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ORIGINAL ARTICLE

Influence of NaCl Salinity on β-carotene, Thiamine, Riboflavin and Ascorbic Acid Contents in the Leaves of *Atriplex hortensis* L. var. Pusa Bathua No. 1

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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. The influence of NaCl salinity on the vitamin content in the leaves of *Atriplex hortensis* was investigated in the present study. *Atriplex hortensis* plants were grown 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 30 day old. Mature leaves of these plants were harvested and used for studies. β - carotene, a precursor of vitamin A and ascorbic acid content were found to increase gradually with increase in the concentrations of NaCl. No significant changes were observed in thiamine and riboflavin content at lower levels of salinity, however significant decrease was observed in thiamine and riboflavin content at higher level of salinity.

Key words: Vitamins, salinity, β - carotene, thiamine, riboflavin, ascorbic acid.

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Key words: Vitamins, salinity, β - carotene, thiamine, riboflavin, ascorbic acid.

Several environmental factors adversely affect plant growth and development and final yield performance of a crop. Drought, salinity, nutrient imbalances and extremes of temperature are among the major environmental constraints to crop productivity worldwide. Currently, there are no economically viable technological means to facilitate crop production under stress conditions (Hamdia and Shaddad, 2010). However, development of crop plants tolerant to environmental stresses is considered a promising approach, which may help satisfy growing food demands of the developing and under-developed countries.

The bulk of the food that we consume provides us with water, and the energy-yielding nutrients, such as carbohydrates, lipids, and proteins. In addition to these major nutrients, our bodies require a variety of other molecules and ions to maintain proper function. These nutrients, which are required in much smaller amounts, are known as vitamins and minerals. 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. Vitamins and minerals are needed by the body to start thousand of chemical reactions necessary to maintain good health (Worthing-Roberts, 2006). They are the functional components of various enzyme-regulated biochemical reactions occurring to create energy (Sarkiyayi and Ikioda, 2010). Vitamins enhance body's use of carbohydrate, proteins and fats and they are critical in the formation of blood cells, hormones, nervous system chemicals known as neurotransmitters and the genetic material deoxyribonucleic acid (Worthing-Roberts, 2006).

Leafy vegetables are consumed on a large scale in India. They are rich sources of calcium, iron, β-carotene, riboflavin, folic acid and vitamin C. Present investigation deals with the influence of varying concentrations of NaCl on β-carotene, thiamine, riboflavin and ascorbic acid contents in the leaves of *Atriplex hortensis*.

MATERIALS AND METHODS

Seeds of *Atriplex hortensis* L. var. Pusa Bathua No. 1 were obtained from the Indian Agricultural Research Institute, Pusa (New Delhi). Morphologically uniform healthy seeds of *Atriplex hortensis* 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. 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 thirty days. The mature leaves of thirty day old plants were used for estimating β-carotene, (a precursor of vitamin A) thiamine (vitamin B_1), riboflavin (vitamin B_6) and ascorbic acid (vitamin C) using standard protocols.

β -carotene was determined according to the protocol of AOAC (1980).

Vitamin B_1 (Thiamine) and B_6 (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.

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). In the present study β -carotene content in the leaves of *Atriplex hortensis* were observed to increase with increasing salt concentration (Table 1). 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, which may be true even in the present study. Similarly, 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. Contrary to this Ayala-Astorga and Alcaraz-Melendez (2010) reported significant decrease in β-carotene content in *Paulownia imperialis* and *P. fortunei* plants grown under NaCl salinity.

Thiamine:

Thiamine or vitamin B_1 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).

In case of *Atriplex hortensis* leaves, no significant changes in thiamine content were observed at 20 and 40 mM NaCl treatment; however at 60 mM NaCl treatment, thiamine content was found to be significantly decreased compared to the leaves of control plants (Table 1). Contrary to this Rapala-Kozik *et al*. (2008) observed an increase in total thiamine content in *Zea mays* under NaCl salinity. 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 also 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 also observed that thiamine accumulation in the plant resulted in enhanced tolerance to oxidative stress.

Table 1. Effect of varying concentrations of NaCl on β-carotene, thiamine, riboflavin and ascorbic acid contents in the leaves of 30 day old *Atriplex hortensis* 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)	0.132 ± 0.003	0.012 ± 0.0005	0.024± 0.0000	5.950 ± 0.065
20	0.175 ± 0.003	0.013 ± 0.0001	0.024 ± 0.0002	10.373 ± 0.404
40	0.228 ± 0.005	0.011 ± 0.0002	0.021 ± 0.0003#	17.106 ± 0.673
60	0.260 ± 0.004	0.009 ± 0.0002 #	0.018 ± 0.0003#	23.573 ± 0.776

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.

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 case of *Atriplex hortensis* leaf, riboflavin content remained same as that of control at lower level of salinity (20 mM NaCl). However, it was observed to decrease significantly at 40 and 60 mM NaCl concentrations (Table 1).

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). Vitamin C (ascorbic acid) is essential to prevent disease associated with connective tissue, improves cardiovascular and immune cell functions, and is used to regenerate α-tocopherol (vitamin E). In contrast to most animals, humans lack the ability to synthesize ascorbic acid as a result of a mutation in the last enzyme required for ascorbate biosynthesis. Vitamin C, therefore, must be obtained from dietary sources and, because it cannot be stored in the body, it must be obtained regularly. Ascorbic acid occurs widely in plant foods, especially in fresh fruits and green leafy vegetables.

In *Atriplex hortensis leaves*, ascorbic acid content increased with increasing salinity (Table 1). Increased ascorbic acid contents in *Hordeum vulgare* plants irrigated with saline water has also 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*. (2009).

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