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Expression of *COR* gene homologues in different plants during cold acclimation

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To investigate orchard plant response to cold stress, homologs of thale cress (*Arabidopsis thaliana* (L.) Heynh.) *COR47* (cold responsive) gene in different plants – herbal and woody – were isolated, and the expression of this gene during the cold acclimation process was examined. Conservative *COR47* DNA fragments typical of dicotyledonous plants were established and specific primers (18–20 nucleotide length) were created. Two strawberry (*Fragaria ananassa* Duch.) cultivars (cold-resistant 'Melody' and cold sensitive 'Holiday'), two sweet cherry (*Prunus avium* L.) cultivars (cold-sensitive 'Kordija' and cold resistant 'Jurgita'), two sour cherry (*Prunus cerasus* L.) cultivars (cold-sensitive 'Erdi Jubileum' and cold-resistant 'Molodiozhnaja') and the M323 hybrid of sour cherry with sweet cherry were used in the study. Our results show that the *COR47* gene homolog transcription is no less than 30 days of cold acclimation at low temperatures. No less than 24–30 days of cold acclimation are necessary for plant hardening. Similar mRNA variations were established in strawberry and cherry plants, implying the similarity of genes involved in the cold acclimation process in orchard plants.

Key words: cherry, cold acclimation, *COR47*, strawberry

INTRODUCTION

As plants are sessile organisms, they are forced to survive in environmental stresses. Many plants exhibit an increase in freezing tolerance in response to low, non-freezing temperatures, a phenomenon known as cold acclimation [1]. A number of genes have been described that respond to cold stress in plants [2, 3], and it is thought that their gene products may play important roles in the acclimation of plants. Recently, 939 cold-regulated genes have been determined using *Arabidopsis thaliana* Affymetrix GeneChips which contain ~24,000 genes [4]. Many *COR* (*cold-responsive*) genes have been cloned from *Arabidopsis* on the basis of induction of expression at low temperatures [5]. Cold-induced mRNAs generally begin to accumulate within a few hours at a low temperature and remain at high levels until plants are returned to a normal growth temperature. The correlation between the expression times of these genes and enhanced freezing tolerance suggests that *COR* gene products could play a role in freezing tolerance [6]. Recently, transgenic plants expressing a transcriptional activator that binds to motifs often found in cold-inducible genes have been constructed. These plants display both constitutively enhanced freezing tolerance and an overexpression of a set of *COR* genes [7, 8]. Expression of *COR* genes in many wintering orchard plants is still rarely investigated. The aim of this study was to examine accumulation of RNA encoded by homologs of thale cress (*Arabidopsis thaliana* (L.) Heynh.) *COR47* genes in different orchard plants: herbal – strawberry (*Fragaria ananassa* Duch.) and woody – sour cherry (*Prunus cerasus* L.), sweet cherry (*Prunus avium* L.) and their hybrids with different winterhardiness, and to evaluate their expression during the cold acclimation process.

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MATERIALS AND METHODS

The work was performed at the Plant Genetic and Biotechnology Department of the Lithuanian Institute of Horticulture. Two strawberry cultivars (cold-resistant 'Melody' and cold-sensitive 'Holiday'), two sweet cherry cultivars (cold-sensitive 'Kordija' and cold-resistant 'Jurgita'), two sour cherry cultivars (cold-sensitive 'Erdi Jubileum' and cold-resistant 'Molodiozhnaja') and the hybrid M323 of sour cherry with sweet cherry were used in this study. Plants were grown *in vitro* in a temperature and humidity controlled culture room at 22 °C and 50 μmol $m^{-2} s^{-1}$ PPF, and a 16-h photoperiod was provided by cool white fluorescent light before investigation. Plants were grown on a sterile medium producing a uniform plant material that was not exposed to additional, uncontrolled stresses such as water stress, nutrient depletion, pathogens, or insect pests, any of which could also induce some level of freezing tolerance. Four-week-old plants were placed to a climatic chamber and cultivated at a temperature of +4 °C for cold-acclimation under the same fluorescent lamps and at the same photoperiod. Total RNA was extracted from the plants acclimated for 6, 12,

18, 24 and 30 days using an RNA extraction kit (Perfect RNA, Eukaryotic Mini, Eppendorf, Germany) according to producer's recommendations. Plants grown *in vitro* at +22 °C were used as control. The copy DNA of the *COR* gene homologs of all the plants was synthesized using the previously isolated RNA, the specific primer COR47X1F1 (Table) and a reverse transcriptase reaction kit (RevertAid™ H Minus First Strand cDNA Synthesis Kit, MBI Fermentas, Lithuania) according to producer's recommendations. Copy DNA fragments were amplified using polymerase chain reaction (PCR) and analysed by agarose gel electrophoresis. Expression of the glyceraldehydephosphate dehydrogenase (GAPDH) gene was used as the internal control in this investigation. The expresion of this gene was uniform during the acclimation process.

PCR reaction. The PCR mixture contained 1 unit of *Taq* DNA polymerase (MBI Fermentas, Lithuania), 1.5 mM $MgCl_2$, 0.2 mM dNTP and 1 μM of COR47X1R1 oligonucleotide primer (Table). The amplification was carried out according to the following scheme: 1 cycle of 94 °C for 3 min, followed by 35 cycles of 94 °C for 1. 15 min, 63 °C for 1 min, 72 °C for 1.30 min and in the end 72 °C for 6 min.

PCR products were analysed using cDNA electrophoresis.

Primer selection The *Arabidopsis thaliana COR47* gene was compared with homolog sequences of different grassy (monocotyledonous and dicotyledonous) and woody (stone fruit) plants supplied in the database (GenBank (NCBI):

Shepherd's-purse *(Capsella bursa-pastoris) COR 29* – AY513787;

wheat (*Triticum aestivum*) *COR39* – AF058794;

peach (*Prunus persica*) *PU1* – DY647091;

apple-tree (*Malus domestica*) *Mdlv2* – CV525037.

Constitutive fragments of *COR47* DNA sequences for the analysed plants were defined and two pairs of degenerated primers for the identification of *COR* gene homologs in orchard plants were developed (Table). Only one pair of degenerated primers provided results (Table, in bold).

Table. **Selected degenerated primers**

Primer	Sequence
COR47X1F1	**(5′-ACRTTCCCGAGCACGAGACH-3′)**
COR47X1R1	**(5′-CTCCAARATACCCTTCTTYTCC-3′)**
COR47X2F1	(5′-ACRTTCCCGAGCACGAGACH-3′)
COR47X2R1	(5′-CCAWRATHCCCTTCTTYTCCKYSG-3′)

RESULTS AND DISCUSSION

Conservative *COR47* DNA fragments typical of dicotyledonous plants were established, and the specific primers (18–20 nucleotide length) were developed for this investigation (Table). Homologs of these fragments were isolated in herbal (strawberry) and woody (sour cherry, sweet cherry and their hybrids) orchard plants. The size of an isolated fragment was 600 bp. Differences in fragment size between species and varieties were not observed.

Our results show that *COR47* gene homolog transcription is no less than 30 days of cold acclimation at low temperatures. These results confirm the results obtained *in vitro* with strawberry, showing that the cold-resistance of strawberry depends on cold acclimation duration. The plants are most resistant after 30–35 days of acclimation [9].

As the transcription is synchronized with the maximum plant acclimation, we can propose that *COR* genes are involved in the orchard plant cold acclimation process, and the acclimation process lasts no less than 24–30 days at low not freezing temperatures. This time is required for plant cell structural alteration to avoid unacceptable lesions related with cell dehydration.

After expression evaluation of *Arabidopsis thaliana COR47* gene homologs in the orchard plants studied (Figs. 1, 2), the *COR47* gene homolog transcription time was similar in both herbal (strawberry) and woody (cherry) plants, allowing an assumption the transcription time similarity of genes involved in cold-acclimation process in herbal and woody orchard plants.

There were no any regularities in respect of different *COR47* homolog expression in cold-resistant and cold-susceptible cultivars. This means that the *COR47* gene structure or other genetic factors may have a stronger impact on the differentiation of orchard plants by this trait than the transcript accumulation velocity of this gene.

Our results confirm the data of other authors [2, 3] regarding the complex nature of acclimation and show the necessity

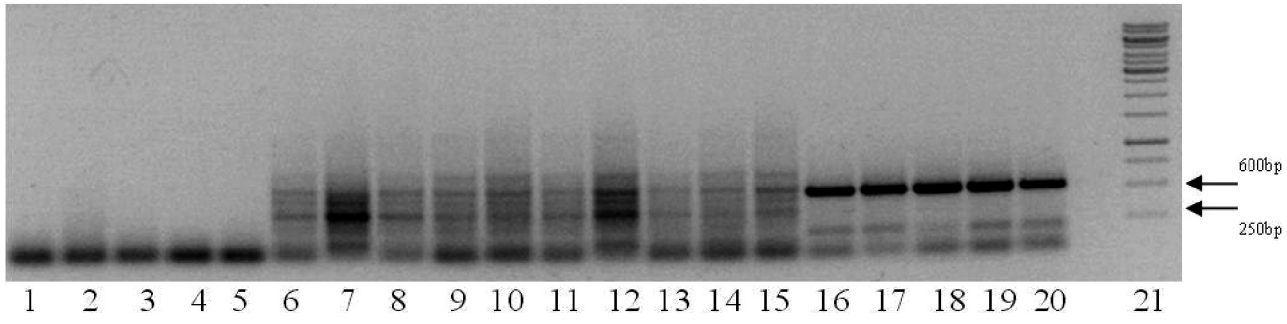

Fig. 1. Transcription of cold-induced *COR47* gene homologues during cold acclimation process in cherry.
1, 6, 11, 16 – cold-sensitive sweet cherry 'Kordija'. 2, 7, 12, 17 – cold-resistant sweet cherry 'Jurgita'. 3, 8, 13, 18 – hybrid of sour cherry with sweet cherry M323. 4, 9, 14, 19 – cold-sensitive sour cherry cultivar 'Erdi Jubileum'. 5, 10, 15, 20 – cold-resistant sour cherry cultivar 'Molodiozhnaja'. 1–5 – not acclimated plants; 6–10 – 6 days cold acclimated plants; 11–15 – 12 days cold-acclimated plants; 16–20 – 30 days cold-acclimated plants; 21 – O'GeneRulerTM 1 kb DNA Ladder

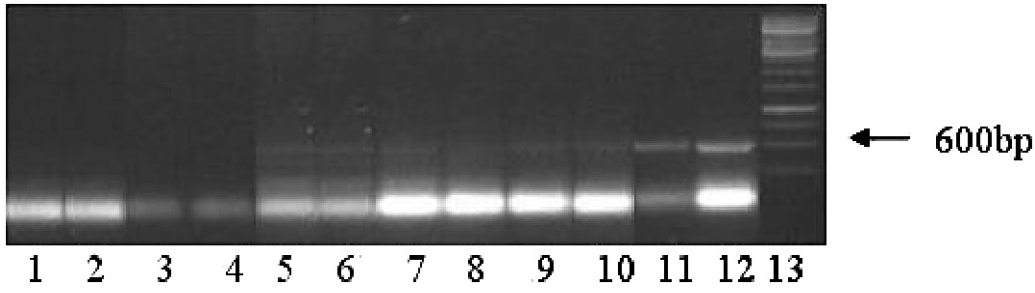

Fig. 2. Transcription of cold-induced *COR47* gene homologues during cold acclimation process in strawberry. 1, 3, 5, 7, 9, 11 – cold-resistant strawberry 'Melody'. 2, 4, 6, 8, 10, 12 – cold-sensitive strawberry 'Holiday'. 1–2 – not acclimated plants; 3–4 – 6 days cold acclimated plants; 5–6 – 12 days cold acclimated plants; 7–8 – 18 days cold-acclimated plants, 8–9 – 24 days cold-acclimated plants 11–12 – 30 days cold-acclimated plants; 13 – O'GeneRulerTM 1 kb DNA Ladder

of further a estimation of the function of *COR* and other cold-responsive genes and determination of their importance in the orchard plant cold-acclimation and cold-resistance formation processes.

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COR GENŲ HOMOLOGŲ RAIŠKA SKIRTINGUOSE AUGALUOSE UŽSIGRŪDINIMO ŠALČIUI METU

S a n t r a u k a

Siekiant ištirti sodo augalų atsaką į žemų temperatūrų stresą, buvo išskirti baltažiedžio vairenio (*Arabidopsis thaliana* (L.) Heynh.) *COR47* geno homologai iš skirtingų žolinių ir sumedėjusių augalų bei ištirta šių genų raiška užsigrūdinimo šalčiui metu. Tyrimu nustatyti konservatyvūs *COR47* DNR fragmentai, tipiški tirtiems dviskilčiams augalams, ir sukurti specifiniai degeneruoti pradmenys tolimesniam tyrimui.

Šiame tyrime naudotos dvi braškių (*Fragaria ananassa* Duch.) veislės – atspari šalčiui 'Melody' ir jautri šalčiui 'Holiday', dvi trešnių (*Prunus avium* L.) veislės – jautri šalčiui 'Kordija', atspari šalčiui 'Jurgita', dvi vyšnių (*Prunus cerasus* L.) veislės – jautri šalčiui 'Erdi Jubileum', atspari šalčiui 'Molodiožnaja', taip pat vyšnių ir trešnių hibridas M323. Mūsų tyrimai patvirtina *COR47* geno homologų transkribciją tirtuose žoliniuose ir sumedėjusiuose sodo augaluose esant ne trumpesniam kaip 30 dienų grūdinimui žemose teigiamose temperatūrose. Todėl galima teigti, kad sodo augalų užsigrūdinimui būtinas ne trumpesnis kaip 24–30 dienų laikotarpis žemose teigiamose temperatūrose. Gautais duomenimis, užsigrūdinimo procese dalyvaujančių sodo augalų genų transkripcijos laikas yra panašus.