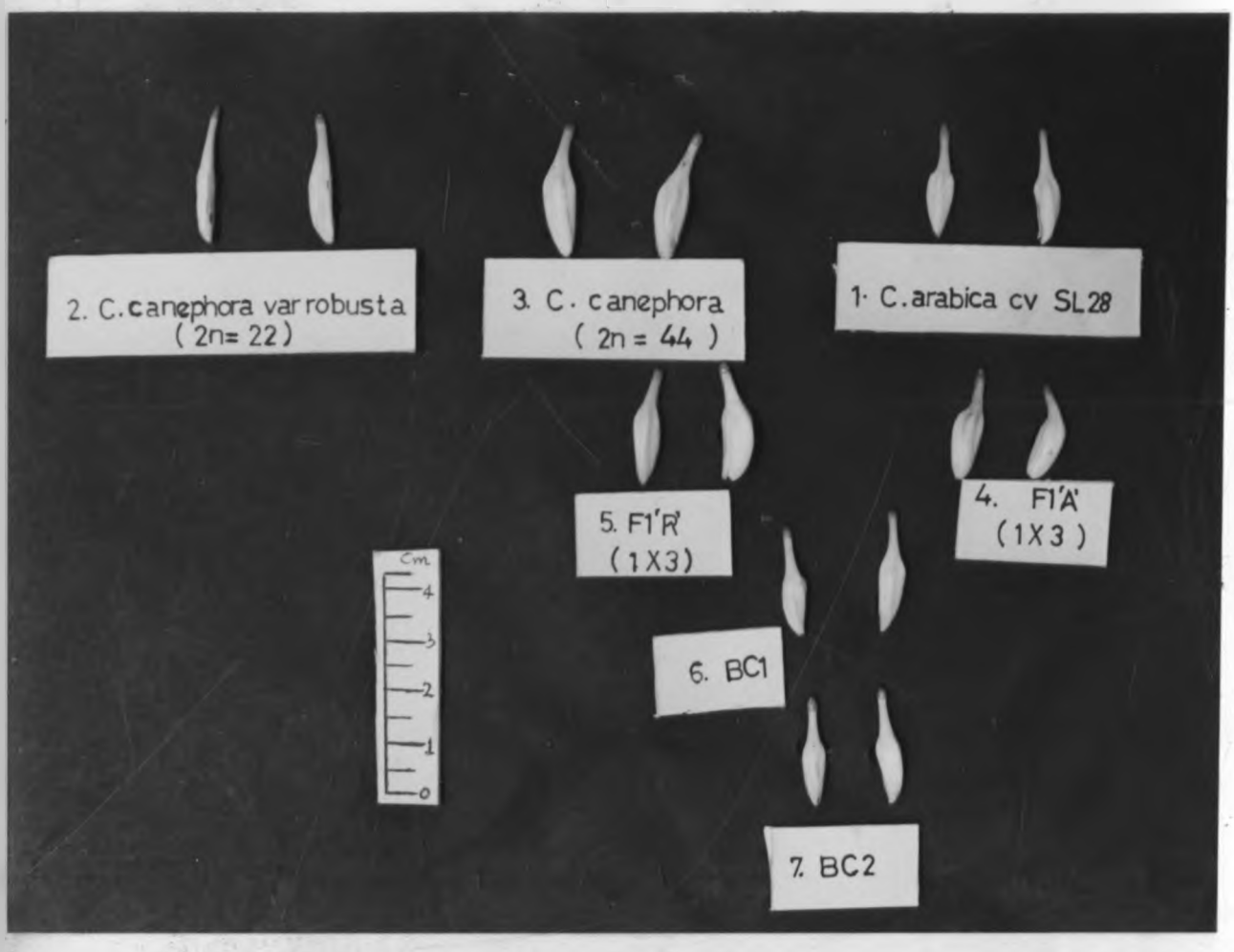

PLATE 5(a)

Flower size in C. arabica and C. canephora (diploid and tetraploid), their arabusta hybrid and backcrosses to C. arabica.

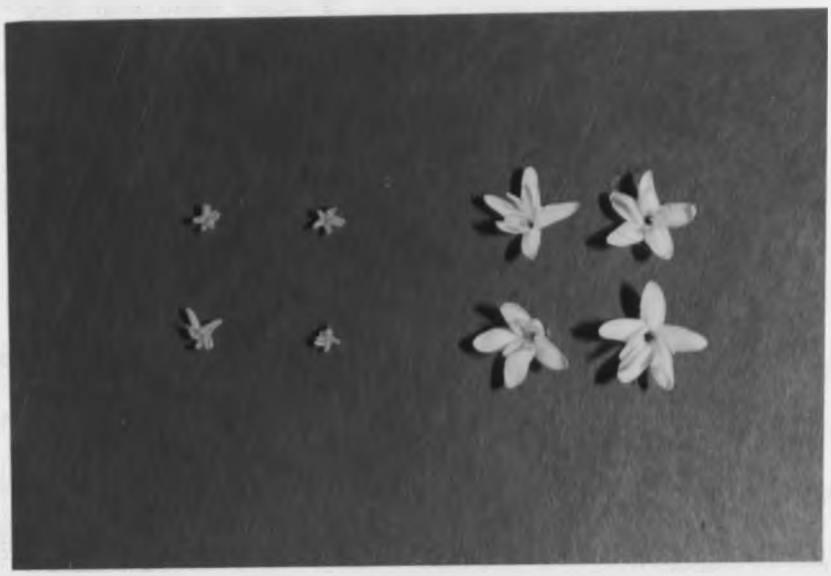

PLATE 5(b)

Star flowers(left) and normal flowers(right) in coffee.

CHAPTER 7

MALE AND FEMALE FERTILITY

7.1 INTRODUCTION

The full use of possibilities offered by polyploidy in plant breeding can only be made after an adjustment in the fertility has been made to the tetraploid level (Sybenga 1972). The same author(Sybenga ,1972) reviewed the work on selection for fertility in autopolyploids and concluded that genotypic improvement for fertility offers promise.

Because of the relative ease and reliability of the method of pollen stainability, the fertility of crop plants is often determined using this method. In such assessment it is essential that fertility of the ovules would be similar. In vitro pollen fertility can also be tested by germination in a sucrose solution. Both methods have been used to determine the fertility of pollen grains in interspecific hybrids of coffee (Krug and Mendes, 1940; Lanaud, 1976; Grassias 1977 and Louarn 1976). In interspecific hybrids and more often in autotetraploids, the major cause of infertility is a disturbed meiotic process. A direct relationship is therefore found between pollen fertility and regular meiosis. Louarn (1976) observed a high correlation between pollen stainability and Metaphase I pairing in hybrid plants of *C. canephora* and *C. eugenioides*. In arabusta hybrids, Grassias (1977) noted high

and significant correlation between pollen fertility and regular Metaphase I pairing as well as distribution of chromosomes over the poles in first anaphase. Similar results were obtained by Lanaud (1976) who observed this in a hybrid of C. canephora and C. kianjavatensis.

Micropollen grains often occur in addition to normal pollen grains where the microsporogenesis is irregular. In potato interspecific hybrids between Solanum polytrichon (2n = 4x = 48) and S. phureja (2n = 2x = 22), Ramana and Abdalla (1970) found complete sterility in accordance with its triploid condition in contrast to the high pollen fertility in the two parent species. The pollen grains were clustered in tetrads when forced out of the anthers and had 1 to 3 additional micropollen grains. Similar observations were made by Krug and Mendes (1940), where up to 9 additional micropollen grains were found in the triploid hybrid of C. arabica and C. canephora. Krug (1937 b) observed low pollen viability 20 - 30% germination as well as 2 - 8 additional micropollen grains per 'tetrad' in the octoploid (2n = 8x = 88) form of C. arabica. In the hexaploid form of this species pollen quality was slightly higher (35% germination) with 3 - 8 additional micropollen grains. Additional micropollen grains apparently occur in coffee hybrids made from crosses between parent species at the same ploidy level. Louarn (1976) observed that, while the pollen production of F_1 hybrids of C. canephora and C. eugenioides was good, its viability was low; 34 - 48% stainability compared to 90% in parents. Up to 7 additional micropollen grains were found per 'tetrad'.

Fruit set has been used to estimate fertility in many crop plants (Roseweir and Rees, 1962; Hermsen and Ramana, 1969; Hardon and Tan 1968; Jansen and Hermsen 1976). The amount of fruit set obtained in arabica coffee is shown in the results of Jimenez-Castano and Castillo Zapata (1976) where 92% fruit set after natural pollination, 80.9% after controlled selfing and 67.2% after controlled cross pollination, were obtained with varieties murta and bourbon. The variation in each case was low, intermediate and high respectively. Orlido and Capinpin (1957) reported differences in fruit set in some diploid and tetraploid coffee species and also noted that a negative correlation existed between % fruit set and % pea berries.

The frequency of pea berries has been used to estimate female fertility. Pea berries (Plate 6), result when one of the two ovules abort and only one continues to develop and grows to a large size in a bid to fill the entire space (Sybenga 1960). Fernamdo (1962) found a linear response in frequency of pea berries to neutron irradiation and an exponential response to X-ray irradiation. He suggested that the high frequency of pea berries obtained following X-ray irradiation was due to chromosomal aberrations. Sybenga (1960) has mentioned a backcross programme to *C. arabica* with a triploid hybrid of *C. arabica* and *C. canephora* which was carried out in India. The reduction in % pea berries from the F_1 to BC_2

generation was found to be a rather slow process. The converse of % pea berries is the % used ovules also called ovular fertility. It is (100 - $\frac{\%\ \text{pea berries}}{2}$) and has also been used to estimate female fertility in coffee. Grassias (1977) tabulated data that indicated a considerable range for ovular fertility in families of arabusta hybrids of magnitude 19 - 85%.

7.2 MATERIALS AND METHODS

Pollen was tested for fertility in three different ways, namely stainability in 1% acetocarmine solution, in vitro germinability in 10% sucrose solution and in fruit set obtained when this pollen was used in crosses to the local C. arabica cv SL 28 as female parent.

Pollen stainability was measured using fresh pollen collected on the day of anthesis during a main flowering period. The number of "good pollen" was noted out of a sample of 200 - 300 pollen grains from each tree. Pollen was classed as "good" only if regularly round and stained uniformly, a classification adopted from the methods of Jansen and Hermsen (1976) and is similar to that used by Louarn (1976) with a hybrid of C. canephora and C. eugenioides.

In vitro germinability of pollen in 10% sucrose solution was determined according to Walyaro and Van der Vossen (1976). During periods of main flowering, flowers from subject

trees were collected early in the morning (before anther dehiscence) on the day of anthesis. The flowers were taken to the laboratory and spread out on sheets of paper. Pollen dehiscence followed shortly, pollen was brushed off the anthers and incubated in a hanging drop in a Van Tieghem cell at 30°C. Pollen germinated and within 2 - 3 hours most of the pollen tubes had elongated considerably in control cells which in each occasion was of the cv SL 28. Pollen was considered germinated ('good pollen') only when the length of the pollen tube was at least twice the diameter of the pollen grain, and was free of any malformation, such as thick, short, bulbous pollen tubes. A net micrometer fitted to the eye piece was used during scoring and 200 pollen grains were examined.

To test pollen viability in crosses, emasculation and bagging for artificial cross pollination was performed on cv SL 28, two or one day before the day of anthesis. Artificial cross pollination was performed according to the methods described by Walyaro and Van der Vossen (1977). The bags were left around the emasculated flowers for 10 - 12 days.

The % used ovules (% u.o.) and % fruit set on natural pollination were used to obtain estimates of female fertility.

A method for determining % u.o. by estimating the frequency of pea berries has been described by Grassias (1977). In this method, 100 - 200 developing fruits, 3 - 4 months old

were cross-sectioned to see whether there were one or two seeds developing. For this purpose berries found to contain more than two developing endosperms were considered to have had only 2 ovules per berry initially, the third bean having arisen from an abnormal development called polycarpy (Sybenga, 1960). The % u.o. was calculated as follows: From the total number of fruits, the number of ovules present initially was determined as twice this number of fruits. The number of one bean fruits (pea berries) were counted. The % pea berries (% pb) is calculated as a fraction of the total number of fruits. Then % u.o. is calculated from the formula on page 6 .

To estimate fruit set from open pollination several nodes with developing flowers at the candle stage were labelled with a loop of string and the number of flowers on these nodes counted. From time to time, newly developing flowers were removed. Counts for fruit set were made after 10 weeks.

For description of the relations between different measurements of fertility, sample linear correlation coefficients (r) were calculated in the usual way.

7.3 R E S U L T S

A summary of the data of pollen fertility is given in Table 18. The results obtained from each of the three methods indicated the same trend of fertility among generations. In vitro

pollen germination gave low values in comparison to stainability. It was also observed that only large differences in germination (10 - 15)% could be discriminated using stainability. Values for fruit set in crosses using the pollen on C. arabica cv SL 28 as female parent, were intermediate. In vitro pollen germination gave the widest range, which in first backcross generation was as low as 5% to as high as 65%. On the other hand stainability was relatively less variable.

The highest proportion of stained pollen grains, germinating pollen grains and % fruit set obtained in crosses was in each case observed in cultivar SL 28. Tetraploid C. canephora was closer to cultivar SL 28, than to second backcross generation. On the other hand, the F_1's had the lowest % stainable pollen, % pollen germinating and also the lowest % fruit set was obtained with crosses using F_1 pollen on cultivar SL 28. At the same time it was noted that F_1A and F_1R were remarkably similar for all three different estimates of pollen fertility. Comparing first backcross and second backcross generations, it was observed that the second backcross generation had significantly higher proportion of 'good' pollen. This was indicated by all the three methods used to test pollen quality.

Pollen fertility data for F_1 progenies from different male parent clones are summarised in Table 19. For these arabusta crosses only a few trees per progeny could be observed

except the progeny from male parent clone, UT6. The results of pollen stainability, in vitro pollen germination and fruit set in crosses with cultivar SL 28 as female parent, all indicated that the male fertility tended to be low in crosses with UT6 as one parent. However, the within progeny variation was large in all cases and was reflected in the range and the standard errors.

High % used ovules estimates were obtained for C. arabica cultivar SL 28 and for tetraploid C. canephora (Table 20). The first backcross generation was very close to the second backcross generation for % used ovules.

The data from fruit set in open pollination gave similar trends, but in backcross generations, the second backcross had a relatively higher % fruit set than first backcross generation. The two estimates of female fertility in general showed that there was a trend of improvement in fertility with each backcrossing generation of the F_1 to C. arabica.

Table 21 gives the data on female fertility for the arabusta. For % used ovules there was little variation among the families just as with the results of pollen fertility. Progeny of clone UT6 however had relatively lower % fruit set from open pollinations in comparison to other progenies. Progenies 49, 50 and 51 had on the other hand, a significantly higher female fertility. However, there was in all cases a large within progeny variation.

Correlation coefficients between various measurements

Note: n = number of trees

used to evaluate male and female fertility are presented in Table 22. Also included is first year cherry yield since this is considered as the best indicator of fertility for all the measurements of fertility. The correlations were calculated from means per progeny or groups of individuals (see Tables 1 - 3). All correlation coefficients were positive, high and significant at $P < 0.01$. Five kinds of relationships could be compared. The correlation between the different measurements of pollen fertility, the correlation between the two female fertility measurements and that between pollen and female fertility measurements. One more relationship is the correlation between fertility measurements and first year cherry yield.

For pollen fertility measurements, it was observed that the correlation between either pollen stainability or pollen germination and fruit set obtained in crosses were equally high. Each of these pollen fertility measurements again had similarly as high correlation with first year cherry yield. Fruit set in crosses with SL 28 as female parent however had a relatively higher correlation with first year cherry yield. The % used ovules and % fruit set from natural pollination each respectively had a high correlation with first year cherry yield, as would be expected.

7.4 D I S C U S S I O N

Pollen stainability is the most frequently used in-vitro method for testing male fertility, since the method is quick and suitable for large numbers of routine observations.

In vitro pollen germination provides a more accurate test of the male fertility but is comparatively more laborious. Depending on the crop, the correlation between % normally stained pollen and % germinable pollen may be low as in the oil palm (Hardon 1969) and potato (Hermsen and Jansen 1976), or it may be high as in coffee, (Lanaud, 1977) and Grassias, 1977). Compared to in vitro germination of the pollen, stainability tends to give an overestimate of fertility, a high % stainability corresponding to relatively lower % germination. This trend was observed in the present study and differences in % germination of the order of (10 - 15)% could sometimes not be discriminated through stainability. However, there existed high and significant correlation between stainability and germination ($r = 0.89^{**}$). Both stainability and germination were also highly and significantly correlated ($r = 0.91^{**}$, $r = 0.89^{**}$) with another measure of pollen fertility; % fruit set in crosses to the locally adapted C. arabica cultivar SL 28.

Ovular fertility as estimated from % used ovules gave high values and covered a range comparable to % stainability. High and significant correlations between pollen fertility and female fertility measurements were also present. These observations agree with those obtained by Grassias (1977). They are indicative of the value of such measurements for evaluation of fertility in arabusta and backcross generations.

From practical considerations, high correlations between stainability with fruit set could indicate the possibility of using pollen stainability alone to obtain a good estimate of pollen fertility. However, the observation that stainability would not discriminate between large differences in fertility as measured by in vitro pollen germination and fruit set in crosses would suggest that stainability should be supplemented with in vitro germination of the pollen.
Stainability would be used in preliminary assessments in which high values (> 90%) would be regarded as indications of 'good pollen', but lower % stainability (60 - 80%) would be further tested in vitro germination to specify differences in pollen quality, whereas much lower % stainabilities would be indicative of very poor pollen to be discarded outright.

The arabusta hybrids were shown to be of particularly low pollen viability in all the three measurements of pollen fertility. The same observation was obtained from the data of female fertility. These findings are in agreement with the observations of Grassias (1977) who reported a low pollen and ovular fertility in arabusta grown at the Ivory Coast. The remarks made by Capot (1972) about the low fruiting of the arabusta as one characteristic limiting their potential in the improvement of robusta coffee in the Ivory Coast, would be viewed on this reduced fertility background. However, it has been shown (Grassias 1977) that arabusta hybrids show a considerable variation for fertility between different families

depending on the level of fertility of their parents. In the present study the between progeny variation was not very large. This could be due to the fact that all the tetraploid robusta clones originated from diploid C. canephora clones which were already selected for high yield. However the data showed that there was a large within progeny variation intimating that selection for genotypes with a high fertility within the arabusta is likely to be effective. It is realised that the variation for fertility within the F_1 generation was not investigated in detail, due to lack of time. However this will be the subject of a further study aimed at selection within arabusta for vigour, fertility and yield. A trend of increasing level of fertility could be observed with every generation of backcrossing of the arabusta to C. arabica. It would therefore seem that much of the low fertility observed in the arabusta is already much improved in two generations of backcrossing to C. arabica. This would be an encouraging situation for an interspecific breeding programme employing the backcross method and using C. arabica as the recurrent parent. The backcrosses compared to the arabusta in the present study were both derived from similar (arabica x robusta) interspecific crosses in which induced tetraploid C. canephora is one of the parents. It is therefore expected that a similarly high level of fertility would be obtained in early generations of backcrosses of the arabusta at present in progress at the Coffee Research Station. The reduction in percentage pea berries in a backcross programme

to C. arabica in which a triploid hybrid of C. canephora and C. arabica was used decreased with successive backcrossing (Sybenga, 1960). The same could apply to advanced generations of backcrosses with the arabusta. In that case several generations of backcrossing may be necessary to reduce the frequency of pea berries to the level in C. arabica.

Table 18: Pollen fertility estimates from stainability, in vitro germination and from fruit set in crosses to the local C. arabica cv. SL 28 as female parent. Means, s.e. of mean, ranges

	POLLEN STAINABILITY (%)		IN VITRO POLLEN GERMINATION (%)		% FRUIT SET IN CROSSES WITH cv. SL 28 AS FEMALE PARENT	
	MEAN	RANGE	MEAN	RANGE	MEAN	RANGE
C. arabica	97.4 ± 0.3	95.5 - 98.5 (n = 9)	72.0 ± 2.6	64.3 - 84.0 (n = 10)	78.8 ± 2.9	70.0 - 92.4 (n = 7)
TETRAPLOID C.canephora	94.4 ± 1.8	86.5 - 98.0 (n = 6)	64.0 ± 6.3	47.0 - 82.5 (n = 6)	61.1 ± 5.7	52.6 - 89.3 (n = 6)
ARABUSTA (F_1R)	51.7 ± 2.3	38.0 - 63.0 (n = 11)	6.0 ± 0.9	0.0 - 18.5 (n = 22)	24.3 ± 2.0	9.6 - 39.1 (n = 19)
ARABUSTA (F_1R)	57.8 ± 1.7	38.5 - 80.0 (n = 32)	5.2 ± 0.5	0.7 - 16.5 (n = 44)	25.6 ± 1.8	11.3 - 42.2 (n = 22)
BC_1F_2	79.9 ± 2.4	60.5 - 98.0 (n = 17)	22.0 ± 4.1	4.7 - 65.0 (n = 19)	48.9 ± 3.8	27.6 - 60.7 (n = 10)
BC_2F_2	91.1 ± 1.1	81.0 - 97.0 (n = 16)	46.2 ± 3.7	27.3 - 67.5 (n = 14)	62.1 ± 5.6	38.6 - 84.2 (n = 8)

Note: n = number of trees

Table 19: Pollen fertility estimates in arabusta progenies of different tetraploid *C. canephora* male parent clones. Means, s.e. of the mean, ranges

ARABUSTA PROGENIES (DETAILS OF CROSSES)	% POLLEN STAINABILITY		IN VITRO POLLEN GERMINATION (%)		% FRUIT SET IN CROSSES WITH cv. SL 28 AS PARENT	
	MEAN	RANGE	MEAN	RANGE	MEAN	RANGE
SL 28, SL 34,		[illegible]		[illegible]		
N39 x UT6 (18,19 & 20)	53.0 ± 1.2	38.5 - 66.0 (n = 28)	2.3 ± 0.4	2.3 - 10.0 (n = 47)	22.2 ± 2.2	9.6 - 36.4 (n = 15)
SL28, N39 x UT3 (49 & 50)	66.0 ± 3.1	54.0 - 80.0 (n= 8)	7.8 ± 1.5	3.0 - 16.5 (n = 11)	24.4 ± 2.5	11.3 - 38.1 (n = 11)
SL28, SL34 x UT8 (51 & 52)	51.8 ± 8.1	38.0 - 65.5 (n = 3)	8.7 ± 3.8	3.5 - 11.5 (n = 4)	27.3 ± 2.1	16.4 - 34.8 (n = 9)
SL34 x UT10 (53)	60.1 ± 2.3	58.2 - 63.3 (n = 4)	8.5 ± 3.8	6.0 - 18.5 (n = 4)	26.7 ± 3.5	16.7 - 39.1 (n = 6)

Table 20: Female fertility estimates from % used ovules and % fruit set in open pollinations. Means, s.e. of the mean, ranges.

	USED OVULES		FRUIT SET FROM NATURAL OPEN POLLINATION	
	MEAN	RANGE	MEAN	RANGE
C.arabica	80.6 ± 1.8	84.7-89.8 (n = 9)	62.9 ± 2.7	47.0-74.6 (n = 12)
TETRAPLOID				
C. canephora	84.6 ± 2.6	78.6-90.1 (n = 4)	55.4	- (n = 1)
ARABUSTA (F_1A)	57.4 ± 1.6	51.4-69.1 (n = 16)	20.1 ± 2.6	5.6-37.9 (n = 17)
ARABUSTA (F_1R)	59.7 ± 1.3	51.4-72.8 (n = 27)	20.7 ± 1.7	5.8-37.7 (n=32)
BC1 F_2	72.5 ± 2.3	60.6-86.2 (n = 14)	36.4 ± 3.6	11.9-54.1 (n = 16)
BC2 F_2	75.1 ± 2.3	59.9-88.3 (n = 14)	52.2 ± 2.5	31.7-71.7 (n = 28)

Table 21: **Female fertility estimates in arabusta progenies of different male parent tetraploid C. canephora clones.** Means, s.e. of mean, ranges.

ARABUSTA PROGENIES (DETAILS OF CROSSES)	USED OVULES (%)		FRUIT SET FROM OPEN POLLINATION (%)	
	MEAN	RANGE	MEAN	RANGE
SL 28, SL 34, N39 x UT6 (18,19 & 20)	56.2 ± 1.1	51.4-72.8 (n = 25)	15.6 ± 1.4	5.6-37.7 (n = 31)
SL 28, N39 x UT3 (49 & 50)	63.9 ± 2.2	58.1-70.2 (n= 8)	25.1 ± 3.1	15.8-37.9 (n = 8)
SL 28, SL 34 x UT8 (51)	62.9 ± 2.3	58.7-70.7 (n = 7)	23.4 ± 6.0	8.6-37.0 (n = 4)
SL 34 x UT10 (53)	58.3 ± 3.0	52.3-62.2 (n = 3)	25.3 ± 3.3	15.5-32.4 (n = 6)

n = number of trees observed

Table 22: Correlation coefficient (r) (based on Means per family using data from families of all generations) between the different methods used to estimate fertility

		2	3	4	5	6
1	POLLEN STAINABILITY	0.89** (n=19)	0.91** (n=12)	0.77** (n=18)	0.75** (n=18)	0.71** (n=19)
2	POLLEN GERMINATION		0.89** (n=12)	0.70** (n=17)	0.77** (n=16)	0.70** (n=19)
3	% FRUIT SET IN CROSSES WITH cv.SL28 AS ♀ PARENT			0.82** (n=12)	0.79** (n=11)	0.82** (n=11)
4	% USED OVULES				0.84** (n=15)	0.84** (n=14)
5	% FRUIT SET OPEN POLLINATION					0.77** (n=15)
6	FIRST YEAR CHERRY YIELD					-

Note: ** r significant at P $<$ 0.01

PLATE 6

Peaberry (left) and normal berry in coffee.

CHAPTER 8

CYTOGENETIC ASPECTS OF FERTILITY

8.1 INTRODUCTION

Once flower buds are formed in coffee they grow slowly for a period of about two months reaching a size of 6 to 8 mm and then they stop growing for many weeks or months depending on the weather. Mes (1957) found that at this point the pollen mother cells are mature. Flower bud dormancy is broken and flowering follows within a few days if sudden changes occur in the weather conditions. Piringer and Borthwick (1955) and Alvim (1960) observed that irrigation following a period of water stress led to blossoming in coffee. Mathew and Chokkana (1964) demonstrated that coffee plants must be subjected to a critical level of soil water stress before the flower buds can respond to irrigation. Cannell cited by Browning (1973) observed that at Ruiru, blossoming in arabica coffee only follows rainfall when this is accompanied by a rapid temperature drop.

The timing for microsporogenesis in coffee and in particular meiosis depends on local environmental conditions tends to be synchronised and takes a short period. The mature and resting pollen mother cells undergo meiosis in a few days after breaking bud dormancy. Leliveld (1940 a) found with robusta coffee in Indonesia meiosis to start about 36 hours after a rainfall and that the process starts an hour earlier

if a long period of drought preceeds the shower. She observed further that meiosis proceeded at the same time in most of the trees but the process was less synchronised in times of regular rainfall. Devreux e.a. cited by Sybenga (1960) observed that meiosis takes place in the period just preceeding flowering when the buds grow from 7 - 8 mm to 20 mm in 48 hours after the shower that stimulates flowering. Kammacher and Capot (1972) found the best sampling time for studying meiosis in a triploid hybrid plant of C. arabica and C. canephora, to be within 32 - 38 hours after irrigation. This was at Bingerville in Ivory Coast. Under the conditions of the east coast of Malagasy, Louarn (1976) found meiosis in pollen mother cells of a hybrid plant of C. canephora and C. eugenioides in samples taken between 40 - 72 hours after breaking bud dormancy by irrigation. Berthaud (1976) noted that flower buds were to be collected between 52 and 64 hours after breaking dormancy to study meiosis in a haploid plant of C. arabica at Bingerville.

Sybenga (1969) has listed several disadvantages in the autopolyploid meiotic system, noting that established natural autopolyploids have evolved mechanisms such as diploidisation for reducing them. Giles and Randolph (1951) reported an increase in the number of bivalents and a decrease in the number of quadrivalents in autotetraploid maize grown over a period of ten years. They suggested therefore that

autopolyploids which form multivalents at the time of origin may later shift to a strictly diploid mode of synapsis as is characteristic of allopolyploids. Grassias and Kammacher (1975) studied first metaphase pairing in three varieties of arabica coffee. They observed that pairing according to strictly diploid mode occurred in two thirds of the metaphase plates examined; and that in one tenth of the metaphase plates, formation of one to three quadrivalents occurred. From these facts, they suggested that the most probable genome structure of C. arabica is that of a segmental allotetraploid formed by the juxta-position of two genomes at x = 11, certain chromosomes of which have maintained their structural similarityin the two parent species.

Considerable work has been done in an attempt to define genome relations between C. arabica and C. canephora. Krug and Mendes (1940) noted that there was good homology between C. arabica and C. canephora. In a triploid hybrid progeny of the two species, the pairing at first metaphase was found to be 14.4 univalents, 5.4 bivalents and 2.6 trivalents on average. However, this analysis was based on only a few number of cells. Monaco (1965) found 10.07 univalents, 9.45 bivalents and 1.33 trivalents in a triploid hybrid plant of these two coffee species. He put forward the suggestion that C. canephora has contributed its genome to the formation of C. arabica. Capot et al (1968) evaluated the ease of obtaining hybrids from crosses between C. arabica

and tetraploid C. canephora. They concluded that the affinity between the genomes of C. arabica and C. canephora was good although the hybrid progeny from such crosses had a poor fruit set which posed a limitation on its utilisation in further breeding programme. Kammacher and Capot (1972) reported 75% pairing in a triploid hybrid progeny of C. arabica and C. canephora. They noted that most of the bivalents were of the regular ring type and that the mean number of bivalents (i.e. bivalents and trivalents together) was close to eleven so that each C. canephora chromosome apparently had a homologous counterpart in C. arabica.

Grassias (1977) noted that the deviation from the normal 22 - 22 Anaphase I distribution of chromosomes in arabusta hybrids was only one in most of the cells examined. She therefore suggested that the occurrence of a high number of univalents at first Metaphase in this hybrid might not be due to a lack of homology between the chromosomes of the two parental genomes but rather point to a poor regulatory system of the meiosis. First metaphase pairing in triploid hybrids between Eucoffea or Mascaracoffea with C. arabica was observed to reflect the allosyndetic pairing potentialities of the eleven chromosomes of C. arabica with those of the diploid species. Charrier (1976) suggested from such observations that the leading diploid meiotic behaviour of the amphidiploid C. arabica would result from a preferential pairing or from a system for regulating synapsis of the Iriticum type. From this account, it once again appears that C. arabica and C. canephora are

related in their genomes.

8.2 MATERIALS AND METHODS

To perform chromosome counts in somatic cells, root tips were obtained from soft wood cuttings of four F_1 plants two of F_1A and the other two of F_1R. For rooting of the cuttings, the method developed by Van der Vossen *et al* (1976b) was used. Root tips were treated in a 0.5% aqueous magnesium sulphate solution saturated with 1, 4 - dichlorobenzene for 2 hours and 30 minutes at 4°C. They were then fixed in a Carnoy's 3:1 alcohol - acetic acid mixture at 4°C for at least 24 hours. This method of fixation in the cold does not require hydrolysis and has successfully been used by Louarn (1976) to study mitosis in hybrid plants of *C. canephora* and *C. eugenioides*. The squash technique for chromosome studies was used, the root tips being stained in ferric-haematoxylin according to the schedules of Henderson and Lu (1968). Counts were made from cells with well spread out chromosomes at prophase stage. Good spreading of the chromosomes was achieved by pressing firmly on the slide held between folds of blotting paper and quickly passing the slide over a flame. Records in the form of photographs and camera lucida tracings were made.

Meiosis was studied in pollen mother cells. Since meiosis has a duration of only about 4-6 hours and tends to be highly synchronised (Leliveld 1940), it was essential to establish an optimum sampling time of the flower buds so

as to obtain pollen mother cells of the right meiotic stage. To determine the right sampling time, the first trial sampling was started after it had rained on 4.11.77. A sample was taken every 2 hours from 36 to 48 hours after the rainfall. Similar sampling trials were held on 16.1.78 and on 14.3.78. On the first trial, sampling intervals were of 2 hours, while on the second and third trials, intervals of 30 minutes were observed but the sampling duration was of 6 and 4 hours respectively. On each occasion, the sampling was held with 3 trees of the hybrid generation. In addition changes in size and colour of the buds over this period were noted.

Flower buds were fixed in a modified Carnoy's 6:3:1 mixture of alcohol, chloroform and glacial acetic acid. To the mixture was added 5% hydrochloric acid by volume. This latter modification has been advanced by Kammacher and Capot (1972) who have noticed that the technique gives a good dispersion of the chromosomes during the meiosis. After fixation for at least 24 hours, the flower buds were transferred to a 70% alcohol solution and stored at about 5^{o}C. Whole anthers were squashed in an acetocarmine-haematoxyline mixture according to Kammacher and Capot (1972); Louarn (1976). As described earlier, spreading of the chromosomes was enhanced by pressing and gently warming over a flame.

For studies on the meiosis, cells with well spread out chromosomes were selected and observed for associations of chromosomes in first metaphase and distribution of the chromosomes to the poles in first anaphase. The charts drawn by Grassias and Kammacher (1975) (see Fig.3) were found useful for recognising and assigning quadrivalent associations. Studies of metaphase I chromosome associations were performed under a phase contrast microscope at a magnification of 1250X. The phase contrast microscope was found invaluable for this work on account of the small sizes of the chromosomes.

The number and frequency of microspores formed per tetrad were scored in at least 75 cells; 4 plants of the F_1 (2 from group F_1A and 2 of F_1R) and 1 plant each of other generations were observed.

8.3 R E S U L T S

Chromosome counts were made from root tip preparations of four F_1 plants (two from group F_1A and the other two from F_1R). The counts were made at prophase stage. In each case 44 chromosomes were counted. Plates 7 and 8 and Figures 1 and 2 are examples of each preparation from which the counts were made.

The results of trials carried out to determine the optimum time in which to sample flower buds at Ruiru for studies of meiosis are given in Table 23. In the trees examined,

flower buds were found to contain meiotic pollen mother cells in samples drawn between 42 - 48 hours after the rainfall that breaks bud dormancy. The length of the flower buds at this time was 4.3 - 7.3 mm, while dormant flower buds were 3.6 - 5.0 mm long (Table 24). In addition, dormant flower buds were green in colour and were held together with a brown gum-like substance, while buds sampled at the above times were slightly white in colour and the gum-like substance that held them initially could be seen to have dislodged.

Meiosis was studied in suitable pollen mother cells in squashed anthers. The following meiotic components which are related to fertility were examined: associations of the chromosomes in first metaphase, distribution of chromosomes to the poles of first anaphase and the frequency and number of microspores additional (to the normal four) per tetrad. The data were collected on at least 15 pollen mother cells per plant. Six plants of the F_1 generation were studied. From the other generations 1 plant was examined in each case. The results obtained are given in Table 25. In general, the F_1 plants had relatively higher frequencies of univalent and trivalent associations than were observed in plants of either parent species and backcrosses. In all plants, bivalent associations were the most frequent covering the range from close to 17.0 in F_1 to 20.0 in the plant of C. arabica. In Table 26 univalents and trivalents have been grouped together and bivalents

and quadrivalents similarly. This was done because univalent and trivalent associations are the ones considered to have the most contribution to chromosomal infertility. For either F_1A or F_1R, the associations were averaged over three plants. It was observed that F_1A and F_1R were similar for metaphase I chromosome associations while there was a decrease in univalent and trivalent associations in plants of backcrosses relative to the F_1's. Both ring and rod type of bivalents were observed in all plants, though C. arabica tended to have more ring bivalents. Most of the trivalents were 'Y' type while the few quadrivalents observed were chains although occasionally ring quadrivalents were also formed (Plates 11 and 12 and Fig. 6 and 7).

Distribution of the chromosomes over the anaphase poles was regular in the plant of C. arabica but also in most of the pollen mother cells in the plant of tetraploid C. canephora (Table 25; Plate 13 and Fig.8). On the other hand, F_1A and F_1R both showed a relatively irregular distribution of the chromosomes (Plate 14, Fig.9) with almost 45% of the poles departing from the normal 22 chromosomes per pole. The highest frequency of laggards were also observed in these plants. However, most of the deviation from the normal 22 chromosomes per pole involved a difference in the disjunction of probably one chromosome (Table 25). In each of the backcross plants examined a much more regular distribution of the chromosomes was observed with a considerable decrease

in frequency of lagging chromosomes.

Tables 25 and 26 gives the data on the number and frequency of microspores per tetrad. A very low frequency of additional microspores per tetrad was observed in the C. arabica plant and the plant of tetraploid C. canephora. In contrast, F_1A and F_1R plants showed a relatively high number of tetrads with more than four microspores. Considering all the six plants examined, a trend could be observed of increasing frequency of tetrads with four microspores with each backcrossing of the F_1 to C. arabica.

The data from cytogenetic observations of arabusta plants in relation to other generation plants tended to indicate for the arabusta in general a considerably more irregular meiotic process with regard to the three components of meiosis examined.

8.4 D I S C U S S I O N

From the work of Lelivald (1940), it has been known that in coffee microsporogenesis is dependent on local climatic conditions as to when it occurs and tends to be synchronous. Sampling of flower buds to study meiosis must be carried out at a specified time in each locality. It seems that at Ruiru, flower buds of arabusta hybrids have to be sampled within 42-48 hours after the rainfall that induces flowering. This time period is slightly longer than that

observed for a triploid hybrid of C. arabica and C. canephora in the Ivory Coast (Kammacher and Capot, 1972) but earlier than for a C. canephora x C. eugenioides hybrid (Louarn 1976), also observed at the Ivory Coast.

The six arabusta plants examined for meiosis in general showed a more irregular meiosis in comparison to the plants from backcrosses and parent species. Only 1 plant per backcross generation could be examined but the plants were specifically chosen because their pollen fertility was close to the mean for the generation and in this way could be regarded to be a representative sample for each backcross generation. For associations of chromosomes in metaphase I, only small differences could be observed between the arabusta hybrids and each backcross plant such as the slightly higher frequency of univalents and trivalents in the F_1 hybrids. This was very much in contrast to the wide differences observed in fertility between the arabusta hybrids and backcross generations. For distribution of chromosomes to the anaphase poles, however, a marked irregularity was noted in the arabusta relative to the situation in backcross plants, much in fashion with the trend already observed with fertility. Grassias (1977) noted this irregular anaphase distribution in two arabusta plants and suggested that a poor regulatory system of meiosis could be responsible. Furthermore, most of the deviation from the regular Anaphase I poles with 22 chromosomes was of $\pm$ 1 chromosome so that when the frequency of normal poles and poles with 1 chromosome deviation were taken together, these

accounted for close to 90% of the poles. The observation that most of the deviation from normal disjunction during anaphase concerns only one chromosome has also been made by Grassias (1977). She concluded therefore that most of the univalents observed in arabusta hybrids could more be the result of precocious separation of bivalents rather than point to a fundamental difference in the chromosome of both genomes. Lack of homology could therefore apply to only one chromosome. The observations with the materials of the present study would similarly indicate a lack of a regulatory mechanism of the meiosis rather than structural differences in the chromosomes in view of the fact that arabusta are also very comparable for chromosome association, particularly bivalent frequency, with plants from generations of backcrosses to C. arabica which already have a markedly improved fertility.

In relation to backcross plants and plants from parent species, arabusta hybrids had a higher frequency of additional micropollen grains per tetrad. In coffee additional micropollen grains have been observed in connection with chromosomal infertility in interspecific hybrids and higher polyploids (Krug and Mendes, 1940; Louarn 1976 and Krug 1937). In this study additional micropollen grains per tetrad followed closely the trend observed with fertility.

Table 23: Trial sampling to determine the optimum time of sampling in order to study meiosis in coffee at Ruiru

DATE	MEAN DAY TEMPS.	RAINFALL	SAMPLING TIME AFTER RAINFALL	NO OF PLANTS SAMPLED	PLANTS WITH PMC'S IN MI-AII
	^{o}C	mm	HOURS		
			36	3	0
			38	3	0
			40	3	1
4-11-77	20.1	45.1	42	3	3
			44	3	3
			46	3	3
			48	3	0
			42	3	3
16-1-78	19.7	75.4	44	3	3
			46	3	3
			42	4	4
14-3-78	19.9	85.1	44	4	4

Notes: PMC'S = pollen mother cells

MI = first metaphase stage of meiosis

AII = second anaphase stage of meiosis

Table 24: Length of flower buds before and after rainfall that induces breaking bud dormancy. Measurements in cm. Means, S E of the Mean.

Generation	Tree No.	Length of Buds before Rainfall		Length of Buds at time of Sampling	
		$\bar{X}$	n	$\bar{X}$	n
C. arabica	B6.1465	4.3 ± 0.2	10	4.7 ± 0.1	10
Tetraploid C. canephora	B1.1806	5.0 ± 0.2	10	7.2 ± 0.2	10
F1 A	B6.1621	4.3 ± 0.3	10	4.7 ± 0.1	10
F1 R	B6.1757	4.1 ± 0.3	10	4.9 ± 0.2	10
BC_1F_1	B6.1254	3.6 ± 0.2	10	4.7 ± 0.1	10
BC_2F_2	B6.1546	3.5 ± 0.2	10	4.3 ± 0.2	10

n = no. of buds

Table 25: Summary of observations of some meiotic components in 6 plants of arabusta hybrids and 1 plant each of backcrosses and parent species C. arabica and C. canephora

		CHROMOSOME ASSOCIATIONS IN MI FREQUENCIES OF				DISTRIBUTION OF CHROMOSOMES OVER AI POLES % POLES WITH			LAGGING CHRS		FREQUENCY OF MICROSPORES PER TETRAD					
	TREE NO	I	II	III	IV	22 CHRS	22±1 CHRS	22±x CHRS	NO.	%	3	4	5	6	7	8
C. arabica cv. SL28	B6.1465	1.75	20.06	0.03	0.50	97.50	0.00	2.30	2.00	0.20	0.00	95.24	2.38	1.19	0.00	0.00
Tetraploid C. canephora	B1.1806	3.75	18.19	0.13	0.88	88.00	11.60	0.00	10.00	1.00	0.00	96.15	3.85	0.00	0.00	0.00
ARABUSTA F 1 A	B6.1391	6.90	16.81	0.86	0.19	45.50	30.60	19.90	39.00	4.00	3.90	43.10	29.40	21.60	2.00	0.00
	B6.1392	6.11	17.84	0.56	0.11	50.00	36.50	11.10	32.00	2.40	0.00	60.00	35.48	4.84	0.00	0.00
	B6.1621	5.22	17.88	0.78	0.11	55.00	29.30	13.60	21.00	2.40	-	-	-	-	-	-
ARABUSTA F 1 R	B6.1357	6.13	17.63	0.60	0.11	59.10	29.80	9.80	17.00	1.20	1.20	40.90	32.53	20.48	3.61	1.21
	B6.1361	5.54	17.45	0.45	0.54	48.00	43.30	7.30	16.00	1.50	0.00	45.05	21.05	21.05	9.20	2.60
	B6.1387	5.73	17.30	0.60	0.30	47.70	37.70	12.20	21.00	2.20	-	-	-	-	-	-
B C 1 F 2	B6.1254	4.80	18.50	0.24	0.29	75.80	17.80	5.40	15.00	1.00	0.00	84.90	13.90	1.20	0.00	0.00
B C 2 F 2	B6.1546	4.95	18.50	0.15	0.40	77.30	21.70	0.00	10.00	1.00	5.77	87.50	5.77	0.00	0.00	0.00

Notes: MI = First metaphase stage of meiosis. X = 2 or >2 NO.,% = number and % lagging chromosomes in >15 cells.

AI = First anaphase stage of meiosis.

Table 26: Summary of observations of meiotic components in arabusta hybrids (means) and 1 plant of each parent species and backcross generations

	CHROMOSOME ASSOCIATIONS IN MI		CHROMOSOME DISTRIBUTION IN AI				MICRO-SPORES PER P M C
	% FREQUENCIES OF		% POLES WITH		LAGGING CHRS		
	I's + II's	II's + IV's	22 CHRS	22 + 1 CHRS	NUMBER	(%)	% 'TETRADS' WITH 4 MICROSPORES
C. arabica	7.97	92.03	97.50	0.00	2.00	0.20	95.24
Tetraploid C. canephora	16.47	83.09	88.00	11.60	10.00	1.00	96.15
ARABUSTA F_1A	27.81	72.19	50.17	31.83	30.67	2.87	51.55
ARABUSTA F_1R	25.93	74.07	51.60	36.93	18.00	1.63	42.96
BC_1F_2	21.10	78.90	75.80	17.80	15.00	1.00	84.90
BC_2F_2	21.20	78.80	77.30	21.70	10.00	1.00	87.50

CHAPTER 9

GENERAL CONCLUSIONS AND PRACTICAL IMPLICATIONS FOR COFFEE BREEDING

1. Diploid and induced tetraploid C. canephora showed differences in a number of morphological characters which could be useful in indirect selection of tetraploids following colchicine treatment of diploid plant material. In general, induced tetraploid plants had broader and thicker leaves than diploid plants. They had a smaller leaf length to width ratio, lower stomata density on the under surface of the leaf while their guard cells were longer. The arabusta progenies showed a variation into two plant types particularly distinctive for leaf size and canopy shape. Observations on the morphology and growth of arabusta showed that although plant type F_1A was markedly different from plant type F_1R in leaf size, canopy shape and vigour, there were hardly any significant differences in characters related to ploidy levels examined in this study. Counts of somatic chromosome numbers in root tips and observations on anaphase in pollen mother cells confirmed the indications from morphological characters that the occurrence of plant types F_1A and F_1R in arabusta were not the result of aneuploidy. The cause for this difference in phenotype remained uncertain but it may well be that other genetic factors, possibly major gene differences are involved.

2. Observations on general growth as well as extension

growth on the primaries indicated that arabusta hybrid progeny of plant type F_1R was vigorous but that in backcrosses to C. arabica there appears a general decrease in vigour. These results clearly showed that F_1R was more vigorous than F_1A intimating that the former should be the one to be used in backcrosses so as to enhance this trait in backcross progenies. Since arabusta progenies were found also to exhibit a considerable variation for vigour, selection for this character within arabusta could furthermore be directed at superior individual F_1R plants which also came from superior families. Long primaries with long internodes were also found to be growth characteristics of plant type F_1R. When such arabusta plants are adopted for commercial planting they would require to be planted at a spacing, normally applied in robusta coffee (3 x 3 m) which is relatively wide.

3. Both diploid and induced tetraploid C. canephora were found to be self incompatible, the artificial doubling of the number of chromosomes in this species apparently being of no consequence on self incompability. These observations would indicate that the genetic system of incompatibility excluding selfing in C. canephora may probably be other than monofactorial gametophytic suggested by Devreux et al (1959) since this type of incompatibility is known to break down with polyploidy in dicotyledons (der Nettancourt, 1977). It is probable that other systems of genetic control such as the bifactorial gametophytic, sporophytic or even the dual gametophytic

sporophytic system operating in Theobroma cacao (Cope, 1962) is the basis of self incompability in C. canephora.

4. From the data on a number of yield components, it was found that arabusta was comparable to other generations studied including the local commercial cultivar SL 28 except in first year cherry yield. Arabusta had very few berries per node and first year cherry yield despite their high flower per node counts. One reason for this low first year cherry yield of arabusta was thought to be their particularly low fertility. The kind of reduced fertility observed in arabusta expresses itself late in the growth of the plant and is confined to microsporogenesis and post anthesis development of the flower. The two arabusta phenotypes were on average very similar for fertility making a choice of either plant type difficult on this ground. Similarly, the between families variation in fertility was small. However, there was a large range in fertility within arabusta families indicating the possibility of effective selection within this material. Unfortunately, this aspect was only insufficiently investigated in the present study owing to lack of time. However, this aspect is part of the subject of further research aimed at selection within arabusta for fertility for possible use in commercial arabusta growing in climatic zones in Kenya, presently under consideration for robusta coffee production. In this context improvement of arabusta fertility becomes an important subject of research of immediate practical relevance since Capot (1972)

noted that drought tolerance, bean size and liquor quality in arabusta disclose a substantial improvement compared to robusta coffee. The arabusta of the Coffee Breeding Unit have, therefore, further gained in practical relevance beyond the scope initially envisaged.

5. An important result for the programme of backcrossing with arabusta at present in progress at the Coffee Research Station, Ruiru is that fertility was found to have been restored to almost normal levels already in the second backcross of the arabusta to C. arabica. The backcross studied presently are similar to those being made at the Coffee Research Station in that they both use arabusta hybrids as a bridge species in the course of introgression of disease resistance and vigour of C. canephora into cultivars of C. arabica. It is apparent therefore that a similar rapid rate of restoration of fertility would be expected in backcrosses of the arabusta presently studied.

6. Concerning the basis of reduced fertility of arabusta hybrids, evidence has recently been obtained (Grassias, 1977) that the main cause is cytogenetic. The present results were in agreement with these findings. In this study it has been shown that in relation to backcross generations, the arabusta hybrid displayed a marked irregularity in all the meiotic components examined except for Metaphase I chromosome associations. The arabusta and backcrosses showed little difference in frequency of bivalents rather disproportionate

to their differences in fertility. However, it was clear that the arabusta had significantly more aneuploid anaphase poles, lagging chromosomes and higher frequency of tetrads with more than four microspores. These findings are in agreement with the results of Grassias (1977) who concluded that the disturbances in arabusta meiosis arise from a poor regulation of the meiosis rather than point to fundamental structural differences in the chromosomes of the genomes hybridized.

7. In the present study it has been found that F_1A and F_1R arabusta were similar for fertility and chromosome number, two criteria important for making the choice of either plant phenotype for use in a backcrossing programme with C. arabica cultivars. A detailed study of the fertility of arabusta with the objectives of selection of the most fertile genotypes is being undertaken and the data obtained will facilitate decision on this matter. Tentatively, it appears that F_1R would be more preferred to F_1A mainly on account of their rather vigorous growth.

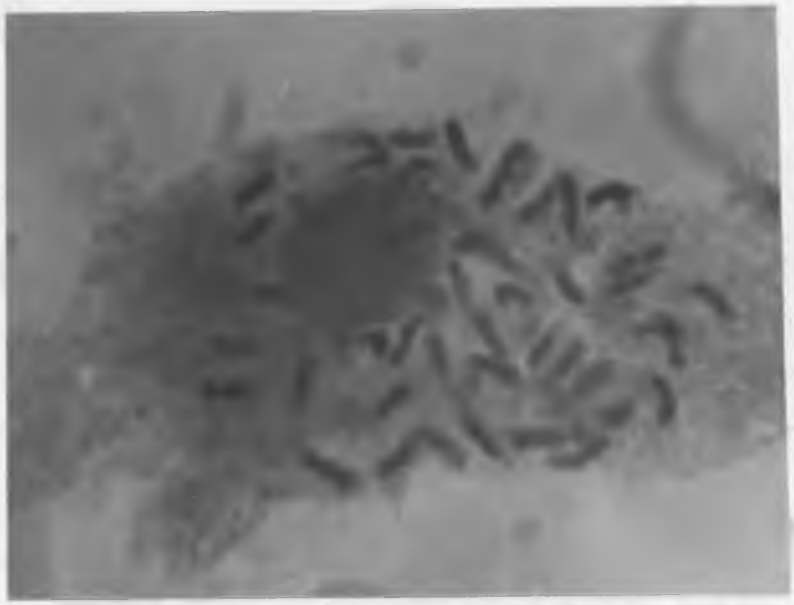

PLATE 7

Mitotic chromosomes in root tip squashes of an arabusta plant of type F_1A (Magnification 1250X). Also see Fig. 1

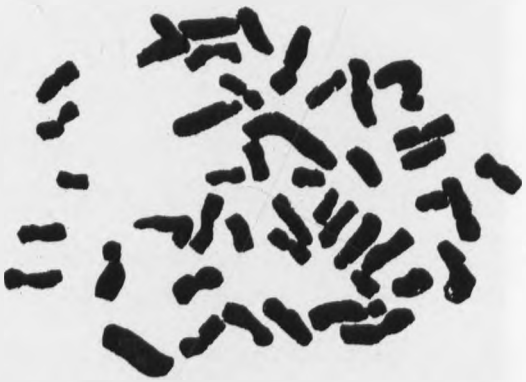

FIG. 1

Line drawings of mitotic chromosomes in Metaphase of plant B 6.1392 (F1A). At a magnification of (X 1250). Also see plate 7.

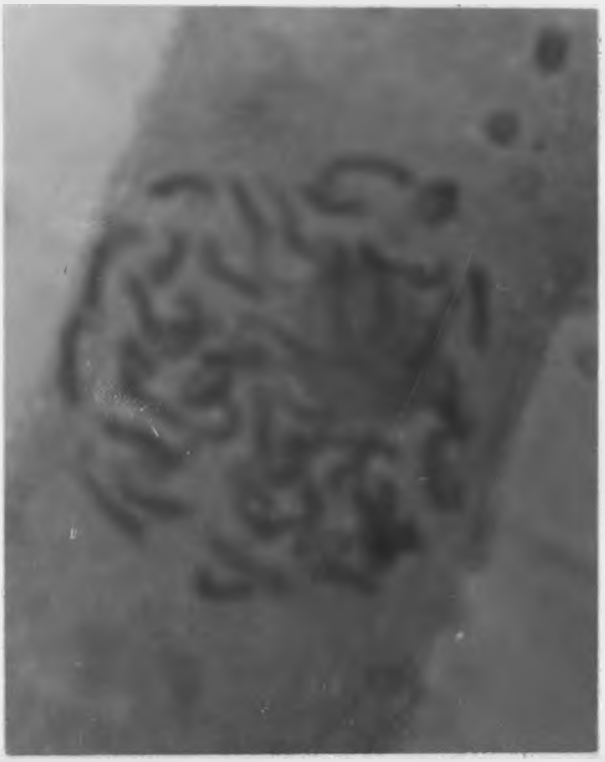

PLATE 8

Mitotic chromosomes in root tip squashes of an arabusta plant of type F_1R. (Magnification 1250X) Also see Fig. 2 .

FIG. 2

Line drawings of mitotic chromosomes in Metaphase of plant B6.1361 (F1R) (x1250). Also see plate 8.

FIG. 3

A scheme for interpretation of some quadrivalent associations in Coffee arabica. Source : Grassias and Kammacher (1975). Cafe' Cacao The' 19(3) : 177-190.

Schematic interpretation of quadrivalent .	Theoretical minimum number of chiasmata .	Example observed and the plate of reference .
	6	—
	5	—
	4	—
	3	12
	3	—
	3	11

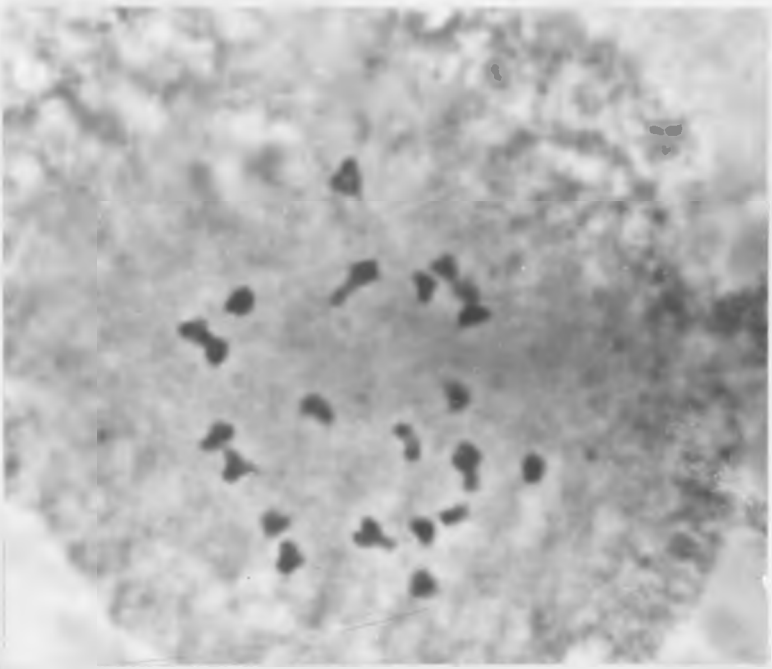

PLATE 9

Chromosome associations in first metaphase of a plant of C. arabica cv. SL 28 All bivalent associations (Magnification 1250 X)
Also see Fig.4,

FIG . 4

Line drawings of Metaphase I chromosome associations in plant B6. 1172 (C. arabica cultivar SL 28). All bivalent associations. (X 1250) under phase contrast microscope. (Also See plate 9).

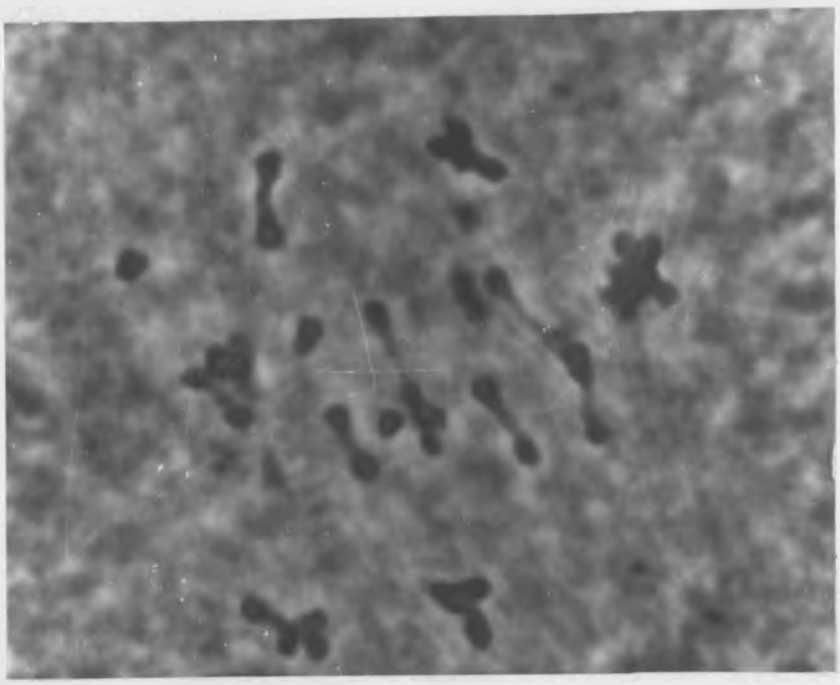

PLATE 10

Chromosome associations in first metaphase of a C. canephora (tetraploid) plant. Magnication (1250X)
Also see Fig.5

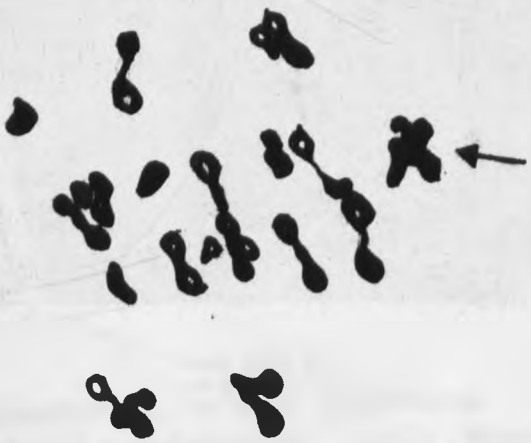

FIG. 5

Line drawings of Metaphase I associations in plant B1.1806 (Tetraploid C. Canephora), under phase contrast, (x1250). Arrow indicates an example of a quadrivalent. (Also see plate 10)

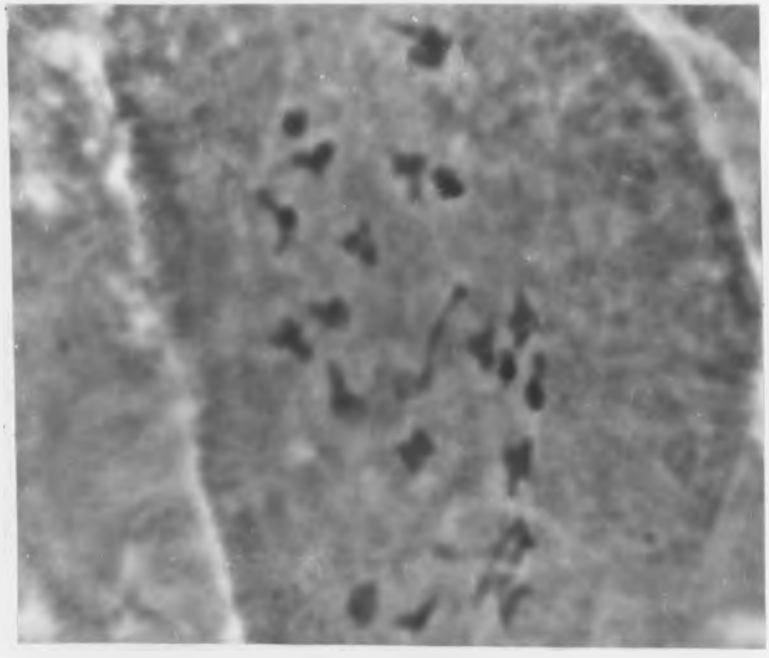

PLATE 11

Chromosome associations in first metaphase of an arabusta plant of type F_1A. (Magnification 1250X) Also see Fig. 6.

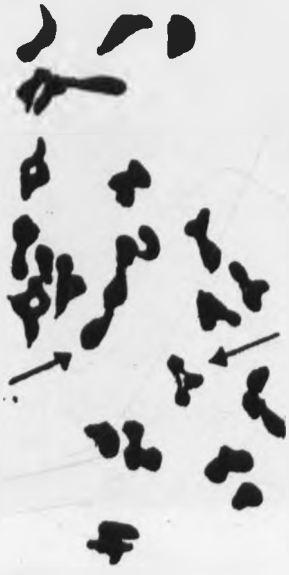

FIG.6

Line drawings of metaphase I associations in plant B6.1392 (F1A), (phase contrast, x 1250). Arrows indicate examples of trivalents and quadrivalents. (Also see plate 11).

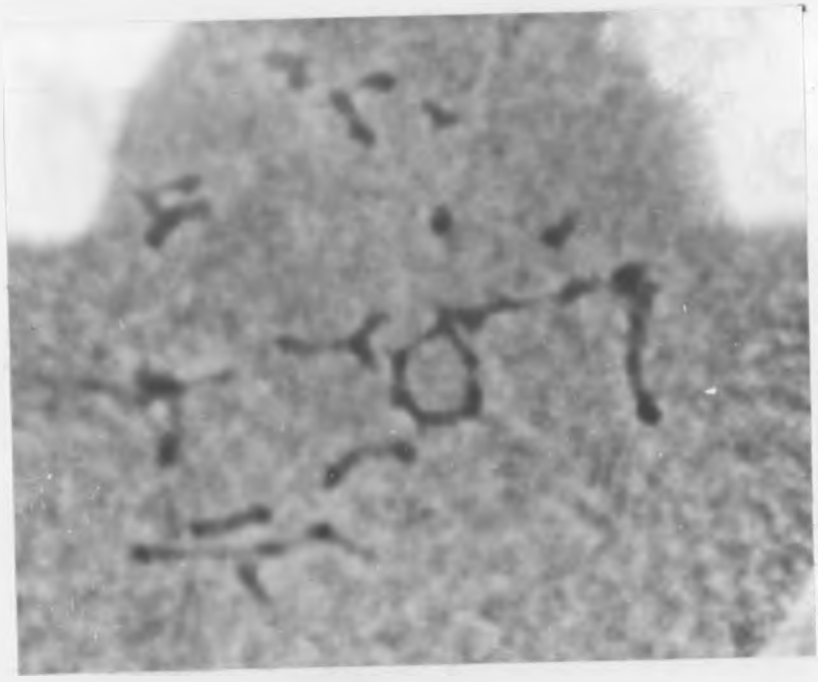

PLATE 12

Chromosome associations in metaphase (first) of an arabusta plant of type F_1R. (Magnification 1250X). Also see Fig. 7

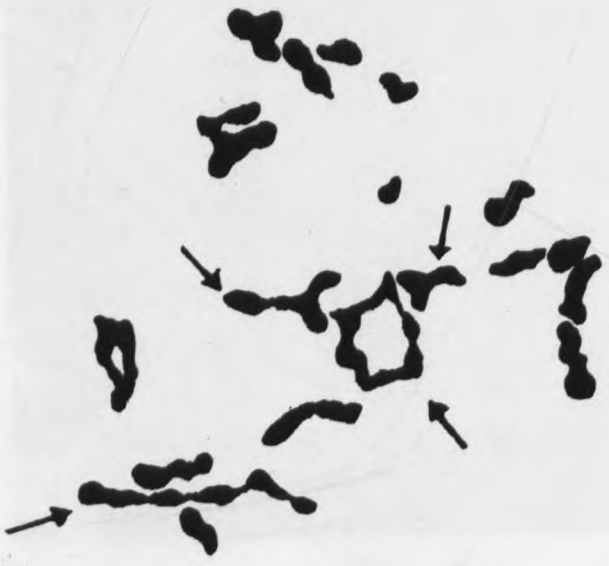

FIG 7

Line drawings of metaphase I associations in plant B6.1361. (F1R)*, (phase contrast, x1250). Arrows show examples of trivalents and quadrivalents. (Also see plate

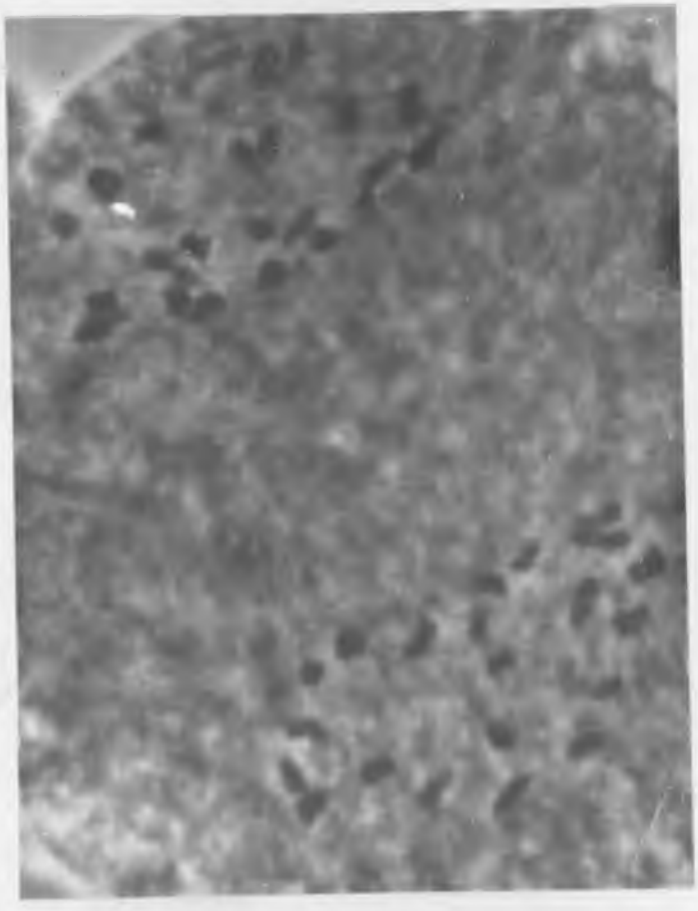

PLATE 13

Normal distribution of chromosomes in first anaphase a plant of C. arabica cv. SL 28 (Magnification 1250 X)
Also see Fig. 8.

FIG. 8.

Line drawings of chromosomes in Anaphase I during Meiosis in plant BB. 1172 (C. arabica cv. SL 28). (see plate 13).

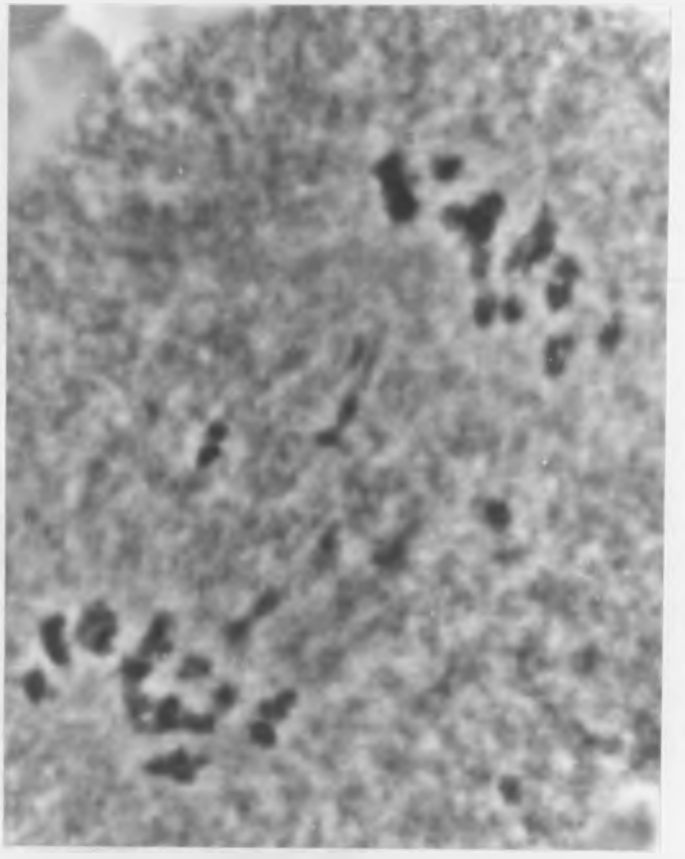

PLATE 14

Distribution of chromosomes over first anaphase poles in a plant of arabusta type F_1A. Note lagging chromosomes. (Mag. 1250 X) Also see Fig. 9.

FIG. 9

Line drawings of Anaphase I chromosomes during Meiosis in plant B6.1392 (F1A). (see also plate 14).

CHAPTER 10

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