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Full Length Article

Root Architectural and Physiological Responses in Contrasting Rice Genotypes to Saline-Alkaline Stress

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Abstract

Saline-alkaline (SA) stress suppress rice growth by severely inhibiting root growth and damaging root system. This study investigated the main limiting factor for root growth in rice under SA stress. Four conventional japonica rice with different saline-alkaline tolerance, Dongdao-4 (D4), Changbai-9 (C9), Jinongda-19 (J19) and Nipponbare (NB) were used in this study. Two-week-old rice seedlings were grown under different types of SA stress simulated by 120 m*M* NaCl, 60 m*M* Na_2SO_4, 30 m*M* $NaHCO_3$ and 15 m*M* Na_2CO_3, respectively. Root growth indices including total root length (TRL), total root surface area (RSA), total root volume (TRV), average root diameter (ARD) and root numbers (RN), and some physiological traits *i.e.*, Na^+, K^+, proline, soluble sugar, superoxide anions ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2) contents were measured in roots. Results showed that all root growth indices significantly decreased by SA stress. The TRL, RSA, TRV and RN of rice seedlings suppressed severely by Na_2CO_3 stress, but the ARD suppressed severely by NaCl stress. The SA stress induced overaccumulation of Na^+, proline, soluble sugar, $O_2^{\cdot -}$ and H_2O_2 in rice roots. More accumulation of Na^+, proline and soluble sugar was observed in NaCl treatment, but Na_2CO_3 treatment induced more accumulation of $O_2^{\cdot -}$ and H_2O_2. Root growth indices showed significant correlations to $O_2^{\cdot -}$, H_2O_2, Na^+, proline and soluble sugar contents under SA stress. Root growth and physiological responses of saline-alkaline tolerant cultivars (D4 and C9) were more superior than sensitive cultivars (J19 and NB). These results suggested that suppression of root growth was a combined effect of osmotic stress, ion toxicity and oxidative stress induced by SA stress. Oxidative stress induced by overaccumulation of $O_2^{\cdot -}$ and H_2O_2 resulted in severe damage to root by inhibiting its elongation and growth of new tips. © 2021 Friends Science Publishers

Keywords: Physiological traits; Rice (*Oryza sativa* L.); Root growth indices; Salt stress; Alkali stress

Introduction

There are over 830 million ha of saline-alkaline soils all over the world (FAO 2016), which result in severe inhibition for growth and yield formation to crops grown in these types of soils. Soil salinization and alkalization is commonly divided into neutral salts which refers to NaCl and Na_2SO_4, and carbonates which refers to $NaHCO_3$ and Na_2CO_3 (Yang *et al.* 2007; Lv *et al.* 2013). Saline stress includes the character of high salinity and high osmotic pressure, and generally plants experience osmotic and high ion toxicity under these stress type (Liu *et al.* 2019, 2020). While, alkaline stress induces high pH stress in addition to salt stress, which damage plants directly (Zhang *et al.* 2017; Liu *et al.* 2019). Consequently, plants grown in saline-alkaline soils suffer from osmotic stress, ion toxicity and high pH stress together.

Roots are primarily exposed to soil or water solution and suffer from various stress conditions (Koevoets *et al.* 2016; Kaashyap *et al.* 2018). Root also absorbs water and various nutrients from soil for plants growth. Hence, the morphological characteristics such as root length, surface and root hairs, as well as the physiological traits play vital role in determining plant growth and yield production (Ghosh and Xu 2014). Plants with higher root length could acquire water and nutrition from deeper soil beneficial for plants to adapt the drought condition (Kim *et al.* 2020). Roots with smaller diameter and higher root length increase the surface area of root in contact with the water in soils, which enhance the volume of soil with water (Hernández *et*

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al. 2010; Comas *et al*. 2012). Roots of diameter 0.5–2.0 mm are "fine" and decrease of diameter contribute to the enhance access to water in soil and production under water stress (Zobel and Waisel 2010; Wasson *et al*. 2012). Root hairs and new root tips are the key indicators determining root continuous growth and vital for the uptake to water and nutrition in soil, responsiveness to different type of abiotic stress (Robinson *et al*. 1991; Bates and Lynch 2001). Therefore, root traits are of great importance in plants for the normal growth, yield formation and adaptive to stress conditions.

Rice is the main food for most the world's population and vulnerable to various abiotic stress including saline-alkaline stress (Munns and Tester 2008; Lv *et al*. 2013). SA stress suppress yield formation of rice plants by inhibiting plant growth (Abbasi *et al*. 2015; Liu *et al*. 2014, 2015), damaging root system (Zhang *et al*. 2017) and disrupting physiological metabolism (Liu *et al*. 2020). Seed germination (Lv *et al*. 2013; Feng *et al*. 2016; Zhao *et al*. 2018), photosynthesis (Xu *et al*. 2019), physiological metabolism (Liu *et al*. 2015, 2020) and yield formation (Wang *et al*. 2016) of rice are significantly suppressed under salt stress conditions, and these suppression range was more serious along with the rise of salt concentration (Lv *et al*. 2013).

Alkaline stress damage rice plants directly (Wei *et al*. 2015) and result in deficiencies of numerous primary nutrient or microelement, such as Fe and P (Tian *et al*. 2016; Liu *et al*. 2019). In addition, alkaline stress severely damage to rice roots by inhibiting root growth (Lv *et al*. 2013), striking out of new roots (Feng *et al*. 2016), damaging root cells and reducing root vigor (Zhang *et al*. 2017; Liu *et al*. 2019). Alkaline stress results in barely new radicles striking out in the germinating rice seeds compared to salt stress (Feng *et al*. 2016). The damaging effects on rice roots by alkaline stress are associated with over accumulation of $O_2^{\bullet-}$ and H_2O_2 induced by alkaline stress (Zhang *et al*. 2017). Thus, certain differences exist in rice plants in response to saline stress or alkaline stress, especially in root growth. However, the mechanisms behind how rice root system response to saline or alkaline stress remains largely unknown.

Numerous studies have demonstrated rice root growth response to multiple managements and stress factors (Lv *et al*. 2013, 2014; Gu *et al*. 2017; Zhang *et al*. 2017; Kim *et al*. 2020). We previously found that severe inhibition of root growth in rice seeds (Feng *et al*. 2016) or seedlings (Lv *et al*. 2013) were showed under NaCl stress, while more severe under alkaline stress stimulated by Na_2CO_3 stress. In addition, alkaline stress caused obvious injury of cell activity and upregulated the gene expression of cell death pathway in rice roots (Lv *et al*. 2013). And root growth indices of rice seedlings showed significant correlation to the saline-alkaline tolerance degree of different rice varieties and represented a series of useful parameters for evaluating the saline-alkaline stress tolerance (Lv *et al*. 2014). Our previous studies showed that excess accumulation of $O_2^{\bullet-}$ and H_2O_2 induced by alkaline stress in rice roots severely damaged root cells and decreased root activity (Zhang *et al*. 2017), which indicated that oxidative stress induced by alkaline stress may be a major factor for root damage under alkaline stress. Furthermore, SA stress induced excess accumulation of osmolyte, such as proline and soluble sugar, and toxic ions, such as Na^+, Cl^- (Liu *et al*. 2020), as well as ROS (Liu *et al*. 2019). Changes in different physiology traits were the results of different stress factor, such as osmolyte was mainly induced by osmotic stress, accumulation of Na^+ and Cl^- resulted in high ion toxicity (Munns and Tester 2008), and ROS accumulation resulted in oxidative stress (Zhang *et al*. 2017; Liu *et al*. 2019). But the correlation between physiology metabolism and root status under SA stress still remains unknown.

This study aimed to investigate the main suppression factor for root growth by analyzing their growth and some physiological traits in rice seedlings under different type of SA stress conditions. This study showed that SA stress caused remarkable inhibition to root growth as shown by decrease of the length, surface area, diameter and volume of rice root, as well as new root tips, and Na_2CO_3 stress caused more injury to root due to its high pH. Inhibition of root growth under SA stress is associated with osmotic stress, ion toxicity and oxidative stress induced by saline-alkaline stress and oxidative stress was main limiting factor which inhibited root growth in rice seedlings. This study would provide theoretical basis for improving rice tolerance to SA stress and breeding strategy of saline-alkaline tolerant rice varieties.

Materials and Methods

Plant materials

Four conventional *japonica* rice (*Oryza sativa* subsp. *japonica*) cultivars, Dongdao-4 (D4), Changbai-9 (C9), Jinongda-19 (J19) and Nipponbare (NB) were used in this study. The rice cultivars Dongdao-4 (D4) and Changbai-9 (C9) are saline-alkaline tolerant rice cultivars and Jinongda-19 (J19) and Nipponbare (NB) are sensitive to saline-alkaline stress (Liu *et al*. 2020).

Rice growth conditions and the stress treatments

Rice seeds were surface-sterilized with 75% (v/v) alcohol for 5 min, and then rinsed with deionized water five times. Full seeds were immersed in distilled water for 2 d, and then sprinkled onto wet filter paper in a petri dish for the germination for 24 h at 28°C in a dark incubator. Eighteen uniformly germinated seeds were transplanted onto a multi-well plate floating on a 320 mL cup containing deionized water for 7 d. After which, the rice seedlings were grown in half-strength Kimura B nutrient solutions for another 7 d

(Miyake and Takahashi 1983) in a controlled growth chamber. The growth condition was as followed: 25°C day/20°C night, and photoperiods of 12 h, 350 μmol m^{-2} s^{-1} of photon flux intensity. Two-week-old rice seedlings were transplanted into black buckets with the space of 4 cm, diameter and height of bucket was 15 and 20 cm, respectively. Rice root fixed with absorbent cotton was inserted into a perforated black plastic foam plate. Nine uniformly rice plants were put into buckets with different type of SA stress, which was simulated by 120 m*M* NaCl, 60 m*M* Na_2SO_4, 30 m*M* $NaHCO_3$ and 15 m*M* Na_2CO_3, respectively. Rice plants grown in the distilled water were set as the control (CK), and the stress solution was replaced once every 2 days. All rice plants were grown in a controlled growth chamber under the growing condition with four biological replicates.

Measurement of root growth traits

Root growth traits were measured at 0, 3, 5 and 7 d of SA stress, respectively. Rice roots were sampled for the measurement of physiology indices after 7 d of different type of SA stress.

Rice seedlings were scanned using a root scanner of Epson Expression 10000XL (Epson America Inc., Long Beach, CA, United States) at 0, 3, 5 and 7 d of different salt stress. The images of rice seedlings were digitized using the *WinRHizo* program, according to the manufacturer's instructions (Regent Instruments Canada Inc., Ville de Québec, QC, Canada), and the total root length (TRL), total root surface area (RSA), total root volume (TRV), average root diameter (ARD) and root number (RN) were determined. The decrease percentage of each root growth index was to evaluate the influence of different salt stress to rice seedlings between 0 and 7 d and calculated by 100*(0–7 d)/0 d.

Measurement of Na^+ and K^+ content

The sodium (Na^+) and potassium (K^+) contents in rice roots were measured according to Liu *et al.* (2020). The dry roots samples were digested completely with the mixture of HNO_3 and $HClO_4$ (v/v = 2:1), and then diluted to 50 mL with deionized water. The Na^+ and K^+ concentrations were determined by flame emission spectrometry (FP6410, Shanghai precision and scientific instrument Co., Ltd., China).

Measurement of proline and soluble sugar content

Dried rice roots of 0.1 g with 10 mL deionized water was placed into a centrifuge tube After centrifuge, and boiling, sample was used for the measurement of proline and soluble sugars in roots. Proline contents were measured by the sulfosalicylic acid method, and soluble sugars was detected with anthrone colorimetry (Liu *et al.* 2020).

Measurement of ROS accumulation

Measurement of the $O_2^{\bullet-}$ contents was followed by the monitoring the nitrite formation from hydroxylamine in the presence of $O_2^{\bullet-}$, described by Elstner and Heupel (1976) and Jiang and Zhang (2001). The H_2O_2 contents were measured by monitoring content of the titanium-peroxide complex at A_{415} according to the Brennan and Frenkel (1977), Zhang *et al.* (2017) and Liu *et al.* (2019).

Experimental design and data analyses

The statistical software SPSS 21.0 (IBM Corp., Armonk, NY) was used in the statistical analyses. Based on the results of one-way analysis of variance (ANOVA), significant difference ($P < 0.05$) was compared among different rice varieties or treatments using Duncan's multiple range test (DMRT).

Results

Root growth traits

Total root length: Root elongation of rice seedlings were significantly inhibited as shown by the decrease of total root length under different type of salt stress conditions (Fig 1). Total root length of the saline-alkaline tolerant cultivars, D4 and C9, were higher than the sensitive cultivars, J19 and NB (Fig 1b–e), during the stress. The average descend percentage compared to 0 d under NaCl, Na_2SO_4, $NaHCO_3$ and Na_2CO_3 conditions were 28.4, 22.6, 21.8 and 32.6%, respectively (Fig. 1f). Na_2CO_3 stress caused the most serious inhibition to root elongation of rice seedlings as shown by the maximal fold change under Na_2CO_3 stress (Fig. 1f).

Total root surface area

Change of total root surface area was similar to root length under different type salt stress factors as shown by salt stress caused a significant downward trend of root surface area of the four rice cultivars (Fig. 2). Total root surface area in the saline-alkaline sensitive cultivars, J19 and NB, were lower, and the descend range were higher than the saline-alkaline tolerant cultivars, D4 and C9, respectively (Fig. 2b–e). The average descend range of root surface area in four cultivars was 26.2, 24.2, 20.6 and 29.3%, under NaCl, Na_2SO_4, $NaHCO_3$ and Na_2CO_3 conditions, respectively (Fig. 2f).

Total root volume

A remarkable decrease of root volume was observed under SA stress conditions compared to CK, and root volume of the saline-alkaline tolerant cultivars were higher than the sensitive cultivars (Fig. 3). Average decrease range of the root volume in four cultivars were 38.7, 37.9, 37.2, and 40.9% under NaCl, Na_2SO_4, $NaHCO_3$ and Na_2CO_3

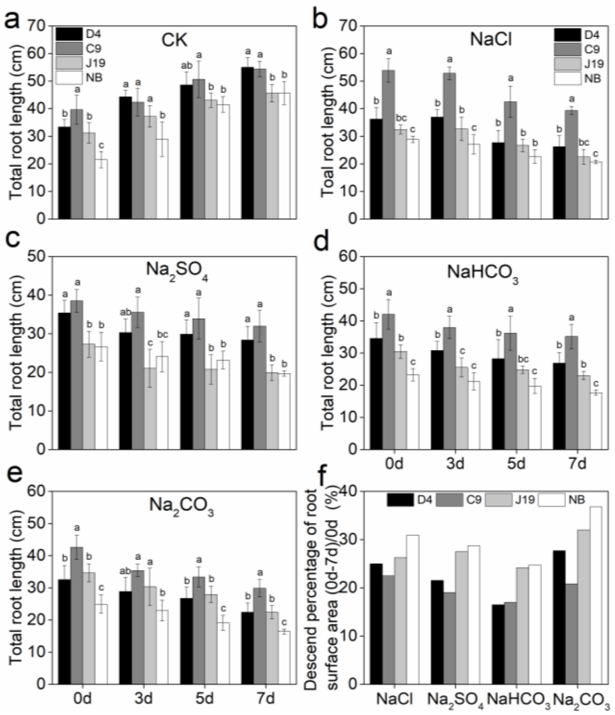

Fig. 1: Total root length of four rice varieties (D4, C9, J19, NB) were measured at 0 d, 3 d, 5 d, and 7 d, under CK (**a**), NaCl (**b**), Na_2SO_4 (**c**), $NaHCO_3$ (**d**), and Na_2CO_3 (**e**) conditions. Descend percentage of root length of 7 d (**f**) was expressed by taking values of 0 d as 1 under different stress conditions (0d-7d/0d). Values are means ± SD, *n=4*. Different letters on the column represent significant difference ($P < 0.05$) between different rice varieties based on Duncan's test

conditions, respectively (Fig. 4f), and it was higher than root length and surface area, which indicated that more serious damage was showed in root volume under stress conditions.

Average root diameter

Saline or alkaline stress caused remarkable decrease of root diameter in the four rice cultivars (Fig 4a–e). The saline-alkaline tolerant cultivars showed higher root diameter than the sensitive cultivars. But the average descend range of the root diameter in four cultivars was 29.7% under NaCl condition, which was higher than other stress factors, while it was 19.0, 21.1 and 21.4% under Na_2SO_4, $NaHCO_3$ and Na_2CO_3 conditions, respectively (Fig 4f). These results indicated that NaCl stress caused more damage on root diameter.

Root numbers

Saline or alkaline stress caused serious damage on root numbers compared to CK (Fig. 5a–e), especially under Na_2CO_3 condition, in which the average descend range was 49.6% (Fig. 5f). Average descend range of root numbers was 41.3, 37.2 and 36.1% under NaCl, Na_2SO_4, and $NaHCO_3$ conditions, respectively (Fig. 5f). Numbers of new roots were lower in the saline-alkaline sensitive cultivars compared with the tolerant cultivars, and the descend range of sensitive cultivars were higher as well (Fig. 5a–e).

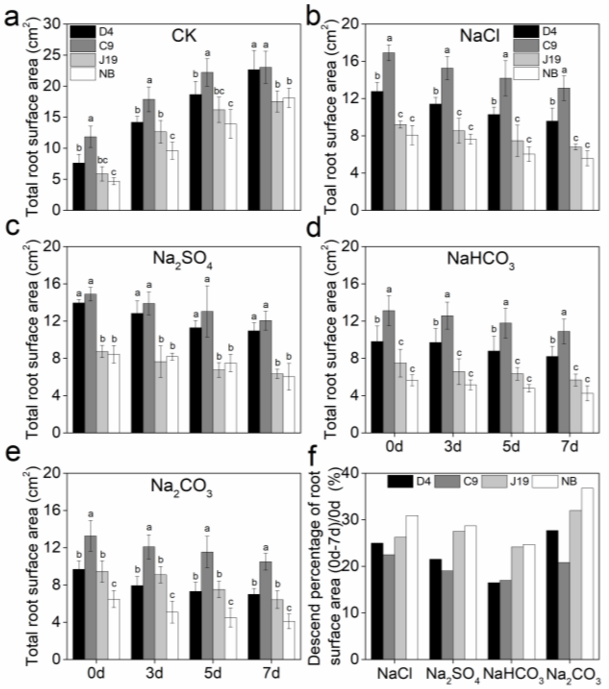

Fig. 2: Total root surface area of four rice varieties (D4, C9, J19, NB) were measured at 0 d, 3 d, 5 d, and 7 d, under CK (**a**), NaCl (**b**), Na_2SO_4 (**c**), $NaHCO_3$ (**d**), and Na_2CO_3 (**e**) conditions. Descend percentage of root surface area of 7 d (**f**) was expressed by taking values of 0 d as 1 under different stress conditions (0d-7d/0d). Values are means ± SD, *n=4*. Different letters on the column represent significant difference ($P<0.05$) between different rice varieties based on Duncan's test

Root physiological traits

Accumulation of osmolytes, ions and ROS in rice roots: Saline or alkaline stress induced excessive accumulation of Na^+ in roots, while K^+ contents significantly decreased under stress conditions compared to CK (Fig 6a–b). Rice roots showed a higher Na^+ and lowed K^+ accumulation under NaCl and Na_2CO_3 treatments, indicating that severe ion toxicity occurred to roots by NaCl and Na_2CO_3 treatment. The total Na^+ content in the saline-alkaline tolerant varieties (D4 and C9) was deceased by 11.3, 9.1, 4.8 and 8.5% compared to the sensitive varieties (J19 and NB) at NaCl, Na_2SO_4, $NaHCO_3$ and Na_2CO_3 treatment (Fig 6a), respectively. And the K^+ content increased by 4.6, 5.8, 9.8 and 1.8% at NaCl, Na_2SO_4, $NaHCO_3$ and Na_2CO_3 treatment (Fig 6b), respectively.

The osmolytes, such as proline and soluble sugar, accumulated under different salt stress conditions, and the accumulation of these osmolytes in saline-alkaline sensitive

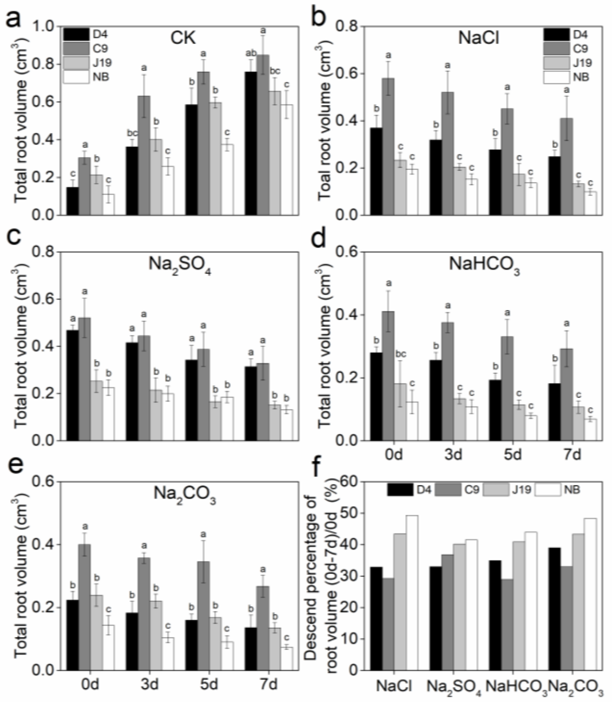

Fig. 3: Total root volume of four rice varieties (D4, C9, J19, NB) were measured at 0 d, 3 d, 5 d, and 7 d, under CK (**a**), NaCl (**b**), Na_2SO_4 (**c**), $NaHCO_3$ (**d**), and Na_2CO_3 (**e**) conditions. Descend percentage of root volume of 7 d (**f**) was expressed by taking values of 0 d as 1 under different stress conditions (0d-7d/0d). Values are means ± SD, *n=4*. Different letters on the column represent significant difference ($P < 0.05$) between different rice varieties based on Duncan's test

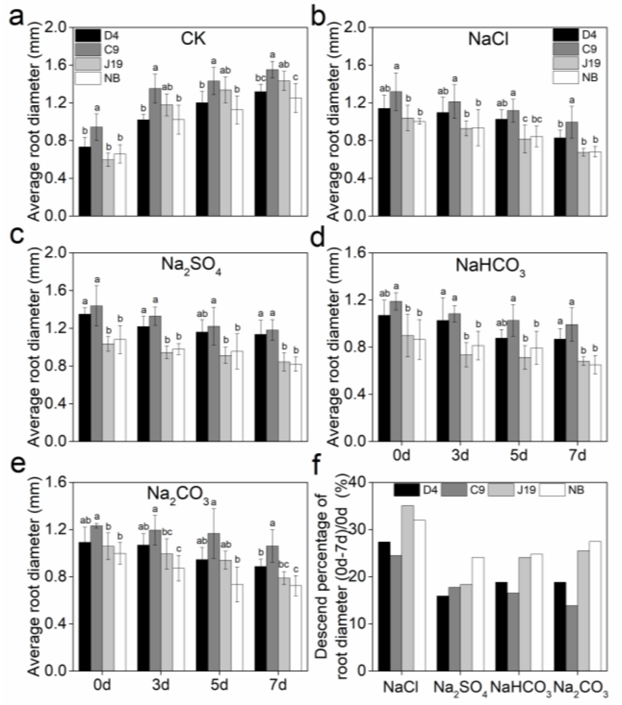

Fig. 4: Average root diameter of four rice varieties (D4, C9, J19, NB) were measured at 0 d, 3 d, 5 d, and 7 d, under CK (**a**), NaCl (**b**), Na_2SO_4 (**c**), $NaHCO_3$ (**d**), and Na_2CO_3 (**e**) conditions. Descend percentage of root diameter of 7 d (**f**) was expressed by taking values of 0 d as 1 under different stress conditions (0d-7d/0d). Values are means ± SD, *n=4*. Different letters on the column represent significant difference ($P < 0.05$) between different rice varieties based on Duncan's test

cultivars, J19 and NB, was higher than tolerant cultivars, D4 and C9 (Fig. 6c–d). The increase of these osmolytes in the NaCl treatment was the highest of all the stress factors compared to CK (Fig. 6c–d).

Consistently, over accumulation of $O_2^{\bullet-}$ and H_2O_2 was observed in rice roots, and more accumulation in the saline-alkaline sensitive cultivars J19 and NB (Fig 6e–f). The $O_2^{\bullet-}$ content in Na_2CO_3 and $NaHCO_3$ treatments increased by 29.5–38.4% compared to the NaCl or Na_2SO_4 treatments, and H_2O_2 content by 16.4–26.2%, respectively (Fig. 6e–f). These results indicated that alkaline stress caused more ROS in rice roots than no salt stress.

Correlation between the growth indices and physiological traits in roots

Correlation analysis showed that the growth indices of roots and physiological traits were statistically significant. The TRL, RSA, and ARD of the NaCl treatment showed significant negative correlation to Na^+, proline, soluble sugar, $O_2^{\bullet-}$ and H_2O_2 content, except for K^+ content (Table 1a). The TRV showed significant negative correlation to Na^+, proline, $O_2^{\bullet-}$ and H_2O_2 content, except for soluble sugar and K^+ content (Table 1a). The RN showed significant negative correlation to proline, soluble sugar, $O_2^{\bullet-}$ and H_2O_2 content, except for Na^+ and K^+ content (Table 1a).

The TRL, RSA, and TRV of the Na_2SO_4 treatment showed significant negative correlation to proline, $O_2^{\bullet-}$ and H_2O_2 content, except for Na^+ soluble sugar and K^+ content (Table 1b). The ARD showed significant negative correlation to Na^+, proline, $O_2^{\bullet-}$ and H_2O_2 content, except for soluble sugar and K^+ content (Table 1b). The RN showed extremely significant or significant negative correlation to soluble sugar, $O_2^{\bullet-}$ and H_2O_2 content, except for Na^+, proline, and K^+ content (Table 1b).

The TRL, RSA and ARD of the $NaHCO_3$ treatment showed significant negative correlation to proline, soluble sugar, $O_2^{\bullet-}$ and H_2O_2 content, except for Na^+ content (Table 1c). The TRL and RSA showed significant positive correlation to K^+ content (Table 1c). The TRV showed significant negative correlation to proline, $O_2^{\bullet-}$ and H_2O_2 content, except for Na^+, K^+ and soluble sugar content (Table 1c). The RN showed significant negative correlation to soluble sugar, $O_2^{\bullet-}$ and H_2O_2 content, except for Na^+, K^+ and proline content (Table 1c).

The TRL and TRV of the Na_2CO_3 treatment showed significant negative correlation to proline, $O_2^{\bullet-}$ and H_2O_2 content, except for Na^+, K^+ and soluble sugar content (Table

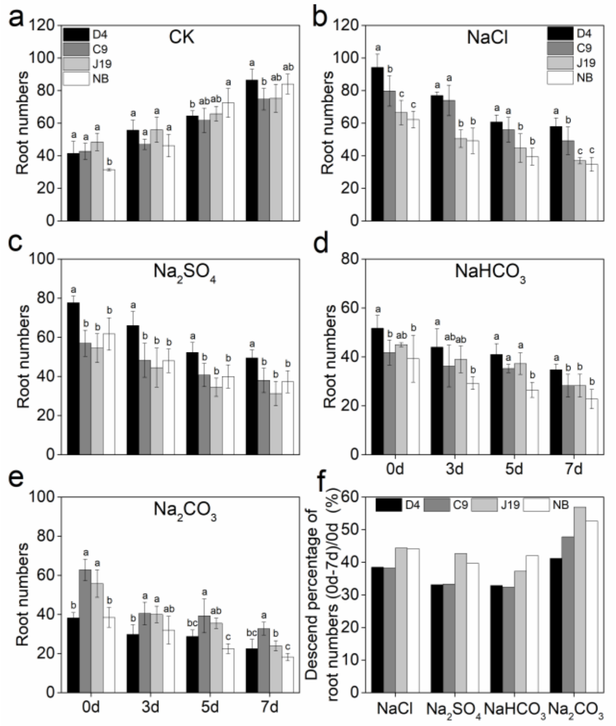

Fig. 5: Root numbers of four rice varieties (D4, C9, J19, NB) were measured at 0 d, 3 d, 5 d, and 7 d, under CK (**a**), NaCl (**b**), Na_2SO_4 (**c**), $NaHCO_3$ (**d**), and Na_2CO_3 (**e**) conditions. Descend percentage of root numbers of 7 d (**f**) was expressed by taking values of 0 d as 1 under different stress conditions (0d-7d/0d). Values are means ± SD, *n=4*. Different letters on the column represent significant difference ($P < 0.05$) between different rice varieties based on Duncan's test

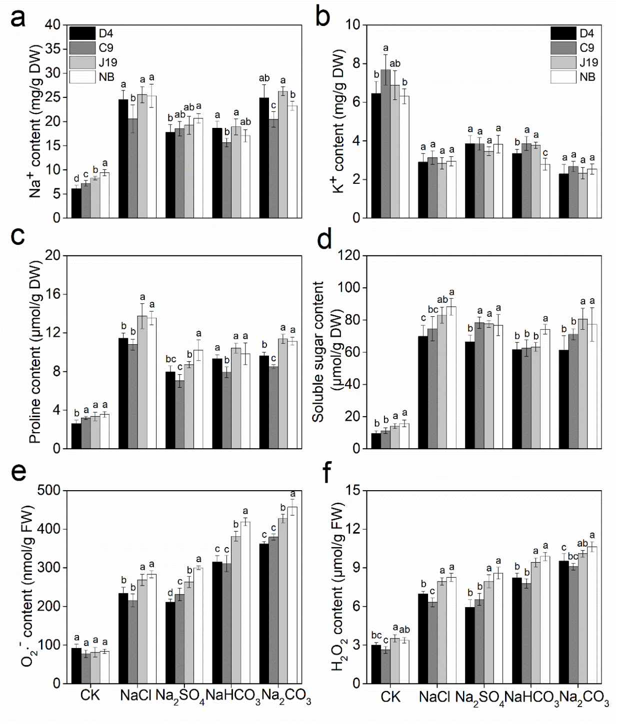

Fig. 6: Content of root Na^+ (**a**), K^+ **(b)**, proline **(c)**, soluble sugar (**d**), $O_2^{\cdot-}$ (**e**) and H_2O_2 (**f**) of four rice varieties (D4, C9, J19, NB) were measured at 7 d under CK, NaCl, Na_2SO_4, $NaHCO_3$, and Na_2CO_3 treatments. Values are means ± SD, *n=4*. Different letters on the column represent significant difference ($P < 0.05$) between different rice varieties based on Duncan's test

1d). The RSA, ARD and RN showed significant negative correlation to Na^+, proline, $O_2^{\cdot-}$ and H_2O_2 content, except for K^+ and soluble sugar content (Table 1d).

Discussion

SA stress is a complex stress factor, including high salinity, osmotic pressure and pH inhibiting plants growth and yield formation by multiple ways (Wei *et al*. 2015; Liu *et al*. 2016; Wang *et al*. 2018). Root plays the key role in the uptake of water and nutrients from soil in plants. SA stress changed root morphology and architecture (Liu *et al*. 2016), as well as root growth as shown by decreasing root length, volume, new root tips and surface area of many crops (Neves *et al*. 2010; Lv *et al*. 2014; Guo *et al*. 2016; Zhang *et al*. 2017), which may be associated to root lignin levels induced by stress (Lin and Kao 2001). In addition, SA stress disturbed root metabolism system by damaging cell activity (Zhang *et al*. 2017), upregulating transcription expression of cell death indication genes (Lv *et al.* 2013), resulting in overaccumulation of Na^+ and ROS (Guo *et al*. 2016; Wei *et al*. 2015). In this study, root growth of rice seedlings was significantly inhibited by multiple SA stress (Fig. 1–5), and SA stress significantly induced excess accumulation of Na^+, proline, soluble sugar, and ROS (Fig. 6). In addition, inhibition of root growth was closely associated with high osmotic pressure, ion toxicity and oxidative stress as shown by overaccumulation of Na^+, proline, soluble sugar, H_2O_2 and $O_2^{\cdot-}$ (Table 1). These data collectively suggest that root growth condition is vital for plants responses to SA stress.

Numerous studies have showed that root growth and physiological metabolism under various stresses conditions (Redjala *et al*. 2011; Lv *et al*. 2013; Shi *et al*. 2015; Liu *et al*. 2016; Kim *et al*. 2020). Rice root growth, architecture and root-to-shoot relationship was changed by water deficit (Pérez-Alfocea *et al*. 2011; Kim *et al*. 2020), and osmoregulatory substances played vital role in the regulation of osmotic pressure in roots (Sharma and Dietz 2006). Under SA stress, differences were showed in the response to neutral salts and carbonates of roots. Seed germination of rice was remarkably inhibited by salt and alkali stress, as well as the growth of shoots or roots, but barely new root tips were sprouted in the germinated seeds (Feng *et al*. 2016). In addition, alkali stress caused severer cell injury of rice seedlings as shown by the more significant expression of cell death-related genes induced by alkali stress (Lv *et al*. 2013; Zhang *et al*. 2017). A significant inhibition in rice root growth was showed in all the SA stress factors by severe

Table 1: Correlation analysis of root growth and physiological traits under stress conditions

Stress	Index	TRL	RSA	TRV	ARD	RN	Na^+	K^+	PC	SSC	$O_2^{\bullet-}$
(**a**)	RSA	0.886**									
NaCl	TRV	0.914**	0.899**								
	ARD	0.753**	0.798**	0.657**							
	RN	0.469	0.596*	0.618*	0.414						
	Na^+	-0.732**	-0.663**	-0.728**	-0.530*	-0.319					
	K^+	0.300	0.141	0.202	0.440	0.202	0.110				
	PC	-0.736**	-0.805**	-0.811**	-0.677**	-0.646**	0.336	-0.349			
	SSC	-0.514*	-0.607*	-0.438	-0.682**	-0.689**	0.186	-0.283	0.635**		
	$O_2^{\bullet-}$	-0.788**	-0.870**	-0.852**	-0.673**	-0.809**	0.537*	-0.313	0.747**	0.633**	
	H_2O_2	-0.854**	-0.904**	-0.941**	-0.720**	-0.696**	0.742**	-0.141	0.767**	0.520*	0.840**
(**b**)	RSA	0.851**									
Na_2SO_4	TRV	0.791**	0.860**								
	ARD	0.780**	0.842**	0.843**							
	RN	0.501*	0.577*	0.427	0.485						
	Na^+	-0.492	-0.398	-0.493	-0.569*	-0.187					
	K^+	0.122	0.145	0.314	0.195	0.174	-0.036				
	PC	-0.742**	-0.643**	-0.693**	-0.562*	-0.041	0.493	-0.254			
	SSC	-0.240	-0.352	-0.311	-0.388	-0.749**	0.370	-0.264	-0.0110		
	$O_2^{\bullet-}$	-0.724**	-0.795**	-0.746**	-0.747**	-0.507*	0.657**	-0.024	0.724**	0.424	
	H_2O_2	-0.807**	-0.792**	-0.815**	-0.753**	-0.600*	0.555*	-0.182	0.706**	0.494	0.864**
(**c**)	RSA	0.947**									
$NaHCO_3$	TRV	0.832**	0.911**								
	ARD	0.815**	0.823**	0.783**							
	RN	0.392	0.476	0.329	0.522*						
	Na^+	-0.390	-0.376	-0.368	-0.295	-0.479					
	K^+	0.646**	0.542*	0.440	0.309	0.217	-0.124				
	PC	-0.661**	-0.676**	-0.622*	-0.517*	-0.136	0.479	-0.322			
	SSC	-0.603*	-0.557*	-0.461	-0.631**	-0.697**	-0.200	-0.457	0.240		
	$O_2^{\bullet-}$	-0.739**	-0.784**	-0.771**	-0.760**	-0.545*	0.139	-0.493	0.646**	0.673**	
	H_2O_2	-0.893**	-0.926**	-0.802**	-0.861**	-0.668**	0.187	-0.500*	0.701**	0.664**	0.847**
(**d**)	RSA	0.879**									
Na_2CO_3	TRV	0.866**	0.868**								
	ARD	0.762**	0.902**	0.780**							
	RN	0.868**	0.853**	0.801**	0.684**						
	Na^+	-0.446	-0.515*	-0.464	-0.597*	-0.508*					
	K^+	0.001	0.275	0.159	0.278	0.116	-0.474				
	PC	-0.739**	-0.839**	-0.767**	-0.826**	-0.715**	0.692**	-0.308			
	SSC	-0.044	-0.263	-0.150	-0.278	-0.036	-0.128	0.106	0.384		
	$O_2^{\bullet-}$	-0.608*	-0.706**	-0.552*	-0.672**	-0.572*	0.168	0.012	0.774**	0.614*	
	H_2O_2	-0.651**	-0.728**	-0.754**	-0.599*	-0.536*	0.184	0.077	0.688**	0.611*	0.689**

[†] TRL: Total root length, RSA: Total root surface area, TRV: Total root volume, ARD: Average root diameter, RN: Root numbers, Na^+: Na^+ content, K^+: K^+ content, PC: Proline content, SSC: Soluble sugar content, $O_2^{\cdot-}$: $O_2^{\cdot-}$ content, H_2O_2: H_2O_2 content

[†] The correlation coefficient (r^2) was showed in the table; ** indicates significant difference at $P < 0.01$ level; * indicates significant difference at $P < 0.05$ level

decrease of TRL (Fig. 1), RSA (Fig. 2), TRV (Fig. 3) and ARD (Fig. 4), RN (Fig. 5) in this study. The TRL, RSA, TRV and RN was inhibited severer by Na_2CO_3 treatment (Fig 1–3, 5), but ARD was influenced more seriously by NaCl treatment (Fig. 4). These data suggested alkali stress caused more serious injury to root system of rice seedlings than salt stress, which was possible due to the high pH induced by carbonates, resulting in disorder or deficiencies of nutritional minerals around root (Tian *et al*. 2016; Liu *et al*. 2019). Furthermore, fewer new root tips observed under alkali stress, which was mainly due to more serious of cell death induced by alkali stress (Zhang *et al*. 2017).

Previous studies have investigated the physiological reaction of plants response to stress, and plants adapt to various environmental stress factors by regulating multiple physiological metabolic processes, such as ion transport, osmoregulation, ROS-scavenging and gene transcription (Kim *et al*. 2020; Liu *et al*. 2020). SA stress caused excessive accumulation of toxic ions, such as Na^+ and Cl^-, which resulted in the damaging of leaf photosynthetic structure and decline in photosynthetic efficiency in rice (Liu *et al*. 2021). While increase of K^+ content contributed to block the Na^+ entrance path into cell and high K^+/Na^+ rate was observed in the salt tolerant rice varieties (Peng *et al*. 2004; Lv *et al*. 2013). In this study, SA stress resulted in a significant increase of Na^+ and decreased K^+ in roots, and more Na^+ content was observed in NaCl and Na_2CO_3 treatments (Fig. 6a–6b), indicating that SA stress caused disbalance of ion homeostasis in cells and overaccumulation of Na^+ induced high ion toxicity to rice roots. Osmoregulation is an important regulation mechanism and physiological response of plants to various stress conditions (Lv *et al*. 2014; Liu *et al*. 2015). Many plants accumulate osmotica, such as proline and soluble sugar, and proline content has been used as a selection parameter to evaluate the stress tolerance of plants (Székely *et al*. 2008). However, in rice, proline content and the fold-change of proline accumulation showed no significant correlation to tolerance of rice varieties under different SA stress factors, indicating that proline accumulation was a result of SA stress (Lv *et al*. 2014). In the present study, high osmotic pressure was induced by SA stress as evident by a remarkable increase of proline and soluble sugar in roots (Fig. 6c–6d), and the most content of proline and soluble was observed in the NaCl treatment. In addition, proline and soluble sugar contents in the sensitive varieties (J19 and NB) were higher than the tolerant varieties (D4 and C9), supposing that the osmotic adjustment system was affected by SA stress. Previous studies showed that overaccumulation of ROS is an important injury factor to rice under alkaline stress and alkaline tolerance in rice was associated to ROS-scavenging capability (Guo *et al*. 2014; Guan *et al*. 2017; Zhang *et al*. 2017). Results of this study showed that SA stress caused overaccumulation of ROS in roots of rice seedlings, such as $O_2^{\bullet-}$ and H_2O_2 (Fig 6e–f), and the most accumulation was in Na_2CO_3 treatment, indicating that severe oxidative stress was induced by alkali stress compared to salt stress.

Plants grown in saline-alkaline soil suffer from a complex abiotic stress, stimulated by single or multiple combinations of sodium salt, such as NaCl, Na_2SO_4, $NaHCO_3$ and Na_2CO_3. Previous studies have showed that response of plants to different sodium stress is a complex network (Ahmed *et al*. 2020; Wang and Jiang 2020). Our results showed that root growth indices were significant correlated to accumulation of ion, osmolytes and ROS in roots (Table 1). The ROS accumulation in root was significant negative correlated to root growth indices under all SA stress factors indicating oxidative stress was induced by different sodium salt stress, which was the same limitation factor damaging rice roots under SA stress conditions. In addition, there were differences under single sodium salt treatment except for ROS accumulation. Under NaCl treatment, root growth was inhibited generally by ion toxicity and osmotic stress as shown by good correlations with Na^+, proline and soluble sugar contents (Table 1a). Under Na_2SO_4 treatment, proline content showed good correlation to root growth indices (Table 1b). But root growth had significant correlation to K^+ accumulation and osmotic adjustment under $NaHCO_3$ treatment, which suggested that osmotic stress also suppressed root growth (Table 1c). While under Na_2CO_3 stress, proline and Na^+ content showed significant correlation to most root growth indices, indicating osmotic stress and ion toxicity caused injury to root system, especially in RSA, ARD and RN (Table 1d). These results suggested that excess accumulation of Na^+ and ROS in root accounted for lower resistance to SA stress in rice seedlings. Overaccumulation of ROS in rice roots caused severe damage to cell membrane at seed germination (Zhao *et al*. 2021) and seedling stage (Zhang *et al*. 2017), which directly inhibited seed germination and seedlings growth; while massive accumulation of Na^+ caused severe damage to the leaf photosynthetic structure in rice (Liu *et al*. 2021). Hence, decrease of ROS accumulation and increase of K^+/Na^+ rate under SA stress conditions by multiple methods will be a potential approach to improve tolerance to stress factor and a focused point for the breeding strategy in the future.

Rice varieties at different degree of saline-alkaline tolerance vary in different growth and physiological traits. Previous studies showed that rice varieties with higher tolerance to SA stress exhibited better growth status and physiological metabolism as shown by higher survival rate, good root growth indices and lower ROS or Na^+ content (Lv *et al*. 2014; Feng *et al*. 2016; Zhang *et al*. 2017). However, proline content was insufficient to serve as reliable physiological traits to evaluate the tolerance to SA stress among rice varieties (Lv *et al*. 2014). In the present study, we selected two saline-alkaline tolerant rice cultivars, D4 and C9, and two saline-alkaline sensitive rice cultivars, J19 and NB (Feng *et al*. 2016; Liu *et al*. 2020). These four rice cultivars exhibited different changes in root growth indices in response to SA stress as evident in higher TRL, RSA, TRV, ARD and RN and lower decrease observed in D4 and C9 under different types of SA stress (Fig 1–5). These results suggest that the saline-alkaline tolerant rice cultivars are based on the differences in root growth indices, as reported earlier (Lv *et al*. 2013, 2014). Significantly lower accumulation of Na^+, $O_2^{\bullet-}$ and H_2O_2, as well as higher K^+ (Fig. 6), in the rice cultivars D4 and C9, improved tolerance to SA stress by decreasing ion toxicity and oxidative stress (Peng *et al*. 2004; Kanawapee *et al*. 2012; Zhang *et al*. 2017; Liu *et al*. 2019). However, accumulation of proline and soluble sugar in the saline-alkaline sensitive rice cultivars was higher than in the tolerant varieties (Fig. 6**c**–6**d**), which may indicate that proline accumulation is a symptom and referent of poor osmotic adjustment capability in the saline-alkaline sensitive rice cultivars (Vaidyanathan *et al*. 2003; Kanawapee *et al*. 2012).

Conclusion

In summary, SA stress caused severe inhibition in root growth of rice seedlings, resulting in overaccumulation of Na^+, proline, soluble sugar and ROS in rice roots, indicated that high ion toxicity, osmotic stress and oxidative stress to rice roots was induced by SA stress. Furthermore, root growth inhibition under SA stress conditions was associated to osmotic stress, ion toxicity and oxidative stress induced by SA stress. In addition, oxidative stress induced by saline-alkaline stress was the main restrictive factor that inhibited root growth in rice seedlings.

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Author Contributions

X-LL, CX, and H-JW designed the study; X-LL, H-TY, P-P S, QS, NC and L-NL performed the laboratory experiments and measurement of the indices; X-LL and CX performed the data collection, analysis and figure mapping; X-LL and CX wrote the manuscript; H-JW participated in the modification of the manuscript; X-LL, Z-AZ and H-JW provided scientific expertise.

Conflict of Interest

The authors declare that they have no competing financial interests.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Ethics Approval

Not applicable to this paper

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