Theor Appl Genet (2004) 108:829–835
DOI 10.1007/s00122-003-1498-3

ORIGINAL PAPER

H. M. Pearl · C. Nagai · P. H. Moore · D. L. Steiger · R. V. Osgood · R. Ming

Construction of a genetic map for arabica coffee

Received: 30 August 2003 / Accepted: 10 September 2003 / Published online: 5 November 2003

Abstract We have used AFLPs to construct a genetic linkage map on a pseudo-F_2 population of arabica coffee (*Coffea arabica* L.) derived from a cross between the cultivars Mokka hybrid and Catimor. Sixty trees from this population were selected on the basis of plant height distribution to construct a linkage map. A total of 456 dominant markers and eight co-dominant markers were generated from 288 AFLP primer combinations. Of the total number of markers generated, 68% were from cv. Catimor, 30% from cv. Mokka hybrid, and 2% were co-dominant. This distribution suggests that the heterozygosity within the cv. Catimor sub-genomes was twice that within the cv. Mokka hybrid sub-genomes. Linkage groups were constructed using MAPMAKER version 3.0, resulting in 16 major linkage groups containing 4–21 markers, and 15 small linkage groups consisting of 2–3 linked markers each. The total length of the map was 1,802.8 cM, with an average distance of 10.2 cM between adjacent markers. This genetic map will serve as the framework for mapping QTL controlling source-sink traits in the same population.

Introduction

Coffee is one of the most important beverage crops in the world; it is grown in over 80 countries in the tropical and subtropical regions. Approximately 100 species of the genus *Coffea* L. (Rubiaceae) are recognized. The most commercially important of these are *C. arabica* L., also known as arabica coffee, and *C. canephora* (Pierre ex Froehner), known as robusta coffee. *C. arabica* is a tetraploid ($2n=4x=44$) and self-fertile species in the *Coffea* genus. All other *Coffea* species, including *C. canephora,* are diploid, and most of them are self-sterile. *C. arabica* is allotetraploid and shows diploid-like meiotic behavior (Grassias and Kammacher 1975; Lashermes et al. 2000b). Genomic in situ hybridization demonstrated clearly that *C. arabica* is derived from hybridization between *C. eugenioides* and *C. canephora* (Lashermes et al. 1999). These authors also compared the restriction fragment length polymorphism (RFLP) patterns of *C. arabica* with those of its two progenitor species to discover low divergence of each of the two constituent genomes of *C. arabica* with those of *C. eugenioides* and *C. canephora.* These results suggest that speciation of *C. arabica* was a recent event. In other research, sequence analyses among 23 *Coffea* taxa of the *trn*L-*trn*F intergenic spacer of chloroplast DNA showed close similarity between cpDNA of *C. arabica* and that of *C. eugenioides* and *C.* sp. Moloundou (Cros et al. 1998). Based on these two studies, *C. eugenioides* would have been the maternal parent when *C. eugenioides* and *C. canephora* hybridized.

The limited genetic diversity detected among arabica coffee cultivars is mostly the consequence of its few introductions and its self-pollinating nature. The lack of genetic diversity in the gene pool of arabica coffee limits the potential for germplasm improvement. The occasionally occurring spontaneous interspecific hybrids have been widely used for improving disease and pest resistance in arabica coffee. One extensively used source is the Timor hybrid, discovered on the island of Timor, that was derived from a spontaneous interspecific cross between *C. arabica* and *C. canephora* based on recent molecular evidence (Lashermes et al. 1993; Orozco-Castillo et al. 1994; Lashermes et al. 2000a). DNA marker analyses of Timor hybrid-derived genotypes revealed a two-fold increase of genetic diversity among these introgression lines (Lashermes et al. 2000a). An example of how the Timor hybrid ($2n=44$) has been successfully used in

Communicated by H.F. Linskens

H. M. Pearl · C. Nagai · D. L. Steiger · R. V. Osgood · R. Ming (✉)
Hawaii Agriculture Research Center, Aiea, HI 96701, USA
e-mail: rming@harc-hspa.com
Tel.: +1-808-4865374
Fax: +1-808-4865020

P. H. Moore
USDA-ARS,
Pacific Basin Agricultural Research Center,
Aiea, HI 96701, USA

coffee breeding programs is that the coffee leaf rust (*Hemileia vastatrix* Berk. & Br.)-resistant Catimor was derived from a cross between Caturra and Timor hybrid (Bisco and Logan 1987). Another spontaneous interspecific hybrid between *C. arabica* and *C. liberica* increased the genetic diversity among *C. liberica* introgressed lines and was used as main source for rust resistance in coffee breeding programs in India (Prakash et al. 2002).

Molecular marker linkage maps are being used successfully in many crop species for directed germplasm improvement. Marker-assisted selection allows screening of large numbers of trees for a gene of interest at an earlier stage and reduces the number of backcrosses required to obtain quality traits (Lashermes et al. 1997). We report here the construction of a genetic linkage map on a pseudo-F_2 population of arabica coffee derived from a cross between the cultivars Mokka hybrid and Catimor to be used for mapping quantitative trait loci (QTL) controlling coffee quality and productivity.

Materials and methods

Plant material

A segregating mapping population H00-20 of arabica coffee (*Coffea arabica L.)* was developed from a cross between the cultivars Mokka hybrid (MA2-7) and Catimor (T 5175-7-1) in 2000. Catimor, T5175-7, is a variety developed at Promecafe in Central America from a hybrid between *C. arabica* and *C. canephora* and backcrossed to *C. arabica,* Catuai. Catimor trees typically have large, round leaves, resistance to rust, high cherry yield, and large-size beans, but are known to have lower cupping quality. Mokka hybrid was developed in Brazil and has small, thin leaves, small beans, is rust susceptible, and has excellent cupping quality. In June 2001, 135 seedlings of the population were planted at the HARC Kunia Substation together with selfed progenies of both parents. Morphological data for tree height and width and leaf characteristics were collected in 2002. For plant height and width, the distribution of the data showed transgressive segregation exceeding parental values at both ends. For leaf length, width, ratio, and area, the distributions of the progeny data were within the range of their parental values. Sixty plants from this population were selected based on their plant heights that formed a normal distribution for constructing the arabica genetic map with amplified fragment length polymorphism (AFLP) markers.

AFLP analysis

Genomic DNA isolation and digestion

Coffee genomic DNA isolation was described previously (Steiger et al. 2002). DNA digestions were performed according to the protocol of Vos et al. (1995) with the modification that 250 ng of DNA was digested at 37°C for 3 h with 2.5 U each of *Eco*RI, *Pst*I, and *Mse*I.

Adapter ligation and pre-amplification

Adapters and primers were synthesized by Integrated DNA Technologies (Coralville, Iowa). Pre-amplification was performed as described by Vos et al. (1995) except that 1 U of *Taq* polymerase (Promega) was used per reaction.

Selective amplification

Infrared dye (IRDye 800 and IRDye 700)-labeled *Eco*RI and *Pst*I primers were purchased from LI-COR Biosciences (Li-Cor, Lincoln, Neb.). Non-labeled *Mse*I primers were obtained from the Research Corporation of University of Hawaii, Manoa. Selective amplification was performed according to the protocol of Qiagen (Valencia, Calif.).

Gel analysis

Ten microliters of sequence loading buffer [95% formamide, 20 mM EDTA, bromophenol blue (Bio-Rad), and deionized water] was added to each selective amplification and the samples were denatured at 95°C for 3 min. Approximately 0.9 μl of each sample was loaded onto a 6.5% polyacrylamide gel. Gels were run for 3 h using a Li-Cor IR2 Automated DNA Sequencer and gel images were stored as tagged image file formats (.tiff) until scored.

Data analysis and map construction

AFLP polymorphic markers were manually scored as numerical data corresponding to dominant parental type. For Mokka hybrid-dominant markers, presence of a band was scored as a "4" and absence of a band was scored as "1." Catimor-dominant markers were scored as "5" for the presence of a band and "3" for the absence of a band. Goodness of fit was tested by chi-square analysis for the expected segregation of dominant (3:1) or (1:1) and co-dominant (1:2:1) markers. Only the most readable markers between the optimally repeatable sizes of 80 bp to 350 bp were used to create the linkage map.

Map construction was carried out using MAPMAKER version 3.0 (Lincoln et al. 1993). Linkage groups were established using two-point analysis with threshold values of 0.25 and LOD=5.0 ($\log_{10}$ of likelihood ratio). Multipoint analysis was then performed to find the most likely order of markers within each linkage group. The Kosambi function was used for converting recombination fractions into map distances in centiMorgans.

Results

Initially, the level of polymorphism was evaluated between parental cultivars of two potential mapping populations derived from the crosses of Maragogipe × Catuai and Catimor × Mokka hybrid. The polymorphism rate between Maragogipe and Catuai was extremely low (0.083 per primer pair) with only two polymorphic markers from 24 AFLP primer combinations surveyed. Therefore, this population was not used for genetic mapping. The polymorphism rate between Catimor and Mokka hybrid was low (1.34 per primer pair) but workable, with 172 markers among 128 primer combinations surveyed. Because the majority of primer combinations generated at least one polymorphic marker in this population, the primer survey was stopped and all additional primer pairs were used for linkage mapping directly.

To produce a more complete coverage of the genome, both methylation-insensitive (*Eco*RI) and sensitive (*Pst*I) restriction enzymes were used for generating AFLP markers. An overall average of 1.6 markers per primer pair was detected. Markers generated from *Eco*RI and *Mse*I produced an average of 2.1 markers per primer pair,

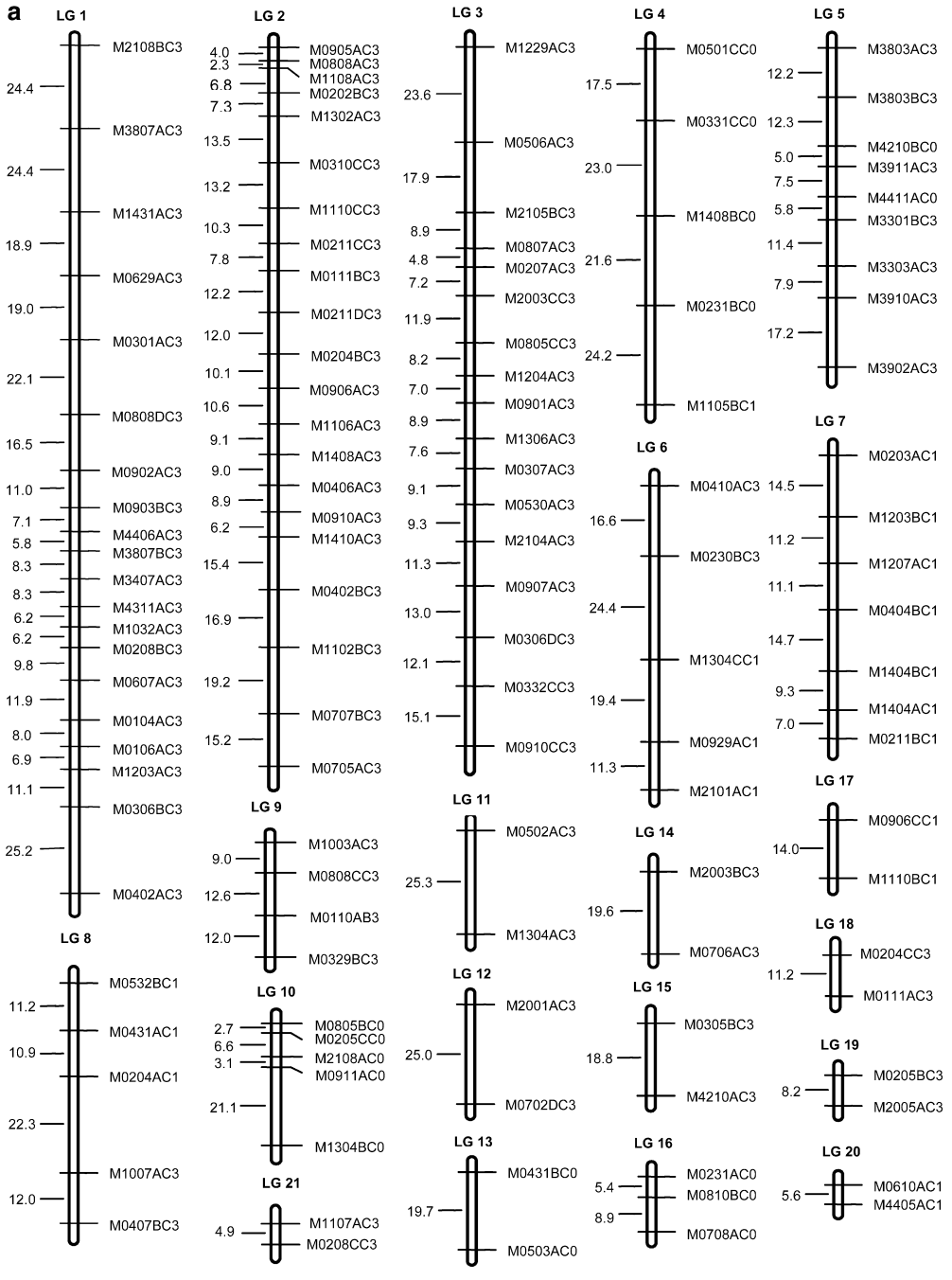

Fig. 1a, b Genetic linkage map of arabica coffee. Catimor linkage groups (LG 1–LG 21) (**a**) and Mokka LGs (LG 22–LG 31) (**b**) have been numbered by size. The AFLP marker names consist of eight characters: the first letter represents the size of the fragments (M = 80–360 bp); the following four numbers represent the E-, P-, and M-primer codes (see Table 1); the sixth character represents the loci detected by each primer set; the seventh character indicates from which parent the marker was derived (C =Catimor, M = Mokka, B = co-dominant); and the last number represents the chi-square test results (3 = 3:1 fit; 1 = 1:1 fit; 0 = no fit)

while those generated from *Pst*I and *Mse*I produced an average of 0.6 markers per primer pair. A total of 464 polymorphic markers were generated from 288 pairs of AFLP primers with two- or three-nucleotide extension for linkage map construction (Table 1). Among the 464 markers, 368 were the optimally repeatable size of 80–350 bp, 82 of the markers scored were smaller than 80 bp, and 14 were larger than 350 bp. Of the total number of markers generated, 319 were Catimor-dominant (68%), 137 were Mokka-dominant (30%), and 8 were found to be co-dominant (2%).

In this pseudo-F_2 population, a segregating dominant marker must be heterozygous in one or both sub-genome(s) of a parental cultivar and homozygous recessive in both sub-genomes of the other parental cultivar. This condition would produce a 1:1 or 3:1 segregation ratio in the progeny. Chi-square analysis revealed 188 markers (41%) fitted a 1:1 segregation ratio and 160 (34%) fitted a 3:1 ratio. The remaining 117 (25%) showed segregation distortion within the population. For Catimor-dominant markers, 40.8%, 37.3%, and 21.9% of markers fitted 1:1, 3:1, or distorted, respectively; for Mokka-

Fig. 1b

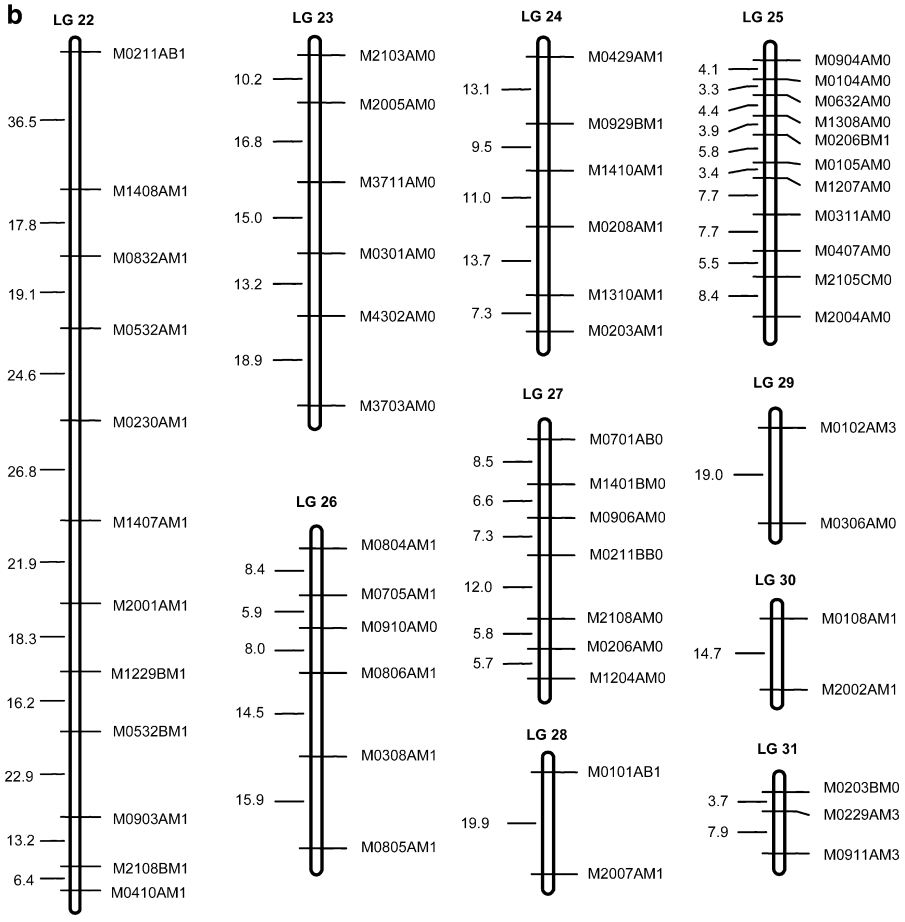

Table 1 List of selective AFLP primers and their codes used in arabica coffee genetic mapping

Primer	Code	Primer	Code	Primer	Code
M-CAA	01	E-AAC	01	P-AAG	33
M-CAC	02	E-AAG	02	P-AAT	34
M-CAG	03	E-ACA	03	P-ACA	35
M-CAT	04	E-ACC	04	P-ACG	37
M-CTA	05	E-ACG	05	P-ACT	38
M-CTC	06	E-ACT	06	P-AGA	39
M-CTG	07	E-AGC	07	P-AGC	40
M-CTT	08	E-AGG	08	P-AGT	42
M-CC	10	E-AAT	09	P-ATA	43
M-CG	11	E-AGT	10	P-ATC	44
M-GA	29	E-ATC	11		
M-GC	30	E-ATT	12		
M-GG	31	E-AGA	13		
M-GT	32	E-ATA	14		

dominant markers, the same three categories were 27.7%, 27.0%, and 45.3%, respectively.

To construct the linkage map, we used markers with optimal size 80–350 bp first to generate linkage groups. Additional markers were added after the initial linkage groups were defined. Of the markers mapped, 145 were linked in 16 major linkage groups ranging in size from 4 to 21 markers each; 32 markers were linked in 15 small linkages each containing only 2 or 3 markers; and 191 markers remained unlinked. Linkage groups (LG) were ranked according to their length in centiMorgans and assigned numbers 1 through 31 (Fig. 1); LG 1 was the longest at 251.12 cM and LG 31 was the smallest at 4.9 cM in length.

Among the 31 mapped LGs, 21 (LGs 1–21) were formed exclusively by Catimor markers along with a single co-dominant marker on LG 9. The remaining 10 LGs (LGs 22–31) consisted of all Mokka hybrid markers, plus four co-dominant markers on LGs 22, 27, and 28. Among the 21 Catimor LGs, 11 of them (52%) consisted of markers fitting a 3:1 segregation ratio and 4 LGs (19%) consisted of markers fitting a 1:1 segregation ratio. In contrast, among the 10 Mokka LGs, only 1 (10%) LG consisted of markers fitting a 3:1 segregation ratio and 5 (50%) consisted of markers fitting a 1:1 segregation ratio. The total length of the linkage map is 1,802.8 cM with average genetic distance of 10.2 cM between adjacent markers.

AFLP markers generated from methylation-sensitive restriction enzyme *Pst*I were mapped on 5 of the 31 LGs (markers named M33 to M44, see Table 1 for primer code). The short LG 5 consisted of *Pst*I markers. Four *Pst*I markers were mapped on LG1 and three of them were clustered. Three *Pst*I markers were mapped on LG

23, and one *Pst*I marker each was mapped on LGs 3 and 20.

Four of the eight co-dominant markers were mapped on LGs 9, 27, and 28.

Discussion

For the arabica coffee allotetraploid species of $2n=4x=44$, 22 LGs are expected to be mapped corresponding to the basic 22 diploid chromosomes. Because our mapping population is the first generation of the cross between two parents, the recombination events detected by DNA markers were the results of previous generations of self-fertilization. There was no recombination between Catimor and Mokka hybrid chromosomes at this true F_1, and pseudo-F_2, population. The high quality of the DNA markers and the accuracy of genetic mapping were exemplified by the clear separation of Catimor and Mokka hybrid LGs, even though all the markers from both parents were combined to construct the map (Fig. 1). One notable feature of this map is the lack of clustering of markers caused by chromosome suppression of recombination, especially around the centromeric regions. For a self-pollinated species, the pairing of highly homologous chromosomes results in a higher rate of recombination, fewer polymorphic markers across the genome, and few, if any, regions of suppression of recombination. Chromosome centromeric regions are usually conserved and may not be polymorphic when self-pollinated. This is in contrast to the clusters of markers mapped on an interspecific segregating population derived from *C. pseudozanguebariae* and *C. liberica* var. *dewevrei*. These results suggest that suppression of recombination occurred in genomic regions involving chromosomal rearrangements since the divergence of these latter two species (Ky et al. 2000).

Any segregating marker in the pseudo-F_2 population would have to be heterozygous in one or both sub-genome(s). If the marker were heterozygous in one sub-genome and homozygous recessive in the other sub-genome, the marker would segregate at 1:1 in the pseudo-F_2 progeny. On the other hand, if the marker were heterozygous in both sub-genomes or dominant in two of the three *C. canephora* homologous chromosomes in an interspecific hybrid derived from *C. arabica* × *C. canephora* 4× from which Catimor derived, it would segregate at 3:1 in the pseudo-F_2 progeny. If the marker were homozygous dominant in one or both sub-genome(s), it would not be polymorphic because it would be present in all individuals of the segregating population. In each of these scenarios, one of the parents would have to be homozygous recessive in both sub-genomes at the particular locus to produce polymorphic markers. The percentages of markers fitting 1:1 and 3:1 segregating ratios were similar in Mokka hybrid (27.0% and 27.7%) and Catimor (40.8% and 37.3%), indicating that approximately equal numbers of polymorphic markers were derived from heterozygous loci in one or both sub-genome(s). Our results are suggestive that a significant portion of the two sub-genomes might be homozygous, supporting the observation of tetrasomic inheritance of RFLP loci and double-reduction genotypes in a arabusta-interspecific hybrid derived from *C. arabica* × *C. canephora* 4× (Lashermes et al. 2000b).

Proportionally more Catimor markers segregating in a 3:1 ratio were mapped into LGs than were the Mokka hybrid markers. Catimor is derived from a cross between Caturra and Hybido de Timor, which in turn is a tetraploid hybrid between *C. arabica* and *C. canephora* 4× (Bisco and Logan 1987). Although the hybrid was backcrossed to *C. arabica*, its *C. canephora* chromosomes may represent a major portion of the Catimor genome and share a high degree of homology with the *C. arabica* chromosomes as reported by Herrera et al. (2002). Catimor markers segregating in a 3:1 ratio might originate from an extra set of *C. canephora* chromosomes that remained in the Catimor genome after backcrossing. The 11 Catimor LGs, consisting of markers fitting a 3:1 segregation ratio, could be the sub-genome of *C. canephora*. In our Mokka hybrid, most of the markers fitting 3:1 segregation ratio were not mapped to LGs; this suggests that some homologous regions scattered on the corresponding chromosomes of the two sub-genomes are at a greater distance than the stringent mapping threshold we set at $\theta=0.25$.

DNA fingerprinting of arabica coffee cultivars has shown very low genetic diversity, which has been attributed to the restricted number of starting ancestral plants and the reproductive consequence of self-pollination (Anthony et al. 2002; Steiger et al. 2002). This is confirmed in our present linkage map. Although Catimor and Mokka hybrid were separated by Catuai and Caturra in the previously published dendrogram (Steiger et al. 2002), only a very low level of polymorphism (an average 1.6 markers per primer pair) was detected between the two parental cultivars. Comparing our two parental cultivars, Catimor is more heterozygous with 68% of the total marker generated as Catimor-dominant; the higher heterozygosity occurs because Catimor was derived from an interspecific cross. Contrarily, Mokka hybrid is derived from a cross of two arabica cultivars, Mokka and Typica (H.P. Medina-Filho, personal communication) and exhibits less heterozygosity with only 30% of the markers generated as Mokka-dominant. Even though our present mapping efforts show the level of polymorphism rate to be low so that large mapping gaps remain (as evidenced by the many small LGs and unlinked markers), the parental cultivars we selected for linkage map construction are possibly the most diverse we could have used among arabica genotypes. The alternative mapping population derived from Maragogipe × Catuai had such a prohibitively low rate of polymorphism that it would have been virtually impossible to construct a genetic map. Our use of hybrid genotypes to develop a mapping population is seen as a valid and efficient approach for genetic and QTL mapping in arabica coffee.

Although the AFLP marker system mainly produces dominant markers, co-dominant markers can be identified

occasionally when the homologous sequences from the two parents differ by only a few nucleotides in length. In the present mapping project, 8 of the 464 markers (1.7%) were co-dominant. In another AFLP mapping project carried out by our group for the diploid species *Carica papaya*, 7.9% of the markers were co-dominant (Ma et al., in preparation). In that case, the two parental cultivars of papaya are known to be closely related and from the same gene pool. The relatively low percentage of co-dominant markers detected in this arabica coffee mapping population compared to that detected in papaya may reflect the degree of genetic difference between the two parental cultivars of coffee than between the papaya parents, or it may reflect the consequences of polyploidization of arabica coffee.

Segregation distortion was detected in a large portion of total markers detected. Specifically, 22% of Catimor markers and 45% of Mokka markers were distorted from expected segregation ratios. Segregation distortion among Catimor markers may be partly due to the multivalent formation of chromosomes: tetrasomic inheritance is reported as prevalent in an interspecific hybrid derived from *C. arabica* and *C. canephora* (Lashermes et al. 2000a) so that subsequent backcrossing might break the balance and generate multivalent formation of chromosomes. Arabica coffee exhibits disomic inheritance (Krug and Mendes 1940; Lashermes et al. 2000b) and a limited amount of segregation distortion was detected when constructing a genetic map of *C. canephora* (Paillard et al. 1996; Lashermes et al. 2001). The high percentage of distorted Mokka hybrid markers was unexpected and could not be explained by occasional multivalent formation of chromosomes reported in *C. arabica* (Grassias and Kammacher 1975; Owuor 1985). Distorted segregation could be caused by gametic, zygotic, and post-zygotic selections and be affected by genetic, physiological, and environmental factors (Gadish and Zamir 1986; Zamir and Tadmor 1986; Burt et al. 1991; Ky et al. 2000; Lashermes et al. 2001). However, our experiments could not address any of these possible influences. In addition, double reduction might have played a role in the segregation distortion observed on both Mokka and Catimor markers (Burnham 1962).

Arabica coffee is a perennial polyploid species. Developing a mapping population would normally take 5–7 years. The time-consuming and costly process in developing a mapping population coupled with the low level of genetic diversity within the species has delayed genetic map construction for this important crop plant. Pseudo-F_2 segregating populations have proved to be an effective and efficient approach for genetic and QTL mapping in polyploid species (Mudge et al. 1996; Ming et al. 1998, 2001). Our use of a coffee pseudo-F_2 mapping population coupled with a high throughput AFLP marker system resulted in the construction of an arabica coffee genetic map. We are in the process of developing a true F_2 mapping population from the same cross for comparative analysis that could reveal insights on genome organization, levels of heterozygosity, and the distribution of QTLs in the two sub-genomes.

The dramatic differences in plant architecture and coffee cupping quality between these two parental cultivars make this segregating population an attractive resource for mapping QTLs controlling these traits. The large differences in leaf and bean sizes between the two parents provide opportunities to study source/sink relationship in coffee. Mapping QTLs controlling source sink traits will identify markers for selection of coffee quality and productivity; the relationship between bean size and cupping quality is also expected to reveal useful markers.

Acknowledgements We thank Susan Ancheta for technical assistance, Andrew Paterson for constructive discussions, Leslie Poland for planting and tending the mapping population at the Kunia substation, and Melinda Moore, Zhiyong Liu, and Stephanie Whalen for reviewing the manuscript. This work was supported by a U.S. Department of Agriculture, Agricultural Research Service Cooperative Agreement (No. CA 58-5320-8-134) with the Hawaii Agriculture Research Center.

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