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Flowering phenology and yield-related traits in an interspecific cross between *Coffea pseudozanguebariae* Bridson and *C. canephora* Pierre

Doffou Sélastique Akaffou[1,*], Ibrahim Konate [1], Raoul Sylvère Sié [2], Valérie Poncet [3], Irié Arsène Zoro Bi [2], Jules Keli [4], Hyacinthe Legnate [4], Alexandre de Kochko [3], Serge Hamon [3] and Perla Hamon [3]

[1]Université Jean Lorougnon Guédé, BP 150 Daloa, Côte d'Ivoire
[2]Université Nangui Abrogoua, 02 BP 801 Abidjan 02, Côte d'Ivoire
[3]IRD, UMR DIADE, BP 64501, 34394 Montpellier cedex 5, France
[4]Centre National de Recherche Agronomique, 01 BP 1740 Abidjan 01, Côte d'Ivoire

***Corresponding author: selastique.akaffou@ujlg.edu.ci**

Abstract

Higher caffeine content and cup beverage bitterness considerably depreciated the commercial value of *Coffea canephora* Pierre (CAN) compared with *C. arabica* L (ARA). Wild caffeine-free species like *C. pseudozanguebariae* Bridson (PSE) offer the opportunity to produce new CAN varieties containing little or no caffeine. F_1 plants resulting from a PSE × CAN cross, and BC_1 individuals, derived from the first backcross generation (PSE × CAN) × CAN) were produced. In order to assess flowering phenology and yield traits in F_1 and BC_1 hybrids, six morphological characters including flowering time, pollen viability (PV), fructification rate (FR), seed set (SSET), flower number per node (NFN) and 100-bean weight (W100) were studied under environmental conditions in Côte d'Ivoire. The results showed that F_1 plants flowered only in February while, for BC_1, 20% and 80% of the plants flowered in January and February, respectively. The fertility and productivity parameters in F1 were much lower than those of the parental species. Indeed, 5-13% of the pollen was viable depending on the year, the fructification rate was 9.6%, seed set was 1.02, the 100-bean weight was 8 g and seven flowers developed per node. These parameters did not differ from those recorded for the BC_1 hybrid. Following multiple linear regression analysis, significant and positive relationships were found between the fructification rate and seed set (r=0.54) and between flower number per node and 100-bean weight (r=0.26), while no significant correlations were computed between these four characters (FR, SSET, NFN and W100) and pollen viability. The principal component analysis revealed that the three principal factors of variability among the BC_1 offspring were female and male fertility as well as yield. Interestingly, two groups of plants (fertile and productive) were identified among the BC_1 individuals and will be used for subsequent backcrosses.

Keywords: *Coffea pseudozanguebariae; Coffea canephora;* flowering; interspecific hybridization; pollen viability.
Abbreviations: ARA_*Coffea Arabica* L., CAN_*Coffea canephora* Pierre, EUG_*Coffea eugenioides* Moore, NFN_Flower number per node, FR_Fructification rate, HET_*Coffea heterocalyx* Stoffelen, LIB_*Coffea liberica* Hiern, PV_Pollen viability, PSE_*Coffea pseudozanguebariae* Bridson, RAC_*Coffea racemosa Lour,* SSET_Seed set, QTL_Quantitative trait loci, W100_ 100-bean weight.

Introduction

The *Coffea* genus consists of 124 species (Davis et al., 2011). However, only two species are widely cultivated, i.e. *Coffea arabica* L. (ARA), tetraploid, autogamous and adapted to highlands (above 1000 m elevation), and *Coffea canephora* Pierre (CAN), diploid, allogamous and adapted to lowlands. The other species are diploid and allogamous, except for *Coffea heterocalyx* Stoffelen (HET) (Stoffelen et al., 1996) and *Coffea anthonyi* Stoffelen (Stoffelen et al., 2009), which are diploid but autogamous. Otherwise all African coffee species contain caffeine, except *Coffea pseudozanguebariae* Bridson (PSE) (Hamon et al., 1984) and, recently, *Coffea charrieriana* (Stoffelen et al., 2008). ARA coffee has good beverage flavor and contains low caffeine in comparison to CAN (Leroy et al., 2011), whose beverage flavor is less appreciated by consumers. Improving CAN organoleptic quality through reducing the caffeine content while increasing the yield is thus of major importance for CAN breeders (Leroy et al., 2011). Since the 1970s, interspecific hybridization has been presented as an encouraging means to improve CAN. This species has thus crossed with ARA (Capot, 1972), *Coffea eugenioides* Moore (EUG) (Louarn, 1976), *Coffea liberica* Hiern (LIB) (Louarn, 1980), *Coffea racemosa* Lour (RAC) (Louarn, 1985), HET and PSE (Louarn, 1992). These crosses were aimed at transferring, into the CAN genome, favorable organoleptic quality traits of ARA, RAC or EUG, the yield components of LIB, the self-compatibility of HET and the absence of caffeine and/or the short fructification time of PSE. However, to date, no trait has been introgressed into CAN from *Arabica* or from the wild diploid species. Obtaining hybrids was difficult (Louarn, 1992). Furthermore, the F_1 plants could not be used directly for breeding since they exhibited a high level of sterility, e.g. the F_1 hybrid pollen viability and fructification rates were very low (less than 10% on average) in crosses with EUG and RAC (Louarn, 1976, 1985, 1992). While, in the parental species, the pollen viability and fructification rate exceeded

80% and 50% on average, respectively (Akaffou, 1999; Louarn, 1992; N'Diaye, 2004). In the first backcross generation progeny (BC_1), pollen viability was partially restored with an average of 30-40% of viable pollen grains. A cross obtained between CAN and LIB was exceptional because the F_1 hybrids were easily produced. In addition, their pollen viability and fructification rate were high, i.e. 54% and 41% on average, respectively (Louarn, 1980). Alien introgressions generally represent a rich source of genes for crop improvement (Gill et al., 2011; Hajjar and Hodgkin, 2007). However, interspecific hybrid sterility represents one of the major barriers to gene transfer (Stebbins, 1958). Hence, understanding mechanisms underlying plant sterility in many crops is broad field of investigation. In coffee trees, hybrid sterility, notably pollen sterility, was thought to be due to genetic divergence between parental species (Louarn, 1992). Evidence of gene involvement in pollen viability was clearly shown by Coulibaly et al. (2003) through pv1, pv2 and pv3 QTLs. However, mechanisms leading to pollen sterility were not accurately identified as in rice where allele interactions or loss-of-function alleles caused pollen sterility in F1 hybrids (Oka, 1974; Yamagata et al., 2010; Win et al., 2010; Zhang et al., 2010). Studies on interspecific *Coffea* hybrids in Côte d'Ivoire were thus focused on trait inheritance, QTL analysis (Ky et al., 2000a; Coulibaly et al., 2003; Akaffou et al., 2003, 2012) and genetic mapping (Ky et al., 2000b; Coulibaly et al., 2001; N'Diaye, 2004). A cross between PSE and CAN—thus involving the only species cultivated in Côte d'Ivoire—is now well under way since the F_1 and BC_1 hybrids have reached the adult stage. Here we investigated: 1) the hybrid's flowering phenology, 2) some fertility parameters such as pollen viability (PV), the fructification rate (FR) and seed set (SSET), yield components such as the number of flowers per node (NFN) and 100-bean weight (W100) in F_1 and BC_1 progeny. These field evaluations will subsequently be used for QTL analysis as molecular genotyping is ongoing.

Results

Flowering phenology

Data recorded over the three consecutive years revealed abundant flowering in F_1 plants only in February, like the PSE parent. Among the eight F_1 plants surveyed, two never induced flower buds, while four to six of the remaining plants flowered depending on the year. For BC1, a total of 15 plants never developed flower buds during the three years, 80 to 90% of the remaining plants flowered each year, while 10 to 20% flowered in alternate years. On average, major flowering occurred at once in February for 80% of the BC1 progeny while the remaining 20% flowered in January. Moreover, flowering time was stable throughout the 3-year experimental period for all plants. Finally, out of the 280 BC1 plants monitored, the cumulated data over the three years resulted in 242 plants for which at least 10 flowers were counted on the whole plant. This plant number was reduced to 164 when a threshold of 100 flowers or more (on the whole plant) was considered.

Number of flowers per node

The number of flowers per node varied significantly between F_1 plants, from 4 to 10.3 ($F_{(4, 45)} = 6.2$; $p = 0.001$) with an average of 6.9±4.3. This average was much lower and departed from the mean parent value: (PSE + CAN)/2 = (4.5 + 36)/2=20.2, i.e. from additivity. Note that the NFN mean was 4.5 in PSE and 36 in CAN (Akaffou, 1999; N'Diaye, 2004). For the BC_1 population, NFN was on average 12.03±7.6. The 2-way ANOVA carried out on eight families consisting of six plants each, revealed that differences between families and between genotypes were highly significant (Table 1). NFN ranged from 8.7±7.0 to 17.6±10.8 between families and from 1.6±0.9 to 30.4±10.8 between plants. The trait distribution deviated from normality according to Shapiro–Wilks tests ($P = 0.004$). Most of the BC_1 fell within the parental range (Fig. 2), meanwhile some individuals scored lower NFN values than the parental PSE, which exhibited the lowest NFN. Figure 2 showed that NFN was highly skewed towards low values. About 13% of BC_1 plants developed less than five flowers per node, i.e. close to PSE (4.5), while 2% developed nearly 30 flowers per node, which was close to CAN (36). The NFN mean in BC_1 (12.03) also deviated from additivity: (F_1 + CAN)/2 = (7 + 36)/2 = 21.5 (Table 2).

Pollen viability

Viable pollen after acetocarmin staining was characterized by a red or pink homogenous cytoplasm, as showed in Figure 3. For F_1, the pollen viability (PV) was on average 13.2±6.8%, 9.7±11.1% and 5.13±3.5% in 2008, 2009 and 2010, respectively. The statistical analysis indicated that the PV varied significantly between years and between genotypes (Table 1). The year x genotype interaction was also significant and explained the high variation of individual PV between years, e.g. the PV of plant D08 522 was 8.67% in 2008, 24.67% in 2009 and 5% in 2010. The mean in 2008 (13.2±6.8%) appeared as the best and under these conditions the between tree (genotypes) variation ranged from 3.33% to 23.67%. In BC_1, the PV was 15.42±18.48% in 2008, 10.43±14.86% in 2009 and 8.84±14% in 2010. These means did not differ significantly from the F_1 ones for one given year. Moreover, 2008 appeared also the best year. The three way-ANOVA performed using a sample of six families and seven plants per family showed that the PV varied significantly between years, families and genotypes (Table 1). The different interactions were also highly significant. However, variation between plants was the highest, it ranged from 0 to 71.33% in 2008, 0 to 54.33% in 2009 and from 0 to 65.67% in 2010. The trait distribution deviated from normality and was highly skewed towards low values. In 2008, for example, 50% of the BC_1 had a PV of less than 5%, while 6% had a PV higher than 50% (Fig. 4). The same trend was noted in 2009 and 2010, in which 54% and 58%, respectively, of BC_1 plants had a PV of less than 5%. The cumulated data for the three years revealed that 19 BC_1 individuals, i.e. 24% of the BC_1, never developed pollen or produced unviable pollen and were thus male sterile. Eight other individuals had a PV that was always over 20%, regardless of the year. Among these latter plants, one was found to be exceptional—its PV was close to the values of the parental CAN or PSE, i.e. 71.33% in 2008, 40% in 2009 and 65.67% in 2010. Finally, even in the best year, the PV means for F_1 (13.2%) and BC_1 (15.42%) were much lower than that noted for the parental species (81-88%) and the mean parent value mid-parent value, 84.5% (Table 2).

Fructification rate

The fructification rate (FR) was on average 9.6±7.8% in F_1, varying significantly between trees from 1.07±1.3 to 22.02±

Table 1. ANOVA results relative to the test of year, family, genotype and interaction effects on flower number per node and pollen viability in BC_1

Morphological characters	Coffee hybrids	Source of variation	df	F
Flower number per node	BC_1	Family	7	9.0***
		Genotype	5	10.1***
		Error	432	
Pollen viability	F_1	Year	2	43.74***
		Genotype	3	25.14***
		Year x Genotype	6	38.04***
		Error	24	
Pollen viability	BC_1	Year	2	231.94***
		Family	5	188.34***
		Genotype	6	327.38***
		Year x Family	10	81.01***
		Year x Genotype	12	42.13***
		Error	252	

*** Significant at p<0.001

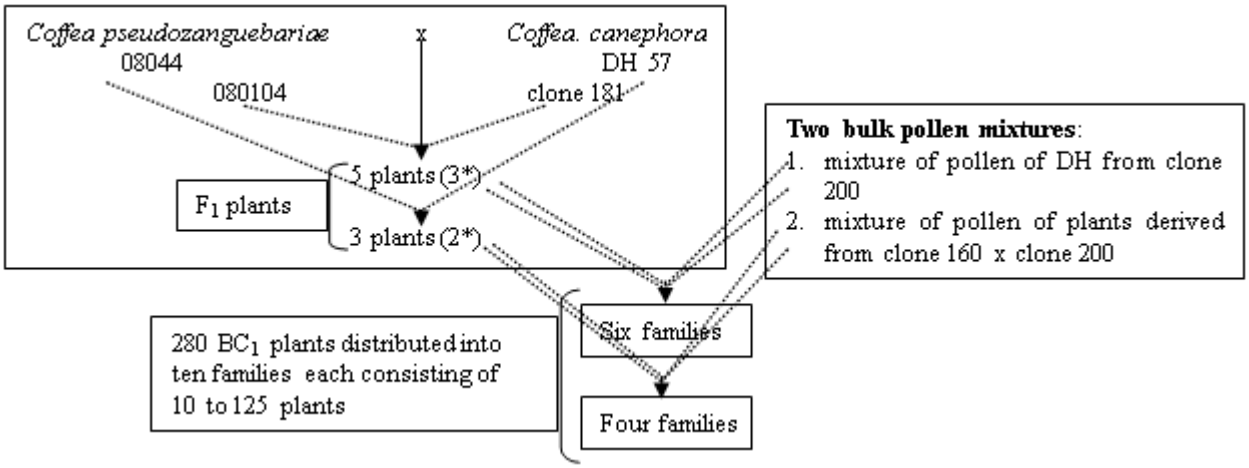

Fig 1. Mating design for the production of F_1 (hybrid between PSE × CAN) and BC_1 (first backcross generation (PSE × CAN) × CAN hybrid); number asterisks (*) correspond to the number of F_1 plants used as female parents to obtain BC_1; CAN and PSE represent *Coffea canephora* Pierre and *Coffea pseudozanguebariae* Bridson, respectively.

22.026.7%. In BC_1, out of the 42 plants used for the FR assessment, data were finally recorded on 33 plants since 9 plants had dead branches. The FR was 8.9±10.4% on average. The two-way ANOVA, performed using a sample of five families containing four plants each, revealed that FR variation between families (6.03±6.2 to 12.78±11.7%) was not significant ($F_{(4,72)} = 1.08$; $p = 0.373$). In contrast, there was a marked variation between trees, ranging from 0 to 30.4±7.0% (Fig. 5(A)). The FR distribution also deviated from normality; 54% of the BC_1 produced less than 5 fruits per 100 flowers in open pollination, whilst few plants (15%) yielded more than 20 fruits per 100 flowers. The FR of BC_1 plants (8.9%) was similar to that of F_1 plants (9.6%), but was substantially lower than the values of the parental species, (52.2% - 51.8%) and the mean parent value , 52.1% (Table 2).

Seed set

Seed set (SSET) in F_1 plants ranged from 1.0±0.0 to 1.1±0.1 and did not differ between the four plants studied. On average, fruit of F_1 plants contained 1.02±0.05 seed instead of 1.5 on average in the parental species. For BC_1 plants, seed set fluctuated significantly from 1.0±0.0 to 1.6±0.5 according to the genotype ($F_{(25, 65)} = 2.62$; $P = 0.001$). The mean value (1.1±0.2) did not differ from the F_1 value (1.02). The trait distribution (Fig. 5(B)) was identical to that of FR plants. Most fruits contained one seed, but three plants exhibited a high SSET, superior to 1.1 (1.4 to 1.6) and close to that of the parental species, i.e. 1.5 (Table 2).

Relationships between fertility and productivity parameters

The regression analysis revealed a significant correlation between FR and SSET ($r = 0.54$; $F_{(1,28)} = 11.90$; $P = 0.002$) on one hand, and between NFN and W100 ($r = 0.26$; $F_{(1,63)} = 4.40$; $P = 0.03$) on the other, while no significant correlation was noted between these four parameters and PV. The principal components analysis, performed on a sample of 26 plants for which data were recorded on PV, NFN, FR, SSET and W100 in 2008, confirmed the previously described relationships between these parameters. Three main factors explained the BC_1 plant diversity. The first factor (33%) explained the female fertility through FR and SSET. The

Table 2. Mean phenotype values for parental species (CAN and PSE), theoretical mid-parent median parent value, F_1 and BC_1 plants (range of variation in parenthesis).

Traits	CAN	PSE	Median parent value	F_1	BC_1
Pollen viability (%)	81 (62-98)	88 (64-96)	84.5	9.3 (0-25)	11.6 (0-71)
Fructification rate (%)	52.4 (41-65)	51.8 (22-86)	51.2	9.6 (0-22)	8.9 (0-30.4)
Seed set	1.5 (1.2-1.6)	1.5 (1.2-1.8)	1.5	1.02 (1-1.1)	1.1 (1-1.6)
Flower number per node	36 (11-76)	4.5 (2.7-8.2)	20.2	7 (4-10)	12 (1.6-30.4)

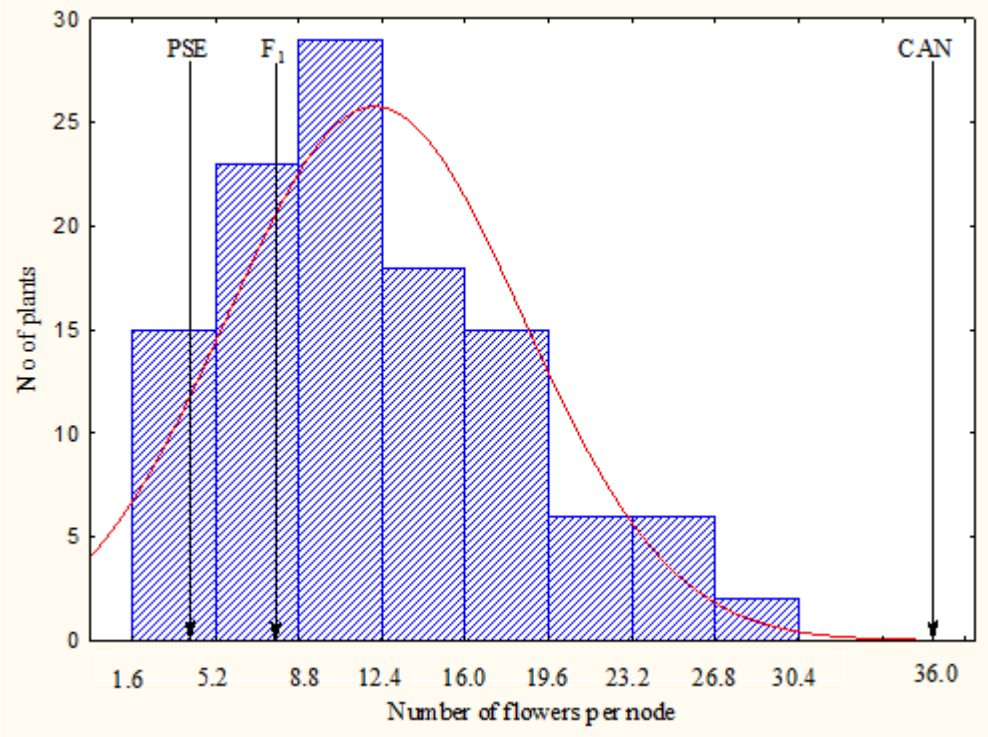

Fig 2. Distribution of the number of flowers per node in BC_1 (first backcross generation (PSE × CAN) × CAN hybrid) plants; the arrays indicate parental species (PSE and CAN) and F_1 (hybrid between PSE × CAN) means; CAN and PSE represent *Coffea canephora* Pierre and *Coffea pseudozanguebariae* Bridson, respectively.

second (29.2%), corresponded to the productivity and was correlated with NFN and W100 and the third factor (21%) was assigned to male fertility (PV). The scatter plot on a 1*2 factorial plane led to the identification of two groups (I and II) of interest (Fig. 6). The first group (I) was characterized by higher NFN and W100 values than obtained in group II: 23.6±4.1 vs. 12.7±3.5 ($F_{(1,9)} = 21.8$; $p = 0.001$) for NFN and 12.10±2.1 g vs. 9.5±1.6 g ($F_{(1,9)} = 5.4$; $p = 0.04$) for W100. In contrast, FR was higher in II than in I: 25.4±3.9 vs. 9.1±3.4 ($F_{(1,9)} = 53.5$; $p = 0.0001$). BC_1 plants simultaneously exhibiting high NFN, W100, FR and SSET were not found. F_1 and BC_1 progenies had intermediary values between the parental species, but the BC_1 individuals formed a continuum between the two parents when considering the productivity parameters (factor 2). While for fertility (factor 1), hybrid plants, F_1 and BC_1 had close values, but they differed markedly from the parental species, i.e. CAN and PSE (Fig. 6).

Discussion

Flowering phenology and inheritance

Hybrid flowering time is quite important from a commercial standpoint, and regarding adaptation, productivity or gene flow (Hao et al., 2008; Campoy et al., 2011; Jones et al., 2011). In coffee trees, male and female plants used in hybridization schemes are selected on the basis of flowering time knowledge, as reported by Louarn (1992): early flowering plants generally being used as male parents. This study revealed that F_1 plants flowered only in February, whereas for BC_1 20% and 80% of the plants flowered in January and February, respectively. Data on the parental species showed that *Coffea canephora* (CAN) flowered in December or January, while *Coffea pseudozanguebariae* (PSE) flowered in February (Louarn, 1992; Akaffou, 1999). The late flowering time of PSE thus seemed dominant. The dominance hypothesis regarding late flowering time in this study was congruent with that observed for F_1, F_2 and BC_1 offspring derived from the cross between CAN and *Coffea eugenioides* (EUG) in Madagascar (Louarn, 1976). However, the 20% early:80% late ratio found in our study deviated from 1:1 segregation and tended to confirm the involvement of more than one gene, indicating that flowering time could be under polygenic control in coffee trees. Genetic inheritance of flowering time therefore certainly involves a complex mechanism, as speculated by Hao et al. (2008), Campoy et al. (2011), Jones et al. (2011) and Carter et al. (2011). In *Coffea* sp., further investigations are needed to gain greater insight into flowering time inheritance. Since the F_1 and most of the BC_1 plants flowered late in the year,

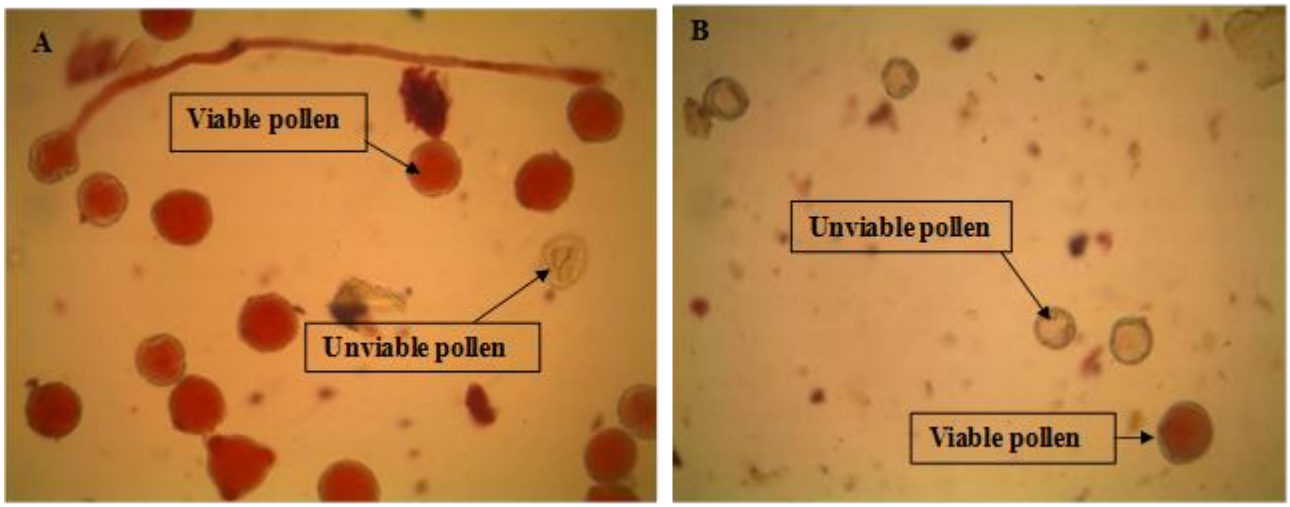

Fig 3. Pollen viability (400x) of a (A) male fertile and (B) semi-sterile plant in BC_1 (first backcross generation (PSE × CAN) × CAN hybrid); viable pollen showed a homogenous pink-coloured cytoplasm while unviable pollen was characterized by an empty or uncoloured cytoplasm; CAN and PSE represent *Coffea canephora* Pierre and *Coffea pseudozanguebariae* Bridson, respectively.

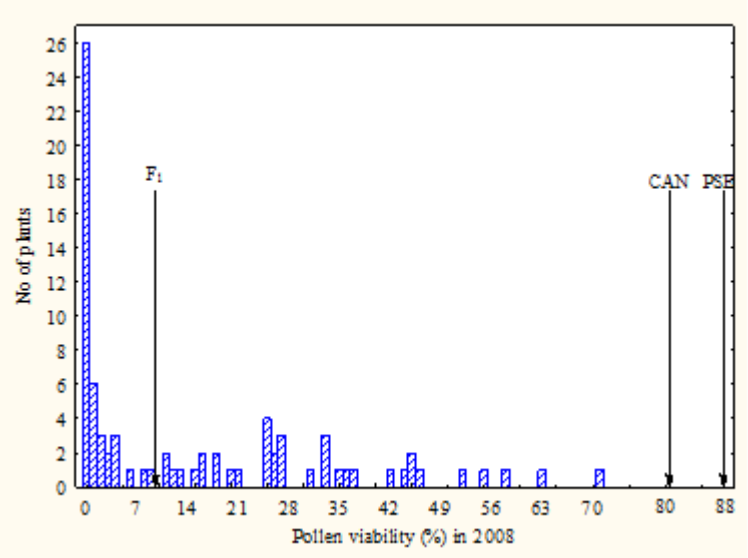

Fig 4. Pollen viability distribution in BC_1 in 2008. The arrays indicate parental species (PSE and CAN) and F_1 (hybrid between PSE × CAN) means.

backcrosses to CAN could be achieved using F_1 or selected BC_1 as female parent. Pollen from CAN could thus be extracted and stored at 4°C until pollination (Louarn, 1992). On the other hand, out of the 280 BC_1 plants studied during the three years, some (15) never induced flower buds or they only developed a few flowers on the whole plant. Others (10 to 20% of the BC_1 plants depending on the year) did not flower over two consecutive years. Finally, 164 plants were potentially interesting for breeding. These anomalies were also observed by Louarn (1992) in the cross between *Coffea kapakata* (from Angola) and *Coffea salvatrix* (from Tanzania), where the F_1 plants did not develop flower buds. Moreover, in the CAN × HET cross, Coulibaly et al. (2003) reported that four out of the seven F_1 plants studied did not flower. In CAN, on an intraspecific progeny, Leroy et al. (2011) observed that seven out of the 273 studied trees never produced any fruit. This phenomenon could thus result from genic dysfunction, as generally reported in plants by Stebbins (1958). In our hybrids, such genic dysfunction could be aggravated by gene incompatibility due to genetic divergence between CAN and PSE, as reported by Noirot et al. (2003) based on genome size and Hamon et al. (2009) based on chromosome organization.

F1 and BC1 hybrid fertility and yield

Data obtained by measuring PV, FR and SSET indicated that the F_1 plants had very low fertility. PV and FR did not reach 10% on average and the fruits contained a single seed, as compared to 1.5 recorded on average in the parental species (Akaffou, 1999). The effects of year, genotype and year x genotype interactions on PV were highly significant. These outcomes were consistent with those previously reported for F_1 plants derived from crosses between CAN and EUG (Louarn, 1976); CAN and RAC (Louarn, 1985), as well as CAN and *Coffea kianjavatensis* (a Malagasy species)

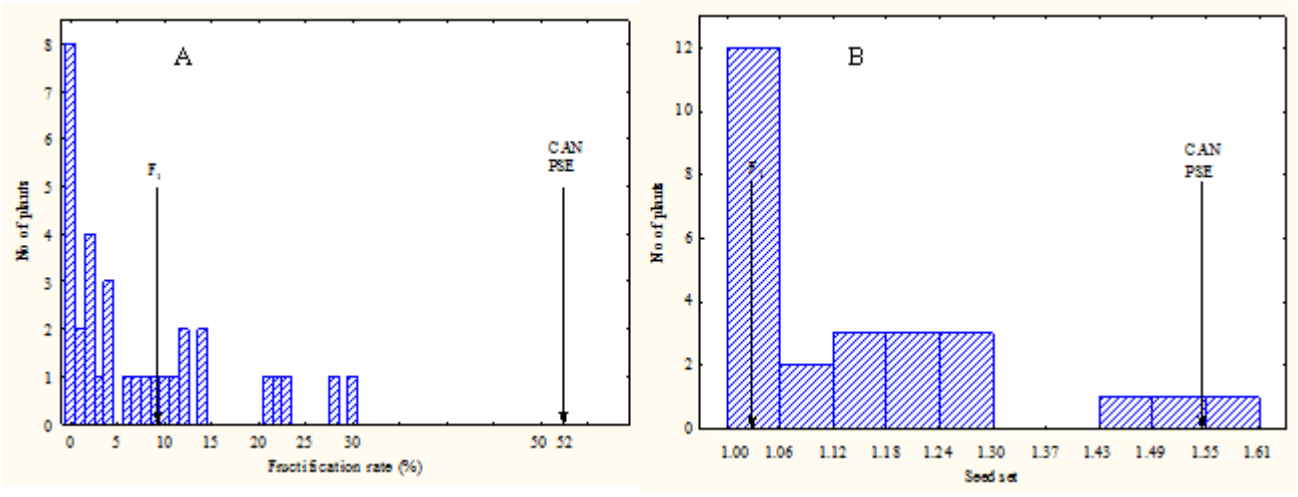

Fig 5. Distribution of the (A) fructification rate and (B) seed set in BC_1 plants. The arrays indicate parental species (PSE and CAN) and F_1 (hybrid between PSE × CAN) means

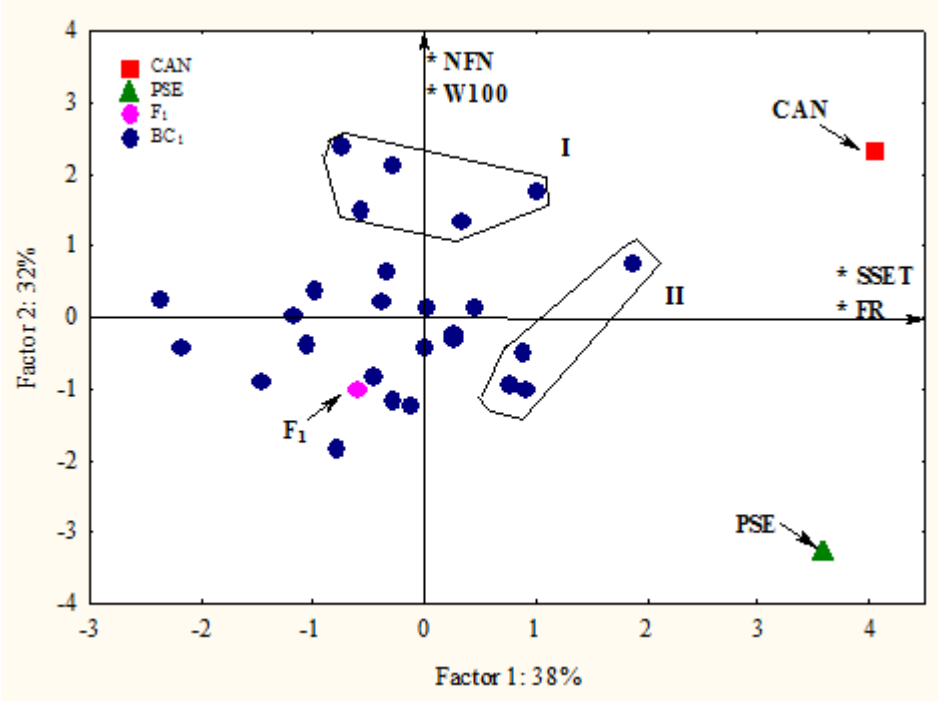

Fig 6. Scatter plots of 26 BC_1 individuals on 1-2 axes of the principal component analysis relative to fertility and productivity parameters; mean F_1 and mean parental species individuals are shown by arrays.

(Lanaud, 1979). PV and FR were slightly higher in progeny of the cross between CAN and HET for which the averages were close to 20% (Louarn, 1992; Coulibaly et al., 2003). In contrast, for F_1 plants derived from CAN × LIB or LIB × DEW crosses (Louarn, 1980, N'Diaye, 2004), the PV was high and close to 50% on average. Our data on PV showed much lower values compared to that observed for interspecific F_1 hybrids by Zhang et al. (2010), Win et al., (2010) and Xangsayasane et al. (2010) in rice, William et al. (2008) in white clover, and by Carter et al. (2011) in wheat. In these cases, even sterile or semi-sterile F_1 plants exhibited a PV of over 25%. The low fertility, considering PV, FR and SSET, recorded in the offspring derived from the PSE × CAN cross thus confirmed the marked genetic differences between CAN and PSE. In this study, fertility was generally not restored in the BC_1 progeny, contrary to that recorded for RAC × CAN, EUG × CAN and CAN × HET BC_1 progenies (Louarn, 1976, 1985, 1992; Coulibaly et al., 2003). Indeed, more than 50% of the BC_1 plants had a PV of under 5%. We deduced that the genetic divergence between CAN and PSE could be much higher than that between CAN and the three following species: RAC, EUG and HET. No one plant simultaneously exhibited high PV, FR and SSET, i.e. some showed high PV while others had high FR or SSET.

However, the observed variability allowed selection of interesting individuals. Environmental and genetic effects, as well as their interaction, were found to be highly responsible for PV variation in this study. Indeed, for most of the F_1 and BC_1 plants, PV differed by one- to two-fold depending on the year, while the PV was constant for a few plants. Otherwise, some plants never produced viable pollen, whereas others maintained a high PV regardless of the year. All of these results were perfectly consistent with those previously reported by Louarn (1992) in interspecific F_1 and BC_1 hybrids obtained between 10 diploid coffee species. Since the environment has a marked influence on PV, a multi-site evaluation covering at least three to five years would be necessary to get a sure PV value for a given plant. Furthermore, selection should primarily be focused on plants with a relatively constant PV throughout the years. Marker-assisted selection (MAS) could be helpful in such selection. Plants that do not produce viable pollen during the three consecutive years should be considered as male sterile. Hence, this stable pollen sterility could be due to particular genes, as reported by Win et al. (2009, 2010) in rice. Precise identification of such genes, as reported by Zhang et al. (2010) who fine-mapped the *S19* gene for pollen semi-sterility in rice, would be essential in *Coffea*. *Coffea* genome sequencing is well under way and should generate interesting results. As the biological cycle (seeds to seeds) length is long (5 years), the identification of these genes could allow early selection of fertile plants. Yield-related traits such as NFN deviated from the normal distribution and their transmission departed from additivity. This result was in agreement with that recorded by Akaffou (1999) for the cross between PSE and *C. liberica* var. *dewevrei* (DEW), where NFN values in F_1 and BC_1 to DEW or PSE were always skewed towards low values, close to the parent exhibiting the low mean. The gene controlling the low NFN values thus seemed dominant. Nevertheless, in *Coffea*, no data is available on NFN inheritance. This trait could involve complex mechanisms, including mixtures of major and poly genes, as recently found for fructification time, caffeine and heteroside content in the same BC_1 plants from a (PSE × CAN) × CAN cross (Akaffou et al., 2012). We also noted that some BC_1 plants showed lower NFN than PSE, i.e. the lowest parent. This could be due to transgressive segregants, generally observed for quantitative traits. In *Coffea*, since NFN is a product of two components, i.e. the number of inflorescences per node and the number of flowers per inflorescence, each component should be investigated separately to gain greater insight into the genetic control of this production component. The polygenic inheritance suggested here has also been reported in other species such as grape (*Vitis vinifera L.*) in which the inflorescence number per shoot, scored as a fertility trait, was assumed to be quantitatively inherited (Doligez et al., 2010). Ongoing molecular genotyping of BC_1 plants and QTL fine mapping analysis should generate further knowledge to elucidate the mechanism that determines NFN. In addition, year effects on NFN should be studied.

Relationships between flowering phenology and yield traits assessed in BC_1

The study on the relationships between PV, FR, SSET, NFN and W100 revealed that FR and SSET were significantly and positively correlated, similar to NFN and W100. PV was independent of the other four traits. The principal component analysis corroborated this relationship, grouping these variables into three independent factors: female fertility (expressed by FR and SSET), yield (NFN and W100) and male fertility (PV). This suggested that traits of each factor could be improved independently. Plants of the two groups (I and II) identified among the BC_1 should thus be backcrossed to CAN. This might result, in BC_2 offspring, in a substantial yield and fertility improvement while also enhancing the PV. The positive and significant correlation between NFN and W100 is of great interest since it could facilitate yield improvement.

Materials and Methods

Plant material

The plant material evaluated consisted of an F_1 hybrid between *C. pseudozanguebariae* (PSE) and *C. canephora* (CAN) and the offspring of the first backcross generation (PSE × CAN) × CAN, designated BC_1. The F_1 were produced in 1988 (Louarn, 1992), but success in the cross with CAN was only achieved when PSE was used as female parent. In these conditions, an average of 0.7 plants per 100 hand-pollinated flowers were obtained. A total of eight F_1 were finally obtained from two crosses: PSE (genotype 08044) × CAN DH 57 (doubled haploid 57 derived from the clone 200) and PSE (08104) × clone 181) (Fig. 1). In 1996, to produce the BC_1, two bulk mixtures of CAN pollen (one pollen mixture of DH from clone 200 and another derived from hybrids of the clone 160 × clone 200 cross) were used to enhance the hand-pollination success rate (Fig. 1). Five F_1 plants were used as female parents. A total of 280 BC_1 progeny (distributed within 10 families) were obtained. F_1 and BC_1 were planted in separate neighboring plots. Some CAN genotypes were mixed with the hybrids in each plot to serve as pollinators. The planting density was 2.5 ×1.25 m. All plants were maintained at the coffee breeding station of the Centre National de Recherche Agronomique (CNRA) at Man (7°40′N, 7°55′W), Côte d'Ivoire. The studies concerned four to six F_1 plants and 30 to 280 BC_1 plants depending on the traits. The high variation in plant number was due to the fact that all plants did not flower, some also developed few flowers or produced few fruits or seeds. Further details on the number of families and plants assessed will be given when presenting the traits.

Flowering phenology

In *Coffea*, flower buds are induced during the dry season, from November to January, under the environmental growing conditions in Côte d'Ivoire. Their progression towards flowering required over 5 mm of rainfall, i.e. 'triggering rainfall'. Note that, the first rainfall at or after the dry season generally occurred between December and January depending on the year. For this study, the principal flowering time of F_1 and BC_1 plants was recorded over three consecutive years, i.e. 2008, 2009 and 2010. This corresponds, for some perennial species and/or their hybrid types, to the period during which more than 50% of the plants flower, as also described by Sié (1999) in cola trees (*Cola nitida* Vent) and Maalouf et al. (2011) in faba bean (*Vicia faba* L.). A plant is considered as flowery when more than 50% of the induced buds have bloomed. Flowering was assessed and recorded from December to April, corresponding to the flowering period of *Coffea* species in Côte d'Ivoire (Louarn, 1992), and concerning all eight F_1 and BC_1 (280) plants. Data on the parental species were obtained from Louarn (1992) and Akaffou (1999).

Fertility parameters

Three parameters, i.e. pollen viability (PV), fructification rate (FR) and seed set (SSET), were assessed. PV was assessed over three consecutive years (2008, 2009 and 2010) by staining mature pollen in 2% acetocarmin of Belling (Grassias, 1980). Technically, the flower buds were harvested the day before blooming and stored at 4°C. At the flowering day, flower stamens were crushed in a drop of acetic carmin on a microscope strip. The preparation was covered with a thin strip and analyzed at 400× magnification under a photonic microscope. 100-pollen grains per flower were monitored. Three flower buds randomly harvested per genotype were analyzed and the mean percentage was calculated. Four F_1 and 80 BC_1 plants were evaluated over three consecutive years (2008, 2009 and 2010). FR was assessed in open pollination conditions in 2008. This corresponds to the ratio between the number of fruits and flowers. The per genotype estimations were based on four different branches chosen randomly the day before blooming. 100-200 flowers were counted per branch, with each branch being monitored once a week from flowering until fruit ripening and harvest. The SSET concerned the number of seeds per fruit. In coffee trees, each ovary contains two ovules, which evolve into two seeds in fertile plants after pollination. The sterility of one of these ovules led to a fruit containing a round seed called a 'caracoli'. For this study, the fruits were harvested at complete ripening, de-pulped by hand and then the number of seeds per fruit was noted. Three samples consisting of 30 fruits each were examined. Four F_1 and 42 BC_1 plants exhibiting abundant flowering in 2008 were used for FR and SSET evaluation.

Yield-related traits

Two components were assessed: the number of flowers per node (NFN) and the 100-bean weight (W100). NFN is an important yield determinant in coffee. CAN exhibits abundant flowering, with an average of 36 flowers developed per node (N'Diaye, 2004), whilst PSE produces an average of 4.5 flowers per node (Akaffou, 1999). The estimation of this parameter was based on 10 nodes (per genotype) from branches randomly chosen the day before blooming. Five F_1 and 114 BC_1 plants were studied in 2008. 100-bean weight data were obtained from Akaffou et al. (2012). This trait is additive and quantitatively inherited.

Statistical analysis

Four statistical analysis methods were applied: ANOVA (one, two and three-way), multiple linear regression analysis, Shapiro-Wilks normality tests and principal component analysis (PCA). A 3-way ANOVA was used to test year, family and genotype effects on PV variation, while FR and NFN variation between families and between genotypes within families was studied using 2-way ANOVA. Years were fixed factors, genotype (random factor) was nested into family (random factor). One-way ANOVA was used for genotype comparisons within F_1 or BC_1, and for comparisons between F_1 and BC_1. Shapiro–Wilks tests (Shapiro and Wilk, 1965) ($p \leq 0.05$) were used to test the trait distribution normality. A multiple linear regression analysis was performed to study relationships between NFN, PV, FR, SSET and W100. Finally, PCA was conducted to identify the principal factor responsible for variability in fertility and productivity traits among BC_1 plants. All analyses were performed using the STATISTICA 7.0 software package (StatSoft, Inc. 2007).

Conclusion

The main outcomes of this study could be summarized in three points: 1) the F_1 and most of the BC_1 plants flowered late in February, like PSE the wild parent; 2) the productivity parameters were very low in F_1 plants and were not totally restored on average in the first backcross generation offspring; and 3) high variability was observed among BC_1 plants, thus allowing selection of interesting plants for further backcrosses. These results were highly promising for *Coffea canephora* improvement through gene transfer from PSE. Molecular genotyping of the BC_1 progeny is well under way and QTL analysis could be performed for a marker-assisted or genome-wide selection scheme.

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