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ORIGINAL ARTICLE

Cold plasma treatment and exogenous salicylic acid priming enhances salinity tolerance of *Oryza sativa* seedlings

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Abstract

The present study was designed to highlight the effects of cold plasma (10 kV) treatment and priming with 2 mM salicylic acid (SA) and their combination (10 kV of plasma + 2 mM SA) on the physiological parameters and metabolism of two cultivars of *Oryza sativa*, i.e., Zhu Liang You 06 (ZY) and Qian You No. 1 (QY), under salinity stress (150 mM NaCl) and normal growth condition (0 mM NaCl). Seed germination and seedling growth were enhanced by SA priming and cold plasma treatment either alone or in combination under salinity stress. Photosynthetic pigments, photosynthetic gas exchange, and chlorophyll fluorescence were improved by cold plasma treatment and SA priming under salinity stress as compared to the untreated seeds. The activities of antioxidant enzymes were significantly improved by the combination of SA priming and cold plasma treatment in both cultivars under salinity stress. There were rapid changes in the cellular content of sodium (Na^+) and calcium (Ca^+), where the plants grown under saline conditions accumulate more Na^+ and less Ca^+ contents resulting in ionic imbalances. Interestingly, cold plasma and SA treatments diminished this action by reducing Na^+ accumulation and increasing K^+ and Ca^+ contents in the plant cell under salinity stress. The activities of enzymes involved in secondary metabolism assimilation were up-regulated with cold plasma and SA priming either alone or combination under salinity stress. An increase in reactive oxygen species (ROS) accumulation and malondialdehyde (MDA) content was also observed under salinity stress condition. On contrast, seed treated with SA and plasma alone or combined resulted in a significant decrease in ROS and MDA contents under salinity stress. Our results indicated that SA priming and cold plasma treatment either alone or combined improved plant uptake of nutrients in both cultivars under stress conditions. The ultrastructural changes were observed to be more prominent in ZY than QY cultivar. Plants without SA priming or cold plasma treatments have a big vacuole due to the movement of ions into the vacuole directly from the apoplast into the vacuole through membrane vesiculation leading to membrane destabilization. However, SA priming and cold plasma treatment alone or combined helped the plants to recover their cell turgidity under salinity stress.

Keywords Salinity · SA · Cold plasma · *Oryza sativa* · Antioxidant · Ultrastructure · Secondary metabolism

Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops that serve as the staple food for almost half of the world's population. Rice is not salt tolerant; however, it is suited to cultivate in affected saline soils because of standing-water tolerance (Sarangi et al. 2015). The standing water benefits rice by diluting the salts; increasing the availability of Fe, Mn, N, P, and Si; and conserving N and eliminating water stress (Lafitte et al. 2004). Recently, it has been reported that seed germination and seedling growth are the two critical stages for the establishment of crops, and these stages are the most sensitive stages to abiotic stress especially salinity stress (Hubbard et al. 2012). As such, Ansari and Sharif-Zadeh (2012) stated that increasing salinity levels

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could decrease the germination percentage and increases the mean germination time. Similarly, Khan and Weber (2008) reported that seed dormancy could be induced by low salt concentration, while high salt concentration would inhibit the seed germination and decrease the germination percentage. Recently, it has been reported that the excess level of NaCl could stimulate reactive oxygen species (ROS) accumulation and adversely effected uptake of water and nutrients, photosynthesis as well as plant growth (Sawada et al. 2008). Furthermore, ionic imbalance occurring in the cells could be due to excessive accumulation of Na^+ and Cl^- ions and reduced uptake of mineral nutrients such as K^+, Ca^{+2}, and Mn^{+2} (Hasegawa et al. 2000). It has been stated that the increase of salinity level in the plant growth media induces the formation of toxic ROS including singlet oxygen ($^1O_2$), superoxide radical ($O_2^{\cdot-}$) and hydroxyl ion ($^{\cdot}OH$) (Mittler 2014). These ROS injures the cellular structure of chloroplasts and mitochondria (Mittler 2014). Recently, it has been reported that salinity stress significantly reduced the photosynthetic pigments such as chl *a*, chl *b*, total chl, and carotenoids as well as carbohydrate contents of *Salicornia prostrata* seedlings (Akcin and Yalcin 2016). Similarly, salinity stress could cause changes in water relations of the plants, and these changes have been reported by a recent study that confirm that common bean plants exposed to salt stress undergo osmotic regulation by increasing the negativity of the osmotic potential of the leaf sap (Gam et al., 2009). In addition, carbohydrate is reported to be a candidate signal of the salt defense mechanism and is controlled by the osmoticum in plant cells subjected to salinity stress (Rosa et al. 2009).

Seed priming technique is a pre-sowing treatment that exposes seeds to a certain solution for a certain time that allows partial hydration, but radicle emergence does not occur, and the seed hydration is controlled during seed priming (Ibrahim 2016; Sheteiwy et al. 2017a). It makes the seed moisture at a level below that needed for actual germination. This level is enough to start the metabolism and physiological processes related with the early stage of germination, but prevent the seed transition toward full germination (Sheteiwy et al. 2015). SA plays an important role in the plant growth regulation, development, ripening, flowering, and responses to different abiotic stresses (Rivas-San and Plasencia, 2011). It has been stated that the low concentrations of SA may enhance the antioxidant capacity in plants, but SA at high concentrations may cause cell death or susceptibility to abiotic stresses (Hara et al. 2012). SA is involved in the regulation of important physiological processes in plants such as photosynthesis, nitrogen and proline metabolism, antioxidant defense system, and plant–water relations under stress conditions and ultimately could provide protection in plants against abiotic stresses (Miura and Tada 2014). Exogenously supplied SA either through seed soaking, adding to the nutrient solution, irrigating, or spraying was reported to induce major abiotic stress tolerance mechanisms (Khan and Nafees 2014). It has been reported that SA treatment is a promising method to the seed industry because it is an important inducer for disease and pest resistance, as well as significantly improved yield and quality of rice seed (Tavares et al. 2014). Other studies have shown that exogenous SA application enhances oxidative damage (Sawada et al. 2008). Moreover, Khan and Nafees (2014) reported that SA application alleviated the adverse effects of salt stress in mungbean through the improvement of plant photosynthesis and growth as well as antioxidant system. Kalaivani et al. (2016) reported that priming rice with methyl salicylate (MeSA) resulted in significant enhancement of germination percentage, germination rate, seedling emergence, root/shoot ratio, and fresh and dry weight of rice seedlings. Another study has reported that priming can increase rice resistance to abiotic stress (Senthil-Nathan et al. 2009).

Earlier study reported that cold plasma treatment is a fast, economic and pollution-free technique to improve seed germination, growth performance, and crop yield under abiotic stress condition (Tong et al. 2014). Cold plasma treatment is an environmentally friendly method because this technique involves dry chemistry and thus does not produce contaminated residues (Dhayal et al. 2006). It has reported that cold plasma treatment could also improve the physiological metabolism of the plant, such as dehydrogenase activity, superoxide dismutase (Yin et al. 2005), and peroxidase activities (Jiang et al. 2014). Seed surface modification in plasma-treated seeds could enhance the transmission of oxygen or water through the seed coat (Bormashenko et al. 2012). This action could increase the water imbibition of the plasma-treated wheat, bean, and lentil seeds as compared with the control samples (Bormashenko et al. 2012). In addition, it has been observed that there are changes of wettability in the seeds' surface under plasma treatment, which might be due to increase in the concentration of oxygen and nitrogen-containing groups at the surface of the plasma-treated seeds (Bormashenko et al. 2012). Earlier, changes in enzyme activity of plants as a result of seed exposure to cold plasma treatment have also been reported by Henselova et al. (2012). It has been reported that the effects of cold plasma treatment using various gases such as carbon fluoride (CF_4), aniline, cyclohexane, helium, and air on the seed germination and growth enhancement of plants were observed (Tong et al. 2014). A previous study has reported that cold plasma improved the activities of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) and decrease the lipid peroxidation (MDA) content of *Andrographis paniculata* seedlings (Tong et al. 2014).

Thus, the purpose of this study is to test the hypothesis that the cold plasma and SA treatments are involved in the regulation of physiological processes and cellular signaling of plants such as antioxidant defense system, photosynthesis, secondary metabolism signaling, and plant–water relations under stress condition which ultimately might provide defense

and protection in rice plants against salinity stress. For this purpose, in the current study, we have investigated the effects of exogenous SA priming and cold plasma treatments and their combination on *O. sativa* seedlings' growth and physiology under 150 mM NaCl by analyzing the activity of enzymes involved in carbohydrate metabolism, enzyme activity of secondary metabolism, antioxidant and non-antioxidant enzyme activities, photosynthesis parameters, and the detection of ROS and cell death as well. In addition, the accumulation of hydrogen peroxide (H_2O_2), $O_2^{\cdot-}$, $^{\cdot}OH$, glutathione reductase (GR), glutathione reduced (GSH), and glutathione oxidized (GSSG) as well as ionic accumulation were also examined to disclose the mechanism underlying the ameliorating role of cold plasma treatment and exogenous SA priming to improve rice tolerance under higher salinity level.

Materials and methods

Seed materials and seed treatments

Seeds of two rice cultivars, i.e., Zhu Liang You 06 (ZY) and Qian You No. 1 (QY), were obtained from the Seed Science Center, College of Agriculture and Biotechnology, Zhejiang University, China. Seeds were surface sterilized according to the method described by Sheteiwy et al. (2015). Thereafter, one part of seeds were primed in petri dishes with blotter paper moistened with 2 mM of SA at 15 °C for 24 h in the dark, and then dried back to their original moisture content (Sheteiwy et al. 2016). Another part of seeds were treated with the cold plasma (non-thermal plasma generator, P) at the College of Environmental and Resource Science, Zhejiang University, Hangzhou, China. The cold plasma frequency used in the present study was 2 kHz, the power was 10 kV (based on our preliminary experiment), and the volume of the discharge chamber was a cylinder with $d = 20$ mm and $L = 12$ cm. The time span of irradiation was 1 min, and the temperature of the discharge was about 25 °C. The plasma gases used for pressure was N_2 (79%) and O_2 (21%) without water vapor. Some of SA-primed seeds were treated with cold plasma and considered as combination treatment (P + SA). Seed soaked in water without both cold plasma treatment and SA priming was used as control (Ck). Salinity treatments, 0 mM (without salinity) and 150 mM NaCl concentrations were supplied to plants with or without SA priming and cold plasma treatment.

Seed germination and seedling growth measurements

Fourteen-day-old seedlings were treated with SA priming and cold plasma either alone or combined and exposed to salinity stress; one part of seedlings was taken out for the germination tests and seedling character measurements according to the method of Almeida et al. (2012). The number of germinated seeds was recorded after 14 days and the final percentage of seed germination was calculated (International Seed Testing Association (ISTA), 2004). The germination energy was calculated at the fourth day as the percentage of germinated seeds to all the tested seeds (Hu et al. 2016). The length of shoots and roots of ten randomly selected seedlings for each treatment were measured manually with a ruler; fresh weights of ten roots and shoots for each treatment were weighed immediately, and their dry weights were recorded after drying at 80 °C for 24 h in an oven. Seed vigor index and leaf surface area were calculated according to the method of Sheteiwy et al. (2015). Another part of seedlings was transplanted into 48-well plastic containers with aerated hydroponic solution (containing 4 mL of 1/4 strength Hoagland's medium, pH 6.0) including 0 and 150 mM NaCl for 1 month for the other remaining analysis. In the first week of hydroculture, half concentration of Hoagland solution was supplied to plants, and then full concentration was supplied from the second week and all solutions were renewed weekly.

Determination of photosynthetic parameters

The photosynthetic pigments including chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids were measured according to the methods of Sheteiwy et al. (2015) with slight modification. Photosynthetic gas exchange parameters such as photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO_2 concentration (Ci), and transpiration rate (Tr) were measured in the seedlings with a LiCor-6400 portable photosynthesis system (Li-Cor Inc., Lincoln, NE, USA) according to the method of Ahmed et al. (2015).

Determination of water potential and relative electrolyte leakage

A piece of fresh leaf (1.0 cm) was cut down and used to determine the water potential (Ψ_p) with a vapor pressure osmometer (Wescor Inc., Logan, UT, USA) (Ahmed et al. 2013). The relative electrolyte leakage (dS m^{-1}) of seedling after salinity treatments was measured according the method of Sheteiwy et al. (2015).

Measurements of chlorophyll fluorescence parameters

Photochemical quenching parameters (F0, Fm, Fm′, and Fv/fm) were measured using an imaging pulse amplitude-modulated (PAM) fluorimeter (IMAG-MAXI; Heinz Walz, Effeltrich, Germany). The F0, Fm, Fm′, and Fv/fm indicated respectively the minimal fluorescence yield when the PSII reaction center is open, the maximal fluorescence yield at the time of PSII reaction center closure, the maximal

fluorescence in the light-adapted state, and the photochemical efficiency of PSII. These four parameters were determined in the expanded leaves after dark adaptation for 15 min according to the method of Ali et al. (2015).

Determination of antioxidant and non-antioxidant enzyme activities

The antioxidant enzyme activities of seedling after salinity treatment such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD) were determined according to the method of Sheteiwy et al. (2015) with some modification. Shoot and root samples (0.5 g each) were taken from each treatment and homogenized in 8 mL of 50 mM potassium phosphate buffer (pH 7.8) under ice-cold conditions. Then, the homogenate was centrifuged at 10,000×*g* for 20 min at 4 °C and the supernatant was used for those four enzymes' activity determination according to the method of Sheteiwy et al. (2015). The activity of GR was assayed according to the method of Schaedle and Bassham (1977) with a little modification. GSH and GSSG activities were determined according to the method of Law et al. (1983).

Element analysis

Root and shoot samples were collected from each treatment after salinity treatments and washed with distilled water thrice. Thereafter, root and shoot samples were dried at 80 °C in an oven for 48 h and were then powdered and ashed at 550 °C for 12 h. Shoot and root powders (0.2 g each) were digested with a mixture of 5 mL of HNO_3 and 1 mL of $HClO_4$, and the concentrations of Na^+, K^+, Ca^{+2}, Mg^{+2}, P, Mn, Fe, Cu, Zn, and Cr were determined using inductively coupled plasma–mass spectrometry (ICP–MS; Agilent, 7500a) according to the standard procedure of Shentu et al. (2008).

Determination of enzyme activity of secondary metabolism

Phenylalanine ammonia-lyase (PAL) enzyme extraction was measured according to the method of Zheng et al. (2005) with slight modification. Two grams of frozen fresh leaves were homogenized with 2.5 mL of solution containing 100 mM K_3PO_4 buffer, 2 mM EDTA, 1% (*m*/*v*) PVP, and 1 mM phenyl-methylsulfonyl fluoride (PMSF). Then, the homogenates were centrifuged at 12,000×*g* for 15 min at 4 °C and the supernatant fractions were used for enzyme analysis. The reaction was terminated by the addition of 6 mol L^{-1} HCl, and its absorbance was measured at 290 nm by a spectrophotometer (Shimadzu UV-Vis spectrophotometer, Japan). The activity of polyphenol oxidase (PPO) was measured by the oxidation of catechol in the presence of H_2O_2 (Ghanati et al. 2002). The shikimate dehydrogenase (SKDH) activity was measured according to the method of Blasco et al. (2013) with slight modification. The activity of cinnamyl alcohol dehydrogenase (CAD) was measured by the oxidation of the appropriate hydroxycinnamyl alcohol at 30 °C according to the method of Wyrambik and Grisebach (1975).

The activities of sucrose synthase (SuSy), sucrose phosphate synthase (SPS), and acid invertase (AI) were measured according to the method of Jiang et al. (2014) with some modifications. In brief, 0.5 g of leaf tissue was ground in 5 mL of grinding buffer and was centrifuged at 10,000×*g*. The supernatant was collected and subjected to dialysis in dialysis bags dipped in dialysis buffer (25 mM Tris–HCl pH 7, 2.5 mM $MgCl_2$, 1 mM EDTA-Na^2, 1% ethylene glycol, and 1 mM DTT) overnight, and then the solution was used for the analysis of SuSy, SPS, and AI activities.

Analysis of MDA and ROS contents

The content of MDA was analyzed according to the method of Sheteiwy et al. (2015). The content of $O_2^{\cdot-}$ in the root and leaf was determined according to the method of Jiang et al. (2014) with slight modification. For the determination of H_2O_2 contents, 0.5 g of root sample was extracted with 5.0 mL of 0.1% trichloroacetic acid (TCA) in an ice bath, and the homogenate was centrifuged at 12,000×*g* for 15 min (Halliwell et al. 1987). Then 0.5 mL of supernatant was mixed with 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0) and 1 mL of 1 M KI. The absorbance was read at 390 nm and the H_2O_2 contents were calculated by using a standard curve. To determine the concentration of $^{\cdot}OH$, shoot and root samples (0.5 g each) were incubated in 15 mM 2-deoxy-D-ribose (pH 7.4) at 37 °C for 2 h (Halliwell et al. 1987). Thereafter, 0.7 mL of mixture was added into the reaction mixture containing 3 mL of 0.5% (*w*/*v*) thiobarbituric acid (TBA, 1% stock solution made in 5 mM NaOH) and 1 mL of glacial acetic acid. Then the mixture was heated at 100 °C in a water bath for 30 min and cooled down to 40 °C for 10 min before measurement.

In vivo detection of ROS and cell death

Hydrogen peroxide (H_2O_2) was measured according to the method of Kwasniewskia et al. (2013) with slight modification. The roots were incubated in 5 μM dichlorodihydrofluorescein diacetate for 15 min, and then washed with excess 20 mM sodium phosphate buffer (pH 6.1) to stop the reaction. The changes in $\Delta\Psi_m$ were analyzed using the tetramethylrhodamine methyl ester assay kit (Immunochemistry Technologies, Bloomington, USA) following the manufacturer's instructions. The viability of root cell was determined by propidium iodide staining (Kwon et al. 2013). The stained roots were mounted on 26 × 76 mm microscope

slides (Citoglas, China) and imaged using laser confocal scan microscope (Zeiss LSM 780, Zeiss, Germany).

Electron microscopy observation

The ultramorphology changes of leaf and root cells were detected according to the method described in our previous study (Sheteiwy et al. 2015) with a little modification. In brief, leaf segments without veins and root tips (8–10 each per treatment) were collected from randomly selected seedlings and fixed overnight in 2.5% glutaraldehyde (*v*/*v*) in 0.1 M PBS (sodium phosphate buffer, pH 7.4). Then the samples were post-fixed in 1% OsO_4 [osmium (VIII) oxide] and dehydrated in a graded series of ethanol concentration from 50 to 100%. Thereafter, the samples were infiltrated and embedded in Spurr's resin overnight. After heating at 70 °C for 9 h, ultrathin sections (80 nm) of specimens were prepared and mounted on copper grids for viewing by a transmission electron microscope (JEOLTEM-1230EX) at an accelerating voltage of 60.0 kV.

Statistical analysis

Treatments were arranged in completely randomized design in factorial experiment. All values were mean of three replicates ± standard deviation (SD). Percentage data were arcsin-transformed before analysis according to $\hat{y} = \arcsin [\mathrm{sqr}\ (x/100)]$. The data were analyzed using SPSS v16.0 (SPSS, Inc., Chicago, IL, USA). Analysis of variance (ANOVA) was carried out, followed by Duncan's multiple range tests ($p < 0.05$).

Results

Changes of seed germination and seedling growth

The results depicted that germination percentage was reduced under salinity stress. However, salt-induced negative effects were significantly diminished by SA priming and cold plasma treatment and their combination (Table 1). The highest germination percentage was obtained from the combination treatment of SA priming and cold plasma treatment under normal growth condition of both cultivars without significant differences between them (Table 1). The main difference in seed germination was observed between the untreated seeds (CK, 63.24%), plasma treatment (P, 71.66%), priming with SA (SA, 66.58%), and the combination treatment (P + SA, 83.58%). Similarly, energy of the germination of the seeds was decreased under salinity stress as compared with normal growth condition (Table 1). The germination energy was the highest in the seed treated with the combination treatment (P + SA, 74.33%) followed by cold plasma-treated seeds (P, 52.91%), SA priming (SA, 43.83%) as compared with the untreated seeds (CK, 21.49%). Our results reported that seeds treated with cold plasma and SA either alone or combined significantly improved the root and shoot length of both cultivars as compared to the control. The highest root length was recorded with P + SA followed by seed treated with plasma alone in both cultivars under normal growth condition (Table 1). Seed treated with cold plasma combined with SA recorded the highest shoot length of both cultivars followed by seed treated with cold plasma alone relative to their respective controls (Table 1). As compared to the control plants, seed treated with the cold plasma and SA alone or combined significantly increased root fresh and dry weight, and shoot fresh and dry weight of both cultivars under both normal and salinity stress conditions (Fig. S1). The combination of plasma treatment and SA priming significantly recorded the highest dry and fresh weight of root and shoot, except for root dry weight of ZY cultivar under 150 mM of salinity, which seem to be highest in SA-primed seeds (Fig. S1). The combination treatment resulted in highest seedling vigor index in both cultivars under normal and salinity stress conditions (Table 1). The leaf surface area was decreased in both cultivars under salinity stress as compared with normal growth condition (Table 1). However, seeds treated with plasma or SA alone or combined improved the leaf surface area in both cultivars under salinity stress. Seeds primed with SA recorded the highest leaf surface area (6.46 cm^2) as compared to the untreated seeds (3.12 cm^2) (Table 1).

Cold plasma and SA improves photosynthetic parameters

Results revealed that salinity stress alone significantly reduced the photosynthetic parameters in both cultivars irrespective to SA priming and cold plasma treatment in both cultivars. However, SA priming and cold plasma treatment significantly increased chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid contents in the leaves under salinity stress of both cultivars compared to those of controls (Fig. 1a–d). Cold plasma treatment recorded the highest photosynthetic pigments followed by priming with SA as compared to those of control.

The results in Fig. 1e–h depicts the changes in photosynthetic gas exchange parameters induced by cold plasma and SA priming, either alone or in combination, in *O. sativa* seedlings under normal and salinity stress conditions. All of the photosynthetic parameters including Pn, Gs, Ci, and Tr were decreased by salinity stress as compared with normal growth conditions. However, cold plasma treatment and SA priming, either alone or combined, significantly enhanced the photosynthetic parameters in both cultivars (Fig. 1e–h). Cold plasma treatment improved Pn by 50.88%, Tr by 35.22%, Gs by 79.88%, and Ci by 22.35% as compared to control. Seeds primed with SA alone significantly improved Pn by 34.53%,

Table 1 Effects of alone and combined treatments of cold plasma and exogenous SA on germination percentage (GP, %), germination energy (GE, %), root length (cm), shoot length (cm), seedling vigor index (SVI), and leaf surface area (LSA) of two cultivars of *Oryza sativa* under salinity stress

Cultivars	Treatments	GP (%)	GE (%)	Root length (cm)	Shoot length (cm)	SVI	LSA (cm^2)
ZY	Ck + 0 mM	76.33 ± 3.21c–f	32.66 ± 3.05f	9.26 ± 0.25g	8.00 ± 0.20ef	1318.5 ± 73.29g	3.30 ± 0.26g
	Ck + 150 mM	53.33 ± 3.05ij	20.00 ± 4.00g	7.03 ± 0.15h	6.70 ± 0.10g	732.67 ± 50.24k	2.48 ± 0.26h
	P + 0 mM	83.66 ± 1.15b	63.00 ± 2.64bc	11.76 ± 0.47b	11.16 ± 0.15b	1918.7 ± 37.71c	6.26 ± 0.25c
	P + 150 mM	63.33 ± 3.05g	49.33 ± 2.30d	10.23 ± 0.25de	8.40 ± 0.36e	1181.0 ± 90.31h	4.86 ± 0.25e
	SA + 0 mM	80.66 ± 1.15bc	56.66 ± 3.05c	10.80 ± 0.10c	9.83 ± 0.25cd	1664.3 ± 16.50e	7.10 ± 0.17a
	SA + 150 mM	56.00 ± 3.46hi	40.00 ± 3.20e	9.43 ± 0.15fg	7.96 ± 0.65ef	973.33 ± 40.01i	5.60 ± 0.40d
	P + SA + 0 mM	94.66 ± 1.52a	88.00 ± 4.0a	14.06 ± 0.37a	11.90 ± 0.36a	2458.0 ± 42.85a	4.26 ± 0.25f
	P + SA + 150 mM	76.00 ± 2.00d–f	69.33 ± 8.32b	11.86 ± 0.45b	10.26 ± 0.25c	1681.3 ± 34.77e	3.10 ± 0.10 g
QY	Ck + 0 mM	74.00 ± 1.00ef	21.33 ± 2.30g	9.40 ± 0.45fg	8.26 ± 0.30e	1306.9 ± 32.05g	4.00 ± 0.10f
	Ck + 150 mM	49.33 ± 3.05j	12.00 ± 4.00h	6.63 ± 0.15h	6.20 ± 0.10g	633.00 ± 47.03l	2.70 ± 0.10h
	P + 0 mM	80.33 ± 2.30b–d	58.00 ± 2.00c	11.50 ± 0.10b	10.86 ± 0.15b	1797.0 ± 61.59d	6.66 ± 0.28b
	P + 150 mM	59.33 ± 3.05gh	41.33 ± 2.30e	9.83 ± 0.25ef	7.90 ± 0.36ef	1052.7 ± 82.88i	4.83 ± 0.20e
	SA + 0 mM	77.66 ± 2.30c–e	46.66 ± 6.11de	10.50 ± 0.10cd	9.46 ± 0.05d	1550.8 ± 51.13f	7.26 ± 0.25a
	SA + 150 mM	52.00 ± 3.46ij	32.00 ± 4.20f	9.06 ± 0.15g	7.46 ± 0.65f	858.67 ± 38.01j	5.90 ± 0.35cd
	P + SA + 0 mM	91.66 ± 2.08a	81.33 ± 6.11a	13.70 ± 0.10a	11.26 ± 0.25b	2289.0 ± 77.35b	4.26 ± 0.25f
	P + SA + 150 mM	72.00 ± 2.00f	58.66 ± 6.11c	11.50 ± 0.40b	9.76 ± 0.25cd	1531.0 ± 54.99f	3.38 ± 0.07 g

Values are mean ± SD ($n = 3$). Different letters following the data within each column mean significant difference at $P < 0.05$

P plasma, *SA* salicylic acid

Tr by 36.77%, Gs by 70.80%, and Ci by 40.09% as compared to control, whereas SA priming combined with cold plasma treatment significantly increased Pn by 59.00%, Tr by 50.71%, Gs by 78.59%, and Ci by 51.87% as compared to control. The highest Pn of the leaf was observed in SA priming combined with cold plasma treated seeds (9.50) followed by cold plasma treatment alone (7.94) as compared to the control plants (3.90). Similarly, Tr and Ci were found to be the highest in SA priming combined with cold plasma treated seeds (6.62 and 531.92, respectively), followed by SA priming alone (5.18 and 427.41, respectively) as compared with control plants (3.28 and 256, respectively). Cold plasma alone resulted in highest Gs in the leaves (0.907) followed by the combination of SA priming and cold plasma treatment (0.850) as compared with the untreated seeds (0.182).

Water potential and electrolyte leakage changes

Our results reported that water potential increased positively in both cultivars as a response to salinity stress (Fig. 1i). The results revealed that cold plasma treatment and SA priming, either alone or combined, significantly decreased water potential in both cultivars under salinity stress. The increase in water potential after cold plasma treatment, SA priming, and their combination was 19.33, 61.18, and 67.24%, respectively, under salinity stress (Fig. 1i). Similarly, the electrolyte leakage of the seed increased in both cultivars under salinity stress (Fig. 1j). However, seeds treated with cold plasma and SA priming alone or combined significantly reduced the electrolyte leakage in both cultivars under salinity stress. The electrolyte leakage was decreased by 35.71, 9.11, and 47.53% in response to cold plasma treatment, SA priming, and their combination, respectively, as compared to control plants.

Cold plasma and SA enhance chlorophyll fluorescence parameters

Results revealed that salinity stress alone significantly reduced the photosynthetic quenching parameters such as F0, Fm, Fm′, and Fv/fm in both cultivars. However, the different treatment of SA and cold plasma significantly improved F0, Fm, Fm′, and Fv/fm of both cultivars under salinity stress. Based on the relevant results, cold plasma treatment improved F0 by 51.57%, Fm by 37.5%, Fm′ by 38.01%, and Fv/fm by 51.89% as compared to control. Seeds primed with SA improved F0 by 31.84%, Fm by 16.66%, Fm′ by 66.22%, and Fv/fm by 65.63% as compared to those of control, whereas the application of SA priming combined with cold plasma treatment improved F0 by 65.08%, Fm by 30.70%, Fm′ by 62.86%, and Fv/fm by 66.79% as compared to those of control. On the other hand, false-color application was useful to understand the changes of Fv/fm induced by cold plasma and SA and their combination treatments under higher salinity level (150 mM). The results showed that Fv/fm content in leaves decreased quickly with prolonged salt stress, but SA priming and cold plasma treatment and their combination inhibited this effect. Leaf color changed from violet blue to

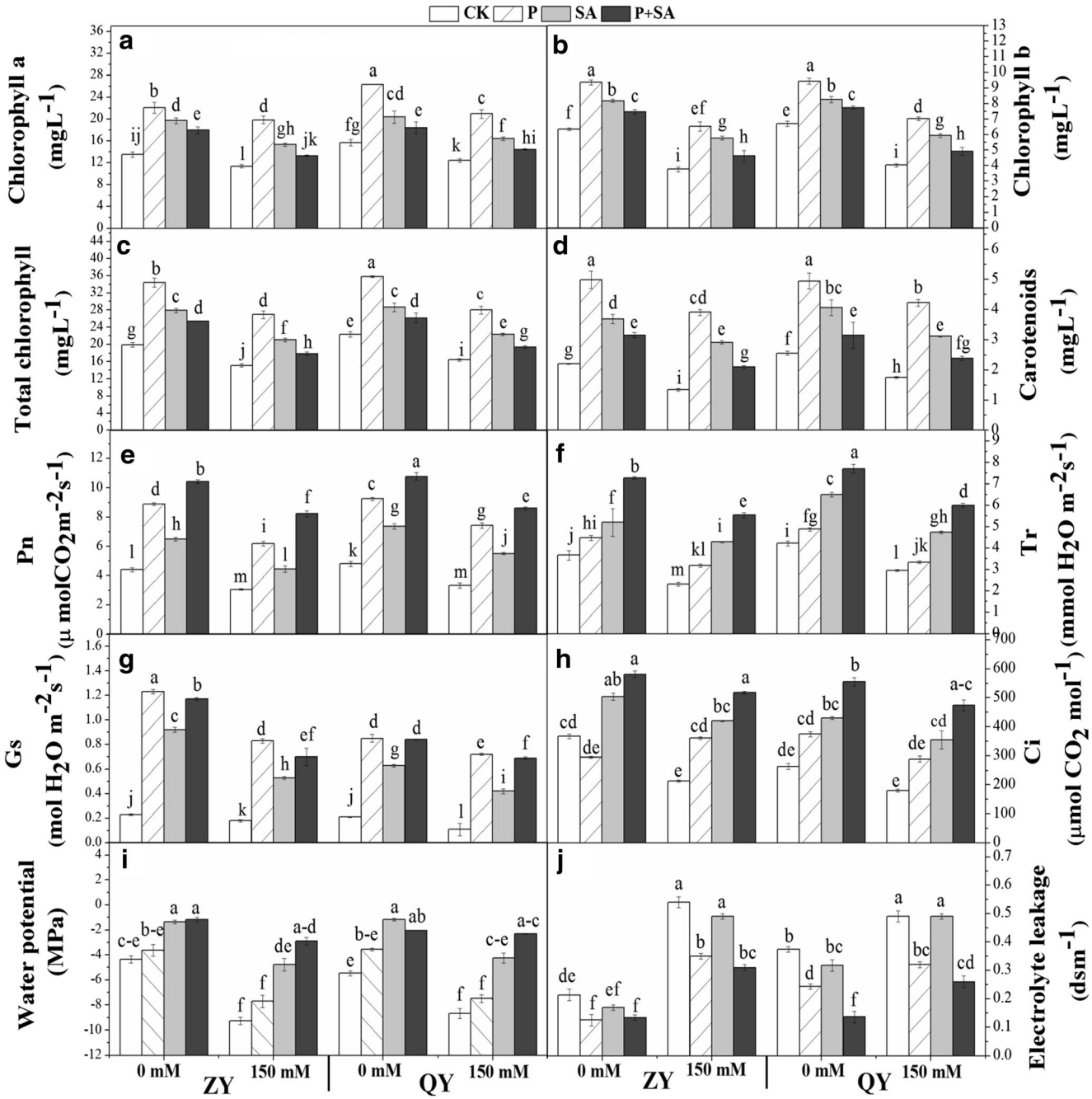

Fig. 1 Effects of alone or combined treatments of cold plasma and exogenous SA on chlorophyll *a* (**a**), chlorophyll *b* (**b**), total chlorophyll (**c**), carotenoid (**d**), net photosynthetic rate (Pn, **e**), transpiration rate (Tr, **f**), stomatal conductance (Gs, **g**), intracellular CO_2 concentration (Ci, **h**), water potential (MPa, **i**), and electrolyte leakage (EL, **j**) of two cultivars of *Oryza sativa* under salinity stress. Values are mean ± SD ($n = 3$). The different letters on top of the bar show significant difference ($P < 0.05$) among different treatments within each cultivar. Ck, control; P, plasma; SA, salicylic acid; and P + SA, plasma + salicylic acid

light blue along with reducing Fv/fm ratio as compared with the control. The false color scale depicted that image ranges from yellow (0.2) to purple (0.1) (Fig. 2e). The false images also reported that salinity stress damage started in the leaf apex and then spread to leaf lamina with the effect of Fv/fm contents in the leaves being least in the basal part of the leaves.

Cold plasma and SA induces changes in enzyme activities

SA priming and plasma treatment resulted in an increase of SOD activity in root and shoot of both cultivars with a more prominent increase in QY as compared with ZY cultivar under

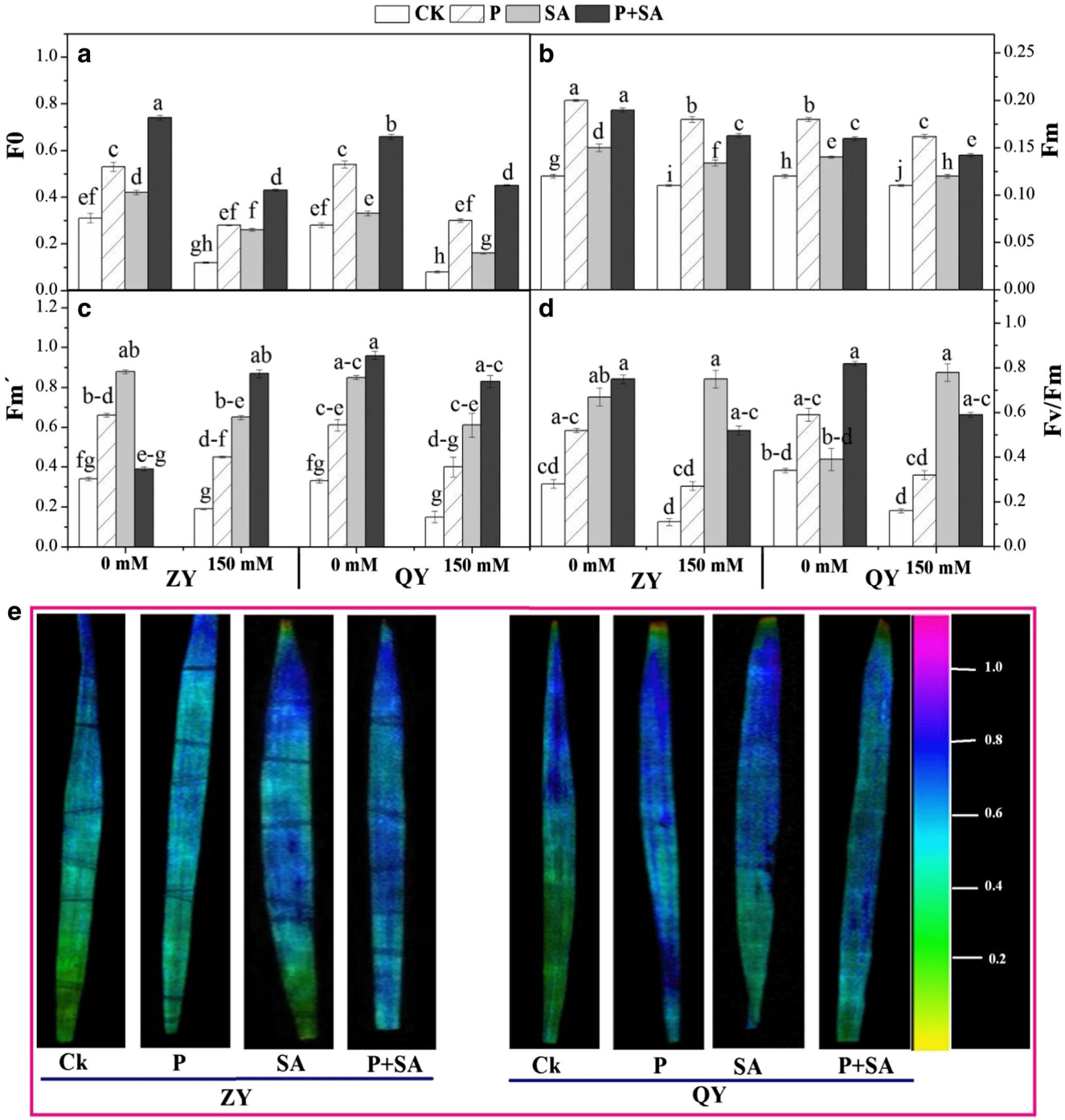

Fig. 2 Effects of alone or combined treatments of cold plasma and exogenous SA on F0 (**a**), Fm (**b**), Fm′ (**c**), Fv/fm (**d**), and false color images of Fv/fm (**e**) of two cultivars of *Oryza sativa* grown under salinity stress (150 mM NaCl). Values are mean ± SD ($n = 3$). The different letters on top of the bar show significant difference ($P < 0.05$) among different treatments within each cultivar. Ck, control; P, plasma; and SA, salicylic acid

normal and salinity stress conditions. The results showed that the combination of SA and cold plasma treatment recorded the highest activity of SOD as compared to SA priming and plasma treatment alone (Table 2). POD activity significantly increased in both cultivars as a response to salinity stress as compared with normal growth condition (Table 2). As compared to the control, seeds treated with cold plasma and SA priming significantly increased the activity of POD in root and shoot of both cultivars. Interestingly, the highest activity of POD recorded in seeds that treated with cold plasma alone in the root and shoots under salinity stress of both cultivars with a more prominent increase in ZY as compared with QY

Table 2 Effects of alone and combined treatments of cold plasma and exogenous SA on SOD (ΔOD 470/g FW-min), POD (ΔOD 470/g FW-min), CAT (ΔOD 240/g FW-min), and APX (μmol min^{-1} mg^{-1} protein) activities in the leaves and roots of two cultivars of *Oryza sativa* under salinity stress

Cultivars	Treatments	SOD		POD		CAT		APX	
		Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
ZY	Ck + 0 mM	673.02 ± 21.9k	1511.1 ± 58.1i	2.16 ± 0.01k	2.33 ± 0.01j	2.04 ± 0.12h	2.48 ± 0.15h	1.43 ± 0.03h	1.74 ± 0.02k
	Ck + 150 mM	888.89 ± 58.1i	1688.8 ± 43.9f–i	2.70 ± 0.01i	2.85 ± 0.10hi	2.86 ± 0.34g	3.33 ± 0.32g	1.97 ± 0.06g	2.29 ± 0.05h–i
	P + 0 mM	965.08 ± 58.1h	1879.4 ± 79.3e–g	4.00 ± 0.01c	4.20 ± 0.05d	2.83 ± 0.11g	3.26 ± 0.03g	2.23 ± 0.03e–g	2.50 ± 0.07f–i
	P + 150 mM	1320.6 ± 21.9f	2069.8 ± 122de	4.70 ± 0.02a	4.90 ± 0.02a	3.37 ± 0.080ef	3.84 ± 0.08ef	2.79 ± 0.03cd	3.09 ± 0.08cd
	SA + 0 mM	761.90 ± 38.0j	1549.2 ± 21.9 hi	2.83 ± 0.02h	2.96 ± 0.20h	3.74 ± 0.36de	4.19 ± 0.33de	1.90 ± 0.05g	2.18 ± 0.03ij
	SA + 150 mM	1015.9 ± 21.9h	1739.7 ± 21.9f–i	3.39 ± 0.05f	3.48 ± 0.11g	4.78 ± 0.03c	5.26 ± 0.04c	2.49 ± 0.03c–f	2.78 ± 0.06d–g
	P + SA + 0 mM	1561.9 ± 38.0d	2298.4 ± 43.9cd	3.60 ± 0.11e	3.79 ± 0.13f	6.89 ± 0.17b	7.33 ± 0.15b	2.52 ± 0.03c–f	2.89 ± 0.03c–f
	P + SA + 150 mM	1904.8 ± 38.0b	2717.5 ± 58.1b	4.27 ± 0.03b	4.47 ± 0.03c	7.85 ± 0.28a	8.31 ± 0.27a	3.24 ± 0.32ab	3.56 ± 0.27ab
QY	Ck + 0 mM	761.90 ± 39.0j	1638.1 ± 38.0g–i	1.99 ± 0.04l	2.13 ± 0.08k	2.25 ± 0.14h	2.75 ± 0.19h	1.10 ± 0.81h	1.91 ± 0.03jk
	Ck + 150 mM	1028.6 ± 30.0h	1803.2 ± 95.8e–h	2.53 ± 0.02j	2.72 ± 0.03i	3.10 ± 0.37fg	3.57 ± 0.36fg	2.10 ± 0.07fg	2.45 ± 0.05f–i
	P + 0 mM	990.48 ± 38.0h	1942.9 ± 166.0ef	3.85 ± 0.01d	4.01 ± 0.03e	2.98 ± 0.08fg	2.72 ± 0.53h	2.42 ± 0.12d–f	2.71 ± 0.03d–h
	P + 150 mM	1473.0 ± 21.9e	2336.5 ± 122.4c	4.52 ± 0.01a	4.72 ± 0.02b	3.60 ± 0.07de	4.08 ± 0.06de	2.88 ± 0.06bc	3.28 ± 0.03bc
	SA + 0 mM	1003.2 ± 21.9h	1866.7 ± 76.1e–g	2.69 ± 0.02i	2.86 ± 0.03h	3.91 ± 0.33d	4.37 ± 0.28f	2.12 ± 0.15fg	2.37 ± 0.09g–i
	SA + 150 mM	1168.3 ± 21.9g	1676.2 ± 43.0f–i	3.21 ± 0.04g	3.42 ± 0.03g	4.98 ± 0.02c	5.46 ± 0.01c	2.65 ± 0.03c–e	2.99 ± 0.03c–e
	P + SA + 0 mM	1688.9 ± 21.9c	2692.1 ± 24.0b	3.32 ± 0.13f	3.50 ± 0.21 g	7.08 ± 0.14b	7.54 ± 0.13b	2.69 ± 0.02d	2.59 ± 0.88e–i
	P + SA + 150 mM	2057.1 ± 38.0a	3060.3 ± 79.3a	4.07 ± 0.04c	4.27 ± 0.04d	8.02 ± 0.28a	8.50 ± 0.29a	3.39 ± 0.31a	3.74 ± 0.29a

Values are mean ± SD ($n = 3$). Different letters following the data within each column mean significant difference at $P < 0.05$

P plasma, *SA* salicylic acid

(Table 2). Similarly, the activities of CAT and APX were increased in both cultivars as a response to salinity stress relative to normal growth condition. As compared with untreated seeds, SA priming and cold plasma treatment alone or combined significantly increased the activities of CAT and APX of both cultivars under salinity stress. The combination of SA priming and cold plasma treatment recorded the highest activities of CAT and APX in root and shoot of both cultivars with a more prominent increase in QY as compared with ZY under salinity stress (Table 2). The correlation analysis showed that the mean length of root and shoot of both cultivars showed a linear and positive correlation with SOD, POD, CAT, and APX activities of both cultivars under salinity stress (Fig. 3) which proved that the antioxidant enzymes' defense system of the plant improved the growth of rice under salinity stress.

The non-antioxidant enzymes in terms of GR, GSH, and GSSG were slightly increased in root and shoot of both cultivars under salinity stress as compared with normal growth condition (Table S1). As compared to the control, seeds treated with cold plasma and SA priming and their combination significantly increased the non-antioxidant enzyme activities under salinity stress in roots and shoots of both cultivars, with a more prominent increase in ZY as compared to QY cultivar. Seeds treated with the combination of cold plasma and SA priming induced the highest activities of enzymes in both cultivars under salinity stress condition followed by SA priming and cold plasma treatment alone, respectively (Table S1).

Uptake of Na^+, K^+, Ca^{2+}, and Mg^{2+} ions and Na^+/K^+ ratio

Seeds treated with cold plasma and SA alone and their combination resulted in a preferential accumulation of Na^+ in leaves and roots of both cultivars under salinity stress (Table 3). Seeds treated with cold plasma and SA and their combination decreased the uptake of Na^+ as compared to the control. Seeds treated with cold plasma alone significantly decreased Na^+ concentration in both roots and leaves as compared to SA either alone or combined with cold plasma treatment. Additionally, seed treated with cold plasma alone recorded the lowest Na^+ concentration in the roots and leaves followed by the combination of cold plasma and SA priming without significant differences between them. The combination of cold plasma treatment and SA priming significantly enhanced the uptake of K^+ and Ca^{2+} in the leaves of both cultivars as compared to cold plasma or SA priming alone. Interestingly, in the root of QY cultivar, cold plasma treatment alone markedly enhanced the uptake of K^+ and Ca^{2+} relative to the control (Table 3). Moreover, seeds treated with cold plasma alone enhanced the uptake of Mg^{2+} higher than those of both SA either alone or combined with cold plasma treatment in roots and leaves of QY cultivar. On the contrary, the combination of cold plasma and SA priming recorded the highest uptake value of Mg^{2+} in roots and leaves of ZY. Further, the results showed that cold plasma and SA priming and their combination enhanced the Na^+/K^+ ratio in the roots and leaves of both cultivars under salinity stress (Table 3).

Uptake of macro- and micronutrients

Cold plasma treatment and SA priming alone and their combination positively improved the concentration of P and Mn in the leaves and roots of both cultivars under salinity stress (Table 4). The SA priming and cold plasma alone or in combination significantly improved the concentration of P and Mn in the leaf and root of both cultivars as compared with control plants. The highest concentration of P and Mn in leaf of both cultivars and root of ZY cultivar was recorded with the combination treatment. Interestingly, the highest concentration of P and Mn in the root of QY cultivar was recorded with cold plasma treatment alone (Table 4). As compared to control, seeds treated with cold plasma or SA priming alone and their combination markedly enhanced Zn concentration in root and leaf of both cultivars. Differential of Fe, Cu, and Cr concentrations were observed in cold plasma treatment and SA priming either alone or combined of roots and leaves of both cultivars under salinity stress (Table 4).

Cold plasma and SA up-regulates secondary metabolism-related enzyme activity

As shown in Fig. 4, the activities of PAL, PPO, SKDH, CAD, SuSy, SPS, and AI were improved by cold plasma and SA alone and in combination under salinity stress as compared with normal condition. The activities of PAL, SKDH, SuSy, and AI were significantly increased ($P > 0.05$) in plasma-treated plants in both cultivars under salinity stress as compared to the control (Fig. 4a, c, e, and g), whereas SA-treated plants significantly increased the activities of PPO and CAD under salinity stress as compared with the untreated seeds (Fig. 4b, d). SPS activity was increased with SA combined with plasma treatment under salinity stress as compared with control (Fig. 4g). It could be concluded that cold plasma treatment improved PAL by 76.76%, PPO by 34.95%, SKDH by 77.84%, CAD by 49.21%, SuSy by 29.27%, SPS by 37.63%, and AI by 46.63%, respectively, as compared to their controls. Seed primed with SA improved PAL by 40.42%, PPO by 61.49%, SKDH by 61.95%, CAD by 59.36%, SuSy by 24.86%, SPS by 28.30%, and AI by 33.18%, respectively, as compared to their controls, whereas the application of SA priming combined with cold plasma treatment improved PAL by 63.15%, PPO by 54.10%, SKDH by 36.36%, CAD by 36.09%, SuSy by 15.03%, SPS by 43.89%, and AI by 30.42%, respectively, as compared to control.

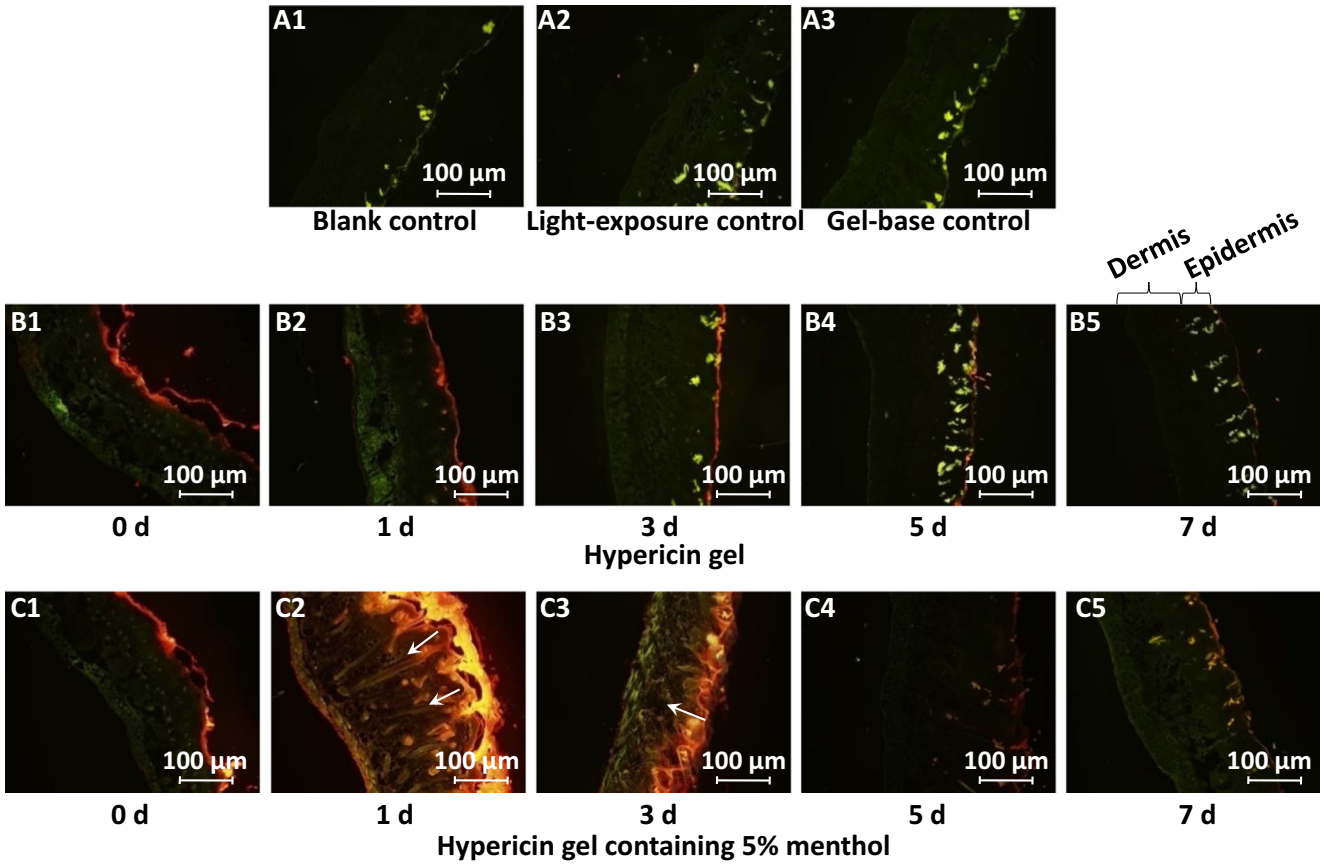

Fig. 3 Relationship between the mean root length of both cultivars and the activities of SOD (**a**), POD (**b**), CAT (**c**), and APX (**d**), and the mean shoot length of both cultivars and the activities of SOD (**e**), POD (**f**), CAT (**g**), and APX (**h**) in *Oryza sativa* seedlings treated with cold plasma and primed with exogenous SA and grown under 100 and 150 mM salinity stress. $^{**}P \geq 0.01$ (significant)

Changes of MDA contents and ROS accumulation and cell death

MDA content and ROS accumulation were increased in response to salinity stress in root and shoot of both cultivars as compared with normal growth condition. As compared to the control, seeds treated with cold plasma and SA and their combination significantly decreased the content of MDA in root and shoot of both cultivars under salinity stress (Table S2). The lowest content of MDA was recorded with SA priming alone followed by the combination treatment and plasma alone, respectively. Higher accumulation of MDA in roots and shoots was observed with ZY as compared with QY under salinity stress (Table S2). Cold plasma treatment alone recorded the lowest $O_2^{\cdot-}$ content in both roots and shoots of both cultivars under salinity stress followed by the combination treatment and SA alone, respectively. The decrease in $O_2^{\cdot-}$ content with cold plasma and SA priming and their combination was more accentuated in QY as compared to ZY (Table S2). Similarly, a linear decrease in $^{\cdot}OH$ content was observed in treatments of cold plasma, SA, and their combination in roots and shoots of both cultivars with a more prominent decrease in QY cultivar as compared to ZY cultivar. The lowest values of $^{\cdot}OH$ content were observed in the combination treatment followed by SA and plasma treatment alone, respectively (Table S2). Our results showed that seeds treated with cold plasma and SA and their combination resulted in a decrease of H_2O_2 content in both cultivars with a more prominent decrease in ZY as compared to QY (Fig. 5a). Seeds treated with the combination of cold plasma and SA priming recorded the lowest values of H_2O_2 followed by SA and plasma treatment alone, respectively, in the roots and shoots of both cultivars under salinity stress. The dichlorodihydrofluorescein diacetate (H2DCFDA) fluorescence staining revealed that H_2O_2 formation was increased by increasing salinity stress (Fig. 5b). The cytotoxicity study showed that the cell death in root tips as revealed by propidium iodide (PI) staining was increased after exposure to salinity stress (150 µm NaCl) as compared to the normal growth condition (Fig. 5c). However, the cell death of root tip was diminished in plants treated with plasma, SA, and their combination.

Ultramorphology of leaf mesophyll and root tip cells

The ultrastructure of leaf mesophyll of hydroponic seedlings treated with cold plasma and SA and their combination and

Table 3 Effects of alone and combined treatments of cold plasma and exogenous SA on Na^+, K^+, Ca^{+2}, and Mg^{+2} contents and Na^+/K^+ ratio in leaves and roots of two cultivars of *Oryza sativa* under higher salinity level (150 mM)

Cultivars	Treatments	Leaf (mg g^{-1} DW)					Root (mg g^{-1} DW)				
		Na^+	K^+	Ca^{2+}	Mg^{2+}	Na^+/K^+	Na^+	K^+	Ca^{2+}	Mg^{2+}	Na^+/K^+
ZY	Ck	1.09a	18.68cd	3.31g	0.44cd	0.05a	1.45a	12.91ef	2.76g	0.41de	0.11a
	P	0.445de	54.02b	5.02d	3.36b	0.008c	0.418c	41.33b	4.24e	3.30b	0.01cd
	SA	0.772b	21.09c	8.11c	0.45c	0.03b	0.896b	20.46c	8.90cd	0.65c	0.04c
	P + SA	0.488d	64.96a	12.54a	4.88a	0.007c	0.465c	72.50a	15.20a	5.79a	0.006f
QY	Ck	1.48a	14.58de	3.75f	0.55fg	0.10a	1.21a	21.08c	3.58fg	0.42g	0.05b
	P	0.416cd	44.37b	4.43e	3.19a	0.009c	0.407d	53.38a	9.92a	3.85a	0.007f
	SA	0.877b	27.09c	8.59bc	0.81e	0.03b	0.964b	41.78b	9.05ab	3.21ab	0.02a
	P + SA	0.468c	54.25a	9.60a	2.90bc	0.008c	0.904b	39.77bc	8.01d	2.99c	0.02a

Different letters following the data within each column mean significant difference at $P < 0.05$

P plasma, *SA* salicylic acid

exposure to salinity stress (150 mM) are shown in Fig. 6. Seed without treatment of cold plasma or SA priming (control) of both cultivars QY and ZY under salinity stress showed unclear cell wall (CW) and undeveloped chloroplast (Chl). A high degree of cytoplasmic vacuolization and mitochondrial tumefaction were also observed, especially in ZY cultivar (Fig. 6a, e). Seed treated with cold plasma treatment alone showed clear cell wall, developed chloroplast, developed mitochondria (M) with visible cristae (C) (Fig. 6b, f). Seed primed with SA alone showed clear cell wall, developed mitochondria with a visible cristae, and chloroplast with developed thylakoids (Fig. 6c, g), whereas seeds treated with the combination treatment showed that cell wall was kept intact and normal organelles were within the cytoplasm as compared with seeds treated with cold plasma or SA alone (Fig. 6d, h).

The ultrastructure of root tips of both cultivars treated with cold plasma and primed with SA under salinity stress is shown in Fig. 6i–p. The TEM microscopy of root tip cells untreated with plasma and SA (control) showed unclear cell wall and undeveloped nucleus with invisible nucleolus. Moreover, the root cell of ZY cultivar was vacuolated and large numbers of vacuolar structures were observed followed by detachment of the cell wall from the plasma membrane (Fig. 6i, m). Seeds treated with cold plasma alone showed clear and smooth cell wall, well-developed nucleus with visible nucleolus, rough endoplasmic reticulum (RER), and plastoglobuli (P) (Fig. 6j, n). Similarly, seeds treated with SA alone resulted in clear cell wall and well-developed nucleus with visible nucleolus and nuclear membrane (NM) (Fig. 6k, o), whereas seeds treated with the combination of cold plasma and SA showed developed and smooth cell wall, mature mitochondria, well-developed nucleus with visible nucleolus and rough endoplasmic reticulum (RER) (Fig. 6l, p).

Table 4 Effects of alone or combined treatments of cold plasma and exogenous SA on macro- and micronutrient contents in leaves and roots of two cultivars of *Oryza sativa* under higher salinity level (150 mM)

Cultivars	Treatments	Leaf (mg g^{-1} DW)						Root (mg g^{-1} DW)					
		P	Mn	Zn	Fe	Cu	Cr	P	Mn	Zn	Fe	Cu	Cr
ZY	Ck	4.13c	0.89f	0.19c	0.03c	0.048de	0.012c	2.88ef	0.81f	0.20d	0.02ef	0.064d	0.010bc
	Plasma	9.37b	1.36c	0.48b	0.63a	0.083b	0.050a	5.19c	1.90cd	0.55a	0.54b	0.084a	0.030a
	SA	3.68de	1.58b	0.17cd	0.02cd	0.051c	0.010c	8.26b	1.94c	0.24c	0.02ef	0.067c	0.010bc
	P + SA	13.2a	2.17a	0.64a	0.60ab	0.112a	0.030b	13.7a	3.22a	0.44b	0.62a	0.075b	0.030a
QY	Ck	5.01c	0.99e	0.18cd	0.04c	0.051cd	0.010c	3.88ef	1.03e	0.21d	0.02ef	0.049d	0.010de
	Plasma	7.16b	1.32c	0.52b	0.55a	0.070b	0.030a	10.5a	2.13a	0.56a	0.60a	0.079a	0.030c
	SA	4.84d	1.50ab	0.22c	0.03cd	0.054cd	0.010c	7.51c	1.88b	0.24b	0.02ef	0.064b	0.010de
	P + SA	8.90a	1.74a	0.63a	0.51ab	0.094a	0.020b	8.44b	1.76bc	0.22c	0.02ef	0.064b	0.090a

Different letters following the data within each column mean significant difference at $P < 0.05$

P plasma, *SA* salicylic acid

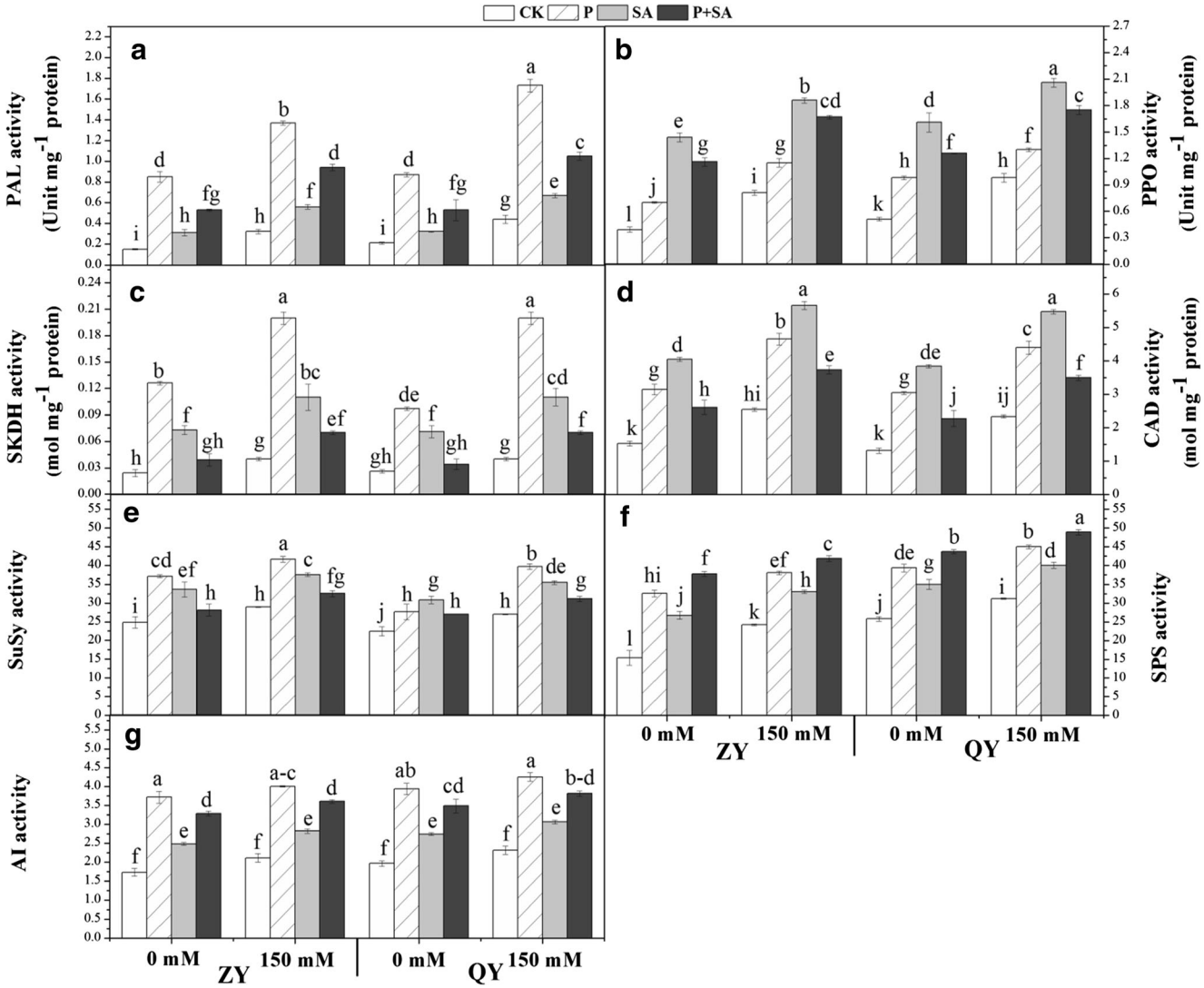

Fig. 4 Effects of alone or combined treatments of cold plasma and exogenous SA on the activities of PAL (**a**), PPO (**b**), SKDH (**c**), CAD (**d**), SuSy (**e**), SPS (**f**), and AI (**g**) of two cultivars of *Oryza sativa* under salinity stress. Values are mean ± SD ($n = 3$). The different letters on top of the bar show significant difference ($P < 0.05$) among different treatments within each cultivar. Ck, control; P, plasma; SA, salicylic acid; and P + SA, plasma + salicylic acid

Discussion

Salinity stress can affect seed germination and seedling growth performance through osmotic stress, ion-specific effects, and oxidative stress. Since seed germination and seedling growth of rice are the most sensitive stages to salinity stress, up to now, the mechanism underlying the effect of cold plasma treatment on rice seedling growth has not been completely clarified. It has been reported that cold plasma treatment can affect seeds through different ways such as seed coat modification, reactions with electrons, ions, and radicals emitted in the discharge, by UV radiation emitted from the plasma (Sera et al. 2010). The enhanced growth and differentiation process motivated by the cold plasma might be attributed to the different bioactive signaling molecules specifically ozone and nitric oxide (NO) originated from the plasma treatments as well as UV radiation. NO, known as a bioactive signaling compound, can modify cell division reaction in the meristem, cell differentiation process, and polar auxin transport via affecting the PIN1 protein levels (Fernández-Marcos et al. 2011). In the present study, germination percentage and root and shoot length were significantly improved by SA priming and cold plasma treatment and their combination relative to their respective controls under salinity stress (Table 1). These effects may be caused by a combination of various factors, such as the inactivation of enzymes as well as the decomposition or modification of endogenous substances such as gibberellic acid which are often induced after plasma treatment (Tappi et al. 2014) or by activating the oxidative pentose phosphate pathway which could improve the germination and plant growth under abiotic stress (Fontaine

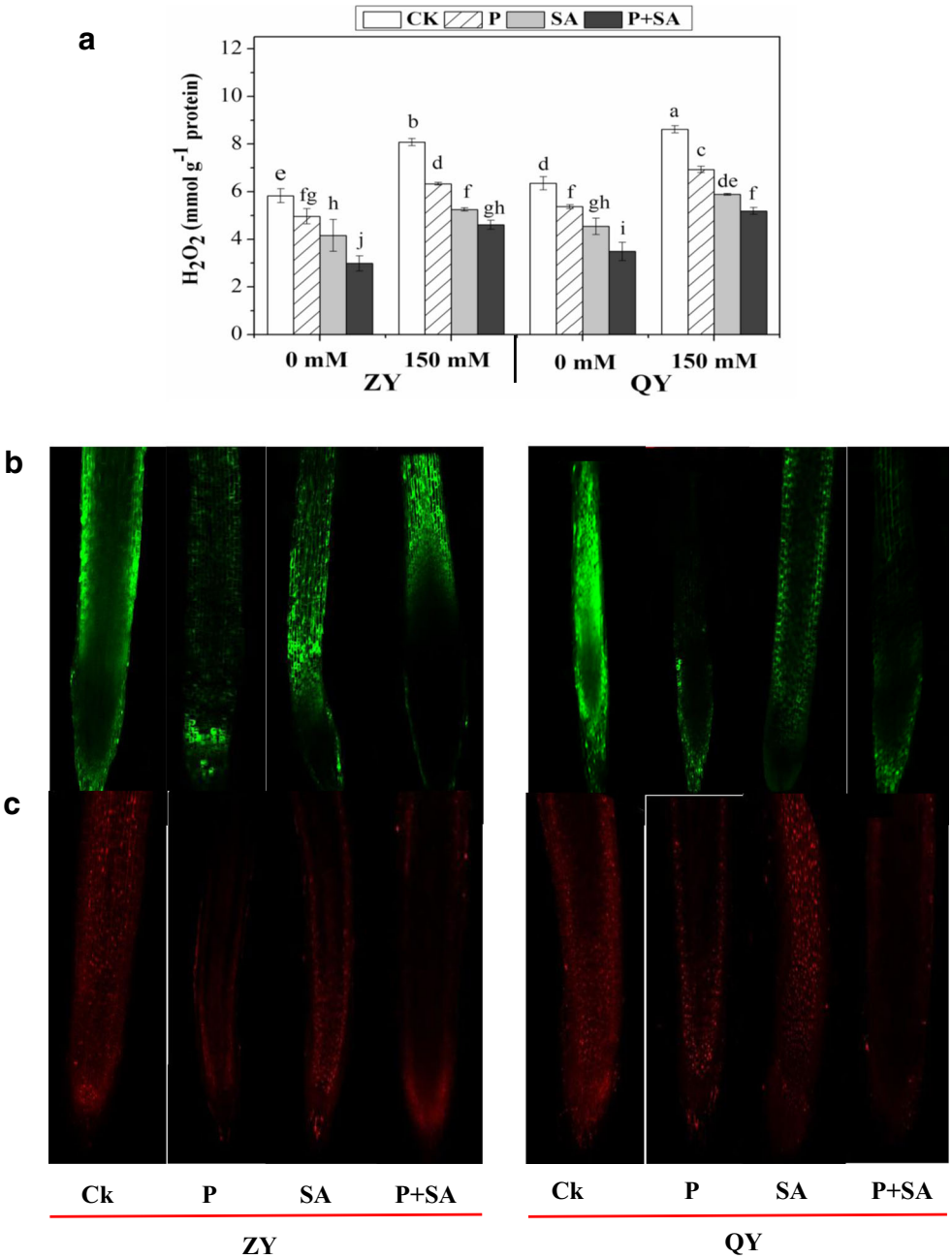

Fig. 5 Effects of alone or combined treatments of cold plasma and exogenous SA on **a** hydrogen peroxide (H_2O_2) content, **b** hydrogen peroxide (H_2O_2) accumulation in roots as revealed by dichlorodihydrofluorescein diacetate fluorescein staining (H2DCFDA; Molecular Probes Inc., Eugene, OR, USA), and **c** propidium iodide (PI) staining showing cell death in root tips of two cultivars of *Oryza sativa* seedlings grown under salinity stress (150 mM NaCl). Ck, control; P, plasma; SA, salicylic acid. Values are mean ± SD ($n = 3$)

et al. 1994). In our previous study, a significant reduction in germination percentage was observed under nano-ZnO concentrations in QY and ZY cultivars, with a more prominent decrease in ZY cultivar (Sheteiwy et al. 2016). In addition, the authors also observed that the shoot length and seedling fresh and dry weight of ZY cultivar under nano-ZnO stress were higher than those of QY cultivar. While the QY had higher root length and seedling vigor index (Sheteiwy et al. 2016). As such, Sookwong et al. (2014) found that seed germination and seedling growth of the rice were improved by cold plasma treatment. Similarly, it has been reported that cold plasma improved the shoot length and dry weight of soybean (Ling et al. 2014). Earlier studies reported that cold plasma treatment markedly improved shoot and root dry weight, shoot and root length, and the number of lateral root of *Brassica napus* (Ling et al. 2015). In addition, the plant height, leaf thickness, stem diameter, and dry weight of plants were significantly higher in cold plasma-treated seeds than those in the control plants (Jiang et al. 2014). Furthermore, Henselova et al. (2012) reported that root length, root fresh weight, and root dry weight were increased in maize treated in a diffuse coplanar surface barrier discharge generated in ambient air as compared with the control treatment. It has

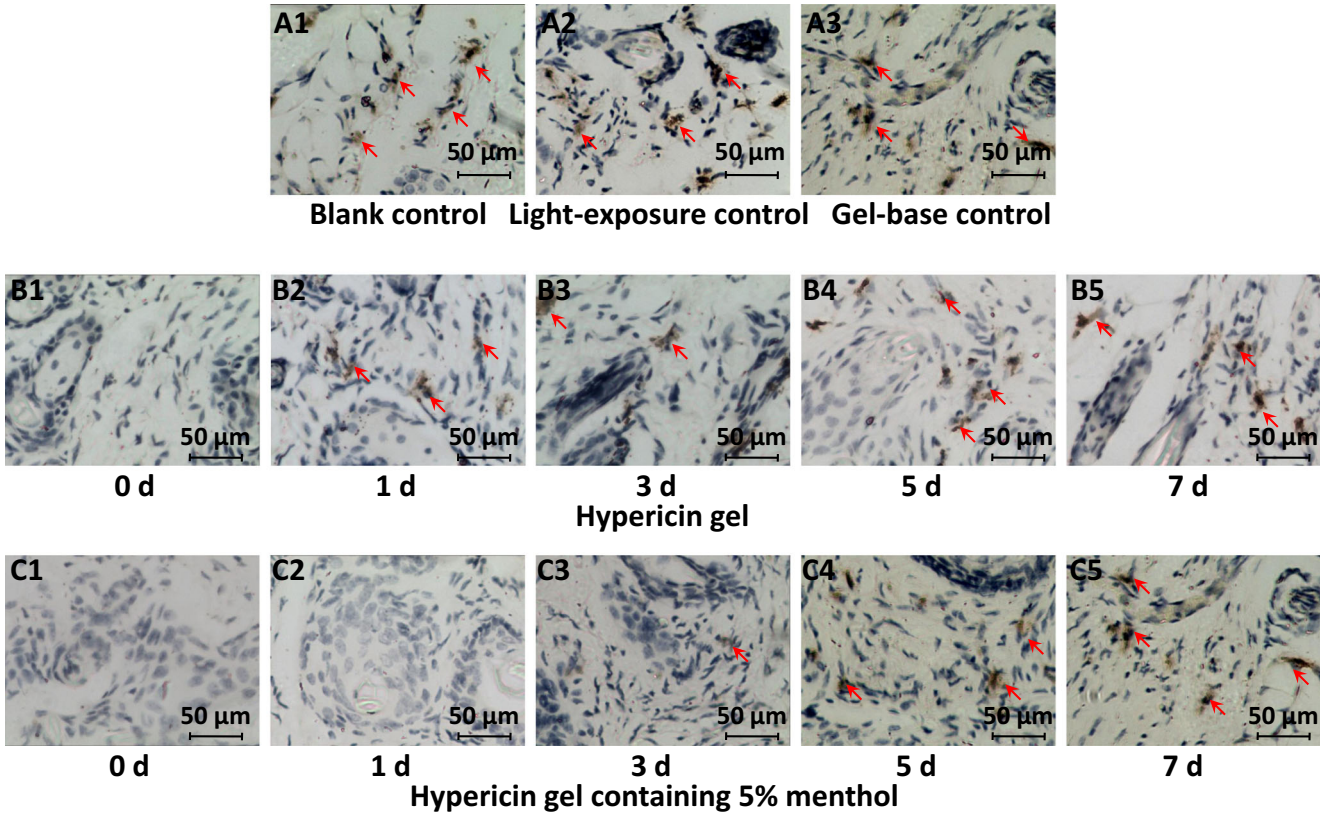

Fig. 6 TEM of 45-day hydroponic treated seedlings with alone and combined cold plasma and SA priming and grown under higher salinity level (150 mM). **a** TEM microscopy of leaf cells of ZY cultivar untreated with cold plasma or SA treatments (CK, control). **b** TEM microscopy of leaf cells of ZY cultivar treated with plasma alone. **c** TEM microscopy of leaf cells of ZY cultivar primed with SA alone. **d** TEM microscopy of leaf cells of ZY cultivar treated with cold plasma and SA combination. **e** TEM microscopy of leaf cells of QY cultivar untreated with cold plasma or SA treatments. **f** TEM microscopy of leaf cells of QY cultivar treated with cold plasma alone. **g** TEM microscopy of leaf cells of QY cultivar primed with SA alone. **h** TEM microscopy of leaf cells of QY cultivar treated with cold plasma and SA combination. **i** TEM microscopy of root tip cell of ZY cultivar untreated with cold plasma or SA treatments. **j** TEM microscopy of root tip cell of ZY cultivar treated with cold plasma alone. **k** TEM microscopy of root tip cell of ZY cultivar primed with SA alone. **l** TEM microscopy of root tip cell of ZY cultivar treated with cold plasma and SA combination. **m** TEM microscopy of root tip cell of QY cultivar untreated with cold plasma or SA treatments. **n** TEM microscopy of root tip cell of QY cultivar treated with cold plasma alone. **o** TEM microscopy of root tip cell of QY cultivar primed with SA alone. **p** TEM microscopy of root tip cell of QY cultivar treated with cold plasma and SA combination. CW, cell wall; Cr, cristae; Chl, chloroplast; ChlM, chloroplast membrane; Po, peroxidase; GT, granule thylakoid; SG, starch grain; Va, vacuole; N, nucleus; Nue, nucleolus, P, plastids; RER, rough endoplasmic reticulum; NM, nuclear membrane

previously been stated that cold plasma treatment increased the absorptive ability, which might certainly contribute to increased seed imbibitions, germination percentage, and seedling growth under abiotic stress condition (Bormashenko et al. 2012). Another study reported that plasma-triggered reactions have been mainly attributed to different mechanisms, including the modifications in seed coat structure (Filatova et al. 2014) and changes in water uptake rates under abiotic stress conditions (Ling et al. 2014). In the current study, rice seedling growth was significantly improved by SA priming under salinity stress (Table 1). These results are in agreement with those obtained by Jayakannan et al. (2013) who found that SA treatment improved seedling fresh and dry weight of *Arabidopsis* under salinity stress condition. The improvement of seedling growth and biomass with SA priming under salinity stress might be due to increased SA in rice plant cell that could promote the seedling growth under salinity stress (Kalaivani et al. 2016). Further, Hussein et al. (2007) stated that rice seeds treated with SA at 200 ppm concentration had improved green leaf number, plant height, stem diameter, and stem dry weight and number of leaves per the whole plant. The vital elicitor and signaling agents which are emitted during the plasma treatment may contribute to the root and shoot tissue growth mainly through regulating auxin transport, cell division process in a meristem region, and cellular differentiation (Iranbakhsh et al. 2018). Recently, Shakirova et al. (2003) reported that presowing treatment with SA leads to the activation of germination of seeds and seedling growth of wheat under salinity stress. The present study reported that SA priming improved leaf area under salinity stress (Table 1), and the promoting effect of SA on the leaf area might be due to SA which could increase cell division, biosynthesis of organic foods, water uptake, and availability of nutrients to the plant (Kalaivani et al. 2016).

The potential effect of the cold plasma treatment on the physiological activity of rice seedlings was estimated by evaluating chlorofluoresence parameters (F0, Fm, Fm′, and Fv/fm). These parameters are a precise tool to determine both stability and capacity of photosynthesis system and its response to abiotic stress (Bjorkman and Demmig 1987). In the current study, the photosynthesis parameters were decreased under salinity stress condition which might be due to low availability of water to plants grown under salinity stress condition. This reduction in Fv/fm under salinity stress might be due to the damage to reaction centers and reducing electron transport capacity in PSII caused by salinity stress (Basu et al. 1998). Our results are in line with the findings obtained by Bulkhov et al. (1999) who reported that increasing of PSII efficiency might be due to the effects of SA on density of reaction centers per PSII antenna chlorophyll, quantum yield for electron transport, and conformational changes in D1 protein. Moreover, SA may protect the photosynthetic system by inducing protein kinase activity and reversible phosphorylation of D1 protein (Hui-Jie et al. 2011). The present study depicted that salinity stress significantly decreased the photosynthetic pigments and fluorescence as well as decreased the leaf water potential (Fig. 1). A previous study has reported that plasma treatment plays an important role in regulating water balance by modulating antioxidant enzymes (Wu et al. 2007). Furthermore, cold plasma treatment might maintain a favorable potential gradient for water uptake into the seedlings and increase energy supply to the plant through the increased accumulation of soluble sugars and soluble proteins, and this action could have alleviated the negative effects of drought stress on oilseed rape seedling growth (Ling et al. 2015). Previously, Lawlor and Cornic (2002) reported that the photosynthetic rate, pigments, and chlorophyll fluorescence were decreased as well as the leaf water potential was decreased in higher plants. Furthermore, the Fv/fm is widely used as a tool to determine photosynthetic activity (Lawlor and Cornic, 2002). In our present study, a significant decrease in F0, Fm, Fm′, and Fv/fm caused by salinity stress inhibiting of PSII photochemistry, which might be due to insufficient energy transfer from light harvesting chlorophyll complex to the reaction center (Guo et al. 2009). Moreover, Sheteiwy et al. (2015) reported that Chl *a*, Chl *b*, total Chl, and carotenoids were significantly reduced in ZY and QY cultivars, and the reduction was more pronounced in ZY cultivar with further application of nano-ZnO stress.

In the present study, the activities of SOD, POD, CAT, and APX were increased in response to salinity stress. However, seed priming with SA and cold plasma treatments improved the antioxidant enzyme activities under salinity stress condition. It has been reported that SA potentially activated plant defenses and regulated the physiological processes in plants under abiotic stress (Wang and Li 2006). SA has been found to improve drought tolerance in wheat (Singh and Usha 2003), salinity tolerance in barley (EL Tayeb et al., 2006), and heat tolerance in mustard (Dat et al. 1998). Our results are consistent with the findings of Ling et al. (2015) who reported that SOD and CAT activities as well as MDA content of *B. napus* seedlings were significantly improved under drought stress (Bormashenko et al. 2012). Furthermore, Jiang et al. (2014) reported that SOD and POD activities of tomato seeds were improved by cold plasma treatment. Our results suggested that SA priming and cold plasma treatment can improve the seedling growth of rice seedlings under salinity stress by improving the antioxidant enzyme activities which can reduce the oxidative damage generating by salinity stress. The current findings stated that the combination of cold plasma and SA priming improved APX activity and improved the rice tolerance to salinity stress which might be due to that APX can catalyze the partitioning of the superoxide radical (O_2·−) into either ordinary molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). The elevated levels of H_2O_2 can act as a signal molecule to activate stress response pathways and increase rice tolerance to abiotic stress (Sheteiwy et al. 2017b). Furthermore, Henselova et al. (2012) reported that cold plasma treatment could significantly increase the activities of antioxidant enzymes such as CAT and SOD. It has also been reported that POD activity in plants was increased upon treatment with cold plasma (Yin et al. 2005). Earlier study showed that low-temperature plasma induced a significant increase of SOD activity in both roots and leaf of maize seedlings (Henselova et al. 2012). Furthermore, SOD catalyzes the dismutation of superoxide to hydrogen peroxide (Sandalio et al. 2001). It was considered that the irradiation of singlet excited oxygen molecules created by cold plasma is a trigger for the growth promotion of the *Brassicaceae* sprouts (Ono and Hayashi 2015). They found that antioxidative substances produced in the leaves upon *Brassicaceae* seeds are irradiated by singlet excited oxygen molecules and cultivated for 4 days. These substances could be transferred into stems leading to improving the tolerance to oxidative stress (Ono and Hayashi 2015).

In the current study, the activities of GR, GSH, and GSSG were improved by cold plasma treatment, SA priming, and their combination under salinity stress. Under abiotic stress condition, plants could develop various defensive mechanisms to reduce the harmful effects of ROS, which were effective at different levels of abiotic stress (Beak and Skinner 2003). Earlier study reported that GSH content gradually increased during drought stress with further increase in GSH observed with exogenous SA application under drought conditions (Kang et al. 2013). Additionally, plants can activate antioxidative defense systems to protect themselves from the detrimental effects of salinity-induced oxidative stress to alleviate the harmful effects of ROS production. Our results reported that SA priming, cold plasma treatment, and their combination improved the activities of antioxidant enzymes as well as GR activity as compared with the control. Seeds

primed with SA and treated with cold plasma significantly decreased the ROS accumulation in terms of H_2O_2, ^{-}OH, and O_2^{-} under salinity stress. It has been reported that excessive amounts of ROS resulted in membrane lipid peroxidation, increased electrolyte leakage, and damaged the chloroplast, thus inhibiting photochemical reactions and decreasing photosynthesis system (Gunes et al. 2007). Furthermore, the maintenance of cellular membrane integrity under salinity stress was considered to be an integral part of the salinity tolerance mechanism (Stevens et al. 2006).

Our outcomes show significant reduction in the uptake of different nutrients under salinity stress. However, cold plasma treatment and SA priming and their combination improved the uptake of different nutrients under salinity stress condition. Cold plasma treatment and SA priming noticeably improved the uptake of K^+, Ca^{+2}, and Mg^{+2}, and decreased Na^+/K^+ ratios in the leaves and roots of both studied cultivars under salinity stress (Table 4). Cold plasma improved the uptake of the nutrients in the present study which might be due to cold plasma having the capability to modify seed coat structure leading to the increase in absorption of essential nutrients as compared with those of the control. SA priming improved the uptake of different nutrients and decreased the Na^+/K^+ under salinity stress. These results are in agreement with the findings obtained by Gunes et al. (2005) who reported that the exogenous application of SA increased the uptake of calcium, copper, magnesium, manganese, potassium, and zinc concentration under salinity stress in maize seedlings. Moreover, the increasing of nutrient uptake and decreases of Na^+ and K^+ ratio in the cytosol by exogenous SA application might be due to the regulation of the expression and activity of K^+ and Na^+ transporters and H^+ pumps that generate the driving force for nutrient uptake (Gunes et al. 2005). Furthermore, the increasing uptake of Ca^{2+} in plants due to exogenous application of SA could maintain membrane integrity and help in reducing the toxicity effects of Na^+ and Cl^- ions under salinity stress condition (Gunes et al. 2005). Earlier, it was reported that Na^+ and K^+ concentrations and Na^+/K^+ ratios had been widely used as indicators of salinity tolerance in barley seedlings under abiotic stress (Munns and Tester 2008). Further, an increase in Na^+ ion concentration and decrease in K^+ ion uptake was observed in most species exposed to salinity stress (Qui et al., 2011). Previously, Ali et al. (2014) found that exogenous application of 5-aminolevulinic acid (ALA) mitigated the effect of Pb stress and restored the capability of plants to accumulate macronutrients (N, P, S) in the leaves and roots and micronutrients (Zn and Fe) in the roots of *B. napus* plant. Moreover, higher concentration of nutrients in seed along with higher seed yield was observed under foliar application of SA that resulted in higher uptake of nutrient (Nafees et al. 2010). Previously, Ahmad et al. (2011) observed that application of exogenous SA improved the nutrient uptake in mustard plants.

In this study, a progressive increase of PPO, PAL, SKDH, and CAD activities was observed in the plants treated with cold plasma and SA priming either alone or combined as compared to the untreated plants under salinity stress. Our findings are in line with the findings obtained by Baque et al. (2010) who reported that salt stress increased the activity of PAL in *Morinda citrifolia*. Jiang et al. (2014) reported that cold plasma-induced activities of PAL, in combination with antioxidant enzymes in tomato plants, have been proposed as key crucial mechanisms, thereby counteracting against *Ralstonia solanacearum* pathogen. In addition, Iranbakhsh et al. (2018) stated that the oligosaccharins were created by cold plasma treatment; these compounds might exert signaling effects and induce various physiological mechanisms by which the defense system is activated against abiotic stress (Fry et al. 1993). Similarly, Kim et al. (2007) reported that SKDH activity increased upon 3-day salinity stress exposure. These phenolic metabolisms may provide an effective defensive tool in tolerance against salinity stress (Kovacik et al. 2009). The activities of SPS, SuSy, and AI were increased with cold plasma and SA priming and their combination under salinity stress (Fig. 4e–g). These results revealed that the increase of SPS, SuSy, and AI activities under salinity stress are necessary to meet the demand of carbohydrate assimilates for plants in response to salinity stress. Our results are consistent with the findings obtained by Hayano-Kanashiro et al. (2009) who reported that the activity of SPS, SuSy, and AI might indeed proceed by excluding the carbon flux out from starch and sucrose into cyclitols; this action regulates leaf carbon allocation and increases phloem sucrose transport. This could increase the growth of lateral roots and the numbers of root hairs under salinity stress condition (Hayano-Kanashiro et al. 2009).

The morphological analyses demonstrated that the cell structure of leaf mesophyll and root tip were strongly affected by the SA priming and cold plasma treatment under salinity stress (Fig. 6). Large vacuoles and cytoplasmic disorganization were observed in the untreated plants and exposure to salinity stress. Earlier study reported that wheat plants treated with 0.05 mM of SA improved the level of cell division within the apical meristem of seedling roots resulting in increase of plant growth and elevated productivity of wheat under salinity stress (Shakirova et al. 2003). The chloroplasts of the untreated plants were deformed, thylakoid membranes were disorganized, and outer membranes of the chloroplasts were totally ruptured. The cold plasma treatment provides a complicated mechanism in which cells are exposed to UV and critical signaling bioactive molecules, among which NO has been reported to be the most important signaling (Iranbakhsh et al. 2018). The improvement of the cell ultrastructure after plasma treatment might be due to oligosaccharins which are created from the plasma, and these compounds caused structural changes in

the cell wall. These oligosaccharin compounds may exert signaling effects and induce various physiological mechanisms by which defense system is activated and, hence, plant resistance against different stresses factors is improved (Fry et al. 1993). It has been reported that the cold plasma triggered accumulations of cyto-protectant enzymes might be the possible potent mechanisms involved in relieving the toxicity signs of nZnO in the treated seedlings (Iranbakhsh et al. 2018). However, we found significantly improved cell form and chloroplast structure with SA priming and cold plasma treatment under salinity stress. These results are consistent with the findings of Zhang et al. (2008) who reported that cell structure was improved by the application of ALA which might be due to the capability of ALA to reduce lipid peroxidation of thylakoids and cell membranes by induction of the antioxidant system under Cd stress. In the present study, the ultrastructural changes were observed to be more obvious in ZY than QY cultivar, which are consistent with our previous study (Sheteiwy et al. 2015) where the leaf mesophyll and root cell ultrastructure were significantly damaged in both QY and ZY cultivars under nano-ZnO stress and more clearly damaged in ZY as compared with QY cultivar.

Conclusions

Seeds treated with cold plasma or primed with SA and their combination enhanced plant growth and uptake of the nutrients under salinity stress while decreasing the ROS production by increasing antioxidant enzyme activities. Thus, it can be deduced that cold plasma treatment and SA priming and their combination have the potential to enhance salinity tolerance via decreasing the oxidative damage of plant membranes by promoting both enzymatic and non-enzymatic antioxidant activities in the rice seedling grown under higher salinity level. The activities of enzymes-related secondary metabolism were up-regulated with plasma and SA priming under salinity stress. Further, the cell structure of root and leaf was improved by both cold plasma and SA priming under salinity stress. Hence, the cold plasma treatment and SA priming could be helpful to improve the rice growth in affected soils with high salinity level. Notwithstanding, the molecular mechanism underlying cold plasma and SA priming improves seedling growth performance, and the uptake of mineral nutrients under higher salinity level conditions still needs further study.

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Compliance with ethical standards

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

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