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ORIGINAL ARTICLE

AsTal1 from *Aquilaria sinensis* regulates ABA signaling-mediated seed germination and root growth in *Nicotiana benthamiana*

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Abstract

Transaldolase, the key enzyme of the pentose-phosphate pathway, plays an important role in plant growth and defense. Seed germination is a key factor that influences the cultivation of *Aquilaria sinensis*, the plant source of agarwood, which is widely used as a traditional medicine, perfume and incenses. However, little is known about the function of transaldolase in abscisic acid (ABA) signaling-mediated seed germination. In the present study, the full-length *AsTal1* gene was isolated and characterized from *A. sinensis* calli. Sublocalization analysis indicated that AsTal1 was localized in the chloroplast. In addition, phenotypic analysis indicated that *AsTal1*-overexpressing *Nicotiana benthamiana* (OE) plants were less sensitive to ABA during seed germination and root growth than wild-type (WT) plants. Overexpression of *AsTal1* regulated the expression of genes involved in ABA metabolism, biosynthesis and signal transduction under ABA treatment. In addition, expression of *NbRbohA* and *NbRbohB* was inhibited in the overexpression lines, whereas the abundance and activities of the antioxidative enzymes were higher in the transgenic plants than in the WT lines after ABA treatment. Taken together, our results indicated that AsTal1 regulates ABA signaling-mediated seed germination and root growth by regulating the expression of genes involved in the ABA signaling pathway and the enzymes responsive to ROS.

Key message

AsTal1 isolated from *Aquilaria sinensis* regulates ABA response during seedgermination and root growth by regulating the expression of genes involved in theABA signaling pathway and the enzymes responsive to ROS in *Nicotiana benthamiana*.

Keywords *Aquilaria sinensis* · Transaldolase · ABA · Seed germination · Root growth

Abbreviations

ABA	Abscisic acid
NAA	Naphtha acetic acid
6-BA	6-Benzyladenim
2,4-D	Dichlorophenoxyacetic acid
KT	Kinetin
TAL	Transaldolase
OE	Overexpression lines
WT	Wild type
OPPP	Oxidative pentose-phosphate pathway
NADPH	Nicotinamide adenine dinucleotide phosphate
G6PDH	Glucose-6-phosphate dehydrogenase
ABI3	ABSCISIC ACID INSENSITIVE 3
ABI5	ABSCISIC ACID INSENSITIVE 5
ROS	Reactive oxygen species
MS	Murashige and Skoog
GFP	Green fluorescent protein
APX	Peroxidase
SOD	Superoxide dismutase
POD	Peroxidase
NCED	9-Cis-epoxycarotenoid dioxygenase

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Introduction

The oxidative pentose-phosphate pathway (OPPP) plays an essential role in carbohydrate metabolism, by both serving as a source of

nicotinamide adenine dinucleotide phosphate (NADPH) for biosynthesis and balance of reactive oxygen intermediates in plant cells and providing precursors for several main biosynthesis pathways (Hawkins et al. 2018; Yang et al. 2015). The OPPP comprises two separate branches: an oxidative branch, in which glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6-PGDH) generate ribulose 5-phosphate (Ru-5-P) and NADPH, and a nonoxidative branch, in which transketolase (TK) and transaldolase (TAL) convert Ru-5-P into xylulose 5-phosphate (Xul-5-P) and ribose-5-phosphate (Rib-5-P) (Caillau and Paul Quick 2005; Hawkins et al. 2018; Stincone et al. 2010). This suggests that the main regulatory steps of the OPPP are catalyzed by TAL (Caillau and Paul Quick 2005). TAL is ubiquitous in prokaryotes and eukaryotes and was first identified as the rate-limiting enzyme in yeast (Yang et al. 2015). In humans, TAL deficiency causes pathological disorders such as hydrops fetalis, liver dysfunction, and neonatal multiorgan disease (Michel et al. 2015; Perl et al. 2011). In plants, TAL appears to be involved in plant defenses and development. For instance, (*TAL*) abundance increased in both cucumber leaves and wheat leaves upon infection by fungi (Caillau and Paul Quick 2005). The expression level of (*TAL*) in potato also increased in response to wounding (Moehs et al. 1996). Yang et al. recently indicated that knockout of TAL in rice resulted in a dwarf phenotype, with narrow, short leaves and an altered vascular pattern (Yang et al. 2015). Zheng et al. reported that GSM2, a TAL from *Arabidopsis thaliana*, is involved in maintaining reactive oxygen species balance in response to glucose during seeding growth (Zheng et al. 2020).

ABA is a key phytohormone that participates in response to environmental stresses and plant growth, such as seed germination and dormancy, and root growth and development (Du et al. 2019; Fujita et al. 2011; Liu et al. 2015). The promoters of ABA-mediated genes contain conserved ABA-responsive elements (ABREs) with the core nucleotide sequence ACGT, which is the binding site for basic leucine zipper (bZIP) transcription factors (Hattori et al. 2002). In *Arabidopsis*, the transcription factors ABSCISIC ACID INSENSITIVE 3 (ABI3) and ABSCISIC ACID INSENSITIVE 5 (ABI5) play important roles in mediating the ABA signaling pathway during seed germination and dormancy (Hattori et al. 2002; Kashiwakura et al. 2016). Recent studies have indicated that the expression of *G6PDH*, the other important gene in the OPPP, can be induced by ABA, and G6PDH regulates seed germination through the ABA signaling pathway (Yang et al. 2019). However, the function of TAL in the ABA response is still poorly understood, despite the first TAL being identified in peas several decades ago.

Aquilaria sinensis is a tropical evergreen tree species that is distributed in Guangdong, Guangxi, Fujian and Hainan provinces in China and in other countries, including Indonesia, India, Vietnam, Malaysia and Thailand (Ding et al. 2020). It is one of the most important plant species for producing agarwood, and *A. sinensis* material has long been used as a sedative, digestive and anti-emetic drug in traditional medicine and as unique perfume and incense (Ding et al. 2020; Xu et al. 2020). Owing to the great demand and high economic value of agarwood, *Aquilaria* forests are facing severe destruction in nearly every country in which agarwood is commercially produced. Therefore, *A. sinensis* is listed in Appendix II of the *Convention on International Trade in Endangered Species of Wild Fauna and Flora*, and *Aquilaria* cultivation has attracted much attention in countries including China, Indonesia, India, Thailand and Malaysia (Wang et al. 2016; Xu et al. 2013). Seeding is currently the main source of cultivated *A. sinensis* sold on the market (Akhsan et al. 2015). The time of seed germination is important for seeding establishment and plant growth. Moreover, the germination ability of *A. sinensis* seeds is easily lost, and the regeneration ability is weak (Chen et al. 2016). Genetic and physiological analyses show that ABA promotes seed maturation and dormancy (Qu et al. 2020). As mentioned above, genes of the TAL family play an important role in plant resistance and plant growth, but little is known about the function of TAL family proteins in seed germination through the ABA signaling pathway. In this study, we isolated the full-length *cDNA* of *AsTal1* and characterized the function of *AsTal1* in seed germination and root growth through the ABA pathway. AsTal1 functions by scavenging reactive oxygen species (ROS) in response to ABA. These findings improve the understanding of the roles of TAL family members in plant development and provide valuable insights for further investigation of the mechanism underlying *A. sinensis* seed germination.

Materials and methods

Plant materials and growth conditions

Aquilaria sinensis calli from plant leaves were obtained as described in previous reports (Wang et al. 2016). The calli were subcultured on fresh Murashige and Skoog (MS) media comprising 4.44 μM 6-benzyladenin (6-BA), 10.74 μM naphthylacetic acid (NAA), 4.65 μM kinetin (KT), and 4.52 μM dichlorophenoxyacetic acid (2,4-D)

for 15 days and then cultured on media supplemented with 100 µM ABA, 200 mM NaCl, 750 mM mannitol, or 500 µM $CdCl_2$. For low-temperature treatment, the calli were cultured in a 4 °C environment. Calli from different treatment groups and the control group were harvested at different time points (0 h, 12 h, 24 h, 36 h, 48 h). Transgenic and wild-type *N. benthamiana* seeds were surface sterilized for 30 s in 75% ethanol, sterilized with 2% NaClO for 10 min, rinsed with sterile water four times, and then cultivated in 1/2 MS media supplemented with different concentrations of ABA in a growth chamber at 25 °C (14 h light/10 h dark).

RNA preparation and cDNA for *AsTal1* analysis

Total RNA was isolated from the samples of *A. sinensis* calli using Total RNA Purification Reagent (Norgen, Cat# 17200) according to the manufacturer's instructions. The quantity and integrity of the RNA samples were determined by a NanoDrop ND-2000 spectrophotometer and 1.2% agarose electrophoresis. Reverse-transcription reactions were performed with 1 µg of total RNA, 200 U of M-MLV transcriptase (Promega, Madison, WI, USA), dNTPs (0.2 mM each), 20 U of RNase inhibitor (Invitrogen) and 0.1 µM oligo dT (18) primer in a final volume of 30 µL. Reverse transcription was carried out at 42 °C for 90 min. The *AsTal1* and *AsTal2* cDNA sequence containing class-specific TAL conserved domains and nucleotide sequences was obtained from the *A. sinensis* callus transcriptomic dataset. To isolate two genes from *A. sinensis* calli, open reading frames (ORFs) were isolated via RT-PCR using specific primers (Table S1). The resulting DNA fragments were ligated into a pMD19-T vector, and three clones were sequenced in both directions. The ORFs of the cDNA sequence of *AsTal1* and *AsTal2* were predicted with the ORF finder online tool (http://www.ncbi.nlm.nih.gov/projects/gorf/). The amino acid alignments were performed by DNAMAN software, and phylogenetic trees were constructed using the neighbor-joining tree algorithm of the MEGA 6.0 program.

Subcellular localization

The ORF of *AsTal1* and *AsTal2* was isolated by PCR amplification using specific primers (Table S1). The fragments were fused to the N-terminus of the green fluorescent protein (GFP) gene in a pCAMBIA1300-35S-EGFP binary vector. The recombinant vector and control vector were transformed into *N.benthamiana* protoplasts for transient expression, and subcellular localization was observed via a Zeiss LSM700 laser scanning confocal microscope.

Quantitative real-time PCR (qRT-PCR) assays

qRT-PCR was performed on a CFX Connect™ Real-time System (Bio-Rad) using TransStart Tip Green qPCR SuperMix (Transgen) according to the manufacturer's protocol. The *A. sinensis* glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene and the *N. benthamiana β-actin* gene were used as internal controls to normalize RNA levels. Real-time PCR was initiated with 30 s of incubation at 94 °C, followed by 40 cycles of 94 °C for 5 s and 60 °C for 30 s. The primers used for qRT-PCR were generated by the Primer 5.0 program (Table S1), and the $2^{-\Delta\Delta CT}$ method was used to analyze the expression level of *AsTal1* gene. Three biological experiments and three experimental replicates were included to analyze gene expression. The primers used for qRT-PCR are listed in Table S1.

N. benthamiana transformation

To construct *AsTal1* overexpression vectors, the AsTal1 ORF was obtained by specific primers and subsequently subcloned into a pCAMBIA1300-35S binary vector to yield pCAMBIA1300-35S-AsTal1 vectors. The pCAMBIA1300-35S-AsTal1 plasmid was then transformed into wild-type *N. benthamiana* using the Agrobacterium-mediated leaf disc transformation method. Positive transgenic *N. benthamiana* plants were identified through RT-PCR. T3 seeds of three independent overexpression lines (OE) and wild type (WT) plants were used to analyze the function of the *AsTal1* gene.

Analysis of ROS

The concentration of total protein extracted from the roots of OE and WT lines was quantified with the BCA method. The H_2O_2 and O_2^- contents and the activities of antioxidant enzymes such as peroxidase (APX), superoxide dismutase (SOD), and peroxidase (POD) were measured using detection kits (Nanjing Jianchen Bioengineering Institute, China) according to the manufacturer's protocols.

Results

Identification and characterization of *AsTal1*

The *AsTal1* cDNA sequence had an ORF of 1 203 bp, encoding polypeptides 401 residues in length, with a calculated MW of 43.32 kDa. The largest *AsTal2* ORF was 1323 nucleotides, and its predicted translation products consisted of 441 amino acids, with calculated molecular mass (MW) of 47.63 kDa. A previous database showed that TAL is putatively encoded by two distinct genes in *Arabidopsis* (AtTal1 and AtTal2). Multiple alignments

indicated that the sequence of AsTal1 was 72.35% identical to that of AtTal1, while AsTal2 shared 77.49% identity with AtTal2. As shown in Fig. 1A, AsTal1 and AsTal2 contained a highly conserved site (KIPAT) (Caillau and Paul Quick 2005). A phylogenetic tree was constructed from a ClustalX multiple amino acid sequence alignment of plant TALs. The results indicated that TAL family proteins from plants existed as two putative isoforms (TAL types I and II) and that AsTal1 was classified as TAL type I, and AsTal2 was grouped into TAL type II (Fig. 1B) (Caillau and Paul Quick 2005).

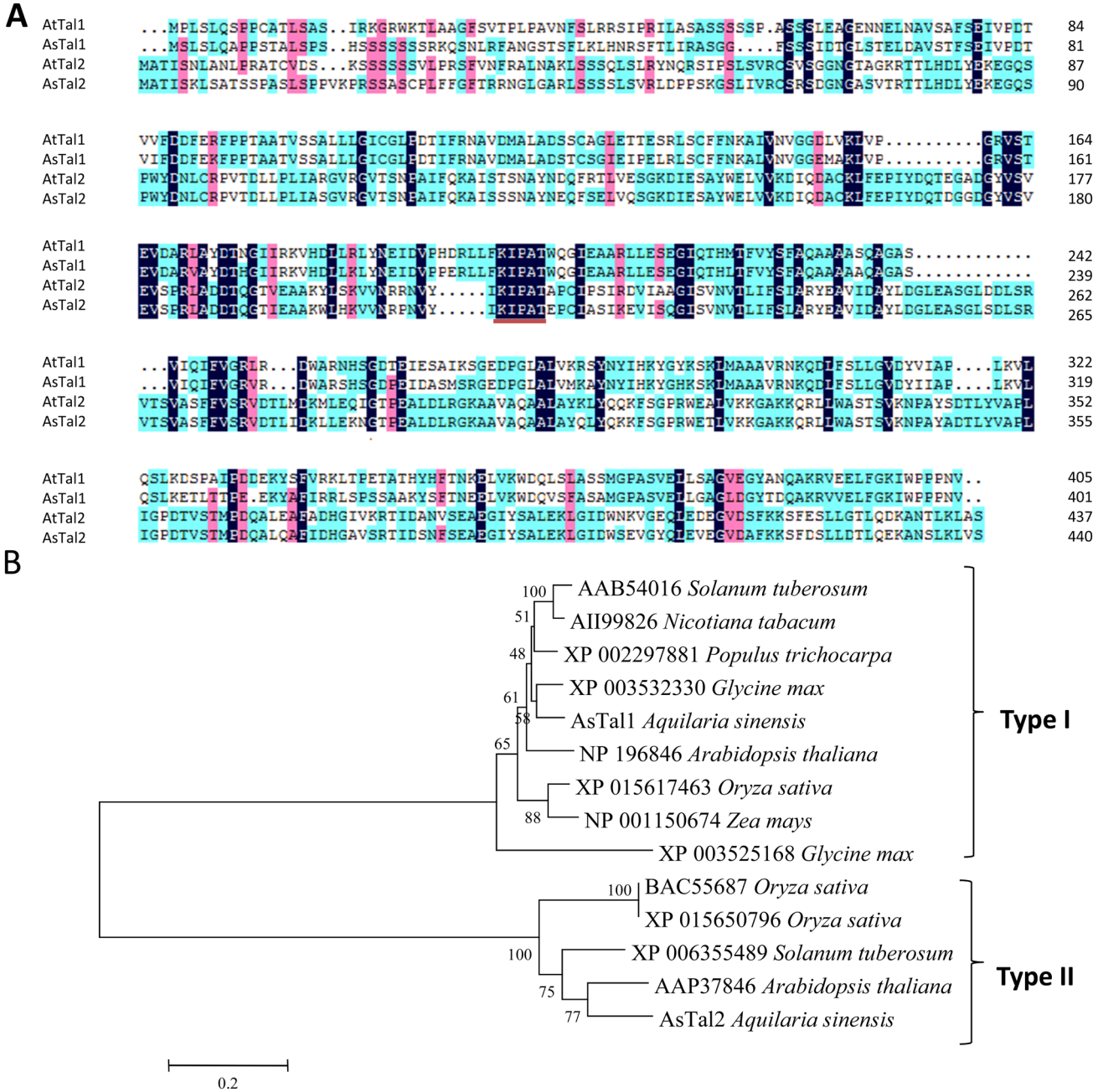

Fig. 1 AsTal1 and AsTal2 sequence analysis. **A** Multiple sequence alignment of AsTal1-2 with AtTal1 and AtTal2 protein. The alignment was made with DNAMAN program. *Black shading* indicates amino acid identities, *red* and *blue shading* indicates amino acid with different similarity. Red line indicates conserved site. **B** Phylogenetic analysis of AsTal1-2 and the TAL protein from other plants. Multiple sequence alignments of TAL sequences were performed using ClustalX, and the phylogenetic tree was constructed using MEGA6 with the neigbor-joining (NJ) method and 1000 bootstrap. The TAL proteins were classified into two groups (I, II). (Color figure online)

Subcellular localization of AsTal1

To determine the subcellular localization of AsTal1 and AsTal2, the coding sequence of AsTal1 and AsTal2 was cloned into a pCAMBIA1300-35S-EGFP vector. The recombinant vectors pCAMBIA1300-35S-AsTal1-EGFP, pCAMBIA1300-35S-AsTal2-EGFP and pCAMBIA1300-35S-EGFP were subsequently transformed into *N.benthamiana* protoplast. In protoplast which expressed GFP alone, the green florescence was distributed across the whole cell, whereas AsTal1:GFP predominantly localized in chloroplast and the signals of AsTal1:GFP co-localized with chlorophyll fluorescence (Fig.2). However, the florescence signals of AsTal2:GFP can not be observed. These results showed that AsTal1 was localized to chloroplast.

Expression profiles of AsTal1 in different tissues and in response to abiotic stresses

To determine the expression of *AsTal1* in different tissues, relative quantitative real-time PCR analysis was performed on total RNA from the roots, stems, leaves and shoot tips (Fig. 3A). The results showed that *AsTal1* was expressed in all of the tested tissues, with tissue-specific expression patterns. *AsTal1* expression was shown to be highest in the shoot tips, followed by the roots and stems (Fig.3A). To investigate the putative role of *AsTal1*, the transcript levels after exposure to ABA, salt, drought, cold temperature and heavy metal stress were measured. As shown in Fig. 3B, the expression of *AsTal1* was slightly induced by drought, cold temperature, salt stress and heavy metal stress, while the transcript level of *AsTal1* was markedly induced by ABA treatment.

Overexpression of *AsTal1* enhances the seed germination and root length of *N. benthamiana* under ABA treatment

Transgenic *N. benthamiana* plants overexpressing *AsTal1* were generated to investigate the functions of the *AsTal1* gene. Three homozygous transgenic lines were evaluated via RT-PCR and selected for functional analysis. To elucidate whether ABA sensitivity was influenced by AsTal1, seeds of OE and WT *N. benthamiana* were germinated on 1/2 MS media supplemented with different concentrations of ABA. As shown in Fig. 4A–B, the germination rates of the OE and WT lines were similar under control conditions. Seed germination was significantly inhibited for both OE and WT lines in the presence of 1 and 2 μM ABA, but the suppression of OE plant germination was much weaker than that of the WT. For instance, the germination of OE lines reached 85.5% compared with 63.6% for WT line after 5 days of treatment with 1 μM ABA, while 62.6% of the OE seeds compared with 36.7% of the WT seeds after 5 days of treatment with 2 μM ABA (Fig. 4B, C). To further confirm the transgenic phenotypes, the primary root length was measured. Under

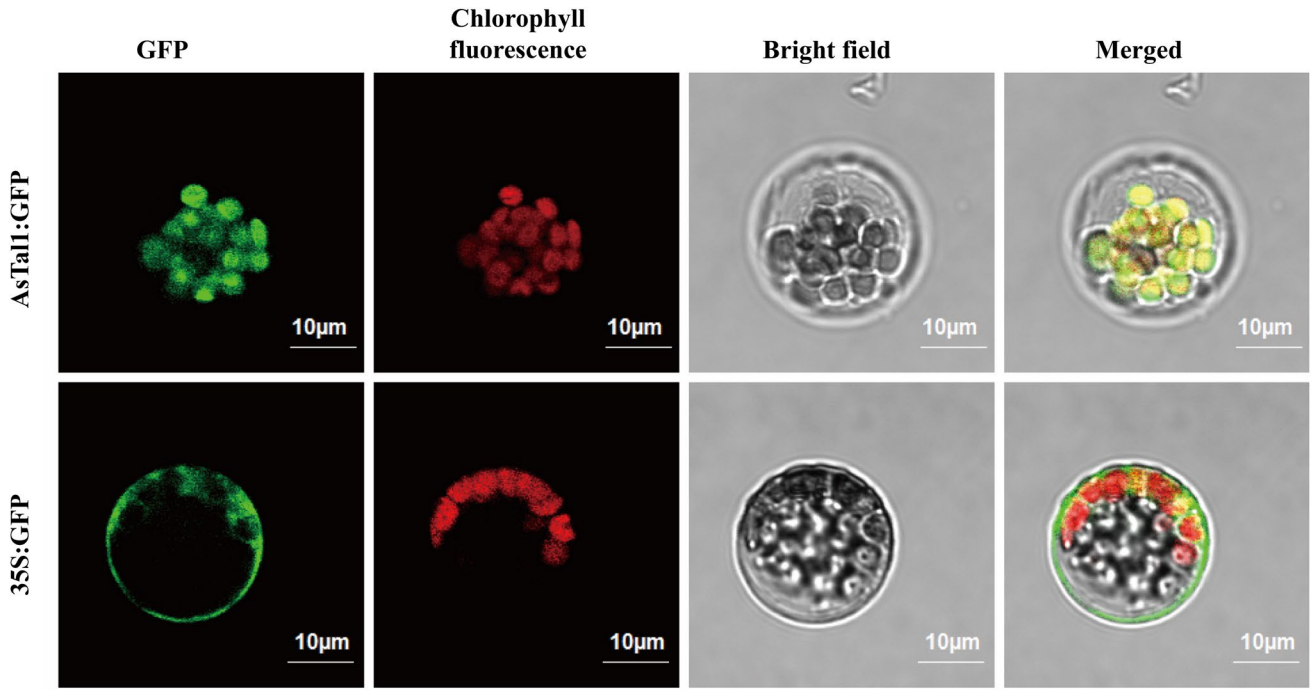

Fig. 2 Subcellular localization of AsTal1 in *N. benthamiana* protoplasts

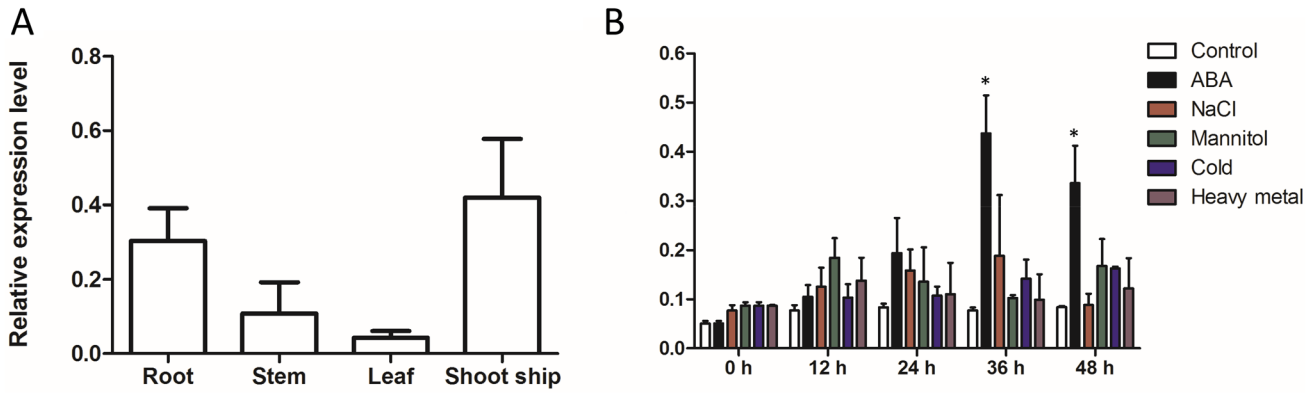

Fig. 3 Expression profiles of *AsTal1* in different tissues and under adverse stress treatments. qRT-PCR was performed with the total RNA which extracted from different tissues and calli treated with ABA, NaCl, mannitol, cold and $CdCl_2$. **A** Expression level of AsTal1 in different tissues; **B** Expression level of AsTal1 under ABA, NaCl, mannitol, cold and $CdCl_2$, respectively. The *GAPDH* gene was used as an internal control, and the experiment was repeated with at least three times. Values are means standard error ($n \geq 3$). Statistical significance was determined using Student's t test ($^*P < 0.05$)

normal growth conditions, no significant difference in primary root growth was detected between the WT and three OE lines (Fig. 4D, E). However, the primary root growth of the OE plants was significantly longer than that of the WT lines after treatment with 10 µM ABA (Fig. 4D, E), indicating that the OE lines were hyposensitive to ABA treatment.

AsTal1 affects genes involved in the ABA signaling pathway

To determine the function of AsTal1 in the ABA signaling pathway, we examined the expression of ABA signaling-related genes, including those involved in ABA biosynthesis, catabolism and signal transduction. The qRT-PCR results showed that the expression level of *9-cis-epoxycarotenoid dioxygenase* (*NCED*), which involved in ABA biosynthesis was significantly lower in the OE lines than that in the WT plants under ABA treatment (Fig. 5A). In contrast, the catabolism-related genes *8'-hydroxylase* (*CYP707A1* and *CYP707A2*) presented remarkably higher expression levels in the OE lines than in the WT plants under ABA treatment (Fig. 5B, C). Furthermore, our results indicated that the transcript levels of the ABA signaling-related transcription factors *ABI3* and *ABI5* significantly decreased in the OE lines after ABA treatment (Fig. 5D, E). These results suggested that *AsTal1* is involved in the regulation of ABA metabolism and signal transduction.

Overexpression of *AsTal1* reduces ROS levels under ABA treatment

Previous investigations showed that ABA causes ROS production and oxidative damage. To further determine the effects of AsTal1 on ABA responses, ROS accumulation was evaluated. Our results indicated that the H_2O_2 content in the OE lines was less than that in WT lines, and the O_2^- content significantly decreased in the OE lines under ABA treatment (Fig. 6A, B). ROS can be generated by amino oxidases, oxygen photoreduction and peroxidases, and the main ROS are produced by NADPH oxidases, which play an important role in primary root growth under different stresses. To investigate whether the function of *AsTal1* in response to ABA is involved in the NADPH oxidase pathway, we analyzed the expression of NADPH oxidase genes in OE and WT lines treated with ABA. As shown in Fig. 6C, D, the expression levels of *NbRbohA* and *NbRbohB* significantly increased after ABA treatment in the WT and OE lines. However, the *NbRbohA* and *NbRbohB* expression levels in the OE lines were markedly lower than those in the WT line under ABA treatment. These results suggested that AsTal1 is involved in NADPH oxidase-dependent ROS production. Antioxidant enzymes remove extra ROS to maintain the balance between ROS production and scavenging under different environmental stresses. Therefore, we measured the expression and activity of antioxidant enzymes in OE and WT lines with or without ABA treatment. ABA-induced transcript levels SOD and APX, and the activity of SOD, APX and POD in the OE lines were significantly higher than those in the WT plants (Fig. 6E–I). These results indicate that *AsTal1* could enhance the capacity to scavenge excess ROS by regulating the expression and activity of antioxidant enzymes.

Discussion

A. sinensis is the main plant species that produced agarwood used as traditional medicine, incense and perfume (Ding et al. 2020). Owing to the economic value and endangered nature of *A. sinensis* plants, cultivation of *A. sinensis* is

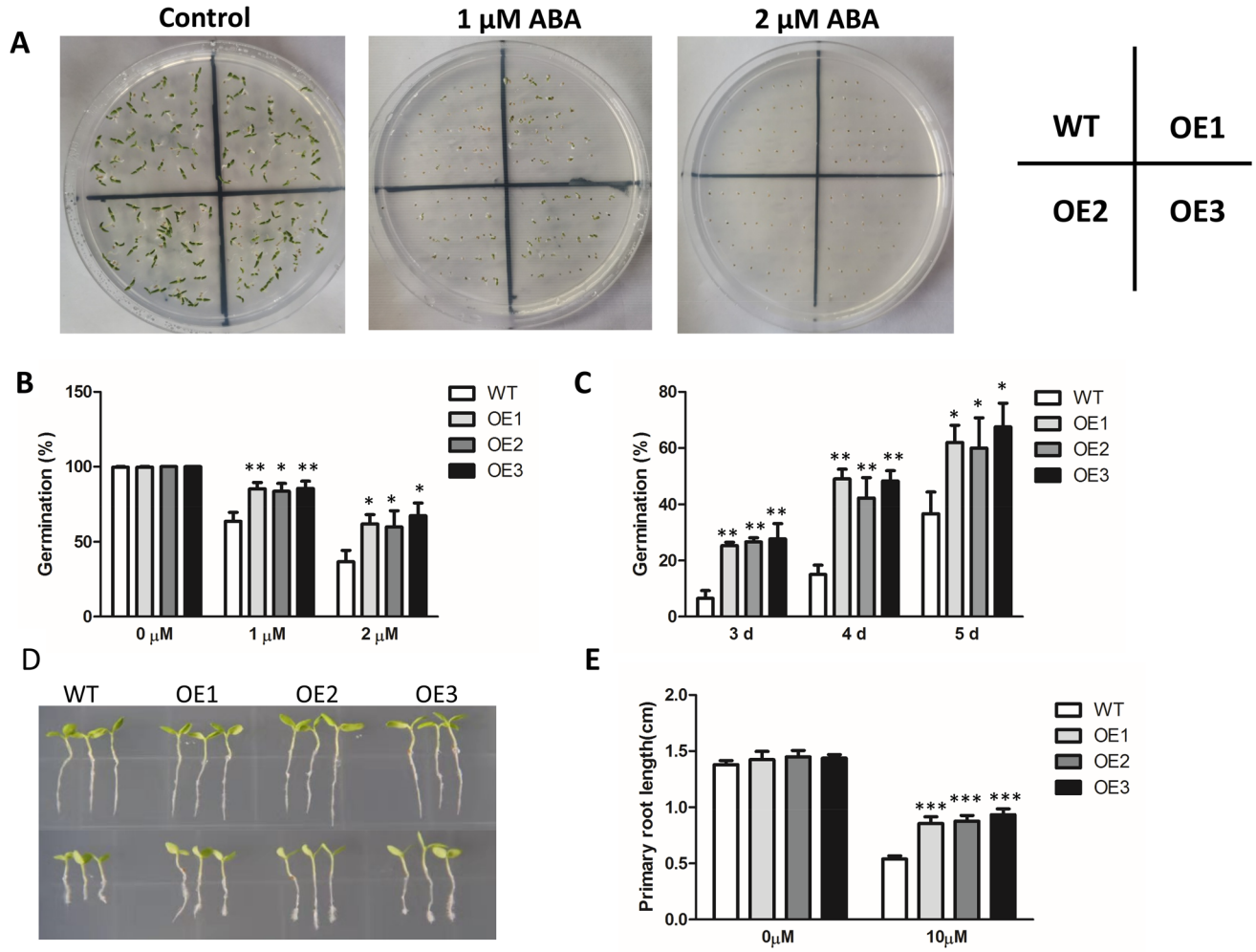

Fig. 4 Seed germination and root growth of wild type and AsTal1 overexpression lines in response to ABA. **A** Seeds were germinated on 1/2 MS plates with or without ABA. Photographs were taken at 5 day under ABA treatment. **B** Seed germination percentage with or without ABA treatment for 5 day. **C** Percentage of seed germination with 2 μM ABA for 3–5 day. **D** Four-day-old seedings were cultured vertically on 1/2 MS medium supplied with 10 μM ABA for 4 days. **E** The root length of WT and OE lines with or without ABA treatment at 4 day. Statistical significance was determined using Student's t test (*$P<0.05$, **$P<0.01$, ***$P<0.001$)

urgently needed. Seed germination is the vital factor that influences the cultivation of *A. sinensis* (Chen et al. 2016). Recent research has demonstrated that TALs also play vital roles in plant development. Here, we isolated a *TAL* gene, *AsTal1*, which belongs to the type I TAL family. Further experiments revealed that *AsTal1* is involved in the response to ABA during seed germination and root development. Compared with WT plants, *AsTal1*-overexpressing lines exhibited a higher seed germination rate and longer primary roots under ABA treatment.

Previous studies have shown that the expression or protein content of TALs could be induced after exposure to biotic stresses. For instance, the expression level of the *TAL* gene in cucumber leaves and the protein content of ToTal1 in potato were significantly induced by fungal infection (Caillau and Paul Quick 2005; Moehs et al. 1996). In this investigation, the transcript level of *AsTal1* was induced by abiotic stresses such as salt, drought, cold and heavy metal stress. Furthermore, the expression level of *AsTal1* was significantly induced by ABA treatment, suggesting that *AsTal1* functions in ABA signaling.

ABA is an important phytohormone that regulates seed dormancy and germination and root development (Chen et al. 2014; Zhong et al. 2015). G6PDH, the other important enzyme of the OPPP, is involved in ABA-inducible repressors of seed germination in the ABA response (Du et al. 2019). Our results first showed that AsTal1 is also involved in the response to ABA during seed germination and root growth. Previous investigations indicated that overexpression of AtNCED3 and LeNCED1 enhances endogenous ABA accumulation (Espasandin et al. 2014; Zhang et al. 2009). CYP707A family proteins, the key

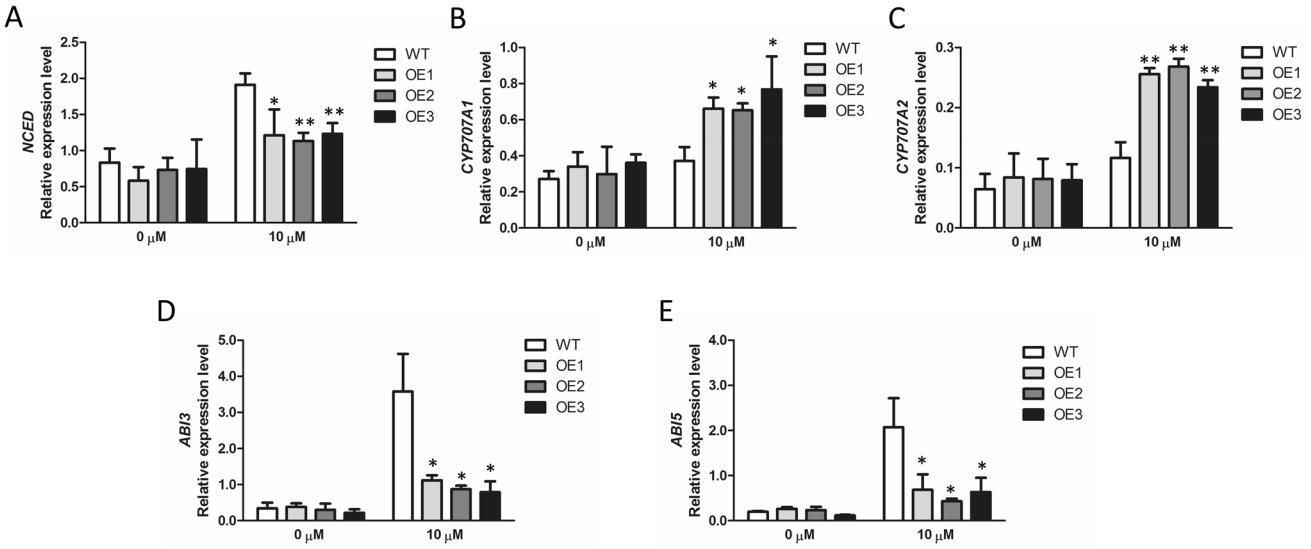

Fig. 5 Expression analysis ABA-associated genes in *AsTal1* OE lines and WT plants with or without ABA treatment. **A** Expression analysis of the gene involving ABA biosynthesis; **B**, **C** Transcript level of ABA catabolic genes *CYP707A1* and *CYP707A2*. **D**, **E** The abundance of ABA-associated genes. All the data were normalized to the actin gene transcript level. 4-day-old seedings were performed vertically on 1/2 MS medium supplemented with 10 μM ABA for 4 days. Mean values obtained from three independent experiments were shown in the histogram. Statistical significance was determined using Student's t test (*$P < 0.05$, **$P < 0.01$)

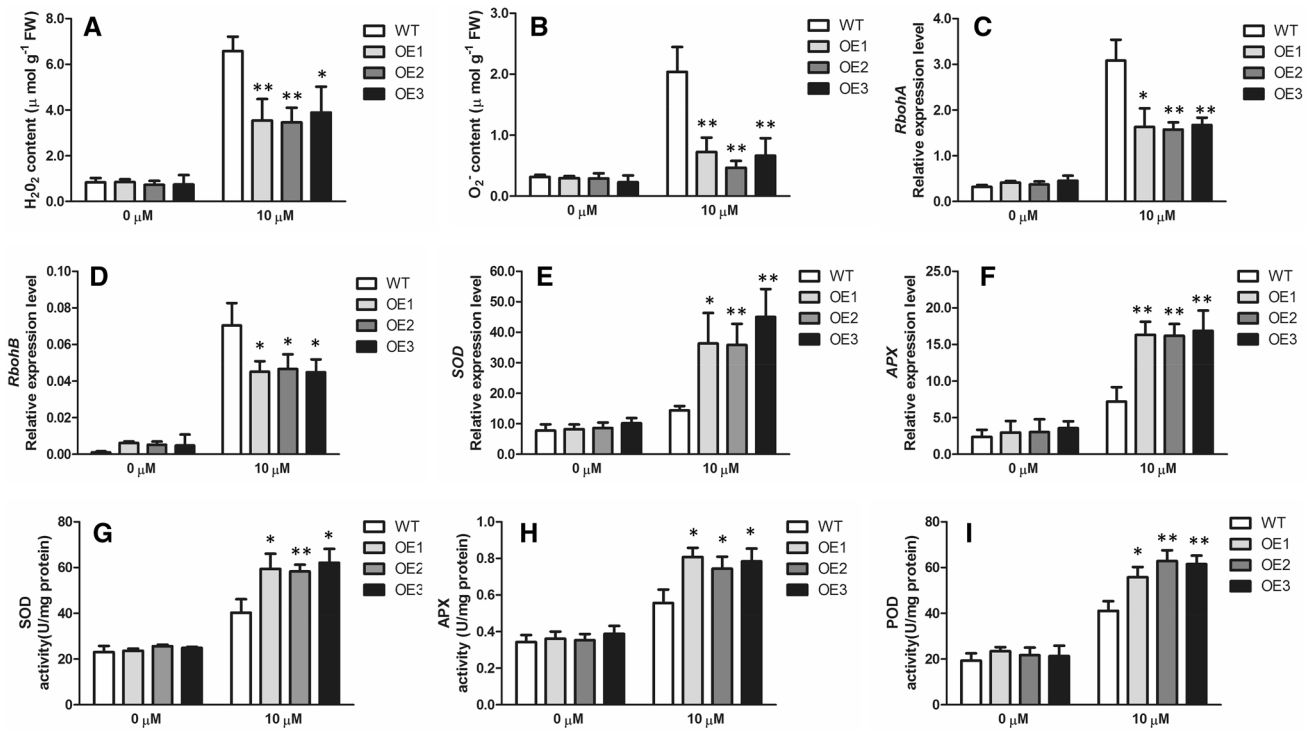

Fig. 6 *AsTal1* influences the ROS level, and the abundance and activities of ROS responsive enzymes with ABA treatment. **A**, **B** The content of H_2O_2 and O_2^- in the *AsTal1* OE and WT lines. **C**, **D** Expression profiles of *NbRbohA* and *NbRbohB*. **E**, **F** Expression analysis of *SOD* and *APX*. **G**–**I** Activities of enzyme SOD, APX and POD. 4-day-old seedings were performed vertically on 1/2 MS medium supplemented with 10 μM ABA for 3 days. Error bars represent in the standard deviation of three biological replicates. Statistical significance was determined using Student's t test (*$P < 0.05$, **$P < 0.01$)

enzymes involved in ABA catabolism, regulate ABA levels in plant cells (Kushiro et al. 2004; Todoroki and Ueno 2010). Our results indicated that overexpression of *AsTal1* could suppress the expression level of *NbNCED* and increase the transcript levels of *NbCYP707A1* and *NbCYP707A2*, suggesting that *AsTal1* is involved in the ABA response by regulating genes involved in ABA biosynthesis and metabolism. The transcription factors ABI3 and ABI5 play vital roles in mediating ABA signaling during seed germination and dormancy (Bi et al. 2017; Monke et al. 2004; Pan et al. 2018). The transcript levels of *ABI3* and *ABI5* were inhibited in the AsTal1-overexpressing lines under ABA treatment in the present study, suggesting that the seed germination in the *AsTal1*-overexpressing lines are mediated by ABA-responsive transcription factors.

Superoxide anion (O_2^-), hydroxyl radical (HO^-) and hydrogen peroxide (H_2O_2) radicals are produced at low concentrations in plant cells during plant growth and development (Mhamdi and Van Breusegem 2018; Zhou et al. 2020). It has been reported that exogenous ABA can induce ROS production in plant cells (Yan et al. 2007). Thus, we measured the influence of oxidative levels in the seeds and roots of *AsTal1* OE lines and WT lines under ABA treatment. Our results showed that the ROS content was lower in the OE lines than in the WT lines under ABA treatment. ROS produce NADPH oxidase, which plays a vital role in the ABA response in maize and in ABA-inhibited primary root growth in *Arabidopsis* (Kwak et al. 2003). Our investigation indicated that overexpressing *AsTal1* inhibited the expression levels of *NbrbohA* and *NbrbohB*. Due to the physicochemical toxicity of ROS, plants have a set of antioxidant enzymes, such as APX, SOD and POD, to scavenge excess ROS and maintain the balance between ROS production and scavenging (Ijaz et al. 2017; Xu et al. 2018). Therefore, we also measured the abundance and activities of antioxidant enzymes such as APX, SOD, and POD in *AsTal1* OE lines and WT lines under ABA treatment. The results suggested that AsTal1 overexpression could enhance antioxidant enzyme activities and increase expression levels to remove excess ROS under ABA treatment.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11240-021-02110-6.

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Author contributions YQR and TZL carried out the experiments and wrote the draft of the paper. XL and SPS participated in the preparation of the manuscript. XHW and PFT designed the research and supervised the work throughout. All the authors read and approved the final manuscript.

Declarations

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

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