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Research article

Cloning and expression profiling of the *PacSnRK2* and *PacPP2C* gene families during fruit development, ABA treatment, and dehydration stress in sweet cherry

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ABSTRACT

Plant SNF1-related protein kinase 2 (SnRK2) and protein phosphatase 2C (PP2C) family members are core components of the ABA signal transduction pathway. SnRK2 and PP2C proteins have been suggested to play crucial roles in fruit ripening and improving plant tolerance to drought stress, but supporting genetic information has been lacking in sweet cherry (*Prunus avium* L.). Here, we cloned six full-length *SnRK2* genes and three full-length *PP2C* genes from sweet cherry cv. Hong Deng. Quantitative PCR analysis revealed that *PacSnRK2.2*, *PacSnRK2.3*, *PacSnRK2.6*, and *PacPP2C1–3* were negatively regulated in fruits in response to exogenous ABA treatment, *PacSnRK2.4* and *PacSnRK2.5* were upregulated, and *PacSnRK2.1* expression was not affected. The ABA treatment also significantly promoted the accumulation of anthocyanins in sweet cherry fruit. The expression of all *PacSnRK2* and *PacPP2C* genes was induced by dehydration stress, which also promoted the accumulation of drought stress signaling molecules in the sweet cherry fruits, including ABA, soluble sugars, and anthocyanin. Furthermore, a yeast two-hybrid analysis demonstrated that PacPP2C1 interacts with all six PacSnRK2s, while PacPP2C3 does not interact with PacSnRK2.5. PacPP2C2 does not interact with PacSnRK2.1 or PacSnRK2.4. These results indicate that PacSnRK2s and PacPP2Cs may play a variety of roles in the sweet cherry ABA signaling pathway and the fruit response to drought stress.

1. Introduction

Sweet cherry (*Prunus avium* L.) is a popular fruit around the world and has a variety of health promoting benefits (Mccune et al., 2010), as well as a high economic value (http://www.fao.org). The external quality of the fruit is one of the most important aspects of its marketability. Fruit quality can be affected by multiple factors before or after harvest, such as soil water content, soil nutrients, environmental temperature, light quality and quantity, and air humidity (Espley et al., 2007; Berdeja et al., 2014; Ferrandino and Lovisolo, 2014). Changes in temperature and air humidity during storage affect the rate at which the sweet cherry fruit loses water, which is the main factor that affects the external quality of fruit during its short postharvest shelf life. The hormone abscisic acid (ABA) plays key roles in the plant's response to drought stress and in the fruit ripening process (Zhu, 2002; Shinozaki and Yamaguchi-Shinozaki, 2007; Jia et al., 2011); therefore, studies focused on the sweet cherry ABA signal transduction pathway are valuable for enriching our understanding of the cherry fruit response to dehydration stress and ultimately improving postharvest longevity.

The plant SNF1-related protein kinase 2 (SnRK2) and protein phosphatase 2C (PP2C) gene families are the core components of the ABA signaling pathway (Ma et al., 2009; Sun et al., 2011). The *SnRK2* genes mainly function as positive regulators, while the *PP2C*

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genes are negative regulators (Fujii et al., 2009; Fujita et al., 2009). When plants are grown under normal well-watered conditions, PP2Cs inhibit the activity of SnRK2s, repressing ABA signal transduction (Park et al., 2009). During drought or salt stress, ABA accumulates, and the ABA-bound receptors inhibit the activity of the PP2Cs, thereby enabling activation of the SnRK2s, which phosphorylate the downstream ABA-responsive transcription factors to activate diverse ABA-responsive genes (Furihata et al., 2006; Gonzalez-Guzman et al., 2014).

Recent studies have suggested that *SnRK2* family members play crucial roles in the plant's response to abiotic stresses (Fujii et al., 2011; Kulik et al., 2011; Cai et al., 2014). The genome of the model plant *Arabidopsis thaliana* contains ten *SnRK2* family members, which can be divided into three groups (Kulik et al., 2011). Group I (*AtSnRK2.1*, *AtSnRK2.4*, *AtSnRK2.9*, *AtSnRK2.5*, and *AtSnRK2.10*) members are involved in the response to osmotic stress and improve plant drought tolerance, but do not respond to ABA (Umezawa et al., 2004; Fujii et al., 2011; Kulik et al., 2011). Group II includes *AtSnRK2.7* and *AtSnRK2.8*, which respond to salt stress and show a weak response to ABA, and can also improve plant drought tolerance (Boudsocq et al., 2004; Kim et al., 2012). In contrast, group III (*AtSnRK2.2*, *AtSnRK2.3*, and *AtSnRK2.6*) members regulate stomatal closure and opening in response to ABA, and play a role in seed germination and early seedling development (Yoshida et al., 2006; Cai et al., 2014; Feng et al., 2014). These *SnRK2* genes showed different sensitivities to ABA, osmotic stress, and drought stress in Arabidopsis, indicating that the functions of these core components of ABA signaling are diverse. The *SnRK2* family members have been isolated and characterized in some fruit crops for which a genome sequence is available, such as tomato (*Solanum lycopersicum*) and strawberry (*Fragaria* sp.) (Han et al., 2015; Chen et al., 2016); however, our knowledge of the full-length coding sequences (CDSs) of many *SnRK2*s and their responses to abiotic stresses in fruit crops is limited.

The *PP2C* family members can be divided into ten groups, comprising 76 members in Arabidopsis (Schweighofer et al., 2004). Among these, the groups A *PP2Cs* have been demonstrated to act as negative regulators of the ABA signal transduction pathway (Ma et al., 2009). The pyrabactin resistance 1-like (PYL) ABA receptors alter their protein conformations when they bind to ABA, inhibiting the activity of the group A PP2Cs (Park et al., 2009). The inactivated PP2Cs detach from the SnRK2-PP2C complex, leaving the SnRK2 proteins to phosphorylate downstream factors (Furihata et al., 2006).

Although the SnRK2s and PP2Cs are core components of the ABA signaling pathway, some members of these families cannot respond to exogenous ABA treatments in both tomato and sweet cherry fruits. These findings indicate that the response mechanism of these gene families is complex and might be different during dehydration treatments of climacteric and non-climacteric fruits.

Sweet cherry is a typical non-climacteric fruit; however, studies on dehydration stress in this crop are limited. In the present work, we isolated six *PacSnRK2* and three *PacPP2C* full-length genes from 'Hong Deng' sweet cherry and investigated their expression levels in developing fruits or in fruits treated with ABA or an ABA biosynthesis inhibitor, as well as in detached fruits under dehydration stress. We also examined several physiological changes related to drought stress in the detached sweet cherry fruits in both the véraison and maturation stages. Finally, we analyzed the interactions between PacSnRK2s and PacPP2Cs in a yeast-two hybrid (Y2H) system. The results of this study facilitate our understanding of the expression patterns of sweet cherry *SnRK2* and *PP2C* genes in response to dehydration stress.

2. Materials and methods

2.1. Cherry fruit harvesting

Materials from 10-year-old sweet cherry (*Prunus avium* L. cv. Hong Deng) rootstock were collected during 2013. Trees were grown in field conditions at the Beijing Academy of Agriculture and Forestry Sciences. Fruits were harvested at different developmental stages, specifically the véraison and maturation stages, between March and May.

2.2. ABA, NDGA, and dehydration stress treatments

To examine the effects of exogenous ABA and the ABA biosynthesis inhibitor nordihydroguaiaretic acid (NDGA) on sweet cherry fruits, two-year-old shoots with fruits and leaves were collected either 27 (just before the fruits turned red; véraison stage) or 42 (maturation stage) days after full blooming (DAFB) and the shoots were placed under a 16-h light/8-h dark regimen for 4 d at 24 °C in 1 mM ABA and 150 μM NDGA solution. Fifteen cherry fruits were collected every day after the initiation of treatment and immediately frozen in liquid nitrogen and stored at −80 °C for subsequent analysis. Each treatment included three biological replicates.

To test the effects of dehydration on sweet cherry fruits, fruits were collected at the véraison (27 DAFB) and maturation (42 DAFB) stages, and divided into two groups: group I (control) fruits were covered with wet gauze to reduce water loss and group II fruits were subjected to dehydration. The fruits of both groups were placed in a growth chamber under a 16-h light/8-h dark cycle at 25 ± 2 °C with 50% relative humidity. Fifteen fruits in each group were sampled daily during the treatment. Each fruit was weighed immediately after collection and then weighed again before sampling to calculate the rate of water loss.

2.3. RNA isolation, gene cloning, and sequence analysis

Total RNA was isolated from 1 g fruit flesh tissue using the improved hot borate method described by Wan and Wilkins (1994). First-strand cDNA was synthesized using M-MLV reverse transcriptase (Promega, Madison, WI, USA), according to the manufacturer's instructions. The cDNA was used as a template to amplify *PacSnRK* and *PacPP2C* homologous genes. Degenerate primers (Supplementary Table S1) were designed based on the conserved domains derived from the sequences of the STKc_SnRK2 and PP2C domains in several rosaceous plants, including apple, peach, strawberry, and peach. Six PCR products encoding STKc_SnRK2 domains and three PCR products encoding PP2C domains were obtained. The full-length cDNAs of these PCR products were cloned using a SMARTERTM RACE cDNA Amplification Kit (Clontech Laboratories, Mountain View, CA, USA). The RACE products were ligated into pEASY-Blunt Zero vectors (Transgen, Beijing, China) and sequenced by Sangon (Shanghai, China). The resulting nine RACE products were named *PacSnRK2.1* (GenBank accession: KY780372), *PacSnRK2.2* (KY780373), *PacSnRK2.3* (KY780374), *PacSnRK2.4* (KY780375), *PacSnRK2.5* (KY780376), *PacSnRK2.6* (KY780377), *PacPP2C1* (KY780378), *PacPP2C2* (KY780379), and *PacPP2C3* (KY780380). All primers were designed using Primer Premier 5.0 (Premier Biosoft, Palo Alto, CA, USA) and are listed in Supplementary Table S1. The phylogenetic trees were generated by MEGA5 (Tamura et al., 2011), while the protein sequence alignments were performed using BioEdit (Thomas, 1999).

2.4. Determination of soluble sugar, anthocyanin, and ABA contents

The soluble sugars content of the fruits was determined by ICS-