GENE NOTE

Isolation and expression analysis of gibberellin 20-oxidase homologous gene in apple

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Abstract

To characterize the gibberellin (GA) 20-oxidase gene in apple, the genomic and cDNA clone from ‘Fujis’ apple (accession no. AB037114) was isolated. The deduced amino acid sequence of this cDNA showed 71% and 66% identity to those of GA 20-oxidase cloned from French bean and Arabidopsis, respectively. The transcript of this gene was detected mainly in immature seeds between 1-3 months after full bloom. These results suggested that this apple GA 20-oxidase gene might be involved in GA biosynthesis in developing apple seed.

Key words: Malus ×domestica, gibberellin, biosynthesis, GA 20-oxidase, immature seed.

In the biosynthetic pathway of active GA, GA 20-oxidase catalyses the successive oxidation from GA13 or GA12 to GA20 or GA20, respectively (Lange, 1994). GA 20-oxidase is thought to be one of the rate-limiting enzymes in GA biosynthesis because the ga5 mutant of Arabidopsis, which is semi-dwarfed has a mutation in the stem GA 20-oxidase gene (Xu et al., 1995).

In apple, it is reported that a chemical identification of GAs in immature seed (Hedden et al., 1993) and that GAs are involved in the spur-type growth habit of ‘McIntosh Wijck’ (Looney et al., 1988). Besides these results, little is known about the genes involved in GA biosynthesis in apple. In this study, the isolation of a GA 20-oxidase gene from apple and the analysis of its expression pattern is reported in order to increase our understanding of the molecular basis of GA biosynthesis in apple.

A 173bp DNA fragment was obtained by PCR of apple genomic DNA (Fig. 1). Sequence analysis revealed that 173bp DNA fragment showed 80% homology compared to that of French bean GA 20-oxidase (U70530; Garcia-Martinez et al., 1997). An apple cDNA library prepared from stem tissue was screened using this PCR product and the tobacco GA 20-oxidase (Kusaba et al., 1998) as probes. As a consequence of cDNA screening, a 5'-truncated GA 20-oxidase homologous cDNA clone of 800 bp was obtained. To isolate the genomic clone, a ‘Fujis’ genomic library was screened with this 5'-truncated GA 20-oxidase cDNA as a probe. Four independent clones were isolated. From the results of mapping and sequencing analysis, these four clones were identical. A full-length cDNA clone of apple GA 20-oxidase was obtained by RT-PCR, the sequence of which is shown in Fig. 1. Sequencing analysis

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from immature seed in various developing stages of apple fruit (Fig. 2B). The mRNA accumulated to high levels in immature seeds 1–3 months after full bloom, and no accumulation was observed in the early developing stages and mature stages of apple fruit. The presence of the transcript coincided with the period of rapid enlargement of the apple fruit (Fig. 2B). Luckwill et al. reported that GA first appeared in apple seeds about 5 weeks after full bloom, increased to a maximum concentration at 9 weeks and subsequently decreased again, disappearing completely by the time the seed was mature (Luckwill et al., 1969). The temporal change in mRNA accumulation levels of apple GA 20-oxidase in immature seed correlates well with the changes in GA levels in immature seed. Pharis et al. reviewed the control of fruit growth by seed-produced GAs (Pharis et al., 1985). For example, fruit growth rate is often correlated with seed number, but exogenous GA can increase fruit growth and it can substitute for the presence of seeds which have been removed. Apple GA 20-oxidase mRNA accumulation was not observed in the flesh of immature fruit at times when high levels of mRNA were detected in immature seeds. It is likely that the expression of this GA 20-oxidase in immature seed may be involved in the development of apple fruit.

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References


