



EFFECT OF SALT STRESS ON GROWTH, CARBOHYDRATE AND PROLINE CONTENT OF TWO FINGER MILLET VARIETIES

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Abstract

Growth, sugar, reducing sugar, non-reducing sugar were decreased, while starch and proline content increase in two finger millet varieties GPU-28 and Intaf-5 under salinity stress. However, Intaf-5 maintained higher contents of carbohydrates as well as proline during the adverse effect of salinity stress, which leads better growth.

Key Words: Carbohydrate; Finger millet; Growth; NaCl.

Introduction

Salinity is currently the major factor which reduces crop yields. World-wide about 33% of the irrigated land is affected by salinity and more land is not being irrigated because of salinity [1, 2]. Growth of the plant is more important which directly affects the photosynthesis and crop yield. Carbohydrate partitioning is highly related to plant metabolism and could alter the whole physiology of a plant. Proline is a compatible solute, function as osmoprotectants for proteins [3]. Finger millet (*Eleusine coracana* L.) is an important minor cereal in the Indian subcontinent, rich in calcium, dietary fiber and known for its health benefits. GPU-28 and Intaf-5 are two important finger millet cultivars grown in southern India. The objectives of the present study was to find out a possible relationship between salinity and plant growth, carbohydrates in finger millet plants. Further an attempt was made to understand the synthesis of proline in two finger millet varieties as influenced by salinity stress.

soil. After 20 days, seedlings were thinned and three plants of uniform vigor were maintained in each pot. The maximum irradiance (PAR, 400-700nm) available during growth was 1800-2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ on clear day. Daily maximum and minimum temperatures were 29-33°C and 20-22°C, respectively. 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 condition which served as control. The watering solution for control plants consists of tap water and one-fourth strength of Hoagland nutrients. Other three group were salinized by daily irrigation to soil capacity (500 ml d⁻¹) with the nutrient medium containing 50mM, 100mM and 150mM NaCl. All the plants used in this study were of comparable size. Young and fully matured leaves were taken at 30 days after salinity treatments for all the experiments described below.

Materials and Methods

The certified finger millet seeds (varieties GPU-28 and Intaf-5) were procured from PASIC, Pondicherry. The plants were raised in pots containing red and clay

The height of the plants was measured with a measuring tape after 30 days of salinity treatments. For fresh weights, mature plants were carefully uprooted and roots were washed, blotted and whole plant was weighed. For dry weights, the whole plant was dried in an oven at 75-80° for 40 h until a constant weight was obtained. For

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alcoholic extraction, the leaf sample was macerated and 25mg of the dried powder was boiled in water bath with 10ml of ethyl alcohol (80%). The homogenate was centrifuged at 1500 g for 15 minutes and the supernatant was made upto 20ml with 80% ethyl alcohol. This alcoholic extract was used for quantitative estimation of total sugar [4], reducing sugar [5] and the residue was saved for starch [6] estimation. Non-reducing sugars were calculated by subtracting the amount of reducing sugars from total sugar content. Proline [7] content was also analysed.

Five independent determinants from individual plants were used for statistical analysis. Student's t-test and analysis of variance (ANOVA) were used for analyzing significant differences between the control and treated plants ($P < 0.05$).

Results and Discussion

Data of the growth parameters such as height of the plant, fresh and dry weight of the whole plants are given in Table 1, which clearly indicate the effect of salinity in two Finger millet varieties. The plant height was 40% lower when subjected to higher salinity (150mM) in Intaf-5, while 52% reduction in GPU-28 under same salinity conditions when compared to control. Fresh and dry weights of the whole plant were also markedly decreased in GPU-28 under high salinity treatments than in Intaf-5. There was a considerable decrease in the contents of total sugar, reducing sugars, non-reducing sugars in both varieties under stress (Table 2). For instance, total sugar was reduced to the tune of 25% in Intaf-5 and 49% in GPU-28 as compared to respective control plants under 150mM salinity. On the other hand, starch content showed reverse trend i.e., increased when increasing salinity concentrations in both varieties (Table 3). However, higher carbohydrate was observed in the Intaf-5 than the GPU-28 under salinity stress. Though proline content showed two fold increase in both varieties, comparatively lower in GPU-28 than in Intaf-5.

The reduction in plant height in the present study under salinity is could be due to its partial closure of stomata which leads to limit in the photosynthetic capacity of the treated plants. Salt accumulation in leaves might first inhibit photosynthesis by increasing stomatal and mesophyll conductance to CO_2 diffusion and is known impair RuBP carboxylase [8]. The reduced plant

height in salinity treated plant was owing to the reduction of photosystem II activity in salt stressed plants might be a crucial factor in determining the photosynthetic productivity in finger millet plants.

Table-1. Effect of salinity stress on plant height, fresh and dry weights in two finger millet varieties.

Varieties and Treatments	Plant height(cm)	F.W. (grams)	D.W. (grams)
GPU - 28			
Control	37.42 \pm 2.10	28.13 \pm 1.55	18.21 \pm 1.82
50mM	29.31 \pm 2.01	22.40 \pm 1.53	15.32 \pm 1.55
100mM	25.75 \pm 1.98	19.17 \pm 1.50	12.19 \pm 1.22
150mM	18.12 \pm 1.95	13.31 \pm 1.49	9.21 \pm 1.19
Intaf - 5			
Control	42.19 \pm 2.17	32.11 \pm 1.65	20.09 \pm 1.85
50mM	36.62 \pm 2.08	28.20 \pm 1.60	18.12 \pm 1.80
100mM	32.43 \pm 1.99	23.52 \pm 1.57	16.72 \pm 1.62
150mM	25.71 \pm 1.98	18.62 \pm 1.27	12.22 \pm 1.24

Each value represents mean \pm S.E. of five independent determinations ($P < 0.05$)

Table-2. Total, reducing and non-reducing Sugars (mg/gdw) in two fingers millet varieties as affected by NaCl stress.

Varieties and Treatments	Total sugar	Reducing sugar	Non-reducing sugar
GPU - 28			
Control	16.72 \pm 1.43	11.37 \pm 1.26	5.35 \pm 0.25
50mM	15.19 \pm 1.30	10.25 \pm 1.20	4.85 \pm 0.29
100mM	12.21 \pm 1.13	8.76 \pm 0.96	3.45 \pm 0.26
150mM	8.62 \pm 1.06	6.71 \pm 0.85	1.91 \pm 0.095
Intaf - 5			
Control	17.52 \pm 1.51	13.74 \pm 1.34	3.78 \pm 0.24
50mM	16.52 \pm 1.34	13.10 \pm 1.22	3.42 \pm 0.23
100mM	15.36 \pm 1.29	12.22 \pm 1.13	3.14 \pm 0.29
150mM	13.21 \pm 1.15	11.01 \pm 1.07	2.20 \pm 0.22

Each value represents mean \pm S.E. of five independent determinations ($P < 0.05$)

The total fresh and dry weights of the salinity treated plant was positively related to the plant height, which was significantly decreased under salinity stressed conditions in Intaf -5 and GPU-28. Salinity inhibits protein synthesis and causes a decrease in fresh and dry weights of plants [9, 10]. The reduction in plant dry weight, can be attributed to the reduced photosynthetic capacity of the leaves under salinity regimes [11]. It is thus possible to predict that decreased photosynthetic rates under salinity condition could have reduced the shoot growth and

development, thus finally yielding lower biomass productions compared to control plants.

Table 3. Influence of salinity stress on starch and proline contents in two finger millet varieties.

Varieties and Treatments	Starch (mg/gdw)	Proline (mg/gfw)
GPU – 28		
Control	33.72 ± 1.93	1.73 ± 0.076
50mM	38.43 ± 2.02	2.09 ± 0.082
100mM	49.62 ± 2.08	2.42 ± 0.092
150mM	61.22 ± 2.10	2.81 ± 0.18
Intaf – 5		
Control	30.41 ± 1.70	2.02 ± 0.080
50mM	33.18 ± 1.80	2.92 ± 0.092
100mM	38.13 ± 1.90	3.38 ± 0.21
150mM	45.49 ± 1.97	4.22 ± 0.31

Each value represents mean ± S.E. of five independent determinations (P<0.05)

The different growth responses to salinity can be presumed and interpreted as resulting from changes in the allocation and partitioning of photoassimilates [12]. In the present study, higher content of sugar was maintained in the Intaf-5 as compared to GPU-28 even under salinity. It is surprising to observe that starch content was inversely related to salinity. The reducing levels of starch in control and low salinity concentration (50mM) treated plant indicate that the export of carbohydrates to various organs is at a faster rate as compared to those with high salinity treated plants. Salinity stress might alter the export of photoassimilates to the growing regions, thus affecting the overall growth and development [13]. The study on carbohydrates clearly indicates that Intaf-5 had an effective carbohydrate partitioning mechanism than GPU-28 which have contributed for efficient photosynthesis. Although the precise role of proline accumulation is still debated, solute involved in osmotic adjustment [14]. The significant increase of proline content in Intaf-5 showed its ability to overcome the salinity injury to the protein structure in cells (Table 3). The accumulation of proline may be through an increase in its synthesis constantly with inhibition of its catabolism [15] and may be a mechanism for stress tolerance.

Conclusion

Finger millet variety Intaf-5 showed tolerance even under high salinity (150mM) than GPU-28 by proper partitioning of carbohydrates and synthesis of proline

which overcome the stress effects and leads better growth and biomass.

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References

1. Marschner, H., 1993. Mineral nutrition of higher plants. Academic Press, London.
2. Chaves, M. M., J. Flexas and C. Pinheiro. 2009. Photosynthesis under drought and salt stress: regulation mechanisms of whole plant to cell. *Annals of Bot.*, 103: 551-560.
3. Bohnert, H. J and R. J. Jenson. 1996. Strategies for engineering water – stress tolerance in plants. *Trends in Biotechnol.*, 14: 89-97.
4. Dubois, M., K. A. Gilles, J.K. Hamilton, P.A. Repers and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, 28: 350-356.
5. Nelson, N. 1944. A photometric adaptation of the somogyi method for the determination of glucose. *J. Biol. Chem.*, 153: 375-380.
6. McCready, R.M., J.Guggole, V. Silveira and H.S. Ownes. 1950. Determination of starch and amylase in vegetables. Application to peas. *Anal. Chem.*, 37: 816-819.
7. Bates, L.S., R. P. Waldern and I.D. Teare. 1973. Rapid determination of free proline for water stress studies. *Plant and Soil.*, 39: 205-207.
8. Delfine, S., A. Alvino, M. Zacchini and F. Loreto. 1998. Consequences of salt stress on conductance to CO₂ diffusion, Rubisco characteristics and

anatomy of spinach leaves. Aust. J. Plant Physiol., 25: 395-402.

9. Hernandez, J.A., E. Olmos, F.J., Corpas, F. Sevilla and L.A., Delrio. 1995. Salt induced oxidative stress in Chloroplasts of pea plants. Sci., 105: 151-117.
10. Kaya, C., H.D., Kirnak Higgs and K. Saltali. 2002. Supplementary calcium enhances plant growth and fruit yield in strawberry cultivars grown at high (NaCl) salinity. Sci. Hort., 93: 65-74.
11. Querghi, Z. G. Cormic, J. Brulfert. 2000. Effect of NaCl on photosynthesis of two wheat species (*Triticum durum* and *T. aestivum*) differing in their sensitivity to salt stress. J. Plant Physiol., 156: 355-446.
12. Poljakoff-Mayber, A and H.R. Lerner. 1994. Plants in saline environments. In: Pessaraki, M., M. Dekker (Eds.), Handbook of plant crop stress. New York. pp. 65-96.
13. Balibrea, M.E., M. Parra, C. Bolarin and F. Perez-Alfocea. 1999. Cytoplasmic sucrolytic activity controls tomato fruit growth under salinity. Aust. J. of Plant Physiol., 26: 561-568.
14. Azooz, M.M., M.A. Shaddad and A.A. Latef. 2004. The accumulation and compartmentation of proline in relation to salt tolerance of three sorghum cultivars. Indian J. Plant Physiol., 9: 1-8.
15. Yoshiba, Y. T. Kiyouse, K. Nakashima, Y. Shinozaki and K. Shinozaki. 1997. Regulation of levels of proline as an osmolyte in plants under water stress. Plant Cell Physiol., 38: 1095-1102.