

A strigolactone signal is required for adventitious root formation in rice

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- **Background and Aims** Strigolactones (SLs) and their derivatives are plant hormones that have recently been identified as regulating root development. This study examines whether SLs play a role in mediating production of adventitious roots (ARs) in rice (*Oryza sativa*), and also investigates possible interactions between SLs and auxin.
- **Methods** Wild-type (WT), SL-deficient (*d10*) and SL-insensitive (*d3*) rice mutants were used to investigate AR development in an auxin-distribution experiment that considered *DR5::GUS* activity, [³H] indole-3-acetic acid (IAA) transport, and associated expression of auxin transporter genes. The effects of exogenous application of GR24 (a synthetic SL analogue), NAA (α -naphthylacetic acid, exogenous auxin) and NPA (*N*-1-naphthylphthalamic acid, a polar auxin transport inhibitor) on rice AR development in seedlings were investigated.
- **Key Results** The rice *d* mutants with impaired SL biosynthesis and signalling exhibited reduced AR production compared with the WT. Application of GR24 increased the number of ARs and average AR number per tiller in *d10*, but not in *d3*. These results indicate that rice AR production is positively regulated by SLs. Higher endogenous IAA concentration, stronger expression of *DR5::GUS* and higher [³H] IAA activity were found in the *d* mutants. Exogenous GR24 application decreased the expression of *DR5::GUS*, probably indicating that SLs modulate AR formation by inhibiting polar auxin transport. The WT and the *d10* and *d3* mutants had similar expression of *DR5::GUS* regardless of exogenous application of NAA or NPA; however, AR number was greater in the WT than in the *d* mutants.
- **Conclusions** The results suggest that AR formation is positively regulated by SLs via the *D3* response pathway. The positive effect of NAA application and the opposite effect of NPA application on AR number of WT plants also suggests the importance of auxin for AR formation, but the interaction between auxin and SLs is complex.

Key words: Adventitious root formation, auxin, IAA, strigolactone, SL, rice, *Oryza sativa*, root growth, plant hormone.

INTRODUCTION

The root system is fundamentally important for plants to efficiently obtain nutrients and water. In contrast to the primary root system of plants, roots of monocot cereals consist almost entirely of a complex fibrous system and a mass of adventitious roots (ARs). AR formation is the process of root initiation from the stem base post-embryonically, which is tightly regulated to prevent the loss of valuable plant resources for non-essential root formation. A lack of stable and credible morphological data makes it difficult to study physiological and molecular mechanisms governing AR growth (Coudert *et al.*, 2010; E *et al.*, 2012). However, comprehensive understanding of AR development should have important implications for manipulating root architecture, which contributes to both improving crop yield and optimizing agricultural land use.

Several plant hormones control AR formation, in which auxin plays a pivotal role (Jiang and Feldman, 2003; E *et al.*, 2012). Indole-3-acetic acid (IAA) is the predominant form of

active auxin in plants and it induces both AR and lateral root (LR) formation (Cooper, 1936; Boerjan *et al.*, 1995; Delarue *et al.*, 1998; Zhao *et al.*, 2001; Negi *et al.*, 2010). Auxin is synthesized predominantly in the shoot apex and young leaves (Ljung *et al.*, 2001), and is redistributed through the cooperative actions of auxin efflux carriers such as PIN-FORMED (PIN) and ABCB/PGP family proteins, and auxin influx carriers including AUX1/LAX family proteins (Friml, 2003; Blakeslee *et al.*, 2005; Zazimalova *et al.*, 2010; Peret *et al.*, 2012). In rice, altered expression of *PIN1b* changes AR development (Xu *et al.*, 2005). Several rice auxin-related mutants show defects in AR formation. For example, CRL1/ARL1 (an AS2/LOB transcriptional factor) acts as a positive regulator for AR formation downstream of the IAA and auxin response factor-mediated auxin signalling pathway. The *crl1/ar1* mutant has been shown to exhibit few LRs (Inukai *et al.*, 2005; Liu *et al.*, 2005). The rice *CROWN ROOTLESS4* (*CRL4*)/*OsGNOM1* mutation affects AR initiation and development by modulating expression of

PIN genes (Kitomi et al., 2008; Liu et al., 2009). Previous studies have demonstrated that AR formation is affected by auxin factors, such as transport, metabolism or the presence of free IAA (Diaz-Sala et al., 1996; Krisantini et al., 2006). Additional signalling pathways may be involved in the regulation of AR induction.

In addition to auxin, studies have shown that strigolactones (SLs) and their derivatives play a key role in modulation of root development (Kapulnik et al., 2011a; Koltai, 2011; Ruyter-Spira et al., 2011; Rasmussen et al., 2012; Sun et al., 2014). Application of GR24 (a synthetic SL analogue) under favourable growth conditions led to a *MORE AXILLARY GROWTH 2* (*MAX2*)-dependent increase in primary root length. The inhibition of primary root length was observed in treatments with relatively higher doses of GR24, in a *MAX2*-independent fashion (Ruyter-Spira et al., 2011). The SL-deficient and response mutants of *Arabidopsis thaliana* and pea (*Pisum sativum*) have enhanced AR development, suggesting, conversely, that SLs suppress AR formation in both species. However, Arite et al. (2012) found that SLs positively regulate the length of ARs in rice. Lateral root densities are increased in SL-deficient and SL-insensitive *Arabidopsis* and tomato (*Solanum lycopersicum*) mutants, suggesting that SLs affect LR formation (Koltai et al., 2010; Kapulnik et al., 2011b; Ruyter-Spira et al., 2011). In contrast to findings in dicots such as *Arabidopsis* and tomato, the number of LRs on the seminal root did not differ among 14-d-old wild-type (WT) and *d10* and *d14* rice mutants (Arite et al., 2012). These results suggest that complex biological mechanisms underlie the regulation of root growth by SLs acting as plant hormones.

In this study, the role of SLs in regulating rice AR formation was examined in SL-deficient and SL-insensitive rice mutants. Rice is an ideal model for SL study, as it has several well-characterized SL synthetic mutants (*d10*, *d17* and *d27*) and response mutants (*d3*, *d14* and *d53*) (Xie et al., 2010; Waters et al., 2012; Jiang et al., 2013; Zhou et al., 2013). The *d* mutants exhibited reduced AR development compared with WT rice plants. Exogenous GR24 restored AR formation in the SL synthesis mutant *d10*, but not in the SL signalling mutant *d3*. In addition, endogenous IAA concentration at the shoot–root junction, SL mutants carrying the auxin reporter construct *DR5::GUS*, polar transport of radiolabeled [³H] IAA and exogenous application of α -naphthylacetic acid (NAA) and the auxin transport inhibitor *N*-1-naphthylphthalamic acid (NPA) revealed that AR formation in rice is regulated by SLs and auxin status.

MATERIALS AND METHODS

Plant growth conditions

The Shiokari ecotype of rice (*Oryza sativa* L.) was the basis for this study. The *d10* (SL synthesis) and the *d3* (SL signalling) mutants were kindly provided by Shinjiro Yamaguchi of the RIKEN Plant Science Center. Rice seedlings were grown in a greenhouse under natural light at day/night temperatures of 30°C/18°C. Seven-day-old seedlings of uniform size and vigour were transplanted into holes in a lid placed over the top of pots (four holes per lid and three seedlings per hole). Nutrient solutions varying from one-quarter to one-half strength were applied for 1 week and a full-strength nutrient solution was

applied thereafter. The full-strength nutrient solution of the International Rice Research Institute (IRRI) was (mM): 1.25 NH₄NO₃, 0.3 KH₂PO₄, 0.35 K₂SO₄, 1.0 CaCl₂, 1.0 MgSO₄·7H₂O and 0.5 Na₂SiO₃, and (μ M) 20.0 Fe-EDTA, 9.0 MnCl₂, 0.39 (NH₄)₆Mo₇O₂₄, 20.0 H₃BO₃, 0.77 ZnSO₄ and 0.32 CuSO₄ (pH 5.5).

The treatments of GR24 (dissolved in acetone), NAA (dissolved in 1 M NaOH) and auxin transport inhibitor NPA (dissolved in dimethyl sulfoxide, DMSO) were applied via the plant-growth media. Seven-day-old rice seedlings were grown in hydroponic medium containing various concentrations of NAA and NPA for 3 weeks. The GR24 treatments were applied in the same IRRI nutrient solution, as described above, in hydroponic or 0.4 % agar medium. Rice seeds were germinated in trays for 3 d and then transferred into plant culture tubes and grown for 3 weeks. The control treatment for GR24 contained 0.1 % acetone (Sun et al., 2014).

Measurement of root system architecture

The average length of ARs was measured using the WinRhizo scanner-based image analysis system (Regent Instruments, Montreal, QC, Canada). The number of ARs was counted manually.

Quantification of IAA levels

The concentration of IAA in the shoot–root junction was determined as described by Song et al. (2013). Fresh weight of samples was determined, followed immediately by freezing in liquid N₂. Sample measurement of free IAA by HPLC was carried out according to Song et al. (2013). A standard IAA sample was obtained from Sigma-Aldrich (St Louis, MO, USA).

Agrobacterium tumefaciens (strain EHA105) contained the construct *pDR5::GUS*, which was kindly provided by Professor Ping Wu's group at Zhejiang University, Hangzhou, China. Tissues were treated with ethanol prior to observation to remove chlorophyll pigmentation. The stained tissues were photographed using an Olympus SZX2-ILLK stereomicroscope with a colour CCD camera (Olympus, Tokyo, Japan).

[³H] IAA-transport assay

To assay the effect of SLs on auxin transport, WT and the *d10* mutant were pre-cultured for 3 weeks under normal nutrition with or without 1.25 μ M GR24 application. Auxin transport was assayed by means of [³H] IAA, as described below.

Shoot-to-root auxin transport in intact plants was assayed according to Song et al. (2013). For this, 20 μ L of [³H] IAA solution was applied to the cut surface after removal of rice shoot 4 cm above the root–shoot junction and plants were kept in darkness for 18 h. The [³H] IAA solution contained 0.5 μ M [³H] (20 Ci mmol⁻¹) in 2 % DMSO, 25 mM MES (pH 5.2) and 0.25 % agar. The root–shoot junction was dissected and weighed before being incubated in scintillation solution for more than 18 h. [³H] IAA radioactivity was detected using a multipurpose scintillation counter (LS6500, Beckman-Coulter, Fullerton, CA, USA) (Song et al., 2013).

qRT-PCR analysis

Total RNA was isolated from the roots of rice seedlings. RNA extraction, reverse transcription and quantitative reverse transcription polymerase chain reaction (qRT-PCR) were performed as described previously (Sun *et al.*, 2014). Primer sets for *PIN* genes are listed in Supplementary Data Table S1.

Data analysis

Data were pooled for calculation of means and standard errors (s.e.) and assessed by one-way analysis of variance, followed by computation of least significant differences to compare means. All statistical analyses were performed using SPSS ver. 11.0 (SPSS Inc., Chicago, IL, USA). In all analyses, the targeted level of statistical significance was $P < 0.05$.

RESULTS

Decreased number of ARs in the *d10* and *d3* mutants compared with WT plants

As reported by Ishikawa *et al.* (2005), the SL synthetic mutant *d10* and signalling mutant *d3* exhibited increased numbers of tillers compared with the WT (Fig. 1A). However, the effective

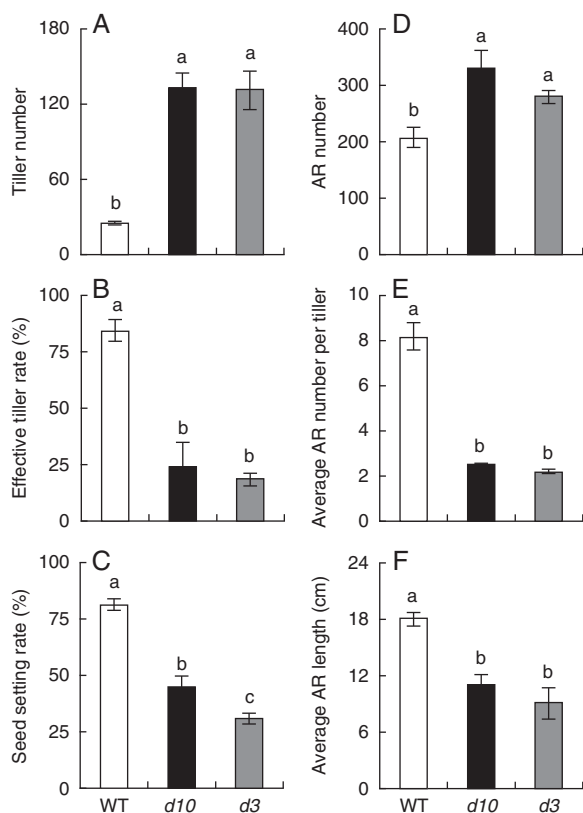


FIG. 1. Phenotype of strigolactone-deficient (*d10*) and strigolactone-insensitive (*d3*) mutants and wild-type (WT) rice plants at maturity. Rice seedlings were grown in soil after germination. (A) Tiller number, (B) effective tiller rate, (C) seed setting rate, (D) adventitious root (AR) number, (E) average AR number per tiller and (F) average AR length of WT, *d10* and *d3*. Data are means \pm s.e. Different lower-case letters indicate significant differences ($P < 0.05$, ANOVA).

tiller rate and seed setting rate were much lower in the *d10* and *d3* mutants compared with the WT (Fig. 1B, C). The reasons for the differences in effective tiller rate and seed setting were unclear. We examined AR growth among the two *d* mutants and the WT at maturity (Fig. 1D–F). Only two ARs per tiller were recorded in the *d10* and *d3* mutants, lower than for the WT. Furthermore, the average length of ARs was lower in the two *d* mutants compared with the WT. These results indicated that the defect in AR growth potentially reduced the effective tillering and seed setting rate in the SL mutants (Fig. 1F).

We examined the time course of development of tillers and ARs at 8 weeks following germination. The outgrowth of tiller buds in the *d10* and *d3* mutants was markedly increased and tiller numbers were greater in both mutants 3 weeks after germination compared with the WT (Fig. 2A). At an early growth stage (from 2 to 4 weeks), the AR numbers in the two mutants were lower compared with the WT (Fig. 2B, D). An increased number of tillers in the *d* mutants led to a greater number of

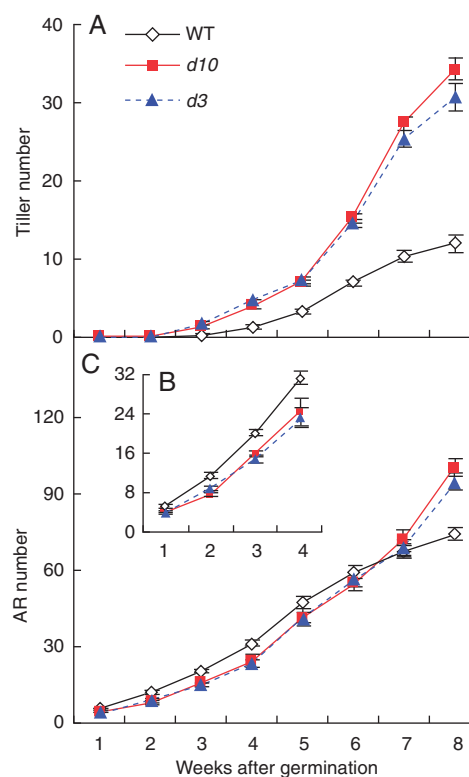


FIG. 2. Time course of development of tillers and adventitious root (AR) in strigolactone-deficient (*d10*) and strigolactone-insensitive (*d3*) mutants and wild-type (WT) rice plants over 8 weeks. Rice seedlings were grown in hydroponic media after germination. (A) Tiller number of WT, *d10* and *d3*. (B, C) AR number of WT, *d10* and *d3* over 4 weeks (B) and 8 weeks (C). (D) ARs of WT, *d10* and *d3* during weeks 2–3. Data are means \pm s.e.

ARs from 8 weeks to maturity (Fig. 2C). Although the total number of ARs in the SL mutants was higher than in the WT at later growth stages, the number of ARs per tiller was three times less than in the WT (Fig. 1E). To further investigate the formation of ARs, we used 3 weeks after germination as the experimental period in subsequent analyses.

Exogenous application of GR24 increased AR numbers in the SL synthetic mutant d10 but not in d3

Endogenous 2'-epi-5-deoxystrigol was detected in the WT and the mutant *d3* but not in the *d10* mutant (Umehara et al., 2008). To determine if SLs affect the growth of ARs in rice, synthetic GR24 at 0–5 μM was applied exogenously to the WT plants and the two *d* mutants (Supplementary Data Fig. S1). Application of GR24 had no effect on the number of ARs in the WT, with the exception of 5 μM GR24, which caused a slight decrease in the number of ARs. Application of 1.25 μM GR24 increased AR number and average AR number per tiller in the *d10* mutant to the level of the WT (Fig. 3). However, root morphology of the *d3* mutant was unaffected by GR24 application, indicating involvement of the *D3* gene in the SL regulatory pathway for AR formation.

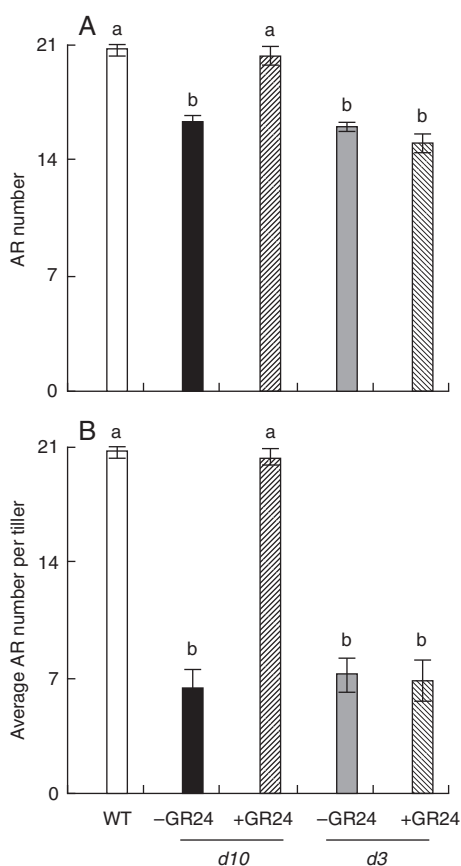


Fig. 3. Effect of the synthetic strigolactone analogue GR24 on the number of adventitious roots (AR) and average AR number per tiller in strigolactone-deficient (*d10*) and strigolactone-insensitive (*d3*) mutants and wild-type (WT) rice plants. Rice seedlings were grown for 3 weeks in agar media containing 1.25 μM GR24. Data are means \pm s.e. Different lower-case letters indicate significant differences ($P < 0.05$, ANOVA).

Auxin levels were significantly higher in the d10 and d3 mutants relative to the WT

To assess whether auxin affected AR formation in the *d10* and *d3* mutants, endogenous IAA concentrations were first analysed in the shoot–root junction. IAA concentration was increased by 100 and 168 %, respectively, in the *d10* and *d3* mutants relative to WT (Fig. 4A). A specific reporter contains seven repeats of a highly active synthetic auxin response element, and changes in *in vivo* auxin levels are monitored via expression of *DR5::GUS* (Ulmasov et al., 1997). Expression of *DR5::GUS* was subsequently examined in the WT and *d10* and *d3* mutants. Expression of *DR5::GUS* in *d10* and *d3* plants was stronger in the first leaf, leaf sheath and junction, in comparison with the WT (Supplementary Data Fig. S2). These results indicated that *d* mutant plants had higher IAA concentrations in the junction relative to the WT.

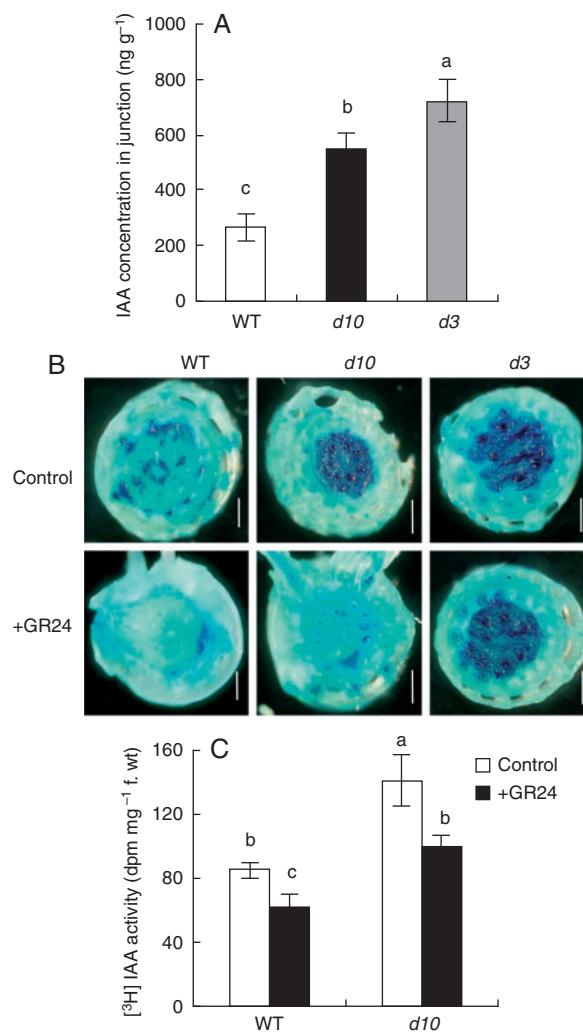


Fig. 4. (A) Auxin concentration and (B) histochemical localization of *DR5::GUS* activity and (C) [³H] IAA transport in junction of wild-type (WT) and strigolactone-deficient (*d10*) and strigolactone-insensitive (*d3*) mutants. Seedlings were grown for 3 weeks in hydroponic media with or without 1.25 μM GR24. Plants were stained for *GUS* activity for 24 h. Scale bar = 1 mm. Data are means \pm s.e. Different lower-case letters indicate significant differences ($P < 0.05$, ANOVA).

To determine whether SLs regulate AR formation through modulating polar auxin transport, the effect of GR24 application on the expression of *DR5::GUS* and [^3H] IAA transport was analysed (Fig. 4B, C). GR24 application significantly decreased expression of *DR5::GUS* in WT and *d10* but not *d3* (Fig. 4B). Application of GR24 markedly reduced [^3H] IAA activity in the junction of WT and *d10* plants, and there were no differences in [^3H] IAA activity in *d10* with GR24 application relative to the WT without GR24 application (Fig. 4C). These results suggested SLs inhibited polar auxin transport in rice plants.

Expression of OsPIN genes in the *d10* and *d3* mutants

Auxin is delivered from the shoot mainly through the polar transport stream, which is facilitated by proteins of the PIN family. The expression pattern of PIN genes in the WT and the *d10* and *d3* mutants was examined (Fig. 5). qRT-PCR showed that the expression levels of *PIN5c*, *PIN9*, *PIN10a* and *PIN10b* increased significantly in the *d10* and *d3* mutants compared

with the WT. The expression levels of *PIN1c*, *PIN5c* and *PIN8* were increased in *d10*, whereas in *d3* the expression levels of *PIN1a*, *PIN1d* and *PIN5b* were increased. The expression level of *PIN5a* was reduced significantly only in *d10*. No differences in the expression of other genes in the PIN family were observed between the WT and the two *d* mutants (Fig. 5A). Application of GR24 markedly downregulated the levels of most PIN family genes in WT (Fig. 5B) and *d10* (Fig. 5C), with the exception of *PIN1b*, *PIN5b* and *PIN9* in WT and *PIN1b* and *PIN2* in *d10*. These results suggested SLs inhibited polar auxin transport by modulating transcriptional level of PIN genes.

NAA and NPA application affected AR formation

The responses of AR number to application of exogenous NAA and the auxin polar transport inhibitor, NPA, were examined to assess whether increased auxin concentrations in the two SL mutants were responsible for the reduced AR formation. WT plants had higher numbers of ARs compared with the

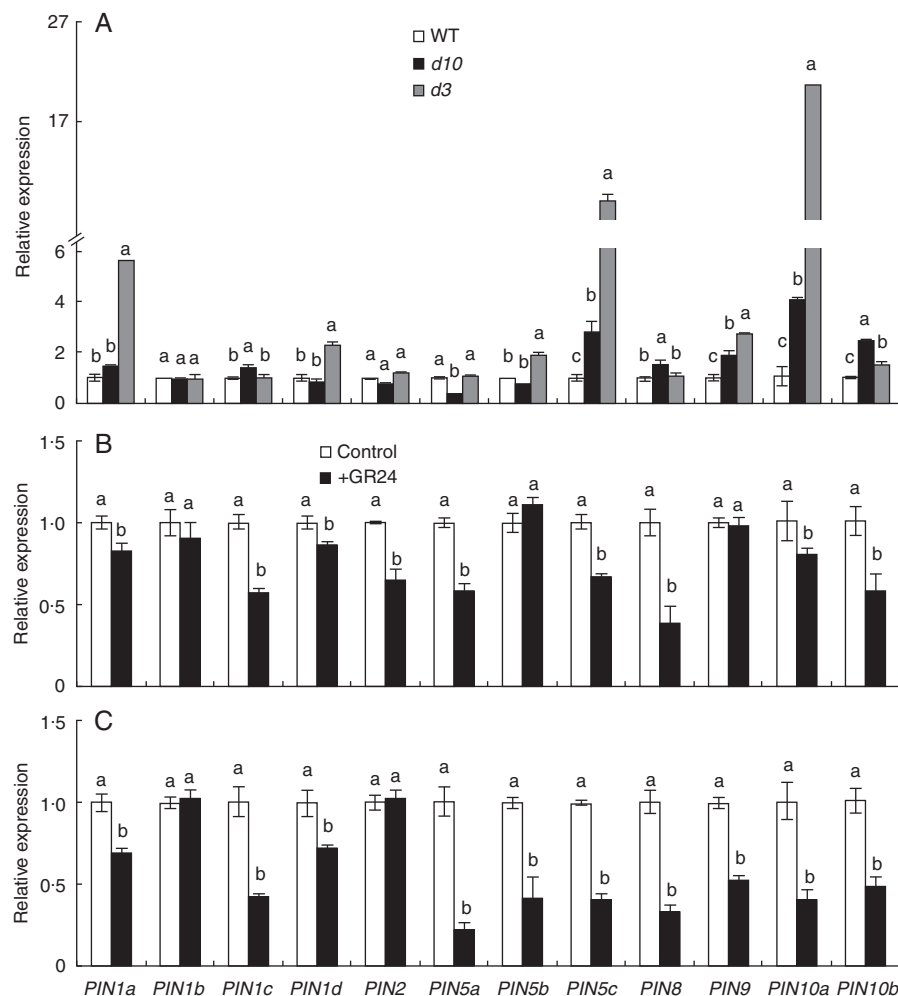


FIG. 5. qRT-PCR analysis of PIN family genes in the junction of wild-type (WT) and strigolactone-deficient (*d10*) and strigolactone-insensitive (*d3*) mutant rice plants. Rice seedlings were grown for 3 weeks in hydroponic media with or without 1.25 μM GR24. (A) WT, *d10* and *d3* mutants. (B, C) WT (B) and *d10* (C) with or without GR24 application. Relative mRNA levels were normalized for individual genes relative to *OsACT*. Data are mean \pm s.e. Different lower-case letters in the same gene indicate significant differences ($P < 0.05$, ANOVA).

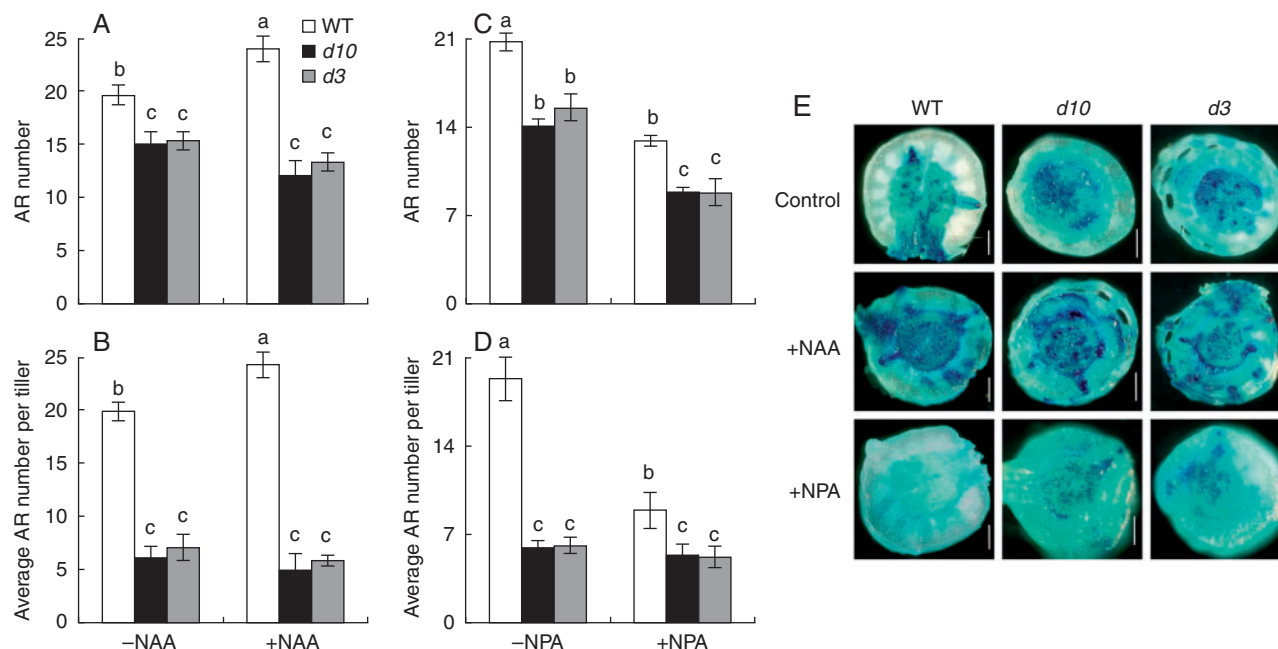


FIG. 6. Effect of synthetic NAA (A and B) and NPA (C and D) on AR number and average AR number per tiller of wild-type (WT) and strigolactone-deficient (*d10*) and strigolactone-insensitive (*d3*) mutant rice plants and *DR5::GUS* activity in the junction of WT and *d10* and *d3* (E). Rice seedlings were grown for 3 weeks in hydroponic media containing 10 nM NAA (α -naphthylacetic acid, exogenous auxin) and 0.5 μ M NPA (*N*-1-naphthylphthalamic acid, polar auxin transport inhibitor). Scale bar = 1 mm. Data are means \pm s.e. Different lower-case letters indicate significant differences ($P < 0.05$, ANOVA).

two *d* mutants regardless of NAA concentration (Supplementary Data Fig. S3A). Application of 10 nM NAA significantly increased AR numbers and average AR number per tiller by 22 % in the WT, but no changes in the two *d* mutants (Fig. 6A, B). Significant decreases were recorded in the number of ARs between the WT and the two *d* mutants after application of various NPA concentrations (Fig. S3B). Adventitious root numbers and average AR number per tiller were greater in the WT than *d10* and *d3* in response to application of 0.5 μ M NPA (Fig. 6C, D). Although the WT and the *d10* and *d3* mutants had similar expression of *DR5::GUS* regardless of exogenous application of NAA or NPA (Fig. 6E), a higher AR number was always recorded in WT than in the *d* mutants under NAA and/or NPA applications over 5 weeks of the experiment (Fig. S4). These results suggested that the increased auxin concentrations in the two *d* mutants might not be responsible for reduced AR production.

DISCUSSION

In this study, we demonstrated that rice *d* mutants with impaired SL biosynthesis and signalling produced lower AR number compared with the WT. The number of ARs in *d10*, an SL-deficient mutant, was complemented by application of lower levels of GR24. This was not the case for *d3*, an SL-insensitive mutant. Based on these results, we concluded that AR formation was positively regulated by SLs in rice. A higher concentration of IAA in the root–shoot junction and [3 H] IAA activity, and stronger expression of *DR5::GUS* were observed in the *d* mutants as comparing with the WT. Exogenous GR24 application decreased the expression of *DR5::GUS* in WT and *d10*, suggesting that SLs

modulate AR formation by inhibiting polar auxin transport. Exogenous application of NAA and NPA induced similar expression levels of *DR5::GUS* in the WT and *d10* and *d3* mutants; however, AR production was still greater in the WT compared with the *d10* and *d3* mutants. These results indicated that the relationship between the SLs and auxin in regulating adventitious root formation appears to be complex in rice.

In rice seedlings, the vigour of shoot growth correlated well with the total length of roots. AR growth in rice is the consequence of shoot growth (E *et al.*, 2012). Tiller numbers of *d10* and *d3* increased significantly and higher tiller numbers were recorded in the *d* mutants compared with the WT 3 weeks after germination. However, lower effective tiller and seed setting rates were found in the two *d* mutants relative to the WT. Arite *et al.* (2012) found that *d* mutants exhibit a shorter AR phenotype. Spike number in rice has a close relationship with the number of elongated ARs (Zhang, 1988). Lower AR numbers were recorded in the *d10* and *d3* mutants during 5 weeks after germination. Because tiller numbers increased more rapidly in the *d* mutants than in WT plants, the more rapid AR outgrowth in the two *d* mutants 6 weeks after germination resulted in a 47 % increase in AR production per plant in the *d* mutants compared with WT plants. Therefore, the higher AR numbers in *d* mutants at the maturity stage observed in this study were probably the consequence of excessive shoot branching. However, the average AR number per tiller was considerably lower in the *d* mutants than in the WT, and these reductions may be another reason for the lower effective tiller and seed setting rates.

Several lines of evidence suggest that the SL pathway is involved in root growth, in a manner that depends on the growth conditions and plant genotypes. LR densities in tomato and *Arabidopsis* are increased in SL-deficient and SL-insensitive

mutants, suggesting that SLs affect LR formation (Koltai *et al.*, 2010; Kapulnik *et al.*, 2011b; Ruyter-Spira *et al.*, 2011). However, rice LR numbers on the seminal root did not differ among 14-d-old WT and *d* mutants. Application of the synthetic SL analogue GR24 decreased LR density via the signalling gene *MAX2/D3*, which suppressed LR outgrowth in *Arabidopsis* and rice (Arite *et al.*, 2012; Sun *et al.*, 2014). Sun *et al.* (2014) found that SLs affect rice LR density in response to nitrogen and phosphorus deficiencies. These results indicate that SLs function as plant hormones to regulate root growth, but the reasons remain obscure. Adventitious rooting was enhanced in SL-deficient and SL-response mutants of *Arabidopsis* and pea, and the *CYCLIN B1* expression results suggested that SLs restrain the number of ARs by inhibiting the first formative divisions of the founder cells (Rasmussen *et al.*, 2012). Arite *et al.* (2012) reported that SLs positively regulate the length of ARs by controlling cell division in the meristematic zone. In this study, rice *d* mutants with impaired SL biosynthesis and signalling produced lower AR number at the seedling stage and a lower number of ARs per tiller at maturity. AR number and average AR number per tiller in *d10*, but not in *d3*, were complemented by application of lower levels of GR24. These results suggest that AR formation is positively regulated by SLs via the *D3* response pathway. Furthermore, the average length of ARs and the tiller number in SL synthetic mutants but not SL response mutants were complemented by application of GR24 (Arite *et al.*, 2012; Jiang *et al.*, 2013; Zhou *et al.*, 2013). These results indicate that application of different concentrations of GR24 were able to compensate for both shoot and root phenotypes in rice.

The importance of auxin in promoting adventitious rooting has been well established (Blakesley *et al.*, 1991; Couderet *et al.*, 2010). AR emergence and development were significantly inhibited in *OsPIN1b* RNA interference (RNAi) transgenic plants, which was similar to the phenotype of NPA-treated WT plants. The NAA treatment rescued the mutated phenotypes in the rice RNAi plants (Xu *et al.*, 2005). In this study, the application of NAA significantly induced AR formation in WT rice plants, while the application of NPA had the opposite effect (Fig. 6), which is consistent with reports of auxin promoting AR formation (Blakesley *et al.*, 1991; Xu *et al.*, 2005).

SLs have been suggested to act as modulators of auxin transport to regulate root growth (Koltai, 2011; Ruyter-Spira *et al.*, 2011; Sun *et al.*, 2014). Ruyter-Spira *et al.* (2011) suggest that SLs can modulate local auxin levels, a function that is dependent on the auxin status of *Arabidopsis*. The effects of SLs on root morphology in the presence of auxin have been shown to involve interference with auxin efflux carriers in tomato (Koltai *et al.*, 2010). In this study, the higher concentration of auxin in the shoot base of SL mutants might result from up-regulation of *PIN5c*, *PIN9* and *PIN10a-b*. Notably, the transcript level of *PIN5c* and *PIN10a* increased by three- to 20-fold in the junction of *d* mutants, which would play pivotal roles in mediating auxin acropetal flow to the junction. Conversely, application of GR24 decreased expression levels of most *PIN* family genes in WT and *d10*. These results confirmed that SLs inhibited polar auxin transport by modulating the expression of *PIN* genes. RT-PCR and promoter-driven GUS expression analysis showed that the high transcript levels of *PIN9* and *PIN10a-b*,

monocot-specific PINs, were observed in the shoot base of rice plants (Paponov *et al.*, 2005; Wang *et al.*, 2009). Furthermore, *PIN9* and *PIN10a* are highly expressed in the primordia of ARs (Wang *et al.*, 2009).

The interaction between the SLs and auxin signalling pathways in regulating adventitious rooting appears to be more complex. In *Arabidopsis* and pea, SLs can at least partially reverse the stimulation of AR formation by auxin, and auxin can further increase the numbers of ARs in *more axillary branching (max)* mutants that are resistant to the inhibitory effects of apical auxin. The above discussion indicates that AR formation is positively regulated by SLs via the *D3* response pathway and modulation of auxin acropetal flow to the junction. Furthermore, the positive effect of NAA application and the opposite effect of NPA application on AR number of WT plants also suggested the importance of auxin for AR formation. In the two *d* mutants, the non-responsiveness of AR to NAA application probably resulted from higher endogenous auxin levels (Supplementary Data Figs S3 and S4). In contrast, *DR5::GUS* expression was similar in the WT and *d10* and *d3* mutants, after NAA or NPA application (Fig. 6E), while the WT had higher AR numbers than the *d10* and *d3* mutants. These data probably suggested that auxin may modulate AR formation depending on SL signalling, i.e. SLs play a role in AR formation downstream of auxin. In conclusion, SLs promoted AR formation in rice via the *D3* component of SL signalling. The relationship between the SLs and auxin in regulating AR formation also appears to be complex.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Table S1: the primers used for qRT-PCR of *PIN* genes. Figure S1: number of ARs produced in response to the synthetic strigolactone analogue GR24. Figure S2: histochemical localization of GUS activity in rice plants. Figure S3: development of AR number in response to varying NAA and NPA concentrations. Figure S4: time course of AR number in response to NAA and/or NPA applications.

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