

Carbon and nitrogen metabolism in rice cultivars affected by salt-alkaline stress

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Abstract. Salt-alkaline stress generally leads to soil compaction and fertility decline. It also restricts rice growth and nutrient acquisition, so reduces rice yield. However, little is known about the effect of salt-alkaline stress on the carbon and nitrogen metabolism of rice. In this study, two relatively salt-alkaline tolerant (Changbai 9 and Dongdao 12) and sensitive (Jinongda 138 and Tongyu 315) rice cultivars were grown in a field experiment conducted with two soil types including black soil and salt-alkaline soil to evaluate the characteristics of yield, carbon and nitrogen metabolism in rice. Results showed that yield and nitrogen use efficiency (NUE) in rice greatly decreased under salt-alkaline stress. Changbai 9 (CB9) and Dongdao 12 (DD12) showed higher NUE than Jinongda 138 (JND138) and Tongyu 315 (TY315) under the salt-alkaline stress. Additionally, carbon and nitrogen metabolism enzyme activity and compound content were significantly affected by salt-alkali stress; different performances were observed between cultivars under salt-alkaline stress. In conclusion, our results indicate that salt-alkaline tolerant rice cultivars may have more stable carbon and nitrogen metabolism than sensitive genotypes under salt-alkaline conditions.

Keywords: agriculture, carbon metabolism, nitrogen metabolism, rice, salt-alkaline soil, yield, sustainable development, genotypes, sensitive, tolerant, global food security.

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Introduction

Globally, more than 900 million ha of land is harmed by salt-alkaline conditions, accounting for 20% of the world's total cultivated land (Flowers and Yeo 1995; Li *et al.* 2020). Due to global climate change, population growth, increased industrial pollution, the development of irrigated agriculture, and improper use of chemical fertilisers and other factors, soil salinisation is becoming an increasingly serious problem (Tian *et al.* 2016). It is estimated that 30% of cultivated land will be salinised in the next 25 years, and it will reach 50% by 2050 (Cheng *et al.* 2007). In China, more than 6.67 million ha of land is damaged by salt-alkaline conditions, and nearly one-fifth of the cultivated land has been salinised (Eraslan *et al.* 2007; Qadir *et al.* 2014). Rice is one of the most important food crops worldwide, and sustainable development of rice agriculture is an essential part to ensure global food security (Qadir *et al.* 2014). Rice cultivation in salt-alkaline land is an effective soil improvement and utilisation measure, as well as an important way to increase food production, as shown by many years of agricultural production practices. Therefore, research on the morphological and physiological characteristics of salt-alkaline tolerant rice is necessary for sustainable development.

Exploring the physiological and biochemical metabolic activities and tolerance mechanisms of salt-alkaline tolerant rice under salt-alkaline stress is the basis for the breeding of

salt-alkaline tolerant rice. In 1939, the strong saline-alkali-tolerant rice variety 'Pokkali' was bred and planted over large areas in Sri Lanka. Studies have found that under salt (NaCl) stress conditions, the salt-alkaline tolerant variety 'Pokkali' can reduce the ratio of ascorbate/dehydroascorbic acid, increase the activity of antioxidant enzymes and increase its salt-alkaline tolerance, by maintaining high activity of key enzymes (glyoxalase I and II) in the methylglyoxaldehyde detoxification system (El-Shabrawi *et al.* 2010; Kabir *et al.* 2016). The salt-alkaline tolerant rice variety 'FL478' was exposed to salt stress by inhibiting the metabolism of organic acid (OA) to inhibit its growth to adapt to salt stress the early stage of salt stress, in the later stage of growth, it accumulates a large amount of soluble sugars, amino acids, and other small molecules that act as osmotic adjustment substances to relieve osmotic stress and improve its own stress resistance (Shobbar *et al.* 2012; Zhao *et al.* 2014). Furthermore, plants can enhance the activities of antioxidant (superoxide dismutase, ferredoxin, ascorbate peroxidase) to ease the damage of reactive oxygen species (Zhang and Mu 2009). Past studies have focussed on the structural stability and metabolic balance of plants. However, salt-alkaline stress also inhibits plant growth through nutrient absorption and utilisation, the mechanism of the effect of salt-alkali stress on nutrient absorption and utilization needs to be further studied. Carbon and nitrogen metabolism are the two basic

metabolism pathways in plants, and their stability under salt-alkaline stress is essential for the stable and high yield of salt-alkaline tolerant rice cultivation.

Salt-alkaline stress seriously affects crop yield (Abdullah *et al.* 2001; Nemati *et al.* 2011); doing so through two important mechanisms. On the one hand, high salt ions cause osmotic stress and ion poisoning, especially the excessive accumulation of Na⁺ in the leaves that destroy the cell membrane structure, affect the production of photosynthetic substances, and cause a decline in yield. On the other hand, high pH values cause ion precipitation and nutrient stress, resulting in low nutrient absorption and utilisation of crops in salt-alkaline soils, resulting in reduced yield. Studies have also shown that under salt-alkaline conditions, the absorption and utilisation of nutrients in rice is significantly inhibited (Dluzniewska *et al.* 2007; Tian *et al.* 2016). Nutrient absorption and utilisation-related gene expression and enzyme activity are also regulated by salt-alkaline stress (Surabhi *et al.* 2008; Abouelsaad *et al.* 2016). Therefore, research on rice photosynthetic characteristics, material production capacity, and nutrient absorption and utilisation under salt-alkaline stress is important. In this study, two relatively salt-alkaline tolerant rice cultivars (Changbai (CB) 9 and Dongdao (DD) 12) and two relatively salt-alkaline sensitive rice cultivars (Jinongda (JND) 138 and Tongyu (TY) 315) were planted into black and salt-alkaline soils. We investigated the effects of salt-alkaline stress on yield, and carbon and nitrogen metabolism in the four rice cultivars. Different changes among the salt-alkaline tolerant and sensitive cultivars are also discussed.

Materials and methods

Plant materials and site description

Field experiments were conducted at a farm belonging to Jilin Agricultural University (43°05'N, 125°38'E) during the rice growing season of 2018, and repeated in 2019. Four *japonica* rice cultivars with similar growth periods, among them, Changbai 9 (CB9) and Dongdao 12 (DD12) are relatively salt-alkaline tolerant cultivars, whereas Jinongda 138 (JND138) and Tongyu 315 (TY315) are relatively salt-alkaline sensitive cultivars. Prior to the experiments, two types of wild soils (Table 1), black soil (BS) and salt-alkaline soil (SAS), soil samples from the upper 20-cm layer were collected to analyse the soil chemical properties.

Experimental design

The experiment was arranged in a completely randomised block design with three replications. The seeds were sown on 12 April 2018 and 9 April 2019. At the four-leaf heart stage, seedlings with the same growth were selected and transplanted on 25 May 2018 and 2019, at a hill spacing of 13.3 × 30.0 cm with two seedlings per hill and a plot size of 16 m² in 2018 and 2019, plots were separated at their perimeters by a cement ridge to prohibit the exchange of irrigation water and fertiliser between plots. Nitrogen (180 kg N ha⁻¹) fertiliser were applied as basal fertiliser, tillering fertiliser, and panicle fertiliser. The proportion of N split was 48, 32 and 20, respectively, at these three stages. Phosphorous (90 kg P₂O₅ ha⁻¹) was used in the basal fertiliser. Potassium (45 kg K₂O ha⁻¹) was used in the basal and panicle fertilizers.

Sampling and measurements

Leaf photosynthesis and physiological parameters

At filling stage, the leaf area of the three plants growing in the three hills selected for each treatment was measured with a leaf area meter (CI-203, CID, USA). The photosynthetic rate (Pn) of latest full-grown leaves on the main plant stems were measured from 09:00 to 11:00 hours using a CIRAS-3 (PP Systems, USA) photosynthetic instrument. Leaf temperature during measurements was maintained at 27°C, leaf chamber humidity during measurements was maintained at 48–50%, with a photosynthetic photon flux density of 1200 μmol m⁻² s⁻¹. Photosynthetic nitrogen use efficiency (PNUE, μmol g⁻¹ s⁻¹) was calculated as follows (Ju *et al.* 2015):

$$\text{PNUE} = \frac{\text{Pn}}{\text{Leaf N content}}$$

At filling stage, the latest full-grown leaves on the main plant stems collected from six representative hills in each plot were sampled to examine the differences in the compound content and the activities of key enzymes. The soluble sugar content was determined according to Maness (2010). The sucrose content was determined according to Zhang and Qu (2003). The leaf nitrogen content was determined by an elemental analyser (Elementar Vario Macro cube, Hanau, Germany). The soluble protein content was determined according to Zhang and Qu (2003). The free amino acids and activity of Rubisco was determined according to Wang *et al.* (2000). The activity of sucrose phosphate synthase (SPS) was determined according to Nakamura *et al.* (1989). The activity of nitrate

Table 1. Basic chemical properties of the two soil types
BS, black soil; SAS, salt-alkaline soil

Years	Soils	Electrical conductivity (μS cm ⁻¹)	pH	Total N (g kg ⁻¹)	Available P (mg kg ⁻¹)	Available K (mg kg ⁻¹)	Organic matter (g kg ⁻¹)
2018	BS	123.6	6.84	1.36	25.9	121.33	33.7
	SAS	380.5	8.96	1.25	18.1	98.3	27.9
2019	BS	114.9	7.02	1.38	24.1	119.2	32.8
	SAS	366.3	9.06	1.24	17.9	98.1	26.5

reductase (NR) was determined according to Gibon *et al.* (2004). The activity of glutamine synthetase (GS) was determined according to Sun *et al.* (2014).

Grain yield and NUE

At maturity, grain yield was determined for except border plants within 1 m² area (three replicates) in each plot and adjusted to a moisture content of 0.14 g H₂O g⁻¹ fresh weight. The plants in the nine representative hills of each plot were sampled to determine the yield components. Plant samples from each hill were separated into straw and panicle. The panicle number of each hill was recorded to determine the panicle number per hectare. Filled and unfilled grains of the panicles were manually separated to measure the grain number per panicle and seed setting rate. Dry weights of rachis, filled and unfilled grains, and straw were determined after oven drying at 80°C to a constant weight. Randomly selected filled grains from each hill were used for 1000-grain weight measurements. Tissue N content was determined by an elemental analyser (Elementar Vario Macro cube) to calculate aboveground N uptake. Total N accumulation (TNA, g m⁻²) was considered the total amount of N accumulated in a plant at maturity. The N utilisation efficiency for grain production (NUEg, g g⁻¹), The N utilisation efficiency for biomass production (NUEb, g g⁻¹), and N harvest index (NHI, g g⁻¹) were calculated as follows:

$$\text{NUEg} = \frac{\text{Grain yield}}{\text{TNA}}$$

$$\text{NUEb} = \frac{\text{Biomass production}}{\text{TNA}}$$

$$\text{NHI} = \frac{\text{Grain N}}{\text{TNA}}$$

Statistical analysis

Data were statistically analysed using Microsoft Excel 2010 and SPSS 19.0 software (Softonic International, Barcelona, Spain). Means were compared using least significant difference at $P < 0.05$ (l.s.d. 0.05). Graphs were drawn with GraphPad Prism 8 software (GraphPad, San Diego, CA, USA).

Results

Grain yield and yield components

As shown in Table 2, the grain yield, effective panicles, grain per panicle and seed setting rate were significantly lower in the salt-alkaline soil than in the black soil. Compared with the black soil treatment, the effective panicles of CB9 (21.59% 2018 same below, 19.20% 2019 same below) and DD12 (23.67%, 20.68%) less relative decrease¹ than JND138 (26.75%, 27.14%) and TY315 (28.24%, 28.11%) under the salt-alkaline soil treatment. The grain per panicle of CB9 (11.74%, 9.66%), DD12 (13.50%, 13.41%) and JND138 (13.50%, 13.41%) less relative decrease than TY315 (23.06%, 23.03%) under the salt-alkaline soil treatment.

The seed setting rate of CB9 (6.60%, 6.10%) and DD12 (7.06%, 7.02%) less relative decrease than JND138 (13.97%, 14.43%) and TY315 (15.35%, 15.81%) under the salt-alkaline soil treatment. The yield of CB9 (31.57%, 31.19%) and DD12 (35.03%, 34.81%) less relative decrease than JND138 (49.18%, 49.07%) and TY315 (50.54%, 51.13%) under the salt-alkaline soil treatment.

N use efficiency and the N harvest index

As shown in Table 3, the TNA, NUEb, NUEg and NHI were significantly lower in the salt-alkaline soil than in the black soil. Compared with the black soil treatment, the TNA of CB9 (27.17%, 24.23%) and DD12 (24.62%, 23.83%) less relative decrease than JND138 (34.29%, 35.56%) and TY315 (34.77%, 36.60%) under the salt-alkaline soil treatment. The NUEb of CB9 (1.88%, 1.79%) and DD12 (10.75%, 9.46%) less relative decrease than JND138 (18.80%, 18.30%) and TY315 (19.49%, 21.13%) under the salt-alkaline soil treatment. The NUEg of CB9 (6.00%, 6.87%) and DD12 (12.18%, 13.87%) less relative decrease than JND138 (22.62%, 22.03%) and TY315 (24.07%, 25.43%) under the salt-alkaline soil treatment. The HNI of CB9 (9.68%, 6.67%) and DD12 (21.21%, 20.00%) less relative decrease than JND138 (29.23%, 26.98%) and TY315 (22.22%, 26.56%) under the salt-alkaline soil treatment.

Photosynthetic characteristics and leaf area index

As shown in Fig. 1, the leaf area index (LAI), Pn and PNUE were significantly lower in plants grown in salt-alkaline soil than in the black soil. Compared with the black soil treatment, LAI of CB9 (17.65%, 18.25%) and DD12 (21.72%, 26.11%) less relative decrease than JND138 (36.11%, 32.68%) and TY315 (29.79%, 31.07%) under the salt-alkaline soil treatment. The Pn of CB9 (23.88%, 23.88%) and DD12 (27.63%, 31.56%) less relative decrease than JND138 (42.34%, 39.98%) and TY315 (45.42%, 37.89%) under the salt-alkaline soil treatment. The PNUE of CB9 (3.16%, 3.25%) and DD12 (13.93%, 13.97%) less relative decrease than JND138 (25.28%, 24.72%) and TY315 (20.30%, 20.57%) under the salt-alkaline soil treatment.

Content of carbon and nitrogen compounds

As shown in Fig. 2, the soluble protein content was significantly lower, whereas the free amino acid, soluble sugar, and sucrose contents were significantly higher in plants grown in the salt-alkaline soil than in the black soil. Compared with the black soil treatment, the soluble protein content of CB9 (10.76%, 10.60%) and DD12 (8.55%, 8.74%) less relative decrease than JND138 (24.27%, 23.78%) and TY315 (26.96%, 27.02%) under the salt-alkaline soil treatment. The free amino acid content of CB9 (31.57%, 31.12%) and DD12 (28.74%, 28.72%) more relative increase than JND138 (14.07%, 14.21%) and TY315 (16.99%, 17.07%) under the salt-alkaline soil treatment. The soluble sugar content of CB9 (175.47%, 178.87%) and

¹Under salt-alkaline conditions, most of the indicators of rice will decrease; the relative decrease of different genotypes is different. The 'less relative decrease' indicates that rice of this genotype is more stable and has a certain tolerance under salt-alkaline conditions.

Table 2. Grain yield and yield components of different rice cultivars in two soil types

The different lowercase letters represent significant differences among treatments and cultivars: * $P < 0.05$; ** $P < 0.01$. BS, black soil; SAS, salt-alkaline soil; RI, relative index; N, nitrogen rate; C, cultivar; Y, year

Year	Cultivar	Effective panicles (number m ⁻²)				Grain per panicle				Seed setting rate (%)				Yield (t ha ⁻¹)			
		BS	SAS	CRI	BBS	BBS	SSAS	CRI	CRI	BS	SAS	CRI	CRI	BS	SAS	CRI	CRI
2018	CB9	360.92	282.99	0.78a	99.30	87.64	0.88a	91.40	85.37	0.93a	7.76	5.31	0.68a				
	DD12	352.84	269.33	0.76ab	118.52	102.52	0.87a	91.91	85.42	0.93a	8.85	5.75	0.65a				
	JND138	329.15	241.11	0.73b	143.50	124.20	0.87a	86.10	74.07	0.86b	9.17	4.66	0.51b				
	TY315	355.32	254.99	0.72b	113.05	86.98	0.77b	89.23	75.53	0.85b	8.35	4.13	0.50b				
2019	CB9	367.54	296.97	0.81a	97.98	88.52	0.90a	91.97	86.36	0.94a	7.63	5.25	0.69a				
	DD12	348.83	276.69	0.79b	118.82	103.98	0.88b	91.17	84.77	0.93a	8.82	5.75	0.65b				
	JND138	329.08	239.78	0.73c	138.80	120.19	0.87b	86.20	73.76	0.86b	9.13	4.65	0.51c				
	TY315	358.42	257.68	0.72c	115.41	88.83	0.77c	89.83	75.63	0.84b	8.41	4.11	0.49c				
Y		18.94*			3.14			1.12					1.42				
	T	2747.57**			279.449**			114.23**					21819.35**				
	C	109.06**			242.23**			127.76**					366.68**				
	Y × T	32.11*			1.17			21.17*					3.86				
Y × C		1.3			1.73			16.57*					2.94				
	T × C	2.48ns			9.63**			28.79**					415.24**				
	Y × T × C	0.37			0.69			0.05					0.44				

Table 3. Nitrogen use efficiency and the nitrogen harvest index of different rice cultivars in two soil types

The different lowercase letters represent significant differences among treatments and cultivars: * $P < 0.05$; ** $P < 0.01$. TNA, total N accumulation; NUEb, nitrogen utilisation efficiency for biomass production; NUEg, nitrogen utilisation efficiency for grain production; NHI, nitrogen harvest index; N, nitrogen rate; C, cultivar; Y, year

Year	Cultivar	TNA (g m ⁻²)				NUEb (g g ⁻¹)				NUEg (g g ⁻¹)				NHI (g g ⁻¹)			
		BS	SAS	CRI	BBS	BS	SAS	CRI	CRI	BS	SAS	CRI	CRI	BS	SAS	CRI	CRI
2018	CB9	13.80	10.05	0.73a	101.72	99.81	0.98a	56.19	52.82	0.94a	0.62	0.56	00.90a				
	DD12	14.30	10.78	0.75a	110.78	98.87	0.89b	61.89	54.35	0.88b	0.66	0.52	00.79b				
	JND138	14.61	90.60	0.66b	114.51	92.98	0.81c	62.78	48.58	0.77c	0.65	0.46	00.71d				
	TY315	14.32	90.34	0.65b	108.48	87.34	0.81c	58.30	44.27	0.76c	0.63	0.49	00.78c				
2019	CB9	13.58	10.29	0.76a	100.14	98.35	0.98a	55.34	51.54	0.93a	0.60	0.56	00.93a				
	DD12	14.81	11.28	0.76a	109.66	99.29	0.91b	61.63	53.08	0.86b	0.65	0.52	00.80b				
	JND138	14.82	90.55	0.64b	113.88	93.04	0.82c	62.47	48.71	0.78c	0.63	0.46	00.73c				
	TY315	14.70	9.32	0.63b	110.26	86.96	0.79c	59.18	44.13	0.75c	0.64	0.47	0.73c				
Y		2.31			0.37			0.21					0.15				
	T	49917.73**			1176.51**			2056.75**					17425.81**				
	C	291.32**			52.09**			135.31**					361.85**				
	Y × T	3.11			0.14			0.03					0.29				
Y × C		0.59			0.81			0.75					0.44				
	T × C	406.28**			184.15**			148.82**					730.62**				
	Y × T × C	0.83			1.14			0.69					1.30				

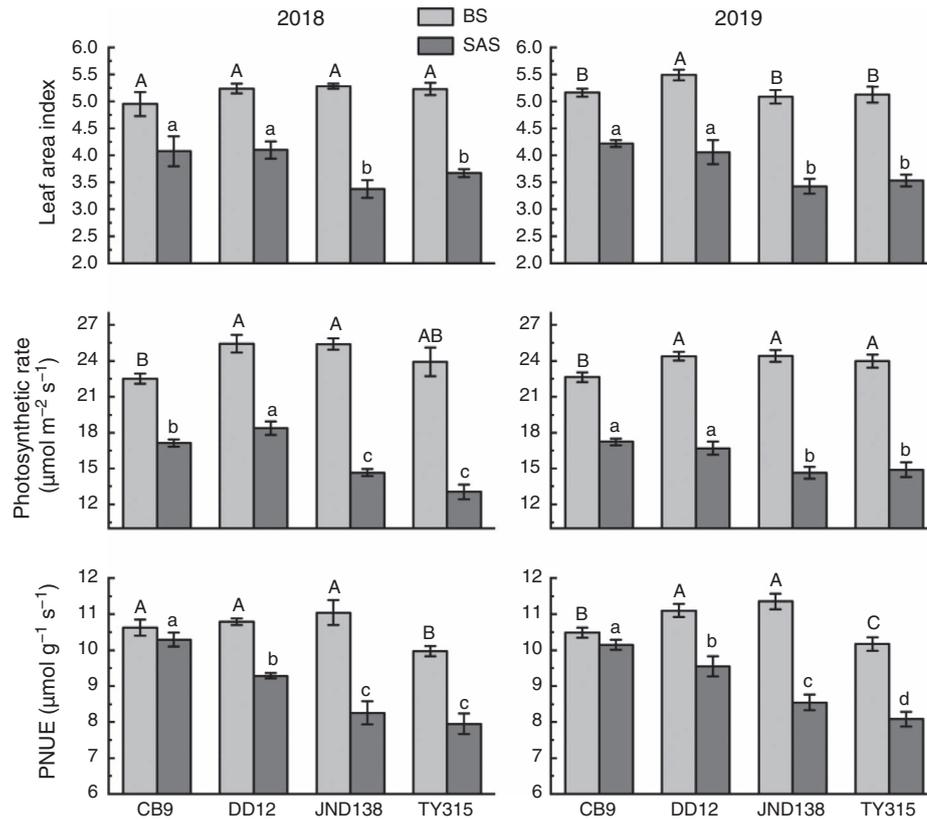


Fig. 1. Leaf area index, photosynthetic rate, and photosynthetic nitrogen use efficiency (PNUE) of four rice cultivars in two soil types. Different letters above columns indicate significant differences ($P < 0.05$) among cultivars. PNUE, BS, black soil; SAS, salt-alkaline soil.

DD12 (136.51%, 138.90%) more relative increase than JND138 (28.47%, 28.22%) and TY315 (71.49%, 68.57%) under the salt-alkaline soil treatment. The sucrose content of CB9 (173.36%, 178.87%) and DD12 (135.61%, 132.48%) more relative increase than JND138 (27.48%, 29.70%) and TY315 (79.31%, 71.82%) under the salt-alkaline soil treatment.

Enzymatic activities related to carbon and nitrogen metabolism

As shown in Fig. 3, the Rubisco activity and NR activity were significantly lower, whereas the SPS activity was significantly higher in plants grown in the salt-alkaline soil than in the black soil. A significant difference among genotypes was observed in the GS activity in the salt-alkaline soil treatment. Compared with the black soil treatment, the Rubisco activity of CB9 (5.65%, 5.41%) and DD12 (9.25%, 8.96%) less relative decrease than JND138 (21.93%, 21.96%) and TY315 (16.67%, 16.82%) under the salt-alkaline soil treatment. The SPS activity of CB9 (112.67%, 121.52%) and DD12 (108.98%, 104.48%) more relative increase than JND138 (96.98%, 85.01%) and TY315 (101.33%, 83.66%) under the salt-alkaline soil treatment. The NR activity of JND138 (14.93%, 14.97%) less relative decrease than CB9 (17.63%, 17.87%), DD12 (16.75%, 16.70%) and TY315 (16.47%,

16.44%) under the salt-alkaline soil treatment. The GS activity of CB9 (12.53%, 12.75%), DD12 (7.67%, 8.98%) and TY315 (1.33%, 1.33%) were increased, and JND138 (4.97%, 3.02%) was decreased under the salt-alkaline soil treatment.

Correlation analysis

The relative effect of the salt-alkaline treatment on Pn, LAI, soluble protein, free amino acid, soluble sugar, sucrose contents, and RuBisco, SPS, and GS activities were significantly and positively correlated with the relative effect on TNA, NUE_b, NUE_g, PNUE and yield for rice under the salt-alkaline soil conditions. The relative effect of the salt-alkaline treatment on NR activity was significantly and negatively correlated with the relative effect on TNA, NUE_b, NUE_g, PNUE and yield for rice under the salt-alkaline soil conditions (Table 4).

Discussion

Rice is the preferred food crops for the development and utilisation of beaches and salt-alkaline land, but it is particularly sensitive to soil salinity (Krishnamurthy *et al.* 2009). The salt-alkaline tolerance of rice is a complex physiological and biochemical process involving the coordination of many genes, proteins, and various salt-

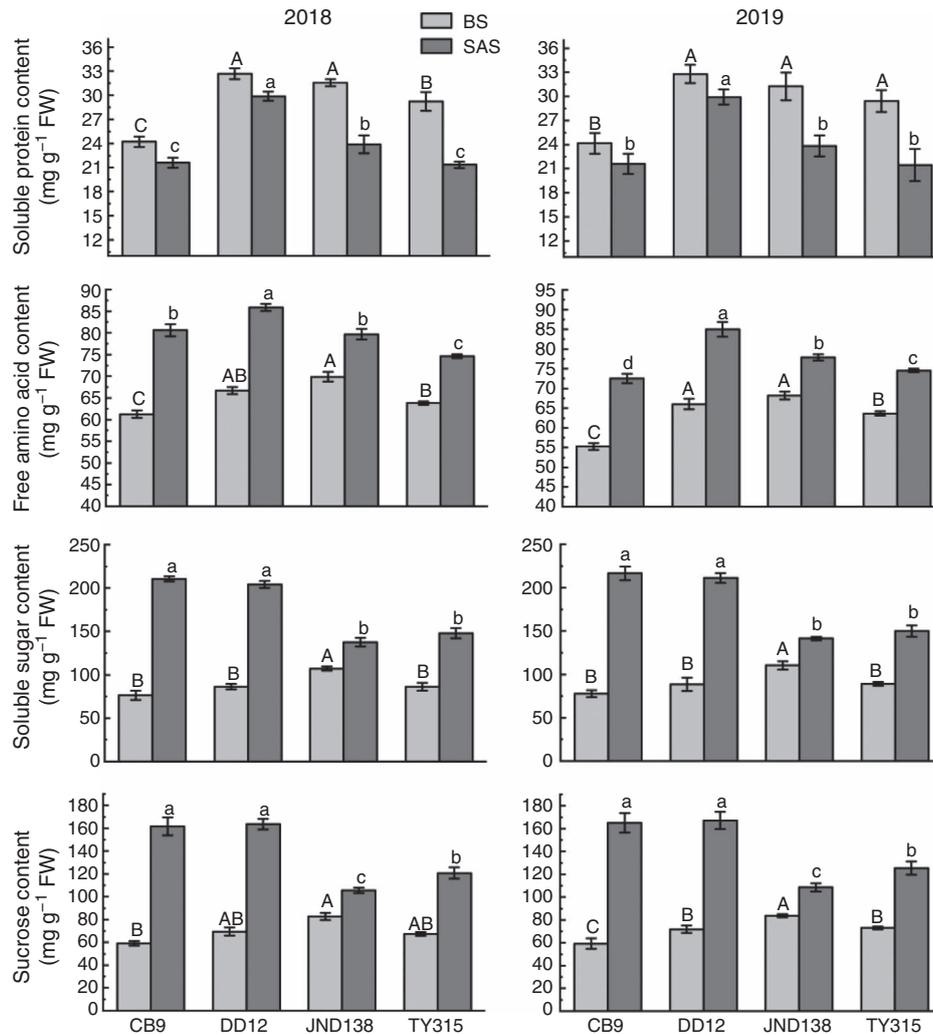


Fig. 2. The soluble protein, free amino acid, soluble sugar and sucrose content of four rice cultivars in two soil types. Different letters above columns indicate significant differences ($P < 0.05$) among cultivars. BS, black soil; SAS, salt-alkaline soil.

alkaline tolerance mechanisms. Different salt tolerance varieties of rice also have differences in saline-alkali tolerance mechanisms (Tian *et al.* 2016). It is vital to explore the physiological and biochemical mechanism of salt-alkaline tolerant rice and to breed salt-alkaline tolerant rice varieties by analysing the effects of salt-alkaline stress on the yield, nitrogen use efficiency, carbon and nitrogen metabolism of different genotype rice varieties.

Yield and NUE

The growth and development of plants in high-salinity environments is restricted, and the leaves are often yellow and curled, which affects cell division and elongation, slowing growth of crops and reducing yield (Han *et al.* 2016). In this study, under the salt-alkaline stress, the yield and components of the four rice cultivars were all affected, and the effective panicles, grain per panicle, seed setting rate, and yield

decreased. Among these measurements, effective panicles were most seriously affected. The decrease in the effective panicles is mainly due to the inhibition of tillering and reduction of productive tiller percentage by salt-alkaline stress (Zeng and Shannon 2000a). This indicates that the decrease in the effective panicles is the main reason for the reduced rice yield under the salt-alkaline stress. Compared with salt-alkaline sensitive cultivars, the yield and component factors of salt-alkaline tolerant cultivars was less affected under salt-alkaline stress. The LAI is closely related to the material production capacity of crops, and is an important parameter of whether the crop population structure is reasonable (Croft *et al.* 2013). Therefore, a reasonable LAI is one of the important conditions to ensure high and stable rice yields. In this study, the LAI and Pn of rice are significantly reduced under salt-alkaline stress, similar to previous studies (Nieman and Clark 1976; Yang *et al.* 2007). It may due to the higher pH under salt-alkaline stress, which damages the

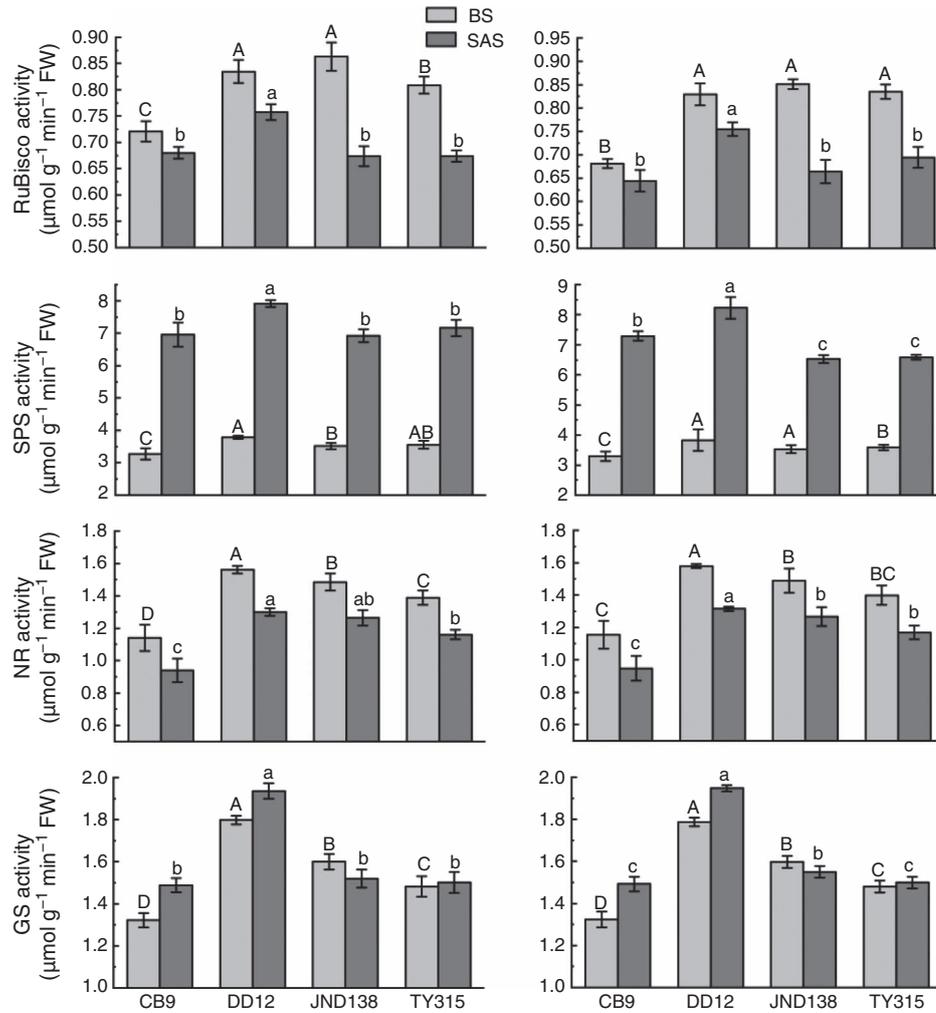


Fig. 3. Rubisco, SPS, NR, and GS activities of four rice cultivars in two soil types. Different letters above columns indicate significant differences ($P < 0.05$) among cultivars. SPS, sucrose phosphate synthase, NR, nitrate reductase, GS, glutamine synthetase, BS, black soil, SAS, salt-alkaline soil.

Table 4. Relationships between the relative effect on nitrogen use efficiency and yield and the relative effect on carbon and nitrogen traits for rice under the salt-alkaline soil conditions

Significant differences are indicated: ** $P < 0.01$. TNA, total N accumulation; NUEb, nitrogen utilisation efficiency for biomass production; NUEg, nitrogen utilisation efficiency for grain production; PUNE, photosynthetic nitrogen use efficiency; SPS, sucrose phosphate synthase; NR, nitrate reductase; GS, glutamine synthetase

Traits	TNA (g m^{-2})	NUEb (g g^{-1})	NUEg (g g^{-1})	PUNE ($\mu\text{mol g}^{-1} \text{s}^{-1}$)	Yield (t ha^{-1})
Photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	0.77**	0.97**	0.96**	0.92**	0.94**
Leaf area index	0.82**	0.95**	0.95**	0.87**	0.95**
Soluble protein content ($\text{mg g}^{-1} \text{FW}$)	0.97**	0.83**	0.87**	0.60**	0.96**
Free amino acid content ($\text{mg g}^{-1} \text{FW}$)	0.92**	0.91**	0.93**	0.76**	0.97**
Soluble sugar content ($\text{mg g}^{-1} \text{FW}$)	0.85**	0.91**	0.91**	0.81**	0.94**
Sucrose content ($\text{mg g}^{-1} \text{FW}$)	0.82**	0.89**	0.89**	0.81**	0.91**
Rubisco activity ($\mu\text{mol g}^{-1} \text{min}^{-1} \text{FW}$)	0.84**	0.89**	0.89**	0.78**	0.92**
SPS activity ($\mu\text{mol g}^{-1} \text{min}^{-1} \text{FW}$)	0.87**	0.91**	0.91**	0.80**	0.94**
NR activity ($\mu\text{mol g}^{-1} \text{min}^{-1} \text{FW}$)	-0.57**	-0.71**	-0.69**	-0.69**	-0.68**
GS activity ($\mu\text{mol g}^{-1} \text{min}^{-1} \text{FW}$)	0.83**	0.89**	0.89**	0.80**	0.92**

plasma membrane system and photosynthetic structure of the cell, leading to aerobic respiration and a decline in photosynthetic function. Additionally, compared with salt-alkaline sensitive cultivars, the LAI and Pn of salt-alkaline tolerant cultivars was less affected under salt-alkali stress. This shows that the salt-alkaline-tolerant cultivars CB9 and DD12 have a greater capacity for growth and material production under the salt-alkaline conditions than the less tolerant cultivars tested.

Salt-alkaline soil affects the normal metabolism and various physiological activities of crops (Woodrow *et al.* 2017). Accumulation of a large amount of Na^+ will destroy the structure and function of the enzyme, leading to hindered free radical scavenging, accelerating the damage of plasma membrane and membrane protein, and increasing the permeability of protoplasm (He and Zhu 2008). At the same time, excessive absorption of Na^+ leads to limited absorption of K^+ and other metal elements necessary to maintain the normal physiological activities of crops. In the protoplast, Na^+ is replaced by K^+ , K^+ is extravagated, and the value of Na^+/K^+ increases, and the ions and nutrients of the cell fluid are out of balance, resulting in insufficient or lack of nutrients (Stevens *et al.* 2006). In current study, the TNA, NUEb, NUEg, and NHI reductions in CB9 and DD12 were significantly lower than JND138 and TY315 under the salt-alkaline conditions. Among them, the TNA has the largest decrease, indicating that nutrient absorption is the main factor limiting the increase in rice yield under the salt-alkaline conditions. Under the black soil conditions, the TNA, NUEb, NUEg, and NHI of CB9 were lower than DD12, JND138 and TY315, whereas under the salt-alkaline soil conditions, these indexes of CB9 decrease less than DD12, JND138, and TY315. These results show that under the salt-alkaline stress, nitrogen absorption and utilisation efficiency can be used as an index to evaluate the salt-alkaline tolerance of rice. Additionally, in order to meet human demand for food, rice cultivars with high NUE under normal cultivation conditions and stable NUE under salt-alkali stress should be selected.

Carbon and nitrogen metabolism

Carbon and nitrogen metabolism are the two most basic metabolic pathways of rice, so their stability under salt-alkali stress is an important guarantee for stable yield and survival of rice (Ball and Anderson 1986; Smirnov and Colombé 1988; Wang *et al.* 2012; Baghel *et al.* 2016). In this study, salt-alkaline stress significantly affected the four cultivars' carbon and nitrogen metabolisms. There were also significant differences between salt-alkaline tolerant rice cultivars and salt-alkaline sensitive rice cultivars under salt-alkaline stress. The results of correlation analysis shown that carbon and nitrogen metabolism traits are closely related to yield and NUE.

Rice yield is the result of the process of production, accumulation, and distribution of photosynthetic products, which are the material basis for the formation of rice yield. Rice leaves are the main source of photosynthesis, whereas rice panicles are organic matter storage and the ultimate expression of rice economic output (Kato *et al.* 2004). In

the current study, Pn and PNUE reductions in CB9 and DD12 were significantly lower than JND138 and TY315 under the salt-alkaline conditions. It may be that the salt-alkaline tolerant cultivars have higher Rubisco activity under the salt-alkaline conditions. Rice grain yield comes from the transportation of storage substances in vegetative organs before heading and the direct transportation of leaf photosynthetic products to the grain after heading (Masoni *et al.* 2007). Under stress conditions, the soluble sugar and sucrose in rice acts as an osmotic regulator and a nutrient that protects the biofilm and its protein (Yildiztugay *et al.* 2014; Guo *et al.* 2018). In this study, the soluble sugar and sucrose contents were increased in CB9 and DD12, and were significantly higher in these cultivars than in JND138 and TY315 under the salt-alkaline conditions: a result that is consistent with findings of previous studies (Ghoulam *et al.* 2002; Li *et al.* 2017). Sucrose is the main product of plant photosynthesis, and is main form of carbohydrate used in transportation. The SPS enzyme is a key regulatory enzyme for sucrose metabolism, and its activity is closely related to the dry matter accumulation and yield formation of rice. In this study, SPS activity significantly increased under salt-alkali stress conditions. Plaut *et al.* (2004) showed that there may be competition between the development of grain and need assimilates of leaves to adapt to stress under water or salt stress. These observations indicate that part of the total assimilate from rice leaves is transported to the panicle under saline-alkali stress, whereas some assimilates need to be retained for osmotic adjustment.

Soluble protein content is an important osmotic adjustment substance in plants, and plays a key role in relieving the damage caused by stress. The level of soluble protein content is closely related to plant nitrogen metabolism and senescence (Xin *et al.* 2019). In this study, the soluble protein content of rice leaves decreased significantly under salt-alkaline stress. Additionally, it may be due to the salt-alkaline stress that the rice is in a state of nitrogen deficiency for a long time, and the plant accumulates free amino acids through protein hydrolysis, which may be an adaptive mechanism of the plant. NR is the rate-limiting enzyme of nitrogen assimilation that is regulated by nitrate, light, and stress. In this study, salt-alkaline stress significantly reduced NR activity in rice, consistent with results of previous studies (Wang *et al.* 2007; Shao *et al.* 2015). This change may be induced by a decrease in nitrate content of leaves caused by salt-alkaline stress. Zeng and Shannon (2000b) showed that salt-alkaline stress significantly increased the NR activity in salt-tolerant rice buds, but significantly reduced the NR activity in salt-sensitive rice buds at the bud stage. In this study, the decrease in NR activity of salt-alkaline tolerant cultivars leaves at the filling stage under saline-alkali stress was less than that of salt-alkaline-sensitive cultivars, showing a certain degree of adaptability to salt-alkaline stress. This indicates that the plant's ability to tolerate salt-alkaline conditions may also be related to its ability to quickly adapt to the environment of nitrogen deficiency. Cao *et al.* (2008) reported that higher GS activities were beneficial for N absorption and accumulation, which are key enzymes involved in plants N metabolism. In this study, compared with the black soil conditions, the GS activity in CB9, DD12, and TY315

increased under the salt-alkaline soil conditions, whereas GS activity of JND138 decreased. These results indicate that higher GS activity is important for maintaining the stability of NUE under the salt-alkaline stress. Salt-alkaline stress produced a significant effect on the grain yield differences between rice cultivars with contrasting salt-alkaline resistance. Overall, compared with salt-alkaline sensitive cultivars, salt-alkaline tolerant cultivars obtained more N from the soil under salt-alkaline conditions. At the same time, salt-alkaline tolerant cultivars NUE and PNUE were significantly higher than salt-alkaline sensitive cultivars, indicating that under salt-alkaline stress, the salt-alkaline tolerant cultivars can obtain more N, provide more basic substances and energy for carbon metabolism, and thus obtain higher yields.

Conclusions

Most previous studies on salt-alkaline effects on plant growth have used chemical agents to simulate salt-alkaline stress. In this study, black soil and saline-alkaline soil were tested in the field, so results can directly guide the selection and breeding of salt-alkaline tolerant rice varieties and the improvement of cultivation measures on these soils. In our study, four cultivars with similar growth periods showed different yields, NUE, and carbon and nitrogen metabolisms under the two soil types. Our results show that the limitation of rice yield was mainly due to changes in effective panicles and N acquisition capacity under salt-alkaline conditions. The results also demonstrated that the salt-alkaline tolerant cultivars CB9 and DD12 have higher carbon and nitrogen compound contents and enzyme activity to maintain a more stable osmotic balance, and maintain carbon and nitrogen metabolisms. This shows that under salt-alkaline stress, higher carbon and nitrogen metabolism capacity is an important guarantee for stable rice yield. The nitrogen use efficiency (NUE_b and NUE_g) of CB9 under black soil conditions was lower than that of TY315 and JND138, whereas the opposite was true under salt-alkaline soil conditions, indicating that nitrogen use efficiency can be used as an important indicator for evaluating the salt-alkaline of rice under salt-alkaline stress. This study provides a basis for breeding salt-alkaline tolerant rice cultivars and the improvement of cultivation measures, which are important for the sustainable development of rice production.

Conflicts of interest

The authors declare no conflicts of interest.

Author contributions

All authors conceived and designed the experiments; Liying Guo and Yanqiu Geng performed the experiments; Liying Guo analysed the data and wrote the paper. All authors have read and approved the final manuscript.

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