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Gene introgression into *Coffea arabica* by way of triploid hybrids (*C. arabica* × *C. canephora*)

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Interspecific triploid hybrid plants between the tetraploid species *Coffea arabica* L. and the diploid species *C. canephora* P. were backcrossed to *C. arabica*. Although characterised by a low production and an important fruit dropping, all attempted crosses (ie, 6) generated BC_1 progenies. Flow cytometric analysis of the nuclear DNA content revealed that most of the BC1 individuals were nearly tetraploid. Among the male gametes produced by the interspecific triploid hybrids, those presenting a high number of chromosomes appeared strongly favoured. Only pollen mother cells having nearly 22 chromosomes were effective, the others leading to deficient endosperm and fruit dropping. Molecular markers (ie, microsatellite and AFLP) combined with evaluations of morphological characteristics and resistance to leaf rust were applied to verify the occurrence of gene transfer from *C. canephora* into *C. arabica*, and to estimate the amount of introgression present in BC_1 individuals. The results reveal a strong deficiency in the *C. canephroa* alleles indicating a severe counter-selection against the introgression of genetic material from *C. canephora* into *C. arabica* by way of triploid hybrids. However, introgressants displaying desirable traits such as a high resistance to leaf rust were obtained. The low level of introgression could be an advantage by facilitating the recovery of the recurrent parent and possibly reducing the number of required backcrosses. On the other hand, this could be a limitation when attempting the transfer of a complex trait or several simply inherited traits.
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Introduction

Coffee-trees (family Rubiaceae) differ greatly in morphology, size and ecological adaptation, thereby leading to the description of a large number of species. The subgenus *Coffea* (genus *Coffea* L.) encompasses more than 80 taxa so far identified, including the two species of economic importance: *C. arabica* L. and *C. canephora* Pierre (Charrier and Berthaud, 1985). Higher quality is associated with *C. arabica*, which accounts for 70% of world coffee production. *Coffea arabica* is the only tetraploid species ($2n = 4x = 44$) in the genus while other species are diploid ($2n = 2x = 22$). Recent investigations established that *C. arabica* is an amphidiploid formed by natural hybridisation between two closely related diploid species, *C. canephora* and *C. eugenioides* (Lashermes *et al*, 1999). In spite of the low divergence between the two constitutive genomes, *C. arabica* displays a diploid-like meiotic behaviour (Krug and Mendes, 1940; Lashermes *et al*, 2000a,b). Furthermore, *C. arabica* is characterised by low genetic diversity and the transfer of desired traits from diploid relative species has been a continuous priority in coffee breeding (Carvalho, 1988; Van der Vossen, 2001).

Plant interspecific hybridisation is a common means of extending the range of variation beyond that displayed by the parental species. However, inherent problems of interspecific introgression such as hybrid instability, infertility, non-Mendelian segregations, and low levels of intergenomic crossing-over can constitute important limitations (Stebbins, 1958). Moreover, features associated with polyploidy or ploidy dissimilarity between species may result in supplementary constraints for interspecific gene flow (Rieseberg *et al*, 2000).

Occurrence of spontaneous hybrids between *C. arabica* and diploid relative species such as *C. canephora* and *C. liberica* is common, especially when these species grow in direct proximity (Cramer, 1957). In artificial conditions, the success in producing viable interspecific hybrids appears to depend on the direction of the cross. When *C. arabica* is used as maternal parent, successful crosses have been obtained with a large number of diploid species. In contrast, attempts to produce hybrids using *C. arabica* as pollen donor have not yet been successful (Carvalho and Monaco, 1968; Le Pierès, 1995). The resulting interspecific hybrids are usually triploid and rather vigorous. While hexaploid hybrids (ie, obtained by duplication of triploid hybrids) and tetraploid hybrids (ie, resulting from the hybridisation between *C. arabica* and auto-tetraploidised diploid parents) appear reasonably fertile (Berthaud, 1978), the triploid hybrids are highly sterile. Meiosis in these triploid hybrids is especially disturbed (Krug and Mendes, 1940; Chinnappa, 1968; Kammacher and Capot, 1972). For instance, a high frequency of meiotic irregularities (eg, 7.8 to 14.4 univalents depending on the study) in chromosome associations at metaphase I has been

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observed. Similarly, anaphase distributions appear very irregular. Nevertheless, viable gametes occur occasionally and these hybrids have been successfully backcrossed to *C. arabica* (Orozco, 1976). The agronomic evaluation of advanced lines (ie, F_4) derived from such triploid hybrids reveal an important potential of production often combined with an enhanced resistance to leaf rust caused by the fungus *Hemileia vastatrix* (Alvarado and Cortina, 1997). The resistance to this serious disease of coffee is assumed to result from the introgression of one or several resistance genes that have been identified in *C. canephora* (Bettencourt and Rodrigues, 1988).

Despite a considerable interest for coffee breeding, genetic study in relation to interspecific triploid hybrids has been very limited. In particular, development of efficient strategies for selection of triploid-derived progenies would require a better knowledge of genome interactions and factors affecting genetic exchange in triploid hybrids. The purpose of the study presented here was to gain insights into the type of viable gametes that are produced in triploid *C. arabica* × *C. canephora* hybrids, and to evaluate the gene flow occurring from *C. canephora* to *C. arabica* in this context. Six progenies resulting from the backcross to *C. arabica* of several interspecific triploid hybrids *C. arabica* × *C. canephora* were produced. The nuclear DNA content of both triploid F_1 and BC_1 individuals was assessed by flow cytometry which is a simple and efficient tool for estimating ploidy level in coffee trees (Barre *et al*, 1996). Molecular markers combined with evaluations of morphological characteristics and resistance to leaf rust were applied to verify the occurrence of gene transfer from *C. canephora* to *C. arabica*, and to estimate the amount of introgression present in BC_1 individuals.

Materials and methods

Plant material

Triploid F_1 hybrids were generated by crossing tetraploid *C. arabica* cv caturra individuals, used as female parents, with three different diploid accessions (ie, EC 40, EC 103 and EC 137) of *C. canephora*. The accessions of *C. canephora* were selected to represent contrasted genotypes and different collecting sites (ie, Angola and Uganda) in the natural distribution area of this species in Africa. A total of six triploid hybrids, two from each *C. canephora* accession, were produced as part of the experiment Meg0635 from the breeding programme of CENICAFE. These plants were named H 880, H 881, H 861, H 866, H 846 and H 855, respectively. Then, the different hybrids were backcrossed as the male parent to the elite line ASIII of *C. arabica*. Six BC_1 progenies were produced (Table 1) and 60 BC_1 plants (10 plants/progeny) were retained for further analysis. The different crosses were performed by means of artificial pollination using standard hybridisation techniques as described by Carvalho (1988). Briefly, flowers of selected healthy branches are emasculed 2 or 3 days before anthesis and protected from pollen contamination. Pollen is collected and stored under dry-condition at 5°C in a hermetically sealed box up, and when required directly put on the style with the help of a small brush.

Determination of nuclear DNA content

The total DNA amount in nuclei of parental species, hybrid and BC_1 individuals was assessed by flow cytometry (Dolezel *et al*, 1989). Segments (5 × 5 cm) from fresh leaves were excised, and chopped with a razor blade in a plastic Petri dish containing 1 ml of nuclei extraction buffer. The cell suspension was then filtered through 48 μm-mesh nylon and the nuclei suspension was incubated for 5 min in propidium iodide (Sigma P4170) at a final concentration of 0.33 mg/ml as described by Barre *et al* (1996). Two independent replicates (ie leaf samples) per individual were prepared, and for each run a minimum of 5000 nuclei per sample were analysed using a FACScan (Becton Dickinson) cytometer equipped with an argon laser (15 mW). Each measurement was repeated at least twice. Coefficients of variation of the 2C fluorescence peaks ranged from 4.3% to 6.2% depending on the individuals. To ascertain the ploidy level of hybrid and BC_1 individuals, the 2C peak values were compared with the values determined (four replicates) for the diploid (*C. canephora* accession EC 37) or tetraploid (*C. arabica* var. Caturra) parental species. Comparison of nuclear DNA content between the mean values of BC_1 progenies was made using the Newman and Keuls test while the BC_1 progenies and the recurrent *C. arabica* parent were compared by the rank-sum test developed by Mann and Whitney (1947).

Molecular marker assay

Nineteen microsatellite loci, previously identified as polymorphic between *C. arabica* and *C. canephora*, were analysed using PCR. Some of these microsatellite loci (Table 2) have been mapped in *C. canephora* (Lashermes *et al*, 2001). The specific primer pairs, amplification conditions, radioactive labelling and polyacrylamide gel electrophoresis were as reported elsewhere (Combes *et al*, 2000). For each hybrid, the microsatellite loci revealing *C. canephora* specific bands (ie alleles) were determined and surveyed in the corresponding BC_1 progeny. The amplified fragment length polymorphism (AFLP) procedure was performed as previously reported (Lashermes *et al*, 2000). Briefly, 500 ng of genomic DNA was digested with the restriction enzymes *Eco*RI and *Mse*I. Restriction fragments were then ligated with double-strand *Eco*RI and *Mse*I adapters. A selective preamplification was performed using the appropriate primers (named E and M, respectively) with one selective nucleotide at the 3′ end (ie E+A/M+C). The reaction mixture was diluted 1/30 and 10tμL was used for the final amplification with two primers, each containing three selective nucleotides (Table 3).

Field characterisation of BC_1 progenies

BC_1 individuals were further evaluated under field conditions at the Naranjal field experimental station of CENICAFE (Chinchina, Colombia). Each individual tree was recorded for three different morphological traits (ie plant height, leaf shape, and branching pattern) that are known (Stoffelen, 1998) to discriminate the two parental species, namely *C. arabica* and *C. canephora*. Subsequently, three morphological types of reference were defined and scored based on a 1–3 scale where 1 designated the 'arabica type', 2 the 'intermediate type', and 3 the 'canephora type', respectively. Furthermore, the resistance to leaf rust (*Hemileia vastatrix*) was scored on a 0–9 field scale as

Table 1 Production of interspecific backcross progenies by pollination of *Coffea arabica* (line ASIII) with pollen originated from different interspecific triploid hybrids (*C. arabica* × *C. canephora*)

BC_1 Progeny	*Pollen donor hybrid*	*No of pollinated flowers*	*Fruit set after 1 month (%)*[a]	*Fruit set after 7 months (%)*[a]	*Final number of recovered seeds*	*Cross success (%)*[b]
BC 4535	H 880	397	78.6	6.0	22	5.5
BC 4530	H 881	318	88.1	9.8	42	13.2
BC 4532	H 861	194	69.1	10.8	23	11.9
BC 4531	H 866	454	92.3	11.9	48	10.6
BC 4533	H 846	389	69.2	7.0	36	9.3
BC 4534	H 855	259	87.3	10.8	28	10.8

[a]Percentage of fruit set was calculated from the number of collected mature fruits/number of pollinated flowers ×100.
[b]Cross-success percentage was calculated from the final number of recovered seeds/number of pollinated flowers ×100.

Table 2 Microsatellite markers showing *Coffea canephora* specific bands in the different triploid interspecific hybrids (*C. arabica* × *C. canephora*)

Microsatellite Loci	*Linkage group*[b]	*Triploid hybrids*[a]					
		H 880	*H 881*	*H 861*	*H 866*	*H 846*	*H 855*
M 12	5	+		+	+	+	+
M 20	9			+	+	+	+
M 24	–	+		+	+	–	–
M 32	–	+		+	+	+	+
M 41	3	+		+	+	–	–
M 42	3			–	–	–	+
M 47	4	–		+	+	+	+
M 66	–	–		–	–	+	+
M 109	–	–		+	+	+	+
M 111	–	+	+	+	+	+	+
M 157	3	–		–	–	+	+
M 160	1	+		+	+	+	+
M 162	–	+	+	+	+	+	+
M 166	–	+	+	+	+	–	–
M 171	5	+	+	+	+	+	+
M 177	9			+	+	+	
M 180	–	+		+	+	+	+
M 181	10	+	+	+	+	+	+
M 189	9	+		–	–	–	–
	Total	12	5	15	15	14	14

[a]+, – indicate the identification or the absence of *C. canephora* specific markers, respectively.
[b]Genetic linkage groups as determined in *C. canephora* (Lashermes *et al*, 2001).

Table 3 Number of *Coffea canephora* specific AFLP markers identified in three interspecific triploid hybrids (*C. arabica* × *C. canephora*). Primer combinations named as E+3 and M+3, represents the 3′ end selective nucleotides of the primers complementary to the *Eco*RI and *Mse*I adapters, respectively

Primer combination		*Triploid hybrids*		
E + 3	*M + 3*	*H 861*	*H 846*	*H 855*
ACT	CTT	3	3	1
AAC	CTA	4	2	2
ACA	CAA	0	2	2
AAC	CAC	4	1	0
ACA	CTT	6	4	3
AAG	CTT	7	5	7
ACA	CAC	5	5	4
ACT	CAT	2	3	3
AGC	CAG	2	4	3
ACC	CAG	4	4	4
AGG	CTA	6	3	2
AAC	CAT	8	9	9
AAC	CAG	4	5	4
AAG	CTA	5	5	5
ACT	CAT	2	5	3
Total		62	60	52

proposed by Eskes and Toma-Braghini (1981), where 0 represents a complete resistance and 9 indicates a high rust susceptibility. Surveys were performed twice a year (March and October) during the most favourable periods for rust development. The final tree score was the average of the separate observations.

Results

Crossability of interspecific triploid hybrids

Results on BC_1 progeny production from 6 different interspecific triploid *C. arabica* × *C. canephora* hybrids are summarised in Table 1. BC_1 progenies were produced for all attempted backcrosses, the triploid hybrids being used as male parent. For all crosses, the number of initiated fruits 1 month after pollination was rather high. The fruit set varied from 69.1 to 92.3% depending the cross considered. However, a large proportion of fruits fell during the subsequent months. Thus, the number of fruits was markedly reduced seven months after pollination, the fruit sets varying from 6.0 to 11.9%. Finally, the frequency of recovered seeds per pollinated flower ranged from 5.5 to 13.2%, which was rather low and constant whatever the interspecific triploid hybrids used.

DNA content variation

2C nuclear DNA contents of parental species, hybrid and BC_1 individuals were successfully assessed (Figure 1). As expected, all interspecific triploid hybrids exhibited a DNA content intermediate between the two parental species, thus confirming their ploidy level (ie 3×) and the possibility of comparing genome sizes by flow cytometry. The DNA content of BC_1 plants varied appreciably around the value of the recurrent tetraploid *C. arabica* parent. The mean value of BC_1 plants (ie 775.3) was not significantly different ($P = 0.398$) from the estimate for *C. arabica* (ie 795.2). On the other hand, the mean value for BC_1 plants was significantly higher ($P > 0.01$) than a hypothetical value (ie 706.7) corresponding to the mean

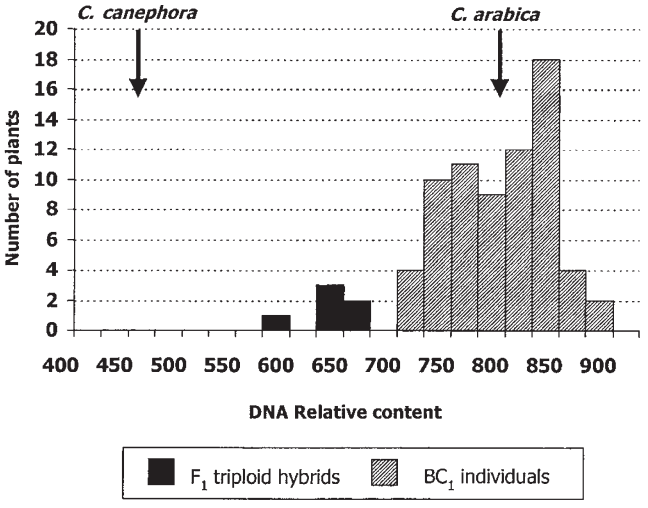

Figure 1 Distribution of interspecific triploid hybrids and BC_1 individuals according to their DNA content as determined by flow cytometry. For comparison, mean values estimated for the diploid (*Coffea canephora*) and recurrent tetraploid (*C. arabica*) parent species are indicated.

between the two parents involved in the backcrosses, namely the interspecific triploid hybrids and the recurrent tetraploid *C. arabica*.

Analyses of variations in DNA content within and between the different BC_1 progenies are given in Table 4. Within progenies, important differences in DNA content were observed. Nevertheless, with the exception of one offspring (ie BC 4530), all analysed BC_1 progenies were not significantly different ($P > 0.05$) from *C. arabica*. Slight differences were observed among progenies. For instance, the mean value varied significantly between BC_1 progenies. It is notable that the two BC_1 progenies (BC 4535 and 4530) exhibiting a lower mean DNA content ($P > 0.05$) were derived from two hybrids involving the same accession (ie EC 037) of *C. canephora* as parent.

Molecular analysis of BC_1 progenies

Molecular analysis was performed to detect *C. canephora*-specific markers in the interspecific triploid hybrids and the BC_1 individuals. A total of 19 microsatellite loci that are distributed on at least six of the 11 linkage groups of the *C. canephora* genetic map were investigated. For two interspecific triploid hybrids (ie H880 and H881), only subsets of 16 and five microsatellite loci, respectively, were used. The number of identified microsatellite loci exhibiting a *C. canephora*-specific allele varied from five to 15 depending on the interspecific triploid hybrids considered (Table 2). Subsequently, all pertinent microsatellite loci were surveyed in the six corresponding BC_1 progenies (Table 5). *Coffea canephora*-specific markers were detected in only eight out of the 58 BC_1 individuals analysed. These introgressed plants (ie showing at least one *C. canephora*-specific marker) belong to four different BC_1 progenies and exhibited between 1 and 10 *C. canephora*-specific markers, the mean number being 3.8 markers. The frequency of *C. canephora*-specific markers was determined for each locus as well as at the plant and backcross level. *Coffea canephora*-specific markers were observed for 16 of the 19 microsatellite loci studied. These loci belong to at least six different linkage groups of the *C. canephora* map. The loci M 20, M 171 and M 189 for which no *C. canephora*-specific markers was observed, are those analysed for only a restricted number of backcrosses. Furthermore, frequency of *C. canephora*-specific markers was 4.1% (range 0 to 13%) depending on the BC_1 progeny considered. The mean percentage of *C. canephora*-specific markers in the introgressed individuals was about 30%.

In complement to microsatellites, AFLP analysis using 15 distinct primer combinations was performed on three BC_1 progenies (Table 3). The number of *C. canephora*-specific bands in the different triploid hybrids varied between 52 and 62. Out of the 30 BC_1 plants (10 per progeny) scored, only two displayed *C. canephora*-specific bands (Table 5). One of these two plants also presented *C. canephora*-specific microsatellite-allele. The mean frequency of *C. canephora* AFLP specific bands (ie, 2.3, ranging between 1 to 8) in BC_1 individuals was similar to the value estimated for the microsatellite loci.

Field observation of BC_1 progenies

Sixty BC_1 individuals were evaluated for tree morphology and resistance to leaf rust (Figure 2). In agreement with the observed absence of *C. canephora*-specific molecular markers, a large majority of BC_1 individuals showed an arabica-like phenotype (type 1). No more than six BC_1 individuals were classified as intermediate or

Table 4 Variation in DNA content among the BC_1 individuals, and between the different BC_1 progenies and the recurrent *Coffea arabica* parent

BC_1 Progeny	*No. of plants*	*Range*	*Mean ± SD**	*Comparison BC_1 vs C. arabica U value†*
BC 4535	10	693.3–861.9	750.2 ± 59.12 ab	8.0 (0.089)
BC 4530	10	697.6–785.7	726.7 ± 28.51 a	2.0 (0.011)
BC 4532	10	677.2–829.9	799.0 ± 44.19 c	17.0 (0.671)
BC 4531	10	773.1–834.9	794.1 ± 22.39 c	18.0 (0.777)
BC 4533	10	768.7–820.5	798.8 ± 17.45 c	19.0 (0.887)
BC 4534	10	710.3–819.0	783.2 ± 39.83 bc	17.0 (0.671)
C. arabica	4	750.6–825.5	795.2 ± 32.33	

*Comparison among progenies was made using the Newman and Keuls test. Values followed by the same letter are not significantly different at $P < 0.05$.
†The U values were determined by using the rank-sum test proposed by Mann and Whitney (1947). For each comparison, probability values are indicated in parentheses.

Table 5 DNA marker analysis of *Coffea canephora* introgression in different BC_1 progenies derived from triploid interspecific hybrids (*C. arabica* × *C. canephora*)

Type of marker	*BC_1 Progeny*	*No. of scored plants*	*No. of loci/bands scored*	*No. of introgressed plants*[a]	*Frequency of C. canephora specific markers (%)*[b]
Microsatellites	BC 4535	10	120	3	13.3
	BC 4530	8	40	1	2.5
	BC 4532	10	150	0	0
	BC 4531	10	150	2	2.0
	BC 4533	10	140	0	0
	BC 4534	10	140	2	7.1
AFLP	BC 4532	10	620	0	0
	BC 4533	10	600	1	0.5
	BC 4534	10	520	1	6.2

[a]A plant was considered as introgressed when at least one *C. canephora* marker was detected.
[b]Percentage of *C. canephora* specific markers was calculated as: number of *C. canephora* loci (or bands)/total number of analysed loci (or bands).

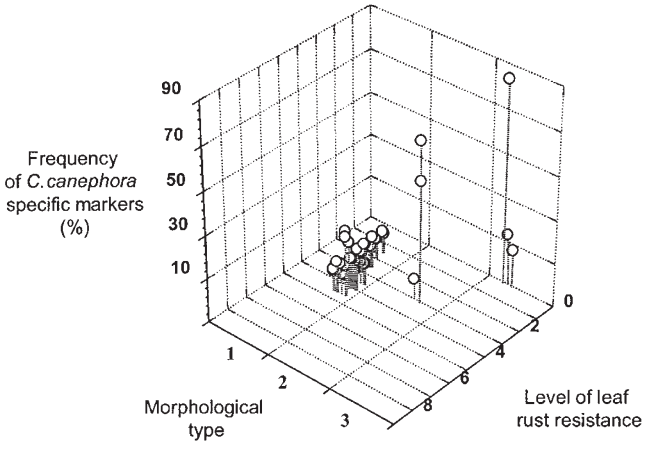

Figure 2 Relationship between phenotype, rust resistance and introgression level among 60 BC_1 individuals derived from different triploid interspecific hybrids (*Coffea arabica* × *C. canephora*). Plant morphological types were designated as 1 'arabica', 2 'intermediate', or 3 'canephora' according to the morphological similarities with parental species. Leaf rust resistance was scored according to the field scale (0–9) proposed by Eskes and Toma-Braghini (1981), where 0 represents complete resistance and 9 indicates high rust susceptibility.

canephora morphological types (types 2 and 3, respectively). Unsurprisingly, these plants displayed *C. canephora*-specific markers.

Regarding leaf rust, most BC_1 individuals appeared either susceptible or moderately resistant. Only three trees exhibited a complete resistance to leaf rust. These trees belonged to the canephora morphological type and appeared introgressed. It is notable that, for two BC_1 individuals, the complete resistance to leaf rust is combined with a low frequency of *C. canephora*-specific markers.

Discussion

In the present study, all crosses performed between interspecific (*C. arabica* × *C. canephora*) triploid hybrids used as male parent and *C. arabica* successfully yielded progeny plants. The mean production of 10 seeds per 100 pollinated flowers clearly reflected the low fertility of triploid hybrids. Fruit setting in coffee tree is affected by many factors including the vegetative state of the mother plant, the pollination conditions and the climatic environment during fruit development (Carvalho, 1988). However, in typical artificial conditions (ie intraspecific), fruit setting varied between 30% and 60% in *C. arabica* (Le Pierres, 1995; Alvarado, unpublished data).

Whatever the origin of the *C. canephora* accessions used to produce the interspecific triploid hybrids, an overall homogeneous behaviour was observed in the BC_1 progenies. The flow cytometric analysis revealed that the generated BC_1 individuals had a DNA content close to that of *C. arabica*. Although allowing only a crude estimation (Barre *et al*, 1996), this observation indicates that most BC_1 plants were nearly tetraploids with about 44 chromosomes. Thus, among the male gametes produced by the interspecific triploid hybrids, only those presenting a high number (ie diploid) of chromosomes could successfully generate viable hybrid seeds. Pollen with about 22 chromosomes would have a selective advantage over gametes presenting a lower number of chromosomes and level of ploidy.

In angiosperms, the successful formation of an embryo is not only dependent on fertilization but also on normal development of the endosperm. Endosperm is a major food and feed source of embryos, playing a capital role in seed formation or failure in interploidy or interspecific crosses (Brink and Cooper, 1947). Analyses in several genera have led to the development of the endosperm balance number (EBN) hypothesis (Johnston *et al*, 1980). The balance of qualitative genetic factors in endosperm (EBN) has been proposed as a determinant mechanism in endosperm development, and thus of the embryo after intra- and inter-ploid interspecific crosses (Ehlenfeldt and Ortiz, 1995; Carputo *et al*, 1999). Although this concept has not yet been fully investigated in coffee, related mechanisms have been invoked to explain the contrasted results obtained in reciprocal crosses between *C. arabica* and several diploid species at different ploidy levels (Carvalho and Monaco, 1968; Berthaud, 1978). It has been suggested (Le Pierres, 1995) that the normal development of the endosperm, and indirectly of the coffee embryo, would be conditioned by the presence of a minimal dosage (ie ploidy) of one parental genome species whatever the endosperm/embryo balance.

In the present study, the reduced fruit set observed in backcrosses of interspecific triploid hybrids to *C. arabica* appeared clearly associated with a severe drop of fruits during the development phase. It is probable that only pollen mother cells having nearly 22 chromosomes generated effective ploidy, the others leading to deficient endosperm and fruit dropping. In addition to this strong postzygotic barrier, fertilisation with aneuploid gametes which are likely to be produced by the interspecific triploid hybrids may also result in embryo abortion and fruit or seed development failure as commonly reported in coffee material derived from interspecific crosses (Carvalho, 1988).

Interspecific hybrids are often characterised by distorted marker-segregations (Lytte, 1991). In the present investigation, the combined use of microsatellite-PCR and AFLP methods were appropriate to mark and ensure a good coverage of the *C. canephora* genome. However, among the different BC_1 progenies, the number of individuals exhibiting *C. canephora*-specific markers appeared particularly small (ie mean frequency of 13.8%). In the same way, an overall low frequency of *C. canephora*-specific markers was observed, even when considering only the identified introgressed plants (ie. observed frequency of 5.3%). Assuming that each of the chromosomes in the sets of three in the triploid hybrid have an equal chance to be included in the diploid gametes formed, the expected proportion of *C. canephora* alleles in a tetraploid backcross would be 0.66. Analysing on average twelve microsatellite loci in a tetraploid backcross, the expected proportion of individuals without any *C. canephora* alleles would be 1:531441 which is much less than the observed value of 1:1.2. Hence, our data revealed a strong deficiency in *C. canephora* alleles indicating a severe counter-selection against the introgression of genetic material from *C. canephora* into *C. arabica* by way of triploid hybrids (*C. arabica* × *C. canephora*). In contrast, the segregation of *C. canephora* alleles transmitted by tetraploid interspecific (*C. arabica* × *C. canephora* 4×) hybrids has been reported to conform with the expected ratio assuming random chromosome segregation and the absence of selection (Herrera *et al*, 2002). The presence of *C. canephora* alleles in the functional gametes of tetraploid hybrids was neither significantly beneficial nor detrimental. This suggests that the deficiency in *C. canephora* alleles is more likely to be due to mechanisms specific to triploid hybrids, associated with the formation of diploid gametes, rather than to the consequence of a counter-selection related to the expression of lethal or sub-lethal *C. canephora* genes. For instance, detailed studies carried out in triploid hybrids of *Lolium* species showed that the orientation and respective position of chromosomes within the multivalents could have a significant effect on the incorporation of specific alleles in the balanced gametes (Thomas *et al*, 1988).

Genetically modified coffee plants including *C. canephora* are approaching commercialisation (Leroy *et al*, 2000). Among the concerns related to genetically engineered crops, the risk of gene escape toward wild relatives is one of the most discussed (Rissler and Mellon, 1996). Regarding coffee species, spontaneous hybridisation between coffee species are known to occur, and the results presented here reveal a potential gene flow, between *C. canephora* and *C. arabica*, which should be studied further.

The use of triploid (*C. arabica* × *C. canephora*) hybrids provides an opportunity to transfer desirable traits for crop improvement. For example, introgressants displaying a high resistance to leaf rust were obtained that constitute a promising starting point for breeding programmes (Alvarado and Cortina, 1997). In comparison with the data reported for tetraploid interspecific (*C. arabica* × *C. canephora* 4×) hybrids (Herrera *et al*, 2002), the level of introgression observed in *C. arabica* when using triploid hybrids appears noticeably low. This could be an advantage by reducing the possibility of introgression of undesirable traits and facilitating the recovery of the recurrent parent. Hence, the triploid hybrid route could recommended for single trait introgressions. On the other hand, this could be a limitation when attempting the transfer of complex traits or several simply inherited traits.

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