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INTERACTIVE EFFECTS OF SALINITY AND PROLINE ON RICE AT THE ULTRASTRUCTURAL LEVEL

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Abstract

The effect of foliar application of proline on the ultrastructure of rice leaves under normal and salt stress conditions was investigated. Thirty-five days old rice seedlings were transplanted into soil with different levels of salinity (0, 50, 100 mM NaCl in the soil) for 20 days, and then the leaves of fifty-five days old seedling were sprayed with proline (0, 15, 30 mM) about 10 ml plant^{-1} once per day for two consecutive days. Three days later, leaf samples were collected for ultrastructure under normal and salt stress conditions. The results showed that salt stress-induced alterations of the ultrastructure of chloroplasts and vascular tissues. Under normal growth conditions, excessive concentrations of proline (30 mM) induced damage of chloroplast ultrastructure, but the two concentrations of proline did not apparently change the vascular tissue. Compared with salt stress alone, exogenous proline markedly suppressed the swelling of chloroplasts, maintained a well-preserved internal lamellar system in the chloroplasts, and apparently increased the number of mitochondria in sieve tube and companion cells under salt stress. The role of foliar application of 30 mM proline in protecting the mitochondria under salt stress was greater than 15 mM proline. These results indicated that the protective role of proline at the ultrastructural level in rice depended on the proline concentration and salt level.

Key words: Rice, Salt stress, Ultrastructure, Chloroplast, Vascular tissue.

Introduction

Proline is a key osmoprotectant, which accumulates rapidly in plants exposed to various stress and has multiple protective effects (Szabados & Savouré, 2010; Hayat *et al*., 2012). Earlier literatures reported the positive effect of proline at the cellular and physiological levels (Ashraf & Foolad, 2007; Hoque *et al*., 2008; Szabados & Savouré, 2010; Shanker & Venkateswarlu, 2011; Kaur & Asthir, 2015; Singh *et al*., 2015; Teh *et al*., 2016). However, the influences of exogenous proline on plant growth under normal and salt stress conditions are actually controversial. Some reports elaborated the toxic effects of proline on plants under normal growth conditions (Verbruggen & Hermans, 2008; Lehmann *et al*., 2010) and exogenous proline exacerbated or did not alleviate the inhibition of plant growth induced by salt stress (Heuer, 2003; Nounjan & Theerakulpisut, 2012). Yet, abundant evidence showed that exogenous proline conferred protection against salt stress in diverse plants (Hoque *et al*., 2008; Athar *et al*., 2009; Ahmed *et al*., 2011; Yan *et al*., 2011; Agami, 2014; Dawood *et al*., 2014; Bhusan *et al*., 2016; Teh *et al*., 2016). The controversial data suggest that the effectiveness of proline applied may be partially explained by the cultivation medium (e.g. nutrition solution, soil or sand with nutrition solution), salt concentration, salt treatment time (or plant development stage) and duration, especially type of species, application time (proline and salt stress applied simultaneously or proline applied before salt stress or vice versa) and frequency, application method (pre-sowing treatment, foliar spray or root irrigation) and proline concentration.

In our previous studies, it was found that at 15 mM and 30 mM exogenous proline induced salt stress tolerance at the physiological and organ levels (Sha *et al*., 2013). However, it is unclear whether proline at the same concentration has the same promotion effect on the plant at the ultrastructural levels. The purpose of our experiment was to provide additional information on the cumulative and interaction effects of foliar application of exogenous proline and salt stress on the ultrastructure of rice leaves.

Materials and Methods

Plant materials and treatments: The trial was conducted in the greenhouse at the Xiang Fang Experimental Station of Northeast Agricultural University (NEAU, China) from April to June 2013. Seeds of (*Oryza sativa* L. sub. *japonica*) cultivar Dongnong 425 (DN425) were surface-sterilized with 5% sodium hypochlorite solutions and washed 5 times with distilled water. After 72 h imbibition at room temperature, the seeds were sown on bed soil in the greenhouse. The basic physical and chemical characteristics of this soil are shown in Table 1. Before transplanting, soil salinization was achieved by incorporating thoroughly 5 L NaCl at various concentrations (0, 50 and 100 mM) into 12 L plastic pot containing 10 kg soil. Thirty-five days of rice seedlings (4-leaf stage) with uniform size was subjected to salinity treatment. Six plants were maintained at each pot. The loss of water by evapotranspiration was compensated for every two days by supplement of tap water (EC= 0.011 dS m^{-1}) to the scale line marked on red chopsticks. After 20 days, seedlings of each salt treatment were divided into three groups and then were given a foliar spray of different concentrations of proline solutions (0, 15 and 30 mM proline in 0.1% (v/v) Tween-20 solution) once per day for two days. The volume of proline spray was 60 ml per pot (10 ml per plant). Each treatment has three replicates.

Table 1. Characteristics of the soil used in the studies.

Soil type	pH	Total salt content (%)	OC (%)	N	P	K	Available K (mg kg^{-1})	Available P (mg kg^{-1})	ECp (dS m^{-1})
				(g kg^{-1})					
Chernozem	7.4	0.059	3.734	1.83	0.75	0.75	168.5	47.6	0.35

EC_P – electrical conductivity of a saturated soil-paste; OC-organic content

Measurements: Soil salinity was examined by measuring the electrical conductivity of a saturated soil paste (ECe) with an EC meter (Delta-T Devices W.E.T Sensors, UK) every seven days till plant harvest to evaluate the sodium consumption by the plants.

The fully expanded leaves (5th leaf on the main stem) were used for electron microscopic studies at three days after the proline treatment following the method of Xing *et al*. (2013). At least forty-five chloroplasts photographs were taken per sample. We used Image J 1.49 Version to determine the length, width, and area of the chloroplast and the area of starch. The swelling extent of chloroplast (SEC) was calculated as follows:

$$SEC=\left(1-\frac{\text{Average of length to width ratio of chloroplast under treatment}}{\text{Average of length to width ratio of chloroplast under non-treatment control}}\right)\times 100\%$$

Table 2. Effect of foliar application exogenous proline on the changes of soil electrical conductivity after rice transplanting into salinity soil (unit: dS m^{-1}).

Treatment		Days after transplanting			
NaCl (mM)	Proline (mM)	7 d	14 d	21 d	28 d
0	0	0.35 ± 0.01c	0.32 ± 0.01c	0.31 ± 0.02c	0.27 ± 0.03c
	15	0.36 ± 0.02c	0.35 ± 0.01c	0.34 ± 0.06c	0.31 ± 0.02c
	30	0.35 ± 0.03c	0.33 ± 0.03c	0.31 ± 0.04c	0.28 ± 0.04c
50	0	4.72 ± 0.04b	4.49 ± 0.03b	4.24 ± 0.12b	4.12 ± 0.04b
	15	4.70 ± 0.03b	4.47 ± 0.06b	4.22 ± 0.13b	4.08 ± 0.05b
	30	4.69 ± 0.04b	4.46 ± 0.03b	4.21 ± 0.16b	4.09 ± 0.04b
100	0	7.84 ± 0.14a	7.26 ± 0.07a	6.66 ± 0.22a	6.24 ± 0.12a
	15	7.81 ± 0.12a	7.25 ± 0.05a	6.64 ± 0.16a	6.22 ± 0.14a
	30	7.82 ± 0.15a	7.24 ± 0.06a	6.63 ± 0.23a	6.21 ± 0.16a

Statistical analysis: Statistical analyses were carried out using SPSS V18.0 software, and the mean comparisons among the treatments were based on the general linear model (GLM) according to Duncan's multiple range test at 0.05 level of probability.

Results and Discussion

To imitate the actual production in the saline field, the NaCl solution was applied just one time before the transplanting of plants into the soil. In order to evaluate the sodium consumption by the plants, we investigated the changes of ECe after rice transplanting (Table 2). ECe declined with days prolonged, but it showed no significant difference at given salt concentration values with or without proline, which indicated that proline foliar spraying did not change the sodium consumption by the plants.

As shown in Fig. 1A, a typical chloroplast of non-stressed seedling possessed a few starch grains, well-developed granum, and stroma thylakoids, which are parallel to the long axis of the plastid. Fig. 1B and 1C show the chloroplasts of a mesophyll cell treated with 15 mM and 30 mM proline. Treatments with proline did not significantly change the length and width of chloroplast; but it caused the swelling of thylakoids, increased the swelling extent of chloroplast (SEC) and the total starch area, especially with 30 mM proline (Fig. 1B, 1C and Table 3). It seems that the toxicity of exogenous proline supply to normal plant only occurred at excessive concentrations at the ultrastructural level. Proline-induced the swelling of thylakoids maybe due to that exogenous proline feedback inhibits *de novo* synthesis of endogenous proline, resulting in an excessive reduction of the photosynthetic electron acceptor pools (Hare *et al*., 2002).

Figs. 1D and 1G show the chloroplasts in the rice leaf treated with 50 mM and 100 mM NaCl solutions respectively. Compared with control, the changes of chloroplasts and thylakoids structure were observed under salt stress. It was observed that the chloroplast and thylakoids swelling, the granum stacking and inter-granal lamellae disintegration, and the number of osmiophilic granules increased in rice leaves under salt stress. These results corresponded with the findings of early reports (Rahman *et al*., 2000; Xing *et al*., 2013; Yamane *et al*., 2008). The shape of chloroplasts changed slightly from elliptical to nearly round, and the SEC changed from 0.195 to 0.279. This is mainly because salt stress reduced the length of chloroplast (Table 3).

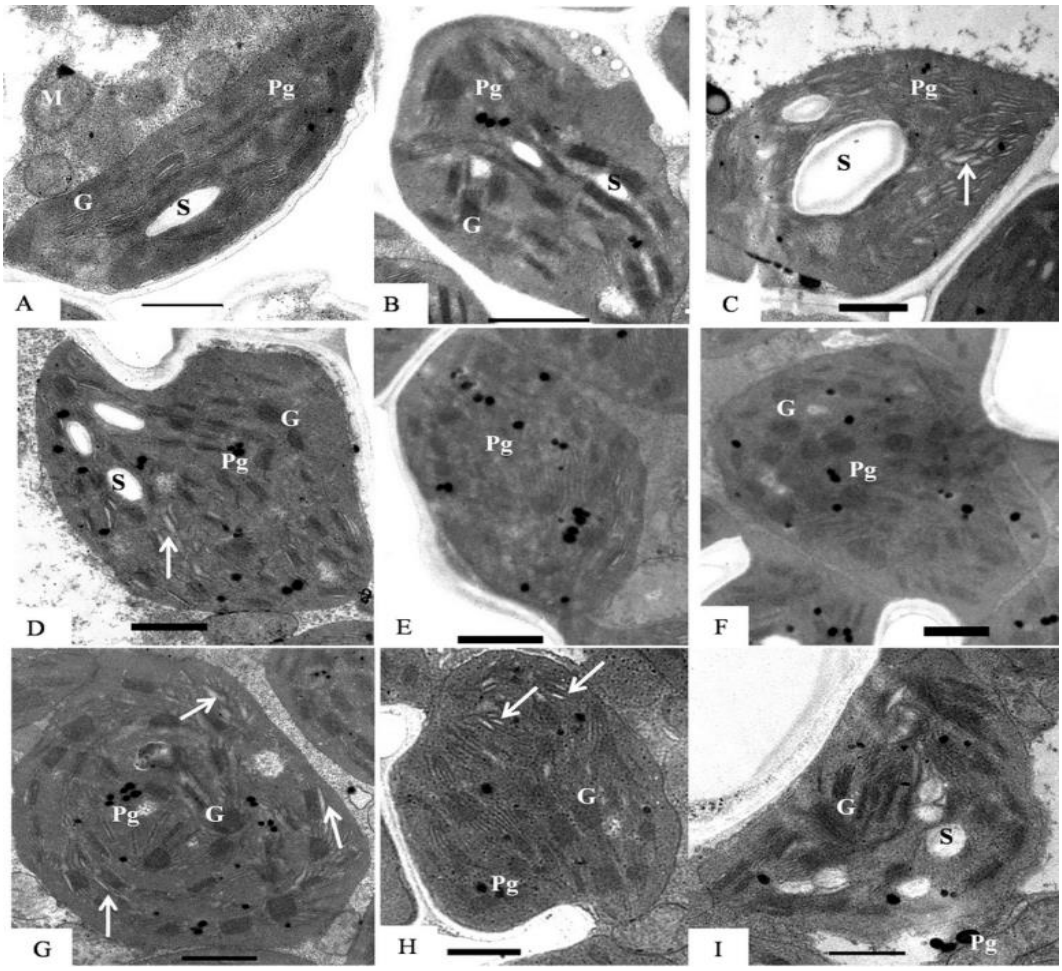

Fig. 1. Electron micrographs of chloroplast in the 5th leaf on the main stem of rice sprayed with or without proline under normal and saline conditions. (A) CK, 0 mM NaCl+0 mM Proline. (B) CP_1, 0 mM NaCl+15 mM Proline. (C) CP_2, 0 mM NaCl+30 mM Proline. (D) S_1, 50 mM NaCl+0 mM Proline. (E) S_1P_1, 50 mM NaCl+15 mM Proline. (F) S_1P_2, 50 mM NaCl+30 mM Proline. (G) S_2, 100 mM NaCl+0mM Proline. (H) S_2P_1, 100 mM NaCl+15 mM Proline. (I) S_2P_2, 100 mM NaCl+30 mM Proline. Abbreviation: S, Starch grain; G, Grana; Pg, plastoglobuli; Arrow, swelling thylakoids; Bar, 1μm.

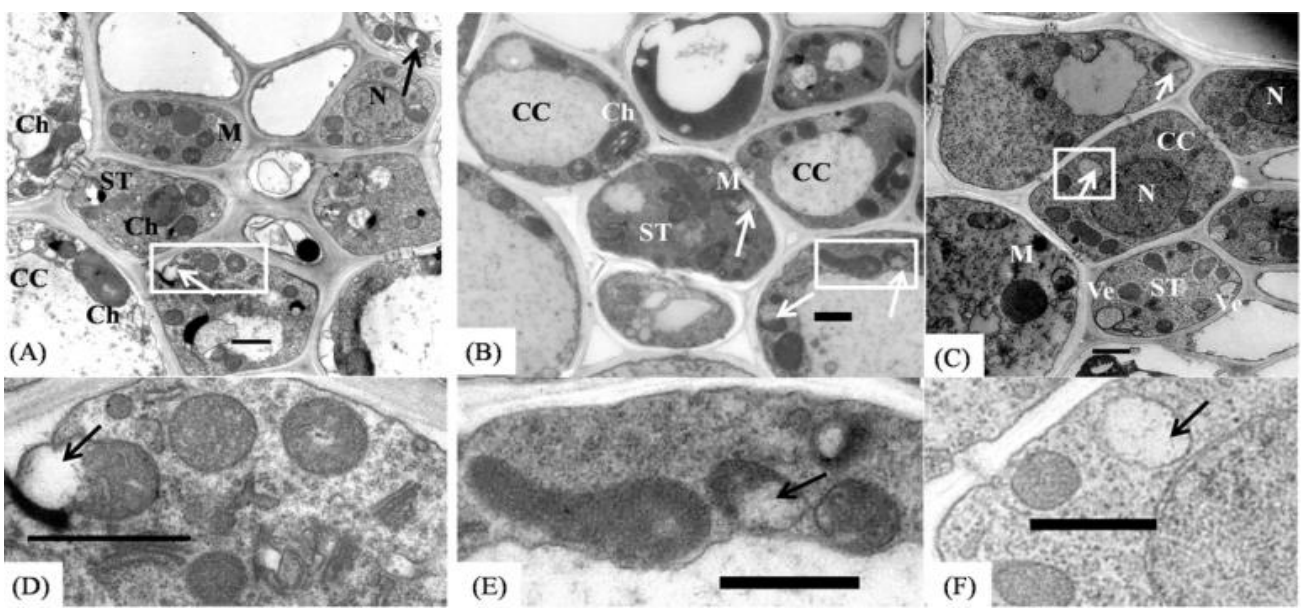

Fig. 2. Electron micrographs of vascular tissues in the 5th leaf on the main stem of rice sprayed with or without proline under normal condition. (A)CK without proline. (B) CK with 15mM proline. (C) CK with 30 mM proline. Figures D, E, F were enlargement of the rectangular area in samples (A, B and C). Abbreviation: CC, companion cell; ST, sieve tube; N, nucleus; M, mitochondria; Ve, vesicles. Arrow, damaged or disappeared mitochondria; Bar, 1μm.

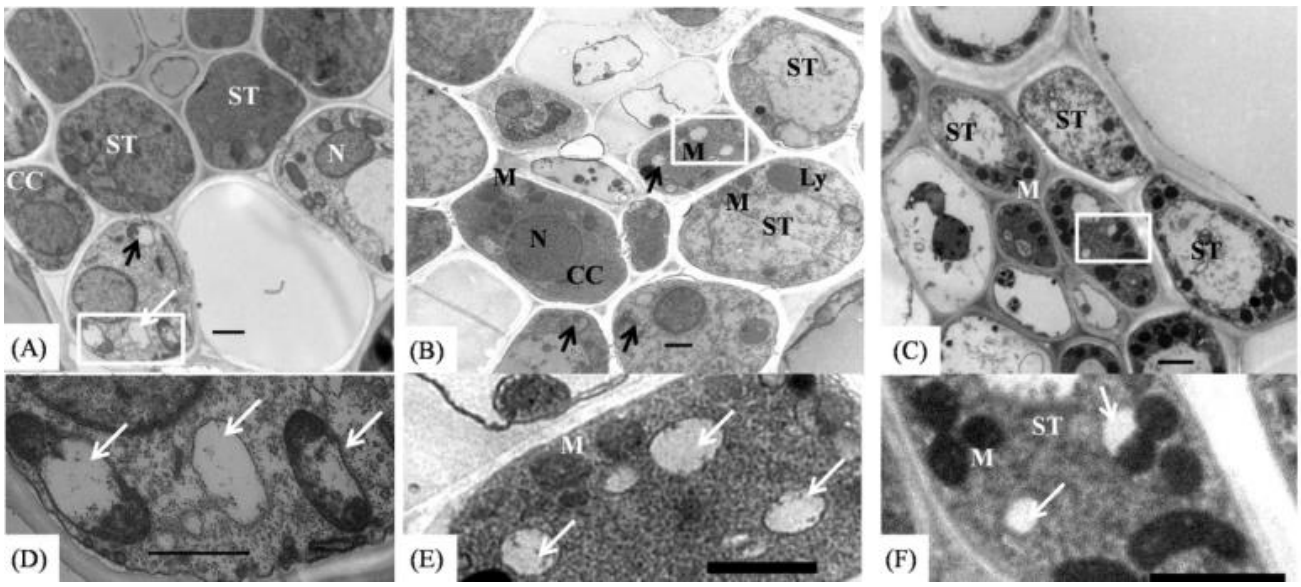

Fig. 3. Electron micrographs of vascular tissues in the 5th leaf on the main stem of rice sprayed with or without proline under 50 mM NaCl stress. (A) 50 mM NaCl without proline. (B) 50 mM NaCl with 15mM proline. (C) 50 mM NaCl with 30 mM proline. Three days later, samples for leaf ultrastructure were collected. Figures D, E, F were enlargement of the rectangular area in samples (A, B and C). Abbreviation: CC, companion cell; ST, sieve tube; N, nucleus; M, mitochondria; Ve, vesicles. Arrow, damaged or disappeared mitochondria; Bar, 1μm.

However, such deterioration was largely alleviated by exogenous proline. Exogenous proline inhibited the swelling of chloroplast and maintained a well-preserved internal lamellar system in the chloroplasts of rice leaves under 50mM and 100mM NaCl stress (Fig. 1E, 1F, 1H, and 1I), but increased the number of osmiophilic plastoglobuli under 50mM NaCl stress (Fig. 1E and 1F). Compared with control group, only 50 mM NaCl without proline increased the total starch area, but salt stress with proline did not significantly change the total starch area in chloroplast (Table 3). The integrity and orderly arrangement of chloroplasts contribute to the conversion of light energy in photosynthesis. According to the findings of Yamane *et al*. (2003 & 2004), salt stress-induced ion toxicity or ionic imbalance results in prominent swelling of thylakoids and induced oxidative stress leads to the changes in thylakoid membrane property. Light plays an important role in salt-induced chloroplast damage, while H_2O_2 and ·OH induced by salt stress lead to alteration of chlorophyll content and chloroplast ultrastructure (Yamane *et al*., 2004). The protective role of proline on chloroplast ultrastructure may be derived from three aspects: firstly, exogenous proline reduced apoplastic flow to suppress sodium ions uptake in rice plants (Sobahan *et al*., 2009), which alleviated the ion toxicity or ionic imbalance of salt stress. Secondly, exogenous proline activated an anti-oxidative defense system that counteracts the deleterious effects of reactive oxygen species by either direct elimination free radicals or by activation of antioxidant systems, which was well reviewed by Rejeb *et al*. (2014). Thirdly, it may be that proline provides more energy by its oxidation process, a kind of exogenous energy which promotes plants can that 'afford' energy-consuming protective mechanisms. When compared with stressed plants that lack energy due to weaker photosynthesis performance, plants with supplemental proline can invest more energy into the synthesis of such compounds that required in the protection mechanisms.

The sieve tube and companion cell in non-stressed seedlings possessed plenty of mitochondria (Fig. 2A), and some loss of cristae can be occasionally observed in mitochondria (Fig. 2D), which may be due to Tween solution detergent treatment. Exogenous proline seems not to alter vascular tissue, even though some mitochondria of companion cells were deficient in cristae or disappeared, especially with 30mM proline (Fig. 2B, C, E, and F). Vascular bundles may be affected first by salt stress as NaCl is transported primarily through vascular tissues. Compared with control, mitochondria of companion cells were deficient in cristae, swelled and often disappeared in the vascular tissue of rice under salt stress (Fig. 3A, 3D, 4A and 4D), which was not consistent with previous studies according to which vascular tissues were not severely damaged as mesophyll cells (Miyake *et al*., 2005). Exogenous proline apparently increased the number of mitochondria in sieve tube and companion cells under salt stress. It appears that 30 mM proline was more efficient than 15mM proline in protecting the mitochondria under salt stress (Fig. 3B, 3C, 4B, and 4C). The role of mitochondria in the sieve tube and companion cells is not precisely known. Gallé *et al*. (2015) reported that electrical signaling within the elongated sieve tubes, which considered as the best pathways of electrical communication between organs and also affects assimilate transport. Thus, plenty of mitochondria with integrity structure observed in this study may be involved in the electrical signal transport and provides sufficient energy for the transport of assimilation in vascular tissues.

Table 3. Effects of exogenous proline on the chloroplast shape and total starch area of a mesophyll cell in leaves of rice under normal and salt conditions. Thirty-five days old seedlings (4-leaf stage) exposed to different saline soil stress for 20 days and then sprayed with 10 ml exogenous proline per plant once per day for two days. Three days later, samples were collected.

Treatment			Chloroplast length CL (μm)	Chloroplast width CW (μm)	CL/CW	Total starch area (μm^2)	Swelling extent of chloroplast SEC
NaCl (mM)	Proline (mM)						
0	0		4.689 ± 0.980a	2.093 ± 0.718bc	2.399 ± 0.628a	0.119 ± 0.259c	0.000
	15		4.761 ± 1.041a	2.166 ± 0.531abc	2.249 ± 0.43ab	0.129 ± 0.112c	0.063
	30		4.896 ± 1.151a	2.356 ± 0.647ab	2.186 ± 0.705ab	0.575 ± 0.778a	0.089
50	0		3.460 ± 1.048b	1.890 ± 0.650c	1.931 ± 0.604cd	0.414 ± 0.634b	0.195
	15		3.896 ± 0.844b	1.954 ± 0.475c	2.042 ± 0.381bc	0.019 ± 0.044c	0.149
	30		4.475 ± 0.782a	2.415 ± 0.726a	2.004 ±0.711bc	0.093 ± 0.144c	0.165
100	0		3.553 ± 0.750b	2.147 ± 0.534abc	1.729 ± 0.497d	0.023 ± 0.039c	0.279
	15		3.747 ± 1.357b	1.939 ± 0.750c	2.008 ± 0.452bc	0.085 ± 0.156c	0.163
	30		3.744 ± 0.750b	2.036 ± 0.424c	1.870 ± 0.334cd	0.062 ± 0.119c	0.221
Statistical analysis		**df**	**Means of square**				
Salt		2	28.621**	0.837^{ns}	6.277**	0.402**	--
Proline		2	1.954^{ns}	2.596**	0.272^{ns}	0.252**	--
Salt×Proline		4	11.466**	5.538**	0.613^{ns}	0.318**	--
Error		408	1.093	0.412	0.277	0.028	--

$$SEC=\left(1-\frac{\text{Average of length to width ratio of chloroplast under treatment}}{\text{Average of length to width ratio of chloroplast under non-treatment control}}\right)\times 100\%$$

*Significance levels at $p<0.05$, ** significance levels at $p<0.01$, ^{ns} not significant

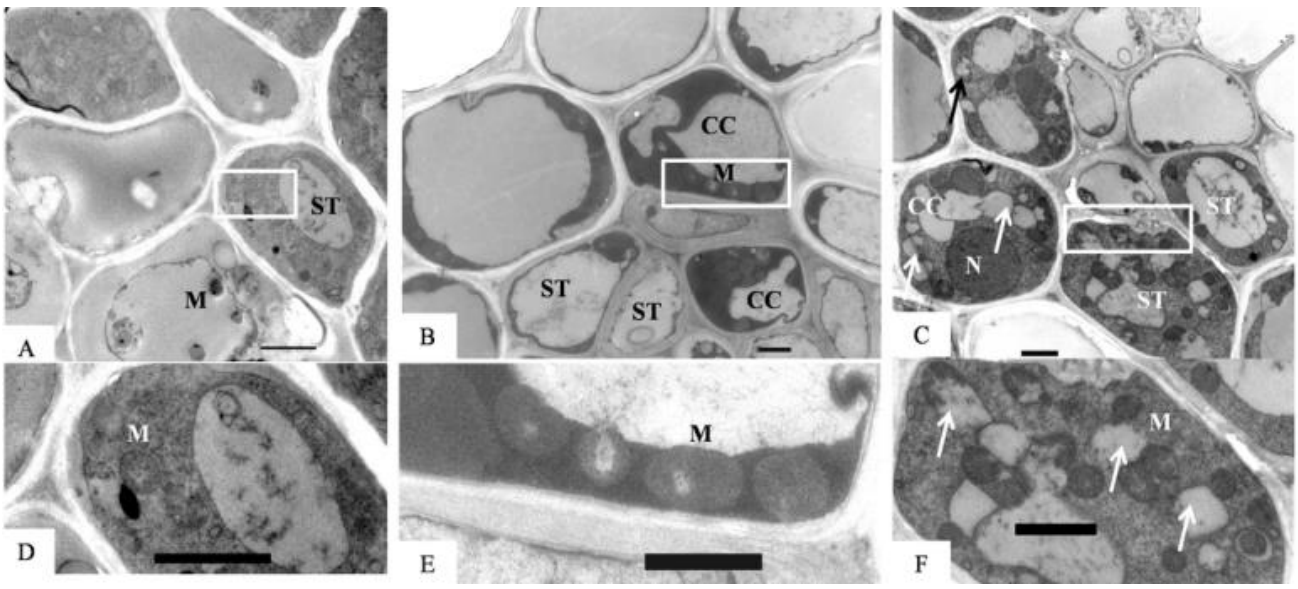

Fig. 4. Electron micrographs of vascular tissues in the 5th leaf on the main stem of rice sprayed with or without proline under 100 mM NaCl stress. (A) 100 mM NaCl without proline. (B) 100 mM NaCl with 15mM proline. (C) 100 mM NaCl with 30 mM proline. Three days later, samples for leaf ultrastructure were collected. Figures D, E, F were enlargement of the rectangular area in samples (A, B and C). Abbreviation: CC, companion cell; ST, sieve tube; N, nucleus; M, mitochondria; Ve, vesicles. Arrow, damaged or disappeared mitochondria; Bar, 1μm.

Conclusion

Our present work demonstrated that the ultrastructure of rice leaves under salt stress was obviously changed. Exogenous proline has a concentration-dependent effect on the chloroplast ultrastructure of rice under normal conditions (i.e., high concentration up to 30 mM) induced toxicity at the ultrastructural level, but does not apparently change the vascular tissue. Exogenous proline at both concentrations alleviated the deterioration effect of salt stress on the ultrastructure of chloroplast and vascular tissue. The role of 30 mM proline in protecting the mitochondria under salt stress was greater than 15 mM proline.

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Reference

Agami, R.A. 2014. Applications of ascorbic acid or proline increase resistance to salt stress in barley seedlings. *Biol. Plant.*, 58(2): 341-347.

Ahmed, C.B., S. Magdich, B.B. Rouina, S. Sensoy, M. Boukhris and F.B. Abdullah. 2011. Exogenous proline effects on

water relations and ions contents in leaves and roots of young olive. *Amino Acids*, 40(2): 565-573.

Ashraf, M. and M.R. Foolad. 2007. Roles of glycinebetaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot*., 59(2): 206-216.

Athar, H.R., M. Ashraf, A. Wahid and A. Jamil. 2009. Inducing salt tolerance in canola (*Brassica Napus* L.) by exogenous application of glycinebetaine and proline: response at the initial growth stages. *Pak. J. Bot*., 41(3): 1311-1319.

Bhusan, D., D.K. Das, M. Hossain, Y. Murata and M.A. Hoque. 2016. Improvement of salt tolerance in rice (*Oryza sativa* L.) by increasing antioxidant defense systems using exogenous application of proline. *Aust. J. Crop Sci*., 10(1): 50-56.

Dawood, M.G., H.A.A. Taie, R.M.A. Nassar, M.T. Abdelhamid and U. Schmidhalter. 2014. The changes induced in the physiological, biochemical and anatomical characteristics of Vicia faba by the exogenous application of proline under seawater stress. *S. Afr. J. Bot.*, 93: 54-63.

Gallé, A., S. Lautner, J. Flexas and J. Fromm. 2015. Environmental stimuli and physiological responses: The current view on electrical signalling. *Environ. Exp. Bot*., 114: 15-21.

Hare, P.D., W.A. Cress, J. van Staden and C.E.J. Botha. 2002. Disruptive effects of exogenous proline on chloroplast and mitochondrial ultrastructure in arabidopsis leaves. *S. Afr. J. Bot.*, 68(3): 393-396.

Hayat, S., Q. Hayat, M.N. Alyemeni, A.S. Wani, J. Pichtel and A. Ahmad. 2012. Role of proline under changing environments: a review. *Plant Signal. Behav*., 7(11): 1456-1466.

Heuer, B. 2003. Influence of exogenous application of proline and glycinebetaine on growth of salt-stressed tomato plants. *Plant Sci*., 165: 693-699.

Hoque, M.A., M.N. Banu, Y. Nakamura, Y. Shimoishi and Y. Murata. 2008. Proline and glycinebetaine enhance antioxidant defense and methylglyoxal detoxification systems and reduce NaCl-induced damage in cultured tobacco cells. *J. Plant Physiol*., 165(8): 813-824.

Kaur, G. and B. Asthir. 2015. Proline: a key player in plant abiotic stress tolerance. *Biol. Plant.*, 59(4): 609-619.

Lehmann, S., D. Funck, L. Szabados and D. Rentsch. 2010. Proline metabolism and transport in plant development. *Amino acids*, 39(4): 949-962.

Miyake, H., S. Mitsuya and M.S. Rahman. 2005. Ultrastructural effects of salinity stress in higher plants. *In:* A. K. Rai and T. Takabe (Eds.) *Abiotic stress tolerance in plants: Toward the improvement of global environment and food*. Springer, The Netherlands, pp. 215-226.

Nounjan, N. and P. Theerakulpisut. 2012. Effects of exogenous proline and trehalose on physiological responses in rice seedlings during salt-stress and after recovery. *Plant Soil Environ*., 58: 309-315.

Rahman, S., T. Matsumuro, H. Miyake and Y. Takeoka. 2000. Salinity-induced ultrastructural alterations in leaf cells of rice (*Oryza sativa* L.). *Plant Prod. Sci*., 3(4): 422-429.

Rejeb, K.B., C. Abdelly and A. Savoure. 2014. How reactive oxygen species and proline face stress together. *Plant Physiol. Biochem*., 80: 278-284.

Sha, H.J., H.L. Liu, J.G. Wang, L. Yang, Y. Liu, D.T. Zou and H.W. Zhao. 2013. Effect of exogenous proline on growth of salt-stressed rice at tillering stage. *Res. Agric. Mod*., 34: 230-234.

Shanker, A.K. and B. Venkateswarlu. (Ed.) 2011. *Abiotic stress in plants - mechanisms and adaptations*. InTech Publishers, Rijeka, Croatia.

Singh, M., J. Kumar, S. Singh, V.P. Singh and S.M. Prasad. 2015. Roles of osmoprotectants in improving salinity and drought tolerance in plants: a review. *Rev. Environ. Sci. Biotechnol*., 14(3): 407-426.

Sobahan, M.A., C.R. Arias, E. Okuma, Y. Shimoishi, Y. Nakamura, Y. Hirai, I.C. Mori and Y. Murata. 2009. Exogenous proline and glycinebetaine suppress apoplastic flow to reduce Na^+ uptake in rice seedlings. *Biosci. Biotechnol. Biochem*., 73(9): 2037-2042.

Szabados, L. and A. Savoure. 2010. Proline: A multifunctional amino acid. *Trends Plant Sci*., 15(2): 89-97.

Teh, C.-Y., N.A. Shaharuddin, C.-L. Ho and M. Mahmood. 2016. Exogenous proline significantly affects the plant growth and nitrogen assimilation enzymes activities in rice (*Oryza sativa*) under salt stress. *Acta Physiol. Plant*., 38:151.

Verbruggen, N. and C. Hermans. 2008. Proline accumulation in plants: A review. *Amino acids*, 35(4): 753-759.

Xing, W., J. Wang, H. Liu, D. Zou and H. Zhao. 2013. Influence of natural saline-alkali stress on chlorophyll content and chloroplast ultrastructure of two contrasting rice (*Oryza sativa* L. *Japonica*). *Aust. J. Crop Sci*., 7(2): 289-292.

Yamane, K., M. Kawasaki, M. Taniguchi and H. Miyake. 2003. Differential effect of NaCl and polyethylene glycol on the ultrastructure of chloroplasts in rice seedlings. *J. Plant Physiol*., 160(5): 573-575.

Yamane, K., M. Kawasaki, M. Taniguchi and H. Miyake. 2008. Correlation between chloroplast ultrastructure and chlorophyll fluorescence characteristics in the leaves of rice (*Oryza sativa* L.) grown under salinity. *Plant Prod. Sci*., 11(1): 139-145.

Yamane, K., S. Rahman, M. Kawasaki, M. Taniguchi and H. Miyake. 2004. Pretreatment with antioxidants decreases the effects of salt stress on chloroplast ultrastructure in rice leaf segments (*Oryza sativa* L.). *Plant Prod. Sci*., 7(3): 292-300.

Yan, Z., S. Guo, S. Shu, J. Sun and T. Tezuka. 2011. Effects of proline on photosynthesis, root reactive oxygen species (ROS) metabolism in two melon cultivars (*Cucumis melo* L.) under NaCl stress. *Afr. J. Biotechnol*., 10(80): 18381-18390.

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