Planta (2012) 235:1081–1089
DOI 10.1007/s00425-012-1639-5

RAPID COMMUNICATION

Rice *PLASTOCHRON* genes regulate leaf maturation downstream of the gibberellin signal transduction pathway

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Received: 29 February 2012 / Accepted: 22 March 2012 / Published online: 5 April 2012
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Abstract Rice *PLASTOCHRON 1* (*PLA1*) and *PLA2* genes regulate leaf maturation and plastochron, and their loss-of-function mutants exhibit small organs and rapid leaf emergence. They encode a cytochrome P450 protein CYP78A11 and an RNA-binding protein, respectively. Their homologs in *Arabidopsis* and maize are also associated with plant development/organ size. Despite the importance of *PLA* genes in plant development, their molecular functions remain unknown. Here, we investigated how *PLA1* and *PLA2* genes are related to phytohormones. We found that gibberellin (GA) is the major phytohormone that promotes *PLA1* and *PLA2* expression. GA induced *PLA1* and *PLA2* expression, and conversely the GA-inhibitor uniconazole suppressed *PLA1* and *PLA2* expression. In *pla1-4* and *pla2-1* seedlings, expression levels of GA biosynthesis genes and the signal transduction gene were similar to those in wild-type seedlings. GA treatment slightly down-regulated the GA biosynthesis gene *GA20ox2* and up-regulated the GA-catabolizing gene *GA2ox4,* whereas the GA biosynthesis inhibitor uniconazole up-regulated *GA20ox2* and down-regulated *GA2ox4* both in wild-type and *pla* mutants, suggesting that the GA feedback mechanism is not impaired in *pla1* and *pla2*. To reveal how GA signal transduction affects the expression of *PLA1* and *PLA2*, *PLA* expression in GA-signaling mutants was examined. In GA-insensitive mutant, *gid1* and less-sensitive mutant, *Slr1-d1*, *PLA1* and *PLA2* expression was down-regulated. On the other hand, the expression levels of *PLA1* and *PLA2* were highly enhanced in a GA-constitutive-active mutant, *slr1-1*, causing ectopic overexpression. These results indicate that both *PLA1* and *PLA2* act downstream of the GA signal transduction pathway to regulate leaf development.

Keywords Rice · *PLASTOCHRON 1* · *PLASTOCHRON 2* · Gibberellin · Leaf development

Abbreviations

GA	Gibberellin/gibberelic acid
2,4-D	2,4-Dichlorophenoxyacetic acid
BR	Brassinosteroid
CK	Cytokinine
24-epiBL	24-Epibrassinolide

Introduction

Leaves are the main photosynthetic organ in plants. In addition, the number and arrangement of leaves greatly contributes to the establishment of plant shape. To understand the genetic mechanism underlying shoot formation, two aspects of leaf primordial formation must be considered: spatial (phyllotaxy) and temporal (plastochron) regulation. Given that the regular phyllotactic pattern has long fascinated plant scientists, a large number of studies have been conducted to investigate this phenomenon (for review, Steeves and Sussex 1989). However, rapid progress has been achieved only recently. In maize, the causal gene of the *abphyl1* mutant, which exhibits decussate phyllotaxy instead of 1/2 alternate, was isolated in 2004 (Giulini et al. 2004). The recent auxin-based model has been widely

Electronic supplementary material The online version of this article (doi:10.1007/s00425-012-1639-5) contains supplementary material, which is available to authorized users.

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accepted (Reinhardt et al. 2000, 2003; Jönsson et al. 2006; Smith et al. 2006).

In contrast, the molecular basis of plastochron regulation remains to be uncovered. *plastochron 1* (*pla1*) is the first mutant that drastically alters plastochron, in which leaf primordia are formed approximately twofold faster than in the wild type (Itoh et al. 1998). Concomitantly, leaves of *pla1* become short, suggesting that *PLA1* regulates organ size. The *PLA1* gene encodes a cytochrome P450 family protein (CYP78A11) (Miyoshi et al. 2004), but its substrate is unknown. An *Arabidopsis* homolog of *PLA*1, *KLUH*, was shown to regulate organ size (Anastasiou et al. 2007). Subsequently, the *PLA2* and *PLA3* genes, loss-of-function mutants of which exhibit similar phenotypes to that of *pla1*, were identified (Kawakatsu et al. 2006, 2009). These encode an RNA-binding protein and glutamate carboxypeptidase, respectively. Interestingly, *PLA1* and *PLA2* are expressed in young leaf primordia, but not in shoot meristems (Miyoshi et al. 2004; Kawakatsu et al. 2006). Therefore, based on analyses of the developmental processes of leaves, the primary functions of *PLA1* and *PLA2* are suppression of precocious leaf maturation, and that some non-cell autonomous signals move from leaf primordia through shoot meristems to suppress the formation of a new leaf primordium (Kawakatsu et al. 2006). Thus, *PLA1* and *PLA2* are key genes for elucidating leaf development. However, the regulation of *PLA1* and *PLA2* expression remains unknown.

pla1 and *pla2* show several phenotypes likely related to phytohormones, such as small leaf size, dwarfism and enlarged SAM. In addition, the phytohormone (CK, abscisic acid and IAA) contents of *pla* mutants differed from those of the wild type (Kawakatsu et al. 2009). These mutant phenotypes suggest that *PLA* genes have some relationship with phytohormones. However, the position of *PLA* genes in the phytohormone-related pathway remains unclear.

Several phytohormones are involved in the regulation of leaf development/growth. Auxin has pleiotropic functions on plant development, including leaf growth (for review, Teale et al. 2006). Rice *tryptophan deficient dwarf 1* (*tdd1*) mutant exhibits low auxin content and dwarfism with small leaves (Sazuka et al. 2009). A gain-of-function mutant of rice, *OsIAA3*, which inhibits auxin signaling, shows an auxin-insensitive phenotype, and produces shorter leaves than the wild type, resulting in dwarfism (Nakamura et al. 2006a). Thus, auxin biosynthesis and signaling is important for normal leaf development and morphological processes in rice. Cytokinine (CK) is also profoundly associated with leaf development. For example, *ABPYL1*, which encodes the A-type response regulator, regulates phyllotaxy in maize (Jackson and Hake 1999; Giulini et al. 2004). Overexpression of a type-A response regulator caused dwarfism in rice (Hirose et al. 2007). Another phytohormone, brassinosteroid (BR), has a role in regulating plant growth. Loss-of-function mutants of BR biosynthetic and signaling genes frequently exhibit dwarfism and a reduced organ size. Rice *D2* and *D11* are BR biosynthesis genes, and regulate grain size and other traits (Hong et al. 2003; Tanabe et al. 2005). A BR-insensitive mutant, *d61*, also exhibits dwarfism, and a severe *d61* allele, *d61-4*, exhibited rolled and twisted leaves (Yamamuro et al. 2000; Nakamura et al. 2006b).

Gibberellin (GA) is the most well-known phytohormone that affects plant height and organ (leaf) size. Semi-dwarf GA mutants were used in the wheat and rice green revolution (Hedden 2003). In rice, many dwarf mutants are associated with GA biosynthesis or signaling. For example, *SEMIDWARF 1* (*SD1*), which encodes GA20 oxidase, was utilized in the rice green revolution (Ashikari et al. 2002; Sasaki et al. 2002). The *d18* dwarf mutant of *GA3ox2* has an extremely dwarf stature with small leaves (Itoh et al. 2001), and a prolonged juvenile phase (Tanaka et al. 2011). Other GA-deficient and GA-insensitive mutants commonly exhibit small leaves and a dwarf stature. In contrast, GA promotes the juvenile-adult phase change (Evans and Poehtig 1995; Teifer et al. 1997; Schwartz et al. 2008). In wild-type rice, plastochron is short in the juvenile compared with the adult phase (Itoh et al. 2005). Thus, GA seems to be associated with *PLA* functions, whose mutants show a short plastochron.

We examined the sensitivities of *pla1* and *pla2* to several phytohormones, and revealed that GA is the major influencer of *PLA* function. Using GA-related genes and mutants thereof, we determined that *PLA1* and *PLA2* function downstream of GA signal transduction.

Materials and methods

Plant materials

We used *pla1-4* and *pla2-1* mutants, which show the most severe phenotypes among their alleles, and share a cv Taichung 65 genetic background (Kawakatsu et al. 2006). We also used a GA-biosynthetic dwarf mutant, *d18-h*, which encodes GA_3 oxidase2 and has a low auxin content (Itoh et al. 2001). Three GA signaling mutants, *gibberellin insensitive dwarf 1* (*gid1*), *slender rice 1-1* (*slr1-1*) and *Slr1-d1* were used (Ikeda et al. 2001; Asano et al. 2009). *GID1* encodes a GA receptor, thus its loss-of-function mutant is GA insensitive (Ueguchi-Tanaka et al. 2005). *SLR1* encodes the DELLA protein and plays an important role in GA signal transduction (Ikeda et al. 2001; Itoh et al. 2002; Gomi et al. 2004). *slr1-1* forms a highly-elongated plant due to constitutive activation of GA signaling, while

Slr1-d1 shows dwarf phenotype and is a dominant allele of *SLR1* (Ikeda et al. 2001; Asano et al. 2009).

Application of phytohormones

Wild-type and mutant seeds were sterilized with 1 % NaClO for 40 min, and washed four times in sterile distilled water. The seeds were then placed on the Murashige and Skoog (1962) medium containing various concentrations of 2,4-D, kinetin, GA_3 24-epiBL or uniconazole. Plants were grown in a growth chamber under continuous light at 28 °C. After 10 or 14 days, plant height and second leaf sheath length were measured for more than five plants of each treatment.

Clearing of leaf sheath and measurement of cell size

To measure cell size, leaf sheaths were fixed with FAA (formalin: acetic acid: 50 % ethanol, 1:1:18) for 24 h at 4 °C. They were then dehydrated in a graded ethanol series and cleared in chloral hydrate at 96 °C in a heat block. We measured epidermal cell sizes on the adaxial side of cleared leaf sheaths under a light microscope. Measurements were performed on at least 100 cells per sample. Significant differences were analyzed by Student's *t* test.

In situ hybridization

Ten-day-old shoot apices of wild-type plants treated with GA_3 or uniconazole and of *slr1-1* plants were fixed with paraformaldehyde in 0.1 M sodium phosphate buffer, dehydrated through a series of butanol extractions, and embedded in paraplast plus. Microtome sections (8 mm thick) were applied to glass slides coated with APS (Matsunami Glasses, Japan). A digoxigenin-labeled antisense probe of *PLA1* was prepared as described previously (Miyoshi et al. 2004). Hybridization and immunological detection with alkaline phosphatase were preformed as described by Kouchi and Hata (1993).

Real-time PCR

Total RNA was extracted from shoot apices using TRIZOL reagent (Invitrogen). After RNase-free DNase I treatment, 1 µg of RNA was used for RT-PCR using High-capacity cDNA Reverse Transcription Kits (Applied Biosystems, USA). To quantify *PLA1*, *PLA2*, *GA20ox2*, *GA3ox2*, *GA2ox4* and *SLR1* expression, real-time PCR was performed using SYBERGREEN (Applied Biosystems, USA) or the TaqMan Fast Universal PCR Master Mix, FAM-labeled TaqMan probes (Applied Biosystems, USA), and the StepOnePlus real-time PCR system (Applied Biosystems, USA). Each gene expression value is the average of three independent real-time PCR assays. Expression levels were normalized to that of an internal control, *ACT1*. The primers and probes for each gene are listed in Supplementary Table S1.

Results

Since the *pla1* and *pla2* mutants exhibit dwarf phenotypes and short plastochron (rapid leaf emergence), it is hypothesized that *pla* phenotypes are related to phytohormones. Of the many *pla1* and *pla2* alleles, we used *pla1-4* and *pla2-1*, both of which are strong alleles with a common genetic background of cv. Taichung 65.

Gibberellin is the major phytohormone associated with *PLA1* and *PLA2* functions

First, we observed the responses of *pla1* and *pla2* seedlings when several phytohormones, which are known to affect plant growth and leaf development, were added to the culture media. Both *pla1-4* and *pla2-1* mutants showed similar responses to auxin (2,4-D) as did the wild type. That is, 2,4-D inhibited the growth of wild-type, *pla1-4* and *pla2-1* seedlings (Fig. 1a). CK (kinetin) application caused similar responses in wild-type and *pla* seedlings (Fig. 1b). Higher kinetin concentrations caused more severe growth inhibition in wild-type, *pla1-4* and *pla2-1* seedlings. BR is also known to affect plant height and leaf development. However, both *pla1-4* and *pla2-1* seedlings responded to external BR (24-epiBL) similarly to wild-type seedlings (Fig. 1c). Thus auxin, CK and BR are not related to *PLA* function. In contrast, GA_3 application induced rapid growth of wild-type plants (Fig. 1d, e). In *pla1-4* and *pla2-1*, however, growth induction was restricted (Fig. 1d, e). Accordingly, GA is the major phytohormone associated with *PLA1* and *PLA2* functions, while *pla1-4* and *pla2-1* seem to be less sensitive to GA than wild-type plants.

Responses of *pla1* and *pla2* to gibberellin application in cell size and leaf elongation

We examined responses to GA in cells and tissues of *pla1-4* and *pla2-1* plants. GA promotes cell elongation and tissue/organ elongation. We measured the length of more than 100 cells on the adaxial surface of the second leaf sheath. In wild-type plants, GA_3 application elongated leaf sheath cells by approximately 18 %, but by at most 6 % in *pla1-4*, and no elongation was observed in *pla2-1* plants (Fig. 2a, b). Leaf sheaths elongate in response to GA_3 application. In wild-type plants, elongation of the 2nd leaf sheath increased with GA_3 concentration, being *circa* threefold longer at 10^{-5} than at 0 M, whereas *pla1-4* and

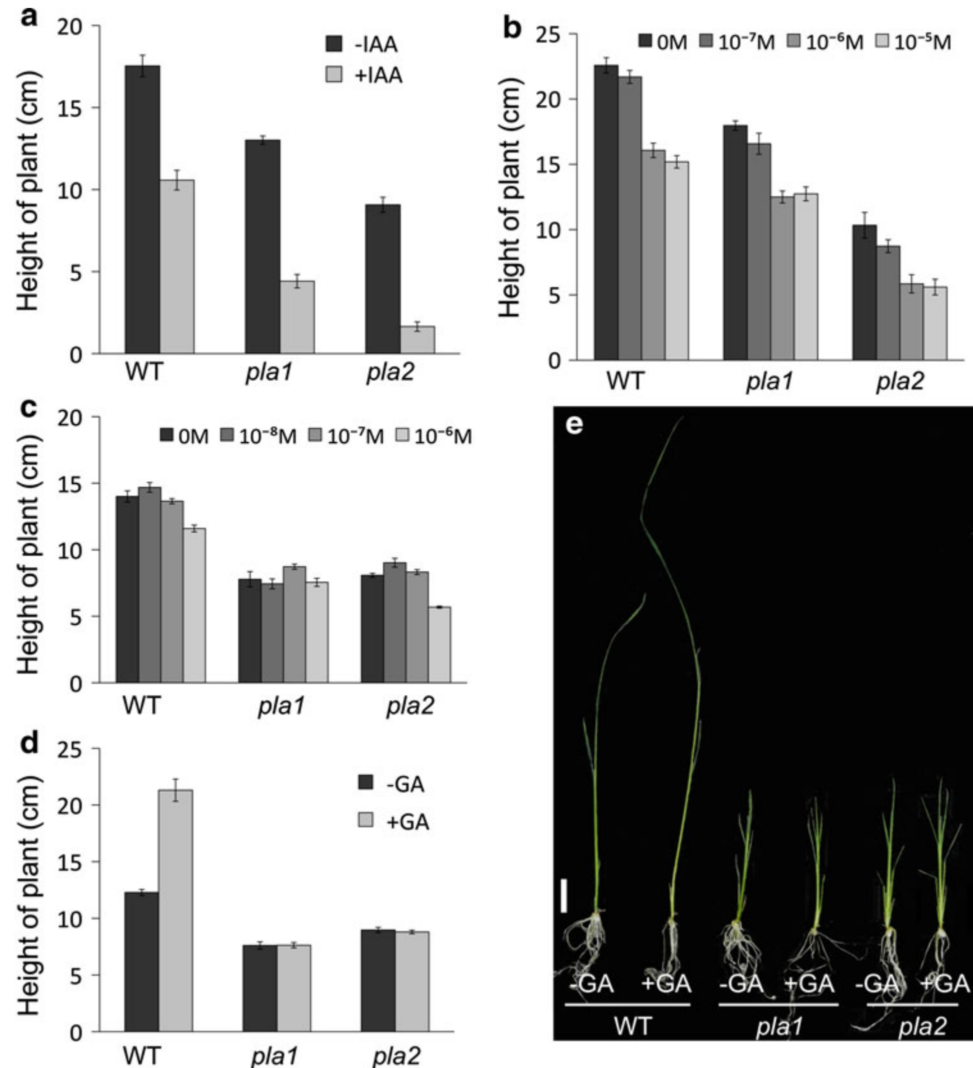

Fig. 1 Effect of several phytohormones on the growth of *pla1* and *pla2* seedlings. Wild-type, *pla1-4* and *pla2-1* seeds were inoculated on culture media containing phytohormones. Plants were grown for 14 days in 2,4-D (**a**) and kinetin (**b**) treatments, and for 10 days in 24-epiBL (BR) (**c**) and GA_3 (**d**) treatments. Data in **a**–**d** represent mean ± SE. **e** Seedlings of wild type, *pla1-4* and *pla2-1* grown for 10 days with or without GA_3 treatment. *Bar* 2 cm

pla2-1 showed a twofold or less elongation at the same GA_3 concentrations (Fig. 2c). In particular, a low GA_3 concentration did not promote *pla1-4* and *pla2-1* leaf elongation.

These results indicate that *pla1-4* and *pla2-1* are insensitive to GA in many traits, suggesting that *PLA1* and *PLA2* act downstream of GA signal transduction.

Induction of *PLA* gene expression by gibberellin

The above results suggest that *PLA1* and *PLA2* gene expression is associated with GA signaling. Thus, we examined the effect of GA on *PLA1* and *PLA2* gene expression. Ten-day-old seedlings were treated with 10 µM GA_3 and *PLA* gene expression was monitored for 24 h by real-time PCR. *PLA1* and *PLA2* expression increased as early as 3 h after treatment, and a high level of expression was maintained for 24 h (Fig. 3a). To investigate the long-term effect of GA, wild-type seeds were inoculated and grown for 8 days on culture media containing 10 µM GA_3. *PLA1* and *PLA2* expression was maintained at a high level for 8 days (Fig. 3b). Next, we examined the effect of uniconazole, a GA biosynthesis inhibitor. Uniconazole treatment markedly suppressed *PLA1* and *PLA2* gene expression (Fig. 3b). Therefore, GA regulates the expressions of both *PLA1* and *PLA2*.

Since GA and uniconazole treatments up- and down-regulated *PLA1* expression, respectively, the *PLA1* expression pattern was investigated using in situ hybridization. *PLA1* is expressed in the basal and abaxial regions of leaf primordia, but not in shoot meristems (Fig. 3c, Miyoshi et al. 2004). When treated with GA_3, strong and ectopic *PLA1* expression was detected in the adaxial region of leaf primordia and in the tip of shoot meristems in addition to the normal expression domain (Fig. 3d). The expression pattern was not unaffected by uniconazole treatment, but hybridization signals were weakened (Fig. 3e).

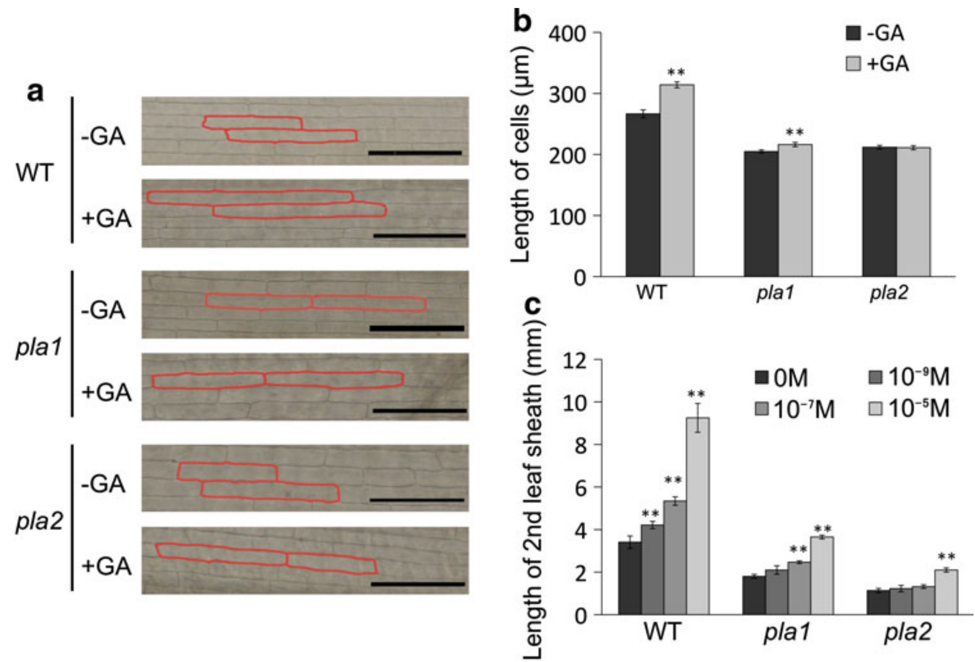

Fig. 2 Response of *pla1* and *pla2* to gibberellin application. **a** Adaxial surface of cleared 2nd leaf sheath of wild type, *pla1-4* and *pla2-1* treated with GA_3. *Bars* 200 μm. **b** Effects of GA_3 treatment on cell length in 2nd leaf sheath. In WT, GA_3 treatment significantly increased cell length. *Double asterisks* significantly longer at 1 % level than in −GA (*t* test). **c** Effects of GA_3 treatment on the length of 2nd leaf sheath. *Double asterisks* significant at 1 % level compared with 0 M (*t* test). Data in **b**, **c** represent mean ± SE

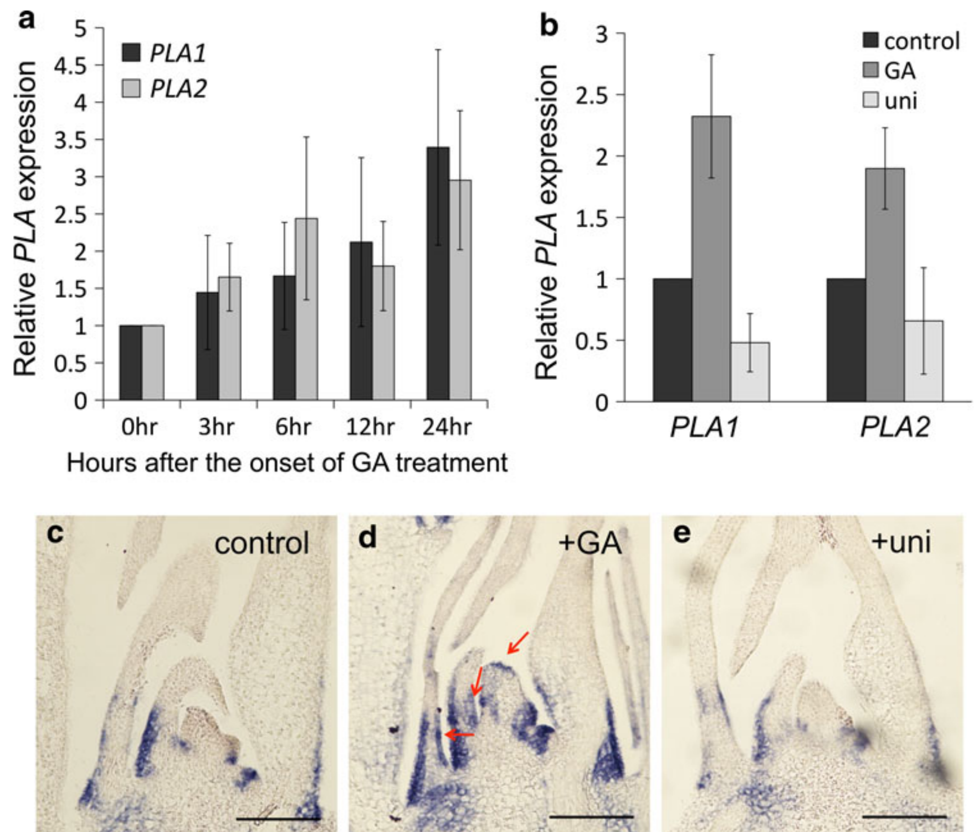

Fig. 3 Induction of *PLA1* and *PLA2* expression by gibberellin. **a**, **b** Real-time PCR assays. **a** Short-term induction of *PLA1* and *PLA2* expression by 10 μM GA_3. Each expression level is represented relative to that at 0 h. **b** Effects of GA_3 and uniconazol treatments for 8 days on *PLA* 1 and *PLA2* expressions. Expression level is represented relative to that in the control. Data in **a**, **b** represent mean ± SE. **c**–**e** In situ hybridization of *PLA1* in wild-type shoot apex (**c**), wild-type shoot apex treated with 10 μM GA_3 for eight days (**d**) and wild-type shoot apex treated with 1 μM uniconazole for 8 days (**e**). *Arrows* indicate ectopic expression. *Bars* 100 μm

Expression of GA-related genes in *pla* mutants

Given that GA regulates *PLA1* and *PLA2* expression, we next examined the expression of GA-related genes in *pla1* and *pla2* mutants. The expression of the GA biosynthesis genes, *GA20ox2* and *GA3ox2*, and the GA-catabolizing gene, *GA2ox4*, did not largely differ among wild-type, *pla1-4* and *pla2-1* plants, although *GA3ox2* expression was somewhat increased in *pla1-4* and *pla2-1* compared with the wild type (Fig. 4a). Similarly, *SLR1* (a gene involved in GA signal transduction) expression was comparable in wild-type, *pla1-4* and *pla2-1* plants (Fig. 4a). These data

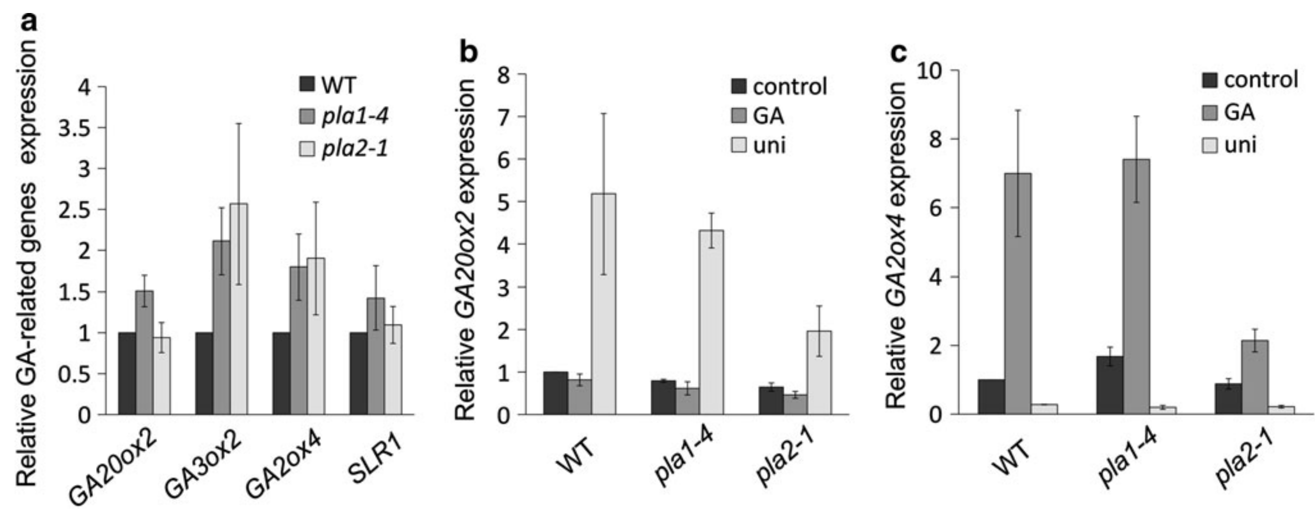

Fig. 4 Expression of gibberellin-related genes in *pla1* and *pla2* seedlings. **a–c** Real-time PCR assays. **a** Expression of GA-biosynthetic (*GA20ox2* and *GA3ox2*), catabolizing (*GA2ox4*) and signaling gene (*SLR1*) genes in wild type, *pla1-4* and *pla2-1*. Expression level in *pla* mutants is represented relative to that in wild type. **b**, **c** Effect of GA_3 and uniconazole treatments on *GA20ox2* (**b**) and *GA2ox4* (**c**) expression in wild type, *pla1-4* and *pla2-1*. Each expression level is represented relative to that in wild-type control. In **b** and **c**, expression level of *GA20ox2*/*GA2ox4* in the control (non-treatment) does not significantly differ among wild type, *pla1-4* and *pla2-1*. Data in **a–c** represent mean ± SE

suggest that *PLA1* and *PLA2* do not affect GA biosynthesis or signal transduction.

In plants, GA content is regulated by a feedback mechanism involving GA signal transduction; e.g., *GA20ox2* expression is enhanced in GA-insensitive mutants such as *gibberellin insensitive dwarf 1* (*gid1*), and downregulated in the GA-constitutive-active mutant *slr1-1* (Yamaguchi 2008). Therefore, we investigated whether GA signal transduction is operating normally in *pla1* and *pla2* by determining the effect of GA and an inhibitor thereof on expression of the above genes. Application of GA slightly decreased the expression of *GA20ox2* in wild-type, *pla1-4* and *pla2-1* plants (Fig. 4b). In contrast, uniconazole treatment markedly enhanced expression in wild type and *pla1-4*, and moderately enhanced it in *pla2-1*, plants (Fig. 4b). The opposite effect was detected for *GA2ox4*, which encodes a GA-catabolizing enzyme. GA treatment strongly enhanced *GA2ox4* expression in wild-type and *pla1-4* plants (Fig. 4c). In *pla2-1* plants, GA also induced the expression, but to a limited extent (Fig. 4c). Uniconazole treatment suppressed *GA2ox4* expression in wild-type, *pla1-4* and *pla2-1* plants (Fig. 4c).

These results show that the feedback mechanism is operating normally in *pla1-4* and *pla2-1* mutants, although somewhat weakened in *pla2-1*. In addition, *PLA1* and *PLA2* may be positioned downstream of GA biosynthesis and signal transduction genes.

PLA gene expression in GA-related mutants

To confirm the relationship between *PLA* genes and GA-related genes, we examined *PLA1* and *PLA2* expression in GA-related mutants. *d18* is a dwarf mutant, whose wild-type gene encodes GA3 oxidase 2. *PLA1* and *PLA2* expression in *d18* was slightly down-regulated compared with the wild type (Fig. 5a). However, this difference in *PLA* expression between the wild type and *d18-h* was not large compared with that between the wild-type and GA-signaling mutants below. In GA-insensitive mutant *gid1* and reduced GA-sensitivity mutant *Slr1-d1*, *PLA1* and *PLA2* expression was severely suppressed, whereas in the GA-constitutive-active mutant *slr1-1*, their expression was markedly increased (Fig. 5b).

To determine the effect of *PLA1* overexpression on the expression domain, we examined the *PLA1* expression pattern by in situ hybridization. Compared with that in the wild-type shoot apex, *PLA1* expression in *slr1-1* was expanded to the adaxial region and the upper regions of leaf primordia and shoot meristems, as well as the normal basal and abaxial regions of leaf primordia (Fig. 5c–e). This ectopic expression of *PLA1* coincided with that induced by GA treatment (Fig. 3d). Since *PLA1* and *PLA2* expression was strongly affected by GA-signaling genes, both *PLA1* and *PLA2* likely act downstream of the GA signal transduction pathway to regulate leaf development.

Discussion

GA is associated with various developmental and physiological processes, such as seed germination, stem elongation, vegetative phase change, flowering and pollen maturation (Olszewski et al. 2002; Yamaguchi 2008). It is

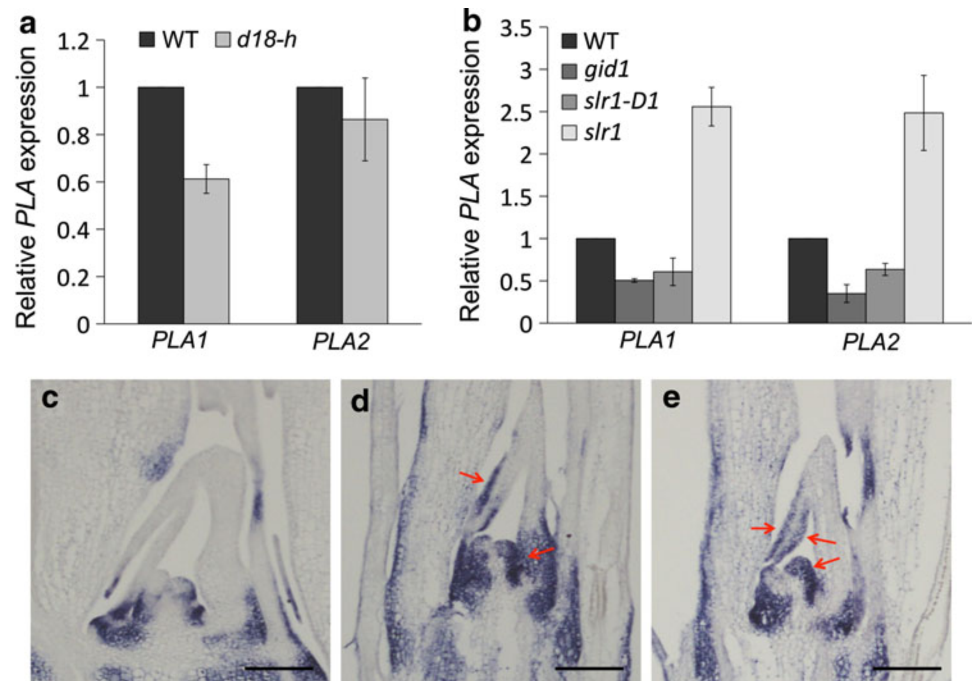

Fig. 5 *PLA1* and *PLA2* gene expression in GA-related mutants. **a**, **b** Real-time PCR assays of *PLA1* and *PLA2* expression in GA-deficient mutant (*d18*-h) (**a**) and in inactive (*gid1* and *Slr1-d1*) and constitutive active (*slr1-1*) mutants of GA signaling (**b**). Expression level in each mutant is represented relative to that in wild type. Data in **a**, **b** represent mean ± SE. **c**–**e** in situ hybridization of *PLA1* in wild-type shoot apex (**c**) and *slr1-1* shoot apex (**d**, **e**). *Arrows* indicate ectopic expression. *Bars* 100 μm

also proposed that GA functions in early leaf development (Olszewski et al. 2002; Yamaguchi 2008). A subset of class I KNOX genes represses GA-biosynthetic gene expression in the SAM by direct transcriptional regulation, resulting in prevention of SAM cells from entering a determinate state (Sakamoto et al. 2001) Once a leaf is initiated from the shoot apex, negative regulation of GA occurs in the leaf primordium, and GA contributes to leaf expansion and differentiation (Olszewski et al. 2002; Yamaguchi 2008). This model is widely accepted, and it is believed that GA is an important regulator of young leaf development. However, the downstream mechanism involved in GA-dependent leaf development remains unknown.

We showed that *PLA1* and *PLA2* expression was positively regulated by GA. Previous studies indicated that both *PLA1* and *PLA2* expression is restricted in the leaf primordia, but not in the SAM (Miyoshi et al. 2004; Kawakatsu et al. 2006). This is consistent with GA synthesis in leaf primordia but not in the SAM. GA-related genes (*GA3ox2*, *GA20ox2*, *SLR1*) are expressed in young leaf primordial, including P0 and P0 primordia (Kaneko et al. 2003). This expression domain overlaps with that of *PLA1* and *PLA2*, supporting the present finding that GA is involved in the regulation of *PLA* gene expression. The proposed primary function of *PLA1* and *PLA2* is precocious leaf maturation (Kawakatsu et al. 2006). Leaf maturation is a complex process involving organized expansion and differentiation of cells/tissues. Because GA also plays a role in this organized expansion and differentiation, it is thought that GA action in leaf primordia could be closely related to *PLA1* and *PLA2* functions. It is assumed that GA regulates leaf development through controlling *PLA* gene expression. Our in situ hybridization experiments revealed that expression of *PLA1* was not only quantitatively enhanced, but was also ectopically expanded to the SAM in GA-signaling mutants and GA-applied plants. These indicate that GA also spatially regulates *PLA1* and *PLA2* expression.

In contrast, expression of GA biosynthetic, and GA-catabolizing and signaling genes was not significantly altered in *pla1* and *pla2* mutants. In addition, feedback regulation of GA-biosynthetic and GA-catabolizing genes after GA application was normal in *pla1* and *pla2* mutants. The rice DELLA protein, SLR1, is a key component of GA signaling, and is a principal factor responsible for feedback regulation of GA biosynthesis (Itoh et al. 2008; Yamaguchi 2008). The normal *SLR1* expression level in the *pla* mutants suggests that SLR1-dependent GA signal transduction is operating normally.

Many GA-related and GA responsive genes have been identified; for example, *PHYTOCHROME INTERACTING FACTOR* in skotomorphogenesis, (Feng et al. 2008; de Lucas et al. 2008) and α-amylase genes in seed germination (Kaneko et al. 2002). In addition, several microarray experiments revealed many GA responsive genes (Yazaki et al. 2003; Yang et al. 2004; Jan and Komatsu 2006). In terms of leaf development, however, less is known about the downstream pathway of GA. Our analyses indicate that *PLA1* and *PLA2* are factors downstream of GA in leaves, and one action of GA is the *PLA*-dependent suppression of precocious leaf maturation.

Although the *pla1* and *pla2* leaf phenotypes were similar, *PLA1* and *PLA2* regulate leaf maturation process through independent genetic pathways. Indeed, a *pla1* and *pla2* double mutant showed a more severe phenotype than that of the single mutants (Kawakatsu et al. 2006). With regard to their molecular function, *PLA1* encodes a member of the cytochrome P450 family thought to be involved in the biosynthetic pathway of an unknown substance (Miyoshi et al. 2004), and *PLA2* encodes a MEI2-like RNA-binding protein that may interact with unidentified RNA molecules (Kawakatsu et al. 2006). Considering the independent expression regulation and molecular functions of *PLA1* and *PLA2,* it would not be surprising if *PLA1* and *PLA2* have functionally diversified. The present results, however, show that both genes are regulated by GA and act downstream of GA signaling. The only difference between *pla1* and *pla2* was in the feedback regulation of GA-related genes. In *pla1*, the effect of GA_3 and uniconazole treatment on *GA20ox2* and *GA2ox4* expression was almost identical to those in the wild type. In contrast, *pla2* showed a weaker response to these treatments. This indicates that *PLA2* is partially involved in the GA feedback mechanism, but *PLA1* is not.

Acknowledgments We thank Dr. H. Kitano (Nagoya University) for kind gift of *d18-h*, *slr1-1*, *Slr1-d1* and *gid1* seeds. This work is supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (23658005 to Y.N.).

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