リンゴには2つのタイプのホメオボックス遺伝子が存在する

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SHORT COMMUNICATION

Two Different Types of Homeobox Genes Exist in Apples

Tomoaki Sakamoto[1], Shinnosuke Kusaba[2], Yuriko Kano-Murakami[2], Masashi Fukumoto[2] and Shuichi Iwahori[3]

[1]*Doctoral Program in Agricultural Sciences, University of Tsukuba, Tsukuba, Ibaraki 305*
[2]*National Institute of Fruit Tree Science, Tsukuba, Ibaraki 305*
[3]*Institute of Agriculture and Forestry, University of Tsukuba, Tsukuba,Ibaraki 305*

Summary

Two different types of partial sequence of homeobox genes, named *APHB1* and *APHB2,* were isolated from apple. From the alignment of amino acid sequences of *KNOTTED1*-like homeobox genes and apple PCR fragments, *APHB1* was considered to be a member of class I type genes because of their similarities. *APHB1* expressed itself only in shoot apical tissues, stems, and flowers, but not in mature leaves and developing fruits which is, again, very similar to class I type genes. Likewise, *APHB2* is considered to be a member of class II type genes because of their homologies. *APHB2* is expressed in all organs involving mature leaves and developing fruits, which is characteristic of class II type homeobox genes. These findings reveal that at least two different types of homeobox genes exist in apple. They probably bind to different genes and play different roles in controlling the developmental switches in apple.

Key Words: homeobox gene, *Malus pumila,* morphogenesis, reverse transcription-PCR, transcription factor.

Introduction

Homeobox genes were first found as the transcriptional regulatory genes from the homeotic mutations of *Drosophila.* Homeobox is a unique 183 bp nucleotide sequence which encodes homeodomain. The homeodomain contains three α-helices which form a helix-turn-helix type DNA binding motif. This motif recognizes and binds to the specific sequences and is believed to regulate the expression of target genes as transcription factors (Laughon, 1991). Homeobox genes were first isolated from three different plant species, maize (Vollbrecht et al., 1991), *Arabidopsis* (Ruberti et al., 1991), and rice (Matsuoka et al., 1993).

From the analysis of the transgenic plants, plant homeobox genes are considered to regulate morphogenesis as in animals, although their mechanisms of regulation may be different. For example, the rice homeobox gene, *OSH1,* causes dramatically altered morphology, such as wrinkled or dissected leaf shapes, reduction of apical dominance, and dwarfness as the result of reduced internode elongation in transgenic *Arabidopsis* (Matsuoka et al., 1993), tobacco and kiwifruit (Kusaba et al., 1995). The levels of endogenous plant hormones also changed in transformed plants with *OSH1* ; especially gibberellin contents were dramatically decreased. Because exogenous GA_3 partially reversed the altered phenotypes, this type of homeobox gene may influence gibberellin biosynthesis, but not its responsiveness (Kusaba et al., 1996).

This study was aimed at isolating homeobox genes from apple. Hence, the isolation of homeobox genes and their characterization, including their relationships in molecular networks, may help solve the developmental mechanisms of fruit trees.

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Materials and Methods

Plant material

Malus pumila Mill. var. *domestica* cv. Fuji trees grown in an orchard at the National Institute of Fruit Tree Science were used.

Isolation of conserved region of homeobox genes

To clone a well-conserved region in homeodomain from apple DNA, polymerase chain reaction (PCR) was performed using synthetic oligonucleotides as primers (sense primer, CCGGATCCCATTACAAGTGGCCGTATCCG; antisense primer, CCAAGCTTTGGTTAATAAACCAGTTGTTGAT). About 220 bp length of PCR product and about 120 bp length of reverse transcription-PCR (RT-PCR) product which was performed with 5 μg of total RNA from shoot apices were cloned, and nucleotide sequences were determined.

The ELK domain, adjacent to homeodomain is one of the highly conserved regions. To clone this region, RT-PCR was performed with degenerate sense primer (CCGGATCCGTIIRICARGARYTIAA). About 290 bp length of fragment was cloned and sequenced.

Expression analysis by RT-PCR

RT-PCR was performed to analyze the specific expression of these genes. The first strand of cDNA was synthesized with total RNA of shoot apices, internodes of elongating shoots, mature leaves, flowers, and developing fruits. Because two different fragments were identified by RT-PCR, specific sense primers were used for PCR-based expression analysis to amplify each type of homeobox gene, (for *APHB1*, CCGGATCCGAGT-CGCAGAAGCTAGC; for *APHB2*, CCGGATC-CGAG CAAGACAAGGCCAG).

Results

Subcloning of the conserved region of homeobox genes

To clone the well-conserved region of homeodomain from apple, PCR was performed. About 200 bp length of a PCR fragment was obtained from apple DNA, whereas about a 110 bp fragment was obtained by RT-PCR as it does not contain intron sequences. A comparison of the nucleotide sequences of both PCR fragments showed that the genomic-PCR fragment contained 54 bp exon and 94 bp intron, whereas another type of nucleotide sequence cloned from RT-PCR product was 54 bp long, and was 40.7% identical with the exon of genomic-PCR fragment.

The deduced amino acid sequences were compared with those of the *Kn-1, KNOX1, KNOX2,* in maize and *OSH1* in rice as shown in Figure 1. From the alignment of amino acid sequences of these genes, the apple PCR fragment of the total DNA, which was defined as *APHB2,* had a 89.7% identity with *KNOX2* and 84.6% identity with *KNOX1*. ELK domain was one of the highly conserved external regions of the homeodomain. Presently, the nucleotide sequence of this region was determined only for that of *APHB2*. The RT-PCR product of the same region, which had a different sequence, was defined as *APHB1* ; it had 92.3% identity with *OSH1,* and 88.5% identity with *Kn-1*.

Expression analysis of two types of homeobox genes

RT-PCR analysis was performed to clarify in which organs these genes are expressed. PCR amplification was conducted with two different oligonucleotides as a specific sense primer. The PCR products derived from *APHB1* were found in shoot apical tissues, internodes of elongating shoots and flowers, but not in mature leaves and developing fruits, whereas products derived from *APHB2* were found in all organs of apple trees, including mature leaves and developing fruits (Fig. 2).

Discussion

In the present study, the conserved region of homeodomain were amplified from both total DNA and mRNA of apple. The nucleotide sequence of PCR product contained 54 bp exon and 94 bp intron. Compared with the same region in Japanese pear (Kano-Murakami et al., 1993), the exon-intron structures of both frag-

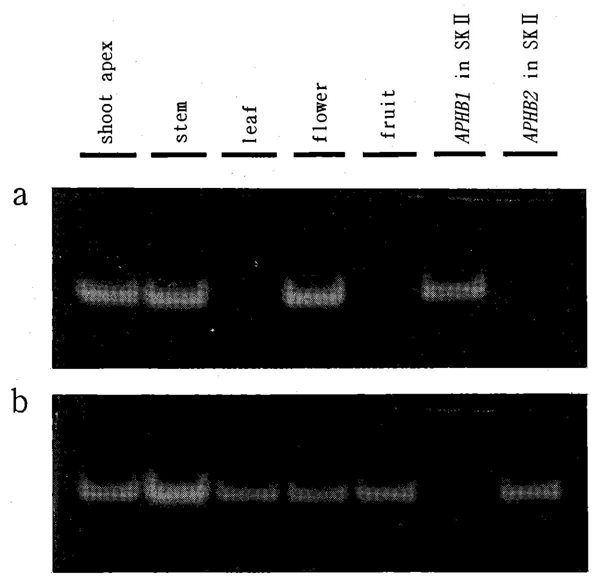

Fig. 2. Gel plates showing the bands of RT-PCR products derived from *APHB1* and *APHB2*.
a) The RT-PCR products of *APHB1* found in shoot apices, stems and flowers; b) RT-PCR products of *APHB2* found in all organs. *APHB1* and *APHB2* in SK II were used as control of PCR.

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         ELK domain        basic end   helix I           helix II  turn   helix III

class1
Kn-1     VDQELKHHLLKKYSGYLSSLKQELSKKKKKGKLPKEARQQLLSWWDQHYKWPYPSETQKVALAESTGLDLKQINNWFINQRKRHWKPS  maize
OSH1     -----------------------------------D------N--EL--------S--------------------------------  rice
APHB1                                                           S--L---------Q--                    apple

class2
KNOX1    -R----LE-KQGFKSRIEDVRE-ILR-RRA----GDTTSI-KQ--QE-S-----T-DD-AK-V-E---Q--------------N-HNN  maize
KNOX2    -R-----E-KQG-RDK-VDIRE-ILR-RRA----GDTAST-KA--QA-S-----T-ED-AR-VQE---Q--------------N-HNN  maize
APHB2      -----E-EQG-KEKIVDIRE-ILR-RRA----GDTTSV-K---QS-S-----T-ED-AR-VQE---Q---                  apple
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Fig. 1. Alignment of homeodomain sequences.
Deduced amino acid sequences of class I (*Kn-1* and *OSH1*), class II (*KNOX1* and *KNOX2*) and two type of Apple homeobox gene were aligned with homeodomain of *Kn-1*. Identical residues are indicated with dashes.

ments are identical, and sequence homology was 96.3% in the exon; therefore, the deduced amino acid sequences completely matched each other. Not only in the exon; also the residues of the intron possessed a high similarity with a 94.8% identity. Besides this fragment, another fragment with a different sequence was obtained by RT-PCR. To distinguish them from each other, the sequence found in genomic PCR fragment was defined as *APHB2,* and the other as *APHB1.*

From the alignment of amino acid sequences of *KNOTTED1*-like homeobox genes and apple PCR fragments, *APHB2* was considered to be a member of class II type genes, because of its high homologies, including the adjacent sequence of the ELK domain. Furthermore, RT-PCR analysis with type specific primers revealed that this type of genes is found in all organs. This pattern is characteristic of class II type homeobox genes (Kerstetter et al., 1994).

APHB1 was considered to be a member of class I type genes, because of their homologies. Moreover the RT-PCR products derived from *APHB1* were found only in shoot apices, internodes of elongating shoots, and flowers, but not in mature leaves and developing fruits. The expression of class I type genes are mainly found in shoot meristem and limited tissues; they are thought to keep meristems in the meristematic state (Jackson et al., 1994). The expression pattern of *APHB1* gene in apple was very similar to that of class I type genes, in that it kept cells in the undifferentiated state.

Our results clearly demonstrate that at least two different types of homeobox genes exist in apple, *APHB1* and *APHB2.* They play an important role as the transcription-regulating factors, but the difference in their expression patterns and amino acid sequences around the putative recognition helices suggest that they could bind to different genes and play different roles in controlling the developmental switches.

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リンゴには2つのタイプのホメオボックス遺伝子が存在する

坂本知昭[1]・草場新之助[2]・村上（嘉納）ゆり子[2]・福元將志[2]・岩堀修一[3]

[1]筑波大学農学研究科　305　つくば市天王台 1-1-1

[2]果樹試験場　305　つくば市藤本 2-1

[3]筑波大学農林学系　305　つくば市天王台 1-1-1

摘　要

リンゴから PCR によってホメオボックス遺伝子の保存領域の単離を試みたところ，塩基配列の異なる2種類の部分配列が得られ，これらを *APHB1*, *APHB2* と名付けた．両者は塩基配列で 40.7%の相同性を示し，それぞれトウモロコシやイネの class I, class II と呼ばれるホメオボックス遺伝子群との間に高い相同性が認められた．実際にリンゴから得られた2つの遺伝子の発現を RT-PCR によって解析したところ，それぞれ class I, class II 型の遺伝子群とよく似た発現パターンを示した．以上の結果から，リンゴには少なくとも2種類のホメオボックス遺伝子が存在し，それぞれ異なる形態形成についてその調節に関与している可能性が示唆された．