The effects of development at chilling temperatures on the function of the photosynthetic apparatus under high and low irradiance in leaves of lettuce (Lactuca sativa L.)

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Abstract: The objective of this study was to evaluate the effects of changes in the photosynthetic photon flux density (PPFD) and temperature on net gas exchange of Lactuca sativa L. Also changes in the content of pigments, lipid peroxidation and chlorophyll fluorescence parameters were measured during growth under low (chilling) and high temperature. Plant production, driven by photosynthesis, is sensitive to abiotic stress. Among all photosynthetic functions, Photosystem II (PSII) is believed to be the most sensitive. The in vivo chlorophyll fluorescence technique is a powerful non-destructive and fast method to detect changes in the photosynthetic activity in leaves influenced by changes in the environment. When PPFD was decreased from 550 to 60 mmol. $m^{-2}s^{-1}$ CO_2 assimilation rate (A) decreased and stabilized in low light and chilling temperatures. Stomatal conductance (gs), and Fv/Fm also decreased significantly. In addition, the significant increase of Tm/Area corresponded to disturbances or damage to the photosynthetic apparatus. Remarkable changes were observed in chlorophyll a+b, fluorescence emission and also accumulation of malondialdehyde (MDA). Our results suggest that lettuce leaves have a great capacity to develop their photosynthetic apparatus to resist chilling-induced photoinhibition through the changes of pigment composition, but low light condition affects this capacity.

Key-words: environment, light, stress, chilling, temperature, Lactuca sativa L., chlorophyll, malondialdehyde, photoinhibition,

1 Introduction

Plants are exposed to various types of environmental stress. Among various stresses, light stress, is a serous problem that limits plant growth and crop productivity in agriculture (Boyer J.S., 1982; Saccardy et al., 1998, Dubey 1999; Behera et al., 2002). This stress is known to bring changes in patterns of leaf development (Deo and Biswal 1998, 2001). Chloroplasts experience irradiance stress when the absorption of light energy exceeds the capacity of utilization. Photoinhibition of photosynthesis will arise when the rate of transfer of excitation energy from the antennae to the photochemical reaction center exceeds the rate of electron transport (Barber and Andersson 1992, Hermann et al., 1997). Photoinhibition is considered as a first stage of irradiance induced thylacoid damage leading to reduction of photosynthetic capacity, (Powles, 1984; Behera et al., 2002). Pigment photooxidation is the second stage which occurs after long-term exposure of the plants to strong irrandiance and concerns the bleaching of the antennae pigments. It is now evident that photoinhibition which results from the conjuction of HIS with other stresses like, water deficit, chilling, salinite e.t.c. has an important impact on the plants under natural conditions (Ramalho et al., 1997). To cope with these stresses, plants must develop protective mechanisms.

However, reductions in the photosynthetic capacity that arise from environmental stresses such as low temperature or different light intensities are more likely to be the results of disturbances in the development of the photosynthetic apparatus rather than of direct effect on the photosynthetic capacity of mature leaves (Baker, 1991).

Chilling stress reduces the capacity of photosynthetic systems to utilize incident light, leading to a photoinhibition process. Photoinhibition of photosynthesis is typically characterized as a reduction in quantum yield of PSII photochemistry and a decrease in chlorophyll fluorescence (Demmig & Bjorkman, 1987). Photoinhibition entails not only the inhibition of PSII (Kyle, 1987) but also the increased thermal de-excitation of excited chlorophyll (Ogren et al., 1984). The latter is often considered a photoprotective process. In the presence of environmental stresses such as low temperature and

drought, plants are more sensitive to light stress (Bjorkman & Powles, 1984; Oquist & Ogren, 1985). Photoinhibition of photosynthesis has long been reported for plant under high light conditions at chilling temperatures of 0-10 oC (Bongi, 1987). Furthermore, for all annual plants of temperate regions have been shown to sometimes undergo photoinhibition even when they are exposed to moderate light at chilling temperatures (Smillie et al., 1988; Somersalo & Krause, 1989).

Growth of lettuce leaves at chilling temperatures leads to a large reduction in the rate of photosynthesis. The xanthophyll cycle has been thought to play an important role in protection of photosynthetic function from photoinhibition (Demmig-Adams, 1990). Non-radiative energy dissipation at PSII has been reported to be mediated by zeaxanthin and also possible by antheraxanthin (Gilmore & Yamamoto, 1993). In the present work we have examined the effect of chilling and light conditions on the function of photosynthetic apparatus during mature phase of lettuce leaves.

2 Materials and Methods

2.1 Plant material and growth conditions

Seeds of two lettuce varieties (Lactuca sativa L., var. Grand rapids) were sterilized with 0.1% HgCl2, 0.03% EDTA and 0.1% KCl for 10 min respectively, rinsed excessively with distilled water and germinated in vermiculite at 21^{o}C. The plants were transplanted individually into plastic pots (15cm in diameter) containing a commercial soil mixture (Terraplant, Basf, Uchte, Germany) and then placed in a computer-controlled environment growth chamber where they remained for 90 days.

Average day and night temperatures inside growth chamber were 21^{o}C /18 ^{o}C respectively with a 12h photoperiod. The PPFD at plant height was 300 μmol $m^{-2}s^{-1}$ and the RH was 70% for 5 days throughout the experiment. The seedlings were watered daily with half strength Hoagland's solution. The 6-week-old plants were acclimated for 5 days under a 12-h photoperiod with an irradiance of 550 μmol $m^{-2}s^{-1}$ at 21/18°C and an RH of 70%. The acclimated plants were exposed to chilling at 3/3°C for 5 days with either low light (60 μmol $m^{-2}s^{-1}$) or hight light (550 μmol $m^{-2}s^{-1}$). After the chilling treatments, all plants were allowed to recover at 21/18°C at 550 μmol $m^{-2}s^{-1}$ PPFD. In the acclimation, chilling and recovery study, chlorophyll fluorescence was measured before the temperature shift and then daily during the chilling and recovery periods. All measurements (gas exchange, chlorophyll fluorescence, and lipid peroxidation) were performed on mature and external leaves (20-25cm length by 10-15 cm wide) in asintomatic areas on the upper zone of the leaves.

2. 2 Pigment estimation

The pigments were extracted in 80% chilled aceton. The amounts of Chl (a+b) and Car were estimated spectrometrically according to Lichtenthaler (1987).

2.3 In Vivo Chlorophyll Fluorescence Measurements

In Vivo Chlorophyll fluorescence was measured on the upper surface of the third fully expanded leaf, after being left for 30 min to dark adaptation, at room temperature. The chlorophyll fluorescence induction curve monitored by a Plant Analyser (PEA, Hansatech Ltd King's Lynn, Norfolk, England) with 600W m^{-} of red (630nm) light intensity (excitation intensity) (Pereira et al., 2000). The initial fluorescence intensity (Fo) when all reactions centres (RCs) are open, Fm maximal fluorescence intensity when all reactions centres (RCs) are close, Fv variable fluorescence, tmax time to reach the maximal fluorescence intensity were calculated. The indicators were measured at room temperature on intact leaves of four replicate plants from the four treatments

2.4 Gas-Exchange Measurements

Gas-exchange measurements were made from the same leaf with a portable photosynthetic apparatus Li-6200 (Licor, Inc., Lincoln, USA) with IRGA. The carbon dioxide (CO_2) analyzer was calibrated with two standard CO_2/air mixtures. The lamina of the leaf was enclosed within a fun stirred ¼ L cuvette. The mean CO_2 concentration and leaf to air vapour pressure deficit for all measurements were 350 μmol mol-1 and 20 mbar respectively. Measurements were made at 25 ^{o}C under a photon flux density of about 600 μmol $m^{-2}s^{-1}$. Calculations of A, Ci and gs from gas-exchange measurements were according to Von Caemmerer and Farquhar (1981).

2.5 Determination of lipid peroxidation

The level of lipid peroxidation in shoots/leaves after five days of chilling stress and 3 days of recovery treatment was measured as malondialdehyde (MDA) content determined by reaction with 2-thiobarbituric acid (TBA) reactive substances according to Heath and Packer (1968). The tissue was homogenized in 0.3% TBA in 10% trichloracetic acid (TCA) at 4 ^{o}C. The concentration of MDA was calculated from the difference of the absorbance at 532 nm and 600 nm

using the extinction coefficient of 155 $mmol^{-1}cm^{-1}$ and expressed as nmol (MDA) g^{-1} of fresh weight.

2.6 Statistical analysis

Significant differences between the stressed and the control samples were analyzed following Duncan's test.

3 Results and Discussion

Chlorophyll a (Chl a) content generally remained constant during high light, but was reduced by low light. The analysis of data of photosynthetic pigment contents of lettuce leaves treated with high light and low light (Table 1) clearly suggest that the stress treatment of low light causes faster rate of loss of chl (a+b) and chl (a/b) compared to high light treatment. The highest pigment loss (39%) in the LL+ CH treated samples suggest that when plants experience two differing stresses simultaneously, because of the additive action the effect was more, compared to plants exposed to single stress (Demmig et al., 1988). Such leaves also have been shown to have a very low photosynthetic capacity (Nie & Baker, 1991; Haldimann et al., 1995). The loss of chl in high light stress condition may be because of photooxidation of pigments (Powles, 1984; Behera and Choudhary, 2001). High irradiance causes formation of reactive O2 species through 3Chl* and due to impairment of electron transport in chloroplasts (Minkov et al., 1999). These highly reactive substances are responsible for faster degradation of chlorophyll.

The effects of temperature and light intensity on the content of carotenoids are depicted in Fig.1. At 3°C the total content of carotenoids expressed on a leaf area basis was higher at high light intensity than at low light intensity. Carotenoids, apart from functioning as accessory light harvesting pigment, play a significant role in photoprotection of Chl and chloroplasts in general against photooxidative damage (Demmig-Adams and Adams 1992; Minkow et al., 1999; Choudhury and Behera, 2001). Choudhury et al., 1993 have suggested that photoprotection is associated with self-destruction of Car due to potentially harmful quanta received from ^{1}Chl* under excess irradiance. The loss of Car was higher (40%) after 5 day of stress treatment (chilling) compared to the control. The decrease of the carotenoids contents was more in low light+ chilling which may suggest synergetic action of two stresses.

The efficiency of photochemistry Fv/Fm declined, showing alterations of PSII reactions centers and an inhibition of enzymatic process in the Calvin cycle. In addition, the significant increase of Tm/Area (Data not shown) corresponding to disturbances or damage to the photosynthetic apparatus. The quantum yield of electron transport decreased as a result of lowered efficiency of photochemistry and highly reduced state of Q_A (the primer acceptor) (Ouzounidou et al., 1997).

The rate of Irradiance saturated CO_2 assimilation rate (Amax) was depressed by lowering the temperature from 21°C to 3°C (Table 2) The reduction in Amax was significant and more severe (49%) at 3°C and low light than high light (38%) at the same chilling temperature. The results in Table 2 demonstrate that: untreated lettuce leaves (21°C) had a much higher stomatal conductunce (gs) but a much lower intercellular CO_2 concentration (Ci) than the treated plants with chilling temperatures under low light.These results point to mesophyll limitation under these conditions.

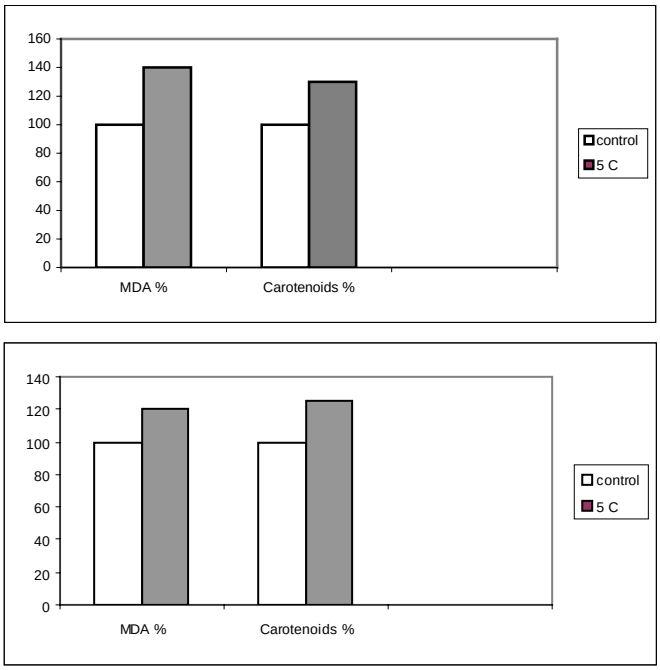

Fig. 1: Changes of carotenoids contents and malondialdehyde (MDA) content (n=5) of lettuce fully expanded leaves in high light and low light treatment respectively

Photoinhibition occurs when the leaves are eposed to irradiances in excess of what can be utilized in photosynthesis (Powles, 1984; Barber & Andersson, 1992). In addition, the suscebility of photosynthesis to inhibition increases with an increasing proportion of reduced Q_A (Ogren et al., 1984). Thus the result that reduction in Fv/Fm occured in 3^0C exposed leaves but not in 21 µmol m-2s-1 PPFD treated ones in 60 and 550 µmol m-2s-1 PPFD could be due to the effects of low temperature on the photosynthetic capacity (Table 2). Consequently, a higher proportion of reduced reaction centres was accumulated in 3°C exposed

leaves. Similar results were found in Zea mays, Zoysia grass where low temperature caused a cocominant reduction in maximum photosynthetic capacity,reductions in chlorophyll fluorescence and in the ability of the plant to utilize high irradiances for photosynthesis (Nie et al., 1991; Okawara & Kaneko, 1995).

Malondialdehyde is formed through autooxidation and enzymatic degradation of polyunsaturated fatty acids in cells during the natural process of ageing or under stress conditions. Hence the occurence of MDA is considered as a useful index of general lipid peroxidation of the plants under stress (Hodges et al., 1999). A linear increase in lipid peroxidation was observed after 5 days of stress treatment in all light intensities (low light, high light). However the increase was highest in (high light + chilling) treatment (about 56%) compared to high light which may suggest synergetic action of the two stresses.

4 Conclusions

From the results of this study it is concluded that growth of lettuce leaves at chilling temperature not only induces a large reduction in the Chl a+b content but also causes important changes in the content of the carotenoids. These changes may be related to low temperature-induced modifications to the polypeptide profile of the thylakoid membranes. Furthermore, it is likely that lettuce leaves treated at chilling temperature at high light intensity accumulate large amounts of the carotenoids on the xanthophyll cycle (zeaxanthin) in order to protect themselves against damage from light. The decrease of Fv/Fm indicated that the efficiency in the energy conversion of PSII was affected. The limitation of photosynthesis appears to be correlated with stomatal closure and a decrease in mesophyll activity as demonstrated by the increase in Ci.

As a conclusion, our study shows that chilling temperature reduced the capacity of photosynthesis of Lactuca sativa causing a increase excitation pressure of the reaction centres of PSII, as expressed by increased reduction state of Q_A, hence increased the susceptibility of PSII to photoinhibition .

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TABLES

Treatment	chlorophyll a		chlorophyll b		chlorophyll a+b	
	High light	Low light	High light	Low light	High light	Low light
21 °C (Control)	8.94a	7.62b	3.52a	2.82a	12.46a	10.44b
3°C	8.64a	6.02c	2.89a	2.05a	11.53a	8.07c

Table 1. Effect of light intensity and temperature on chlorophyll content of lettuce. Mean values ± s.e of five replicates. Means within columns followed by the same letter are not significantly different at the 0.05 probability level using Duncan Multiple Range Test.

Treatment	A_{max}		g_s		C_i		Fv/Fm	
	High light	Low light	High light	High light	Low light	Low light	High light	Low light
21°C (Control)	14.5a	12.4a	0.323a	273.8a	268.5a	0.282a	0. 83 a	0.8a
3°C	8.64b	6.02c	0.234b	342.6b	339.1b	0.152b	0.71b	0.67b

Table 2. Effect of light intensity and temperature on gas exchange and chlorophyll fluorescence parameters of lettuce. Mean values ± s.e of five replicates. Means within columns followed by the same letter are not significantly different at the 0.05 probability level using Duncan Multiple Range Test.