Auxin Regulates Sucrose Transport to Repress Petal Abscission in Rose (Rosa hybrida) [OPEN]

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Developmental transitions in plants require adequate carbon resources, and organ abscission often occurs due to competition for carbohydrates/assimilates. Physiological studies have indicated that organ abscission may be activated by Suc deprivation; however, an underlying regulatory mechanism that links Suc transport to organ shedding has yet to be identified. Here, we report that transport of Suc and the phytohormone auxin to petals through the phloem of the abscission zone (AZ) decreases during petal abscission in rose (Rosa hybrida), and that auxin regulates Suc transport into the petals. Expression of the Suc transporter RhSUC2 decreased in the AZ during rose petal abscission. Similarly, silencing of RhSUC2 reduced the Suc content in the petals and promotes petal abscission. We established that the auxin signaling protein RhARF7 binds to the promoter of RhSUC2, and that silencing of RhARF7 reduces petal Suc contents and promotes petal abscission. Overexpression of RhSUC2 in the petal AZ restored accelerated petal abscission caused by RhARF7 silencing. Moreover, treatment of rose petals with auxin and Suc delayed ethylene-induced abscission, whereas silencing of RhARF7 and RhSUC2 accelerated ethylene-induced petal abscission. Our results demonstrate that auxin modulates Suc transport during petal abscission, and that this process is regulated by a RhARF7-RhSUC2 module in the AZ.

INTRODUCTION

Abscission, a common process in plants, involves the detachment of organs from the main body and is triggered by developmental and environmental cues (Bleecker and Patterson, 1997; Roberts et al., 2002; Lewis et al., 2006; Sawicki et al., 2015; Tucker and Kim, 2015). Organ abscission often occurs due to lack of nutrients and competition for carbohydrates (van Doorn, 2002). Carbohydrate transport is thus thought to play a critical role in regulating abscission.

Suc can serve as the principal long-distance transport form of carbohydrates and energy (Riesmeier et al., 1994). Suc distribution between source and sink organ depends on the sink strength, which in turn is determined by competition for nutrition between different organs (Marcelis et al., 2004; Sawicki et al., 2015; Yu et al., 2015). Plants sense Suc distribution, and the associated signaling pathways regulate development. As an example in Arabidopsis (Arabidopsis thaliana), Suc accelerates the transition from the juvenile to the adult stage by reducing levels of the micro-RNA miR156 (Yu et al., 2013). In pea (Pisum sativum), Suc is considered as the initial regulator of apical dominance (Mason et al., 2014), and in rose (Rosa hybrida), Suc mediates the light-mediated control of bud burst (Henry et al., 2011). A major decline in Suc levels in abscising organs has been observed in several plant species, including rose (Borochov et al., 1976), pepper (Capsicum annuum; Aloni et al., 1997), citrus (Gómez-Cadenas et al., 2000), and apple (Malus domestica; Zhu et al., 2011). However, the significance of a reduced Suc supply and the mechanism by which it is regulated have not been characterized.

Suc translocation is mainly mediated by two Suc transporter families: Suc Carrier or Suc Transporter (SUC/SUT), and Sugar Will Eventually be Exported Transporter (SWEET). SUC proteins function as the principal mediators of long-distance Suc transport, while SWEET proteins mainly play roles in Suc loading and unloading (Chen et al., 2012; Peng et al., 2014). Dictoc-specific Type I and monocot-specific Type IIB members are associated with Suc loading and transport (Sauer, 2007), whereas Type III proteins localize to the tonoplast and plasma membrane and are thought to facilitate Suc release (Payavula et al., 2011). In Arabidopsis, the Type I member SUC2 localizes to the phloem and plays a key role in Suc loading and long-distance transport. In agreement, mutation of Arabidopsis SUC2 results in an impaired nutrition phenotype, as evidenced by stunted growth and the accumulation of carbohydrates...
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Background: Abscission, a common process in plants, involves the detachment of organs from the main body. Abscission often occurs due to the lack of nutrients and competition for carbohydrates. Accordingly, carbohydrate transport is thought to play a critical role in regulating abscission. Sucrose can serve as the main long-distance transport form of carbohydrates and energy. A major decline in sucrose levels in abscising organs has been observed in several plant species, including rose, pepper, citrus, and apple. However, the significance of a reduced sucrose supply and the mechanism by which sucrose transport is regulated have not been characterized. A better understanding of the role of sucrose during abscission has direct applications for the cut flower industry, where one goal is to delay petal abscission and wilting, one method being the addition of sucrose to vase water. Abscission also plays a critical role during fruit maturation, and farmers will favor certain cultivars based on yield, time to maturity and the presence of pedicels that might damage other fruits during harvesting.

Question: We wished to identify the factors that regulate sucrose transport during petal abscission in rose. We used two rose cultivars whose petals fall off quickly as our experimental system.

Findings: We determined that the phytohormone auxin controls sucrose transport during petal abscission. In roses, sucrose transport and auxin levels gradually decrease as flower buds mature. Petal abscission can be delayed by adding sucrose or auxin to vase water, while auxin transport inhibitors hasten abscission. This process is regulated by the auxin response factor RHARF7 via direct binding to the promoter of the sucrose transporter RhsUC2 and activation of RhsUC2 expression in the petal abscission zone. As rose flower buds age, RhsARF7 transcript levels diminish, resulting in a decrease in RhsUC2 expression and lower sucrose transport through the petal AZ. Silencing of RhsUC2 or RhsARF7 accelerated petal abscission.

Next steps: We are now working on the role of the abscission accelerator ethylene in sucrose transport during abscission. Indeed, ethylene accelerates abscission but only after auxin levels and sucrose transport potential have decreased in the petal AZ.

RESULTS

Auxin Regulates Suc Transport during Petal Abscission

To investigate the roles of Suc and auxin in rose petal abscission, we chose an abscission-prone rose cultivar (Rosa hybrida cv Golden Shower) and divided the process of flower opening and abscission into 6 stages (Gao et al., 2016; Supplemental Figure 1). As determined by scanning electron microscopy, petal AZ cells appeared to be more densely packed than adjacent cells from stage 1 to stage 3, with looser packing from stage 4 to stage 5 and clear intercellular separation at stage 6 (Supplemental Figure 1). Accordingly, we selected stage 3 and stage 5 as marker stages before and after the initiation of abscission, respectively.

Here, we investigate the regulatory mechanism of Suc transport during rose petal abscission. We show that auxin regulates Suc transport during petal abscission. We found that auxin regulates Suc transport during petal abscission, and that a decrease in Suc transport enhances ethylene sensitivity of the petal AZ. The molecular mechanism regulating this process is described.

in source organs (Gottwald et al., 2000). However, to date SUC proteins have not been shown to be involved in organ abscission. The abscission process is tightly regulated by endogenous phytohormones, with auxin acting as a major inhibitor of abscission and ethylene as an accelerator (La Rue, 1936; Roberts et al., 2002; Ma et al., 2015). A continuous polar auxin flow passing through the abscission zone (AZ) inhibits abscission, and auxin depletion in the AZ results in abscission initiation as a consequence of enhancing the sensitivity of the AZ to ethylene (La Rue, 1936; Roberts et al., 2002; Ma et al., 2015). In addition, ethylene sensitivity can be significantly reduced by auxin during organ ripening and detachment (Sexton and Roberts, 1982; Olsson and Butenko, 2018; Shin et al., 2019). The regulatory genes involved in the auxin and ethylene signal transduction pathways have been shown to be involved in the regulation of abscission, and include AUXIN RESPONSE FACTOR (ARF)1, ARF2, ARF7, and ARF19 (Ellis et al., 2005; Lombardi et al., 2015), and the ethylene signaling gene ETHYLENE RESPONSE 1 (ETR1) and ETHYLENE INSENSITIVE 2 (Patterson and Bleecker, 2004). However, many aspects of the interaction between auxin and ethylene in organ abscission are not well understood.

Here, we investigate the regulatory mechanism of Suc transport during rose petal abscission. We show that auxin regulates Suc transport during petal abscission, and that a decrease in Suc transport enhances ethylene sensitivity of the petal AZ. The molecular mechanism regulating this process is described.
metabolic controls, and with mannitol as an osmotic control sugar (Supplemental Figures 2A and 2B). Suc application caused the most substantial delay in petal abscission relative to the other treatments, although Glc also caused a delay due to an increased energy supply (Supplemental Figures 2A and 2B). Treatment with the synthetic auxin naphthalene acetic acid (NAA) at 10 μM or 100 μM caused petals to wilt, but they barely abscised by the end of the experiment (15 d; Figures 1E and 1F; Supplemental Figure 3). By contrast, flowers treated with the auxin transport inhibitor naphthalene acetic acid (NPA) showed accelerated petal abscission, with only 3.0 ± 0.7 d from fully opened flowers to complete petal abscission (Figures 1E and 1F). Notably, following a combined treatment with Suc and NPA, the time from fully opened flowers to abscission of all petals was 5.0 ± 0.7 d, which was similar to the mock treatment (Figures 1E and 1F). These results therefore strongly suggest that Suc and auxin act in the same pathway in petal abscission. We also tested petal abscission in response to the auxin signaling inhibitor auxinole, which binds the auxin receptors TRANSPORT INHIBITOR RESPONSE1/AUXIN-SIGNALING F-BOX to inhibit auxin-responsive gene expression (Hayashi et al., 2012). The flowers treated with auxinole exhibited accelerated petal abscission (average time to abscission 4.6 ± 0.5 d), whereas a combined treatment with Suc and auxinole restored the accelerated petal abscission by auxinole alone (average time to abscission 7.2 ± 1.3 d; Supplemental Figure 3).

To further investigate the interaction between auxin and Suc during petal abscission, we determined the effects of NAA treatment on the fluorescence signal resulting from staining with

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**Figure 1.** Auxin Regulates Suc Transport During Petal Abscission.

(A) Simulation of Suc transport, using esculin fluorescence, in the petal AZ at stage 3 or 5 of flower opening (MOCK), or after 100 μM NAA treatment at stage 5 of flower opening. Images of longitudinal sections were captured by laser scanning confocal microscopy, and transverse sections were imaged using a fluorescence microscope. Bars = 100 μm.

(B) Immunolocalization of IAA in the petal AZ at stage 3 or 5 of flower opening, or after treatment with 50 mM Suc (SUC) at stage 3 of flower opening. Immunofluorescence assays were performed using an anti-IAA monoclonal antibody to investigate auxin distribution.

(C) and (D) Suc (C) and IAA (D) levels in the petal AZ at stage 3 and stage 5 of flower opening, as measured by LC-MS/MS. Results are the means of three biological replicates from independent pools of petal AZ with standard deviations. Statistical significance between stage 5 and stage 3 was determined by two-tailed Student’s t test (**P < 0.01).

(E) and (F) Phenotypes (E) and rose petal (F) abscission time following different treatments. Statistical results are the means of five flower samples harvested from different plants with standard deviations. SUC, 50 mM Suc; NAA, 100 μM; NPA, 100 μM; N.A., no abscission. Every experiment was repeated three times with similar results. Arrows indicate the petal AZ; P, petal; R, receptacle. Statistical significance between different treatments and mock was determined by Dunnett test (**P < 0.01). Bar = 5 cm.
esculin. Images of longitudinal and transverse sections showed a much higher esculin signal after NAA treatment compared to the signal in stage 5 flowers without NAA treatment (Figure 1A). In addition, the IAA signal was similar to the mock control after Suc treatment (Figure 1B). These results suggest that auxin regulates Suc transport during petal abscission, but that Suc does not affect the distribution of auxin.

**Repression of Suc Transport by Silencing of RhSUC2 Promotes Petal Abscission**

To begin to elucidate the molecular mechanism behind long-distance Suc transport in the petal AZ, we identified three members of the rose SUC gene family (RhSUC2, RhSUC3, and RhSUC4), all of which have been previously reported to be expressed in rose flowers (Henry et al., 2011). We examined the expression patterns of these SUC genes in the petal AZ during flower opening and abscission by real-time quantitative RT-PCR (RT-qPCR). RhSUC2 expression peaked at stage 3, before significantly decreasing at stage 5 (Supplemental Figure 4A), whereas the expression of RhSUC3 and RhSUC4 remained constant during flower opening and abscission (Supplemental Figure 4A). An analysis of RhSUC2 expression in specific tissues further revealed a more than twofold decrease in expression in the petal AZ at stage 5 compared to stage 3, whereas the adjacent petal and receptacle tissues experienced no such change (Figure 2A). In addition, NAA treatment significantly induced the expression of RhSUC2 in the petal AZ (Figure 2B). We therefore selected RhSUC2 for further analysis of its potential function in petal abscission.

We tested the transport function of RhSUC2 in a complementation assay, involving its heterologous expression in the Suc uptake-deficient yeast strain SUSY7/ura3, which cannot grow efficiently on medium with Suc as the sole carbon source (Riesmeier et al., 1994; Wieczorke et al., 1999). A drop test showed that transformants with pDR196-RhSUC2 or empty vector (pDR196) grew on medium with 2% Glc, but only pDR196-RhSUC2 transformants grew well on medium with 2% Suc (Figure 2C), suggesting that RhSUC2 is a functional Suc transporter. Transmembrane domain prediction using transmembrane hidden Markov model (TMHMM) indicated a functional Suc transporter. Transmembrane domain prediction medium with 2% Suc (Figure 2C), suggesting that pDR196-RhSUC2 showed that the

In petal abscission. We further tested the expression of RhSUC2 in the petal AZ. Of the 7 candidate ARFs, the expression of RhSUC2 increased in the petal AZ by over twofold at stage 3 relative to stage 1 and decreased sharply at stage 5 and stage 6 (Figure 5B), thereby following an expression pattern similar to that of RhSUC2 during petal abscission (Figure 2A). We further tested the expression of all RhARF members in response to Suc treatment, and did not find any with greater than twofold change in expression (Figure 5C), indicating that Suc does not substantially alter their expression.

We next conducted a dual-luciferase (LUC) reporter assay to assess the regulation of the RhSUC2 promoter by RhARF7 and
RhARF8 in vivo. We determined that *N. benthamiana* leaf cells expressing RhARF7 dramatically activated the expression of the RhSUC2pro:LUC reporter when compared to those expressing other RhARF genes (Figure 5D). In addition, expressing the RhARF7 effector resulted in a 9.3-fold increase in LUC activity of the RhSUC2pro:LUC reporter, whereas RhARF6 as effector failed to raise LUC activity over control levels (Figure 5E). We then conducted a yeast one-hybrid assay to analyze the interaction of RhARF7 with the RhSUC2 promoter: RhARF7 bound to all three AuxRE elements, but not to a mutated AuxRE motif (Figure 5F). We confirmed these results in an electrophoretic mobility shift assay (EMSA), where again RhARF7 bound to all three AuxRE biotin-labeled probes. Increasing concentrations of unlabeled probes gradually attenuated the extent of binding, indicative of efficient competition. As in the dual-luciferase assays, we observed no binding using a mutated labeled AuxRE probe in EMSA (Figure 5G). Finally, a subcellular localization assay by coinfiltrating a RhARF7-GFP construct and the nuclear marker construct NF-YA4-mCherry in *N. benthamiana* leaves demonstrated that RhARF7 accumulates in the nucleus (Supplemental Figure 7B). Taken together, these results are consistent with RhARF7 functioning as a direct regulator of RhSUC2 expression.

To investigate whether RhARF7 plays a role in regulating petal abscission, we first evaluated its expression and measured higher transcript levels in the AZ than in petals or the receptacle (Supplemental Figure 7C). Notably, silencing of RhARF7 in rose plants using VIGS resulted in reduced RhSUC2 expression compared to the TRV control (Figure 3A). RhARF7-silenced plants also had smaller flower diameters and petal sizes for fully opened flowers compared to TRV control plants (Figure 3C; Supplemental Figure 5B), phenotypes similar to those of RhSUC2-silenced plants (Figure 3C; Supplemental Figure 5A). The time from fully opened flowers to complete petal abscission was 3.6 ± 0.5 d in the RhARF7-silenced plants, whereas it was 7.0 ± 0.7 d in TRV control plants (Figure 3B). With NAA treatment, the time from fully opened flower to abscission of half of all petals in RhARF7-silenced plants was 7.0 ± 0.7 d, compared to no abscission in TRV control (Figures 4A and 4B). In addition, flowers treated with NPA showed similar times to those genotypes, with 3.8 ± 0.4 d in RhARF7-silenced plants and 3.6 ± 0.5 d in the TRV control (Figures 4A and 4C). Suc transport to the petals substantially decreased in the silenced plants compared to the TRV control, as revealed by esculin transport assays (Figure 3E). LC-MS/MS analysis confirmed the lower Suc content of RhARF7-silenced petals relative to the TRV control (Figure 3D).

**RhSUC2 Expression Rescues Accelerated Petal Abscission Caused by RhARF7 Silencing**

To further dissect the genetic interaction between RhSUC2 and RhARF7, we silenced both RhSUC2 and RhARF7 (TRV-RhSUC2 + RhARF7) in rose plants using VIGS, and separately transiently overexpressed RhSUC2 in the petal AZ of the RhARF7-silenced background (TRV-RhARF7 + RhSUC2OX). As expected, TRV-RhSUC2 + RhARF7 plants exhibited the same low RhARF7
transcript levels as RhARF7-silenced plants, and RhSUC2 transcript levels reached even lower levels than in RhARF7-silenced plants (Figure 3A). The time from fully opened flower to abscission of all petals in TRV-RhSUC2-RhARF7 plants was 4.2 ± 0.4 d, thus faster than in TRV control plants and not statistically significantly different from RhSUC2-silenced or RhARF7-silenced plants (Figures 3B and 3C).

In TRV-RhARF7-RhSUC2OX plants, the expression of RhSUC2 increased 3.2 times over that seen in RhARF7-silenced plants (Figure 3A). The time to complete petal abscission was 6.6 ± 0.5 d in TRV-RhARF7-RhSUC2OX, which represented a significant delay compared to RhARF7-silenced plants. This time to complete petal shedding was not significantly different from that of the TRV control (Figures 3B and 3C).

Silencing of RhSUC2 and RhARF7 Increases in Petal Sensitivity to Ethylene

Ethylene is the main accelerator of petal abscission. To examine the influence of Suc and auxin on ethylene-induced rose petal abscission, we therefore treated flowers with Suc and auxin in an air-tight chamber containing 10 ppm ethylene. This treatment delayed ethylene-induced petal abscission compared to the mock control, and a combination of Suc and auxin treatments had a greater retardation effect compared to either treatment alone (Figure 6A; Supplemental Video). When we tested the effects of RhSUC2 and RhARF7 silencing on ethylene-induced petal abscission, we observed that the time of petal abscission following ethylene treatment in RhSUC2-silenced and RhARF7-silenced plants was 10.3 ± 2.1 h and 7.6 ± 1.0 h, respectively, compared with 21.5 ± 2.5 h for the TRV control plants (Figure 6B). In addition, RT-qPCR analysis showed that the expression of genes related to ethylene biosynthesis and signaling was upregulated in RhSUC2 and RhARF7-silenced plants compared to TRV control plants (Supplemental Figure 8). These results indicate that Suc and auxin repress ethylene sensitivity during petal abscission.

Finally, RT-qPCR analyses indicated that the expression levels of RhSUC2 and RhARF7 in the petal AZ decreased in response to ethylene treatment (Figure 6C). Esculin transport assays corroborated reduced Suc transport in the petal AZ under ethylene treatment (Figure 6D). However, immunolocalization of IAA indicated that auxin distribution in the area from AZ to petal did not change following the ethylene treatment (Figure 6E).
DISCUSSION

The shedding of superfluous organs is necessary for normal plant development and survival (Patterson, 2001), and the abscission process often occurs due to deprivation of, or competition for, carbohydrates/assimilates (Addicott and Lynch, 1955). Here, we report the role and regulatory mechanism of Suc transport during petal abscission in rose flowers.

The exogenous application of Suc is well-known to suppress organ abscission or senescence in plants. In Dendrobium and pepper, Suc feeding was reported to inhibit flower abscission (Aloni et al., 1997; Patteraravayo et al., 2013), and in citrus, branch girdling (that is, the removal of the bark and phloem) can lead to an increase in sugar content and a reduction in fruit abscission rates (Iglesias et al., 2006), whereas defoliation reduces carbohydrate levels and increases fruit abscission (Mehouachi et al., 1995). In this study, we determined the dynamics of Suc transport during petal abscission in vivo, and showed that Suc import into petals was impaired during petal abscission (Figures 1A and 1C). Sugar transport includes symplastic and apoplastic pathways, with Suc transporters mainly functioning in the apoplastic pathway. SUC proteins with high-affinity Suc activity serve in phloem loading, long-distance transport, and unloading (Sauer, 2007; Kühn and Grof, 2010). The Arabidopsis SUC2 transporter belongs to the high Suc-affinity type and plays an essential role in Suc transport and distribution between source and sink tissues (Durand et al., 2018). In the context of rose bud growth, RhSUC2, RhSUC3, and RhSUC4 have been reported to be expressed in buds. However, only RhSUC2 transcript levels displayed an upregulation in response to light in the buds of beheaded plants, hinting at the special role of RhSUC2 in light-induced bud break (Henry et al., 2011). Here, we also observed the downregulation of RhSUC2 expression during petal abscission, whereas the expression of RhSUC3 and RhSUC4 did not change (Supplemental Figure 4A).

Notably, RhSUC2 expression exhibited a downregulation in the AZ but not in the receptacle or petals (Figure 2A). Moreover, RhSUC2 silencing led to decreased Suc import to the petals and promoted petal abscission (Figure 3). Taken together, these results demonstrate that RhSUC2 plays a role in the regulation of petal abscission, in addition to bud outgrowth.

Auxin is a well-known inhibitor of abscission, and its depletion is a prerequisite for abscission (Lombardi et al., 2015). In Arabidopsis, overexpression of an auxin synthesis gene in the AZ was reported to effectively reduce petal abscission (Basu et al., 2013),
Figure 5. RhARF7 Binds to the RhSUC2 Promoter.

(A) Positions of AuxRE cis-elements in the RhSUC2 promoter and conservation of AuxRE cis-elements in the SUC2 promoters from the different species: Arabidopsis (AtSUC2-1 and AtSUC2-2), strawberry (FvSUC2-1), and peach (PpSUC2-1).
and in tomato (*Solanum lycopersicum*), depletion of auxin by the removal of flowers resulted in promoting pedicel abscission (Meir et al., 2010). In our study, auxin distribution decreased from the receptacle to the petal at stage 5 compared to stage 3 in rose (Figures 1B and 1D), and auxin application inhibited petal abscission (Figures 1E and 1F).

The influence of Suc on auxin metabolism and signaling has been described in previous studies. In Arabidopsis, Suc application led to elevated auxin levels and increased polar auxin transport in seedlings (Lilley et al., 2012). Suc has also been shown to promote auxin signaling to regulate the iron-deficiency response in Arabidopsis (Lin et al., 2016). In maize (*Zea mays*), sugar levels regulate auxin biosynthesis in developing seeds (LeClere et al., 2010), and in rose, Suc regulates bud outgrowth as an early modulator of relative phytohormone content during bud development (Barbier et al., 2015). In particular, the expression of genes encoding auxin biosynthesis and efflux carriers was induced during bud outgrowth (Barbier et al., 2015). However, we observed that Suc had no effect on the distribution of auxin during petal abscission (Figure 1B), whereas auxin promoted Suc transport during abscission (Figure 1A), indicating that Suc regulates Suc distribution during petal abscission. The expression of RhSUC2 was reported to be reduced in response to auxin in rose buds (Henry et al., 2011), but we saw an upregulation in RhSUC2 expression by auxin in the petal AZ (Figure 2B). These differences may reflect distinct auxin-Suc regulatory relationships during bud outgrowth and petal abscission. Moreover, we observed that flower diameter and petal size of fully opened flowers were smaller in both RhSUC2- and RhARF7-silenced plants compared to TRV control (Supplemental Figure 5). Previous studies suggested that defects in Suc transport or auxin signaling resulted in smaller size of flower. In Arabidopsis, the sizes of petals were significantly reduced in the *arf6 arf8* double mutant (Nagpal et al., 2005). In cucumber (*Cucumis sativus*), silencing of the Suc transporter *CssSUT1* resulted in smaller male flowers (Sun et al., 2019).

It has been reported that ARF1, ARF2, ARF7, and ARF19 contribute to petal abscission in Arabidopsis (Ellis et al., 2005; Okushima et al., 2005), whereas we found that RhARF7 is involved in the regulation of petal abscission in rose (Figure 3), and directly regulates the expression of RhSUC2 (Figures 5D to 5G). Sequence analysis identified auxin responsive cis-elements (AuxRE) in the SUC2 promoter that are conserved in the promoters of homologs from Arabidopsis, strawberry, and peach (Figure 5A). This suggests that the regulation of Suc transport in the AZ by auxin may be a common phenomenon in plants.

The balance of auxin and ethylene constitutes a major organ abscission regulatory module in plants (Patterson, 2001), with auxin inhibiting ethylene sensitivity (Taylor and Whitelaw, 2001). However, the mechanistic details of this antagonistic mechanism remain unknown. Previous studies had demonstrated that sugar can influence ethylene metabolism and signaling. In carnation (*Dianthus caryophyllus*), Suc delayed the senescence of cut flowers by delaying ethylene biosynthesis and regulating ethylene sensitivity (Verlinden and Garcia, 2004; Hoeberichts et al., 2007; Pun et al., 2016). In Arabidopsis, Glc enhanced the degradation of the transcription factor ETHYLENE INSENSITIVE 3, a key regulator in ethylene signaling (Zhou et al., 1998; Shi et al., 2003). Our results showed that Suc treatment can suppress ethylene-induced petal abscission in rose (Figure 6A; Supplemental Video), and that the strongest suppression of ethylene-induced petal abscission resulted from a cotreatment with auxin and Suc (Figure 6A; Supplemental Video). These results indicate that Suc acts as a mediator of the interaction between auxin and ethylene in organ abscission. Intriguingly, we observed that ethylene-induced abscission can be accelerated by silencing of RhSUC2 or RhARF7 (Figure 6B). Our results suggest that auxin and Suc may have other functions in petal abscission, bypassing ethylene sensitivity during abscission.

Ethylene was reported to affect Suc distribution and accumulation as a feedback regulation. In carnation, ethylene promotes Suc mobilization from petals to other organs in the flower (Nichols and Ho, 1975), whereas in rice (*Oryza sativa*), ethylene inhibitors enhance Suc biosynthesis during grain filling (Naik and Mohapatra, 2000). Moreover, ETHYLENE RESPONSIVE FACTOR 72 (ERF72) suppresses Suc biosynthesis in cassava (*Manihot esculenta* Crantz; Liu et al., 2018). Here, we showed that an ethylene treatment reduced Suc transport in the petal AZ (Figure 6D), but did not affect auxin distribution (Figure 6E), suggesting that ethylene reduces Suc transport as part of a feedback mechanism during petal abscission.

In conclusion, our results demonstrate that auxin induces Suc transport to repress petal abscission in the early stages of flower development. The reduction of auxin levels in the petal AZ leads to

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**Figure 5.** (continued).

(B) and (C) Expression of ARF genes in the petal AZ during flower opening and abscission (B), or at stage 3 of flower opening after 50 mM Suc treatment for 24 h (C), as determined by RT-qPCR. RhUBI2 was used as the internal control.

(D) Transactivation assays using different combinations of ARF proteins and RhSUC2 promoter constructs in *N. benthamiana* leaves. LUC activities were recorded 3 d after infiltration.

(E) Interaction of ARF proteins and the RhSUC2 promoter, as shown using dual luciferase assays. The *Renilla luciferase* (*REN*) gene driven by the 3SS promoter was used as an internal control.

(F) Interaction between RhARF7 and the RhSUC2 promoter AuxRE cis-elements, as shown by yeast one-hybrid assays. A mutated AuxRE cis-element was used as a negative control. Interactions between bait and prey were determined by cell growth on synthetic dropout medium lacking Ura, Leu, and containing 200 ng mL⁻¹ aureobasidin A (AbA).

(G) Interactions of RhARF7 and biotin-labeled RhSUC2 promoter AuxRE motifs, as shown by EMSA. One microgram of purified protein was incubated with 20 nM wild type or mutated biotin-labeled probes. Ten-, 100- and 1,000-fold concentrations of unlabeled probes were used for the competition tests. Letters indicate significant differences according to Tukey-Kramer test (P < 0.05). Asterisks indicate statistically significant differences between Suc treatment and mock determined by two-tailed Student’s t test (**P < 0.01).
an attenuation of Suc transport and promotes the initiation of petal abscission. In this process, the \( \text{RhARF7-RhSUC2} \) module mediates the regulation by auxin of Suc transport (Figure 7).

**METHODS**

**Plant Materials and Growth Conditions**

We used the abscission-prone rose (Rosa hybrida cv Golden Shower) as material for the experiments in this study, except for the VIGS assays. We harvested flowers at flower opening stage 2 from plants grown in a mixture of vermiculite and nutritive soil (1:1) in a greenhouse at the China Agricultural University (Beijing, China), and transported them to the laboratory within 1 h. We cut the stems again to a length of 16 cm under water and immediately placed them in distilled water. Vase solutions contained 200 mg L\(^{-1}\) 8-hydroxyquinoline sulfate as anti-microbial agent.

Preliminary tests demonstrated that R. hybrida cv Samantha shows a high VIGS silencing efficiency and tissue-cultured R. hybrida cv Samantha plants can bloom in 40 d under our conditions. In addition, vase life experiments showed that R. hybrida cv Samantha and R. hybrida cv Golden Shower behaved similarly with respect to auxin and Suc treatments, such that petal abscission was inhibited by auxin, and delayed by Suc (Supplemental Figure 3). We therefore used Rosa hybrida cv Samantha for VIGS assays. R. hybrida cv Samantha plants were propagated by tissue culture as previously described by Wu et al. (2017). We used Nicotiana benthamiana plants as material for transient expression assays. N. benthamiana and rose plants were grown in a mixture of vermiculite and nutritive soil (1:1) at 23 ± 1°C, 40% to 60% relative humidity, and 100–120 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) illumination with fluorescent lamps (SINOL, SN-T5, 16W) under a 16-h light/8-h dark photoperiod. We conducted all flower longevity analyses at 23 ± 1°C, 60% to 80% relative humidity, 50–60 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) illumination with fluorescent lamps under a 16-h light/8-h dark photoperiod. The phenotype of the flowers was photographed every day or monitored by time-lapse image capture.

The distal and proximal sides of the petal AZ are connected to the petal and receptacle, respectively. We collected petal AZ samples by excising both sides at the base of the petals (<2 mm in length) and the area of the receptacles adjacent to the petals (<2 mm in length; Gao et al., 2016).
We measured Suc levels as previously described by Ma et al. (2017). Briefly, we ground freeze-dried petals or AZs using a mixer mill with zirconia beads for 1.5 min at 30 Hz. We then extracted 100 mg powder overnight at 4°C for 15 min, we filtered the supernatants through a 0.22 μm pore size, ANPEL) for LC-MS/MS analysis.

For auxin extraction, we collected 120 mg fresh rose AZ tissue, followed by flash-freezing and grinding in liquid nitrogen, before extraction with 1.2 mL methanol/water (8/2, v/v) at 4°C overnight. We centrifuged the extract at 12,000g at 4°C for 15 min. We collected the supernatant and allowed it to evaporate to dryness under a nitrogen gas stream. We dissolved the final pellet in 100 μL methanol/water (3/7, v/v), centrifuged the solution at 12,000g at 4°C for 15 min, and collected the supernatant for LC-MS/MS analysis.

We performed LC-MS/MS using an LC-electrospray ionization (ESI)-MS/MS system (HPLC, Shim-pack UFLC SHIMADZU CBM30A system; MS, Applied Biosystems 6500 Triple Quadrupole). HPLC parameters were as follows: column, Waters ACQUITY UPLC HSS T3 C18 (1.8 μm, 2.1 mm×100 mm); solvent system, water (0.04% [v/v] acetic acid) and acetonitrile (0.04% [v/v] acetic acid); gradient program, 95:5 (v/v) at 0 min, 5:95 (v/v) at 9.5 min, 95:5 (v/v) at 12.0 min, 95:5 (v/v) at 12.1 min, 95:5 v/v at 15.0 min; flow rate, 0.4 mL min⁻¹ for Suc and 0.35 mL min⁻¹ for auxin; temperature, 40°C; injection volume: 2 μL for Suc and 5 μL for auxin. The effluent was alternatively connected to an ESI-triple quadrupole-linear ion trap-MS. The ESI source operation parameters were as follows: ion source, turbo spray; source temperature 500°C; ion spray voltage (IS) 5500 V; curtain gas (CUR) were set at 25 psi for Suc and 35 psi for auxin; the collision gas (CAD) setting was high for Suc and medium for auxin. Declustering potential and collision energy for individual multiple reaction monitoring transitions was done with further declustering potential and collision energy optimization. A specific set of multiple reaction monitoring transitions was monitored for each period according to the metabolites eluted within this period.

**Esculin Feeding and Confocal Microscopy**

We diluted esculin hydrate (Sigma-Aldrich) to 10 mM in deionized water. We performed vacuum infiltration by immersing leaves adjacent to the rose flower in esculin solution under vacuum at 0.7 MPa. We sectioned petal AZ by hand with a razor blade 10 h after esculin treatment and immediately immersed the sections in 80% (v/v) glycerol before mounting on glass slides. We recorded fluorescence using a FV1000 laser scanning confocal microscope (Olympus), with an excitation wavelength of 405 nm and emission wavelength of 454 nm.

**Immunolocalization of IAA**

We examined IAA distribution as previously described (Sakata et al., 2010). We prefixed freshly prepared AZ samples for 2 h in 3% (w/v) 1-ethyl-3-(3-dimethyl-aminopropyl)-carbodiimide hydrochloride (Sigma-Aldrich) at room temperature and transferred them to 4% (w/v) parafomaldehyde (Sigma-Aldrich) and 2% (v/v) glutaraldehyde (Sigma-Aldrich). We dehydrated the samples using a gradient series of ethanol solutions (30%, 50%, 70%, 80%, 90%, 100% [v/v]). After dehyrdration, we transferred the samples to xylene and then paraaffin (Thermo Fisher Scientific), each for 1 h, followed by embedding in 100% (v/v) paraaffin. We cut the embedded samples into 10 μm sections using a microtome (HistoCore BIOCUT, Leica Biosystems). We incubated the sections with 1:100 (v/v) dilutions of anti-IAA monoclonal antibody (Sigma-Aldrich, A0855) overnight at 4°C, and then with DyLight™ 488-labeled anti-mouse IgG antibody (1:500 [v/v], KPL, 5230-0391) for 4 h at room temperature in the dark. We recorded the fluorescence signal using a SP8 laser scanning confocal microscope (Leica), with an excitation wavelength of 488 nm and emission wavelength of 518 nm. The negative controls were specimens not incubated with the anti-iaa antibody.

**RNA Extraction and RT-qPCR**

We extracted total RNA using the hot borate method as previously described by Gao et al. (2016). We synthesized first-strand cDNAs using 1 μg of total RNA with oligo d(T) and random primers in a final volume of 20 μL. For RT-qPCR, we used 1 μL cDNA as the template. We performed RT-qPCR (40 cycles of denaturation for 5 s at 95°C and annealing for 30 s at 60°C) using the Step One Plus™ real-time PCR system (Applied Biosystems) with KAPATM SYBR® FAST quantitative PCR kits (Kapa Biosystems). We used RrhUBI2 as the reference gene (Meng et al., 2013). Each experiment was performed independently three times. The primers used in this study are listed in Supplemental Data Set 1. The statistical tests are shown in Supplemental Data Set 2.

**Subcellular Localization of RhSUC2 and RhARF7**

We determined the subcellular localization of RhSUC2 and RhARF7 in N. benthamiana leaf epidermal cells. We PCR-amplified the open reading frames (ORFs) for RhSUC2 and RhARF7, and inserted the PCR products at the SaII/SpeI restriction sites in the pSuper1300 vector to form SUPERpro:RhSUC2-GFP and SUPERpro:RhARF7-GFP, respectively. We introduced the resulting vectors into Agrobacterium (Agrobacterium tumefaciens) strain GV3101. We grew Agrobacterium cultures overnight at 28°C, pelleted the cells by centrifugation at 3214g for 5 min at room temperature and resuspended the pellet in infiltration buffer (10 mM MgCl₂, 10 mM MES, 200 μM acetosyringone, pH 5.6) to a final OD₆₀₀ = 0.8. We infiltrated Agrobacterium cell suspensions into N. benthamiana leaves. After 3 d, we detached infiltrated leaves and recorded fluorescence signal in tobacco epidermal cells on a laser confocal fluorescence microscope (Olympus Fluo View FV1000). 35Spro:PM-mCherry (CD3-1007) and NF-YA4 (SUPERpro:NF-YA4-mCherry) were used as plasma membrane and nuclear markers, respectively (Nelson et al., 2007; Zhou et al., 2015). The GFP fluorescence signals were observed at an excitation wavelength of 488 nm and emission wavelength of 506-538 nm, and mCherry signals were detected using excitation with a 597-nm laser and emission with...
a 575- to 675-nm band pass filter. The primers used in this study are listed in Supplemental Data Set 1.

VIGS
We performed VIGS-mediated gene silencing as previously described (Wu et al., 2017). Briefly, we PCR-amplified 534-bp and 414-bp fragments specific to RhSUC2 and RhARF7 3’ UTR regions, respectively, and inserted them separately into the pTRV2 vector, which we then introduced into Agrobacterium strain GV3101. For double silencing of RhSUC2 and RhARF7, we fused the 3’ UTR regions of RhSUC2 and RhARF7 by fusion PCR, and then inserted the fused product into the pTRV2 vector. The primers used in this study are listed in Supplemental Data Set 1.

We grew the relevant Agrobacterium colonies overnight in Luria-Bertani medium containing 10 mM MES (pH 6.3), 20 μM acetoxyringoine, 50 μg mL⁻¹ kanamycin, and 50 μg mL⁻¹ gentamycin sulfate. We collected the cells by centrifugation at 2057 g for 10 min, and resuspended the pellet in infiltration buffer (10 mM MgCl₂, 10 mM MES, 200 μM acetoxyringoine, and 5.6) to a final OD₆₀₀ of 1.5. We mixed the pTRV1 and pTRV2 cultures in a 1:1 (v/v) ratio and then placed the mixes in the dark for 3 h at room temperature.

We vacuum-infiltrated rose plants by immersing whole plants propagated by tissue culture for 60 d in the bacterial suspension and infiltrated under a vacuum at 0.7 MPa. We then transplanted plants into a mixture of sterile soil and sifted leaves from young N. benthamiana leaves. After 3 d, we harvested the infiltrated leaves and sprayed them with 50 mg L⁻¹ D-luciferin (Promega). We captured images of LUC signals with a charge-coupled device camera (CHEMIPROHT1300B/LND, 16 bits, Roper Scientific) at −110°C.

For dual luciferase reporter assays, we coinfiltrated different bacterial mixtures described above with Agrobacterium cells harboring a 35Spro::REN construct [where the Renilla luciferase gene (REN) is under the control of the 35S promoter] into N. benthamiana leaves as previously described by Wei et al. (2017). After 3 d, we measured LUC and REN activities with the dual-luciferase reporter assay reagents (Promega) and a GloMax 20/20 luminometer (Promega). The primers used in this study are listed in Supplemental Data Set 1. The statistical tests are shown in Supplemental Data Set 2.

Transient Expression in Rose Petal AZ
We introduced the SUPERpro:RhSUC2-GFP construct into Agrobacterium as above for transient overexpression of RhSUC2 in the flowers of RhARF7-silenced plants. We performed vacuum infiltration by immersing flowers at opening stage 1 in the bacterial suspension and infiltration under a vacuum at 0.7 MPa. We first measured the accumulation of GFP transcripts in the petals at the flower opening stage 2 of each plant. The plants with downregulated target gene expression were used for further assays. Three independent experiments were performed with 50 plants in each experiment. The statistical tests are shown in Supplemental Data Set 2.

Suc Uptake Assay
We cloned the ORF for RhSUC2 into the yeast expression vector pDR196 and transformed the resulting construct into the Suc uptake-deficient yeast strain SUSY7/ura3 by the lithium acetate method. The pDR196 empty vector was used as the control. We spotted transformed pDR196-RhSUC2 and pDR196 colonies as serial dilutions and cultured them on synthetic dropout medium (SD-Ura) plus 2% (w/v) Glc or 2% (w/v) Suc as the sole carbon source at 30°C for 3 d. We recorded the growth of the various yeast strains by taking photographs.

Yeast One-Hybrid Assay
We inserted RhSUC2 promoter fragments into the pAbaI vector (Clontech) and introduced the resulting constructs into the yeast one-hybrid Y1H gold strain. The transformed cells were grown on synthetic dropout medium (SD-Ura) plus 100 μg mL⁻¹ aureobasidin A (Aba). We then introduced the recombinant vector pGADT7-RhARF7 into a yeast strain harboring the pRhSUC2 (AuxRE)-AbaI and pRhSUC2 (mAuxRE)-AbaI. The transformed cells were grown on synthetic dropout medium (SD-Ura) plus 200 ng mL⁻¹ Aba for 3 d. The primers used in this study are listed in Supplemental Data Set 1.

Transactivation and Dual Luciferase Reporter Assays
We analyzed the transactivation of the RhSUC2 promoter by ARFs as previously described by Kong et al. (2015) and Wang et al. (2018). We inserted the ORFs for the ARF genes into the pGreenII0029 62-SK vector (Hellens et al., 2005) to generate effector constructs under control of the 35S promoter; we also cloned the RhSUC2 promoter into the pGreenII0800-LUC vector (Hellens et al., 2005) to drive expression of the luciferase reporter. We used empty vectors as negative controls. We introduced all constructs into Agrobacterium strain GV3101 harboring the pSoup plasmid, and grew bacterial cultures overnight as described above. We collected cells by centrifugation at 3214g for 5 min at room temperature and resuspended the pellets in infiltration buffer (10 mM MgCl₂, 200 μM acetoxyringoine, 10 mM MES, pH 5.6) to a final OD₆₀₀ of 0.8 before mixing cell suspensions in a 1:1 (v/v) ratio. We then infiltrated into young N. benthamiana leaves. After 3 d, we harvested the infiltrated leaves and sprayed them with 50 mg L⁻¹ D-luciferin (Promega). We captured images of LUC signals with a charge-coupled device camera (CHEMIPROHT1300B/LND, 16 bits, Roper Scientific) at −110°C.

We performed EMSA assays according to the instructions of the Light Shift chemiluminescent EMSA kit (Thermo Fisher Scientific). We cloned the RhARF7 ORF into the GST vector pGEX-4T-2 (GE Healthcare) and transformed the resulting construct into E. coli Rosetta cells. We induced the RhARF7 ORF, and transformed the resulting construct into E. coli Rosetta cells. We induced the RhARF7 ORF into the GST vector pGEX-4T-2 (GE Healthcare) and transformed the resulting construct into E. coli Rosetta cells. We induced the production of the GST-RhARF7 fusion protein by the addition of 0.6 mM isopropyl β-D-1-thiogalactopyranoside in 100 mL in Luria-Bertani medium at 16°C for 12 h. We extracted and purified the fusion protein with glutathione Sepharose 4B beads (GE Healthcare) following the manufacturer's instructions. The biotin-labeled probes were designed as described in Supplemental Data Set 1.

Sequence Analysis
We aligned amino acid sequences with default parameters using ClustalW (http://www.genome.jp/tools-bin/clustalw). We constructed the phylogenetic tree based on the alignment result using the neighbor-joining method with MEGA version 5.05 (Tamura et al., 2011) with the following parameters: 1000 bootstrap replicates, Poisson correction, partial deletion, and uniform rates. The protein transmembrane prediction was performed on the TMHMM server (http://www.cb.s.dtu.dk/services/TMHMM-2.0/; Möller et al., 2001). The files of phylogenetic analysis and protein alignment are shown in Supplemental Data Sets 3 and 4.
Accession Numbers
Rose gene sequences from this article can be found in GenBank (http://www.ncbi.nlm.nih.gov) under the following accession numbers: RhSUC2 (HQ403679), RhARF7 (MN850677), RhSUC3 (CF349302), RhSUC4 (HG762758), and RhUBI2 (JK618216).

Supplemental Data

Supplemental Figure 1. Flower opening stages and scanning electron micrographs of the petal abscission zone in rose.

Supplemental Figure 2. The effects of different sugars on rose petal abscission.

Supplemental Figure 3. Phenotypes and rose petal abscission times, respectively, following different treatments in Rosa hybrida cv. Samantha.

Supplemental Figure 4. The expression of RhSUC genes in the petal abscission zone (AZ) during rose flower opening (A) and in the petals of RhSUC2-silenced (TRV-RhSUC2) and TRV control plants (B).

Supplemental Figure 5. Flower diameters of RhSUC2- (A) and RhARF7- (B) silenced plants.

Supplemental Figure 6. Effects of RhSUC2 and RhSUC4 silencing on petal abscission.

Supplemental Figure 7. Phylogenetic analysis of ARF proteins and characterization of RhARF7.

Supplemental Figure 8. Expression of genes related to ethylene synthesis and signaling transduction in petals of RhSUC2-, RhARF7-silenced and TRV control plants at stage 5.

Supplemental Video. The petal abscission process after co-treatment with ethylene and different solutions.

Supplemental Data Set 1. List of primers used in this study.

Supplemental Data Set 2. Results of statistical analyses.

Supplemental Data Set 3. Text file of phylogenetic analysis used in Supplemental Figure 7. in Newick format.

Supplemental Data Set 4. Alignment of proteins used for the phylogenetic tree in Supplemental Figure 7.

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AUTHOR CONTRIBUTIONS

C.M. and J.G. conceived and designed the experiments; Y. Liang performed most of the experiments; Y. Liu, Y.G., J.L., and P.A. contributed to the VIGS assay; C.J. contributed to the immunolocalization assay; Z.F., C.-Z.J., and B.H. provided technical support and conceptual advice; Y. Liang, C.M., and J.G. analyzed the data and wrote the article.

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REFERENCES

Addicott, F.T., and Lynch, R.S. (1955). Physiology of abscission. Annu. Rev. Plant Biol. 6: 211–238.
Aloni, B., Karni, L., Zaidman, Z., and Schaffer, A.A. (1997). The relationship between sucrose supply, sucrose-cleaving enzymes and flower abortion in pepper. Ann. Bot. 79: 601–605.
Barbier, F., et al. (2015). Sucrose is an early modulator of the key hormonal mechanisms controlling bud outgrowth in Rosa hybrida. J. Exp. Bot. 66: 2569–2582.
Basu, M.M., González-Carranza, Z.H., Azam-Alli, S., Tang, S., Shahid, A.A., and Roberts, J.A. (2013). The manipulation of auxin in the abscission zone cells of Arabidopsis flowers reveals that indoleacetic acid signaling is a prerequisite for organ shedding. Plant Physiol. 162: 96–106.
Bleecker, A.B., and Patterson, S.E. (1997). Last exit: Senescence, abscission, and meristem arrest in Arabidopsis. Plant Cell 9: 1169–1179.
Borochov, A., Mayak, S., and Haley, A.H. (1976). Combined effects of abscisic-acid and sucrose on growth and senescence of rose flowers. Physiol. Plant. 36: 221–224.
Chandran, D., Reinders, A., and Ward, J.M. (2003). Substrate specificity of the Arabidopsis thaliana sucrose transporter AtSUC2. J. Biol. Chem. 278: 44320–44325.
Chen, L.Q., Qu, X.Q., Hou, B.H., Sosso, D., Osorio, S., Fernie, A.R., and Frommer, W.B. (2012). Sucrose efflux mediated by SWEET proteins as a key step for phloem transport. Science 335: 207–211.
Durand, M., Mainson, D., Porcheron, B., Maurousset, L., Lemoine, R., and Pourtau, N. (2018). Carbon source-sink relationship in Arabidopsis thaliana: The role of sucrose transporters. Planta 247: 587–611.
Ellis, C.M., Nagpal, P., Young, J.C., Hagen, G., Guilfoyle, T.J., and Reed, J.W. (2005). AUXIN RESPONSE FACTOR1 and AUXIN RESPONSE FACTOR2 regulate senescence and floral organ abscission in Arabidopsis thaliana. Development 132: 4563–4574.
Eom, J.S., Chen, L.Q., Sosso, D., Julius, B.T., Lin, I.W., Qu, X.Q., Braun, D.M., and Frommer, W.B. (2015). SWEETs, transporters for intracellular and intercellular sugar translocation. Curr. Opin. Plant Biol. 25: 53–62.
Gao, Y., Liu, C., Li, X., Xu, H., Liang, Y., Ma, N., Fei, Z., Gao, J., Jiang, C.Z., and Ma, C. (2016). Transcriptome profiling of petal abscission zone and functional analysis of an Aux/IAA Family gene RhIAA16 involved in petal shedding in rose. Front Plant Sci 7: 1375.
Gómez-Cadenas, A., Mehouachi, J., Tadeo, F.R., Primo-Millo, E., and Talon, M. (2000). Hormonal regulation of fruitlet abscission induced by carbohydrate shortage in citrus. Planta 210: 636–643.
Gora, P.J., Reinders, A., and Ward, J.M. (2012). A novel fluorescent assay for sucrose transporters. Plant Methods 8: 13.
Gottwald, J.R., Krysan, P.J., Young, J.C., Evert, R.F., and Sussman, M.R. (2000). Genetic evidence for the in planta role of phloem-specific plasma membrane sucrose transporters. Proc. Natl. Acad. Sci. USA 97: 13979–13984.
Hayashi, K., Neve, J., Hirose, M., Kuboki, A., Shimada, Y., Kepinski, S., and Nozaki, H. (2012). Rational design of an auxin

Printed on Arch A4 paper.
antagonist of the SCF^{RIN} auxin receptor complex. ACS Chem. Biol. 7: 590–598.

Hellens, R.P., Allan, A.C., Friet, E.N., Bolitho, K., Grafton, K., Templeton, M.D., Karunairetnam, S., Gleave, A.P., and Laing, W.A. (2005). Transient expression vectors for functional genomics, quantification of promoter activity and RNA silencing in plants. Plant Methods 1: 13.

Henry, C., Rabot, A., Laloi, M., Morteau, E., Sigogne, M., Leduc, N., Lemoine, R., Sakr, S., Vian, A., and Pelleschi-Travier, S. (2011). Regulation of RhSUC2, a sucrose transporter, is correlated with the light control of bud burst in Rosa sp. Plant Cell Environ. 34: 1776–1780.

Hoeberechts, F.A., van Doorn, W.G., Vorst, O., Hall, R.D., and van Woudragen, M.F. (2007). Sucrose prevents up-regulation of senescence-associated genes in carnation petals. J. Exp. Bot. 58: 2873–2885.

Iglesias, D.J., Tadeo, F.R., Primo-Millo, E., and Talon, M. (2006). Carbohydrate and ethylene levels related to fruitlet drop through abscission zone A in citrus. Trees (Berl.) 20: 348–355.

Knox, K., and Oparka, K. (2005). Identification and validation of reference genes for gene expression studies in postharvest rose flower (Rosa hybrida). Sci. Hortic. (Amsterdam) 158: 16–21.

Kühn, C., and Grof, C.P. (2010). Sugar transporters of higher plants. Curr. Opin. Plant Biol. 13: 288–295.

Lilley, J.L., Gee, C.W., Sairanen, I., Ljung, K., and Nemhauser, J.L. (2010). Sugar levels regulate tryptophan-dependent auxin biosynthesis in developing maize kernels. Plant Physiol. 153: 306–318.

La Rue, C.D. (1936). The effect of auxin on the abscission of petioles. Proc. Natl. Acad. Sci. USA 22: 254–259.

Lalonde, S., Wipf, D., and Frommer, W.B. (2004). Transport mechanisms for organic forms of carbon and nitrogen between source and sink. Annu. Rev. Plant Biol. 55: 341–372.

LeClerre, S., Schmelz, E.A., and Chourey, P.S. (2010). Sugar levels regulate tryptophan-dependent auxin biosynthesis in developing maize kernels. Plant Physiol. 153: 306–318.

Lewis, M.W., Leslie, M.E., and Lilijegren, S.J. (2006). Plant separation: 50 Ways to leave your mother. Curr. Opin. Plant Biol. 9: 59–65.

Lilley, J.L., Gee, C.W., Sairanen, I., Ljung, K., and Nemhauser, J.L. (2012). An endogenous carbon-sensing pathway triggers increased sucrose synthase activity and promoted grain filling of barley kernels. Funct. Plant Biol. 39: 997–1008.

Liu, C., Chen, X., Ma, P., Zhang, S., Zeng, C., Jiang, X., and Wang, W. (2018). Ethylene responsive factor MeERF72 negatively regulates sucrose synthase 1 gene in cassava. Int. J. Mol. Sci. 19: 19.

Lombardi, L., Arron, L., Mariotti, L., Battelli, R., Picciarelli, P., Kille, P., Stead, T., Munné-Bosch, S., and Rogers, H.J. (2015). Auxin involvement in tepal senescence and abscission in Lilium: A tale of two lilies. J. Exp. Bot. 66: 945–956.

Ma, C., Meir, S., Xiao, L., Tong, J., Liu, Q., Reid, M.S., and Jiang, C.Z. (2015). A KNOTTED1-LIKE HOMEOBOX protein regulates abscission in tomato by modulating the auxin pathway. Plant Physiol. 170: 907–920.

Mason, M.G., Ross, J.J., Babst, B.A., Wienclaw, B.N., and Beveridge, C.A. (2014). Sugar demand, not auxin, is the initial regulator of apical dominance. Proc. Natl. Acad. Sci. USA 111: 6092–6097.

Mehouachi, J., Serna, D., Zaragoza, S., Agusti, M., Talon, M., and Primomillo, E. (1995). Defoliation increases fruit abscission and reduces carbohydrate levels in developing fruits and woody tissues of Citrus unshiu. Plant Sci. 107: 189–197.

Meir, S., Philosoph-Hadas, S., Sundaresan, S., Selvaraj, K.S., Burd, S., Ophir, R., Kochanek, B., Reid, M.S., Jiang, C.Z., and Lers, A. (2010). Microarray analysis of the abscission-related transcriptome in the tomato flower abscission zone in response to auxin depletion. Plant Physiol. 154: 1929–1956.

Meng, Y.L., Li, N., Tian, J., Gao, J.P., and Zhang, C.Q. (2013). Increased sucrose synthase activity and promoted grain filling of barley kernels. Funct. Plant Biol. 27: 997–1008.

Möller, S., Croning, M.D., and Apweiler, R. (2001). Evaluation of methods for the prediction of membrane spanning regions. Bioinformatics 17: 646–653.

Naik, P.K., and Mohapatra, P.K. (2000). Ethylene inhibitors enhanced sucrose synthase activity and promoted grain filling of barley kernels. Funct. Plant Biol. 27: 997–1008.

Nelson, B.K., Cai, X., and Nebenfuhr, A. (2007). A multicolored set of in vivo organelle markers for co-localization studies in Arabidopsis and other plants. Plant J. 51: 1126–1136.

Nichols, R., and Ho, L.C. (1975). An effect of ethylene on the distribution of 14C-sucrose from the petals to other flower parts in the senescent cut inflorescence of Dianthus Caryophyllus. Ann. Bot. 39: 433–438.

Okushima, Y., Mitina, I., Quach, H.L., and Theologis, A. (2005). AUXIN RESPONSE FACTOR 2 (ARF2): A pleiotropic developmental regulator. Plant J. 43: 29–46.

Olsson, V., and Butenko, M.A. (2018). Abscission in plants. Curr. Biol. 28: R338–R339.

Pattarayavu, R., Ketsa, S., and van Doorn, W.G. (2013). Sucrose feeding of Cut Dendrobium inflorescences promotes bud opening, inhibits abscission of open flowers, and delays tepal senescence. Postharvest Biol. Technol. 77: 7–10.

Patterson, S.E. (2001). Cutting loose. Abscission and dehiscence in Arabidopsis. Plant Physiol. 126: 494–500.

Patterson, S.E., and Bleecker, A.B. (2004). Ethylene-dependent and -independent processes associated with floral organ abscission in Arabidopsis. Plant Physiol. 134: 194–203.

Payavula, R.S., Tsai, K.H., Tsai, C.J., and Harding, S.A. (2011). The sucrose transporter family in Populus: The importance of a tonoplast PtsSUT4 to biomass and carbon partitioning. Plant J. 65: 757–770.

Peng, D., Gu, X., Xue, L.J., LEEbens-Mack, J.H., and Tsai, C.J. (2014). Bayesian phylogeny of sucrose transporters: Ancient origins, differential expansion and convergent evolution in monocots and dicots. Front Plant Sci 5: 615.

Pun, U.K., Yamada, T., Azuma, M., Tanase, K., Yoshioka, S., Shimizu-Yumoto, H., Satoh, S., and Ichimura, K. (2016). Effect of sucrose on sensitivity to ethylene and enzyme activities and gene expression involved in ethylene biosynthesis in cut carnations. Postharvest Biol. Technol. 121: 151–158.

Reinders, A., Sivitz, A.B., and Ward, J.M. (2012). Evolution of plant sucrose uptake transporters. Front Plant Sci 3: 22.
Auxin Regulates Sucrose Transport in Abscission 3499

Riesmeier, J.W., Willmitzer, L., and Frommer, W.B. (1994). Evidence for an essential role of the sucrose transporter in phloem loading and assimilate partitioning. EMBO J. 13: 1–7.

Roberts, J.A., Elliott, K.A., and Gonzalez-Carranza, Z.H. (2002). Abscission, dehiscence, and other cell separation processes. Annu. Rev. Plant Biol. 53: 131–158.

Sakata, T., Oshino, T., Miura, S., Tomabechi, M., Tsunaga, Y., Higashitani, N., Miyazawa, Y., Takahashi, H., Watanabe, M., and Higashitani, A. (2010). Auxins reverse plant male sterility caused by high temperatures. Proc. Natl. Acad. Sci. USA 107: 8569–8574.

Sauer, N. (2007). Molecular physiology of higher plant sucrose transporters. FEBS Lett. 581: 2309–2317.

Sawicki, M., Alt Barka, E., Clément, C., Vaillant-Gaveau, N., and Jacquard, C. (2015). Cross-talk between environmental stresses and plant metabolism during reproductive organ abscission. J. Exp. Bot. 66: 1707–1719.

Sexton, R., and Roberts, J.A. (1982). Cell biology of abscission. Annu. Rev. Plant Biol. 33: 133–162.

Shi, Y., Evans, J.E., and Rock, K.L. (2003). Molecular identification of a danger signal that alerts the immune system to dying cells. Nature 425: 516–521.

Shin, J.H., Mila, I., Liu, M., Rodrigues, M.A., Vernoux, T., Pirrello, J., and Bouzayen, M. (2019). The RIN-regulated Small Auxin-Up RNA SAUR69 is involved in the unripe-to-ripe phase transition of tomato fruit via enhancement of the sensitivity to ethylene. New Phytol. 222: 820–836.

Sun, L., Sui, X., Lucas, W.J., Li, Y., Feng, S., Ma, S., Fan, J., Gao, L., and Zhang, Z. (2019). Down-regulation of the sucrose transporter CsSUT1 causes male sterility by altering carbohydrate supply. Plant Physiol. 180: 986–997.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28: 2731–2739.

Taylor, J.E., and Whitelaw, C.A. (2001). Signals in abscission. New Phytol. 151: 323–340.

Tucker, M.L., and Kim, J. (2015). Abscission research: What we know and what we still need to study. Stewart Postharvest Rev. 11: 1–7.

van Doorn, W.G. (2002). Effect of ethylene on flower abscission: A survey. Ann. Bot. 89: 689–693.

Verlinden, S., and Garcia, J.J. (2004). Sucrose loading decreases ethylene responsiveness in carnation (Dianthus caryophyllus cv. White Sim) petals. Postharvest Biol. Technol. 31: 305–312.

Wang, Q.L., Sun, A.Z., Chen, S.T., Chen, L.S., and Guo, F.Q. (2018). SPL6 represses signalling outputs of ER stress in control of panicle cell death in rice. Nat. Plants 4: 280–288.

Wei, Q., Ma, C., Xu, Y., Wang, T., Chen, Y., Lü, J., Zhang, L., Jiang, C.Z., Hong, B., and Gao, J. (2017). Control of chrysanthemum flowering through integration with an aging pathway. Nat. Commun. 8: 829.

Wieczorke, R., Krampe, S., Weierstall, T., Freidel, K., Hollenberg, C.P., and Boles, E. (1999). Concurrent knock-out of at least 20 transporter genes is required to block uptake of hexoses in Sakaromyces cerevisiae. FEBS Lett. 464: 123–128.

Wu, L., Ma, N., Jia, Y., Zhang, Y., Feng, M., Jiang, C.Z., Ma, C., and Gao, J. (2017). An ethylene-induced regulatory module delays flower senescence by regulating cytokinin content. Plant Physiol. 173: 853–862.

Yu, S., Cao, L., Zhou, C.M., Zhang, T.Q., Lian, H., Sun, Y., Wu, J., Huang, J., Wang, G., and Wang, J.W. (2013). Sugar is an endogenous cue for juvenile-to-adult phase transition in plants. eLife 2: e00269.

Yu, S.M., Lo, S.F., and Ho, T.D. (2015). Source-sink communication: regulated by hormone, nutrient, and stress cross-signaling. Trends Plant Sci. 20: 844–857.

Zhou, L., Jiang, J.C., Jones, T.L., and Sheen, J. (1998). Glucose and ethylene signal transduction crosstalk revealed by an Arabidopsis glucose-insensitive mutant. Proc. Natl. Acad. Sci. USA 95: 10294–10299.

Zhou, S.F., Sun, L., Valdés, A.E., Engström, P., Song, Z.T., Lu, S.J., and Liu, J.X. (2015). Membrane-associated transcription factor peptidase, site-2 protease, antagonizes ABA signaling in Arabidopsis. New Phytol. 208: 188–197.

Zhu, H., Dardick, C.D., Beers, E.P., Callahan, A.M., Xia, R., and Yuan, R. (2011). Transcriptomics of shading-induced and NAA-induced abscission in apple (Malus domestica) reveals a shared pathway involving reduced photosynthesis, alterations in carbohydrate transport and signaling and hormone crosstalk. BMC Plant Biol. 11: 138.
Auxin Regulates Sucrose Transport to Repress Petal Abscission in Rose (*Rosa hybrida*)
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