Volume 55(1):59-61, 2011
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

ARTICLE

Characterization of gene expression in apple, connected potentially to cuticular wax production

Zsolt Albert[1], Balázs Ivanics[1], Attila Molnár[1], Csilla Deák[1], András Miskó[1], Magdolna Tóth[2], István Papp[1]*

[1]Department of Plant Physiology and Plant Biocehmistry, Corvinus University of Budapest, Budapest, Hungary, [2]Department of Pomology, Corvinus University, Budapest, Hungary

ABSTRACT The plant cuticle takes part in several important processes of plant's life, e.g. controling peristomal transpiration, attenuating short-wave irradiation or discouraging the attachment of invading microorganisms. These functions are mainly fulfilled by the apolar cutin matrix and waxes of the cuticle layer on the epidermis. In *Arabidopsis thaliana*, the biosynthetic pathway of wax components have been mostly described, altough many steps are still unknown at this time. It is known that an endoplasmic reticulum (ER) associated elongase enzyme-complex is involved in the production of very long chain fatty acids (VLCFAs), and several other enzymes are responsible for further modifications on these molecules that build up surface waxes. We were looking for potential homologs of three *Arabidopsis* genes shown to be involved in wax biosynthesis (KCR1, CER2, WAX2). Primers were designed based on apple sequences which are potential orthologs of these *Arabidopsis* genes. Then RT-PCR experiments were carried out in order to identify the expression of these candidate genes. Our work was performed on two scab-resistant apple cultivars, the early-ripening Prima, and the late-ripening Florina. In both cases leaf, fruit peel and pulp tissues were examined. Our results show that the genes targeted have different expression levels in the tissues sampled, some show most preferential expression in the fruit peel. This means that several of the selected genes could be involved in wax-biosynthesis in the apple fruit epidermis.
Acta Biol Szeged 55(1):59-61 (2011)

KEY WORDS

apple
gene expression
RT-PCR
wax
cuticle

The plant cuticle consists of the polymer matrix formed by cutin as well as intracuticular and epicuticular waxes. This structure serves vital functions for the plants by creating a repellent layer against water soaking, protects against ultraviolet radiaton, limits non-stomatal water-loss, plays important role in plant-pathogen or plant-insect interactions or even has functions in plant development. Most of these functions are dependent on the apolar wax-compounds of the cuticle layer (Riederer and Müller 2006). Suberin and sporopollenin are similar structures to cutin in roots and on the surface of pollens respectively. Many experiments show that the biosynthesis of these layers are connected to each other (Pollard et al. 2008).

In *Arabidopsis thaliana* L. (Heynch.) the biosynthetic pathway of surface waxes has been described to some detail. Long chain fatty acids are synthesized in the plastid by a fatty-acid synthase complex, called FAS. The resulting fatty acids are elongated by the ER-localized fatty-acid elongase enzyme complex (FAE), which consists of four large enzymes, having condensing, reducing and dehydrating functions. One cycle produces a longer chain-length lipid by two carbons, that finally creates very long-chain fatty acids (VLCFAs) (Samuels et al. 2008). These VLCFAs can be modified further, yielding alkanes, aldehydes, ketones or alcohols, although many of the reactions involved are still unknown (Kunst and Samuels 2009). Some functions however have already been described. WAX2 seems playing important role in the so called decarbonylation pathway of cuticular wax-biosynthesis (Kunst and Samuels 2003). A mutation in the *WAX2* gene resulted in a decrease of the products of this pathway, and the plants also showed postgenital fusions (Chen et al. 2003). The KCR1 gene seems more important for plant development, the loss of KCR1 function in Arabidopsis resulted in embryo lethality. Suppressed KCR1 activity resulted in reduced wax load, and altered VLCFA composition of seed triacylglycerols or root glycerolipids (Beaudoin et al. 2009).

Materials and methods

The plant material was two scab resistant apple (*Malus domestica* Borkh.) cultivars, the early ripening 'Prima' and the late-ripening 'Florina'. Both cultivars were grown in the Experimental and Research Farm of Corvinus University of Budapest, Soroksár. The apple fruits were harvested at full ripening, at 120 DAP (days after pollination) of 'Prima' and

Accepted July 11, 2011
*Corresponding author. E-mail: istvan.papp2@uni-corvinus.hu

Table 1. Primers that were used in the experiments.

Gene name	Sequence origin	Primer sequence	Amplicon length
EF1	AJ223969	5′ GCTCAAGGCTGAGCGTGAACGT 5′CAGAAATGGGGACAAAGGGGAT	398 bp / 600 bp
KCR1	GO523303	5′GACCTGCCAAGAATCTCAAAAAG 5′AATCAAAAGTCCCACATCCAACC	281 bp
WAX2	DR997009	5′CCTTTATATTACTGGGTGC 5′TTTGCGTTTAGTGTCTTCC	413 bp
CER2	ES789986	5′CTTCTGCTTTTAATGGTCTTG 5′GAAATGTATGCGTCTTCGTCT	351 bp

145 DAP of the 'Florina' cultivars. Leaves were collected earlier, at 90 DAP. Collected tissues were placed into liquid nitrogen, and stored at -80°C. The RNA isolation was carried out as described by Asif and his co-workers (Asif et al., 2006). The gDNA contamination was eliminated by DNase I treatment (Fermentas) using the protocol of the manufacturer. Normalization of RNA levels was done by measuring RNA concentration by NanoDrop (NanoDrop Technologies Inc). Reverse transcription was performed by a First Strand cDNA Synthesis Kit (Fermentas). RT-PCR was done by using GoTaq Polymerase (Pharmacia), applying the following protocol: 95°C 2'30", 95°C 30", 55°C 30", 72°C 1' 30 cycles followed by 72°C 7' and coolled to 4°C. The homologs for the Arabidopsis genes were searched in the apple genome database, then the mRNAs were aligned to the NCBI EST (Expressed Sequence Tag) database. The primers designed are listed in Table 1. The amplification products were run on ethydium-bromide containing 1,2% agarose gel (Invitrogen).

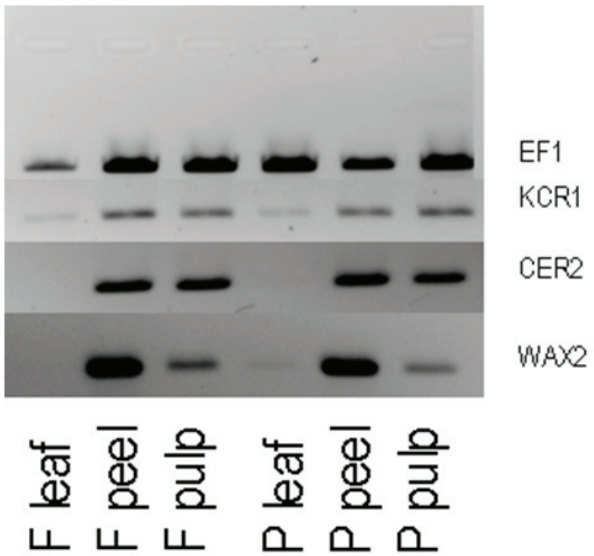

Figure 1. Agarose gel electrophoresis of RT-PCR products on cDNAs from F – Florina, P – Prima tissues.

Results and Discussion

Figure 1 shows that the isolated RNA is free of genomic DNA contamination. The EF1 specific primer pair amplified only a single band in all cases, while the genomic EF1 region would contain an extra small intron. The RNA levels were equal to each other (Fig. 1 and data not shown).

While the CER2 and WAX2 apple homologs show fruit-specific expression, the KCR1 specific primer pair also amplified products from the leaves. The bands from peel and pulp seem of equal intensity, with less product amplified from leaves. The targeted apple WAX2 homolog shows tissue-specific expression in these cultivars, moreover we find that this gene is mostly expressed in the peel. Our results show, that we could identify at least two wax-biosynthesis associated genes in apple, that have tissue specific expression. One of them, a WAX2 homolog also shows peel-specific expression. The products of KCR1 specific amplification appear in all tissues examined in both cultivars. This means that it probably has a function which is needed in both fruits and leaves. This result is consistent with the indispensable role of KCR1 in *Arabidopsis thaliana* plant development (Beaudoin et al. 2009).

Acknowledgements

Our work was funded by TÁMOP-4.2.1/B-09/1/KMR/-2010-0005 project. A part of this study was presented on the 10th Congress of the Hungarian Society for Plant Biology, August 31 - September 2, 2011, Szeged, Hungary.

References

Asif M, Trivedi P, Solomos T, Tucker M (2006) Isolation of high-quality RNA from apple (Malus domestica) fruit. J Agric Food Chem 54:5227-5229.

Beaudoin F, Wu X, Li F, Haslam RP, Markham JE, Zheng H, Napier JA, Kunst L (2009) Functional characterization of the Arabidopsis β-ketoacyl-coenzyme A reductase candidates of the fatty acid elongase. Plant Physiology 150:1174-1191.

Chen X, Goodwin SM, Boroff VL, Liu X, Jenks MA (2003) Cloning and characterization of the WAX2 gene of Arabidopsis involved in cuticle membrane and wax production. Plant Cell 15:1170-1185.

Kunst L, Samuels AL (2003) Biosynthesis and secretion of plant cuticular wax. Prog Lipid Res 42:51-80.

Kunst L, Samuels AL (2009) Plant cuticles shine: advances in wax biosynthesis and export. Curr Opin Plant Biol 12:721-727.

Pollard M, Beisson F, Li Y, Ohlrogge JB (2008) Building lipid barriers: biosynthesis of cutin and suberin. Trend in Plant Science 13:236-246.

Riederer M (2006) Introduction: biology of the plant cuticle. In Riederer M, Müller C ed., Biology of the plant cuticle. Blackwell Publishing, Oxford, pp. 1-10.

Samuels L, Kunst L, Jetter R (2008) Sealing plant surfaces: cuticular wax formation by epidermal cells. Annu Rev Plant Biol 59:683-707.