Modifications of water status, growth rate and antioxidant system in two wheat cultivars as affected by salinity stress and salicylic acid

Naglaa Loutfy[1] · Yoh Sakuma[2] · Dharmendra K. Gupta[2,3] · Masahiro Inouhe[2]

Abstract

Salicylic acid (SA) has an important role in drought-tolerance in wheat (*Triticum aestivum* L.) but its relevance to the salinity-tolerance is not well understood. In the present study, possible roles of SA and salinity responses were examined using two wheat cultivars i.e., drought-tolerant Sakha-69 and drought-sensitive Gemaza-1, exposed to 150 mM NaCl. Parameters were determined for growth i.e. fresh or dry mass (FM, DM), osmotic concentration (OC) of organic/inorganic solute, leaf relative water content (LRWC), photosynthesis pigment content (PPC), and selective antioxidant system (AOS) enzyme/molecule that might be involved in the stress remediation. Sakha-69 exhibited salinity tolerance greater than Gemaza-1 and SA ameliorated their salinity stresses like drought stress, suggesting that a common tolerant mechanism might be involved in the stresses. Salinity decreased root growth by 44–52% more strongly than shoot (36–41%) in FM or those in DM (32–35%). SA ameliorated root growth (40–60%) more efficiently than shoot (6–24%) for DM/FM. These results suggested that salinity and SA might target sensitive roots and hence influencing shoot functions. In fact, salinity reduced PPC by 10–18%, LRWC by 16–28%, and more sensitively, OC of inorganic solutes (K^+, Ca^{2+}, Mg^{2+}) in shoot (19–36%) and root (25–59%), except a conspicuous increase in Na^+, and SA recovered all the reductions near to control levels. SA and salinity increased additively most parameters for OC of organic solutes (sugars and organic acids) and AOS (glutathione and related enzyme activities), like drought responses. However, SA decreased the Na^+ and proline contents and catalase activity in a counteracting manner to salinity. It is concluded from this experiment that SA-mediated tolerance might involve two mechanisms, one specific for minerals in root and the other related to drought/dehydration tolerance governed in the whole module systems.

Keywords Antioxidant enzymes · Glutathione · Organic and inorganic solutes · Salicylic acid · Salinity stress · Sodium chloride · Wheat

✉ Masahiro Inouhe
inouhe@sci.ehime-u.ac.jp

1 Department of Botany, Faculty of Science, South Valley University, Qena, Egypt

2 Department of Biology, Faculty of Science, Ehime University, Matsuyama 790-8577, Japan

3 Ministry of Environment, Forest and Climate Change, Indira Paryavaran Bhavan, Aliganj, Jorbagh Road, New Delhi 110003, India

Introduction

Wheat (*Triticum aestivum* L.) is one of the typical glyco-phytes and an important cereal crop grown throughout the globe. For example, its kernels are used in staple foods like bread, pasta, noodles and sweets and its straw as animal feed and also in manufacturing paper (Milad et al. 2013). Contrarily, effects of salinization in lowering crop yields have been a major problem especially in arid and semiarid areas (Al-Naggar et al. 2015; Tuna et al. 2008). In arid regions crops are cultivated on low quality soils deficient of major nutrients, under extreme severe conditions of high temperature, water deficit (drought) and sometimes combinations of both. These conditions occurred due to the unnecessary uses of chemical fertilizers, which result in accumulation of extra salts in the soils due to evaporation especially after

unsuitable irrigation (Mariani and Ferrante 2017; Negrao et al. 2017; Silva et al. 2011). Salty soils result in disturbance of osmotic regulation and ion imbalance in roots, which leads to impair metabolism, growth and yield of the cultivated plant. Combinational stresses induced by drought and salinity rather than each single one might cause much stronger damage to the entire plant, therefore these plants need to develop more complex resistant mechanisms for survival against both dehydration and high salinity stresses occurring at the same time. Conversely, if plants had acquired stable tolerance mechanisms against stress, they could adjust to more variable and complex environmental changes. However, details for the mechanisms against both the stresses and their physiological interrelations have not been fully understood (Isayenkov and Maathuis 2019; Miura and Tada 2014).

Isayenkov and Maathuis (2019) reported that salinity stress might cause a rapid influence on young leaf growth through osmotic effects and a slower response on mature leaves to accelerate the senescence due to ionic effects. They suggested that interaction between different organs or sites might be involved in a spatial or temporal course effect of the stress expression via certain transport and/or signal transduction systems. Glycophytes cannot tolerate strong levels of salt stress in nature and their developmental stages such as seed germination, growth and vigor, and vegetative growth are adversely affected by the presence of high salt concentration (Sairam and Tyagi 2004). Salinity stress affects various physiological and biochemical processes, for instance, NaCl stress inspires oxidative stress through the creation of reactive oxygen species (ROS) that activate lipid peroxidation and also injure the photosynthetic pigments. Salinity effects on photosynthetic level include stomatal or non-stomatal confines and also inhibition of photochemical routes by disturbing the water balance and nutrient uptake. Excess salts might affect plant development either via osmotic inhibition of water absorption by roots or specific ion effects. Specific ion effects may cause a direct chemical toxicity, or otherwise the insolubility or competitive absorption of ions affects plant nutritional balances (Hafsi et al. 2010; Karlidag et al. 2009). Plants face deficiencies by several nutrients and nutritional imbalance that may be caused by high salt (NaCl) uptake that competes with uptake of other nutrient ions such as K^+, Ca^{2+} and Mg^{2+}, resulting in nutritional disorder and eventually, reduced yield and quality (Grattan and Grieve 1999). The multiple factors above are considerable for salinity and related stress even in a selected glycopyhte species.

Salicylic acid (SA) has a role in plant protection against stress. It is usually used in plant agriculture by reason of its ability to regulate the plant resistance response to different abiotic stresses (Hayat et al. 2010 for review). SA acts as a plant growth regulator and plays a significant role

in modulating the plant's responses to abiotic stresses such as salinity (Nazar et al. 2015). For example, SA lightens salt stress by improving photosynthetic characteristics and increases salt tolerance by inducing antioxidant metabolism in *Vigna radiata* (Nazar et al. 2011). El-Beltagi et al. (2017) found that SA increases soluble sugars in salinized plants. SA treatment increases the growth of radicle cells by the division and expansion of meristem cells (Boukraâ et al. 2013). Samaras et al. (1994) reported that plant species adjust to high salt concentrations by lowering tissue osmotic potential with the accumulation of inorganic as well as organic solutes. Khan et al. (2010) also reported that application of SA (0.5 mmol l^{-1}) enhanced salinity tolerance in mung bean by reducing Na^+ and increasing K^+ content. SA can increase the concentration of abscisic acid (ABA) in plant tissues, furthermore, both SA and ABA may mutually inhibit each other's signaling (Khan et al. 2015). Positive effects of SA treatment were reported in different plant species such as rice (Jini and Joseph 2017), wheat (Wang and Zhang 2017), mung bean (Ghassemi-Golezani and Lotfi 2015), *Phaseolus vulgaris* L. (Rady and Mohamed 2015), and *Pisum sativum* (Singh et al. 2016), under salinity stress. It is known that SA treatment (0.5 mM) increases the endogenous SA level by four times and the salinity stress (100 mM NaCl) reduces it below 50% in a salt-sensitive variety of rice (Jini and Joseph 2017). However salinity effects at lower level on the endogenous level of SA are more complex and expressions of genes for SA biosynthesis and signaling are controlled by a self-amplifying feedback loop between SA and ROS (Jayakannan et al. 2015; Miura and Tada 2014; Vlot et al. 2009).

In our previous study, we have examined the effect of drought stresses on different wheat cultivars (Gemaza-1, Gemaza-3, Sakha-69 and Geza-164) and found that drought tolerance was controlled by SA that is involved in the enhanced accumulation of organic and inorganic solutes and enhanced water content in wheat leaves (Loutfy et al. 2012). In the present study, we elucidate whether or not the drought tolerance characteristics can be involved in the salinity stress tolerance, and/or whether the tolerance mechanisms can be expressible under imbalanced ionic conditions around the roots disturbed by high NaCl concentration in medium. In the present study, we found that the drought tolerant cultivar exhibited much stronger salinity tolerance, and that SA-pretreatment enhanced salt tolerance by increasing contents of organic solutes (sugars and organic acids) and some of the major detoxification enzymes (APOX, GPOX) activities coupling with a bio-reduction/oxidation (redox) cycle named as the glutathione-ascorbate cycle (Noctor and Foyer 1998). We further revealed that salinity stress caused a significant increase in the proline content and catalase activity as well as Na^+ content but a decrease in the nutritional solute K^+, Ca^{2+}, Mg^{2+} contents. All of these changes were strongly

relieved by SA treatment and vice versa. We also checked multiple effects of SA on various physiological parameters including accumulation and translocation of organic solutes, uptake and transports of Na$^+$ and other inorganic solutes, activities of AOS enzymes and their role in LRWC and PPC, in two different wheat cultivars.

Material and methods

Plant material and growth conditions

Seeds of two cultivars (Sakha-69 and Gemaza-1) of wheat (*Triticum aestivum* L.) were obtained from the agriculture research center, Shandaweel Agriculture Research Station, Sohag, Egypt. Wheat seeds were sterilized and divided into two groups; the first group was soaked in 0.5 mM SA for 12 h; the other group was soaked in distilled water for 12 h. After soaking, seeds were transferred on to a sterilized Petri dish that had been mounted with filter paper and then cultured for 15 days hydroponically using 1/1-strength Hoagland medium (Tawussi et al. 2017). Experiments were conducted under four different treatments i.e. (1) Control without SA or NaCl, (2) salinity treatment with 150 mM NaCl, (3) SA treatment with 0.5 mM SA, and (4) Na + SA treatment with both 0.5 mM SA and 150 mM NaCl. This NaCl concentration was selected since it had been shown to cause significant growth inhibitions (ca. 40–60%) in both wheat cultivars but with no apparent toxic symptoms in the external traits or morphology (data not shown).

After 15 days of the treatment mentioned above, harvested plant seedlings were washed three times with double distilled water and then divided into roots/shoots and the fresh mass (FM) of each plant sample was measured. The harvested plant materials were quickly frozen and stored at –20 °C for further biochemical investigation. Some plant samples were dried in an oven at 80 °C to estimate constant weight for the determination of dry mass (DM). The water content (WC) of each organ was also estimated by the subtraction of DM from FM (i.e. WC = FM – DM).

Leaf relative water content and determination of photosynthetic pigments

Leaf relative water content (LRWC) was calculated based on the methods from Yamasaki and Dillenburg (1999). Briefly, two leaves from two randomly chosen plants per replicate were collected from the middle section of the plant in order to minimize age effects. Individual leaves were first removed from the stem and then weighed to obtain FM. In order to determine the turgid mass (TM), leaves were floated on distilled water inside a closed Petri dish. Maximum turgidity was determined by weighing leaves (after gently wiping the water from the leaf surface with tissue paper) until no further weight increase occurred. At the end of the inhibition period, leaves were placed in a pre-heated oven at 80 °C for 48 h, in order to obtain DM. Values of FM, TM and DM were used to calculate LRWC using the equation: LRWC (%) = 100 × (FM – DM)/(TM – DM).

The photosynthetic pigments (chlorophyll *a*, *b* and carotenoids) were extracted from fresh leaves and determined according to Metzner et al. (1965). The results have been presented as the average of four observations over three replications for each treatment.

Determinations of soluble sugars by high performance liquid chromatography

Plant tissues, which had been frozen at –20 °C, were extracted with an equal volume (1 ml g FM^{-1}) of 80% ethanol for 30 min at 80 °C. The extracts were kept at 0 °C for 30 min prior to centrifugation at 10,000g for 5 min. The supernatants were dried in vacuo at 50 °C. The dried materials were dissolved in 50% acetonitrile, and the impurities were removed by filtration (Millipore Millex-GV, pore size 0.22 μm). The samples (20 μl) were injected into a carbohydrate analysis column (Shodex NH2P-50 4E, 250 mm × 4.6 mm i.d., Showa Denko, Tokyo, Japan) connected to a high performance liquid chromatography (HPLC) pump (L-7000, Hitachi, Tokyo), and the column was eluted with 75% acetonitrile (v/v) at a flow rate of 0.5 ml min^{-1}. Monosaccharides and oligosaccharides eluted from the column were quantified with a refractive index (RI) detector (L7490, Hitachi) equipped with a chromatographic-data processor (D-2500, Hitachi).

Determinations of organic acids by HPLC

Plant tissues which had been frozen at –20 °C were extracted with an equal volume (1 ml g FM^{-1}) of 1% HClO$_4$. The extracts were centrifuged at 15,000g for 10 min, the centrifugation was repeated two times and the filtrates were collected. The samples (50 μl) were injected into organic acid analysis columns (Inertsil CX connected to Inertsil Ph-3 in series, 250 mm × 4.6 mm i.d. each) and eluted with 3 mM HClO$_4$ at a flow rate of 0.5 ml min^{-1} using an HPLC pump (L-7110 Hitachi, Tokyo). Standard solution of malic acid, citric acid and acetic acid (1 mg ml^{-1} each) were prepared in 3 mM HClO$_4$. Reaction solution used was 0.1 mM bromothymol blue (BTB) in 30 mM Na$_2$HPO$_4$ (pH 7.6) and the reaction temperature as well as the column temperature was maintained at 35 °C. Mixture solution contain organic acid was measured at the wave length 440 nm. After chromatography of samples and standards, the retention times and areas of identified organic acid peaks were used for the

determination of the contents and the acid contents were expressed as µmol g FM^{-1}.

Determination of total glutathione

Total glutathione (GSH + GSSG) was determined using 150 µl of a solution containing 0.5 ml buffer (50 mM HEPES–KOH pH 7.6 and 330 mM betaine), 0.3 ml of 10% (w/v) sulfosalicylic acid (SSA) and 0.1 ml of extract was mixed with 700 µl of 0.3 mM NADPH, 100 µl of 6 mM 5,5-O-dithiobis-(2-nitrobenzoic acid) and 50 µl of glutathione reductase (10 units ml^{-1}). The reaction was followed by the increase in absorbance at 412 nm due to the formation of 2-nitro-5-mercaptobenzoic acid.

Estimation of proline and some inorganic solute contents

Free proline contents were determined according to Bates et al. (1973). Briefly, 100 mg of powdered tissue was homogenized in 10 ml of 3% SSA for 10 min followed by filtration. Two ml of the filtrate were mixed with 2 ml of glacial acetic acid and 2 ml of acid ninhydrin for 1 h at 90 °C. The developed color was extracted in 4 ml toluene and measured colorimetrically at 520 nm against toluene. A standard curve with proline was used for the final calculations. The results are presented as the average of three replicate estimations for each treatment. The concentrations of Na$^+$, Ca^{2+}, Mg^{2+} and K$^+$ were determined by atomic absorption spectrophotometry according to the method described by Loutfy et al. (2012).

Assays of enzymes activities

About 100 mg of plant tissue was homogenized in 1 ml K-phosphate buffer pH 7.5, 0.2 M sucrose 2 mM DTT and 1 mM MnCl$_2$, and then centrifuged at 10,000 rpm at 4 °C for 30 min. The supernatant was used for enzyme assay. Catalase (CAT) activity was determined following Aebi (1984). The rate of H$_2$O$_2$ decomposition at 240 nm was measured spectrophotometrically. The reaction mixture consisted of 0.05 M K-phosphate buffer pH 7.0 (2 ml), 0.1 mM H$_2$O$_2$ (1 ml), and supernatant (100 µl). One unit of catalase activity (U) was assumed to be the amount of enzyme that decomposed 1 mol H$_2$O$_2$ mg^{-1} soluble protein min^{-1} at 30 °C. Guaiacol peroxidase (GPOX) activity was determined according to Adam et al. (1995). The assay contained 1.5 ml of 100 mM sodium acetate buffer (pH 5.5), 1 ml of 1 mM guaiacol, 10 µl of tissue extract, and 190 µl of water. The reaction was started by addition of 300 µl of 1.3 mM H$_2$O$_2$. The increase in absorption was recorded at 470 nm. Ascorbate peroxidase (APOX) was determined according to the method described by Nakano and Asada (1981). The

reaction mixture consisted of 0.05 M K-phosphate buffer (pH 7.0) (1.8 ml), 5 mM sodium ascorbate (20 µl), 0.1 mM H$_2$O$_2$ (100 µl), and supernatant (100 µl). Total ascorbate peroxidase activity was determined as the decrease in absorbance of ascorbate at 290 nm and calculated using a molar extension coefficient $e = 2.8$ mM^{-1} cm^{-1}. The enzyme activity (U) was calculated as the amount of the enzyme that oxidizes 1 mol ascorbate consumed mg^{-1} soluble protein per min at 30 °C.

Statistical analysis

All data were subjected to the analyses of variance (ANOVA) to test the significant difference between the mean ($n = 3$) of measured variables. Tukey's multiple comparison tests were performed to compare means at $P < 0.05$, different letters (a, b, c, d) indicate significant differences at $P < 0.05$ based on Tukey's test. The effect of studied factors (cultivars, NaCl, and SA) and interactions were assessed by multivariate analysis at $P < 0.05$. The main effect of a factor was investigated pooling the effects of the other factors/covariates. Shoot/root factors were pooled for investigating other factors. All statistical analyses were carried out using IBM-SPSS version 23.0 for Mac OS (McInnes 2017; O'Brien and Kaiser 1985).

Results

Effects of salinity and SA treatments on growth of root/shoot, leaf relative water contents, and photosynthetic pigments in wheat

The FM and DM of root or shoot were determined in 2 wheat cultivars (Gemaza-1 and Sakha-69) as affected by salinity stress (150 mM NaCl) and SA (Table 1). Under control condition, Sakha-69 had higher FM and DM in root and shoot than Gemaza-1 to different extents (by 15–62%), indicating that Sakha-69 has relatively higher biomass than Gemaza-1. Especially, FM of root was greater in Sakha-69 than in Gemaza-1 by 62%, and its water content (WC = FM – DM), as shown in Table S1 was also conspicuously higher than that in Gemaza-1 (by 69%). These results suggested that the root capacity superior to water storage or absorption in Sakha-69 might be involved in the greater drought tolerance in the seedling (Loutfy et al. 2012). Salinity stress decreased FMs of root and shoot by 42 and 36% in Gemaza-1 and by 52 and 41% in Sakha-69, respectively. Salinity stress also deceased their DMs but to a smaller extent (by ca. 33%) than in FMs. These results indicated that salinity stress was expressed in FM (or WC) more sensitively than in DM of the seedlings in two wheat cultivars. In the absence of NaCl, SA treatment increased FMs of root and shoot by 29 and 17%

Table 1 Effects of salicylic acid and salinity stress (150 mM NaCl) on fresh mass, dry mass, and leaf relative water content in 15-day old seedlings of two wheat cultivars

Treatment	Root (g)		Shoot (g)		LRWC (%)
	FM	DM	FM	DM	
Gemaza-1					
Control	0.119 ± 0.001^b	0.015 ± 0.001^a	0.442 ± 0.008^b	0.055 ± 0.004^b	88.3 ± 0.8^b
NaCl	0.067 ± 0.003^d	0.010 ± 0.000^b	0.283 ± 0.004^d	0.037 ± 0.001^d	65.5 ± 0.5^d
SA	0.154 ± 0.005^a	0.021 ± 0.005^a	0.516 ± 0.004^a	0.066 ± 0.002^a	92.3 ± 0.3^a
SA + NaCl	0.094 ± 0.003^c	0.014 ± 0.005^a	0.346 ± 0.007^c	0.046 ± 0.001^c	78.5 ± 0.5^c
Sakha-69					
Control	0.193 ± 0.004^b	0.017 ± 0.005^a	0.553 ± 0.006^b	0.069 ± 0.002^b	91.4 ± 0.4^b
NaCl	0.093 ± 0.002^d	0.011 ± 0.005^a	0.325 ± 0.006^d	0.046 ± 0.001^c	81.7 ± 0.2^d
SA	0.223 ± 0.004^a	0.028 ± 0.005^a	0.655 ± 0.004^a	0.086 ± 0.003^a	94.4 ± 0.5^a
SA + NaCl	0.134 ± 0.005^c	0.018 ± 0.005^a	0.379 ± 0.001^c	0.049 ± 0.001^c	89.0 ± 0.1^c

FM fresh mass, *DM* dry mass, *LRWC* leaf relative water content

Values not followed by the same letter are significantly different at $P < 0.05$ (Tukey's test) within the same parameter (FM, DM, LRWC) in each cultivar

in Gemaza-1, and 16 and 18% in Sakha-69, respectively. In the presence of 150 mM NaCl, SA significantly recovered the decreased FMs of root and shoot by 40 and 23% in Gemaza-1, and by 44 and 17% in Sakha-69, respectively, suggesting that its amelioration effect on FM was greater in root (40–44%) rather than shoot (17–23%) in both cultivars under the influence of 150 mM NaCl. Similar amelioration effects were found in DM of root (42–62%) or shoot (7–24%) in two cultivars. These results suggested that salinity and SA might be targeting the sensitive roots and hence influence
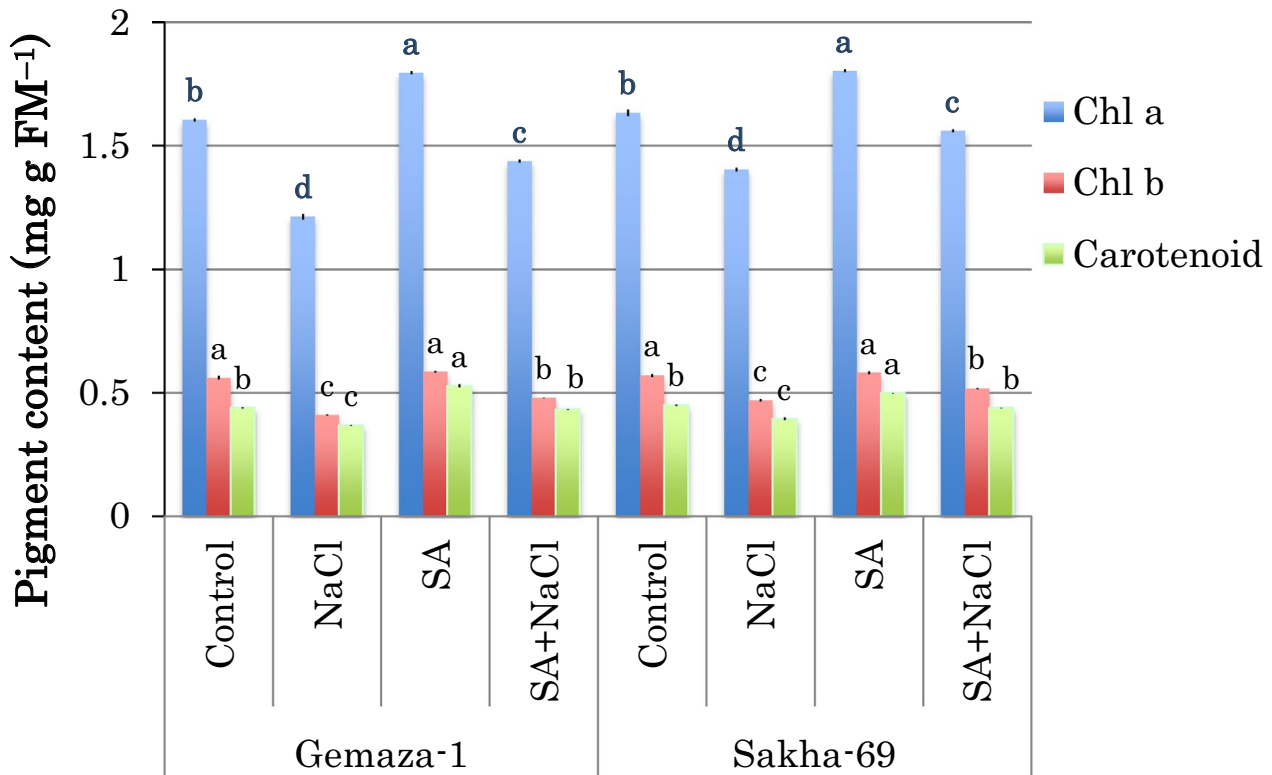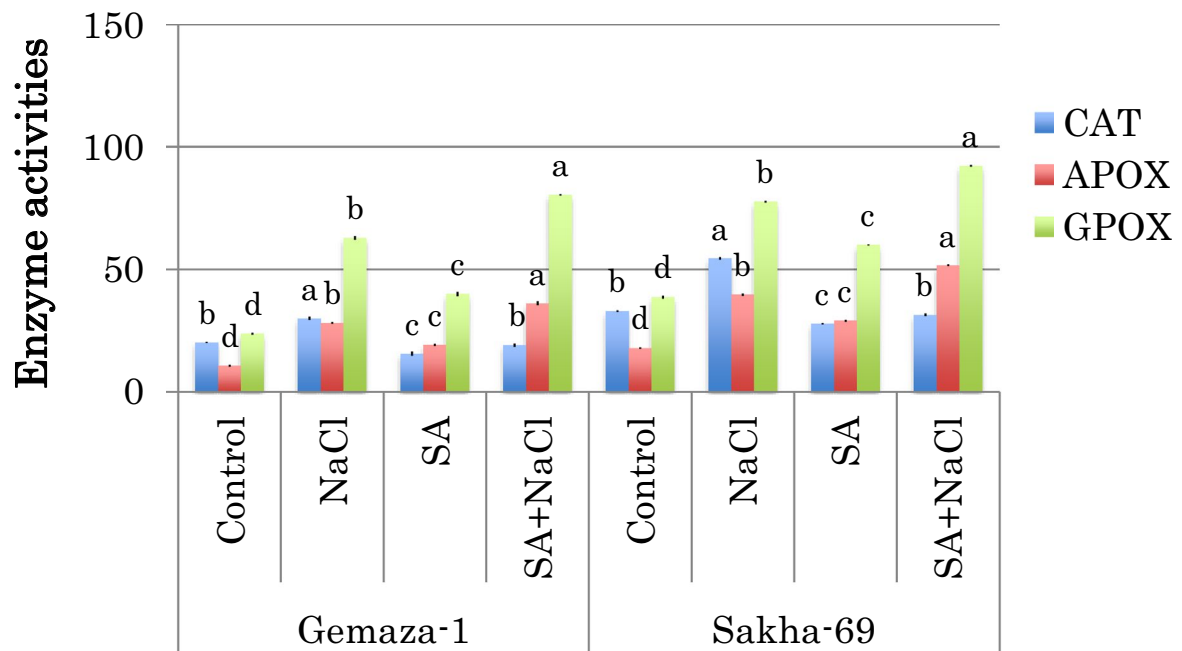

Fig. 1 Effects of salicylic acid and salinity stress (150 mM NaCl) on leaf chlorophyll and carotenoid contents in 15-day old seedlings of two wheat cultivars. The results are presented as the average of four observations ($n = 4$) over three replications for each treatment. The values are expressed as mg g FM^{-1}. Means followed by different letters indicate significant differences at $P < 0.05$ level (Tukey's test) within the same pigment in each cultivar
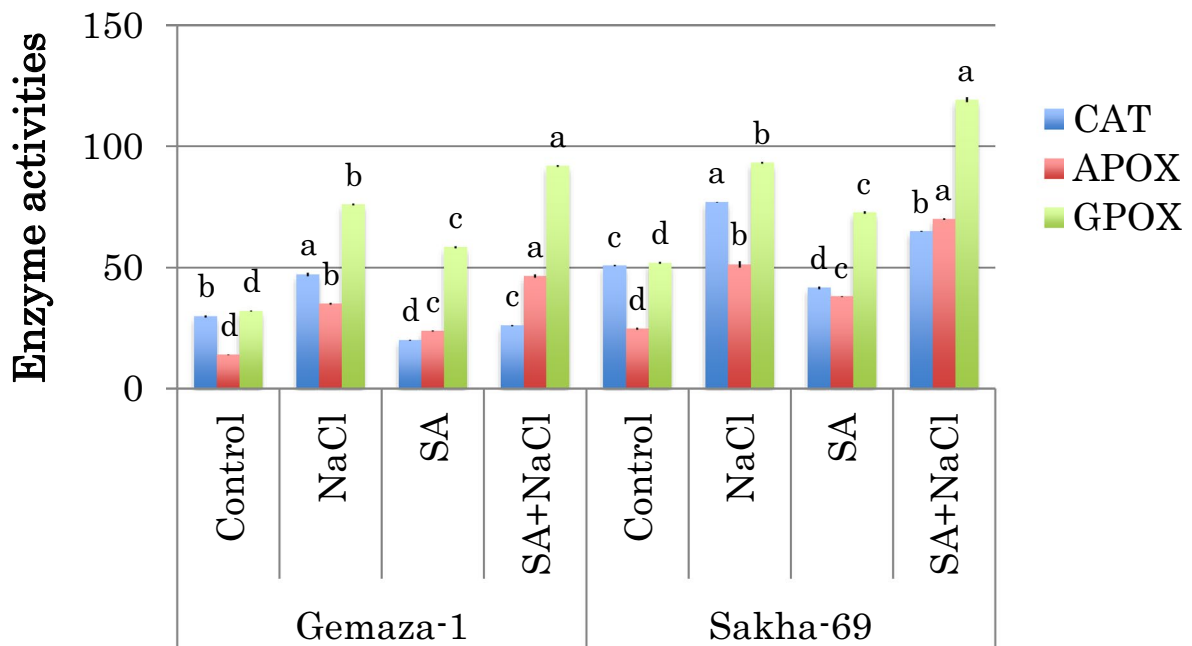
a Root

b Shoot

Fig. 2 Effects of salicylic acid and salinity stress (150 mM NaCl) on the activities of three enzymes involved in anti-oxidation systems in root (**a**) and shoot (**b**) in two wheat cultivars. *CAT* catalase, *APOX* ascorbate peroxidase, *GPOX* guaiacol peroxidase. *The values for CAT, APOX and GPOX are expressed as µg H_2O_2 min^{-1} µg protein^{-1}, µg ascorbate min^{-1} µg protein^{-1}, and µg guaiacol min^{-1} µg protein^{-1}, respectively. Data are expressed as means ± SD ($n = 3$). Means followed by different letters indicate significant differences at $P < 0.05$ level (Tukey's test) within the same enzyme in each cultivar

Table 2 Effects of salicylic acid and salinity stress (150 mM NaCl) on the total glutathione and proline contents in two wheat cultivars

Treatment	Root		Shoot	
	TGS	Proline	TGS	Proline
Gemaza-1				
Control	0.133 ± 0.005^d	6.84 ± 0.02^c	0.163 ± 0.005^d	19.12 ± 0.13^c
NaCl	0.263 ± 0.005^b	33.67 ± 0.07^a	0.296 ± 0.005^b	69.37 ± 0.10^a
SA	0.163 ± 0.005^c	5.68 ± 0.02^d	0.183 ± 0.005^c	16.12 ± 0.07^d
SA + NaCl	0.293 ± 0.005^a	18.40 ± 0.10^b	0.353 ± 0.005^a	36.58 ± 0.19^b
Sakha-69				
Control	0.200 ± 0.009^c	4.93 ± 0.02^c	0.296 ± 0.015^d	21.33 ± 0.18^c
NaCl	0.336 ± 0.005^b	20.13 ± 0.17^a	0.450 ± 0.010^b	42.66 ± 0.28^a
SA	0.233 ± 0.005^c	4.46 ± 0.02^d	0.340 ± 0.010^c	15.16 ± 0.13^d
SA + NaCl	0.413 ± 0.005^a	14.00 ± 0.16^b	0.603 ± 0.015^a	24.42 ± 0.23^b

The values for TGS and proline are represented as µmol g FM^{-1}, respectively

Values not followed by the same letter are significantly different at $P < 0.05$ (Tukey's test) within the same substance in each cultivar

TGS total glutathione that represents summation of reduced form glutathione (GSH) plus oxidized form glutathione (GS-SG)

the various functions in shoots by interactions. Leaf relative water content (LRWC) was also affected by salinity and SA treatments (Table 1). Under control condition, Gemaza-1 had the lower LRWC (88%) than Sakha-69 (91%), as reported previously. Salinity stress decreased LRWC more severely in Gemaza-1 (to 65.5%) than in Sakha-69 (to 82%). SA treatments increased these values over control (to 92 and 94%) in the absence of NaCl, and near to control (79 and 89%) in the presence of NaCl.

Photosynthetic pigment contents (PPCs) in Gemaza-1 were similar to or slightly lower than those in Sakha-69 (Fig. 1). Salinity stress caused a decrease of 16–24% in PPC in Gemaza-1 and a 12–18% decrease in Sakha-69, where the strongest effects were found in Chl *b* (18–27%) and weakest in carotenoid (12–16%). SA increased PPCs in both cultivars, by 10–20% for carotenoid or Chl *a*, but only 2–4% for Chl *b*. SA also recovered the decreased PPCs, near to control levels for carotenoid (99–98%), or to 90–96% and 85–91% of control for Chl *a* and Chl *b*, respectively.

Effect of salinity and SA treatments on the activities of three enzymes involved in anti-oxidation systems in wheat

Effects of salinity and SA treatments on the activities of three enzymes involved in antioxidation system (AOS) in wheat were examined (Fig. 2). Under control conditions, all enzyme activities were detected in both root and shoot tissues. Their activities were higher in shoot than in root and 63–76% higher in Sakha-69 than in Gemaza-1, indicating that these cultivars have distinct levels of AOS enzymes constitutively expressing in the organs. Salinity stress, however, further increased the enzyme levels, by 49–65% for

CAT and higher by 108–164% and 80–164% for APOX and GPOX, respectively. These enzymes might be regarded as inducible and thus can be involved in the sequestration of ROS in the tissues emerged in response to salinity stress or increased intracellular free ions. SA treatment increased APOX and GPOX activities by 68–82% in Gemaza-1 and 40–62% in Sakha-69 under no salinity condition. SA further increased those activities additively with NaCl under salinity condition. Here it can be noted interestingly that the SA treatment significantly decreased the CAT activities by 23–16% in root and 33–18% in shoot in both cultivars. SA also inhibited salinity-induced increase in CAT activity. These results indicated that CAT is not a mediator of SA amelioration of salinity damage or increase in the salinity tolerance in wheat. Cytological distribution and isozyme analysis for CAT are therefore necessary to disclose the further functions; however, it can be possible to conclude from the present experiment that the total CAT activities might be reflective for the tissue conditions as affected by salts and/or remediated by SA and may be not be directly targeting enzymes induced by SA signals.

Effect of salinity and SA treatments on proline and glutathione contents in wheat

Contents of proline (Pro) and total glutathione (TG, TG = reduced from GSH + oxidized form GSSG) were determined in two wheat cultivars (Table 2). The molecules Pro and GSH have been reported to play important roles as the compatible and/or protective amino acid and the antioxidant thiol-peptide that protects against oxidative stress in plant cells, while both of the molecules have been widely recognized also as key molecules involving detoxification and
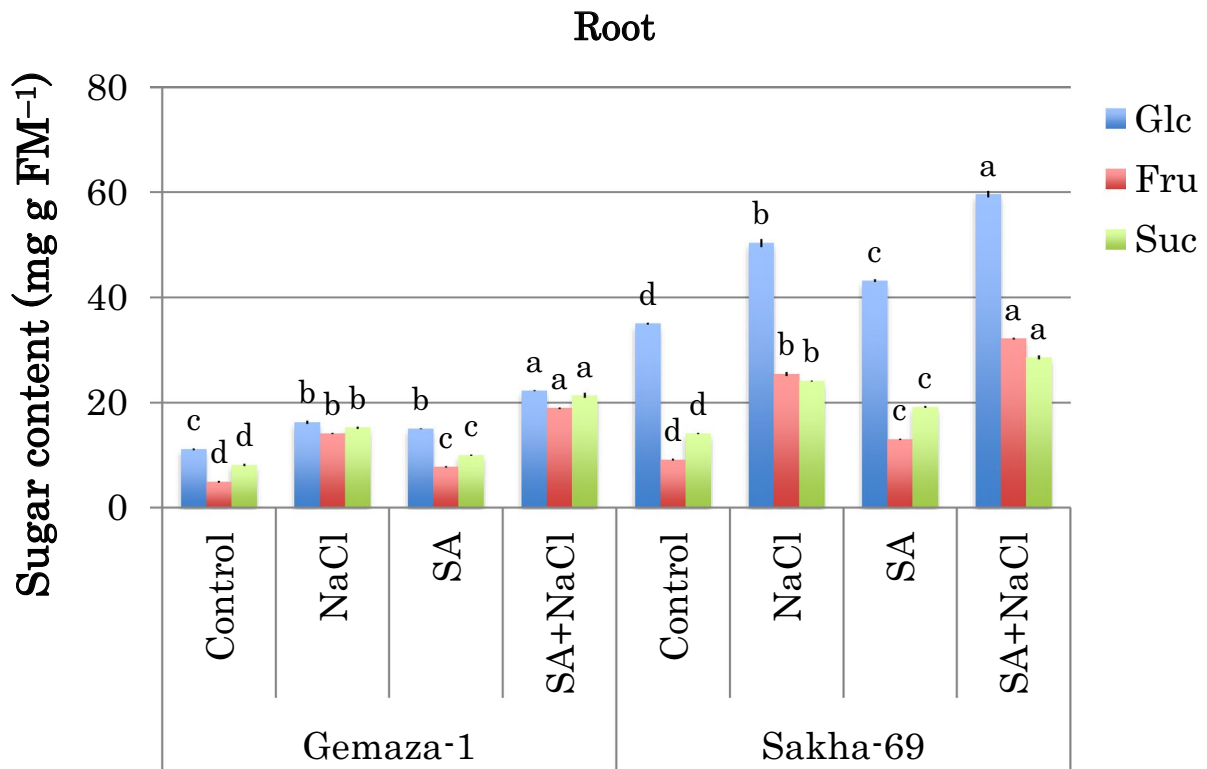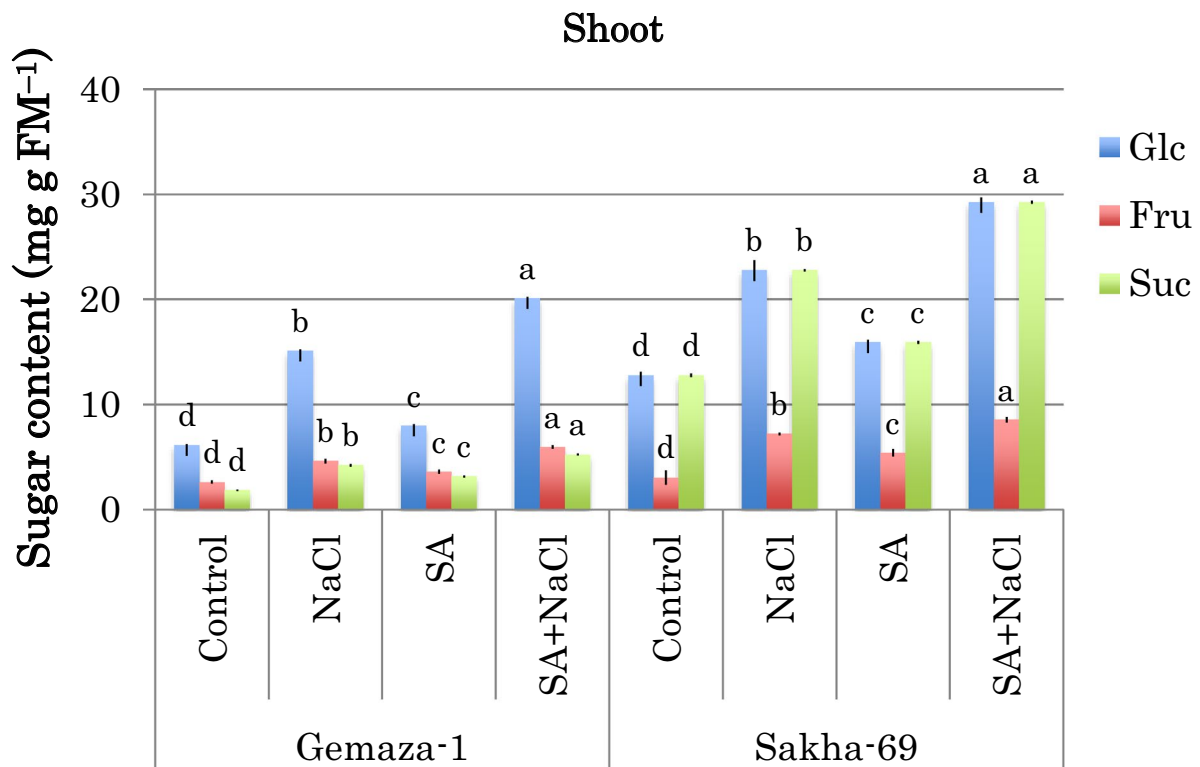
a Root

b Shoot

◄**Fig. 3** Effects of salicylic acid and salinity stress (150 mM NaCl) on contents of soluble sugars in root (**a**) and shoot (**b**) in two wheat cultivars. *Glc* glucose, *Fru* fructose, *Suc* sucrose. The values are expressed as mg g FM^{-1}. Data are expressed as means ± SD ($n = 3$). Means followed by different letters indicate significant differences at $P < 0.05$ level (Tukey's test) within the same sugar in each cultivar

neutralization of toxic metal ions and free radicals in plant cells. Both are synthesized under the amino acid assimilation metabolism after photosynthesis in chloroplasts and some of them are long-distantly transported from shoot to root and some are re-synthesized in root from amino acids and organic acids. Interestingly, for roots, TG contents were high in Sakha-69 but Pro contents were in Gemaza-1, a similar relationship was also found in the case of shoots. Pro was present at 5–21 mM order if assumed that 1 g (FM) of tissues contained 1 ml of the tissue sap, while TG was at 0.13–0.30 mM order, indicating that the former can act as osmotic protectants in a certain compartment of cells although the latter may not. Rather GS can function as an intracellular signal messenger or redox recycling under fast turning over. Salinity stress increased both TG and Pro contents in root and shoot, more conspicuously for Pro in shoots rather than roots in both cultivars. SA treatment also increased TG contents, but significantly decreased Pro contents in both cultivars, irrespective for the presence or absence of salinity stress effects. Degrees of the SA-induced inhibition (%) to the NaCl-increased Pro contents [100 × (NaCl + SA)/(NaCl)] in root and shoot were 57–65% for Gemaza-1 and 40–85% for Sakha-69, respectively, which suggested that SA serves as an effective hormonal mediator that might reduce the metabolic costs for excessive Pro production and/or accumulation in the different plant bodies of both wheat cultivars.

Effects of salinity and SA treatments on the soluble sugar and organic acid contents in wheat

Major soluble sugar contents in two wheat cultivars as affected by salinity stress and SA treatments were determined (Fig. 3). As a total, soluble sugar concentrations were 2.3–2.8 times higher in roots than in shoots in both cultivars. Under control conditions, the root and shoot in Sakha 69 contained Glc (60–62%), Fru (15–16%) and Suc (23–24%), 2–3 times higher concentrations than those in Gemaza-1 (Glc: 46–58%, Fru: 20–25%, Suc: 17–34%). Especially, Glc (ca. 35 mg g FM^{-1}) in Sakha-69 root that was compatible for 194 mM of osmotic solute concentration at a maximum was beyond threefold of Glc in Gemaza-1 root. This can be beneficial for its constitutive response to an external osmotic pressure of the root. Salinity stress (150 mM NaCl, which brings a possible raise of 280 mM of osmotic concentration in the medium) caused a noticeable rise in the concentrations

of Glc (43–46%), Fru (178–186%), Suc (70–86%) in roots, and also Glc (78–147%), Fru (76–136%), Suc (71–128%) in shoots, respectively, suggesting that the total increases in the concentrations of soluble sugars can compensate the rising osmotic pressures around the roots and in the apoplastic compartments of the shoots. The prominent increase in Fru in roots as compared to other sugars or those in shoots suggested that salinity stress might facilitate conversion to Fru (and Glc-phosphates) from Suc that had been transported from shoots by translocation. SA treatments caused an almost uniform increase (by ca. 50% in average) for all the sugars in both organs of two cultivars under the absence of salinity stress. Effects of SA and salinity stress on the soluble sugar concentrations were additive (counted as 1.7–3.8 folds of controls) and thus their influences appeared to be expressed in the same way in both organs and cultivars of wheat.

Unlike in the case of soluble sugars, total concentrations of major organic acids were 2.0–2.3 times higher in shoots than roots (Fig. 4). Under control condition, both root and shoot of Sakha-69 contained 2–2.3 times higher concentration of organic acids than those of Gemaza-1. Of the total organic acids detected, the most abundant are 59–65% of acetic acid (Ace), 22–31% of malic acid (Mal) and 8–13% of citric acid (Cit) in root and shoot of each wheat cultivar. Salinity stress caused an increase of such organic acids to 1.8 to 3.8-fold levels of controls, especially in the roots and also 1.5 to 3.0 fold levels in the shoots of both cultivars. SA treatment also significantly increased those organic acid levels but to 1.2 to 2.4-fold level of controls. Salinity and SA treatments increased organic acid levels additively up to 2.4 times for Ace, 3.3 times for Mal, 4.2 times for Cit in both wheat cultivars, on average. The magnitudes of increment of organic acids were almost equivalent to those in the case of soluble sugars (Fig. 3).

Effects of salinity and SA treatments on the inorganic solute contents in wheat

The inorganic solute contents including Na$^+$ and other major nutritional cations (K$^+$, Ca^{2+}, Mg^{2+}) were measured in two wheat cultivars as affected by salinity and SA treatments (Fig. 5). Under control conditions, Sakha-69 contained those 4 cations in the root and shoot to larger extents than Gemaza-1. Especially Ca^{2+} contents in shoots were excessively present in Sakha-69 than Gemaza-1. However, under salinity stress, contents of Na$^+$ were nearly similar between Gemaza-1 and Sakha-69, indicating their absorption rates for Na$^+$ ions are almost in the same range. Furthermore, salinity stress caused a 25–59% inhibition of uptakes of the nutritional cations in roots and a 19–36% inhibition of their accumulations by shoots, to a similar extent between Sakha-69 and Gemaza-1. These results indicated that Na$^+$
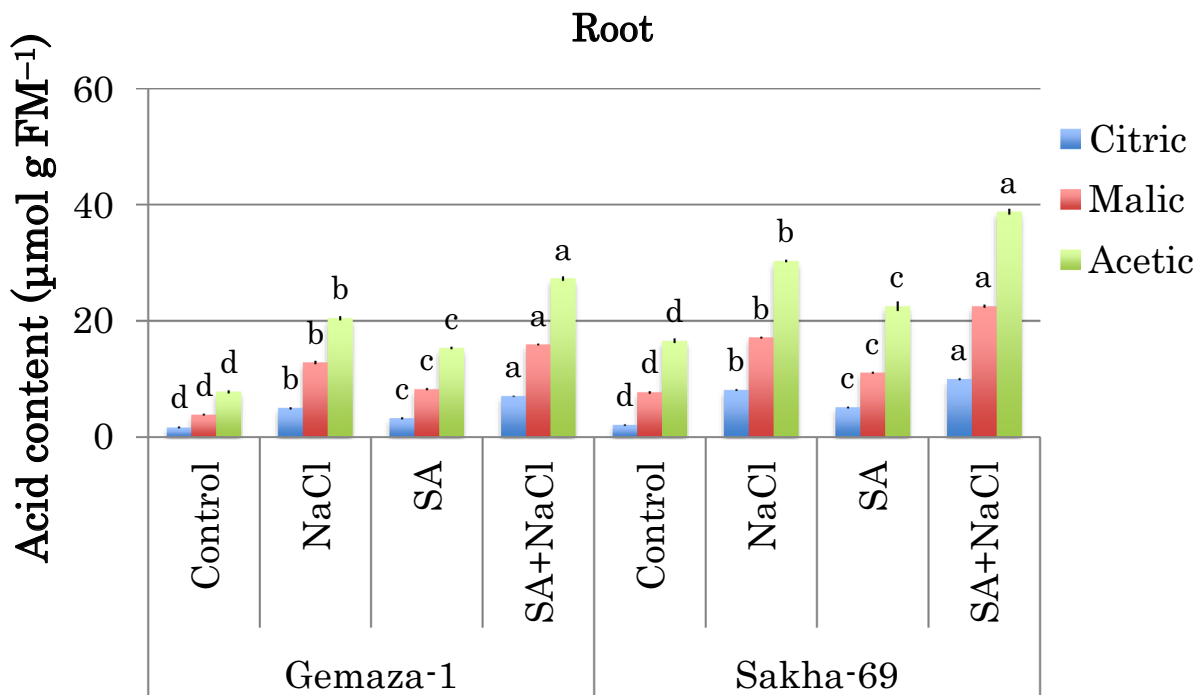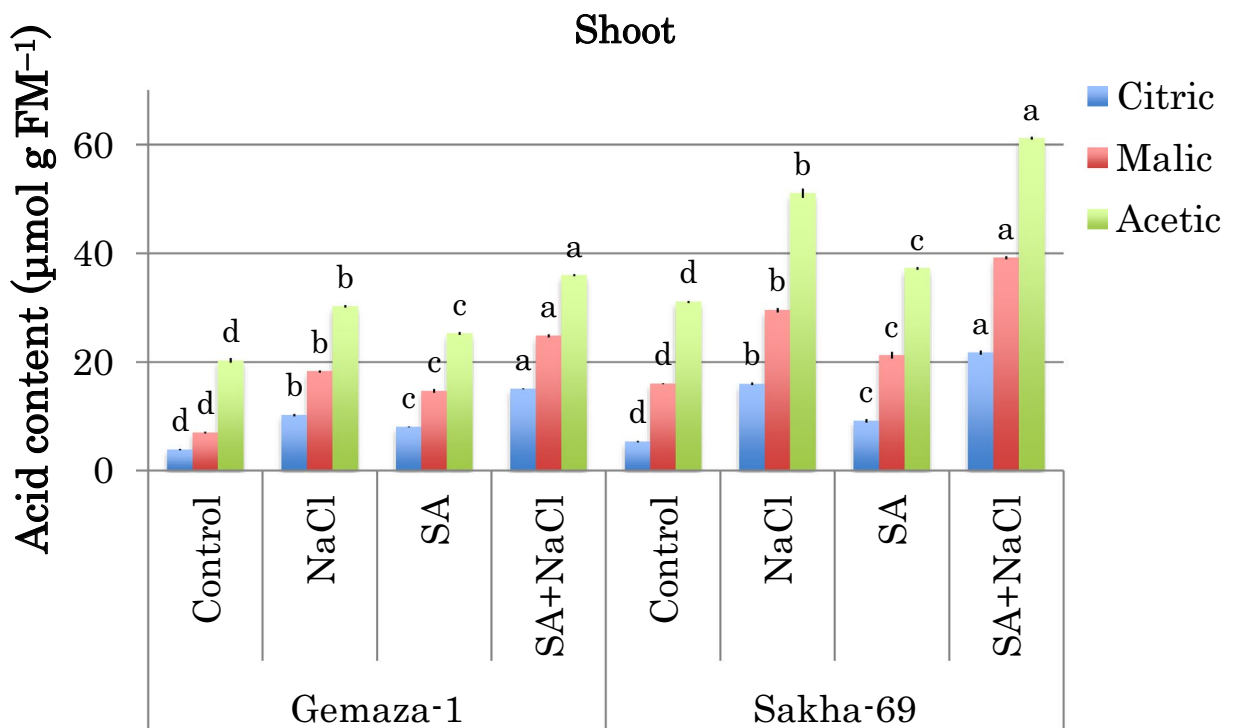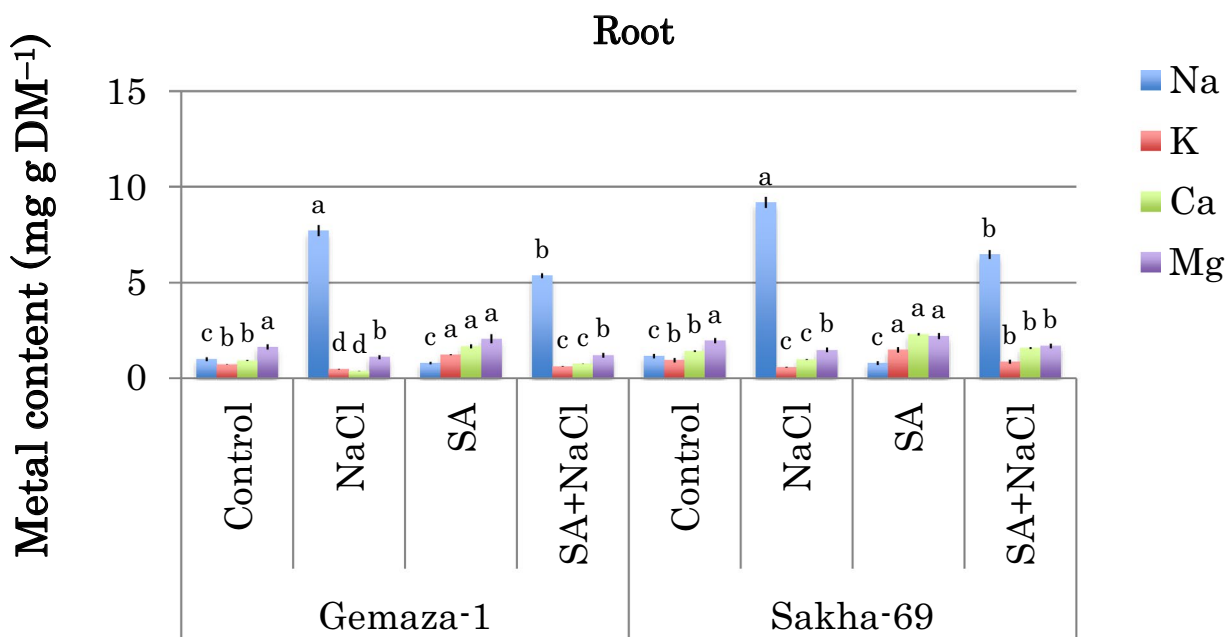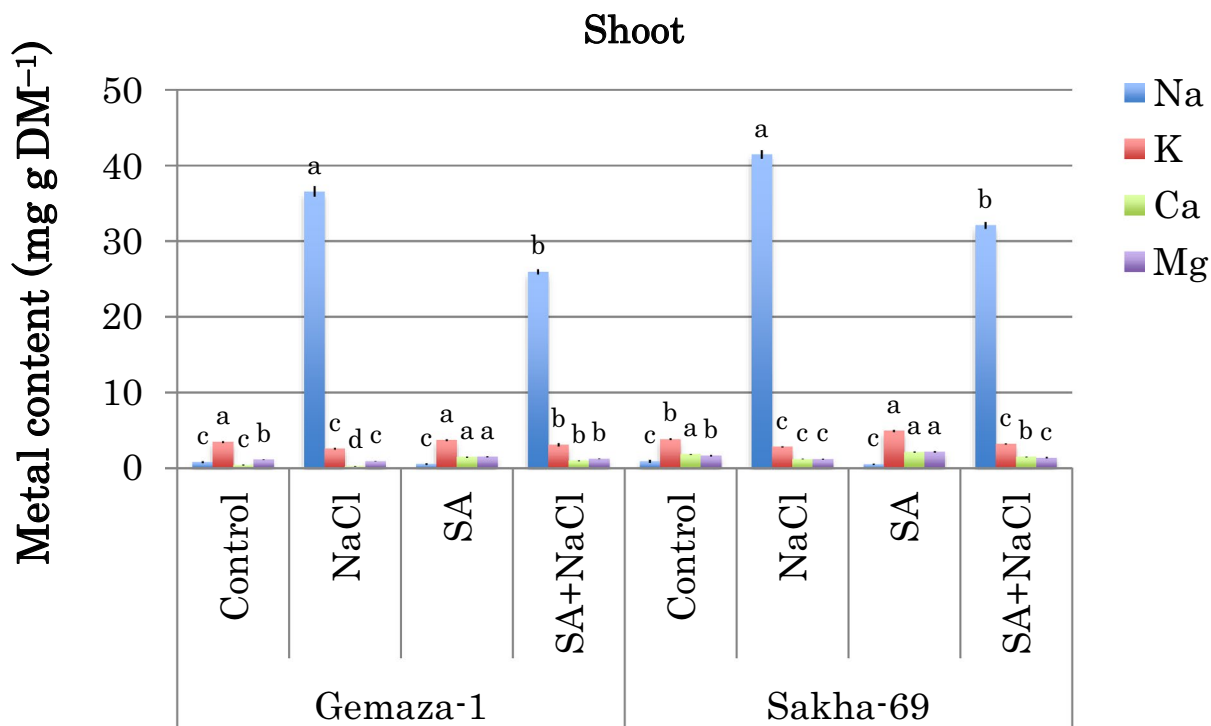
a Root

b Shoot

Fig. 4 Effects of salicylic acid and salinity stress (150 mM NaCl) on contents of organic acids in root (**a**) and shoot (**b**) in two wheat cultivars. *Citric* citric acid, *Malic* malic acid, *Acetic* acetic acid. The values are expressed as μmol g FM^{-1}. Data are expressed as means ± SD ($n = 3$). Means followed by different letters indicate significant differences at $P < 0.05$ level (Tukey's test) within the same acid in each cultivar

a Root

b Shoot

Fig. 5 Effects of salicylic acid and salinity stress (150 mM NaCl) on contents of inorganic cations in root (**a**) and shoot (**b**) in two wheat cultivars. The values are expressed as mg g DM^{-1}. Data are expressed as means ± SD ($n=3$). Means followed by different letters indicate significant differences at $P<0.05$ level (Tukey's test) within the same ion in each cultivar
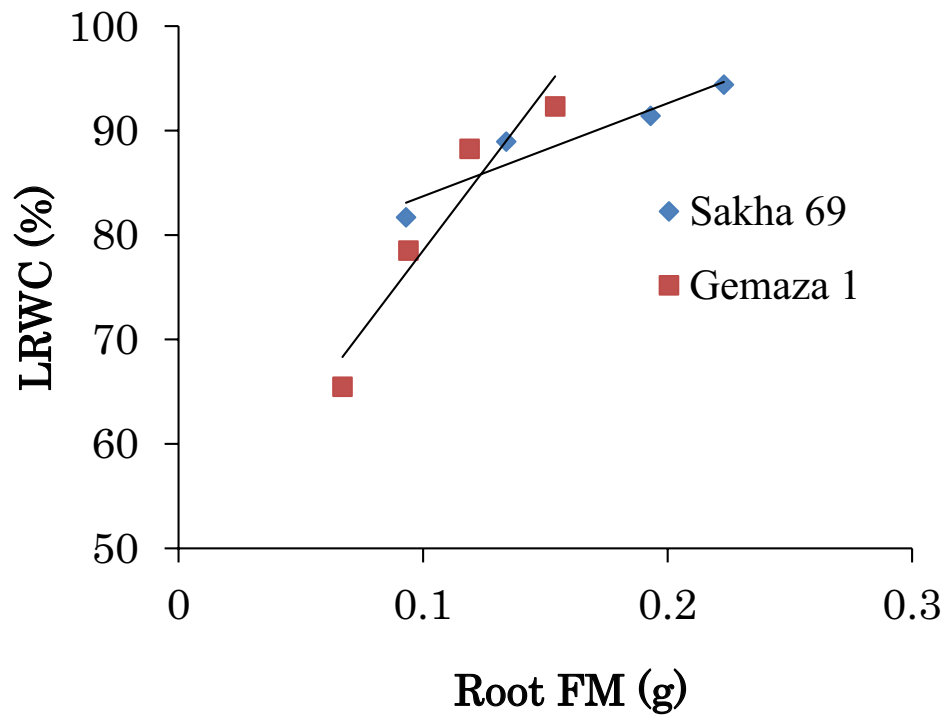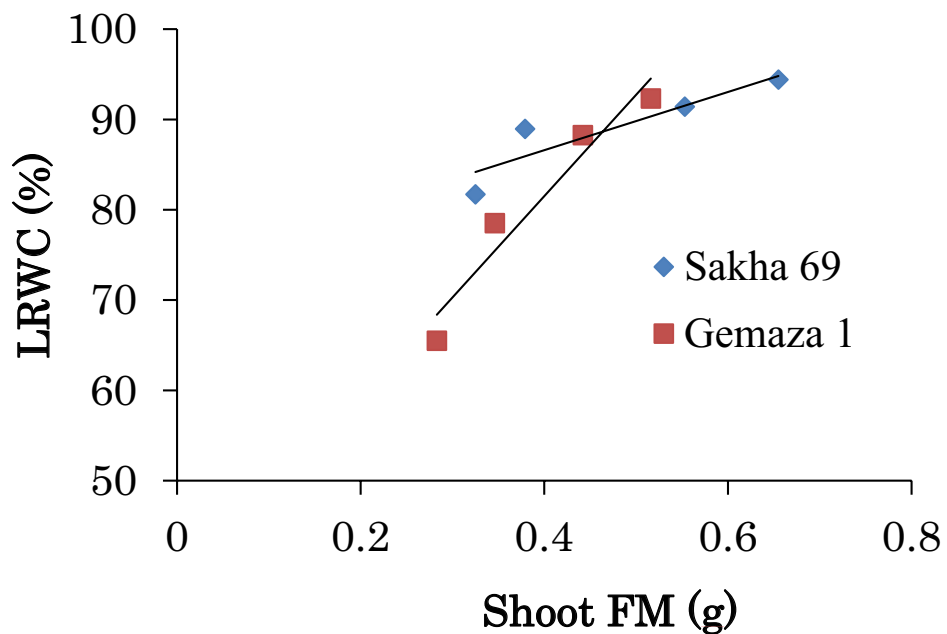
Fig. 6 Relationships between LRWC and FM of root (**a**) or shoot (**b**) in two wheat cultivars. The LRWC values (*y*-axis) were plotted against the FM values (*x*-axis) using data in Table 1. The linear regression curve analysis was carried out on the basis of the corresponding data (*n*=4) for respective cultivars. The correlation coefficients (degree of freedom: $m = n - 2 = 2$) calculated for Gemaza-1 and Sakha-69 were $r = 0.958$ and 0.958 in (**a**), and $r = 0.968$ and 0.908 in (**b**), respectively. The *r*-values more than 0.950 are significant at the 5% level

ion-induced inhibition for the activity of transporters for other cations are similar. SA treatment also increased K^+, Ca^{2+} and Mg^{2+} contents in root and shoot in both cultivars, especially more strongly for K^+ and Ca^{2+} in roots in both cultivars (60–80%) and Ca^{2+} in shoots of Gemaza-1 (270%), otherwise in a range of 10–37% increment. However, it conversely decreased Na contents in the organs of both cultivars by 20–33%, while some of the decreases in the absence of salinity stress were not significant at a 5% level. These results suggested that SA could improve the salinity tolerance of both cultivars by either inhibiting Na^+ uptake or promoting Na^+ exclusion or the other nutritional ion's imports, or both. Taken together, SA amelioration of salinity stress might be substantially dependent on the resumed nutritional balance in the root/shoot tissues at the uptake levels of solute ions. Here remains a possibility that residual inhibition of growth of both cultivars after Na^+ plus SA treatments as compared to control is due to an imperfect repairing of internal ions balances caused by competence between Na^+ and the other cations.

According to multivariate statistical analysis presented in Table S2, the main effects of cultivars, NaCl, and SA and their interactions were assessed at $P < 0.05$ level. The effects of previous factors were assessed by also pooling the effects of the other factors/covariates. Shoot/root factors were pooled for investigating other factors. According to multivariate analysis in Table S2, cultivars (Gemaza-1, Sakha-69) induced highly significant differences ($P < 0.05$) in various growth and physiological consequences. Moreover, cultivars and NaCl treatment induced significant differences in all tested growth and physiological parameters. However, interaction between cultivars and SA induced significant differences in growth and physiological parameters, except in DM and Cit, Mal and Mg contents ($P > 0.05$ each, Table S2). The interaction between NaCl and SA induced significant differences in measured parameters except in Fru content ($P > 0.05$). The interaction between cultivars, NaCl and SA showed a general significant effect except in Chl b, Glc and Na. These data revealed that the two cultivars of wheat plants exhibited different growth and physiological responses either to salinity and SA.

Discussion

Salinity response on two wheat cultivars and the relevance to drought stress

In the present study, we demonstrated that two cultivars of wheat plants grown in the presence of 150 mM NaCl exhibited different growth responses to salinity stress. Sakha-69 had greater salinity tolerance than Gemaza-1 (Table 1). Previously we showed that Sakha-69 had greater drought tolerance than Gemaza-1 (Loutfy et al. 2012). In drought stress, difference among wheat cultivars were well established and recognized using the LRWC values and the relationships to the biomass such as FMs of root and shoot (Loutfy et al. 2012). Figure 6 shows relationships between LRWC and FMs of root and shoot in two wheat cultivars as affected by salinity stress and SA. The data shows that the LRWC values in each cultivar are highly positively correlated to the FM values in root and shoot, respectively, which suggests that the water status in the leaves of each cultivar are dependent basically on the respective organ biomass, even under the salinity stress. The slopes in the graphs represent the other traits of cultivars apart from the growth and biomass (Loutfy et al. 2012), that is, Sakha-69 has higher ability of water maintenance than Gemaza-1, thus can be tolerant against higher salinity conditions by avoiding the water stress in the leaves.

Physiological parameter changes in wheat cultivars due to salinity stress

Salt stress is one of the major environmental stresses that affect plant growth and development by altering various physiological processes, including photosynthesis, transpiration, antioxidant system, osmotic regulation, and water and/or solute uptakes in many plants (Jini and Joseph 2017; Ma et al. 2017; Manaa et al. 2014; Negrao et al. 2017). In the present study, salinity stress induced physiological parameters tested in two wheat cultivars. Mainly it decreased water content in root and shoot by 50 and 40%, LRWC and PPC in leaves by 20% and contents of nutritional inorganic solutes (K^+, Mg^{2+}, Ca^{2+}) in root/shoot by ca. 40 and 30%, respectively, on average. On the other hand, salinity stress increased the contents of organic solutes (sugars, organic acids, Pro and GSH) by 50–300% and the activities of AOS enzymes (catalase and peroxidases) by 50–160%, both in root and shoot. The NaCl at 150 mM can be dissociated to 140 mM each of Na^+ and Cl^- ion particles in solution at 25 °C, thus the resulting osmotic concentration (280 mOsmol l^{-1}, equivalent to − 0.70 MPa of water potential) is enough to cause a moderate water stress to the wheat plants. Drought stress has shown to mimic those changes above in the salinity stress in wheat (Loutfy et al. 2012) and many other plants (Bartels and Sunkar 2005; Golldack et al. 2014; Silva et al. 2011). In fact, salinity stress causes a compensational rise of total concentrations of osmotic solutes (or compatible solutes), as found in wheat as affected by drought stress (Loutfy et al. 2012). In the present study, total molar concentrations of organic solutes (summation of Glc, Fru, Suc, Pro, Cit, Mal, and Ace) in roots against 150 mM NaCl can be counted as 151 mM (or − 0.38 MPa) for Gemaza-1 and 248 mM (− 0.62 MPa) for Sakha-69, respectively. These could account for 50 and 83% effectiveness for the
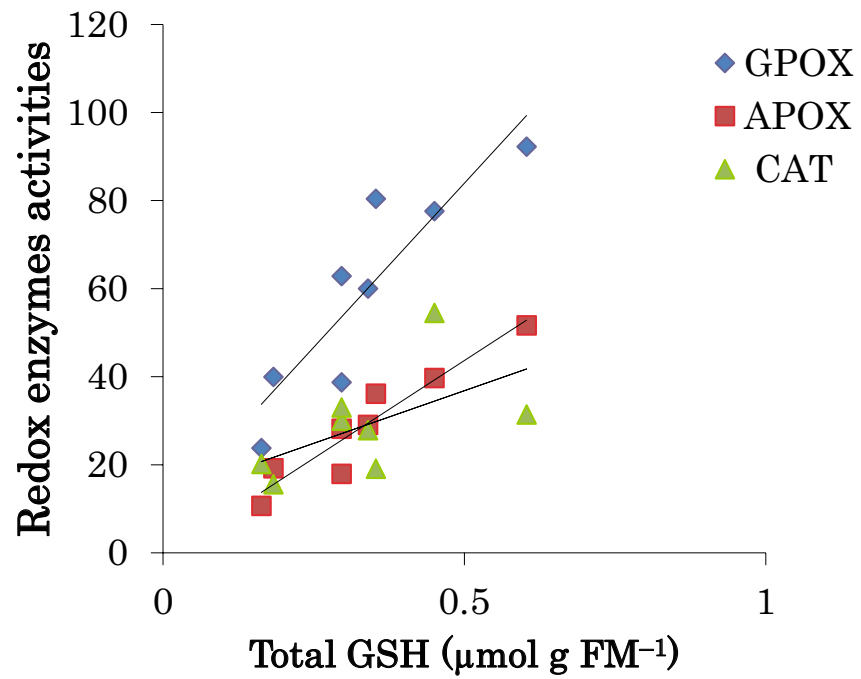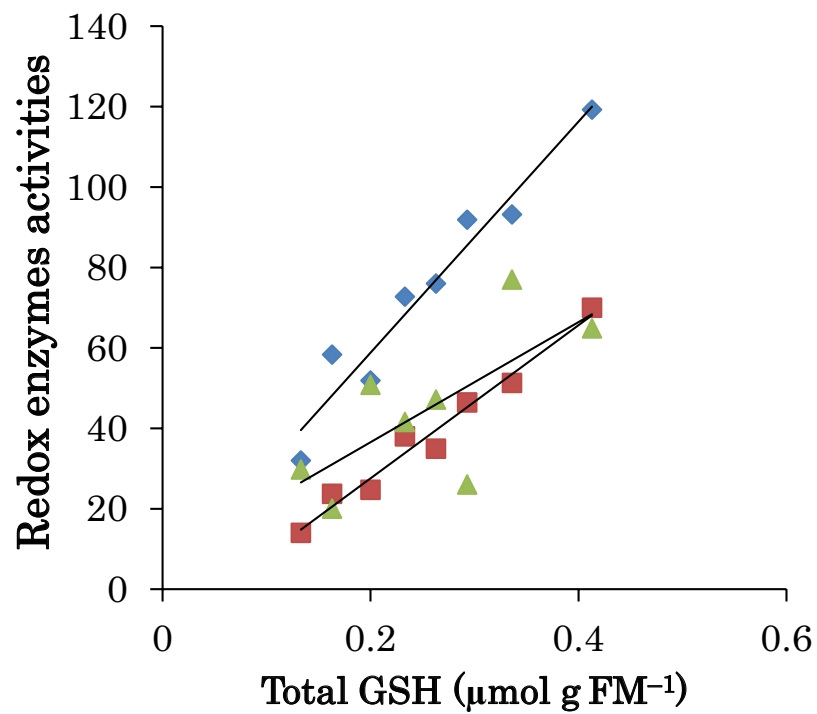
a Root

b Shoot

elimination or protection of water loss from the respective tissues to medium. Other factors still need to be examined for changes in other possible solutes like amino acids and changes in Na^+ and Cl^- concentrations in media during salinity treatments before drawing a detail diagram of the relationships.

Na^+ caused a total decrease in contents of the nutritional cations such as K^+, Ca^{2+}, and Mg^{2+} in the root and shoot tissues (Fig. 5). Similar results have been found in basil (*Ocimum basilicum*), broad bean (Azooz et al. 2011; Delavari et al. 2010), strawberry (Karlidag et al. 2009), lettuce (Neocleous et al. 2014), soybean (Essa 2002), cucumber (Yildirim et al. 2008), maize (Gunes et al. 2007), and rice (Jini and Joseph 2017). Essa (2002) has reported that NaCl may create great ratios of Na/Ca and Na/K in plants, causing them to be susceptible to both osmotic and ion-specific injury, in addition to nutritional disorders. These changes in K^+, Ca^{2+}, and Mg^{2+} uptake were observed significantly in both organs of both wheat cultivars tested.

From this experiment, we notice that after 150 mM NaCl treatment, the total Na^+ contents (not concentrations) in root and shoot tissues increased to near or over 50 and 250 mmol kg^{-1} in FM basis, respectively, while the uptakes of the counter ion (Cl^-) were not determined. These Na^+ ions incorporated in the tissues might be toxic for many glycophyte plants including wheat, if they have no special defense mechanisms against free Na^+ ions and Na^+-induced free radicals by binding for detoxification, sequestration, neutralization, or compartmentalization by transporters. All of these mechanisms, however, appeared to be set in two wheat cultivars examined, because of the marked rise in concentrations of Pro, organic acids, and GSH, the activities of AOS enzymes and the high Na^+-translocation rate from root to shoot (ca. 5) were observed. Salinity and drought stress have been reported to cause a rise in AOS enzymes as well as the GSH and AsA cycle systems in many plants. These are critical mechanisms in preventing oxidative stress in plants and usually improve response to stressful conditions in many plants, such as *Lupimus termis* (Salem and Abu Alhamd 2009), mustard (Nazar et al. 2015), *Torreya grandis* (Liu et al. 2016), and others (Gressel and Galun 1994, for review). These results correspond with the results in our present study. Furthermore, we found GSH levels are positively well correlated with APOX and GPOX activities

but not to CAT activity (Fig. 7). These results imply that the former enzymes coupling to the GSH and ASA redox cycle systems might be cooperatively operating in reducing the direct salinity toxicity and the secondary oxidative damages in the tissues. Shim et al. (2003) also reported that salinity and oxidative stresses inhibit CAT activities in wheat, rice and cucumber seedlings.

Drought stress caused an increase in Pro content and a decrease in inorganic solute contents at the same time (Loutfy et al. 2012). In this experiment, we found that similar changes to these were caused by salinity stress (Fig. 8). Pro content was correlated to increase of Na^+ positively in the roots of Gemaza-1 ($r = 0.977$) and Sakha-69 ($r = 0.998$) at 5% level ($r \geq 0.950$, $n = 4$) and to some extent in the shoots ($r = 0.942$ and 0.850). By contrast, it showed a negative correlation to other nutritional ions (K^+, Ca^{2+} and Mg^{2+}) in the root and shoot tissues, although some of the correlation coefficients (r values) were not significant at 5% level ($n = 4$). Pro can be accounted to bind 75 and 41% of Na^+ in roots and 35 and 17% of Na^+ in shoots, for Gemaza-1 and Sakha-69, respectively. This implied that Pro might play an important role, if produced once, for Na^+-bindings in root and shoot and also Na^+-transport to vacuole in roots and its long distance transport to shoot in wheat cultivars. It is not yet directly answered that the rise in Pro contents is due to a signal of osmotic shock rather than Na^+ ion in cytoplasm, but this is likely because a substantial increase in Pro with no apparent rise of Na^+ occurred in wheat after drought treatment (Loutfy et al. 2012, and unpublished results). Furthermore, inverse correlations between Pro and nutritional salts (K^+, Mg^{2+}, Ca^{2+}) in wheat were observed but not strong (Naglaa Loutfy unpublished, data not shown).

Figure 9 shows relationships between Pro content and catalase activity that are up or down regulated by salinity or SA. We found that there was a positive high correlation significant at 1% level between them in each cultivar ($r = 0.929$ and 0.942 for Gemaza-1 and Sakha-69, respectively, $n = 8$), while the correlation ($r = 0.473$) for all the data ($n = 16$) was not significant even at 5% level. Great value of the catalase and/or its ratio to Pro content (ca. three time higher than in Gemaza-1) could be an outstanding trait of the tolerant cultivar of Sakha-69 that might be independent on SA function. The Pro content and catalase activity correlated with Na^+ content in the both cultivars at 1% level, as shown in Fig. S1.

SA amelioration of salinity stress in wheat cultivars

In the present study, SA was tested for their efficiency on growth of two wheat cultivars (Sakha-69 and Gemaza-1) under the influence of 150 mM of NaCl. The results indicated that the SA significantly improved the seedling growth and related seedling parameters and diminishes the inhibitory effect of NaCl (Tables 1, S2, Fig. 1). These results
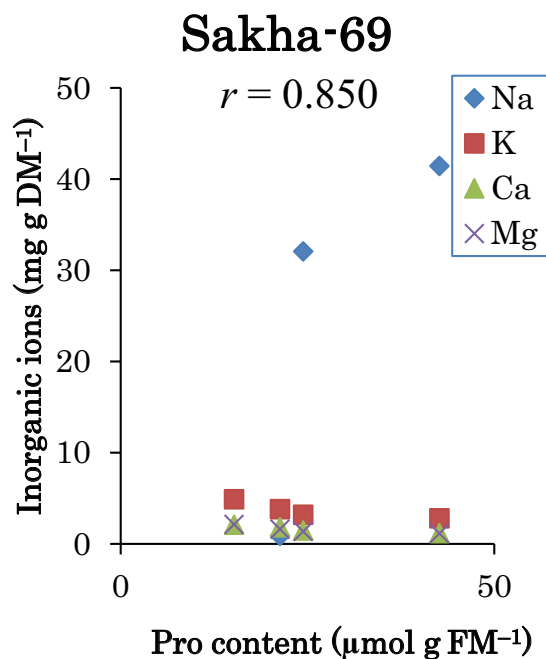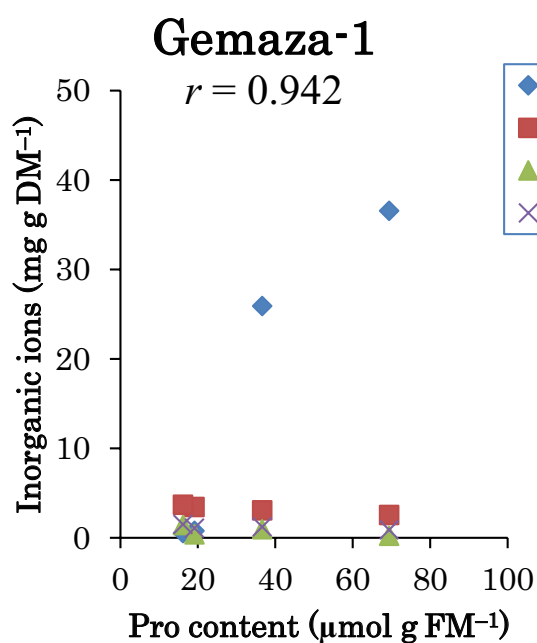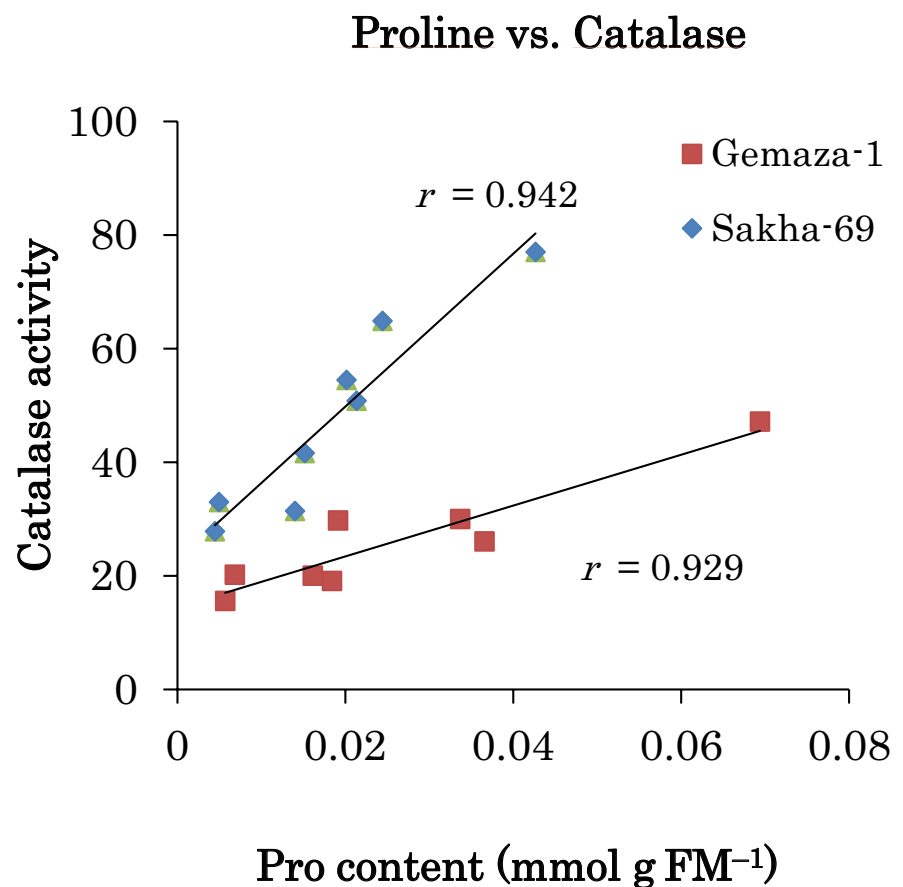
a Root
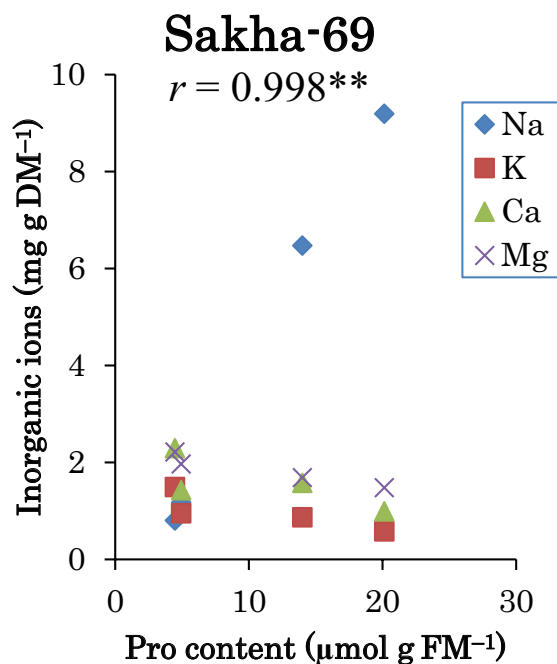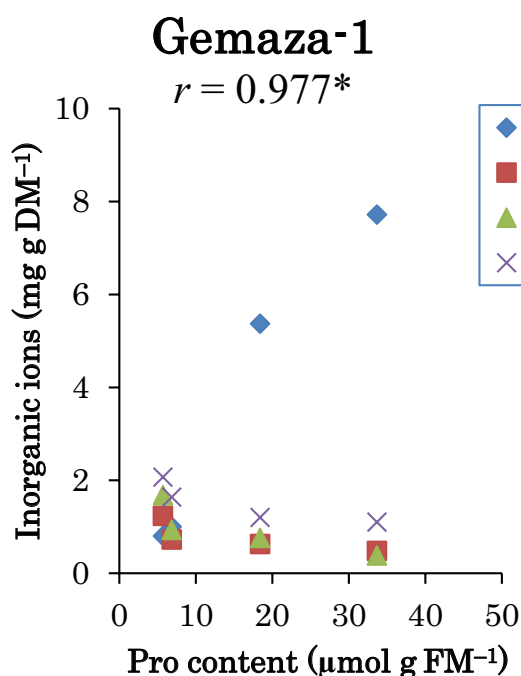
b Shoot

Fig. 8 Relationships between proline contents and inorganic ion (Na^+, K^+, Ca^{2+} and Mg^{2+}) contents in roots and shoots of two wheat cultivars as affected by SA and/or salinity stresses. **a** root, **b** shoot. The correlation coefficients between Pro and Na^+ contents in roots are $r = 0.977$ and 0.998 for Gemaza-1 and Sakha-69, which are sig- nificant at 5% and 1% levels, respectively ($n = 4$), however those in shoots ($r = 0.942$ and 0.850) are not at 5% level. The contents of K^+, Ca^{2+} and Mg^{2+} ions tended to decrease as the Pro or Na^+ contents increase, while some of the r-values were not significant at 5% level (data not shown)

Fig. 9 Relationships between proline contents and catalase activities in roots and shoots of two wheat cultivars as affected by SA and/or salinity stresses. The correlation coefficients ($n = 8$) were calculated for Gemaza-1 and Sakha-69, separately. The r-values more than 0.834 are significant at the 1% levels

Proline vs. Catalase

$r = 0.942$

$r = 0.929$

Catalase activity

Pro content (mmol g FM^{-1})

■ Gemaza-1

◆ Sakha-69

were in accordance with those reported in *Brassica juncea* (Yusuf et al. 2008), cotton plant (El-Beltagi et al. 2017), and *Dianthus surperbus* (Ma et al. 2017). SA recovered salinity-induced reduction in growth of roots and shoot of both wheat cultivars near to control levels. This remediation effect of SA resembles that in drought stress, in its remediation effects on the biomass, LRWC, and PPC in leaves (Loutfy et al. 2012). SA further increased most of the organic solute contents (except Pro) and AOS enzyme activities (except CAT) in an additive manner to those increments caused by salinity stress (Table 2, Figs. 2, 3, 4). SA also caused a substantial increase in contents of nutritional mineral ions such as K$^+$, Ca^{2+} and Mg^{2+} (except Na$^+$), which are significantly decreased by salinity stress (Fig. 5). All of these changes in osmotic solutes and antioxidant enzyme activities are thought to contribute to recovery for osmotic shock stress caused by salinity stress. In contrary, SA caused a significant decrease in Pro content, CAT activity, and Na$^+$ content in both root and shoot of two cultivars, which can explain that SA can be effective in lowering Na$^+$ levels in tissues and hence effective in lowing of the catalytic enzyme activity and origination of protective molecules in the cytoplasm. Therefore, SA effects on salinity stress are diversified as they are combined with water stress dependent on osmotic

potential changes and saline specific chemical stress due to Na$^+$-triggered intrinsic toxicity.

If considered more, each possible factor that is working in adjustment of mineral uptake by SA treatment may be a direct mechanism for the reduction of salinity stress. The increased seedling growth in SA-treated wheat seedlings might be due to its effects and regulations on the stomatal openings and decrease water loss under stress condition facilitating the plants to maintain turgor as shown in Kentucky bluegrass (He et al. 2005) and/or enhanced increase in the net photosynthetic rate in *Torreya grandis* under salt stress (Li et al. 2014). Photosynthetic pigment is one of the acute factors determining photosynthetic efficiency and plant growth in *Anoectochilus roxburghii* (Shao et al. 2014). In the present study, NaCl salt noticeably decreased the Chl *a*, Chl *b* and carotenoids, while SA treatment reduced this reduction of the chlorophyll contents in both wheat cultivars under salinity stress. These results are consistent with study on *D. superbus* L. (Ma et al. 2017). They described that SA might stimulate the activity of enzymes related to chlorophyll biosynthesis or might release the damage of the photosynthetic system, thus decreasing chlorophyll degradation. One can suggest that the plants actively produce antioxidant system to deal with toxic oxygen products that result from salinity

a Osmotic solute concentration (mmol g FM^{-1}) in root and shoot.
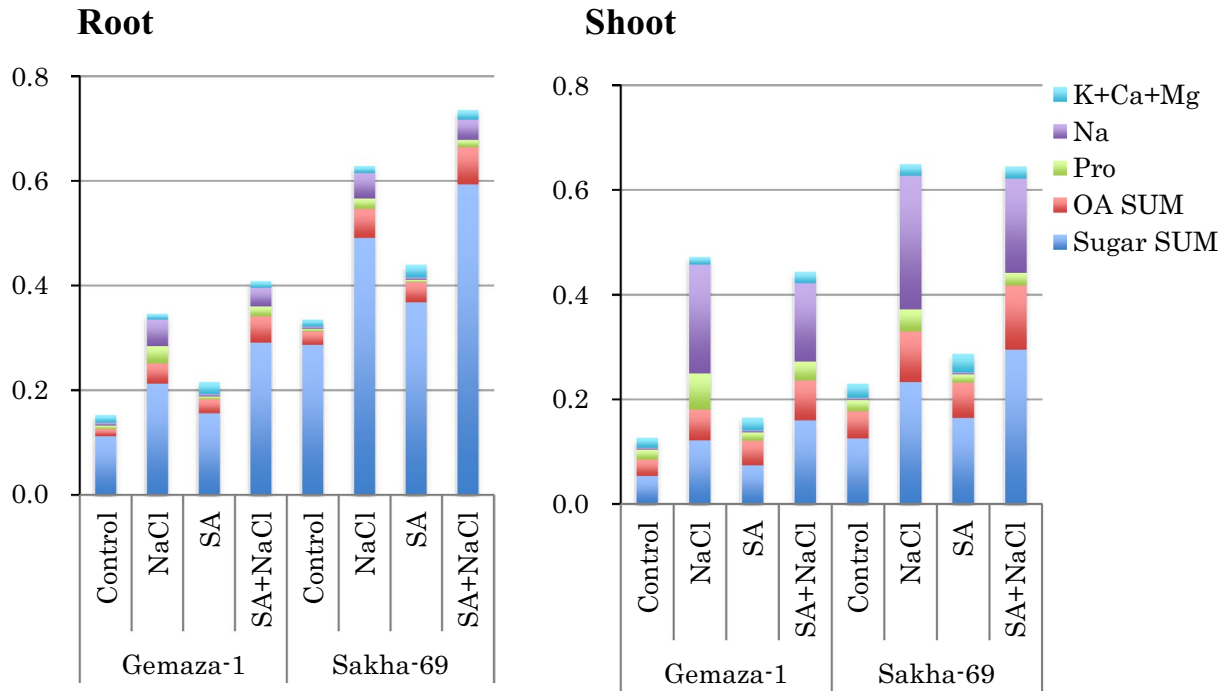
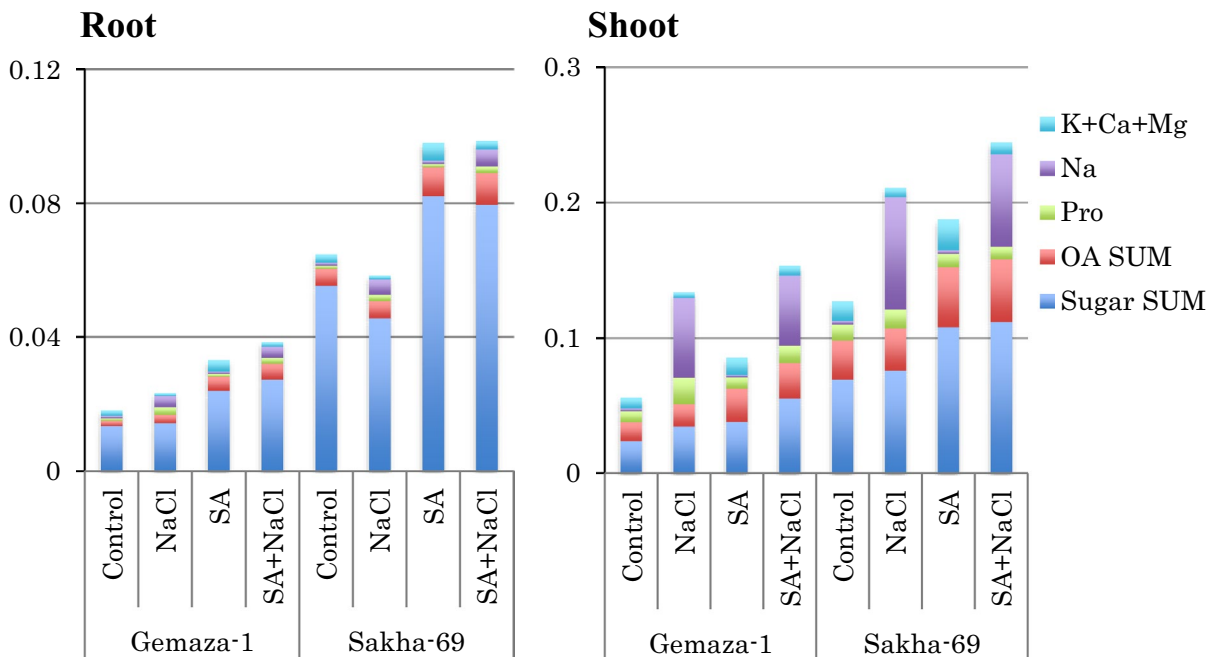## b Total solute accumulation (mmol organ^{-1}) in root and shoot.

◄**Fig. 10** Changes in organic and inorganic solutes concentrations and the total solutes accumulations in root and shoot of two wheat cultivars as affected by SA and salinity stress. **a** Osmotic solute concentrations (mmol g FM^{-1}) in roots and shoots. All the concentrations of solutes were expressed as mmol g FM^{-1} basis of molecules or ions and simply integrated in leading to an apparent total concentration value of the osmotic solutes. **b** Total solute contents of organic and inorganic osmotic solutes in root and shoot in 2 wheat cultivars. The total solute contents (TS) of respective osmotic solutes were calculated with the data of the FM of the organs (Table 1) and the respective solute concentrations (SC) in Table 2 and Figs. 3, 4, 5, using the following equation: TS (mmol solute per organ) = SC (mmol g FM^{-1}) × (g FM). Note that the salinity-stress treatment generally increases the total solute contents for sugars especially roots and other organics especially shoots, but decreases for the inorganic solutes other than Na^+ ions. SA treatments caused the increase in total solute contents for sugar, organic acids, and inorganic solutes but did not for proline. Wheat cultivars are apparent Na accumulators in shoots rather than in roots

or osmotic stresses and both. It can be concluded that SA alleviates the adverse effects of salinity stress by activating photosynthesis and enhancing antioxidant systems as a common mechanism in plants.

Of organic solutes increased by SA treatment, soluble sugars are osmotic regulators, and of which disaccharides and oligosaccharides have a role in membrane stability in cucumber (Dong et al. 2011). Our data revealed that SA greatly increased sugars (Glc, Fru and Suc) especially in roots under effect of salinity (Fig. 10). So, one could conclude that SA regulates the metabolism of sugar and hence alters sugars levels in cells, which reflects plants enhanced tolerance to salinity stress. This hypothesis is in agreement with Poor et al. (2011), they reported that SA treatment might reduce hexokinase activity, resulting in higher content of Glc and Fru in the leaf and higher Suc in the root of tomato plants, thus, alleviating the influence of salt stress through osmotic adjustment. Organic acids have various
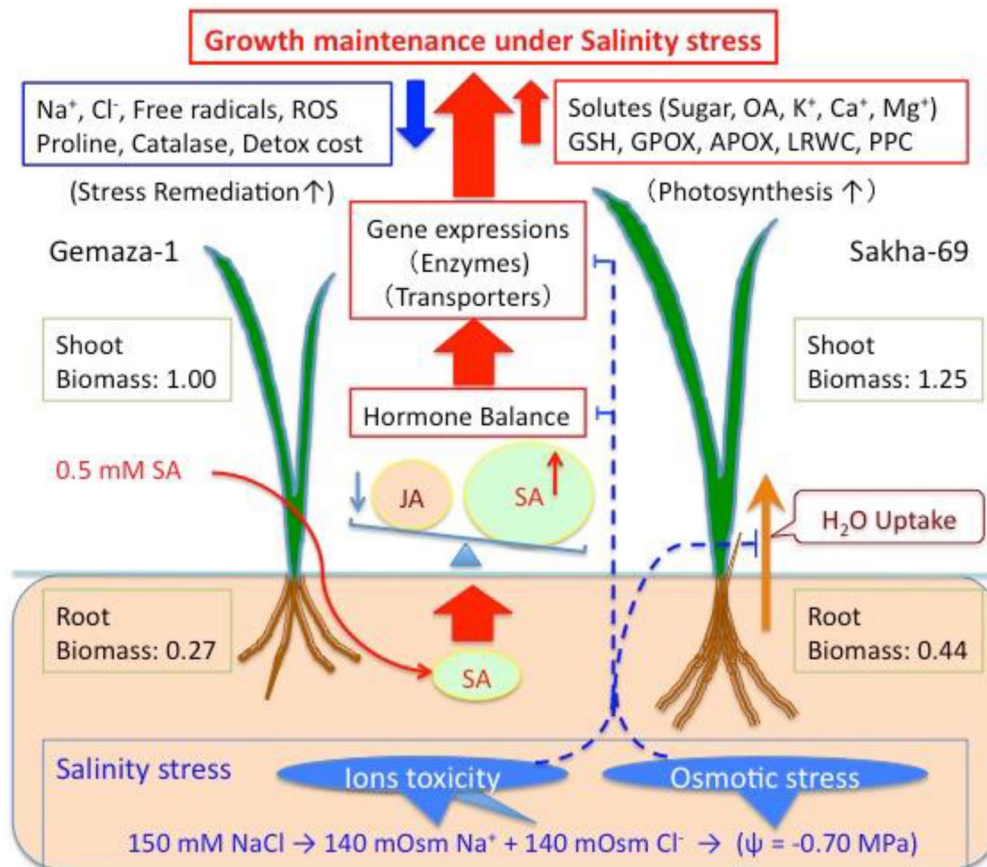

Fig. 11 A proposed model of possible SA action on salinity stress in wheat. Salinity stress induced by 150 mM NaCl may cause high impacts to both wheat cultivars through the ionic toxicity due to Na^+ and Cl^- ions and the osmotic stress near to –0.70 MPa. Salinity also induces toxic radicals and ROS evolutions in tissues. These may inhibit the water uptake, hormone balance, and a series of gene expression for enzymes, transporters and other proteins that participate in the photosynthesis, solute translocation, stress-relaxation and

many other biochemical and physiological processes. In contrast, externally applied SA (0.5 mM) may increase the endogenous level of SA and hence prime or establish the ordered balance for this and other hormones (such as JA, ABA, GA and IAA) that take part in the integrative regulations of gene expressions. These changes can be coped with the multiple disorders due to salinity stress above mentioned. SA application can be useful for improving the growth and yield of wheat in both salty and drought areas

roles in the metabolic and physiological responses of plants to water stress (Franco et al. 1992). Our result indicates that organic acids (Cit, Mal and Ace) in both tested plants highly increased under salt stress or in the presence of SA, especially in shoots (Fig. 10), where an apparent accumulation of Na^+ ions occurs too. Treatment of plants with SA causes an array of protection reactions, including the accumulation of organic acids, which have auto (ox-reduction) properties and act as cofactors for dismutases, catalases and peroxidases which catalyze break of the toxic H_2O_2, OH, O^-_2 (Sadak and Orabi 2015). Foliar applications of citric and oxalic acids improve thermo-tolerance of wheat plants (Sadak and Orabi 2015). The synthesis and breakdown of organic acids serve as a mechanism for pH regulation in plant cell as shown in cotton (Timpa et al. 1986) and other plants (Marschner 1995).

Based upon the data in the present study and evidence form the other research literatures, possible actions of SA on salinity stress in wheat are summarized (Fig. 11). Exogenously applied SA at a moderate concentration (0.5 mM) can be effective to increase the endogenous level of SA, as reported earlier in rice, soybean and other plants (Hamayun et al. 2010; Jini and Joseph 2017), and also in changing the balance of other hormones such as JA, ABA, GA and IAA, of which functions and relationships to plant salinity stress responses have been reviewed (Miura and Tada 2014; Jayakannan et al. 2015). These and other signals such as ROS and Ca^{2+} induced as second messengers may activate and/or regulate the gene expressions of various proteins that participate in the solute metabolism, transport, signaling and detoxification in wheat. However, detailed mechanisms for the SA-induced priming and maintenance of the multiple tolerance characteristics await further experimental research.

Conclusion

In summary, we found that salinity and SA increased additively most parameters for OC and AOS, but SA decreased Na^+ and Pro contents as well as CAT activity, and increased K^+, Ca^{2+}, and Mg^{2+} contents in a counteracting manner to salinity. We concluded that SA-mediated tolerance might involve two distinct mechanisms; one specific for salts and the other related to drought or dehydration stress response in wheat root and shoot module systems. SA application can be useful for improving the growth and yield of wheat in both the salty and drought areas.

References

Adam AL, Bestwick CS, Barna B, Mansfield JW (1995) Enzymes regulating the accumulation of active oxygen species during the hypersensitive reaction of bean to *Pseudomonas syringae* pv. Phaseolicola Planta 197:240–249

Aebi H (1984) Catalase in vitro. Method Enzymol 105:121–126

Al-Naggar AMM, Sabry SRS, Atta MMM, El-Aleem OMA (2015) Effects of salinity on performance, heritability, selection gain and correlations in wheat (*Triticum aestivum* L.) doubled haploids. Scientia Agriculturae 10:70–83

Azooz MM, Youssef AM, Ahmad P (2011) Evaluation of salicylic acid (SA) application on growth, osmotic solutes and antioxidant enzyme activities on broad bean seedlings grown under diluted seawater. Int J Plant Physiol Biochem 3:253–264

Bartels D, Sunkar R (2005) Drought and salt tolerance in plants. Crit Rev Plant Sci 24(1):23–58

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39(1):205–207

Boukraâ D, Benabdelli K, Belabid L, Bennabi F (2013) Effect of salinity on chickpea seed germination pre-treated with salicylic acid. Sci J Biol Sci 2:86–93

Delavari PM, Baghizadeh A, Enteshari SH, Kalantari KM, Yazdanpanah A, Mousavi EA (2010) The effects of salicylic acid on some of biochemical and morphological characteristic of *Ocimum basilicucm* under salinity stress. Aust J Basic Appl Sci 4:4832–4845

Dong C, Wang X, Shang Q (2011) Salicylic acid regulates sugar metabolism that confers tolerance to salinity stress in cucumber seedlings. Scitia Horti 129:629–636

El-Beltagi H, Ahmed S, Namich SH, Abdel-Sattar AA (2017) Effect of salicylic acid and potassium citrate on cotton plant under salt stress. Fresenius Environ Bull 26:1019–1100

Essa TA (2002) Effect of salinity stress on growth and nutrient composition of three soybean (*Glycine max* L. Merrill) cultivars. J Agron Crop Sci 188:86–93

Franco AC, Ball E, Luttge U (1992) Differential effects of drought and light levels on accumulation of citric acids during CAM in Clusia. Plant Cell Environ 15:21–829

Ghassemi-Golezani K, Lotfi R (2015) The impact of salicylic acid and silicon on chlorophyll a fluorescence in mung bean under salt stress. Russ J Plant Physiol 62:611–616

Golldack D, Li C, Harikrishnan M, Probst N (2014) Tolerance to drought and salt stress in plants: unraveling the signaling networks. Front Plant Sci 5:151

Grattan SR, Grieve CM (1999) Salinity-mineral nutrient relations in horticultural crops. Sci Hortic 78:127–157

Gressel J, Galun E (1994) Genetic controls of photooxidant tolerante. In: Foyer CH, Mullineaux PM (eds) Causes of photooxidative stress and amelioration of defence systems in plants. Taylor & Francis Inc, CRC Press, Boca Raton, pp 237–273

Gunes A, Inal A, Alpaslan M, Eraslan F, Bagci EG, Cicek N (2007) Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zea mays* L.) grown under salinity. J Plant Physiol 164:728–736

Hafsi C, Romero-Puerta MC, Gupta DK, del Rio LA, Sandalio LM, Abdelly C (2010) Moderate salinity enhances the antioxidative response in the halophyte *Hordeum maritimum* L. under potassium deficiency. Environ Exp Bot 69:129–136

Hayat Q, Hayat S, Irfan M, Ahmad A (2010) Effect of exogenous salicylic acid under changing environment: a review. Environ Exp Bot 68:14–25

He Y, Liu Y, Cao W, Huai M, Xu B, Huang B (2005) Effects of salicylic acid on heat tolerance associated with antioxidant metabolism in Kentucky bluegrass. Amer J Crop Sci 45:988–995

Isayenkov S, Maathuis FJM (2019) Plant salinity stress: many unanswered questions remain. Front Plant Sci. https://doi.org/10.3389/fpls.2019.00080

Jayakannan M, Bose J, Babourina O, Rengel A, Shabala S (2015) Sli-cylic acid in plant salinity stresss signaling and tolerance. Plant Growth Regul. https://doi.org/10.1007/s10725-015-0028-z

Jini D, Joseph B (2017) Physiological mechanism of salicylic acid for alleviation of salt stress in rice. Rice Sci 24:97–108

Karlidag H, Yildirim E, Turan M (2009) Salicylic acid ameliorates the adverse effect of salt stress on strawberry. Sci Agric 66:180–187

Khan N, Syeed S, Masood A, Nazar R, Iqbal N (2010) Application of salicylic acid increases contents of nutrients and antioxidative metabolism in mung bean and alleviates adverse effects of salinity stress. Int J Plant Biol 1:1–8

Khan MIK, Fatma M, Per TS, Anjum NA, Khan NA (2015) Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. Front Plant Sci 6:462

Li T, Hu YY, Du XH, Tang H, Shen CH, Wu JS (2014) Salicylic acid alleviates the adverse effects of salt stress in *Torreya grandis* cv. Merrillii seedlings by activating photosynthesis and enhancing antioxidant systems. PLoS ONE 9:e109492

Liu W, Zhang Y, Yuan X, Xuan Y, Gao Y, Yan Y (2016) Exogenous salicylic acid improves salinity tolerance of *Nitraria tangutorum*. Russ J Plant Physiol 63:132–142

Loutfy N, El-Tayeb MA, Hassanen AM, Moustafa MFM, Sakuma Y, Inouhe M (2012) Changes in the water status and osmotic solute contents in response to drought and salicylic acid treatments in four different cultivars of wheat (*Triticum aestivum* L.). J Plant Res 125:173–184

Ma X, Zheng J, Zhang X, Hu Q, Qian R (2017) Salicylic acid alleviates the adverse effects of salt stress on *Dianthus superbus* (Caryophyl-laceae) by activating photosynthesis, protecting morphological structure, and enhancing the antioxidant system. Front Plant Sci 8:600

MacInnes J (2017) An introduction to secondary data analysis with IBM SPSS statistics. Sage Publications Ltd, Los Angeles, p 2017

Manaa A, Gharbi E, Mimouni H, Wasti S, Aschi-Smiti S, Lutts S, Ben Ahmed H (2014) Simultaneous application of salicylic acid and calcium improves salt tolerance in two contrasting tomato (*Sola-num lycopersicum*) cultivars. S Afr J Bot 95:32–39

Mariani L, Ferrante A (2017) Agronomic management for enhancing plant tolerance to abiotic stresses—drought, salinity, hypoxia, and lodging. Horticulture 3:52

Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic Press, London, New York. ISBN: 9780124735439

Metzner H, Rau H, Senger H (1965) Untersuchunge zur synchronisi-erbarkeit einzelner- pigmentmangle- mutanten von chlorella. Planta 65:186–194

Milad SI, El-Banna MN, El-Sheikh MH, Ebaid ME (2013) Effect of genotypes and medium protocols on callus formation and plant regeneration from mature embryos of Egyptian wheat (*Triticum aestivum* L.) varieties. Int J Adv Agric Res 18:874–889

Miura K, Tada Y (2014) Regulation of water, salinity, and cold stresss responses by salicylic acid. Front Plant Sci 5(4):1–12. https://doi.org/10.3389/fpls.2014.00004

Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol 22:867–880

Nazar R, Iqbal N, Syeed S, Khan NA (2011) Salicylic acid alleviates decreases in photosynthesis under salt stress by enhancing nitrogen and sulfur assimilation and antioxidant metabolism differentially in two mung bean cultivars. J Plant Physiol 168:807–815

Nazar R, Umar S, Khan NA (2015) Exogenous salicylic acid improves photosynthesis and growth through increase in ascorbate glutathione metabolism and S assimilation in mustard under salt stress. Plant Signal Behav 10:e1003751–e1003810

Negrao S, Schmockel SM, Tester M (2017) Evaluating physiological responses of plants to salinity stress. Ann Bot 119:1–11

Neoclous D, Koukounaras A, Siomos AS, Vasilakakis M (2014) Assessing the salinity effects on mineral composition and nutritional quality of green and red baby lettuce. J Food Qual 37:1–8

Noctor G, Foyer CH (1998) Ascorbate and glutathione: keeping active oxygen under control. Annu Rev Plant Physiol Plant Mol Biol 49:249–279

O'Brien RG, Kaiser MK (1985) MANOVA method for analyzing repeated measures designs: An extensive primer. Psychol Bull 97:316–333

Poor P, Gemes K, Szepesi A, Horvath F, Simon ML, Tari I (2011) Salicylic acid treatment via the rooting medium interferes with the stomatal response, CO_2 fixation rate and carbohydrate metabolism in tomato and decreases the harmful effects of subsequent salt stress. Plant Biol 13:105–114

Rady MM, Mohamed CF (2015) Modulation of salt stress effects on the growth, physio chemical attributes and yields of *Phaseolus vulgaris* L. plants by the combined application of salicylic acid and *Moringa oleifera* leaf extract. Sci Hortic 193:105–113

Sadak MS, Orabi SA (2015) Improving thermo tolerance of wheat plant by foliar application of citric acid or oxalic acid. Int J Chem Tech Res 8:333–345

Sairam RK, Tyagi A (2004) Physiological and molecular biology of salinity stress tolerance in plants. Curr Sci 86:407–421

Salem WM, Abu Alhamd MF (2009) Alteration in seedling growth and antioxidant enzyme activities in *Lupinus termis* under the influence of H_2O_2 with native *Azotobacter sp.* strain. Egypt J Bot 49:35–51

Samaras Y, Bressan RA, Csonka LN, Garcia-Rios M, Paino D, Urzo M, Rhodes D (1994) Proline accumulation during drought and salinity. In: Smirnoff N (ed) Environment and plant metabolism: flexibility and acclimation. BIOS Scientific Publisher, Oxford, pp 161-187. ISBN 1-872748-93-7

Shao QS, Wang HZ, Guo HP, Zhou AC, Huang YQ, Sun YL (2014) Effects of shade treatments on photosynthetic characteristics, chloroplast ultrastructure, and physiology of *Anoectochilus rox-burghii*. PLoS ONE 9:e85996

Shim IS, Momose Y, Yamamoto A, Kim DW, Usui K (2003) Inhibition of catalase activity by oxidative stress and its relationship to salicylic acid accumulation in plants. Plant Growth Regul 39:285–292

Silva EC, Nogueira RJMC, Silva MA, Albuquerque MB (2011) Drought stress and plant nutrition. Plant Stress 5 (*Special Issue 1*):32–41. Global Science Books, Print ISSN: 1749–0359

Singh H, Singh AS, Hussain I, Yadav V (2016) Physiological and biochemical effects of salicylic acid on *Pisum sativum* exposed to isoproturon. Arch Agron Soil Sci 62:1425–1436

Tawussi F, Walther C, Gupta DK (2017) Does low uranium concentration generates phytotoxic symptoms in *Pisum sativum* L. in nutrient medium? Environ Sci Pollut Res 24:22741–22751

Timpa JD, Burke JJ, Quisenberry JE, Wendt CW (1986) Effects of water stress on the organic acid carbohydrate compositions of cotton plants. Plant Physiol 82:724–731

Tuna L, Kaya C, Higgs D, Murillo-Amador B, Aydemir S, Girgin R (2008) Resistance gene stacking in wheat breeding programs and further genetic analysis. Silicon improves salinity tolerance in wheat plants. Environ Exp Bot 62:10–16

Vlot AC, Dempsey DMA, Klessig DF (2009) Salicylic acid, a multi-faceted hormone to combat disease. Annu Rev Phytopathol 47:177–206. https://doi.org/10.1146/annurev.phyto.050908.135202

Wang C, Zhang Q (2017) Exogenous salicylic acid alleviates the toxicity of chlorpyrifos in wheat plants (*Triticum aestivum*). Ecotox Environ Saf 137:218–224

Yamasaki S, Dillenburg LR (1999) Measurements of leaf relative water content in *Araucaria angustifolia*. R Bras Fisiol Veg 11:69–75

Yildirim E, Turan M, Guvenc I (2008) Effect of foliar salicylic acid applications on growth, chlorophyll and mineral content of

cucumber (*Cucumis sativus* L.) grown under salt stress. J Plant Nutr 31:593–612

Yusuf M, Hasan SA, Ali B, Hayat S, Fariduddin O, Ahmad A (2008) Effect of salicylic acid on salinity-induced changes in *Brassica juncea*. J Integr Plant Biol 50:1096–1102