



EFFECT OF NA₂CO₃ SALINITY ON B-CAROTENE, THIAMINE, RIBOFLAVIN AND ASCORBIC ACID CONTENTS IN THE LEAVES OF *AMARANTHUS POLYGAMOUS* L. VAR. PUSA KIRTI

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Abstract: Vitamins are considered as the accessory nutrients, required in minute quantities. Most of the vitamins are involved in enzyme systems. Vitamin contents of plants are also known to show altered metabolism under the influence of salinity. Not much of work has been done on the influence of salinity on the vitamin content in higher plants. Present study was carried out to study the influence of NaCl salinity on vitamin content in the leaves of *Amaranthus polygamous*. The β -carotene, a precursor of vitamin A, thiamine, riboflavin and ascorbic acid content were found to decrease gradually with increase in the concentrations of NaCl.

Keywords: Ascorbic acid, β - carotene, Riboflavin, Salinity, Thiamine, Vitamins.

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INTRODUCTION

According to Lal (2007), about 952 million hectares of land throughout the world is affected by salinity and sodicity, and this area is increasing year by year. The salts restrict the plant growth and hence salt stress has become an ever increasing threat to agriculture (Zhu, 2007; Pattanagul and Thitisaksakul, 2008). Salinity results in delayed germination, high rate of seedling mortality, stunted growth and reduced yield (Muhammad and Hussain, 2010). Vitamins are substances which are indispensable for growth and maintenance of the organism, and are required in very small amounts. Vitamins function in the body with carbohydrates, proteins and fats to supply energy. They are the functional components of various enzyme-regulated biochemical reactions occurring to create energy (Sarkiyayi and Ikioda, 2010). There are various reports indicating the influence of NaCl salinity on vitamin contents in plants (Seth *et al.*, 2007; Emam and Helal, 2008 and Mandhania *et al.*, 2010). With this background, present investigation deals with the influence of varying concentrations of NaCl on β -carotene, thiamine, riboflavin and ascorbic acid contents in the leaves of *Amaranthus polygamous*.

MATERIAL AND METHODS

Seeds of *Amaranthus polygamous* L. var. Pusa Kirti were obtained from the Indian Agricultural Research Institute, Pusa (New Delhi). Morphologically uniform healthy seeds of *Amaranthus polygamous* were soaked in water overnight. These seeds were sown in earthen pots of 20 cm diameter and 29 cm height. The pots were filled with 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 (Figure 1). 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. The mature leaves of forty five day old plants were used for estimating β -carotene, (a precursor of vitamin A) thiamine (vitamin B₁), riboflavin (vitamin B₆) and ascorbic acid (vitamin C) using standard protocols. β -carotene was determined according to the protocol of AOAC (1980). Vitamin B₁ (Thiamine) and B₆ (Riboflavin) were estimated according to the method of Strohecker and Henning (1966). Estimation of vitamin C was done as per the protocol of Birch *et al.* (1933). F test was used to test for the statistical significance and Student's *t*-test was use to compare the treatment means.



Figure 1: Effect of varying concentrations of NaCl (0mM, 20 mM, 40 mM and 60 mM) on the growth of *Amaranthus polygamous* L. var. Pusa Kirti.

RESULTS AND DISCUSSION

β -carotene

Vegetables contain yellow pigments called carotenes which are converted to vitamin A in the human body. Carotenes are accordingly called as provitamin A. There are different types of carotenes, namely α (alpha), β (beta) and γ (gamma). The rich sources of β -carotene are vegetables like spinach, amaranth, coriander, drumstick, radish etc. (Gopalan *et al.*, 1971). In the present investigation, β -carotene content in the leaves of *Amaranthus polygamous* showed a decrease with increasing concentration of NaCl in the growth medium (Table 1). Similarly Ayala-Astorga and Alcaraz-Melendez (2010) reported significant

decrease in β -carotene content in *Paulownia imperialis* and *P. fortunei* plants grown under NaCl salinity. Fathi and Asem (2013) while working with *Chlorella*, also reported that high salinity (50 gL⁻¹) leads to decrease in β -carotene content. Pisal and Lele (2005) on the basis of their experiments on unicellular green alga *Dunaliella salina* suggested that the cells show unbalanced physiological conditions under the influence of various stresses leading to increased β -carotene content. They observed increased production of β -carotene in cells with increasing NaCl in the growth medium. They suggested that, β -carotene is a secondary metabolite and these molecules are produced by the cells under stress for protection. They observed that 3 M NaCl concentration was the optimum for carotenoid production. Rad *et al.* (2011) while studying the effect of salinity on cell growth and β -carotene production in a unicellular microalga *Dunaliella* reported increased β -carotene content with increase in the salinity of the culture medium. Sarmad *et al.* (2007), while studying the relationship between endogenous abscisic acid and β -carotene synthesis in different species of *Dunaliella*, observed that at 3.5 M NaCl concentration, β -carotene accumulated in salt stressed cells of *Dunaliella salina* after a decrease at initial hours of stress, as compared to control (1.5 M NaCl). In contrast, they have reported no noticeable change in β -carotene production in *Dunaliella viridis* under similar experimental conditions.

Table 1: Effect of varying concentrations of NaCl on β -carotene, thiamine, riboflavin and ascorbic acid contents in the leaves of 45 day old *Amaranthus polygamous* plants

NaCl Concentration (mM)	β -carotene (mg/100 g DW) •	Thiamine (mg/100g DW) *	Riboflavin (mg/100g DW) *	Ascorbic Acid (mg/100g DW) *
0 (Control)	0.604 ± 0.006	0.013 ± 0.0001	0.045 ± 0.0003	8.930 ± 0.208
20	0.530 ± 0.006	0.013 ± 0.0002	0.036 ± 0.0000#	4.630 ± 0.063#
40	0.433 ± 0.005	0.012 ± 0.0001	0.035 ± 0.0003#	2.777 ± 0.055#
60	0.269 ± 0.004	0.0106 ± 0.001 #	0.035 ± 0.0002#	2.586 ± 0.074#

Results are the mean of three determinants.

* One-way ANOVA was carried out and the F ratios were significant at 5% level of significance.

Significant at $p < 0.05$ (*t*-test was carried out to test whether there is significant difference between control and individual salt concentration).

• No significant difference was observed in the group, so data is not analyzed further for pair-wise comparisons among the treatments.

Thiamine

Thiamine or vitamin B₁ is an important member of the vitamins of B-group. The richest source of thiamine is yeast where it occurs as coenzyme, thiamine pyrophosphate. In general, the foods that contain carbohydrates as a major source of energy contain a higher level of thiamine which is required for carbohydrate metabolism (Gopalan *et al.*, 1971). Daily thiamine requirement in human being ranges between 0.5-2.0 mg depending upon the age, physiological status and the level of daily activities of an individual (Gopalan *et al.*, 1971). In addition, thiamine can also function to alleviate environmental stresses in plants, as it can directly act as an antioxidant (Tunc-Ozdemir *et al.*, 2009). Thiamine content in the leaves of *Amaranthus polygamous* was observed to reduce with increase in salt concentration of the growth medium (Table 1). Rapala-Kozik *et al.* (2008) observed an increase in total thiamine content in *Zea mays* under NaCl salinity. According to them, thiamine metabolism is dependent upon pools of individual thiamine compounds such as free thiamine, thiamine monophosphate and thiamine diphosphate; the sum of which is the total thiamine level. These pools are determined by the activity of the various biosynthetic pathways that generate thiamine monophosphate and thiamine pyrophosphokinase, which activates thiamine to thiamine diphosphate and the numerous phosphatases capable of dephosphorylating thiamine diphosphate

and/or thiamine monophosphate. Rapala-Kozik *et al.* (2008) suggested that such an increase in thiamine content can be taken as a link between thiamine metabolism and abiotic stresses such as salinity which affect plants. An increase in total thiamine content has been observed in *Arabidopsis thaliana* seedlings subjected to saline conditions by Tunc-Ozdemir *et al.* (2009). Their study showed that salt stress induced accumulation of thiamine, was at least in part due to the increased expression of thiamine biosynthesis genes. They have also observed that thiamine accumulation in the plant resulted in enhanced tolerance to oxidative stress. In their experiment, thiamine was applied exogenously to *Arabidopsis* seedlings and their degree of tolerance was observed to be enhanced and was proportional to the amount of thiamine added.

Riboflavin

Riboflavin is a vitamin of B-group. These are essential for the metabolism and proper utilization of energy, carbohydrates, proteins and fats. It is essential for several oxidative processes occurring inside the cell. Green leafy vegetables are a good source of riboflavin. The daily requirement of riboflavin in human being ranges from 0.7-2.2 mg depending upon age, physiological status and the level of activity (Gopalan *et al.*, 1971). In the present investigation, 20 percent decrease in riboflavin content at 20 mM NaCl concentration was recorded in *Amaranthus polygamous* leaves, as compared to control (Table 1). At 40 mM and 60 mM NaCl, the decrease in riboflavin content was observed to be around 23 percent compared to control.

Ascorbic Acid

Ascorbic acid is a small, water soluble, antioxidant molecule which acts as a primary substrate in the cyclic pathway of enzymatic detoxification of hydrogen peroxide (Beltagi, 2008). Ascorbic acid or vitamin C is an essential nutrient. It is a strong reducing agent. It is involved in collagen synthesis as well as bone and teeth calcification. Vitamin C deficiency causes scurvy characterized by weakness, bleeding gums and defective bone growth. Vitamin C also helps in absorption of dietary iron by keeping it in the reduced form. Ascorbic acid occurs widely in plant foods, especially in fresh fruits and green leafy vegetables. In the leaves of *Amaranthus polygamous*, the ascorbic acid content was observed to decrease with increase in salt concentration (Table 1). Decreased ascorbic acid content has also been reported in wheat under the influence of salinity (Seth *et al.*, 2007). Emam and Helal (2008) observed that salinity is capable of inducing significant decrease in ascorbic acid content in *Linum usitatissimum* plants. According to them, decrease in ascorbate content might be one of the reasons why salinised flax seedlings were unable to tolerate salt stress. Similarly, a decrease in ascorbic acid content has also been recorded in wheat seedlings under NaCl salinity by Mandhania *et al.* (2010). Increased ascorbic acid contents in *Hordeum vulgare* plants irrigated with saline water has been recorded by Sarwat and El-Sherif (2007). A 30 percent increase in ascorbic acid content in tomato fruits grown under saline conditions has been reported by Kim *et al.* (2008). Similarly, in the leaves *Cicer arietinum* cv. Abrodhi, the ascorbic acid content has been reported to increase with increasing NaCl concentration (Mishra *et al.*, 2009). Increased ascorbate content in tomato fruit with increase in NaCl salinity in the growth medium has also been reported by Gautier *et al.* (2010).

CONCLUSION

On the basis of above findings it is clear that increasing concentrations of NaCl has lead to gradual decrease in β -carotene, thiamine, riboflavin and ascorbic acid contents in the leaves of *Amaranthus polygamous* L. var. Pusa Kirti plants. Decrease in vitamin content indicates the susceptibility of plant to salinity, leading to reduction in growth of the plants.

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CONFLICT OF INTEREST: Nothing