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ORIGINAL PAPER

Isolation and Characterization of *IaYABBY2* Gene from *Incarvillea arguta*

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Abstract The establishment of ad-abaxial polarity is an important characteristic of the development of lateral organs in plants. YABBY genes encode higher plant-specific nuclear proteins which play critical roles in promoting abaxial cell fate. *IaYABBY2* (*IaYAB2*, Genbank accession no. KF250432), isolated from *Incarvillea arguta*, is a member of YABBY gene family. Sequence characterization and phylogenetic analyses show that *IaYABBY2* is a member of the *YAB2* subfamily of *Arabidopsis thaliana*. Subcellular localization analysis indicates that IaYABBY2 is localized in the nucleus. Ectopic expression of *IaYABBY2* in *Arabidopsis* plants resulted in the partial abaxialization of adaxial epidermises of leaves and sepals and development defect of florescence. The transgenic lines also showed higher level of anthocyanin content and photosynthesis capability after differential environment stress. These results indicate that the *IaYABBY2* functions in the ad-abaxial polarity, development of shoot apical meristem, and environmental stress.

Keywords *IaYABBY2* · Ad-abaxial polarity · *Incarvillea arguta* · *Arabidopsis thaliana*

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Introduction

Leaves and floral organs of seed plants are generated on the periphery of shoot apical meristems (SAM). These lateral organs possess three developmental axes: the adaxial–abaxial (ad-abaxial) axis, the proximal–distal axis, and the lateral axis. Among these axes, the ad-abaxial axis may be the most important (Bowman et al. 2002; Matsumoto and Okada 2001).

YABBY genes have been identified in all seed plants examined and encoded putative transcriptional factors (Lee et al. 2005b; Bowman and Smyth 1999). These are characterized by two conserved domains: a C2C2 zinc finger domain and a YABBY domain similar to the high mobility group (HMG) box (Siegfried et al. 1999; Bowman and Smyth 1999). YABBY genes of extant angiosperms can be divided into five subfamilies based on phylogenetic analyses: CRABS CLAW (CRC), FILAMENTOUS FLOWER (FIL)/YABBY3 (YAB3), INNER NO OUTER (INO), YABBY2 (YAB2), and YABBY5 (YAB5) (Yamada et al. 2011). In *Arabidopsis*, *FIL*, *YAB2*, *YAB3*, and *YAB5* are expressed in the adaxial tissue of lateral organs and have redundant functions that are essential for suppressing shoot apical meristem, activating laminar programs, and forming the marginal domain in leaves. Similar functions for these gene classes were independently found in *Antirrhinum majus*, and their role has been demonstrated to have overlapping functions in patterning of all lateral organs (Golz et al. 2004). Therefore, YAB genes are considered to be powerful candidates for participating in the evolutionary stem-to-leaf transformation. Moreover, YABBY genes can down-regulate the *KNOTTED1*-like homeobox (*KNOX*) and *WUSCHEL* (*WUS*) genes in either an indirect or direct way. *WUS* and *shoot* meristemless (*STM*) play key roles in SAM initiation and maintenance although both act independently (Sarkar et al. 2007; Sieber et al. 2004; Mayer et al. 1998; Dodsworth 2009). *WUS* is expressed in the organizing center

and maintains stem cell identity in the overlying cells forming the SAM. *WUS* expression is downregulated by *CLAVATA3* (*CLV3*) signaling, and the interaction between WUS and CLV forms a feedback loop that controls meristem size (Muller et al. 2006; Schoof et al. 2000; Brand et al. 2000). WUS-CLV similar signaling pathways were also found in differentiation of stem cell in popular vascular (Miyashima et al. 2012; Yang et al. 2013; Zheng et al. 2013).

Incarvillea arguta is an endemic and constructive species in the alpine steppe and alpine meadow, widely distributed in alpine areas of six provinces in China including Tibet, Qinghai, Gansu, Xinjiang, Yunnan, and Sichuan. The extensive distribution has very important meaning in the safeguard of soil and water, windbreak, and sand fixation. In addition, *I. arguta* has been widely used as a herbal medicine of Yi nationality (known as "Wabuyou") to treat hepatitis and diarrhea in China. Therefore, *I. arguta* not only is an important natural forage resource but also has an important ecological and economic value. In this study, *IaYABBY2* was cloned from young leaves of *I. arguta*. We generated 35S: *IaYABBY2-GFP Arabidopsis* plants to investigate the effect of *IaYABBY2* overexpression on the phenotype of this species.

Materials and Methods

Plant Materials

Seedlings of *I. arguta* were collected from the Tibetan plateau. The *A. thaliana* (ecotype: Col-0) plants used for ectopic expression experiments were grown on solid Murashige and Skoog (MS) medium for about 14 days before being transferred to soil. All plants were incubated in a growth chamber at 21 °C and a photoperiod of 16-h light/8-h darkness.

Isolation of *I. Arguta* YABBY Gene

Total mRNA was extracted from young leaves using the RNeasy kit (Qiagen). First strand complementary DNA (cDNA) was generated using Superscript III reverse transcriptase (Invitrogen, USA). Two degenerate primers targeting the zinc finger domain (5′ GTIACIGTIMGITGYGGICAYTG 3′) and the YABBY domain (5′ GCCCARTTYTTIGCIGC 3′) were used to amplify *I. arguta* partial cDNA sequences. Products were cut from the gel and TA cloned using the pMD-18T vector kit (Takara). To obtain the 3′ and 5′ partial cDNA end of *IaYABBY2*, a combination of 3′- and 5′-RACE PCR was used according to Xu et al. (2008). The full-length *IaYABBY2* sequence was generated using primers *YAB2F* (5′ ATGTCAGAGGAAATGAACTCG 3′) and *YAB2R* (5′ TTAA TTATCTGATATTCCTGCTGC 3′).

DNA Sequencing and Phylogenetic Analysis

DNASTAR software was used to carry out the sequence analyses. Multiple protein sequence alignment was created using the amino acid domain of IaYABBY2. Phylograms were generated using MEGA 4.0 software with 1,000 bootstrap trials.

Binary Vector Construction and *Agrobacterium*-Mediated Transformation of *Arabidopsis*

The full-length cDNA of *IaYABBY2* without end codon was inserted into a binary vector pBIN, with a GFP and driven by

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1      CTTTTTCTTGTTTTGTGAAATTCTTTTTGCCATCTCCGGAGATCATCCGATCCGGCGAAA
61     ATGTCAGAGGAAATGAACTCGGAACGTGTTTGTTACCTTCACTGCAACTTCTGCAACACC
1       M  S  E  E  M  N  S  E  R  V  C  Y  L  H  C  N  F  C  N  T
121    ATTTTAGTGGTTAATGTTCCATGCAACAGTATGCTGAGCTTAGTGACAGTAAGATGTGGG
21      I  L  V  V  N  V  P  C  N  S  M  L  S  L  V  T  V  R  C  G
181    CATTGTTCAAATTTGCTGTCTGTAAATATGGGAGCTTTGCTTCATCATTCCCTTCATCTC
41      H  C  S  N  L  L  S  V  N  M  G  A  L  L  H  H  S  L  H  L
241    CAAGATCATTTTCAGAGACAACAATCTTTGACCGATGTTCATGCTGCCATAAAAGACAGC
61      Q  D  H  F  Q  R  Q  Q  S  L  T  D  V  H  A  A  I  K  D  S
301    GGCTCATCATCTTCAAAGCCTGAAGATGAACTACCTAAGCCGACTCCAATTCGTCCCCCA
81      G  S  S  S  S  K  P  E  D  E  L  P  K  P  T  P  I  R  P  P
361    GAGAAAAGACAACGTGTCCCTTCAGCATACAACCGCTTCATAAAGGAGGAAATCCAGAGG
101     E  K  R  Q  R  V  P  S  A  Y  N  R  F  I  K  E  E  I  Q  R
421    ATAAAAGCTAGCAATCCTGAAATCACTCACAGGGAAGCTTTTAGCGCAGCTGCAAAAAAT
121     I  K  A  S  N  P  E  I  T  H  R  E  A  F  S  A  A  A  K  N
481    TGGGCACATCTTCCTCACATTCATTTTGGAATGAAGTTAGAGGGCAACAAACAAGCTAAG
141     W  A  H  L  P  H  I  H  F  G  M  K  L  E  G  N  K  Q  A  K
541    ATGGACCACGCGGTTGCAGCAGGAATATCAGATAATTAAAGGGACTCACAACTCCCTTGA
161     M  D  H  A  V  A  A  G  I  S  D  N  *
601    ATAAGATATATATGTATTTATAAATAAGGGTAATTTTTACATTTTTTTGCACCCTGAATT
661    ATAGAGCTCATGGCATTTTGATCCCTCTTTAGTCTTTAATCTTTAACTGAAAAGAGTGAT
721    GTTAGTTTACTAACTAATTCTTAACTAACTGGAAAATGAGGGATTAAATGCCAAATATTA
781    GAACGTTGGACTCTAGGGTTGCAAAAAGTGTAGTTCTTTGCCCTTAGTTTATGTTCTCTA
841    TATGTAGTACTAAACAGATTAATGATTATGTATTGGACATTTATGTAGTATGTATACTAT
901    TATAATATATAAATGTGGCATGTATTTGTGATTCAAAAAAAAAAAAAAAAAA
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Fig. 1 Nucleotide and deduced amino acid sequences of the *IaYABBY2* cDNA. The DNA sequence included the putative coding region and 5′ and 3′ non-coding regions. The amino acid of the putative coding region is shown beneath the DNA sequence

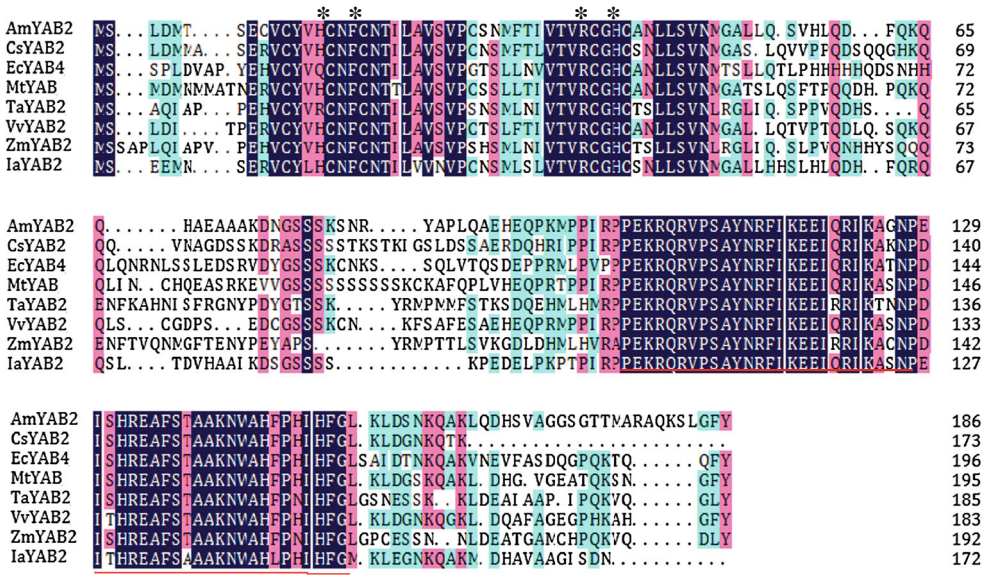

Fig. 2 Amino acid sequence alignment of IaYABBY2 and homology gene proteins. Conserved cysteine residues in the zinc finger domain are indicated with *asterisks*. The YABBY domains are *underlined with red line. Dark shading with white letters* and *pink shading with dark letters* indicate 100 and 75 % similarity sequence conservation, respectively

Cauliflower mosaic virus (*CaMV*) 35S promoter, forming a *35S:IaYABBY2-GFP* construct. The recombinant plasmid was then introduced into *Agrobacterium tumefaciens* GV3101 and used for wild-type *Arabidopsis* transformation via the floral dip method (Clough and Bent 1998).

Screening of the Transgenic Plants

T0 seeds were germinated on MS medium supplemented with 50 mg L^{-1} kanamycin for screening. All overexpressing *35S: IaYABBY2-GFP* transgenic lines (T1) were verified by PCR

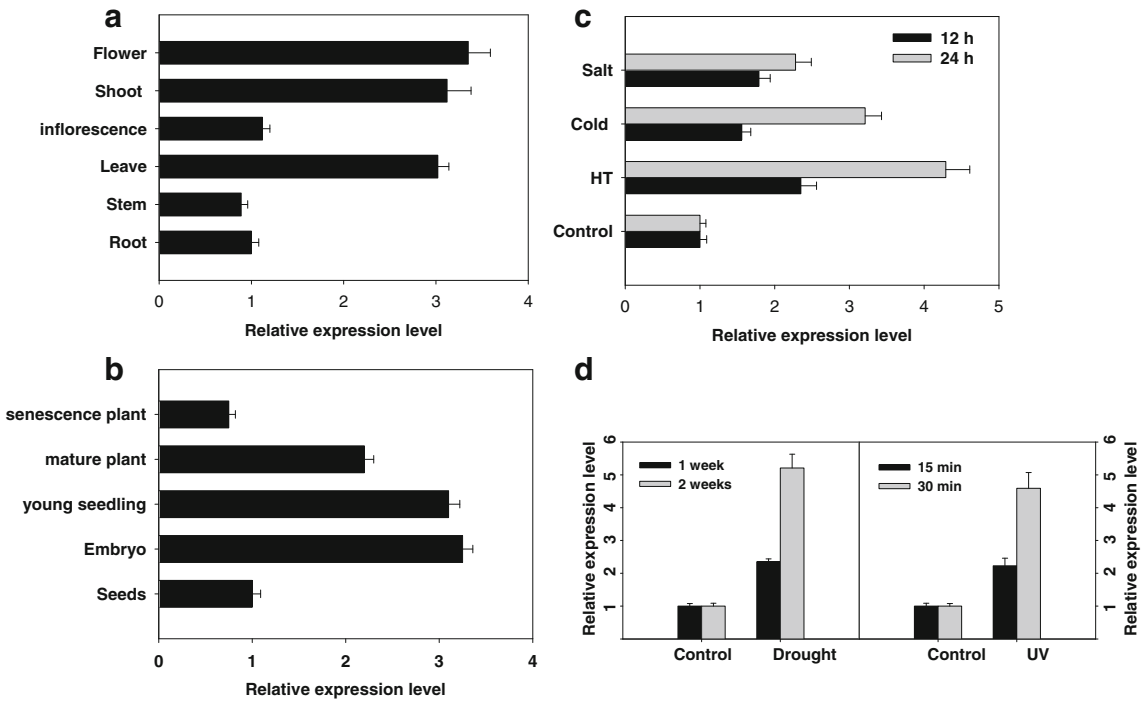

Fig. 3 Measuring the expression patterns of *IaYABBY2* at different tissues and different development statuses. **a** The expression levels of *IaYABBY2* at different tissues (root, stem, leave, inflorescence, shoot, and flower) by real-time qPCR analysis. **b** The expression level of *IaYABBY2* at different development statuses (seeds, embryo, young seedling, mature plant, and senescence plant) by real-time qPCR analysis. **c** The expression levels of *IaYABBY2* in response to different abiotic stress, including high temperature (HT), cold and salt, respectively, for 12 and 24 h, and the expression level was measured by real-time PCR. **d** The expression level of *IaYABBY2* in response to drought for 1 and 2 weeks or UV stress for 15 or 30 min respectively and the expression levels were measured by real-time PCR

using an npt II primers (5′ gaggctattcggctatgact 3′ and 5′ aatctcgtgatggcaggttg 3′) according to Sun et al. (2012). Fluorescence images were captured using an Olymplus FV1000 laser confocal microscopy (Olymplus, Japan). GFP was excited with the 488-nm laser line, and emission was captured using a 505-530 band-pass filter. For propidium iodide staining, seedlings were stained with 10 mg/mL propidium iodide for 5 min and washed once in water. Propidium iodide was visualized using wavelengths of 600 to 640 nm.

Expression Analysis

For measuring the expression pattern of *IaYABBY2* at different tissues, 4-week-old plants were used and different tissues (root, leave, stem, flower, and inflorescence) were collected for total RNA extract. For measuring the expression of *IaYABBY2* at different development times, the seeds, embryo, 1-week-old young seedling, 4-week-old mature plant, and 10-week-old senescence plant were collected for RNA extract. For abiotic stress, the 1-week-old young seedlings were treated with high temperature at 45 °C, cold treatment at 4 °C, or salt treatment at 150 mM salt solution for 12 and 24 h respectively. For UV treatment, the seedlings were irradiated with UV treatment at UV-B (5.3 mW/cm^2) for 15 and 30 min respectively. For drought stress, the soil was gradually dried for 1 and 2 weeks without watering. For real-time RT-PCR analysis of the *IaYABBY2* expression, total RNA was extracted using TRIzol regent (Invitrogen) and treated with RNase-free DNaseI (Fermentas) according to the manufacturer's instructions. Total RNA (about 2 μg) was reverse-transcribed in a 20-μL reaction mixture suing Superscript II (Invitrogen). After the reaction, 1-mL aliquots were used as a template for PCR amplification, and SYBR green was used to monitor the kinetics of PCR productions in real-time PCR. Three

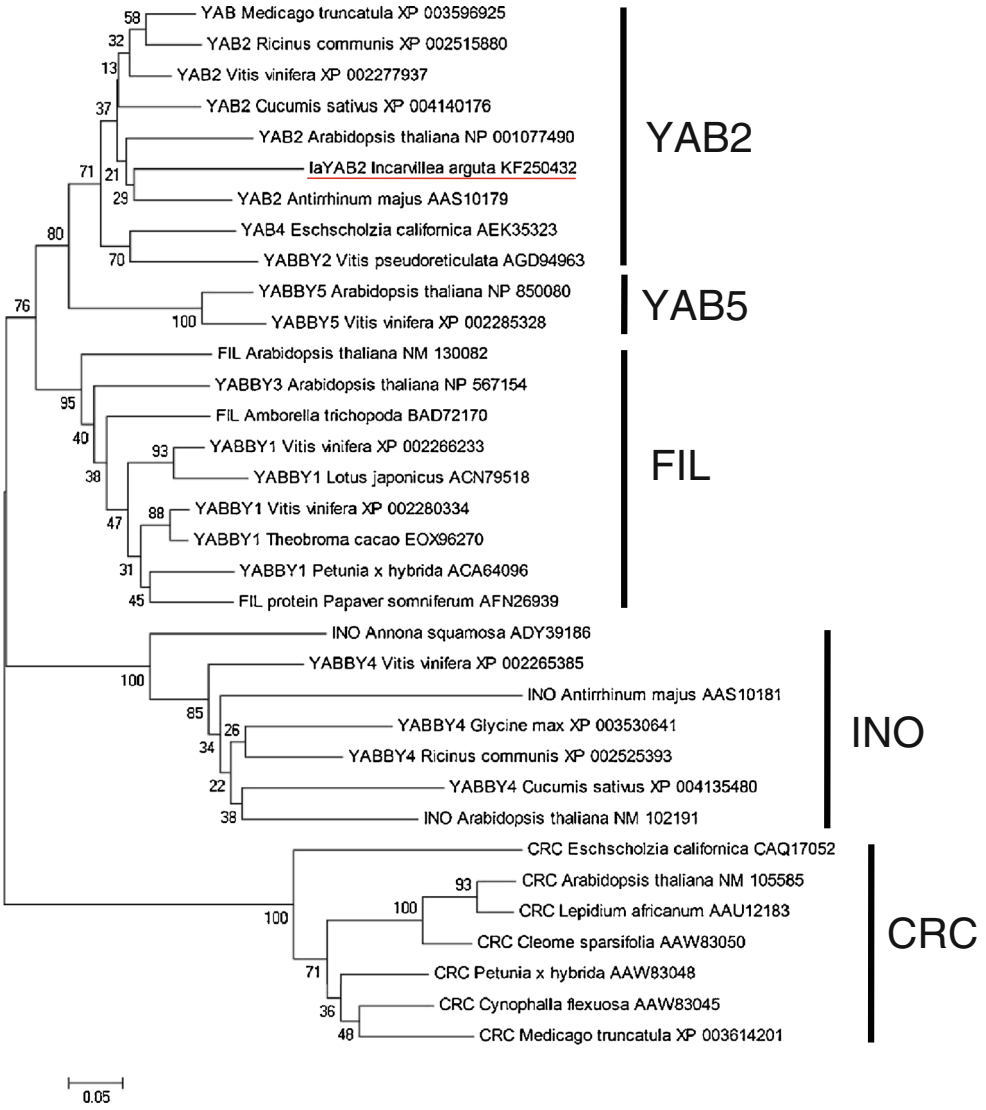

Fig. 4 The phylogenetic tree of YABBY family proteins. An unrooted neighbor-joining tree was generated using MEGA 4.0 neighbor-joining software. The results show the relative similarity of the full-length IaYABBY2 protein in *Incarvillea arguta* and other species, suggesting their evolutionary relationships. Bootstrap values (1,000 replicates) are given. *Bar* (0.1) represents 10 % amino acid substitution per site per million years

biological replicates were conducted. The prime pair (forward prime: AGACAGCGGCTCATCATCTT, reserve prime: AAGCGGTTGTATGCTGAAGG) was used for amplification of *IaYABBY2*. The prime pair (forward prime: GCTG GATTCTGGAGATGGTGTC, reverse prime: CAGCCGTA GTGGTGAATGAGTAA) was used for amplification of *ACTIN* as the control.

Scanning Electron Microscopy

For scanning electron microscopy, the leaves of *35S: IaYABBY2* transgenic plants and wild-type plants were fixed and dehydrated according to Sun et al. (2013). Finally, the samples were observed and photographed under the scanning electron microscope (SEM, JSM-6360LV SEM, JEOL Ltd., Japan) at an accelerating voltage of 10 kV.

Anthocyanin and Photosynthesis Capability Measurement

The transgenic and control *Arabidopsis* seedlings were treated with different environment stress for 1 week, and then the leaves were collected for anthocyanin measurement with the method described previously (Qi et al. 2013). To show the quantity of anthocyanin (A_{535}-A_{560}), per gram fresh weight was used. For measuring the leave photosynthesis capability, the ratio of Fv/Fm was measured to reflect the leave photosynthesis capability (Bai et al. 2011). All experiments were repeated at least three times.

Results and Discussion

Cloning and Structural Analysis of *IaYABBY2* Gene

The full-length ORF of *IaYABBY* gene was amplified using the RACE technique and designated as *IaYABBY2* (Genbank accession no. KF250432). The full-length ORF of *IaYABBY2* was 519 bp, encoding a polypeptide of 172 amino acids (Fig. 1). The reading frame shown was the longest open-reading frame in the cDNA and had both a start and stop codon, thus indicating that the sequence contained the complete coding region. IaYABBY2 is characterized by having two conserved domains, a C2C2 zinc finger-like domain in the N terminus and a helix-loop-helix in the C terminus (Fig. 2), which were referred to as the YABBY domain (Bowman and Smyth 1999). We also measured the transcriptional pattern of *IaYABBY2* gene at different tissues and development time. As shown in Fig. 4, we found that high transcriptional level of *IaYABBY2* in the leave tip, flower, and shoot tip, but lower transcriptional level in the inflorescence and stem (Fig. 3a). We also found that high transcriptional level of *IaYABBY2* in the embryo and young seedling and lower level of transcription in the senescence plant (Fig. 3b). We also checked the gene transcriptional level of *IaYABBY2* in response to different abiotic stress and found that all of the various environment stresses, including high temperature, cold, salt, drought, and UV stress, could induce the transcriptional level of *IaYABBY2* at different degrees (Fig. 3c, d). The phylogenetic analysis of

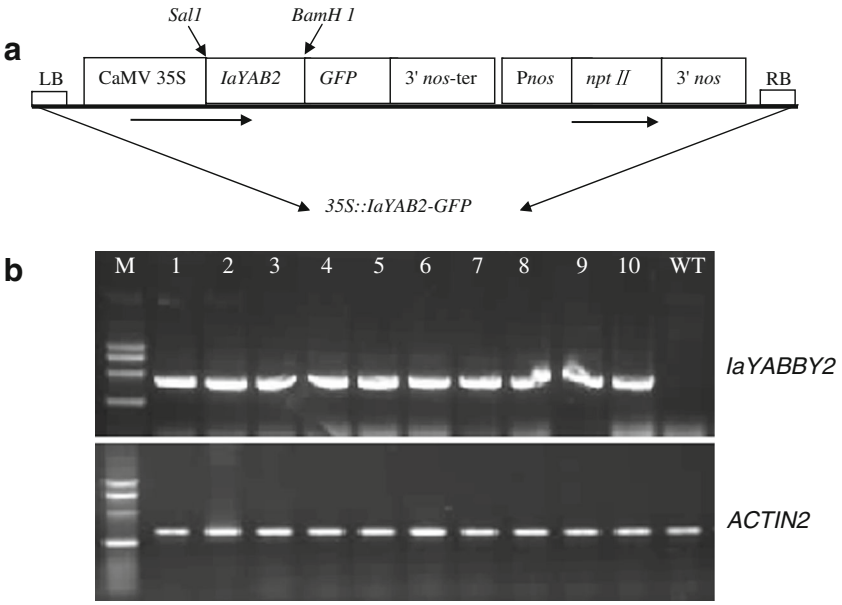

Fig. 5 Construction of the expression vectors and RT-PCR analysis of transgenic plants. **a** Schematic diagram of the plant expression vectors. The vector used for the introduction of the 519-bp *Incarvillea arguta IaYABBY2* cDNA sense orientation into *Arabidopsis*. The sense *IaYABBY2* cDNA inserted is flanked by the cauliflower mosaic virus 35S promoter at the 5′ end and by the transcriptional terminator at the 3′ end. *LB* left border, *RB* right border, *npt II* neomycin phosphotransferase gene whose expression confers plant resistance to kanamycin; *IaYABBY2* -F and *IaYABBY2* -R, primers for RT-PCR. **b** The RT-PCR analysis of the transgenic plants and the wild-type plants. *1–9* transgenic *Arabidopsis* plants, *10* positive control, *WT* wild type plants, *M* DNA molecular marker

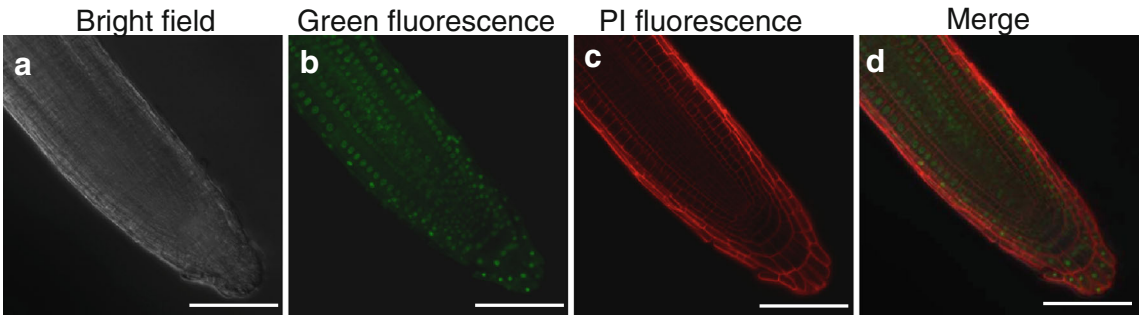

Fig. 6 Subcellular localization of 35S:*IaYABBY2-GFP* in transgenic plants. IaYABBY2-GFP localized in the nucleus. *Bars*=50 μm

amino acid sequences with other YABBY proteins showed that IaYABBY2 was a member of the YAB2 subfamily (Fig. 4). In *Arabidopsis*, *FIL*, *YAB2*, and *YAB3* are expressed in abaxial domains of lateral organs when primordial emerge and begin to differentiate from the meristem. The three genes also encode biochemically similar proteins and proposed that they act redundantly to promote abaxial cell fate in lateral organs (Bowman 2000). These results indicate that *IaYABBY2* is a putative member of *YABBY* family gene in *I. arguta* and might function in the establishment of abaxial lateral organ polarity.

Identification of Ectopic-Expressing *35S:IaYABBY2-GFP* Transgenic *Arabidopsis*

To elucidate the role of *IaYABBY2* gene in the growth and development of *Arabidopsis*, we constructed the sense expression of *IaYABBY2* gene under the control of *CaMV 35S*. The *IaYABBY2* sequence was introduced into a binary vector (pBIN) along with the green fluorescent protein gene (GFP) (Fig. 5a). Transgenic plants were generated by introducing the *35S: IaYABBY2 -GFP* into the wild-type *Arabidopsis* plants. Using specific PCR primers, the presence of the *npt II* gene was detected in all of the kanamycin-resistant transgenic lines, while this gene was not detected in the wild-type *Arabidopsis* (data not shown). Twenty-six transgenic plants were obtained. RT-PCR analysis confirmed the expression of *IaYABBY2*, whereas no signal was detected in the wild-type plants (Fig. 5b). GFP has become an ideal visual marker to select transgenic plants (Sun et al. 2010). Fluorescence microscopy of the roots revealed that IaYABBY2-GFP fusion protein was specifically localized in the nuclei (Fig. 6). These results demonstrate that IaYABBY2 is a nuclear protein, which is consistent with its role as a putative transcriptional factor.

IaYABBY2 May Play a Role in the Establishment of Ad-Abaxial Polarity and Flower Organ Differentiation

The phenotype of the transgenic *Arabidopsis* plants was dramatically different from the wild-type plants (Fig. 7a, b). The

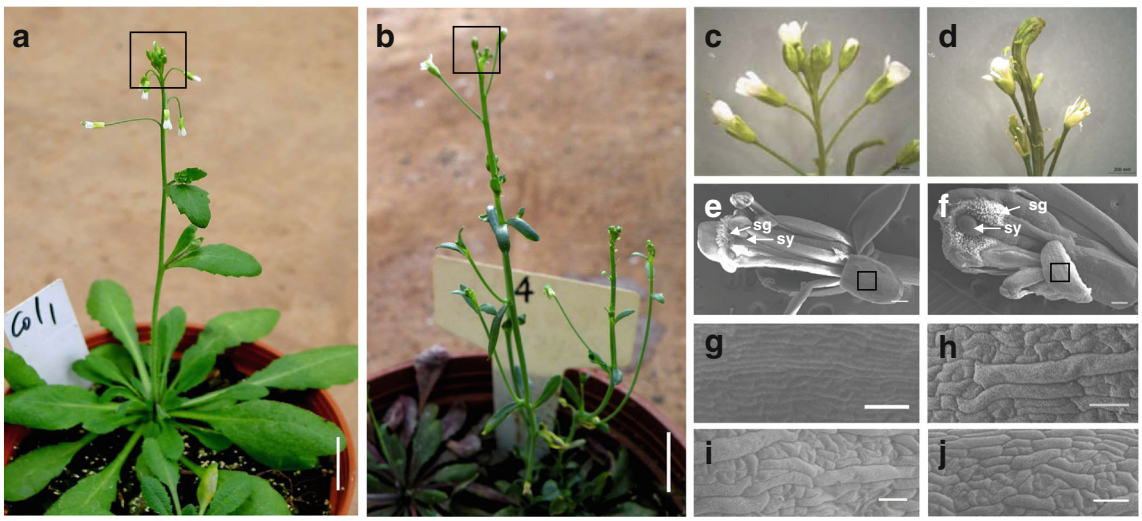

Fig. 7 Phenotypic analysis of *35S:IaYABBY2* transgenic *Arabidopsis* plants. **a** 6-week-old wild-type plant. **b** 6-week-old transgenic *Arabidopsis* plants. **c** The magnified view of region *boxed* in **a. d** The magnified view of region *boxed* in **b**. Scanning electron micrograph of flowers of **e** wild type and **f** transgenic *Arabidopsis*. **g** Scanning electron micrograph of adaxial epidermis of *box* in **e. h** Scanning electron micrograph of adaxial epidermis of box in **f. i** Scanning electron micrograph of abaxial epidermis of *box* in **e. j** Scanning electron micrograph of abaxial epidermis of *box* in **e**. stigma (*sg*), style (*sy*). *Bars* 1 cm (**a**, **b**), 200 μm (**e**, **f**), 50 μm (**i**, **j**)

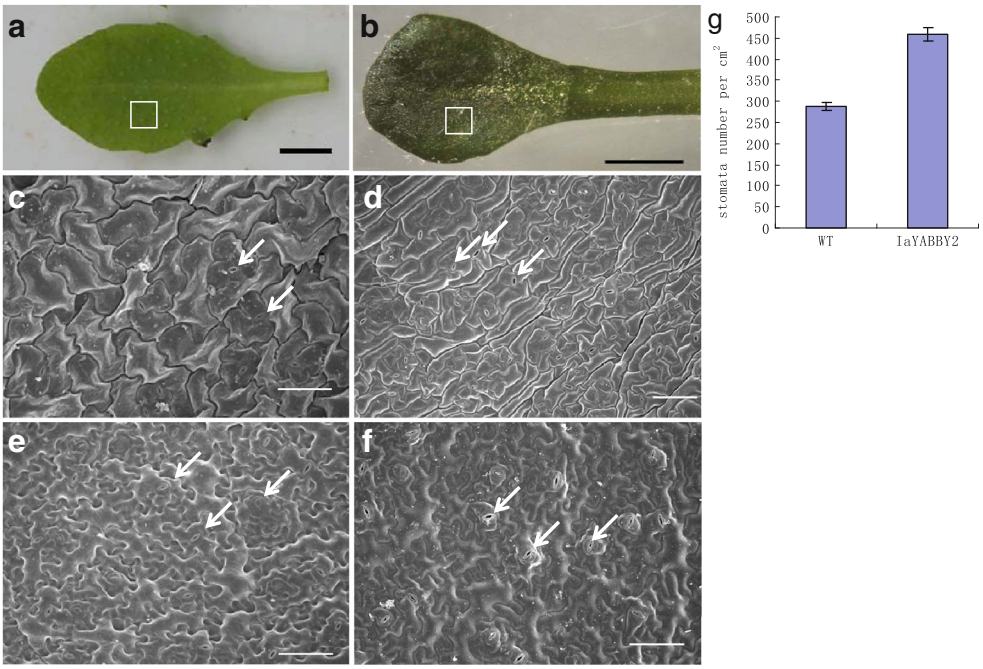

Fig. 8 Scanning electron microscope analysis of leaves of *35S:IaYABBY2* transgenic *Arabidopsis* plants. **a** Abaxial epidermis of a wild-type rosette leaf. **b** Abaxial epidermis of a *35S::IaYABBY2* transgenic rosette leaf. **c** Scanning electron micrograph of adaxial epidermis of *box* in **a.** **d** Scanning electron micrograph of adaxial epidermis of *box* in **b.** **e** Scanning electron micrograph of abaxial epidermis of *box* in **a.** **f** Scanning electron micrograph of abaxial epidermis of *box* in **b.** **g** Stomata densities of wild-type and transgenic plants. *Arrows* indicate stomata. *Bars* in **a** and **b** 0.2 cm, **c**–**f** 50 μm

phenotypes of transgenic plants varied but typically showed small, narrow leaves that are curled outward (Fig. 7b), and the phenotype of sepals were also shown to be narrow and curled outward (Fig. 6d). To confirm whether the ectopic expression of *IaYABBY2* can alter the ad-abaxial polarity of sepals, the sepal epidermises of *IaYABBY2* transgenic plants were examined with a SEM. The adaxial epidermises of wild-type sepals are flat with uniform-sized cells (Fig. 7g), while the abaxial epidermises have uneven surfaces with irregularly sized cells. Interestingly, the adaxial epidermises of *IaYABBY2* transgenic sepals show similar characteristics to the abaxial epidermises of wild-type sepals (Fig. 7h). Overexpression of *IaYABBY2* resulted in aberrant ad-abaxial polarity of sepals suggesting that the *IaYABBY2* might be responsible for the identity of the abaxial epidermis cells. Strong divergence in the proposed functions of the YAB2 subfamily genes has been reported. *AtYAB2* is expressed in both leaf and floral organ primordia. It acts redundantly to promote abaxial cell fate in lateral organs (Siegfried et al. 1999). In contrast, *OsYABBY1*, a member of *YAB2* subfamily genes in *Oryza sativa*, is specifically expressed in the palea and lemma of the flower organs. It is functioned in the differentiation of several specific cell types and is not associated with polar regulation of lateral organ development. (Toriba et al. 2007). *VvYAB2* expression was detected in young petals, anthers, and carpels without polar partitioning, and it might play a role in grape berry

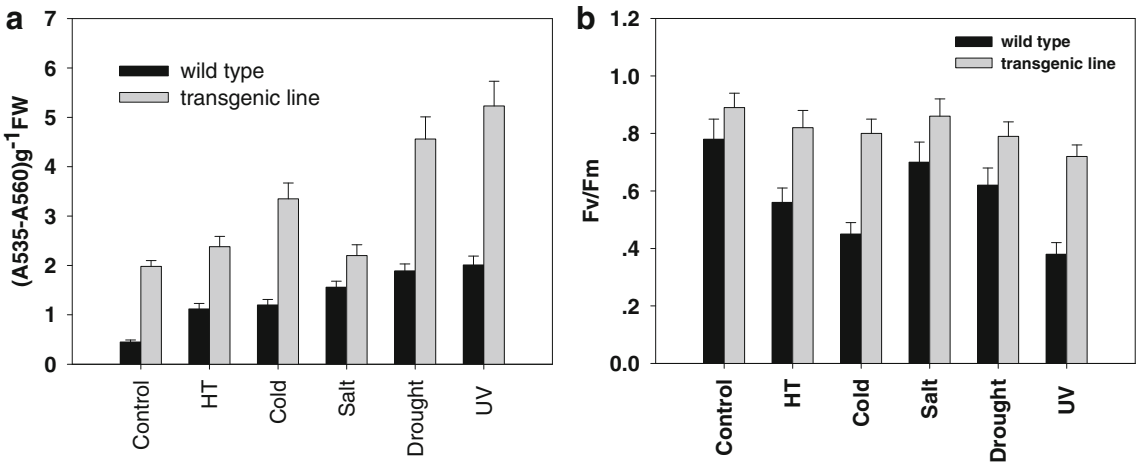

Fig. 9 The effects of different environment stress on anthocyanin and leave photosynthesis capability in the transgenic *35S:IaYABBY2* and the control wild type of *Arabidopsis* plants. **a** The leave anthocyanin content in the transgenic *35S:IaYABBY2* and the control wild type of *Arabidopsis* plants after high temperature (*HT*), cold, salt, drought, and UV stress for 1 week. **b** The leave photosynthesis capability (Fv/Fm) in the transgenic *35S:IaYABBY2* and the control wild type of *Arabidopsis* plants after high temperature (*HT*), cold, salt, drought, and UV stress for 1 week

morphogenesis (Fernandez et al. 2007). In addition, the defects of flower development were observed in *IaYABBY2* transgenic plants (Fig. 7f). YAB1 activity stimulates signals from the organs to the meristem, which are essential for organized growth of the SAM (Goldshmidt et al. 2008). It would seem that *IaYABBY2* is responsible for the differentiation of inflorescence meristem (IM).

To confirm *IaYABBY2* functions in the ad-abaxial polarity, the sixth rosette leaves of transgenic plants were examined by SEM. As shown in Fig. 8, the adaxial surfaces of wild-type leaves are flat with uniform-sized cells and low stomatal densities (Fig. 8c) while the abaxial epidermises have uneven surfaces with irregularly sized cells and high stomatal densities (Fig. 8e). The adaxial cells of the *IaYABBY2* transgenic leaves varied greatly in size, showed a mosaic of large cells among smaller cells, and the leaves were irregularly shaped, appearing from above as a mixture of adaxial and abaxial surfaces (Fig. 8b, d). These results indicated that these properties might be responsible for the leaf curling and the "rough" appearance of the *IaYABBY2* transgenic plants. In addition, high densities of stomata were observed on the adaxial epidermises of transgenic leaves (Fig. 7d, g). Due to special geographical environment and great day–night temperature difference in the Tibetan plateau, plants growing at higher altitude had higher net photosynthetic rates, photosynthesis parameters, and sensitivities to CO_2 enhancement than plants growing at lower altitude (Fan et al. 2011). High densities of stomata indicated the high photosynthetic capacities.

The diversity of gene function could result from the changes in the regulatory regions, especially in some cis-acting elements of the promoters (Simon et al. 2012). *CRC* expression is regulated by a combination of positive and negative regulatory elements in the modules of 5′ upstream regions. These modules function in conjunction with specific factors in the activation of *CRC* in the nectarines and carpels, but not in leaves of *Arabidopsis*. The specific tissue expression of the *CRC* might explain why *CRC* does not work in leaf development (Lee et al. 2005a). Therefore, in order to elucidate the regulation mechanism of *IaYABBY2*, it is important to investigate the cis-acting elements of the *IaYABBY2* and their respective roles in gene regulation.

The Transgenic 35S:IaYABBY2-GFP *Arabidopsis* Shows High Level of Photosynthesis Capability and Anthocyanin Accumulation

To investigate the possible role of IaYABBY2 in plant response to alpine environment stress, such as cold, drought, and UV stress, we then measured the response of the transgenic *35S:IaYABBY2-GFP Arabidopsis* against these environment stresses. We found that a higher level of anthocyanin accumulated in the transgenic line after 2 weeks of high temperature, cold, salt, and drought stress compared with the control wild type of *Arabidopsis*. The strong UV irradiation also induced more anthocyanin accumulation in the transgenic line compared with the wild-type *Arabidopsis* (Fig. 9a). The ratio of Fv/Fm is the important index to reflect the plant photosynthesis capability. We compared the Fv/Fm ratio between transgenic *35S:IaYABBY2-GFP Arabidopsis* and wild-type line under cold, drought, and UV stress; we found that the higher Fv/Fm ratio in the transgenic *35S:IaYABBY2-GFP Arabidopsis* compared with the wild type after high temperature, cold, salt, drought, and UV stress (Fig. 9b) suggests the tolerance role of IaYABBY2 in plant adaptation to alpine environment stress.

In summary, our data indicate that the IaYABBY2-GFP proteins execute their function in the nucleus, consistent with a putative function as transcription factor. Clearly, the protein plays an important role in plant development and morphogenesis.

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