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SHORT COMMUNICATION

Yield and nutritional quality of greenhouse lettuce as affected by shading and cultivation season

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Abstract

Glasshouse experiments were conducted in winter and spring growing seasons in order to evaluate the effects of shading on production and nutritional quality of lettuce (*Lactuca sativa* L. cv. Parris Island), under Mediterranean climate conditions. In both seasons, plants were cultivated hydroponically under four different levels of photosynthetically active radiation intensities (26, 47, 73 and 100 % of incident light intensity). The results showed that stomatal conductance and photosynthetic rate significantly decreased in shaded plants. This strong negative correlation of leaf physiological parameters with light deficiency resulted in lower biomass yield production in both growing seasons. Moreover, the nutritional value (ascorbic acid concentration) was also significantly decreased in relation to incident light intensity decrease. In contrary, a strong positive correlation of leaf total chlorophyll content and nitrate content with light deficiency was detected. However, nitrate concentration in all treatments remained within the European Union's permissible levels being significantly lower in plants produced in spring compared to winter.

Key words: *Lactuca sativa*, Light intensity, Nitrate content

Introduction

Lettuce consists one of the most important cultivated vegetable in Greece, contributing significantly to national economy. Lettuce leaves are usually consumed raw and without any restriction to daily intake. However, lettuce is characterized by its great ability to accumulate nitrate in leaves which can be harmful to human health (Cometti et al., 2011). Thus, nitrate concentration in lettuce is considered one of the more important quality parameters.

Nitrate accumulation in plants is quite complex since it is influenced by both genetic and environmental factors (Novo et al., 2008). Among environmental factors light intensity is reported to strongly affect nitrate accumulation in plants (Novo et al., 2008). Taking into consideration the recent results which conclude that plants subjected to lower light intensities tend to accumulate more nitrates, nitrate levels are expected to be lower in plants grown in the Mediterranean zone which is characterized by relatively higher radiation intensities. However, the fact that nitrate accumulation in plants does not solely depend on irradiation intensity make it difficult the extrapolation of the results obtained in areas characterized by different environmental conditions. Furthermore, little information is available concerning the interaction of light intensity and growing season on the nitrate concentration. Thus, a more comprehensive study concerning the effects of different light intensities in each production area is needed.

Besides nitrates, ascorbic acid concentration is also considered as an important quality indicator in lettuces which is also influenced by both abiotic and biotic parameters (Cometti et al., 2011). However, the effects of different light intensities in ascorbic acid concentration in lettuces remained more or less obscure.

The aim of the present study was to examine the effects of different light intensities in nitrate concentration and nutritional value in lettuce plants

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grown in hydroponic system in two different growing seasons.

Materials and methods

Two experiments in different seasons (winter–spring) were conducted in a heated greenhouse of Technological Institute of Mesolonghi (lat. $38^0 21$'N, long. $20^0 56$'E), using a hydroponic system. The first experiment, which conducted during the winter period, started on November 10th, 2007 while the second experiment (spring season) started on March 8th, 2008. In both experiments, lettuce seedlings (*Lactuca sativa* L. cv. Parris Island) of three true leaves were obtained from a nursery and transplanted in 4 liters pots filled with perlite. Immediately after transplanting the seedlings were randomly divided into four groups (each one consisting of 80 plants). All plants were uniformly irrigated with a nutrient solution containing: 8 mM KNO_3, 4 mM $Ca(NO_3)_2$, 2mM $MgSO_4$, 1 mM KH_2PO_4, 4 mM $MgCl_2$, 42 μM NaFe-EDTA, 45 μM H_3BO_3, 9 μM $MnCl_2$, 0.7 μM $ZnSO_4$, 0.3 μM $CuSO_4$, 0.12 μM Na_2MoO_4. During the experimental period the nutrient solution's pH ranged between 5.6 and 6.0.

Four different light intensity treatments were applied using shade nets: (1) 100% of full ambient light (FAL) (control); (2) 73% of FAL; (3) 47% of FAL and (4) 26% of FAL. Light interception by different type nets, expressed as percentage of incident photosynthetic active radiation (PAR), was calculated at midday by taking 10 readings of PAR in rapid succession above the each type nets and 10 below the nets using a 60-cm Sunfleck Ceptometer (Decagon Devices, Pullman, WA, USA). The maximum daily PAR values measured in a sunny day in the control treatments ranged between 1290 μmol $m^{-2}s^{-1}$ and 1000 μmol $m^{-2}s^{-1}$ in spring and winter season respectively.

Gas exchange measurements

Gas exchange measurements were conducted on twenty fully expanded, mature leaves per treatment in both experimental periods. All measurements were performed one day before harvest, at the same daytime (10.00–11.30 a.m.), using a portable gas exchange system (LCpro+, ADC BioScientific Ltd). Fresh weight was measured at harvest on twenty plants per treatment.

Chlorophyll content

Chlorophyll concentration was determined on twenty leaf discs obtained from twenty randomly selected leaves per treatment. Leaf samples were collected at harvest, wrapped in plastic bags and transferred immediately in the laboratory for chlorophyll content estimation. The concentration of chlorophyll a (Chl a) was measured at 665nm and chlorophyll b (Chl b) 648nm, using a spectrophotometer (Shimadzu UV-1601 VIS) (Shinano et al., 1996). The content of the chlorophylls was calculated using the following equations (Lichtenthaler and Wellburn, 1983):

$$Chla = 14{,}85xA_{665} – 5{,}14xA_{648} \text{ (mg Chl a/ml)}$$
$$Chlb = 25{,}4xA_{648} – 7{,}36xA_{665} \text{ (mg Chl b/ml)}$$

Ascorbic acid

Ascorbic acid content was determined in the same leaves that were previously used for chlorophyll determinations according to the method described by Bajaj and Kaur (1981), using a Spectrophotometer (Shimadzu UV-1601 VIS).

Nitrates content

Nitrate concentration was measured at harvest using twenty randomly selected leaves obtained from twenty plants per treatment. Each leaf sample was homogenized in a blender and the resulting slurry was filtered through two paper filters. The filtrate was diluted (1: 10-1:200) in the eluent used and filtered through a 0.2-μm membrane filter. This solution was injected into the chromatograph according to Pentchuk et al. (1986).

Statistical data analysis

Mean values of all treatments were compared by two-way ANOVA test and significant differences were determined using Duncan's test ($p<0.05$). All data were analyzed using SPSS 15.0 program for Windows.

Results and Discussion

In both seasons (winter and spring) the accumulation of nitrates in the leaf tissues was significantly higher in lower irradiance intensity treatments compared to the control (Table 1). Furthermore, nitrate concentration was significantly higher in plants grew during the winter period compared to those in spring (Table 1) irrespective the light intensity applied, indicating that different growing seasons affect nitrate content. These higher values of nitrate concentration during the winter season could be attributed to lower PAR intensity exhibited during that period and/or to differences in other microclimatic parameters. However, the fact that there was no significant interaction between growing season and PAR intensity (Table 1) provide evidences that the above mentioned differences in nitrate concentration could be mainly attributed to changes in PAR intensity. These results clearly suggest that light intensity should be considered as the main factor influencing of nitrate concentrations in lettuces. Novo et al. (2008) growing lettuce in hydroponic system reported that only a 18% shading resulted in an significant increase in nitrate concentration in plants. In our results the maximum concentration of nitrates found (even at lowest levels of PAR intensity in winter) does not exceed the European union permissible levels for winter season suggesting that under the studied area conditions the nitrate levels do not represent any risk for human consumption of hydroponics lettuces. Similar results concerning the significant role of light intensity in nitrate concentration have also been reported for a variety of vegetables (Blom-Zandstra and Lampe, 1985; Drews et al., 1997; Proietti et al., 2004).

Except nitrates, ascorbic acid concentration is considered as one of the most important nutritional quality factors in many vegetable crops (Lee and Kader, 2000). In our results, the higher levels of shading seemed to negatively affect ascorbic acid concentration (Table 1). Furthermore, the ascorbic acid content was significantly lower in lettuces grown during winter compared to those grown during spring (Table 1). The statistical analysis of all data indicates that ascorbic acid concentration in lettuce seemed to be light dependent. Several reports concerning the mechanism of light induced ascorbic acid synthesis tend to conclude an indirect effect of light intensity in ascorbic acid concentration rather a direct one. In particular, a close relationship between photosynthetically produced sugars and ascorbic acid concentration is suggested (Lee and Kader, 2000). If this is true, then it would be expected that photosynthetic rate would be significantly higher in those treatments exhibiting the higher ascorbic acid content. Indeed, in our results the treatments characterised by higher accumulation in ascorbic acid i.e the control treatments also exhibited the higher photosynthetic rates (Figure 1). Thus, our results tend to conclude that the amount and intensity of light during the growing season might have a considerable effect on the concentration of ascorbic acid probably via its influence on the plants photosynthetic performance. Similar results were also observed in other leafy greens where ascorbic acid concentration was also increased in relation to light exposure (Gruda, 2005; Oyama et al., 1999; Weerakkody, 2003).

Table 1. Effect of different light intensities (expressed as percentage of the control) and growing seasons on nitrates, chlorophyll and ascorbic acid content as well as on fresh weight in lettuces plants*.

Season	PAR (% Control)	Nitrates mg/ Kg f.w	Chlorophyll a+b mg /g f.w	Ascorbic acid mg /100g f.w	Fresh weight (f.w) g.plant^{-1}
Winter	100	2571.3±97.83^{a}	1.51±0.01^{a}	67.77±0.91^{d}	290.41±5.77^{d}
	73	2931.3±50.21^{b}	1.67±0.03^{a}	61.37±0.73^{c}	240.67±5.81^{c}
	47	3203.9±49.65^{c}	1.91±0.06^{b}	55.47±0.52^{b}	208.33±9.28^{b}
	26	3616.7±61.51^{d}	2.13±0.09^{c}	48.20±0.26^{a}	167.67±6.49^{a}
Spring	100	1991.1±61.26^{a}	1.37±0.03^{a}	73.57±0.88^{d}	326.67±12.02^{d}
	73	2357.1±49.8^{b}	1.57±0.03^{b}	65.47±0.58^{c}	270.33±2.91^{c}
	47	2749.7±73.76^{c}	1.77±0.03^{c}	58.43±0.56^{b}	242.67±4.33^{b}
	26	3028.3±52.74^{d}	1.97±0.02^{d}	52.47±0.61^{a}	201.67±4.41^{a}
Two-way ANOVA (p-values)					
Season (S)		<0.0001	<0.001	<0.0001	<0.0001
PAR (P)		<0.001	<0.001	<0.0001	<0.0001
S X P		0.692	0.910	0.241	0.966

*Values are means ± S.E. from 20 replicates. Means followed by different letters are significantly different ($p < 0.05$) according to the Duncan's test. Probability (p-value) of two way analysis for season and PAR is shown.

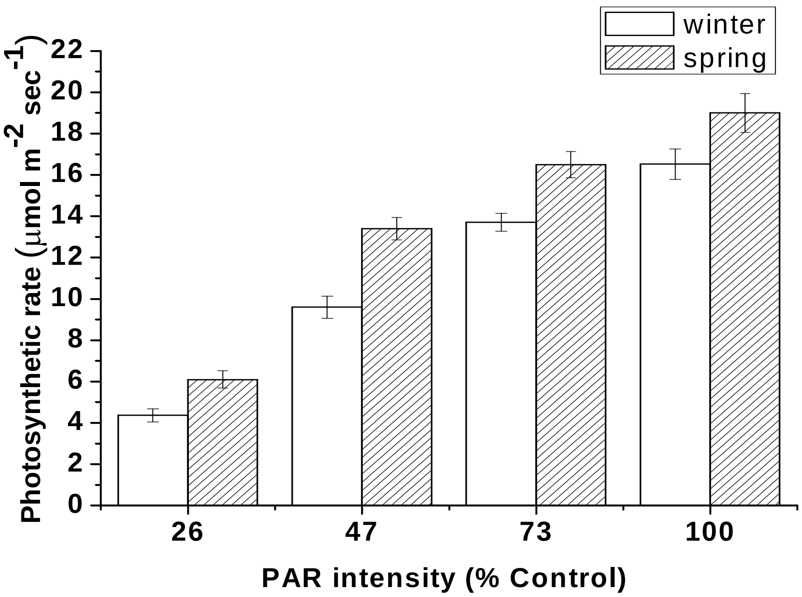

Figure 1. Effect of different light intensities (expressed as percentage of the control) and growing seasons in plants photosynthetic rate.
Data are means ± S.E of twenty replicates.

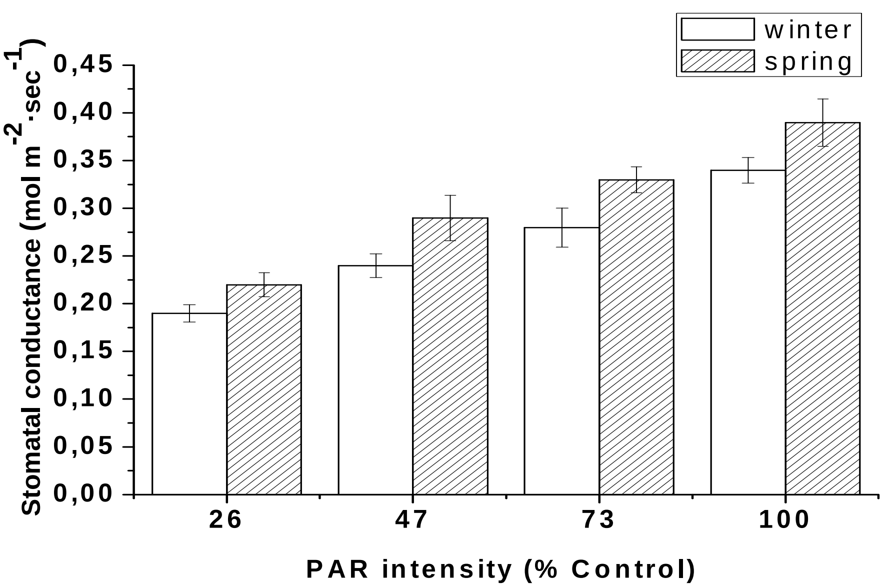

Figure 2. Effect of different light intensities (expressed as percentage of the control) and growing seasons in plants stomatal conductance.
Data are means ± S.E of twenty replicates.

As it was probably expected the increase in photosynthetic rate in plants grown under the higher light intensity was also positively related to plants fresh weight (Table 1). Similar results were also observed by Caruso et al. (2004), who reported that shading caused a reduction in the fresh weight and the content of the main taste-determining compounds (sugar, acids, mineral elements except nitrates) in strawberries, resulting in a poor taste sometimes associated with autumn-grown vegetables. Higher fresh weight was due to largest leaves per plant rather to different leaf number per plant (data not shown). Craker and Seibert (1983) have suggested a light-dependent photo-regulatory mechanism within the plants that controls the development of leaf size. Our data with lettuce seem to support these interpretations. Higher photosynthetic rate under higher irradiance could be attributed to higher stomatal conductance since the latter exhibit a linear relationship in response changes in photosynthetic rate (Figure 2). In contrary, changes in total chlorophyll concentration in relation to photosynthetic rate followed an opposite pattern exhibiting higher values under reduced radiation intensities (Table 1). This is consistent with the results already reported for various species which indicated higher chlorophyll content in the plants grown under shaded condition (Vandana and Bhatt, 1999). This increased pigment content in shaded leaves has been attributed to the increase in number and size of chloroplast, the amount of chlorophyll per chloroplast and/or better granna development and is considered to be an efficient plants adaptive acclimation process to low light intensities.

Conclusions

Our study indicated that the reduction of light intensity represented the main environmental factor affecting lettuce quality indicators. However, although nitrate increase due to low light intensity was significant, nitrate accumulation in leaves remained within European Union's permissible levels. Furthermore, a direct light-intensity effect on fresh weight and an indirect one on nutritional value (ascorbic acid) of lettuce were also evident.

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