A MADS-box gene associated with protocorm-like body formation in *Rosa canina* alters floral organ development in *Arabidopsis*

Journal:	<i>Canadian Journal of Plant Science</i>
Manuscript ID	CJPS-2017-0027.R2
Manuscript Type:	Article
Date Submitted by the Author:	23-Jul-2017
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Keywords:	protocorm-like body, somatic embryogenesis, RcAGL15<i></i>, MADS-box

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A MADS-box gene associated with protocorm-like body formation in *Rosa canina* alters floral organ development in *Arabidopsis*

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Abbreviations: **ABA**, abscisic acid; **AG**, AGAMOUS; **AGL15**, AGAMOUS-LIKE15; **AP2**, APETALA2; **ARF**, AUXIN RESPONSE FACTOR; **CaMV**, cauliflower mosaic virus; **CAL**, CAULIFLOWER; **CTK**, cytokinin; **ELISA**, enzyme-linked immunosorbent assay; **FLC**, FLOWERING LOCUS C; **FUL**, FRUITFULL; **GA**, gibberellic acid; **GFP**, green fluorescent protein; **IAA**, indole-3-acetic acid; **MADS**, MCM1-AGAMOUS-DEFICIENS-SRF; **ORF**, open reading frame; **PCR**, polymerase chain reaction; **PBS**, phosphate-buffered saline; **PLB**, protocorm-like body; **RACE**, rapid amplification of cDNA ends; **RT-PCR**, reverse transcriptase-PCR; **SE**, somatic embryogenesis; **SEP**, SEPALLATA; **SHP**, SHATTERPROOF; **SOC**, SUPPRESSOR of OVEREXPRESSION of CONSTANS; **SVP**, SHORT VEGETATIVE PHASE; **TIR**, TRANSPORT INHIBITOR RESPONSE; **TDZ**, thidiazuron; **UTR**, untranslated region.

Abstract: We isolated a gene encoding a putative MADS-box protein designated *RcAGL15* from PLB (Protocorm-like body, a somatic embryogenetic structure) of *Rosa canina*. *RcAGL15* encodes a predicted protein of 251 amino acid residues with a molecular weight of 28 kDa and theoretical isoelectric point of 7.5. RT-PCR shows that *RcAGL15* is mainly expressed in PLB and regenerated PLB, but not in leaves of aseptic seedlings, incubated leaflets before callus formation, calli, rhizoids or leaves of regenerated shoots. Transgenic, ectopic expression of *RcAGL15* in *Arabidopsis* changes numbers of floral organs and reduces contents of indole-3-acetic acid, but increases levels of abscisic acid. Thus *RcAGL15* appears to be a new MADS-box gene that plays regulatory roles in the morphological development and numbers of flower organ, as well as somatic embryogenesis.

Key words: MADS-box, protocorm-like body, somatic embryogenesis, *RcAGL15*.

Introduction

MADS-box genes are defined by a highly-conserved motif encoding 56 amino acids known as the MADS (MCM1-AGAMOUS-DEFICIENCY-SRF)-box and are present in animals, fungi, and plants (Schwarz-Sommer 1990). In land plants, MADS-box genes were first identified as floral homeotic selector genes (Sommer et al. 1990; Yanofsky et al. 1990). And all floral homeotic genes then known, except *APETALA2* (*AP2*), were shown to be MADS-box genes (Weigel and Meyerowitz 1994). Two types of land plant MADS-box genes are recognized: MIKC^C- and MIKC* (Henschel et al. 2002). MIKC^C-type genes are composed of a MADS (M) domain, an intervening (I) domain, a keratin-like (K) domain, and a C-terminal (C) domain, and they have been detected in diverse land plants, including seed plants, pteridophytes, and bryophytes (Theißen et al. 2000; Henschel et al. 2002). Plant MADS-box genes form a large family of

transcription factors and are involved in various aspects of developmental processes, *inter alia*: flowering time control, floral meristem identity, floral organogenesis (Riechmann and Meyerowitz 1997), fruit formation, seed pigmentation, and endothelium development (Parenicová et al. 2003). During flower development, MADS-box genes have pivotal roles, for example all Arabidopsis floral organ identity genes, except *AP2*, are MADS-box genes (Guo et al. 2007). However, MADS-domain transcriptional regulators, *AGAMOUS-LIKE15* (*AGL15*) and *AGL18*, along with *SVP* (*SHORT VEGETATIVE PHASE*) and *AGL24*, are also required to block initiation of floral programs in vegetative organs (Fernandez et al. 2014). *AGL15* reportedly enhances somatic embryo development when constitutively expressed (Thakare et al. 2008). *AGL15* was initially identified in *Brassica napus* (Heck et al. 1995) and Arabidopsis (Rounsley et al. 1995), where it was found to be preferentially expressed in embryonic tissues. In Arabidopsis, there is evidence that *AGL15* acts *via* control of Gibberellic acid (GA) metabolism. Chromatin immunoprecipitation analysis showed that AtGA₂ox6 (GA₂-oxidase) is a downstream target of *AGL15* and affects seed germination and somatic embryogenesis (SE) (Wang et al. 2004). GA₂-oxidases convert biologically active GAs to inactive forms. A decrease in biologically active GA caused an increase in SE, whereas addition of active GA (GA₃) reduced SE (Wang et al. 2004). Recent studies have shown that *GhAGL15s* is preferentially expressed during SE and promotes embryogenic callus formation in *Gossypium hirsutum* (Yang et al. 2014), while *AGAMOUS-Like 15* may enhance SE by promoting a dedifferentiated state in *Glycine max* (Perry et al. 2016). *AGL15* negatively regulates auxin signaling in both Arabidopsis and soybean, at many levels of the pathway, including repression of *AUXIN RESPONSE FACTOR (ARF)6* and *ARF8*, and *TRANSPORT INHIBITOR RESPONSE 1 (TIR1)*, as well as indirectly controlling components via direct

expression of a microRNA-encoding gene (Zheng et al. 2016).

PLBs are somatic embryonic structures that develop from intermediary callus, and differ in terms of developmental process, morphological characters and microscopic structure from other kinds of somatic embryos of higher plants (Tian et al. 2008). In this study, to elucidate the function of *RcAGL15*, we investigated its expression pattern in *Rosa canina*, and effects of its transgenic, ectopic expression in *Arabidopsis* (which included alterations in the development of floral organs and hormone levels).

Materials and methods

Plant materials preparation and growth conditions

We have successfully established a highly efficient SE system in *R. canina*, in which protocorm-like body (PLB) formation is induced in rhizoids by treatment with medium supplemented with thidiazuron (TDZ) under light (Tian et al., 2008). *R. canina* seedlings were grown and PLB development was induced, following Tian et al. (2008). Samples including leaves of aseptic *R. canina* seedlings, incubated leaflets before callus formation, callus, rhizoid, PLB, regenerated PLB and leaves of regenerated shoots were collected and immediately frozen in liquid nitrogen, then stored at -80 °C until RNA extraction.

Arabidopsis thaliana (Col-0) seeds were sterilized and sown after vernalization, then seedlings were transplanted onto culture medium at 20 °C, with 16 h light (120 $\mu\text{mol.m}^{-2}\text{s}^{-1}$)/ 8 h dark cycles, following published methods (Xu et al. 2014a; Xu et al. 2014b). In each cultivation cycle, seeds were sown simultaneously, the resulting plants were simultaneously harvested and stored under similar conditions, and their seeds were stored in paper bags for at least a month before germination.

RNA isolation, DNase treatment and Isolation of the *RcAGL15* gene

Total RNA of various tissues (leaves of aseptic seedlings, incubated leaflets before callus formation, calli, rhizoids, PLB, regenerated PLB, leaves of regenerated shoots) was extracted using an RN38-EASY spin Kit (Aidlaid, China), according to the manufacturer's instructions. Total RNA preparations were subjected to on-column DNase digestion and simultaneously cleaned using a Qiagen RNase-Free DNase-Set and Qiagen RNeasy RNA Clean-up Midi Kit (Qiagen, Germany). First-strand cDNA was synthesized with 1 µg total RNA and 1 µl superscript II enzyme (Invitrogen, USA) according to the manufacturer's protocol.

A partial sequence of *RcAGL15* was then obtained by nested polymerase chain reaction (PCR) amplification, using the first-strand cDNA as template. Two degenerate primers based on multiple sequence alignments of homologous *AGL15* sequences, FAG₁ and RAG₁ (Supplementary table S1), were used for the first PCR round, and FAG₁ plus RAG₂ (Supplementary table S1) in the nested round. Flanking sequences were then obtained by rapid amplification of cDNA ends (RACE). For 3'-RACE the first-round primers were AUAP and FAG₃₋₁ (Supplementary table S1), while the second-round primers were AUAP and FAG₃₋₂ (Supplementary table S1). For 5'-RACE, first-round primers were AAP and RAG₅₋₁ (Supplementary table S1), while second-round primers were AUAP and RAG₅₋₂ (Supplementary table S1).

The RACE reactions were performed according to Xu et al. (2011). The full-length cDNA sequence was obtained by combining the 5'-RACE fragment, partial fragment and C-terminal fragment using a pair of primers FG₁ and RG₁ (Supplementary table S1) designed from the putative 5' and 3' untranslated region (UTR) of the *RcAGL15* gene. A 756 bp putative fragment was generated for the full-length cDNA. The resultant DNA fragments and RACE products were

purified by agarose gel electrophoresis, then cloned into the pMD18-T vector (Takara) and sequenced (Invitrogen, Beijing). The nucleotide sequence was submitted to the GenBank.

Sequence alignment and phylogenetic tree analysis

The *RcAGL15* sequence was aligned with other *AGL15* sequences using DNAMAN (ver. 5.2.2) and a phylogenetic tree was constructed using the neighbor-joining method implemented in MEGA program (ver 4.0) (Xu et al. 2011).

Semi-quantitative RT-PCR

To assess *RcAGL15* expression patterns, semi-quantitative reverse transcription polymerase chain reaction amplification (RT-PCR) was applied, using the 18s rRNA gene (Genbank No.: FM164424.1) as a control and the following primers: F-rt and R-rt (Supplementary table S1). The PCR program consisted of pre-denaturation at 94 °C for 5 min, followed by 35 cycles (for *RcAGL15*, 28 cycles for 18s rRNA) of 45 s at 94 °C, 45 s at 58 °C, 1 min at 72 °C, and a final extension of 10 min at 72 °C. The amplified products were resolved on 1.2 % agarose gel, then detected by agarose gel electrophoresis. All the RT-PCR experiments were repeated at least three times.

Transient expression vector construction and subcellular localization of *RcAGL15*

The open reading frame (ORF) of *RcAGL15* was used to construct a transient expression vector using the pSAT6-GFP-N1 vector (Xu et al. 2014a), which includes a sequence encoding modified green fluorescent protein (GFP) between *NcoI* and *XbaI* restriction enzyme cutting sites. An upstream primer F-agS and downstream primer R-agS (Supplementary table S1) were designed by adding *XhoI* and *BamHI* restriction enzyme cutting sites to F-rt and R-rt, respectively. The PCR program for *RcAGL15* amplification consisted of pre-denaturation at 94 °C for 5 min, followed by

28 cycles of 45 s at 94 °C, 45 s at 58 °C, 1 min at 72 °C, and a final extension of 10 min at 72 °C. The amplified products were checked by 1.2 % agarose electrophoresis, purified with a DNA Purification Kit (Biomed, China) and ligated into the T vector, then the vector was transformed and amplified in chemically competent *Escherichia coli* strain Trans T1 (TransGen, China). The target gene was extracted from *E. coli* plasmids and transferred, by digestion with *Xho*I and *Bam*HI, into the pSAT6-GFP-N1 vector, and the resulting construct was named pSAT6-GFP-RcAGL15.

The recombination plasmid RcAGL15-GFP was transformed into onion epidermal cells by particle bombardment, as previously described (Xu et al. 2011), and an *Agrobacterium*-mediated *in planta* transient transformation protocol (Xu et al. 2014a). Effects of the transformations were examined, and images of the cells were acquired, using a Zeiss LSM 510 META confocal microscope and Zeiss 340, 363, and 3100 oil objectives (Plan-Apochromat; numerical aperture 1.4).

Overexpression vector construction and ectopic expression in Arabidopsis

An upstream primer F-agB and downstream primer R-agB (Supplementary table S1) were designed according to the *RcAGL15* sequence information and the enzyme cutting sites on pCAMBIA2300. Then, following procedures applied to construct the transient expression vector (described above), the ORF of *RcAGL15* was cloned into the *Kpn*I and *Xba*I sites of pCAMBIA2300, and the resulting construct was named pCAMBIA2300-RcAGL15. pCAMBIA2300-RcAGL15 was introduced into GV3101 by the freeze-thawing method, and Arabidopsis was transformed by the *Agrobacterium*-mediated floral dip method (An et al. 1986).

Microscopic investigation

For observations of cell morphology, 7 d old transgenic *Arabidopsis* seedlings were incubated in destaining solution (ethanol:glacial acetic acid, 3:1) for 30 minutes, followed by basic solution (7 % NaOH in 60 % ethanol) for 30 minutes at room temperature, then rehydrated in a 40 %, 20 % and 10 % ethanol series (15 minutes per step). The samples were subsequently immersed in 5 % ethanol and 25 % glycerol for 30 minutes, and finally pavement cells of cotyledons and first euphylla were observed using an Olympus BX 41 microscope (4× to 100× magnification) equipped with a color CCD camera (DP 70, Olympus, Japan).

Quantification of endogenous hormones by enzyme-linked immunosorbent assays

N₂-dried extracts of sampled rosette leaves were dissolved in 2 ml of phosphate-buffered saline (PBS) containing 0.1 % (v/v) Tween-20 and 0.1 % gelatin (w/v, pH 7.5) to quantify endogenous hormones IAA (indole-3-acetic acid), CTK(cytokinin), GA and ABA(abscisic acid) in them by enzyme-linked immunosorbent assays (ELISAs), following previously published protocols (Weiler et al. 1981; Zhao et al. 2006; Wang et al. 2012).

Results and Discussion

Identification of *RcAGL15* gene

A partial cDNA fragment of *RcAGL15* was amplified from cDNA generated from total RNA (OD₂₆₀/OD₂₈₀=1.9) extracted from PLBs of *R. canina* using degenerate PCR primers based on homologous domains of *AGL15* genes, then full-length *RcAGL15* cDNA (GenBank no. KM083102) was obtained by 3'- and 5'-RACE. Sequence analysis showed that *RcAGL15* cDNA is 1570 bp long, including a complete ORF of 756 bp flanked with a 5'-UTR of 392 bp and 3'-UTR of 422 bp (Fig. 1A). The predicted protein of *RcAGL15* consists of 251 amino acids with a calculated molecular mass of 28 kDa and theoretical isoelectric point of 7.5. The deduced amino

acid sequence of RcAGL15 includes a MADS-box domain, I domain, K domain and C domain (Fig. 1B). In a phylogenetic tree of AGL15 proteins from diverse plants constructed using the neighbor-joining method, RcAGL15 clustered with proteins from *Dimocarpus longan* and *Glycine max* in one clade, proteins from *Arabidopsis thaliana* and *Brassica napus* clustered in another clade, and TaAGL15 from the monocotyledonous plant wheat was separated from the AGL15 proteins of the included dicotyledonous plants (Fig. 1C).

RcAGL15 expression profile according to semi-quantitative RT-PCR

The spatial-specific expression of *RcAGL15* in different tissues in the developmental stages in PLB were determined by RT-PCR. The result shows that *RcAGL15* mRNA is more abundant in PLBs and regenerated PLBs of *R. canina*, but not in leaves of aseptic seedlings, incubated leaflets before callus formation, callus, rhizoids or leaves of regenerated shoots (Fig. 2).

Subcellular localization of RcAGL15

The RcAGL15-GFP fusion protein was preferentially localized in the nucleus of transformed onion epidermal cells, whereas the control GFP was distributed throughout the cytoplasm. This suggests that RcAGL15 protein is a nucleus-localized protein, so *RcAGL15* may have transcription activity (Fig. 3).

Transgenic expression of RcAGL15 affects the morphological development and number of floral organs in Arabidopsis

We analyzed the function of *RcAGL15* in Arabidopsis, because no *R. canina* transformation system is available yet. We detected no evidence that *RcAGL15* induces, or plays any other role in somatic embryogenesis of Arabidopsis, although we isolated it from PLBs of *R. canina*. However, we found that transgenic, ectopic expression of *RcAGL15* alters the morphological development

(Fig. 4 G and H) and numbers of floral organs, including sepals, petals and stamens in Arabidopsis. As shown in Table 2, numbers of petals in flowers of two transgenic lines (designated *RcAGL15*-OE-3 and *RcAGL15*-OE-21) varied from 0 to 5, and numbers of stamens from 3 to 6 (Fig. 4), while there were consistently 4 petals and 4 sepals in flowers of wild-type (WT) controls. In total, we examined 3000 flowers (30 replicate sets of 100 flowers) of WT, *RcAGL15*-OE-3 and *RcAGL15*-OE-21 plants, respectively, and found that 2612 and 2613 of the *RcAGL15*-OE-3 and *RcAGL15*-OE-21 flowers had less-than-WT numbers of floral organs. In contrast, just 0.1-0.13 % of the *RcAGL15*-OE flowers had 5 petals and 0.23 % had 4 petals. A few *RcAGL15*-OE flowers (6.13-6.60 %) had no petals. However, most had some, but fewer than normal (1, 2 or 3). Thus, *RcAGL15* expression apparently perturbed, but did not prevent, petal whorl formation.

Transgenic, ectopic expression of *RcAGL15* alters shapes of pavement and stomata cells

Microscopic examination of the *RcAGL15*-OE Arabidopsis plants showed that the transgenic, ectopic expression of *RcAGL15* in them resulted in many abnormalities in cell directional expansion, morphology of stomata cells, and both the shape and size of pavement cells in the abaxial surfaces of cotyledons and the first euphylla. Pavement cells of WT cotyledons usually have irregular shapes, like pieces of a jigsaw puzzle (Fig. 5A1 and C1), while those of *RcAGL15*-OE plants were wider and flatter (Fig. 5B1 and D1). Numbers of stomata in *RcAGL15*-OE and WT plants were similar, but the guard cells were much thinner in the former (Fig. 5 B1 and D1) than the latter (Fig. 5A1 and C1).

Transgenic, ectopic expression of *RcAGL15* changes endogenous hormone contents

GA and IAA contents were lower, while CTK and ABA contents were higher, in *RcAGL15*-OE plants than in WT counterparts (Table 1).

MADS-box genes are homeotic genes that are widely distributed in plants and play key roles in regulating the transcription of other genes with specific recognition sequences, and thus numerous aspects of diverse (if not all) physiological and developmental processes. These processes include, *inter alia*: flowering time, e.g. *FLOWERING LOCUS C* (*FLC*), *SUPPRESSOR of OVEREXPRESSION of CONSTANS 1* (*SOC1*) and *SHORT VEGETATIVE PHASE* (*SVP*) (Michaels et al. 2003), meristem characteristics, e.g. *CAULIFLOWER* (*CAL*), *API*, and *FRUITFULL* (*FUL*), floral organ decisions, e.g. *AGAMOUS* (*AG*), *API*, *AP3* and *SEPALLATA1-3* (*SEPI-3*) (Saedler and Huijser 1993), formation of meristematic zones, and development or maturation of fruits and ovules, e.g. *SHATTERPROOF1* (*SHP1*) and *FUL* (Rounsley et al. 1995; Battaglia et al. 2006), embryos (e.g. *AGL15*) and vegetative organs such as leaves (e.g. *AGL16* and *AGL19*) (Alvarez-Buylla et al. 2000). In plants, genes of the *AGL15*-like clade of MADS domain regulatory factors are expressed both in embryos and developing endosperm (Lehti-Shiu et al. 2005). Constitutive expression of *AGL15* promotes maintenance of embryonic identity and enhances formation of somatic embryos from the shoot apical meristem in culture (Harding et al. 2003). Moreover, plants that constitutively overexpress *AGL15* reportedly exhibit perturbations in various traits, including changes in leaf morphology, reduction in fertility, and delays in flowering, floral organ abscission and senescence (Fernandez et al. 2000).

In the present study, we isolated a MADS-box gene *RcAGL15* from PLBs of *R. canina*, using primers derived from conserved sequences of *AGL15* genes, and characterized it. Sequence analysis shows that it contains a MADS-box domain, I domain, K domain, and C-terminal domain. These results strongly indicate that *RcAGL15* is an ortholog of *AGL15s*, and a novel member of the MADS-box family. Subcellular localization of *RcAGL15* showed that 35S::*RcAGL15*-GFP is

mainly located in the nucleus, indicating that *RcAGL15* has transcription activity, and *RcAGL15* is thus a transcription factor. Phylogenetic analysis showed that *RcAGL15* is closely related to *D. longan AGL15* (*DIAGL15*) and *G. max AGL15* (*GmAGL15*), which promote formation of somatic embryos (Kaufmann et al. 2005). Moreover, *RcAGL15* is mainly expressed in PLBs and regenerated PLBs, implying that *RcAGL15* participates in their development.

We also found that transgenic expression of *RcAGL15* reduces IAA and GA contents, but increases CTK and ABA contents, in accordance with previous findings that AGL15 directly and indirectly controls components of the auxin signaling pathway, in both *Arabidopsis* and soybean in a manner, that would limit auxin signaling (Zheng et al. 2016).

In addition, we found that *RcAGL15* expression affects floral organ morphology and numbers in *Arabidopsis*, indicating that further elucidation of *RcAGL15*'s roles will facilitate understanding of both PLB formation and floral organ development mechanisms. *RcAGL15* also alters the morphology of flower organs, especially the androecium and perianth, as well as perturbing hormone levels and shapes of both pavement cells and guard cells in *Arabidopsis*. These findings suggest that *RcAGL15* could have pleiotropic functions, which will be explored in further studies involving (*inter alia*) analysis of genes that interact with *RcAGL15* during the development of PLBs in *R. canina*.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (grant nos. 30871733 and 31272168) (<http://www.nsf.gov.cn/>), National Training Program of Innovation and Entrepreneurship for Undergraduates of China (grant no. 201610478040) (<http://www.moe.gov.cn/>), Science and Technology Research Major Projects of Department of

Education of Henan Province (grant no. 14A180003) (<http://www.haedu.gov.cn/>), Department of Science and Technology Planning Project of Henan Province (grant no. 144300510064) (<http://www.hnkjt.gov.cn/>), International Science and Technology Cooperation Key Project of Henan Province (grant no. 152102410074) and Open Laboratory Project of Zhoukou Normal University (grant no. K201601) (<http://www.zknu.edu.cn/>).

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Fig. 1. Nucleotide, deduced amino acid sequence and phylogenetic tree of *RcAGL15*. A. Nucleotide and amino acid sequences, with the MADS box, and Intervening, Keratin-like and C-terminal domains indicated by black, red, brownish red and blue underlining, respectively. B. Alignment of deduced amino acid sequence, with AGL15 proteins from *Arabidopsis thaliana* (AtAGL15), *Brassica napus* (BnAGL15-1 and -2), and *Glycine max* (GmAGL15), again showing the MADS-box and other domains, with positions occupied by identical and conservative residues shaded in dark blue and green, respectively. C. Phylogenetic tree, with bootstrap values indicating the divergence of each branch and scale indicating branch length. Genbank Accession Nos. of BnAGL15-1, BnAGL15-2, AtAGL15, DIAGL15 (from *Dimocarpus longan*), GmAGL15 and TaAGL15 (from *Triticum aestivum*) are U22665, U22681, UM121382, GU584088, AY370659 and DQ512361, respectively. Note, we would use the same color coding for the domains in A and B.

Fig. 2. Semi-quantitative RT-PCR analysis of expression profile of *RcAGL15* in tissues of *R. canina*. *RcAGL15*⁺ (top), *RcAGL15*⁻ (middle) and 18S rRNA (bottom) rows show results of RT-PCR amplification of *RcAGL15*, negative control, and amplification of 18S rRNA. LAS, leaf of aseptic seedling; ILC, incubated leaflet before callus formation; CAL, callus; RHI, rhizoid; PLB, protocorm-like body; RPL, regenerated PLB; LRS, leaf of regenerated shoot.

Fig. 3. Subcellular localization of GFP-*RcAGL15* expressed from pSAT6-GFP-*RcAGL15* (E-H) and GFP control expressed from pSAT6-GFP (A-D) in onion epidermal cells, as shown by: bright field microscopy (A and E), dark field microscopy (B and F), dark field microscopy following DAPI nuclear staining (C and G), dark field microscopy with GFP fluorescence detection (D), merger of A, B and C images (D) and merger of E, F and G images (H).

Fig. 4. Morphology and numbers of floral organs of *RcAGL15*-OE *Arabidopsis* plants. A and A1, WT; B and B1, C and C1, D and D1, E and E1, F and F1, *RcAGL15*-OE flowers; G, Inflorescence of WT; H, Inflorescence of

RcAGL15-OE; I, apetalous flower of *RcAGL15*-OE; A-F, scale bar=500 μ m; A1-F1, scale bar=3 mm; G and H, scale bar=4 mm; I, scale bar=1 mm.

Fig. 5. Morphology of pavement cells in abaxial surfaces of cotyledons and first euphylla of *Arabidopsis*.

Pavement cells of: WT cotyledons (A and A1), *RcAGL15*-OE cotyledons (B and B1), WT first euphylla (C and C1) and *RcAGL15*-OE first euphylla (D and D1). Scale bar=30 μ m.

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Table 1. Endogenous hormone contents of WT and *RcAGL15*-OE Arabidopsis plants according to ELISAs

Endogenous hormone	Concentration (ng.g ⁻¹ FW)		
	WT	<i>RcAGL15</i> -OE-8	<i>RcAGL15</i> -OE-16
IAA	94.43 ± 0.33 aA	68.94 ± 0.24 bB	68.78 ± 0.14 bB
CTK	7.94 ± 0.02 aA	9.50 ± 0.05 bB	9.50 ± 0.08 bB
GA	14.27 ± 0.06 aA	10.49 ± 0.08 bB	10.43 ± 0.04 bB
ABA	120.57 ± 0.41 aA	200.70 ± 0.66 bB	200.48 ± 0.76 bB

Note: Values are means and standard errors obtained from separate analyses of 30 plants of each line. Upper and lower case letters indicate significant differences at the 1 % and 5 % probability levels, respectively, according to the Duncan test, as implemented in SPSS 16.0.

Table 2. Percentages of flowers with indicated numbers of petals in WT and *RcAGL15*-OE Arabidopsis plants

Petal number	Percentage (%)		
	WT	<i>RcAGL15</i> -OE-3	<i>RcAGL15</i> -OE-21
0	0.00 ± 0.00 bB	6.13 ± 0.24 aA	6.60 ± 0.25 aA
1	0.00 ± 0.00 bB	15.23 ± 0.29 aA	14.60 ± 0.43 aA
2	0.67 ± 0.05 bB	42.47 ± 0.32 aA	42.90 ± 0.35 aA
3	0.20 ± 0.07 bB	23.30 ± 0.31 aA	23.00 ± 0.37 aA
4	99.53 ± 0.10 aA	12.83 ± 0.31 bB	12.77 ± 0.29 bB
5	0.20 ± 0.07 aA	0.10 ± 0.03 aA	0.13 ± 0.06 aA

Note: Values are means and standard errors obtained from examination of 3000 flowers (30 replicates of 100 flowers) of each line. Upper and lower case letters indicate significant differences at the 1 % and 5 % probability levels, respectively, according to the Duncan test, as implemented in SPSS 16.0.

83x37mm (300 x 300 DPI)

176x159mm (300 x 300 DPI)

47x33mm (300 x 300 DPI)

58x19mm (300 x 300 DPI)

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