Journal of Plant Growth Regulation (2020) 39:941–953
https://doi.org/10.1007/s00344-019-10035-w

Silencing of the MADS-Box Gene *SlMADS83* Enhances Adventitious Root Formation in Tomato Plants

Anzhou Li[1] · Guoping Chen[1] · Yunshu Wang[1] · Honglian Liang[1] · Zongli Hu[1,2]

Received: 8 March 2019 / Accepted: 30 September 2019 / Published online: 11 October 2019

Abstract

Adventitious roots (ARs) are important for the growth of plants and the improvement in their stress resistance and survival capacity. Although many genes have been confirmed to be involved in adventitious root (AR) formation in *Arabidopsis* and tomato plants, MADS-box genes have rarely been mentioned. Here, we isolated a MADS-box gene named *SlMADS83*, which may negatively regulate AR formation in tomato plants, as the number of the ARs formed in the transgenic lines in which the *SlMADS83* gene was silenced by RNA interference (RNAi) was increased. The above phenotype was further confirmed by the analysis of the macroscopic, anatomical, and molecular features and related statistical data. Previous Studies have proven that auxin can stimulate early AR primordium initiation. Interestingly, in the RNAi transgenic lines, the concentration of auxin in the hypocotyl base was increased, resulting in early induction of AR primordia initiation, promoting the formation of ARs. Briefly, *SlMADS83* may play an important role in AR formation.

Keywords Adventitious roots · Tomato · *SlMADS83* · Auxin biosynthesis

Abbreviations

RNAi	RNA interference
IAA	Indole-3-acetic acid
ACC	1-Aminocyclopropane-1-carboxylate

Introduction

Roots are vital for plants to acquire nutrients and water and, thus, for plant growth, survival, and development (Petricka et al. 2012; Vidoz et al. 2010). The study of root development is of great significance to the adaptation of plants to environmental changes and to ensure crop yield under low soil fertility (Kell 2011). The root system is divided into embryo roots and ARs. An AR is defined as a root that forms on the non-root tissue of a plant. ARs share developmental characteristics with lateral roots and have strong plasticity (Verstraeten et al. 2014). They are the post-embryonic roots that generally form on the hypocotyl, and their primordia initiate from pericycle cells (Correa et al. 2012; Falasca and Altamura 2003). ARs enlarge the root system of plants and are widely used in clonal propagation, including growing plant cuttings and for tissue cultures (Ahkami et al. 2009), and they also improve the plant's stress resistance (Vidoz et al. 2010). The study of ARs is important for agriculture, horticulture, and forestry (Puri and Thompson 2003; Yamauchi et al. 2014).

AR formation is influenced by multiple factors. Wounding, flooding, light, and plant hormones can all induce AR formation (De Klerk et al. 1999; Vidoz et al. 2010). Auxin plays a central role in AR formation. The report indicates that the excised leaves and stem segments in *Arabidopsis* only rooted after short IBA (auxin analogs) treatment times, which suggests auxin stimulation is a central factor for adventitious rooting (Ludwig-Muller et al. 2005). When free and conjugated IAA (the main endogenous auxin) accumulated in the stem base of the aerial roots (aer) mutant of tomato, a profuse and precocious formation of AR primordia was observed, which suggests auxin induced AR primordial initiation (Mignolli et al. 2017). Silencing of *PIN1* in rice

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00344-019-10035-w) contains supplementary material, which is available to authorized users.

✉ Zongli Hu
huzongli71@163.com

[1] Laboratory of Molecular Biology of Tomato, Bioengineering College, Chongqing University, Chongqing 400044, People's Republic of China

[2] Bioengineering College, Chongqing University, Room 523-1, Campus B, 174 Shapingba Main Street, Chongqing 400030, People's Republic of China

plants, which acts as an auxin efflux carrier and transport auxin from the stem apex to the root, led to inhibition of AR emergence and development (Xu et al. 2005). Thus, auxin is necessary for AR formation, and acts as an inducer of AR primordium formation. AR formation is also controlled by related genes. For example, the auxin-response factors *ARF7* and *ARF19*, which are involved in auxin signaling, positively regulate AR formation (Gutierrez et al. 2009); the gene adventitious rootless1 (*ARL1*), an auxin- and ethylene-responsive gene, whose protein contains a lateral organ boundaries (LOB) domain, is characteristically expressed in lateral and AR primordia in rice and promotes AR formation (Liu et al. 2005); *OsAGAP*, which encodes an ARF (ADP-ribosylation factor)-GTPase-activating protein, regulates ARs formation by interfering auxin influx in rice (Zhuang et al. 2005, 2006); the gene *OsCKI1* encoding a casein kinase I (CKI) in rice is involved in auxin metabolism or that it may affect auxin levels, and promoting AR formation (Liu et al. 2003). However, these related genes were relating to auxin. In addition, AR formation is also regulated by other plant hormones. However, auxin is a central player in the hormone cross-talk that controls adventitious rooting (Pacurar et al. 2014). For example, ethylene is a plant hormone that promotes AR formation by affecting auxin transport (Negi et al. 2010). Thus, auxin determines the formation of AR.

MADS-box transcription factors, such as those related to the development of floral organs, fruits, leaves, and stems have been explored for a long time and have been identified as being involved in the development of plants (Xuhu et al. 2017; Honma and Goto 2001; Kater et al. 2006; Koo et al. 2010; Pelaz et al. 2000; Vrebalov et al. 2002; Zhang and Forde 1998). Recently, MADS-box genes have been reported to regulate lateral root development (Chunyan et al. 2015; Liu et al. 2015; Sun et al. 2017). However, studies on the MADS-box gene in the regulation of AR formation have scarcely been reported. Here, we report a novel gene named *SlMADS83* that encodes a member of the tomato MADS-box transcription factors, belonging to a *SlMBP9* group, which functions in root development and auxin regulation (Li et al. 2019). Silencing of *SlMADS83* enhanced AR formation, and we explored how the *SlMADS83* gene negatively regulated ARs, as described in this report.

Materials and Methods

Plant Materials and Growth Conditions

Seeds of wild-type and transgenic tomato (*Solanum lycopersicum* Mill. Cv. Ailsa Craig, AC^{++}) plants were sterilized and cultured in 28 °C at 100 rpm for 2 days in the dark. All seeds were sterilized by incubation for 2 min in 75% ethanol, then for 10 min in 10% bleach, and then washed with sterile water. After seeds germinated, they were transferred to Murashige and Skoog (MS) medium (Murashige and Skoog 1962) and cultured in a greenhouse. All the seedlings were grown in a greenhouse with a 16-h-day (26 °C) and 8-h-night (18 °C) cycle, and 70% humidity and 250 μM m^{-2} s^{-1} light intensity. Flowers were tagged at anthesis, and the fruits of immature green and mature green were defined as 28 and 35 days post-anthesis (DPA), respectively. The breaker fruits were defined as the fruits' color changed from green to yellow, and the breaker fruits at 4 days after breaking and 7 days after breaking were also labeled. All the samples were collected at the same time and immediately frozen in liquid nitrogen and stored at − 80 °C until further use.

Total RNA Extraction and Quantitative Real-Time PCR Analysis

Total RNA of tomato was extracted using the RNAiso plus kit (Takara) according to the manufacturer's instructions. The total RNA (2 μl) was used to synthesize first-strand cDNA using M-MLV reverse transcriptase (Takara) with a tailed oligo-d(T_{20}) primer (Supplementary Table S1). The cDNA was used to clone the specific DNA fragment of *SlMADS83* (GenBank accession number: XM_019211854, SGN number: Solyc01g106170) for construction of the RNAi vector and for quantitative real-time PCR (qPCR).

qPCRs were performed using the SYBR PremixEx Taq II Kit (Takara) in a 10-μl total sample volume (5.0 μl of 2 × SYBR PremixEx Taq, 0.5 μl of primers, 1.0 μl of cDNA, and distilled deionized water to a final volume of 10 μl) on the CFX96™ Real-Time System (Bio-Rad). The *SlCAC* gene (Exposito-Rodriguez et al. 2008) was used as an internal standard. No-template and no-reverse transcription controls were performed for each gene analysis. Three biological repeats were performed in the analyses of gene expression. All primers used in this study are listed in Supplementary Table S1.

Construction of the *SlMADS83* RNAi Vector and Plant Transformation

A 480-bp specific DNA fragment of *SlMADS83* was amplified with SlMADS83i-F and SlMADS83i-R primers tailed with *Hind*III/*Kpn*I and *Xho*I/*Xba*I restriction sites at the 5′ end, respectively. The purified fragment was digested and linked into the PHANNIBAL (Dong et al. 2013) plasmid at the *Hind*III/*Xba*I restriction site in the sense orientation and at the *Kpn*I/*Xho*I restriction site in the antisense orientation. The double-stranded RNAi-expression unit was digested with *XbaI* and *Sac*I and linked into the plant binary vector pBIN19. The pBIN19 vector carries the CaMV (cauliflower mosaic virus) 35S promoter, the *SlMADS83* fragment in the

sense orientation, a *PDK* intron, the *SlMADS83* fragment in the antisense orientation, and an OCS terminator. Finally, the plant binary vector pBIN19 was transferred into the *Agrobacterium* LBA4404 strain, and then the LBA4404 strain was transferred into wild-type tomato cotyledon explants through the *Agrobacterium*-mediated transformation method (Guoping et al. 2004).

Phylogenetic Analyses and Gene Expression Prediction

A neighbor-joining tree was constructed using MEGA 5.2 software with a bootstrap analysis of 1000 replicates. All of the proteins used in neighbor-joining tree were acquired from the NCBI database. *SlMADS83* gene expression prediction was performed using Tomato eFP Browser (http://bar.utoronto.ca/efp_tomato/cgi-bin/efpWeb.cgi).

Inhibitors Treatment and Chemical Solutions

Germinated wild and transgenic seeds were cultured on medium with different doses of 5-(4-chlorophenyl)-4H-1,2,4–triazole-3–thiol (Yucasin, Sigma, US) (0, 10, 20 and 100 μM) and L-Kynurenine (Kyn, APExBIO, US) (0, 1, 10 and 100 μM), respectively. Nine days later, the number of ARs was counted. Yucasin and Kyn were dissolved in DMSO (Nishimura et al. 2014).

Quantification of ARs

All of the seedlings for the quantification of ARs were grown on the medium for 9 days, including the seedlings with treatment of silver nitrate. The seedlings for adventitious rooting were grown on medium for 8 days. Then the top of these seedlings was removed at a position 5 mm from the stem apex and the roots at a position 5 mm above the hypocotyl/root junction. Finally, the excised hypocotyls were transferred to a new medium for rooting for 9 days, and then the ARs were quantified.

Anatomical Analysis of the Segment of Hypocotyl Base

The segments of the hypocotyl base from 5 mm above the hypocotyl/root junction to the hypocotyl/root junction of the 5-day-old seedlings were collected and immediately fixed in FAA liquid (38% formaldehyde/acetic acid/70% ethanol = 1:1:18, by volume). Subsequently, the samples underwent dehydration, fixation, sectioning, and dewaxing. Longitudinal sections were made by cutting the samples along the middle of the segment. The entire longitudinal sections were visualized under a microscope and photographed.

Application of 1-Aminocyclopropane-1-Carboxylate (ACC) and Silver Nitrate

The germinated wild-type seeds were respectively sowed on medium containing 0, 1, 5, and 10 μM ACC. Subsequently, the entire stem (containing the shoot apex) of wild-type seedlings was collected for total RNA extraction and qPCR analysis after 9 days. Both transgenic and wild-type seeds were grown on medium containing 50 μM silver nitrate ($AgNO_3$) for 9 days, and then the number of ARs was counted and the entire stems (containing the stem apex) were collected for qPCR.

Quantification of Free Indole-3-Acetic Acid (IAA) in Hypocotyl Base

The samples for free IAA quantification were the segments of the 9-day seedlings of the three Ri lines (Ri-4, Ri-5, and Ri-9) and three wild type excised from 5 mm above the hypocotyl/root junction to the hypocotyl/root junction where ARs formed. Free IAA was extracted using an IAA kit (IAA-4-C Comin Biotechnology Co., Ltd., China). Finally, the free IAA content was quantified by high-performance liquid chromatography.

Results

Analysis of the Expression Pattern of *SlMADS83* in Wild-Type Tomato Plants

In this study, we cloned and isolated a new tomato MADS-box transcription factor gene, *SlMADS83*, whose sequence was searched from the NCBI database. Phylogenetic tree analysis showed that SlMADS83 belongs to a SlMBP9 clade, which includes the other two MADS-box proteins, AtAGL21 and AtAGL17 (Fig. S1a). All of the proteins of the SlMBP9 clade play important roles in root development (Li et al. 2019), and it is speculated that SlMADS83 may play a role in root development. To explore the putative function of *SlMADS83* in tomato development, qPCR was performed to analyze its transcript accumulation in different tissues and organs. *SlMADS83* transcripts were detectable at very low levels in senescent leaves and ripe fruits, but its transcripts accumulated mostly in root tissue, sepals, and floral organs (Fig. 1). This result was consistent with our prediction expression profile of the *SlMADS83* gene using Tomato eFP browser (Fig. S1b, c). Transcripts also accumulated in stems, young leaves, mature leaves, and immature green fruits. Notably, during leaf senescence and fruit ripening, the *SlMAD83* transcript level displayed a decreasing trend (Fig. 1). Ethylene acts as a positive regulator of leaf senescence and fruit ripening,

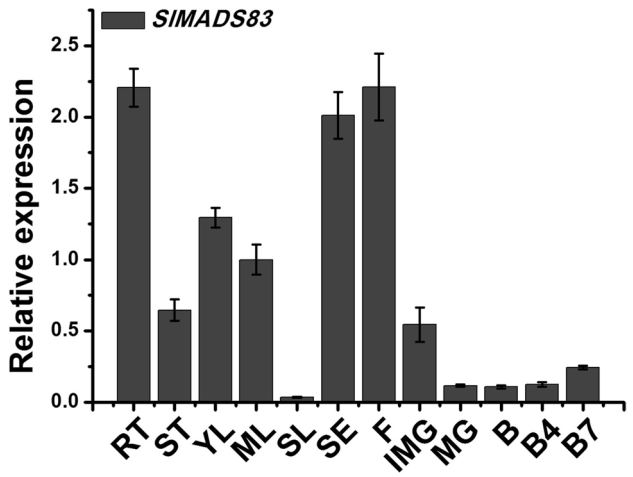

Fig. 1 Expression pattern analysis of *SlMADS83* in wild-type tomato plants. RT, root; ST, stem; YL, young leaf; ML, mature leaf; SL, senescent leaf; SE, sepal; F, flower; IMG, immature green fruit; MG, mature green fruit; B, breaker fruit; B4, breaker fruit after 4 days; B7, breaker fruit after 7 days

and ethylene biosynthesis improves with the processes of leaf senescence and fruit ripening (Aharoni and Lieberman 1979; Alexander and Grierson 2002; Barry and Giovannoni 2007; Bleecker and Patterson 1997; John et al. 2010; Picton et al. 1993). During leaf senescence and fruit ripening, the expression of *SlMADS83* showed an opposite trend to ethylene biosynthesis. We thus inferred that *SlMADS83* may mediate the regulation of ethylene.

Anatomical and Macroscopic Analysis of Phenotype of Enhanced ARs

We selected three RNAi transgenic tomato lines (Ri-4, Ri-5, and Ri-9) with low transcript accumulation in roots tissue for further exploration of *SlMADS83* function (Fig. 2a). Seeds of transgenic lines and wild-type tomato were grown on the MS medium for 9 days. A phenotype of enhanced ARs on the basal region from 5 mm above the hypocotyl/root junction to the hypocotyl/root junction was observed in the RNAi transgenic lines (Fig. 2b). Statistical analysis showed that the number of ARs was enhanced by 2.5- to 3.7-fold in the 9-day transgenic lines compared to the wild-type plants (Fig. 2c). Additionally, we anatomically analyzed the segment of the hypocotyl base (from 5 mm above the hypocotyl/root junction to the hypocotyl/root junction) of 5-day-old seedlings, which showed no emergence of macroscopic ARs (Fig. 2d). At ×40 magnification, several early AR primordia were found in the hypocotyl base segment of the transgenic line (Fig. 2f), whereas no early AR primordium had initiated in the wild type (Fig. 2e).

Adventitious Rooting Analysis

Based on the enhanced ARs forming on the hypocotyl base, we determined the free IAA (the main endogenous auxin) content in the hypocotyl base segment (from 5 mm above the hypocotyl/root junction to the hypocotyl/root junction). The free IAA level in the hypocotyl base of the RNAi lines was elevated over twofold compared to the wild type (Fig. 3a).

Next, we investigated whether silencing *SlMADS83* enhanced auxin signaling to promote AR formation. After seeds were cultured on MS medium for 8 days, the top of the shoots (where auxin is produced) and the hypocotyl base (where auxin accumulated) of the seedlings were removed. The decapitated and de-rooted hypocotyls and the de-rooted seedlings were transferred to new MS medium for 9 days. Only the de-rooted seedlings produced more ARs than the wild-type plants, while the decapitated and de-rooted seedlings produced a similar number of ARs in both the transgenic and wild-type tomato (Fig. 3b, c).

Silencing of *SlMADS83* Upregulated Auxin Biosynthesis and Altered Auxin Transport Capacity

Figure 4a shows a tryptophan-dependent IAA biosynthesis pathway in *Arabidopsis* to better understand how IAA is synthesized in stem apex. The genes of *YUC* (*YUCCA*) encode proteins of flavin monooxygenase that catalyze the rate-limiting step of the conversion of IPA to IAA, which is the main IAA biosynthesis pathway (Mashiguchi et al. 2011). The *ToFZY* tomato genes are the orthologs or potential orthologs of *YUC* genes and encode proteins with the same enzymatic activity as YUC and are involved in the IAA biosynthesis pathway (Exposito-Rodriguez et al. 2007, 2011). By qPCR, the transcripts of *ToFZY1*, *ToFZY4,* and *ToFZY5* were significantly increased—among which, *ToFZY5* was upregulated by fourfold to tenfold—while neither *ToFZY2* nor *ToFZY3* were significantly changed in the stem apex of the RNAi plants (Fig. 4b). Polar transport and distribution of auxin are dependent on the auxin efflux carrier PINs (Naramoto 2017). Tomato *PIN1*, which mediates basipetal polar auxin transport in the stem (Shi, et al. 2017), and *PIN4* and *PIN7,* which are named after *AtPIN4* and *AtPIN7* of *Arabidopsis* (Pattison and Catala 2012), were unaffected by the silencing of *SlMADS83* (Fig. 4c). However, the transcript level of tomato auxin transporter gene *PIN8*, which is an ortholog of *AtPIN8* (Pattison and Catala 2012), was upregulated by 50% to 80% (Fig. 4c), which indicates that the silencing of *SlMADS83* may positively alter auxin transport capacity.

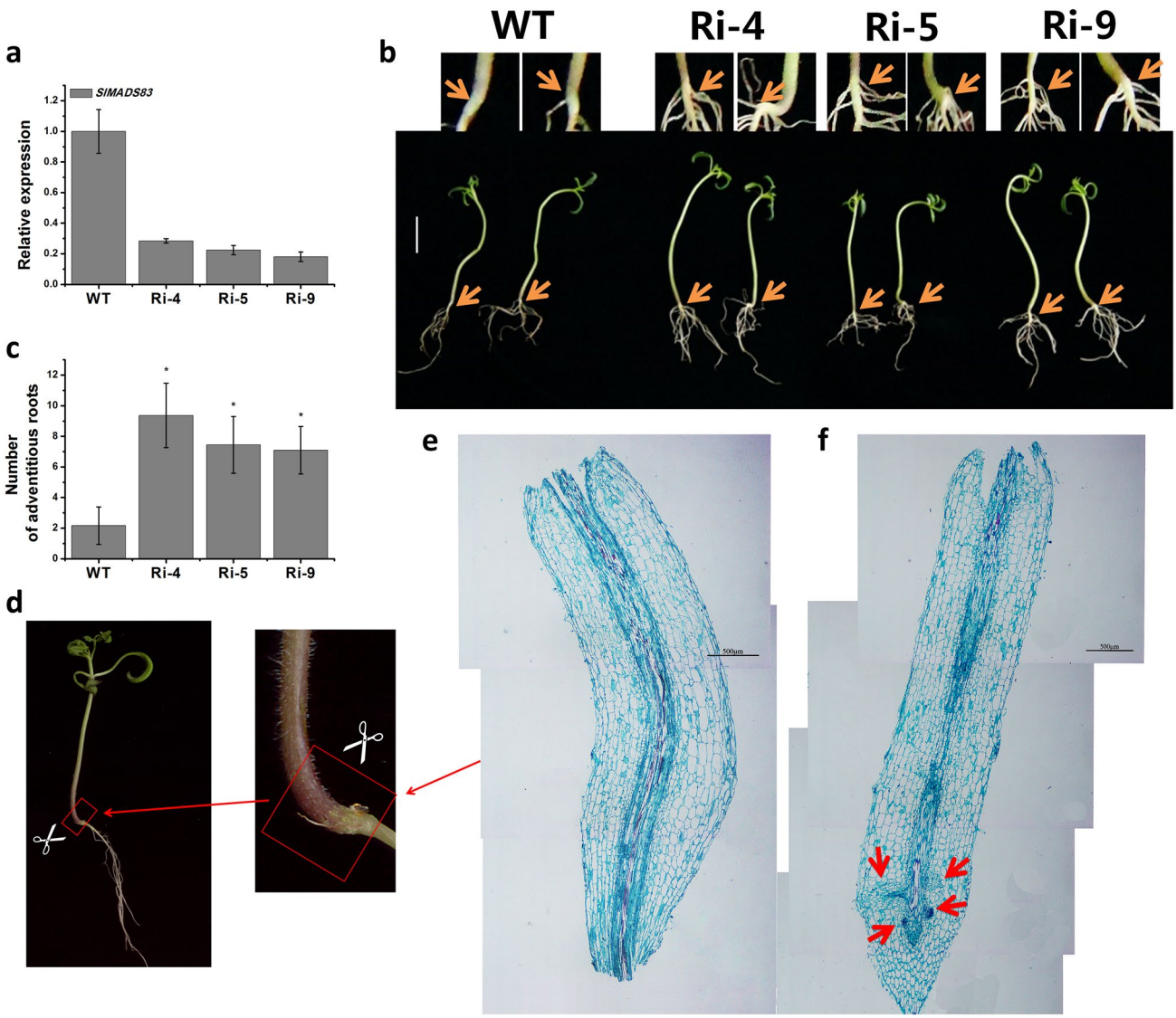

Fig. 2 Anatomical and macroscopic analysis of phenotypes. **a** Expression level of *SlMADS83* in the root tissue of transgenic lines and wild type of 9-day-old seedlings. **b** Phenotype of enhanced ARs of 9-day-old seedlings. Orange arrows indicate ARs in the basal region of the hypocotyl. **c** Statistical analysis of the number of ARs of 9-day-old seedlings. Means ± SE with $n > 20$ per group. **d** Segments for longitudinal sections excised at a position 5 mm above the hypocotyl/root junction and at the site of the hypocotyl/root junction. Red boxes indicate the basal region of segments that were excised for anatomical analysis. e, f Anatomical analysis of the segment of hypocotyl base from wild-type (**e**) and transgenic line Ri-4 (**f**) of 5-day-old seedlings. Red arrows show the early adventitious root primordia. Asterisks indicate a significant difference ($P < 0.05$)

Silencing of *SlMADS83* Increased the Transcript of Ethylene Biosynthesis Genes

As mentioned above, *SlMADS83* may mediate the regulation of ethylene, and we thus examined the expression of ethylene biosynthesis genes, including *ACS2* [1-aminocyclopropane-1-carboxylate (ACC) synthase 2], *ACS4* (ACC synthase 4), *ACO1* (ACC oxidase 1), and *ACO3* (ACC oxidase 3) (Blume et al. 1997; Eum et al. 2009), which are expressed in root tissue. By qPCR analysis, the expression levels of *ACO1* and *ACS6* in transgenic lines were all increased by 70–130% and *ACS2* was increased by 140–260% in root tissue compared to the wild type (Fig. 5).

Treatment with 1-Aminocyclopropane-1-Carboxylate (ACC) and Silver Nitrate

Based on the significant upregulation of IAA biosynthesis and ethylene biosynthesis genes, we treated wild-type tomatoes with the ethylene precursor ACC to simulate the increased endogenous ethylene biosynthesis of RNAi plants.

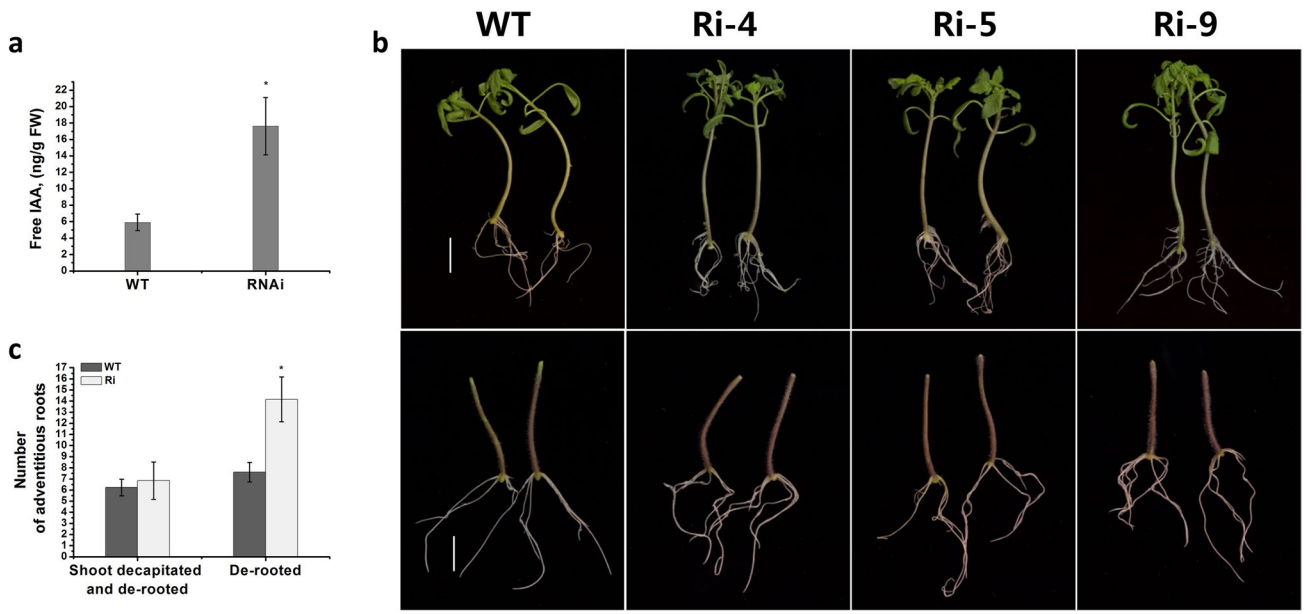

Fig. 3 Analysis of adventitious rooting and free IAA content**. a** Quantification of free IAA in the basal region of the hypocotyl (from 5 mm above the hypocotyl/root junction to the hypocotyl/root junction) where the enhanced AR formation occurs. **b** The pictures above show ARs of seedlings grown on medium for 8 days and de-rooted for subsequent adventitious rooting for 9 days. The pictures in the bottom show the ARs of seedlings grown on medium for 8 days and then capitated and de-rooted (excised at a position 5 mm above the hypocotyl/root junction) for adventitious rooting for 9 days. Bars = 10 mm. **c** Statistical analysis of ARs in decapitated and de-rooted seedlings and de-rooted only seedlings. Means ± SE with $n > 15$ per group. *$P < 0.05$

After the wild-type seeds were respectively cultured on MS medium with different doses (0, 1, 5, and 10 μM) of ACC for 9 days, the expression of *ToFZY1*, *ToFZY4,* and *ToFZY5* was examined in the stem apex. Of these, only *ToFZY5* was upregulated (onefold–tenfold) at high concentrations of ACC treatment (Fig. 6a). In addition, when transgenic and wild-type tomatoes were treated with 50 μM silver nitrate, which is an ethylene signaling antagonist, for 9 days, the expression level of *ToFZY5* in RNAi plants was restored and even downregulated below the original expression level of the wild type (Fig. 6b). In contrast to untreated seedlings, the number of ARs of seedlings after 50 μM silver nitrate treatment was decreased (Fig. 6c, d).

Treatment with Auxin Biosynthesis Inhibitors Yucasin and Kyn

To further confirm whether SIMADS83-mediated elevated auxin biosynthesis is involved in the increase in AR formation, we cultured wild and transgenic seedlings grown on medium with different doses of the auxin biosynthesis inhibitors Yucasin (0, 10, 20, and 100 μM) and Kyn (0, 1, 10, and 100 μM). Nine days later, we counted the AR number of these seedlings. Statistical analysis suggests that under treatment with high concentration of Yucasin (100 μM), the AR numbers of both WT and transgenic seedlings were significantly decreased compared to that of the control. The wild-type tomato hardly had ARs, whereas a small number of ARs were formed in the transgenic seedlings that were similar to the wild type under normal growth conditions (Fig. 7a, e). Although the ARs of transgenic seedlings were obviously suppressed under the treatment of 20 μM Yucasin, the number of ARs was still higher than that of the untreated wild-type seedlings (Fig. 7a, e).

Kyn is a co-inhibitor of ethylene response and auxin biosynthesis (He et al. 2011). In contrast to the Yucasin treatment, a high concentration (100 μM) of Kyn treatment resulted in an increase in the number of ARs in both wild-type and transgenic seedlings (Fig. 7a). However, low doses of Kyn (1 and 10 μM) did not significantly reduce the number of ARs (data not shown).

Discussion

Recently, we reported a MADS-box gene *SlMBP9*, which suppresses lateral root formation by regulating local auxin accumulation (Li et al. 2019). In this study, a novel tomato MADS-box gene, *SlMADS83*, which is homologous to *SlMBP9* (Fig. S1a) and may play an important role in root development, was isolated from wild-type tomato roots based on a cDNA clone. Silencing of *SlMADS83* enhanced AR formation. Because the enhancement of AR formation could be used in breeding programmes to improve plant

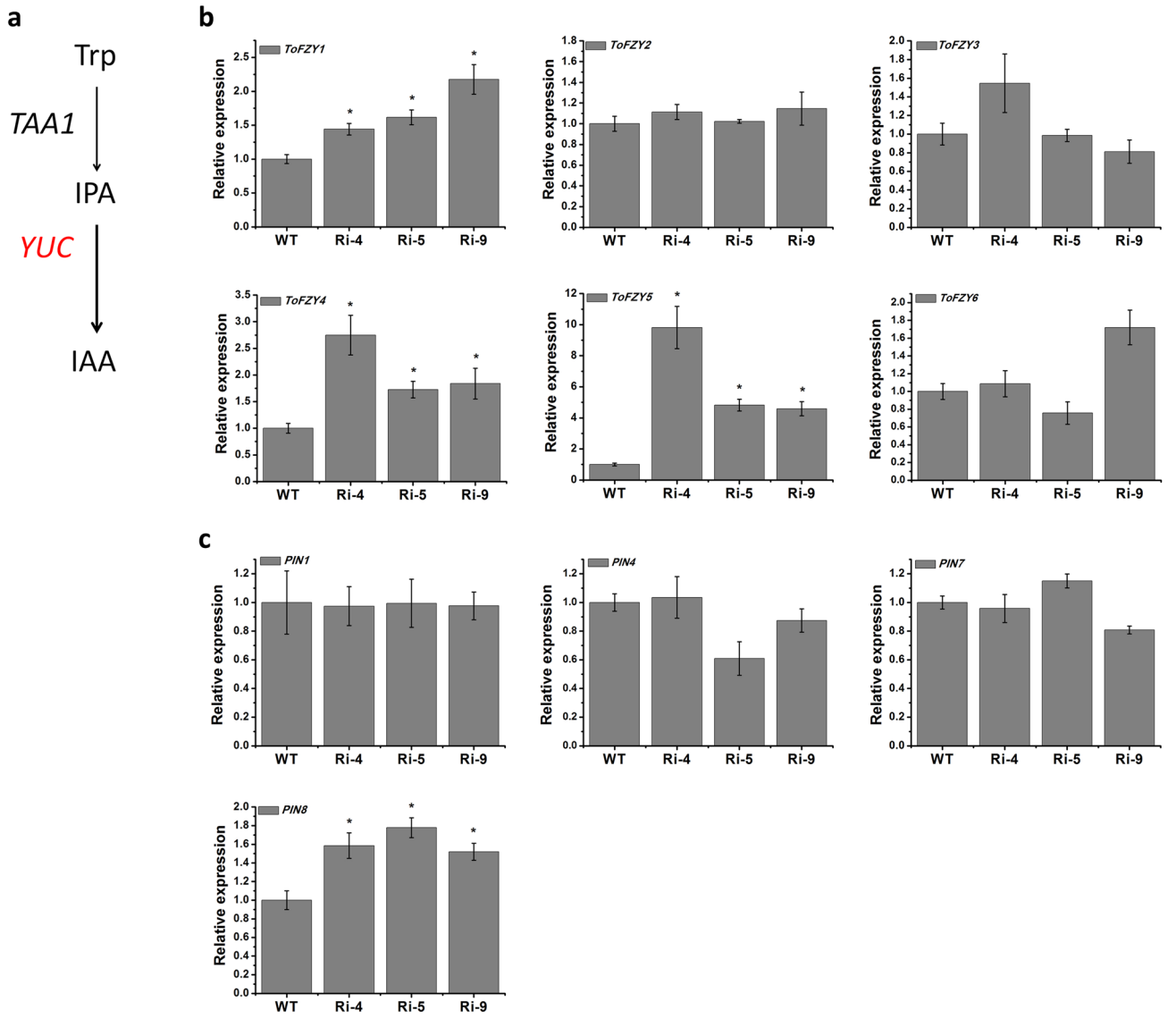

Fig. 4 Analysis of the expression levels of auxin biosynthesis and transport genes. **a** A tryptophan-dependent IAA biosynthesis pathway in *Arabidopsis*. **b** The transcript levels of auxin biosynthesis genes in the stem apex. **c** The transcript levels of auxin transport genes in stem. The samples used for the examination of gene expression were 9-day-old seedlings. Each value represents the mean ± SE of three replicates. $^{*}P < 0.05$

survival, we examined how the silencing of *SlMADS83* positively regulates AR formation.

SlMADS83 was expressed mostly in roots, sepals, and flowers, and presented a trend of downregulation during leaf senescence and fruit ripening, which indicates that *SlMADS83* may function in root regulation and ethylene-mediated progress as mentioned above. Subsequently, we constructed a *SlMADS83*-RNAi vector and produced *SlMADS83*-RNAi transgenic plants, and three RNAi transgenic lines (Ri-4, Ri-5, and Ri-9) were selected for further experiments (Fig. 2a). Based on the statistical analysis, 2.5- to 3.7-fold more ARs were produced in the region of the hypocotyl base of the transgenic plants than in that of the wild-type tomato plants (Fig. 2b, c). AR development is a normal part of plant development, but generally, only external stimulation such as wounding can induce the formation of additional ARs (Ahkami et al. 2009). Silencing of *SlMADS83* resulted in an increase in ARs in the transgenic seedlings grown under normal conditions without external stimuli. Thus, we inferred that *SlMADS83* is a key factor that negatively regulates AR formation.

Segments of the hypocotyl base of 5-day-old seedlings, which had no macroscopic AR emergence, were excised from a position 5 mm above the hypocotyl/root junction to the hypocotyl/root junction (Fig. 2d) and used for anatomical analysis. Several early AR primordia were initiated in the

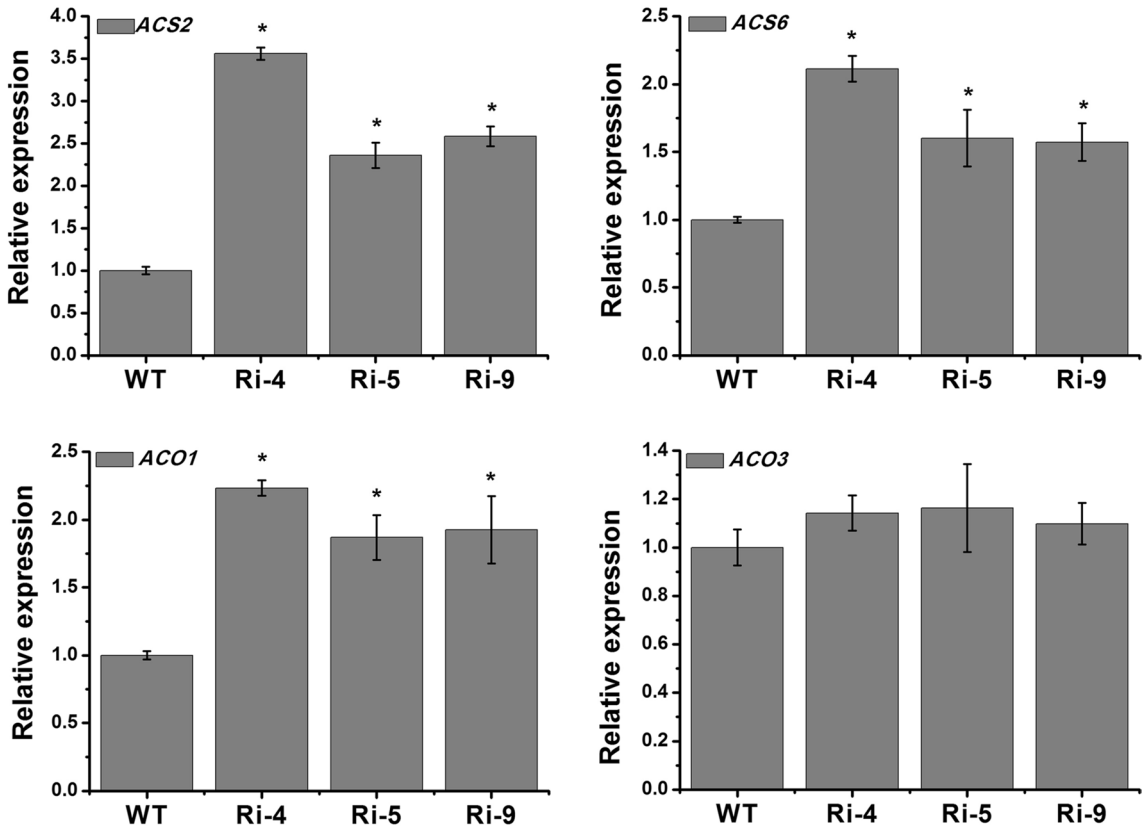

Fig. 5 Relative mRNA transcription levels of tomato ethylene biosynthesis-related genes. Each value represents the mean ± SE of three replicates. Asterisks indicate a significant difference ($P < 0.05$) between the wild-type and transgenic lines

hypocotyl base of the transgenic line (Ri-4), while no AR primordia were observed in the wild-type plants (Fig. 2e, f). Auxin is an inducer of AR formation, and early AR primordium formation is strictly dependent on auxin (Correa et al. 2012). Thus, these results suggest that silencing *SlMADS83* accelerates early AR primordium formation, which may be ultimately caused by the enhanced auxin or auxin signaling that we predicted.

To explore whether silencing of *SlMADS83* leads to increased auxin or auxin signaling, we determined the free IAA content in the region of the hypocotyl base where the increased number of ARs formed. The free IAA content in RNAi lines was twofold greater than that in the wild type (Fig. 3a). After adventitious rooting for 9 days, the de-rooted transgenic seedlings produced more ARs than wild-type de-rooted seedlings (Fig. 3b, c). However, the AR formation in decapitated and de-rooted hypocotyls, which may be induced by residual auxin, did not significantly increase in RNAi lines. These data indicate that the silencing of *SlMADS83* induced the accumulation of auxin in the hypocotyl base but did not enhance auxin signaling.

The expression levels of *ToFZY1*, *ToFZY4,* and *ToFZY5* were upregulated, which suggests that the silencing of *SlMADS83* may increase auxin biosynthesis in the shoot apex. The transcript of the auxin transporter gene *PIN8* was also increased, which indicates that the silencing of *SlMADS83* may increase auxin transport capacity in the stem (Fig. 4c). However, *PIN1*, the main basipetal polar auxin transporter gene (Shi et al. 2017), as well as *PIN4* and *PIN7,* was unaffected (Fig. 4c). Although only *PIN8* of the auxin transport genes was upregulated, we cannot rule out the possibility that basipetal polar auxin transport was enhanced. The aerial roots (aer) mutant of tomato showed that PIN1 and PIN4 were more expressed in shoot apices than in the hypocotyl (Mignolli et al. 2017). In our study, the samples for the detection of *PINs* were the entire shoots (containing the shoot apex), and thus, this may have led to the expression of *PINs* being apparently unchanged. The detailed pathway of polar auxin basipetal transport in the stem requires further experimental exploration.

It is well known that there is cross-talk between ethylene and auxin. Ethylene promotes auxin biosynthesis and regulates auxin transport and enhances AR formation (Barry et al. 2001; Negi et al. 2010; Swarup et al. 2007). Enhancing ethylene synthesis or signaling may elevate auxin biosynthesis. For example, the evidence proved that exogenous ACC

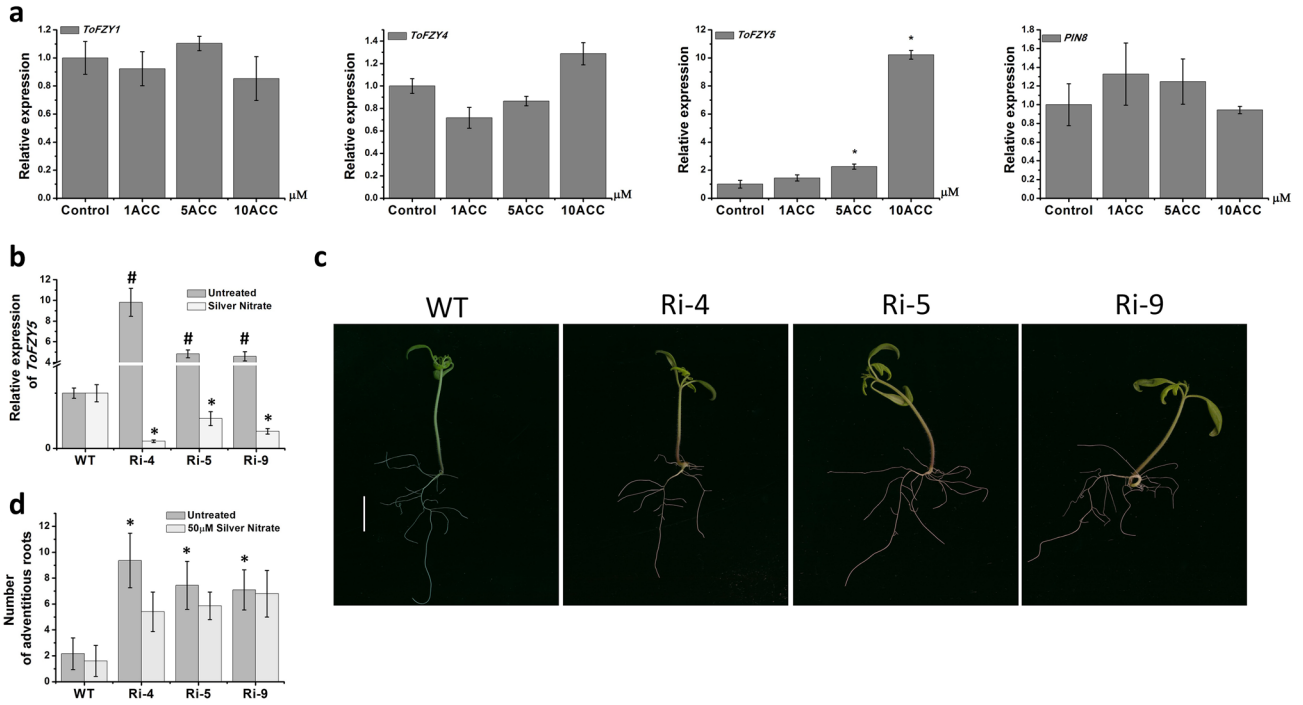

Fig. 6 Analysis of treatment with ACC and silver nitrate. **a** The expression levels of auxin biosynthesis- and transport-related genes in the stems (containing the stem apex) under different doses (0, 1, 5, and 10 μM) of ACC treatment. **b** Relative expression of *ToFZY5* in the stem apex of seedlings treated with 50 μM silver nitrate and untreated seedlings. Each value represents the mean ± SE of three replicates. **c** ARs in 9-day-old seedlings treated with 50 μM silver nitrate. Bars = 10 mm. **d** Statistical analysis of ARs of the seedlings treated with 50 μM silver nitrate and untreated seedlings. The seedlings were 9 days old. Means ± SE with $n > 20$. The asterisk (*) indicates statistically significant differences between silver nitrate-treated wild-type and silenced lines as determined by a Student's *t*-test. The hash (#) indicates statistically significant differences between untreated wild-type and silenced lines as determined by Student's *t*-test

addition improved auxin synthesis (Swarup et al. 2007). The mutant *ctr1*, which showed enhanced ethylene signaling, significantly increased auxin biosynthesis (Muday et al. 2012). Ethylene upregulated the tryptophan (Trp, the substrate for auxin biosynthesis) biosynthesis genes WEAK ETHYLENE INSENSITIVE2 (*WEI2*) and *WEI7*, resulted in an increase in auxin (Stepanova et al. 2005). In addition, a molecular mechanism by which ethylene promotes auxin biosynthesis is via activation of the transcription of *TAA1* and its close homologs *TARs* (Stepanova et al. 2008), which are involved in Trp into IPA pathway of IAA biosynthesis pathway (Fig. 4a). Furthermore, ethylene increases the capacity for auxin transport by upregulating the genes of auxin carriers such as *AUX1* in roots (Ruzicka et al. 2007). Ethylene also promotes auxin transport in stems (Ivanchenko et al. 2010), e.g., the mutant *epi*, which overproduces ethylene, significantly increased basipetal auxin transport in stems and formed more ARs (Negi et al. 2010). In contrast, auxin also elevates ethylene biosynthesis by upregulating ACS genes encoding ACC synthase (Stepanova et al. 2007). Moreover, ethylene affects root development, including root elongation, lateral roots, and AR formation. However, the combination of the description above and other reports (Muday et al. 2012) suggests that ethylene regulates root development by modulating auxin biosynthesis and transport. Mutations that impair ethylene perception (*etr1*), or signaling (*ein2* and *ein3*) reduced root growth inhibition regulated by elevated ethylene (Muday et al. 2012), suggesting that ethylene-mediated auxin regulation occurred in the downstream of the ethylene pathway.

During leaf senescence and fruit ripening, the expression of *SlMADS83* presented a trend of downregulation, which contrasted with the trend of ethylene biosynthesis (Fig. 1). We suspected that silencing *SlMADS83* would improve ethylene biosynthesis. To determine whether the silencing of *SlMADS83* mediates the progress of ethylene biosynthesis, we examined the genes involved in ethylene biosynthesis in root tissue. The results showed that *ACS2*, *ACS6,* and *ACO1* were upregulated in the root tissue of transgenic plants compared to that of wild-type plants (Fig. 5), which suggests that the silencing of *SlMADS83* may promote ethylene biosynthesis.

Subsequently, we examined the expression levels of *ToFZY1*, *ToFZY4*, *ToFZY5,* and *PIN8* in wild-type tomato

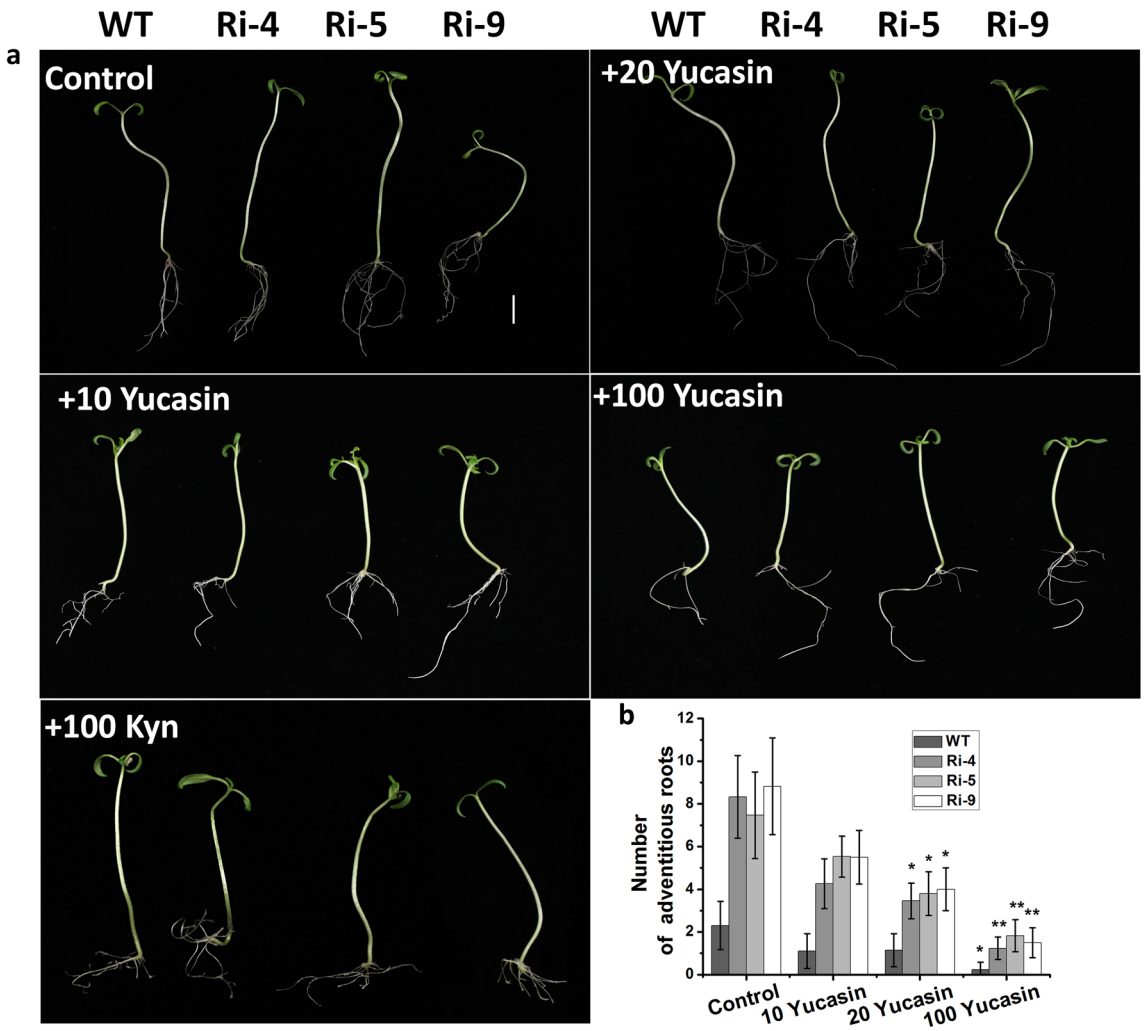

Fig. 7 Inhibition treatment. **a** The phenotype of the 9-day-old seedings treated with different doses of Yucasin (0, 10, 20, and 100 μM) and 100 μM Kyn. **b** The number of ARs in the 9-day-old seedlings treated with Yucasin. Means ± SE with $n > 15$. *$P < 0.05$, **$P < 0.01$

after ACC treatment, which simulates endogenous ethylene biosynthesis in transgenic plants, to explore whether these genes in transgenic lines were regulated by enhanced ethylene biosynthesis. Only the transcript of *ToFZY5*, whose level of gene upregulation was similar to that of RNAi plants grown on untreated medium, was increased (onefold to tenfold) under high concentrations of ACC (Fig. 6a). In addition, the transcript abundance of *ToFZY5* in transgenic lines treated with a high concentration (50 μM) of silver nitrate (an ethylene signaling antagonist) was downregulated to below the original expression level of the wild type, which is in contrast to that in the untreated transgenic plants (Fig. 6b). Silver ions replace the copper cofactor binding site of ethylene receptors and interact with ethylene to resist the ethylene response, but silver ions also have potential off-target effects (Strader et al. 2009; Schaller and Binder 2017). However, the high concentration (50 μM) of silver nitrate still suppressed the expression of *ToFZY5* in transgenic lines without making a larger impact. These results suggest that ethylene may affect not only the upstream processes of IAA biosynthesis but also the transcription of the YUC-like gene *ToFZY5*. The increased *ToFZY5* may be regulated by enhanced ethylene biosynthesis in transgenic plants but not directly by *SlMADS83*. The upregulation of *ToFZY1* and *ToFZY4* may be regulated by another pathway via the silencing of *SlMADS83*. After treatment with 50 μM silver nitrate, the expression of *ToFZY5* returned to the wild-type level, and it was inferred that the ethylene signaling of transgenic lines probably also returned to the wild-type level. This high-concentration silver nitrate treatment may lead to normal levels of ethylene-stimulated auxin biosynthesis and transport. Simultaneously, the number of ARs in transgenic plants was decreased compared to the number in untreated transgenic plants, but was still much higher than the original AR number of the wild-type tomato plants (Fig. 6d). These data suggest that the increase in ARs by SlMADS83

might be mainly achieved through a non-ethylene dependent pathway, and the improved transcript level of ethylene biosynthesis genes may increase ethylene biosynthesis and partially increase AR formation by regulating auxin biosynthesis and transport.

To further confirm whether *SlMADS83*-induced AR formation was mainly regulated by the auxin pathway, we performed Yucasin and Kyn treatments. Yucasin is a potent inhibitor of YUC protein activity that is involved in the key step of IAA biosynthesis (Fig. 4a), and Yucasin significantly reduced IAA levels. Simultaneously, high concentration (> 25 μM) of Yucasin restored the high-auxin phenotype of *YUC*-overexpression seedlings (Nishimura et al. 2014). Kyn is also an inhibitor of IAA biosynthesis, through the repression of EIN3 nuclear accumulation which involved ethylene signaling and then targeted to inhibit TAA1 activity (He et al. 2011). In our results, treatment with 20 μM Yucasin decreased the AR number in transgenic lines, but the AR number is still higher than that of the untreated wild-type. This suggests that this dose of Yucasin is not enough to abolish the auxin accumulation in the hypocotyl base by *SlMADS83*, but it still proved that Yucasin suppressed the effects of *SlMAS83*-induced AR formation. Furthermore, a high concentration (100 μM) of Yucasin restored the AR phenotype, demonstrating that *SlMADS83* may mainly regulate auxin biosynthesis and transport, resulting in the accumulation of auxin in the hypocotyl base and finally enhancing AR formation. In addition, 10 μM Kyn inhibitor treatment did not affect AR formation; it was speculated that the deficiency in auxin biosynthesis by Kyn may be compensated by increased auxin transport regulated by *SlMADS83*. Surprisingly, 100 μM Kyn was found to increase the ARs in both transgenic and wild-type tomato plants. This is the opposite of what we would expect if Kyn reduced ethylene signaling and auxin biosynthesis. We speculate that the high concentration of Kyn may cause damage to the primary root and thus increase AR formation.

To date, we have not been able to determine whether SlMADS83 directly or indirectly regulates these upregulated genes, including the genes involved in auxin biosynthesis and transport, and ethylene biosynthesis. The MADS domain of the MADS-box gene could recognize and bind to a DNA sequence called a GArG-box motif (Tang and Perry 2003; Aerts et al. 2018). A recent report demonstrated that the chrysanthemum MADS-box gene *CmANR1* directly binds to the recognition site GArG motif of *CmPIN2* through ChIP-PCR. In vivo, *CmANR1* directly activates the transcription of *CmPIN2* and regulates auxin transport (Sun et al. 2018). Our phylogenetic analysis suggests that SlMADS83 is homologous to SlMBP9. SlMBP9 was a member of the AtANR1 clade (Hileman et al. 2006). *SlMADS83* may also recognize and bind some GArG-box motifs of auxin biosynthesis and transport genes. We thus explored whether some putative GArG motifs exist in the promoter sequence of the genes upregulated by *SlMADS83*. We found that all of these genes have one or several potential GArG-box motifs in the 1000 bp sequence upstream of the initiation codon ATG (Supplementary Table S2). *ToFZY4*, *ToFZY5*, and *ACS2* have potential GArG motifs 500 bp upstream of the ATG (Supplementary Table S2). This suggests that these genes may be directly regulated by some MADS-box genes, including the MADS-box gene *SlMADS83*. However, further investigation is required in the future to determine whether increased ethylene biosynthesis is induced by increased auxin or directly activated by *SlMADS83*.

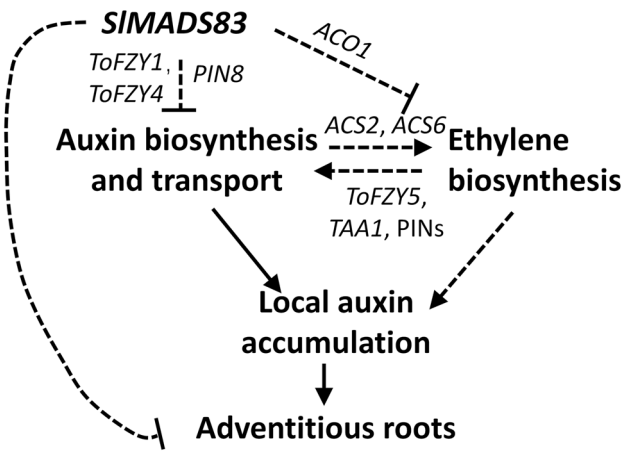

Fig. 8 Schematic of AR formation regulated by *SlMADS83*

In conclusion, the silencing of *SlMADS83* enhances AR formation. Our study suggests that silencing *SlMADS83* leads to more free IAA accumulation in the hypocotyl base. The high concentration of endogenous IAA accumulation may promote early AR primordia initiation, thereby enhancing AR formation. The increased auxin in transgenic plants is regulated by the silencing of *SlMADS83*, and the increased transcript of ethylene biosynthesis genes may enhance endogenous ethylene biosynthesis, positively regulating auxin biosynthesis and transport, thus leading to auxin accumulation in the hypocotyl base. Together, the silencing of *SlMADS83* might, in cooperation with the increased transcript levels of ethylene biosynthesis genes, lead to auxin accumulation in the hypocotyl base and increased AR formation (Fig. 8).

Acknowledgements This work was supported by National Natural Science Foundation of China (No. 31572129) and the Natural Science Foundation of Chongqing of China (No. cstc2015jcyjA80026).

Author Contributions Z.H. and G.C. designed the research; A.L., Y.W., and H.L. performed the research; A.L. wrote the paper; Z.H. modified the paper. All authors have read and approved the final version of the manuscript.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Aerts N, De Bruijn S, Van Mourik H, Angenent GC, Van Dijk AD (2018) Comparative analysis of binding patterns of MADS-domain proteins in *Arabidopsis thaliana*. BMC Plant Biol 18:131. https://doi.org/10.1186/s12870-018-1348-8

Aharoni N, Lieberman M (1979) Ethylene as a regulator of senescence in tobacco leaf discs. Plant Physiol 64:801–804

Ahkami AH, Lischewski S, Haensch KT, Porfirova S, Hofmann J, Rolletschek H, Melzer M, Franken P, Hause B, Druege U, Hajirezaei MR (2009) Molecular physiology of adventitious root formation in *Petunia hybrida* cuttings: involvement of wound response and primary metabolism. New Phytol 181:613–625

Alexander L, Grierson D (2002) Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening. J Exp Bot 53:2039–2055

Barry CS, Giovannoni JJ (2007) Ethylene and fruit ripening. J Plant Growth Regul 26:143–159

Barry CS, Fox EA, Yen H, Lee S, Ying T, Grierson D, Giovannoni JJ (2001) Analysis of the ethylene response in the epinastic mutant of tomato. Plant Physiol 127:58–66

Bleecker AB, Patterson SE (1997) Last exit: senescence, abscission, and meristem arrest in Arabidopsis. Plant Cell 9:1169–1179

Blume B, Barry CS, Hamilton AJ, Bouzayen M, Grierson D (1997) Identification of transposon-like elements in non-coding regions of tomato ACC oxidase genes. Mol Gen Genet 254:297–303

Chunyan Y, Yihua L, Aidong Z, Sha S, An Y, Linli H, Imran A, Yu L, Forde BG, Yinbo G (2015) MADS-box transcription factor OsMADS25 regulates root development through affection of nitrate accumulation in rice. PLoS ONE 10:e0135196. https://doi.org/10.1371/journal.pone.0135196

Correa LD, Troleis J, Mastroberti AA, Mariath JEA, Fett AG (2012) Distinct modes of adventitious rooting in *Arabidopsis thaliana*. Plant Biol 14:100–109

De Klerk GJ, Van der Krieken W, De Jong JC (1999) Review the formation of adventitious roots: new concepts, new possibilities. Vitro Cell Dev-Pl 35:189–199

Dong TT, Hu ZL, Deng L, Wang Y, Zhu MK, Zhang JL, Chen GP (2013) A tomato MADS-box transcription factor, SlMADS1, acts as a negative regulator of fruit ripening. Plant Physiol 163:1026–1036

Eum HL, Kim HB, Sang BC, Lee SK (2009) Regulation of ethylene biosynthesis by nitric oxide in tomato (*Solanum lycopersicum* L.) fruit harvested at different ripening stages. Eur Food Res Technol 228:331–338

Exposito-Rodriguez M, Borges AA, Borges-Perez A, Hernandez M, Perez JA (2007) Cloning and biochemical characterization of ToFZY, a tomato gene encoding a flavin monooxygenase involved in a tryptophan-dependent auxin biosynthesis pathway. J Plant Growth Regul 26:329–340

Exposito-Rodriguez M, Borges AA, Borges-Perez A, Perez JA (2008) Selection of internal control genes for quantitative real-time RT-PCR studies during tomato development process. BMC Plant Biol 8:131–142

Exposito-Rodriguez M, Borges AA, Borges-Perez A, Perez JA (2011) Gene structure and spatiotemporal expression profile of tomato genes encoding YUCCA-like flavin monooxygenases: the ToFZY gene family. Plant Physiol Bioch 49:782–791

Falasca G, Altamura MM (2003) Histological analysis of adventitious rooting in *Arabidopsis thaliana* (L.) Heynh seedlings. Plant Biosyst 137:265–273

Guoping C, Rachel H, David W, Andy T, Zhefeng L, Donald G (2004) Identification of a specific isoform of tomato lipoxygenase (TomloxC) involved in the generation of fatty acid-derived flavor compounds. Plant Physiol 136:2641–2651

Gutierrez L, Bussell JD, Pacurar DI, Schwambach J, Pacurar M, Bellini C (2009) Phenotypic plasticity of adventitious rooting in Arabidopsis is controlled by complex regulation of AUXIN RESPONSE FACTOR transcripts and MicroRNA abundance. Plant Cell 21:3119–3132

He W, Brumos J, Li H, Ji Y, Ke M, Gong X, Zeng Q, Li W, Zhang X, An F, Wen X, Li P, Chu J, Sun X, Yan C, Yan N, Xie DY, Raikhel N, Yang Z, Stepanova AN, Alonso JM, Guo H (2011) A small-molecule screen identifies L-kynurenine as a competitive inhibitor of TAA1/TAR activity in ethylene-directed auxin biosynthesis and root growth in Arabidopsis. Plant Cell 23:3944–3960

Hileman LC, Sundstrom JF, Litt A, Chen M, Shumba T, Irish VF (2006) Molecular and phylogenetic analyses of the MADS-box gene family in tomato. Mol Biol Evol 23:2245–2258

Honma T, Goto K (2001) Complexes of MADS-box proteins are sufficient to convert leaves into floral organs. Nature 409:525–529

Ivanchenko MG, Muday GK, Dubrovsky JG (2010) Ethylene-auxin interactions regulate lateral root initiation and emergence in Arabidopsis thaliana. Plant J 55:335–347

John I, Drake R, Farrell AW, Lee P, Horton P, Grierson D (2010) Delayed leaf senescence in ethylene-deficient ACC-oxidase antisense tomato plants—molecular and physiological analysis. Plant J 7:483–490

Kater MM, Dreni L, Colombo L (2006) Functional conservation of MADS-box factors controlling floral organ identity in rice and Arabidopsis. J Exp Bot 57:3433–3444

Kell DB (2011) Breeding crop plants with deep roots: their role in sustainable carbon, nutrient and water sequestration. Ann Bot 108:407–418

Koo SC, Bracko O, Park MS, Schwab R, Chun HJ, Park KM, Seo JS, Grbic V, Balasubramanian S, Schmid M, Godard F, Yun DJ, Lee SY, Cho MJ, Weigel D, Kim MC (2010) Control of lateral organ development and flowering time by the *Arabidopsis thaliana* MADS-box gene AGAMOUS-LIKE6. Plant J 62:807–816

Li A, Chen G, Yu X, Zhu Z, Zhang L, Zhou S, Hu Z (2019) The tomato MADS-box gene SlMBP9 negatively regulates lateral root formation and apical dominance by reducing auxin biosynthesis and transport. Plant Cell Rep 38:951–963

Liu W, Xu ZH, Luo D, Xue HW (2003) Roles of OsCKI1, a rice casein kinase I, in root development and plant hormone sensitivity. Plant J 36:189–202

Liu HJ, Wang SF, Yu XB, Yu J, He XW, Zhang SL, Shou HX, Wu P (2005) ARL1, a LOB-domain protein required for adventitious root formation in rice. Plant J 43:47–56

Liu W, Han X, Zhan G, Zhao Z, Feng Y, Wu C (2015) A novel sucrose-regulatory MADS-box transcription factor GmNMHC5 promotes root development and nodulation in soybean (*Glycine max* [L.] Merr.). Int J Mol Sci 16:20657–20673

Ludwig-Muller J, Vertocnik A, Town CD (2005) Analysis of indole-3-butyric acid-induced adventitious root formation on Arabidopsis stem segments. J Exp Bot 56:2095–2105

Mashiguchi K, Tanaka K, Sakai T, Sugawara S, Kawaide H, Natsume M, Hanada A, Yaeno T, Shirasu K, Yao H, McSteen P, Zhao YD, Hayashi K, Kamiya Y, Kasahara H (2011) The main auxin biosynthesis pathway in Arabidopsis. Proc Natl Acad Sci USA 108:18512–18517

Mignolli F, Mariotti L, Picciarelli P, Vidoz ML (2017) Differential auxin transport and accumulation in the stem base lead to profuse adventitious root primordia formation in the aerial roots (aer)

mutant of tomato (*Solanum lycopersicum* L.). J Plant Physiol 213:55–65
Muday GK, Rahman A, Binder BM (2012) Auxin and ethylene: collaborators or competitors? Trends Plant Sci 17:181–195
Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497
Naramoto S (2017) Polar transport in plants mediated by membrane transporters: focus on mechanisms of polar auxin transport. Curr Opin Plant Biol 40:8–14
Negi S, Sukumar P, Liu X, Cohen JD, Muday GK (2010) Genetic dissection of the role of ethylene in regulating auxin-dependent lateral and adventitious root formation in tomato. Plant J 61:3–15
Nishimura T, Hayashi K, Suzuki H, Gyohda A, Takaoka C, Sakaguchi Y, Matsumoto S, Kasahara H, Sakai T, Kato J, Kamiya Y, Koshiba T (2014) Yucasin is a potent inhibitor of YUCCA, a key enzyme in auxin biosynthesis. Plant J 77:352–366
Pacurar DI, Perrone I, Bellini C (2014) Auxin is a central player in the hormone cross-talks that control adventitious rooting. Physiol Plant 151:83–96
Pattison RJ, Catala C (2012) Evaluating auxin distribution in tomato (*Solanum lycopersicum*) through an analysis of the PIN and AUX/LAX gene families. Plant J 70:585–598
Pelaz S, Ditta GS, Baumann E, Wisman E, Yanofsky MF (2000) B and C floral organ identity functions require SEPALLATA MADS-box genes. Nature 405:200–203
Petricka JJ, Winter CM, Benfey PN (2012) Control of Arabidopsis root development. Annu Rev Plant Biol 63:563–590
Picton S, Barton SL, Bouzayen M, Hamilton AJ, Grierson D (1993) Altered fruit ripening and leaf senescence in tomatoes expressing an antisense ethylene-forming enzyme transgene. Plant J 3:469–481
Puri S, Thompson FB (2003) Relationship of water to adventitious rooting in stem cuttings of Populus species. Agrofor Syst 58:1–9
Ruzicka K, Ljung K, Vanneste S, Podhorská R, Beeckman T, Friml J, Benková E (2007) Ethylene regulates root growth through effects on auxin biosynthesis and transport-dependent auxin distribution. Plant Cell 19:2197–2212
Schaller GE, Binder BM (2017) Inhibitors of ethylene biosynthesis and signaling. Methods Mol Biol 1573:223–235
Shi Z, Jiang Y, Han X, Liu X, Cao R, Qi M, Xu T, Li T (2017) SlPIN1 regulates auxin efflux to affect flower abscission process. Sci Rep 7:14919–14931
Stepanova AN, Hoyt JM, Hamilton AA, Alonso JM (2005) A Link between ethylene and auxin uncovered by the characterization of two root-specific ethylene-insensitive mutants in Arabidopsis. Plant Cell 17:2230–2242
Stepanova AN, Jeonga Y, Likhacheva AV, Alonso JM (2007) Multilevel interactions between ethylene and auxin in Arabidopsis roots. Plant Cell 19:2169–2185
Stepanova AN, Robertson-Hoyt J, Yun J, Benavente LM, Xie DY, Doležal K, Schlereth A, Jürgens G, Alonso JM (2008) TAA1-mediated auxin biosynthesis is essential for hormone crosstalk and plant development. Cell 133:177–191
Strader LC, Beisner ER, Bonnie B (2009) Silver ions increase auxin efflux independently of effects on ethylene response. Plant Cell 21:3585–3590
Sun CH, Yu JQ, Wen LZ, Guo YH, Sun X, Hao YJ, Hu DG, Zheng CS (2017) Chrysanthemum MADS-box transcription factor CmANR1 modulates lateral root development via homo-/heterodimerization to influence auxin accumulation in Arabidopsis. Plant Sci. https://doi.org/10.1016/j.plantsci.2017.09.017
Sun CH, Yu JQ, Duan X, Wang JH, Zhang QY, Gu KD, Hu DG, Zheng CS (2018) The MADS transcription factor CmANR1 positively modulates root system development by directly regulating CmPIN2 in chrysanthemum. Hortic Res 5:52. https://doi.org/10.1038/s41438-018-0061-y
Swarup R, Perry P, Hagenbeek D, Van Der Straeten D, Beemster GT, Sandberg G, Bhalerao R, Ljung K, Bennett MJ (2007) Ethylene upregulates auxin biosynthesis in Arabidopsis seedlings to enhance inhibition of root cell elongation. Plant Cell 19:2186–2196
Tang W, Perry SE (2003) Binding site selection for the plant MADS domain protein AGL15: an in vitro and in vivo study. J Biol Chem 278:28154. https://doi.org/10.1074/jbc.M212976200
Verstraeten I, Schotte S, Geelen D (2014) Hypocotyl adventitious root organogenesis differs from lateral root development. Front Plant Sci 5:495–507
Vidoz ML, Loreti E, Mensuali A, Alpi A, Perata P (2010) Hormonal interplay during adventitious root formation in flooded tomato plants. Plant J 63:551–562
Vrebalov J, Ruezinsky D, Padmanabhan V, White R, Medrano D, Drake R, Schuch W, Giovannoni J (2002) A MADS-box gene necessary for fruit ripening at the tomato ripening-inhibitor (Rin) locus. Science 296:343–346
Xu M, Zhu L, Shou HX, Wu P (2005) A PIN1 family gene, OsPIN1, involved in auxin-dependent adventitious root emergence and tillering in rice. Plant Cell Physiol 46:1674–1681
Xuhu G, Guoping C, Naeem M, Xiaohui Y, Boyan T, Anzhou L, Zongli H (2017) The MADS-box gene SlMBP11 regulates plant architecture and affects reproductive development in tomato plants. Plant Sci 258:90–101
Yamauchi T, Abe F, Kawaguchi K, Oyanagi A, Nakazono M (2014) Adventitious roots of wheat seedlings that emerge in oxygen-deficient conditions have increased root diameters with highly developed lysigenous aerenchyma. Plant Signal Behav 9:28506–28509
Zhang HM, Forde BG (1998) An Arabidopsis MADS box gene that controls nutrient-induced changes in root architecture. Science 279:407–409
Zhuang X, Xu Y, Chong K, Lan L, Xue Y, Xu Z (2005) OsAGAP, an ARF-GAP from rice, regulates root development mediated by auxin in Arabidopsis. Plant, Cell Environ 28:147–156
Zhuang XL, Jiang JF, Li JH, Ma QB, Xu YY, Xue YB, Xu ZH, Chong K (2006) Over-expression of OsAGAP, an ARF-GAP, interferes with auxin influx, vesicle trafficking and root development. Plant J 48:581–591