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**ORIGINAL ARTICLE**

# Salinity Induced Changes in β-carotene, Thiamine, Riboflavin and Ascorbic Acid Content in *Spinacia oleracea* L. var. All Green

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Vitamins are the functional components of various enzyme-regulated biochemical reactions occurring to create energy. Vitamin contents of plants are 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 *Spinacia oleracea*. *Spinacia oleracea* plants were grown in earthen pots and were subjected to different concentrations 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 studies. Thiamine and riboflavin content were found to increase with increase in NaCl concentration, however, β-carotene was found to decrease with increasing level of NaCl in the growth medium.

*Key words: ascorbic acid, β-carotene, riboflavin, salinity, thiamine, vitamins*

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*Key words: ascorbic acid, β-carotene, riboflavin, salinity, thiamine, vitamins*

Salinity is one of the environmental factors limiting crop productivity of marginal agricultural soils in many parts of the world (Nabati *et al*., 2011). Nazarbeygi *et al*. (2011) suggested that the adverse effect of salinity on plant productivity is due to the decrease in vegetative growth of the plant. Currently, there are no economically viable technological means to facilitate crop production under stress conditions (Hamdia and Shaddad, 2010). Vitamins are biologically active organic compounds that are essential micronutrients involved in metabolic and physiological functions in the human body. There are thirteen vitamins identified, these compounds greatly differ in their chemical composition, physiological action and nutritional importance in the human diet. They are the functional components of various enzyme-regulated biochemical reactions occurring to create energy (Sarkiyayi and Ikioda, 2010). Not much of work has been done on the influence of salinity on vitamin content in higher plants.

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 *Spinacia oleracea.*

## MATERIALS AND METHODS

Seeds of *Spinacia oleracea* L. var. All Green were obtained from the Indian Agricultural Research Institute, Pusa (New Delhi). Morphologically uniform healthy seeds of *Spinacia oleracea* 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 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 $B_1$), 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:

In the leaves of *Spinacia oleracea* β-carotene content were observed to decrease with increasing concentration of NaCl in the growth medium (Table 1). Similar to the present finding, Ayala-Astorga and Alcaraz-Melendez (2010) reported significant decrease in β-carotene content in *Paulownia imperialis* and *P. fortunei* plants grown under NaCl salinity. Ratnakar and Rai (2013a) also reported decrease in β-carotene content under the influence of salinity in *Amaranthus polygamous*.

Pisal and Lele (2005) 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. Rad *et al*. (2011) while studying the effect of salinity on cell growth and β-carotene production in a unicellular microalga *Dunaliella* have 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*, have 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.

### Thiamine:

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. Similarly in the present study thiamine content was observed to increase slightly in *Spinacia oleracea* leaves with increasing NaCl concentration (Table 1). 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:**

A gradual increase in riboflavin content was observed in the leaves of *Spinacia oleracea* with increase in the concentration of NaCl (Table 1). At 60 mM NaCl concentration riboflavin content were found to be 12.3 percent higher compared to control. Contrary to the present finding, Ratnakar and Rai (2013a; 2013b)) reported decrease in riboflavin content under the influence to NaCl salinity in *Amaranthus polygamous* and *Atriplex hortensis* leaves.

**Ascorbic Acid:**

In the present investigation, an initial increase in ascorbic acid content has been observed in *Spinacia oleracea* leaves at 20 mM NaCl as compared to control, which decreased thereafter with further increase in the concentration of NaCl, although at 40 mM NaCl, ascorbic acid content remained much higher level than that of control (Table 1) and at 60 mM NaCl it was found to be lower than that of control.

Decreased ascorbic acid content has been reported in wheat under the influence of salinity (Seth *et al*., 2007). Emam and Helal (2008) observed that salinity induced 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). They have reported higher ascorbate content in a salt-tolerant cultivar (KRL-19) as compared to a salt-sensitive cultivar (WH-542) under control conditions, which decreased with increased salt stress.

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.* (2010).

**Table 1** Effect of varying concentrations of NaCl on β-carotene, thiamine, riboflavin and ascorbic acid contents in the leaves of 45 day old *Spinacia oleracea* 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) | 0.521 ± 0.002 | 0.020 ± 0.0001 | 0.035 ± 0.0003 | 3.239 ± 0.163 |
| 20 | 0.443 ± 0.004 | 0.020 ± 0.0003 | 0.039 ± 0.0002 | 6.481 ± 0.655# |
| 40 | 0.347 ± 0.003 | 0.021 ± 0.0001 | 0.040 ± 0.0000 | 3.495 ± 0.247# |
| 60 | 0.365 ± 0.002 | 0.022 ± 0.0003 | 0.043 ± 0.0006 | 2.839 ± 0.161 |

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.

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