RESEARCH ARTICLE

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

Yue Liang, Chuyan Jiang, Yang Liu, Yuerong Gao, Jingyun Lu, Palinuer Aiwaili, Zhangjun Fei, Cai-Zhong Jiang, Bo Hong, Chao Ma, Junping Gao

State Key Laboratory of Agrobiotechnology, Beijing Key Laboratory of Development and Quality Control of Ornamental Crops, Department of Ornamental Horticulture, College of Horticulture, China Agricultural University, Beijing 100193, China.
Robert W. Holley Center for Agriculture and Health, United States Department of Agriculture, Agricultural Research Service, Ithaca, NY, 14853, USA.
Boyce Thompson Institute, Ithaca, NY, 14853, USA.
Crops Pathology and Genetic Research Unit, United States Department of Agriculture, Agricultural Research Service, Davis, CA, 95616, USA.
Department of Plant Sciences, University of California at Davis, Davis, CA, 95616, USA.
Corresponding Authors: mac@cau.edu.cn and gaojp@cau.edu.cn

Short title: Auxin regulates sucrose transport in abscission

One-sentence summary: Auxin modulates sucrose transport to repress petal abscission in rose, and that this process is regulated by a RhARF7-RhSUC2 module in the abscission zone.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantcell.org) is: Chao Ma (mac@cau.edu.cn) and Junping Gao (gaojp@cau.edu.cn).
ABSTRACT

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 sucrose deprivation; however, an underlying regulatory mechanism that links sucrose transport to organ shedding has yet to be identified. Here, we report that transport of sucrose 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 sucrose transport into the petals. Expression of the sucrose transporter *RhSUC2* decreased in the AZ during rose petal abscission. Similarly, silencing of *RhSUC2* reduced the sucrose 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 sucrose 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 sucrose delayed ethylene-induced abscission, while silencing of *RhARF7* and *RhSUC2* accelerated ethylene-induced petal abscission. Our results demonstrate that auxin modulates sucrose 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.

Sucrose can serve as the principal long-distance transport form of carbohydrates and energy (Riesmeier et al., 1994). Sucrose 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 sucrose distribution, and the associated signaling pathways regulate development. As an example in Arabidopsis (Arabidopsis thaliana), sucrose 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), sucrose is considered as the initial regulator of apical dominance (Mason et al., 2014), and in rose (Rosa sp.), sucrose mediates the light-mediated control of bud burst (Henry et al., 2011). A major decline in sucrose 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 (Gomez-Cadenas et al., 2000), and apple (Malus domestica) (Zhu et al., 2011). However, the significance of a reduced sucrose supply and the mechanism by which it is regulated have not been characterized.

Sucrose translocation is mainly mediated by two sucrose transporter families: Sucrose Carrier or Sucrose Transporter (SUC/SUT), and Sugar Will Eventually be Exported Transporter (SWEET). SUC proteins function as the principal mediators of long-distance sucrose transport, while SWEET proteins mainly play roles in
sucrose loading and unloading (Chen et al., 2012; Eom et al., 2015). SUC proteins have 12 transmembrane domains (Lalonde et al., 2004) and are classified into 3 types, based on their structure and function (Reinders et al., 2012; Peng et al., 2014). Dicot-specific Type I and monocot-specific Type IIB members are associated with sucrose loading and transport (Sauer, 2007), while Type III proteins localize to the tonoplast and plasma membrane and are thought to facilitate sucrose release (Payyavula et al., 2011). In Arabidopsis, the Type I member SUC2 localizes to the phloem and plays a key role in sucrose 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 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 (EIN2) (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 sucrose transport during rose petal abscission. We show that auxin regulates sucrose transport during petal abscission, and that a decrease in sucrose transport enhances ethylene sensitivity of the petal AZ. The molecular mechanism regulating this process is described.

RESULTS

Auxin Regulates Sucrose Transport during Petal Abscission

To investigate the roles of sucrose 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.

To gain a better understanding of sucrose dynamics during petal abscission, we used the fluorescent coumarin glucoside esculin, which is recognized by sucrose transporters (Chandran et al., 2003; Gora et al., 2012; Knox and Oparka, 2018), to simulate the transport of sucrose through the petal AZ. Images of longitudinal sections showed that the strength of the esculin signal in the area from the AZ to the petal was lower at stage 5 compared to stage 3 (Figure 1A). Transverse sections of petal AZs also revealed a much weaker esculin signal in the phloem of the AZ at stage 5 relative to stage 3 (Figure 1A). To investigate auxin distribution during petal abscission, we performed immunolocalization studies of longitudinal sections with an anti-indole-3-acetic acid (IAA) monoclonal antibody,
and observed a lower immunofluorescence signal in the area from the AZ to petal at stage 5 when compared to stage 3 (Figure 1B). Liquid chromatography-mass spectrometry (LC-MS)/MS analysis of endogenous sucrose and auxin contents in the AZ confirmed that their abundance significantly decreased in the AZ at stage 5 compared to stage 3 (Figures 1C and 1D).

We then examined the effects of applying exogenous sucrose or auxin to rose petals. Following sucrose treatment, the time from fully opened flowers (stage 5) to complete petal abscission was 7.6 ± 0.5 d, and 5.0 ± 0.7 d for the mock control (Figures 1E and 1F). To test whether this repressive effect of sucrose was dependent on sucrose hydrolysis or osmosis, we treated flowers with glucose or fructose as metabolic controls, and with mannitol as an osmotic control sugar (Supplemental Figures 2A and 2B). Sucrose application caused the most substantial delay in petal abscission relative to the other treatments, although glucose 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 sucrose 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 sucrose 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 (TIR1)/AUXIN-SIGNALING F-BOX (AFB) 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), while a combined treatment with sucrose 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 sucrose during petal abscission, we determined the effects of NAA treatment on the fluorescence signal resulting from staining with 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 sucrose treatment (Figure 1B). These results suggest that auxin regulates sucrose transport during petal abscission, but that sucrose does not affect the distribution of auxin.

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

To begin to elucidate the molecular mechanism behind long-distance sucrose 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-qPCR. RhSUC2 expression peaked at stage 3, before significantly decreasing at stage 5 (Supplemental Figure 4A), while 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 two-fold 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 sucrose uptake-deficient yeast strain SUSY7/ura3, which cannot grow efficiently on medium with sucrose 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% glucose, but only pDR196-RhSUC2 transformants grew well on medium with 2% sucrose (Figure 2C), suggesting that RhSUC2 is a functional sucrose transporter. Transmembrane domain prediction using TMHMM indicated that RhSUC2 contains 12 transmembrane domains (Figure 2D). Subcellular localization assays in Nicotiana benthamiana leaves showed that the RhSUC2-green fluorescent protein (GFP) signal overlapped with the signal derived from a plasma membrane marker protein tagged with mCherry (PM-marker, CD3-1007), indicating that RhSUC2 localizes to the plasma membrane (Figure 2E).

We then tested the consequences of RhSUC2 silencing using virus-induced gene silencing (VIGS). To this end, we inserted 534 bp of the RhSUC2 3′ untranslated region (UTR) into the pTRV2 VIGS vector. The RhSUC2-silencing construct reduced the expression of RhSUC2 in transformed petals compared to the TRV empty vector control (Figure 3A), while it did not alter the expression of the related genes RhSUC3 or RhSUC4 (Supplemental Figure 4B), confirming the specificity of the gene silencing. We noticed that the flower diameter and petal size of fully opened flowers were significantly smaller in RhSUC2-silenced plants compared to the TRV control plants (Figure 3C; Supplemental Figure 5A). In the RhSUC2-silenced plants, the time from fully opened flowers to abscission of all the petals was 3.8 ± 0.8 d, compared to 7.0 ± 0.7 d in the TRV control (Figures 3B and 3C). In addition, LC-MS/MS analysis showed that sucrose levels in the petals of
RhSUC2-silenced plants were lower than in the TRV control (Figure 3D). To further examine whether the decrease of sucrose levels in the RhSUC2-silenced petals was caused by suppression of sucrose transport, we simulated the transport of sucrose using esculin. We observed a dramatic decrease in esculin transport into the petals of the silenced plants compared to the TRV control (Figure 3E). Collectively, these results indicated that sucrose transport is associated with petal development and abscission.

We next tested the effects of NAA and NPA treatments on petal abscission in RhSUC2-silenced plants. In TRV control plants, NAA treatment strongly inhibited petal abscission, and maintained wilting status at the end of the experiment (15 d), while the time from fully opened flower to abscission of half of all petals was 5.8 ± 0.4 d in RhSUC2-silenced plants, although a few petals remained on flowers at the end of the experiment (Figure 4A and 4B). By contrast, NPA treatment did not affect the time taken by fully opened flowers to complete petal abscission in either RhSUC2-silenced or TRV control plants, with 3.0 ± 0.7 d and 3.6 ± 0.5 d in RhSUC2-silenced and TRV control plants, respectively (Figure 4A and 4C).

We also tested whether RhSUC3 and RhSUC4 function in petal abscission by silencing each separately using VIGS. RT-qPCR analysis confirmed the specificity of RhSUC3 and RhSUC4 silencing in plants (Supplemental Figure 6A). We observed no differences in the time of petal abscission for the RhSUC3- or RhSUC4-silenced plants when compared to the TRV control (Supplemental Figures 6B and 6C).

**RhARF7 Modulates Sucrose Transport by Regulating RhSUC2 Expression**

To investigate the regulation of RhSUC2, we analyzed 1,773 bp of the RhSUC2 promoter region upstream from the transcription start site using PLACE software (https://www.dna.affrc.go.jp/PLACE/?action=newplace). This analysis revealed a
number of cis-elements, including three auxin responsive cis-elements, AuxRE (TGTCTC), which are typical binding sites for auxin response factor (ARF) proteins (Figure 5A). We also analyzed the promoter regions of SUC2 in other plant species, including Arabidopsis, strawberry (Fragaria vesca), and peach (Prunus persica), and found that they all contained AuxRE elements (Figure 5A). We next identified ARF genes in the rose genome database (https://lipmbrowsers.toulouse.inra.fr/pub/RchiOBHm-V2/) with similarity to those reported as regulators of petal abscission in Arabidopsis (Ellis et al., 2005) (Supplemental Figure 7A). We cross-validated this gene list against their expression patterns in the rose petal AZ. Of the 7 candidate ARFs, the expression of RhARF7 and RhARF8 increased in the petal AZ by over 2-fold 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 sucrose treatment, and did not find any with > 2-fold change in expression (Figure 5C), indicating that sucrose 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 RhARF8 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 co-infiltrating 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, while it was 7.0 ± 0.7 d in TRV control plants (Figures 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 (Figure 4A and 4B). In addition, flowers treated with NPA showed similar times to in both genotypes, with 3.8 ± 0.4 d in RhARF7-silenced plants and 3.6 ± 0.5 d in the TRV control (Figure 4A and 4C). Sucrose 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 sucrose 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 (Figure 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 (Figure 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 sucrose and auxin on ethylene-induced rose petal abscission, we therefore treated flowers with sucrose 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 sucrose and auxin treatments had a greater
retardation effect compared to either treatment alone (Figure 6A; Supplemental video 1). 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- 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 up-regulated in RhSUC2 and RhARF7-silenced plants compared to TRV control plants (Supplemental Figure 8). These results indicate that sucrose 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 sucrose 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 sucrose transport during petal abscission in rose flowers.

The exogenous application of sucrose is well-known to suppress organ abscission or senescence in plants. In Dendrobium and pepper, sucrose feeding was reported to inhibit flower abscission (Aloni et al., 1997; Pattaravayo 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), while defoliation reduces carbohydrate levels and increases fruit abscission (Mehouachi et al., 1995). In this study, we determined the dynamics of sucrose transport during petal abscission in vivo, and showed that sucrose import into petals was impaired during petal abscission (Figures 1A and 1C). Sugar transport includes symplastic and apoplastic pathways, with sucrose transporters mainly functioning in the apoplastic pathway. SUC proteins with high-affinity sucrose activity serve in phloem loading, long-distance transport and unloading (Sauer, 2007; Kuhn and Grof, 2010). The Arabidopsis SUC2 transporter belongs to the high sucrose-affinity type and plays an essential role in sucrose 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 up-regulation 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 down-regulation of RhSUC2 expression during petal abscission, whereas the expression of RhSUC3 and RhSUC4 did not change (Supplemental Figure 4A). Notably, RhSUC2 expression exhibited a down-regulation in the AZ but not in the receptacle or petals (Figure 2A). Moreover, RhSUC2 silencing led to decreased sucrose 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), 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 sucrose on auxin metabolism and signaling has been described in previous studies. In Arabidopsis, sucrose application led to elevated auxin levels and increased polar auxin transport in seedlings (Lilley et al., 2012). Sucrose 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 (Le et al., 2010), and in rose, sucrose 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 sucrose had no effect on the distribution of auxin during petal abscission (Figure 1B), whereas auxin promoted sucrose transport during abscission (Figure 1A), indicating that auxin regulates sucrose 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 up-regulation in RhSUC2 expression by auxin in the petal AZ (Figure 2B). These differences may reflect distinct auxin-sucrose 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 sucrose 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
sucrose transporter CsSUT1 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), while 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-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 sucrose 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), sucrose 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, glucose enhanced the degradation of the transcription factor ETHYLENE INSENSITIVE 3 (EIN3), a key regulator in ethylene signaling (Zhou et al., 1998; Shi et al., 2003). Our results showed that sucrose treatment can suppress ethylene-induced petal abscission in rose (Figure 6A; Supplemental video 1), and that the strongest suppression of ethylene-induced petal abscission resulted from a co-treatment with auxin and sucrose (Figure 6A; Supplemental video 1). These results indicate that sucrose 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 sucrose may have other functions in petal abscission, bypassing
ethylene sensitivity during abscission.

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

In conclusion, our results demonstrate that auxin induces sucrose transport to repress petal abscission in the early stages of flower development. The reduction of auxin levels in the petal AZ leads to an attenuation of sucrose transport and promotes the initiation of petal abscission. In this process, the RhARF7-RhSUC2 module mediates the regulation by auxin of sucrose transport (Figure 7).

**METHODS**

**Plant Materials and Growth Conditions**

We used the abscission-prone rose cultivar R. 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 sucrose treatments, such that petal abscission was inhibited by auxin, and delayed by sucrose (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 (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, 40-60% relative humidity, and 100-120 μmol m⁻² 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, 60-80% relative humidity, 50-60 μmol m⁻² 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).

### Quantification of Endogenous Sucrose and Auxin Levels

We measured sucrose levels as previously described (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 with 1 mL 70%
(v/v) methanol. Following centrifugation at 10,000g for 10 min, we filtered the supernatants through a 0.22 μm membrane filter (SCAA-104, 0.22 μm pore size, ANPEL) for liquid chromatography tandem mass spectrometry (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-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% acetic acid) and acetonitrile (0.04% acetic acid); gradient program, 95:5 v/v at 0 min, 5:95 v/v at 11.0 min, 5:95 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 sucrose and 0.35 mL min⁻¹ for auxin; temperature, 40°C; injection volume: 2 μL for sucrose and 5 μL for auxin. The effluent was alternatively connected to an ESI-triple quadrupole-linear ion trap (Q TRAP)-MS. The electrospray ionization (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 sucrose and 35 psi for auxin; the collision gas (CAD) setting was high for sucrose and medium for auxin. Declustering potential (DP) and collision energy (CE) for individual multiple reaction monitoring (MRM) transitions was done with further DP and CE optimization. A specific set of MRM transitions was monitored for each period according to the metabolites eluted.
within this period.

**Esclulin Feeding and Confocal Microscopy**

We diluted esclulin hydrate (Sigma-Aldrich) to 10 mM in deionized water. We performed vacuum infiltration by immersing leaves adjacent to the rose flower in esclulin solution under vacuum at 0.7 MPa. We sectioned petal AZ by hand with a razor blade 10 h after esclulin treatment and immediately immersed the sections in 80% (v/v) glycerol before mounting on glass slides. We recorded fluorescence using a laser scanning confocal microscope (Olympus FV1000, Japan), 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-dimethylamino(propyl))-carbodiimide hydrochloride (EDAC, Sigma-Aldrich) at room temperature and transferred them to 4% paraformaldehyde (Sigma-Aldrich) and 2% glutaraldehyde (Sigma-Aldrich). We dehydrated the samples using a gradient series of ethanol solutions (30%, 50%, 70%, 80%, 90%, 100%, v/v). After dehydration, we transferred the samples to xylene and then paraplast (Thermo Scientific), each for 1 h, followed by embedding in 100% paraplast. We cut the embedded samples into 10 μm sections using a microtome (HistoCore BICUT, 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 laser scanning confocal microscope (Leica SP8, Germany), with an excitation
wavelength of 488 nm and emission wavelength of 518 nm. The negative controls were specimens not incubated with the anti-auxin antibody.

**RNA extraction and RT-qPCR**

We extracted total RNA using the hot borate method as previously described (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 sec at 95ºC and annealing for 30 sec at 60ºC) using the Step One Plus™ real-time PCR system (Applied Biosystems) with KAPATM SYBR® FAST quantitative PCR kits (Kapa Biosystems). We used RhUBI2 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.

**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 Sall/Spel 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 28ºC, 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 OD$_{600}$ = 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 microscopy (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 587 nm laser and emission with a 575-675 nm band pass filter. The primers used in this study are listed in Supplemental Data Set 1.

**Virus Induced Gene Silencing (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 (LB) medium containing 10 mM MES (pH 6.3), 20 μM acetosyringone, 50 μg mL⁻¹ kanamycin, and 50 μg mL⁻¹ gentamycin sulfate. We collected the cells by centrifugation at 2,057g for 10 min, and resuspended the pellet in infiltration buffer (10 mM MgCl₂, 10 mM MES, 200 μM acetosyringone, pH 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 mixtures 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 vermiculite and nutritive soil (1:1). Prior to further functional analyses, we measured the expression of the target genes (RhSUC2 or RhARF7) by RT-qPCR in the petals at flower opening stage 2 in each plant. The plants with down-regulated target gene expression were used for further assays. Three independent experiments were performed with 50 plants in each experiment.

**Transplant 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. Prior to further functional analyses, we first measured the accumulation of GFP transcripts in the petals at the flower opening stage 2 of inoculated plants by semi-quantitative RT-PCR. We first identified successfully inoculated plants by PCR for the presence of the GFP transgene. We then measured the expression of the target genes (RhSUC2 or RhARF7) in the same petals of these successfully inoculated plants by RT-qPCR. Plants with up-regulated RhSUC2 and downregulated RhARF7 expression were used for further assays.

**Sucrose Uptake Assay**

We cloned the ORF for RhSUC2 into the yeast expression vector pDR196 and transformed the resulting construct into the sucrose 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) with
2% glucose or 2% sucrose 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 ng 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 (Kong et al., 2015; 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 acetosyringone, 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 CDD camera (CHEMIPROHT 1300B/LND, 16 bits, Roper Scientific) at –110°C.

For dual luciferase reporter assays, we co-infiltrated 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 (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.

**Electro Mobility Shift Assay (EMSA)**

We performed EMSA assays according to the instructions of the Light Shift chemiluminescent EMSA kit (Thermo 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 production of the GST-RhARF7 fusion protein by the addition of 0.6 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) in 100 mL LB 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 (https://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: 1,000 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/) (Moller et al., 2001).

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 (HO762758), 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 RhSUC3 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 1. 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; J.L., Y. Liu, P.A., and Y.G. contributed to the VIGS assay; C.J. contributed to the immunolocalization assay; C.Z.J., Z.F., 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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Figure 1. Auxin Regulates Sucrose Transport During Petal Abscission.

(A) Simulation of sucrose transport, using esculin fluorescence, in the petal abscission zone (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. Scale 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 sucrose (SUC) at stage 3 of flower opening. Immunofluorescence assays were performed using an anti-IAA monoclonal antibody to investigate auxin distribution.

(C) Sucrose and (D) IAA levels in the petal AZ at stage 3 and stage 5 of flower opening, as measured by liquid chromatography-mass spectrometry (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) Phenotypes and (F) rose petal abscission time following different treatments. Statistical results are the means of five flower samples harvested from different plants with standard deviations. SUC, 50 mM sucrose; NAA, 100 μM 1-naphthaleneacetic acid; NPA, 100 μM N-1-naphthylphthalamic acid; 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). Scale bar, 5 cm.
Figure 2. Characterization of RhSUC2.

(A, B) Expression of RhSUC2 in different tissues during flower opening (A), or in the petal abscission zone (AZ) at stage 3 of flower opening treated with 100 μM NAA for 24 h (B), based on RT-qPCR analysis. RhUBI2 was used as the internal control. Letters indicate significant differences according to Tukey-Kramer test (P<0.05). Asterisks indicate statistically significant differences between NAA treatment and mock determined by two-tailed Student’s t-test (**P<0.01).

(C) RhSUC2 functions as a sucrose transporter, and can rescue the growth of the sucrose uptake-deficient yeast strain SUSY7/ura3 on 2% sucrose medium. The empty vector pDR196 was used as control, and 2% glucose served as the medium control.

(D) Transmembrane domains of RhSUC2, as predicted by TMHMM.

(E) Subcellular localization of RhSUC2 in Nicotiana benthamiana leaves. Green fluorescent protein (GFP) was fused to the C terminus of RhSUC2. A mCherry-labelled plasma membrane marker (PM-marker, CD3-1007) was co-infiltrated with RhSUC2-GFP to indicate membrane localization. Scale bars, 100 μm.
Figure 3. Silencing of RhSUC2 and RhARF7 Promotes Petal Abscission.

(A) Expression of RhSUC2 and RhARF7 in the petals of TRV control plants and silenced plants: RhSUC2-silenced (TRV-RhSUC2), RhARF7-silenced (TRV-RhARF7), RhSUC2 and RhARF7 double silenced (TRV-RhSUC2+RhARF7), and RhSUC2 overexpression in RhARF7-silenced (TRV-RhARF7+RhSUC2OX) plants. RhUBI2 was used as the internal control.

(B) Time from fully opened flowers to abscission of all the petals in TRV control and silenced plants.

(C) Petal abscission phenotypes of TRV control and different silenced plants, recorded daily. Scale bars, 5 cm.

(D) Sucrose levels in the petals of RhSUC2-, RhARF7-silenced and TRV control plants at stage 5 of flower opening.

(E) Simulation of sucrose transport, using esculin fluorescence, in the petal AZ at stage 3 of RhSUC2- and RhARF7-silenced and TRV control plants. Images were captured by laser scanning confocal microscopy. Arrows indicate the petal abscission zone; P, petal; R, receptacle. Scale bars, 100 μm.

For (A, B, D) results are the means of five biological replicates from different plants with standard deviations. Letters indicate significant differences according to Tukey-Kramer test (P<0.05). Asterisks indicate statistically significant differences between TRV-RhSUC2 or TRV-RhARF7 and TRV determined by Dunnett test (**P<0.01).
Figure 4. The Effects of Exogenous NAA and NPA Treatments on Petal Abscission of RhSUC2- and RhARF7-Silenced Plants.

(A) Petal abscission phenotypes in RhSUC2-, RhARF7-silenced and TRV control plants treated with 10 μM NAA or 100 μM NPA, recorded daily. Scale bars, 5 cm.

(B) Time from fully opened flowers to abscission of half of all petals upon NAA treatment in RhSUC2-, RhARF7-silenced and TRV control plants. N.A., no abscission.

(C) Time from fully opened flowers to abscission of all the petals upon NPA treatment in RhSUC2-, RhARF7-silenced and TRV control plants. Results are the means of five biological replicates from different plants with standard deviations. Asterisks indicate statistically significant differences between TRV-RhSUC2 or TRV-RhARF7 and TRV determined by Dunnett test (**P<0.01).
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).

(B, C) Expression of ARF genes in the petal abscission zone (AZ) during flower opening and abscission (B), or at stage 3 of flower opening after 50 mM sucrose 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 35S 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 electrophoretic mobility shift assays (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 sucrose treatment and mock determined by two-tailed Student’s *t*-test (***P*<0.01).
Figure 6. The Effects of Sucrose and Auxin on Ethylene-Induced Petal Abscission.

(A) Timeline of petal abscission after co-treatment with ethylene and different solutions. SUC, 50 mM sucrose; NAA, 100 μM 1-naphthaleneacetic acid. Statistical significance between different treatments and mock was determined by Dunnett test (**P<0.01).

(B) Timeline of petal abscission in response to ethylene in RhSUC2-, RhARF7-silenced (TRV-RhSUC2, TRV-RhARF7) and TRV control plants. Statistical significance between TRV-RhSUC2 or TRV-RhARF7 and TRV was determined by Dunnett test (**P<0.01).

(C) Expression of RhSUC2 and RhARF7 in the petal abscission zone (AZ) at stage 3 following ethylene (ETH) treatment, as determined by RT-qPCR. RhUBI2 was used as the internal control. The differential significances between ethylene treatment and mock were determined by two-tailed Student's t-test (**P<0.01).

(D) Simulation of sucrose transport, using esculin fluorescence, in the petal AZ at stage 3 under ethylene treatment. Scale bar, 100 μm.

(E) Immunolocalization of IAA in the petal AZ at stage 3 following 12 h ethylene treatment. Immunofluorescence assays were performed by probing with an anti-IAA monoclonal antibody. Arrows indicate the petal AZ; P, petal; R, receptacle. Scale bar, 100 μm.
Figure 7. Model of auxin and sucrose interaction during petal abscission.
During the early developmental stages of flower opening, auxin and sucrose are transported from the receptacle to the petal through the petal abscission zone via the phloem to repress petal abscission. In this process, \textit{RhARF7} mediates the regulation by auxin of sucrose transport by affecting the transcription of the \textit{RhSUC2} sucrose transporter.
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