

Development of EST-SSR and TRAP markers from transcriptome sequencing data of the mango

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ABSTRACT. Mango is one of the most commercially important fruit crops in tropical and subtropical regions. To increase the efficiency of breeding strategies, two EST-derived marker systems were developed in the present study using information from the mango fruit transcriptome. Using simple sequence repeats, 218 of 230 primer pairs showed stable amplification for 7 mango genotypes with amplicons ranging from 84 to 160 bp; 93 of the primer pairs yielded polymorphic products. The proportion of polymorphic bands ranged from 16.67 to 100%, with a mean of 55.64%. In contrast, 86 primer pairs exhibited good amplification with clear bands for target region amplification polymorphism analysis, and a total of 66 primer combinations were polymorphic. These two novel sets of EST-derived markers will be of use in future studies of genetic diversity, genetic map construction, and marker-assisted selection in mango.

Key words: Mango; EST-SSR; TRAP; Genetic analysis

INTRODUCTION

Mango (*Mangifera indica* Linn.) is a very valuable commercial fruit crop in tropical and subtropical regions. During the past century, a large number of mango cultivars have been developed in different countries; these cultivars vary in the shape of the fruit, size, skin color, and flavor. However, crosses between these cultivars may result in homonymy and synonymy and may cause cultivar confusion (Parfitt et al., 1991). Traditionally, morphological characteristics have been used to discriminate different cultivars but this approach had limited success. More effective markers are needed to overcome these problems of identification.

The mango is considered a difficult species to improve through breeding programs because of some inherent characteristics including long juvenile phase, high level of heterozygosity, only one seed per fruit, and heavy fruit drop leading to low retention of crossed fruits (Iyer and Schnell, 2009). Many economically important traits are controlled by multiple loci and it will be necessary to develop a comprehensive genetic linkage map to investigate them. In recent years, DNA molecular markers have been widely applied in plant genetic research. Identification of mango cultivars and study of genetic diversity have been carried out using DNA marker methods, such as random amplified polymorphic DNA, amplified fragment length polymorphism, inter-simple sequence repeat, and simple sequence repeat (SSR). To date, however, the number of markers developed is insufficient to build a high-density genetic map.

The development of next generation sequencing methods has enabled the production of data that can be used for developing SSR (microsatellite) and targeted region amplified polymorphism (TRAP) molecular markers. SSRs are widespread and randomly distributed in animal and plant genomes. Two types of SSR are recognized, namely genomic SSRs and expressed sequence tag (EST)-SSRs. In general, identifying genomic SSRs is a time-consuming and labor-intensive process (Thiel et al., 2003). However, it is simpler and cheaper to develop EST-SSRs; additionally, EST-SSRs from one species can be applied to others in the same taxon (Varshney et al., 2005). The TRAP approach also makes use of expression data and uses anchored and arbitrary primers to target functional gene regions (Hu and Vick, 2003). It is simple to operate and easy to build, and the repeatability and efficiency of TRAP markers are high. Because of these beneficial characteristics, TRAP marker have now been successfully applied to many horticultural applications, such as in genetic map construction in wheat (Menzo et al., 2013) and genetic diversity analysis in spinach (Hu et al., 2007).

The main purpose of the present study was to develop abundant EST-SSR and TRAP markers from the mango transcriptome sequencing data. Through development of these two marker systems, it should be feasible in future to investigate genetic relationships among wild species and cultivars of mango, construct a genetic map, carry out functional mapping, and perform marker-assisted selection.

MATERIAL AND METHODS

Plant materials and DNA extraction

Seven mango cultivars were used here, namely, Macheso, Guire No. 82, Nam Dok Mai, Renong No. 1, Zillate, Irwin, and Jin-hwang. The plants were obtained from the South Subtropical Crops Research Institute, Zhanjiang, China. Genomic DNA isolation was performed using fresh leaves by the improved CTAB method (Kashkush et al., 2001).

Primer design

EST-SSR primers were designed using the mango EST database that was constructed from transcriptome sequencing in our laboratory (Wu et al., 2014). The primers were designed with Primer Premier 5.0 (Lalitha, 2000). To generate TRAP markers, we designed 13 fixed primers and selected 7 arbitrary primers (Table 1). The fixed primers were derived from mango disease-resistance genes identified in a search of the non-redundant NCBI nucleotide database. The arbitrary primers were taken from Li and Quiros (2001).

Table 1. TRAP primer sequences used in this study.

Primer	Type	Sequences (5'-3')
MI1	Fixed	GCTTGGGTTTCCGTTTCT
MI2	Fixed	CAATAGAAAGACTTAGCCGTAA
MI3	Fixed	AACAACCATTGCGGGAGC
MI4	Fixed	TGACACGAGGTCTGAATG
MI5	Fixed	AAAGTTCTTATTGTGCTGGATG
MI6	Fixed	CTACCCTTGCTCAACTCG
MI7	Fixed	GCTTGGGTTTCCGTTTCT
MI8	Fixed	ATGTGGCTAAGGTTGCTG
MI9	Fixed	CGGCAAACCCAGCAAAGAGT
MI10	Fixed	CCACCACTCACCTGGAAGCA
MI11	Fixed	ATGACAAGGACAAAGGAGGAG
MI12	Fixed	CGACAGGGAAGGAAGGAGTA
MI13	Fixed	TCACTTGTTTGGGTCTCAG
Em1	Arbitrary	GACTGCGTACGAATTAAT
Em2	Arbitrary	GACTGCGTACGAATTTGC
Em3	Arbitrary	GACTGCGTACGAATTGAC
Em4	Arbitrary	GACTGCGTACGAATTTGA
Em5	Arbitrary	GACTGCGTACGAATTAAC
Em6	Arbitrary	GACTGCGTACGAATTGCA
Me1	Arbitrary	TGAGTCCAAACCCGGATA

EST-SSR development

Two hundred and thirty EST-SSR primer pairs were employed. The PCR mixtures (20 μ L total volume) contained 40 ng genomic DNA, 0.4 mM dNTPs, 1X buffer, 1.8 mM $MgCl_2$, 0.75 U *Taq* DNA polymerase and 0.2 μ M of each primer. The amplifications were carried using a TaKaRa PCR Thermal Cycler Dice (Takara, Japan) with the following program: 5 min at 94°C; 35 cycles of 30 s at 94°C, 45 s at 58°C, and 1 min at 72°C; and then a final elongation step of 5 min at 72°C. PCR fragments were separated on 6% denaturing polyacrylamide gels followed by silver staining for visualization of the amplicons (Bassam et al., 1991; Charters et al., 1996).

TRAP development

Ninety-one different primer combinations were employed using 13 fixed and 7 arbitrary primers. Amplification was performed in 15 μ L PCR mixtures containing 40 ng genomic DNA, 1X reaction buffer, 1.7 mM $MgCl_2$, 0.2 mM dNTPs, 0.8 μ M fixed primer, 0.04 μ M arbitrary primer, and 1.7 U *Taq* DNA polymerase. The following amplification program was used: initial denaturation of the template DNA at 94°C for 4 min; then 5 cycles at 94°C for 45 s, 40°C for 45 s and 72°C for 1 min; followed by 35 cycles at 94°C for 45 s, 52°C for 45 s, and

72°C for 1 min; and a final extension step at 72°C for 7 min. PCR products were separated on 2% agarose gels.

Analysis of polymorphic loci

Seven mango accessions were used to evaluate the degree of polymorphism with the newly developed EST-SSR and TRAP markers. All visible and clear polymorphic bands were scored in EST-SSR and TRAP profiles as 0 and 1 on the basis of absence or presence of the band, respectively. Each polymorphic band at a particular position on the gel was considered a unique locus in the genome.

RESULTS AND DISCUSSION

To verify that the 230 pairs of EST-SSR primers designed from transcription data were effective and could identify polymorphisms, they were used to screen genomic DNA from seven mango genotypes (Figure 1). In total, 218 pairs of primers showed stable PCR amplification with products of the expected sizes; 93 pairs of primers identified polymorphisms in the 7 mango genotypes ([Table S1](#)). The rate of polymorphism in mangoes (43.12%) was lower than the 64.7% reported in oak (Torre et al., 2014), 63.23% in persimmon (Luo et al., 2014), and 46.5% in field pea (Kaur et al., 2012), but higher than the 21.2% in Asian lotus (Zhang et al., 2014a), 27.3% in cotton (Zhang et al., 2014b), and 36% in tea (Wu et al., 2013). The sequences of the 93 pairs of primers that identified polymorphisms and the characteristics of their amplification products are given in [Table S1](#). The number of alleles detected in the 93 EST-SSR loci ranged from 2 to 8 with a mean of 3.91. The proportion of polymorphic bands ranged from 16.67 to 100% with a mean of 55.64%.

The 13 fixed primers derived from mango disease-resistance genes and the 7 arbitrary primers were combined into 91 primer pairs and used to screen the 7 mango genotypes. Overall, 66 primer combinations exhibited robust and polymorphic amplifications; 5 primer combinations produced either unclear or no amplification (MI3, MI4 or MI10 combined with Em1; MI4 or MI10 combined with Me1); 20 primer combinations gave an amplified product without polymorphism (MI1, MI6, MI7, MI11, and MI12 combined with Em1; MI2, and MI7 combined with Em2; MI4, MI7, and MI11 combined with Em3; MI1, MI7, MI9, and MI10 combined with Em4; MI2 and MI6 combined with Em5; MI1, MI11, and MI12 combined with Em6; MI3 combined with Me1). The TRAP markers developed here can therefore be used to study genetic diversity in mangoes and be of value for marker-assisted breeding.

Mango trees can be affected by diseases during flowering, fruit setting, and fruit development and, as a result, can have severely reduced production. The development of disease resistant varieties using marker-assisted selection offers an effective strategy to solve this problem. The TRAP markers developed here from disease-resistance gene sequences, and the polymorphic markers between different mango genotypes are candidate markers for such selection strategies. For example, the Jin-hwang cultivar is highly resistant to anthracnose whereas the Irwin cultivar is highly susceptible. In the TRAP analysis, we identified loci that were deleted in Irwin (MI3, MI4 or MI6 combined with Em4) (Figure 2) compared with Jin-hwang, which suggests that these loci might be linked to anthracnose disease resistance.

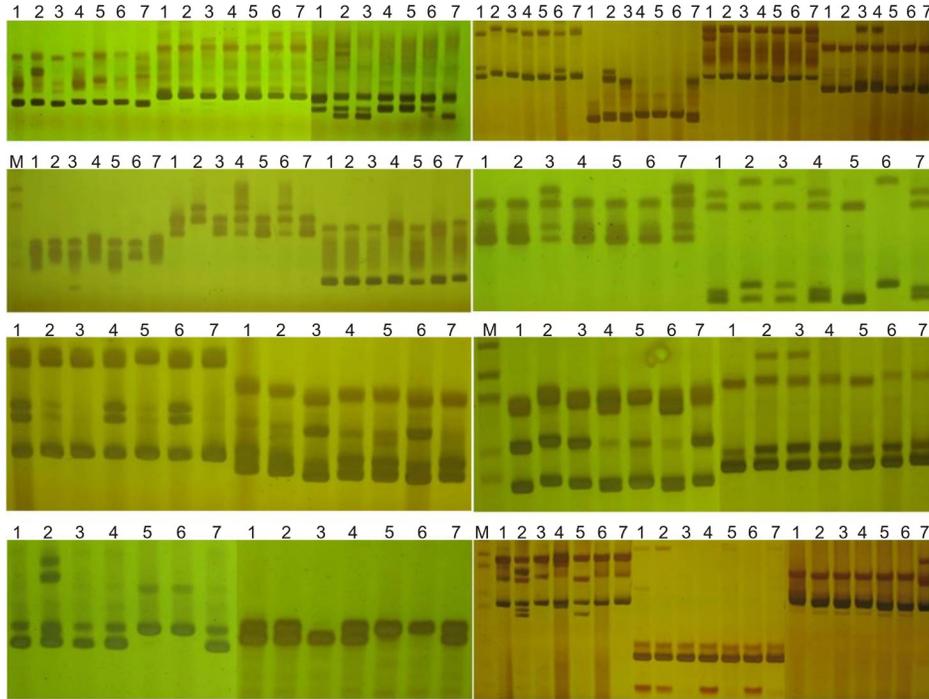


Figure 1. Representative examples of PCR products amplified using several EST-SSR primer pairs and separated by electrophoresis on 6% denaturing polyacrylamide gels. *Lanes 1, 2, 3, 4, 5, 6, and 7* represent DNA samples from mango genotype Macheso, Guire No. 82, Nam Dok Mai, Renong No. 1, Zillate, Irwin, and Jin-hwang, respectively; *Lane M*: pBR322 DNA/*MspI* marker.

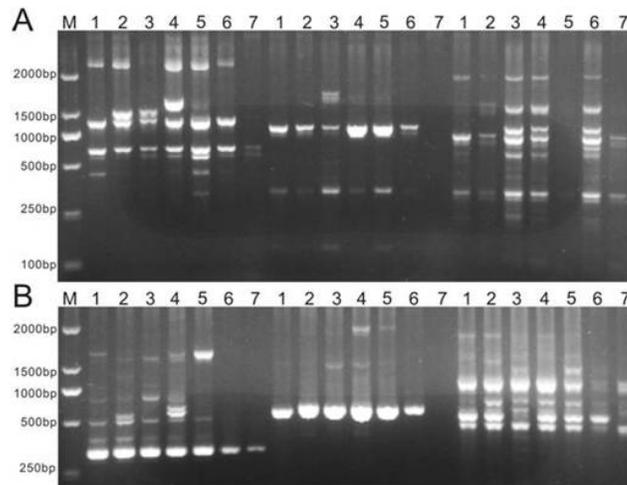


Figure 2. Profile of TRAP amplification products using primer combinations MI3/MI4/MI5+Em4 (**A**) and MI6/MI7/MI8+Em4 (**B**). *Lanes 1, 2, 3, 4, 5, 6, and 7* represent DNA samples from mango genotypes Macheso, Guire No. 82, Renong No. 1, Zillate, Jin-hwang, Irwin, and Nam Dok Mai. *Lane M*: Trans 2K marker.

Conflicts of interest

The authors declare no conflict of interest.

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[Supplementary material](#)

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