A *DELLA* gene, *RhGAI1*, is a direct target of EIN3 and mediates ethylene-regulated rose petal cell expansion via repressing the expression of *RhCesA2*

Jing Luo, Nan Ma, Haixia Pei, Jiwei Chen, Jing Li and Junping Gao*

Department of Ornamental Horticulture, China Agricultural University, Beijing 100193, China

* To whom correspondence should be addressed. E-mail: gaojp@cau.edu.cn

Received 28 May 2013; Revised 2 August 2013; Accepted 6 August 2013

Abstract

Ethylene plays an important role in organ growth. In *Arabidopsis*, ethylene can inhibit root elongation by stabilizing *DELLA* proteins. In previous work, it was found that ethylene suppressed cell expansion in rose petals, and five unisequences of *DELLA* genes are induced by ethylene. However, the mechanism of transcriptional regulation of *DELLA* genes by ethylene is still not clear. The results showed that the expression of *RhGAI1* was induced in both ethylene-treated and *ETR1* gene-silenced rose petals, and the promoter activity of *RhGAI1* was strongly induced by *RhEIN3-3* in *Arabidopsis* protoplasts. What is more, *RhEIN3-3* could bind to the promoter of *RhGAI1* directly in an electrophoretic mobility shift assay (EMSA). Cell expansion was suppressed in *RhGAI1*-Δ*17*-overexpressed *Arabidopsis* petals and promoted in *RhGAI1*-silenced rose petals. Moreover, in *RhGAI1*-silenced petals, the expression of nine cell expansion-related genes was clearly changed, and *RhGAI1* can bind to the promoter of *RhCesA2* in an EMSA. These results suggested that *RhGAI1* was regulated by ethylene at the transcriptional level, and *RhGAI1* was a direct target of *RhEIN3-3*. Also, *RhGAI1* was shown to be involved in cell expansion partially through regulating the expression of cell expansion-related genes. Furthermore, *RhCesA2* was a direct target of *RhGAI1*. This work uncovers the transcriptional regulation of *RhGAI1* by ethylene and provides a better understanding of how ethylene regulates petal expansion in roses.

Key words: Cell expansion, ethylene, petal, *RhGAI1*, rose, transcriptional regulation.

Introduction

Rose is one of the most important commercial flowers worldwide. In China, 4.1 billion stems of cut roses were sold in 2010, accounting for 38% of total sales of cut flowers (data from the Ministry of Agriculture of China). Cut flowers of roses are usually harvested at the bud stage, which is helpful in reducing physical damage to the petals in post-harvest handling, such as packing and transportation. It is reported that post-harvest loss of cut roses is mainly caused by ethylene, which can result in abnormal flower opening (Ma et al., 2006; Xue et al., 2008). Therefore, investigation of the underlying mechanism of how ethylene regulates rose flower opening has been an important issue in post-harvest biology of ornamental plants worldwide.

Ethylene plays an important role in multiple aspects of plant growth and development, including seed germination, seedling growth, flower opening, fruit ripening, and senescence (Zhao and Guo, 2011). In regulation of plant organ growth, ethylene can function in opposite ways (Pierik et al., 2006). Ethylene plays an important role in multiple aspects of plant growth and development, including seed germination, seedling growth, flower opening, fruit ripening, and senescence (Zhao and Guo, 2011). In regulation of plant organ growth, ethylene can function in opposite ways (Pierik et al., 2006). In most cases, ethylene inhibits organ growth, such as root elongation (Swarup et al., 2007), hypocotyl...
elongation, and cotyledon expansion (Hall and Bleecker, 2003) in Arabidopsis, and internode elongation in rice (Qi et al., 2011). In some other cases, ethylene stimulates organ growth, such as cell elongation in cotton fibre (Shi et al., 2006) and internode elongation in rice in response to deep water (Hattori et al., 2009). However, the mechanisms underlying petal growth regulation by ethylene are not well understood.

Transcription factors are widely involved in phytohormone signalling, and regulate plant growth and development. In terms of ethylene-regulated organ growth, BOLITA, an AP2/ERF1 transcription factor, represses both cell proliferation and cell expansion in Arabidopsis (Marsch-Martinez et al., 2006).

DELLA proteins, a subfamily of GRAS transcription factors, also play important roles in plant growth and development. In Arabidopsis, there are five DELLA proteins: GA INSENSITIVE (GAI), REPRESSOR OF ga1-3 (RGA), and three RGA-LIKE proteins (RGL1, RGL2, and RGL3) (Harberd et al., 2009). Among them, GAI and RGA have high redundancy in their function, and they both repress gibberellic acid (GA)-regulated organ growth (Wang et al., 2009).

Plant organ growth is coordinated in two processes, cell proliferation and cell expansion (Geitmann and Ortega, 2009; Križek, 2009). In leaf growth of Arabidopsis, DELLA proteins not only inhibit the cell division rate during the proliferation phase, but also repress the cell expansion rate during the expansion phase (Achard et al., 2009; Gonzalez et al., 2012). To date, however, whether the DELLA proteins are involved in petal expansion remains unclear.

Petal growth depends on cell division during early development stages, but is regulated by cell expansion during late flower development (Varaūd et al., 2011). In Arabidopsis, it undergoes the transition from cell division to cell expansion during stage 9–11 of flower opening. After stage 12, flower opening is mainly attributed to cell expansion (Irish, 2008). In cut roses, flowers are harvested commercially at stage 2—the bud-opened stage—and the petal expansion depends on cell expansion (Yamada et al., 2009).

Cell expansion is accompanied by modification of the cell wall, changes of turgor pressure, and remodelling of the cytoskeleton (Winship et al., 2010). It is reported that ethylene influences cell expansion partly through regulating the expression of cell expansion-related genes. Ethylene induces the expression of XTH and EXPANSIN7, which results in cell wall loosening and root hair initiation in Arabidopsis (Sánchez-Rodríguez et al., 2010). Ethylene can repress the expression of an aquaporin gene, RhPIP2;1; thus, it partly contributes to the inhibition of water absorption and cell expansion in rose petals (Ma et al., 2008). However, to date, how these genes are regulated by ethylene and whether DELLA genes mediate this process remains unclear.

Here, an ethylene-responsive DELLA gene, RhGAI1, was isolated through microarray analysis. The results indicated that RhGAI1 was regulated by ethylene at the transcriptional level, and RhGAI1 was a direct target of RhEIN3-3. In addition, RhGAI1 was found to be involved in cell expansion partly through regulating the expression of several cell expansion-related genes, and RhCesA2 was a direct target of RhGAI1.

### Materials and methods

#### Plant materials and growth conditions

The flowers of cut roses (Rosa hybrida) cv. Samantha were harvested at stage 2 from the greenhouse, and transported to the laboratory within 1 h. The flower stems were cut to 25 cm length in distilled water and then placed in vases with distilled water. The ethylene and 1-methylcyclopropene (1-MCP) treatment were according to Ma et al. (2008).

Seeds of Arabidopsis thaliana were surface sterilized and sown on Murashige and Skoog (MS) medium. After vernalization, the seeds were transferred to a growth chamber for 7 d. The 7-day-old seedlings were transplanted into growth mixture (vermiculite/nutritive soil=1:1). For protoplast extraction, the wild-type (Col-0) seedlings were grown under conditions with a photoperiod of 12 h light/12 h dark at 23 °C, low light intensity (50–75 mE m$^{-2}$ s$^{-1}$), and a relative humidity of 40–60%.

#### Plasmid construction and plant transformation

For silencing of RhGAI1, a 321 bp RhGAI1 3’-untranslated region sequence was amplified from the cDNA sample of rose flowers, and inserted into the multiple cloning site (MCS) of pTRV2 to construct the pTRV2-RhGAI1 vector. For silencing of RhEIN3 genes, a fragment of 852 bp, possessing the conserved domain of RhEIN3, RhEIN3, and RhETR5, was amplified from rose cDNA, and inserted into pTRV2 to construct the pTRV2-RhETRs vector. The silencing of RhGAI1 and RhETRs genes in petals by virus-induced gene silencing (VIGS) was performed according to the procedures described by Dai et al. (2012).

The pSuper::GFP-RhGAI1-A17 vector was constructed by overlapping PCR. In RhGAI1-A17, the 51 bp fragment encoding the DELLA domain was deleted, mimicking the mutation of gai. The recombinant pSuper::GFP-RhGAI1-A17 vector was transformed into Agrobacterium strain GV3101 and then introduced into Arabidopsis plants using the floral dip method (Clough and Bent, 1998).

To obtain the pUC-35S mini::GUS vector, the sequence harbouring the 35S mini promoter and β-glucuronidase (GUS) gene was excised from the pHBI-89 vector (Liu et al., 2003), and subcloned into the pUC19 vector. The promoter regions of RhCesA2 and RhPIP2;1 were inserted into the pUC-35S mini::GUS vector to replace the 35S mini promoter, and the pUC-proRhCesA2::GUS and pUC-proRhPIP2;1::GUS reporter vectors were generated.

For prokaryotic expression of RhEIN3-3 and RhGAI1, the N-terminus (amino acids 141–356) containing the binding domain of RhEIN3-3 (Shi et al., 2012) and the C-terminus (amino acids 238–618) containing the binding domain of RhGAI1 (Hirano et al., 2010) were inserted into the pGEX-2T vector.

#### RNA extraction and quantitative real-time PCR (qRT-PCR) analysis

The total RNA of rose petals was extracted using the hot borate method, according to Ma et al. (2006). The primer sequences are listed in Supplementary Table S1 available at JXB online. RhACT5 was used as the internal control. The qRT-PCR analysis was done with the KAPA SYBR® FAST qPCR Kits (KAPA BIOSYSTEMS, USA) on an ABI7500 machine (Applied Biosystems by Life Technologies, USA).

#### Microscopic examination and cell counting

Observation of abaxial subepidermis (AbsE) cells was performed according to Ma et al. (2008). The number of AbsE cells in a 261 × 261 μm area was counted and statistical analysis was performed with SPSS software.

For Arabidopsis, petals of flowers at stage 14 (Smyth et al., 1990) were fixed by FAA and used to count cell numbers. The number of AbsE cells in a 261 × 261 μm area was counted, and statistical analysis was performed with SPSS software.
Sequence analysis
Alignment of the deduced amino acid sequence was performed using ClustalX (default values were used) and DNAMAN (default values were used), and phylogenetic analysis was performed using ClustalX and MEGA. The phylogenetic trees were computed using the Neighbor–Joining algorithm with 10 000 bootstrap replicates.

Subcellular localization
The pSuper::GFP-RhGAI1 and pSuper::GFP vectors were bombarded into onion bulb scale epidermal cells. After incubation in the dark at 23 °C for 24 h, the onion epidermis was stained with 2.5 μg ml⁻¹ 4',6-diamidino-2-phenylindole (DAPI) for 30 min. Fluorescence signals were detected using a Nikon T1 confocal laser-scanning microscope (Nikon, Japan). The excitation wavelengths for green fluorescent protein (GFP) and DAPI were 488 nm and 408 nm, respectively, and the emission filter wavelengths were 505–530 nm for GFP and 420–480 nm for DAPI.

Transient expression in Arabidopsis mesophyll protoplasts
The Arabidopsis mesophyll protoplasts were prepared according to Yoo et al. (2007). The plasmids were extracted with MACHEREY-NAGEL kits; 10 μg of effector plasmid and 10 μg of reporter plasmid were introduced into ~2 × 10⁶ protoplasts with polyethylene glycol (PEG). The GUS activity was determined according to Li et al. (2009).

Electrophoretic mobility shift assay (EMSA)
The EMSA was performed according to Wang et al. (2011). Recombinant pGEX-RhEIN3-3 and pGEX-RhGAI1 proteins were produced in Escherichia coli strain BL21. The E. coli cells were lysed by sonication, and purified with glutathione-Sepharose 4B beads (GE Healthcare).

The proRhGAI1 probe 5'-CGTTTTTATACATTTCTTGGATTAA-3’, the proRhCesA2 probe 5'-GCAGAGGGCTAA TTAGTGCTGGAGAT-3’, and their complementary probes were labelled with biotin. A 1 μg aliquot of recombinant protein and 2 nM biotin-labelled probe were used for the binding reaction for each sample. The LightShift chemiluminescent EMSA kit (Pierce, IL, USA) was used for EMSA.

Results
Isolation of RhGAI1
In order to investigate the molecular mechanism underlying the ethylene-regulated flower opening in rose, a microarray database (http://bioinfo.bti.cornell.edu/rose) was built. Five out of eight unsequences, annotated as DELLA proteins, were found to be significantly up-regulated by ethylene at early times (Supplementary Table S2 at JXB online).

By using rapid amplification of cDNA ends (RACE), these five unsequences were found to be different parts of the same gene, which encoded a DELLA protein with 618 amino acid residues. Phylogenetic analysis showed that this DELLA protein had high homology with MdGAI1, MhGAI1, and AtGAI. Therefore, the gene was named RhGAI1 (GenBank accession no. AGK07287) (Supplementary Figs S1, S2 at JXB online). The pSuper::GFP-RhGAI1 plasmid was then transferred into onion (Allium cepa) epidermal cells by particle bombardment. The result indicated that RhGAI1 was located in the nucleus (Fig. 1A).

RhGAI1 is transcriptionally regulated by ethylene
The expression of RhGAI1 in petals was determined by qRT-PCR. The results showed that, compared with untreated control, ethylene significantly enhanced the expression of RhGAI1 by 1.9-fold at 1 h and by 2.75-fold at 6 h. 1-MCP, an inhibitor of ethylene action, significantly reduced the expression of RhGAI1 within 12 h of treatment (Fig. 1B).

Ethylene receptors (ETRs) function as negative regulators in ethylene signalling, and five members have been identified in rose flowers (Tan et al., 2006). To check whether RhGAI1 acted downstream of ethylene signalling, the RhETR family was silenced in rose petals through the VIGS approach. The expression of RhGAI1 was clearly increased in RhETR gene-silenced petals when compared with the TRV control (Fig. 2A). The results above suggested that RhGAI1 was regulated by ethylene at a transcriptional level.

Through sequence analysis of the 1507 bp promoter sequence of RhGAI1, an EIN3 binding site (EBS) ‘AATTCCAA’ was found in the region from −518 bp to −511 bp of proRhGAI1 (Fig. 3A).

EIN3 is known as the primary transcription factor in ethylene signalling (Zhao and Guo, 2011). Based on previous transcriptome sequencing, seven unsequences of EIN3 genes were obtained (Supplementary Table S3 at JXB online). The unsequence RU03799, which had higher homology with EIN3 in other plants, was cloned by RACE. The full length of RU03799 was 1857 bp, encoding a deduced protein with 618 amino acids. Phylogenetic analysis showed that the deduced
protein had higher homology with CsEIN3 and RcEIN3, and the gene was named RhEIN3-3 (GenBank accession no. AGK07288) (Supplementary Fig. S3), since two rose EIN3 genes, RhEIN3-1 and RhEIN3-2, have been deposited in GenBank.

To test whether RhGAI1 was regulated by RhEIN3-3, a transactivation test was performed in Arabidopsis mesophyll protoplasts. The promoter activity of RhGAI1 was strongly induced by RhEIN3-3, compared with the vector control (Fig. 2B). These results indicated that RhGAI1 might be a downstream gene of EIN3-3.

Further, an EMSA was carried out to test the direct binding of proRhGAI1 and RhEIN3-3. The biotin-labelled probe was designed according to the EBS element in the promoter of RhGAI1 (Fig. 3A). The EMSA result showed that RhEIN3-3 could bind to the biotin-labelled probe of proRhGAI1. Also, this binding was attenuated gradually by increasing the concentration of unlabelled probe, whereas it could not be competed by the mutated probe (Fig. 3B). This result indicated that RhGAI1 was a direct target of RhEIN3-3.

RhGAI1 is involved in cell expansion in both Arabidopsis and rose petals

To understand whether RhGAI1 functions in cell expansion, RhGAI1 was overexpressed in Arabidopsis, and the function of RhGAI1 in expansion of petals was investigated. As DELLA proteins are easily degraded by GA, gain-of-function mutagenesis of RhGAI1 was carried out, and RhGAI1-Δ17 was obtained, in which 17 degradation-related amino acids in the DELLA domain were deleted (Dill et al., 2001). Compared with the vector control, the expansion of petals and AbsE cells was markedly repressed in RhGAI1-Δ17-overexpressing (OX) lines (Fig. 4A, B, E). In a 261 × 261 μm area of petals, the average cell number was 308.3 ± 18.7 in the vector control, and 388.6 ± 15.7 and 402.7 ± 20.5 in OX-1 and OX-2 petals, respectively (Fig. 4D). These results indicated that RhGAI1 was involved in inhibition of cell expansion in petals of Arabidopsis.

RhGAI1 was also silenced in rose petals by VIGS. The expansion of petals was substantially promoted in RhGAI1-silenced samples when compared with the TRV control (Fig. 5A, E). The average size in control petals was...
RhGA11 regulates petal expansion in rose petals. (A) Petal and (B) AbsE cell phenotype of RhGA11-silenced samples and the TRV control. Determination of (C) the petal area and (D) cell numbers in RhGA11-silenced samples and the TRV control. Each plot represents the mean ±SD, n=18 for both. Student's t-test, **P < 0.01. (E) qRT-PCR analysis of RhGA11 in silenced petals and the TRV control. The results show the mean ±SD from three biological replicates.

10.52 ± 1.02 cm², and 12.72 ± 0.80 cm² in RhGA11-silenced petals (Fig. 5C). In a 388 × 388 μm area of petals, the average cell number was 1032.3 ± 12.23 in control petals, and 107.5 ± 4.8 in RhGA11-silenced petals (Fig. 5B, D). These results indicated that RhGA11 was involved in repression of cell expansion in rose petals.
RhGAI1 regulates several cell expansion-related genes

In order to explore the possible mechanism of RhGAI1-regulated petal expansion in rose, 25 cell expansion-related genes were selected from the microarray database (Supplementary Table S4 at JXB online), and their expression was checked by qRT-PCR in RhGAI1-silenced rose petals. Among them, three genes were up-regulated, six genes were down-regulated, and the remaining 16 genes were unchanged (Fig. 6). The three up-regulated genes were RhCesA2, RU03736, and RU25443. The six down-regulated genes were RU23321, RU06171, RU10722, RU20002, RU20999, and RU06247.

To test whether the genes whose expression was changed in RhGAI1-silenced rose petals were downstream genes of RhGAI1, a transactivation analysis was performed in Arabidopsis mesophyll protoplasts. Since the expression of RhCesA2 was apparently enhanced in RhGAI1-silenced petals, while the expression of RhPIP2;1 was not clearly changed compared with the TR V control, the promoters of RhCesA2 and RhPIP2;1 were used to construct the reporters (Fig. 7A). The promoter activity of RhCesA2 was strongly repressed by RhGAI1, while the promoter activity of RhPIP2;1 was barely changed by RhGAI1 (Fig. 7B).

RhCesA2 is a direct target of RhGAI1

It is reported that a GRAS transcription factor NSP1 can bind to the AATTT element in the promoter of OND11 by EMSA during nodulation signalling in Medicago truncatula (Hirsch et al., 2009). Since DELLA proteins are a subfamily of GRAS transcription factors, and the binding region in the GRAS domain is conserved among GRAS transcription factors, AATTT may also be the binding site of DELLA proteins.

Through sequence analysis of the RhCesA2 promoter, six putative AATTT elements were found to be located in the promoter of RhCesA2 (Supplementary Fig. S4A at JXB online). After truncation, two fragments, proRhCesA2 D1 and proRhCesA2 D2, were generated. The results of a transactivation assay in protoplasts showed that the activities of proRhCesA2 D1 and proRhCesA2 D2 dropped to 51% and 46%, respectively, when compared with proRhCesA2. In addition, these fragments were also strongly repressed by RhGAI1.

Fig. 6. qRT-PCR analysis of 25 cell expansion-related genes in RhGAI1-silenced rose petals. RhACT5 was used as internal control. The results show the mean ±SD from three biological replicates. The relative expression of the 25 genes in the TRV control was defined as 1.
RhGAI1 regulates petal expansion in rose by RhGAI1 (Supplementary Fig. S4B). These results suggested that there were important binding sites in \textit{proRhCesA2} D2. Therefore, a second truncation was carried out to determine the positive binding site, and \textit{proRhCesA2} D3 and \textit{proRhCesA2} D4 were obtained (Supplementary Fig. S4A). The promoter activities of these fragments were gradually reduced, and still notably repressed by RhGAI1 (Supplementary Fig. S4C). These results indicated that \textit{RhCesA2} might be a downstream gene of \textit{RhGAI1}, and the AATTT element in \textit{proRhCesA2} D4 might partly account for the repression of the promoter activity of \textit{RhCesA2} by RhGAI1.

To verify whether RhGAI1 can bind directly to the promoter of \textit{RhCesA2}, an EMSA was carried out. The biotin-labelled probe was designed according to the AATTT element in \textit{proRhCesA2} D4 (Fig. 8A). The EMSA result showed that RhGAI1 could bind to the biotin-labelled \textit{proRhCesA2} probe. Also, this binding diminished gradually with an increasing concentration of unlabelled probe, whereas it could not be competed by the mutated probe (Fig. 8B), indicating that \textit{RhCesA2} is a direct target of RhGAI1.

The promoters of \textit{RU25443} and \textit{RU10722} were also cloned, and it was found that there were eight putative AATTT elements in the 1255 bp promoter region of \textit{RU25443} and two putative AATTT elements in the 1347 bp promoter region of \textit{RU10722}. The promoter activity of \textit{RU25443} was strongly repressed by RhGAI1, while the promoter activity of \textit{RU10722} was induced by RhGAI1 (Supplementary Fig. S5 at JXB online), which was consistent with the expression level of \textit{RU25443} and \textit{RU10722} in \textit{RhGAI1}-silenced petals. They might also be downstream genes of \textit{RhGAI1}. It is speculated that RhGAI1 may function in cell expansion through directly targeting multiple downstream genes related to cell expansion.

### Discussion

**RhGAI1 is regulated by ethylene at the transcriptional level in rose petals**

It is well known that ethylene regulates plant organ growth, such as elongation of root and hypocotyls in \textit{Arabidopsis} and internode elongation in rice (Hall and Bleecker, 2003; Swarup et al., 2007; Qi et al., 2011). In addition, DELLA proteins are involved in regulation of organ growth, including cotyledon expansion and hypocotyl elongation in \textit{Arabidopsis} (de Lucas et al., 2008; Josse et al., 2011). In terms of involvement of DELLA proteins in ethylene-regulated organ growth, it is reported that ethylene inhibits root elongation by enhancing the stability of DELLA proteins (Achard et al., 2003). In the present work, a DELLA gene, \textit{RhGAI1}, was found to be induced by ethylene, and it was a direct downstream gene of \textit{RhEIN3-3}. This work uncovers the transcriptional regulation of \textit{RhGAI1} by ethylene, which has not been reported before, and provides a better understanding of how ethylene regulates petal expansion in roses.

Regarding regulation of DELLA proteins, most work has focused on the post-transcriptional regulation of DELLA proteins, such as ubiquitination, phosphorylation, and O-GlcN acylation (Hauvermale et al., 2012).
At the transcription level, it is reported that PIL5, a basic helix–loop–helix (bHLH) transcription factor, can bind directly to the promoter of DELLA genes, and therefore regulates GA responsiveness (Oh et al., 2007).

Based on the post-transcriptional regulation of DELLA proteins by ethylene reported before and the transcriptional regulation in this work, it is speculated that, on one hand, ethylene induces the expression of RhGAII; on the other hand, ethylene enhances the stability of RhGAII, and consequently the expansion of petals is repressed.

RhGAII can regulate ethylene-responsive genes that are related to cell expansion

Since DELLA proteins play important roles in cell expansion, researchers have spent much effort in identifying its downstream genes (Cao et al., 2006; Hou et al., 2008). It is widely accepted that DELLA proteins do not have DNA binding capacity, and they regulate the downstream genes by interacting with other transcription factors such as PIF3 (Feng et al., 2008), PIF4 (de Lucas et al., 2008), JAZs (Hou et al., 2010), MYC2 (Hong et al., 2012), BZR1 (Gallego-Bartolomé et al., 2012), EIN3 (An et al., 2012), and SPL3 (Yu et al., 2012).

With regard to the regulation of downstream genes by DELLA proteins, except for indirect regulation through interaction of DELLA with other transcription factors, there also exists direct transcriptional regulation (Gallego-Bartolomé et al., 2011). Using ChIP-qPCR, several direct targets of DELLA proteins were found, such as SCL3, MYB, and GID1b (Zentella et al., 2007; Zhang et al., 2011).

In this work, it was found that the expression of nine cell expansion-related genes was clearly changed in RhGAII-silenced rose petals when compared with the TRV control. Furthermore, RhCesA2 was a direct downstream gene of RhGAII. These genes are widely involved in loosening and synthesis of the cell wall, changes of turgor pressure, and remodelling of the cytoskeleton, which suggests that RhGAII plays a comprehensive role in cell expansion.

In terms of synthesis of new cell wall materials, it is widely reported that CESAs genes are involved in this process (Mutwil et al., 2008). The null allele of CESAs6, pre1-8, showed increases in width in both epidermal and cortical cells (Fagard et al., 2000). What is more, in the cesa2 cesa6 cesa9 triple mutant, irregular wall thickness is observed, which results in shrinkage of pollen grains (Persson et al., 2007). These reports indicate that CESAs genes play an important role in synthesis of the cell wall and cell expansion, and RhCesA2 may be an important downstream gene in RhGAII-regulated cell expansion of rose petals.

Nevertheless, the current data are insufficient to provide a complete view of the role of RhGAII during cell expansion. Through a transactivation assay, the promoter activity of RU25443 (PE) and RU10722 (XTH) was regulated by RhGAII, which suggested that they might also be downstream genes of RhGAII. Whether there are any other downstream genes of RhGAII and what their regulatory mechanisms are need further investigation.

Accession numbers

Sequence data from this article can be found in the EMBL/GenBank data libraries under accession numbers: RhGAII (AGK07287), RhEIN3-3 (AGK07288), MdGAI1 (ADW85805), MgGAI1 (ABL61270), PtGAI/RGA1 (XP_002314799), GbGAI (ABG26370), RzGAI (XP_002527794), GhGAI/RGA (ACR58455), VvGAI1 (AEK06229), BoGAI (BAG16374), BnRGA (ADD71137), BrGAI (BAG16380), SsGAI (ACM47244), GmGAI1 (NP_001240948), LsDELLA1 (BAG71200), SIGAI (NP_001234365), RIDEELLA (AF88481), PvEIN2 (BAF62637), AmGRAS (ADA84480), PsDELLA (ABI30654), DmGAI (AAM15884), AaGAI-like1 (ABL97842), LiGAI-like 1 (ABL97941), WgGAI (AAM15886), DcGAI (AAM15882), ApGAI-like1 (ABL97840), DaGAI (AAM15880), VrGAI-like 1 (ABL97934), MsGAI (AAM15891), DrGAI (AAM15885), VaGAI-like 1 (ABL97928), CsGAI-like 1 (ABL97886), YtGAI-like 1 (ABL97940), VpGAI-like 1 (ABL97932), VtGAI-like 1 (ABL97938), VIGAI-like 1 (ABL97930), CmGAI (AAM15892), AkGAI (AAM15901), AtRGA (NP_178266), AtGAI (NP_172945), AtRGL1 (NP_176809), AtRGL2 (NP_186995), AtRGL3 (NP_197251), ReEIN3 (XP_002530192), AdEIL2 (ACI70675), VrEIL2 (AAL76271), NtEIL2 (AAP30998), NtEIL1 (AAP30997), VvEIL (XP_002275284), LeEIL (ACP56697), MtEIL1 (ACX54782), NtEIL4 (AAP40000), SIEIL1 (NP_001234541), SIEIL2 (NP_001234721), AtEIN3 (NP_188713), PpEIL2 (ABK35086), CsEIN3 (AKF80347), DcEIL (BAI44821), AtEIL1 (AAC49746), AtEIL2 (NP_197611), AtEIL3 (NP_177514), SIEIL3 (NP_001234546), ZmEIL1 (NP_001152035), PeEIN3a (XP_002312841), PeEIN3b (XP_002328098), MdEIN3 (ADE41155), OsEIL (BAB78462), RhETR1 (AY953869), RhETR3 (AF154119), RhETR5 (AF414283), RhEIN3-1 (AF443783), RhEIN3-2 (AY919867), and RhCesA1 (JQ001775).

Supplementary data

Supplementary data are available at JXB online.

Figure S1. Alignment of deduced amino acid sequence of RhGAII with nine DELLA proteins of other plants.

Figure S2. Phylogenetic tree of RhGAII and other DELLA proteins.

Figure S3. Phylogenetic tree of RhEIN3-3 and other EIN3/EILs proteins.

Figure S4. Transactivation of truncated proRhCesA2 by RhGAII.

Figure S5. Regulation of proRU25443 and proRU10722 by RhGAII.

Table S1. Oligonucleotide primer sequences.

Table S2. Expression of unisequences of DELLA genes in rose petals by microarray analysis.

Table S3. Expression of unisequences of EIN3 genes in rose petals by microarray analysis.

Table S4. Expression of unisequences of cell expansion-related genes in rose petals by microarray analysis.
Acknowledgements

We thank Dr Susheng Gan (Cornell University, Department of Horticulture, USA) and Dr Cai-zhong Jiang (USDA/ARS, Crops Pathology and Genetics Research Unit) for their helpful suggestions. We also thank Dr Zhizhong Gong (China Agricultural University) for providing the super1300 vector. This work was supported by the National Natural Science Foundation of China (grant nos 31071827 and 31130048).

References


Ma N, Tan H, Liu XJ, Xue JQ, Li YH, Gao JP. 2006. Transcriptional regulation of ethylene receptor and CTR genes involved in


