CHILEANJAR
CHILEAN JOURNAL of AGRICULTURAL RESEARCH

RESEARCH

Effects of exogenous salicylic acid and abscisic acid on growth, photosynthesis and antioxidant system of rice

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Received: 30 June 2021; Accepted: 11 November 2021; doi:10.4067/S0718-58392022000100021

ABSTRACT

The key function of plant growth regulators is to adjust the growth and development of plants to adapt to the changing environment, which plays a vital role in the growth and yield of crops. This study was aimed to evaluate the ability of foliar application of salicylic acid (SA) and abscisic acid (ABA) to regulate rice (*Oryza sativa* L.) growth quality at seedling stage and reproductive stage. The two rice cultivars Wanshengyoutianhong-4 (W4) and Huanghuazhan (HZ) were sprayed foliar with SA (100 mg L^{-1}) and ABA (4 mg L^{-1}) at two-leaf stage, and their effects on morphological growth, physiological characteristics, malondialdehyde (MDA), osmotic substances, protective enzyme activities, photosynthesis and yield components were studied. Results indicated that SA and ABA significantly increased morphological growth, osmotic substances, antioxidant enzyme activities; MDA content dropped significantly ($p < 0.05$) in W4. While the two regulators inhibited morphological growth and physiological characteristics in HZ. Additionally, net photosynthesis, stomatal conductance, transpiration rate, water use efficiency and apparent mesophyll conductance were improved in both of rice cultivars at heading stage as well as grain weight. In conclusion, foliar spray of SA and ABA enhanced plant growth, induced an increase in protective enzyme activities and reduced the oxidative damage of the hybrid rice cultivar but not of the conventional rice cultivar at seedling stage. Meanwhile, photosynthetic parameters and grain weight were slightly enhanced at reproductive stage. These results served as a theoretical basis and technical support for the chemical regulation and cultivation practices of rice.

Key words: Abscisic acid, antioxidant enzyme activities, photosynthesis, salicylic acid, yield components.

INTRODUCTION

Salicylic acid (SA) is a small molecule phenolic substance and secondary metabolite, whose chemical composition is *o*-hydroxybenzoic acid, which is commonly found in different prokaryotes and eukaryotes including plants. As an endogenous natural signalling molecule, SA plays a pivotal role in affecting a range of plant growth processes, including various physiological and biochemical processes, development and yield. Besides, it was recognized as participating in interacting with other organisms and responding to environmental stress so that providing systemic acquired resistance (SAR) and tolerance defence mechanisms (Janda and Ruelland, 2015). Previous studies have demonstrated that exogenous SA treatment enhanced plant growth, photosynthesis and a variety of antioxidant enzymes or non-antioxidant enzymes (Hayat et al., 2012). Khan et al. (2003) shown that application of SA or its close analogues increased leaf area and dry biomass of maize and soybean. Hayat et al. (2012) pointed out that spraying SA increased dry biomass accumulation, stomatal conductance and net photosynthesis, as well as enhancement of superoxide dismutase (SOD), peroxidase

(POX) and catalase (CAT) level. On the other hand, SA could act as an inhibitor, which induced reactive oxygen system (ROS) accumulation, increased oxidative stress, which inhibited the accumulation of dry biomass, chlorophyll content and intensified the membrane lipid peroxidation as well as the decrease of antioxidant enzymes activity such as SOD, ascorbate peroxidase (APX) and CAT (Tirani et al., 2013).

Abscisic acid (ABA), one of the five recognized plant hormones, was first isolated from immature cotton boll in 1963. Since its role in promoting shedding, it is called abscisin II. The compound was officially named abscisic acid in 1965. As an endogenous hormone, ABA was widely used in agricultural production for its regulation ability of plant growth, such as participation in seed dormancy and germination, flowering and fruiting, storage protein and lipid synthesis, grain filling process and plant-microbial interactions to regulate root structure, leaf senescence and stomatal opening and closing (Sah et al., 2016). Meanwhile, plants could acquire various resistance under abiotic stress with exogenous ABA application. According to most of previous researches, exogenous ABA induced the increase of H_2O_2 and O_2^- generation, which had an inhibitory effect on seedling growth under normal condition (Lin and Kao, 2001). Wang et al. (2013) shown that exogenous spraying with 5 mg L^{-1} ABA resulted in a significant reduction of shoot dry biomass and total chlorophyll content. Similar study was observed in Arabidopsis and rice (Meguro and Sato, 2014). However, evidences shown that etiolated tissues of grasses such as rice or oat were significantly stimulated by low ABA concentrations, or a negative correlation between ABA biosynthesis and light signals was found (Humplík et al., 2017). Recently some reports also pointed out that ABA could enhance plant growth and development. Thus, ABA could be a stimulator of plant growth (Yoshida et al., 2019). Additionally, ABA can induce the expression of antioxidant genes and enhance the ability of antioxidant defence system so that confer tolerance to plants against environmental stress.

Since previous studies have reported the effect of SA and ABA application on plant growth and development, most of the trials were under abiotic or biotic stress condition (Dar et al., 2017); only a few results were reported with regard to the effect of SA and ABA application under normal condition. Even so, different results were observed. For instance, 0.25 mM exogenous SA treatment slightly increased the content of soluble protein and significantly increased SOD, peroxidase (POD), CAT and APX activity, however, higher concentration (< 1 mM) of exogenous SA to plants were usually led to oxidative stress (Chen et al., 2016). Janda et al. (2014) pointed out that exogenous SA treatment whether increase or decrease the photosynthetic activity of plants may depend on species. Additionally, the mode of application could also influence the results (Chen et al., 2016). Otherwise, the application of the two regulators in rice is less reported.

In the present study, we investigated the effects of spraying leaf with SA and ABA on morphological, physiological photosynthetic characteristics and some yield components in two rice cultivars, a hybrid cultivar and a conventional cultivar at the seedling stage and heading stage, which aimed to appraise the difference between the two rice cultivars under regulation of SA and ABA. Moreover, to ascertain the dual role of SA and ABA in plant growth under the normal condition.

MATERIALS AND METHODS

Experimental design

The hybrid rice (*Oryza sativa* L.) 'Wanshengyoutianhong-4' (W4) and the conventional rice 'Huanghuazhan' (HZ) were obtained from Guangdong Tianhong Seed Company Limited, Zhanjiang. Seeds were surface sterilized with a 2.5% sodium hypochlorite solution for 15 min, followed by rinsing several times with distilled water, and then the seeds were soaked with distilled water for 24 h and vernalized for 24 h in an incubator at 30 °C. After that uniformly germinated seeds were used as experimental materials for growing under control condition in small plastic containers (upper diameter 8.5 cm, lower diameter 6.7 cm and 9.1 cm depth) that contained sand:Latosol:nutrient soil 1:1:1 v/v/v (30 seeds per container). The rice was incubated in a greenhouse at 30 °C daytime and 25 °C in dark. In this study, two experimental periods were carried out.

Seedling stage: Treatments were carried out after 7 d naturally growth. Seedlings of the two rice cultivars were treated with salicylic acid (SA; 100 mg L^{-1}) and abscisic acid (ABA; 4 mg L^{-1}) (provided by the research group on physiological and ecological regulation of Guangdong Ocean University) through foliar spraying using a small hand-held sprayer. The spray standard was that the front and back of the blade were evenly sprayed wet to saturated drip. Therefore, the experiment included three treatments: Control, SA, and ABA. Both SA and ABA were dissolved in a small amount of absolute ethanol, but the ethanol should control below a final concentration of 0.1% in the solutions. Finally added 0.05%

Tween 20 to the solution and diluted with distilled water. The same amount of absolute ethanol, Tween 20 and distilled water were added to control. From the first day after foliar spraying was accounted. Samples (green leaves) were taken after 7 d for the measurement of morphology.

Reproductive stage: The 4 leaf/1 heart seedlings of treatments were transplanted to plastic containers (sand:Latosol 1:3 v/v; container diameter was 28 cm and depth was 38 cm), three holes per container with two plants per hole (three replicates per treatment). The amount of N fertilizer (urea, converted to pure N), P fertilizer (ammonium phosphate, converted to P_2O_5) and K fertilizer (potassium chloride, converted to K_2O) were 270, 120 and 150 kg hm^{-2}, respectively. Nitrogen fertilizer according to base fertilizer, tiller fertilizer and panicle fertilizer at the quality ratio 4:3:3; P fertilizer all as base fertilizer; K fertilizer according to base fertilizer and panicle fertilizer at the quality ratio 7:3. Samples were taken at 43rd day after transplanting (heading stage). Plant height, chlorophyll content and photosynthetic parameters were measured. Samples were taken for yield components measurement till the maturity stage.

Growth response measurements

During seedling and heading stages, plant height, stem base width, leaf area, plant height ratio, seedling index, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight were measured. Before determining dry weight, shoots and roots were separately placed in paper bags, and then were placed in an oven at 80 °C for 48 h. All the values for each parameter were mean of four independent replicates. Plant height ratio and seedling index were calculated according to the following formulas:

$$\text{Plant height ratio (mg cm}^{-1}) = \text{Shoot dry weight/Plant height} \times 1000$$

$$\text{Seedling index} = \text{Stem base width/Plant height} \times \text{Shoot dry weight}$$

Determination of chlorophyll content

SPAD values were measured from the second fully expanded leaf at seedling stage, and the flag leaf at heading stage by using portable chlorophyll meter (SPAD-502, Konica Minolta, Tokyo, Japan). The SPAD values of the apex of the leaves were measured with four independent replicates per treatment. The average was calculated and analyzed.

Determination of photosynthetic parameters

Forty-three days after transplanting, flag leaf in each treatment was measured for net photosynthesis (P_n), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and transpiration rate (T_r) by using portable photosynthesis system LI-6400 (LI-COR, Lincoln, Nebraska, USA). The parameters of instantaneous water use efficiency (WUE) and apparent mesophyll conductance (AMC) were calculated following Dias et al. (2017). The AMC, representing instantaneous carboxylation efficiency, was used to estimate ribulose bisphosphate carboxylase (RuBPCase) activity. The measurements were made between 09:00 and 11:00 h. The CO_2 concentration in the leaf chamber was 400 μmol mol^{-1}, air velocity was 500 μmol s^{-1}, light intensity was 1000 μmol m^{-2} s^{-1}, leaf temperature was 32 ± 1 °C, and relative air humidity was between 70% and 80%. The data was for three replicates with six measurements per replicate.

$$\text{WUE} = \text{Net photosynthesis/Transpiration rate}$$

$$\text{AMC} = \text{Net photosynthesis/Intercellular CO}_2\text{ concentration}$$

Determination of yield components

Rice was harvested at maturity stage. Panicle length, number of effective panicles, grain weight, 1000-grain weight, stem and leaf biomass and harvest index were measured.

Determination of malondialdehyde content

Samples of equal weight (0.5 g) were homogenized in liquid nitrogen for physiological measurement. The malondialdehyde (MDA) level was measured using the thiobarbituric acid (TBA) method as described by Calmak and Horst (1991) with a slight modification. Briefly, 0.5 g leaf tissue was homogenized with 10 mL phosphate buffer (pH 7.8) and extracted in 2 mL 0.6% TBA made in 10% trichloroacetic acid (TCA). The extract was incubated at 100 °C for 15 min and then quickly cooled on ice. After centrifugation at 5000×g for 20 min, absorbance of the supernatant was measured at 450, 532 and 600 nm. The MDA-TBA complex was quantified using the extinction coefficient as 155 mM^{-1} cm^{-1}.

Determination of enzyme activities

Peroxidase (POD) activity was assayed as described by Machly and Chance (1955) slightly modified. Briefly, enzyme was assayed with guaiacol as the substrate in a total volume of 3 mL. The reaction mixture consisted of 200 mM phosphate buffer (pH 6.0), 1% guaiacol, 30% H_2O_2, and enzyme extract. The increase in the absorbance due to the oxidation of guaiacol was measured at 470 nm. One unit of POD activity was defined as the OD_{470nm} value reduced by 0.01 in 1 min.

Catalase (CAT) activity was measured at 25 °C according to the method of Ekinci et al. (2020). The reaction mixture contained 100 µL enzyme extract, 30% H_2O_2 in 150 mM phosphate buffer (pH 7.0). CAT activity was estimated by following the decrease in absorbance of H_2O_2 at 240 nm and was expressed as mmol H_2O_2 decomposed min^{-1} mg^{-1} protein using the extinction coefficient 39.4 mM^{-1} cm^{-1}.

Ascorbate peroxidase (APX) activity was determined by following the rate of oxidation of ascorbate (2.8 mM^{-1} cm^{-1}) leading to decrease in absorbance at 290 nm, which is observed spectrophotometrically at 25 °C (Nakano and Asada, 1981). The reaction mixture (2.9 mL) comprised of 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA, 5 mM ascorbate and 20 mM H_2O_2. The reaction was started by addition of 100 µL enzyme extract in a quartz cuvette. One unit of enzyme activity was calculated as the amount of enzymes required to oxidise 1 µmol ascorbate min^{-1} mg^{-1} protein.

Determination of soluble protein content

The level of soluble protein was measured using the Coomassie brilliant blue method (Bradford, 1976); 0.5 g leaf tissue was homogenized with 10 mL phosphate buffer (pH 7.8) and extracted in 2.9 mL react mixture contained 0.1 g Coomassie brilliant blue G-250, 50 mL 95% ethanol and 100 mL 85% (w/v) phosphoric acid. The absorbance of the supernatant was measured at 595 nm after 2 min reaction, and then figure out the protein content in the sample according to the standard curve with bovine serum albumin.

Statistical analysis

Data analysis and graphical presentation was performed using the Microsoft Excel 2019. A factorial experiment based on completely randomized design was carried out with four replicates (n = 4) and statistical significance of the mean was compared by Duncan's multiple range tests at the 5% probability level using SPSS Statistics software version 19.0 (IBM, Armonk, New York, USA).

RESULTS

Effect of SA and ABA on plant height, stem base width and leaf area

As shown in Table 1, the treatments ABA and SA exhibited greater plant height values and leaf area compared with the control in hybrid rice W4, especially treated with SA, which increased by 6.99% and 10.50%, respectively. Whereas SA and ABA significantly decreased the three morphological indexes in conventional rice cultivar HZ, which decreased by 12.50%, 6.25%, 27.79% and 9.89%, 31.25%, 37.40% of plant height, stem base width and leaf area, respectively.

Rice seedling stage

Effect of SA and ABA on plant height ratio and seedling index. Under SA and ABA treatment, plant height ratio and seedling index of the two rice cultivar seedlings did not exhibit exactly the same (Figure 1). Leaf spraying with

Table 1. Effect of leaf spraying with salicylic acid (SA) and abscisic acid (ABA) on plant height, stem base width and leaf area on 14-d old hybrid rice 'Wanshengyoutianhong-4' (W4) and conventional rice 'Huanghuazhan' (HZ).

	W4			HZ		
Treatment	Plant height	Stem base width	Leaf area	Plant height	Stem base width	Leaf area
	cm	cm	cm^2	cm	cm	cm^2
Control	28.20 ± 0.56b	0.16 ± 0.003ab	64.37 ± 1.29a	28.32 ± 0.61a	0.16 ± 0.002a	61.61 ± 2.68a
SA	30.17 ± 0.37a	0.17 ± 0.004a	71.13 ± 3.33a	24.78 ± 0.46b	0.15 ± 0.007a	44.49 ± 2.52b
ABA	28.50 ± 0.75ab	0.15 ± 0.003b	65.42 ± 1.20a	25.52 ± 0.33b	0.11 ± 0.004b	38.57 ± 0.85b

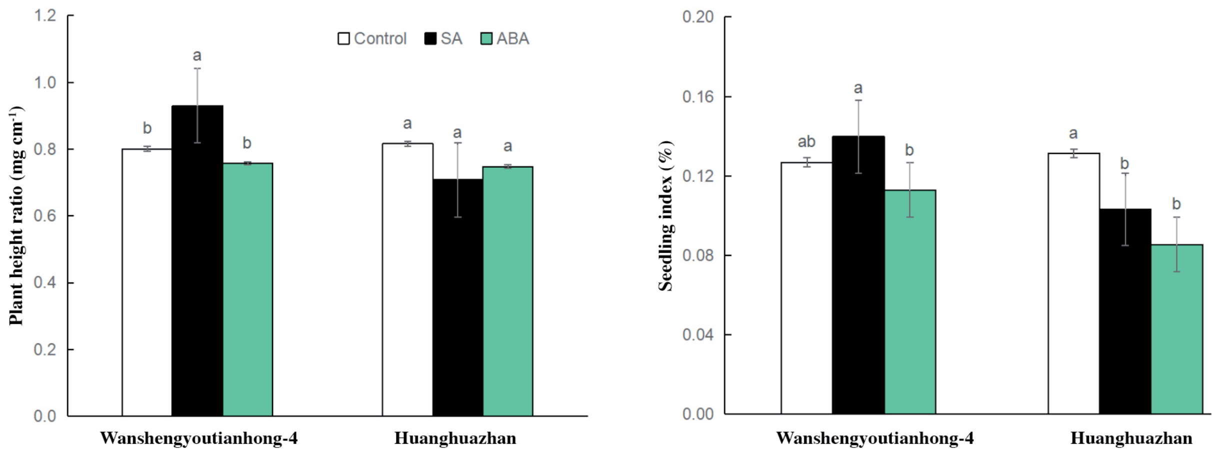
Data are mean ± standard error of four replicates (n = 4). Within each column, different letters indicate significant difference at the 5% significance level according to Duncan's multiple range tests.

SA increased plant height ratio (16.10%) and seedling index (10.25%) in comparison of the control in W4 (Figure 1); however, this effect was opposed whether SA or ABA treatment, with regard to the cultivar HZ, which decreased by 13.26%, 21.4% and 8.41%, 34.88% of plant height ratio and seedling index, respectively.

Effect of SA and ABA on chlorophyll content. Treatments with SA and ABA increased chlorophyll content in hybrid rice W4, though the difference was not prominent (Figure 2). Nevertheless, the two regulators had an inhibitory effect on chlorophyll content with regard to the cultivar HZ, the SPAD value under SA treatment exhibited the less compared to that under ABA treatment, namely, they significantly decreased the SPAD value by 15.51% and 9.78%, respectively, in comparison with the control.

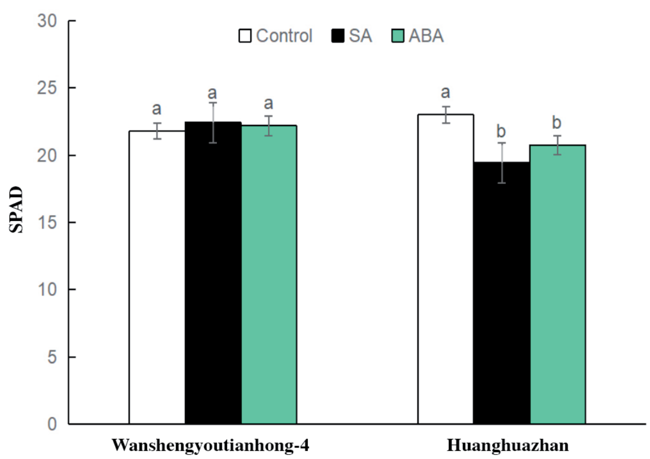
Effect of SA and ABA on biomass accumulation. Following SA and ABA treatments, shoot biomass accumulation exhibited a different trend from that of root compared to the control (Figure 3); SA had a positive effect on shoot fresh weight and shoot dry weight in W4. Opposed was the trend with SA and ABA treatment in HZ, where the decrease in

Figure 1. Effect of leaf spraying with salicylic acid (SA) and abscisic acid (ABA) on plant height ratio and seedling index of 14-d old hybrid rice 'Wanshengyoutianhong-4' and conventional rice 'Huanghuazhan'.



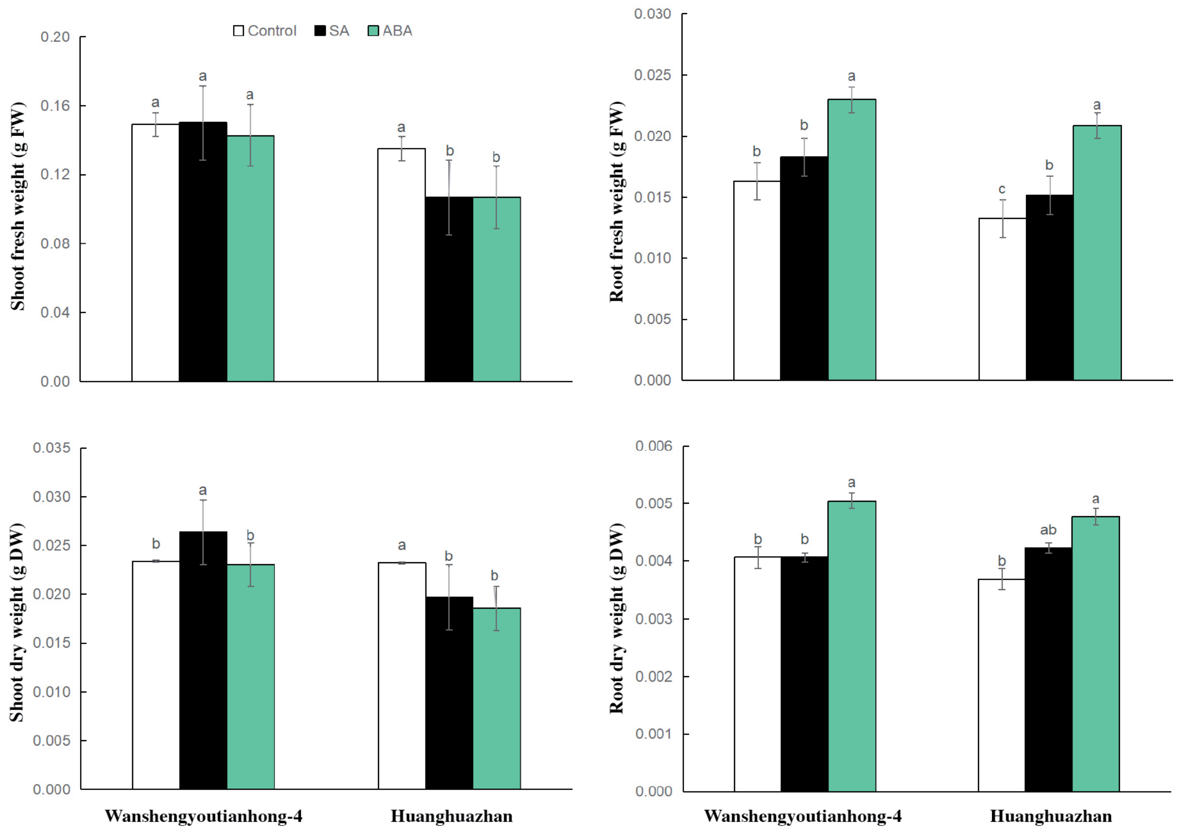
Data are shown as the mean ± standard and derived from four replicates (n = 4). Bars indicate standard errors.

Figure 2. Effect of leaf spraying with salicylic acid (SA) and abscisic acid (ABA) on chlorophyll content of 14-d old hybrid rice 'Wanshengyoutianhong-4' and conventional rice 'Huanghuazhan'.



Data are shown as the mean ± standard and derived from four replicates (n = 4). Bars indicate standard errors.

Figure 3. Effect of leaf spraying with salicylic acid (SA) and abscisic acid (ABA) on shoot fresh weight, shoot dry weight, root fresh weight and root dry weight of 14-d old hybrid rice 'Wanshengyoutianhong-4' and conventional rice 'Huanghuazhan'.



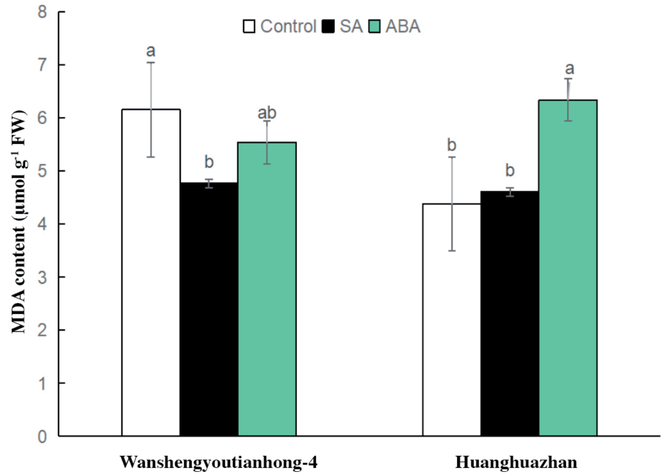
Data are shown as the mean ± standard and derived from four replicates (n = 4). Bars indicate standard errors.

shoot fresh weight and shoot dry weight was 21.01%, 15.09% and 21.04%, 20.01%, respectively. Relative to the control, both SA and ABA application enhanced root fresh weight and root dry weight in the two rice cultivar seedlings, being higher in ABA treatment, which prominently increased by 40.96% and 23.17% in W4 and 57.67%, 29.40% increase in HZ, respectively, compared with lesser increase in SA treatment.

Effect of SA and ABA on malondialdehyde (MDA). The MDA content decreased under SA and ABA treatments in W4, particularly, SA significantly decreased (by 22.55%) the MDA level in comparison with control (Figure 4). Similar was the trend with ABA treatment, where the decline percentage in MDA was 10.06% in W4. Compared to the control, MDA content was enhanced under SA (by 5.06%) and ABA (by 44.57%) application in HZ in contrast to the hybrid rice cultivar.

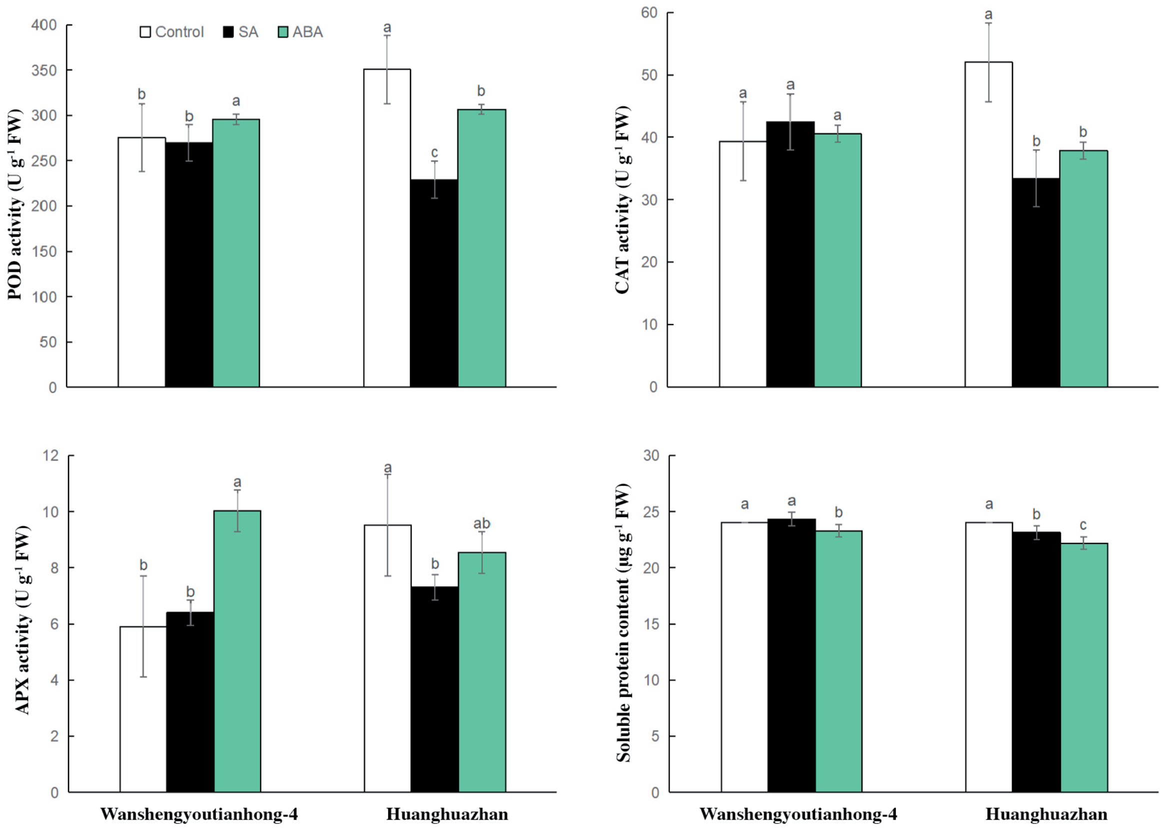
Effect of SA and ABA on some antioxidant enzymes and soluble protein. The antioxidant enzymes activity and soluble protein content exhibited an opposed trend following the SA and ABA treatment, in comparison of the control between W4 and HZ. The SA treatment decreased POD activity, while ABA treatment prominently increased the level of POD in W4. However, the level of POD significantly declined under SA (34.74%) or ABA (12.54%) application in the conventional rice HZ. The SA and ABA treatments induced higher CAT and APX activities than control, which increased by 7.82%, 8.27% in SA and 3.05%, 69.74% in ABA, respectively, in W4; with regard to HZ, CAT and APX activities under SA and ABA treatments exhibited a similar trend as POD. As shown in Figure 5, soluble protein content increased slightly in W4 treated with SA. Nevertheless, a detectable lowering in the level of soluble protein on SA and ABA application were observed in HZ, which reached a significant level.

Figure 4. Effect of leaf spraying with salicylic acid (SA) and abscisic acid (ABA) on malondialdehyde (MDA) of 14-d old hybrid rice 'Wanshengyoutianhong-4' and conventional rice 'Huanghuazhan'.



Data are shown as the mean ± standard and derived from four replicates (n = 4). Bars indicate standard errors.

Figure 5. Effect of leaf spraying with salicylic acid (SA) and abscisic acid (ABA) on peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX) activities and soluble protein content of 14-d old hybrid rice 'Wanshengyoutianhong-4' and conventional rice 'Huanghuazhan'.



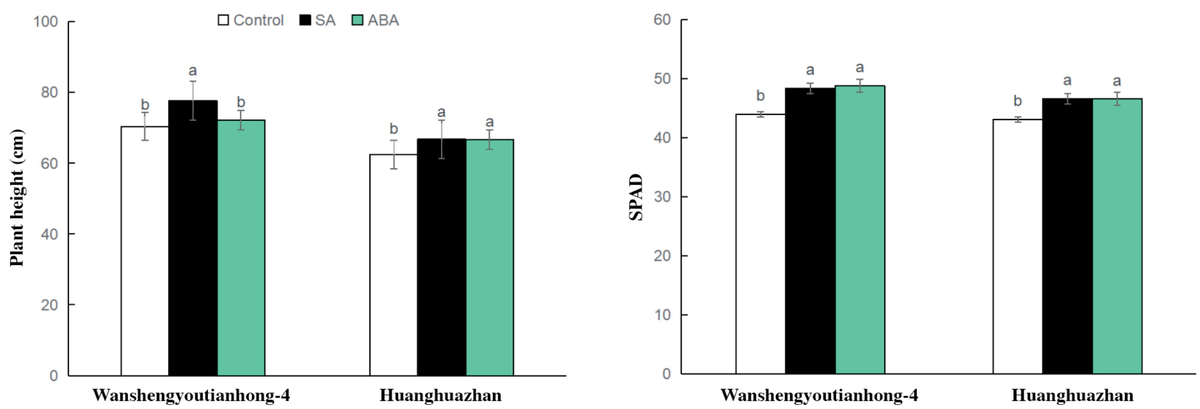
Data are shown as the mean ± standard and derived from four replicates (n = 4). Bars indicate standard errors.

Rice heading and maturity stages

Effect of SA and ABA on plant height and chlorophyll content. As shown in Figure 6, SA and ABA application had an enhancement effect on plant height, particularly for SA treatment (increased by 10.28%), which reached a significant level in W4. Similar trend was observed with SA and ABA treatment, which increased by 6.87% and 6.64% in comparison of the control in HZ. The SPAD value was prominently enhanced under SA and ABA treatment in both of the rice cultivars. Moreover, the SPAD value in W4 was higher than that of HZ.

Effect of SA and ABA on photosynthesis and yield components. Treated with SA slightly enhanced the P_n, g_s, Tr, WUE and AMC, while ABA treatment showed a better enhancement in all the photosynthetic parameters, and g_s exhibited a 31.61% increment among them, reaching a significant level in W4 (Table 2). Similarly, both SA and ABA treatment increased P_n, g_s, C_i, T_r, WUE, AMC and P_n (by 18.34%) compared to the control in HZ. There was nonsignificant effect of SA and ABA on panicle length, number of effective panicles and grain weight to the two rice cultivars (Table 3). Notably, SA and ABA enhanced grain weight and 1000-grain weight; moreover, ABA improved harvest index (15.63%) in comparison with the control in HZ. Leaf sprayed with SA and ABA significantly decreased the biomass of stem and leaf in W4, however, they improved the harvest index compared with the non-regulator treatment, particularly, reaching a prominent level with regard to SA treatment.

Figure 6. Effect of leaf spraying with salicylic acid (SA) and abscisic acid (ABA) on plant height and chlorophyll content at heading stage of hybrid rice 'Wanshengyoutianhong-4' and conventional rice 'Huanghuazhan'.



Data are shown as the mean ± standard and derived from four replicates (n = 4). Bars indicate standard errors.

Table 2. Effect of leaf spraying with salicylic acid (SA) and abscisic acid (ABA) on photosynthetic characteristic of hybrid rice 'Wanshengyoutianhong-4' (W4) and conventional rice 'Huanghuazhan' (HZ) at heading stage.

Variety	Treatment	P_n	g_s	C_i	T_r	WUE	AMC
		μmol m^{-2} s^{-1}	mol m^{-2} s^{-1}	μmol mol^{-1}	mmol m^{-2} s^{-1}	μmol mmol^{-1}	mmol m^{-2} s^{-1}
W4	Control	14.08 ± 0.63a	0.348 ± 0.011b	319.00 ± 6.25a	7.87 ± 0.14a	1.74 ± 0.10a	43.69 ± 3.23a
	SA	14.78 ± 0.53a	0.361 ± 0.022b	318.25 ± 2.39a	7.84 ± 0.31a	1.94 ± 0.07a	46.64 ± 2.58a
	ABA	15.83 ± 1.04a	0.458 ± 0.004a	328.20 ± 3.14a	8.34 ± 0.13a	1.90 ± 0.15a	48.11 ± 3.95a
HZ	Control	14.18 ± 0.32b	0.398 ± 0.054a	316.00 ± 6.67a	9.08 ± 0.69a	1.56 ± 0.10a	44.89 ± 0.68a
	SA	16.78 ± 1.06a	0.497 ± 0.041a	318.60 ± 3.27a	10.64 ± 0.57a	1.58 ± 0.05a	52.75 ± 3.66a
	ABA	14.75 ± 0.62ab	0.485 ± 0.063a	321.25 ± 6.06a	9.33 ± 0.82a	1.60 ± 0.10a	45.90 ± 1.56a

P_n: Net photosynthesis; g_s: stomatal conductance; C_i: intercellular CO_2; T_r: transpiration rate; WUE: water use efficiency; AMC: apparent mesophyll conductance.
Data are mean ± standard error of three replicates (n = 3) with six measurements per replicates. Within each column, different letters indicate significant difference at the 5% significance level according to Duncan's multiple range tests.

Table 3. Effect of leaf spraying with salicylic acid (SA) and abscisic acid (ABA) on yield components of hybrid rice 'Wanshengyoutianhong-4' (W4) and conventional rice 'Huanghuazhan' (HZ) at maturity stage.

Variety	Treatment	Panicle length	Number of effective panicles	Grain weight	1000-grain weight	Stem and leaf biomass	Harvest index
		cm		g	g	g	%
W4	Control	23.30 ± 1.11a	29.67 ± 1.45a	47.51 ± 3.84a	20.84 ± 0.51ab	71.60 ± 1.55a	0.40 ± 0.02b
	SA	22.00 ± 0.15a	26.00 ± 1.16a	46.01 ± 2.22a	21.50 ± 0.42a	40.75 ± 0.87b	0.53 ± 0.01a
	ABA	21.20 ± 0.29a	28.00 ± 1.00a	40.13 ± 3.38a	19.34 ± 0.71b	42.86 ± 3.40b	0.48 ± 0.04ab
HZ	Control	22.43 ± 0.52a	27.33 ± 0.67a	27.54 ± 3.04a	19.65 ± 0.46a	70.45 ± 4.20a	0.32 ± 0.05a
	SA	22.44 ± 0.20a	28.00 ± 3.51a	29.10 ± 6.14a	20.44 ± 0.76a	65.09 ± 7.30a	0.31 ± 0.05a
	ABA	21.93 ± 1.03a	24.33 ± 2.40a	34.08 ± 0.60a	20.54 ± 0.46a	59.92 ± 7.25a	0.37 ± 0.03a

Data are mean ± standard error of three replicates (three holes as one replicate). Within each column, different letters indicate significant difference at the 5% significance level according to Duncan's multiple range tests.

DISCUSSION

The current research confirmed that SA and ABA affected a range of plant processes, including plant growth and yield (Sah et al., 2016), regulation of plant physiological and biochemical functions (Janda and Ruelland, 2015). In present study, we evaluated the effects on morphology and physiology with exogenous SA and ABA in two rice cultivars. Our results indicated that SA and ABA significantly improved plant growth (plant height, stem base width, leaf area, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight) in W4 seedlings. However, inhibitory effects of SA and ABA were observed in HZ. Here, we found that the dual role of SA and ABA were probably in a cultivar-dependent manner. Kang et al. (2012) showed that 0.5 mM SA increased plant height and aboveground biomass of wheat seedlings. These results were consistent with the phenomenon of W4 in our study. Interestingly, SA exhibited an inhibitory effect in conventional cultivar HZ. In fact, whether the inhibited or enhanced effect of SA on plants under normal conditions mainly depended on a number of factors, including dose, plant species, developmental stages and application patterns (Poór et al., 2019). In contrast to the above statement about positive effect of SA, it was reported that 1 mM SA could reduce root length and bud length in 7-d old wheat seedlings (Sahu and Sabat, 2011). Notably a significant enhancement in root biomass was observed in HZ, which was similar to that in previous research of soybean (Gutiérrez-Coronado et al., 1998). In rice and *Arabidopsis*, ABA has been shown to inhibit both root and bud growth of seedlings (Meguro and Sato, 2014). As shown in our study, the repression of growth in HZ seedlings, possibly because ABA was located upstream of gibberellin biosynthesis pathway and negatively regulated the expression of gibberellin biosynthesis (Qi et al., 2019). Nevertheless, it was proved that ABA could also exhibit a stimulating effect in plants (Humplík et al., 2017). The enhancement of shoot and root growth was mainly via ethylene pathway, namely, via inhibiting the ethylene production (LeNoble et al., 2004). Besides, Finkelstein et al. (2002) pointed out that stimulation of ABA application on shoot growth may be due to the improvement of cell extension and stimulation of cell division, which could be attributed to the stagnation of cell cycle G1, since cell development in G1 stage is closely related to shoot growth. In this study, the hybrid cultivar W4 shown a similar result, whose aboveground morphology and root biomass were promoted via ABA application. It was noteworthy that shoot biomass was reduced while root biomass increased to a prominent level under ABA treatment in HZ, this phenomenon was well recorded in a study of Chen et al. (2006).

Determination of SPAD in plant leaves is a simple and valuable technique that can immediately assess chlorophyll content in plants, which is often used as a stress indicator (Shah et al., 2017). Fariduddin et al. (2003) pointed out that 10^{-2} mM SA prominently increased chlorophyll content in *Brassica juncea*, while 1 mM SA repressed the chlorophyll. The increased chlorophyll content by SA may be due to the protection of chloroplast pigments from harmful effects and thus promote photosynthesis. Likewise, under the concentration from 10^{-2} to 1 mM SA, the repression of chlorophyll content was demonstrated in *Brassica napus* (Tirani et al., 2013). In another research, Chandra and Bhatt (1998) pointed out that whether SA treatment increased or decreased chlorophyll content mainly depended on genotype. All of these results were in agreement with our study about the two rice cultivars. Similar to SA treatment, ABA application shown an enhancement of chlorophyll in W4, whereas an inhibitory effect in HZ. This was in agreement with Wang et al. (2013).

Studies showed that plenty of phenolic compounds play a vital role in regulating different physiological processes, including plant growth and development, ion absorption and photosynthesis (Denaxa et al., 2020). The SA is involved in the regulation of physiological and biochemical processes throughout the life cycle of plants. It is considered an effective plant hormone because of its different regulation in plant metabolism. In addition, ABA is an important signal to trigger plant response to adverse environmental conditions (Sah et al., 2016). Both SA and ABA could induce the occurrence of antioxidant systems in plants using an appropriate range of concentrations. In the current study, application of SA and ABA enhanced the CAT, POD and APX activity, significantly reduced the MDA content in W4, which is in agreement with many existing results (Hayat et al., 2012; Wang et al., 2013). These results could be attributed to that SA and ABA reduced oxidative damage to plants caused by increased production of reactive oxygen species under pressure, which may maintain the integrity of the photosynthetic apparatus by reducing membrane lipid peroxidation, increasing antioxidant enzyme activity, and protecting the membrane structure from damage, which is essential for the normal growth of rice seedlings (Park et al., 2004). Nevertheless, effects of exogenous SA and ABA on plant physiological processes under normal environmental conditions are controversial. Although they are inducers of various physiological and biochemical actions in plants, a negative effect on activity of enzymatic antioxidants was found (Sahu and Sabat, 2011). According to previous studies, SA and ABA could result in accumulation of ROS, induction of oxidative stress, stimulation of SOD, POD, APX and other antioxidant enzymes, while reducing the CAT activity (Krantev et al., 2008). In the present study, with SA and ABA application POD and APX level were significantly inhibited in addition to CAT while it was accompanied by an increase in lipid peroxidation in HZ. This may be attributed to the insufficiency of antioxidant enzymes or decrease of enzymes synthesis remove the accumulating ROS so that caused a negative endogenous protective effect as well as the high level of MDA. Last but not least, ABA-induced ROS production coordinates the antioxidant enzyme activity at different subcellular levels, whether the enzyme activity in hybrid rice increases or the activity in conventional rice decreases needs further study.

Photosynthesis is directly related to crop production. So far, previous study has demonstrated that SA and ABA could regulate plant photosynthetic processes (Fariduddin et al., 2003). Particularly, a dual role of SA was reported by previous researches. Pancheva and Popova (1998) shown that SA could inhibit the synthesis of ribose-1,5-diphosphate carboxylase/oxygenase (RuBisCO). On the other hand, many studies reported that SA could enhanced photosynthesis (Nazar et al., 2015). In the current study, the slightly improvement of photosynthetic parameters (P_n, g_s, Tr, WUE and AMC) mediated by SA and ABA were recorded in both rice cultivars. It may be due to the low doses of SA in photosynthesis that related to the repression of auxin oxidation, since the increase of auxin level increases the net photosynthetic rate and nitrate reductase activity (Ahmad et al., 2001). Khan et al. (2003) pointed out that the increase of photosynthetic rate after application of SA was not always accompanied by the increase of stomatal conductance level or transpiration rate. Instead, the concentration of intercellular CO_2 was generally lower than that in control plants. This suggested that the increase in photosynthetic rate after spray some phenolic compounds such as SA may be the result of an increase in enzyme activity related to CO_2 absorption at the chloroplast level, rather than an increase in simple stomatal opening. Similar result was observed in W4 in our study.

The P_n/C_i ratio can be considered as RuBisCO activity in the Calvin cycle, which catalyses the rate-limiting step during CO_2 fixation process. In our study, we found that both SA and ABA application possessed higher AMC than that in control, which indicated that the application helped plants effectively use existing CO_2 to maintain their growth. Additionally, water use efficiency (WUE) means how a plant effectively uses limited available water for biomass production, which is important in determining crop productivity (Medrano et al., 2015). As shown in the current research, enhancement of WUE was observed with SA and ABA in the two cultivars, which contributed to improve photosynthetic efficiency and crop productivity by providing optimal conditions for plants themselves.

One important factor in controlling physiological responses is tissue sensitivity to regulatory compounds (Humplík et al., 2017). Trewavas (2010) pointed out that any effect of plant hormones is mainly tissue sensitivity, followed by compound concentration. Overall, application of SA and ABA result the differences in growth and physiology between the two different rice varieties during seedling stage, which can be explained by differences in experimental design, development and environmental condition, since each operation of ABA content leads to changes in plant water status, which in itself affects growth.

CONCLUSIONS

Foliar spraying of exogenous salicylic acid (SA) and abscisic acid (ABA) at seedling stage could exhibit differences due to the different rice genotypes. Multiple growth and developmental processes may accompany this phenomenon. For instance, increasing seedling index and leaf area as well as the level of chlorophyll, besides, enhanced the protective enzyme activity in leaves of hybrid rice cultivar (W4), while these morphophysiological processes were suppressed in conventional rice cultivar (HZ). On the other hand, SA and ABA improved growth quality of both rice cultivars at reproductive stage, these was mainly related to the enhancement of photosynthesis in leaves. These results indicated that the effect of exogenous SA and ABA on seedling stage mainly depended on plant growth stages and cultivars. This study can provide reference for plant growth regulators in rice cultivation management.

ACKNOWLEDGEMENTS

We would like to acknowledgement the financial supports from "Ling Hang" Program of Zhanjiang Innovation and Entrepreneurship Team Introduction (2020LHJH01); Research start-up project of Guangdong Ocean University (R20046); Research start-up project of Guangdong Ocean University (060302052012); "Innovation and Strong School Project" of Guangdong Ocean University in 2020 (230420006). Wanshengyoutianhong-4 and Huanghuazhan cultivars were furnished by Guangdong Tianhong Seed Company Limited, China.

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