Octa Journal of Environmental Research ***Apr. – Jun., 2015***
International Peer-Reviewed Journal ISSN 2321 3655
Oct. Jour. Env. Res. Vol. 3(2): 163-166
Available online http://www.sciencebeingjournal.com

Research Article

Octa Journal of Environmental Research
(Oct. Jour. Env. Res.) ISSN: 2321-3655
Journal Homepage: http://www.sciencebeingjournal.com

Scientific Planet Society

INFLUENCE OF NACL SALINITY ON THE ACTIVITY OF RIBULOSE 1, 5-BISPHOSPHATE CARBOXYLASE IN THREE LEAFY VEGETABLES

Anjali Ratnakar[a] and Aruna Rai[b]
a. Department of Botany, K. J. Somaiya College of Science and Commerce, Vidyavihar, Mumbai-400077.
b. Department of Botany, Smt. C. H. M. College, Ulhasnagar-421003, Thane, Maharashtra.
*Corresponding author's E-mail: **aru_r17@hotmail.com**
Received: 19th June 2015 **Revised**: 25th June 2015 **Accepted**: 30 June 2015

Abstract: Salinity is one of the major factors which have an adverse effect on crop growth. Stress caused due to excessive accumulation of salts results in various physiological and biochemical changes in plants. Due to their nutritional value, leafy vegetables constitute an important part of our daily diet. In the present investigation, effect of NaCl salinity on the activity of ribulose 1,5–bisphosphate carboxylase in three leafy vegetables was studied. Seeds of leafy vegetables were sown in earthen pots and were subjected to different levels of saline water (NaCl) treatment. Control plants were irrigated with tap water. Treatments started after the seedling emergence and continued till the plants were 45 day old. Mature leaves of these plants were harvested and used for the studies. The activity of ribulose 1,5-bisphosphate in all the three leafy vegetables was observed to decrease gradually with increase in the concentration of NaCl in the growth medium.
Keywords: Leafy vegetables; Ribulose 1,5–Bisphosphate Carboxylase; Salinity.
Postal Address: 1704, 2C, Dreams, L.B.S. Marg, Bhandup (W), Mumbai 400078, Maharashtra, India, Ph-09821774412

INTRODUCTION

Abiotic stress is the principal cause of crop failure, decreasing the average yields of most major crops by more than 50 % and threatening the sustainability of agriculture worldwide (Galmes *et al*., 2013). Photosynthesis is the source of energy which sustains life on earth. It is the most severely affected process under various abiotic stresses including salt stress (Stoeva and Kaymakanova, 2008). Abiotic stresses interfere with photosynthesis at various points such as carbon dioxide diffusion, photosystem II (PS II) efficiency, electron transport, formation of reactive oxygen species, ribulose 1,5-bisphosphate content, activity of enzyme ribulose 1,5–bisphosphate carboxylase/ oxygenase and photorespiration (Saibo *et al*., 2009). Abiotic stresses can negatively affect Calvin cycle, by reducing the concentration and the activity of enzymes involved in the photosynthetic reduction of carbon, including ribulose 1,5-bisphosphate carboxylase thereby limiting carbon dioxide assimilation (Saibo *et al*., 2009). The plants undergoing saline stress, synthesize certain organic solutes in the cytoplasm which serve as osmoprotectants, such as sugars, glycine betaine and proline. The synthesis of these organic solutes require source of carbon and energy which is derived mainly from photosynthesis (Heuer, 2005). Ribulose 1,5-bisphosphate carboxylase/ oxygenase (E.C. 4.1.1.39) is the most abundant protein in plants (Sharkey *et al*., 1991). It is about 30 percent of total proteins distributed in green leaves (Parry *et al*., 2003; Sinha, 2004). Ribulose 1,5-bisphosphate carboxylase is the main enzyme in plants, responsible for the initial step in the C_3 photosynthetic carbon reduction cycle (Heuer, 2005). It is a unique enzyme catalyzing carboxylation of ribulose 1,5-bisphosphate to form two molecules of phosphoglyceric acid and oxidation of ribulose 1,5-bisphosphate to form phosphoglyceric acid and phosphoglycolic acid. The latter is metabolized via photorespiratory cycle. Thus, this enzyme has a dual role of carboxylation as well as oxidation. Carboxylase/oxygenase property of the enzyme is regulated by carbon dioxide/oxygen ratio in the atmosphere. If this ratio is high carboxylase activity is favoured, whereas low ratio favours oxygenase activity (Sinha, 2004). Ribulose 1,5-bisphosphate carboxylase catalyses the covalent attachment of carbon dioxide to the five carbon sugar ribulose 1,5-bisphosphate and cleaves the unstable 6-C intermediate (2-carboxy-3-keto-arabinitol-1,5-bisphosphate) to form two molecules of 3-phosphoglycerate, one of which bears the carbon introduced as carbon dioxide in its carboxyl group.

In the present study variations in the activity of ribulose 1,5-bisphosphate carboxylase under the influence of various salinity levels were observed in three leafy vegetables

EXPERIMENTAL

Source of plant material: Seeds of the three leafy vegetables, *Amaranthus polygamous* L. var. Pusa Kirti, *Spinacia oleracea* L. var. All Green and *Trigonella foenum-graecum* L. var. PEB were obtained from the Indian Agricultural Research Institute, Pusa (New Delhi).

Salinity treatment to plants: To obtain the material for physiological studies, morphologically uniform seeds of *Amaranthus polygamous*, *Spinacia oleracea* and *Trigonella foenum-graecum* were soaked separately in tap water for six hours. The seeds were then sown in earthen pots of 20 cm diameter and 29 cm height containing about 3 kg soil:manure mixture in 3:1 proportion. The pots were watered with tap water everyday till the fourth day. At this stage, thinning of seedlings was done so as to maintain uniform spacing amongst them. These pots were divided in four sets. One set of pots, watered with tap water, served as control. Remaining three sets of pots were watered with 20 mM, 40 mM and 60 mM NaCl solutions respectively. In order to acclimatize the plants, the concentrations of NaCl were raised every 3-4 days in a step-wise manner. The plants were grown for forty five days (Photoplates 1, 2 and 3). The leaves of forty-five day old plants were used for the studies.

Ribulose 1,5-bisphosphate carboxylase (E.C. 4.1.1.39): The activity of enzyme ribulose 1,5-bisphosphate carboxylase was estimated according to the method of Kluge and Osmond (1972). Soluble protein content of the enzyme source was estimated by the method of Lowry *et al*. (1951). Activity of enzyme is expressed as mM NADH oxidized/mg protein/min. F test was used to test for the statistical significance and Student's *t*-test was use to compare the treatment means.

RESULTS AND DISCUSSION

It is evident from table 1, that the activity of enzyme ribulose 1,5-bisphosphate carboxylase has been affected adversely in all the selected leafy vegetables under the influence of various NaCl concentrations. In *Amaranthus polygamous* leaves, the activity of enzyme ribulose 1,5-bisphosphate carboxylase reduced by 84 percent at 60 mM NaCl concentration (0.016 mM NADH oxidized/mg protein/min) as compared to control (0.106 mM NADH oxidized/mg protein/min). In the leaves of *Spinacia oleracea*, the activity of enzyme ribulose 1,5-bisphosphate carboxylase was observed to decrease gradually, reaching the final reduction of about 27 percent at 60 mM NaCl concentration (0.064 mM NADH oxidized/mg protein/min) as compared to control (0.088 mM NADH oxidized/mg protein/min). A reduction in the activity of enzyme ribulose 1,5-bisphosphate carboxylase by about 16 percent was observed in *Trigonella foenum-graecum* at 60 mM NaCl concentration (0.021 mM NADH oxidized/mg protein/min) as compared to control (0.025 mM NADH oxidized/mg protein/min). Significant decrease in the activity of ribulose 1,5-bisphosphate carboxylase has been reported in *Vigna sinensis* under saline conditions (Nemat Alla *et al*., 2001). Similarly, Desingh and Kanagaraj (2007) recorded a decrease in photosynthetic rate and ribulose 1,5-bisphosphate carboxylase activity at a concentration of 150 mM NaCl in a cotton variety 'Arya-Anubam'. Garg and Singla

(2004) observed a reduction in leaf chlorophyll content as well as an inhibition of ribulose 1,5-bisphosphate carboxylase activity in chickpea under varying levels of NaCl salinity, suggesting that the magnitude of ribulose 1,5-bisphosphate carboxylase inhibition was dependent on salt concentration.

Table 1. Effect of varying concentrations of NaCl on activity of ribulose 1,5-bisphosphate carboxylase in leaves of vegetables

NaCl Conc. (mM)	Activity of enzyme ribulose 1,5-bisphosphate carboxylase (mM NADH oxidized/mg protein/min)		
	***Amaranthus polygamous* •**	***Spinacia oleracea* ***	***Trigonella foenum-graecum* ***
0	0.106 ± 0.007	0.088 ± 0.004	0.025 ± 0.000
20	0.060 ± 0.002	0.085 ± 0.005	0.024 ± 0.001
40	0.038 ± 0.001	0.067 ± 0.000	0.022 ± 0.002
60	0.016 ± 0.001	0.064 ± 0.007	0.021 ± 0.003

Results are the mean of three determinants.
* One-way ANOVA was carried out and the F ratio was significant at 5% level of significance.
• No significant difference was observed in the group, so data is not analyzed further for pair-wise comparisons among the treatments.

Figure 1. *Amaranthus polygamous* grown at different levels of salinity

Figure 2. *Spinacia oleracea* grown at different levels of salinity

Figure 3. *Trigonella foenum-graecum* grown at different levels of salinity

According to Seemann and Sharkey (1986), salinity reduces the capacity for the synthesis of adenosine triphosphate and hence, under saline conditions, ribulose 1,5-bisphosphate pool size is generally lowered. They recorded a lower rate of photosynthesis in the leaflets of salinised plants of *Phaseolus vulgaris* as compared to non-salinised ones. Even when ribulose 1,5-bisphosphate pool size became equal, a difference was observed in the photosynthetic capacity. They suggested that this reduction in photosynthetic capacity must be due to a reduction in the activity of ribulose 1,5- bisphosphate carboxylase under saline environment. Khodary (2004) also reported a significant reduction in the activity of enzyme ribulose 1,5-bisphosphate carboxylase and photosynthetic activity in salinised maize plants. Seemann and Critchley (1985) observed that in the leaves of *Phaseolus vulgaris*, with increasing leaf chloride concentration, the efficiency rather than concentration of ribulose 1,5-bisphosphate carboxylase reduces, leading to decrease in the photosynthesis with increasing salinity. Kafi (2009) while experimenting with *Triticum aestivum* with NaCl and $CaCl_2$ salt treatments recorded a decrease in carbon dioxide assimilation rate with increasing concentrations of salts. He reported a decrease in stomatal conductance as well as the rate of photosynthesis in *Triticum aestivum* on long term exposure to salinity. According to Shafi *et al*. (2010), under saline conditions, net photosynthetic carbon dioxide uptake decreases mainly because sodium chloride treatment decreases the stomatal conductance and as a result less carbon dioxide is available for carboxylation reaction. They also recorded a decrease in the rate of ribulose 1,5-bisphosphate carboxylase/oxygenase activity under NaCl salinity.

CONCLUSION

As the enzyme ribulose 1,5-bisphosphate carboxylase is the enzyme of photosynthetic activity, the adverse effect on the enzyme will influence the organic matter production leading to reduced yield. Based on the findings of present study, it can be said that all the three leafy vegetables are sensitive to salinity.

REFERENCES

Desingh, R., Kanagaraj, G. (2007). Influence of salinity stress on photosynthesis and antioxidative systems in two cotton varieties. *General and Applied Plant Physiology*, 33(3-4) 221-234.

Galmes, J., Aranjuelo, I., Medrano , H. and Flexas, J. (2013). Variation in Rubisco content and activity under variable climatic factors. Photosynthesis Research, Springer Science + Business Media Dordrecht.

Garg, N., Singla, R. (2004). Growth, photosynthesis, nodule nitrogen and carbon fixation in the chickpea cultivars under salt stress. *Brazilian Journal of Plant Physiology*, 16(3):137-146.

Heuer, B. (2005). Photosynthetic carbon metabolism of crops under salt stress. *In*: Handbook of Photosynthesis. (2nd Edition) Taylor and Francis Group, LLC. U.S.

Kafi, M. (2009). The effects of salinity and light on photosynthesis, respiration and chlorophyll fluorescence in salt-tolerant and salt-sensitive wheat (*Triticum aestivum* L.) cultivars. *Journal of Agriculture, Science and Technology*, 11: 535-547.

Khodary, S. E. A. (2004). Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt stressed maize plants. International Journal of Agriculture and Biology, 6(1): 05-08.

Kluge, M., Osmond, C. B. (1972). Studies on phosphoenol-pyruvate carboxylase and other enzymes of crassulacean acid metabolism of *Bryophyllum tubiflorum* and *Sedum pracaltum*. Z. Pflanzenphysiology, 66: 97-105.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951). Protein measurement with Folin Reagent. *Journal of Biological Chemistry*, 193: 265-275.

Nemat Alla, M. M., Younis, M. E., El-shihaby, O. A., El-Bastawisy, Z. M. (2001). Effect of kinetin on photosynthetic activity and carbohydrate content in waterlogged or seawater treated Vigna sinensis and *Zea mays*. *Journal of Biological Sciences*, 1(10): 918-924.

Parry, M. A. J., Andralojc, P. J., Khan, S., Jea, P. J., Keys, A. J. (2002). Rubisco activity: Effects of drought stress. *Annals of Botany*, 89: 833-839.

Saibo, N. J. M., Lourenco, T., Oliveira, M. M. (2009). Transcription factors and regulation of photosynthetic and related metabolism under environmental stresses. *Annals of Botany*, 103: 609-623.

Seemann, J. R., Critchley, C. (1985). Effects of salt stress on the growth, ion content, stomatal behavior and photosynthetic capacity of a salt-sensitive species, *Phaseolus vulgaris* L. *Planta*, 164:151-162.

Seemann, J. R., Sharkey, T. D. (1986). Salinity and nitrogen effects on photosynthesis, ribulose 1,5-bisphospahte carboxylase and metabolite pool sizes in *Phaseolus vulgaris* L. *Plant Physiology*, 82: 555-560.

Shafi, M., Bakht, J., Khan, M. J., Khan, M. A., Anwar, S. (2010). Effect of salinity on yield and ion accumulation of wheat genotypes. Pakistan Journal of Botany, 42(6): 4113-4121.

Sharkey, T. D., Vassey, T. L., Vanderveer, P. J., Vierstra, R. D. (1991). Carbon metabolism enzymes and photosynthesis in transgenic tobacco (*Nicotiana tobaccum* L.) having excess phytochrome. *Planta*, 185: 287-296.

Sinha, R. K. (2004). Photosynthesis. *In*: Modern Plant Physiology. Narosa Publishing House. pp: 176-255.

Stoeva, N., Kayamakanova, M. (2008). Effect of salt stress on the growth and photosynthesis rate of bean plants (*Phaseolus vulgaris* L.). *Central European Journal of Agriculture*, 9(3): 385-392.

Source of Financial Support: Nil
Conflict of Interest: None. Declared.