S Nutrition Is Involved in Alleviation of Damage of Photosynthetic Organelles by Salt Stress in Kentucky Bluegrass (*Poa pratensis* L.)

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S nutrition is involved in alleviation of damage of photosynthetic organelles by salt stress in Kentucky Bluegrass (*Poa pratensis* L.)

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Introduction:

Salt-stress is considered as one of the major environmental factor limiting plant growth and productivity. It has been well reported that salt stress induce the reduction of stomatal density and number leading to poor gaseous exchange which resulted in decrease of photosynthesis is associated with inhibition of several enzymes related to the Calvin cycle such as RuBisCo. In addition, salt stress decreases photosynthetic pigments such as chlorophyll and carotenoid which has important role in photosynthesis.

Sulfur (S) is one of six macronutrients needed for proper plant growth and development. In our previous work, we found that sulfur nutrition has significant role in ameliorating the damaged in photosynthetic organelles caused by Fe-deficiency in oilseed rape (Muneer *et al*., 2014). In addition, application of sulfur mitigated the adverse effects of heavy metals stress by enhancing plant growth, chlorophyll content and net photosynthetic rate. Despite extensive researches attempting to elucidate the interactions between external sulfur supplies and stress tolerance, to our knowledge, the responses of the photosynthetic mechanism to combined S deficiency and salt stress have not yet been fully investigated.

In this study, therefore, we hypothesized that S nutrition affects photosynthetic organs to salt stress, so that may involve in alleviating negative impact of salt stress in Kentucky bluegrass. To test this hypothesis, the responses of photosynthetic parameters, thylakoid protein complexes and ion uptake were compared for 21 days of four S and salt stress combined treatments; sulfur sufficient without salt stress (+S/non-salt, control), present of sulfur with salt stress (+S/salt), sulfur deprivation without salt stress (-S/non-salt) and sulfur deprivation and salt stress (-S/salt).

Materials and Methods

Plant culture and sampling: The experimental plant, Kentucky bluegrass (*Poa pratensis* L.) were taken from the local golf course and were transferred to mixture of soil and sand (50 : 50, v/v), and divided in to 2 groups; one group was supplied with complete nutrient solution, and another set of plant were supplied with S-free nutrient solution during 4 weeks. After 4 weeks of S-treatment, each of S-supplied or S-deprived plants were exposed to salt stress with 100 mM NaCl or non-salt stress, respectively, for 3 weeks. Four groups of treatment thus were designated as sufficient S without salt stress (+S/non-salt, control), sufficient S with salt stress (+S/salt), deprived S without salt stress (-S/non-salt), and deprived S with salt stress (-S/salt) with three replicates. The samplings were done at 0, 7, 14 and 21 days of salt stress.

Measurements: The content of chlorophyll and carotenoid was estimated by the method of Hiscox and Israelstam (1979). Ribulrose-1, 5-bisphosphate carboxylase / oxygenase (RuBisCo) activity was determined spectrophotometrically by monitoring NADH oxidation at 340 nm. BN-PAGE of integral thylakoid proteins was performed according to Kügler *et al*. (1997).

Results and Discussion

Salt stress more quickly (*i.e*. from day 7) reduced chlorophyll and carotenoid content both in the presence and absence of S (Fig.1). However, at day 21, the extent of the decrease in chlorophyll and carotenoid content by salt stress was much higher in the absence of S (-50% and -42%, respectively, compared to control) than in the presence of S (-28% and -23%) (Fig. 1). This result can be explained by the S-demand for taking part in building chlorophyll content and regulation of protein synthesis, since S is an integral constituent of S-rich amino acids, cysteine and methionine, which act as structural and functional elements of proteins. Similarly, Juszczuk and Ostaszewska (2011) reported that a strong correlation between chlorophyll content and S concentration was observed in chlorotic bean leaves. In recent our works, an additional loss of photosynthetic pigments in Fe-deprived leaves was observed under S-deficient condition (Muneer *et al*., 2014).

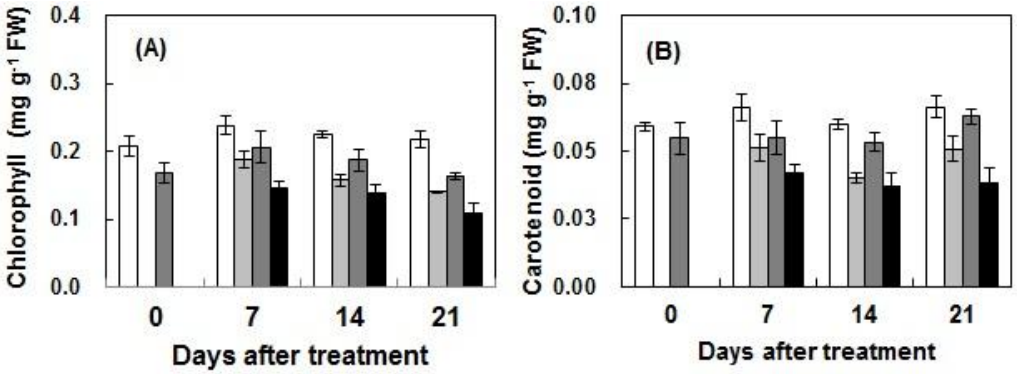

Fig 1: Changes in photosynthetic pigments such as total chlorophyll (A) and (B) carotenoid of four sulfur and salt stress combined treatments: sulfur sufficient (+S/non-salt, control white bar), presence of sulfur with salt stress (+S/salt light grey bar), absence of sulfur without salt stress (-S/non-salt dark grey bar) and absence of sulfur with salt stress (-S/salt black bar) in Kentucky bluegrass for 21 days. Each value is the mean ± SE for n = 3.

In this study BN-PAGE was used as a proteomic tool to better define the change in thylakoidal multi-protein complexes to salt stress and understand the significance of S. The Kentucky bluegrass BN gel profile showed an interesting band identified as sub-complexes of Photosystem (PS) I, PSII and RuBisCo at 21 days (Fig. 2A). Salt stress showed a considerable impact on the proteomic structure of photosystems in Kentucky bluegrass. Under salt-stressed condition, Kentucky bluegrass showed a considerable reduction of multi-protein complexes, such as PSI, PSII and cytochrome *b6/f* and RuBisCo band volumes. PSII core dimer and PSI (RCI-LHCI) complex bands were more affected. It was widely reported that salt stress affected the functionality of both PSII and PSI which has role of electron transport (Akram and Ashraf, 2011). On the other hand, the losses of most thylakoidal protein complexes under salt stress in the absence of S were largely restored in the presence of S (+S/salt) (Fig. 2A). RuBisCo activity was decreased both by S-nutrition and salt stress from day 7 (Fig. 2B). Sulfur-deprivation for 4 weeks before salt stress (*i.e.* day 0) resulted in a reduction of RuBisCo activity (-43.7%). At day 21, salt stress in the absence of S (-S/salt) decreased RuBisCo activity up to 80% compared to control, while much lower (62%) in the presence of S (+S/salt). It has been reported that S nutrition alleviates the negative responses of RuBisCo and photosynthesis activity to Fe-deficiency (Muneer *et al*. 2014)

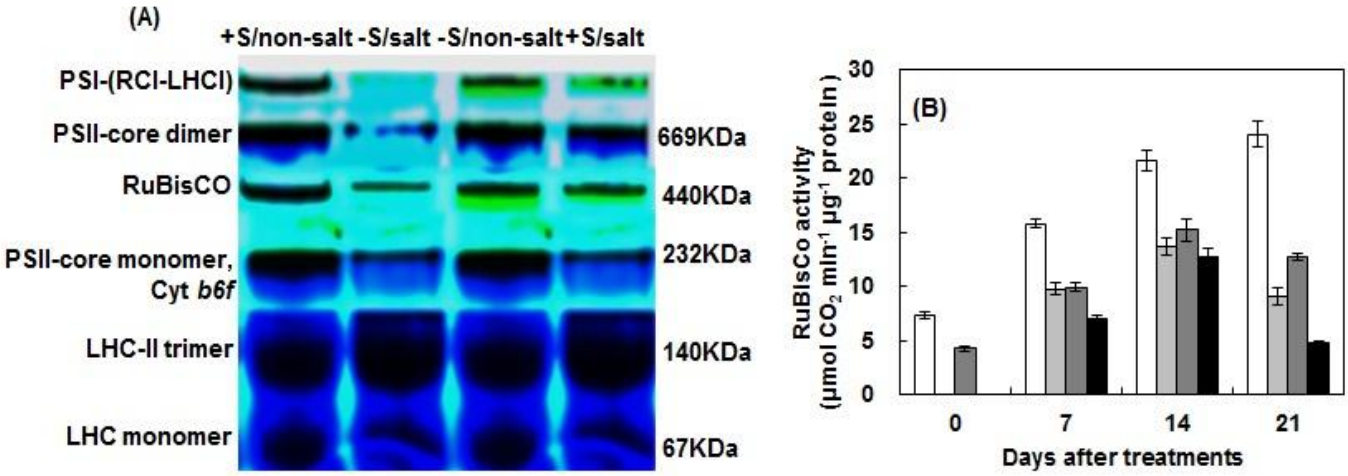

Fig 2: Analysis of thylakoid protein complex by BN-PAGE (A) at 21 day and changes in enzyme activity of RuBisCo (B) of four sulfur and salt stressed combined treatments: sulfur sufficient (+S/non-salt, control white bar), presence of sulfur with salt stress (+S/salt light grey bar), absence of sulfur without salt stress (-S/non-salt dark grey bar) and absence of sulfur with salt stress (-S/salt black bar) in Kentucky bluegrass. Freshly thylakoid membranes from mature leaves were solubilized in 2 % BDM at chlorophyll concentration of 1µg µl^{-1}, and the protein sample separated by 7-10 % gradient BN-PAGE. Each value is the mean ± SE for n = 3.

Conclusion

The decrease of photosynthetic pigments occurred in the absence of S with the highest loss of thylakoid multi-protein complexes, mainly PSI (RCI-LHCI) complex protein, PSII core dimer and RuBisCo. These negative responses to salt stress were significantly recovered by S nutrition. It thus concludes that S nutrition involves in tolerance mechanism by alleviating the salt stress-induced damages in photosynthetic organelles.

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