Auxin signaling is closely associated with Zn-efficiency in rice (Oryza sativa L.)

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ABSTRACT
This study elucidates the involvement of auxin with Zn-efficiency (ZE) in Zn-efficient rice var. Pokkali. Pokkali showed no significant decrease in morpho-physiological features, electrolyte leakage and total soluble proteins due to Zn deficiency as compared with Zn-sufficient seedlings. However, auxin inhibitor under Zn deficiency severely affected these characteristics, suggesting that ZE is associated with auxin signaling in rice. Further, the expression of several genes (OsIRT1, OsZIP4 and OsZIP1), Zn chelation and phytochelatin in roots due to auxin inhibitor. It implies that auxin signaling may trigger Zn uptake, transport and chelation in rice seedlings to withstand Zn-deficiency. Further, significant reduction of major S-metabolites (cysteine, methionine, glutathione) and antioxidant enzymes (superoxide dismutase and glutathione reductase) along with increased H2O2 content, due to auxin inhibitor under Zn deficiency compared with controls. Taken together, these findings reveal that mechanisms associated with ZE in Pokkali are dependent on auxin signaling.

Introduction
Zn deficiency causes leaf bronzing, delayed maturity and yield loss in rice (Dobermann & Fairhurst 2000; Fageria et al. 2002). High pH and precipitation lead to Zn deficiency in soil (Kabir et al. 2014). Zn deficiency in crops causes malnutrition in children and birth problems in pregnant women (Prasad 2009; Graham et al. 2012). However, few plant species are able to withstand Zn deficiency, which is known as Zn-efficiency (ZE) by modulating biochemical and molecular mechanisms. Most of the mechanisms conferring Fe and Zn deficiency tolerance are common (Wissuwa et al. 2006; Höller et al. 2014). Zn homeostasis in plants is tightly regulated by Zn sensors and metal chelators involved in Zn acquisition and sequestration (Clemens 2001). In response to abiotic stresses, different signals get activated and transmitted between roots and shoots. Plants undergo complex signaling pathways for sensing nutrient status (Liu et al. 2009; Kabir et al. 2013). These deficiency signals can either originate in the root or shoot leading to both direct and local responses in other tissues through signal transmission. Hormone signaling plays crucial roles in regulating the response to variable mineral availability. Auxin is transported throughout the plant both locally and systemically. Indole-3-acetic acid (IAA), the predominant auxin in plants, plays a critical role in many growth and developmental processes (Teale et al. 2006). Furthermore, previous works revealed the mechanisms of IAA-mediated gene expression, signaling and transport in the model dicot Arabidopsis (Leyser 2002; Paponov et al. 2005). Cross-talk between auxin and Fe homeostasis suggests that auxin plays a role in the root morphology in response to Fe deficiency and Fe deficiency leads to increased auxin synthesis in Arabidopsis (Schmidt et al. 2000; Chen et al. 2010) and rice (Qi et al. 2012; Shen et al. 2015). Oxidative stress or increase in reactive oxygen species (ROS) is a consequence of several abiotic stresses. Auxin and ROS are rapidly altered by environmental stress factors as evidenced by the dual role of auxin in H2O2 production under stress (Tyburski et al. 2009). Further, enzymes having antioxidant properties are crucial for controlling radicals and peroxides at the cellular level and to protect plants from abiotic stresses (Rai 2009; Kabir et al. 2014). In a recent study, evidence demonstrated the cross-talk between Zn and auxin in Arabidopsis (Rai et al. 2015). However, a link between auxin and Zn deficiency has not yet been studied in rice.

Pokkali is a highly tolerant cultivar to Zn deficiency, and Zn deficiency signal is originated in roots before modulating the tolerance mechanisms in shoots (Na. et al. 2014; Naher 2015). However, information on what type of signal is involved and how it affects ZE mechanisms remains still unclear in rice. Therefore, we investigated whether auxin inhibitor affects Zn concentration, root-shoot development and chlorophyll concentration in Zn-efficient Pokkali. In addition, changes of biochemical activities and metabolites were studied upon triiodobenzoic acid (TIBA) treatment. We further studied the expression of several genes (OsIRT1, OsZIP4, OsZIP1 and OsDMAS1) associated with Zn efficiency in roots of Pokkali to elucidate if auxin drives ZE signal in rice seedlings.

Materials and methods

Plant materials and growth conditions
Seeds of Zn-efficient Pokkali (Oryza sativa L.) were surface sterilized in 70% ethanol and 5% sodium hypochlorite for 1 and 15 min, respectively. After germination, healthy and uniform seedlings were transferred to a solution culture...
samples were then kept at room temperature for 10 min. at 75°C for 10 min, followed by 109°C for 15 min. The ZnSO4 at two levels viz. 0.01 µM (Zn-deficient) and Zn concentrations in roots and leaves were determined in 1-week-old seedlings as previously described (Kabir et al. 2015). Briefly, harvested roots and shoots were washed with deionized water to remove surface contaminants and submerged in a vial containing 20 mL of deionized water. The samples were then incubated at 25°C on a shaker (3000g) for 2 h. Afterwards, electrical conductivity of the solution was measured with the meter.

**Enzymatic assays**

Superoxide dismutase (SOD) and glutathione reductase (GR) enzymes were extracted in roots of 1-week-old seedlings as previously described with slight modifications (Goud & Kac-hole 2012). Briefly, 100 mg root tissue was ground in 5 ml of phosphate buffer (100 mM). The homogenate was then centrifuged for 10 min before separating the supernatant in Eppendorf tubes. SOD assay mixture contained 50 mM sodium carbonate/bicarbonate buffer (pH 9.8), 0.1 mM EDTA, 0.6 mM epinephrine and enzyme (Sun et al. 1978). Once epinephrine is added, adrenochrome formation for 4 min was then read at 475 nm in a UV-Vis spectrophotometer (UV-1650PC, Shimadzu). Per unit SOD activity is expressed as the amount of enzyme required for 50% inhibition of epinephrine oxidation. Lastly, to 100 µL of the root extract was added the assay mixture containing 1 mL of 0.2 M phosphate buffer (pH 7.0) with 1 mM EDTA, 0.75 mL distilled water, 0.1 mL of 20 mM oxidized glutathione and 0.1 mL of 2 mM NADPH for GR analysis. Oxidation of NADPH by GR was then monitored at 340 nm. The GR activity rate (µmol min⁻¹ g⁻¹) was then calculated using the extinction coefficient of 6.12 mM⁻¹ cm⁻¹ (Halliwell & Foyer 1978). For hydrogen peroxide (H₂O₂) determination, tissues were homogenized in 0.1% trichloroacetic acid at 4°C. The samples were then centrifuged 10,000g for 15 min to separate the supernatant. The samples were then mixed with phosphate buffer (10 mM, pH 7.0) and potassium iodide (1 M) for 1 h. The absorbance of the solution mixture was recorded at 390 nm (Alexieva et al. 2001).

**Quantitative real-time polymerase chain reaction analysis**

Expression analysis of Actin (housekeeping gene; AY212324), OsIRTI (Zn transporter; AB070226.1), OsZIP4 (Zn transporter; AB126089.1), OsZIP1 (Zn transporter; AY302058.1) and OsDMAS1 (deoxyxymugenic acid synthase; AB269906.1) was performed by quantitative real-time polymerase chain reaction (PCR) in roots of Pokkali. Briefly, root tissues (50–100 mg) were ground with a mortar and pestle to a fine powder in liquid nitrogen. Afterwards, total RNA was isolated according to the protocol supplied by SV Total RNA Isolation Kit (Zymo Research). The isolated RNA was then treated with DNase I to remove any contaminating DNA. The quality and concentration of RNA were assessed using a Nanodrop spectrophotometer (Thermo Scientific). Reverse transcription was performed using a high capacity cDNA reverse transcription kit (Applied Biosystems) following the manufacturer’s protocol. The cDNA was then used as a template for quantitative PCR using a Bio-Rad CFX96 real-time PCR system with the appropriate primers and probe. The relative expression levels were calculated using the 2⁻³⁴⁰ equation and normalized to the expression of Actin as an internal control. The PCR products were also separated on a 1% agarose gel to verify the specificity and size of the amplified product.

**Measurement of electrolyte leakage**

Electrolyte leakage (EL) of roots and shoots was measured by an electrical conductivity meter (Lutts et al. 1996). Briefly, harvested roots and shoots were washed with deionized water to remove surface contaminants and submerged in a vial containing 20 mL of deionized water. The samples were then incubated at 25°C on a shaker (3000g) for 2 h. Afterwards, electrical conductivity of the solution was measured with the meter.

**Measurement of morphological growth parameters**

Seedlings grown under hydroponic conditions were harvested after 1 week. Root length and shoot height were measured using scale. Further, root tissues were washed in deionized water and quickly blotted in tissue paper. Both root and leaf tissues were then dried at 75°C for 3 days before measuring the dry weight with a digital balance.

**Determination of chlorophyll concentrations**

Leaf chlorophyll concentration (a and b) was determined using spectrophotometer on 1-week-old seedlings as previously described (Lichtenthaler & Wellburn 1985) with some modifications. Briefly, 100 mg leaf tissues were poured into a 5 mL falcon tube having 95% acetone. The tissues were then ground with the mortar–pestle, homogenates were centrifuged at 2500 rpm for 10 min. After centrifugation, the supernatant was placed in another falcon tube. The absorbance was then read at 662 (chlorophyll a) and 646 (chlorophyll b) on a spectrophotometer (UV-1650PC, Shimadzu) and chlorophyll concentrations were calculated.

**Analysis of Zn in tissues**

Zn concentrations in roots and leaves were determined in 1-week-old seedlings as previously described (Kabar et al. 2015). Briefly, harvested seedlings were washed with CaSO4 and deionized water before oven drying at 75°C for 3 days. Afterwards, 3 mL HNO3 was mixed with samples (1 g) and heated at 75°C for 10 min, followed by 109°C for 15 min. The samples were then kept at room temperature for 10 min. Then, 1 mL of H₂O₂ was added to each vessel through the ventilation hole and subsequently heated at 109°C for 15 min. The samples were then analyzed for Zn concentration by Flame atomic absorption spectroscopy outfitted with ASC-6100 auto sampler and air–acetylene atomization gas mixture system (AA-6800, Shimadzu). Standard solutions of Zn were separately prepared from their respective concentration of stock solutions (Shimadzu).

**Determination of total soluble protein**

Total soluble proteins were extracted from the roots with few modifications (Guy et al. 1992). Calibration curve of different concentrations of bovine serum albumin (BSA) was prepared before being mixed with 1 mL of Coomassie Brilliant Blue G 250. The root samples were homogenized with a chilled mortar and pestle using a buffer containing ice-cold 50 mM Tris-HCl, pH 7.5; 2 mM EDTA and 0.04% (v/v) 2-mercaptoethanol. The homogenate was centrifuged at 4000 rpm for 30 min at room temperature. Supernatant was then re-centrifuged for 20 min and transferred (100 µL) to cuvette containing 1 mL of Coomassie Brilliant Blue G 250. The absorbance was read at 595 nm and the concentration of total soluble protein was calculated using the calibration curve of BSA.
System (cat. no. Z3100), Promega Corporation, U.S.A. Integrity of isolated RNA was then checked by denaturing agarose gel electrophoresis and quantified by a NanoDrop 2000 UV-Vis Spectrophotometer. The first-strand cDNA was then synthesized by using GoScript™ Reverse Transcription System (Cat no. A5001), Promega Corporation. Before real-time analysis, the cDNA samples were treated with RNase-free, removing RNA contamination. Real-time PCR was performed using gene specific primers (Actin-fw: 5′-GAGCCTATCGACACATC-3′, Actin-rev: 5′-AACTCCAGAC ACATTGACCT-3′, OsZIP4-fw: 5′-AGGGTTGTCTCCCA GGTTCCT-3′, OsZIP4-rev: 5′-GGGCTTTAAATTTGCG TGAA-3′, OsIPTI-fw: 5′-CAAGATTTCAACCGCAGC A-3′, OsIPTI-rev: 5′-GGCTATCTGACAGCAGTGAA-3′), OsZIP1-fw: 5′-CAGCGGCGCAAGTTGACGACCAGCC GGA-3′, OsZIP1-rev: 5′-GAAGAAGTCTCAGGAACATGG TGGATGGAATC-3′, OsDMAS1-fw: 5′-CACGATGAGCG CGCGGCGCCAGGGCCG-3′, OsDMAS1-rev: 5′-TCATA TCTCGCCGCTCCATAAGTGTCGTC-3′) in triplicate using the Eco™ real-time PCR system (Illumina, U.S.A.) and GoTaq® qPCR Master Mix (Promega) programmed by Eco Software (v.0.7.0). Actin was used as an internal control for standardizing the amount of input cDNA template and as a reference to normalize the relative expression of target mRNA. Relative mRNA expression of target genes was calculated as \( \Delta \Delta CT \) in comparison to Zn-sufficient seedlings (Livak & Schmittgen 2001). The real-time PCR program used was as follows: 3 min at 95°C, 40 cycles of 30 s at 94°C, 15 s at 58°C and 30 s at 72°C.

**Analysis of plant metabolites**

Metabolites and IAA were analyzed in roots of 1-week-old seedlings by the high-performance liquid chromatography (HPLC) system (Binary Gradient HPLC System, Waters Corporation, Milford, MA, U.S.A.) with Empower2™ software as previously described with some modifications (Lindberg et al. 2007; Kabir et al. 2015). Briefly, samples were ground in a mortar–pestle using deionized water and were centrifuged at 1500g for 10 min before separating the supernatant in microcentrifuge tubes. The HPLC systems comprised a Waters 515 HPLC pump and Waters In-line degasser AF. For compound separation, a C18 reverse-phase-HPLC column (particle size: 5 μm, pore size: 300 A, pH range: 1.5–10, dimension: 250 mm × 10 mm) was attached. In mobile phase, buffer A (water and 0.1% trifluoroacetic acid (TFA)) and buffer B (80% acetonitrile and 0.1% TFA) were used at the gradient of 1–24 min 100% A, 25–34 min 100% B and 35–40 min 100% A. Both standards and samples were diluted (100×) and subsequently filtered using 0.22 µm Minisart Syringe Filters (Sartorius Stedim Biotech, Germany) before injection. Phytochelatin (PC) were then detected with a Waters 2489 dual absorbance detector (Waters Corporation, Milford, MA, U.S.A.) at 280 and 360 nm. Furthermore, the PC peak was detected with Thlaspi arvensis in comparison with the retention times (glycin-PCn). Glutathione (GSH)-equivalents of each PC were further used for PC quantification (Lindberg et al. 2007).

**Statistical analysis**

All experiments had at least three independent replicates for each sample following by completely randomized block design. Data were subjected to one-way ANOVA using the IBM SPSS package v. 20 (IBM Corp., New York, NY, U.S.A.) and means were compared with Duncan’s multiple range test at 5% significance level (P < .05). Further, graphical presentation was prepared using GraphPad Prism 6.

**Results**

Pokkali seedlings grown without TIBA treatment under Zn deficiency showed no significant changes in root length, root dry weight, shoot height, shoot dry weight and total chlorophyll (a and b) concentrations in comparison with the seedlings grown under Zn-sufficient conditions (Table 1 and Figure 1). However, the application of auxin inhibitor under Zn deficiency reduced significantly the above characteristics compared with the seedlings grown under Zn+ or Zn– conditions. In addition, supplementation of TIBA in Zn-sufficient seedlings ceased lateral root formation leading to significant reduction in root length and root dry weight but had no effect on shoot features compared with the seedlings grown in either Zn+ or Zn– conditions (Table 1).

Similarly, Zn concentrations in roots and shoots were not affected under Zn deficiency compared with Zn-sufficient seedlings in Pokkali seedlings (Figure 2). However, auxin inhibitor under Zn deficiency decreased significantly Zn concentrations in both roots and shoots compared with Zn+ and Zn– hydroponic seedlings. Similarly, total soluble protein showed a significant decrease in roots when seedlings were grown with auxin inhibitor under Zn deficiency compared with Zn+ and Zn– conditions (Figure 2). However, TIBA treated in Zn-sufficient plants showed no significant decrease in tissue Zn concentration and root-soluble protein compared with the seedlings treated without TIBA under Zn deficiency (Figure 2). Although EL was not increased due to Zn deficiency in Zn-efficient Pokkali, the application of auxin inhibitor treated under Zn-deficient showed a significant increase compared with control and Zn-deficient seedlings in both roots and shoots (Figure 2).

Activities of SOD and GR increased significantly due to Zn deficiency compared with Zn-sufficient seedlings. However, the application of TIBA on either Zn– or Zn+ seedlings showed an adverse effect on both enzymes and showed a significant decrease compared with Zn-deficient seedlings (Figure 3). However, the application of TIBA under Zn-sufficiency showed similar SOD and GR activities to those of controls (Zn+). Further, application of TIBA along with or without Zn stress caused a significant increase in H2O2 concentration in both roots and shoot compared with control and Zn-deficient seedlings (Figure 3).

**Table 1.** Effect of auxin inhibitor on morphological features of rice plants grown under hydroponic conditions.

| Features         | Zn+ (mg) | Zn– (mg) | Zn– TIBA (mg) | Zn+ TIBA (mg) |
|------------------|---------|---------|--------------|--------------|
| Root length (cm) | 8.3 ± 0.95 | 8.0 ± 0.45 | 4.3 ± 0.32 | 6.5 ± 0.61 |
| Root dry weight  | 4.8 ± 0.37 | 4.0 ± 1.13 | 1.7 ± 0.46 | 2.7 ± 0.07 |
| Shoot height (cm)| 14.4 ± 1.81 | 13.3 ± 0.52 | 5.2 ± 1.17 | 12.8 ± 1.05 |
| Shoot dry weight | 11.6 ± 0.50 | 10.3 ± 1.72 | 5.5 ± 0.61 | 9.5 ± 1.28 |
| Chlorophyll concentrations (a + b) in leaves (mg/g FW)| 6.4 ± 0.25 | 6.2 ± 0.53 | 2.3 ± 0.20 | 5.7 ± 0.36 |

Note: Different letters indicate significant differences between means ± SD of treatments (n = 3). Data were taken on 1-week-old plants.
Under Zn-sufficient conditions, expression of OsIRT1, OsZIP4 and OsZIP1 transcripts showed similar expression patterns (Figure 4). However, these three genes were significantly upregulated under Zn deficiency compared with Zn-sufficient seedlings. Interestingly, expression of the genes decreased significantly when seedlings were treated with auxin inhibitor under Zn deficiency compared with Zn-deficient seedlings. In addition, expression of OsDMAS1 gene increased significantly due to Zn deficiency compared with Zn-sufficient seedlings. Auxin inhibitor caused a significant decrease in OsDMAS1 expression under Zn deficiency compared with Zn-deficient seedlings. Seedlings treated with TIBA under Zn-sufficiency showed similar expression for OsDMAS1 to that of controls (Zn+) and seedlings grown under Zn-sufficiency along with TIBA supplementation (Figure 4).

All three S-metabolites (cysteine, methionine and glutathione) and proline increased significantly in roots of Pokkali under Zn deficiency compared with Zn-sufficient seedlings (Table 2). Furthermore, the application of auxin inhibitor caused a significant decrease in all metabolites excluding proline compared with Zn-deficient seedlings. PC showed a non-significant increase in concentration when seedlings were grown under Zn deficiency compared with Zn-sufficient plants seedlings. Similarly, auxin inhibitor treated under Zn-deficiency caused a significant decrease in PC concentration compared with the seedlings grown in either Zn+ or Zn− conditions (Table 2). Zn deficiency caused a significant increase in IAA concentration in both roots and shoots compared with the seedlings grown under Zn-sufficient conditions. Auxin inhibitor in either Zn-deficient or Zn-sufficient seedlings caused a significant decrease in IAA compared with the seedlings grown under Zn+ or Zn− conditions. However, the application of TIBA showed a significant increase in shoot IAA concentration compared with the seedlings grown under Zn+ or Zn− conditions (Table 2).

**Discussion**

Auxin plays important roles in plant development and contributes to protective mechanisms in responses to abiotic stresses. Although few reports provide some clues on the involvement of auxin signaling in stress responses (Song et al. 2009; Kabir et al. 2013), the exact mechanism of auxin-mediated stress responses remains to be elucidated. Though Zn-efficient Pokkali showed no significant reduction in growth parameters other than slight leaf bronzing under Zn deficiency, these parameters were affected significantly due to TIBA application. Being consistent with these morpho-physiological features, tissue (roots and shoots) Zn concentration and shoot total protein also decreased due to auxin inhibitor treated under Zn deficiency in comparison with Zn-sufficient and Zn-deficient seedlings. However, auxin inhibitor applied under Zn-sufficiency caused reduced lateral root formation and significant decrease in root growth parameters, suggesting that endogenous auxin is required for the normal growth and development of root in rice. In a previous study, polar auxin inhibitor inhibited rice seminal roots and auxin regulated cell division by controlling the expression
of various cyclin genes in rice root tips (Guo et al. 2007). HPLC analysis showed decreased IAA level in roots due to TIBA application on both Zn-sufficient and Zn-deficient seedlings compared with either Zn-sufficient or Zn-deficient seedlings. It does confirm that TIBA ceased polar transport of auxin from shoot to root and thus affected root IAA concentration regardless of the Zn conditions. The increase in IAA in the shoot due to TIBA treatment further confirms that root IAA biosynthesis is not affected by Zn deficiency and/or TIBA treatment. To maintain optimal concentrations of IAA and IAA derivatives in plant tissues, auxin homeostasis is regulated by degradation, conjugation to amino acids and transport (Lindberg et al. 2007).

In this present study, SOD and GR activities notably increased in roots due to Zn deficiency in Pokkali, suggesting that scavenging ROS are associated with the active involvement with these enzymes. Increased GR activity due to Zn shortage may activate both antioxidant enzyme activities and the ASC–GSH cycle to utilize antioxidant metabolites. Further, changes in Zn-dependent regulation of key Zn requiring enzymes could play a role in ZE in Pokkali. Similarly, variations in antioxidant activities (SOD, Guaiacol peroxidase and GR) associated with differential ZE were reported in contrasting rice genotypes (Frei et al. 2010). However, a significant reduction in SOD and GR activities due to TIBA under Zn deficiency indicated crosslink between auxin signaling and elevated antioxidant activities in roots of Pokkali. To withstand abiotic stresses, plants adapt antioxidant defense for restoring redox metabolism and cellular turgor (Subba et al. 2014; Kabir et al. 2015). Our results also indicated that auxin might be helpful for keeping SOD balance in plants. The decrease in antioxidant activities is consistent with the increase in H$_2$O$_2$ observed in both roots and shoots of Pokkali following auxin treatment under Zn-deficiency. It does confirm that elimination of auxin signal affects the ROS scavenging mechanism of Zn-efficient Pokkali seedlings.

To confirm the role of auxin at the molecular level, we performed a real-time PCR analysis on Zn transporters (OsIRT1, OsZIP4 and OsZIP1). In the present study, these Zn transporters were significantly upregulated due to Zn deficiency compared with Zn-sufficient seedlings, and thus flagged an interaction of Zn transporters genes to withstand Zn deficiency in Zn-efficient Pokkali. Interestingly, blocking of auxin signal showed significant downregulation of OsIRT1, OsZIP4 and OsZIP1 in roots under Zn deficiency suggesting the involvement of auxin in Zn uptake and transport in rice under Zn-deficiency. Further, the OsDMAS1 gene showed significant upregulation under Zn-deficiency compared with Zn-sufficient seedlings, while this gene was not induced in roots due to TIBA treatment under Zn deficiency. HPLC analysis showed a similar pattern in PC synthesis in roots suggesting that higher accumulation of deoxymugeneic acid and PC is closely linked with ZE in Pokkali. As a result, blocking auxin signal may inhibit higher Zn chelation when Zn is limited.

Elevated glutathione, methionine, cysteine and proline contents in roots of Pokkali under Zn-deficiency indicated that a higher accumulation of these metabolites can trigger ZE and, in particular, can function as an antioxidant compound to protect cells from Zn-deficiency-induced oxidative injury. Further, blocking of auxin signal under Zn deficiency caused a significant decrease in these metabolites in root tissue, suggesting that auxin signaling is closely linked with the antioxidant defense underlying ZE in rice seedlings. However, regulation of antioxidant defense appeared to be a major point of regulation of the auxin pathway, while emerging

**Table 2.** Changes of metabolites (µg g$^{-1}$ FW) in roots of rice plants grown under different growth conditions.

| Metabolites | Zn$^+$ | Zn$^-$ | Zn$^-$ TIBA$^+$ | Zn$^+$ TIBA$^+$ |
|-------------|--------|--------|----------------|----------------|
| Cysteine    | 2.11 ± 0.64$^a$ | 24.2 ± 2.02$^b$ | 1.16 ± 0.14$^c$ | 2.0 ± 0.10$^d$ |
| Methionine  | 3.33 ± 0.35$^a$ | 5.24 ± 0.81$^b$ | 1.83 ± 0.28$^c$ | 3.1 ± 0.21$^d$ |
| Glutathione | 0.44 ± 0.15$^a$ | 1.10 ± 0.09$^b$ | 0.27 ± 0.06$^c$ | 0.39 ± 0.01$^d$ |
| Proline     | 1.31 ± 0.47$^a$ | 3.08 ± 0.47$^b$ | 0.29 ± 0.05$^c$ | 1.66 ± 0.30$^d$ |
| PC          | 3.20 ± 0.20$^a$ | 4.73 ± 0.24$^b$ | 2.09 ± 0.74$^c$ | 2.7 ± 0.24$^d$ |
| IAA in roots| 0.80 ± 0.12$^a$ | 2.2 ± 0.23$^b$ | 0.17 ± 0.06$^c$ | 0.23 ± 0.03$^d$ |
| IAA in shoots| 0.91 ± 0.07$^a$ | 2.41 ± 0.10$^b$ | 2.70 ± 0.12$^c$ | 1.32 ± 0.09$^d$ |

Note: Different letters indicate significant differences between means ± SD of treatments (n = 3). Data were taken on 1-week-old plants.

**Figure 3.** Effect of auxin inhibitor (TIBA) on the activity of SOD and GR, H$_2$O$_2$ content of rice plants cultivated under different combination of Zn and TIBA in hydroponic conditions. Different letters (a and b) indicate significant differences (P < 0.05) between means ± SD of treatments (n = 3). Data were taken on 1-week-old plants.

**Figure 4.** Quantitative real-time PCR analysis of OsIRT1, OsZIP4, OsZIP1 and OsDMAS1 transcripts in roots of rice plants grown under different growth conditions. Different letters (a and b) indicate significant differences (P < 0.05) between means ± SD of treatments (n = 3). Expression of OsIRT1, OsZIP4, OsZIP1 and OsDMAS1 was relative to that of the rice Actin gene used as an internal control.
evidence for interaction of mechanisms suggests the existence of additional mechanisms by which auxin signaling may integrate ZE in rice.

Taken together, our findings suggest that auxin signaling may be closely associated with ZE in rice and open a perspective to alter crop/cultivar tolerance to stress by engineering auxin synthesis and signaling. Also, new uses for exogenous auxin to optimize crop performance under stress could be explored.

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Disclosure statement
No potential conflict of interest was reported by the authors.

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