Short title: PuWRKY31 is involved in sucrose accumulation

Corresponding author: Aide Wang, College of Horticulture, Shenyang Agricultural University, Shenyang, 110866 China. Tel: 86-24-88487143.
Fax: 86-24-88487146. E-mail: awang@syau.edu.cn.

Histone Acetylation at the Promoter for the Transcription Factor PuWRKY31 Affects Sucrose Accumulation in Pear Fruit

Xinyue Li[1], Wei Guo[1], Juncai Li[2], Pengtao Yue[1], Haidong Bu[1,3], Jing Jiang[1], Weiting Liu[1], Yaxiu Xu[1], Hui Yuan[1], Tong Li[1], Aide Wang[1]*

[1]College of Horticulture, Shenyang Agricultural University, Shenyang, 110866 China
[2]Liaoning Institute of Pomology, Xiongyue, 115009 China
[3]Mudanjiang Branch of Heilongjiang Academy of Agricultural Sciences, Mudanjiang, 157000 China
*Corresponding author: Aide Wang, E-mail: awang@syau.edu.cn

One sentence summary:
High acetylation in the promoter of a transcription factor gene is associated with sucrose accumulation in pear fruit by activating the expression of a sucrose transport gene.

Author contributions:
A.W. and X.L. designed this project and wrote the manuscript. X.L. performed most of the experiments. W.G. extracted the RNA. J.L. provided the plant materials. W.L., H.B. and Y.X. measured the sugar content. X.L., H.Y., J.J., T.L. and P.Y. analyzed the data and discussed the article.
Corresponding author: Aide Wang, E-mail: awang@syau.edu.cn

ABSTRACT

Sugar content is an important trait of fleshy fruit, and elevating sucrose levels is a major goal in horticultural crop breeding. Here, we examined the sugar content in two varieties of the Ussurian pear (*Pyrus ussuriensis*), Nanguo (NG) and its bud sport (BNG), and we found that sucrose content was higher in BNG fruit than in NG fruit. We compared the transcriptomes of the two varieties using RNA-seq and identified a *SWEET* (Sugars Will Eventually be Exported Transporter) gene, *PuSWEET15*, expressed at higher levels in BNG fruit. Heterologous expression of *PuSWEET15* in a SUSY7/ura yeast (*Saccharomyces cerevisiae*) strain showed that PuSWEET15 is an active sucrose transporter. Overexpression of *PuSWEET15* in NG pear fruit increased sucrose content, while silencing of *PuSWEET15* in BNG fruit decreased sucrose content. The WRKY transcription factor *PuWRKY31* was also expressed more highly in BNG fruit than in NG fruit, and we found that PuWRKY31 bound to the *PuSWEET15* promoter and induced its transcription. The histone acetylation level of the *PuWRKY31* promoter was higher in BNG fruit, suggesting a mechanism by which sucrose levels can be elevated.

INTRODUCTION

In plants, the three major soluble sugars are sucrose, glucose and fructose. Of these, sucrose is the main carbohydrate transported from the photosynthetic source tissues to heterotrophic sink tissues, and so is central to the resource allocation system (Rennie and Turgeon, 2009; Eom et al., 2012; Braun et al., 2014). Sucrose represents a metabolic resource for carbon skeleton construction and energy, allowing growth and development, and is also an important contributor to the sweetness and flavor of many fleshy fruits (Braun et al., 2014). Sweetness is one of the main factors of fruit quality and it has been recognized as an important driver of consumer preference (Jaeger et al., 1998). Thus, an understanding of the mechanisms involved in sucrose transport and the enhancement of sugar accumulation in fruit is of both fundamental and applied importance.

Sucrose accumulation in fruits depends on its transportation and metabolism. The enzymes involved in the metabolism of sucrose are sucrose phosphate synthase (SPS), sucrose synthase (SS) and invertase (INV) (Stitt et al., 1988; Moriguchi et al., 1992; Sturm et al., 1999). Sucrose movement between cells can be passive, through plasmodesmata along a concentration gradient, or active involving transporters, such as membrane-localized sucrose transporters (SUTs) that translocate sucrose from mesophyll cells into the phloem in leaves (Riesmeier et al., 1992; Lemoine, 2000). It has also been shown that intracellular sucrose is transported from mesophyll cells to the apoplast by the SWEET (Sugars Will Eventually be Exported Transporters) proteins (Chen et al., 2012). The role of *SWEET* genes in sucrose transport was first identified in Arabidopsis (*Arabidopsis thaliana*), in which a double mutation of *AtSWEET11* and *AtSWEET12* caused severe growth retardation and reduced sucrose content in the vascular bundles but increased sucrose levels in the leaves (Chen et al., 2012). These results demonstrated that the *SWEET* genes play important roles in sucrose phloem loading, and led to subsequent identification and characterization of *SWEET* family members in other plant species, including rice (*Oryza sativa*), soybean (*Glycine max*), grape (*Vitis vinifera*), apple (*Malus domestica*), sorghum (*Sorghum bicolor*) and pear (*Pyrus bretschneideri*) (Yuan and Wang, 2013; Chong et al., 2014; Wei et al., 2014;

Patil et al., 2015; Mizuno et al., 2016; Li et al., 2017a). SWEET transporters are predicted to have seven transmembrane segments (TMSs) with two distinct repeated units of three TMSs and a connecting fourth TMS (Xuan et al., 2013). There are 21 *SWEET* genes in the rice genome, among which *OsSWEET11* (also named *Os8N3/Xa13*) and *OsSWEET14* (*Os11N3*) encode proteins that are localized to the plasma membrane and so likely affect sugar levels in the apoplast. Knocking out *OsSWEET11* causes smaller seeds, reduced pollen viability, defective stamens and decreased sucrose content (Chu et al., 2006; Yang et al., 2006; Ma et al., 2017; Gao et al., 2018). In Arabidopsis, AtSWEET15 localizes to the plasma membrane, and its transcript levels are significantly higher during water stress, suggesting a role in sucrose apoplastic unloading (Durand et al., 2016). AtSWEET17 is a fructose transporter (Guo et al., 2014). In soybean, GmSWEET15 mediates sucrose export from endosperm to early embryo, and in the *gmsweet15* mutant, the sucrose and glucose contents are significantly decreased in all seed parts compared with the wild type (Wang et al., 2019). Moreover, a *Medicago truncatula* MtSWEET1b transporter supplies glucose for *Arbuscular mycorrhizal* (An et al., 2019a). These findings suggest a broad role for *SWEET* genes in sugar transportation.

Pear is a very important horticultural crop in the world. With the published pear genome, genes related to many quality traits such as stone cells, sugar, acid, volatiles, color and ripening have been identified (Wu et al., 2013; Dong et al., 2019; Chagne et al., 2014). This provides plenty of resources to study the formation of quality traits in pear. However, information regarding the sugar accumulation in pear fruit is still lacking. In this study, we characterized the basis of sweetness in the Nanguo (NG) clonal variety of Ussurian pear (*Pyrus ussuriensis*). NG is highly valued by growers and consumers because of its cold resistance, taste and aroma (Huang et al., 2014). In perennial fruits, a new variety that derives from shoot cells of the parent, presumably through genetic or epigenetic alterations, is called a bud sport variety (Furiya et al., 2009). A bud sport variety of NG (herein referred to as BNG) was identified on a NG tree in 1980s on a farm in the Anshan region in Liaoning province. The skin color of BNG fruit is similar to NG fruit in the early stage (before 40 days after full bloom), and

thereafter turns to brown (Fig. 1A), and this phenotype is stable after being propagated clonally (Supplemental Fig. 1A-B). More interestingly, BNG is sweeter tasting than NG, but the underlying mechanism is unknown. We compared the sugar content of NG and BNG fruits and found that the sucrose content was higher in BNG fruit. A sucrose transporter *PuSWEET15* was more highly expressed in BNG fruit than in NG fruit. We also determined that a WRKY transcription factor PuWRKY31, which was also expressed at higher levels in BNG fruit, bound to the *PuSWEET15* promoter and upregulated its expression.

Plant WRKY proteins participate in developmental processes and respond to various biotic and abiotic stresses (Zhou et al., 2008; Ren et al., 2010; Sun et al., 2019). The expression of *WRKY* genes is also strongly induced during senescence; for example, overexpression of *AtWRKY45* significantly accelerates the expression of SENESCENCE ASSOCIATED GENEs (SAGs) (Chen et al., 2017). AtWRKY57 interacts with repressors of the jasmonate (JA) and auxin signaling pathways, affecting JA-induced leaf senescence in Arabidopsis (Jiang et al., 2014). In addition, AtWRKY75 interacts with DELLA proteins and may function as a component of the gibberellins (GA)-mediated signaling pathway to positively regulate Arabidopsis flowering (Zhang et al., 2018). WRKY proteins are also reported to participate in regulation of quality traits in proanthocyanidin and anthocyanin biosynthesis (Lloyd et al., 2017). For example, a WRKY transcription factor (TRANSPARENT TESTA GLABRA2, TTG2) interacts with the MBW (MYB-bHLH-WD40) complex to regulate proanthocyanidin biosynthesis in Arabidopsis seed (Gonzalez et al., 2016). Overexpressing *MdWRKY11* significantly promotes anthocyanin accumulation and increases the expression of *MYB* transcription factors and structural genes of anthocyanin in apple (Liu et al., 2019). MdWRKY40 interacts with MdMYB1 physically, thus enhancing the binding of MdMYB1 to its target genes to induce wounding-induced anthocyanin biosynthesis in apple fruit (An et al., 2019b). WRKY transcription factors respond to sugar treatment by activating the expression of sugar-responsive genes in Arabidopsis (Chen et al., 2019). However, to date, involvement of WRKYs in sugar transport has not been reported. We show here that increased histone acetylation in the *PuWRKY31* promoter

is associated with its higher expression in BNG fruit.

RESULTS

Sucrose Levels are Significantly Higher in BNG Fruit than in NG Fruit

To investigate the basis of the sweeter taste of BNG fruit, we first compared the content of total soluble solids in BNG and NG fruits. Based on measurements from two years (2014 and 2018), we found that the total soluble solid content was higher in BNG fruit than in NG fruit (Supplemental Fig. S2A and S2B). To determine which sugars were present at higher levels in BNG, sucrose, glucose, fructose and sorbitol levels in fruit at different developmental stages were measured using high performance liquid chromatography (HPLC). We observed a significantly higher sucrose content in BNG fruit than in NG fruit from 105 to 134 DAFB (days after full bloom) (Fig. 1B), while no significant differences were observed for glucose and fructose (Fig. 1C and 1D). These results were consistent with data from a 2018 study (Supplemental Fig. S2C-E). Sorbitol content was significantly higher in BNG than in NG fruit only at the time of commercial harvest (134 DAFB) (Fig. 1E), while no difference was found in the 2018 samples (Supplemental Fig. S2F). These findings suggested that the higher BNG sugar content and sweeter taste was due to a higher accumulation of sucrose.

The Sugar Transporter, *PuSWEET15*, is Highly Expressed in BNG Fruit

To identify genes that might contribute to the higher sucrose accumulation in BNG fruit, we compared the transcriptomes of NG and BNG fruits harvested at 134 DAFB (commercial harvest) using RNA-seq. Genes known to be involved in sucrose transport and metabolism, such as *SUT*, *SPS*, *SS* and *INV*, did not show differential expression between NG and BNG fruits. However, the RNA-seq analysis revealed that a *SWEET* gene was expressed ~11 fold higher in BNG fruit than in NG fruit (Supplemental Dataset S1; Supplemental Fig. S3). We cloned this gene from both varieties and in both cases the coding region of *PuSWEET* was completely identical with 918 bp. The predicted amino acid sequence was most similar to the AtSWEET15 protein, with 50%

identity (Supplemental Fig. S4), and so it was named PuSWEET15.

The expression profile of *PuSWEET15* was investigated in NG and BNG fruits during development, and we found that it was expressed at significantly higher levels in BNG fruit from 105 to 134 DAFB (Fig. 2A; Supplemental Fig. S5), consistent with the change in sucrose content (Fig. 1B). To determine the intracellular localization of PuSWEET15, its coding sequence (CDS) was fused downstream of a green fluorescent protein (GFP) tag driven by the *CaMV35S* promoter (*35S:GFP-PuSWEET15*) in the pRI101 vector. The recombinant plasmid (*35S:GFP-PuSWEET15*) or a plasmid encoding GFP alone, were transiently expressed in protoplasts of maize (*Zea mays*) leaves. GFP alone was detected in both the membrane and nucleus, while GFP-PuSWEET15 only localized to the plasma membrane (Fig. 2B).

Functional Characterization of *PuSWEET15* by Heterologous Expression in Yeast Cells

To investigate whether *PuSWEET15* encodes a functional sucrose transporter, we ligated its CDS into the pDR196 vector and expressed it in a yeast (*Saccharomyces cerevisiae*) mutant strain SUSY7/ura which is deficient in the wild-type sucrose uptake mechanism in yeast (invertase-mediated hydrolysis of sucrose with uptake of the resulting monosaccharides) and has a plant-derived sucrose synthase activity to metabolize any sucrose taken up by foreign sucrose transporters. The mutant strain carrying an empty pDR196 vector was used as a control. All transformants were grown on SD (synthetic deficient) solid medium containing glucose or sucrose as the sole carbon source without uracil. The yeast cells containing *PuSWEET15* survived well on SD/-uracil solid medium containing sucrose as the sole carbon source compared with control (Fig. 3A), suggesting that PuSWEET15 is a typical sucrose transporter.

PuSWEET15 Is Essential for Sucrose Accumulation in Pear Fruit

To identify the function of PuSWEET15 in pear fruit, we overexpressed *PuSWEET15* under the control of the *CaMV35S* promoter in NG fruit using *Agrobacterium tumefaciens*-mediated infiltration. The empty pRI101 vector was used as a control.

196 Higher expression of *PuSWEET15* was detected in *PuSWEET15*-overexpressing fruit
197 (PuSWEET15-OE) (Fig. 3B), and the sucrose content was significantly higher than in
198 control fruit, while no significant difference was observed for the other three sugars
199 investigated (Fig. 3C). Then we silenced *PuSWEET15* expression in BNG pear fruit
200 using *A. tumefaciens*-mediated infiltration. Lower expression of *PuSWEET15* was
201 detected in *PuSWEET15*-silenced fruit (PuSWEET15-AN) (Fig. 3D), and the sucrose
202 content was significantly lower than in control fruit, while no significant difference was
203 observed for the other three sugars investigated (Fig. 3E), suggesting that PuSWEET15
204 is essential for sucrose accumulation in pear fruit.

205 To provide further evidence for *PuSWEET15* functioning as a sucrose transporter,
206 we examined the putative role of *PuSWEET15* in sucrose transport using *A.*
207 *tumefaciens*-mediated infiltration of *Nicotiana benthamiana* leaves. Following
208 treatment with 1% sucrose for 6 d, *PuSWEET15* was highly expressed (Fig. 3F), and
209 significantly higher sucrose levels were detected in PuSWEET15-OE leaves than in
210 those of wild type (Fig. 3F). These results were all consistent with PuSWEET15
211 contributing to sucrose transport.

212

213 **The Transcription Factor *PuWRKY31* is Highly Expressed in BNG Fruit**

214 To elucidate the *PuSWEET15* expression profiles in NG and BNG fruits, we compared
215 the *PuSWEET15* CDS from each; however, no difference was found. Moreover, no
216 differences were observed in the *PuSWEET15* promoter regions (1,177 bp from the
217 translation initiation site) from NG and BNG, and the methylation levels (+1 to -1107)
218 and the histone acetylation levels (-60 to -409; -895 to -1167) of the promoters were
219 also almost identical (Supplemental Fig. S6).

220 We then analyzed the *cis*-elements of the *PuSWEET15* promoter (1,177 bp) and
221 identified binding sites of transcription factors such as WRKY, DOF (DNA-binding one
222 finger) and MYB. In combination with the RNA-seq results, a WRKY transcription
223 factor, *PuWRKY31*, was more highly expressed in BNG fruit than in NG fruit
224 (Supplemental Fig. S7). This was confirmed by reverse transcription quantitative PCR
225 (RT-qPCR) (Fig. 4A; Supplemental Fig. S8). We then focused on the characterization

of *PuWRKY31*.

To investigate the function of *PuWRKY31*, we cloned the corresponding CDS into the pRI101 vector to allow its expression under the control of the *CaMV35S* promoter and as a fusion with a MYC peptide tag. This construct was overexpressed in NG fruit (PuWRKY31-OE), and the higher expression of *PuWRKY31* in PuWRKY31-OE fruit was verified by RT-qPCR (Fig. 4B). We detected that the sucrose content in PuWRKY31-OE fruit was significantly higher than control fruit (Fig. 4C). Notably, the expression level of *PuSWEET15* was also higher in PuWRKY31-OE fruit (Fig. 4B), suggesting that PuWRKY31 might play a role in sucrose transport by regulating the expression of *PuSWEET15*.

PuWRKY31 Binds to the Promoter of *PuSWEET15* and Upregulates its Transcription

To investigate whether *PuSWEET15* is a direct target of PuWRKY31, we performed an electrophoretic mobility shift assay (EMSA) with three biotin-labeled fragments of the *PuSWEET15* promoter containing four W-box motifs (TGAC, binding site of WRKY) as the labeled probe. His-tagged PuWRKY31 (PuWRKY31-His) was purified and used for DNA-binding assays. As shown in Fig. 5A, PuWRKY31 bound to the *PuSWEET15* promoter (Fig. 5A, lanes 2, 5 and 8). When an unlabeled probe was added as a competitor, the binding of PuWRKY31 to the *PuSWEET15* promoter was reduced (Fig. 5A, lanes 3, 6 and 9), confirming that PuWRKY31 bound to the *PuSWEET15* promoter *in vitro*.

Next, we used chromatin immunoprecipitation (ChIP) PCR to investigate the *in vivo* binding of PuWRKY31 to the *PuSWEET15* promoter. Cross-linked chromatin samples were extracted from the PuWRKY31-OE fruit (Fig. 4B) and precipitated with an anti-MYC antibody. Eluted DNA was used to amplify the sequences neighboring the W-box by qPCR. Fruits overexpressing the GFP sequence were used as negative controls. Fig. 5B shows that the presence of PuWRKY31 substantially enhanced the PCR-based detection of the *PuSWEET15* promoter, indicating *in vivo* binding of PuWRKY31 to the *PuSWEET15* promoter.

We then investigated the regulation by PuWRKY31 of the *PuSWEET15* promoter using a β-glucuronidase (GUS) activation assay in *N. benthamiana* leaves, following co-infiltration with *Pro35S:PuWRKY31* and *ProPuSWEET15:GUS*. *Pro35S:GUS* was used as a control. When *Pro35S:PuWRKY31* was co-infiltrated with *ProPuSWEET15:GUS*, *PuSWEET15* promoter activity increased significantly compared with the control (Fig. 5C), suggesting that PuWRKY31 is a transcriptional activator of *PuSWEET15*. Collectively, these results suggested that PuWRKY31 binds to the *PuSWEET15* promoter and promotes its transcription.

The Expression Profile of *PuWRKY31* Correlates with Histone Acetylation Levels

To investigate the *PuWRKY31* expression profiles in NG and BNG fruits, we compared its CDS, promoter sequences (1,550 bp from the translation initiation site) and methylation levels of the promoter regions (Supplemental Fig. S9). However, no significant differences were observed.

We hypothesized that the *PuWRKY31* expression pattern might correlate with a change in histone modification, and so examined the *PuWRKY31* histone acetylation levels in NG and BNG fruits by ChIP-PCR, using anti-acetyl-histone H3 (H3ac) and H4 (H4ac) antibodies. As a control, the change in histone acetylation (H3ac and H4ac) of the *PuActin* housekeeping gene was also analyzed. No significant changes in H3ac and H4ac were found in NG or BNG for the *PuActin* gene (Fig. 6A), and it was used to normalize the subsequent ChIP-PCR results. Three regions (S1-S3) of pear genomic DNA including the *PuWRKY31* promoter and CDS were examined, which were predicted to be easily acetylated (Zhou et al., 2013; Han et al., 2016). The acetylation levels of regions S1 and S3 detected by histone H3ac, and region S1 detected by H4ac were significantly higher in BNG than in NG fruit (Fig. 6B).

To investigate what causes the higher acetylation level of *PuWRKY31* in BNG fruit, we identified a histone acetyltransferase gene *HLS1* (*HOOKLESS 1*) (Liao et al., 2016), *PuHLS1*, from the RNA-seq results (Supplemental Dataset S1). *PuHLS1* expression was higher in BNG fruit than in NG fruit (Fig. 6C; Supplemental Fig. S10). A previous report has shown that HLS1 binds to the TSS (transcription start site) and the 3'-CDS

of the *AtWRKY33* in Arabidopsis (Liao et al., 2016). We investigated whether PuHLS1 directly interacts with the CDS of *PuWRKY31* using EMSA analysis with two biotin-labeled fragments of the CDS of *PuWRKY31* (probe 1: 1 to 50; probe 2: 50 to 100) as the hot probe. GST-tagged PuHLS1 (PuHLS1-GST) was purified and used for DNA-binding assays. As shown in Fig. 6D, the GST alone did not bind to the *PuWRKY31* CDS (Fig. 6D, lanes 4 and 8), but PuHLS1 did (Fig. 6D, lanes 1 and 5). When an unlabeled probe was added as a competitor, the binding of PuHLS1 to the *PuWRKY31* CDS was reduced (Fig. 6D, lanes 2 and 6), confirming that PuHLS1 bound to the *PuWRKY31* CDS *in vitro*. To elucidate the *PuHLS1* expression profiles in NG and BNG fruits, we compared its CDS, promoter sequences (2,032 bp from the translation initiation site) and methylation levels of the promoter regions, however, they were almost identical in NG and BNG (Supplemental Fig. S11).

DISCUSSION

Sucrose is the main photosynthesis product transported in most plants (Ayre, 2011). By comparing the contents of different sugars in NG and BNG fruits, we found that only the sucrose content was significantly higher in BNG fruit than in NG fruit (Fig. 1). Although sorbitol is important for transport of photosynthesis products in tree fruit crops of the *Rosaceae* family (Priestley, 1983; Zhang et al., 2014), our data showed that the sorbitol content was higher in BNG fruit than in NG fruit only in samples collected on 134 DAFB in 2014 (Fig. 1E), and no difference was observed in samples collected in 2018 (Supplemental Fig. S2F). The difference of sorbitol content between years might be caused by climatic conditions such as rainfall, light or temperature. However, importantly, the difference in sucrose content between BNG and NG fruits did not vary between years. These results suggested that BNG is a bud sport variety of NG pear with higher sucrose accumulation.

SWEET proteins have been widely identified as sugar transporters in plants, especially for sucrose transport (Chen et al., 2012). Here, PuSWEET15 was observed to transport sucrose in pear fruit tissue and *N. benthamiana* leaves when expressed heterologously (Fig. 3). This is consistent with the function of *AtSWEET11*,

316 *AtSWEET12* and *AtSWEET15* from Arabidopsis, in which a double mutation of
317 *AtSWEET11* and *AtSWEET12* causes defects in phloem sucrose loading (Chen et al.,
318 2012). AtSWEET15 was shown to transport sucrose by expressing *SWEET15* in
319 *Xenopus laevis* oocytes and measuring [^{14}C]-sucrose uptake (Chen et al., 2015).

320 In plants, the WRKY family is one of the largest transcription factors families
321 (Zhang and Wang, 2005; Rushton et al., 2010), but functional characterization has
322 mostly focused on their roles in various biotic and abiotic stresses and developmental
323 processes (Rushton et al., 2010). For example, WsWRKY1 regulates nitrogen stress
324 tolerance through modulation of phytosterol and defense pathways in *Withania*
325 *somnifera*, and soybean GmWRKY16 enhances drought and salt tolerance in
326 Arabidopsis through an ABA-mediated pathway (Pal et al., 2017; Singh et al., 2017;
327 Ma et al., 2018). Another recent report showed that VaWRKY33 is involved in cold
328 tolerance in Amur grape (*Vitis amurensis*) (Sun et al., 2019). A more recent study
329 reported that AtWRKY18 and AtWRKY53 directly bind to the promoter of sugar
330 response genes and activate their expression in response to glucose treatment in
331 Arabidopsis (Chen et al., 2019). In our study, we showed that *PuWRKY31* was
332 expressed at significantly higher levels in BNG fruit than in NG fruit (Fig. 4A).
333 Moreover, PuWRKY31 positively regulated the expression of *PuSWEET15* by binding
334 to its promoter (Fig. 5). Importantly, overexpression of *PuWRKY31* in pear fruit led to
335 increased sucrose content (Fig. 4C), suggesting the involvement of PuWRKY31 in
336 sucrose transport in pear fruit.

337 Bud sport varieties occasionally occur in tree fruit crops and are usually caused by
338 a small number of presumably genetic or epigenetic alterations (Whitham and
339 Slobodchikoff, 1981; Furiya et al., 2009). BNG was found by our colleague in the 1980s
340 on a NG tree. BNG showed phenotypes similar to those of NG in leaf, flower and fruit
341 shape (Fig. 1A; Supplemental Fig. 1). Unfortunately we do not have the original picture
342 showing both NG and BNG fruits on different branches of the same tree. However,
343 BNG maintained stable phenotypes when grafted on a NG tree (Supplemental Fig. 1A)
344 or when propagated clonally and cultivated in different regions (Supplemental Fig. 1A-
345 B). Moreover, we analyzed the genomic DNA of both NG and BNG by using 17 pairs

of SSR (simple sequence repeat) primers, but failed to detect any polymorphic bands between two varieties (Supplemental Fig. 1C). These findings indicated that BNG and NG share high similarity in genetic background. In addition to sweetness and sucrose content, BNG differs from NG in other characteristics, such as fruit skin color (Fig. 1A; Supplemental Fig. 1). It will be quite interesting to explore if the skin color is related to the high sucrose content in BNG fruit.

We set out to determine why *PuWRKY31* was expressed differentially in NG and BNG fruits. We investigated the CDS and promoter sequence, as well as the promoter methylation levels of two varieties, but found no differences. So we compared the *PuWRKY31* histone acetylation level, and found that the higher *PuWRKY31* expression level in BNG fruits is associated with a higher level of histone acetylation in its promoter and CDS region (Fig. 6B). A high histone acetylation level can be regulated by various coactivators, which recruit histone acetyltransferases (HATs) to enhance the acetylation of lysine residues, which in turn can neutralize the positive charge of histone proteins. This causes an unwinding of the chromatin structure and exposure of binding sites in the promoter, thereby increasing the accessibility for transcription factors (Shahbazian and Grunstein, 2007). In Arabidopsis, a histone acetyltransferase HLS1 mediates histone acetylation on *AtWRKY33* chromatin, and the histone H3 acetylation level at *AtWRKY33* chromatin is significantly lower in the *hls1* mutant than that in the wild type (Liao et al., 2016). Moreover, in Arabidopsis, *hls1* mutants accumulate less total soluble sugar than the wild type (Ohto et al., 2006). In our study, a histone acetyltransferase *PuHLS1* was expressed significantly higher in BNG fruit than in NG fruit, and PuHLS1 could bind to the CDS of PuWRKY31 (Fig. 6C and 6D). Therefore, we propose that the higher expression level of PuHLS1 might cause the higher histone acetylation level of PuWRKY31, resulting in higher sucrose accumulation in BNG fruit.

In conclusion, *PuSWEET15* was expressed higher in BNG fruit than in NG fruit and PuWRKY31 bound to the *PuSWEET15* promoter to induce its expression. Moreover, the high acetylation level of the *PuWRKY31* promoter was associated with its high expression level in BNG fruit (Fig. 7).

MATERIALS AND METHODS

Plant Material and Treatments

Fruits of Ussurian pear (*Pyrus ussuriensis*) cv. Nanguo (NG) and its bud sport variety (BNG) were sampled from mature trees growing in the experimental farm of the Liaoning Pomology Institute (Xiongyue, China). Fruits were harvested at 60, 90, 105, 120 and 134 DAFB (commercial harvest day) in 2014, and 60, 90, 120 and 137 DAFB (commercial harvest day) in 2018, and immediately transported to the laboratory. At each sampling point, three fruits of each variety were selected for measuring sugar content. The flesh of those fruits was cut into pieces, frozen in liquid nitrogen and stored at -80 °C for further use.

Nicotiana benthamiana plants used in this study were grown with potting medium in a growth chamber (25 °C, 16-h-light, 8-h-dark).

Measurements of Soluble Solids and Sugar Content

At each sampling point, the fruit flesh was homogenized with a homogenizer, filtered through a cell strainer (Cat. no. CSS010040, Jet Biofil, https://www.jetbiofil.com), and the soluble solids content of the filtrate was measured with a sugar meter (PAL-1, ATAGO, Japan). The soluble sugar content was measured by HPLC (Agilent Technologies 1260 Series) as described in Jia et al. (2011). Briefly, samples were ground to a fine powder in liquid nitrogen. Three grams of the powder was mixed with 10 ml of 80% (v/v) ethanol, incubated in a water bath for 30 min at 80 °C, and then centrifuged at 10,000 g for 5 min in a 50-ml centrifuge tube and the supernatant was collected. The above step was repeated twice to re-extract the pellets, the supernatants were combined and the samples were evaporated in boiling water. After drying in a 50-ml centrifuge tube, the samples were dissolved in 1 ml of ultrapure water and passed through a 0.45 μm membrane and the soluble sugar content of the filtrate was measured. HPLC (Agilent 1260) was then performed with the following components and parameters: a 7.8 × 300 mm Carbomix Ca-NP column (Sepax); ultrapure water as the mobile phase, at a flow rate of 1 ml min^{-1}; a column temperature of 80 °C; a refractive index detector temperature of 35 °C; and an injection volume of 10 μl. At each sampling

406 point, at least nine fruits were randomly selected and divided into three groups as three
407 biological replicates. The flesh in each group was pooled for measuring soluble solids
408 and sugar content.
409
410 **RNA-seq**
411 Total RNA was extracted from NG and BNG fruits harvested at the commercial harvest
412 day (134 DAFB in 2014). RNA-seq analysis, including library construction, sequencing
413 and bioinformatics analysis was performed as in Huang et al. (2014) by Biomarker
414 (www.biomarker.com.cn). Sequencing was performed on an Illumina HiSeq^{TM} 2000
415 system. Total RNA was extracted from the three groups of fruit as mentioned above as
416 three biological replicates for RNA-seq. All the raw data were deposited into NCBI
417 Sequence Read Archive (SRA) under accession number PRJNA545020. The heat maps
418 for differentially expressed genes between NG and BNG fruits were constructed using
419 an online software
420 (https://console.biocloud.net/static/index.html#/drawtools/intoDrawTools/heatmap/inp
421 ut) according to the log2 (FC) value from the RNA-Seq data.
422
423 **Gene Cloning and Expression Analysis**
424 Total RNA extraction was conducted as in Li et al. (2015), and first strand cDNA was
425 synthesized from 700 ng of total RNA using the M-MLV RTase cDNA Synthesis Kit
426 (Cat. no. D6130, TaKaRa). The cDNA was then used as template for reverse
427 transcription quantitative PCR (RT-qPCR) and standard RT-PCR assays, using
428 sequence information for each gene derived from the RNA-seq data. Standard RT-PCR
429 was performed according to Li et al. (2015), with 4 μl of each PCR product separated
430 on a 1% agarose gel and imaged on a GelDoc XR System (Bio-Rad). RT-qPCR was
431 performed using the SYBR Premix ExTaq II Kit (Cat. no. RR820, TaKaRa) on an
432 Applied Biosystems 7500 Real-Time PCR System as previously described (Li et al.,
433 2015). The pear *Actin* gene was used as an internal control and total RNA was extracted
434 from the three groups of fruit as mentioned above as three biological replicates. All
435 primers were designed using the Primer3 software (http://frodo.wi.mit.edu/) and are

436 listed in Supplemental Dataset S2.

437

438 **SSR analysis of NG and BNG pear fruit**

439 Genomic DNA was isolated from the fruit samples harvested in 2014 as described in
440 Wang et al. (2013). SSR (simple sequence repeat) primers with polymorphism were
441 selected from previous reports (Yamamoto et al. 2002; Jiang et al. 2009). Standard PCR
442 was conducted and the PCR products were analyzed on 6% denaturing polyacrylamide
443 gel with silver staining according to Bao et al. (2007).

444

445 **Subcellular Localization of PuSWEET15**

446 The protoplasts of maize (*Zea mays*) leaves were prepared as described previously (Yoo
447 et al., 2007). The *PuSWEET15* coding region was cloned into the *Bam*HI and *Sac*I sites
448 downstream of GFP in the pRI101 vector (TaKaRa) to form the
449 *Pro35S:GFP:PuSWEET15* construct. *Pro35S:GFP* was used as a control. The
450 constructs were transformed into the protoplasts of maize leaves according to a previous
451 report (Yoo et al., 2007). The fluorescence was observed using a fluorescence
452 microscope 16 h after transformation under a confocal microscope (TCS SP8, Leica,
453 Germany). FM4-64 (*N*-(3-triethylammoniumpropyl)-4-(6-(4-(diethylamino) phenyl)
454 hexatrienyl) pyridinium dibromide, Cat. no. T3166, Thermo Fisher Scientific) was used
455 as a cell membrane dye. All transient expression assays were repeated at least three
456 times. The primers used are listed in Supplemental Dataset S2.

457

458 **Heterologous Expression of *PuSWEET15* in Yeast Cells**

459 For the complementation assay in yeast (*Saccharomyces cerevisiae*) cells, the CDS of
460 PuSWEET15 was cloned into the yeast expression vector pDR196 (Cat. no. VT8007,
461 YouBio, http://www.youbio.cn/) using *Sma*1 and *Sal*1 restriction enzyme sites to form
462 the pDR196-PuSWEET15 construct. Empty pDR196 vector was used as a negative
463 control. The constructs were transformed into yeast mutant strain SUSY7/ura (Li et al.,
464 2017c; Riesmeier et al., 1992) using the lithium acetate method (Soni et al., 1993). The
465 transformants were cultured in liquid SD (synthetic deficient) (Cat. no. PM2271,

Coolaber, http://www.coolaber.com/) medium containing 2 % (w/v) glucose (Sigma) as the sole carbon source without uracil by shaking at 180 rpm under 30 °C to OD_{600} 0.5. The culture was then diluted by different fold (×10, ×100, ×200, ×1000), and 6 μl of dilution was dropped on SD/-uracil solid medium containing 2% (w/v) glucose or 2% (w/v) sucrose (Sigma) as sole carbon source at pH 4.0. Yeast cells on medium with glucose were grown at 30 °C for 2 d, and those on medium with sucrose were grown at 30 °C for 4 d.

EMSA

The *PuWRKY31* CDS was cloned and inserted into the pEASY-E1 vector (Transgen Biotech, http://www.transgen.com.cn/) resulting in its fusion to a His-tag, and the CDS of *PuHLS1* was cloned and inserted into the downstream of GST in the pGEX4T-1 (GE Healthcare, http://www3.gehealthcare.com/) vector, before being transformed into *Escherichia coli* BL21 (DE3) (Transgen Biotech) competent cells. The purification of the His-tagged and GST-tagged fusion proteins were performed as previously described (Li et al., 2016). For EMSA, the 3' biotin end-labeled double-stranded DNA probes were prepared by annealing complementary oligonucleotides. The oligonucleotides were heated at 95 °C for 5 min, then at 72 °C for 20 min, and left to cool to room temperature before use. The biotin-labeled *PuSWEET15* promoter and *PuWRKY31* CDS sequences are shown in Fig. 5A and Fig. 6D. EMSA was performed as previously described (Li et al., 2016) using the LightShift Chemiluminescent EMSA Kit (Cat. no. 20148, Thermo Scientific).

GUS Analysis

The *PuSWEET15* promoter sequence (1,177 bp upstream of the translation start site) was cloned into the *Sal*I and *Sma*I sites upstream of the GUS reporter gene in the pBI101 vector to generate a reporter construct. The *PuWRKY31* CDS was introduced into the pRI101 vector through restriction enzyme sites (*Sal*I and *Kpn*I) to form the effector construct. The infiltration of the reporter and effector constructs into *N. benthamiana* leaves and measurement of GUS activity were performed as previously

496 described (Li et al., 2016). The infiltration was repeated independently at least three
497 times. The primers used are listed in Supplemental Dataset S2.
498
499 **Methylation Analysis**
500 Genomic DNA was isolated from the fruit samples harvested in 2014 as described in
501 Wang et al. (2013). McrBC-PCR was used to analyze the methylation of relative
502 sequences. One μg DNA isolated from fruit was digested with McrBC (Cat. no. M0272,
503 New England Biolabs) according to the manufacturer's instruction. Three biological
504 replicates were analyzed. For the control, water was added instead of GTP. After
505 digestion, DNA was used as a template for standard PCR analysis. The thermal cycling
506 conditions were 3 min at 95 °C; 27 cycles of 30 s at 95 °C, 30 s at 55 °C and 1 min at
507 72 °C; followed by 72 °C 5 min for a final extension. The PCR product was separated
508 in 0.5 % agarose gel and photographed with GelDoc XR System (BioRad). Four regions
509 of the *PuSWEET15* promoter or the *PuWRKY31* promoter and five regions of the
510 *PuHLS1* promoter were examined (Supplemental Fig. S6, S9 and S11). The amount of
511 PCR product was used to estimate the degree of methylation of the promoter region.
512 The PCR bands were quantified by ImageJ software.
513
514 **Agrobacterium-mediated infiltration**
515 To overexpress *PuSWEET15* in *N. benthamiana* leaves, its CDS was cloned into the
516 pRI101 plant transformation vector using *Bam*HI and *Sac*I restriction enzyme sites to
517 form the *Pro35S:PuSWEET15* construct. The recombinant plasmid was transformed
518 into *Agrobacterium tumefaciens* strain EHA105 for infiltration of *N. benthamiana*
519 leaves as previously described (Li et al., 2017b). Briefly, the suspension for infiltration
520 was injected into mature leaves of *N. benthamiana* which were grown on potting
521 medium when the plants were 5 weeks old. After infiltration, the potting medium was
522 irrigated with 1% sucrose every 2 d. The plants were collected 6 d after infiltration for
523 further use.
524 To overexpress *PuSWEET15* or *PuWRKY31* in NG pear fruit, the CDS regions
525 were separately cloned into the *Sal*I and *Kpn*I sites upstream of the MYC tag in the

pRI101 vector to form *Pro35S:PuSWEET15-MYC* and *Pro35S:PuWRKY31-MYC*, respectively. To silence *PuSWEET15* expression in BNG pear fruit, a partial *PuSWEET15* CDS (686-898 bp) was ligated into the pRI101 vector in the reverse direction to generate the antisense *PuSWEET15* construct (PuSWEET15-AN). These plasmids were transformed into *A. tumefaciens* strain EHA105, and the preparation of infiltration buffer and fruit infiltration were performed as previously described (Li et al., 2016). Briefly, 100 µl of the infiltration buffer was taken with a 1-ml sterile syringe and injected into on-tree fruit at a depth of 0.3 cm at 120 DAFB. For each fruit, one side was used for infiltrating target constructs, and the other side for infiltrating empty pRI101 as control. Three injections were performed on each side of fruit. The infiltrated fruits were harvest 6 d after infiltration, and the fruit flesh around the infiltrated area was sampled for further use. One fruit was used as a biological replicate and at least three biological replications were performed. The overexpression of *PuSWEET15* and *PuWRKY31* was performed on NG fruit, and silencing of *PuSWEET15* on BNG fruit.

ChIP-PCR

The recombinant *Pro35S:PuWRKY31-MYC* construct was transformed into NG pear fruit as described above and ChIP assays were performed using the EpiQuik™ Plant ChIP Kit (Cat. no. P-2014, Epigentek, https://www.epigentek.com/) according to the manufacturer's instructions. An anti-MYC antibody (Transgen Biotech) was used to verify the binding of PuWRKY31 to the *PuSWEET15* promoter *in vivo* as previously described (Li et al., 2017b). The amount of immunoprecipitated chromatin was determined by qPCR as previously described (Li et al., 2017b) with 0.5 µl of immuno-precipitated chromatin as template. Each ChIP assay was repeated three times and the enriched DNA fragments in each ChIP sample were used as one biological replicate for qPCR. Three regions of the *PuSWEET15* promoter were analyzed to assess enrichment. Primers used are listed in Supplemental Dataset S2.

Analysis of Histone Acetylation Levels

NG and BNG fruits harvested at commercial harvest day in 2014 were used for

analyzing the histone acetylation levels. The chromatin was prepared as above and immunoprecipitated with specific antibodies including anti-acetyl-histone H3 and H4 (Millipore, Billerica, MA). ChIP-PCR analysis to measure the histone acetylation level of the *PuWRKY31* or *PuSWEET15* promoter was performed as described by Li et al. (2017b). *PuActin* was used as an internal control to normalize the ChIP enrichment signal. Three regions of the *PuWRKY31* or *PuSWEET15* promoter were analyzed to assess enrichment. Primers used are listed in Supplemental Dataset S2.

Accession Numbers

Sequence data from this article can be found in GenBank libraries under accession numbers *PuSWEET15* (MK940530), *PuWRKY31* (MK940531), *PuHLS1* (MN201566) and *PuActin* (AF386514).

Supplemental Data

Supplemental Figure S1. Phenotype of Nanguo pear fruit (NG) and its bud sport (BNG).

Supplemental Figure S2. Sugar contents of NG and BNG fruits during development.

Supplemental Figure S3. Heat map of sugar transporter genes with differential expression between NG and BNG fruits from the RNA-seq data.

Supplemental Figure S4. Sequence alignment of PuSWEET15 and AtSWEET15 amino acid sequences.

Supplemental Figure S5. Expression of *PuSWEET15* in NG and BNG fruits sampled in 2018 as determined by reverse transcription quantitative PCR.

Supplemental Figure S6. Methylation and histone acetylation levels of *PuSWEET15* promoter regions between NG and BNG fruits.

Supplemental Figure S7. Heat map of transcription factors with differential expression between NG and BNG fruits from the RNA-seq data.

Supplemental Figure S8. Expression of *PuWRKY31* in NG and BNG fruits sampled in 2018 as determined by reverse transcription quantitative PCR.

Supplemental Figure S9. Methylation level of *PuWRKY31* promoter regions in NG

and BNG fruits determined using McrBC-PCR.

Supplemental Figure S10. Expression of *PuHLS1* in NG and BNG fruits sampled in 2018 as determined by reverse transcription quantitative PCR.

Supplemental Figure S11. Methylation level of *PuHLS1* promoter regions in NG and BNG fruits determined using McrBC-PCR.

Supplemental Dataset S1. Differentially expressed genes between NG and BNG fruits from RNA-seq data.

Supplemental Dataset S2. List of primers used in this study.

ACKNOWLEDGMENTS

We thank the Editors and the anonymous reviewers for their efforts on our manuscript. This work was supported by the National Natural Science Foundation of China (31722047), the National Key Research and Development Program of China (2018YFD1000105), and the LiaoNing Revitalization Talents Program (XLYC1802019). We also thank PlantScribe (http://www.plantscribe.com) for editing this manuscript.

604

Figure Legends

Figure 1. Phenotype and sugar content of NG and BNG fruits during development.

A. Flowers and fruits of NG and BNG. Pictures were taken at different days after full bloom (DAFB) in 2014. Scale bars, 1 cm.

B-E. Sugar content of NG and BNG fruits during development. High performance liquid chromatography (HPLC) was used to measure the content of sucrose (B), glucose (C), fructose (D) and sorbitol (E) in fruit collected at the indicated day after full bloom (DAFB) in 2014. Commercial harvest day was 134 DAFB (September 4, 2014). Numbers under the x axes indicate the DAFB. Three biological replicates were analyzed, and the error bars represent SE. Asterisks indicate significant difference as determined by a Student's *t*-test (**P < 0.01).

Figure 2. Expression of *PuSWEET15* in NG and BNG fruits and its subcellular localization.

A. Relative expression of *PuSWEET15* during NG and BNG fruits development as determined by reverse transcription quantitative PCR (RT-qPCR). Fruit samples were collected in 2014. Numbers under the x axes indicate days after full bloom (DAFB). Three biological replicates were analyzed, and the error bars represent SE. Asterisks indicate significant difference as determined by a Student's *t*-test (**P < 0.01).

B. Subcellular localization of PuSWEET15. *35S:GFP-PuSWEET15* was transiently expressed in protoplasts of maize leaves. Transient expression of GFP alone (*35S:GFP*) was used as a control. FM4-64 was used as a plasma membrane marker. Scale bars, 5 μm.

Figure 3. Functional analysis of *PuSWEET15*.

A. Heterologous expression of *PuSWEET15* in yeast strain SUSY7/ura. Yeast cells with pDR196-PuSWEET15 or pDR196 vector (as a negative control) were grown on SD (synthetic deficient)/-uracil solid medium containing 2% (w/v) glucose or sucrose as sole carbon source. The numbers under the panel indicate the dilution fold.

635 **B** and **C**. *PuSWEET15* was overexpressed in NG pear fruit using *Agrobacterium*
636 *tumefaciens*-mediated infiltration. The expression of *PuSWEET15* was detected by
637 reverse transcription quantitative PCR (RT-qPCR) (B) and the sugar content was
638 measured by high performance liquid chromatography (HPLC) (C). PuSWEET15-OE,
639 *PuSWEET15* overexpressing fruit; Empty vector, control fruit overexpressing empty
640 pRI101 vector.
641 **D** and **E**. *PuSWEET15* was silenced in BNG pear fruit using *A. tumefaciens*-mediated
642 infiltration. The expression of *PuSWEET15* was detected by RT-qPCR (D) and the sugar
643 content was measured by HPLC (E). PuSWEET15-AN, *PuSWEET15* silenced fruit;
644 Empty vector, control fruit expressing empty pRI101 vector.
645 **F**. *PuSWEET15* was overexpressed in *N. benthamiana* leaves using *A. tumefaciens*-
646 mediated infiltration. The expression of *PuSWEET15* (left) was detected by RT-qPCR,
647 and the sugar content (right) was measured by HPLC. PuSWEET15-OE, *PuSWEET15*
648 overexpressing leaves; Empty vector, pRI101 overexpressing leaves. Three biological
649 replicates were analyzed, and the error bars represent SE. Asterisks indicate significant
650 difference as determined by a Student's *t*-test (**P < 0.01).
651
652 **Figure 4. Functional analysis of *PuWRKY31*.**
653 **A**. Expression of *PuWRKY31* during NG and BNG fruit development. Fruit samples
654 were the same as in Figure 1. Numbers under the x axis indicate days after full bloom
655 (DAFB).
656 **B** and **C**. *PuWRKY31* was overexpressed in NG pear fruit using *Agrobacterium*
657 *tumefaciens*-mediated infiltration. The relative expression of *PuWRKY31* and
658 *PuSWEET15* was detected by reverse transcription quantitative PCR (RT-qPCR) (B)
659 and the sugar contents in PuWRKY31-OE and control fruit were measured by high
660 performance liquid chromatography (HPLC) (C). PuWRKY31-OE, PuWRKY31
661 overexpressing pear fruit; Empty vector, pear fruit overexpressing empty pRI101 vector.
662 Three biological replicates were analyzed, and the error bars represent SE. Asterisks
663 indicate significant difference as determined by a Student's *t*-test (**P < 0.01).
664

Figure 5. PuWRKY31 promotes *PuSWEET15* transcription.

A. Electrophoretic mobility shift assay (EMSA) analysis of PuWRKY31 binding to the *PuSWEET15* promoter. The hot probe was a biotin-labeled *PuSWEET15* promoter, while the cold probe was a non-labeled competitive probe (with a 100-fold higher concentration than the hot probe). His-tagged PuWRKY31 (PuWRKY31-His) was purified and used for DNA-binding assays. The sequence of the biotin labeled probe is shown and the W-box motif is highlighted in bold.

B. Chromatin immunoprecipitation-PCR (ChIP-PCR) showing the *in vivo* binding of PuWRKY31 to the *PuSWEET15* promoter. Cross-linked chromatin samples were extracted from *PuWRKY31-MYC* overexpressing NG pear fruit and precipitated with an anti-MYC antibody. Eluted DNA was used to amplify the sequences neighboring the W-box by quantitative PCR (qPCR). Six regions (S1–S6) were analyzed. Fruit overexpressing green fluorescent protein (GFP) were used as negative controls. The ChIP assay was repeated three times and the enriched DNA fragments in each ChIP were used as one biological replicate for qPCR.

C. Schematic representation of the β-Glucuronidase (GUS) reporter vector containing the *PuSWEET15* promoter and the effector vector containing *PuWRKY31*. The effector reporter vectors were infiltrated into *Nicotiana benthamiana* leaves to analyze the regulation of GUS activity. Three independent infiltrations were performed, and the error bars represent SE. Asterisks indicate significant difference as determined by a Student's *t*-test (**$P < 0.01$).

Figure 6. Differences of H3ac and H4ac in *PuWRKY31* chromatin and *PuHLS1* expression between NG and BNG fruits.

A. Differences of H3ac and H4ac in *PuActin* chromatin. The results were normalized to the amount of input DNA.

B. Differences of H3ac and H4ac at different regions of the *PuWRKY31* chromatin by ChIP-PCR. Fruit harvested at commercial harvest day in 2014 were used. The results were normalized relative to the amount of *PuActin*. Each experiment was repeated three times. The ChIP assay was repeated three times and the enriched DNA fragments in

each ChIP were used as one biological replicate for qPCR, and the error bars represent SE. Asterisks indicate significant difference as determined by a Student's *t*-test (**P < 0.01).

C. Expression of *PuHLS1* during NG and BNG fruits development by reverse transcription quantitative PCR (RT-qPCR). Fruit samples were the same as in Figure 1. Numbers under the x axis indicate days after full bloom (DAFB). Three biological replicates were analyzed, and the error bars represent SE. Asterisks indicate significant difference as determined by a Student's *t*-test (**P < 0.01).

D. Electrophoretic mobility shift assay (EMSA) analysis of PuHLS1 binding to the CDS of *PuWRKY31*. The hot probe was biotin-labeled *PuWRKY31* CDS, while the cold probe was a non-labeled competitive probe (with a 100-fold higher concentration than the hot probe). GST-tagged PuHLS1 (PuHLS1-GST) was purified and used for DNA-binding assays. The sequence of the biotin labeled probe is shown.

Figure 7. Model showing the molecular mechanism of differential sucrose accumulation in NG and BNG fruits.

In the fruit of BNG, a highly sucrose-accumulated bud sport of NG, the high acetylation level of the *PuWRKY31* promoter is associated with its high expression, and PuWRKY31 binds to the promoter of *PuSWEET15*, an active sucrose transporter, to induce its expression, resulting in high levels of sucrose.

LITERATURE CITED

An J, Zeng T, Ji C, de Graaf S, Zheng, Z, Xiao TT, Pan Z (2019a) A Medicago truncatula SWEET transporter implicated in arbuscule maintenance during arbuscular mycorrhizal symbiosis. New Phytol (in press)

An JP, Zhang XW, You CX, Bi SQ, Wang XF, Hao YJ (2019b) Md WRKY 40 promotes wounding-induced anthocyanin biosynthesis in association with Md MYB 1 and undergoes Md BT 2-mediated degradation. New Phytol (in press)

Ayre BG (2011) Membrane-transport systems for sucrose in relation to whole-plant carbon partitioning. Mol plant **4(3):** 377-394

Bao L, Chen K, Zhang D, Cao Y, Yamamoto T, Teng Y (2007) Genetic diversity and similarity of pear (Pyrus L.) cultivars native to East Asia revealed by SSR (simple sequence repeat) markers. Genet Resour Crop Ev **54:** 959-971

Braun DM, Wang L, Ruan YL (2014) Understanding and manipulating sucrose phloem loading, unloading, metabolism, and signalling to enhance crop yield and food security. J Exp Bot **65:** 1713-1735

Chagne D, Crowhurst RN, Pindo M, Thrimawithana A, Deng C, Ireland H, Fiers M, Dzierzon H, Cestaro A, Fontana P, Bianco L, Lu A, Storey R, Knabel M, Saeed M, Montanari S, Kim YK, Nicolini D, Larger S, Stefani E, Allan AC, Bowen J, Harvey I, Johnston J, Malnoy M, Troggio M, Perchepied L, Sawyer G, Wiedow C, Won K, Viola R, Hellens RP, Brewer L, Bus VG, Schaffer RJ, Gardiner SE, Velasco R (2014) The draft genome sequence of European pear (Pyrus communis L. 'Bartlett'). PLoS One **9:** e92644

Chen LQ, Qu XQ, Hou BH, Sosso D, Osorio S, Fernie AR, Frommer WB (2012) Sucrose efflux mediated by SWEET proteins as a key step for phloem transport. Science **335:** 207-211

Chen L, Xiang S, Chen Y, Li D, Yu D (2017) Arabidopsis WRKY45 Interacts with the DELLA Protein RGL1 to Positively Regulate Age-Triggered Leaf Senescence. Mol Plant **10:** 1174-1189

Chen LQ, Lin IW, Qu XQ, Sosso D, McFarlane HE, Londono A, Samuels AL, Frommer WB (2015) A cascade of sequentially expressed sucrose transporters in the seed coat and endosperm provides nutrition for the Arabidopsis embryo. Plant Cell **27:** 607-619

Chen Q, Xu X, Xu D, Zhang H, Zhang C, Li G (2019) WRKY18 and WRKY53 coordinate with HISTONE ACETYLTRANSFERASE1 to regulate rapid responses to sugar. Plant Physiol **180(4):** 2212-2226

Chong J, Piron MC, Meyer S, Merdinoglu D, Bertsch C, Mestre P (2014) The SWEET family of sugar transporters in grapevine: VvSWEET4 is involved in the interaction with Botrytis cinerea. J Exp Bot **65:** 6589-6601

Chu Z, Yuan M, Yao J, Ge X, Yuan B, Xu C, Li X, Fu B, Li Z, Bennetzen JL, Zhang Q, Wang S (2006) Promoter mutations of an essential gene for pollen development result in disease resistance in rice. Genes Dev **20:** 1250-1255

Dong X, Wang Z, Tian L, Zhang Y, Qi D, Huo H, Xu J, Li Z, Liao R, Shi M, Wahocho SA, Liu C, Zhang S, Tian Z, Cao Y (2019) De novo assembly of a wild pear (Pyrus betuleafolia) genome. Plant Biotechnol J (in press)

Durand M, Porcheron B, Hennion N, Maurousset L, Lemoine R, Pourtau N (2016) Water Deficit Enhances C Export to the Roots in Arabidopsis thaliana Plants with Contribution of Sucrose Transporters in Both Shoot and Roots. Plant Physiol **170:** 1460-1479

Eom JS, Choi SB, Ward JM, Jeon JS (2012) The mechanism of phloem loading in rice (Oryza sativa). Mol Cells **33:** 431-438

Furiya T, Suzuki S, Sueta T, Takayanagi T (2009) Molecular characterization of a bud sport of Pinot gris bearing white berries. Am J Enol Viticult **60(1):** 66-73

Gao Y, Zhang C, Han X, Wang ZY, Ma L, Yuan P, Wu JN, Zhu XF, Liu JM, Li DP, Hu YB, Xuan YH (2018) Inhibition of OsSWEET11 function in mesophyll cells improves resistance of rice to sheath blight disease. Mol Plant Pathol **19:** 2149-2161

Gonzalez A, Brown M, Hatlestad G, Akhavan N, Smith T, Hembd A, Moore J, Montes D, Mosley T, Resendez J, Nguyen H, Wilson L, Campbell A, Sudarshan D, Lloyd A (2016) TTG2 controls the developmental regulation of seed coat tannins in Arabidopsis by regulating vacuolar transport steps in the proanthocyanidin pathway. Dev Biol **419:** 54-63

Guo WJ, Nagy R, Chen HY, Pfrunder S, Yu YC, Santelia D, Frommer WB, Martinoia E (2014) SWEET17, a facilitative transporter, mediates fructose transport across the tonoplast of Arabidopsis roots and leaves. Plant Physiol **164:** 777-789

Han YC, Kuang JF, Chen JY, Liu XC, Xiao YY, Fu CC, Wang JN, Wu KQ, Lu WJ (2016) Banana Transcription Factor MaERF11 Recruits Histone Deacetylase MaHDA1 and Represses the Expression of MaACO1 and Expansins during Fruit Ripening. Plant Physiol **171:** 1070-1084

Huang G, Li T, Li X, Tan D, Jiang Z, Wei Y, Li J, Wang A (2014) Comparative transcriptome analysis of climacteric fruit of Chinese pear (Pyrus ussuriensis) reveals new insights into fruit ripening. PLoS One **9:** e107562

Jaeger SR, Andani Z, Wakeling IN, MacFie HJ (1998) Consumer preferences for fresh and aged apples: a cross-cultural comparison. Food Qual Prefer **9:** 355-366

Jia HF, Chai YM, Li CL, Lu D, Luo JJ, Qin L, Shen YY (2011) Abscisic acid plays an important role in the regulation of strawberry fruit ripening. Plant Physiol **157:** 188-199

Jiang Y, Liang G, Yang S, Yu D (2014) Arabidopsis WRKY57 functions as a node of convergence for jasmonic acid- and auxin-mediated signaling in jasmonic acid-induced leaf senescence. Plant Cell **26:** 230-245

Jiang Z, Tang F, Huang H, Hu H, Chen Q (2009) Assessment of genetic diversity of Chinese sand pear landraces (Pyrus pyrifolia Nakai) using simple sequence repeat markers. HortScience **44(3):** 619-626

Lemoine R (2000) Sucrose transporters in plants: update on function and structure. BBA-Biomembranes **1465:** 246-262

Li J, Qin M, Qiao X, Cheng Y, Li X, Zhang H, Wu J (2017a) A New Insight into the Evolution and Functional Divergence of SWEET Transporters in Chinese White

804 Pear (Pyrus bretschneideri). Plant Cell Physiol **58:** 839-850
805 **Li T, Jiang Z, Zhang L, Tan D, Wei Y, Yuan H, Li T, Wang A** (2016) Apple (Malus
806 domestica) MdERF2 negatively affects ethylene biosynthesis during fruit
807 ripening by suppressing MdACS1 transcription. Plant J **88:** 735-748
808 **Li T, Tan D, Liu Z, Jiang Z, Wei Y, Zhang L, Li X, Yuan H, Wang A** (2015) Apple
809 MdACS6 Regulates Ethylene Biosynthesis During Fruit Development
810 Involving Ethylene-Responsive Factor. Plant Cell Physiol **56:** 1909-1917
811 **Li T, Xu Y, Zhang L, Ji Y, Tan D, Yuan H, Wang A** (2017b) The jasmonate-activated
812 transcription factor MdMYC2 regulates ETHYLENE RESPONSE FACTOR
813 and ethylene biosynthetic genes to promote ethylene biosynthesis during apple
814 fruit ripening. Plant Cell **29:** 1316-1334
815 **Li Y, Feng S, Ma S, Sui X, Zhang Z** (2017c) Spatiotemporal expression and substrate
816 specificity analysis of the cucumber SWEET gene family. Front Plant Sci **8:**
817 1855
818 **Liao CJ, Lai Z, Lee S, Yun DJ, Mengiste T** (2016) Arabidopsis HOOKLESS1
819 Regulates Responses to Pathogens and Abscisic Acid through Interaction with
820 MED18 and Acetylation of WRKY33 and ABI5 Chromatin. Plant Cell **28:**
821 1662-1681
822 **Liu W, Wang Y, Yu L, Jiang H, Guo Z, Xu H, Zhang Z** (2019) MdWRKY11
823 participates in anthocyanin accumulation in red-fleshed apples by affecting
824 MYB transcription factors and the photoresponse factor MdHY5. J Agr Food
825 Chem **67(32):** 8783-8793
826 **Lloyd A, Brockman A, Aguirre L, Campbell A, Bean A, Cantero A, Gonzalez A**
827 (2017) Advances in the MYB-bHLH-WD Repeat (MBW) Pigment Regulatory
828 Model: Addition of a WRKY Factor and Co-option of an Anthocyanin MYB for
829 Betalain Regulation. Plant Cell Physiol **58:** 1431-1441
830 **Ma L, Zhang D, Miao Q, Yang J, Xuan Y, Hu Y** (2017) Essential Role of Sugar
831 Transporter OsSWEET11 During the Early Stage of Rice Grain Filling. Plant
832 Cell Physiol **58:** 863-873
833 **Ma Q, Xia Z, Cai Z, Li L, Cheng Y, Liu J, Nian H** (2018) GmWRKY16 Enhances
834 Drought and Salt Tolerance Through an ABA-Mediated Pathway in Arabidopsis
835 thaliana. Front Plant Sci **9:** 1979
836 **Mizuno H, Kasuga S, Kawahigashi H** (2016) The sorghum SWEET gene family: stem
837 sucrose accumulation as revealed through transcriptome profiling. Biotechnol
838 Biofuels **9:** 127
839 **Moriguchi T, Abe K, Sanada T, Yamaki S** (1992) Levels and role of sucrose synthase,
840 sucrose-phosphate synthase, and acid invertase in sucrose accumulation in fruit
841 of Asian pear. J Am Soc Hortic **117:** 274-278
842 **Ohto MA, Hayashi S, Sawa S, Hashimoto-Ohta A, Nakamura K** (2006)
843 Involvement of HLS1 in sugar and auxin signaling in Arabidopsis leaves. Plant
844 Cell Physiol **47:** 1603-1611
845 **Pal S, Yadav AK, Singh AK, Rastogi S, Gupta MM, Verma RK, Nagegowda DA,**
846 **Pal A, Shasany AK** (2017) Nitrogen treatment enhances sterols and withaferin
847 A through transcriptional activation of jasmonate pathway, WRKY transcription

factors, and biosynthesis genes in Withania somnifera (L.) Dunal. Protoplasma **254:** 389-399

Patil G, Valliyodan B, Deshmukh R, Prince S, Nicander B, Zhao M, Sonah H, Song L, Lin L, Chaudhary J, Liu Y, Joshi T, Xu D, Nguyen HT (2015) Soybean (Glycine max) SWEET gene family: insights through comparative genomics, transcriptome profiling and whole genome re-sequence analysis. BMC Genomics **16:** 520

Priestley C (1983) Interconversions of 14C-labelled Sugars in Apple Tree Tissues12. J Exp Bot **34:** 1740-1747

Ren X, Chen Z, Liu Y, Zhang H, Zhang M, Liu Q, Hong X, Zhu JK, Gong Z (2010) ABO3, a WRKY transcription factor, mediates plant responses to abscisic acid and drought tolerance in Arabidopsis. Plant J **63:** 417-429

Rennie EA, Turgeon R (2009) A comprehensive picture of phloem loading strategies. Proc Natl Acad Sci USA **106:** 14162-14167

Riesmeier JW, Willmitzer L, Frommer WB (1992) Isolation and characterization of a sucrose carrier cDNA from spinach by functional expression in yeast. EMBO J **11:** 4705-4713

Rushton PJ, Somssich IE, Ringler P, Shen QJ (2010) WRKY transcription factors. Trends Plant Sci **15:** 247-258

Shahbazian MD, Grunstein M (2007) Functions of site-specific histone acetylation and deacetylation. Annu Rev Biochem **76:** 75-100

Singh AK, Kumar SR, Dwivedi V, Rai A, Pal S, Shasany AK, Nagegowda DA (2017) A WRKY transcription factor from Withania somnifera regulates triterpenoid withanolide accumulation and biotic stress tolerance through modulation of phytosterol and defense pathways. New Phytol **215:** 1115-1131

Soni R, Carmichael JP, Murray JA (1993) Parameters affecting lithium acetate-mediated transformation of Saccharomyces cerevisiae and development of a rapid and simplified procedure. Curr Genet **24(5):** 455-459

Stitt M, Wilke I, Feil R, Heldt HW (1988) Coarse control of sucrose-phosphate synthase in leaves: alterations of the kinetic properties in response to the rate of photosynthesis and the accumulation of sucrose. Planta **174:** 217-230

Sturm A, Hess D, Lee HS, Lienhard S (1999). Neutral invertase is a novel type of sucrose-cleaving enzyme. Physiol Plant **107(2):** 159-165

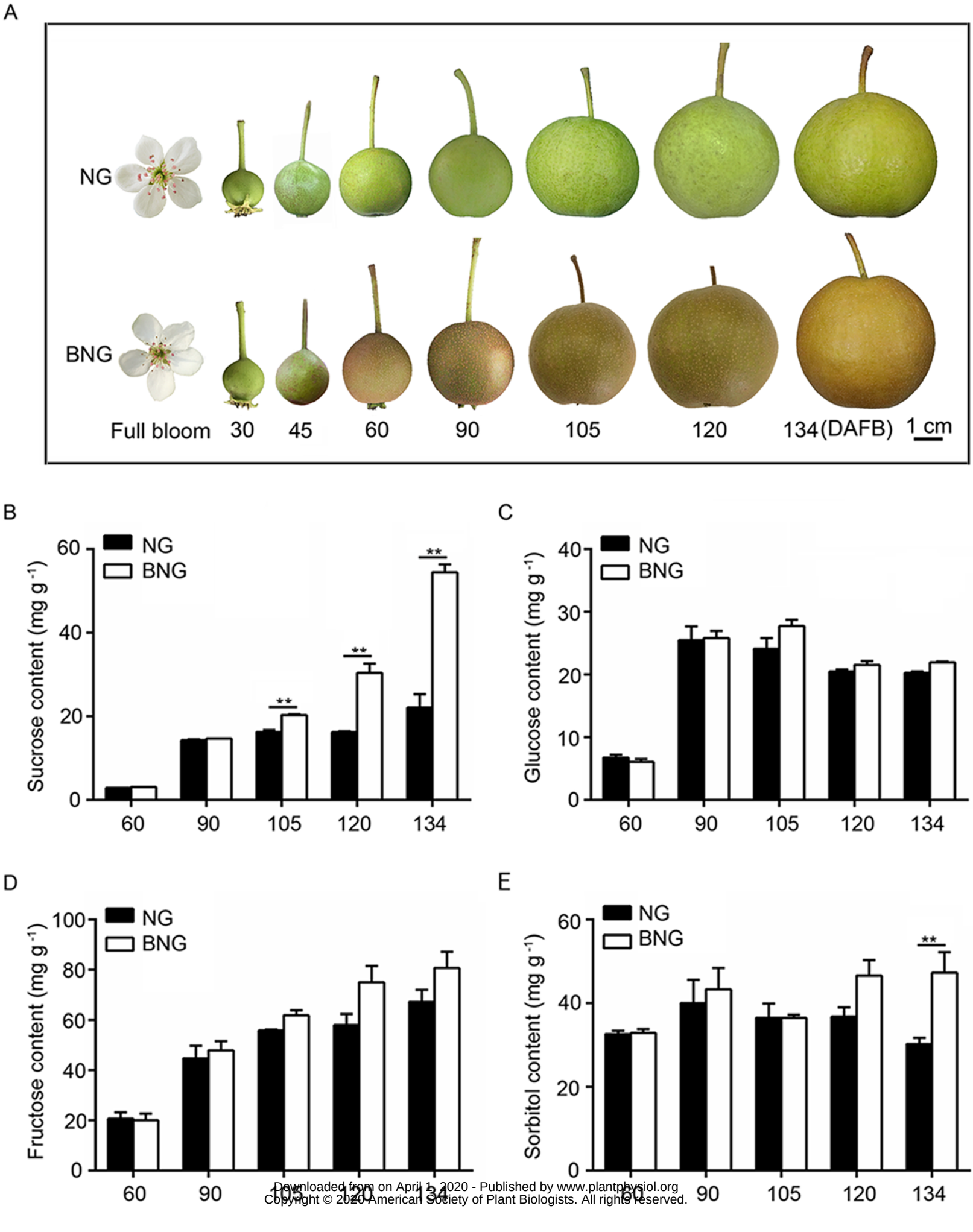
Sun X, Zhang L, Wong DCJ, Wang Y, Zhu Z, Xu G, Wang Q, Li S, Liang Z, Xin H (2019) The ethylene response factor VaERF092 from Amur grape regulates the transcription factor VaWRKY33, improving cold tolerance. Plant J (in press)

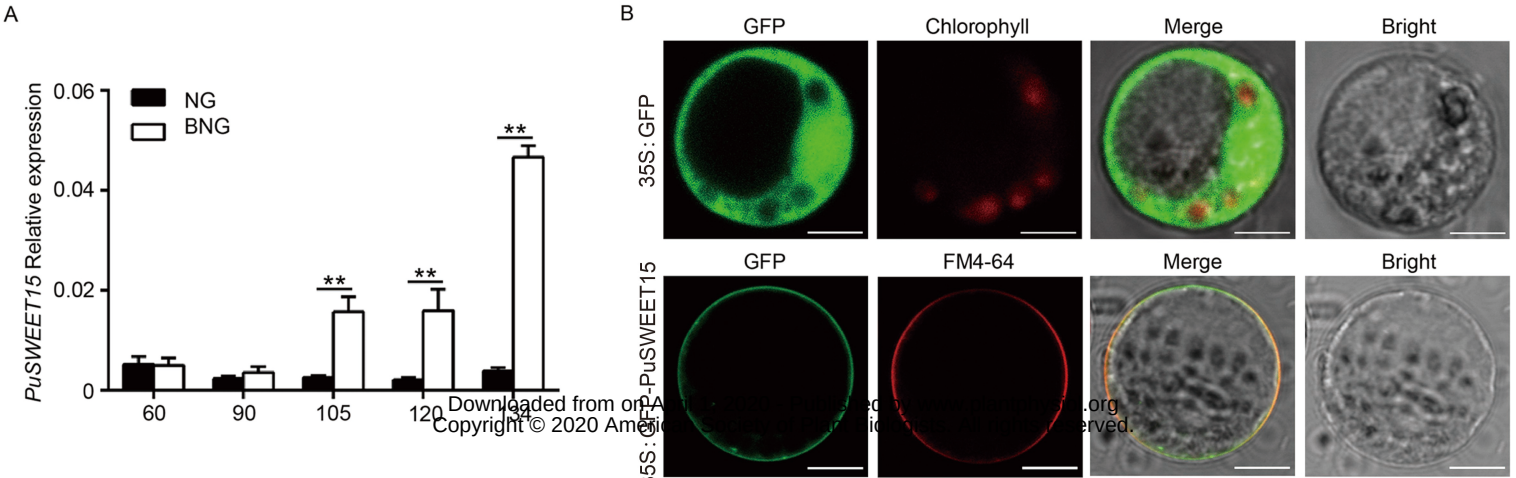
Wang S, Yokosho K, Guo R, Whelan J, Ruan YL, Ma JF, Shou H (2019) The soybean sugar transporter gmsweet15 mediates sucrose export from endosperm to early embryo. Plant Physiol **180(4):** 2133-2141

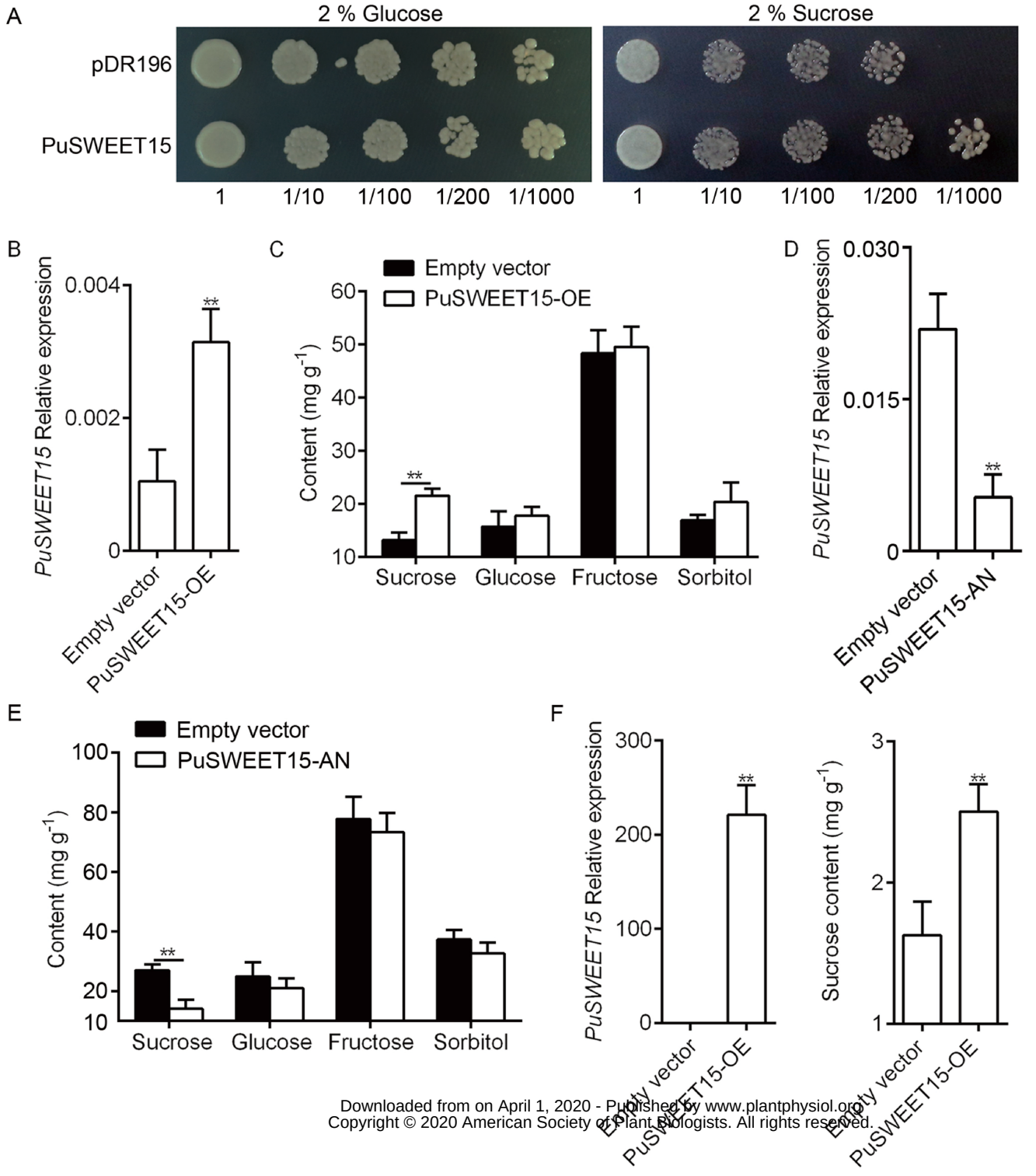
Wang Z, Meng D, Wang A, Li T, Jiang S, Cong P, Li T (2013) The methylation of the PcMYB10 promoter is associated with green-skinned sport in Max Red Bartlett pear. Plant Physiol **162:** 885-896

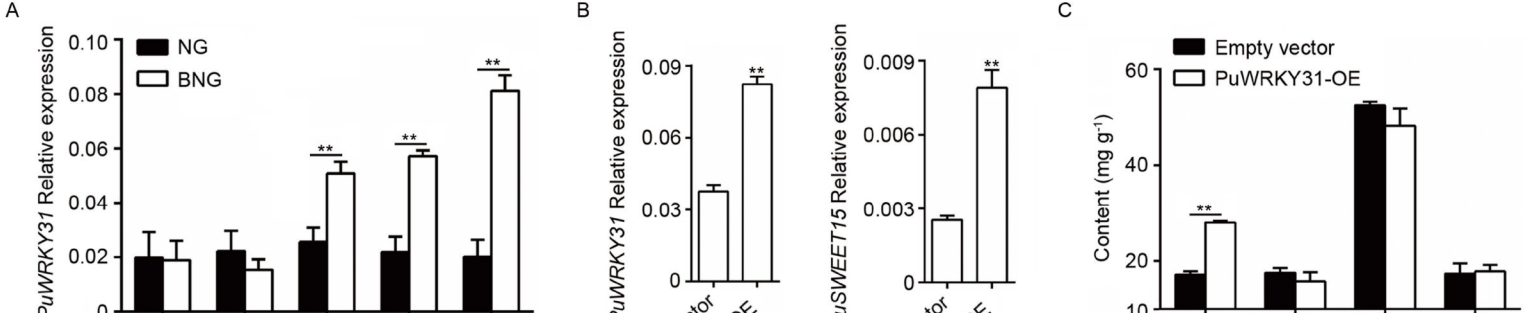
Wei X, Liu F, Chen C, Ma F, Li M (2014) The Malus domestica sugar transporter gene family: identifications based on genome and expression profiling related

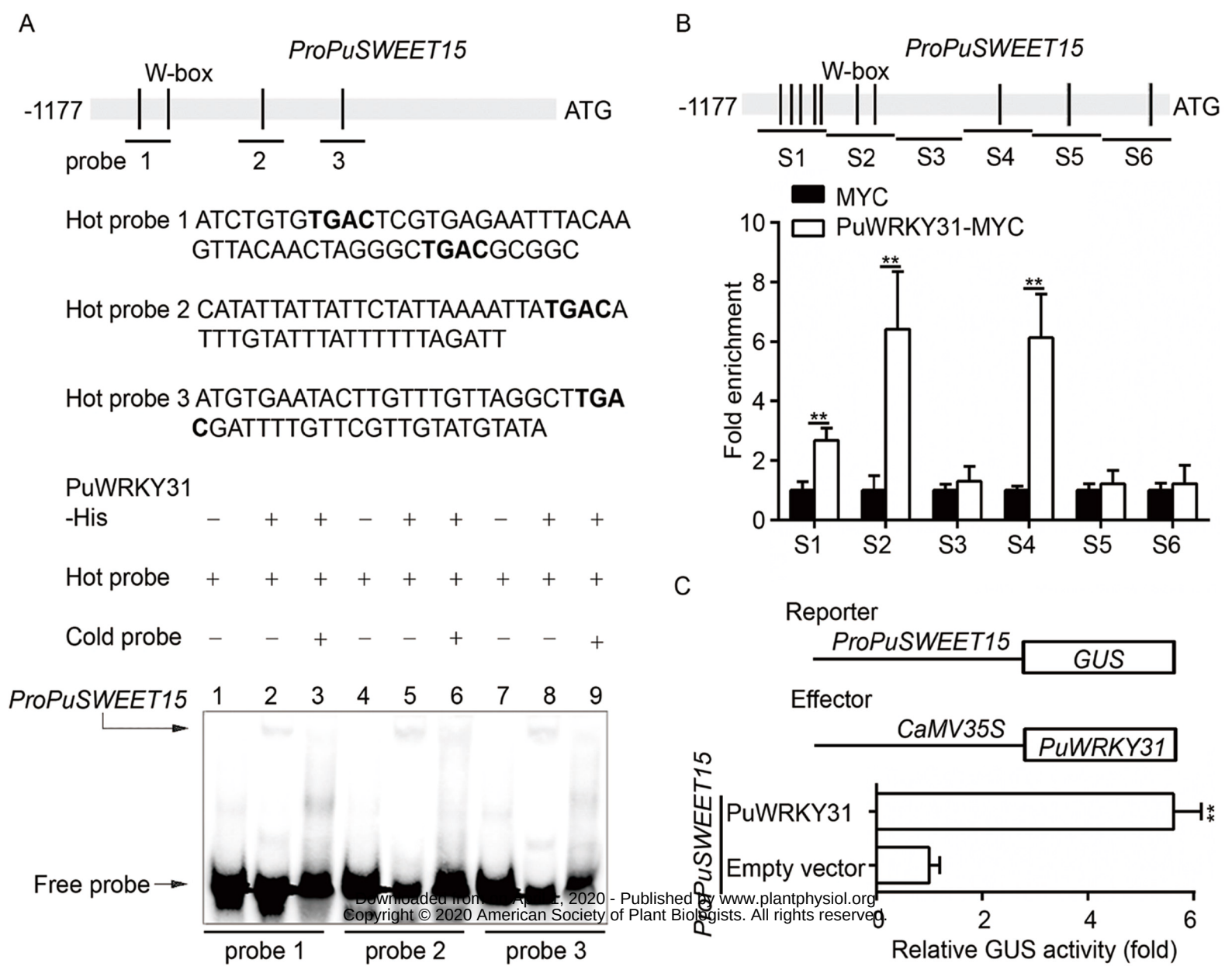
892 to the accumulation of fruit sugars. Front Plant Sci **5:** 569
893 **Whitham TG, Slobodchikoff C** (1981) Evolution by individuals, plant-herbivore
894 interactions, and mosaics of genetic variability: the adaptive significance of
895 somatic mutations in plants. Oecologia **49:** 287-292
896 **Wu J, Wang Z, Shi Z, Zhang S, Ming R, Zhu S, Khan MA, Tao S, Korban SS,**
897 **Wang H, Chen NJ, Nishio T, Xu X, Cong L, Qi K, Huang X, Wang Y, Zhao**
898 **X, Wu J, Deng C, Gou C, Zhou W, Yin H, Qin G, Sha Y, Tao Y, Chen H,**
899 **Yang Y, Song Y, Zhan D, Wang J, Li L, Dai M, Gu C, Wang Y, Shi D, Wang**
900 **X, Zhang H, Zeng L, Zheng D, Wang C, Chen M, Wang G, Xie L, Sovero**
901 **V, Sha S, Huang W, Zhang S, Zhang M, Sun J, Xu L, Li Y, Liu X, Li Q,**
902 **Shen J, Wang J, Paull RE, Bennetzen JL, Wang J, Zhang S** (2013) The
903 genome of the pear (Pyrus bretschneideri Rehd.). Genome Res **23:** 396-408
904 **Xuan YH, Hu YB, Chen LQ, Sosso D, Ducat DC, Hou BH, Frommer WB** (2013)
905 Functional role of oligomerization for bacterial and plant SWEET sugar
906 transporter family. Proc Natl Acad Sci USA **110:** E3685-3694
907 **Yang B, Sugio A, White FF** (2006) Os8N3 is a host disease-susceptibility gene for
908 bacterial blight of rice. Proc Natl Acad Sci USA **103:** 10503-10508
909 **Yamamoto T, Kimura T, Sawamura Y, Manabe T, Kotobuki K, Hayashi T, Ban Y,**
910 **Matsuta N** (2002) Simple sequence repeats for genetic analysis in pear.
911 Euphytica **124(1):** 129-137
912 **Yoo SD, Cho YH, Sheen J** (2007) Arabidopsis mesophyll protoplasts: a versatile cell
913 system for transient gene expression analysis. Nature Protoc **2(7):** 1565-1572
914 **Yuan M, Wang S** (2013) Rice MtN3/saliva/SWEET family genes and their homologs
915 in cellular organisms. Mol Plant **6:** 665-674
916 **Zhang HP, Wu JY, Tao ST, Wu T, Qi KJ, Zhang SJ, Wang JZ, Huang WJ, Wu J,**
917 **Zhang SL** (2014) Evidence for apoplasmic phloem unloading in pear fruit.
918 Plant Mol Biol Rep **32:** 931-939
919 **Zhang L, Chen L, Yu D** (2018) Transcription Factor WRKY75 Interacts with DELLA
920 Proteins to Affect Flowering. Plant Physiol **176:** 790-803
921 **Zhang Y, Wang L** (2005) The WRKY transcription factor superfamily: its origin in
922 eukaryotes and expansion in plants. BMC Evol Biol **5:** 1
923 **Zhou QY, Tian AG, Zou HF, Xie ZM, Lei G, Huang J, Wang CM, Wang HW,**
924 **Zhang JS, Chen SY** (2008) Soybean WRKY-type transcription factor genes,
925 GmWRKY13, GmWRKY21, and GmWRKY54, confer differential tolerance
926 to abiotic stresses in transgenic Arabidopsis plants. Plant Biotechnol J **6:** 486-
927 503
928 **Zhou Y, Tan B, Luo M, Li Y, Liu C, Chen C, Yu CW, Yang S, Dong S, Ruan J, Yuan**
929 **L, Zhang Z, Zhao L, Li C, Chen H, Cui Y, Wu K, Huang S** (2013) HISTONE
930 DEACETYLASE19 interacts with HSL1 and participates in the repression of
931 seed maturation genes in Arabidopsis seedlings. Plant Cell **25:** 134-148
932

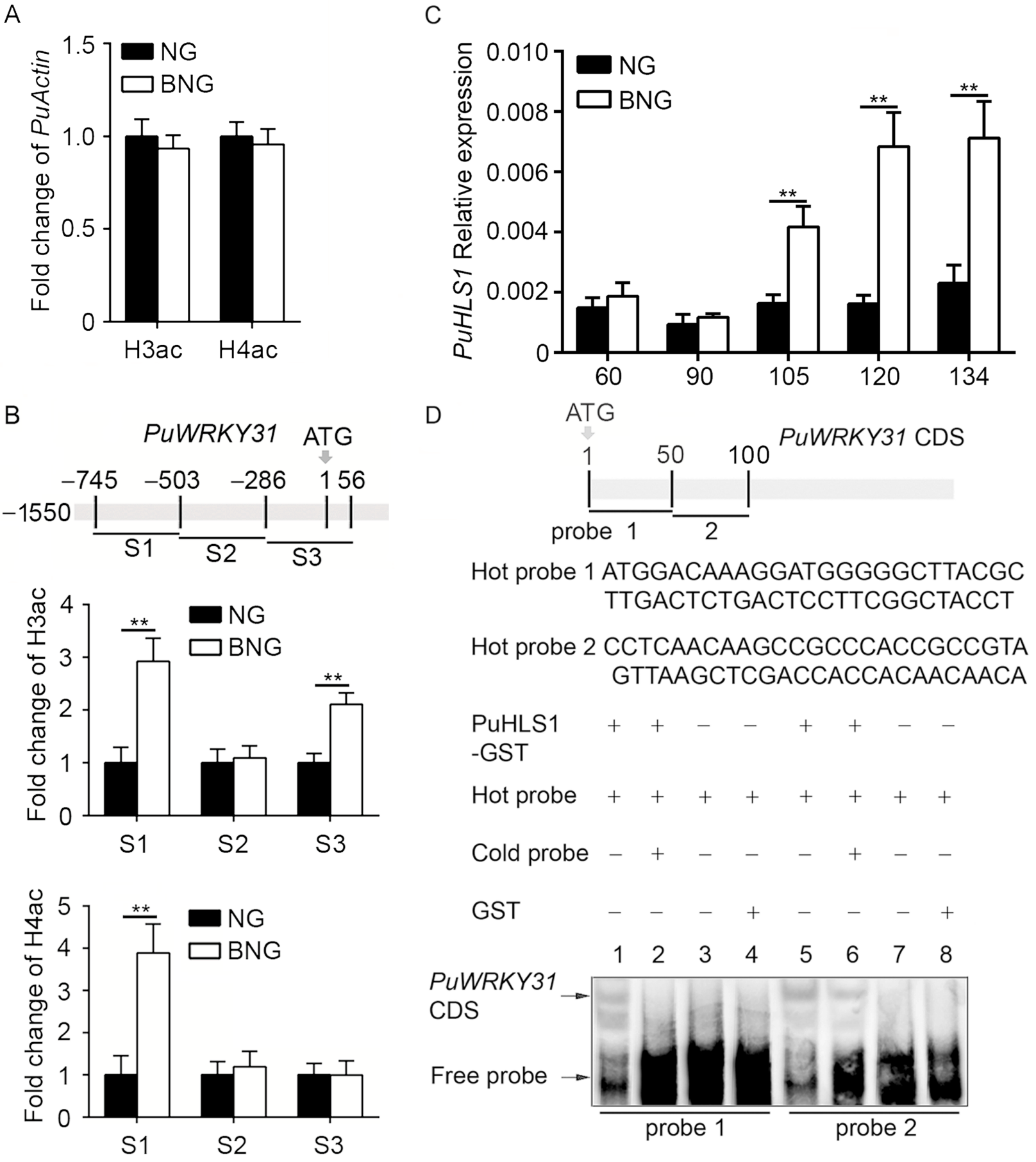


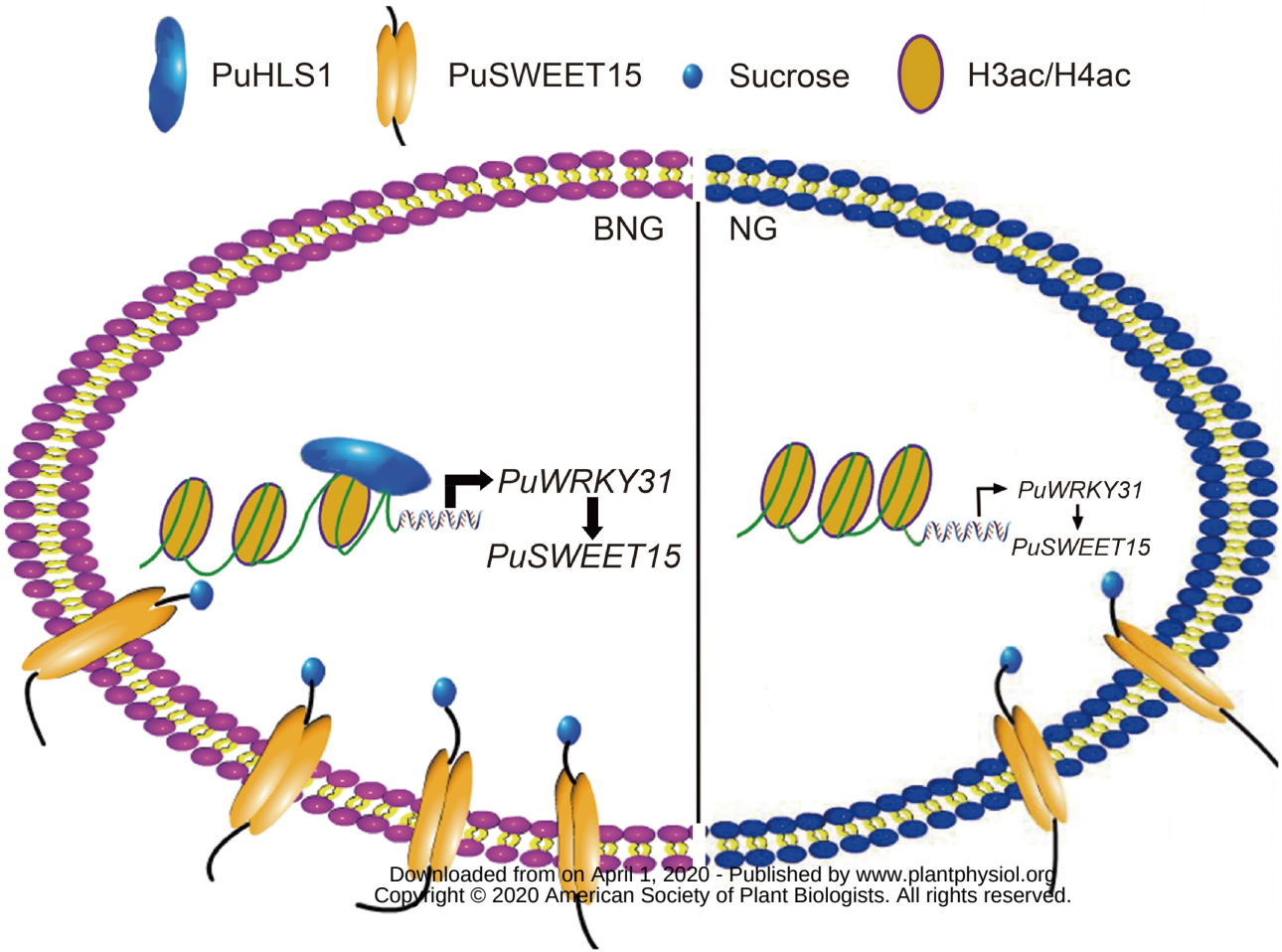












Parsed Citations

An J, Zeng T, Ji C, de Graaf S, Zheng, Z, Xiao TT, Pan Z (2019a) A Medicago truncatula SWEET transporter implicated in arbuscule maintenance during arbuscular mycorrhizal symbiosis. New Phytol (in press)

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

An JP, Zhang XW, You CX, Bi SQ, Wang XF, Hao YJ (2019b) Md WRKY 40 promotes wounding-induced anthocyanin biosynthesis in association with Md MYB 1 and undergoes Md BT 2-mediated degradation. New Phytol (in press)

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Ayre BG (2011) Membrane-transport systems for sucrose in relation to whole-plant carbon partitioning. Mol plant 4(3): 377-394

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Bao L, Chen K, Zhang D, Cao Y, Yamamoto T, Teng Y (2007) Genetic diversity and similarity of pear (Pyrus L.) cultivars native to East Asia revealed by SSR (simple sequence repeat) markers. Genet Resour Crop Ev 54: 959-971

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Braun DM, Wang L, Ruan YL (2014) Understanding and manipulating sucrose phloem loading, unloading, metabolism, and signalling to enhance crop yield and food security. J Exp Bot 65: 1713-1735

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Chagne D, Crowhurst RN, Pindo M, Thrimawithana A, Deng C, Ireland H, Fiers M, Dzierzon H, Cestaro A, Fontana P, Bianco L, Lu A, Storey R, Knabel M, Saeed M, Montanari S, Kim YK, Nicolini D, Larger S, Stefani E, Allan AC, Bowen J, Harvey I, Johnston J, Malnoy M, Troggio M, Perchepied L, Sawyer G, Wiedow C, Won K, Viola R, Hellens RP, Brewer L, Bus VG, Schaffer RJ, Gardiner SE, Velasco R (2014) The draft genome sequence of European pear (Pyrus communis L. 'Bartlett'). PLoS One 9: e92644

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Chen LQ, Qu XQ, Hou BH, Sosso D, Osorio S, Fernie AR, Frommer WB (2012) Sucrose efflux mediated by SWEET proteins as a key step for phloem transport. Science 335: 207-211

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Chen L, Xiang S, Chen Y, Li D, Yu D (2017) Arabidopsis WRKY45 Interacts with the DELLA Protein RGL1 to Positively Regulate Age-Triggered Leaf Senescence. Mol Plant 10: 1174-1189

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Chen LQ, Lin IW, Qu XQ, Sosso D, McFarlane HE, Londono A, Samuels AL, Frommer WB (2015) A cascade of sequentially expressed sucrose transporters in the seed coat and endosperm provides nutrition for the Arabidopsis embryo. Plant Cell 27: 607-619

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Chen Q, Xu X, Xu D, Zhang H, Zhang C, Li G (2019) WRKY18 and WRKY53 coordinate with HISTONE ACETYLTRANSFERASE1 to regulate rapid responses to sugar. Plant Physiol 180(4): 2212-2226

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Chong J, Piron MC, Meyer S, Merdinoglu D, Bertsch C, Mestre P (2014) The SWEET family of sugar transporters in grapevine: VvSWEET4 is involved in the interaction with Botrytis cinerea. J Exp Bot 65: 6589-6601

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Chu Z, Yuan M, Yao J, Ge X, Yuan B, Xu C, Li X, Fu B, Li Z, Bennetzen JL, Zhang Q, Wang S (2006) Promoter mutations of an essential gene for pollen development result in disease resistance in rice. Genes Dev 20: 1250-1255

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Dong X, Wang Z, Tian L, Zhang Y, Qi D, Huo H, Xu J, Li Z, Liao R, Shi M, Wahocho SA, Liu C, Zhang S, Tian Z, Cao Y (2019) De novo assembly of a wild pear (Pyrus betuleafolia) genome. Plant Biotechnol J (in press)

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Durand M, Porcheron B, Hennion N, Maurousset L, Lemoine R, Pourtau N (2016) Water Deficit Enhances C Export to the Roots in Arabidopsis thaliana Plants with Contribution of Sucrose Transporters in Both Shoot and Roots. Plant Physiol 170: 1460-1479

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Eom JS, Choi SB, Ward JM, Jeon JS (2012) The mechanism of phloem loading in rice (Oryza sativa). Mol Cells 33: 431-438

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Furiya T, Suzuki S, Sueta T, Takayanagi T (2009) Molecular characterization of a bud sport of Pinot gris bearing white berries. Am J Enol Viticult 60(1): 66-73
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Gao Y, Zhang C, Han X, Wang ZY, Ma L, Yuan P, Wu JN, Zhu XF, Liu JM, Li DP, Hu YB, Xuan YH (2018) Inhibition of OsSWEET11 function in mesophyll cells improves resistance of rice to sheath blight disease. Mol Plant Pathol 19: 2149-2161
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Gonzalez A, Brown M, Hatlestad G, Akhavan N, Smith T, Hembd A, Moore J, Montes D, Mosley T, Resendez J, Nguyen H, Wilson L, Campbell A, Sudarshan D, Lloyd A (2016) TTG2 controls the developmental regulation of seed coat tannins in Arabidopsis by regulating vacuolar transport steps in the proanthocyanidin pathway. Dev Biol 419: 54-63
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Guo WJ, Nagy R, Chen HY, Pfrunder S, Yu YC, Santelia D, Frommer WB, Martinoia E (2014) SWEET17, a facilitative transporter, mediates fructose transport across the tonoplast of Arabidopsis roots and leaves. Plant Physiol 164: 777-789
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Han YC, Kuang JF, Chen JY, Liu XC, Xiao YY, Fu CC, Wang JN, Wu KQ, Lu WJ (2016) Banana Transcription Factor MaERF11 Recruits Histone Deacetylase MaHDA1 and Represses the Expression of MaACO1 and Expansins during Fruit Ripening. Plant Physiol 171: 1070-1084
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Huang G, Li T, Li X, Tan D, Jiang Z, Wei Y, Li J, Wang A (2014) Comparative transcriptome analysis of climacteric fruit of Chinese pear (Pyrus ussuriensis) reveals new insights into fruit ripening. PLoS One 9: e107562
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Jaeger SR, Andani Z, Wakeling IN, MacFie HJ (1998) Consumer preferences for fresh and aged apples: a cross-cultural comparison. Food Qual Prefer 9: 355-366
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Jia HF, Chai YM, Li CL, Lu D, Luo JJ, Qin L, Shen YY (2011) Abscisic acid plays an important role in the regulation of strawberry fruit ripening. Plant Physiol 157: 188-199
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Jiang Y, Liang G, Yang S, Yu D (2014) Arabidopsis WRKY57 functions as a node of convergence for jasmonic acid- and auxin-mediated signaling in jasmonic acid-induced leaf senescence. Plant Cell 26: 230-245
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Jiang Z, Tang F, Huang H, Hu H, Chen Q (2009) Assessment of genetic diversity of Chinese sand pear landraces (Pyrus pyrifolia Nakai) using simple sequence repeat markers. HortScience 44(3): 619-626
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Lemoine R (2000) Sucrose transporters in plants: update on function and structure. BBA-Biomembranes 1465: 246-262
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Li J, Qin M, Qiao X, Cheng Y, Li X, Zhang H, Wu J (2017a) A New Insight into the Evolution and Functional Divergence of SWEET Transporters in Chinese White Pear (Pyrus bretschneideri). Plant Cell Physiol 58: 839-850
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Li T, Jiang Z, Zhang L, Tan D, Wei Y, Yuan H, Li T, Wang A (2016) Apple (Malus domestica) MdERF2 negatively affects ethylene biosynthesis during fruit ripening by suppressing MdACS1 transcription. Plant J 88: 735-748
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Li T, Tan D, Liu Z, Jiang Z, Wei Y, Zhang L, Li X, Yuan H, Wang A (2015) Apple MdACS6 Regulates Ethylene Biosynthesis During Fruit Development Involving Ethylene-Responsive Factor. Plant Cell Physiol 56: 1909-1917
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Li T, Xu Y, Zhang L, Ji Y, Tan D, Yuan H, Wang A (2017b) The jasmonate-activated transcription factor MdMYC2 regulates ETHYLENE

RESPONSE FACTOR and ethylene biosynthetic genes to promote ethylene biosynthesis during apple fruit ripening. Plant Cell 29: 1316-1334
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Li Y, Feng S, Ma S, Sui X, Zhang Z (2017c) Spatiotemporal expression and substrate specificity analysis of the cucumber SWEET gene family. Front Plant Sci 8: 1855
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Liao CJ, Lai Z, Lee S, Yun DJ, Mengiste T (2016) Arabidopsis HOOKLESS1 Regulates Responses to Pathogens and Abscisic Acid through Interaction with MED18 and Acetylation of WRKY33 and ABI5 Chromatin. Plant Cell 28: 1662-1681
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Liu W, Wang Y, Yu L, Jiang H, Guo Z, Xu H, Zhang Z (2019) MdWRKY11 participates in anthocyanin accumulation in red-fleshed apples by affecting MYB transcription factors and the photoresponse factor MdHY5. J Agr Food Chem 67(32): 8783-8793
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Lloyd A, Brockman A, Aguirre L, Campbell A, Bean A, Cantero A, Gonzalez A (2017) Advances in the MYB-bHLH-WD Repeat (MBW) Pigment Regulatory Model: Addition of a WRKY Factor and Co-option of an Anthocyanin MYB for Betalain Regulation. Plant Cell Physiol 58: 1431-1441
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Ma L, Zhang D, Miao Q, Yang J, Xuan Y, Hu Y (2017) Essential Role of Sugar Transporter OsSWEET11 During the Early Stage of Rice Grain Filling. Plant Cell Physiol 58: 863-873
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Ma Q, Xia Z, Cai Z, Li L, Cheng Y, Liu J, Nian H (2018) GmWRKY16 Enhances Drought and Salt Tolerance Through an ABA-Mediated Pathway in Arabidopsis thaliana. Front Plant Sci 9: 1979
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Mizuno H, Kasuga S, Kawahigashi H (2016) The sorghum SWEET gene family: stem sucrose accumulation as revealed through transcriptome profiling. Biotechnol Biofuels 9: 127
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Moriguchi T, Abe K, Sanada T, Yamaki S (1992) Levels and role of sucrose synthase, sucrose-phosphate synthase, and acid invertase in sucrose accumulation in fruit of Asian pear. J Am Soc Hortic 117: 274-278
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Ohto MA, Hayashi S, Sawa S, Hashimoto-Ohta A, Nakamura K (2006) Involvement of HLS1 in sugar and auxin signaling in Arabidopsis leaves. Plant Cell Physiol 47: 1603-1611
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Pal S, Yadav AK, Singh AK, Rastogi S, Gupta MM, Verma RK, Nagegowda DA, Pal A, Shasany AK (2017) Nitrogen treatment enhances sterols and withaferin A through transcriptional activation of jasmonate pathway, WRKY transcription factors, and biosynthesis genes in Withania somnifera (L.) Dunal. Protoplasma 254: 389-399
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Patil G, Valliyodan B, Deshmukh R, Prince S, Nicander B, Zhao M, Sonah H, Song L, Lin L, Chaudhary J, Liu Y, Joshi T, Xu D, Nguyen HT (2015) Soybean (Glycine max) SWEET gene family: insights through comparative genomics, transcriptome profiling and whole genome re-sequence analysis. BMC Genomics 16: 520
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Priestley C (1983) Interconversions of 14C-labelled Sugars in Apple Tree Tissues12. J Exp Bot 34: 1740-1747
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Ren X, Chen Z, Liu Y, Zhang H, Zhang M, Liu Q, Hong X, Zhu JK, Gong Z (2010) ABO3, a WRKY transcription factor, mediates plant responses to abscisic acid and drought tolerance in Arabidopsis. Plant J 63: 417-429
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Rennie EA, Turgeon R (2009) A comprehensive picture of phloem loading strategies. Proc Natl Acad Sci USA 106: 14162-14167
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Riesmeier JW, Willmitzer L, Frommer WB (1992) Isolation and characterization of a sucrose carrier cDNA from spinach by functional expression in yeast. EMBO J 11: 4705-4713
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Rushton PJ, Somssich IE, Ringler P, Shen QJ (2010) WRKY transcription factors. Trends Plant Sci 15: 247-258
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Shahbazian MD, Grunstein M (2007) Functions of site-specific histone acetylation and deacetylation. Annu Rev Biochem 76: 75-100
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Singh AK, Kumar SR, Dwivedi V, Rai A, Pal S, Shasany AK, Nagegowda DA (2017) A WRKY transcription factor from Withania somnifera regulates triterpenoid withanolide accumulation and biotic stress tolerance through modulation of phytosterol and defense pathways. New Phytol 215: 1115-1131
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Soni R, Carmichael JP, Murray JA (1993) Parameters affecting lithium acetate-mediated transformation of Saccharomyces cerevisiae and development of a rapid and simplified procedure. Curr Genet 24(5): 455-459
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Stitt M, Wilke I, Feil R, Heldt HW (1988) Coarse control of sucrose-phosphate synthase in leaves: alterations of the kinetic properties in response to the rate of photosynthesis and the accumulation of sucrose. Planta 174: 217-230
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Sturm A, Hess D, Lee HS, Lienhard S (1999). Neutral invertase is a novel type of sucrose-cleaving enzyme. Physiol Plant 107(2): 159-165
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Sun X, Zhang L, Wong DCJ, Wang Y, Zhu Z, Xu G, Wang Q, Li S, Liang Z, Xin H (2019) The ethylene response factor VaERF092 from Amur grape regulates the transcription factor VaWRKY33, improving cold tolerance. Plant J (in press)
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Wang S, Yokosho K, Guo R, Whelan J, Ruan YL, Ma JF, Shou H (2019) The soybean sugar transporter gmsweet15 mediates sucrose export from endosperm to early embryo. Plant Physiol 180(4): 2133-2141
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Wang Z, Meng D, Wang A, Li T, Jiang S, Cong P, Li T (2013) The methylation of the PcMYB10 promoter is associated with green-skinned sport in Max Red Bartlett pear. Plant Physiol 162: 885-896
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Wei X, Liu F, Chen C, Ma F, Li M (2014) The Malus domestica sugar transporter gene family: identifications based on genome and expression profiling related to the accumulation of fruit sugars. Front Plant Sci 5: 569
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Whitham TG, Slobodchikoff C (1981) Evolution by individuals, plant-herbivore interactions, and mosaics of genetic variability: the adaptive significance of somatic mutations in plants. Oecologia 49: 287-292
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Wu J, Wang Z, Shi Z, Zhang S, Ming R, Zhu S, Khan MA, Tao S, Korban SS, Wang H, Chen NJ, Nishio T, Xu X, Cong L, Qi K, Huang X, Wang Y, Zhao X, Wu J, Deng C, Gou C, Zhou W, Yin H, Qin G, Sha Y, Tao Y, Chen H, Yang Y, Song Y, Zhan D, Wang J, Li L, Dai M, Gu C, Wang Y, Shi D, Wang X, Zhang H, Zeng L, Zheng D, Wang C, Chen M, Wang G, Xie L, Sovero V, Sha S, Huang W, Zhang S, Zhang M, Sun J, Xu L, Li Y, Liu X, Li Q, Shen J, Wang J, Paull RE, Bennetzen JL, Wang J, Zhang S (2013) The genome of the pear (Pyrus bretschneideri Rehd.). Genome Res 23: 396-408
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Xuan YH, Hu YB, Chen LQ, Sosso D, Ducat DC, Hou BH, Frommer WB (2013) Functional role of oligomerization for bacterial and plant SWEET sugar transporter family. Proc Natl Acad Sci USA 110: E3685-3694
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Yang B, Sugio A, White FF (2006) Os8N3 is a host disease-susceptibility gene for bacterial blight of rice. Proc Natl Acad Sci USA 103:

10503-10508
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Yamamoto T, Kimura T, Sawamura Y, Manabe T, Kotobuki K, Hayashi T, Ban Y, Matsuta N (2002) Simple sequence repeats for genetic analysis in pear. Euphytica 124(1): 129-137
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Yoo SD, Cho YH, Sheen J (2007) Arabidopsis mesophyll protoplasts: a versatile cell system for transient gene expression analysis. Nature Protoc 2(7): 1565-1572
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Yuan M, Wang S (2013) Rice MtN3/saliva/SWEET family genes and their homologs in cellular organisms. Mol Plant 6: 665-674
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Zhang HP, Wu JY, Tao ST, Wu T, Qi KJ, Zhang SJ, Wang JZ, Huang WJ, Wu J, Zhang SL (2014) Evidence for apoplasmic phloem unloading in pear fruit. Plant Mol Biol Rep 32: 931-939
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Zhang L, Chen L, Yu D (2018) Transcription Factor WRKY75 Interacts with DELLA Proteins to Affect Flowering. Plant Physiol 176: 790-803
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Zhang Y, Wang L (2005) The WRKY transcription factor superfamily: its origin in eukaryotes and expansion in plants. BMC Evol Biol 5: 1
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Zhou QY, Tian AG, Zou HF, Xie ZM, Lei G, Huang J, Wang CM, Wang HW, Zhang JS, Chen SY (2008) Soybean WRKY-type transcription factor genes, GmWRKY13, GmWRKY21, and GmWRKY54, confer differential tolerance to abiotic stresses in transgenic Arabidopsis plants. Plant Biotechnol J 6: 486-503
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Zhou Y, Tan B, Luo M, Li Y, Liu C, Chen C, Yu CW, Yang S, Dong S, Ruan J, Yuan L, Zhang Z, Zhao L, Li C, Chen H, Cui Y, Wu K, Huang S (2013) HISTONE DEACETYLASE19 interacts with HSL1 and participates in the repression of seed maturation genes in Arabidopsis seedlings. Plant Cell 25: 134-148
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title