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Changes in Photosynthesis, Yield, and Quality of Baby Lettuce under Salinity Stress

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ABSTRACT

The objective of this work was to study the responses of two baby lettuce (*Lactuca sativa* L.) cultivars, namely, green Paris Island and red Sanguine, to different NaCl concentrations (0, 5, 10, and 20 mM). The study was conducted in a floating system and plants were harvested at the stage of 5-6 leaves, outside leaf 6-10 cm in length (25 and 31 days after sowing for green and red lettuce, respectively). Photosynthetic parameters (photosynthesis rate, stomatal conductance, transpiration rate, and intercellular CO_2 concentration) and yield (leaf fresh weight) as well as color [L* (lightness), C* (saturation), Hº (object's color)], pigments (*Chla*, *Chlb*, total chlorophyll, carotenoids and anthocyanins) and quality indices (appearance, freshness, taste and texture) were determined. The results indicated that, in both cultivars, leaf fresh weight was reduced at 20 mM NaCl; the reduction depended on the limited water accessibility and photosynthetic rate was suppressed in high salt concentration through stomatal limitations aiming at the retention of water but resulting in restricted availability of CO_2. Nevertheless, no limitations were observed in photosynthetic pigments and, thus, stomatal closure was the dominant factor limiting photosynthesis. On the other hand, salinity improved anthocyanin content and coloration in red lettuce and enhanced freshness in green lettuce.

Keywords: Gas exchange, Leafy vegetables, NaCl, Plant growth, Sensory quality.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is an important crop of Mediterranean agro-ecosystems. However, its growth is sensitive to salinity, a well known problem not only in Mediterranean countries but also in arid and semi-arid regions worldwide (Kohler *et al*., 2009; Dadkhah and Grrifiths, 2006). Plant growth as biomass production is a measure of net photosynthesis; therefore, environmental stresses affect growth as well as photosynthesis. Although there are lots of reports about photosynthesis inhibition by salt stress, there are also reports indicating that photosynthesis is either not slowed down by salinity or is even stimulated by low salt concentration (Parida and Das, 2005). Also, the ability of a plant to live under stress has been shown to be related to gas exchange of the plant (Klamkowski and Treder, 2006).

Green color, an obvious indicator of the leafy vegetable quality having a great impact on consumer preference, is associated with chlorophyll content (Roura *et al*., 2000). However, leaf chlorophyll and carotenoid content may decrease under salt stress (Parida and Das, 2005). On the other hand, there is a growing interest in red lettuce, because it is colorful and contributes to the color variety of mixed salads. Red lettuce contains larger amounts of phenolics than the green one (romaine or butterhead lettuce), which

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contribute to higher antioxidant capacity satisfying the demand for healthy foods (Liu *et al*., 2007). Moreover, anthocyanins constitute one of the primary phenols in red lettuce (Llorach *et al*., 2008), that are elicited though the phenylpropanoid pathway by environmental stresses (Kim *et al*., 2008).

In recent years, baby vegetables are becoming more and more popular as they are easy in preparation for the market, with a low degree of injury. Therefore, consumers demand has been increasing and many farmers have oriented their production plans towards baby leaf vegetables, such us lettuce, spinach, rocket and Swiss chard (Fallovo *et al*., 2009).

The effects of NaCl salinity on cultivated lettuce plants are well known (Shannon and Grieve, 1999). However, baby lettuce is harvested during the early stages of growth when metabolism is intense and this may alter salinity responses (Läuchli and Grattan 2007). Moreover, the available information regarding the influence of salinity on baby lettuce is currently limited with emphasis on yield and quality. Scuderi *et al*. (2009) reported the salinity effect on fresh-cut romaine lettuce and determined yield and nitrate content at harvest. Also, the effect of salinity on enzymatic browning of fresh-cut baby green lettuce during storage was investigated by Chisari *et al*. (2010). Fallovo *et al*. (2009) reported about the fertilizer concentration and, indirectly, the EC effect on yield and color of green leafy lettuce. As red lettuce is characterized by higher phenolics content and antioxidants properties than green lettuce (Caldwell, 2003; Liu *et al*., 2007) salinity effects could be different between red and green lettuce.

In view of the above background, the aim of the present work was to evaluate the responses of two lettuce cultivars (one green and one red-pigmented) to different salinity levels during the initial stages of growth and to understand the mechanisms underlying the responses of baby lettuce to salinity, photosynthetic parameters, yield, color, pigments and quality indices.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Lettuce (*Lactuca sativa* L. green cv. Paris Island and red-pigmented cv. Sanguine) plants were cultivated under natural conditions of light and temperature in a glass greenhouse at the experimental farm of Aristotle University, Thessaloniki, Greece, in a floating system. Hoagland solution (pH 6.5) was mixed up with zero, 5, 10, or 20 mM NaCl to establish four salinity solutions, which corresponded to EC 2.2, 2.8, 3.6, and 4.6 dS m^{-1}, respectively. Seeds of both lettuce cultivars were sown on 26 March 2010 in polystyrene 128-cell plug trays (480 plants m^{-2}) and 3 trays (replicates) of each cultivar were floated on a stagnant solution (200 l) of each tank (one for each salinity solution) 5 days later (Figure 1). Average temperature and relative humidity inside the greenhouse during the experiment were 18.2±7.3°C and 62.7±20.4 %, respectively.

Photosynthetic Parameters

Photosynthetic rate (A), stomatal conductance (g_s), transpiration rate (E) and intercellular CO_2 concentration (Ci) measurements were performed on the outside leaf of six plants per replication, when plants reached harvesting stage (5-6 leaves, outside leaf 6-10 cm in length), 25 and 31 days after sowing for green and red lettuce, respectively, using an LCi portable photosynthesis system (ADC Bioscientific Ltd., Hoddesdon, England). Intrinsic water use efficiency (WUEi) was defined as the ratio of A/g_s (Escalona *et al*., 1999).

Yield, Color, Pigments and Quality Data

After measurements of photosynthetic parameters, harvesting was carried out. From each replication, 35 plants were

Figure 1. Seedlings of baby lettuce in polystyrene 128-cell plug trays floating on a stagnant nutrient solution.

harvested and weighed. Plant yield was expressed as leaf fresh weight per plant (g $plant^{-1}$).

The color was determined on the three outer leaves in four plants per replication. In each leaf, two measurements were taken on the upper leaf surface with a Minolta CR-200 chromameter (Minolta, Osaka, Japan) equipped with an 8 mm measuring head and a C illuminant (6,774 K) and calibrated with a white plate. In the red cultivar (Sanguine), color readings were taken in both green and red leaf parts. Leaf color changes were quantified in the *L**, *a** and *b** color space. Hue angle (Hº), an indicator of the objects color and chroma (C*), a measure of saturation, were calculated [($H^o = 180+tan^{-1}(b^*/a^*)$ and $C^* = (a^{*2}+b^{*2})^{1/2}$] (McGuire, 1992). After the color measurement, at the same position, leaf disks with a diameter of 9 mm were taken for pigments determination. For chlorophyll and carotenoid determination, leaf disks were extracted with acetone and the absorbance of leaf extracts was measured spectro-photometrically at 470, 648, and 664 nm, respectively (Lichtenthaler, 1987). Results were expressed in μg g^{-1} FW. For the total anthocyanins determination, leaf disks were extracted with acidified ethanol and their concentration was estimated with the pH differential method (Cheng and Breen, 1991). Results were expressed in mg cyanidine-3-glucoside equivalents 100 g^{-1}

FW. Chemicals were of analytical grade and purchased from Sigma (Sigma Chemicals, St. Louis, MO, USA). Assays were performed using an automated UV/Vis spectrophotometer (Shimadzu UV-1700 PharmaSpec, Shimadzu Corporation, Kyoto, Japan).

Finally, quality of six plants were evaluated in the laboratory by a trained panel (8 judges, aged 27-55 years). Quality indices, such as appearance, freshness, taste, and texture were assessed and scored following a 9 point rating scale with 9: Excellent, 7: Very good, 5: Good (marketability limit), 3: Fair, and 1: Poor.

Statistical Analysis

A randomized block design was adopted. Analysis of variance (ANOVA) of the data was performed by the Statistical Analysis System (SAS ver. 9.2, Cary, NC, USA). ANOVA for the main effects (cultivar and salinity) and their interaction showed that all treatments had a significant effect on the measured parameters, but most of the total variance was accounted for by differences between cultivars. For this reason, ANOVA was performed again for each one cultivar separately. Duncan's Multiple Range Test at the 0.05 level was employed to determine the statistical significance of the differences between the means.

Results and Discussion

Yield

In both cultivars, yield expressed as leaf fresh weight (Table 1) and dry weight (data not shown) were significantly affected by salinity. Compared to the control, yield was reduced at 20 mM NaCl by 13 and 17% in the green and the red cultivar, respectively. Previous results (Tesi *et al*., 2003) also indicated that 10 mM NaCl decreased the fresh weight of commercial butterhead lettuce in a floating system. In general, it is known that weight and height of leaves, stems, and roots in different plant species is reduced by salinity (Kim *et al*., 2008; Dadkhah and Grrifiths, 2006; Alomran *et al*., 2012). This could be because of the decreased photosynthetic capacity (Morsy, 2003), linked with water stress in the root zone, or salt toxicity in the plants tissue (Ho, 2003). In accordance, the greater reduction in leaf fresh weight compared to leaf dry weight at the highest salt concentration observed (data not shown) concurs with the notion that the reduction of growth depended on the limited water accessibility in baby leaves. Similar results have been reported when baby romaine lettuce was

Table 1. Effects of salinity (0, 5, 10, and 20 mM NaCl) on leaf fresh weight (FW), photosynthetic rate (A), stomatal conductance (g_s), transpiration rate (E), intercellular CO_2 (Ci), and intrinsic water use efficiency (WUEi,) in green (cv. Paris Island) and red (cv. Sanguine) baby lettuce harvested at the stage of 5-6 leaves, outside leaf 6-10 cm in length (25 and 31 days after sowing, respectively).

Cultivar[a]	NaCl (mM)	FW (g plant^{-1})	A (µmol CO_2 m^{-2} s^{-1})	g_s (cm s^{-1})	E (mmol H_2O m^{-2} s^{-1})	Ci (µmol CO_2 mol^{-1})	WUEi (A/g_s)
Paris Island	0	6.63^{a}	17.06^{a}	0.391^{a}	7.87^{a}	301^{a}	37.6^{b}
	5	6.64^{a}	16.27^{ab}	0.443^{ab}	7.06^{a}	294^{a}	39.9^{b}
	10	6.14^{ab}	15.25^{bc}	0.425^{bc}	5.24^{b}	274^{ab}	44.5^{b}
	20	5.75^{b}	13.84^{c}	0.220^{c}	5.76^{b}	250^{b}	63.8^{a}
Sanguine	0	9.10^{a}	7.26^{a}	0.168^{a}	3.84^{a}	272^{a}	51.1^{b}
	5	8.73^{ab}	7.05^{a}	0.138^{a}	3.64^{ab}	259^{ab}	57.1^{ab}
	10	8.38^{ab}	6.71^{a}	0.127^{a}	2.84^{ab}	254^{ab}	58.9^{ab}
	20	7.56^{b}	3.71^{b}	0.053^{b}	2.17^{b}	237^{b}	73.7^{a}

[a] In each cultivar, means within a column followed by the same superscript letters are not significantly different (Duncan's multiple range test, P≤ 0.05 level).

cultivated in soil (Kim *et al*., 2008) or commercial butterhead lettuce in a floating system (Tesi *et al*., 2003).

Leaves of both cultivars did not show any visual symptoms of salinity damage and this may be attributed to the short-term growing period in combination with the fast growth rates of baby leaves.

Photosynthetic Parameters

In the green cultivar, photosynthesis rate (A) and stomatal conductance (g_s) were reduced at the highest salinity level (20 mM) by 19 and 44% compared to the control, respectively. The same parameters were reduced by 49 and 69% in the red cultivar, respectively (Table 1). Noteworthy, the reduction trend of photosynthesis was in accordance with the respective yield results (Table 1). There is still no unified concept of the events which reduce the photosynthetic efficiency (Kafi, 2009; Dadkhah, 2011). However, it is generally believed that stomatal limitations are the main determinant for decreased photosynthesis under mild and moderate stress (Cornic, 2000; Flexas *et al*., 2004; Bolla *et al*., 2009). In the current experiment, salinity at 20 mM NaCl had a negative effect on the photosynthetic parameters (Table 1). The reduction in g_s was accompanied by a reduction in *Ci*, indicating that there were stomatal limitations for CO_2 supply. It is reasonable to conclude that plants reacted by the closure of stomata in order to avoid transpirational water loss and, consequently, CO_2 diffusion into the leaf was restricted (Table 1). As a result, stomatal limitations decreased photosynthetic rate inhibiting metabolism (Cornic, 2000) and, finally, growth as it occurs in other plant species and genotypes under mild and moderate salinity conditions (Parida and Das, 2005). Moreover, due to the non-linear relationship between *A* and g_s (Figures 2-a and -b), the concomitant *WUEi* (A/g_s) was increased at the highest salt concentration in both cultivars (Table 1). This concurs with the notion that there were water restrictions rather than the inhibition of photosynthesis (Flexas *et al*., 2004). Such results indicated that stomatal closure, aiming at the retention of water, was the dominant factor limiting photosynthesis and, thereby, biomass production in baby lettuce under salinity conditions. Finally, the absolute values of photosynthetic parameters in Table 1 could be used to explain the shorter growing period until baby size of green lettuce Paris Island (25 days after sowing) compared with red leaf Sanguine (31 days after sowing).

Pigments and Color Indices

Chla, *Chlb*, total chlorophyll (Chl *a+b*),

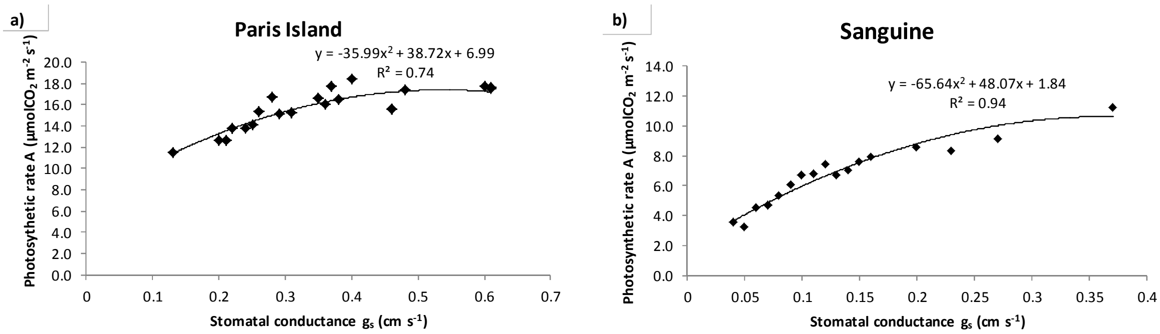

Figure 2. Relationship of photosynthetic rate (A) with stomatal conductance (g_s) in (a) Green baby lettuce (cv. Paris Island) and (b) Red baby lettuce (cv. Sanguine). Regression lines (the best fitted model), equations and coefficients of determination (R^2) are presented. Points represent measurements at different levels of salinity.

and carotenoid content were not affected by salinity either in green or in red lettuce (total chl from 0.95 to 0.99 μg g^{-1} and 0.19 to 0.22 μg g^{-1} FW, respectively), although it is known from the literature that chlorophyll and carotenoid content of leaves may decrease under salt stress (Parida and Das, 2005). These findings support the idea that the reduction in photosynthesis in the current experiment was due to factors affecting stomatal closure rather than damages in the photosynthetic apparatus.

In the green cultivar 'Paris Island' color indices $L*$ (lightness) and $C*$ (saturation) were reduced, whereas H^o (object's color) was increased. In the green leaf part of red cultivar 'Sanguine' $L*$ was reduced, while $C*$ and H^o were not affected (Table 2). Darker green leaves are reported to be the first symptom of high osmotic pressure and is attributed to the reduced water uptake (Tesi *et al*., 2003). Similarly, Kim *et al.* (2008) observed that the color in baby romaine lettuce cv. Clemente changed with NaCl treatments. In the red leaf part of red cultivar Sanguine, $L*$ and $C*$ were reduced, whereas H^o was increased by 1.8-fold in the highest salt concentration, compared to the control (Table 2).

The latter change was matched to the observed increase in the anthocyanin content (2.4-fold) (Figure 3) and it was likely due to

Table 2. Effects of salinity (0, 5, 10 and 20 mM NaCl) on lightness, chroma, and hue angle in the green leaf part of green (cv. Paris Island) and red (cv. Sanguine) baby lettuce and the red leaf part of red (cv. Sanguine) baby lettuce.

Cultivar[a]	NaCl mM	Lightness (Green part)	Chroma (Green part)	Hue (Green part)	Lightness (Red part)	Chroma (Red part)	Hue (Red part)
Paris Island	0	49.39[a]	37.87[a]	125.9[b]			
	5	48.66[ab]	36.82[ab]	126.7[a]			
	10	49.14[a]	36.37[b]	126.6[a]			
	20	47.96[b]	35.65[b]	127.1[a]			
Sanguine	0	72.51[a]	38.06[a]	118.4[a]	44.50[a]	27.42[a]	107.1[b]
	5	71.41[ab]	37.40[a]	118.1[a]	48.22[a]	30.97[a]	119.2[b]
	10	71.73[ab]	35.93[a]	118.2[a]	46.83[a]	28.36[a]	144.4[b]
	20	69.20[b]	36.76[a]	117.4[a]	38.03[b]	17.13[b]	188.1[a]

[a] In each cultivar, means within a column followed by the same superscript letters are not significantly different (Duncan's multiple range test, P≤ 0.05 level).

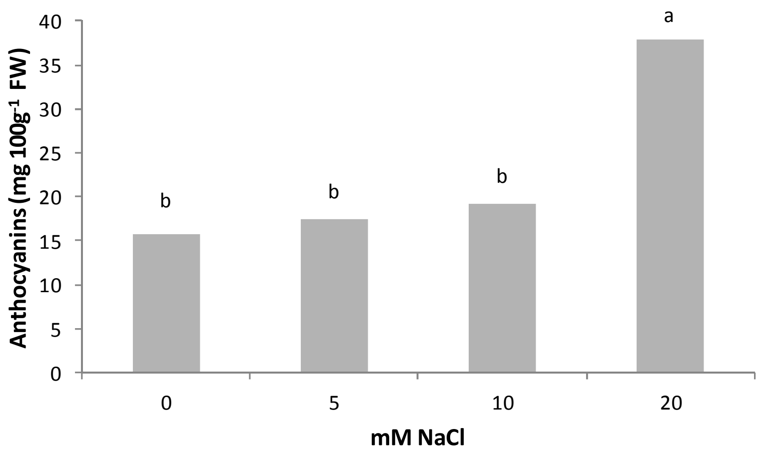

Figure 3. Effects of salinity (0, 5, 10 and 20 mM NaCl) on anthocyanin content (mg cyaniding-3-glucoside 100g^{-1} FW) of red (cv. Sanguine) baby lettuce harvested at the stage of 5-6 leaves, outside leaf 6-10 cm in length (31 days after sowing). Columns with the same letter are not significantly different (Duncan's multiple range test, P= 0.05 level).

higher solute concentration (Kim *et al*., 2008). Anthocyanins in red lettuce cultivars are considered to be one of the most important phenolic compounds correlated with powerful antioxidant activities (Caldwell, 2003; Li and Kubota, 2009). In line with previous findings (Parida and Das, 2005), our results suggest that salinity enhances anthocyanins and a more attractive color in red baby lettuce.

Quality Indices

Quality indices results accounted for final scores of all NaCl concentrations higher than the marketability limit (5.0) all over the experiment. No significant effect of salinity on appearance, taste, and texture of both 'Paris Island' and 'Sanguine' was observed (Table 3). On the contrary, freshness of 'Paris Island' lettuce was evaluated with significantly higher score at 20 mM NaCl than the control, while freshness score for Sanguine at 20 mM NaCl was significantly lower that the control (Table 3). Similarly, Mizrahi and Pasternak (1985) reported no difference for iceberg lettuce both in taste and consumer preference among control and saline treatments. On the contrary, in the same study, melon fruits from plants irrigated with saline water scored higher in taste than their controls. Probably, the different type of tissue (fruit-leaves) could explain the different response of each crop to salinity.

CONCLUSIONS

Using saline water to prepare nutrient solution for green and red leaf baby lettuce negatively affected yield and photosynthesis. The reduction of yield, expressed as leaf fresh weight, depended on the limited water accessibility in baby leaves and photosynthesis was reduced upon salt stress through stomatal limitations resulting in restricted availability of CO_2. The reduced yield could be at least partly compensated by improved anthocyanin content and coloration in the red cultivar and enhanced freshness in green lettuce.

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Table 3. Effects of salinity (0, 5, 10, and 20 mM NaCl) on appearance, freshness, taste, and texture of green (cv. Paris Island) and red (cv. Sanguine) baby lettuce harvested at the stage of 5-6 leaves, outside leaf 6-10 cm in length (25 and 31 days after sowing, respectively).

Cultivar	NaCl	Appearance	Freshness	Taste	Texture
Paris Island	0	7.33 ± 0.53[a]	6.67 ± 0.41	6.33 ± 0.50	7.22 ± 0.32
	5	7.56 ± 0.41	7.00 ± 0.33	7.22 ± 0.43	7.22 ± 0.36
	10	7.33 ± 0.47	7.22 ± 0.36	7.33 ± 0.50	7.78 ± 0.40
	20	8.11 ± 0.26	7.56 ± 0.29	7.11 ± 0.51	7.89 ± 0.31
Sanguine	0	7.13 ± 0.35	7.38 ± 0.32	6.88 ± 0.58	7.13 ± 0.30
	5	7.25 ± 0.49	7.00 ± 0.38	7.25 ± 0.45	7.13 ± 0.35
	10	7.25 ± 0.41	7.38 ± 0.38	6.88 ± 0.40	7.25 ± 0.41
	20	6.38 ± 0.38	6.63 ± 0.32	6.63 ± 0.32	7.38 ± 0.32

[a] Means±SE are presented; each parameter was assessed and scored following a 9 point rating scale with 9: Excellent; 7: Very good; 5: Good (marketability limit); 3: Fair, and 1: Poor.

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تغییرات فتوسنتز، عملکرد، و کیفیت بچه کاهو در تنش شوری

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چکیده

هدف این پژوهش بررسی پاسخ دو کولتیوار بچه کاهو (*Lactuca sativa* L.) به نام های Paris Island جزیره پاریس (سبز رنگ) و Sanguine سنگویین (قرمز رنگ) به غلظت های مختلف نمک طعام (صفر،۵، ۱۰، و ۲۰ mM) بود. پژوهش در یک سامانه شناور انجام شد و بوته ها در مرحله ۶-۵ برگی در زمانی که طول برگ بیرونی آنها به ۱۰-۶ سانتی متر رسید (برای کاهو سبز و قرمز به ترتیب ۲۵ روز و ۳۱ روز بعد از کاشت) برداشت شدند. شاخص های اندازه گیری شده عبارت بودند از پارامتر های فتوسنتز(نرخ فتوسنتز، نرخ تعرق، غلظت CO_2 بین سلولی) ، عملکرد (وزن تر برگ) و نیز رنگ برگ ها(روشنی L^* ، درجه اشباع C^*، و هیو (رنگ H^o)، رنگ دانه ها (کلروفیل آ، کلروفیل ب، کلروفیل کل، کاراتینوید ها وآنتوسیانین ها) و نمایه های کیفیت (ظاهر برگ ها، تر و تازگی، مزه، و بافت). نتایج نشان داد که در هر دو کولتیوار وزن برگ تردر تیمار ۲۰ میلی مولار نمک طعام کم شد و این کاهش به محدودیت دسترسی به آب وابسته بود و در غلظت بالای نمک طعام به علت محدود شدن (بسته شدن نسبی) روزنه ها که در تلاش برای حفظ و نگهداری آب رخ داد فرایند فتوسنتز کم شد زیرا فراهمی و دسترسی به CO_2 محدود شد. با این همه، هیچ محدودیتی در رنگدانه های فتو سنتز مشاهده نشد و به این قرار، بسته شدن روزنه ها عامل چیره در محدود کردن فتوسنتز بود. از سوی دیگر، شوری باعث بهبود مقدار آنتوسیانی و رنگ در کاهو های قرمز و افزایش تر وتازگی کاهو سبز شد.