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Relationship between parental chromosomic contribution and nuclear DNA content in the coffee interspecific hybrid *C. pseudozanguebariae* × *C. liberica* var 'dewevrei'

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Abstract F_1 hybrids were obtained between two coffee species with the same chromosome number (2n = 22) but with different nuclear DNA contents [*C. pseudozanguebariae* (PSE) 2C = 1.13 pg and *C. liberica* var 'dewevrei' (DEW) 2C = 1.42 pg]. G2 hybrids were obtained by open-pollination of the F_1 hybrids. Genomic in situ hybridisation (GISH) and flow cytometry were used on six F_1 hybrids and seven G2 hybrids to determine their parental chromosomic contribution and their nuclear DNA content (qDNA), respectively. GISH efficiently identified chromosomes from both species. F_1 hybrids had a qDNA intermediate between that of the parental species and contained the expected 11 chromosomes from each species. There was a linear relationship between the number of PSE chromosomes and the nuclear DNA content, which indicates that flow cytometry can be used to give a rough estimate of the parental chromosomic contribution in G2 hybrids.

Key words *Coffea* · Interspecific hybrids · Genomic in situ hybridisation (GISH) · Flow cytometry

Introduction

Coffee trees belong to the genus *Coffea* subgenus *coffea* (Rubiaceae), which contains approximately 80 taxa. Evaluations of coffee species for different characteristics have discovered many wild species with interesting traits for breeding purposes (Berthaud and Charrier 1988). Interspecific hybrids offer the possibility of transferring these traits from wild coffee species to cultivated ones, namely *C. arabica* and *C. canephora* (Carvalho and Monaco 1967; Louarn 1992, 1993). Success in introgression depends mainly on the genetic control of the trait, possible links with other traits, level of recombinations between the different genomes and the restoration of fertility. To evaluate these factors, we crossed *C. liberica* var 'dewevrei' (DEW) from Central Africa with *C. pseudozanguebariae* (PSE) from Kenya. The second generation of hybrids (G2) was obtained by open-pollination of the F_1 hybrids.

Coffee species are diploid with 2n = 22 chromosomes, except for *C. arabica* which is tetraploid (2n = 4x = 44). In diploid African species, nuclear DNA content (qDNA) ranges from 2C = 0.95 to 1.78 pg (Cros et al. 1995). The two diploid species used for this study differ greatly in their genome size: 2C = 1.42 pg and 2C = 1.13 pg for DEW and PSE, respectively.

We considered it interesting to investigate whether a relationship exists between genome size and parental chromosomic contribution. Genomic in situ hybridisation (GISH) is an efficient technique for identifying parental chromosomes in plant interspecific hybrids and for determining their genome composition (Jiang and Gill 1994; D'Hont et al. 1996; Leblanc et al. 1996). However, this technique is time-consuming and cannot be used on large progenies. Flow cytometry, in contrast, is a rapid technique for estimation of qDNA (Bennett and Leitch 1995). A relationship between genome size and parental chromosomic contribution would allow the genome composition of hybrids to be predicted by flow cytometry.

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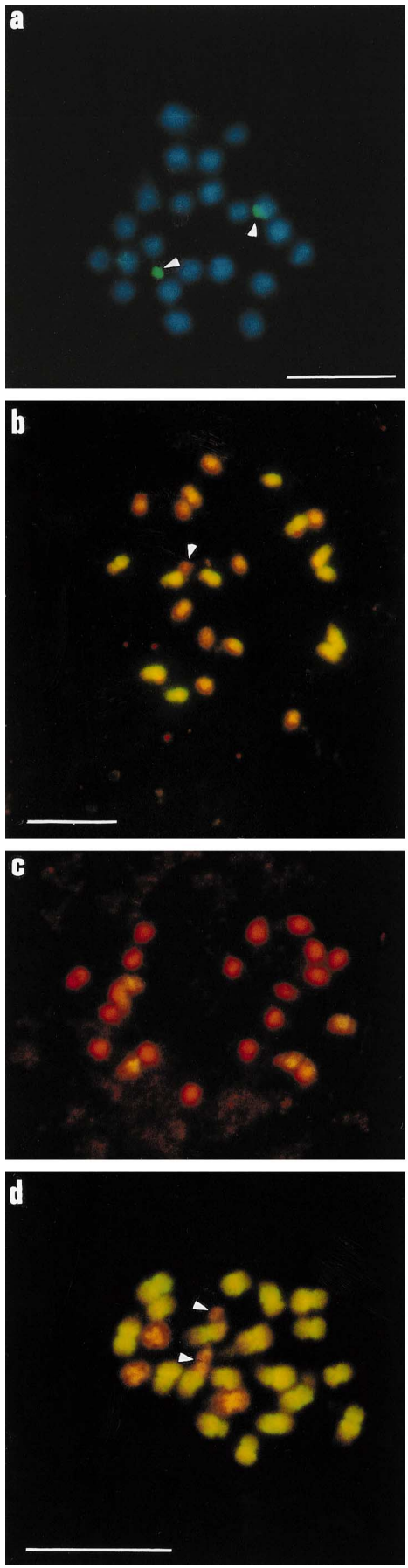

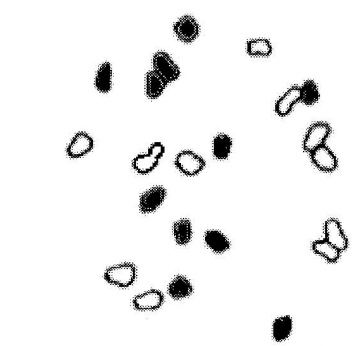

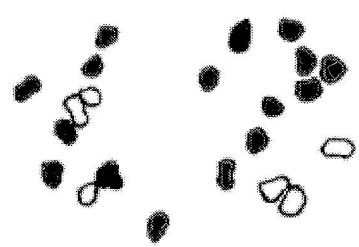

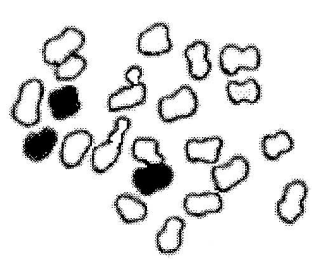

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Materials and methods

Plant material

Six F_1 hybrids and seven G2 hybrids were studied. The G2 hybrids consisted of four $F_1 \times$ DEW (BCDEW) and three $F_1 \times$ PSE (BCPSE). G2 hybrids were chosen in order to cover uniformly the range of qDNA between the parental species: 1.13–1.42 pg (unpublished data and Barre et al. 1996). Plants were grown in a greenhouse held at tropical climate (24°C during the day, 18°C at night, relative humidity of 70%).

Flow cytometric measurements

qDNA was estimated as described in Barre et al. (1996). Nuclei were extracted by the leaf chopping method (Galbraith et al. 1983). A modified (0.5% Triton X-100 and pH = 9) version of the lysis buffer of Dolezel et al. (1989) was used. The solution was filtered through nylon cloth (50-μm mesh size) and kept on ice. Just before measurement, saturating propidium iodide (330 μg/μl) was added. *Petunia hybrida* was used as the internal standard (2C = 2.85 pg; Marie and Brown 1993). Three nuclei extractions were done for each plant.

A FACScan cytometer (Becton Dickinson) was used with an argon laser (15 mW) at 488 nm, taking a pulse area of emission at 585 ± 22 nm.

Fluorescent in situ hybridisation

Chromosome preparations, rDNA in situ hybridisations and genomic in situ hybridisations (GISH) were performed as described in D'Hont et al. (1996). For rDNA in situ hybridisation, 5 ng/μl of the probe pTA 71 (Gerlach and Bedbrook 1979), labelled with digoxigenin-11-dUTP (Boehringer, Mannheim), was used with 50 ng/μl of sheared herring sperm DNA. The probe was detected with FITC and the chromosome counterstained with DAPI.

For GISH, 6.5 ng/μl of DEW genomic DNA labelled with digoxigenin-11-dUTP and 6.5 ng/μl of PSE genomic DNA labelled with biotin-14-dUTP (Gibco BRL) were used. Sequences homologous to PSE total DNA fluoresced red, a result of the detection of the biotin-labelled total DNA from PSE with Texas Red. Sequences homologous to DEW total DNA fluoresced yellow green, a result of the detection of the digoxigenin-labelled total DNA from DEW with FITC. The use of a specific filter to detect FITC or Texas Red showed that all chromosomes were labelled yellow-green and red, respectively, because of sequence homologies between the genomes of the two species. However, sequence differences between the genomes of the two species differentially enhanced the fluorescence of the two groups of chromosomes. A double exposure, one using the FITC filter and one using the Texas Red filter, allowed detection of two types of chromosomes, one red-orange and one yellow.

Fig. 1 **a** In situ hybridisation of the rDNA probe (pTA 71) detected with FITC to metaphase of *Coffea pseudozanguebariae* counterstained with DAPI. **b** Genomic in situ hybridisation to metaphase of the F_1 hybrid between *C. pseudozanguebariae* (fluoresced in *red*) and *C. liberica* var 'dewevrei' (fluoresced in *yellow*). **c, d** GISH to metaphase of G2 hybrids with 17 and 3 chromosomes of *C. pseudozanguebariae*, respectively. *Arrows* indicate rDNA sites. *Bar*: 10 μm. **b', c'** and **d'** Schematic representation of **Fig. 1b, c** and **d**, respectively, showing the specific origin of the chromosomes, after correction for staining of rDNA sites. The *black* and *white* chromosomes correspond to chromosomes of *C. pseudozanguebariae* and *C. liberica* var 'dewevrei', respectively

Results

rRNA genes

In PSE and DEW, two 18S-5.8S-25S rDNA sites were localised on terminal segments of 2 chromosomes (Fig. 1a). In both species, signals appeared that were associated with a nucleolar constriction. These terminal segments were often broken and separate from the chromosomes in our preparations.

F_1 hybrids

qDNA of F_1 hybrids varied from 1.28 to 1.32 pg, with an average of 1.30 pg, which was close to the average value of the parental species, 1.29 pg. No significant difference between hybrids was noted ($F_{5,12} = 2.22$, $P = 0.12$). The six F_1 hybrids analysed had 22 chromosomes. Their genomic composition was determined using GISH. Eleven red-orange chromosomes originating from PSE and 11 yellow chromosomes originating from DEW, 1 of which had a red terminal signal on a nucleolar constriction, were detected (Fig. 1b).

G2 hybrids

qDNA ranged from 1.20 to 1.39 pg in G2 hybrids ($F_{6,14} = 110$, $P = 0.000$). The seven G2 hybrids had 22 chromosomes. The three BCPSE hybrids had more than 13 chromosomes from PSE (Fig. 1c) and four BCDEW had fewer than 9 chromosomes from PSE (Fig. 1d). The average numbers of chromosomes from PSE, in BCPSE (15) and in BCDEW (6.25), were close to the number expected in backcrosses, 16.5 and 5.5, respectively.

A linear relationship, $y = -71.41,\ x + 102.42$ ($r = 0.98$***), was found between the number of PSE chromosomes versus qDNA in the species and their hybrids (Fig. 2).

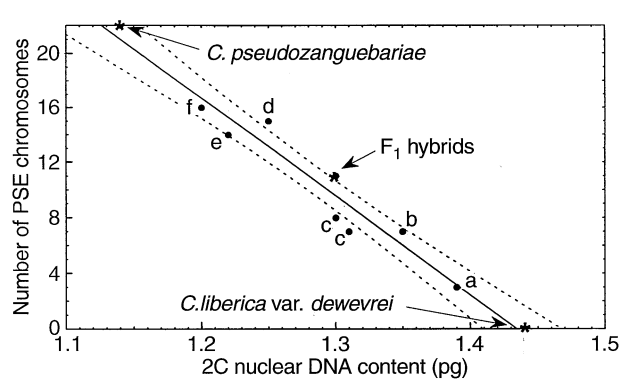

Fig. 2 Linear relationship ($y = -71.41,\ x + 102.42,\ r = 0.98$) between the nuclear DNA content and the number of chromosomes on DEW and PSE, F_1 and G2 hybrids. Results of the Newmann and Keuls test on qDNA are indicated by *letters*

Discussion

Identification of parental chromosomes

GISH efficiently identified the parental origin of all the chromosomes in F_1 and G2 hybrids between DEW and PSE. Yellow chromosomes with a red terminal signal on a nucleolar constriction were interpreted to be chromosomes from DEW. Indeed, it has been shown in sugarcane using the same technique that major rDNA sites fluoresce red even on a yellow chromosome (D'Hont et al. 1996). The highly conserved sequences of rDNA hybridised to DNA of both species, but the red fluorescence appeared more intense than the yellow-green fluorescence. Moreover, the shape and the position of the terminal red signal on the yellow chromosome was comparable with those of rRNA genes localised by FISH. This chromosome can thus be identified as the chromosome from DEW bearing rRNA genes.

Phylogenetic studies on *Coffea* based on variations in chloroplast DNA and internal transcribed spacer sequences (ITS 2) of nuclear ribosomal DNA indicate that DEW and PSE belong to different groups (Lashermes et al. 1996, 1997). This was confirmed by the fact that the genomes of the two species were differentiated by a double exposure with both FITC and Texas Red filters. This indicates that the two species have diverged for repeated DNA sequences. These sequences could be involved in the difference in nuclear DNA content between the two species.

Recombination between DEW and PSE

All F_1 hybrids were euploid with 22 chromosomes. Despite a low level (7–43%) of pollen mother cells with 11 bivalents in F_1 hybrids (Louarn 1992), all our G2 hybrids were also euploid with 22 chromosomes. Moreover, despite the low number of G2 hybrid investigated, the average parental chromosomic contributions in the two backcrosses with PSE and DEW were close to the expected values.

No recombination was detected in the seven G2 hybrids, even though GISH is an efficient tool for observing interspecific recombination (Thomas et al. 1994; D'Hont et al. 1996). This is possibly explained by the fact that as in other species with very small genome sizes, such as *Arabidopsis* and *Brassica* species, in coffee species genome-specific labelling could be limited to heterochromatic segments near the centromere (Bennett 1995). Indeed, we noted that coffee chromosomes fluoresced predominantly near the centromere, a phenomenon that could impede the detection of distal recombinations. Another explanation may be the low recombination rate often observed in interspecific crosses due to chromosomal rearrangements between species or genic factors (Reiseberg et al. 1995). In this case, it would be difficult to dissociate the interesting and unfavourable traits of wild species from each other and an analysis of large progenies would be required.

Nuclear DNA content and number of PSE chromosomes

The between-species difference in qDNA was equally distributed on all chromosomes, irrespective of the chromosome size, as in *Allium cepa* × *A. fistulosum* hybrids (Narayan 1988). In our G2 hybrids, the fact that qDNA varied gradually and all chromosomes were differentiated by GISH seems to indicate a distribution of the between-species difference (about 0.288 Mpb) on all chromosomes. The linear relationship between qDNA and the number of PSE chromosomes in G2 hybrids enables estimation of the number of PSE chromosomes by flow cytometry. The imprecision of the estimation could be due either to an unequal distribution of the between species-difference of qDNA on all DEW chromosomes or to a bias in qDNA estimation.

Breeding purposes

In introgression programmes of wild traits into the cultivated species, the aim is to produce plants with the introgressed trait and the background of the cultivated species. This is usually done by backcrossing the hybrids with the favourable trait on the cultivated species. This is time-consuming, especially for coffee trees which yield only after 4 years. The use of molecular markers and flow cytometry for early detection of hybrids having the expected trait with the minimum number of chromosomes from the wild species could accelerate this process.

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