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Heterologous expression of a novel *Poa pratensis* gibberellin 2-oxidase gene, *PpGA2ox*, caused dwarfism, late flowering, and increased chlorophyll accumulation in *Arabidopsis*

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Abstract

Gibberellin 2-oxidases (GA2oxs) irreversibly convert bioactive gibberellins (GAs) and their immediate precursors into inactive GAs *via* 2-β hydroxylation and so regulate gibberellin content in plants. However, to the best of our knowledge, little has been known about the GA2oxs and its function in cool season turfgrass *Poa pratensis*. In this study, rapid amplification of cDNA end (RACE) was employed to isolate *PpGA2ox* from *P. pratensis*. The open reading frame of *PpGA2ox* was 1 047 bp in length, corresponding to 348 amino acids. PpGA2ox was localized in both nucleus and cytoplasm. The expression of *PpGA2ox* could be up-regulated by 10 µM gibberellic acid, 5 µM methyl jasmonate, or 10 µM indole-3-acetic acid. In addition, its native promoter could drive *GUS* expression in both leaf apex and shoot apical region. Moreover, overexpression of *PpGA2ox* in *Arabidopsis* led to GA-deficiency leading to dwarf phenotype, delayed flowering time, and increased chlorophyll content. Our study suggests that *PpGA2ox* could be a candidate gene for breeding new cultivars of *P. pratensis*.

Additional key words: gibberellic acid, indole-3-acetic acid, methyl jasmonate, transgenic plants.

Introduction

Bioactive gibberellins (GAs) play crucial roles in plant growth and development including stem elongation, leaf expansion, and flower development (Yamaguchi 2008, Hedden and Thomas 2012, Yin *et al*. 2017). In plants, geranylgeranyl diphosphate, a common C_{20} precursor for diterpenoids, is converted into GAs by several reactions using terpene synthases, cytochrome P_{450} monooxygenases, and 2-oxo-glutarate-dependent dioxygenases (Yamaguchi 2008). To achieve a dynamic balance, plants have also evolved an effective deactivation system and GA2-oxidases (GA2oxs) irreversibly inactivate GAs and its immediate precursors *via* 2-β hydroxylation (Hedden and Phillips 2000, Wuddineh *et al*. 2015).

Recently, *GA2oxs* have been increasingly characterized in various plant species including *Prunus salicina* (El-Sharkawy *et al.* 2012), *Panicum virgatum* (Wuddineh *et al*. 2015), *Camellia lipoensis* (Xiao *et al*. 2016), and *Brassica napus* (Yan *et al.* 2017). Heterologous overexpression of plum *GA2ox* in *Arabidopsis thaliana* causes characteristic GA deficient phenotypes including smaller leaves, shorter stems, and delayed reproductive events than in wild-type (WT) plants. In transgenic switchgrass, expression of *PvGA2ox5* and *PvGA2ox9* improves architecture and reduces biomass recalcitrance.

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Abbreviations: ABA - abscisic acid; *FLC - FLOWERING LOCUS C*; *FT- FLOWERING LOCUS T*; GA - gibberellin; GA2oxs - GA2-oxidases; GA_3 - gibberellic acid; GUS - β-glucuronidase; IAA - indole-3-acetic acid; MeJA - methyl jasmonate; MS - Murashige and Skoog; PAC - paclobutrazol; qPCR - quantitative polymerase chain reactions; *SOC1 - SUPPRESSOR OF OVEREXPRESSION OF CO1;* WT - wild type.
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Ectopic expression of *ClGA2ox1* or *ClGA2ox3* from camellia in *Nicotiana tabacum* induces dwarfism and smaller flowers. Accordingly, *Arabidopsis* over-expressing *BnGA2ox6* from rapeseed shows dwarfism, late flowering, and enhanced chlorophyll accumulation. Previous studies demonstrated that *GA2oxs* could be valuable factors in plant architecture engineering breeding.

Poa pratensis (Kentucky bluegrass), a typical C_3 cool season grass, is widely used in home lawns, landscaping, football pitches, and golf courses (Puyang *et al.* 2015). Semi-dwarf or dwarf *P. pratensis* is becoming increasingly popular around the world to save the intensive labour and energy costs in turf maintenance. GA2oxs were reported to fine tune the endogens GA content resulting in dwarf phenotypes of plants. However, little has been known about the GA2oxs in *P. pratensis*. The objectives of this study were to isolate a gibberellin 2-oxidase gene from *P. pratensis,* to determine its promoter activity and its subcellular localization pattern, and finally, to uncover its function utilizing transgenic *Arabidopsis*.

Materials and methods

Plants and growth conditions: *Poa pratensis* L. cv. Baron seeds were purchased from the *TopGreen* seed company (Beijing, China). The seedlings were cultivated in a greenhouse at day/night temperatures of 20/18 °C, a relative humidity of approximately 60 %, a 12-h photoperiod, and an irradiance of 400 μmol m^{-2} s^{-1} . *Nicotiana benthamiana* Domin was cultivated in a growth chamber at temperatures of 22/20 °C, a relative humidity of 65 %, a 16-h photoperiod, and an irradiance of 200 μmol m^{-2} s^{-1}. *Arabidopsis thaliana* L. ecotype Columbia plants were kept at day/night temperatures of 24/22 °C, a relative humidity of 65 %, a 16-h photoperiod, and an irradiance of 200 μmol m^{-2} s^{-1} to generate transgenic lines. Seeds utilized for the phenotype observation were harvested from individual lines, which grew under the identical growth condition. The plants were fertilized weekly using a half-strength Hoagland's solution (Hoagland and Arnon 1950).

Isolation of *PpGA2ox*: Total RNA was isolated from *P. pratensis* leaves using the plant RNA kit (*OMEGA*, Norcross, GA, USA). Full-length 3'-5' sequences of *PpGA2ox* were obtained using rapid amplification of cDNA end (RACE) with a *SMARTer RACE* kit (*Clontech*, Palo Alto, CA, USA). Primer sequences for 3'-5' for RACE amplification were designed using a known cDNA sequence fragment screened from *RNA-seq* database (Table 1 Suppl.). The PCR products were purified and then inserted into the pMD19-T cloning vector (*TaKaRa*, Dalian, China) before being sequenced at *Tianyi Huiyuan Company* (Beijing, China). The obtained sequences were used to design specific primers, PpGA2ox-F and PpGA2ox-R, for amplification of full-length complementary DNA and genomic DNA sequences.

Isolation of the *PpGA2ox* promoter: Genomic DNA was extracted from leaves using a plant DNA kit (*OMEGA*). Using the gDNA as a template, the genome walking was carried out with a genome walking kit (*TaKaRa*) according to the instructions. Three specific primers, GA2ox-R1, GA2ox-R2, and GA2ox-R3, were used. The purified PCR products were sequenced using the GA2ox-R3 primer. Based on the sequencing data, the promoter-specific primers, Prom-F and Prom-R, were used to amplify the upstream sequence of *PpGA2ox*.

Bioinformatics: The PpGA2ox amino acid sequence was derived from the corresponding cDNA sequence using *DNAMAN* software (*v. 7.0*). *BLAST* analysis of the *NCBI* database was used to identify homologs. A phylogenetic tree was built using *MEGA* (*v. 5.0*) with the neighbor-joining method (Tamura *et al.* 2011). A bootstrap analysis was performed with 1 000 replicates excluding positions with gaps. The molecular masses (Mr) and theoretical isoelectric points (pI), potential signal peptide cleavage sites, subcellular localization pattern, and *cis*-regulatory elements in promoter were analyzed by *Compute pI/MW* tool (http://web.expasy.org/compute_pi/), *SignalP 4.1* server (http://www.cbs.dtu.dk/services/SignalP/) (Petersen *et al.* 2011), *ProtComp 9.0* (http://www.softberry.com), and *PlantCARE* database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/), respectively.

Real-time quantitative PCR: The expression pattern of *PpGA2ox* in *P. pratensis* was analyzed in the leaves at different developmental stages: young (3-week-old), fast-growing (6-week-old), and mature (3-month-old), as well as in roots and stems using real-time qPCR. *PpGA2ox* expression profiles were examined in 3-month-old *P. pratensis* after 24-h induction with 10 μM gibberellic acid (GA_3), 5 μM methyl jasmonate (MeJA), or 10 μM indole-3-acetic acid (IAA). *GUS* gene expression analysis was determined in 2-week-old transgenic *A. thaliana* under treatments with 10 μM GA_3 or 10 μM paclobutrazol (PAC). *Arabidopsis FLOWERING LOCUS C* (*AtFLC*), *SUPPRESSOR OF OVER-EXPRESSION OF CO1* (*AtSOC1*), and *FLOWERING LOCUS T* (*AtFT*) were selected to monitor the flowering mechanisms. All the gene specific primers could be found in Table 1 Suppl. Reactions were performed in 96-well blocks with a *CFX Connect* PCR system (*BIO-RAD*, Hercules, CA, USA) using *SYBR Premix* (*TaKaRa*) in a total volume of

0.025 cm^3. The two-step program was set: initial denaturation at 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, and 60 °C for 30 s. The *P. pratensis actin* (GenBank accession No. KX342945) and *A. thaliana UBQ10* (GenBank accession No. NM116771) were selected as the internal reference genes (Table 1 Suppl.). The relative expression was calculated using the comparative ΔΔCt method (Livak and Schmittgen 2001). All data were presented as the means of at least three independent biological replicates (each including three technical replicates) with corresponding standard deviations (SDs).

Binary vector construction and generation of transgenic plants: The *35S::PpGA2ox:YFP* fusion construct was created by inserting the complete *PpGA2ox* open reading frame (ORF) into plasmid 3302Y(Jia *et al*. 2016). Primers 3302Y-GA2ox-F and 3302Y-GA2ox-R were used to amplify the *PpGA2ox* coding sequence (Table 1 Suppl.), which was then purified and inserted into 3302Y vector digested with *Bgl*II (*TaKaRa*) using an *In-fusion HD* cloning kit (*TaKaRa*).

The $PpGA2ox_{pro}$*::GUS* fusion construct contained a 1 109 bp *PpGA2ox* promoter region. The promoter region was amplified from the plasmid containing the target sequence using primers 1 391-GA2ox-F and 1 391-GA2ox-R (Table 1 Suppl.). The pCAMBIA1391Z vector was digested with *Nco*I (*TaKaRa*). Then the purified *PpGA2ox* promoter was inserted into the digested vector using an In-fusion *HD Cloning* kit (*TaKaRa*) to produce the $PpGA2ox_{pro}$*::GUS* fusion construct.

Using the floral dip method, *Agrobacterium* strain GV3101 transformed with the constructed plasmids was used to infect *Arabidopsis* plants to generate transgenic plants expressing *PpGA2ox* or $PpGA2ox_{pro}$*::GUS*. Transformed *Arabidopsis* plants (T_2) were screened using 60 mg dm^{-3} glufosinate. Positive transgenic plants were verified by PCR. Representative T_3 transgenic lines that exhibited 100 % resistance to glufosinate were harvested for further phenotype observation or GUS staining assays.

Subcellular localization of PpGA2ox and GUS assay: To examine the subcellular localization pattern of PpGA2ox, *Agrobacterium* strain EHA105 transformed with the *35S::PpGA2ox:YFP* fusion construct was used to transform 3-week old *N. benthamiana* plants according to the method of Sparkes *et al*. (2006). After dark inducement for 48 h, tobacco leaf cells were examined and photographed using a *SP-5* laser confocal scanning microscope (*Leica*, Mannheim, Germany) according to the method of Teng *et al.* (2016).

The GUS staining was carried out using a GUS kit (*O'BioLab*, Beijing, China) according to the instruction provided by the manufacturer. After removing the chlorophyll with 70 % (v/v) ethanol, the tobacco seedlings were photographed using a *M205FA* stereomicroscope (*Leica*, Mannheim, Germany).

Phenotype observation: *Arabidopsis* line-35 and line-36 with the highest amount of transcripts among the T_3 transgenic lines were selected as the representative lines for the phenotype observation. The seeds of the representative lines and wild type (WT) were sterilized with 70 % (v/v) ethanol and 1 % (m/v) sodium hypochlorite and then sowed on Murashige and Skoog (MS) medium. The phenotype was photographed using an *EOS 60D* digital camera (*Canon*, Tokyo, Japan) after growing for 14 d. The chlorophyll content was measured using in 95 % ethanol extract according to the protocol of Teng *et al*. (2016). To examine whether exogenous application of gibberellin could rescue the dwarfism phenotype of the *PpGA2ox*-overexpressing lines, sterilized seeds of both the transgenic lines and WT were sowed on the MS medium in culture flasks with or without GA_3. After 4 weeks, the seedlings were photographed. For the determination of flowering time, the 12-d-old seedlings of line-35 and line-36 were transplanted from MS medium to nutrition medium containing peat, *Vermiculite*, and *Perlite* (1:1:1 in volume) and grown in growth chambers until the primary open flowers were observed.

Results

The *PpGA2ox* cDNA sequence was deposited in the *NCBI* database with the accession number of KX254272. The ORF of *PpGA2ox* was 1 047 bp in length, corresponding to 348 amino acids (Fig. 1*A* Suppl.). PpGA2ox belonged to the GA2ox superfamily. The theoretical pI was 6.21, and the molecular mass was 37.89 kDa. No potential signal peptide was found in the PpGA2ox protein. Phylogenetic analysis showed that PpGA2ox was most closely related to the proteins from *Hordeum vulgare* or *Triticum aestivum* (Fig. 1*B* Suppl.).

A 1109 bp upstream fragment in front of the ATG start codon containing CAAT-box and TATA-box was examined for promoter elements identification (Fig. 1*C* Suppl.). Two MeJA-responsive motifs were also identified, including CGTCA-motif and TGACG-motif. In addition, one *cis*-acting regulatory element related to meristem expression (CAT-box) and two involved in endosperm expression (GCN4-motif and Skn-1 motif) were found.

Real-time qPCR was carried out to investigate the expression characters of *PpGA2ox*. The transcripts were found in all investigated tissues including leaf, stem, and root, and the highest transcription was observed in the leaves (Fig. 1*A*). Moreover, the expression of *PpGA2ox*

was higher in young leaves than in fast-growing and in mature leaves (Fig. 1*B*). The *PpGA2ox* expression was up-regulated significantly by 10 µM GA_3 with the highest transcript abundance after 24 h (122-fold higher than before application; Fig. 1*C*). In addition, *PpGA2ox* was induced by 5 µM MeJA or 10 µM IAA, with the highest expression found after 6 and 1 h, respectively (Fig. 1*D,E*).

Transient overexpression of *35S::PpGA2ox:YFP* in *N. benthamiana* leaf cells allowed us to study the subcellular localization of PpGA2ox. The strong yellow florescent protein (YFP) signal was detected in the whole cell of the control plants whereas the YFP signal was only found in nucleus and cytoplasm in *35S::PpGA2ox:YFP* overexpressing cells (Fig. 2). It indicated that PpGA2ox was localized in nucleus and cytoplasm.

The native promoter of *PpGA2ox* was infused with *GUS* gene to generate the *PpGA2oxpro::GUS* construct. Then transgenic *Arabidopsis* lines were generated to explore the promoter activity. Histochemical analysis showed that strong GUS activity was in both the leaf apex and shoot apical region of the *PpGA2oxpro::GUS* transgenic seedlings (Fig. 3*B-D*). However, no GUS signal was found in the seedlings transformed with pCambia1391Z empty vector (the control) (Fig. 3*A*). It could be supposed that *PpGA2ox* expressed most abundantly in leaf apex and shoot apical region.

Furthermore, real-time qPCR analysis was also employed to investigate the *GUS* gene expression under the treatments with 10 µM GA_3 or 10 µM PAC using the *PpGA2oxpro::GUS* transgenic seedlings. It showed that the *GUS* expression was 1.92-fold higher than control under GA_3 treatment for 3 d. However, it was lower than control after PAC inducement (Fig. 3 Suppl.).

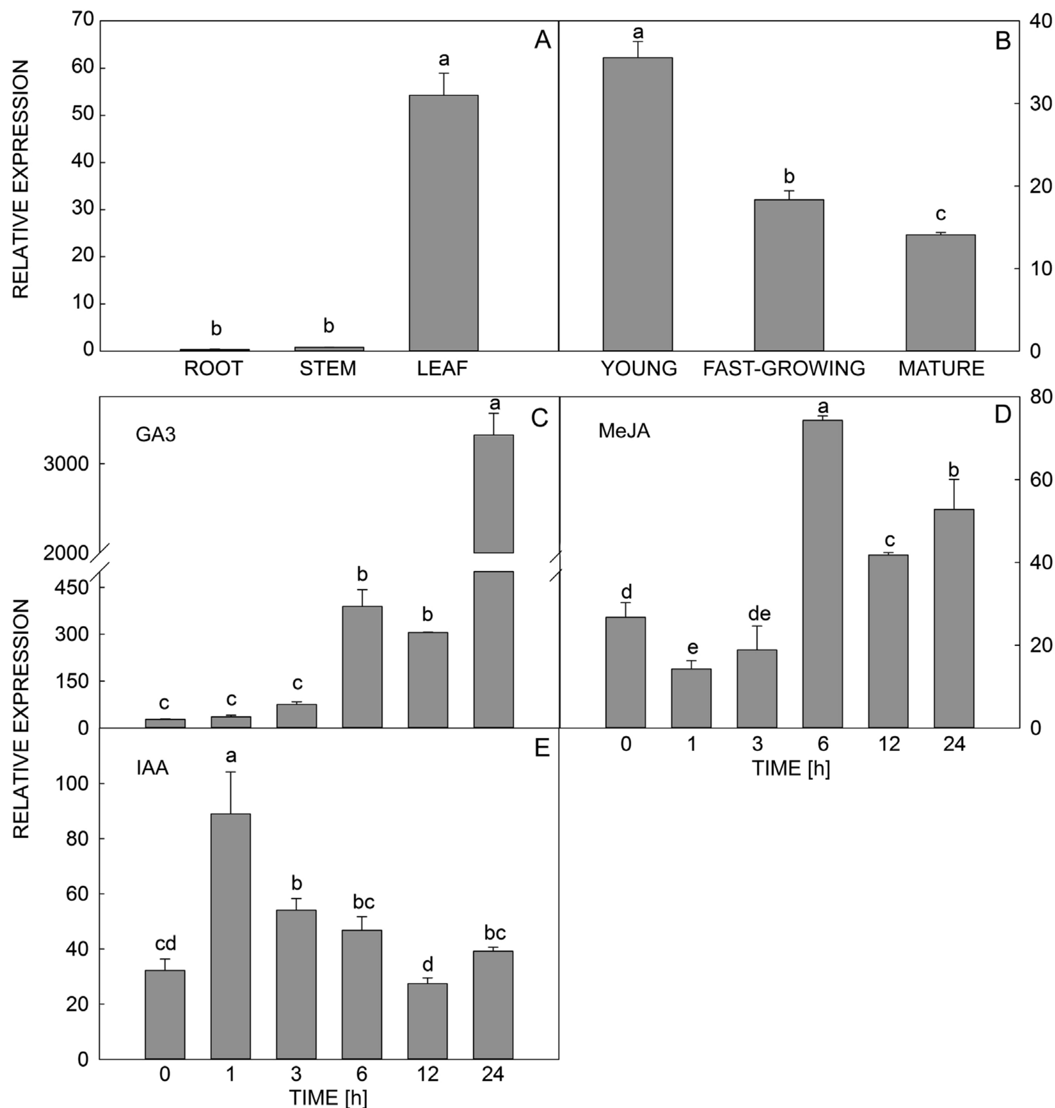

Fig. 1. Expression profiles of *PpGA2ox*. *A* - *PpGA2ox* expression in root, stem, and leaf of *Poa pratensis*. *B* - Expression of *PpGA2ox* in leaves at different developmental stages. *C, D, E* - *PpGA2ox* expression in plants exposed to 10 µM GA_3 (*C*), 5 µM MeJA (*D*), and 10 µM IAA (*E*). Means ± SDs (n = 3). Different letters indicate significant differences at 5 % level of probability.

Transgenic *Arabidopsis* plants overexpressing *PpGA2ox* were generated to further explore the function of *PpGA2ox*. Two representative T_3 homozygous lines, line-35 and line-36, were selected based on their higher expression of *PpGA2ox* than in other lines. Transgenic *Arabidopsis* seedlings exhibited gibberellin deficiency leading to dwarf phenotype with smaller leaves and shorter stems compared with WT after being grown on the MS medium for 14 d (Fig. 4*A,B*). The fresh mass of the transgenic lines was also lower than that of WT (data not shown).

To investigate the underlying genetic mechanism that caused the dwarfism of the transgenic lines, the relative expressions of *AtGA2ox1*, *AtGA2ox2*, and *AtGA2ox3* were

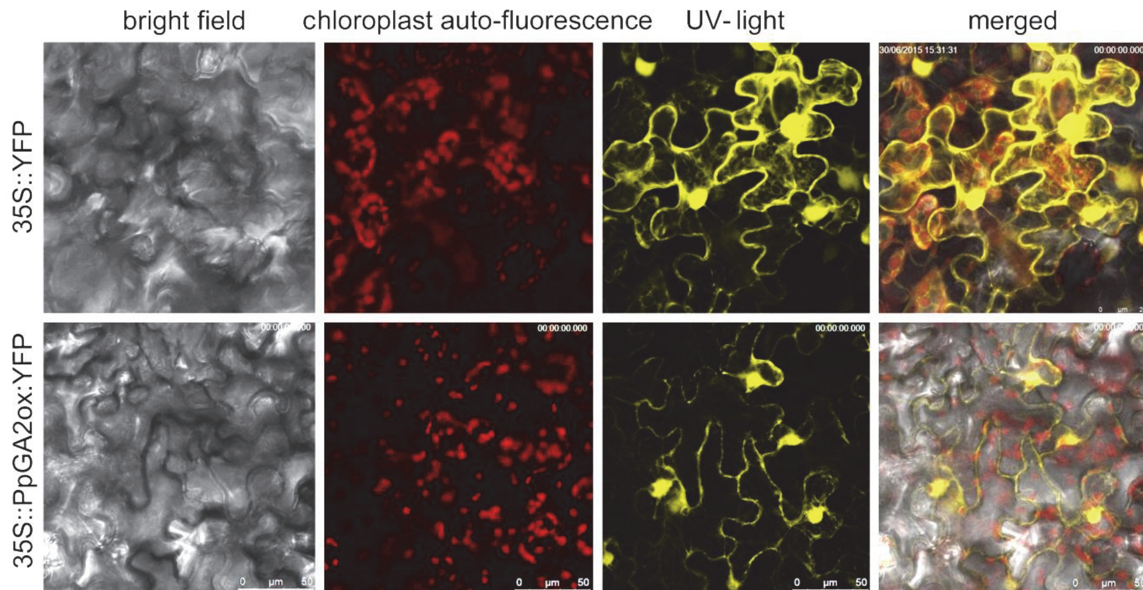

Fig. 2. Subcellular localization of PpGA2ox under UV radiation in *Nicotiana benthamiana* leaf epidermis. Fluorescence due to the 35S:: PpGA2ox:YFP was strongly distributed in both the nucleus and cytoplasm. The *scale bar* is 50 µm.

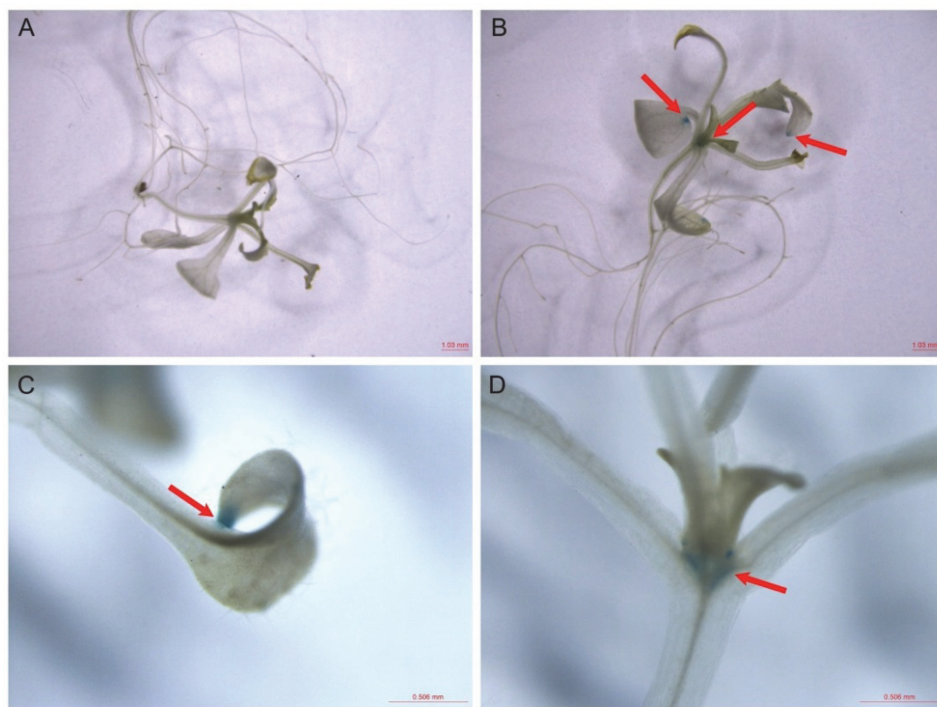

Fig. 3. GUS staining (blue colour) in 2-week-old *Arabidopsis* seedlings: control (*A*), *PpGA2ox*$_{Pro}$*::GUS*-overexpressing lines (*B*), leaf apex (*C*), and shoot apical region (*D*). The *scale bar* is 1 mm (*A*,*B*) or 0.5 mm (*C*,*D*).

examined using real-time qPCR. The results showed that the expression of the selected marker genes related to gibberellin catabolism were higher in the transgenic lines than in the WT plants (Fig. 4*C-F*). In particular, the relative expressions of *AtGA2ox1, AtGA2ox2*, and *AtGA2ox3* in line-35 increased by 117.8, 68.3, or 31.7 % compared to WT, respectively.

After 4 weeks of cultivation on MS medium without GA_3, line-35 and line-36 exhibited obvious dwarf phenotype compared with the WT grown in MS medium without GA_3 (Fig. 2 Suppl.). Nevertheless, the plant height difference between the transgenic lines and WT disappeared when they were grown on MS medium containing GA_3, indicating that application of 100 µM GA_3 rescued the dwarf phenotype in a period of 4 weeks.

A surprising phenotype which *PpGA2ox*-overexpressing lines exhibited later flowering than the control drew our attention (Fig. 5*A*). To quantify the flowering time, we recorded the time when the first open flowers were observed and we found that overexpression of *PpGA2ox* delayed flowering for 10 to 14 d in comparison with WT plants. Three marker genes related to plant flowering time including *AtFLC*, *AtSOC1*, and *AtFT* were selected to monitor the underlying mechanisms. The

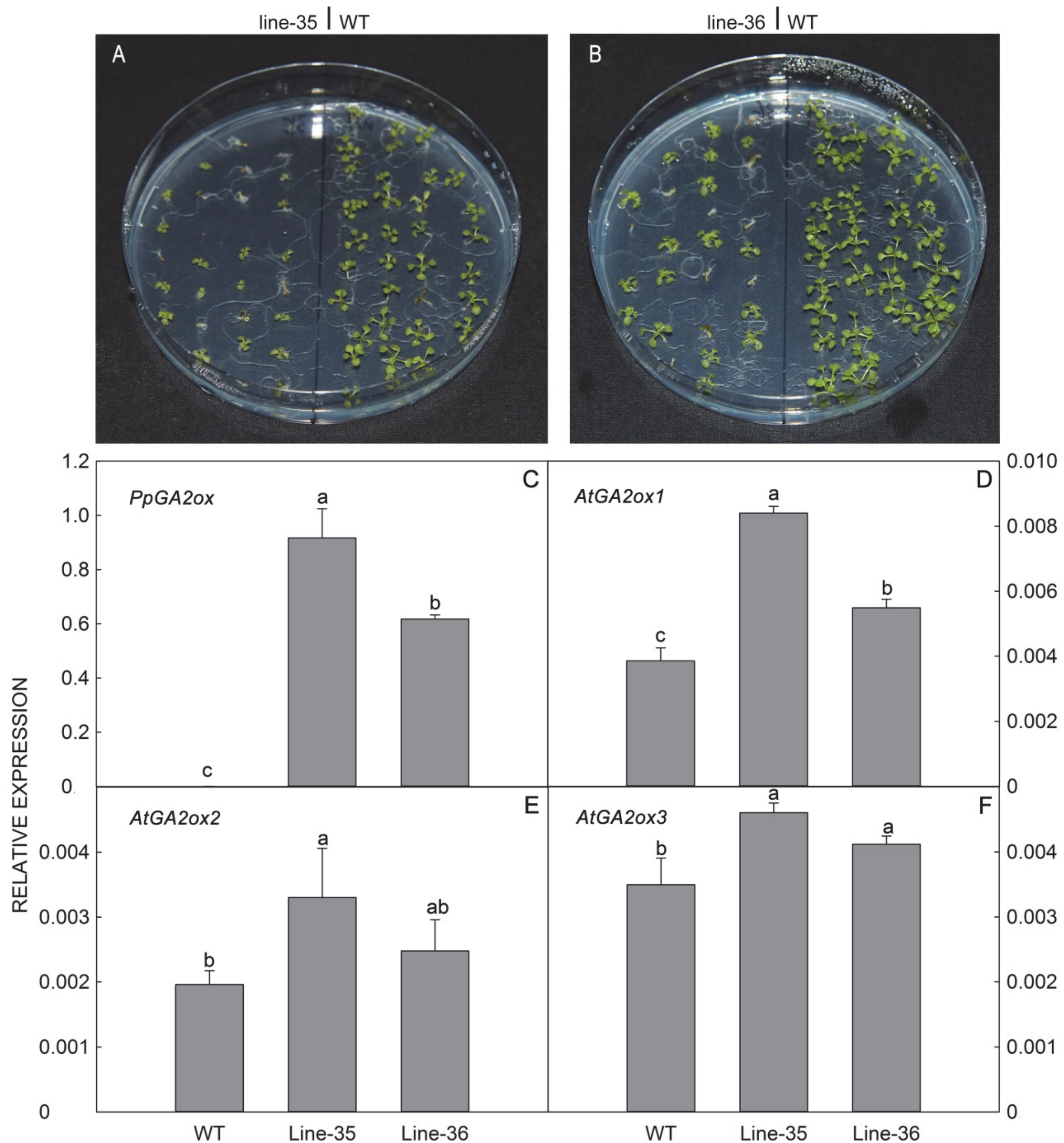

Fig. 4. Phenotype observation of 2-week-old *PpGA2ox*-overexpressing lines 35 (*A*) and 36 (*B*), Relative expressions of *PpGA2ox* (*C*), *AtGA2ox1* (*D*), *AtGA2ox2* (*E*), and *AtGA2ox3* (*F*). Means ± SDs (n = 3). Different letters indicate significant differences at 5 % level of probability.

results showed that the relative transcription of *AtFLC* in line-35 or line-36 was increased by 83.1 and 28.3 % compared to that of WT, respectively (Fig. 5*D*). On the contrary, the expression of *AtSOC1* in line-35 and line-36 was reduced by 41.6 or 31.0 %, respectively, and expression of *AtFT* was reduced by 77.6 or 63.6 %, respectively, relative to control (Fig. 5*E,F*). Moreover, the leaf colour of the transgenic lines was greener than that of WT but the leaf size was obviously smaller than in the WT (Fig. 5*B*). Chlorophyll content in line-35 and line-36 was 1.57- or 1.55-fold higher than in the WT (Fig. 5*C*).

Discussion

The GA2oxs, a kind of 2-oxoglutarate-dependent dioxygenases, catalyses the deactivation of bioactive GAs or their precursors. In this study, a novel gibberellin 2-oxidase gene, *PpGA2ox*, was isolated from *P. pratensis* with the aim of genetic engineering *P. pratensis* for improved architecture. PpGA2ox belonged to the GA2ox superfamily and contained three Fe^{2+} binding sites and one 2-oxoglutarate binding site. It was localized in both nucleus and cytoplasm which was consistent with the localization AtGA2ox4 in *Arabidopsis* (Lee *et al.* 2014), OsGA2ox6 in rice (Ueguchi-Tanaka *et al.* 2005), and PslGA2ox in plum (El-Sharkawy *et al.* 2012). Previous studies proved that GA2oxs play diverse roles in regulating gibberellin metabolism compared with the cytosol localized GA20ox and GA3ox (Hedden and Phillips 2000, Olszewski *et al.* 2002, Wuddineh *et al.* 2015).

Examination of *PpGA2ox* expression revealed that *PpGA2ox* was widely distributed in different tissues of *P. pratensis*, but the highest expression was in leaves. In addition, *PpGA2ox* was expressed most abundantly in young leaves, implying that *PpGA2ox* actively

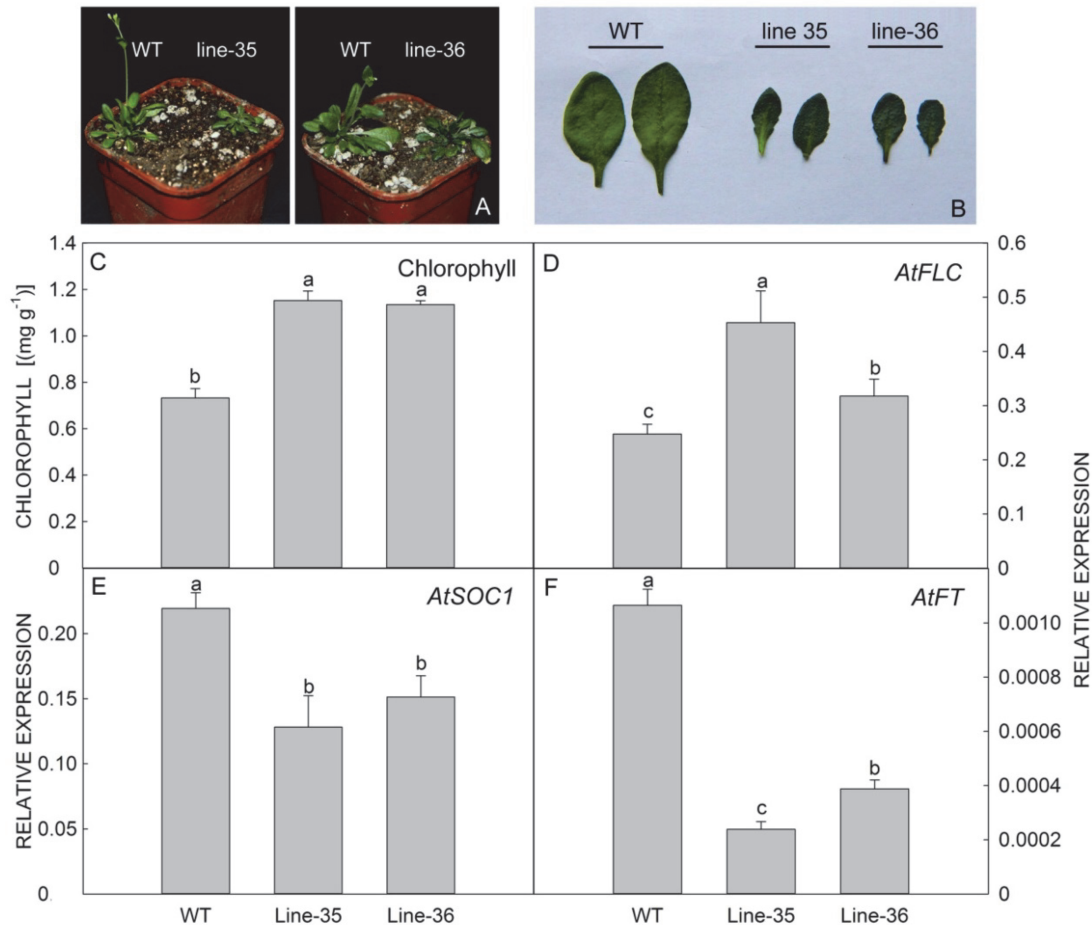

Fig. 5. *A* - phenotype of WT and transgenic *Arabidopsis* plants line-35 and line-36. *B*, *C* - Leaf colour (*B*) and chlorophyll content per fresh mass basis (*C*) in WT and transgenic plants. *D, E, F* - Relative expressions of genes regulating flowering time: *AtFLC* (*D*), *AtSOC1*(*E*), and *AtFT* (*F*). Means ± SDs (n = 3). Different letters indicate significant differences at a 5 % level of probability.

participated in early leaf development. The motifs identified in *PpGA2ox* promoter implied that its expression might be regulated by different hormones. Application of 10 µM GA_3 induced the significant expression of *PpGA2ox* in this study. It is consistent with the expression pattern of *AaGA2ox1*, *AaGA2ox2* and *AaGA2ox3* from *Artocarpus altilis* (Zhou and Underhill 2016). Exogenous GA_3 might cause drastic fluctuation of endogenous GA due to activation of the GA biosynthetic and degradation mechanisms. Induction experiments proved that *PpGA2ox* could be up-regulated by 5 µM MeJA, which is supported by the MeJA-responsiveness *cis*-elements in the promoter sequence. The often reported regulation of GA by auxin led us to examine the expression of *PpGA2ox* after IAA application. In this study, IAA application induced the *PpGA2ox* expression during the first hour which is in accordance with the findings of Frigerio *et al.* (2006) that auxin up-regulates *GA2ox* expression very quickly after application.

To gain insight into the actual localization of *PpGA2ox* in regulating GA metabolism in plant, we examined the spatial regulation of *PpGA2ox* using GUS staining. Histochemical staining of GUS activity in transgenic *Arabidopsis* confirmed that *PpGA2ox* was expressed in both leaf apex and shoot apical region. This is in accordance with *AtGA2ox2* (Frigerio *et al*. 2006). Further, real-time qPCR revealed that exogenous GA enhanced the expression of *GUS* while PAC application caused a decline in the *GUS* expression. Together with the relative expression character of *PpGA2ox* under GA treatment, it is rational to rule out a scenario that plants could activate the expression of *PpGA2ox* to deal with the abrupt increase of GA, but suppress the expression of *PpGA2ox* to respond to the decrease of GA. This finding not only uncovered the actual functional localization of *PpGA2ox* in plants, but also interpreted the possible physiological roles of *PpGA2ox* in regulating GA metabolism.

GA2oxs promote the inactivation of bioactive GAs or their precursors. Previous studies reported that *GA2oxs* overexpression resulted in typical GA-deficiency phenotype in plants (Wuddineh *et al*. 2015, Xiao *et al.* 2016, Yan *et al*. 2017). In this study, overexpression of *PpGA2ox* caused a dwarf phenotype in *Arabidopsis*. *AtGA2ox1*, *AtGA2ox2*, and *AtGA2ox3* were reported to generate inactive GA forms by catalyzing their 2β-hydroxylation (Olszewski *et al.* 2002, Gallego-Giraldo *et al.* 2008, Yamaguchi 2008, Hedden and Thomas 2012). The relatively high expressions of *AtGA2ox1*, *AtGA2ox2*, and *AtGA2ox3* reflected a programed GA catabolism at least partially contributing to the dwarf phenotype of *PpGA2ox*-overexpressing plants. Furthermore, the dwarf phenotype of the transgenic lines was reversed by exogenous GA_3. It supported the hypothesis that it was the overexpression of *PpGA2ox* that inactivated the bioactive GAs in transgenic lines and resulted in the GA-deficiency leading to dwarfism.

Ectopic expressions of *BnGA2ox6* (Yan *et al.* 2017), *ClGA2ox1* (Xiao *et al.* 2016), and *NtGA2ox* (Gargul *et al.* 2013) caused late flowering in *A. thaliana*, *N. tabacum*, and *Petunia* hybrid, respectively. Consistent with these results, the heterologous expression of *PpGA2ox* caused delayed flowering time in transgenic *Arabidopsis* compared with that of the control. *SOC1* (Hepworth *et al.* 2002) and *FT* (Hisamatsu and King 2008) were reported as key genes in accelerating flowering time, while *FLC* (Kim *et al.* 2007) was reported as a floral repressor. The relatively higher amount of *FLC* transcripts together with the relatively lower amount of *SOC1* and *FT* transcripts in *PpGA2ox*-overexpressing lines interpreted the mechanism of the delayed flowering phenotype. In addition, the transgenic lines also showed dark green and small leaves, indicating a potential role of *PpGA2ox* in regulating chlorophyll biosynthesis and leaf development. The increased chlorophyll content in transgenic *Arabidopsis* lines was supported by the similar findings in plants overexpressing *BnGA2ox6* (Yan *et al.* 2017), and *ClGA2ox1* and *ClGA2ox3* (Xiao *et al*. 2016).

In conclusion, *PpGA2ox* was successfully isolated from *P. pratensis*. Its expression was most abundant in young leaves. PpGA2ox was localized in both nucleus and cytoplasm and its native promoter could drive *GUS* expression in both the leaf apex and shoot apical region. Overexpression of *PpGA2ox* caused GA-deficient phenotype in *Arabidopsis* including dwarf plants, delayed flowering time, and increased chlorophyll content. Our study suggested that *PpGA2ox* could be a valuable gene for breeding new cultivars of *P. pratensis*.

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