IPA1 enhances rice drought tolerance mainly through activating the ABA pathway. It endows rice seedlings with a more developed root system, smaller leaf stomata aperture, and enhanced carbon metabolism.

IPA1 (IDEAL PLANT ARCHITECTURE 1)/OsSPL14 encodes a transcription factor and has been reported to function in both rice ideal plant architecture and biotic resistance. Here, with a pair of IPA1 and ipa1-NILs (Near Iso-genic Lines), we found that ipa1 could significantly improve rice drought tolerance at seedling stage. The ipa1 plants had a better-developed root system and smaller leaf stomatal aperture. Analysis of carbon–nitrogen metabolism-associated enzyme activity, gene expression, and metabolic profile indicated that ipa1 could tip the carbon–nitrogen metabolism balance towards an increased carbon metabolism pattern. In both the control and PEG-treated conditions, ABA content in the ipa1 seedlings was significantly higher than that in the IPA1 seedlings. Expression of the ABA biosynthesis genes was detected to be up-regulated, whereas the expression of ABA catabolism genes was down-regulated in the ipa1 seedlings. In addition, based on yeast one-hybrid assay and dual-luciferase assay, IPA1 was found to directly activate the promoter activity of OsHOX12, a transcription factor promoting ABA biosynthesis, and OsNAC52, a positive regulator of the ABA pathway. The expression of OsHOX12 and OsNAC52 was significantly up-regulated in the ipa1 plants. Combined with the previous studies, our results suggested that ipa1 could improve rice seedling drought tolerance mainly through activating the ABA pathway and that regulation of the ipa1-mediated ABA pathway will be an important strategy for improving drought resistance of rice.

Keywords Rice (Oryza sativa L.) · Drought tolerance · ipa1 · Carbon–nitrogen metabolism · Abscisic acid (ABA)

Introduction

Plants live in fixed locations and face diverse abiotic stresses (such as drought, salinity, and cold) negatively affecting plant growth and seed production. To survive, plants have evolved high plasticity and complex mechanisms to respond to these stimuli over a long period of time (Hu and Xiong 2014). The understanding of plant responses to stresses in physiology, genetics, and molecular biology will be greatly helpful in improving the tolerance of plants to abiotic stresses through genetic engineering (Huang et al. 2009).

Metabolic adaption to abiotic stress is important to plant surviving under unfavorable conditions (Barnaby et al. 2019; Ma et al. 2016). Generally, nitrogen promotes plant shoot growth rather than root, while carbon does oppositely, and high carbon/nitrogen (C/N) ratio enhances plant root development with a high root/shoot ratio (Osuna et al. 2015), which allows plant root access to water profoundly and decreases shoot water losses. Accumulation of carbohydrates resulting from enhanced photosynthesis protects plants from membrane damage and accounts, in part, for the more vigorous growth during stress (Garg et al. 2002). On the contrary, increasing nitrogen levels increased the degree of water stress, resulting in decreased leaf water potential, especially when the total water applied was minimal (Aragon...
and De Datta 1982). Moreover, high C/N status may act as a stress condition, which induces a series of stress-related genes, including transcription factors such as OsMYB4, CHS (encoding key enzyme in flavonoid biosynthesis) and genes involved in the jasmonate signaling pathway (Hwang et al. 2016).

Abscisic acid (ABA) is a multifunctional plant hormone that regulates many physiological processes, including seed dormancy and germination, stomatal movement, and plant responses to abiotic stress. NCED (9-cis-epoxy-carotenoid dioxygenase) is the key rate-limiting enzyme in ABA biosynthetic pathway. Overexpression of OsNCED3 in Arabidopsis results in increased accumulation of ABA, reduced relative water loss, delayed seed germination, and greater drought tolerance relative to that of wild-type (Hwang et al. 2010). Rice need3 mutants had increased sensitivity to water and H2O2 stress, increased stomata aperture, delayed leaf senescence, and decreased ABA content, while overexpression of OsNCED3 could enhance rice water stress tolerance, promote leaf senescence and increase ABA content (Huang et al. 2018). ABA 8'-hydroxylase is considered as the main ABA catabolic enzyme. OsABA8ox3 RNAi lines showed significant improvement in drought stress tolerance with increased ABA content. In contrast, overexpression seedlings were hypersensitive to drought stress with decreased ABA content, indicating OsABA8ox3 gene plays an important role in controlling ABA level and drought stress resistance in rice (Cai et al. 2015).

Stress response at the molecular level involves induction of stress-responsive and stress-tolerant genes. Many transcription factors have been identified to be involved in plant adaptation to abiotic stresses (Baillo et al. 2019). SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) family transcription factors sharing a highly conserved SQUAMOSA PROMOTER BINDING PROTEIN (SBP) domain are plant specific, and their functions are surprisingly diverse, covering virtually every aspect of plant growth and development and response to stresses (Wang and Wang 2015). In rice genome, about 19 SPL genes have been identified. Among these, as a multifunctional gene in regulating plant development, IPA1/OsSPL14 has attracted extensive attention. It was reported first in function in rice “Ideal Plant Architecture (IPA)” characterized by fewer unproductive tillers, larger panicles and stronger culms (Jiao et al. 2010; Miura et al. 2010). Since then, IPA1 was also identified to play a vital role in rice biotic resistance (Liu et al. 2019; Wang et al. 2018b). Overexpressing of IPA1 could enhance rice resistance to Xanthomonas oryzae pv. oryzae, partially through gibberellin signaling, including interacting with SLR1 and enhancing GA metabolism by activating EUI1 expression (Liu et al. 2019).

Although great progress has been made in understanding the roles of IPA1 in rice plant development and biotic resistance, its function in rice abiotic stress tolerance is still unknown. Here, using a pair of the IPA1- and ipa1-NILs, we found that ipa1 could significantly improve rice drought tolerance at the seedling stage mainly through activating ABA pathway.

**Materials and methods**

**Plant materials**

A pair of the ipa1 and IPA1-NILs was developed from a cross of an indica cv. Yuetai B (IPA1/IPA1) and a japonica cv. Shaoniejing (SNJ) (ipa1/ipa1). Yuetai B was crossed with SNJ to develop F1 plants; then, the F1 plants were backcrossed to Yuetai B to develop BC1F1 plants. The BC1F1 plants were self-crossed for six generations to develop F7 plants, from which a plant with a genotype IPA1/ipa1 was identified. Let this plant self-cross, and from its offspring, the plants with genotype IPA1/IPA1 were identified as a IPA1-NIL, while the plants with genotype ipa1/ipa1 as a ipa1-NIL. The degree of genomic similarity for the NILs is 95.8%. The ipa1 plants showed fewer tillers and leaves, but more panicle branches (Supplementary table 1), as reported previously (Jiao et al. 2010).

**Hydroponic culture conditions**

Seeds were disinfected in 20% sodium hypochlorite solution for 30 min, thoroughly washed with deionized water. Sterilized seeds were germinated in distilled water for 48–72 h at 30 °C in darkness, and then transferred to hydroponic culture solution as described previously (Wang et al. 2018c). Fresh solution was changed every 3 days, and pH was adjusted to 5.5 every day.

**Drought stress and osmotic stress experiments**

In soil drought experiments, two-leaf stage seedlings cultivated in sandy soil were treated with dehydration by removing water with PVC pipes for 7 days and then re-watered for 5 days. For PEG treatment, two-leaf stage seedlings were transferred to culture solution containing 25% (w/v) PEG4000 for 5 days, and then recovered for 4 days.

**Imaging of rice leaf stomata**

Imaging of rice leaf stomata was conducted as described previously (Wang et al. 2016) with some modifications. Leaves of 15 days seedlings under control and 6 h 25% PEG treatment conditions were immediately fixed by 2.5% glutaraldehyde, and stomatal pictures were obtained by scanning electron microscopy (JSM-6390LV, JEOL, Tokyo, Japan).
Carbon–nitrogen metabolism-associated indexes measurement

After cultured for 20, 28, 36, 44 and 52 days in nutrient solution under outdoor conditions, the leaves of IPA1 and ipa1 seedlings were collected for carbon–nitrogen metabolism-associated indexes measurement. Soluble sugar and sucrose contents were determined using the anthrone method (Shields and Burnett 1960) and the resorcinol method (Han et al. 2015), respectively. FBP (Fructose 1,6-bisphosphatase) activity was determined according to kit instructions (Solarbio, Beijing, China). PEPC (Phosphopyruvate carboxylase) activity was measured by the previously method (Blanke and Ebert 1992). SPS (Sucrose Phosphate Synthase) and SS (Sucrose Synthase) activities were measured as described previously (Shi et al. 2016).

Soluble protein and free amino acid contents were determined using the Bradford assay (Bradford 1976) and the ninhydrin method (Sun et al. 2006), respectively. Nitrate content was measured as described previously (Doane and Horwáth 2003). NR (Nitrate reductase), GS (Glutamine synthase), GOGAT (Glutamate synthase), and GDH (Glutamate dehydrogenase) activities were estimated based on the previous methods (Li et al. 2016).

Metabolomics analysis

The shoot bases of IPA1 and ipa1 seedlings grown for 21 days in nutrient solution under outdoor conditions were collected for metabolomics analysis. The sample preparation, extract analysis, metabolite identification and quantification were performed as described previously (Chen et al. 2014) at Wuhan Metware Biotechnology Co., Ltd., Wuhan, China.

Phytohormone measurement

Leaves of 15-day seedlings under control and 6 h 25% PEG treatment conditions were collected for phytohormone measurement. Plant materials were ground into powder in liquid nitrogen, and extracted with 80% methanol at 4 °C. The extract was centrifuged at 12,000xg under 4 °C for 15 min. The supernatant was collected and evaporated to dryness under nitrogen gas stream, and then reconstituted in 30% methanol. The solution was centrifuged, and the supernatant was collected for LC–MS analysis. The LC–MS analysis was conducted with the API6500 QTRAP LC/MS/MS system, equipped with an ESI Turbo Ion-Spray interface, operating in a positive ion mode and controlled by Analyst 1.6 software (AB Sciex).

RNA extraction and real-time PCR

The plants grew in culture room at 28 °C under a 16-h light/8-h dark photoperiod. After treated with 25% PEG for 0, 3, 6 and 9 h, leaves of 15 days seedlings were collected for RNA extraction. Total RNA was extracted with TRIzol (Invitrogen, Carlsbad, CA, USA) reagent. According to the manufacturer, RNA sample (~2 μg) was treated with DNaseI and then used for cDNA synthesis with the Super-Script III first-strand cDNA synthesis system (Invitrogen, Carlsbad, CA, USA). Real-time PCR was performed using 2 × SYBR Green PCR Master Mix (Takara, Dalian, China) in a CFX96TM Real-Time System (BIO-RAD, Hercules, China). Each experiment included three technical replicates and three biological replicates. OsActin was used as an internal control for normalization. Primers used are listed in Supplementary Table 2.

Dual-luciferase assays

The promoters of OsHOX12, OsNAC52, OsNCED1 and OsNCED3 were amplified by PCR from genomic DNA and cloned into pGreenII 0800-LUC reporter vectors in front of luciferase (LUC) gene. Besides, the Renilla luciferase (REN) reporter gene was driven by the CaMV 35S promoter as a control in each transformation. The coding regions of IPA1, OsHOX12 and OsTB1 were cloned behind the Ubi promoter into the effector vector pRGV (He et al. 2018). The reporter and effector were transformed into rice protoplasts. The luciferase activities were measured using the Dual-Luciferase Reporter Assay System (Promega, Beijing, China) and compared with empty vector-transformed plants.

Yeast one-hybrid assay

The coding regions of IPA1_SBp and OsHOX12 were amplified and cloned into the pGADT7 prey vector. The promoters of OsHOX12 and OsNAC52 were amplified and cloned into pAbAi bait vectors. The prey vectors, respectively, co-transformed with bait vector into Y1HGOLD strain. These transformed cells were grown on SD/-Leu/-Ura plates and then grown on SD/-Leu/-Ura/500 ng/ml AbA plates at 28 °C for 3–5 days.

Results

ipa1 enhances rice drought tolerance at seedling stage

To investigate the effect of ipa1 on rice drought tolerance, a soil drought experiment was performed with a pair of the IPA1 and ipa1-NILs. The ipa1-NIL obtained
a survival rate of 83.5%, while it was only 20.8% for the IPA1-NIL (Fig. 1a, c). As treated with 25% PEG4000 (osmotic stress-simulating drought stress), the ipa1 seedlings exhibited a survival rate of 62.1%, while the IPA1-NIL showed a significantly lower survival rate of 43.5% (Fig. 1b, d). These results indicated that ipa1 could significantly improve rice drought tolerance at seedling stage.

As compared to that of the IPA1 seedlings, the ipa1 seedlings had significantly increased root length, root and shoot dry weight as well as the dry weight ratio of root to shoot (Fig. 2a–e).

As for leaf stomata, the ipa1 seedlings showed a decreased stoma size as compared with that of the IPA1 seedlings (Fig. 2f, g), despite no significant difference in respectively. Each experiment has three replicates, and 24 (c) or 36 (d) seedlings were tested in each replicate. Data represent the means ± SE. *P < 0.05, t test. **P < 0.01, t test.
stoma density between the NILs (Supplementary Fig. 1). Moreover, the ipa1 plants displayed more stomata completely close (29.3%) and less stomata completely open (18.5%) than that of the IPA1-NIL (14.0% and 34.7%, respectively). When treated with PEG, more leaf stomata tended to close for both the IPA1 and ipa1 seedlings. Even so, there were still more stomata completely close (56.6%) and less stomata completely open (nearly 0.0%) for the ipa1 seedlings than that for the IPA1 seedlings (45.9% and 8.3%, respectively) (Fig. 2h). Consequently, the ipa1 seedlings were found to lose less water (Fig. 2i).

The ipa1 plants had a better-developed root system conducive to enhancing their ability to absorb water from the soil and their leaves with smaller stomatal aperture were beneficial to enhance their moisturizing function, which could play a vital physiological role in improving their drought tolerance.

**The ipa1 seedling could adjust its carbon–nitrogen metabolism balance to a metabolic pattern with a relatively strong carbon metabolism**

It was found that the ipa1 plants had a higher content for soluble sugar and sucrose than the IPA1 plants in both leaves (Fig. 3a, b) and sheaths (Supplementary Fig. 2). Then, we measured the activity of several carbon metabolism-related enzymes. Except for FBP, three carbon metabolism-related enzymes (PEPC, SPS, and SS) showed a activity level higher in the ipa1 plants than in the IPA1 plants (Fig. 3c–f).

As for the nitrogen metabolism, soluble protein and free amino acid contents for the ipa1 seedlings were significantly lower than that for the IPA1 plants (Fig. 4a, b). Meanwhile, the ipa1 plants were found to be significantly higher in inorganic nitrate–nitrogen content than the IPA1 plants (Fig. 4c). Four key nitrogen assimilation enzymes (NR, GS, GOGAT and GDH) were investigated and each of them showed a significantly lower activity level in the ipa1 plants than in the IPA1 plants (Fig. 4d–g). Accordingly, the expression of genes involved in nitrogen absorption, transport and assimilation was detected to be down-regulated, especially in the roots of the ipa1 plants (Supplementary Fig. 3).

Combined with the above findings, it seems that the ipa1 plant could change its carbon–nitrogen metabolism balance to a metabolic pattern with a relatively stronger carbon metabolism by enhancing its carbon metabolic activity and down-regulating its nitrogen metabolism, thus benefiting the accumulation of carbohydrates in the plant, which could

![Fig. 3 Carbon metabolism-associated indices of the IPA1 and ipa1 seedlings.](image-url)
provide a stronger material and energy basis for the plants to tolerate external abiotic stress.

**Metabolic profile analysis of the IPA1 and ipa1 plants**

To further explore the effect of ipa1 on plant metabolism, we analyzed the metabolic profiles with the IPA1/ipa1-NILs using a liquid chromatography-electrospray ionization-tandem mass spectrometry. There were 357 compounds to be identified. These compounds covered the key components involved in metabolic pathways of sugars, amino acids, nucleotide, organic acids, fatty acids and others (Supplementary Table 3).

The majority of carbohydrates such as sucrose, trehalose 6-phosphate (T6P), glucosamine and glucarate o-phosphoric acid were detected to accumulate more in the ipa1 plants than in the IPA1 plants, except glucose (Fig. 5a). For the key metabolites involved in nitrogen assimilation, the ipa1 plants showed a significantly decrease in the contents of amino acids Tyr and Trp (Fig. 5b) while their precursor shikimic acid mainly accumulated in the ipa1 plants (Fig. 5c). Similarly, the levels of organic acids (2-OG, succinic acid, and malic acid) and amino acids (Gln, Glu, Asn and Asp, as the major forms of nitrogen in xylem sap of rice plant) were significantly decreased in the ipa1 plants (Fig. 5b, c) while their precursor aconitic acid (a major element in TCA cycle) also showed to accumulate significantly in the ipa1 plants (Fig. 5c). These results indicated that the carbon flux to nitrogenous compounds was depressed in the ipa1 plants as compared to the IPA1 plants. Besides that, most of the other identified amino acids, amino acid derivates and nucleotides were decreased to different degrees in the ipa1 plants (Supplementary Fig. 4a, b). Therefore, all these results suggested that the gene ipa1 could significantly influence the balance of carbon–nitrogen metabolism, tipping the carbon/nitrogen metabolism balance towards increased carbon metabolism.

Cysteine is the first carbon/nitrogen-reduced sulfur product resulting from the sulfate assimilation pathway. As a sulfur donor, it plays a major role in the growth and development of plant. Glutathione derived from cysteine protects plants from reactive oxygen species (ROS) damage caused by abiotic stress (Droux 2004). In this study, a significantly increased cysteine content was found in the ipa1 plants, coupled with an increase of reduced glutathione content and a decrease of oxidized glutathione content (Fig. 5d). Moreover, contents of the other antioxidants such as coumarin and curcumin raised dramatically in the ipa1 plants (Supplementary Fig. 4c). The same situation also happened to glycerophospholipids (Supplementary Fig. 4d), the cell membrane major components. Therefore, ipa1 may activate sulfate assimilation and the related defense mechanism, which plays an essential role in protecting the ipa1 plants from ROS damage under abiotic stresses. In addition, ferulic
Acid is reported to be a marker metabolite for plant drought resistance and high photosynthesis (Ma et al. 2016). The contents of ferulic acid-related metabolites were significantly up-regulated in the ipa1 seedlings (Supplementary Fig. 4e).

The enhanced drought tolerance in the ipa1 plants could be mediated by ABA accumulation

ABA and GAs are known to be primary phytohormones that antagonistically regulate plant abiotic stress resistance (Vishal and Kumar 2018). In this study, exogenous ABA application led to an obvious inhibition on plant height, whereas GA3 treatment significantly promoted the trait for both the NILs under non-drought stress conditions (Supplementary Fig. 5). When PEG was applied to simulate drought conditions (osmotic stress), the ABA application significantly improved survival rates of seedlings, whereas GA3 decreased survival rates for both the NILs (Fig. 6a, b). Although so, the two NILs showed significant differences in degree of response to ABA and GA3 treatments. Comparatively, the ipa1 seedlings were less sensitive to ABA or GA3 treatment. Exogenous ABA treatment improved PEG resistance of the IPA1 plants to a greater extent, resulting in a fact that survival rate of the IPA1 plants was no longer different from that for the ipa1 plants (Fig. 6a, b).

Then, we measured ABA and GAs contents of the NILs. In both control and PEG-treated conditions, ABA content in the ipa1 seedlings was significantly higher than that in the IPA1 seedlings (Fig. 6c). Meanwhile, the ABA biosynthesis genes such as OsNCED1, OsNCED3, and OsNCED4 were detected to be up-regulated, whereas ABA catabolism genes were down-regulated in ipa1-NIL (Fig. 6e). OsABI5, OsLEA3, OsLIP9, and OsRAB16A are marker genes of the ABA pathway involved in abiotic stress response (Zhang et al. 2015). In our study, the expression of OsLEA3 (under PEG-treated condition) and the other marker genes (under both the control and PEG-treated conditions) was up-regulated significantly in the ipa1 plants (Fig. 6f).

As for GAs, with an exception of a remarkable increase of the GA4 content in ipa1-NIL under the control condition, no significant difference has been observed in the contents of GAs investigated between the two NILs under the control or PEG-treated conditions (Fig. 6d), although several genes for GA biosynthesis and catabolism showed some differences.
in expression levels between the two NILs (Supplementary Fig. 6).

The above results suggested that the enhanced drought tolerance of the ipa1-NIL could mainly result from a high level of ABA accumulation in the ipa1 seedlings.

**IPA1 directly activated the expression of OsHOX12 and OsNAC52**

OsHOX12 is a transcription factor homologous with Arabidopsis HOMEBOX PROTEIN 21 (HB21), HOMEBOX PROTEIN 40 (HB40) and HOMEBOX PROTEIN 53 (HB53). It was reported to activate expression of *OsNCED1*, and promote ABA biosynthesis in rice (Liu et al. 2020). OsNAC52, a transcription factor belonging to NAC family, potentially responds to ABA and confers drought tolerance in transgenic plants (Gao et al. 2010). In addition, as a transcription activator, IPA1 can regulate its target gene by directly binding to the core motif GTAC or indirectly to the core motif TGGGCC/T of the target gene promoter (Lu et al. 2013). Bioinformatics analysis identified twelve and three GTAC motifs in the promoters of *OsHOX12* and *OsNAC52*, respectively (Fig. 7a, b). We searched previously published ChIP-seq data of IPA1 (Lu et al. 2013), and found that *OsHOX12* and *OsNAC52* were potential targets of IPA1, suggesting that IPA1
may directly activate the expression of *OsHOX12* and *OsNAC52*.

To test the hypothesis, we conducted a yeast one-hybrid assay. Cells co-transformed with bait vectors and prey vectors grew well on SD/-Leu/-Ura/AbA plates, indicating that IPA1 can directly bind to the promoters of *OsHOX12* and *OsNAC52* (Fig. 7c, d). Then, we carried out a dual-luciferase assay using the full length of the *OsHOX12* and *OsNAC52* promoters in rice protoplasts. Co-transformed reporter vectors and effector vectors activated the expression of LUC gene, suggesting that IPA1 can significantly enhance the activity of the *OsHOX12* and *OsNAC52* promoters (Fig. 7e, f). Accordingly, the expression of *OsHOX12* was increased in the *ipa1* seedlings under PEG-treated conditions (Fig. 7h). Moreover, the expression of *OsNAC52* was also significantly up-regulated in the *ipa1* plants under both the control and PEG-treated conditions (Fig. 7i). In addition, we also found that *OsHOX12* could directly bind to the promoter of *OsNCED3* and activate its activity (Supplementary Fig. 7a–c). We also tested expression of the other genes involved in abiotic stresses in the NIL plants. *OsNAC5*, *OsNAC6*, and *OsNAC19* are three other NAC family transcription factors, and overexpression of each of those was reported to enhance rice resistance to abiotic stresses (Hu et al. 2006; Takasaki et al. 2010). The results depicted that these genes’ expression showed a significantly higher level
in the ipa1 plants than in the IPA1 plants under control and PEG-treated conditions (Fig. 7j–l).

**Discussion**

IPA1/OsSPL14 is one of the most concerned genes in current studies of rice functional genomics due to its multifunctions in regulating plant development (Wang et al. 2018a). In this study, we found that ipa1 could significantly improve rice drought tolerance at seedling stage. The ipa1 seedlings demonstrated a better-developed root system in terms of phenotypes, which helped to enhance their ability to absorb water from the soil. Their leaves with smaller stomatal aperture improved their moisturizing ability, which could play a crucial physiological role in enhancing their resistance to drought tolerance.

ABA is induced in response to adverse environmental conditions, and it plays a critical role in regulating abiotic stress response in plants (Cutler et al. 2010). Deficit of ABA in ncd3 mutants increased rice sensitivity to water stress, while accumulation of ABA in OsNCED3-overexpressing seedlings enhanced rice water stress tolerance (Huang et al. 2018). In this study, the ipa1 seedlings had a significantly higher ABA content than the IPA1 plants (Fig. 6c). The ABA pathway marker genes were up-regulated by ipa1 (Fig. 6f). Exogenous ABA treatment largely promoted PEG resistance for the IPA1 plants (Fig. 6a, b). These results seem to suggest that the improved drought tolerance might result from activation of the ABA pathway in the ipa1 seedlings.

OsHOX12 and OsNAC52 are two of the transcription factors involved in ABA pathway in rice (Gao et al. 2010; Liu et al. 2020). Our yeast one-hybrid assay and dual-luciferase test indicated that IPA1 can directly bind to the promoters of OsHOX12 and OsNAC52 and significantly enhance the activity of the OsHOX12 and OsNAC52 promoters (Fig. 7c–f). Therefore, IPA1 may directly activate OsHOX12, thus promoting ABA biosynthesis. Meanwhile, our study indicated that ipa1 could enhance the ABA pathway by directly regulating OsNAC52, which was reported to be a positive regulator of the ABA pathway (Gao et al. 2010). In addition, Gonzalez-Grandio et al. (2017) reported that the TCP (TEOSINTE BRANCHED1, CYCLOIDEA, PCF) transcription factor BRANCHED1 (BRC1) in Arabidopsis binds to and positively regulates the transcription of three related Homeodomain leucine zipper protein (HD-ZIP) encoding genes HB21, HB40 and HB53, which together with BRC1, enhances NCED3 expression, leading to ABA accumulation and triggering hormone response. In rice, Lu et al. (2013) revealed that IPA1 directly targets OsTB1 (an ortholog of BRC1). Here, we showed that OsTB1 was up-regulated significantly in the ipa1 seedlings, and that OsTB1 could activate the promoter activity of OsHOX12, OsNCED1 and OsNCED3 (Supplementary Fig. 7d–g). Therefore, we speculate that, as in Arabidopsis, IPA1 could also target OsTB1 to enhance the expression of the ABA biosynthesis genes, thus leading to ABA accumulation in ipa1 seedlings.

ABA is also a key regulator of plant stomatal aperture and root development. In response to drought stress, plants can synthesize ABA, which triggers closing of stomatal pores, thus reducing water loss (Schroeder et al. 2001). The foliage-derived ABA promoted root growth relative to shoot growth but inhibited the development of lateral roots (McAdam et al. 2016). Moreover, a very recent paper showed that moderate enhancement of ABA signaling helps maintain the RM (root meristem) size, sustaining root growth by antagonizing the GA-promoted degradation of OsSHR1 through the SnRK2-APC/CTE regulatory module, while mutants of OsABA1 (a ABA biosynthesis gene) displayed a short root phenotype (Lin et al. 2020). Therefore, the smaller stomatal and better-developed root system in ipa1 seedlings could result from the accumulation of ABA.

The ABA pathway also plays a vital role in regulating plant metabolism. The presence of ABA releases and activates the SNF1-RELATED KINASES2 (SnRK2s), which phosphorylate the downstream targets to induce ABA responses (Fujii et al. 2009; Nakashima et al. 2009). Overexpression of SnRK2.6 promotes plant carbon assimilation with drastically boosted sucrose and total soluble sugar levels in the leaves through increasing SPS activity (Zheng et al. 2010). SnRK2s-mediated phosphorylation of NRT1.1 was reported to be involved in the inhibitory effect of ABA on nitrate uptake in Arabidopsis (Su et al. 2021). In rice, the effect of ABA on plant metabolism was found to be concentration-dependent. Lower concentrations of ABA significantly stimulated the accumulation of sucrose and total soluble sugars, and increased SPS and SS activity, while higher concentrations of ABA exerted inhibitory effects on SPS and SS activities (Liang et al. 1996; Tang et al. 2009). In addition, exogenous ABA incubation decreased the GS and GDH activities, and soluble protein contents in rice leaves in a dose-dependent manner (Zakari et al. 2020). Our results indicated that ipa1 plants could adjust the balance of carbon–nitrogen metabolism by enhancing their carbon metabolic activity, but relatively down-regulating their nitrogen metabolism (Fig. 3, 4). Metabolic profile analysis further supported such a change of carbon–nitrogen metabolism in ipa1 plants (Fig. 5a, b). Thus, it seems that, for the ipa1 seedlings, tipping the carbon/nitrogen balance towards increased carbon metabolism might also, at least partially, be associated with the enhancement of ABA signaling, which contributes to their enhanced drought tolerance. Further detailed analyses are required to understand the interaction of ABA pathway and C/N metabolism balance in the ipa1 plants.
It should be noted that the results of the current study revealed the effect of the gene *ipa1* on drought tolerance of rice plants only at seedling stage. At this stage, *OsTB1* is the most important target of IPA1, and this target gene is mainly expressed at seedling stage (Lu et al. 2013). As rice plants develop into panicle differentiation stage, the main targets of IPA1 turn into genes such as *OsDEP1* (Lu et al. 2013). Accordingly, rice plants’ hormone regulation and metabolic pattern could be changed remarkably, which is worthy of further study in the future. In addition, the gene *ipa1* is one of the most essential yield-increasing genes reported in rice so far (Wang et al. 2018a; Yu et al. 2020). It increases rice yield mainly by shaping ideal plant type with fewer tillers and larger panicles (Jiao et al. 2010). In this study, the *ipa1* seedlings were observed to have a larger dry weight per plant (Fig. 2c, d), although their nitrogen metabolism was down-regulated relative to its WT seedlings. Obviously, the *ipa1* rice plants with larger biomass at the seedling stage would be more likely to develop larger panicles later, thus contributing to increased yields.

In conclusion, this study elucidated that *ipa1* could significantly improve rice drought tolerance at seedling stage. The *ipa1* plants had a better-developed root system and smaller leaf stomatal aperture. They could tip the carbon–nitrogen metabolism balance towards an increased carbon metabolism pattern. Meanwhile, the ABA biosynthesis genes were up-regulated, whereas the ABA catabolism genes were down-regulated in the *ipa1* seedlings, resulting in accumulation of endogenous ABA. Based on yeast one-hybrid assay and dual-luciferase assay, IPA1 was found to directly activate the expression of *OsHOX12* and *OsNAC52*, a transcription factor promoting ABA biosynthesis and a positive regulator of the ABA pathway, respectively. These results suggested that *ipa1* could improve rice seedling drought tolerance mainly through activating the ABA pathway, and that it may has potential to improve rice drought resistance.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00299-021-02804-3.

Acknowledgements The authors thank Dr. Q. Qian of the China National Rice Research Institute for providing the *ipa1* donor rice cv. Shaoniejing used in this particular research. This work was financed by the Special Transgenic Program of the Ministry of Agriculture in China (No. 2016ZX08001004-002), the National Natural Science Foundation of China (No.31901522), and the Collaborative Innovation Center of Hubei Province for Hybrid Rice.

Author contribution statement MZ and ZZ designed the experiments. MZ performed most experiments. MZ, YH and ZZ analyzed the data. MZ, AA, SX, ZH, SJ, JH, ZL and SL assisted in the materials and data collection. MZ, XH and ZZ drafted the manuscript.

Declarations

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

References

Aragon EL, De Datta SK (1982) Drought response of rice at different nitrogen levels using line sprinkler system. Irrig Sci 3:63–73

Baillo EH, Kimoto RN, Zhang Z, Xu P (2019) Transcription factors associated with abiotic and biotic stress tolerance and their potential for crops improvement. Genes (basel) 10:771–793

Barnaby JY, Rohila JS, Henry CG, Sicher RC, Reddy VR, McClung AM (2019) Physiological and metabolic responses of rice to reduced soil moisture: relationship of water stress tolerance and grain production. Int J Mol Sci 20:1846

Blanke MM, Ebert G (1992) Phosphoenolpyruvate carboxylase and carbon economy of apple seedlings. J Exp Bot 43:965–968

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Cai S, Jiang G, Ye N, Chu Z, Xu X, Zhang J, Zhu G (2015) A key ABA catabolic gene, *OsABA8ox3*, is involved in drought stress resistance in rice. PLoS ONE 10:e0116646

Chen W, Gao Y, Xie W, Gong L, Lu K, Wang W, Li Y, Liu X, Zhang H, Dong H, Zhang W, Zhang L, Yu S, Wang G, Jin X, Luo J (2014) Genome-wide association analyses provide genetic and biochemical insights into natural variation in rice metabolism. Nat Genet 46:714–721

Cutler SR, Rodriguez PL, Finkelstein RR, Abrams SR (2010) Abscisic acid: emergence of a core signaling network. Annu Rev Plant Biol 61:651–679

Doane TA, Horwáth WR (2003) Spectrophotometric determination of nitrate with a single reagent. Anal Lett 36:2713–2722

Droux M (2004) Sulfur assimilation and the role of sulfur in plant metabolism: a survey. Photosynth Res 79:331–348

Fujii H, Chinnusamy V, Rodrigues A, Rubio S, Antoni R, Park SY, Cutter SR, Sheen J, Rodriguez PL, Zhu JK (2009) In vitro reconstitution of an abscisic acid signalling pathway. Nature 462:660–664

Gao F, Xiong A, Peng R, Jin X, Xu J, Zhu B, Chen J, Yao Q (2010) *OsNAC52*, a rice NAC transcription factor, potentially responds to ABA and confers drought tolerance in transgenic rice. Plant Cell Tiss Organ Cult 100:255–262

Garg AK, Kim JK, Owens TG, Ranwala AP, Choi YD, Kochian LV, Wu RJ (2002) Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. Proc Natl Acad Sci USA 99:15898–15903

Gonzalez-Grandio E, Pajoro A, Franco-Zorrilla JM, Tarancón C, Immink RG, Cubas P (2017) Abscisic acid signaling is controlled by a *BRANCHED1/HD-ZIP I* cascade in *Arabidopsis* auxillary buds. Proc Natl Acad Sci USA 114(2):E245–E254

Han H, Tian Z, Fan Y, Cui Y, Cai J, Jiang D, Cao W, Dai T (2015) Water-deficit treatment followed by re-watering stimulates seminal root growth associated with hormone balance and photosynthesis in wheat (*Triticum aestivum L.*) seedlings. Plant Growth Regul 77:201–210

He F, Zhang F, Sun W, Ning Y, Wang GL (2018) A versatile vector toolkit for functional analysis of rice genes. Rice (NY) 11:27

Hu H, Xiong L (2014) Genetic engineering and breeding of drought-resistant crops. Annu Rev Plant Biol 65:715–741
Hu H, Dai M, Yao J, Xiao B, Li X, Zhang Q, Xiong L (2006) Over-expressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. Proc Natl Acad Sci USA 103:12987–12992

Huang XY, Chao DY, Gao JP, Zhu MZ, Shi M, Lin HK (2009) A previously unknown zinc finger protein, DST, regulates drought and salt tolerance in rice via stomatal aperture control. Genes Dev 23:1805–1817

Huang A, Sang Y, Sun W, Fu Y, Yang Z (2016) Transcriptional analysis of responses to imbalanced carbon: nitrogen availabilities in rice seedlings. PLoS ONE 11:e0165732

Huang Y, Guo Y, Liu Y, Zhang F, Wang H, Fang W, Li D, Mao D, Luan S, Liang M, Chen L (2018) 9-cis-epoxy-carotenoid dioxygenase 3 regulates plant growth and enhances multi-abi st stress tolerance in rice. Front Plant Sci 9:162

Hwang SG, Chen HC, Huang WY, Chu YC, Shih CT, Cheng WH (2010) Ectopic expression of rice OsNACD3 in Arabidopsis increases ABA level and alters leaf morphology. Plant Sci 178:12–22

Jiao Y, Wang Y, Xue D, Wang J, Yan M, Liu G, Dong G, Zeng D, Lu Z, Zhu X, Qian L, Li J (2010) Regulation of OsSPL14 by OsMri156 defines ideal plant architecture in rice. Nat Genet 42:541–544

Li H, Liang Z, Ding G, Shi L, Xu F, Cai H (2016) A natural light/dark cycle regulation of carbon-nitrogen metabolism and gene expression in rice shoots. Front Plant Sci 7:1318

Liang J, Cao X, Zhu Q (1996) Abscisic acid may involve in the regulation of grain filling in water stressed rice (Oryza sativa L.). Chin J Rice Sci 10:29–36

Lin Q, Zhang Z, Wu F, Feng M, Sun Y, Chen W, Zheng Z, Xue Y, Lei C, Zhu S, Wang J, Zhao Z, Guo X, Wang H, Han J (2020) The APCI(C)TE3 ubiquitin ligase complex mediates the antagonistic regulation of root growth and tillering byABA andGA. Plant Cell 32:1973–1987

Liu M, Shi Z, Zhang X, Wang M, Zhang L, Zheng K, Liu J, Hu X, Di C, Qian Y, He Z, Yang DL. (2019) Inducible overexpression of Ideal Plant Architecture1 improves both yield and disease resistance in rice. Nat Plants 5:389–400

Liu X, Hu Q, Yan J, Sun K, Liang Y, Jia M, Meng X, Fang S, Wang Y, Jing Y, Liu G, Wu D, Chu C, Smith SM, Chu J, Wang Y, Li J, Wang B (2020) ζ-carotene isomerase suppresses tillering in rice through the coordinated biosynthesis of strigolactone and abscisic acid. Mol Plant 13:1784–1801

Lu Z, Yu H, Xiong G, Wang J, Jiao Y, Liu G, Jing Y, Meng X, Hu X, Qian Q, Fu X, Wang Y, Li J (2013) Genome-wide binding analysis of the transcription activator IDEAL PLANT ARCHITECTURE1 reveals a complex network regulating rice plant architecture. Plant Cell 25:3743–3759

Ma X, Xia H, Liu Y, Wei H, Zheng X, Song C, Chen L, Liu H, Luo L (2016) Transcriptional and metabolomic studies disclose key metabolism pathways contributing to well-maintained photosynthesis under the drought and the consequent drought resistance in rice drought-resistant. Front Plant Sci 7:1886

McCadam SA, Brodribb TJ, Ross JJ (2016) Shoot-derived abscisic acid promotes root growth. Plant Cell Environ 39:652–659

Miura K, Ikeda M, Matsubara A, Song XJ, Ito M, Asano K, Matsuoka M, Kitano H, Ashikari M (2015) Control of seed germination and plant development by carbon and nitrogen availability. Front Plant Sci 6:1023

Osuna D, Prieto P, Aguilar M (2015) Control of seed germination and plant development by carbon and nitrogen availability. Front Plant Sci 6:1023

Schroeder JK, Kwak JM, Allen GJ (2001) Guard cell abscisic acid signalling and engineering drought hardness in plants. Nature 410:327–330

Shi H, Wang B, Yang P, Li Y, Miao F (2016) Differences in sugar accumulation and mobilization between sequential and non-sequential senescence wheat cultivars under natural and drought conditions. PLoS ONE 11:e0166155

Shields R, Burnett W (1960) Determination of protein-bound carbohydrate in serum by modified anthrone method. Anal Chem 32:885–886

Su H, Wang T, Ju C, Deng J, Zhang T, Li M, Tian H, Wang C (2021) Abscisic acid signaling negatively regulates nitrate uptake via phosphorylation of NRT1.1 by SnRK2s in Arabidopsis. J Integr Plant Biol 63:597–610

Sun S-W, Lin Y-C, Weng Y-M, Chen M-J (2006) Efficiency improvements on ninyhydrin method for amino acid quantification. J Food Compos Anal 19:112–117

Takasaki H, Maruyama K, Kidokoro S, Ito Y, Fujita Y, Shinozaki K, Yamaguchi-Shinozaki K, Nakashima K (2010) The abiotic stress-responsive NAC-type transcription factor OsNAC5 regulates stress-inducible genes and stress tolerance in rice. Mol Genet Genom 284:173–183

Tang T, Xie H, Wang Y, Lu B, Liang J (2009) The effect of sucrose and abscisic acid interaction on sucrose synthase and its relationship to grain filling of rice (Oryza sativa L.). J Exp Bot 60:2641–2652

Vishal B, Kumar PP (2018) Regulation of seed germination and abiotic stresses by gibberellins and abscisic acid. Front Plant Sci 9:838

Wang H, Wang H (2015) The miR156/SPL module, a regulatory hub and versatile toolbox, gears up crops for enhanced agronomic traits. Mol Plant 8:677–688

Wang L, Yu C, Xu S, Zhu Y, Huang W (2016) OsDi9-19-4 acts downstream of OsCDPK14 to positively regulate ABA response in rice. Plant Cell Environ 39:2740–2753

Wang B, Smith SM, Li J (2018a) Genetic regulation of shoot architecture. Annu Rev Plant Biol 69:437–468

Wang J, Zhou L, Shi H, Chern M, Yu H, Yi H, He M, Yin J, Zhu X, Li Y, Li W, Liu J, Wang J, Chen X, Qing H, Wang Y, Liu G, Wang W, Li P, Wu X, Zhu L, Zhou J-M, Ronald PC, Li S, Li J, Chen X (2018b) A single transcription factor promotes both yield and immunity in rice. Science 361:1026–1028

Wang Q, Nian J, Xie X, Yu H, Zhang J, Bai J, Dong G, Hu J, Bai B, Chen L, Xie Q, Feng J, Yang X, Peng J, Chen F, Qian L, Li J, Zuo J (2018c) Genetic variations in ARE1 mediate grain yield by modulating nitrogen utilization in rice. Nat Commun 9:735

Yu S, Ali J, Zhang C, Li Z, Zhang Q (2020) Genomic breeding of green super rice varieties and their deployment in Asia and Africa. Theor Appl Genet 133:1427–1442

Zakari SA, Asad M-A-U, Han Z, Zhao Q, Cheng F (2020) Relationship of nitrogen deficiency-induced leaf senescence with ROS generation and ABA concentration in rice leaf flag leaves. J Plant Growth Regul 39:1503–1517

Zhang DP, Zhou Y, Yin JF, Yan XJ, Lin S, Xu WF, Baluska F, Wang YP, Yuan H, Shields R, Shinozaki K, Yamaguchi-Shinozaki K, Nakashima K (2010) The abiotic stress-responsive NAC-type transcription factor OsNAC5 regulates stress-inducible genes and stress tolerance in rice. Mol Genet Genom 284:173–183

Zhu X, Qian Q, Li J (2010) Regulation of OsSPL14 by OsMri156 defines ideal plant architecture in rice. Nat Genet 42:541–544

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