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Cultivar identification and genetic map of mango (*Mangifera indica)*

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Summary

Amplified Fragment Length Polymorphism (AFLP) information was used for identification of mango (*Mangifera indica L.*) cultivars, for studying the genetic relationship among 16 mango cultivars and seven mango rootstocks and for the construction of a genetic linkage map. Six AFLP primer combinations produced 204 clear bands and on the average 34 bands for each combination. The average Band-Sharing between cultivars and rootstocks was 83% and 80%, respectively. The average Band-Sharing for mango is 81%. The probability of obtaining a similar pattern for two different mango cultivars and rootstocks is 6×10^{-3} and 2×10^{-3}, respectively. A preliminary genetic linkage map of the mango genome was constructed, based on the progeny of a cross between 'Keitt' and 'Tommy-Atkins'. This linkage map consists of 13 linkage groups and covers 161.5 cm defined by 34 AFLP markers.

Introduction

DNA markers have the potential to be used in mango for marker assisted selection (MAS) and for cultivar identification (Lavi et al., 1993). Morphological markers (Singh, 1969) as well as isozymes (Degani et al., 1990, 1992) have been used for this purpose. However, the small number of available markers of these kinds, as well as their low level of polymorphism has limited their application.

DNA fingerprint information based on minisatellite loci and RAPD markers was used for identification of mango cultivars (Adato et al., 1995; Schnell et al., 1995), for genetic relatedness analysis of 20 mango cultivars and for genetic analysis of a family structure. Several other genomes of fruit trees including apple, citrus and avocado have been mapped using DNA markers (Kijas et al., 1997; Sharon et al., 1997).

Amplified Fragment Length Polymorphism (AFLP) was developed by Zabeau & Vos (1993). AFLP is considered to be a powerful, reliable and rapid assay with potential application in genome mapping, fingerprinting and marker-assisted breeding (Thomas et al., 1995; Vos et al., 1995). The AFLP technique is based on the detection of genomic restriction fragments by PCR amplification, and has been applied to various plant species. Band patterns are produced without prior sequence knowledge (Vos et al., 1995) using a limited set of generic primers. This PCR-based technique permits inspection of polymorphism at a large number of loci within a very short period of time and requires very small amounts of DNA. In this study, AFLP information was used for identification of mango trees ($2n$ = 20), and for the construction of a mango genetic map.

Materials and methods

Plant material

The plant material for the present study consists of: a. 16 cultivars: 'Austin', 'Carabao', 'Edward', 'Haden', 'Irvin', 'Keitt', 'Kensington', 'Langra', 'Mabruka', 'Manzanillo', 'Maya', 'Naomi', 'Palmer', 'Shelly', 'Tango', 'Tommy- Atkins'. b. Seven rootstocks:

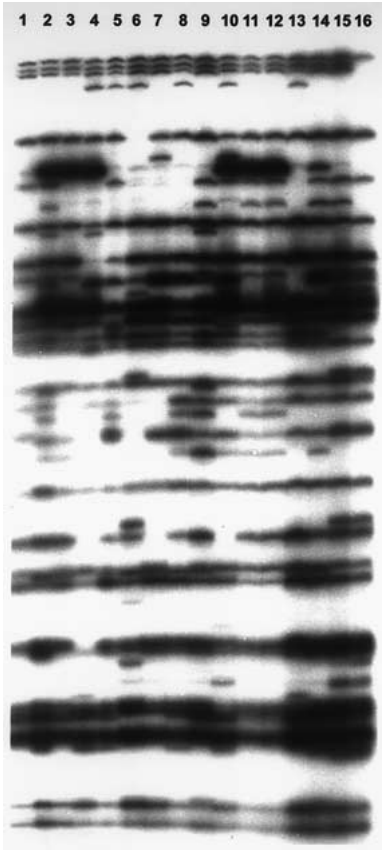

Figure 1. AFLP band pattern derived by the primer combination AG/TG. Lanes: 1) 'Tango', 2) 'Shelly', 3) 'Naomi', 4) 'Langra', 5) 'Mabruka', 6) 'Carabao', 7) 'Edward', 8) 'Austin', 9) 'Manzanillo', 10) 'Kensington', 11) 'Tommy-Atkins', 12), 'Irvin' 13) 'Palmer', 14) 'Haden', 15) 'Maya', 16). 'Keitt'.

'Colombo Kidney', 'Gumera 1', 'Gumera 3′, 'Gumera 4', 'Sabre', '7/32' and '13/1' and c. Sixty progeny originated from the cross 'Keitt' × 'Tommy-Atkins', grown at the Volcani Center orchard in Israel. The cross was performed by caging the two trees under a net cage in the presence of a beehive (Lavi et al., 1993). It is noteworthy that crossing mango cultivars is quite difficult due to the large amount of fruit drop and the small flower size (Lavi et al., 1993; Schnell et al., 1995). In the current cross only 29 out of the 60 progeny turned out to be true hybrids, while the remaining 31 seedlings were found to be either the result of selfing of one parent or the result of a foreign pollen donor.

DNA extraction

DNA was extracted from (2gr) young fresh leaves by modification of cetyltrimethyl ammonium bromide (CTAB) method (Murray & Thompson, 1980). The extraction buffer (20 ml) contained 2% CTAB, 0.1 M Tris-HCl (pH-8), 0.02 M ethylenediamine tetraacetic acid (EDTA), 1.4 M NaCl and 15 mM sodium bisulfate ($Na_2S_2O_5$). Chloroform extraction (20 ml) was performed two times. Two volumes of precipitation buffer were added to the final aqueous phase. The precipitation buffer contained 1% CTAB, 0.05 M Tris-HCl (pH-8), 0.01 M EDTA and 15 mM sodium bisulfate. The DNA samples were dissolved in 1 M cesium chloride (CsCl), precipitated with ethanol and redissolved in ddH_2O. DNA quantity and quality was assessed on an agarose gel.

AFLP reaction

The AFLP procedure was performed according to the protocol of GIBCO BRL, after Zabeau and Vos (1993) with minor modifications. Genomic DNA (0.5 μg) was digested at 37 °C for 2 h. The Restriction-Ligation reaction contained 12 U *MseI*, 10 U *EcoRI*, 1 U T4-DNA ligase, 5 pmole *EcoRI*-adaptor, 50 pmole *MseI* adaptor, 0.5 M NaCl, 5 μg BSA (bovine serum albomine), and 10 × DNA ligase buffer in a final volume of 10 μl. The digested-ligated DNA was diluted 1:10 with 0.1 TE (10 mM Tris-HCl (pH-8), 0.1 mM EDTA). The adaptor sequences were:

MseI adaptors:
5′ TAC TCA GGA CTC AT 3′
5′ GAC GAT GAG TCC TGA G 3′
EcoRI adaptors:
5′ CTC GTA GAC TGC GTA CC 3′
5′ AAT TGG TAC GCA GTC TAC 3′

Pre-selective amplification was performed with primers complementary to the core of the adaptor sequences. The PCR reaction contained 50 ng of restricted-ligated DNA, 50 ng *EcoRI* primer, 50 ng *MseI* primer, 1 U *Taq* DNA polymerase (Promega), 2 μl of 10 × *Taq* DNA polymerase buffer (Promega), 2 μl of 25 mM $MgCl_2$, and 2.5 mM dNTPs in a final volume of 20 μl. The PCR conditions: 20 cycles of 30 sec at 94 °C, 60 sec at 56 °C, and 60 sec at 72 °C. After pre-amplification, the PCR products were diluted 1:20 with 0.1 TE. The pre-selective amplification primers sequences were:

EcoRI pre-selective amplification primer:
5′ GAC TGC GTA CCA ATT CA 3′
MseI pre-selective amplification primer:
5′ GAT GAG TCC TGA GTA AC 3′

Selective-amplification was performed by end-labeling

the *EcoRI* primer using γ-^{33}P ATP. The labeling reaction contained, 0.5 μg *EcoRI* primer, 100 μCi γ-^{33}P ATP, 10 U T4 polynucleotidekinase, and 10 μl of 5 $\times$ polynucleotide kinase buffer, in a final volume of 50 μl. Samples were incubated at 37 °C for 1h then heated to 70 °C for 10 min. Several selective amplifications were run from a single pre-selective amplification.

The selective-amplification reaction contained: 50 ng template DNA from the preselective amplification, 1ng labeled*EcoRI* primer, 5ng *MseI* primer, 1 U *Taq* DNA polymerase (Promega), 2 μl of *Taq* DNA polymerase buffer (Promega), 2 μl of 25 mM $MgCl_2$ and 2.5 mM dNTPs, in a final volume of 20 μl. The PCR cycles were: one cycle of 2 min at 94 °C, 30 sec at 65 °C and 2 min at 72 °C, followed by 10 cycles each of annealing temperature of 1 °C less than the former one and 25 cycles of 1sec at 94 °C, 30 sec at 56 °C, and 2 min at 72 °C. The sequence of the selective amplification primers were:

EcoRI selective amplification primers:
5′ GAC TGC GTA CCA ATT CA ***NN*** 3′
MseI selective amplification primers:
5′ GAT GAG TCC TGA GTA AC ***NN*** 3′

The first 17 nucleotides of these primers are identical to the pre-selective amplification primers, and the NN are random nucleotides added to the selective primers (see details in the Results). The AFLP primers were named by the last 2 specific nucleotides added to the pre-selective amplification primers, by their order on the gel and in parenthesis, their bp size.

The PCR products were mixed with 20 μl of formamide dye (98% formamide, 10 mM EDTA, bromophenol blue, xylene cyanol). The denatured PCR products were separated on 5% denaturing polyacrylamide (20:1 acrylamide: bis, 7.5 M urea, 1 $\times$ Tris borate EDTA-TBE buffer) standard sequencing gel (43 cm long) at 55 Watts for 1.5 hr (after 40 min of pre-run in the same conditions). The gel was then dried on a gel drier for 40 min at 80 °C and exposed to X-ray film (Fuji) for about 72 h at room temperature.

AFLP analysis

All AFLP bands were assessed for presence (1) or absence (0) in each analyzed lane (either cultivars or progeny of a cross). In each genotype, only clear and unambiguous bands were scored. Band-Sharing was defined as: Band-Sharing = 2(Nab)/(Na+Nb), where Na and Nb are the total number of scored bands in lanes a and b, respectively, and Nab is the number of bands common to both lanes (Hillel et al., 1990). Band-Sharing was calculated to estimate the level of similarity between two multi-band patterns. The probability of obtaining a similar pattern of bands for two different cultivars was defined as: $P = X^T$, where X is average Band-Sharing of among various cultivars and T is the average total bands of the various cultivars (Hillel et al., 1990). Grouping was carried out by unweighted pair-group method using arithmetic averages (UPGMA) cluster analysis.

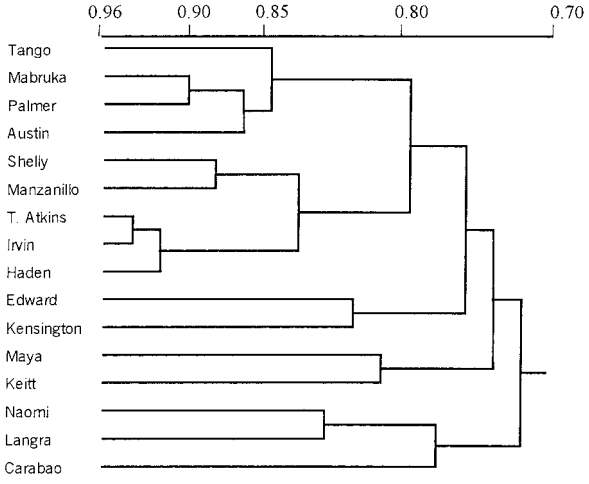

Figure 2a. Analysis of 16 mango cultivars using 42 various AFLP bands.

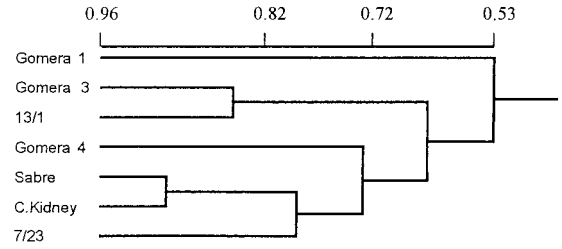

Figure 2b. Analysis of 7 mango rootstocks using 30 various AFLP bands.
The analysis was carried out using the UPGMA cluster analysis method for the bands and a pairwise difference between genotypes.

Map construction

Each of the two parents and 29 progeny were genotyped using 105 AFLP bands. The segregation of the alleles from each marker was examined for deviation

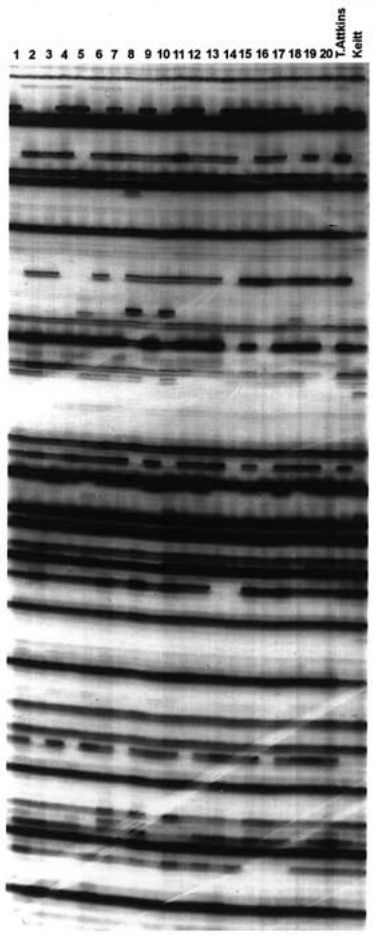

Figure 3. AFLP band pattern derived by the primer combination GG/TT. The right two lanes are the parents 'Keitt' and 'Tommy-Atkins'. The remaining lanes are 20 of their progeny.

from Mendelian expectations using the LINKEM software (Vowden et al., 1994). Map construction was performed using both the LINKEM and the MAPMAKER 3.0 software (Lander & Green 1987), which analyzes data from controlled crosses with a known linkage phase.

Reproducibility of the results was demonstrated by obtaining identical band patterns from independent DNA samples of the same plant (data not shown).

Results

AFLP analysis of 16 mango cultivars and seven rootstocks with six primer combinations: AG/TG, GG/AT, AC/TG, AG/AT, AC/TA, CC/AC, identified 204 clear bands. On the average, there were 34 bands for each primer combination. Figure 1 shows AFLP patterns of various cultivars produced by one primer combination. The size of the polymorphic amplified fragments ranged from 50–500 bp.

AFLP analyses of mango cultivars

The average number of cultivar specific bands was 4. The average Band-Sharing between the 16 cultivars was 83%, and ranged between 70% and 94%, depending on the primer combination (Table 1). The lowest Band-Sharing was between 'Mabruka' and 'Carabao', and the highest Band-Sharing was between 'Maya' and 'Keitt'.

AFLP analyses of mango rootstocks

The average number of rootstock specific bands was 5. The average Band-Sharing between the seven rootstocks was 80%, and ranged between 53% and 96% (Table 2). The lowest Band-Sharing was between 'Sabre' and '7/32', and the highest Band-Sharing was between '13/1' and 'Sabre'. The probability of obtaining a similar pattern for two different mango cultivars is 6×10^{-3} and for two mango rootstocks is 2×10^{-3}.

Genetic relationships among 16 mango cultivars and seven mango rootstocks represented in a dendrogram using UPGMA cluster analysis (Figures 2a and b, respectively).

AFLP segregation

Twenty-nine progeny of the cross 'Keitt' × 'Tommy Atkins' were analyzed to study the segregation of the AFLP markers. Eighty five percent (85%) of the polymorphic AFLP bands showed Mendelian segregation and 15% showed distorted segregation. The Mendelian segregating bands included two groups: 1. Bands common to both parents which segregated in the progeny in a 3:1 ratio (presence/absence) and therefore represent heterozygous loci in both parents. 2. Unique bands in one of the parents, which segregated in the progeny in a 1:1 ratio and therefore represent heterozygous loci. The first group included 78.6% of the bands and the second 21.4%.

Mango linkage map

The genotypes of the two parents ('Tommy-Atkins' and 'Keitt') and 29 progeny were determined using 105 AFLP bands, derived from 14 primer combinations: AG/TG, CT/TT, AC/TA, GC/AA, GG/TT,

Table 1. Band-Sharing values among 14 pairs of sixteen mango cultivars

Cultivar pairs		Total number of bands		Common	Band-
A	B	A	B	bands	Sharing
'Maya'	'Keitt'	37	33	33	0.94
'Edward'	'Austin'	31	35	30	0.90
'Palmer'	'Haden'	30	35	29	0.89
'Irvin'	'Palmer'	37	30	30	0.89
'Austin'	'Manzanillo'	35	37	31	0.86
'Shelly'	'Naomi'	38	32	30	0.86
'Haden'	'Maya'	35	37	31	0.86
'Kensington'	'T.Attkins'	38	37	32	0.85
'Tango'	'Shelly'	30	38	28	0.82
'Langra'	'Mabruka'	32	35	27	0.80
'Manzanillo'	'Kensington'	37	38	30	0.80
'Naomi'	'Langra'	32	32	25	0.78
'Carabao'	'Edward'	33	31	24	0.70
'Mabruka'	'Carabao'	35	33	27	0.70
Total		585[a]		–	–
Average		34		29	0.83

[a] Total bands of the various cultivars analyzed in this study.

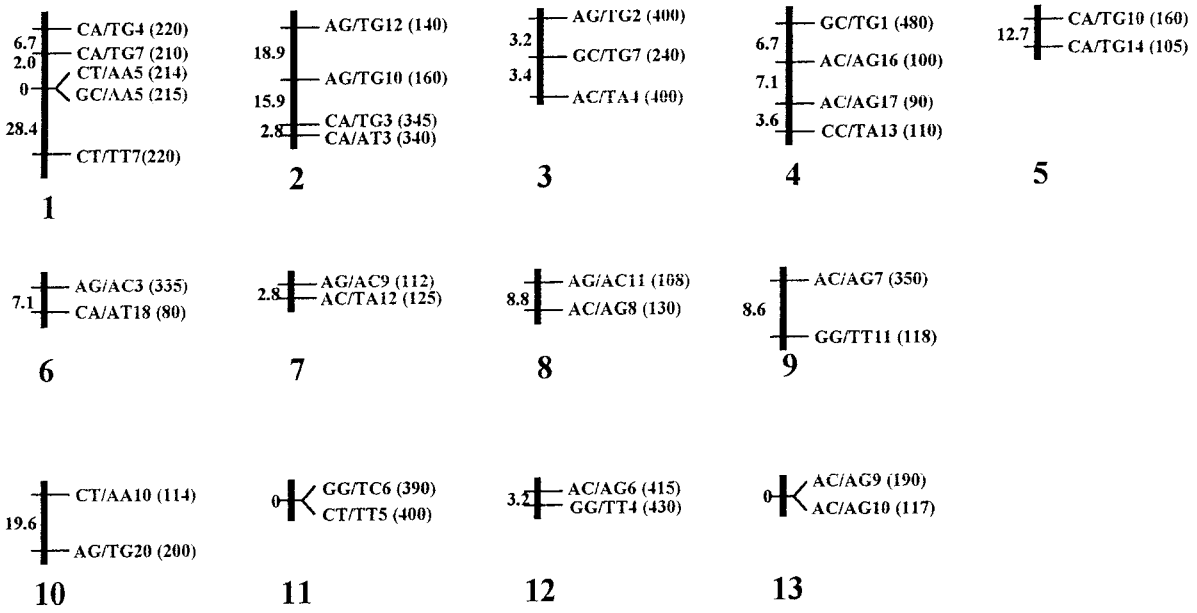

Figure 4. Mango Genetic Linkage Map.
The various marker names refer to the primer combinations used in the AFLP (see Material and methods). The numbers in parentheses indicated the size of the markers in base pairs (bp). Distances between markers are in centiMorgans.

Table 2. Band-Sharing values among six pairs of seven mango rootstocks

Rootstock pairs		Total number of bands		Common	Band-
A	B	A	B	bands	Sharing
'13/1	'Sabre'	30	24	13	0.96
'Gomera 1'	'Gomera 3′	27	30	27	0.94
'Gomera 3'	'Gomera 4'	30	26	23	0.82
'Gomera 4'	'13/1'	26	30	20	0.71
'7/32'	'C. Kidney'	28	34	26	0.54
'Sabre'	'7/32'	24	28	14	0.53
Total		199[a]		–	–
Average		28		20	0.8

[a] Total bands of the various rootstocks analyzed in this study.

AC/AG, CC/TA, GC/TG, CA/AT, AG/AC, CT/AA, CA/TG, GG/TC, AC/AA. Of the 105 AFLP markers that were defined, 90 markers behaved in a Mendelian fashion and were analyzed. Thirty-four were mapped in linkage groups and 56 markers remain unlinked. Figure 3 shows AFLP patterns produced by one primer combination. The combined map consists of 13 linkage groups and 34 markers (Figure 4). It is noteworthy, that the genetic map consists only of markers that behave in a Mendelian fashion. The number of markers per linkage group varies between 2 and 5, and the length of the 13 linkage groups varies from 0 cM to 37.1 cM. The genetic map spans 161.5 cM with an average distance of 4.7 cM between two markers. Linkage group 1 is the largest group containing 5 markers. This group alone spanned over 37.1 cM. Linkage analyses were carried out using both the MAPMAKER and LINKEM softwares. The two programs provided identical results.

Discussion

Currently, most of the mango cultivars are being identified on the basis of leaf, fruit and stone characteristics. These traits may be affected by environmental conditions (Lakshminarayana, 1980). Therefore, AFLP markers seem to be a useful tool for identification of mango cultivars.

The AFLP technique allows the detection of polymorphisms at multiple loci, generating large number of reproducible molecular markers within a short period of time. This method is robust for efficient DNA fingerprinting of the mango genome. A great majority of the AFLP markers (85%) are transmitted in a Mendelian fashion thus these markers could be used for genetic analysis.

In this study, AFLP markers were found to be useful in identification of mango cultivars and rootstocks. No identical AFLP patterns were detected between two different cultivars or rootstocks. Minisatellite loci markers (DNA fingerprint) were used by Adato et al. (1995) for identification of mango cultivars. Based on the minisatellite markers, the probabilities of obtaining a similar pattern for two different mango cultivars and rootstocks were calculated to be 8×10^{-6} and 1.2×10^{-6}, respectively (Adato et al., 1995). In the current study based on AFLP these probabilities are lower and were calculated to be: 6×10^{-3} for cultivars and 2×10^{-3} for rootstocks. Although cultivars and rootstocks were analyzed separately, their Band-Sharing values are not significantly different (0.80 for cultivars and 0.81 for rootstocks), the average Band-Sharing for mango is therefore, 0.81. The probabilities for obtaining similar pattern for two mango trees are somewhat higher for rootstocks mainly due to lower number of total bands in the rootstocks. However, this number could results from the lower number of analyzed rootstocks (seven) compared with that of the cultivars (sixteen). The DFP system is much more polymorphic although less easy to handle. The AFLP probabilities are satisfactory for most practical applications and if necessary, can be improved by using more primer combinations. RAPD markers, which were used to identify mango cultivars (Schnell et al., 1995), are limited in their level of polymorphism and the technique is not highly reproducible (Jones et al., 1997). AFLPs, on the other hand, are PCR based, reliable and multi-loci, detecting a significant level of polymorphism.

Because of the difficulty in performing controlled crosses, horticulturists have analyzed open-pollinated seeds or Maternal Half-Sib families-MHS (Schnell et al., 1995). AFLP technique can be used to determine the pollen donor in crosses performed in cages.

The current AFLP map is the first genetic map of mango. Both the minisatellite (Adato et al., 1995) and the RAPD (Schnell et al., 1995) were used for identification and estimation of genetic relatedness only. The current AFLP map consists of 34 markers. The proportion of unlinked markers (62%) is higher than in other maps but this is not surprising considering the number of true progeny that could be analyzed. In *Eucalyptus globulus,* 606 AFLP markers were analyzed, 338 of them (55%) remained unlinked and the other 268 AFLP markers were mapped to 14 linkage groups using F1 progeny (Marques et al., 1998). In rice, 33% of the AFLP markers remained unlinked, using F2 progeny as a mapping population (Mackill et al., 1996), while in another rice population (doubled haploid), all the 208 AFLP markers were mapped (Maheswaran et al., 1997).

The mapping approach we used in the current research is based on transforming the mango mapping data to F2 intercross data in order to meet the MAPMAKER and the LINKEM requirements. These two programs provided identical results. The genetic map is based on a minimum Lod Score of 3.0 to determine linkage between two markers. The mango family used in the current study consists of 29 progeny, which were selected from 60 progeny (see Materials and Methods). The current map resulted from analysis of Mendelian bands only. Linkage analysis of all bands (Mendelian and non-Mendelian) result in a major change namely, the appearance of a large linkage group consisting of 203 cM. After reexamination of the data, we believe that the non-Mendelian markers do not result from technical reasons. Therefore, the most plausible explanations are either selection against certain progeny or some chromosomal aberrations such as duplication which could hamper the linkage analysis.

The mango genome consists of 4.39×10^8 bp (Arumuganathan & Earle, 1991) and has 20 chromosomes, most of them small. There is no estimation for the mango genome size in cM. In humans 1 cM is about 1 Mbp (Ott 1991), in tomato it is estimated that 1 cM equals to 750 kb (Pillen et al., 1996) and in rice it is estimated that 1 cM equals about 1 Mbp (Maheswaran et al., 1997). The mango genome size is therefore estimated to range between 440 cM to 590 cM of which the current map covers 161.5 cM. To increase the reliability of the map there is a need to increase the number of markers and the number of progeny.

This preliminary research suggests that the AFLP markers are suitable for cultivar identification, estimating genetic relationships and mapping QTLs in mango.

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