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

Farwa Basit
Zhejiang University
Liu Jiaxin
Zhejiang University
An Jianyu
Zhejiang University
Chen Min
Zhejiang University
He Can
Zhejiang University
Zhu Xiaobo
Zhejiang University
Li Zhan
Zhejiang University
Hu Jin
Zhejiang University
Guan yajing (vcguan@zju.edu.cn)
Zhejiang University College of Agriculture and Biotechnology

Research Article

Keywords: Aluminum, Brassinosteroids, Rice, antioxidant enzyme activity, heavy metals, phytohormones

Posted Date: February 24th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-212038/v1

Version of Record: A version of this preprint was published at Environmental Science and Pollution Research on September 13th, 2021. See the published version at https://doi.org/10.1007/s11356-021-

16209-y.

Article

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

Farwa Basit[1a], Liu Jiaxin[1a], An Jianyu[1], Chen min[1], He Can[1], Zhu Xiaobo[1], Li Zhan[1], Hu Jin[1], Guan Yajing*

[1]Institute of Crop Sciences, College of Agriculture and Biotechnology, Zhejiang University, P.R, China

[a]These authors contributed equally to this work.

***Corresponding author**

Dr. Guan Yajing
Associate professor
College of Agriculture and Biotechnology,
Zhejiang University, P.R, China
Email ID: vcguan@zju.edu.cn

Abstract

Brassinosteroids (BRs) are growth-promoting hormones that exhibit high biological activities inside numerous plant species. BRs play a protective role in plants against various stresses. In the present study, seed priming of 24-epibrassinolide (0.01µM) was used to demonstrate the mitigation effect of Aluminum (400µM) in rice plants. BRs application was found effective in control beside plants under aluminum (Al) toxicity. It enhanced seed germination energy, germination percentage as well as root length, shoots length, fresh and dry weight as well under the stressed condition as well as inside control (primed with BRs). Although, Aluminum toxicity induced reduction in seed growth parameters, also in photosynthetic pigments, increased MDA content, and H_2O_2 production by enhancing the activities of antioxidative enzymes such as ascorbate peroxidase, catalase, and peroxidase in both roots and shoots. These changes were noticed higher inside tolerant variety (YLY-689) as compared to sensitive cultivar (CY-927). Interestingly, 24-EBL copped the stress through decreasing MDA content as well as H_2O_2 production by more stimulating antioxidant activities to modulate the stress condition under Al stress. Gene expression analysis of *SOD-Cu-Zn*, *SOD-Fe*, *CATa*, *CATb*, *APX02*, and *APX08* also supported the data related to antioxidant activities. These findings led us toward the conclusion that more induction of antioxidant activities with effective response toward other seed growth parameters with low uptake of Aluminum under 400µM Aluminum stress, 24-EBL was responsible for the mitigation of aluminum stress in rice seedlings.

Keywords: Aluminum, Brassinosteroids, Rice, antioxidant enzyme activity, heavy metals, phytohormones

1. Introduction

Rice is considered a more edible crop in most countries of Asia and its overall production is estimated 90% world widely (Dawe et al. 2010). China is one of the leading rice-producing countries. In southern China,

Rice is the main source to meet the hunger of underprivileged people who fulfill their nutrients from rice uptake (Huang et al. 2013). Lately, soil pollution concern is arising due to industrialization and heavy metal contamination such as cadmium (Cd), mercury (Moghaddasi et al.) and lead (Pb) in soil, water and air (Fu &Kane 2008) is the biggest issue inside all over the world Aluminum is one of the most growths inhibiting factor inside acidic soils. Almost 30-50% of soil is polluted with aluminum word widely and 21% inside China as well (Agha et al. 2018). It directly causes influences root length as well as affects membrane lipids and other peroxidation like Fe (Mannon et al. 2004). It increases the peroxidation of lipids and enhances the action of antioxidant enzymes such as catalase, peroxidase, superoxide dismutase, and glutathione reductase (Liu et al. 2003) that lead to the plant stress stage. It is investigated that Al toxicity is also observed in shoots which are observed as an upshot of root system damage (Vitorello et al. 2005). There are some nastiest effects caused by Al toxicity in plants such as water relation, reduces stomatal opening, reductions of photosynthetic activity besides it; reasons chlorosis as well as necrosis of leaves. Nevertheless, it surges the level of proline (Nandi &Neogy 2002) which performs itself as an osmoprotectant, membrane stabilizer as well as ROS vulture (Apel &Hirt 2004).

Brassinosteroids (BRs) are polyhydroxy steroidal phytohormones that have great capability to ardently demonstrate the plant growth-promoting effect (Latha &Vidya Vardhini 2018). It was firstly discovered in the rape plant, Brassica napus pollen based on its capability to stimulate growth (Leaska 1970). Afterward, 70 various types of BRs steroidal growth-promoting hormones were identified from virtually all plants like gymnosperms, monocots, dicots, pteridophytes, and alga from various parts of plants such as pollen, flower buds, fruits, seeds, vascular cambium, leaves, shoots, and roots (Bajguz &Hayat 2009, Haubrick &Assmann 2006, Piotrowska-Niczyporuk &Bajguz 2014). Wide research on BRs indicates that it plays an important role to mitigate various plant stresses including biotic and abiotic stresses (Wu et al. 2014).

24-Epibrassinolide is the most biologically active BR compound that is involved in developmental processes, cell division, elongation, gene expression, and vascular differentiation, etc. (Bergonci et al. 2014). It improves the plant growth by enhancing the chlorophyll contents which have a crucial role to increase photosynthetic capability, improves antioxidant system capacity, surges enzymatic activity, and up-regulates stress response genes [superoxide (SOD), peroxide (POD), catalase (CAT), glutathione reeducates (GR) and ascorbate peroxide (APX)] (Liu et al. 2016, Yuan et al. 2012). Various mutants of BRs showed many kinds of growth flaws such as dwarfism, deep green leaves, late flowering, and male sterility (Chakraborty et al. 2015, Hou et al. 2017) in ideal plants Arabidopsis and Brassica (Clouse et al. 1996, Russinova et al. 2004). These investigations specify that BR has an optimistic response toward numerous types of stresses as well as it stimulates various physiological and molecular mechanisms to improve plant growth by inducing stress tolerance.

The current study is aimed to deliberate the role of Brassinosteroids in lessening aluminum stress in rice plants as well as to drill the association of antioxidant system and capability of brassinosteroids to produce resistance against aluminum stress in rice plants. In this study, the application of brassinosteroids (EBL) to alleviate the aluminum stress in rice plants is scrutinized

2. Materials and methods

2.1. Brassinosteroids (BRs) preparation

24-Epibrassinolide (EBL) was obtained from the Institute of crop science, Zhejiang University, China. The BR was liquefied in an adequate quantity of ethanol and a stock solution of 10^{-5} M was prepared by adding ddH_2O comprising 0.05% Tween-20 as a surfactant.

2.2. Plant materials and growth conditions

89 The seeds of two cultivars of *Oryza sativa, L.* (cvs. CY-927 and YLY-689) were obtained from the Center
90 of Seed Science, College of Agriculture and Biotechnology, Zhejiang University, China. Seeds were surface
91 sterilized by using 0.5% NaClO solution for 15 minutes and then washed several times through tap water
92 followed by washing with sterilized distilled water thrice to eradicate the smidgens of the disinfectant. Priming
93 of sterilized seeds was done at 15 °C in darkness for 24 hrs with 0.01µM BRs. Then, seeds were dried back to
94 their original moisture contents at room temperature. The unprimed dry seeds were used as control (CK). After
95 priming, seed germination tests were carried out. Fifty seeds were used for each treatment positioned in a plastic
96 germination box (12 cm × 18 cm) as well as each treatment was replicated three times. Then, incubation of
97 seeds was carried out in a germination chamber at 25 °C under an interchanging cycle of 8 hr lighting and 16 hr
98 darkness for 14 days (Zeng et al. 2006). The incubated seeds were treated with 400µM concentration of
99 Aluminum with nutrient media solution. The composition of nutrient solution was 0.5µM $MKNO_3$, 0.5 µM
100 $MCa(NO_3)_2$, 0.5µM $MgSO_4$, 2.5µM MKH_2PO_4, 2.5 µM NH_4Cl, 100µM Fe–K–EDTA, 30µM MH3BO_3, 5µM
101 $MnSO_4$, 1µM $CuSO_4$, 1µM $ZnSO_4$ and 30µM H_3BO_3, 5µM $MnSO_4$, 1µM $CuSO_4$, 1µM $ZnSO_4$ and 1µM
102 $(NH_4)_6Mo_7O_{24}$ per liter. The pH of the nutrient solution was adjusted to 5.0 with HCl and NaOH. The aluminum
103 concentration was based on findings from a primary experiment which was conducted on the bases of various
104 concentrations of Aluminum i.e. 0, 100, 200, 300, 400, 500, 600, 700, and 800µM. The aluminum
105 concentrations (100-300) µM exhibited slight damage to plant growth. Although, aluminum concentration
106 400µM was exhibited substantial damage to plant growth. However, concentrations greater than 500µM were
107 excessively toxic for the growth of the plant.

108 **2.3. Measurement of physiological parameters**

Counting of germinated seeds was carried out daily for 14 days. Total germinated seeds were counted on the 5th day of germination and were considered as germination energy. Then, the germination percentage was calculated on day 14th. Germination Index, Mean Germination Time, as well as Vigour Index, was carried out by following formulas (Hu et al. 2005)

$$GI = \Sigma(Gt/Tt) \quad (1)$$

$$MGT = \Sigma(Gt \times Tt)/\Sigma Gt \quad (2)$$

$$VI = \text{Germination (\%)} \times [\text{Shoot length (Clouse et al.)} + \text{Root length (Clouse et al.)}] \quad (3)$$

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

2.4. Experimental design and treatment pattern

The two-week-old seedlings (primed with water as well as primed with 0.01µM 24-epibrassinolide (EBL)) were treated with a 400µM concentration of aluminum. The way of the experimental pattern was wholly randomized design (CRD) in addition to the position of the pots, inside the growth chamber was altered every day. The plants were sampled at 21 days to make the various observations.

2.5. Plant growth investigation

The plants were detached and immersed in a bucket, occupied with water, to confiscate the smidgens of the disinfectant, confirming the security of roots. The plants were impaired and the lengths of roots and shoots were measured, followed by their later weighing to record their fresh mass. The roots in addition to shoots were formerly dried out in an oven, run at 80 ºC for 24 hr, and assessed to record their dry mass.

2.6. Measurement of photosynthetic pigments

Investigation of photosynthetic pigments such as chlorophyll-a, b, and total chlorophyll was determined by following the methodology of Hartmut *et al*. In short, Fresh leaf tissues (0.2 g) were standardized in 3 mL ethanol (95%, v/v). The homogenate was centrifuged at 5000 g for 10 min and then, the supernatant was extracted. 9 mL ethanol (95%, v/v) was further supplemented with 1 mL aliquot of the supernatant. Afterward, the mixture was determined by observing the absorbance at the wavelengths 665, 649, and 470 nm through an exhausting spectrophotometer (LICHTENTHALER &Wellburn 1983). The following equations were utilized for the cunning of pigment amounts:

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

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

$$\text{Total chlorophyll content} = Ca + Cb \quad (6)$$

The quantities of pigments were premeditated as milligrams per liter of plant excerpt.

2.7. Measurement of metal contents in plant tissues

Aluminum scrutiny was performed on dried roots and shoots. Dry plant samples (0.2 g) for each treatment, were assimilated by using 5 mL concentrated HNO_3 and $HCLO_4$ (5:1, v/v) on a hot plate at 70ºC for almost 5 hr. The digested sample was diluted with 2% HNO_3 to a final volume of 10 ml and sifted. The filtrate was used for the analysis of Al and the microelements Fe^{2+}, Zn^{2+}, Mn^{2+}, and macro elements calcium (Ca^{2+}), potassium (K^{+}), and magnesium (Mg^{2+}) with an atomic absorption spectrometer (iCAT-6000-6300, Thermo Scientific, USA) (Khan et al. 2013).

2.8. Measurement of MDA contents and H_2O_2 measurements

MDA concentration was investigated as 2-thiobarbituric acid (TBA) volatile metabolites 20. Approximately 1.5 mL excerpt was homogenized in 2.5 mL of 5% TBA prepared in 5% trichloroacetic acid (TCA). The mixture was intense at 95 °C for 15 min, besides then hastily chilled on ice. Afterward, centrifugation at 5,000 g for 10 min was carried out; the absorbance of the supernatant was calculated at 532 nm. Amendment of nonspecific turbidity was through by subtracting the absorbance value measured at 600 nm. The concentration of MDA was intended in terms of (nmol mg^{-1} protein). To measure the concentration of hydrogen peroxide (H_2O_2), shadowed the protocol designated by (Velikova et al. 2000).

2.9. Antioxidant enzyme activity assay

Fresh samples (0.5 g) of both shoots and roots were homogenized in 8 ml of 50mM potassium phosphate buffer (pH 7.0 containing 1mM $EDTANa_2$ as well as 0.5% PVP W/V) on ice. Then and there, centrifugation of the homogenate was done for 20 minutes at 12000rpm at 4ºC. Supernatants were unruffled in discrete tubes and stored at 80ºC. The method of Giannopolitis & Ries, (1977) was followed to examine the activity of superoxide dismutase (SOD). Peroxidase (POD) activity was assessed as designated by (Dobson &Wilson 1992) exhausting the elimination coefficient 25.5 mM-1 cm-1. Catalase (CAT) activity was inspected conferring to (Aebi, 1984) by the extermination constant of 39.4 mM-1 cm-1, whereas for the fortitude of ascorbate peroxidase (APX) activity scrutiny was done by the method of (Nakano &Asada 1981).

2.10. RNA extraction and gene expression analysis

Antioxidant gene expression was scrutinized by quantitative real-time PCR (qRTPCR). Total RNA was extracted grounded on the Trizol reagent as described by Gunia, Barnes, & Sah, (2014). For cDNA synthesis, reverse transcription from 1 μg of total RNA through PrimeScript™ RT reagent kit was utilized as well as

q-PCR was performed according to (Zhang et al. 2016). *OsActin* was used as an inner standard. Primers used for qPCR are specified in Table S1.

2.11. Statistical Analysis

One-way analysis of variance treatments with the least significant differences (LSD) was applied as a posthoc test at a 95% assurance interlude amongst numerous data sets, using SPSS v16.0 (SPSS, Inc., Chicago, IL, USA). Three samples for one treatment were conducted from three different pots. Variance (Oberdörster et al.) analysis was done through Duncan's multiple range tests amongst the treatment's mean to conclude the significant difference at $p < 0.05$ and 0.01 levels between mean values. Principle component analysis (PCA) and Agglomerative hierarchical clustering (AHC) was accomplished to scrutinize the classification of two different cultivars of rice grounded on their vulnerability toward Al by using XLSTAT.

3. Results

3.1. Supplement of BRs significantly enhanced seed vigor and plant growth

The current study has demonstrated that germination energy, percentage, vigor index, and germination index were significantly declined under 400µM Al toxicity in both rice cultivars as compared to control (Table 1). More reduction was observed in cultivar CY-927 as compared to cultivar YLY-689. As concerned, priming with 0.01µM concentration of BRs resulted in the resistance against 400µM Al stress as well as it significantly enhanced the germination energy, percentage, vigor index, and germination index under toxicity as compared to the unprimed seeds. The latent study demonstrated that mean germination time is reduced with priming of 0.01µM BRs under toxicity of 400µM Al in both cultivars (Table 1).

Disclosure of Al has shown the phenotypically changes in the shoot as well as root length in both cultivars (Fig. 1, Fig. 2). It was observed that there was a significant difference between shoot length, root length, fresh

191 weight, and dry weight at 400µM Al exposure in both cultivars. More reduction is observed in cultivar CY-927
192 as compared to cultivar YLY-689 and significant improvement in shoot length, root length, fresh weight, and
193 dry weight under 400µM Al exposure were noticed with seed priming with 0.01µM BRs (Table 2).

194 **3.2. Seed Priming with BRs significantly increased photosynthetic pigments**

195 The latent study represented that alone treatment of Al caused a significant reduction in Chl a, b and total
196 chlorophyll contents as compared to control. (Fig. 3). This reduction inside photosynthetic pigments was
197 observed in both cultivars under Al stress. The decrease was more noticeable in CY-927 as compared to
198 YLY-689. Seed priming with 0.01µM BRs increased photosynthetic pigments such as Chl a, b, and total
199 chlorophyll contents as compared to control inside both treatments alone as well as under 400µM Al stresses in
200 both cultivars. Plants treated with BRs alone exhibited more photosynthetic pigment than control in both
201 cultivars (Fig. 3).

202 **3.3. Supplement of BRs reduced Al accumulation under Al stress**

203 Roots are the main part that interacts first with heavy metals and the main source of uptake for a nutrient
204 solution as well as heavy metals. A recent study revealed that Al uptake was more in those plants which were
205 primed by water as compared to the plants primed with 0.01µM BRs under Al toxicity. In roots, Al
206 accumulation was observed higher as compared to shoots (Table 3-4). More Al accumulation was pragmatic in
207 cultivar YLY-689 as compared to CY-927. More interestingly, it was observed that K, Ca, Fe and Mn contents
208 decreased by exposure of Al toxicity in both roots and shoots whereas Zn was increased. The same trend was
209 noticed in both cultivars (Table 3-4).

210 **3.4. Supplement of BRs ameliorated Al generated oxidative stress**

The presence of Al caused an enhancement in MDA contents in both cultivars as compared to control and also increased the production of H_2O_2. This increase was observed more significant in CY-927 as compared to YLY-689 cultivar. The application of BRs seed priming reduced the MDA contents as well as H_2O_2 production significantly inside both cultivars (Fig. 4). The MDA contents were observed higher in shoots as compared to roots under alone treatment of Al was noticed 64.7% in CY-927 and 55.4% in YLY-689. Whereas it was observed 56% and 42% in roots respectively. Moreover, 44.5%, 45% in shoots, and 26%, 39.7% decreased were recorded in roots of both cultivars under primed seeds with 0.01µM BRs respectively.

3.5. Determination of Antioxidant enzyme activities

Under alone treatment of Al, stimulated behavior of antioxidant activity was noticed. A recent investigation showed that under 400µM Al concentration; SOD, CAT, POD as well as APX was enhanced more in stressed plants as compared to the control and this effect was observed higher in YLY-689 than CY-927 (Fig. 5). These activities were observed higher in roots as compared to shoots. SOD activity under alone treatment of Al was noticed at 22.5% in CY-927 and 43.6% in shoots of YLY-689 cultivar. Whereas it was observed 47.7% and 58.2% in roots respectively. Interestingly, 44.6%, 46.3% in shoots, and 57.4%, 58.2% increment were recorded in roots of both cultivars under primed seeds with 0.01µM BRs respectively. For CAT activity, under separate treatment of Al was noticed 45% in CY-927 and 59% in YLY-689. Whereas it was observed 39% and 45% in roots respectively. Stimulatingly, 51%, 61% in shoots, and 46%, 58% increment were recorded in roots of both cultivars under primed seeds with 0.01µM BRs respectively. In the presence of Al alone, POD and APX were also noticed higher but with BRs priming this effect was observed further greater however POD activity was not

231 more enhanced. Under Al treatment, 14.8% in CY-927, 37.4% in YLY-689 POD was recorded in shoots as well
232 as 30.2% and 46.8 % were observed in roots respectively (Fig. 5).

233 **3.6. Determination of gene expression analysis**

234 A significant difference was noted in the expression of *APX02* in both roots and shoots as compared to
235 control inside both cultivars. Transcriptional level of *APX02* was higher under the stress of 400µM Al as well as
236 with the treatment of 0.01 µM BRs under 400µM toxicity. Expression of *APX02* was higher in the YLY-689
237 cultivar as compared to the CY-927 rice cultivar ($p < 0.01$). Interestingly, the transcriptional level of *APX02* in
238 plants that were treated with seed priming of 0.01 µM BRs were higher than stressed plants and data was
239 supporting the results of APX activity (Fig. 5). Correspondingly, the transcription level of *APX08* was also
240 prominent in both roots and shoots inside both cultivars as compared to control but inside roots, the expression
241 level of *APX08* was observed higher in YLY-689 as compared to CY-927 cultivar (Fig. 6a). Additionally, gene
242 expression of *CATa* and *CATb* in both cultivars is observed higher in both roots and shoots as well as compared
243 to the gene expression level of the control condition. It was investigated that increase was more pronounced
244 inside YLY-689 as compared to CY-927 cultivars. As a result, under µM Al stress, the gene expression level of
245 *CATa* and *CATb* was higher in both roots and shoots in both cultivars as compared to the control (Fig. 6b).
246 Significant up-regulation of *SOD Cu-Zn* and *SOD-Fe*$_2$ gene expression was observed in both cultivars under
247 stressed conditions but it was noticed higher in YLY-689. *SOD Cu-Zn* and *SOD-Fe*$_2$ gene transcriptional level
248 was observed higher in roots as compared to shoots. *SOD Cu-Zn* gene transcriptional level was more clearly
249 up-regulated in roots as compared to control condition irrespective of 400µM Al stress (Fig. 6c). Interestingly,
250 seeds primed with 0.01µM BRs showed higher expression of *SOD Cu-Zn* and *SOD-Fe*$_2$ genes as compared to
251 seeds primed with water. It may be happened to modulate the stress condition inside both cultivars by

252 up-regulating specific genes expression momentarily irrespective of 400μM Al toxicity concentration (Fig. 6c).
253 Our data also supports the resultant behavior of antioxidant activities (Fig. 5). It clearly showed that 0.01μM
254 BRs have a significant role to cope with the stress condition as compared to control plants by modulating and
255 regulating the transcriptional expression level of specific genes

256 **3.7. Determination of cluster and correlation analysis between observations**

257 Based on physiological traits of two different rice varieties; biplot graphs of Principle component analysis were
258 constructed to investigate the sensitive and tolerant groups through F1 and F2 of numerous parameters under
259 distinct treatments for example control primed with water (CY927-H2O, YLY689-H2O), control primed with
260 BRs (CY927-BRs, YLY689-BRs), seed primed with BRs, and treatment under Al stress (CY927-BRs+Al,
261 YLY689-BRs+Al), seed primed with H2O under Al stress(CY92-Al+ H2O, YLY689- Al+ H2O) (Fig7A-C).
262 MDA, MGT, and H2O2 were grouped and represented a positive correlation between each other. Although,
263 MGT, H2O2, and MDA showed a significantly negative correlation with V.I, F/W, D/W, S.L, R.L, G.E, and
264 G.P but simultaneously exhibited a negative correlation with SOD, POD, CAT, and APX as well. A similar
265 trend was noticed in both varieties (Fig. 7A, and B). PCA analysis of both cultivars (CY927 and YLY689)
266 demonstrated that YLY689 is a tolerant genotype to Al and CY927as a sensitive genotype. It demonstrated the
267 maximum contribution of F1 (84.92) followed by F2 (10.20), with total contribution of 95.12% in CY927 and
268 for YLY689 the maximum contribution of F1 (86.39) followed by F2 (10.69), with total contribution of 97.09%
269 is noticed. ACH outcomes also confirmed the same response of both varieties under distinct treatments (Fig.
270 7C). It represented the close correlation between both cultivars (CY927 and YLY689) primed with BRs as well
271 as primed with water than cultivars primed with water under Al stress. Cultivars primed with BRs under Al

stress showed a close correlation with both controls (primed with water and BRs) as compared to plants primed with water under Al toxicity (7C).

Discussion

Aluminum is more 3rd abundant element in the earth's crust which is present in high quantity but slightly available in a soluble form which causes severe damage to the biological systems (da Silva Leite 2012). At pH less than 5.5, Al is present in an available form which can cause toxicity in plants especially inhibit the roots of plants; (Barcelo &Poschenrieder 2002) reduce shoots length, fresh weight, and dry weight (Table 2) because it has direct exposure to roots and cause inhibition of cell elongation at an early stage and later on cause damage in plants growth and development as well (Čiamporová 2002, Silambarasan et al. 2019). Al also causes nutritional imbalance inside plant because the intrusion in water retention besides membrane permeability (Olivares et al. 2009) such as in recent study; K, Ca, Fe and Mn contents decreased by exposure of Al in both roots and shoots (Table 3-4). Saliva *et al*., (2010) reported that K, Ca, Fe and Mn contents were reduced by the toxicity generated by Al in rice plants as its effect was more stated in sensitive variety as compared to tolerant. In tomato and maize, Al caused inhibitory effects on K, Ca, Fe, and Mn contents in both roots and shoots (Castro et al. 2010, Simon et al. 1994). As a consequence of it, photosynthetic pigments and associated processes are also affected (Fig. 3) resultant in a caused reduction in plant growth by Al exposure (Table 2). Nevertheless, seeds treated with brassinosteroids (24 EBL) priming showed better response toward Al stress resistance as compared to seeds primed with water (Table 1-2) and enhanced the photosynthetic pigments inside both sensitive, as well as tolerant variety under Al toxicity (Fig. 3) due to the brassinosteroids, stress resistance behavior in amendments and manipulations in plasma membrane under stressed environment (Hamada &Tsuruo 1986) as well as with stimulating the antioxidant enzyme activity (Fig. 5) Moreover, Brassinosteroids act as a proton pump which can enhance water uptake (Mei et al. 2005), regulate suppression and up-regulation

294 of genes, protein synthesis (Brand et al. 2003) besides nucleic acid stimulation as well as improvement in
295 antioxidant enzyme activity (Khripach et al. 2003). All of these modified effects of brassinosteroids play an
296 important role to stimulate plant growth under stressed conditions. It was reported that Al reduces the stress
297 effect on plants and improves plant growth as well as photosynthetic pigments and water uptake (Abdullahi et
298 al. 2003, Rajewska et al. 2016). Likewise, It inhibited toxicity caused by cadmium in cowpea plants (Santos et
299 al. 2018), reduced salinity stress (Guinney et al. 2015, O¨ zdemir &Edwards 2004), Chromium stress in
300 Brassica Juncea L. (Arora et al. 2010), temperature stress (Di Angelantonio et al. 2016, Finlayson &Van der
301 Valk 1995), drought (FILOVÁ 2014, Talaat &Shawky 2016, Talaat et al. 2015) and heavy metal stresses (Li et
302 al. 2016, Singhal et al. 2015, Swamy et al. 2014) as well by improving antioxidant system.
303 The results obtained from present studies that antioxidant enzyme activities such as catalase (CAT), ascorbate
304 peroxidase (APX), superoxide dismutase (SOD) which acts as a defense system during plant stress periods are
305 increased by aluminum stress (Fig. 5) because of interference inside ROS activity (Jones et al. 2006, Yamamoto
306 et al. 2003b). In a likewise recent study, SOD, CAT, and APX have increased in both sensitive as well as
307 tolerant varieties in maize after exposure to Al treatment alone (Liu et al. 2008, Yamamoto et al. 2003a). In
308 tobacco plants, SOD, POD, APX, and CAT activity was increased after Al disclosure (Ghanati et al. 2005).
309 Interestingly, in latent investigation brassinosteroids stimulated more antioxidant activities in both pensive and
310 resistant cultivars (Fig. 5). The role of antioxidant activities i.e. SOD, POD, and CAT, etc. is very important and
311 it is enhanced by BRs after Al exposure to reduce the toxicity induced by Al (Padmanabhan et al. 2010). It's
312 found consistent in various studies that BRs increase the antioxidant enzymatic activity in stress conditions to
313 mitigate various stresses and to (Arora et al. 2008, Bajguz &Hayat 2009) regulate plant's normal behavior (Ali
314 et al. 2008, Sharma et al. 2007). Al stress causes damage inside the membrane as a result hydrolytic enzyme
315 activity reduces and causes enhancement of ROS activity (Fig. 4). It is strongly believed that an increase in ROS

316 activity causes severe damage inside cellular structure as well as to macromolecules (Halliwell 1999). It is noted
317 that the application of BRs reduces H_2O_2 production as well as MDA content under a stress environment (Fig.
318 4) to protect the plant's membrane from oxidative damage. It lessens the rate of superoxide radicles and as a
319 result enhances the antioxidant activity in plants (Singh et al. 2008).
320 Gene expression study by checking the transcriptional level of plants under heavy metal stress provides a more
321 precise approximation of antioxidant genes toward the behavior of antioxidant enzyme activities. Hence, we
322 investigated multiple genes related to antioxidant activities to estimate both enzymatic as well as transcriptional
323 responses of both rice cultivars under stress conditions as compared to control. Transcriptional levels of *APX02*
324 and *APX08* were higher in our study. This expression has gone toward up-regulation because of NaCl treatment
325 (Matsumoto et al. 2001). Likewise, APX transcripts influence was up-regulated through augmented levels of
326 H_2O_2 in tobacco chloroplasts as consequences of *Cu-Zn*-superoxide dismutase overexpression (Gupta et al.
327 1993). Moreover, significant up-regulation of catalase genes (*CATa* and *CATb*) was also observed in both roots
328 and shoots inside both cultivars. There is no significant change in catalase gene expression of leaves were
329 investigated in Arabidopsis thaliana (Mol et al. 2008). It may have occurred because of the presence of multiple
330 allo- or isozymes. In contrast, Al causes an increase of catalase gene expression because of the breakdown of
331 proteins which leads to up-regulation of the transcriptional level. In the present investigation, it is also examined
332 that gene expression *SOD-Cu-Zn* and *SOD-Fe*$_2$ were higher under Aluminium stress which indicated that
333 oxidative damage inside various cellular compartments were induced by Al toxicity. The pattern of gene
334 regulation was different because its expression was more up-regulated in roots as compared to shoots in both
335 cultivars as compared to control. It may happen because roots are the plant's primary interaction point which
336 first interacts with toxicity caused by Al stress. These interpretations reinforced the opinion of Smeets *et al*. who
337 specified that the fundamental mechanism of oxidative stress was dissimilar in the roots as compared to leaves.

Furthermore, the generation of superoxide besides the lipoxygenase activity is the foremost reason for oxidative stress inside roots, although inside the leaves H_2O_2 was appeared to be an imperative contestant. However, H_2O_2 was formed close by as a product of augmented Cd content of the leaves, or maybe it attained as an indication from roots, rests to be illuminated (Mol et al. 2008).

Principle component analysis (PCA) is a multivariate method which frequently used to categorize the values based on various biological statuses, quality as well as origins. To identify and categorize the large data set into a small number of vigorously correlated variables PCA is utilized (Shan et al. 2013). ACH disclosed the interaction between different genotypes of rice-based on distinct treatments (Fig 7C). On physiological traits based, various treatments were utilized to distinguish the sensitive and tolerant genotype and to represent the correlation between various traits by using the amalgamation of both PCA and ACH (Fig. 5). V.I, F/W, D/W, S.L, R.L, G.E, and G.P were showed a group and significantly positive correlation between each other but instantaneously negative relation with MGT, H2O2, and MDA. This investigation contributes to cognize the response mechanism of both cultivars (CY927, YLY689) under Al stress as well as seed priming with BRs under Al toxicity in both varieties on morphophysiological, biochemical, and molecular bases.

Conclusion

The latest study investigated the clear phytotoxic effect of aluminum (Al) on the physiological, antioxidant system as well as on the molecular mechanism of rice seedling. Data exposed that seed priming with brassinosteroids (BRs) mitigated the venomous effect of aluminum to *Oryza sativa* seeds besides enhanced both germination as well as early seedling growth under toxicity of Al. Antioxidant system (SOD, POD, CAT, and APX) was increased by the response of Al stress in rice plants which was remarkably further persuaded by BRs. Furthermore, a transcriptional study of antioxidant genes also confirmed the same pattern in both cultivars. Consequently, it may be suggested that the modified level of the antioxidant system, however in part, was the

reason for the improvement of resistance level against Al in rice seedlings. A recent study illustrated that YLY-689 proved as a tolerant variety as compared to CY-927 against Al toxicity. Furthermore, the application of BRs increased the degree of resistance by enhancing plant growth, photosynthetic pigments, and other associated processes under aluminum stress inside rice seedlings.

Author contribution

FB, HJ, and GY involved in conceptualization, GY and FB design experiment. FB, AJ, LZ performed experiment and writing manuscript. HC, LJ and CM writing and editing assistance the manuscript. HJ and ZX perform statistical analysis. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding

The authors did not receive support from any organization for the submitted work.

Data availability statement

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

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

Abdullahi I, Winter S, Atiri G, Thottappilly G (2003) Molecular characterization of whitefly, Bemisia tabaci (Hemiptera: Aleyrodidae) populations infesting cassava. Bull Entomol Res 93:97

383 Agha RA, Borrelli MR, Farwana R, Koshy K, Fowler AJ, Orgill DP, Zhu H, Alsawadi A, Noureldin A, Rao A
384 (2018) The SCARE 2018 statement: updating consensus Surgical CAse REport (SCARE) guidelines.
385 Int J Surg 60:132-136
386 Ali M, Schiedt B, Healy K, Neumann R, Ensinger W (2008) Modifying the surface charge of single
387 track-etched conical nanopores in polyimide. Nanotechnology 19:085713
388 Apel K, Hirt H (2004): Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu.
389 Rev Plant Biol 55:373-399
390 Arora A, Ceccagnoli M, Cohen WM (2008) R&D and the patent premium. INT J IND ORGAN 26:1153-1179
391 Arora P, Bhardwaj R, Kanwar MK (2010) 24-Epibrassinolide induced antioxidative defense system of
392 Brassica juncea L. under Zn metal stress. PHYSIOL MOL BIOL PLA 16:285-293
393 Bajguz A, Hayat S (2009) Effects of brassinosteroids on the plant responses to environmental stresses. Plant
394 Physiol Biochem 47:1-8
395 Barcelo J, Poschenrieder C (2002) Fast root growth responses, root exudates, and internal detoxification as
396 clues to the mechanisms of aluminium toxicity and resistance: a review. ENVIRON EXP BOT
397 48:75-92
398 Bergonci T, Ribeiro B, Ceciliato PH, Guerrero-Abad JC, Silva-Filho MC, Moura DS (2014) Arabidopsis
399 thaliana RALF1 opposes brassinosteroid effects on root cell elongation and lateral root formation. J.
400 Exp Bot 65:2219-2230
401 Brand S, Felner R, Shim M, Seitsinger A, Dumas T (2003) Middle school improvement and reform:
402 Development and validation of a school-level assessment of climate, cultural pluralism, and school
403 safety. J Educ Psychol 95:570
404 Castro SSd, Abdala K, Silva AA, Bôrges VMS (2010) A expansão da cana-de-açúcar no cerrado e no estado
405 de Goiás: elementos para uma análise espacial do processo.
406 Chakraborty N, Sharma P, Kanyuka K, Pathak RR, Choudhury D, Hooley R, Raghuram N (2015) G-protein
407 α-subunit (GPA1) regulates stress, nitrate and phosphate response, flavonoid biosynthesis, fruit/seed
408 development and substantially shares GCR1 regulation in A. thaliana. Plant Mol Biol
409 89:559-576
410 Čiamporová M (2002) Morphological and structural responses of plant roots to aluminium at organ, tissue,
411 and cellular levels. Biol Plant 45:161-171

412 Clouse SD, Langford M, McMorris TC (1996) A brassinosteroid-insensitive mutant in Arabidopsis thaliana
413 exhibits multiple defects in growth and development. Plant Physiol 111:671-678
414 da Silva Leite SA (2012): Afetividade nas práticas pedagógicas. Temas em psicologia 20:355-368
415 Dawe D, Pandey S, Nelson A (2010) Emerging trends and spatial patterns of rice production. Rice in the
416 global economy: strategic research and policy issues for food security. Los Banos, Philippines: Int
417 Rice Res (IRRI)
418 Di Angelantonio E, Bhupathiraju SN, Wormser D, Gao P, Kaptoge S, de Gonzalez AB, Cairns BJ, Huxley R,
419 Jackson CL, Joshy G (2016) Body-mass index and all-cause mortality: individual-participant-data
420 meta-analysis of 239 prospective studies in four continents. Lancet 388:776-786
421 Dobson J, Wilson M (1992) Sensitization of oral bacteria in biofilms to killing by light from a low-power
422 laser Arch Oral Biol 37:883-887
423 FILOVÁ A (2014) Production of secondary metabolities in plant tissue cultures. J Agric Sci 46
424 Finlayson C, Van der Valk A (1995) Wetland classification and inventory: a summary. Vegetatio
425 118:185-192
426 Fu L, Kane CL (2008) Superconducting proximity effect and Majorana fermions at the surface of a
427 topological insulator. Phys Rev Lett 100:096407
428 Ghanati F, Morita A, Yokota H (2005) Effects of aluminum on the growth of tea plant and activation of
429 antioxidant system. Plant Soil 276:133-141
430 Guinney J, Dienstmann R, Wang X, De Reyniès A, Schlicker A, Soneson C, Marisa L, Roepman P,
431 Nyamundanda G, Angelino P (2015) The consensus molecular subtypes of colorectal cancer. Nat
432 Med 21:1350-1356
433 Gupta AS, Webb RP, Holaday AS, Allen RD (1993) Overexpression of superoxide dismutase protects plants
434 from oxidative stress (induction of ascorbate peroxidase in superoxide dismutase-overexpressing
435 plants). Plant Physiol 103:1067-1073
436 Halliwell B (1999) Antioxidant defence mechanisms: from the beginning to the end (of the beginning). Free
437 Radic 31:261-272
438 Hamada H, Tsuruo T (1986) Functional role for the 170-to 180-kDa glycoprotein specific to drug-resistant
439 tumor cells as revealed by monoclonal antibodies. Proc Natl Acad Sci USA 83:7785-7789

440 Haubrick LL, Assmann SM (2006) Brassinosteroids and plant function: some clues, more puzzles. Plant Cell
441 Environ 29:446-457
442 Hou S, He J, Parikh A, Kahl D, Bertulani C, Kajino T, Mathews G, Zhao G (2017) Non-extensive statistics to
443 the cosmological lithium problem. Astrophys J 834:165
444 Hu J, Chen G, Lo IM (2005) Removal and recovery of Cr (VI) from wastewater by maghemite nanoparticles.
445 Water Res 39:4528-4536
446 Huang Z, Pan X-D, Wu P-G, Han J-L, Chen Q (2013) Health risk assessment of heavy metals in rice to the
447 population in Zhejiang, China. PloS one 8:e75007
448 Jones D, Blancaflor E, Kochian L, Gilroy S (2006) Spatial coordination of aluminium uptake, production of
449 reactive oxygen species, callose production and wall rigidification in maize roots. Plant Cell Environ
450 29:1309-1318
451 Khan HAA, Shad SA, Akram W (2013) Resistance to new chemical insecticides in the house fly, Musca
452 domestica L., from dairies in Punjab, Pakistan. Parasitol Res 112:2049-2054
453 Khripach VA, Zhabinskii VN, Khripach NB (2003) New practical aspects of brassinosteroids and results of
454 their ten-year agricultural use in Russia and Belarus, Brassinosteroids. Springer pp. 189-230
455 Latha P, Vidya Vardhini B (2018) Effect of homobrassinolide on the growth of mustard crops grown in
456 semi-arid tropics of nizamabad. Int J Curr Res 7:2320-2326
457 Leaska MA (1970) Virginia Woolf's Lighthouse: a study in critical method. Chatto & Windus
458 Li J, Galley M, Brockett C, Spithourakis GP, Gao J, Dolan B (2016) A persona-based neural conversation
459 model. arXiv preprint arXiv:1603.06155
460 LICHTENTHALER HK, Wellburn AR (1983) Determinations of total carotenoids and chlorophylls a and b
461 of leaf extracts in different solvents. Portland Press Ltd.
462 Liu C-W, Lin K-H, Kuo Y-M (2003) Application of factor analysis in the assessment of groundwater quality
463 in a blackfoot disease area in Taiwan. Sci Total Environ 313:77-89
464 Liu Q, Yang J, He L, Li Y, Zheng S (2008) Effect of aluminum on cell wall, plasma membrane, antioxidants
465 and root elongation in triticale. Biol Plant 52:87-92
466 Liu XS, Wu H, Ji X, Stelzer Y, Wu X, Czauderna S, Shu J, Dadon D, Young RA, Jaenisch R (2016) Editing
467 DNA methylation in the mammalian genome. Cell 167:233-247. e17

Mannon PJ, Fuss IJ, Mayer L, Elson CO, Sandborn WJ, Present D, Dolin B, Goodman N, Groden C, Hornung RL (2004) Anti–interleukin-12 antibody for active Crohn's disease. N Engl J Med 351:2069-2079

Matsumoto Y, Murakami M, Shono T, Hasegawa T, Fukumura T, Kawasaki M, Ahmet P, Chikyow T, Koshihara S-y, Koinuma H (2001) Room-temperature ferromagnetism in transparent transition metal-doped titanium dioxide. Science 291:854-856

Mei H, Li Z, Shu Q, Guo L, Wang Y, Yu X, Ying C, Luo L (2005) Gene actions of QTLs affecting several agronomic traits resolved in a recombinant inbred rice population and two backcross populations. THEOR APPL GENET 110:649-659

Moghaddasi S, Fotovat A, Khoshgoftarmanesh AH, Karimzadeh F, Khazaei HR, Khorassani R (2017) Bioavailability of coated and uncoated ZnO nanoparticles to cucumber in soil with or without organic matter. Ecotoxicol 144:543-551

Mol SE, Bus AG, De Jong MT, Smeets DJ (2008) Added value of dialogic parent–child book readings: A meta-analysis. Early Educ Dev 19:7-26

Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. PLANT CELL PHYSIOL 22:867-880

Nandi A, Neogy S (2002) Modelling of a beam with a breathing edge crack and some observations for crack detection. J Vib Control 8:673-693

O¨ zdemir AT, Edwards L (2004) Through-thickness residual stress distribution after the cold expansion of fastener holes and its effect on fracturing. J Eng Mater Technol 126:129-135

Oberdörster G, Maynard A, Donaldson K, Castranova V, Fitzpatrick J, Ausman K, Carter J, Karn B, Kreyling W, Lai D (2005) Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. PART FIBRE TOXICOL 2:8

Olivares A, Daza A, Rey A, Lopez-Bote C (2009) Interactions between genotype, dietary fat saturation and vitamin A concentration on intramuscular fat content and fatty acid composition in pigs. Meat Sci 82:6-12

Padmanabhan S, Melander O, Johnson T, Di Blasio AM, Lee WK, Gentilini D, Hastie CE, Menni C, Monti MC, Delles C (2010) Genome-wide association study of blood pressure extremes identifies variant near UMOD associated with hypertension. PLoS Genet 6:e1001177

496 Piotrowska-Niczyporuk A, Bajguz A (2014) The effect of natural and synthetic auxins on the growth,
497 metabolite content and antioxidant response of green alga Chlorella vulgaris (Trebouxiophyceae).
498 Plant Growth Regul 73:57-66
499 Rajewska I, Talarek M, Bajguz A (2016) Brassinosteroids and response of plants to heavy metals action.
500 FRONT PLANT SCI 7:629
501 Russinova E, Borst J-W, Kwaaitaal M, Caño-Delgado A, Yin Y, Chory J, de Vries SC (2004)
502 Heterodimerization and endocytosis of Arabidopsis brassinosteroid receptors BRI1 and AtSERK3
503 (BAK1). Plant Cell 16:3216-3229
504 Santos L, Batista B, Lobato A (2018) Brassinosteroids mitigate cadmium toxicity in cowpea plants.
505 Photosynthetica 56:591-605
506 Shan Y, Arkhipov A, Kim ET, Pan AC, Shaw DE (2013) Transitions to catalytically inactive conformations
507 in EGFR kinase. Proc Natl Acad Sci USA 110:7270-7275
508 Sharma R, Duveiller E, Ortiz-Ferrara G (2007) Progress and challenge towards reducing wheat spot blotch
509 threat in the Eastern Gangetic Plains of South Asia: is climate change already taking its toll? Field
510 Crops Res 103:109-118
511 Silambarasan S, Logeswari P, Cornejo P, Kannan VR (2019) Role of plant growth–promoting rhizobacterial
512 consortium in improving the Vigna radiata growth and alleviation of aluminum and drought stresses.
513 ENVIRON SCI POLLUT R 26:27647-27659
514 Simon L, Smalley T, Jones Jr JB, Lasseigne F (1994) Aluminum toxicity in tomato. Part 1. Growth and
515 mineral nutrition. J Plant 17:293-306
516 Singh N, Kamath V, Narasimhamurthy K, Rajini P (2008) Protective effect of potato peel extract against
517 carbon tetrachloride-induced liver injury in rats. ENVIRON TOXICOL PHAR 26:241-246
518 Singhal R, Chung S-H, Manthiram A, Kalra V (2015) A free-standing carbon nanofiber interlayer for
519 high-performance lithium–sulfur batteries. J Mater Chem A 3:4530-4538
520 Swamy M, Norlina W, Azman W, Suhaili D, Sirajudeen K, Mustapha Z, Govindasamy C (2014) Restoration
521 of glutamine synthetase activity, nitric oxide levels and amelioration of oxidative stress by propolis
522 in kainic acid mediated excitotoxicity. AFR J TRADIT COMPLEM 11:458-463

523 Talaat NB, Shawky BT (2016) Dual application of 24-epibrassinolide and spermine confers drought stress
524 tolerance in maize (Zea mays L.) by modulating polyamine and protein metabolism. J Plant Growth
525 Regul 35:518-533
526 Talaat RM, Mohamed SF, Bassyouni IH, Raouf AA (2015) Th1/Th2/Th17/Treg cytokine imbalance in
527 systemic lupus erythematosus (SLE) patients: Correlation with disease activity. Cytokine 72:146-153
528 Velikova V, Yordanov I, Edreva A (2000) Oxidative stress and some antioxidant systems in acid rain-treated
529 bean plants: protective role of exogenous polyamines. PLANT SCI 151:59-66
530 Vitorello VA, Capaldi FR, Stefanuto VA (2005) Recent advances in aluminum toxicity and resistance in
531 higher plants. Braz J Plant Physiol 17:129-143
532 Wu Z, Wang S, Zhao J, Chen L, Meng H (2014) Synergistic effect on thermal behavior during co-pyrolysis of
533 lignocellulosic biomass model components blend with bituminous coal. Bioresour Technol
534 169:220-228
535 Yamamoto M, Sato S, Hemmi H, Hoshino K, Kaisho T, Sanjo H, Takeuchi O, Sugiyama M, Okabe M,
536 Takeda K (2003a) Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling
537 pathway. Science 301:640-643
538 Yamamoto Y, Kobayashi Y, Devi SR, Rikiishi S, Matsumoto H (2003b) Oxidative stress triggered by
539 aluminum in plant roots, Roots: The Dynamic Interface between Plants and the Earth. Springer pp.
540 239-243
541 Yuan L, Yuan Y, Du J, Sun J, Guo S (2012) Effects of 24-epibrassinolide on nitrogen metabolism in
542 cucumber seedlings under Ca (NO3) 2 stress. J Plant Physiol Biochem 61:29-35
543 Zeng M-H, Wang B, Wang X-Y, Zhang W-X, Chen X-M, Gao S (2006) Chiral magnetic metal-organic
544 frameworks of dimetal subunits: magnetism tuning by mixed-metal compositions of the solid
545 solutions. Inorg Chem 45:7069-7076
546 Zhang J, Terrones M, Park CR, Mukherjee R, Monthioux M, Koratkar N, Kim YS, Hurt R, Frackowiak E,
547 Enoki T (2016) Carbon science in 2016: Status, challenges and perspectives. Carbon 98:708-732

548

549

550

551 Table 1. Seed priming effect with 0.01µM BRs on germination energy, germination percentage, germination

552 index, mean germination time as well as vigour index of two rice cultivars under 400µM Al toxicity.

Varieties Name	G.E	G.P	G.I	MGT	**V.I**
CY-927-H_2O	88.00a±2.00	94.67b±1.15	20.13b±1.17	2.91b±0.17	0.88b±0.17
CY-927-Al	46.00d±2.00	60.67d±1.15	10.87c±0.50	3.81a±0.08	0.15c±0.07
CY927-BRs	90.67a±3.06	100.00a±0.00	31.62a±0.92	2.17c±0.07	1.47a±0.16
CY927-BRs+Al	69.33c±3.06	85.33c±5.03	21.49b±1.82	2.91b±0.20	0.64b±0.06
YLY689- H_2O	90.00b±2.00	99.33a±1.15	23.72b±0.43	2.59bc±0.26	1.26b±0.15
YLY-689-Al	69.33c±1.15	72.67c±2.31	16.53c±0.58	2.88a±0.37	0.37d±0.19
YLY689-BRs	98.00a±2.00	100.00a±0.00	32.44a±1.12	1.93c±0.14	2.06a±0.03
YLY689-BRs+Al	80.67b±3.06	88.00b±2.00	23.57b±0.56	2.63b±0.08	0.74c±0.03

553 Each value is demonstrating the mean of three repeats of every treatment. The similar letters inside a column

554 specify that there was no significant difference at a 95% probability level at the $p < 0.05$ level,

555 correspondingly.

556 Table 2. Seed priming effect with 0.01µM BRs on shoot length, root length, fresh weight and dry weight of

557 two rice cultivars under 400µM Al toxicity.

Varieties Name	S.L	R.L	F/W	D/W
CY-927-H_2O	14.26b±0.12	13.85b±0.37	0.41b±0.01	0.04a±0.01
CY-927-Al	5.52c±0.23	6.85d±0.15	0.16d±0.01	0.01c±0.01
CY927-BRs	16.40a±0.08	15.29a±0.44	0.53a±0.01	0.05a±0.00
CY927-BRs+Al	8.38b±0.72	8.93c±0.46	0.29c±0.01	0.03b±0.00
YLY689-H_2O	15.26a±0.35	18.96b±0.47	0.48b±0.01	0.06a±0.00
YLY-689-Al	8.56d±0.15	9.19d±0.32	0.18d±0.01	0.02c±0.01
YLY689-BRs	18.23a±0.11	19.54a±0.28	0.59a±0.01	0.06a±0.01
YLY689-BRs+Al	11.43c±0.22	11.48c±0.18	0.31c±0.01	0.04b±0.00

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

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

Treatment	Mg	Al	K	Ca	Mn	Fe	Zn
	mg/g	mg/g	mg/l	mg/l	mg/l	mg/l	mg/l
CY927-H_2O	2.32c±0.13	-	31.72d±1.06	1.59ab±1.04	0.27ab±0.10	0.13b±0.10	0.05b±0.02
CY927-BRs	2.37b±0.74	-	32.43b±2.06	2.80a±0.99	0.43a±0.19	0.20a±0.19	0.05b±0.02
CY927-Al	2.45a±0.46	0.61a±0.06	37.48a±1.78	1.18ab±0.24	0.09b±0.01	0.08b±0.01	0.07a±0.01
CY927-BRs+Al	2.31d±0.17	0.43b±0.07	32.08c±1.10	0.80b±0.09	0.12b±0.02	0.11b±0.02	0.06b±0.00
YLY689-H_2O	1.77c±0.78	-	26.36c±1.99	0.71b±0.48	0.15a±0.48	0.07a±0.04	0.02c±0.01
YLY689-BRs	2.20b±0.65	-	32.09bc±1.93	0.75ab±0.21	0.14a±0.23	0.09a±0.02	0.03c±0.00
YLY689-Al	2.24ab±0.21	0.82a±0.34	40.43b±2.21	0.80ab±0.23	0.10b±0.21	0.07a±0.01	0.05a±0.01
YLY689-BRs+Al	3.04a±0.30	0.24b±0.26	49.60a±0.52	1.29a±0.27	0.13a±0.27	0.10a±0.01	0.04b±0.00

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

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

Treatment	Mg	Al	K	Ca	Mn	Fe	Zn
	mg/g	mg/g	mg/l	mg/l	mg/l	mg/l	mg/l
CY927-H_2O	2.34a±0.17	-	19.69a±1.42	0.88a±0.34	0.08a±0.01	6.16a±0.30	0.08b±0.03
CY927-BRs	1.52c±0.53	-	14.21ab±2.73	0.51b±0.33	0.07b±0.02	4.19ab±1.06	0.12a±0.05
CY927-Al	1.14d±0.49	5.02a±2.34	9.81b±0.29	0.21bc±0.05	0.03b±0.01	0.52b±0.23	0.14a±0.05
CY927-BRs+Al	1.19b±0.42	3.92b±1.23	12.60ab±1.10	0.11c±0.04	0.04b±0.02	0.46b±0.17	0.07b±0.01

YLY689-H_2O	1.77b±0.50	-	13.48b±1.29	1.31b±1.25	0.07a±1.25	4.03a±0.94	0.02c±0.01
YLY689-BRs	2.13a±0.43	-	17.46a±2.10	0.76b±0.17	0.05ab±0.17	3.63a±1.95	0.03c±0.00
YLY689-Al	1.28cd±0.34	8.78a±0.25	13.47b±2.94	0.30b±0.17	0.04ab±0.17	1.53b±0.17	0.04b±0.00
YLY689-BRs+Al	1.32c±0.76	7.50ab±2.45	11.02bc±1.74	1.50a±0.43	0.03ab±0.43	0.71b±0.24	0.05a±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 ($p<0.05$)

Figure legends

Fig. 1. Physiological effect of Al toxicity on rice cultivar CY-927 and mitigation effect by 0.01µM BRs under 400µM Al stress

Fig. 2. Physiological effect of Al toxicity on rice cultivar YLY-689 and mitigation effect by 0.01µM BRs under 400µM Al stress

Fig.3. Seed priming effect with 0.01µM BRs on (A) Chlorophyll a, (B) Chlorophyll b (C) Chlorophyll a+b in leaves of two different cultivars of *Oryza sativa* under 400µM Al concentration

Fig. 4. Seed priming effect with 0.01µM BRs on MDA contents and H_2O_2 production in shoots and roots of two rice cultivars under 400µM Al toxicity.

Fig. 5. Seed priming effect with 0.01µM BRs on SOD, CAT, APX and POD contents in both shoots and roots of two rice cultivars under 400µM Al toxicity.

Fig. 6a. Effect of seed priming 0.01µM BRs on gene expression of (A) *APX02*, (B) *APX08* in shoots and roots of both cultivars of rice under toxicity of 400µM Al.

Fig. 6b. Effect of seed priming 0.01µM BRs on gene expression of (C) *CATa*, (D) *CATb* in shoots and roots of both cultivars of rice under toxicity of 400µM Al.

584 Fig. 6c. Effect of seed priming 0.01µM BRs on gene expression of (E) *SOD Cu-Zn*, (F) *SOD-Fe₂* in shoots
585 and roots of both cultivars of rice under toxicity of 400µM Al.

586 Fig.7. Bioplot of principle component of 1 and 2 of the PCA extracted from results obtained from
587 physiological data of two different rice cultivars (CY927, YLY689) under various treatments such as control
588 primed with water (CY927-H_2O, YLY689-H_2O), control primed with BRs (CY927-BRs, YLY689-BRs), seed
589 primed with BRs, and treatment under Al stress (CY927-BRs+Al, YLY689-BRs+Al), seed primed with H_2O
590 under Al stress(CY92-Al+ H_2O, YLY689- Al+ H_2O). Sharp angle represented positive, obtuse angle showed
591 a negative correlation, as well as a right angle, demonstrated a correlation between parameters. (A)
592 Physiological parameters of rice variety CY927 illustration through Pearson's correlation coefficients under
593 different treatments. (I) contains POD, CAT, APX, and SOD, (II) Showed G.I, F/W, D/W, G.E, G.P, V.I, and
594 S.L, (III) Illustrated MDA, MGT, and H2O2; while (IV) represented R.L. (B) Physiological parameters of rice
595 variety YLY689 representation via Pearson's correlation coefficients under different treatments. Distance
596 between each circle represented the strength of correlation. . (I) contains POD, CAT, APX, and SOD, (II)
597 Showed G.I, F/W, D/W, G.E, G.P, and V.I, (III) Illustrated MDA, MGT, and H2O2; while (IV) represented
598 R.L and S.L (C) Dendrogram of two different rice cultivars under various treatments obtained through
599 Agglomerative hierarchical clustering using ward’s method on basis of physiological traits.

Fig. 1. Physiological effect of Al toxicity on rice cultivar CY-927 and mitigation effect by 0.01µM BRs under 400µM Al stress

Fig. 2. Physiological effect of Al toxicity on rice cultivar YLY-689 and mitigation effect by 0.01µM BRs under 400µM Al stress

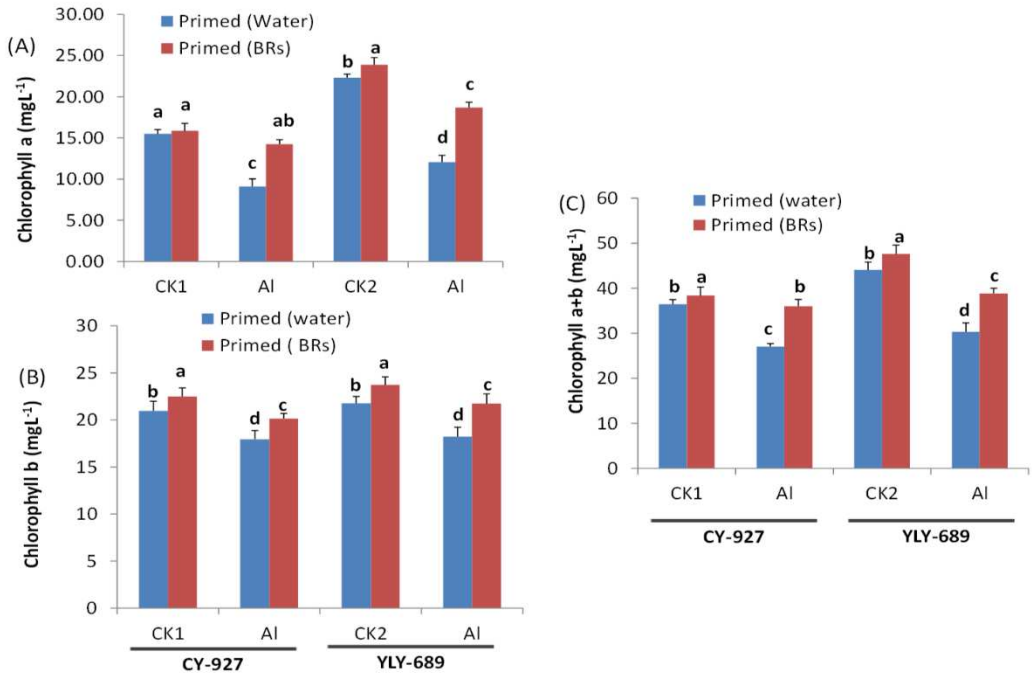
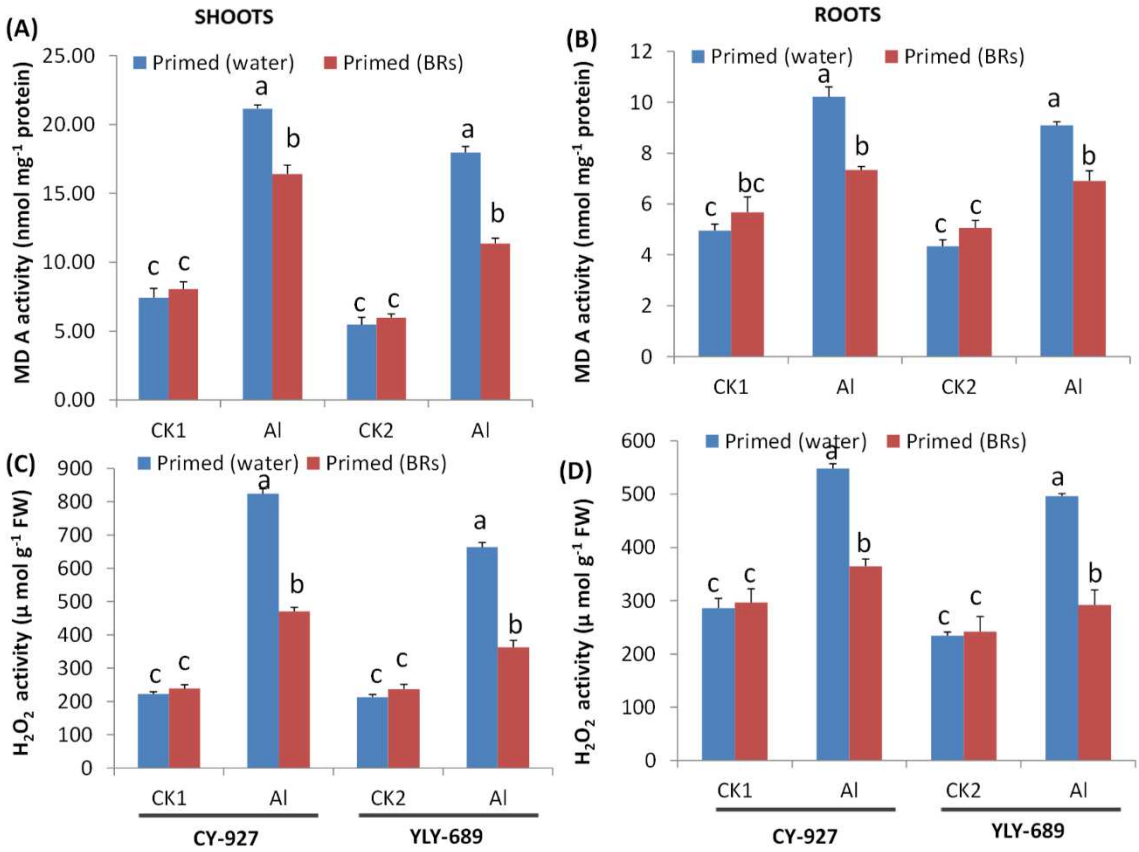


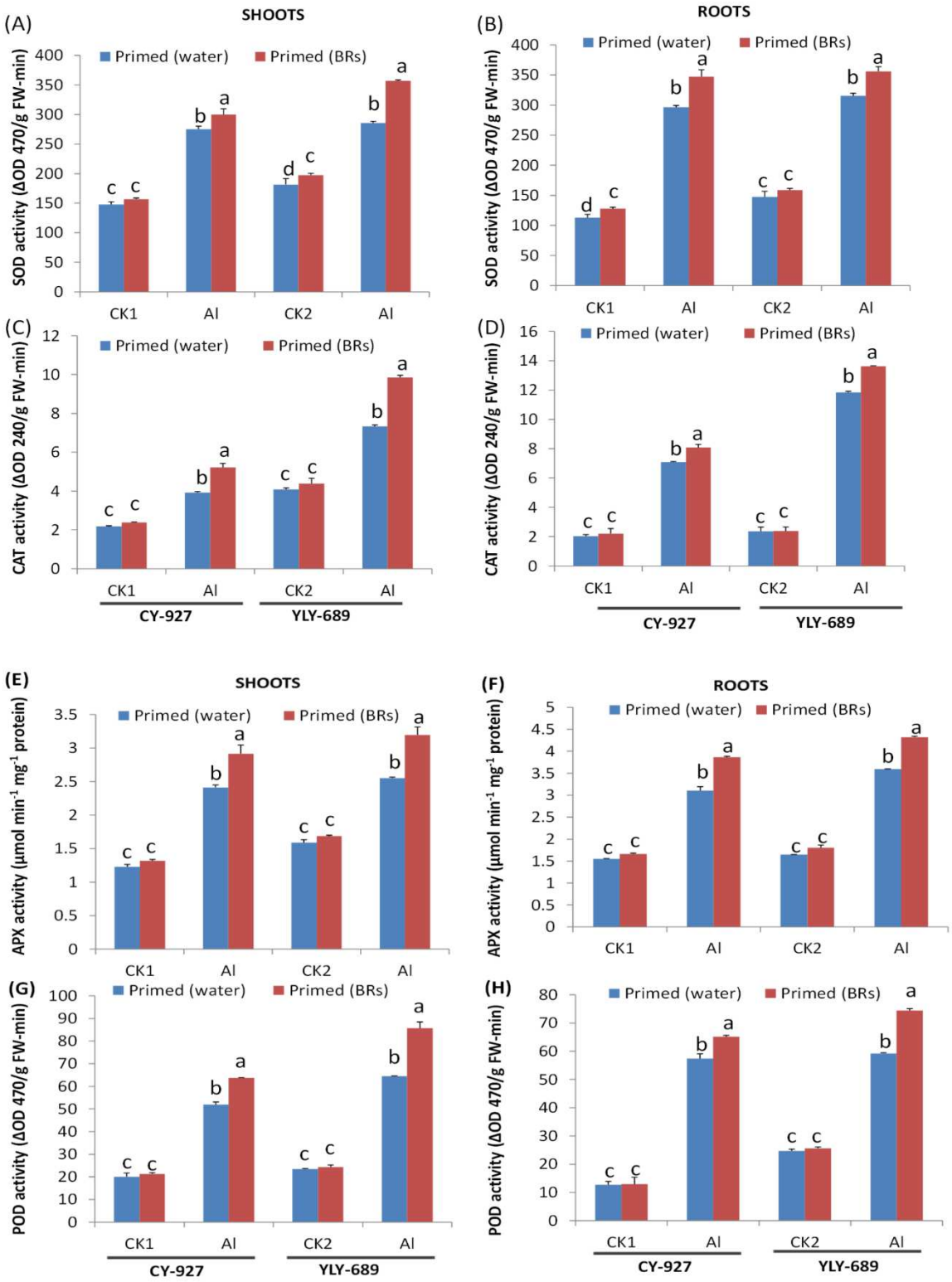
Fig.3. Seed priming effect with 0.01µM BRs on (A) Chlorophyll a, (B) Chlorophyll b (C) Chlorophyll a+b in leaves of two different cultivars of *Oryza sativa* under 400µM Al concentration



607

608 Fig. 4. Seed priming effect with 0.01µM BRs on MDA contents and H_2O_2 production in shoots and roots of

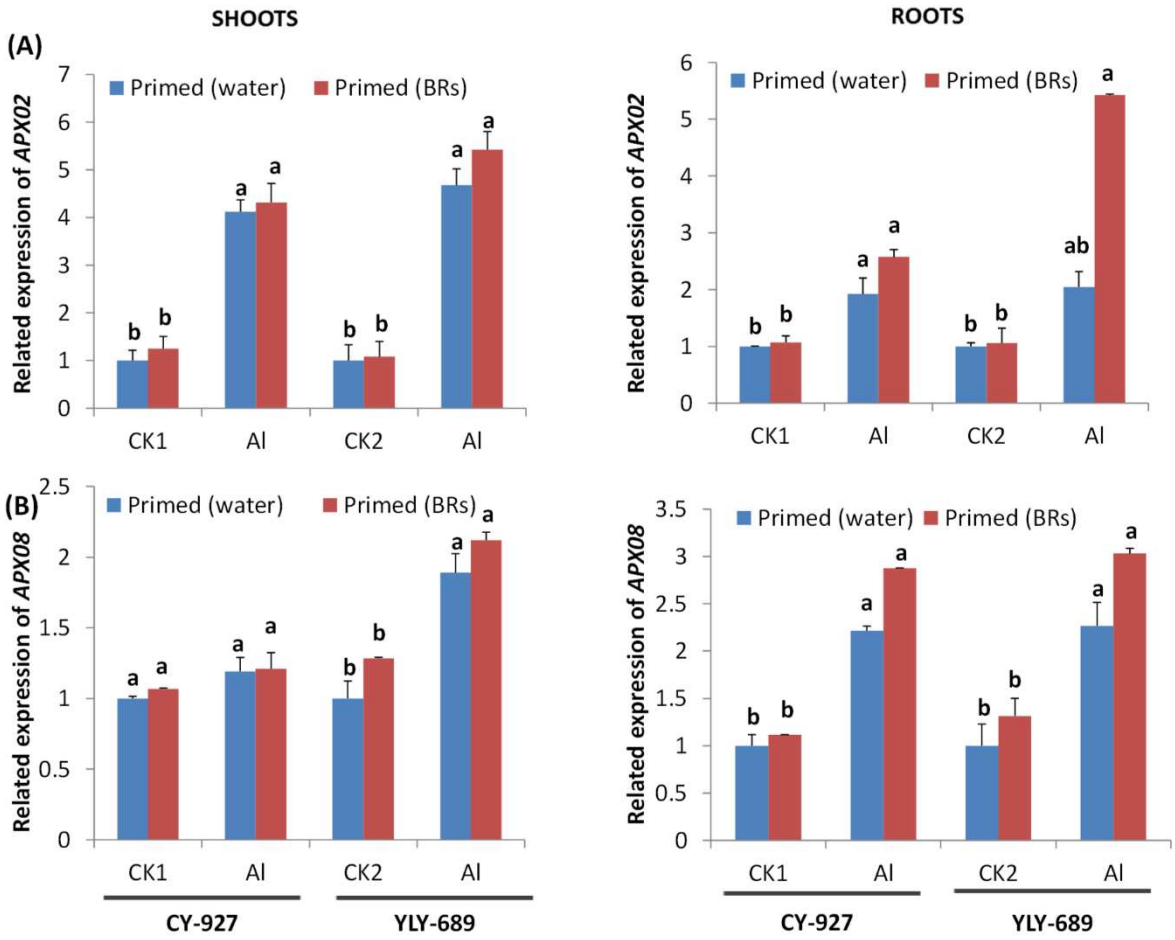
609 two rice cultivars under 400µM Al toxicity.



610

611 Fig. 5. Seed priming effect with 0.01μM BRs on SOD, CAT, APX and POD contents in both shoots and

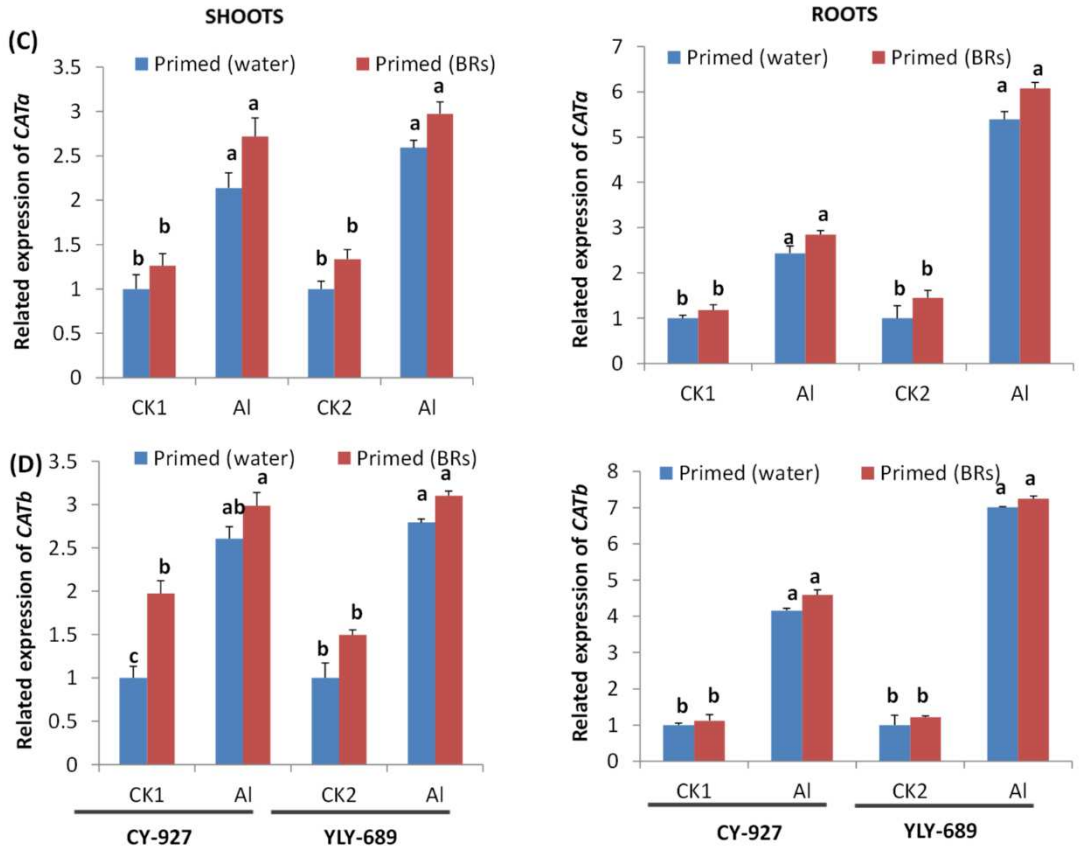
612 roots of two rice cultivars under 400μM Al toxicity.



613

614 Fig. 6a. Effect of seed priming 0.01µM BRs on gene expression of (A) *APX02*, (B) *APX08* in shoots and roots

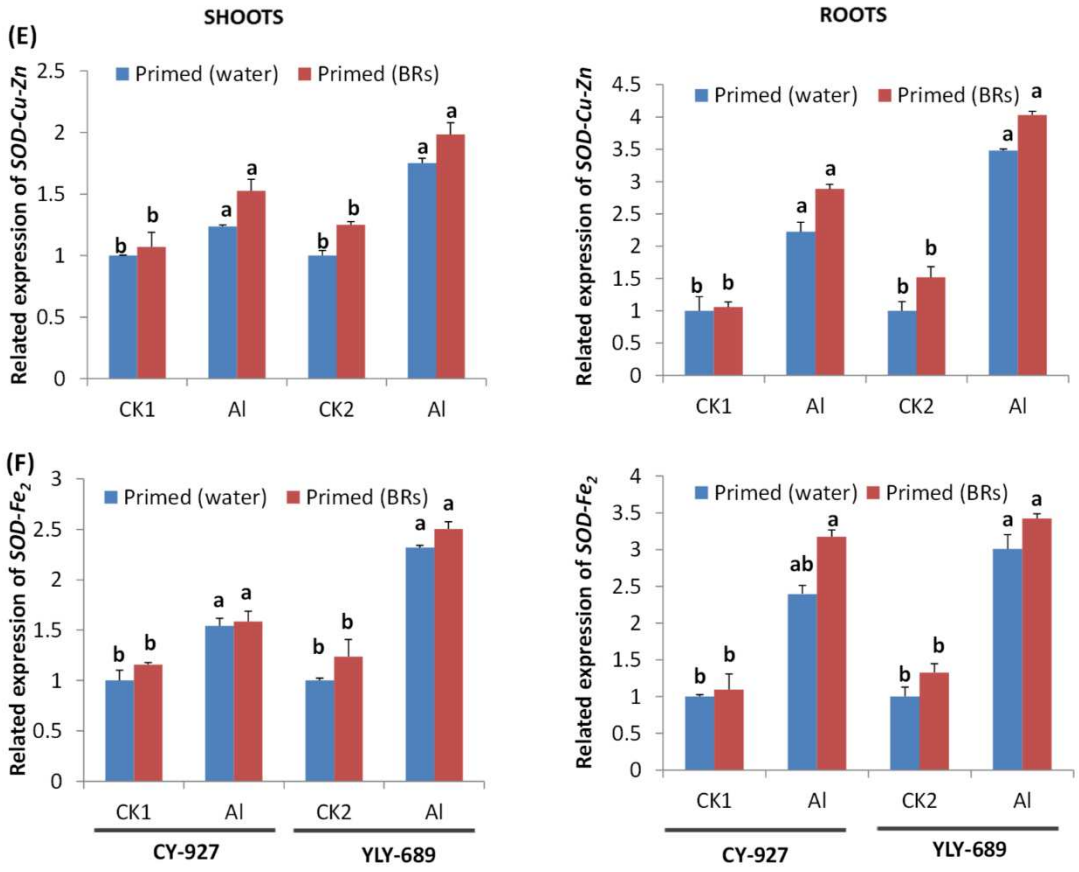
615 of both cultivars of rice under toxicity of 400µM Al.



616

617 Fig. 6b. Effect of seed priming 0.01µM BRs on gene expression of (C) *CATa*, (D) *CATb* in shoots and roots

618 of both cultivars of rice under toxicity of 400µM Al.



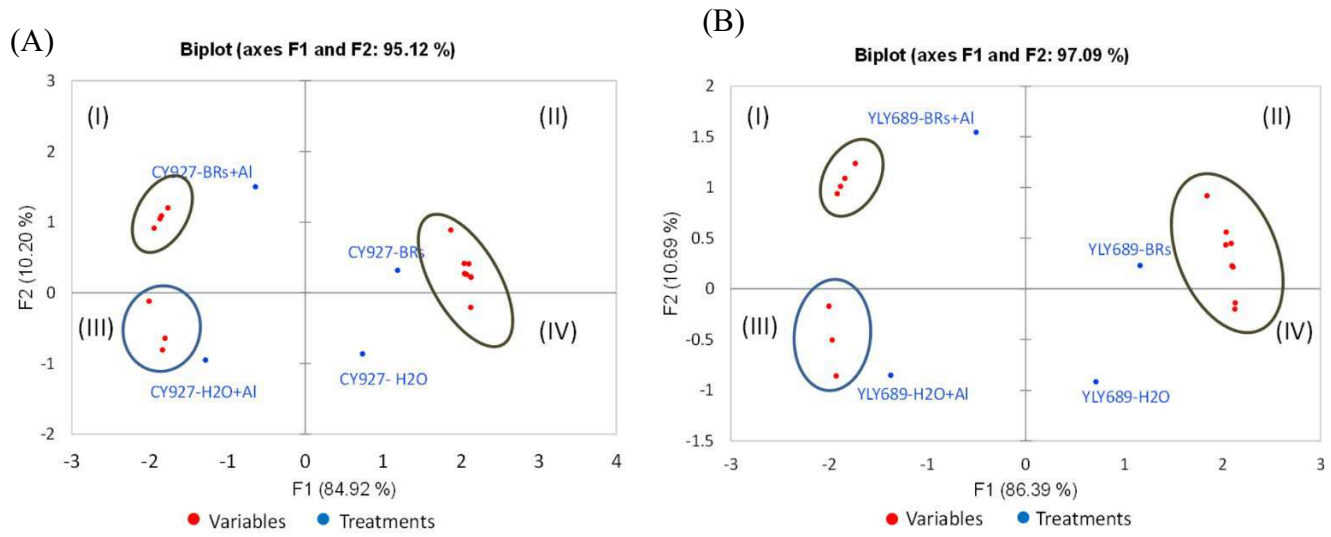
619

620

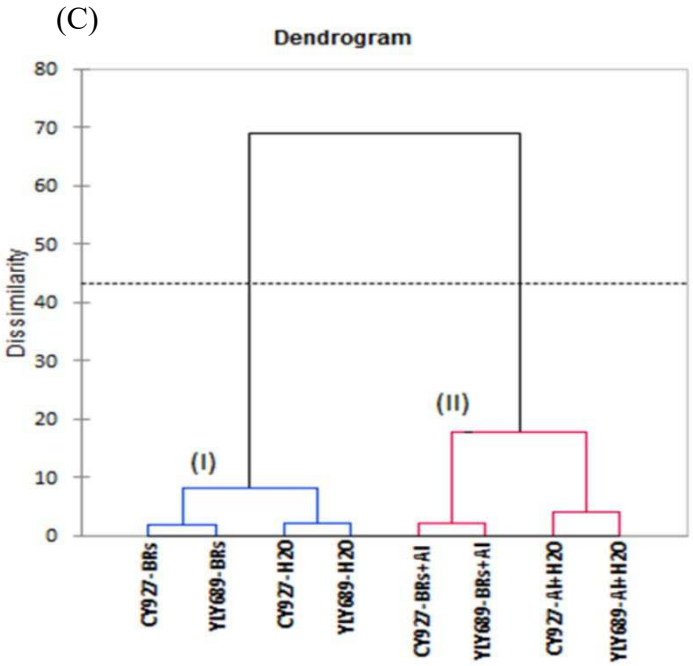
621 Fig. 6c. Effect of seed priming 0.01µM BRs on gene expression of (E) *SOD Cu-Zn*, (F) *SOD-Fe₂* in shoots

622 and roots of both cultivars of rice under toxicity of 400µM Al.

623



624



625

626 Fig.7. Bioplot of principle component of 1 and 2 of the PCA extracted from results obtained from
627 physiological data of two different rice cultivars (CY927, YLY689) under various treatments such as control
628 primed with water (CY927-H_2O, YLY689-H_2O), control primed with BRs (CY927-BRs, YLY689-BRs), seed
629 primed with BRs, and treatment under Al stress (CY927-BRs+Al, YLY689-BRs+Al), seed primed with H_2O

630 under Al stress(CY92-Al+ H_2O, YLY689- Al+ H_2O). Sharp angle represented positive, obtuse angle showed
631 a negative correlation, as well as a right angle, demonstrated a correlation between parameters. (A)
632 Physiological parameters of rice variety CY927 illustration through Pearson's correlation coefficients under
633 different treatments. (I) contains POD, CAT, APX, and SOD, (II) Showed G.I, F/W, D/W, G.E, G.P, V.I, and
634 S.L, (III) Illustrated MDA, MGT, and H_2O_2; while (IV) represented R.L. (B) Physiological parameters of rice
635 variety YLY689 representation via Pearson's correlation coefficients under different treatments. Distance
636 between each circle represented the strength of correlation. . (I) contains POD, CAT, APX, and SOD, (II)
637 Showed G.I, F/W, D/W, G.E, G.P, and V.I, (III) Illustrated MDA, MGT, and H_2O_2; while (IV) represented
638 R.L and S.L (C) Dendrogram of two different rice cultivars under various treatments obtained through
639 Agglomerative hierarchical clustering using ward’s method on basis of physiological traits.

640

641

Figures

Figure 1

Physiological effect of Al toxicity on rice cultivar CY-927 and mitigation effect by 0.01µM BRs under 400µM Al stress

Figure 2

Physiological effect of Al toxicity on rice cultivar YLY-689 and mitigation effect by 0.01µM BRs under 400µM Al stress

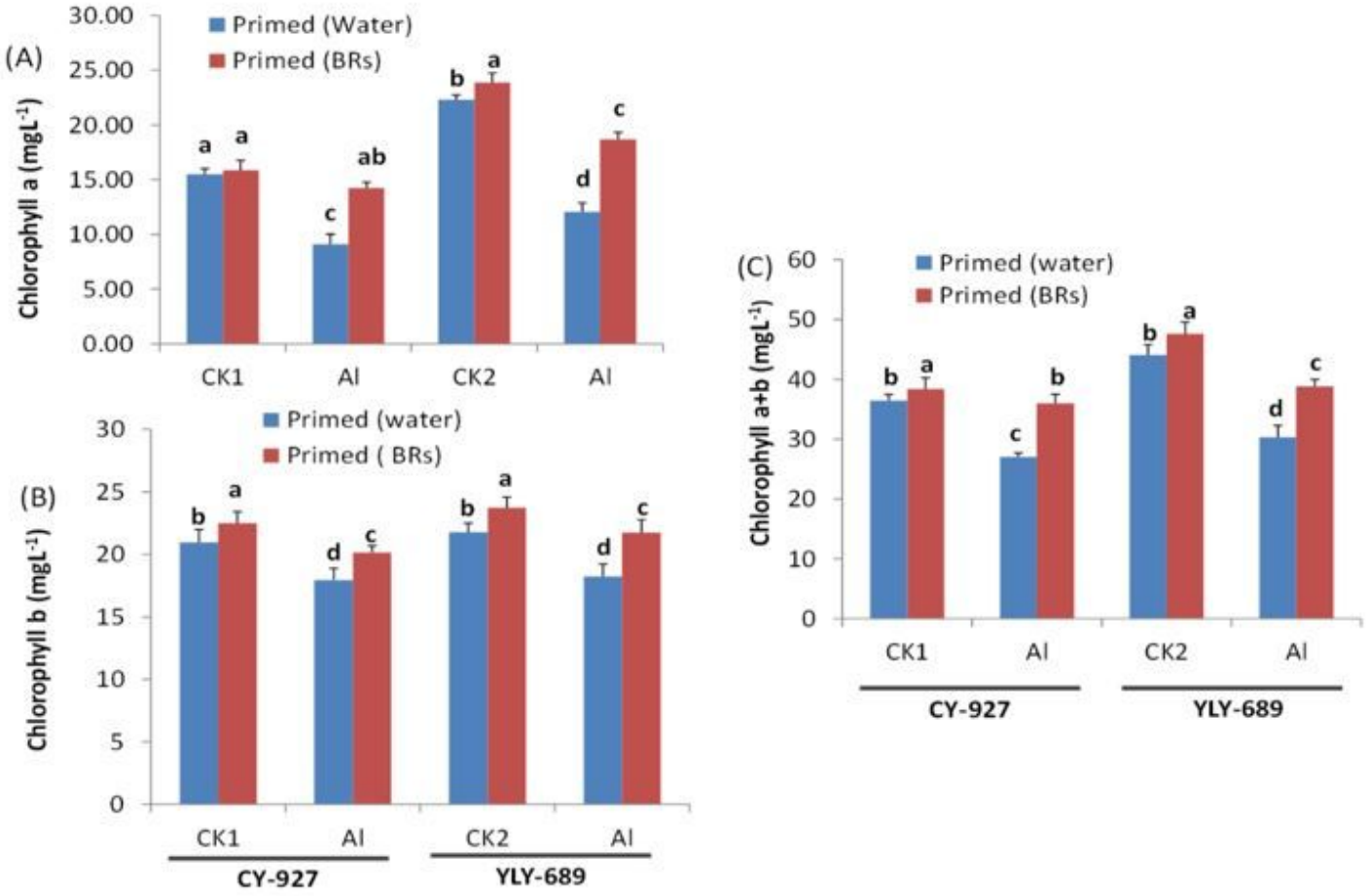


Figure 3

Seed priming effect with 0.01µM BRs on (A) Chlorophyll a, (B) Chlorophyll b (C) Chlorophyll a+b in leaves of two different cultivars of Oryza sativa under 400µM Al concentration

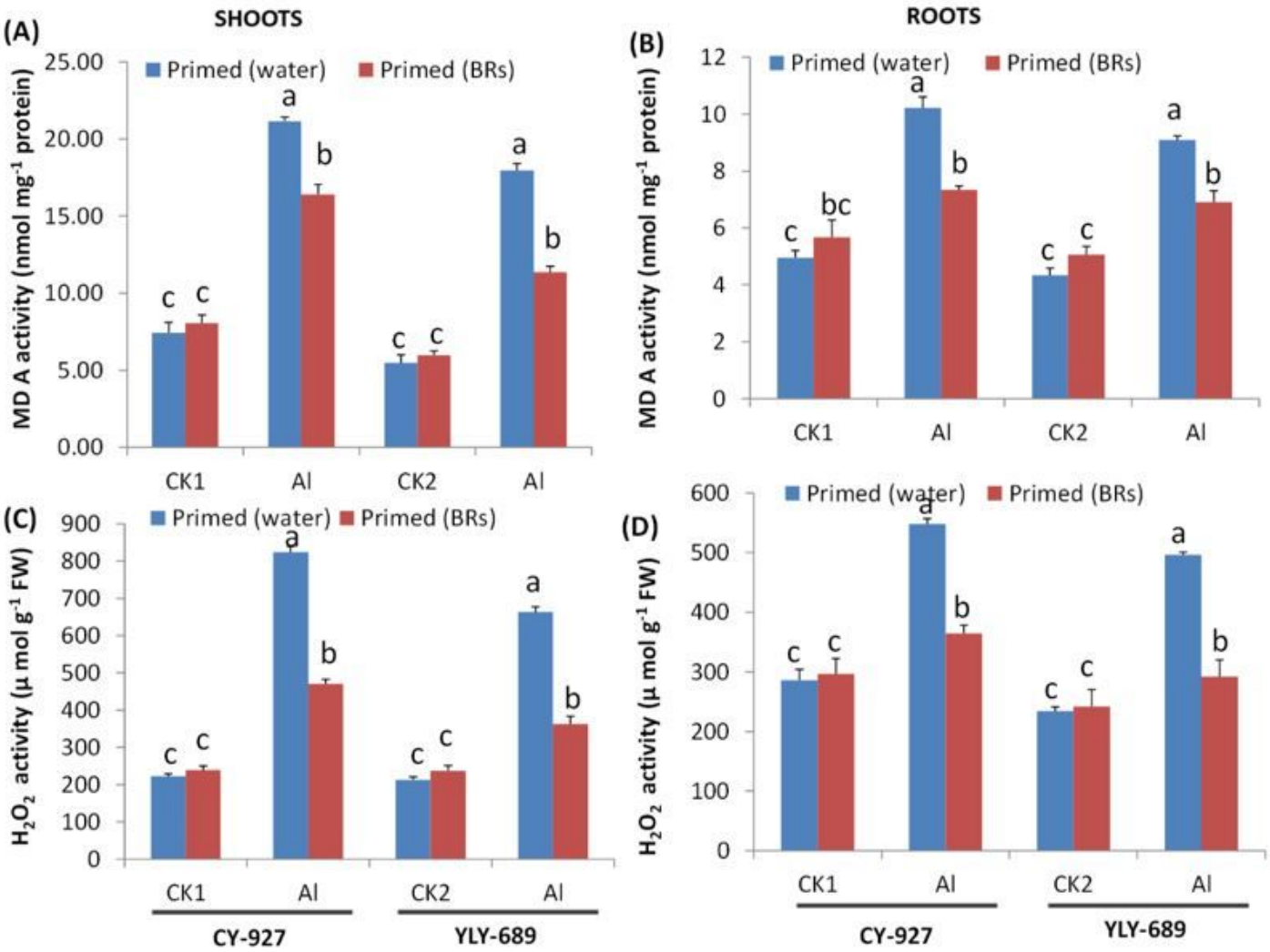


Figure 4

Seed priming effect with 0.01μM BRs on MDA contents and H2O2 production in shoots and roots of two rice cultivars under 400μM Al toxicity.

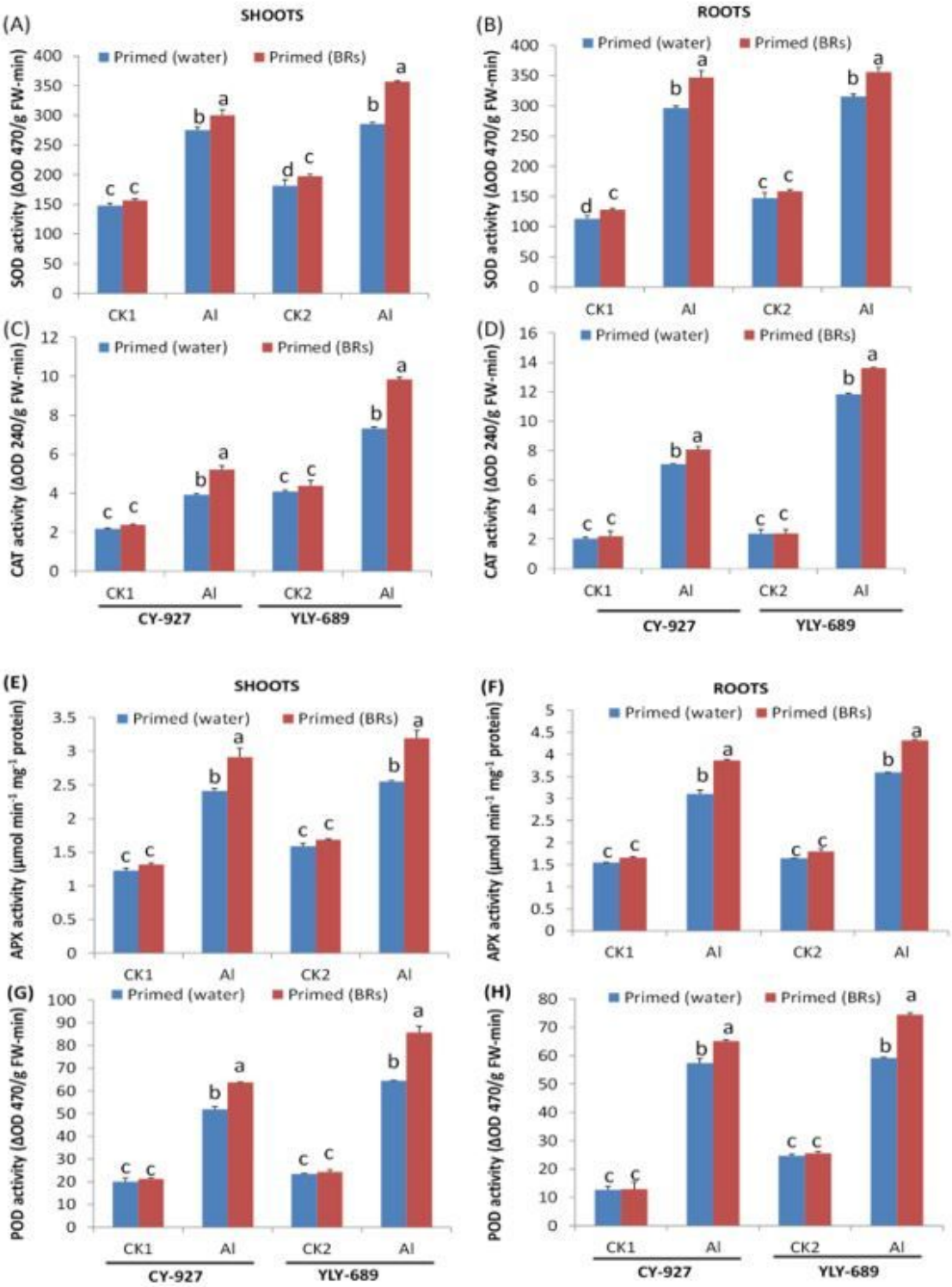


Figure 5

Seed priming effect with 0.01μM BRs on SOD, CAT, APX and POD contents in both shoots and roots of two rice cultivars under 400μM Al toxicity.

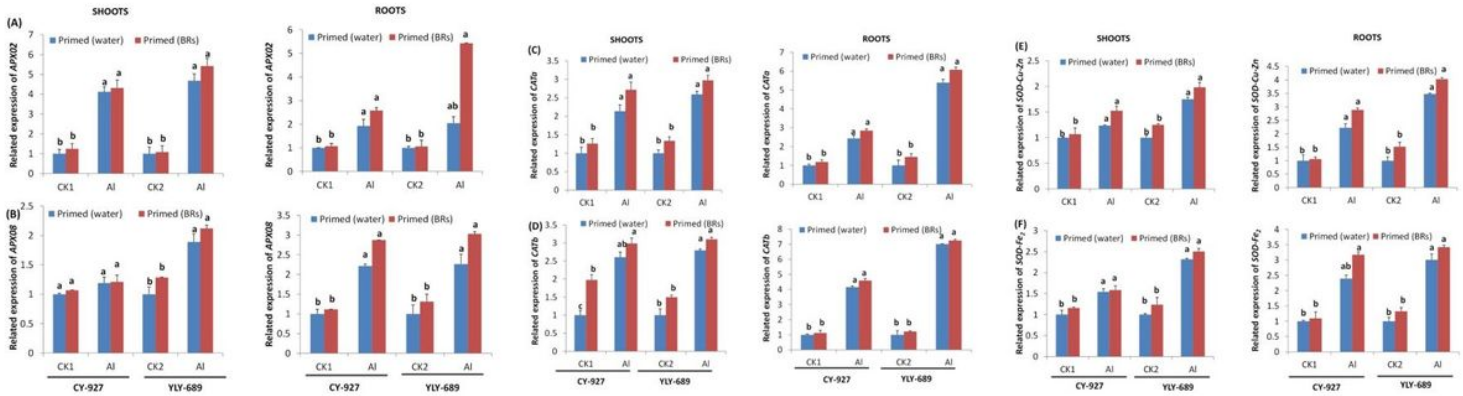


Figure 6

Fig. 6a. Effect of seed priming 0.01μM BRs on gene expression of (A) APX02, (B) APX08 in shoots and roots of both cultivars of rice under toxicity of 400μM Al. Fig. 6b. Effect of seed priming 0.01μM BRs on gene expression of (C) CATa, (D) CATb in shoots and roots of both cultivars of rice under toxicity of 400μM Al. Fig. 6c. Effect of seed priming 0.01μM BRs on gene expression of (E) SOD Cu-Zn, (F) SOD-Fe2 in shoots and roots of both cultivars of rice under toxicity of 400μM Al.

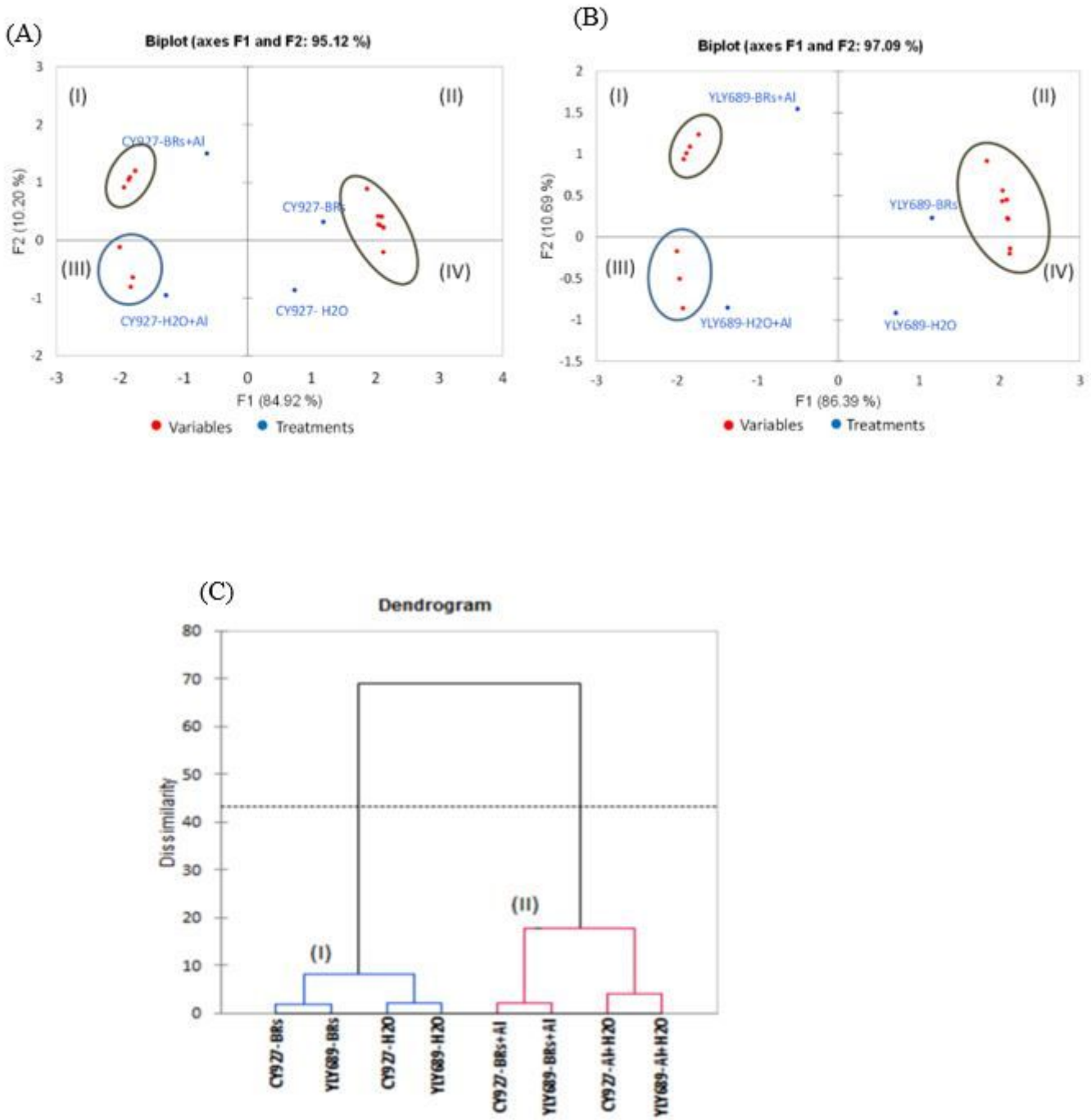


Figure 7

Bioplot 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-H2O, YLY689-H2O), 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 H2O under Al stress(CY92-Al+ H2O, YLY689- Al+ H2O). 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 G.I, F/W, D/W, G.E, G.P, V.I, and S.L, (III) Illustrated MDA, MGT, and H2O2; while (IV) represented R.L. (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 G.I, F/W, D/W, G.E, G.P, and V.I, (III) Illustrated MDA, MGT, and H2O2; while (IV) represented R.L and S.L (C) Dendrogram of two different rice cultivars under various treatments obtained through Agglomerative hierarchical clustering using ward’s method on basis of physiological traits.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementaryFile.docx