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Influence of natural saline-alkali stress on chlorophyll content and chloroplast ultrastructure of two contrasting rice (*Oryza sativa* L. japonica) cultivars

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Abstract

This study examined the changes of chlorophyll content and chloroplast ultrastructure in mesophyll cells of two rice (*Oryza sativa* L. spp. japonica) cultivars with different saline-alkali tolerance (Suijing NO.5: salinity-alkalinity-tolerant, and Songjing NO.6: salinity-alkalinity-sensitive). Both rice cultivars were grown under three natural occurring saline-alkali conditions, as well as the normal condition as control. All types of soils were obtained from Zhaodong city Heilongjiang province in their natural forms. The chlorophyll content of both rice cultivars was significantly higher than control under mild saline-alkali stress, and the chloroplasts showed no significant structural distortion. In contrast, the content of chlorophyll was significantly reduced in two cultivars and the chloroplasts swelled under moderate and severe saline-alkali stress, compared to the control. The grana lamellae of the chloroplasts were distorted and the chloroplast membrane suffered different degrees of disintegration. When the stress intensified, the chlorophyll content of Songjing NO.6 decreased from 2.671mg/g FW to 3.57mg/g FW and the chlorophyll content of Suijing NO.5 decreased from 3.456mg/g FW to 4.105mg/g FW. The chloroplast ultrastructure appeared to be severely destroyed in both cultivars. However, cultivar Suijing NO.5 exhibited less decrease in the chlorophyll contents and less damage in the chloroplasts ultrastructure than Songjing NO.6, which is consistent with the known difference in the salinity-alkalinity tolerance of the two rice cultivars.

Keywords: chloroplast; chlorophyll; rice; saline-alkali stress; ultrastructure.
Abbreviations: HAS_Heavy saline-alkali soil; MSA_Medium saline alkali soil; LSA_Light saline-alkali soil; CK_Control soil; SEC_Swelling extents of chloroplast.

Introduction

Soil salinization is a worldwide problem that has been threatening the survival of mankind by destroying the limited soil resources (Zhang et al. 2004). According to incomplete statistics from the United Nations Educational, Scientific and Cultural Organization (UNESCO) and the Food and Agriculture Organization (FAO) of the United Nations, over one hundred countries in the world possess different types of saline-alkali soil. The entire saline soil area is about 9.5438 billion hm^2, which accounts for 10% of the arable land in the world. China is in possession of 1 / 16 of the entire world's saline-alkali land, which constitutes about 21% of the cultivated land in China. In Heilongjiang province alone, the saline soil area is about 1.8873 million hm^2. Developing and utilizing the saline alkali soil, and improving crop yield in the saline alkali soil are very important tasks of agriculture. Rice is one of the most important crops in the world and is the primary food source for over two billion people. The salt tolerance of different rice cultivars is different (Yan and Tan, 1991). Rice needs a special water environment for its growth, which can play a role in leaching soluble salts out of the soil. Furthermore, the biological effect of rice on soil can also reduce the salt content of the soil (Zhang and Shao, 2006). Therefore, rice is often the primary choice for saline-alkali land improvement. The effects of salinity-alkalinity stress on the physiological and biochemical characteristics of rice have been reported by many researchers. However, most of the existing researches were done under artificially controlled conditions such as water planting, sand culture or manual simulation. Several previous researches indicate that the different stress conditions such as changes in water (Bourque et al., 1975), temperature (Tang et al., 2012), light intensity (Zhen and Zhang, 2000) and oxidative stress (Hernandez et al., 1995) can affect the ultrastructural organization of the chloroplasts. The effect of salt on chloroplast ultrastructural organization has been reported but the studies were limited to only a few plants such as wheat (Diana and Thorpe, 1986), corn (Hasan et al., 2005), tomato (Mäkelä et al., 2000), and sorghum (Netondo et al., 2004). The effect of salt on the chloroplast ultrastructure in rice has never been systematically studied, although there have been reports in which salt solutions were used (Xue and Liu, 2008). In this paper, we used three types of saline-alkali soils to study the effect of different saline-alkali soils on chlorophyll content and chloroplast ultrastructure in different rice cultivars. We also extended our study to the relationship between the chlorophyll content and the chloroplast ultrastructure under saline-alkali stress. In this study, we revealed the variation of chlorophyll content in the functional leaves of rice, explore the physiological mechanism of chloroplast ultrastructure changes under saline-alkali stress, and elucidate the physiological basis of saline tolerance in rice.

Table 1. Soil Basic situation used in the experiment.

Soil type	pH	Total salt content (%)	CO_3^{2-} (%)	HCO_3^- (%)	SO_4^{2-} (%)	Na^+ (%)	K^+ (%)	Ca^+ (%)
LSA	8.4	0.131	13	698	28.2	317.6	32.3	56.4
MSA	8.75	0.139	27	747	33.4	398.7	45.4	48.6
HSA	9.15	0.327	274	2013	36.1	853.1	127.6	14.7
CK	7.4	0.059	6	297	13.7	52.1	14.6	73.1

HSA: Heavy saline-alkali soil; MSA: Medium saline alkali soil; LSA: Light, saline-alkali soil; CK: Control soil.

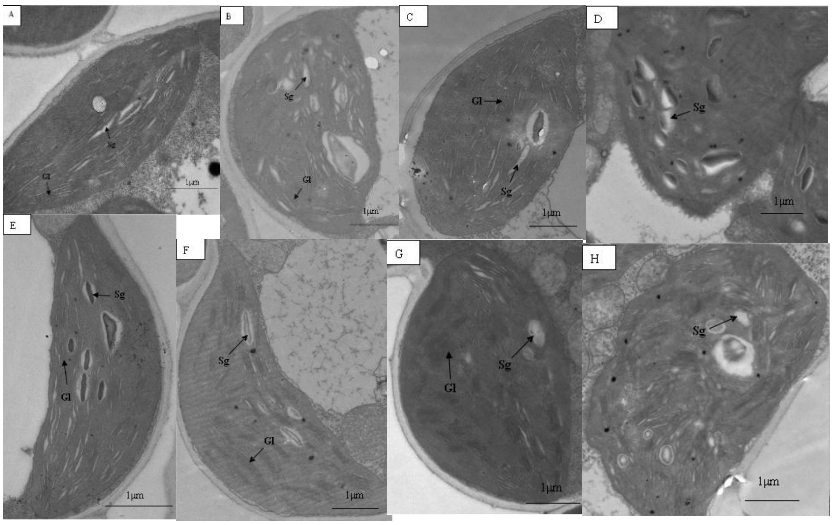

Fig 1. Chloroplast ultrastructural structure, (a) chloroplast ultrastructure of Song-jing NO.6 (CK), (b) chloroplast ultrastructure of Song-jingNO.6 (LSA), (c) chloroplast ultrastructure of Song-jingNO.6 (MSA), (d) chloroplast ultrastructure of Song-jing NO.6 (HAS). (e) chloroplast ultrastructure of Sui-jingNO.5 (CK), (f) chloroplast ultrastructure of Sui-jingNO.5 (LSA), (g) chloroplast ultrastructure of Sui-jing NO.5 (MSA), (h) chloroplast ultrastructure of Sui-jing NO.5 (HAS)

Results

Changes in the chlorophyll content

Table 2 shows the changes of chlorophyll content in two cultivars of rice (*Oryza sativa* L. spp. japonica) with different tolerance to salinity and alkalinity planted in four natural saline-alkali soils. Chlorophyll contents in both cultivars showed a similar trend as saline-alkali soil increased. The content of chlorophyll in rice plant under mild saline-alkali stress (soil type LSA) was significantly higher than the control (soil type CK). However, the chlorophyll content was significantly reduced when the rice is under medium or severe saline-alkali stress (soil types MSA and HAS) as compared to control. The chlorophyll content decreased as the stress intensified. Furthermore, chlorophyll content of Sui-jing NO.5 only slightly decreased under medium saline-alkali stress, while the content of chlorophyll in Song-jing NO.6 was significantly reduced. This is consistent with the different tolerance in saline-alkali stress between the two rice cultivars.

Alterations in the chloroplast ultrastructure under salinity and alkalinity stress

Fig 1 A-D and E-H show the chloroplast ultrastructure of mesophyll cells in two cultivars of rice treated with four natural saline-alkali soils, respectively. Fig 1A and 1E show a typical chloroplast ultrastructure in mesophyll cells of the control rice plants of both cultivars. The elongated-oval-shaped chloroplasts distribute along the inside of cell wall of mesophyll cells and are parceled by the bilayer membrane. The chloroplast stroma lamellae and grana lamellae are clearly arranged at a position paralleled to the long axis of the chloroplast. These healthy chloroplasts have abundant stroma and the thylakoids are stacked closely. Very small amount of plastoglobule was observed in these chloroplasts. Fig 1B and 1F show chloroplast ultrastructure in mesophyll cells of both rice plants under light saline-alkali stress. The chloroplasts from both rice plants appeared to be slightly swollen. The chloroplasts in Song-jing NO.6 are slightly separated from the cell wall and the starch grains swelled mildly, with the internal cellular structure remained intact. Compared to Song-jing NO.6, chloroplasts in rice cultivar Sui-jing NO.5 had no significant difference other than the slightly swollen chloroplasts. Fig 1C and 1G show chloroplast ultrastructure in two rice plants under medium saline-alkali stress. The medium saline-alkali stress induced changes in the ultrastructure of chloroplasts. Chloroplasts appeared severely swollen; the grana lamellae became sparse and were expanded. The orientation of grana was also altered. The chloroplast membrane of Song-jing NO.6 disintegrated gradually, while the chloroplast membrane remained intact in Sui-jing NO.5. The swelling and distortion of grana lamellae in Song-jing NO.6 is more severe than that in Sui-jing NO.5. Fig 1D and 1H show chloroplast ultrastructure in two rice plants under heavy saline-alkali stress. Heavy saline-alkali stress caused a striking disruption of chloroplasts in both rice cultivars. In saline-alkali treated plants, the chloroplasts were extremely swollen and the chloroplast membrane disintegrated. The starch grains were distorted and the lamellar structure was evacuated. The overall internal cellular structure appeared to be simplified and the chloroplasts were scattered in the cytoplasm.

Table 2. The influence of saline-alkali stress on rice chlorophyll content.

Treatment	Variety	
	Song-jing NO.6	Sui-jing NO.5
CK	3.407±0.022A	3.745±0.042A
LSA	3.570±0.086B	4.105±0.069B
MSA	3.102±0.05C	3.660±0.033A
HSA	2.671±0.025D	3.456±0.026C

Values are means ±SD，significant different at P<0.01

Table 3. The influence of saline-alkali stress on swelling extents of chloroplast.

Song-jingNO.6	With-length ratio	SEC	Sui-jingNO.5	With-length ratio	SEC
CK	0.4339±0.12a	0	CK	0.3382±0.02a	0
LSA	0.5188±0.11a	0.1957	LSA	0.3491±0.05a	0.0322
MSA	0.637±0.04ab	0.4681	MSA	0.5136±0.08b	0.5186
HAS	0.7531±0.11b	0.7357	HAS	0.6196±0.01c	0.8321

Chloroplasts of the two rice cultivars in control soil didn't show any swollen, and the SEC is zero. Values are means ±SD, significant different at P<0.05

Discussion

NaCl stress decreases chlorophyll content even at the lowest concentration (Santos, 2004). Salt stress was demonstrated to decrease sunflower growth (Santos, 1999), chlorophyll content and fluorescence (Santos et al., 2001). In leaves of tomato, the contents of total chlorophyll (Chla+b), Chl a, and Chl b carotene decreased due to NaCl stress (Khavarinejad and Mostofi, 1998). But Wang and Nil, (2000) have reported that chlorophyll content was increased under conditions of salinity in Amaranthus. In our experiment, the content of chlorophyll in two cultivars are slightly higher than control under mild saline-alkali stress and the chloroplast ultrastructure under mild saline-alkali stress was not drastically different from control. This observation is consistent with rice trying to compensate the lower binding affinity between chlorophyll and its protein partners caused by high salt concentration. Under moderate and severe saline-alkali stress, the chlorophyll content of the two varieties was decreased. In addition, the severe saline-alkali stress destroyed the thylakoid membrane structure and made the stack of thylakoid membrane loose. The lamellar structure also evacuated or even collapsed. With the degree of saline-alkali stress increased, the injury severity of the thylakoid and grana piece also become increased. In potato, salt stress reduced the numbers and depth of the grana stacks and caused a swelling of the thylakoid. Starch grains also became larger in the chloroplasts (Bruns and Hecht-Buchholz, 1990). Transmission electron microscopy showed that in leaves of NaCl-treated tomato plants the chloroplasts are aggregated and the cell membranes are distorted and wrinkled (Khavarinejad and Mostofi, 1998). In addition, there are no signs of grana or thylakoid structures in chloroplasts (Khavarinejad and Mostofi 1998). Chlorophyll is an important part of chlorophyll protein complexes on the thylakoid membranes. It is the key photosynthetic pigment and its content directly reflects the photosynthetic efficiency and assimilation capacity. As a result, chlorophyll content is an important index in determining salt stress level (Munns, 1993). Chloroplast is the site of photosynthesis in plants, the structural integrity of which is the premise of photosynthesis in plants. The membrane system of chloroplast can be destroyed by salt stress (Yang et al., 2008), which suggests that the chloroplast is the most susceptive organelle to high salt in plant cells. Our research shows that moderate and severe saline-alkali stress destroyed the thylakoid membrane structure, lowered the affinity between the chlorophyll and the chloroplast protein, and decreased the activity of the chlorophyll enzyme, which in turn, promoted chlorophyll break down. The stability of the plant chloroplast ultrastructure is closely related to salt resistance (Xia et al., 2002). Our research shows the same result that in the same saline-alkali stress, the extent of damage in salt tolerant rice cultivar (Sui-jing NO.5) is less than that of the salt sensitive rice cultivar (Song-jing NO.6).

Materials and methods

Plant materials and soil saline-alkali treatments

Two rice cultivars were used in this study. Suijing NO.5 is a salt tolerant cultivar, while Songjing NO.6 is a salt sensitive cultivar, which is used as control. Seeds of rice were surface sterilized with a 5% sodium hypochlorite solution for five minutes. After washing several times with distilled water, seeds were imbibed in a beaker containing distilled water and incubated in a culture room with a temperature of 24±2ºC until the white tip of the coleoptiles appear. After imbibition, the seeds were sown on bed soil in the greenhouse. When the seedlings of rice reached the three-leaf stage, they were transplanted into soil containing three levels of saline-alkali soil (HAS: Heavy saline-alkali soil; MSA: Medium saline alkali soil; LSA: Light saline-alkali soil;) and normal soil (CK: Control soil).

Experimental design and data analysis

The experiments were laid out in a complete randomized design with three replications. Rice was grown in pots filled with four different types of soil and the physicochemical properties of the soils were sampled (Table 1). The height of the pot was 21.5 cm and the inside diameter 20.0 cm. Each pot was filled with water to approximately 3 cm above the top of the soil. Urea (0.75 g/pot), Phosham (0.6 g/pot), and potassium sulfate (0.56 g/pot) were supplemented to the soil in order to supply nitrogen, phosphorus, and potassium for the rice growth. The mean day temperatures ranged between 23-32˚C. The light intensity and CO_2 concentration ranged between35000-45000 lx and 350-380 μmol mol^{-1}, respectively. The data was statistically analyzed using analysis of variance. Statistical analysis were performed using the statistical software package SPSS 17.0. Windows and significant difference were analyzed based on $p < 0.05$ and $p < 0.01$.

Measurement of chlorophyll content

A 0.5 g of the first piece of fully expanded leaves at the tillering stage was cut into uniform pieces, mixed with an organic solvent containing 5 mL of 80% acetone and 5 mL of ethanol and left standing overnight in darkness. The leaf tissues turned white on the next day, indicating complete extraction of the chlorophyll by the organic solvent. The supernatant from the extraction was collected and the leaf debris discarded. The chlorophyll content was determined by measuring the absorbance at 645 nm and 663 nm with a UV 1800 spectrophotometer.

Transmission electron microscopy (TEM)

The first piece of fully expanded leaves at the tillering stage was also used for the electron microscopy experiments. Leaf pieces (1×1 mm) were fixed in 2.5% glutaraldehyde at 4 ℃ overnight and washed three times for 15 minutes with phosphate buffer (0.1 M, pH7.2). Samples were post-fixed for 1.4 h in 1% osmium tetroxide and then rinsed three times for 10 minutes with the same phosphate buffer. Samples were gradient dehydrated in a series of solutions containing 50%, 70%，90%, or 100% of ethanol for 10min every time. Then the samples were soaked in acetone-ethanol (1:1) solution for 10 minutes, followed by soaking in pure acetone for 5 minutes. The Epon812 resin was mixed with acetone at a 1.5: 1 ratio to make the embedding medium. The dehydrated leaf samples were molded into the embedding medium and incubated at 30 ℃ for five hours. The embedding medium was then polymerized at 60℃ for 36-48 hours. The samples were then embedded in pure embedding medium till the next day. Ultrathin sections (70 nm) were then cut from the cured medium with a diamond knife and mounted on grids using a LKB5 microtome. The samples were examined with a Hitachi 7650 transmission electron microscope. Three photographs of chloroplasts per sample were taken and analyzed for the calculation of swelling extents of chloroplast（SEC）. The swelling extents of chloroplast were calculated as follows:

$$\text{SEC}=\frac{T_n - CK_n}{CK_n}\times 100\%$$

The n was representation of width to length ratio and the width and length of chloroplast were estimated with an Image J program, a free, Java-based image-processing package.

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References

Bourque DP, McMillan PN, Clingenpeel WJ, and Naylor AW (1975) Ultrastructural Effects of Water Stress on Chloroplast Development in Jack Bean. Plant Physiol. 56, 160-163.

Bruns S, Hecht-Buchholz C (1990) Light and electron-microscope studies on the leaves of several potato cultivars after application of salt at various developmental stages. Potato Res. 33, 33–41.

Diana MR, Thorpe JR (1986) Some Observations on the Effects of Salinity on Ion Distributions and Cell Ultrastructure in Wheat Leaf Mesophyll Cells. J Exp Bot. 37(1), 1-7.

Hasan R, Ohnuki Y, Kawasaki M, Taniguchi M, Miyake H (2005) Differential Sensitivity of Chloroplasts in Mesophyll and Bundle Sheath Cells in Maize, an NADP-Malic Enzyme-Type C4 Plant, to Salinity Stress. Plant Prod Sci. 8(5), 567-577.

Hernandez JA, Olmos E, Corpas FJ, Sevilla F, Del Rio LA (1995) Salt-induced oxidative stress in chloroplasts of pea plants. Plant Sci. 105, 151–167.

Khavarinejad RA, Mostofi Y (1998) Effects of NaCl on photosynthetic pigments, saccharides, and chloroplast ultrastructure in leaves of tomato cultivars. Photosynthetica. 35, 151–154.

Mäkelä P, Kärkkäinen J, Somersalo S (2000) Effect of Glycinebetaine on Chloroplast Ultrastructure, Chlorophyll and Protein Content, and RuBPCO Activities in Tomato Grown under Drought or Salinity. Biologia Plantarum. 43(3), 471-475.

Munns R (1993) Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. Plant Cell Environ. 16,15-24.

Netondo GW, Onyango J, Beck E (2004) Sorghum and salinity: II. Gas exchange and chlorophyll fluorescence of sorghum under salt stress. Crop Sci. 44, 806-811.

Santos C, Azevedo H, Caldeira G (2001) In situ and in vitro senescence induced by KCl stress: nutritionalimbalance, lipidperoxidationandantioxidantmetabolism. J Exp Botany. 52, 351–360.

Santos C, Caldeira G (1999) Comparative responses of Helianthus annuus plants and calli exposed to NaCl: I. Growth rate and osmotic regulation in intact plants and calli. J Plant Physiol. 155, 769–777.

Santos CV (2004) Regulation of chlorophyll biosynthesis and degradation by salt stress in sunflower leaves. Scientia Horticulturae.103, 93–99.

Tang T, Zheng GW, Li WQ (2012) Defense Mechanisms of Plants Photosystem to Heat Stress. China J Biol Chem Mole Biol. 28(2), 127-132.

Wang Y, Nil N (2000) Changes in chlorophyll, ribulose bisphosphate carboxylase–oxygenase, glycine betaine content, photosynthesis and transpiration in Amaranthus tricolor leaves during salt stress. J Hortic Sci. Biotechnol.75, 623–627.

Xia FC, Jiang GQ, Lu JM (2002) Development of the Study on Halophyte's Structure of Resisting Saline-alkali. J TongHua Teachers' College. 23(2), 67-69.

Xue YF, Liu ZP (2008) Effects of NaCl and Na_2CO_3 stresses on photosynthesis and parameters of chlorophyll fluorescence in Helianthus Tuberosus seedlings. J Plant Ecol. 32(1), 161-167.

Yang CW, Li CY, Yin HJ, Ju M, Shi DC (2008) PH and ion balance in wheat-wheatgrass under salt-or alkali stress. Chinese J Applied Ecol. 19(5), 1000-1005.

Yan XL, Tan KZ (1991) Screening rice varieties for salt tolerance in greerrekuse. IRRN. 16(1), 16-17.

Zhang JF, Xing SJ, Li JY, Makeschin F, Song YM (2004) Agroforestry and its application in amelioration of saline soils in eastern China coastal region. For Stud China. 6(2), 27-33.

Zhang RZ, Shao XW (2006) Effect of Saline-Alkali Stress on Source-Sink and Yield of Rice. Chinese J Rice Sci. 20 (1), 116-118.

Zhen W, Zhang FM (2000) The effects of low light intensity on photosynthetic characteristics and ultrastructure of cucumber functional leaves. Acta Hort Sinica. 4, 30-32.