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RESEARCH ARTICLE

Seed priming with brassinosteroids alleviates aluminum toxicity in rice via improving antioxidant defense system and suppressing aluminum uptake

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Abstract
Brassinosteroids (BRs) are growth-promoting hormones that exhibit high biological activities across various plant species. BRs shield plants against various abiotic stresses. In the present study, the effect of BRs against aluminum (Al) toxicity was investigated through seed priming with 24-epibrassinolide (0.01 μM) in two different rice cultivars. BRs application was found effective in confronting plants from Al toxicity (400 μM). The rice seeds primed with BRs showed enhancement in seed germination energy, germination percentage, root and shoot length, as well as fresh and dry weight under Al-absence and Al-stressed conditions as compared to water-priming. Especially under Al stress, BRs priming promoted the growth of rice seedlings more obviously. Al toxicity significantly increased the Al contents in seedling root and shoot, as well as the MDA concentration, H_2O_2 production, and the activities of antioxidative enzymes including ascorbate peroxidase, catalase, and peroxidase. Meanwhile, the photosynthetic pigments of seedling reduced under Al stress. When compared to sensitive cultivar (CY-927), these modifications were more obvious in the tolerant variety (YLY-689). Surprisingly, BRs were able to alleviate the Al injury by lowering MDA and H_2O_2 level and increasing antioxidant activities and photosynthetic pigments under Al stress. The results on antioxidant activities were further validated by gene expression study of *SOD-Cu-Zn*, *SOD-Fe$_2$*, *CATa*, *CATb*, *APX02*, and *APX08*. It suggested that BRs were responsible for the mitigation of Al stress in rice seedlings by inducing antioxidant activities with an effective response to other seed growth parameters and reduced Al uptake under induced metal stress.

Keywords Aluminum · Antioxidants · Brassinosteroids · Heavy metals · Rice

Introduction

Rice (*Oryza sativa* L.) is an important staple food of the developing world due to its high protein, vitamin, and mineral contents (Ahmed et al. 2021a). Almost 85% of rice is cultivated in Asia, whereby China is the world's leading producer of rice. In southern China, rice is the main cereal crop to fulfil the nutritional requirements of underprivileged people (Ahmed et al. 2020; Huang et al. 2013). During past few years, soil properties have been changed due to rapid industrialization and excessive release of heavy metals (Ahmed et al. 2021b), such as aluminum (Al), cadmium (Cd), mercury (Hg), and lead (Pb), in environmental system (Ahmed et al. 2021c; Noman et al. 2020a; Noman et al. 2020b). In low pH soils, Al contamination has devastating impacts that catastrophically disturbed the agronomic traits, such as yield and quality, of cereal crops. It was estimated that almost 30–50% of soil is polluted with Al globally, whereby 21% of total arable land in China is Al-affected (Xu et al. 2012). Al has been found to alter the morphological (such as growth and biomass production) as well as physiological (such as oxidative stress) states of crop plants (Silva 2012). It boosts lipid peroxidation by inhibiting antioxidant enzymes including catalase, peroxidase, superoxide dismutase, and glutathione reductase, which ultimately trigger plant stress (Rout et al. 2001). It was discovered that Al toxicity can also be found

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in shoots that develop as a result of root system damage (Vitorello et al. 2005). Al toxicity has a number of negative effects in plants, including altered water balance, reduced stomatal conductance and photosynthetic activity, chlorosis, and necrosis of leaves (Ali et al. 2008a; Ma 2007). The excess amount of Al in soil or growth medium perturbs the entry of essential ions such as magnesium (Mg), potassium (K), calcium (Ca), manganese (Mn), and zinc (Zn) into the plant system, thereby disturbing the mineral balances and retarding the overall growth and development (Basit et al. 2021b; Manzoor et al. 2021; Mendonça et al. 2003). However, it raises proline levels, which acts as an osmoprotectant, membrane stabilizer, and ROS scavenger (Apel and Hirt 2004).

Brassinosteroids (BRs) are polyhydroxy steroidal phytohormones that have a strong ability to promote plant growth under diverse environmental conditions (Basit et al. 2021a; Latha and Vidya Vardhini 2018). 24-Epibrassinolide is the most biologically active BR compound, which is involved in a variety of developmental processes such as cell division, elongation, gene expression, and vascular differentiation and others (Basit et al. 2021a; Bergonci et al. 2014). BRs have been shown to help plants cope with a variety of stresses, including biotic and abiotic stresses (Bhandari and Nailwal 2020). Exogenously applied BRs improve the resistance mechanisms to low and high temperatures, drought, chilling, and various metal stresses. Similarly, BRs treatment improved tomato plant resistance to chromium stress by modulating physiological and molecular pathways (Jan et al. 2020). It has been discovered that BRs strengthen nitrogen fixation, secondary metabolites, and protection mechanisms in *Cicer arietinum* (Ali et al. 2005) and *Brassica juncea* under salinity and heavy metal stresses such as nickel (Ali et al. 2008b). It has also been documented that exogenous application of BRs improves the antioxidant system, photosynthesis, and growth characteristics of mung bean plants under aluminum stress (Ali et al. 2008a). Numerous studies have shown that BRs enhance plant growth under various heavy metal stresses by increasing chlorophyll contents, which play an important role in increasing photosynthetic capability, improving antioxidant system performance, boosting enzymatic activity, and upregulating stress-responsive genes [superoxide (SOD), peroxide (POD), catalase (CAT), glutathione reeducates (GR), and ascorbate peroxide (APX)] (Cominelli et al. 2008).

Hence, based on the hypothesis that BRs could boost the biomass of rice plants by reducing the uptake of Al and alleviating the oxidative stress under Al toxicity, we explored the impact of seed priming with BRs on rice seed germination, morphophysiological and biochemical parameters, nutrient acquisition, and Al uptake under Al toxicity as well as proposed the possible mechanism of Al stress mitigation in rice plants through seed priming with BRs in this study.

Materials and methods

Brassinosteroids (BRs) preparation

24-Epibrassinolide was obtained from the Shanghai Aladdin Biochemical Technology Co., Ltd. It was liquefied in sufficient ethanol, and a stock solution of 10^{-5} M was prepared by adding ddH_2O with 0.05% Tween 20 for use as the priming reagent of BRs.

Plant materials and growth conditions

The seeds of two cultivars of *Oryza sativa* L. (cv. CY927 and YLY689) were obtained from the Zhejiang Nongke Seeds CO., LTD. Hangzhou, Zhejiang Province, China (Salah et al. 2015). Seeds were surface sterilized for 15 min with a 0.5% sodium hypochlorite (NaClO) solution and then washed several times with tap water before being washed three times with sterilized distilled water to remove any remaining disinfectant. Sterilized seeds were primed with 0.01-μM BRs at 15 °C in the dark for 24 h. The seeds were then dried at room temperature to their original moisture content. The seeds primed with water (H_2O) and BRs without Al stress were used as the control group (CK). After seed priming, germination tests were performed. Each treatment consisted of fifty seeds that were placed in a plastic germination box (12 cm × 18 cm) and repeated three times. The seeds were then incubated for 14 days at 25 °C in a germination chamber with an 8/16 h light/darkness period (Zhang et al. 2007). The 400-μM concentration of Al was supplied to seeds with nutrients solution. The nutrient solution was made up of the following ingredients: 0.5-μM potassium nitrate (KNO_3), 0.5-μM calcium nitrate ($Ca(NO_3)_2$), 0.5-μM magnesium sulfate $MgSO_4$, 2.5-μM monopotassium phosphate (KH_2PO_4), 2.5-μM ammonium chloride (NH_4Cl), 100-μM ferric EDTA (Fe–K–EDTA), 30-μM boric acid (H_3BO_3), 5-μM manganese monosulfate ($MnSO_4$), 1-μM copper sulfate ($CuSO_4$), 1-μM zinc sulfate ($ZnSO_4$), and 1-μM ammonium heptamolybdate ($(NH_4)_6Mo_7O_{24}$) per 1000 mL ddH_2O. The pH of the nutrients solution was adjusted to 5.0 with hydrochloric acid (HCl) and sodium hydroxide (NaOH). The Al concentration was determined using data from a primary experiment involving different Al concentrations of 0, 100, 200, 300, 400, 500, 600, 700, and 800 μM. Plant growth was slightly harmed at low aluminum concentrations (100–300 μM). Despite this, plant growth was significantly harmed by aluminum concentrations of 400 μM. Concentrations greater than 500 μM, on the other hand, were excessively toxic to the plant's growth.

Measurement of physiological parameters

For 14 days, the number of germinated seeds was counted every day. On the 5th day of germination, total germinated seeds were counted, and germination energy was calculated. On the 14th day, the germination percentage was determined. The following formulas were used to calculate the germination index (GI), mean germination time (MGT), and vigor index (VI) (Hu et al. 2005).

$$GI = \Sigma(G_t/T_t) \quad (1)$$

$$MGT = \Sigma(G_t \times T_t)/\Sigma G_t \quad (2)$$

$$VI = GI \times [\text{Shoot height} + \text{Root length}] \quad (3)$$

G_t is the total calculated number of germinated seeds on day t, and T_t is the time conforming to G_t in days (Hu et al. 2005).

Experimental design and treatment pattern

The 2-week-old seedlings were treated with a 400-μM Al concentration after being primed with water (H_2O) and 0.01-μM BRs. The experimental pattern was constituted through a completely randomized design (CRD), and the location of the pots within the growth chamber was changed every day. The plants were sampled 21 days after being treated with Al for 7 days to observe alterations morphophysiological parameters.

Plant growth investigation

The plants were harvested with intact roots and immersed in a bucket filled with water to remove any traces of media contents. The plants were divided into roots and shoots, and the lengths of the roots and shoots were measured, followed by determination of their fresh weight using electronic weighing balance. The roots and shoots were dried in an oven at 80 °C for 24 h and then weighed to determine their dry mass.

Measurement of photosynthetic pigments

Photosynthetic pigments, such as chlorophyll a and b and total chlorophyll, were investigated by following the methodology of Ahmed et al. (2021b). Briefly, fresh leaf tissues (0.2 g) were homogenized in 3 mL ethanol (95%, v/v). The supernatant was removed after centrifuging the homogenate at 5000 *g* for 10 min. 1 mL aliquot of the supernatant was added to 9 mL of ethanol (95% v/v). After that, the mixture was subjected to spectrophotometry to determine the absorbance at 665- and 649-nm wavelengths (Lichtenthaler and Wellburn 1983). To quantify chlorophyll pigments, the following equations were used:

$$\text{Chlorophyll a } (C_a) = 13.95 \text{ A665}-6.88 \text{ A649} \quad (4)$$

$$\text{Chlorophyll b } (C_b) = 24.96 \text{ A649}-7.32 \text{ A665} \quad (5)$$

$$\text{Total chlorophyll content} = C_a + C_b \quad (6)$$

The pigment concentrations were calculated in milligrams per liter of plant extract.

Measurement of metal contents in plant tissues

To determine Al contents, dried plant roots and shoots (0.2 g) for each treatment were acid digested by using 5 mL concentrated HNO_3/HCLO4 (5:1, v/v) on a hot plate at 70 °C for 5 h. The digested samples were diluted with 2% HNO_3 to a final volume of 10 mL and filtered through Whatman filter paper. The filtrate was used to estimate the concentrations of Al, other microelements [iron (Fe^{2+}), zinc (Zn^{2+}), manganese (Mn^{2+})], and macroelements [(calcium (Ca^{2+}), potassium (K^+), and magnesium (Mg^{2+})] by using atomic absorption spectrometer (iCAT-6000-6300, Thermo Scientific, USA) (Khan et al. 2013).

Measurement of MDA contents and H_2O_2 measurements

MDA concentration was examined in terms of production of 2-thiobarbituric acid (TBA) metabolites. Approximately 1.5 mL plant extract was homogenized in 2.5 mL of 5% TBA prepared in 5% trichloroacetic acid (TCA). The mixture was heated to 95 °C for 15 min followed by incubation on ice. After that, the supernatant was centrifuged for 10 min at 5,000*g*. Finally, the absorbance of the supernatant was determined at 532 nm using the abovementioned unit of spectrophotometer. To reduce nonspecific turbidity, the absorbance value of reaction mixture at 600 nm was subtracted from the value at 532 nm (Rao and Sresty 2000). The MDA concentration was measured in nmol mg^{-1} protein. To measure hydrogen peroxide (H_2O_2) concentration, the plant tissues were homogenized in phosphate buffer followed by centrifugation at 6000g. The supernatant was mixed with 0.1% titanium sulfate containing 20% (v/v) H_2SO_4 followed by centrifugation. The intensity of yellow color was estimated calorimetrically at 410nm using abovementioned unit of UV-vis spectrophotometer (Velikova et al. 2000). H_2O_2 concentration was calculated by using standard curve constructed using known concentrations of H_2O_2.

Enzymatic antioxidants activity assay

Fresh plant tissues were homogenized in 8 mL of 50 mM potassium phosphate buffer (containing 1-mM $EDTANa_2$ as well as 0.5% PVP W/V, pH 7.0) on ice. After that, centrifugation of the homogenate was performed at 12000rpm for 20 min at 4°C. After centrifugation, supernatant was collected in separate tube and stored at 80°C until further processing. The superoxide dismutase (SOD) activity was determined according to Giannopolitis and Ries (1977), by examining the capability of the enzyme to restrict the photochemical reduction of nitroblue tetrazolium chloride (NBT). The absorbance was measured at 560 nm, and one unit of SOD activity, represented as EU/mg protein, was shown to be the enzyme quantity required for inhibiting the rate of NBT photoreduction by up to 50% (Giannopolitis and Ries 1977). Peroxidase (POD) activity was examined according to the method of Zhang (1992) exhausting the elimination coefficient 25.5 mM^{-1} cm^{-1}. The homogenization of both root and shoot fresh tissues was done in phosphate buffer (50 mM and pH 7.8) followed by centrifugation at 8000*g* at 25 °C. The OD of resulting mixture was measured at 470 nm. Catalase (CAT) activity was recorded as described by Aebi (1984) by the extermination constant of 39.4 mM^{-1} cm^{-1}. The absorbance was calculated at 240 nm, and CAT activity was expressed as EU/mg protein, whereby for the determination of ascorbate peroxidase (APX) activity, the decrease in absorbance at 290 nm for 3 min was observed, and APX activity was expressed as EU/mg protein (Nakano and Asada 1981).

RNA extraction and gene expression analysis

Antioxidant gene profiling was performed by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). Total RNA from plant samples was extracted using Trizol reagent as described by Sah et al. (2014). For cDNA synthesis, 1 μg of total RNA was reverse transcribed through PrimeScript™ RT reagent kit, and the resulting product was used as template for qRT-PCR. The qRT-PCR reaction was prepared in SYBR Premix Ex Taqkit (TaKaRa, Dalian, China), containing 10 mL 2× SYBR Premix Ex Taq buffer, 1-μg synthesized cDNA, and 10 μmol of each of gene-specific primers in a final volume of 20 mL (Livak and Schmittgen 2001). *OsActin* was used as an internal control to normalize the data to measure the relative transcript abundance for target genes. Relative expressions of target genes were calculated using the 2-ΔΔCT method. Primers used for qRT-PCR are listed in Table S1.

Statistical analysis

Experimental data were analyzed using one-way analysis of variance treatments. The significance among means of different dataset was determined using least significant difference (Fisher's LSD) test with 95% confidence level using SPSS v16.0 (SPSS, Inc., Chicago, IL, USA). Principle component analysis (PCA) and agglomerative hierarchical clustering (AHC) were performed to classify two different rice cultivars used in the current study according to their vulnerability toward Al by using XLSTAT.

Results

BRs promotes seed vigor and plant growth

The current study has demonstrated that Al toxicity (400 μM) caused a significant decrease in seed germination energy, germination percentage, vigor index, as well as germination index in both cultivars as compared to control; more reduction was observed in cultivar CY927. However, seed priming with BRs revealed a significant resistance against Al stress as compared to control (seed priming with water) in both cultivars. MGT was significantly enhanced in plants treated under Al stress as compared to untreated plants. However, seed priming with BRs reduced MGT in both cultivars (Table 1). More reduction was observed in cultivar CY927 as compared to cultivar YLY689. Current study demonstrated that 0.01-μM concentration of BRs significantly enhanced the GE, germination %, VI, and GI under metal toxicity as compared to the unprimed seeds in both cultivars (Table 1).

Al exposure reduced the morphological parameters of rice seedlings (Fig. 1 and Fig. 2). Plants treated with Al toxicity caused a significant decrease in root and shoot length of rice plants as compared to untreated control plants. A significant reduction was observed in seedling fresh weight of CY927 cultivar after exposure to Al stress, whereby dry weight was significantly decreased in both cultivars under Al toxicity. More reduction was observed in cultivar CY927 as compared to cultivar YLY689. It was noticed that seed priming with BRs significantly improved the shoot/root length as well as fresh/dry weight as compared to control plants without BRs treatment under Al-spiked conditions (Table 2).

Seed priming with BRs increases photosynthetic pigments

The negative effect of Al stress on photosynthetic pigment concentration was observed in both rice cultivars (Fig. 3). The present study demonstrated that Al treatment alone resulted in a significant drop in chlorophyll pigments such as Chl a and b and total chlorophyll content as compared to control. Under Al stress, both cultivars showed a decrease in photosynthetic pigments. In comparison to YLY689, the decline was more obvious in CY927. Seed priming with 0.01-μM BRs mitigated Al toxicity in both genotypes to some extent. In the case of seed priming with BRs, the total chlorophyll pigment was increased by 37.2% on average across both cultivars. Seed priming with BRs improved Chl a and b and total chlorophyll concentrations in both cultivars when compared to respective controls in both treatments without and with Al stress. In both cultivars, plants treated with BRs alone showed more photosynthetic pigments than non-treated control plants (Fig. 3).

Table 1 Seed priming effect with 0.01-μM BRs on germination energy, germination percentage, germination index, mean germination time, as well as vigor index of two rice cultivars under 400-μM Al toxicity

Varieties	Treatments	GE (%)	GP (%)	MGT (*d*)	GI	VI
CY927	H_2O	88.00b±2.00	94.67b±1.15	2.91b±0.17	20.13b±1.17	0.88b±0.17
	BRs	90.67a±3.06	100.00a±0.00	2.17c±0.07	31.62a±0.92	1.47a±0.16
	H_2O+Al	46.00d±2.00	60.67d±1.15	3.81a±0.08	10.87c±0.50	0.15d±0.07
	BRs+Al	69.33c±3.06	85.33c±5.03	2.91b±0.20	21.49b±1.82	0.64c±0.06
YLY689	H_2O	90.00b±2.00	99.33a±1.15	2.59c±0.26	23.72b±0.43	1.26b±0.15
	BRs	98.00a±2.00	100.00a±0.00	1.93d±0.14	32.44a±1.12	2.06a±0.03
	H_2O+Al	69.33d±1.15	72.67c±2.31	2.88a±0.37	16.53c±0.58	0.37d±0.19
	BRs+Al	80.67c±3.06	88.00b±2.00	2.63b±0.08	23.57b±0.56	0.74c±0.03

Each value is demonstrating the mean of three repeats of every treatment. The similar letters inside a column specify that there was no significant difference at a 95% probability level at the $p < 0.05$ level, correspondingly

BRs supplementation reduces Al accumulation in rice seedling

Roots are the main part that interacts with heavy metals first, as well as the primary source of nutritional solution and heavy metal uptake. Al accumulation was found to be higher in roots than in shoots (Tables 3, 4). However, Al accumulation was noticeable in the YLY689 cultivar than in the CY927 cultivar. Seed priming with BRs reduced Al content by 10.90% and 6.140% in the CY927 and YLY689 cultivars, respectively. More interestingly, when exposed to Al toxicity, the concentrations of K^+, Ca^{2+}, Fe^{2+}, and Mn^{2+} in both roots and shoots reduced, whereas Zn^{2+} concentration increased in both cultivars (Tables 3–4). Seed priming with BRs maintained the nutritional balance of both cultivars under Al stress and significantly improved the nutrients availability such as Mn^{2+} and Fe^{2+} inside shoots of cultivar CY927 and elevated K^+, Ca^{2+}, Mn^{2+}, and Fe^{2+} accessibility in shoots of YLY689 cultivar (Tables 3–4).

BRs ameliorates Al-induced oxidative stress

In both cultivars, the presence of Al raised MDA and H_2O_2 contents when compared to the control. In comparison to the YLY689 cultivar, this rise was more pronounced in CY927. The application of BRs dramatically lowered MDA levels as well as H_2O_2 generation in both cultivars (Fig. 4). MDA levels were found to be greater in shoots (64.70% and 55.40%) than that in roots (56% and 42%) in both CY927 and YLY689 cultivars under induced Al stress as compared to respective healthy controls, respectively. However, seed priming with BRs reduced the MDA levels in shoots (44.50% and 45%) and roots (26% and 39.70%) of both cultivars (CY927 and YLY689, respectively) as compared to corresponding controls under Al-spiked conditions.

Determination of antioxidant enzyme activities

Antioxidant activity was shown to be increased when Al was treated alone. A recent study found that stressed plants had

Table 2 Seed priming effect with 0.01-μM BRs on shoot length, root length, fresh weight, and dry weight of two rice cultivars under 400-μM Al toxicity

Varieties	Treatments	SL (cm)	RL (cm)	FW (g/10 seedlings)	DW (g/10 seedlings)
CY927	H_2O	14.26b±0.12	13.85b±0.37	0.41b±0.01	0.04b±0.01
	BRs	16.40a±0.08	15.29a±0.44	0.53a±0.01	0.05a±0.00
	H_2O+Al	5.52d±0.23	6.85d±0.15	0.16d±0.01	0.01d±0.01
	BRs+Al	8.38c±0.72	8.93c±0.46	0.29c±0.01	0.03c±0.00
YLY689	H_2O	15.26b±0.35	18.96b±0.47	0.48b±0.01	0.06a±0.00
	BRs	18.23a±0.11	19.54a±0.28	0.59a±0.01	0.06a±0.01
	H_2O+Al	8.56d±0.15	9.19d±0.32	0.18d±0.01	0.02c±0.01
	BRs+Al	11.43c±0.22	11.48c±0.18	0.31c±0.01	0.04b±0.00

Each value is demonstrating the mean of three repeats of every treatment. The similar letters inside a column specify that there was no significant difference at a 95% probability level at the $p < 0.05$ level, correspondingly

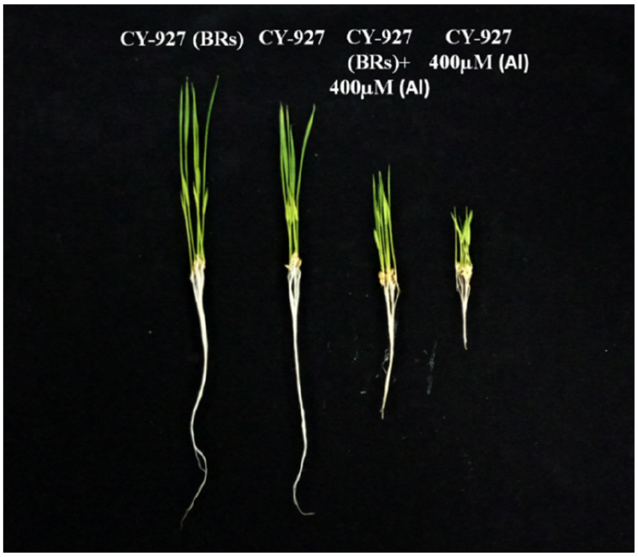

Fig. 1 Physiological effect of Al toxicity on rice cultivar CY927 and mitigation effect by 0.01-μM BRs under 400-μM Al stress

higher levels of SOD, CAT, POD, and APX than control plants when exposed to 400-μM Al and that this effect was stronger in YLY689 than that in CY927 (Fig. 5). These activities were found to be more prevalent in roots than that in shoots. SOD activity was found to be 22.5% in CY927 and 43.6% in YLY689 cultivar shoots when Al was applied, whereby in roots of both cultivars, it was found in 47.7% and 58.2%, respectively. Both cultivars under combined treatment of Al and BRs showed 44.6% and 46.3% increase in shoot SOD activity and 57.4% and 58.2% increase in root SOD activity in contrast to Al-affected plants. Under independent treatment of Al, CAT activity in the shoots was found to be 45% in CY927 and 59% in YLY689. In roots, it was found

Fig. 2 Physiological effect of Al toxicity on rice cultivar YLY689 and mitigation effect by 0.01-μM BRs under 400-μM Al stress

to be 39% and 45%, respectively. The seed priming with BRs increased the CAT activity by 51% and 61% in shoots and by 46% and 58% in roots as compared to Al-stressed plants, respectively. Similarly, POD and APX levels were higher in the presence of Al alone, but this impact was more prevalent with BRs priming; however, POD activity was not increased significantly. However, 14.8% and 37.4% increase in POD activity was observed in shoots of CY927 and YLY689, whereby 30.2% and 46.8% boost in roots was observed in both cultivars after Al treatment, respectively (Fig. 5).

Determination of gene expression analysis

In both cultivars, there was a substantial difference in the expression of *APX02* in both roots and shoots when compared to control. Under both independent Al stress and combined treatment of Al/BRs, the transcriptional level of *APX02* was increased. The YLY689 rice cultivar had higher *APX02* expression than the CY-927 rice cultivar ($p < 0.01$). Interestingly, the transcriptional level of *APX02* in rice seedlings emerged from BRs primed seeds was found to be higher than plants under Al stress alone and supported the results of APX activity (Fig. 5). Similarly, the transcription level of *APX08* was high in both roots and shoots of either cultivar when compared to control under independent and combined treatments of Al and BRs; however, the expression level of *APX08* was found to be higher in the roots of YLY689 cultivar than that of CY927 cultivar (Fig. 6). Additionally, in contrast control condition, the expression levels of *CATa* and *CATb* were found to be higher in both roots and shoots of both cultivars under independent and combined treatments of Al and BRs. However, the rise was more pronounced in YLY689 cultivar than that in CY927 cultivar. Al stress also triggered the expression levels of *CATa* and *CATb* in both roots and shoots of both cultivars as compared to the non-treated control (Fig. 6). Under stressful conditions, both cultivars showed significant upregulation of *SOD Cu-Zn* and *SOD-Fe2* genes, where YLY689 cultivar showed a greater increase. The transcriptional level of the *SOD Cu-Zn* and *SOD-*Fe_2 genes was found to be higher in roots than that in shoots in both cultivars. Regardless of Al stress, the transcriptional level of the *SOD Cu-Zn* gene was much higher in roots than in non-treated control (Fig. 6). In contrast to seeds primed with water, seedlings primed with BRs depicted higher expression of *SOD Cu-Zn* and *SOD-*Fe_2 genes. Under Al toxicity, it may be possible to modify the stress condition inside both cultivars by temporarily upregulating particular gene expression (Fig. 6). This data further confirms the BRs-based alterations in the concentrations of enzymatic antioxidants (Fig. 5). It was clearly demonstrated that BRs play a substantial role in stress tolerance in rice plants by modifying and regulating the transcriptional level of particular genes under induced Al stress.

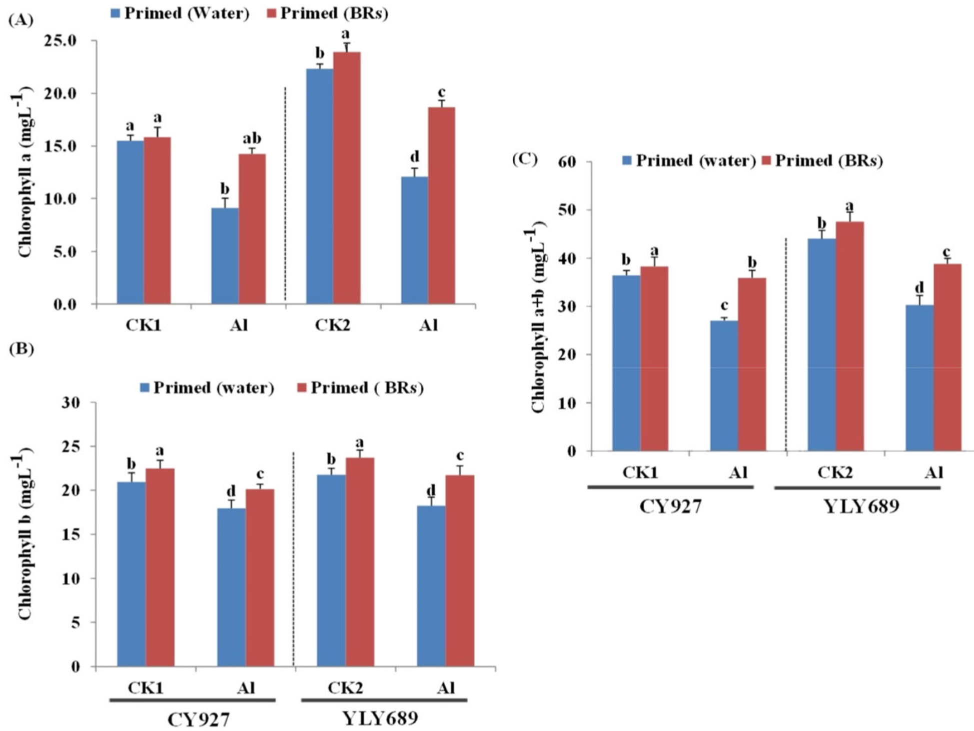

Fig. 3 Seed priming effect with 0.01-µM BRs on **A** chlorophyll a, **B** chlorophyll b, **C** chlorophyll a+ b in leaves of two different cultivars of rice under 400-µM Al concentration

Determination of cluster and correlation analysis between observations

Biplot graphs of PCA were generated based on physiological features of two different rice varieties to study the sensitive and tolerant groups through F1 and F2 of multiple parameters under diverse treatments, such as seeds primed with water (CY927-H_2O, YLY689-H_2O) and BRs (CY927-BRs, YLY689-BRs) under normal conditions and seed primed with water and BRs under Al stress (Figs. 7A, B, 8). MDA, MGT, and H_2O_2 were pooled together and were found to have a positive correlation. Although MGT, H_2O_2, and MDA had a negative relationship with VI, F/W, D/W, SL, RL, GE, and GP, they also had a negative relationship with SOD, POD, CAT, and APX in both cultivars (Fig. 7A, B). PCA analysis of both cultivars (CY927 and YLY689) demonstrated that YLY689 is a tolerant genotype, whereby CY927 is a sensitive genotype for Al stress. In CY927, the largest contribution of F1 (84.92) was observed, followed by F2 (10.20), with a total contribution of 95.12%, whereas in YLY689, the largest contribution of F1 (86.39) was observed, followed by F2 (10.69), with a total contribution of 97.09%. ACH results also confirmed the identical response of both varieties to distinct treatments (Fig. 8). It represented the close relationship between both cultivars (CY927 and YLY689) primed with BRs and primed with water under Al stress, as well as cultivars primed

Table 3 Seed priming effect with 0.01-µM BRs on Al uptake and accumulation in shoots of two rice cultivars under 400-µM Al toxicity

Varieties	Treatments	Mg mg/g	Al mg/g	K mg/g	Ca mg/g	Mn mg/g	Fe mg/g	Zn mg/g
CY927	H_2O	2.32a±0.13	-	39.72a±1.06	2.69a±1.04	0.27ab±0.10	0.23b±0.10	0.05b±0.02
	BRs	2.37a±0.74	-	39.43a±2.06	2.80a±0.99	0.43a±0.19	0.39a±0.19	0.07ab±0.02
	H_2O+Al	2.45a±0.46	0.61a±0.06	31.48b±1.78	0.18c±0.24	0.09b±0.01	0.08c±0.01	0.10a±0.01
	BRs+Al	2.31a±0.17	0.43b±0.07	37.08a±1.10	1.80b±0.09	0.22ab±0.02	0.21b±0.02	0.06b±0.00
YLY689	H_2O	1.77b±0.78	-	26.36c±1.99	0.71a±0.48	0.55a±0.48	0.77a±0.04	0.02c±0.01
	BRs	2.20ab±0.65	-	32.09bc±1.93	0.75a±0.21	0.64a±0.23	0.89a±0.02	0.03bc±0.00
	H_2O+Al	2.24ab±0.21	0.82a±0.34	40.43ab±2.21	0.20b±0.23	0.10b±0.21	0.07b±0.01	0.05a±0.01
	BRs+Al	3.04a±0.30	0.24b±0.26	49.60a±0.52	1.29a±0.27	0.53a±0.27	0.70a±0.01	0.04b±0.00

Each value is demonstrating the mean of three repeats of every treatment. Same letters are representing no significant differentiation at 95% probability level (p<0.05)

Table 4 Seed priming effect with 0.01-µM BRs on Al uptake and accumulation in roots of two rice cultivars under 400-µM Al toxicity

Varieties	Treatments	Mg mg/g	Al mg/g	K mg/g	Ca mg/g	Mn mg/g	Fe mg/g	Zn mg/g
CY927	H_2O	2.34a±0.17	-	19.69a±1.42	0.88a±0.34	0.08a±0.01	6.16a±0.30	0.07a±0.03
	BRs	1.52a±0.53	-	14.21ab±2.73	0.81a±0.33	0.07a±0.02	5.79a±1.06	0.12a±0.05
	H_2O+Al	1.14a±0.49	5.02a±2.34	9.81b±0.29	0.21b±0.05	0.03b±0.01	3.52b±0.23	0.14a±0.05
	BRs+Al	1.19a±0.42	3.92b±1.23	12.60ab±1.10	0.75a±0.04	0.04b±0.02	3.46b±0.17	0.07a±0.01
YLY689	H_2O	1.77a±0.50	-	13.48b±1.29	1.31a±1.25	0.07a±1.25	6.49a±0.94	0.02a±0.01
	BRs	2.13a±0.43	-	17.46a±2.10	0.76ab±0.17	0.05a±0.17	7.03a±1.95	0.03a±0.00
	H_2O+Al	1.28a±0.34	8.78a±0.25	13.47b±2.94	0.30b±0.17	0.04a±0.17	2.53b±0.17	0.05a±0.00
	BRs+Al	1.32a±0.76	7.50b±2.45	11.02c±1.74	1.50a±0.43	0.03a±0.43	4.71ab±0.24	0.04a±0.01

Each value is demonstrating the mean of three repeats of every treatment. Same letters are representing no significant differentiation at 95% probability level ($p<0.05$)

with water and BRs under normal condition. When compared to plants primed with water under Al toxicity, cultivars primed with BRs exhibited a close association with both controls (primed with water and BRs) (Fig. 8).

Discussion

Al is the 3rd most prevalent metal in the earth's crust, and while it is abundant, it is only minimally soluble, causing serious harm to biological systems (Bolt et al. 2020). Al is present in plant-accessible form at pH value less than 5.5, which can cause toxicity in plants, particularly the roots (Barcelo and Poschenrieder 2002). Because it has direct exposure to roots, Al toxicity affects shoot length, fresh weight, and dry weight (Table 2), causing cell elongation inhibition at an early stage and later causing damage to plant growth and development (Čiamporová 2002; Silambarasan et al. 2019). In addition to membrane permeability, Al promotes nutritional imbalance in plants by altering osmotic balance (Olivares et al. 2009). Similarly, Al treatment lowered the levels of K^+, Ca^{2+}, Fe^{2+}, and Mn^{2+} in both roots and shoots in the

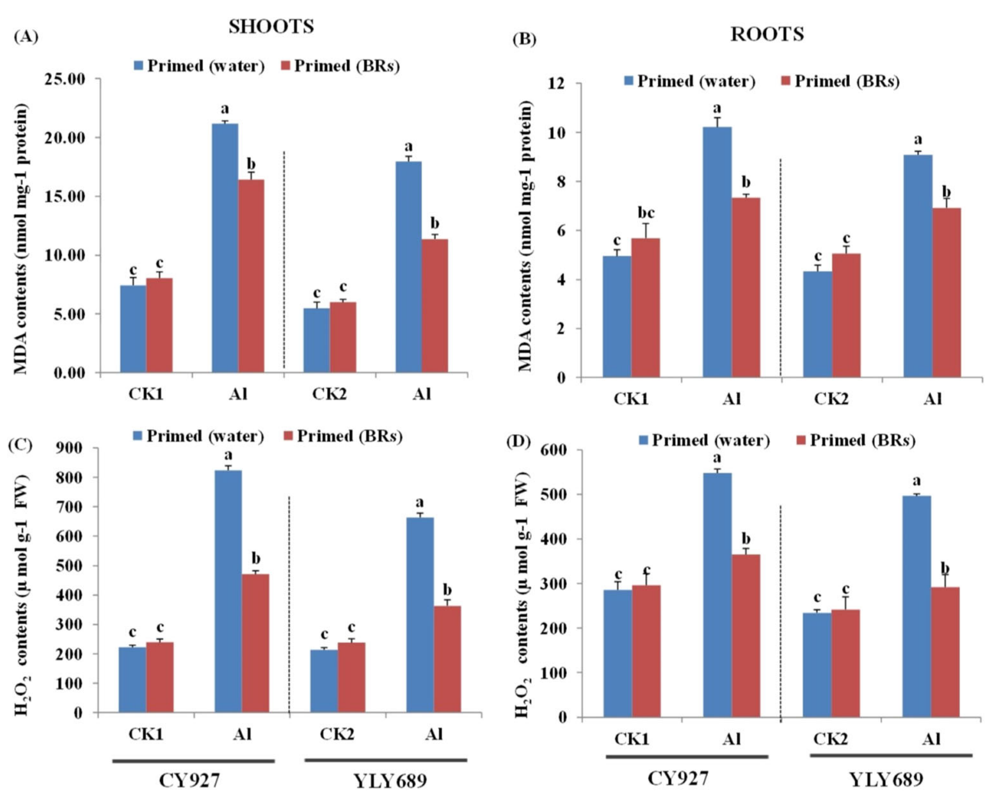

Fig. 4 Seed priming effect with 0.01-µM BRs on MDA contents and H_2O_2 production in shoots and roots of two different rice cultivars under 400-µM Al toxicity. **A** MDA contents in shoots of both rice cultivars, **B** MDA contents in roots of both rice cultivars, **C** H_2O_2 contents in shoots of both rice cultivars, **D** H_2O_2 contents in roots of both rice cultivars

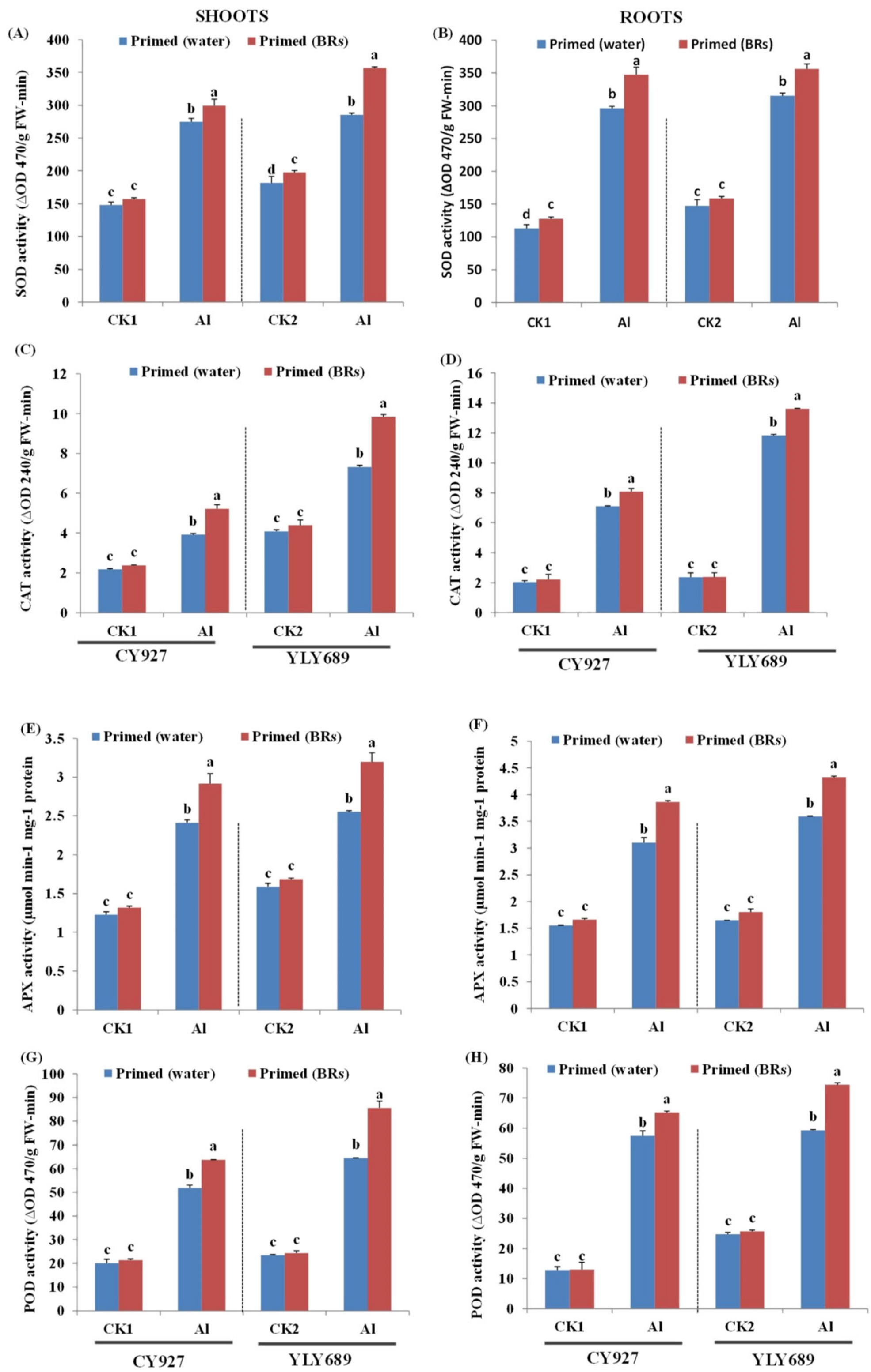

Fig. 5 Seed priming effect with 0.01-μM BRs on SOD, CAT, APX, and POD contents in both shoots and roots of two rice cultivars under 400-μM Al toxicity. **A** Superoxide dismutase (SOD) in shoots of both rice cultivars, **B** SOD in roots of both rice cultivars, **C** Catalase (CAT) in shoots of both rice cultivars, **D** CAT in roots of both rice cultivars, **E** Ascorbate peroxidase (APX) in shoots of both rice cultivars, **F** APX in roots of both rice cultivars, **G** Peroxidase (POD) in shoots of both rice cultivars, **H** POD in roots of both rice cultivars

current study (Tables 3–4). Al toxicity lowered K^+, Ca^{2+}, Fe^{2+}, and Mn^{2+} concentration in rice plants, with the effect being stronger in sensitive varieties than tolerant varieties. Similarly, Al inhibited the level of K^+, Ca^{2+}, Fe^{2+}, and Mn^{2+} in the roots and shoots of tomato and maize plants (Giannakoula et al. 2008; Simon et al. 1994). Consequently, photosynthetic pigments and associated processes were also disrupted (Fig. 3), resulting in a reduction in plant growth caused by Al exposure (Table 2). In comparison to seeds primed with water, seeds treated with BRs demonstrated a

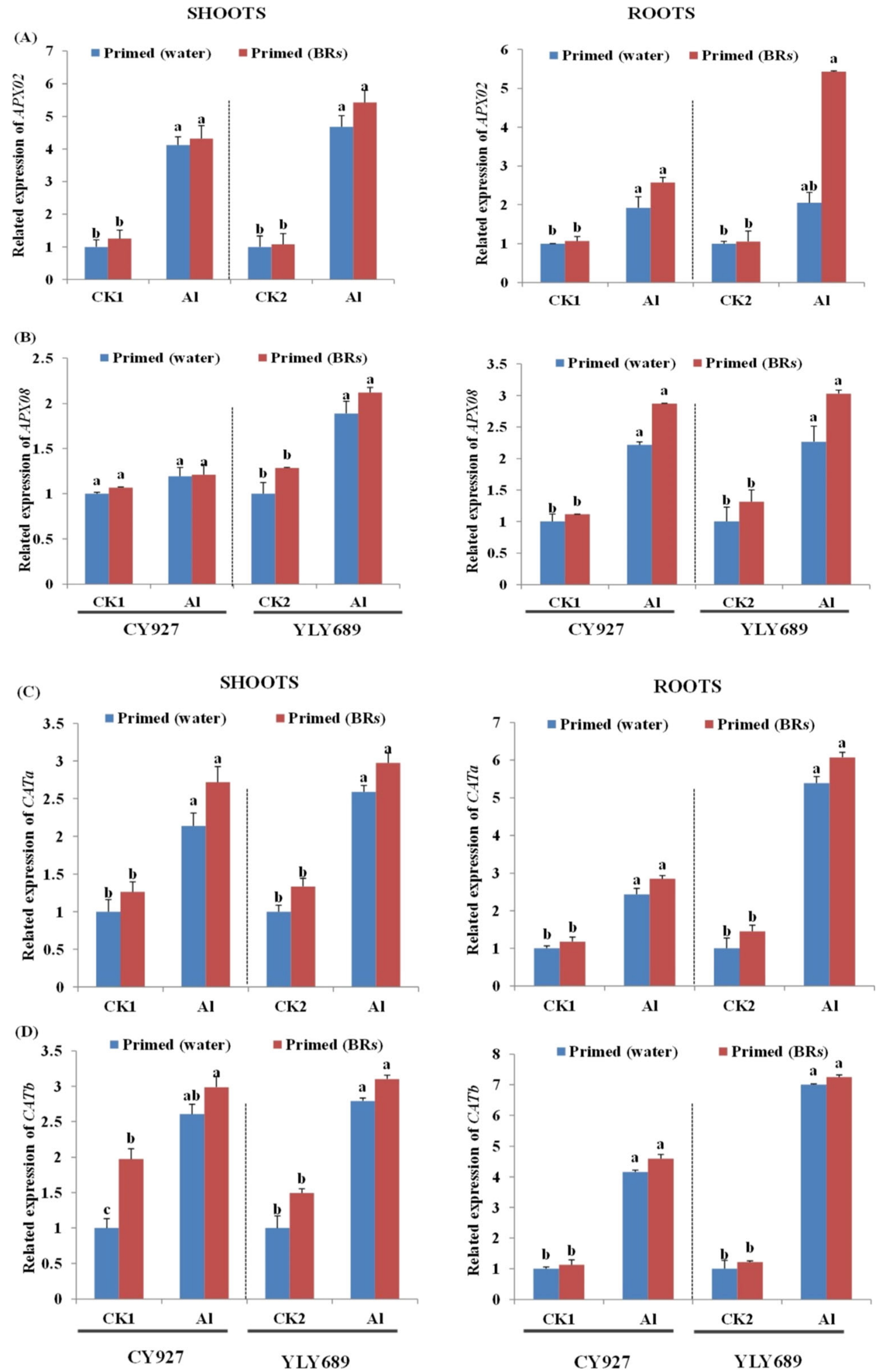

Fig. 6 Effect of seed priming 0.01-µM BRs on gene expression of **A** *APX02*, **B** *APX08*, **C** *CATa*, **D** *CATb*, **E** *SOD Cu-Zn*, **F** *SOD-Fe*$_2$ in shoots and roots of both cultivars of rice under toxicity of 400-µM Al

stronger shielding response to Al stress in rice seedlings (Tables 1–2). The concentration of Al in roots was higher than that in shoots (Tables 3–4). It could be due to the possibility that the roots are the main source of Al uptake, and the plants activate various mechanisms to minimize Al translocation from roots to shoots (Mendonça et al. 2003; Ali et al. 2008c).

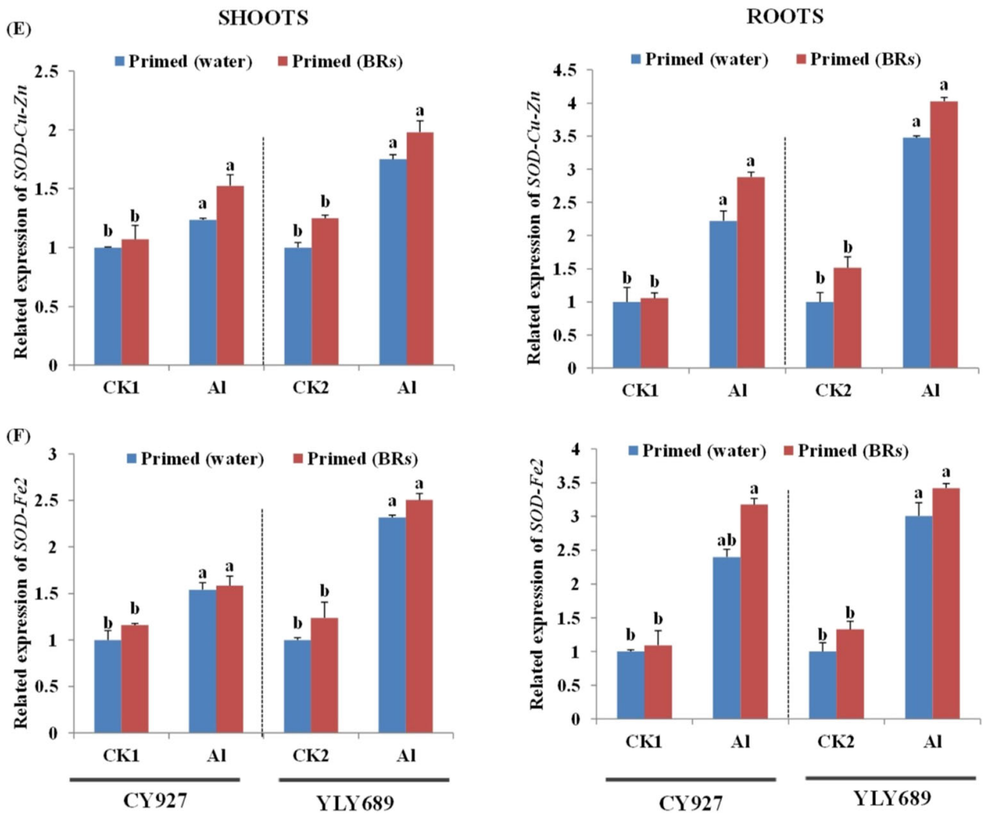

Fig. 6 continued.

Current study demonstrated that Al absorption was dramatically reduced in both cultivars, particularly in those plants primed with BRs over those primed with water. This reduction was more noticeable in tolerant variety (cv. YLY689). Furthermore, seed priming with BRs significantly maintained the nutrient balance in both cultivars. The regulation of

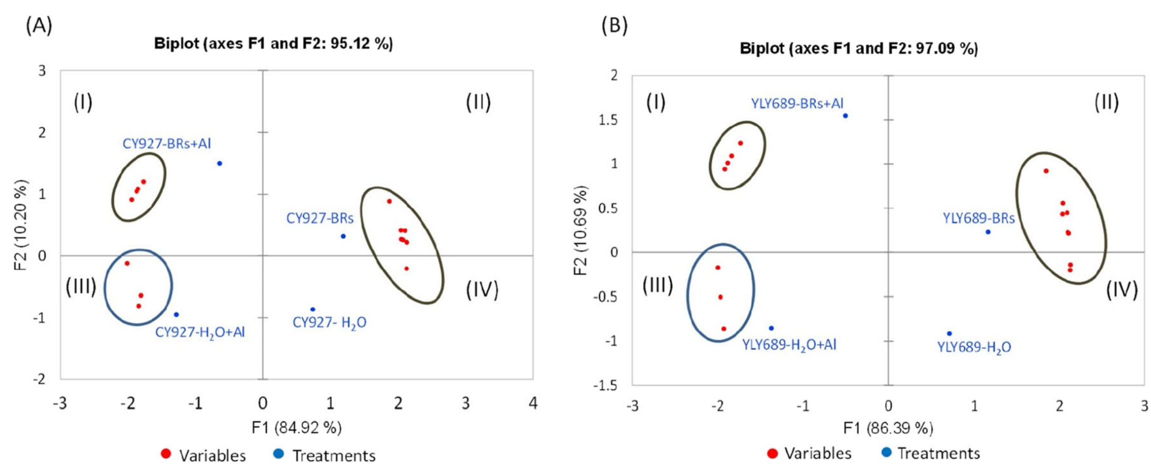

Fig. 7 Biplot of principle component of 1 and 2 of the PCA extracted from results obtained from physiological data of two different rice cultivars (CY927, YLY689) under various treatments such as control primed with water (CY927-H_2O, YLY689-H_2O), control primed with BRs (CY927-BRs, YLY689-BRs), seed primed with BRs, and treatment under Al stress (CY927-BRs+Al, YLY689-BRs+Al), seed primed with H_2O under Al stress(CY92-Al+ H_2O, YLY689-Al+ H_2O). Sharp angle represented positive, obtuse angle showed a negative correlation, as well as a right angle demonstrated a correlation between parameters. **A** Physiological parameters of rice variety CY927 illustration through Pearson's correlation coefficients under different treatments. (I) contains POD, CAT, APX, and SOD; (II) showed GI, F/W, D/W, GE, GP, VI, and SL; (III) illustrated MDA, MGT, and H_2O_2; while (IV) represented RL. **B** Physiological parameters of rice variety YLY689 representation via Pearson's correlation coefficients under different treatments. Distance between each circle represented the strength of correlation. (I) contains POD, CAT, APX, and SOD; (II) showed GI, F/W, D/W, GE, GP, and VI; (III) illustrated MDA, MGT, and H_2O_2; while (IV) represented RL and SL

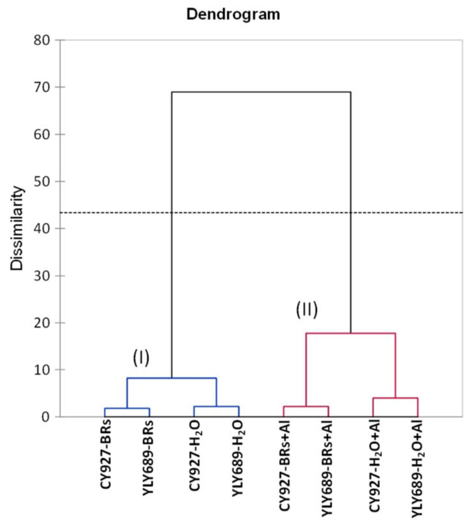

Fig. 8 Dendrogram of two different rice cultivars under various treatments obtained through agglomerative hierarchical clustering using ward's method on the basis of physiological traits

nutrient balance was more prominent in tolerant variety under Al stress. Consequently, both rice cultivars primed with BRs showed considerably increased biomass, root length, shoot length, fresh weight, and dry weight when compared to unprimed plants, where tolerant cultivar showed more pronounced rise in morphological parameters than sensitive cultivar (Tables 2–3). BRs seed priming further boosted photosynthetic pigments within both sensitive and tolerant varieties under Al toxicity (Fig. 3). This boost in photosynthetic pigments corresponds to better stress resistance behavior and alterations in plasma membrane as well as to activation of antioxidant enzymes under Al-stressed environment (Fig. 5) (Divi and Krishna 2009). Furthermore, BRs serve as a proton pump, enhancing water uptake; regulating gene suppression and activation, protein synthesis (Nawaz et al. 2017), and nucleic acid stimulation; and triggering antioxidant enzyme activity (Khripach et al. 2003). BRs-based modifications in plant behavior play a vital role in stimulating plant growth in stressful environments. According to a report, BRs relieve plants from stress by promoting plant growth, photosynthetic pigments, and water uptake (Rajewska et al. 2016). Similarly, the ameliorating roles of BRs in reducing cadmium toxicity in cowpea plants (Santos et al. 2018), salinity stress (Anuradha and Rao 2001, 2003), zinc metal stress in *B. juncea* (Arora et al. 2010), heat stress (Wu et al. 2014), and heavy metal stress (Anuradha and Rao 2007; Vardhini 2016; Vázquez et al. 2013) are well-established.

The results acquired from present investigations showed antioxidant enzyme activities, such as CAT, APX, and SOD, which serve as a defense system during plant stress periods and are raised under Al stress (Fig. 5) because of elevation in ROS (Jones et al. 2006; Yamamoto et al. 2003b). These results coincide with previously reported studies that suggested increase in SOD, CAT, and APX levels in sensitive and tolerant maize after exposure to Al treatment (Liu et al. 2008; Yamamoto et al. 2003a; Yamamoto et al. 2003b). After Al exposure, the activity of SOD, POD, APX, and CAT in tea plants increased (Ghanati et al. 2005). BRs induced greater antioxidant activities in both sensitive and resistant cultivars in the present study (Fig. 5). Antioxidant activities, such as SOD, POD, and CAT, are critical, and they are boosted by BRs after Al exposure to lessen the toxicity caused by Al (Ali et al. 2008a). BRs have been discovered to improve antioxidant enzymatic activity in stress situations to ameliorate various stresses (Nawaz et al. 2017; Rattan et al. 2020) and to regulate plant normal behavior in a number of studies (Ali et al. 2008b; Sharma et al. 2007). Al stress damages the membrane, resulting in a decrease in hydrolytic enzyme activity and an increase in ROS activity (Fig. 4). An increase in ROS activity is thought to cause significant damage to the cellular structure as well as macromolecules (Halliwell 1999). Under a stress situation, the use of BRs reduced H_2O_2 generation as well as MDA concentration (Fig. 4) to protect the plant's membrane from oxidative damage. It reduced the rate of superoxide radicle formation and boosted plant antioxidant activity (Anuradha and Rao 2007; Mazorra et al. 2002; Ogweno et al. 2008).

A more precise approximation of antioxidant genes to the behavior of antioxidant enzyme activities can be obtained by studying gene expression at the transcriptional level of plants during heavy metal stress. As a result, we did the expression profiling of a number of genes related to antioxidant activity in order to evaluate both enzymatic and transcriptional responses of both rice cultivars under normal as well as stressful conditions. Higher transcriptional levels of *APX02* and *APX08* were recorded in the present study (Yamamoto et al. 2001). Similarly, *APX* expression was triggered by high levels of H_2O_2 in tobacco chloroplasts under heavy metal stress (Gupta et al. 1993). Moreover, significant upregulation of *CAT* genes (*CATa* and *CATb*) was also observed in both roots and shoots in both cultivars. Previously, no significant change in *CAT* gene expression was observed in leaves of *Arabidopsis thaliana* under varying light qualities (Cominelli et al. 2008). It could have happened because there were several allo- or isozymes present. Al, on the other hand, induces a rise in *CAT* gene expression as a result of protein breakdown, resulting in transcriptional upregulation. Under Al stress, gene expression of *SOD-Cu-Zn* and *SOD-Fe$_2$* was increased, indicating that oxidative damage inside diverse cellular compartments was produced by Al toxicity. When compared to control, the pattern of gene regulation was different because its expression was more upregulated in roots than that in shoots in both cultivars. It is possible since the plant roots are the initial site of contact with the toxicity generated by Al stress. Furthermore, superoxide production, rather than lipoxygenase activity, appears to be the primary cause of oxidative stress in roots, despite H_2O_2 being an important competitor in leaves. However, the knowledge regarding whether

H_2O_2 is produced as a result of increased heavy metal content in the leaves or as a signal molecule from the roots is still elusive (Cominelli et al. 2008).

PCA is a multivariate method that is commonly used to categorize values based on biological state, quality, and origins. PCA is used to detect and categorize a big data collection into a small number of strongly associated variables (Aziz et al. 2018). Based on different treatments, agglomerative clustering hierarchy (ACH) revealed the interaction between different rice genotypes (Fig. 8). Using a combination of PCA and ACH, several treatments were used to separate the sensitive and tolerant genotypes and to show the association between various attributes based on physiological traits (Fig. 5). VI, F/W, D/W, SL, RL, GE, and GP were found to have a group and showed positive association with each other, but a negative relationship with MGT, H_2O_2, and MDA. On morphophysiological, biochemical, and molecular levels, this study adds to understanding the mechanistic response of both cultivars (CY927, YLY689) under Al stress, as well as seed priming with BRs under Al toxicity in metal-tolerant and metal-sensitive rice cultivars.

Conclusions

In the current study, Al has demonstrated phytotoxic effects on the physiological, antioxidant system, and molecular mechanism of rice seedlings, according to this study. Results of this study depicted that seed priming with BRs reduced the negative effects of Al on *O. sativa* seeds and improved both germination and early seedling growth in the presence of Al toxicity. The exposure of Al stress in rice plants boosted the antioxidant system (SOD, POD, CAT, and APX), which was substantially further activated by BRs. A transcriptional analysis of antioxidant genes in both cultivars demonstrated the similar pattern. As a result, it is possible that the improved resistance to Al in rice seedlings corresponded to the altered level of the antioxidant system. In this study, YLY689 was found to be a more resistant variety against Al stress than CY927. Furthermore, the use of BRs boosted resistance by improving plant growth, photosynthetic pigments, and other related processes in rice seedlings under Al stress.

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Author contribution FB, JH, and GY are involved in the conceptualization, and GY and FB in design experiment. FB and AJ performed the experiment and wrote the manuscript. HC, LZ, and LJ assisted in writing and editing the manuscript. CM, JH, and ZX perform statistical analysis. All authors read and approved the final manuscript.

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Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

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