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Introgression of self-compatibility from *Coffea heterocalyx* to the cultivated species *Coffea canephora*

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Abstract Self-compatibility segregation was assessed in two successive backcross progenies originating from an interspecific cross between *Coffea canephora* (self-incompatible) and *Coffea heterocalyx* (self-compatible). After self- and cross-pollination, pollen tube behaviour in styles was observed under ultraviolet fluorescence microscopy and fruit-set was determined at harvesting time. Segregation ratios in the two progenies were consistent with monofactorial control of self-compatibility. Self-compatible plants exhibited higher fruit-set than self-incompatible ones in open-pollination conditions. Segregation of AFLP markers was scored in the first backcross progeny. By molecular linkage analysis, the S locus could be mapped to a short linkage group.

Keywords *Coffea canephora* · Back-cross · Self-compatibility · Fruit-set · S locus

Introduction

Self-incompatibility (SI) is a widespread genetically controlled mechanism that prevents self-fertilization and promotes cross-fertilization in flowering plants (de Nettancourt 1977). In the SI gametophytic system, the incompatibility reaction results from the matching of the pollen S allele with one of the two alleles of the diploid pistil.

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All but *Coffea arabica* L and *Coffea heterocalyx* are self-incompatible among more than 80 described coffee taxa (Stoffelen et al. 1996). In *Coffea canephora*, self-incompatibility (SI) is due to a gametophytic system controlled by a single multiallelic gene (Devreux et al. 1959; Berthaud 1980) that was mapped on a short linkage group by Lashermes et al. (1996).

From an agronomic viewpoint, as for many self-incompatible fruit tree species, *C. canephora* plant productivity is strongly influenced by: (1) field association of at least two cross-compatible trees, (2) synchronous flowering trees, and (3) the availability of pollinating agents such as wind and insects. In addition, SI is a key drawback for obtaining homozygous lines. In coffee, some haploids, arising from polyembryonic seeds, had given homozygous dihaploids after colchicine treatment, but only from four parental trees (Couturon and Berthaud 1982; Lashermes et al. 1994a). In this context, self-compatibility introgression in *C. canephora* from self-compatible species could be of potential interest.

We investigated an interspecific cross between *C. canephora* and *C. heterocalyx,* for which F1 and backcrossed hybrids BC1 and BC2 were available (Louarn 1992), and assessed the introgression potential of self-compatibility in *C. canephora*. The first results concerned the inheritance of self-compatibility, the location of the gene on a genetic map and the impact of self-compatibility on fruit-set. The potential use of marker-assisted selection in the next BC generations is also discussed.

Materials and methods

Plant material

Plant materials were obtained and are maintained at the IRD [1] coffee breeding station of Man in the Côte-d'Ivoire. *C. heterocalyx* (HET) is only represented by a homozygous seed-derived line from a single wild tree of uncertain origin (probably the Cameroon).

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Interspecific F1 hybrids were obtained by crossing the *C. canephora* (CAN) genotype # IF182 (used as female) and the *C. heterocalyx* plant as described by Louarn (1992).

The most-fertile F1 hybrid # 2C017 had been pollinated with a mixture of pollen originating from four CAN plants, generating the backcross-1 (BC1) progeny. The other backcross progeny (BC2) was obtained from three controlled crosses involving BC1 self-compatible hybrid pistil parents and CAN pollinators: D160436 × IF181; D160437 × IF444; and D160538 × IF444.

Self-compatibility tests

Pollen-tube growth and fruit setting were used to determine the self-compatibility of each hybrid.

Pollen-tube growth

Pollen–pistil interactions were assessed in cross-pollination and self-pollination under ultraviolet fluorescence microscopy according to the ABF (aniline blue fluorescence) method (Martin 1959; Rêgo et al. 2000). About 36 h after pollination, pistils were harvested from SP and OP branches. After fixation in a FAA solution [formaldehyde at 40%, pure acetic acid and 95% ethanol (at a 1:1:8 ratio)], they were stored at 4 °C. Later, pistils were: (1) rinsed in water; (2) softened by submerging in a NaOH solution of 1 N for 24 h; (3) washed again; and (4) stained for 12 h with 1% aniline blue containing 0.1 M of K_2PO_4. Pistils were observed by fluorescence microscopy under ultra-violet light at 350–400 nm. Pollen tube growth was visualised by callose fluorescence on walls. Five pistils were observed per branch. Pollen germination on the stigma and the extent of growth of pollen tubes into styles were recorded.

Fruit setting

The day before flowering, two branches per tree having at least 100 flowers were bagged. On the flowering day, branches were shaken to allow self-pollination (SP). The bags were removed 2 days later. All new floral buds were also removed. For each tree, two similar branches were also monitored as open-pollination (OP) controls. Fruit-set was counted at maturity.

It was important, in SP conditions, to separate the effect of meiotic sterility (male and/or female) from self-incompatibility, which could influence the results. We thus set a fertility threshold in order to avoid low female- and male-fertility plants. Male fertility was estimated by the percentage of viable pollen grains using the acetic carmine procedure of Belling (Grassias 1980), and female fertility was estimated by fruit-set on open-pollinated branches. A hybrid was classified as self-compatible only when the fruit-set was >0% in SP conditions; a hybrid was classified as self-incompatible when the fruit-set was 0% in SP and >10% in OP conditions, and when pollen viability was >20% (data not shown). Thus, 26 BC1s and 23 BC2s were analysed.

Mapping of the self-compatibility gene

The mapped BC1 progeny consisted of a larger BC1 population, i.e. 74 individuals. The map construction and mapping strategy were previously described for a similar interspecific cross, *Coffea pseudozanguebariae* × *Coffea liberica dewevrei* (Ky et al. 2000). AFLP (amplified fragment length polymorphism) markers specific to HET (absent in CAN) were used for mapping. For mapping, self-compatibility was scored as a qualitative trait (presence or absence).

Data analysis

The software program Mapmaker/Exp version 3.0b (Lander et al. 1987) was used to determine linkage groups and order loci. Analyses were performed with a LOD score threshold of 5.0 and a maximum recombination value of 30% for grouping and ordering markers. A Jacknife procedure was applied to estimate a confidence interval for the autogamy gene location (Lebart et al. 1982). It consisted of computing data each time with a different one of the individuals observed being ignored.

Statistical analyses were carried out using Statistica software (5.1 version 1997 for Microsoft Windows). The two-way ANOVA model was used to test differences between BC1 and BC2, and between self-compatible and self-incompatible hybrids for fruit-set.

Results

Hybrid self-compatibility

Evaluation of hybrid self-compatibility using two complementary procedures reduced the risk of plant misclassing.

Pollen-tube behaviour

The ABF method allowed early determination of self-compatibility. We observed one of the two following patterns on selfed pistils:

(1) bulbous-shaped short pollen tubes in the stigma area and rarely in the third upper of the style (Fig. 1),
(2) long pollen tubes up to at least two-thirds of the style transmitting-tract-length towards the ovary (Fig. 2) and the concomitant presence of inhibited pollen tubes in the stigmatic region.

These patterns corresponded to self-incompatibility and self-compatibility, respectively. Thus, in BC1 12 hybrids were classified as self-compatible and 14 hybrids were classified as self-incompatible. In BC2, 13 hybrids were classified as self-compatible and 12 hybrids were classified as self-incompatible.

Fruit setting

The results obtained with the ABF method were checked against the observations of fruit setting. All trees classified as self-compatible by the ABF method set fruit in SP conditions. Thus in BC1, the same 12 hybrids, versus 14, did not set fruit in SP conditions (Table 1). This segregation did not differ from the 1:1 ratio and was confirmed in BC2, with 13 plants setting fruit versus 12 in SP conditions (Table 2).

A two-way ANOVA had been performed in order to test both generation (BC1 vs BC2) and compatibility (self-compatible vs self-incompatible) effects on fruit-set in OP conditions. No significant difference was noted between BC1 and BC2 for fruit-set in open-pollination conditions ($F_{1,42}$ = 2.34; P = 0.134). On average, self-compatible hybrids set 27.3% of fruit. This fruit-set is significantly higher than that of self-incompatible ones which is 18.4% ($F_{1,42}$ = 7.04; P = 0.0112). No interaction was detected between the tested effects ($F_{1,42}$ = 0.03;

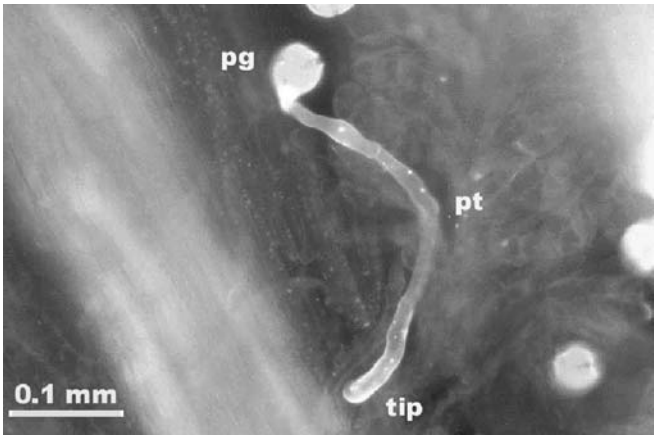

Fig. 1 Incompatible pollen-pistil reaction: pollen grain (*pg*) germinated in a short pollen tube (*pt*), the *tip* of which is vesiculated in the stigmatic area

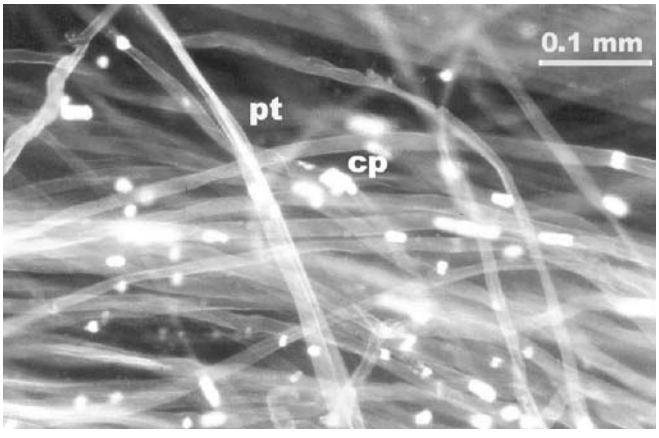

Fig. 2 Compatible pollen-pistil reaction: long pollen tubes (*pt*) with regular callose plugs (*cp*) run through the style-transmitting tract towards the ovary

Table 1 Self-compatibility in BC1 hybrids determined by fruit-set comparison in self-pollination (SP) and open-pollination (OP)

Self-compatible			Self-incompatible		
BC1 hybrid	SP	OP	BC1 hybrid	SP	OP
D15-0938	4%	22%	D15-0635	0%	11%
D16-0133[a]	9%	4%	D15-0734	0%	16%
D16-0319	13%	24%	D15-0839	0%	16%
D16-0333	6%	20%	D15-1035	0%	14%
D16-0436	12%	25%	D16-0117	0%	13%
D16-0437	14%	16%	D16-0339	0%	10%
D16-0527	45%	49%	D16-0425	0%	24%
D16-0538	17%	28%	D16-0522	0%	10%
D16-0623	8%	18%	D16-0526	0%	11%
D16-0814[a]	5%	3%	D16-0531	0%	19%
D16-0817[a]	4%	9%	D16-0620	0%	17%
D16-0831[a]	6%	4%	D16-0926	0%	32%
D16-0921	11%	34%			
D16-1038	16%	30%			
Mean	12%	20%		0%	16%

[a] Indicates plants in which crossed fruit-set was affected by ovary fall or branch breaking during fruiting. These values were not taken into account for further analysis

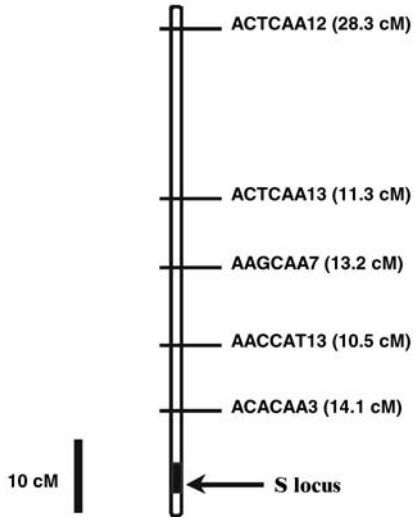

Fig. 3 S locus location in a linkage group constructed with AFLP markers. Marker names are symbolised on the right side of the linkage group: the *first three letters* correspond to selective nucleotides of *Eco*RI primers and the *second three letters* designate selective nucleotides of *Mse*I primers. The *number* designates the size of the marker, with *smaller numbers* representing fragments of greater sizes. The *number in brackets* indicates the genetic distance in cM (Kosambi) separating a marker and the marker below. The *arrow* indicates the most-likely position estimated by the Jacknife process with the Mapmaker/Exp version 3.0b software program

Table 2 Self-compatibility in BC2 hybrids determined by fruit-set comparison in self-pollination (SP) and open-pollination (OP)

Self-compatible			Self-incompatible		
BC2 hybrid	SP	OP	BC2 hybrid	SP	OP
D14-1701[a]	11%	9%	D14-1607	0%	18%
D14-1907[a]	13%	7%	D14-1608	0%	25%
D14-1908	25%	12%	D14-1610	0%	16%
D14-1936	24%	18%	D14-1630	0%	18%
D14-2010	38%	38%	D14-1707	0%	13%
D14-2030	37%	52%	D14-1708	0%	56%
D14-2110	9%	42%	D14-1825	0%	14%
D14-2207	10%	31%	D14-1830	0%	22%
D14-2225	3%	38%	D14-2007	0%	21%
D14-2237	2%	13%	D14-2108	0%	17%
D14-2330	19%	20%	D14-2307	0%	16%
D14-2402	29%	50%	D14-2437	0%	12%
D14-2525	21%	18%			
Mean	19%	27%		0%	21%

[a] Indicates plants in which crossed fruit-set was affected by ovary fall or branch breaking during fruiting. These values were not taken into account for further analysis

$P = 0.87$). However, mean fruit-set in the self-compatible hybrids was lower than half of that of the recipient self-incompatible parent CAN (50.9%).

Self-compatibility gene location

The self-compatibility locus was mapped at the end of a short linkage group comprising five markers for a total length of 63.2 cM (Fig. 3). The Jacknife method showed

Table 3 Genotype and phenotype frequencies at the S locus, and pollen self-compatibility behaviour in CAN and HET parents, and F1, BC1 and BC2 hybrids. NB: F1 self-compatibility behaviour is assumed. Their reduced fertility was too low for our experiments. In order to simplify the scheme, CAN heterozygosity at the S locus was not represented

Item	CAN	HET	F1 CAN × HET	BC1		BC2	
				F1 × CAN		BC1 × CAN	
Genotype at the S locus	S_IS_I[a]	S_CS_C[b]	S_IS_C	S_IS_C	S_IS_I	S_IS_C	S_IS_I
	100%	100%	100%	50%	50%	50%	50%
Phenotype	$[S_I]$[c]	$[S_C]$[d]	$[S_C]$	$[S_C]$	$[S_I]$	$[S_C]$	$[S_I]$
	100%	100%	100%	50%	50%	50%	50%
Pollen self-compatibility behaviour	–[e]	+[f]	–50%	–50%	–	–50%	–
	+100%	100%	+50%	+50%	100%	+50%	100%

[a] S_I= self-incompatible alleles
[b] S_C = self-compatible allele
[c] $[S_I]$ = self-incompatible phenotype
[d] $[S_C]$ = self-compatible phenotype
[e] – = self-incompatible pollen
[f] + = self-compatible pollen

a 5.4 cM-long interval most likely bearing the S locus. An AFLP marker (ACACAA3) located about 10 cM from the S locus could be used in marker-assisted selection.

Discussion

Model of self-compatibility inheritance

The self-compatibility 1:1 segregation ratio is consistent with the assumption of Mendelian monofactorial control of self-compatibility. This inheritance agrees with Berthaud's (1980) results on *C. canephora*, confirmed by Lashermes et al. (1996), i.e. gametophytic polyallelic monogenic control of self-incompatibility.

In many self-incompatible species, with a gametophytic system and monogenic inheritance, S alleles code for glycoproteins, with specific stylar ribonuclease activity acting against pollen-tube growth (Bredemeijer and Blaas 1981; Mau et al. 1986; McClure et al. 1989; Ai et al. 1990; Bošković et al. 1997; Ishimizu et al. 1998). Self-compatible variants appear when this activity disappears or strongly decreases. This is the case in *Prunus dulcis* (Dicenta and Garcia 1993; Socias i Compani et al. 1995; Bošković et al. 1999), *Lycopersicon peruvianum* (Kowyama et al. 1994), *Petunia inflata* (Huang et al. 1994) and *Pyrus pyrifolia* (Sassa et al. 1997). Although little is known about the biochemical basis of self-incompatibility in *Coffea*, one could reasonably argue that self-compatibility in *C. heterocalyx* could comply with such a model. In other words, in *C. heterocalyx*, alleles at the S locus could have no inhibition effect against self-pollen. This hypothesis is supported by the self-pollen behaviour on the pistil of hybrids, which represented S-allele segregation. Self-incompatible hybrids displayed only inhibited self-pollen tubes on their stigma. All these gametes could have inherited self-incompatibility alleles (S_I) from CAN; while on selfed pistils of self-compatible hybrids we could see both non-inhibited pollen tubes and inhibited pollen tubes. These two types of gametes have inherited the self-compatibility allele (S_C) from HET and self-incompatibility alleles S_I from CAN, respectively. Hence, self-compatible hybrids and self-incompatible hybrids could be S_CS_I and S_IS_I, respectively, at the S locus. According to this pattern, we propose a crossing scheme between CAN and HET with a theoretical gene frequency in the offspring (Table 3).

Self-compatibility and fruit-set

This work has highlighted the positive impact of self-fertility on fruit-set. As abundantly described in the literature, self-compatible species have a higher fruit set than self-incompatible ones (Sutherland and Delph 1984; Sutherland 1986). In self-incompatible plants, self-incompatibility adverse action inhibits all self-pollen and some allo-pollen, thus reducing the fertilisation rate. The lack of such a system in self-compatible plants allows them to take part of the self-pollen and allo-pollen to increase fruit-set. This was confirmed in coffee trees: fruit-set in the self-compatible *C. arabica* and in the self-incompatible *C. canephora* was 61% (Le Pierres 1995) and 40% (de Reffye 1974), respectively. Fruit-set in our BC1 and BC2 self-compatible hybrids was 27% vs 18% in self-incompatible hybrids, representing a similar fruit-set gain (about 50%). After fertility restoration, a fruit-set improvement could be expected in introgressed *C. canephora* varieties, i.e. a productivity gain.

S locus genetic mapping and marker-assisted selection (MAS)

Despite the small-sized segregating BC1 population used for self-compatibility, the S locus was relatively accurately mapped. The limit of accuracy was mainly due to the low frequency of fertile hybrids in the BC1. A larger population with higher fertility, i.e. BC2 or BC3 progenies, would improve the location accuracy. Bulk segregation analysis could be applied to the two sets of compatible and incompatible BC1 and BC2 hybrids to identify additional markers linked to the S locus.

Lashermes et al. (1996), using RFLP markers in a *C. canephora* dihaploid population, had previously

mapped the S locus on a short linkage group comprising three markers: probably a conservative chromosomal segment with low polymorphism. These RFLP markers, of which two bracketed the S locus and the other co-segregated with it, could be sequenced, primer synthesized and converted to PCR-based markers as described by Kolchinski et al. (1997) and Ghareyazie et al. (1996) in soybean and rice, respectively. Screening our segregating BC1 population with these markers could enable identifying their location on the AFLP map and then estimate the possible co-localisation of the S locus.

The availability of map- and S locus-linked markers should facilitate a marker-assisted selection. MAS efficiency was studied by simulation in relation to the total number of markers, their distribution on the genome and around the gene to introgress (Hospital et al. 1992). These authors showed that the use of markers mapped at least 12 cM from the trait locus was more efficient in the early generations. In these conditions, the ACACAA3 AFLP marker could be used to screen potential introgressed hybrids in the BC1 and BC2, and further backcross progenies. Nevertheless, a second marker located on the other side of the S locus would improve MAS efficiency. Another screening criterion could be the percentage of HET-specific AFLP markers, which should be lower in hybrid genomes. It should thus be possible to speed up genome recovery of the recurrent parent and to detect self-compatible hybrids at the plantlet stage, therefore avoiding field planting of potentially useless hybrids.

Self-compatibility could enable mass production of CAN fixed lines. Although hampered by inbreeding depression, homozygous lines could be useful for genetic studies and CAN breeding, as demonstrated by Berthaud et al. (1987) and Lashermes et al. (1993, 1994b).

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