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EFFECT OF SALT (NaCl) ON PHOTOSYNTHESIS AND CARBOHYDRATES IN LEAVES OF TWO RAGI (*ELEUSINE CORACANA* (L.) GAERTN) VARIETIES

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ABSTRACT

Salinity is one of the most widespread environmental threats to global crop production, especially in arid and semi-arid regions. Photosynthesis and carbohydrates were determined in two ragi (*Eleusine coracana* (L.) Gaertn) varieties (CO-13 and PAIYUR-1), subjected to salt stress of different concentrations (0, 40, 80 and 120mM). Salinity was given as a basal dose and sampling was done in leaves on 30th Days. After Treatment (DAT). There was a marked variation in the photosynthetic rates and ribulose-1, 5-bisphosphate carboxylase activity between the two ragi varieties subjected to salt stress. Photosystem II (PSII) and sucrose phosphate synthase activities were also significantly reduced as measured by salt stressed conditions. The quantity of glucose and sucrose decreased with increasing salt stress while starch showed a reverse trend under salt-stressed conditions. The results revealed that CO-13 exhibits higher photosynthetic rates and activities of ribulose-1,5-bisphosphate carboxylase, sucrose phosphate synthase with photochemical efficiency of PSII compared to PAIYUR-1

Keywords: Carbohydrates, photosynthesis, photosystem II, ragi, RuBP carboxylase, salt, sucrose phosphate synthase

INTRODUCTION

Agriculture is expanding into regions that are affected by salinity (Causin *et al.*, 2020). Soil salinity in agricultural areas is increasing world-wide due to irrigation with brackish water and to seawater encroachment on low-lying coastal regions and its impact on crop production is further increasing as the global demand for food means agriculture extends into naturally salt-affected lands (Munns *et al.*, 2020; El-Hendawy, *et al.*, 2021)). Photosynthesis is the single most vital metabolic processes that not only regulates plant growth and development, but also directly controls overall productivity (Hohner *et al.*, 2019). Soil salt prevents plants from taking up water, exposing them to drought stress. To conserve water, they close their stomata and this simultaneously restricts the entry of CO₂ into the leaf, reducing photosynthesis (Soda *et al.*, 2018). Higher photosynthetic rates would require an increase of RuBPCarboxylase activity, whereas lower photosynthetic rates would indicate a reduction of RuBPCarboxylase characteristics in salt-stressed leaves (Delfine *et al.*, 1999). In higher plants, salt stress inhibits PSII activity and was decreased in response to salt stress due to the dissociation of 23 kDa polypeptide extrinsically bound to PSII (Kao *et al.*, 2003). Khan (2003) found out that saline stress slows down the production of photosynthetic pigments. SPS is an important cytosolic enzyme that is known to control the flux of carbon fixation into sucrose and starch (Penella *et al.*, 2016). Sugars (i.e. starch and sucrose) are primary products of photosynthesis in higher plants (Lunn and Hatch, 1995) and are highly sensitive to environmental stresses and the major forms of carbohydrates. Starch is also emerging as a key molecule in mediating plant

responses to abiotic stresses, such as water deficit, high salinity or extreme temperatures (Thalman and Santelia, 2017). A better understanding of the mechanisms that enable plants to adapt to salt stress and maintain growth and productivity during the salt stressed conditions will ultimately help in the selection of salt tolerant varieties. Ragi (*Eleusine coracana* (L.) Gaertn) is an important minor cereal in India, rich in calcium, dietary fiber and known for its health benefits and salinity is a major factor limiting its productivity and hence the profitability of the farmers in India. The main objective of the present study was to assess the photosynthetic performance and carbohydrate levels in two different ragi varieties (CO-13 and PAIYUR-1) under salt-stressed conditions.

MATERIALS AND METHODS

The certified ragi (*Eleusine coracana* (L.) Gaertn) seeds (Varieties: CO-13 and PAIYUR-1) were procured from PASIC, Pondicherry. Seeds with uniform size were selected and the plants were raised in pots containing red and clay soil and pH of the soil was 7.2 with EC of 0.2 dsm⁻¹. After 20 days, seedlings were thinned and three plants of uniform vigor were maintained in each pot. Plants were grown under natural climatic conditions. Plants were watered for the first 20 days after germination. The seedlings were divided into four groups. One group of seedlings was maintained under non-salinized conditions which served as control plants. The watering solution for control plants consists of tap water and one-fourth strength of Hoagland nutrients (Hoagland and Arnon, 1950). Other three groups were salinized

Table 1. Influence of salt stress on rate of photosynthesis, activity of RuBP carboxylase and sucrose phosphate synthase, DCPIP reduction, chlorophyll content in two ragi varieties

Variety and parameters	Salinity treatments (mM)			
	control	40	80	120
Photosynthesis (nmolCO ₂ /gfw/s)				
CO-13	4.59	3.84	3.67	3.12
	±0.39	±0.28	±0.24	±0.21
PAIYUR-1	4.03	2.44	1.95	1.53
	±0.37	±0.12	±0.092	±0.054
RuBP carboxylase (µmolCO ₂ /mgprotein/min)				
CO-13	252.67	215.90	202.19	180.32
	±5.11	±4.98	±4.84	±4.66
PAIYUR-1	245.51	170.11	115.24	94.38
	±5.03	±4.37	±3.89	±3.18
Sucrose phosphate synthase (µmolUDP/mgprotein/min)				
CO-13	36.89	32.15	29.62	27.09
	±3.44	±3.36	±3.11	±3.03
PAIYUR-1	34.76	25.63	19.98	14.37
	±3.23	±3.09	±2.27	±1.95
DCPIP reductions (µmol/mgprotein/min)				
CO-13	164.52	145.08	137.44	125.25
	±7.30	±7.24	±7.16	±6.83
PAIYUR-1	162.17	117.61	85.29	63.28
	±7.81	±7.63	±7.55	±7.49
Total chlorophyll (mg/gfw)				
CO-13	1.49	1.34	1.25	1.15
	±0.045	±6.030	±0.024	±0.021
PAIYUR-1	1.42	1.01	0.89	0.65
	±0.043	±0.022	±0.012	±0.011

The data are expressed as mean ± s.e. for five independent determinations (P<0.05).

by irrigation daily to soil capacity (500 ml d⁻¹) with the nutrient medium containing 40mM, 80mM and 120mM NaCl. Young and fully matured leaves were taken from control and salinity treated plants on 30th Days After Treatment (DAT), for all the experiments described below.

Photosynthetic rates were determined by following the incorporation of ¹⁴CO₂ by young and fully expanded leaves as described by Sundar and Ramachandra Reddy, (2000). Chloroplast isolation and estimation of photosystem (II) activity measured according to the method of Leegood and Walker, (1993).

Ribulose-1,5-bisphosphate carboxylase activity was assayed at 30 °C by the incorporation of ¹⁴CO₂ in to acid stable products (Lorimer *et al.*, 1977) and the radioactivity was measured in the liquid scintillation counter. Sucrose phosphate synthase (SPS) was assayed at 30 °C by measuring the production of UDP (Huber, 1981). Chlorophyll content was determined in 80% acetone extract (Arnon, 1949). The contents of starch and sucrose in the leaf tissues were estimated enzymatically according to the method of Ramachandra Reddy *et al.*, (1996). The glucose content in the 80% ethanolic extract was determined using the anthrone method (Dubois *et al.*,

Table 2. Effect of salt stress on the content of glucose, sucrose and starch in two ragi varieties.

Variety and parameters	Salinity treatments (mM)			
	control	40	80	120
Glucose (mg/gdw)				
CO-13	19.78	17.23	15.17	14.66
	±2.37	±2.19	±1.11	±1.09
PAIYUR-1	18.67	14.84	11.47	8.34
	±2.23	±1.97	±1.07	±0.99
Sucrose (mg/gdw)				
CO-13	36.20	33.46	29.88	26.82
	±3.10	±3.04	±2.55	±2.32
PAIYUR-1	31.06	23.32	16.34	13.51
	±2.90	±2.63	±2.25	±1.19
Starch (mg/gdw)				
CO-13	26.81	29.33	33.62	36.28
	±2.71	±2.92	±3.03	±3.18
PAIYUR-1	28.56	36.28	48.54	60.23
	±2.84	±3.13	±3.22	±3.80

The data are expressed as mean ± s.e. for five independent determinations ($P < 0.05$).

1956).

For statistical analysis, five samples were taken for each treatment from five individual plants. Student's t-test and Analysis of Variance (ANOVA) were applied for analyzing significant differences between the control and treated plants ($P < 0.05$).

RESULTS AND DISCUSSION

Salt stress causes decrease in plant growth and productivity by disrupting physiological processes, especially photosynthesis (Sudhir and Murthy, 2004; Galic *et al.*, 2020). The ability of plants to maintain a reasonable photosynthetic rate under environmental stress is fundamental for the maintenance of plant growth and development (Eisa *et al.*, 2012). Photosynthetic rate was measured under different salinity concentrations to assess the photosynthetic potential and there was a marked variation between two ragi varieties (Fig. 1). CO-13 exhibited significantly high rates of photosynthesis both in control (4.59 nmolCO₂/gfw/s) and salt-stressed plants (3.12 nmolCO₂/gfw/s) even under higher salinity treatments (Table 1). Qin *et al.*, (2010) found that decrease in photosynthetic rate was mainly due to serious cellular dehydration, inhibited synthesis of chlorophyll and ionic imbalance and toxicity. There are several ways in which the CO₂ assimilation and activity of photosynthetic enzymes could decrease in the plants subjected to salt stress. These include stomatal closure, the differences in the activation

states of enzymes, a decrease in the total protein content per leaf area or specific transcriptional and translational control of synthesis of specific proteins (Gomes *et al.*, 2011). In our study, Ribulose-1,5 bisphosphate carboxylase and sucrose phosphate synthase decreased significantly under salt stressed conditions (Table 1). However, CO-13 maintained higher level of photosynthetic enzyme activities at all the salinity treatments. For instance, less than 120mM salinity, reduction of activity of RuBP carboxylase (29%) and SPS (27%) was noticed in CO-13, while 62% (RuBpcarboxylase activity) and 59% (SPS activity) in PAIYUR-1 when compared to respective control plants. This reduction of photosynthetic enzymes in the ragi varieties under salt stress is presumed to be due to reduced de novo synthesis of these enzymes. The effect of salt stress on the activities of the photosynthetic enzymes is a secondary effect mediated by the reduced CO₂ partial pressure inside the leaf caused by stomatal closure (Pattanagul and Thitisaksakul, 2008).

Salt stress affects the biochemistry of photosynthesis by causing the disorientation of lamellar system of the chloroplasts and loss of integrity of the chloroplasts leading to a decrease in the activities of PSII (Zhang *et al.*, 2018). Activity of photosystem II as measured by the photo reduction of DCP/IP was also high in CO-13 (125.25 µmol/mgprotein/min) even under salt stressed conditions when compared to PAIYUR-1 (63.28 µmol/mgprotein/min) (Table 1). Under salt stressed conditions, limitation of carbon assimilation within the plant cell results in

exposure to excess excitation energy. Chlorophyll is the principal agent responsible for photosynthesis and under adverse conditions, chlorophyll level is a good indicator of photosynthetic activity (XinWen *et al.*, 2008) and thus, we quantified the content of photosynthetic pigments in order to infer the effect of salt concentrations on the photosynthetic activity of two ragi varieties. Our data indicate that CO-13 recorded high chlorophyll value (1.15 mg/gfw) even under higher salinity compared to PAIYUR-1 (Table 1). Most studies show that salinity adversely affects chlorophyll content (Meloni *et al.*, 2003) and the observed decrease in chlorophyll contents in the leaves of ragi varieties grown under saline conditions may be attributed to both an inhibited synthesis of that pigment and damaged PS antenna system (Youssef and Awad, 2008).

Sugars play roles in osmotic adjustment, stabilizing membranes upon stress (Ahmed *et al.*, 2017), and affecting sugar-sensing system that regulates the expression of a variety of genes involved in photosynthesis, respiration, and synthesis and degradation of starch and sucrose. Salt stress also affected the carbohydrate metabolism in ragi leaves and the quantity of glucose and sucrose decreased with increasing salt stress (Table 2). PAIYUR-1 showed more reduction of glucose (55%) and sucrose (57%) at 120mM salinity treatment than compared to CO-13. The decrease in the glucose content may be attributed to the decreased photosynthetic rate as well as reduced sucrose phosphate synthase activity under salt stress in ragi leaves. Sucrose with glucose was reported to act as substrates for cellular respiration, osmolytes to maintain homeostasis and stabilize membrane (Norwood *et al.*, 2000) and soil salinity promoted sucrose exportation in the ragi varieties, lowering sucrose content in leaves of ragi varieties.

Under salinity stress, considerable amounts of starch were accumulated in two ragi varieties and the increase in starch content was greater in PAIYUR-1 (53%) than CO-13 (26%) when compared to control plants (Table 2). This may be an indication of changing carbon partitioning between sucrose and starch to retain greater amounts of fixed carbon in the ragi varieties under soil salinity stress. Limited CO₂ availability can alter leaf carbohydrate content and source-to-sink translocation pattern and it is possible that utilization of carbohydrate could be a limiting factor of growth under salinity and the accumulation of starch in the salt-sensitive cultivar is probably due to reduced utilization in the actively growing tissue (Thalman and Santelia, 2017). In the present study, starch accumulated under salt stress conditions which might be one of the reasons for reduced photosynthetic rates under salt stress.

CONCLUSION

Although salt stress elicited considerable variations between the two varieties of ragi, the varieties that show less susceptibility to salt stress is expected to improve the ragi crop production in semi-arid areas. The present study clearly shows that variety CO-13 is superior in

photosynthetic performance under salt stressed conditions and such studies can be useful in ragi breeding programs to generate plants with high photosynthetic performance even under salt stressed regimes.

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REFERENCES

- Ahmad, A, A. Ismun and M. Taib (2017). Effects of salinity stress on carbohydrate metabolism in *Cryptocoryne elliptica* cultures. *J. Trop. Plant Physiol.*, 9: 1-13.
- Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenol oxidases in *Beta vulgaris*. *Plant Physiol.*, 24: 1–15.
- Causin, H.F, D.A.E. Bordon and H. Burrieza (2020). Salinity tolerance mechanisms during germination and early seedling growth in *Chenopodium quinoa* Wild. genotypes with different sensitivity to saline stress. *Environ. Exp. Bot.*, 172: 1-11.
- Delfine, S, A. Alvino, M.C. Villani and F. Loreto (1999). Restrictions to carbon dioxide conductance and photosynthesis in spinach leaves recovering from salt stress. *Ameri. Soci. Plant Physiol.*, 119: 1101-1106.
- Dubois, M, K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith (1956). Colorimetric method for the determination of sugars and related substances. *Analyt. Biochem.*, 28: 350–356.
- Eisa, S, S. Hussin, N. Geissler and H.W. Koyro (2012). Effect of NaCl salinity on water relations, photosynthesis and chemical composition of Quinoa (*Chenopodium quinoa* Willd.) as a potential cash crop halophyte. *Aust. J Crop Sci.*, 6(2): 357-368.
- El-Hendawy, S., S. Elsayed, N. Al-Suhaibani, M. Alotaibi, M. U. Tahir, M. Mubushar, Attia and W. M. Hassan (2021). Use of Hyperspectral Reflectance Sensing for Assessing Growth and Chlorophyll Content of Spring Wheat Grown under Simulated Saline Field Conditions. *Plants.*, 10: 101-126.
- Galic, V, M. Mazur, D. Simic, Z. Zdunic and M. Franic (2020). Plant biomass in salt-stressed young maize plants can be modelled with photosynthetic performance. *Photosyn.*, 58: 194-204.
- Gomes, M.A.C, M.S. Suzuki, M. Cunha and C.F. Tullii (2011). Effect of salt stress on nutrient concentration, photosynthetic pigments, proline and foliar morphology of *Salvinia auriculata* Aubl. *Acta Limno. Brasilien.*, 23(2): 164-176.

- Hoagland, D.R. and D.I. Arnon (1950). The water-culture method for growing plants without soil (CAES, circular, 347). *Califor. Agri. Exp. Station*, California, p.32.
- Hohner, R, V.C. Galvis, D.D. Strand, C. Volkner, M. Kramer, M. Messer, F. Dinc, I. Sjuts, B. Bolter, D.M. Kramer, U. Armbruster and H. Kunz (2019). Photosynthesis in *Arabidopsis* Is Unaffected by the Function of the Vacuolar K⁺ Channel TPK3. *Plant Physiol.*, 180(3): 1322-1335.
- Huber, S.C. (1981). Interspecific variation in the activity and regulation of leaf sucrose phosphate synthase. *Z. Pflanzenphysiol.* 102: 443–450.
- Khan, N.A. (2003). NaCl-inhibited chlorophyll synthesis and associated changes in ethylene evolution and antioxidative enzyme activities in wheat. *Biol. Plantarum.*, 47: 437-440.
- Kao, W.Y, T.T, Tsai and C.N. (2003). Shih Photosynthetic gas exchange and chlorophyll a fluorescence of three wild soybean species in response to NaCl treatments. *Photosyn.*, 41: 415-419.
- Leegood, R.C. and D.A. Walker (1993). Chloroplasts and protoplasts. In: Hall D.O., Scurlock J.M.O., Bolharnordenkamp H.R., Leegood R.C. and S.P. Long (eds), *Photosynthesis and Production in Changing Environment - a Field and Laboratory Manual*. Chapman and Hall, London, pp. 268–282.
- Lorimer, G.H, M.R. Badger and T.J. Andrews (1977). D-ribulose 1,5-bisphosphate carboxylase-oxygenase. Improved methods for activation and assay of catalytic activities. *Analyt. Biochem.*, 78: 66–75.
- Lunn, J.E. and M.D. Hatch (1995). Primary partitioning and storage of photosynthate in sucrose and starch in leaves of C₄ plants. *Planta.*, 197(2): 385–391.
- Meloni, D.A, M.A. Oliva, C.A. Martinez and J. Cambraia (2003). Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. *Environ. Exp. Bot.*, 49: 69–76.
- Munns R, J.B. Passioura, T.D. Colmer and C.S. Byrt (2020). Osmotic adjustment and energy limitations to plant growth in saline soil. *New Phytologist.*, 225: 1091–1096.
- Norwood, M, M.T. Truesdale, A. Richter and P. Scott (2000). Photosynthetic carbohydrate metabolism in the resurrection plant, *Craterostigma plantagineum*. *J. Exp. Bot.*, 51: 159-165.
- Pattanagul, W. and M. Thitisaksakul (2008). Effect of salinity stress on growth and carbohydrate metabolism in three rice (*Oryza sativa* L.) cultivars differing in salinity tolerance. *Indian J. Exp. Biol.*, 46: 736-742.
- Penella, C, M. Landi and L. Guidi (2016). Salt-tolerant rootstock increases yield of pepper under salinity through maintenance of photosynthetic performance and sinks strength. *J. Plant Physiol.*, 193: 1-11.
- Qin, J, W.Y. Dong, K.N. Hel, Y. Yu, G.D. Tan, L. Han, M. Dong, Y.Y. Zhang, D. Zhang, A.Z. Li and Z. Wang (2010). NaCl salinity-induced changes in water status, ion contents and photosynthetic properties of *Shepherdia argentea* (Pursh) Nutt. Seedlings. *Plant Soil Environ.*, 56(7): 325–332.
- Ramachandra Reddy, A, K.R. Reddy and H.F. Hodges (1996). Mepiquat chloride (PIX)- induced changes in photosynthesis and growth of cotton. *Plant Growth Regul.*, 20: 179–183.
- Soda, N, B.K. Gupta, K. Anwar, A. Sharan, Govindjee, S.L. Singla-Pareek and A. Pareek (2018). Rice intermediate filament, OsIF, stabilizes photosynthetic machinery and yield under salinity and heat stress. *Sci. Reports.*, 8: 1-13.
- Sundar, D. and A. Ramachandra Reddy (2000). Low night temperature-induced changes in photosynthesis and rubber accumulation in Guayule (*Parthenium argentatum* Gray). *Photosyn.*, 38: 421–427.
- Sudhir, P. and S.D.S. Murthy (2004). Effects of salt stress on basic processes of photosynthesis. *Photosyn.*, 42 (4): 481-486.
- Thalmann, M. and D. Santelia (2017). Starch as a determinant of plant fitness under abiotic stress. *New Phytol.* 214: 943–951.
- Xinwen, X, X. Hailiang, Yangling, Wang, W. Xiaojing, Q. Yongzhi and X. Bo (2008). The effect of salt stress on the chlorophyll level of the main sand – binding plants in the shelterbelt along the Tarim Desert Highway. *Chinese Sci. Bulletin.*, 53: 109-111.
- Youssef, T. and M. Awad (2008). Mechanisms of enhancing photosynthetic gas exchange in date palm seedlings (*Phoenix dactylifera* L.) under salinity stress by a 5-aminolevulinic acid-based fertilizer. *J. Plant Growth Regul.*, 27: 1–9.
- Zhang, Y, E. Kaiser, Y. Zhang, Q. Yang and L. Li (2018). Short-term salt stress strongly affects dynamic photosynthesis, but not steady-state photosynthesis, in tomato (*Solanum lycopersicum*). *Env. Exp. Bot.*, 149: 109-119.