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Molecular cloning, identification, and chromosomal localization of two *MADS* box genes in peach (*Prunus persica*)

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Abstract

MADS box proteins play an important role in floral development. To find genes involved in the floral transition of *Prunus* species, cDNAs for two *MADS* box genes, *PpMADS1* and *PpMADS10*, were cloned using degenerate primers and 5′- and 3′-RACE based on the sequence database of *P. persica* and *P. dulcis*. The full length of *PpMADS1* cDNA is 1,071 bp containing an open reading frame (ORF) of 717 bp and coding for a polypeptide of 238 amino acid residues. The full length of *PpMADS10* cDNA is 937 bp containing an ORF of 633 bp and coding for a polypeptide of 210 amino acid residues. Sequence comparison revealed that *PpMADS1* and *PpMADS10* were highly homologous to genes *AP1* and *PI* in *Arabidopsis*, respectively. Phylogenetic analysis indicated that *PpMADS1* belongs to the euAP1 clade of class A, and *PpMADS10* is a member of GLO/PI clade of class B. RT-PCR analysis showed that *PpMADS1* was expressed in sepal, petal, carpel, and fruit, which was slightly different from the expression pattern of *AP1*; *PpMADS10* was expressed in petal and stamen, which shared the same expression pattern as *PI*. Using selective mapping strategy, *PpMADS1* was assigned onto the Bin1:50 on the G1 linkage group between the markers MCO44 and TSA2, and *PpMADS10* onto the Bin1:73 on the same linkage group between the markers Lap-1 and FGA8. Our results provided the basis for further dissection of the two *MADS* box gene function.

Keywords: peach (*Prunus persica*); flower development; *MADS* box gene; selective mapping

Introduction

MADS box genes regulate several developmental processes in plants (Shore and Sharrocks, 1995). Extensive studies in *Arabidopsis thaliana*, *Antirrhinum majus*, *Petunia hybrida*, and in a number of other species have revealed that specific *MADS* box genes, alone or in combination, serve as regulators for formation of flower organs (Weigel, 1995; Theissen and Saedler, 1999; Theissen et al., 2000). Based on the studies of homeotic flower mutants in *Arabidopsis*, *Antirrhinum*, and *Petunia*, the ABCDE model of floral organ identity was established (Coen and Meyerowitz, 1991; Weigel and Meyerowitz, 1994; Colombo et al., 1995; Pelaz et al., 2000, 2001; Pinyopich et al., 2003). Class A genes specify sepals, and together with class B genes specify petals, class B and C genes specify stamens, class C alone determines the identity of carpels, class D genes are necessary for ovule development, and class E genes (*SEP* genes) are indispensable for the determination of petal, stamen, carpel, and ovule identity. The majority of classes A, B, C, D, and E homeotic genes belong to the *MADS* box transcription factor family (Theissen et al., 2000). Recent studies demonstrated that the function of *MADS* box genes are not restricted to flower organ development; some *MADS* box genes were seen to be involved in the initiation of flowering, determination of meristem identity (Weigel, 1995), embryonic development (Perry et al., 1999), root formation (Alvarez-Buylla et al., 2000), development of vascular tissue, and seed and fruit formation (Buchner and Boutin, 1998; Gu et al., 1998; Liljegren et al., 1998).

Peach (*Prunus persica* (L.) Batsch) is one of the most economically important species in the Rosaceae family, which includes a wide range of fruit and ornamental trees (OECD, 2002). Owing to the relatively small genome size,

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which is about twice that of *Arabidopsis*, peach is becoming an important model species for comparative genomics (Georgi et al., 2003) and identification of genes for fruit improvement (Abbott et al., 2002). Compared with several fruit trees, the juvenile phase of peach is relatively short (2–3 years), which facilitates genetic studies. The initiation and development of peach flowers are characterized by a number of distinct stages that occur throughout the year (Warriner et al., 1985; Reinoso et al., 2002). The transition from the vegetative to reproductive meristem coincides with cytological events that appear to reflect general changes in gene expression (Dermen and Stewart, 1973; Bitonti et al., 2002). During the summer months, the reproductive buds develop in the leaf axils, but are indistinguishable from the vegetative buds. The floral organs begin to differentiate in autumn and then go into dormancy over winter, in which the androecium continues to develop slowly; however, the gynoecium does not develop until spring when the organs mature rapidly (Monet and Bastard 1968; Dermen and Stewart 1973; Bitonti et al., 2002). The regulatory genes involved in this process have not been explored. Previous study by our group identified five *MADS* box genes related to floral bud, floral organ, and fruit formation, one *AGAMOUS* homologue *PpMADS4*, one *FRUITFULL* homologue *PpMADS6*, and three *SEPALLATA* homologues *PpMADS2*, *PpMADS5*, and *PpMADS7* (Wu et al., 2004; Xu et al., 2008a, 2008b). Genetic analysis has identified dozens of loci that regulate floral and fruit development in *Prunus* species in recent years (Dirlewanger et al., 2004; Silva et al., 2005; Sánchez-Pérez et al., 2007), but the genes responsible for these loci still remain unknown. The *MADS* box genes mentioned above can be the candidates for controlling these loci, as in the case of apple (*Malus domestica*) and almond (*Prunus dulcis*) (Yao et al., 2001; Silva et al., 2005,).

For a woody plant like peach, understanding of the mechanisms controlling the transition from vegetative to reproductive growth and the initiation of flowering is of great interest for the breeding program. In this study, two peach *MADS* box genes were identified and mapped onto the *Prunus* linkage group. The significance of the genes for molecular manipulation of *Prunus* species was further discussed.

Materials and methods

Plant materials

Peach materials were collected from the cultivar 04-01 grown in the field in Beijing Academy of Agriculture and Forestry Sciences, Beijing. The mapping materials were provided by Prof. Pere Arús of Department of Plant Genetics, CSIC-IRTA Laboratory of Plant Molecular Genetics, Barcelona, Spain.

Methods

RNA extraction and cDNA synthesis

Total RNA was isolated from floral buds by TE-3D method (Jacobsen-Lyon et al., 1995), and cDNA was synthesized using BD SMART^{TM} RACE cDNA Kits (Cat. No. 1804-1, BD Biosciences Clontech, Palo Alto, CA, USA).

Isolation of PpMADS1 and PpMADS10 cDNAs

The putative protein sequences of the class A gene *AP1* (GenBank accession no. Z16421) and the class B gene *PI* (GenBank accession no. D30807) were used as the queries to search against EST (Expressed sequence tags) database in the GenBank through tBLASTN (http://www.ncbi.nih.gov/BLAST), and there was no hit sequence homologous to *AP1* and only one peach EST (AY773012) homologous to *PI* in the database. To obtain the sequence homologous to *AP1* gene, two primers PP1-1, 5′-GCTCATGAGATCTCTGTCTTGT-3′ and PP1-2, 5′-CTGCRGCCTTCTCCTTCTCCT-3′ specific to the MADS-box and the K-box, respectively, were then designed based on the conserved sequences of several *AP1/SQUA* genes. After amplification and sequence analysis, a homologous sequence of 445 bp in length was obtained. The 5′-and 3′-RACE (Rapid amplification of cDNA ends) were used to amplify the 5′-and 3′-ends of the cDNA, respectively, and the full-length of cDNA was named *PpMADS1*. The primers for 5′-RACE were PP1-5′ GSP1, 5′-GCTCCTGCATCGCCCTTTCCTTTCTCT-3′ and PP1-5′ GSP2, 5′-CGAGCTGGTGCTCCAAACTTTGGATCTC-3′, and the primers for 3′-RACE were PP1-3′ GSP1, 5′-AGAGAAAGGAAAGGGCGATGCAGGAGC-3′ and PP1-3′ GSP2, 5′-CAGGAGCAAAATAACTTGTTGGCAAAGAAGAT-3′. Similarly, the full length of *PpMADS10* cDNA homologous to *PI* gene was then obtained starting from the EST sequence. The 5′-RACE primers were PP10-5′ GSP1, 5′-GCTGGACACGAAAGGCAAACGGTATCTGG-3′ and PP10-5′ GSP2, 5′-GGCGCTTATGCTCCTCTTCCAGTG CTC-3′, and the 3′-RACE primers were PP10-3′ GSP1, 5′-GAAAGAGCACTGGAAGAGGAGCATAAGCGC-3′ and PP10-3′ GSP2, 5′-CCAGATACCGTTTGCCTTTCGTGTCCAGC- 3′.

For the first round of PCR reaction with a total volume of 20 μL, the following reagents were mixed: 0.4 μL of 10 mmol/L dNTP, 2 μL of 10 × ExTaq buffer, 2 μL of 0.4 μmol/L UPM (Universal Primer Mix, provided by the kit), 0.4 μL of 10 μmol/L GSP1, 2 μL of 5′- or 3′-RACE cDNA product, 0.2 μL of ExTaq (5 U/μL), and 13 μL of ddH_2O. The second round of PCR was performed with the NUP (Nested Universal Primer, provided by the kit) and GSP2 primers using 1–2 μL of the first round PCR products as templates. The amplifications were carried out as follows: