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INTERSPECIFIC HYBRIDIZATION BETWEEN *COFFEA ARABICA* L. AND TETRAPLOID *C. CANEPHORA* P. EX FR. II. MEIOSIS IN F_1 HYBRIDS AND BACKCROSSES TO *C. ARABICA*

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INDEX WORDS

Coffea arabica, coffee, induced tetraploid *C. canephora*, interspecific hybrids, arabusta hybrids, meiosis, chromosome association, chromosome distribution, fertility, backcrossing.

SUMMARY

Meiosis was studied in *Coffea arabica*, in induced tetraploid *C. canephora*, in their F_1 hybrid (arabusta hybrid) and in backcross generations of the hybrid with *C. arabica* as recurrent parent. Irregularities were observed, consisting of univalents (especially in the arabusta hybrid), multivalents (especially in tetraploid *C. canephora*) and uneven distribution of chromosomes at first anaphase. Chromosome distribution was improved by backcrossing. Meiotic irregularities were negatively correlated with pollen fertility.

INTRODUCTION

The interspecific hybrid between *C. arabica* and induced tetraploid *C. canephora*, the arabusta hybrid was created with the objective of improving the liquor quality of robusta coffee (CAPOT et al., 1968). Despite its superior liquor quality with respect to robusta (CAPOT, 1972), the hybrid has not yet gained general commercial adoption. The main drawback lies in its reduced fertility which has justifiably drawn considerable attention (DE REFFYE, 1974; GRASSIAS, 1977; DUCEAU, 1980; OWUOR & VAN DER VOSSEN, 1981). Considerable effort geared to the improvement of the ferility of the arabusta hybrid has been directed at selection within the induced tetraploid *C. canephora* base population (IFCC – SATMACI, 1977). It has also been suggested that restoration of fertility could be achieved by backcrossing the arabusta hybrid to *C. arabica* (OWUOR & VAN DER VOSSEN, 1981). Studies on the meiosis of triploid hybrids have shown high pairing between the chromsomes of the two species indicating that the two species have a common genome (MONACO, 1965; KAMMACHER & CAPOT, 1972; KAMMACHER, 1980). GRASSIAS (1977) observed high univalent frequency in two arabusta hybrid plants. She attributed most of this to precocious separation of bivalents, because in subsequent stages very few chromosomes went astray and distribution in anaphase was comparatively less irregular. She concluded that the low fertility of the arabusta hybrids had a cytogenetic background, though results of selection for regular meiosis were not available. Since backcrossing of arabusta hybrids to *C. arabica* resulted in a rapid restoration of fertility (OWUOR & VAN DER VOSSEN, 1981) a study of meiosis was undertaken.

MATERIALS AND METHODS

Meiosis was studied in three plants of induced tetraploid *C. canephora,* in six plants of arabusta (F_1) hybrid and in three plants of the first backcross of F_1 to *C. arabica* (BC_1). In addition a check was made on the meiosis of one plant of BC_2 and *C. arabica.* A detailed description of the generations has been made earlier (OWUOR & VAN DER VOSSEN, 1981).

Flower buds were sampled at the growth stage optimal for observing meiosis (OWUOR, 1980) and were fixed in a 6:3:1 mixture of ethanol, chloroform and glacial acetic acid. The mixture also contained 5% hydrochloric acid. After fixation for at least 24 hours the buds were stored in 70% ethanol solution at 5 °C. Whole anthers were squashed in aceto-carmine-haematoxylin stain according to KAMMACHER & CAPOT (1972). Pollen mother cells with well individualised Metaphase I and Anaphase I stages were chosen for analysis of chromosome association and distribution. Pollen fertility was estimated from pollen germination in 10% sucrose solution as described in WALYARO & VAN DER VOSSEN (1977). Sample correlation coefficients between components of meiosis and pollen fertility were calculated.

RESULTS AND DISCUSSION

Observations of chromosome associations at Metaphase I showed that univalents and multivalents (trivalents and quadrivalents) occurred in all plant types (Table 1). The induced auto-tetraploid *C. cenephora* had a high frequency of multivalents as might be expected. In the arabusta hybrid, a high frequency of univalents was found which however decreased significantly ($P < 0.05$) in the first backcross generation mainly at the expense of a slight increase in the frequency of bivalents. With bivalents and quadrivalents, rings were generally more preponderant over rods and chains, while with trivalents, various configrations including often 'Y' types and occassional 'frying pans' were seen. Figures 1–4 show examples of some of the configurations of chromosomes at Metaphase I.

Distribution of chromosomes at Anaphase I was analysed, viz: the percentage of PMCs with even (22/22) or uneven ($22 \pm x$) distribution of chromosomes, or with lagging chromosomes. Anaphase poles with 22 chromosomes were generally low in tetraploid *C. canephora* and in the arabusta hybrid, though the latter had significantly ($P < 0.05$) more poles with 22 chromosomes than *C. canephora* and showed a higher variation (Table 1). In this respect the BC_1 showed more than 60% increase over the hybrids. Figure 5 and 6 show examples of disjunctions at Anaphase I.

Considering both Metaphase I association and distribution at Anaphase I it was clear that the induced tetraploid *C. canephora* and the arabusta hybrids showed a highly disturbed meiosis. However, whereas in *C. canephora* it is the multivalent associations (trivalents and quadrivalents) that apparently largely contributed to the irregular disjunctions in Anaphase I and subsequent stages, in the arabusta hybrids this anomaly mainly appeared to arise from univalents. Despite the high univalent frequency, Anaphase I distribution was better regulated in the arabusta hybrid than in tetraploid *C. canephora.* This observation lends further support to that of GRASSIAS (1977) who attributed the high univalent frequency in the arabusta hybrid to preco-

Table 1. Summary of results of observations on meiotic components in a cultivar of *Coffea arabica*, in induced tetraploid *C. canephora*, in interspecific arabusta hybrids of *C. arabica* and tetraploid *C. canephora* (F_1) and in backcrosses of the hybrid to *C. arabica* (BC_1 and BC_2).

Species/Generation	Number of plants	Number of cells	Frequency of chromosome assocation per pollen mother cell at Metaphase I[1]			
			I	II	II	IV
C. arabica cv. SL28	1	16	1.8	19.9	0.1	0.5
Tetraploid *C. canephora*	3	38	2.4 ± 1.8	14.9 ± 0.9	0.8 ± 1.7	2.3 ± 1.0
Arabusta F_1 hybrid	6	75	6.1 ± 0.8	17.4 ± 0.4	0.8 ± 0.1	0.2 ± 0.2
BC_1–F_2	3	27	1.8 ± 2.5	18.8 ± 0.3	0.2 ± 0.01	0.9 ± 0.6
BC_2–F_2	1	15	4.9	18.5	0.1	0.4
			Frequency of pollen mother cells at Anaphase I-2			Pollen fertility (% germ.)
			22/22	22 ± x	laggs.	
C. arabica cv. SL28	1	20	97.6	2.2	0.2	77.5
Tetraploid *C. canephora*	3	55	40.3 ± 3.0	56.1 ± 2.4	3.3 ± 0.3	5.6 ± 0.9
Arabusta F_1 hybrid	6	104	48.4 ± 5.8	49.6 ± 5.5	2.5 ± 1.1	9.3 ± 1.9
BC_1–F_2	3	55	79.5 ± 5.4	19.6 ± 5.3	0.9 ± 0.1	33.0 ± 4.0
BC_2–F_2	1	22	77.3	21.7	0.8	56.5

[1] I = univalent, II = bivalent, III = trivalent, IV = quadrivalent.
[2] Cells with even (22/22) or uneven (22 ± x) distribution of chromosomes, or cells with lagging chromosomes (laggs.).

cious separation of bivalents and quadrivalents and with the inevitable increase in the poles disjoining regularly.

In Table 2, sample correlation coefficients between a number of meiotic components and pollen fertility are given. All correlation coefficients are high and higly significant ($P < 0.01$). It is concluded that the low fertility of the induced tetraploid *C. canephora* and arabusta hybrids are mainly the result of meiotic disturbances. This result is consistent with previous observations (GRASSIAS, 1977). Since KAMMACHER (1980) has

Table 2. Coefficients of correlation between components of meiosis and pollen fertility in tetraploid *Coffea canephora*, in interspecific arabusta hybrids of *C. arabica* and tetraploid *C. canephora*, and in the first backcross of this hybrid to *C. arabica* (n = 9, ** = significant at $P \leq 0.01$)

Item	3	4	5
1. Frequency of univalents per PMC at Metaphase I	-0.831**	0.741**	-0.831**
2. Frequency of bivalents per PMC at Metaphase	0.808**	-0.789**	0.866**
3. Frequency of PMCs at Anaphase I with even chromosome distribution	–	–	0.888**
4. Frequency of PMCs at Anaphase I with lagging chromosomes	–	–	-0.728**
5. Pollen fertility (% germination)	–	–	–

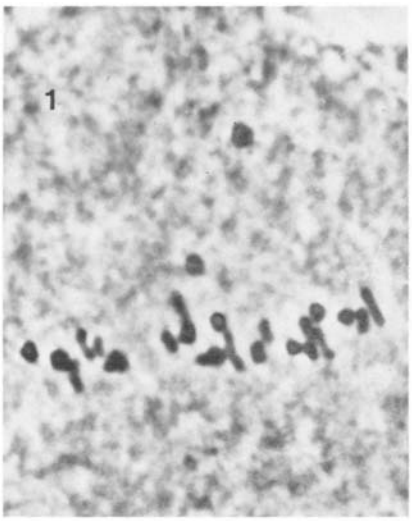
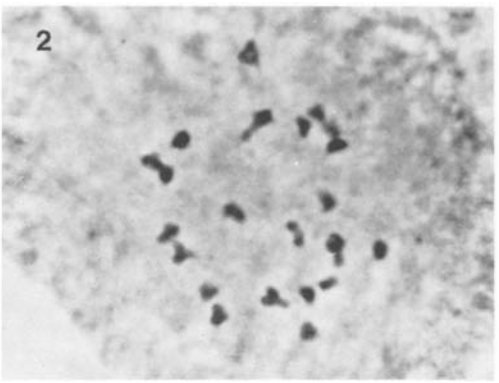
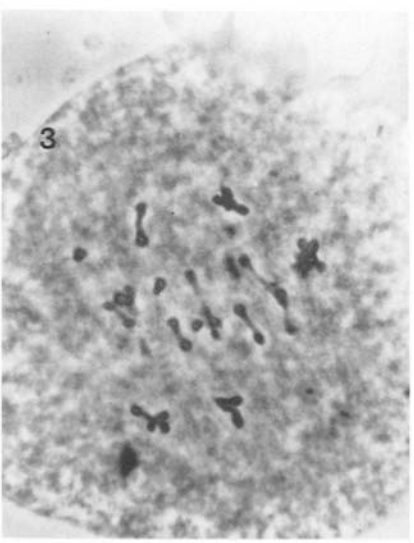


Fig. 1. MI in tetraploid *C. canephora*.





Figs 2 and 3. MI in Arabusta hybrids.

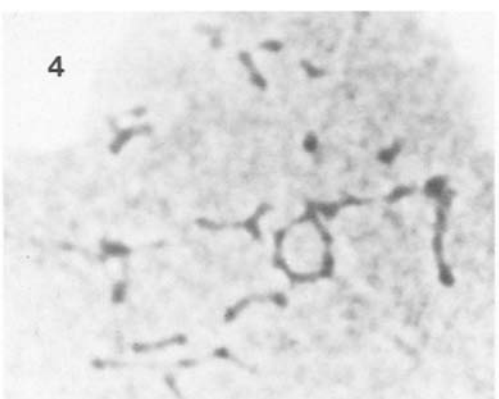


Fig. 4. MI in BC_1–F_2.

observed significant homeologous pairing and also owing to the fact that the two species hybridised have a genome in common (KAMMACHER & CAPOT, 1972), the dramatic response of meiosis to backcrossing might occur through elimination of non-homologous chromosomes, without any attendant deleterious effects due to compensation (SYBENGA, 1975). Backcrossing interspecific hybrids in *Lolium* species has been observed to lead to improved pairing (REES & JONES, 1967). The favourable response to backcrossing observed in the present study parallels that reported on fertility (OWUOR & VAN DER VOSSEN, 1981) and lends further support to the suggestion that genotypes selected in the first backcross generation (BC_1 of the arabusta hybrids to *C. arabica*) are more likely to be adopted for commercial planting than the arabusta hybrids.

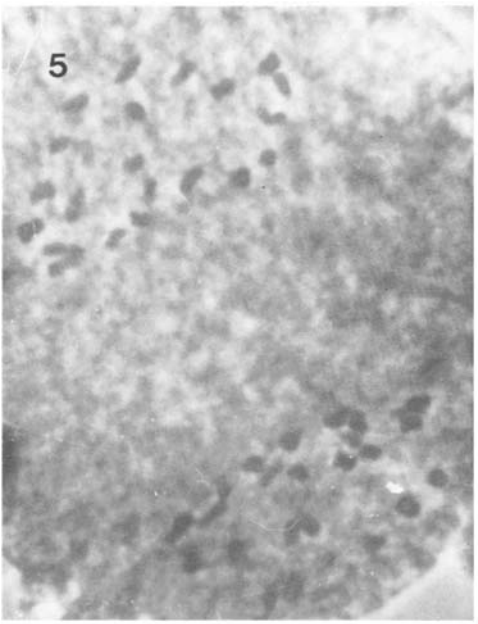


Fig. 5. Anaphase I in BC_1–F_2.

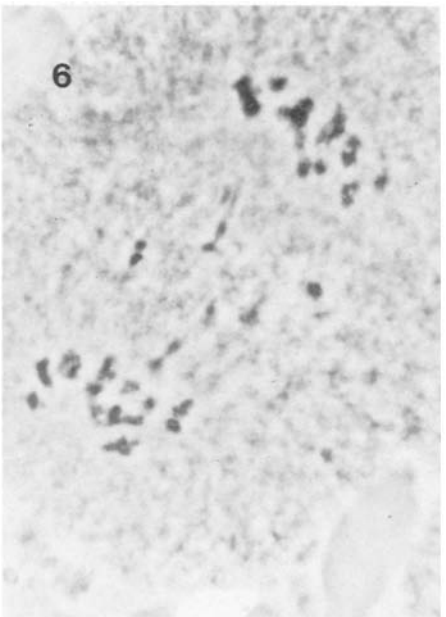


Fig. 6. Anaphase I in Arabusta hybrid.

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