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Full Length Research Paper

Cloning and characterization of an ascorbate peroxidase gene regulated by ethylene and abscisic acid during banana fruit ripening

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An ascorbate peroxidase (APX) cDNA, designated *MaAPX1*, was isolated from banana fruit by suppression subtractive hybridization (SSH). *MaAPX1* shares an extensive sequence identity (79 to 83%) with other plant APX homologues. Southern blot analysis revealed only two copies of the APX gene in the banana genome. Reverse-transcriptase PCR analysis of *MaAPX1* expression confirmed its expression in the root, leaf, flower and fruit, with higher levels detected in the leaf compared to other organs. Real-time quantitative polymerase chain reaction was used to explore expression patterns of *MaAPX1* in banana postharvest. In naturally ripened banana fruits, *MaAPX1* expression gradually peaked at day 6 after harvest, and subsequently decreased. In ethylene-treated fruits, *MaAPX1* expression increased to a maximum at day 3 and then decreased. Meanwhile, in banana treated with abscisic acid, *MaAPX1* levels were suppressed from day 0 to 8. These data suggest that *MaAPX1* may play distinct roles in the multiple mechanisms that underlie banana fruit ripening.

Key words: Banana, postharvest ripening, ascorbate peroxidase, gene expression, ethylene, abscisic acid.

INTRODUCTION

The oxidative process that occurs during fruit ripening is accompanied by obvious alterations in fruit metabolism and the activity of a number of enzymatic systems, including those related to the regulation of reactive oxidative species (ROS) (Masia, 1998). ROS, which include superoxide anion radical, hydrogen peroxide and hydroxyl radical production, are by-products of cellular metabolism in the mitochondria (Masaki et al., 1999; Terman et al., 2006). These factors have been implicated in the cellular oxidation, and consequent membrane lipid peroxidation, related to fruit ripening (Esterhazy et al., 2008). To minimize and/or protect against the toxic effects of these damaging ROS, cells have evolved highly regulated enzymatic and non-enzymatic mechanisms to balance ROS production and scavenging in order to maintain cellular redox homeostasis. ROS-scavenging enzymes include superoxide dismutase, ascorbate peroxidase (APX), glutathione reductase and catalase (Scandalios, 2002; Mittler et al., 2004).

Hydrogen peroxide (H_2O_2) is one of the ROS generated as a by-product in plant tissues during normal metabolism, as well as under different stress conditions, such as oxidative stress, pathogen attacks, extreme temperatures, drought, ozone, wounding and senescence (Blokhina et al., 2003). H_2O_2 is also considered important in fruit metabolism, and in many fruits it is reportedly

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Abbreviations: ABA, Abscisic acid; **APX,** ascorbate peroxidase; **bp,** base pair; **cDNA,** complementary DNA; **ORF,** open reading frame; **PCR,** polymerase chain reaction; **RACE,** rapid amplification of cDNA ends

associated with fruit development, ripening and senescence (Brennan and Frenkel, 1977; Woods et al., 2005). In addition, treatment with ethylene has been linked to elevated hydrogen peroxide content in the initiation of banana fruit softening (Yang et al., 2008).

A major hydrogen peroxide detoxifying system in plant chloroplasts and cytosol is the ascorbate-glutathione cycle, in which APX functions as the key enzyme (Asada, 1992). This enzyme uses ascorbate as an electron donor to reduce H_2O_2 to water. APX has been identified in many higher plants, with different isozymes distributed in at least four cellular compartments, including the cytosol, chloroplasts, mitochondria and peroxisomes (Shigeoka et al., 2002). During the ripening of pepper fruits, the elevated APX activity in red fruit might play a role in preventing the accumulation of any activated oxygen species generated in the mitochondria (Jimenez et al., 2002). Thus far, studies of APX gene transcription have only been performed in bell pepper (Schantz et al., 1995) and strawberry fruit (Kim and Chung, 1998), and previous studies in different fruits have shown varying patterns of APX expression during fruit ripening.

The primary focus of this study is the banana (*Musa* spp.), one of the most important fruit crops in the world in terms of production and consumption (Aurore et al., 2009). The pattern of ethylene production during ripening in banana fruit differs from other climacteric fruits, with a sharp rise and fall of ethylene production during the early climacteric rise of respiration (Liu et al., 1999). Exogenous ethylene can induce fruit ripening by advancing and increasing the release of ethylene during banana ripening (Pathak et al., 2003; Barry and Giovannoni, 2007). Exogenous abscisic acid (ABA) can also regulate the ripening physiology by stimulating the activity of cell wall hydrolases and pectate lyase, and may enhance the sensitivity to ethylene during banana ripening (Lohani et al., 2004; Jiang et al., 2000). However, the effects of these hormones on the expression of *MaAPX1* during the ripening process have not been elucidated.

To understand the molecular basis of fruit ripening in banana, we isolated genes that were differentially expressed at the early stage of postharvest banana ripening using suppression subtractive hybridization (SSH) (Xu et al., 2007). A cDNA fragment of an APX upregulated at the early stage of postharvest banana ripening was obtained. In the present study, we cloned and analyzed the APX gene from banana (designated as *MaAPX1*), and examined gene expression during fruit ripening and in response to exogenous ethylene and ABA treatment.

MATERIALS AND METHODS

Plant materials and treatments

Banana (*Musa acuminata* L. AAA group, cv. Brazilian) fruits obtained from the banana plantation of the Institute of Tropical Bioscience and Biotechnology (Chengmai, Hainan) were harvested at mature green stage (100 to 110 days after flower shooting). Banana hands at similar development stages were selected, and three fingers from each hand were divided into three groups for different treatments. For natural ripening, the group of bananas was kept at 25°C and allowed to ripen naturally. For ethylene treatment, the group of bananas was treated with 100 µl ethylene for 18 h and then ripened at 25°C (Scott et al., 1970). For ABA treatments, the group of bananas was treated with 100 µM ABA in 0.2% Teepol (Pathak and Sanwal, 1999) for 24 h at 25°C. The treated materials were then allowed to ripen at 25°C, and were subsequently frozen in liquid nitrogen and stored at -80°C for extraction of total RNA and subsequent analysis.

RNA extraction and cDNA synthesis

Total RNA was extracted from the roots, rhizomes, flowers or leaves of the plant from which the fruits were obtained, as described previously (Wan and Wilkins, 1994). For cloning of full-length cDNA, total RNA from banana fruit tissues (including peel and pulp) was first isolated 2 days after harvest using a modified cetyltrimethylammonium bromide (CTAB) method. First-strand cDNA was then synthesized using the SMART™ PCR cDNA Synthesis Kit and SMARTScribe reverse transcriptase (Clontech, Palo Alto, CA, USA), according to the manufacturer's instructions.

Cloning and sequence analysis of *MaAPX1*

Rapid amplification of cDNA ends (RACE) was used to obtain the full-length cDNA, based on the partial sequence previously cloned by SSH. For 5′ RACE, the forward primer was 5′-aagtccaagctccctcgaaacc-3′, and the reverse primer was 5′-ctccgagatctggacgagc-3′ (provided in the SMART PCR cDNA Synthesis Kit as 3′ SMART CDS primer IIA). For 3′ RACE, the forward primer was 5′-tgggtctcagcgatcaggatat-3′, and the reverse primer was 5′-taatacgactcactcactataggg-3′ (provided in the SMART PCR cDNA Synthesis Kit as the SMARTIIA oligonucleotide). The amplified products of the 5′ and 3′ cDNA ends were inserted into the pGEM-T easy vector (Promega, Madison, WI, USA). Nucleotide sequences of the inserted cDNA fragments were determined on an ABI PRISM310 Genetic Analyzer (Perkin Elmer Applied Biosystems, Foster City, CA, USA) using the BigDye Termination Cycle Sequencing Ready Reaction Kit (Perkin Elmer Applied Biosystems, Foster City, CA, USA). A pair of primers was designed based on the 5′ and 3′ end sequences of the cDNA (5′-cgggccatggactagacgaagaccaga-3′ and 5′-cgaacactagtgtcctccgcaaatccta-3′) and used for the amplification of the entire open reading frame.

The PCR conditions were as follows: 94°C for 5 min, followed by 35 cycles of amplification (94°C for 30 s, 57°C for 40 s, and 72°C for 60 s), and then 72°C for 10 min. The amplified product was inserted into the PMD-18T (Takara) vector and sequenced. Nucleotide sequences of the inserted cDNA fragments were determined with an ABI PRISM310 Genetic Analyzer (Perkin Elmer Applied Biosystems, Foster City, CA, USA) using the BigDye Termination Cycle Sequencing Ready Reaction Kit (Perkin Elmer Applied Biosystems, Foster City, CA, USA). The sequences were compared to those in the NCBI database using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The cDNA sequence was designated as *MaAPX1*. Putative conserved domain detection tools at NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and ExPASy (Predotar, TargetP V1. 03, PSORTb version 3.0, and iPSORT, at http://www.expasy.ch/) were used to analyze the sequence.

Southern blot analysis

Genomic DNA was isolated from developing leaves of banana using

the method of Doyle and Doyle (1987). For Southern Blot analysis, 100 µg of genomic DNA was digested overnight with the NcoI and SpeI restriction enzymes. The DNA was separated by gel electrophoresis and transferred overnight onto a positively charged nylon membrane. The membrane was then cross-linked with UV radiation, pre-hybridized at 42°C with digoxigenin (DIG)-labeled cDNA probe obtained from RT-PCR clones for *MaAPX1*, and hybridized overnight in the same buffer containing the probe. The membrane was then washed once in 2x SSPE and 0.1% (w/v) sodium dodecyl sulphate (SDS) for 15 min at 55°C, and then twice in 0.5x SSPE and 0.1% (w/v) SDS for 30 min at 55°C. The probe was labeled with digoxigenin (DIG) and detected using the luminescent detection kit according to the manufacturer's instructions (Roche Molecular Biochemicals, Mannheim, Germany).

Semi-quantitative RT-PCR

Total RNA was extracted from the roots, rhizomes, leaves, flowers and fruit tissues (including peel and pulp) using a modified CTAB method. First strand cDNA was synthesized from 2 µg of poly $(A)^+$ RNA from each sample using AMV Reverse Transcriptase (Promega, Heidelberg, Germany). Primers were constructed (forward primer, 5′-TCCGCCTTGCTTGACACT-3′ and reverse primer, 5′-GGAACTGCCTCCTTGATTGG-3′) to amplify an RT-PCR product of 151 bp, and designed to exclude the highly conserved APX domain to ensure specific amplification of *MaAPX1*. The PCR conditions were as follows: 94°C for 4 min, followed by 29 cycles of amplification (94°C for 7 s, 56°C for 15 s, and 72°C for 20 s), and then 72°C for 10 min. As an internal control, actin transcripts were amplified using forward, (5´-CGAGGCTCAATCAAAGA-3´) and reverse (5´-ACCAGCAAGGTCCAAAC-3´) primers. The experiments were repeated at least three times with similar results.

Real-time RT-PCR

Total RNA was isolated from the pulp of banana fruit at different ripening stages. Samples of 200 ng Poly$(A)^+$mRNA were converted into cDNA using the SMART PCR cDNA Synthesis Kit (Clontech Laboratories, Palo Alto, CA) in a final volume of 20 µl, which subsequently served as the template for real-time PCR. SYBR® Premix Ex Taq™ (TaKaRa) was used in 25 µl reactions with 0.5 µl ROX reference dye. Primers (100 nM each) were mixed with the equivalent of 100 ng reverse-transcribed RNA template per reaction. In all experiments, negative controls containing no template RNA were subjected to the same procedure, to exclude or detect any possible contamination.

Moreover, before proceeding with the actual experiments, a series of template dilutions was performed to determine the optimal template concentration for the experiments to obtain maximal amplification of the target. Each quantitative real-time PCR was performed on a Stratagene Mx3000P (Stratagene, CA, USA) machine using SYBR chemistry. The thermal cycling conditions were as follows: 94°C for 3 min, followed by 40 cycles of 94°C for 7 s, 55°C for 10 s, and 72°C for 15 s. Reactions were performed in triplicate, and data were analyzed using the MxProTM QPCR software (Stratagene, CA, USA). Actin was used as the control sample. The differences in Ct value between the *MaAPX1* and *MaActin* transcripts were expressed as fold-changes relative to actin.

RESULTS

Cloning and identification of *MaAPX1*

We selected and used the constructs directly from the SSH cDNA library, circumventing the need to clone the full-length cDNAs. Inner and outer fragments were used to amplify full-length cDNAs of APX using the 5′- and 3′ -RACE method. The 650 bp 5′ -RACE and 500 bp 3′ -RACE products were amplified (data not shown), purified, cloned and sequenced. These sequences were then compared to sequences in the GeneBank NCBI-Blastn database; the closest matches were *Zea mays* (86%), *Zantedeschia aethiopica* (81%), *Gossypium hirsutum* (78%) and *Vitis vinifera* (78%). The resulting full-length banana APX cDNA was 962 bp, and designated as *MaAPX1*; the cDNA included a 750 bp ORF, a 15 bp 5′ UTR, and a 197 bp 3′ UTR (Figure 1). The deduced amino acid sequence of *MaAPX1* is shown in Figure 2, and contains 250 amino acid residues with a predicted molecular weight of 27.97 kD and a *pI* of 5.15.

Sequence homology and phylogenetic analysis of APX

The deduced amino acid sequence of *MaAPX1* was aligned with homologous sequences from various species, including *Capsicum annuum, Arabidopsis thaliana, Oryza sativa* and *Glycine max*, using BLAST (http://blast.ncbi.nlm.nih.gov/) and the Molecular Evolutionary Genetics Analysis (MEGA) software. The APX active-site signature (depicted in Figure 2) includes the A region located at positions 33-44 (AP-LMLPLAWHSA), together with the proximal heme-li-Band motif (the H region) between residues 155 and 163: DIVALSGGH (Bairoch, 1991). A neighbor-joining bootstrap tree constructed based on the homologous APX proteins revealed that the sequences could be divided into four groups; comparative analysis categorized *MaAPX1* as belonging to the cytosolic APX proteins (Figure 3). Analysis using the available prediction programs (Predotar, TargetP V1. 03, PSORTb version 3.0, and iPSORT) did not detect any signal, mitochondrial targeting, or chloroplast transit peptides in *MaAPX1*. Instead, the APX clone from banana is predicted to code for a cytosolic isoform. Southern blot analysis was conducted to determine the relative copy number of the APX gene in the banana genome. Results show that the *MaAPX1* probe hybridized to two DNA bands, suggesting that *MaAPX1* has a low copy number in banana (Figure 4).

Transcript expression of *MaAPX1* in banana tissues

To assess the expression levels of *MaAPX1* in different organs in the banana, semi-quantitative RT-PCR was carried out using cDNA isolated from various organs. The results show a wide range of *MaAPX1* transcript expression levels among the different tissues, with relatively strong expression observed in leaves and roots. In comparison, lower levels of the *MaAPX1* transcript were

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1    AAGTGGACGACGGCGATGGGGAAGTCGTACCCGGCGGTGAGCGAGGAGTACCAGAAGGCG
                   M  G  K  S  Y  P  A  V  S  E  E  Y  Q  K  A
61   GTGGAGAAGGCCAGGAGGAAGCTCCGCGGCCTCATCGCCGAGAAGAACTGCGCCCCTATC
      V  E  K  A  R  R  K  L  R  G  L  I  A  E  K  N  C  A  P  I
121  ATGCTCCGCCTTGCTTGGCACTCGGCGGGGACGTATGACGTGAGCACGAAAACGGGAGGC
      M  L  R  L  A  W  H  S  A  G  T  Y  D  V  S  T  K  T  G  G
181  CCGTTCGGGACGATAAGGTTCGCGGCGGAGCTCGCCCACGGCGCCAACAACGGCCTCGAC
      P  F  G  T  I  R  F  A  A  E  L  A  H  G  A  N  N  G  L  D
241  ATCGCCCTCCGGCTCCTGGAGCCAATCAAGGAGCAGTTCCCCACTCTCTCCTTCGCTGAC
      I  A  L  R  L  L  E  P  I  K  E  Q  F  P  T  L  S  F  A  D
301  TTCTACCAGCTCGCCGGAGTCGTCGCCGTCGAAGTCACCGGAGGGCCGGAGATCCCTTTC
      F  Y  Q  L  A  G  V  V  A  V  E  V  T  G  G  P  E  I  P  F
361  CATCCCGGCAGAGAGGACAAGACCCAACCTCCTGAGGAAGGCCGCCTGCCTGATGCCACA
      H  P  G  R  E  D  K  T  Q  P  P  E  E  G  R  L  P  D  A  T
421  AAAGGTTCGGACCACCTCAGGGATGTGTTTGGCAAGCACATGGGTCTCAGCGATCAGGAT
      K  G  S  D  H  L  R  D  V  F  G  K  H  M  G  L  S  D  Q  D
481  ATCGTTGCACTCTCTGGTGGCCACACGCTGGGGAGATGCCACAAGGAGCGCTCCGGTTTC
      I  V  A  L  S  G  G  H  T  L  G  R  C  H  K  E  R  S  G  F
541  GAGGGAGCTTGGACTTCCAATCCTCTTATTTTCGACAACTCCTATTTCAAGGAGCTCCTG
      E  G  A  W  T  S  N  P  L  I  F  D  N  S  Y  F  K  E  L  L
601  AGCGGCGAGAAAGACGACGTCATCCAGCTCCCGTCCGATAAGGCTCTCCTCACCGATCCT
      S  G  E  K  D  D  V  I  Q  L  P  S  D  K  A  L  L  T  D  P
661  GTTTTCCGTCCCCTGGTCGAGAAATACGCTGCCGATGAGGATGCCTTCTTTGCTGACTAT
      V  F  R  P  L  V  E  K  Y  A  A  D  E  D  A  F  F  A  D  Y
721  GCTGAAGCACACCTGAAGCTCTCCGAACTAGGATTTGCGGAGGACTAGATCGTTCGTCCA
      A  E  A  H  L  K  L  S  E  L  G  F  A  E  D  *
781  TACATGAAGTCACTTCAGTAAGCATATTTCCAATAAACCTTTCCGAGTGTTAATGCATGG
841  ATGTCTGGACACATCGAATGTTTCATATGTTTCTGCTCTCCGTTTTCCCTTTATGAATTT
901  CACATGAAAATTCCGATTCTCGGTTTGTGGACAAAAAAAAAAAAAAAAAAAAAAAAAAA
961  AA
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Figure 1. Nucleotide sequence of *MaAPX1* and its deduced amino acid sequence.

detected in fruit, stem and flower organs (Figure 5).

Exogenous ethylene and ABA treatments promoting ripening of banana fruit with different effects on the expression of *MaAPX1*

Expression levels of *MaAPX1* were first examined at various time points during the natural ripening process in the banana; results demonstrate that *MaAPX1* showed a specific expression pattern during ripening. Expression increased starting from day 0, with the highest peak at day 6, when its relative expression was 5.08; 1.5 times higher than that at day 0 (12.83 to 5.08). Following this peak, *MaAPX1* expression dramatically decreased, with levels at day 10 approximately; 5.6 fold less compared to day 6 (5.08 compared to 0.91) (Figure 6). This result demonstrates that *MaAPX1* expression negatively correlated with banana fruit ripening. Moreover, in order to assess whether the gene expression of APX was responsive to treatment with external stimuli of fruit ripening in banana, we next treated banana fruit with

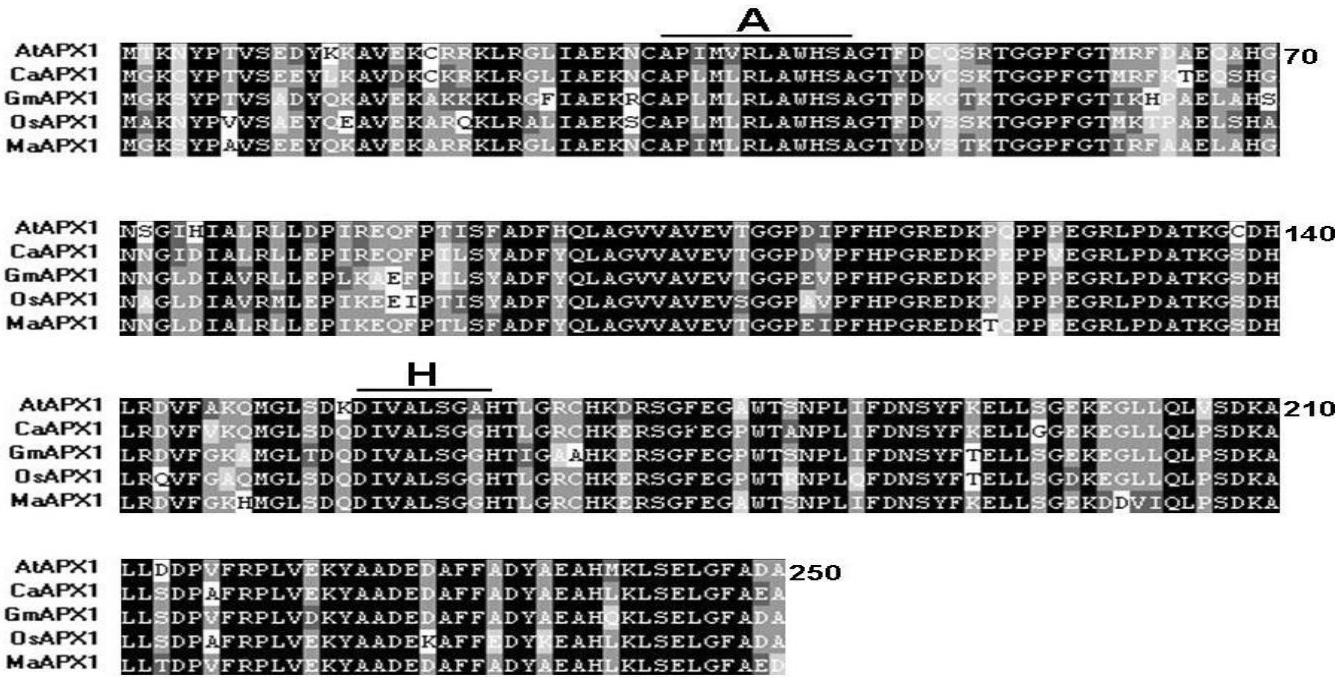

Figure 2. Sequence alignment of *MaAPX1* and related proteins. Amino acid sequences were deduced from putative full-length cDNAs available in the NCBI database. Ca, *Capsicum annuum* (AAL83708); At, *Arabidopsis thaliana* (NP_172267); Os, *Oryza sativa* (japonica cultivar group) (P93404); Gm, *Glycine max* (BAC92739). Black shading indicates strictly conserved residues, and grey shading indicates regions of less strict conservation. Regions A and H indicate the highly conserved ascorbate peroxidase (APX) active-site signature.

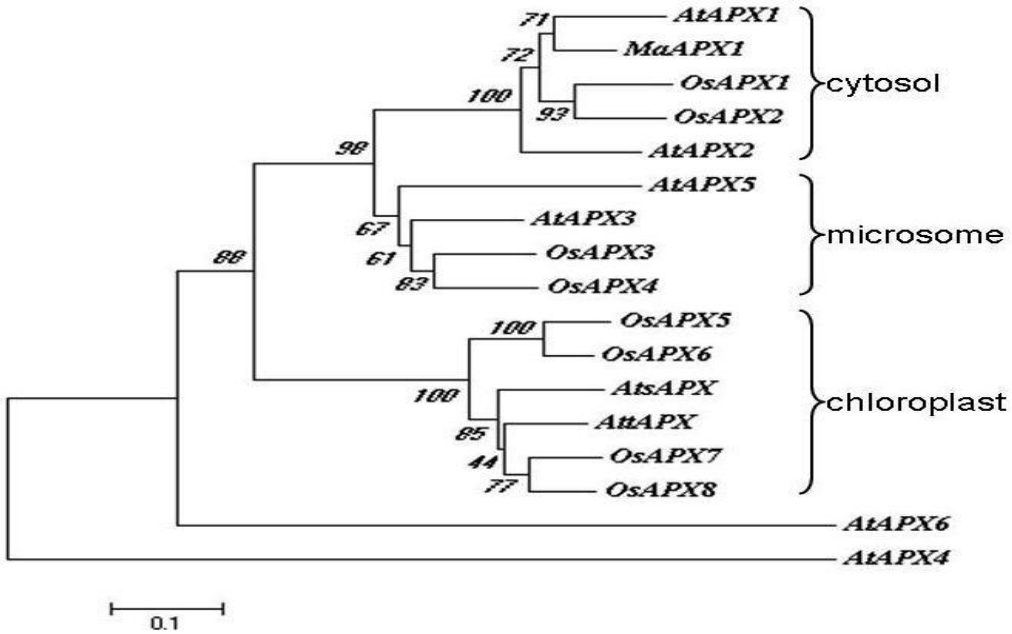

Figure 3. Phylogenetic analysis of *MaAPX1* and related proteins. A neighbor-joining bootstrap tree was constructed from the alignment of *MaAPX1* with the two best-known APX families in Arabidopsis thaliana and Oryza sativa using molecular evolutionary genetics analysis (MEGA) software with 1000 bootstrap replicates. The scale bar corresponds to 0.1 estimated amino acid substitutions per site. AtAPX1, NP_172267; AtAPX2, NP_187575; AtAPX3, NP_195226; AtAPX4, P82281; AtAPX5, Q7XZP5; AtAPX6, Q8GY91; AtsAPX, Q42592; AttAPX, Q42593; OsAPX1, P93404; OsAPX2, Q9FE01; OsAPX3, Q6TY83; OsAPX4, Q6ZJJ1; OsAPX5, P0C0L0; OsAPX6, P0C0L1; OsAPX7, Q7XJ02; OsAPX8 and Q69SV0.

Figure 4. Genomic Southern analysis of *MaAPX1* in banana. Genomic DNA isolated from banana leaf tissue was digested overnight with NcoI and SpeI, and probed with DIG-labeled *MaAPX1*.

exogenous ethylene or ABA, and evaluated *MaAPX1* expression in response to either hormone. In ethylene-treated banana fruits, the expression of *MaAPX1* increased more quickly, from day 0 to 3; the highest levels detected at day 3 showed an elevated relative expression of 59.52. *MaAPX1* levels then decreased until day 6 (Figure 7). In comparison to naturally ripened fruits, the average expression level of *MaAPX1* in ethylene-treated banana was notably higher (17.78 with ethylene and 2.02 under natural conditions) (Figures 7 and 8). Further, treatment with exogenous ethylene caused a marked increase in *MaAPX1* expression compared to naturally ripened fruit (≥ 8.8 fold). Additionally, the maximal expression of *MaAPX1* during postharvest ripening in ethylene-treated banana was much higher than naturally ripened fruits (59.52 and 5.08, respectively). In ABA-treated banana fruits, the highest level of *MaAPX1* was at day 0, with a relative expression of 5.85; transcript levels then decreased in the days following treatment. The largest decrease of *MaAPX1* relative expression occurred between day 4 and day 5 (11.51 to 3.91) (Figure 8). Furthermore, in ABA-treated fruits, the average and maximal expressions of *MaAPX1* were not significantly different than those in naturally ripened fruits; notably, however, the expression of *MaAPX1* was suppressed at every time point during ABA-induced ripening (Figure 8).

DISCUSSION

The genes encoding members of the ascorbate peroxidase enzyme family have been characterized in a number of plants, including pea (Mittler and Zilinskas, 1992), bell pepper (Schantz et al., 1995), spinach (Webb and Allen, 1995), Arabidopsis (Santos et al., 1996), maize (Bresegem et al., 1995), tobacco (Ovar and Ellis, 1997), strawberry (Kim and Chung, 1998), tomato (Gadea et al., 1999), potato (Kawakami et al., 2002), cotton (Li et al., 2007), melon (Cheng et al., 2009) and citrus (Kunta et al., 2010). In this study, we characterized the APX gene from banana; the cDNA sequence showed very high identity to other known cytosolic APX proteins, with the closest match to *Z. mays* (86%), *Z. aethiopica* (81%), *G. hirsutum* (78%) and *V. vinifera* (78%). Analysis of the amino acid sequence encoding MaAPX1 revealed only a common core catalytic region without any organelle-specific N-terminus transit peptide sequences or the C-terminus trans-membranous region found in membrane-bound APX isoforms, suggesting that MaAPX1 is a cytosolic soluble APX. Consistent with this prediction, phylogenetic analysis with amino acid sequences of other plant APXs revealed that MaAPX1 belongs to the cytoplasmic APX1 evolutionary lineage (Figure 1B). Southern analysis also revealed that the MaAPX1 probe was strongly hybridized to two fragments of genomic DNA. In addition, the APX gene exhibited a distinct and wide range of expression in different plant tissues. Although the APX transcripts were detected in all tissues, elevated levels were observed in leaves and roots. In contrast, low levels of APX transcripts were expressed in flowers (Figures 3 and 4), suggesting a functional differentiation in plant tissues.

Fruit ripening is known to be a complex process as it involves many physiological and chemical changes. This process is inevitably affected by oxidative stress. Notably, oxidative stress can induce the expression of the APX gene. We found that the expression of *MaAPX1* in natural ripening fruit gradually increased and reached its peak around day 6, after which it subsequently decreased (Figure 6); this suggests that the regulation of the expression of *MaAPX1* correlated with the process of banana fruit ripening. This result is consistent with those reported by a previous study (Clendennen and May, 1997). Studies of APX gene expression in bell pepper, a climacteric fruit, linked its expression to fruit ripening (Schantz et al., 1995). In addition, elevated expression of cAPX gene in strawberry was observed during fruit ripening (Kim and Chung, 1998).

Ethylene and ABA play important roles in the ripening of banana fruit. In ethylene-treated banana fruits, the release of ethylene occurred earlier and to higher levels, which further accelerated the fruit ripening (Liu et al., 2008). In our study, we observed a marked response and change of the expression of *MaAPX1* in response to exogenous ethylene treatment during postharvest banana ripening. Similar results have also been reported,

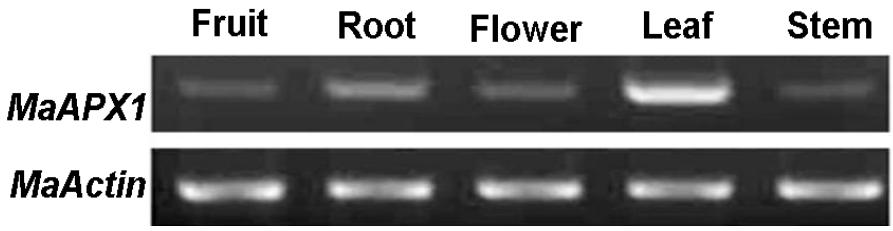

Figure 5. RT-PCR analysis of *MaAPX1* expression in different organs in banana.

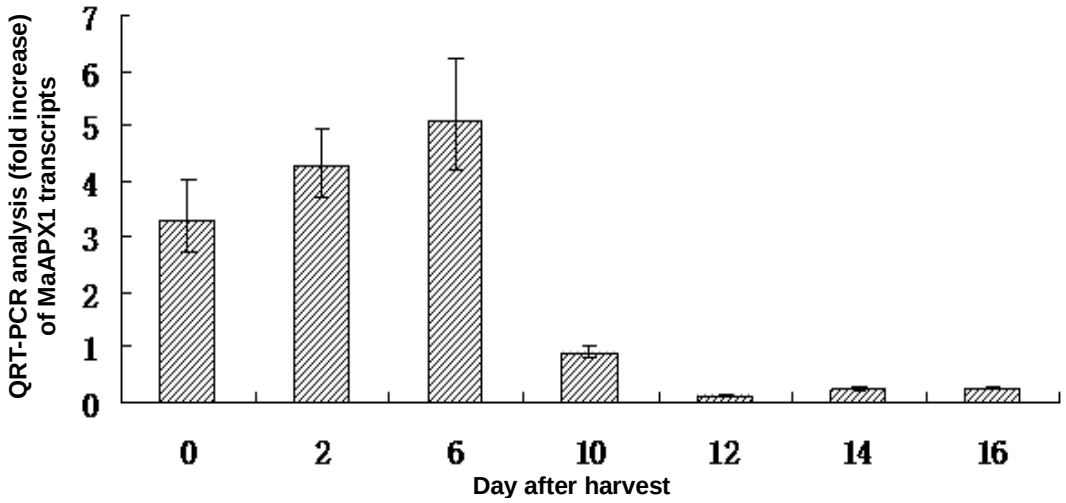

Figure 6. Relative expression of *MaAPX1* in naturally ripened banana at various post-harvest stages. Expression was determined using real-time RT-PCR. The mean were determined from three independent measurements, and the vertical bars indicate the standard error.

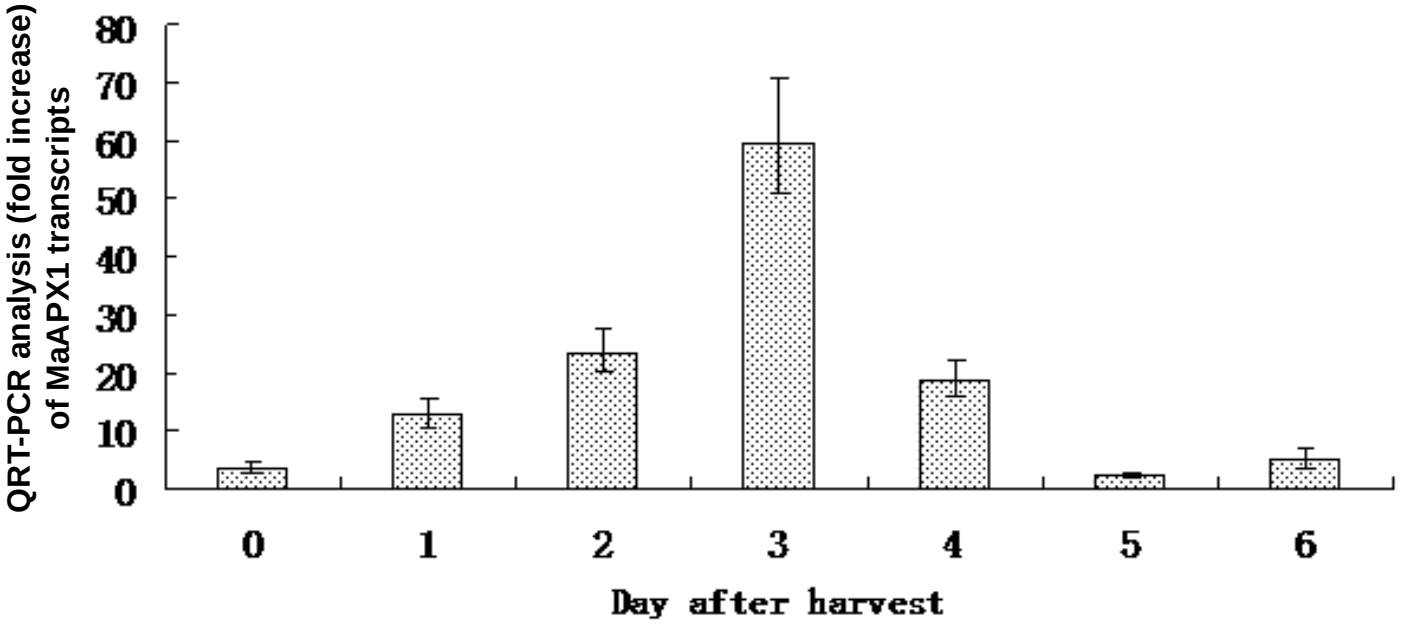

Figure 7. Relative expression of *MaAPX1* in ethylene-treated bananas at various post-harvest stages. Expression was determined using real-time RT-PCR. The mean were determined from three independent measurements, and the vertical bars indicate the standard error.

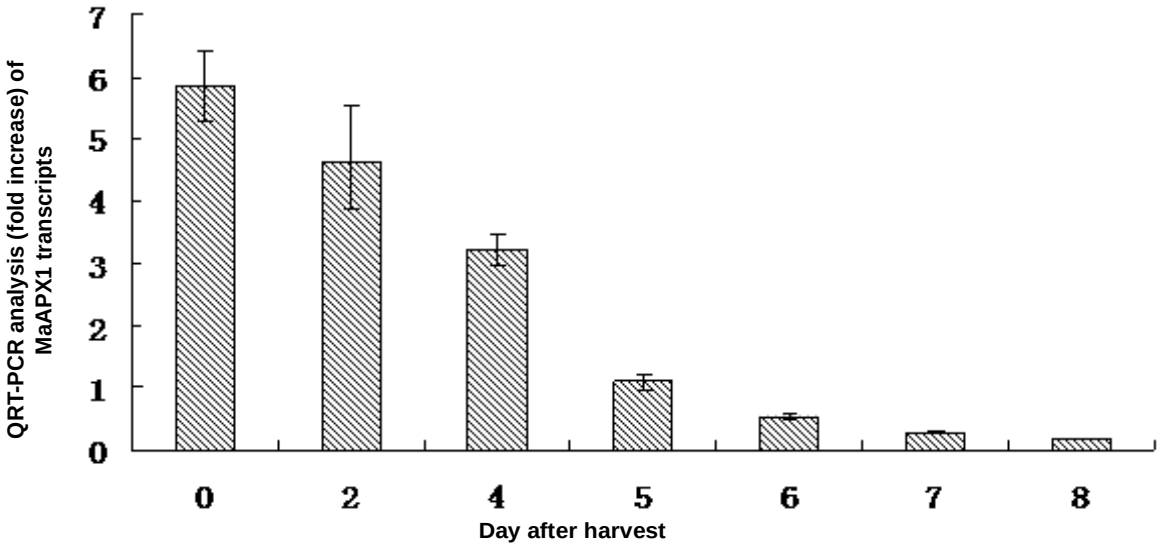

Figure 8. Relative expression of *MaAPX1* in ABA-treated bananas at various post-harvest stages. Expression was determined using real-time RT-PCR. The mean were determined from three independent measurements, and the vertical bars indicate the standard errors.

in which *GhAPX1* transcripts were increased by exogenous ethylene in cotton (Li et al., 2007). Together, these results suggest that the *MaAPX1* gene might function in the pathway by which ethylene regulates the ripening of banana fruit. Exogenous ABA also modulates ripening (Jiang et al., 2000) and enhances the softening of banana fruit (Lohani, 2004). Notably, however, the expression of *MaAPX1* was suppressed by exogenous ABA treatment (Figure 8). This result implies that exogenous ethylene and ABA regulate banana fruit ripening via distinct mechanistic pathways. In other plant species, the expression of APX also showed different responses to exogenous ABA. In *Pimpinella brachycarpa*, application of ABA induced the expression of APX (Sohn et al., 2002), while it had no effect on the *CaAPX1* transcript levels in hot pepper (Yoo et al., 2002).

In this study, we demonstrated that expression of the *MaAPX1* gene showed a distinct expression pattern during banana fruit ripening. Furthermore, this gene was induced and stimulated by exogenous ethylene, but suppressed by exogenous ABA. Together, this suggests that *MaAPX1* gene might be involved in the pathway by which ethylene regulates the ripening of banana fruit.

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