Leaf plasticity to light intensity in Italian cypress (*Cupressus sempervirens* L.): Adaptability of a Mediterranean conifer cultivated in the Alps

Paolo Baldi [a], Krishnasamy Muthuchelian [b,c], Nicola La Porta [b,*]

[a] *Genomics and Biology of Fruit Crops Department, IASMA Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, 38010 San Michele all'Adige (TN), Italy*
[b] *Sustainable Agro-Ecosystems and Bioresources Department, IASMA Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, 38010 San Michele all'Adige (TN), Italy*
[c] *Centre for Biodiversity and Forest Studies, School of Energy, Environment and Natural Resources, Madurai Kamaraj University, Madurai 625 021, India*

ABSTRACT

Italian cypress (*Cupressus sempervirens* L.) is native to the eastern Mediterranean, an area characterised by hot, dry summers and mild winters. Over the centuries, however, the species has been introduced into more northerly regions, a long way from its native range. The current, generally warmer climatic conditions brought about by global warming have favoured its cultivation in even more northerly areas in the Alps and other European alpine regions. Given that not only temperature, but also light availability are limiting factors for the spread of cypress in these environments, it is important to ascertain how this species copes with low light conditions. The photosynthetic characteristics of cypress leaves collected from different portions of the crown with contrasting light availability were evaluated by several methods. Chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoid (Car) content was found to be higher in shade leaves than in sun leaves when measured on a fresh mass basis, although enzymatic activities of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) and nitrate reductase were lower in shade leaves. When the efficiency of PSII was measured by chlorophyll fluorescence, a marked reduction in F_m was found in shade leaves, while F_o remained unchanged. The use of exogenous electron donors diphenyl carbazide (DPC) and NH_2OH actually improved the photosynthetic efficiency of shade leaves, and the same effect was found when PSII electron transport activity was measured as O_2 evolution. Altogether, these results seem to indicate lesser photosynthetic efficiency in shade leaves, probably an impairment on the donor side of the PSII. At the same time, analysis by SDS–PAGE revealed differences in the polypeptide composition of the thylakoid membranes of sun and shade leaves: the bands corresponding to 23 kDa, 28–25 kDa and 33 kDa polypeptides were less intense in the thylakoid membranes extracted from shade leaves. These results were further confirmed by an immunological study showing that the content of the 33 kDa protein, corresponding to the extrinsic PSII protein PsbO, was significantly diminished in shade leaves. The high plasticity of cypress leaves appears to be an advantageous trait in the plant's response to variations in environmental conditions, including global change. Implications for the management of this Mediterranean species at the northern edge of its distribution are discussed.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Light availability is highly variable in all natural environments. Light fluctuations can occur both within the crown of a single plant and within plant canopies, so it is crucial for all plant species to be able to cope with differences in this key environmental factor [1].

Physiological plasticity allows plants to adapt to different light gradients, and photosynthetic acclimation may occur at the whole-plant, leaf and chloroplast levels [2]. The plasticity index, in the sense of Valladares et al. [3], is much higher for physiological than

for morphological and anatomical leaf traits in Mediterranean evergreen tree species, while in Norway spruce, light absorption by tree crowns is only a slightly better predictor of stem volume increment than crown leaf area.

Furthermore, tree photosynthesis and growth rates decline with increasing age and size, while support biomass in roots, stems and branches accumulates and the concentrations of non-structural carbohydrates increase, collectively resulting in an increase in non-structural carbon pools [4].

Light harvesting can be adjusted by changes in leaf morphology and in exposure of foliage to incident irradiance, as well as by alteration of the chlorophyll a/b ratio and carotenoid composition [5]. Both the quantity and quality of light regulate the activity and composition of the photosynthetic apparatus [6]. The light environment therefore plays a critical role during leaf growth and gives

* Corresponding author. Address: Fondazione Edmund Mach, Via E. Mach 1, 38010 San Michele all'Adige (Trento), Italy. Tel.: +39 0461 615 396, mobile: +39 338 7888 736; fax: +39 0461 650 956.
E-mail address: nicola.laporta@fmach.it (N. La Porta).

rise to morphological as well as biochemical adaptations that will greatly influence the photosynthetic properties of the mature leaf.

Plants adapted to high light conditions may differ considerably from those adapted to low light conditions. The main differences lie in the amount and composition of photosynthetic pigments, electron carriers, chloroplast ultrastructure and rates of photosynthesis [7]. Sun leaves are generally defined as those requiring a higher light saturation photon flux density and having a higher saturated photosynthetic rate and increased light compensation point compared with corresponding shade leaves [8]. However, the way in which the photosynthetic rate is measured will affect the comparison. Because sun leaves are thicker than shade leaves [9,10], their pigment levels are higher when based on leaf area but lower when based on fresh weight. Sun leaves, with their sun-type chloroplasts, have a much higher photosynthetic rate when assessed on leaf area and Chl, higher Chl a/b ratio, higher saturation irradiance of photosynthetic CO_2 fixation, lower concentrations of light-harvesting Chl proteins (LHCPII), as well as smaller grana stacks and more exposed thylakoid membranes than shade leaves with their low-irradiance chloroplasts [7].

Light energy is a limiting factor for plants growing in the shade. In order to maximise photon absorption, plants produce shade leaves with a greater surface area and a higher chlorophyll content per chloroplast. Their chloroplasts are characterised by large grana stacks, and most of the chlorophyll is contained in the outer antenna, the light-harvesting complex LHCII. Other elements of their photosynthetic apparatus, such as electron-transport chains, are present in relatively low amounts [1,11].

Extensive research on sun-to-shade adaptation in leaves has shown that plants grown in low light adapt to these conditions by increasing their light harvesting ability and they alter their chloroplast anatomy by increasing the area of appressed regions in the thylakoid membranes [12–14]. Exposure to high light conditions triggers inactivation of PSII reaction centres in shade-adapted leaves, leading in turn to oxidative stress [15]. The most evident symptoms of oxidative damage are bleaching, chlorosis and necrosis of leaves [16,17]. Adjustments in the levels of Chl b and light-harvesting complex proteins, and additional changes in Rubisco and Q_B protein levels are mostly regulated in shade-adapted leaves by changes in gene expression at the transcriptional or post-transcriptional level [18,19].

According to Boardman [20], the productivity of a leaf is primarily governed by its position in the canopy. Although several factors may vary with position in the leaf canopy, the most important and limiting one, especially where photosynthesis is concerned, is light quantity and quality [21]. In natural conditions, leaves of the same plant growing in different canopy positions may be subject to different stresses and consequently forced to respond with individual adaptation strategies [3]. Therefore it would be useful to determine the changes in chlorophyll concentrations of leaves exposed to contrasting light conditions, and whether this is related to other photosynthetic activities. Most studies of plant response to light have been concerned with investigating the morphological and physiological mechanisms and ecological implications of tolerance to extreme sun or shade, but noticeably less effort has been invested in exploring trends in the plastic response to light [3].

Italian cypress (*Cupressus sempervirens*) is a medium-sized evergreen tree with a crown that is usually narrow and conical, and small, scale-like, ovate, dark green leaves. It is widely cultivated in forests and as an ornamental plant. The species is native to the eastern Mediterranean region [22], which is characterised by hot, dry summers and mild winters, but over the centuries it has been introduced into regions far north of its native range.

The Pre-Alps, the alpine foothills and the valley bottoms of the southern slopes of the Alps are suitable natural vegetation areas for cypress and there is considerable potential for further spread of this conifer in these regions. The northward shift is assisted by two important contingent factors: overall climate warming, bringing with it tree migration and changes in species distributions [23], and the alarming and progressive decline of the European black pine (*Pinus nigra* Arnold) caused by *Sphaeropsis sapinea* and *Cenangium ferruginosum* [24]. These pathogens are causing the disappearance of the only conifer present at low altitude in the forests and landscapes of the Italian Alps.

The alpine region of Trentino (eastern Italian Alps) has recently been surveyed for the presence and performance of cypress. The cypress was introduced to this region a long time ago [25] and is currently present at altitudes ranging from 66 m a.s.l. (upper Garda Lake) to 985 m a.s.l. in the Fiemme Valley, usually in small groups or in woods, distributed over around 1900 sites [26], more than 90% of them between 66 and 350 m a.s.l. and with predominantly S, SW and W aspects (unpublished data). Having long since established itself in Trentino, the cypress continues to spread northwards, although climatic conditions in the region, like several other regions in the Alps, are considerably different from those in which the cypress originally developed [22]. While cold stress [27,28] and the pathogen *Seiridium cardinale* (Wag) Sutton [29] may be considered the main factors limiting cypress diffusion, quality and quantity of light also play a role, especially in mountainous regions where the hours of direct sun are usually greatly reduced due to topography and the greater number of cloudy days [30–32]. Cypress has been able to adapt to a certain extent to the reduced photoperiod [33], but more needs to be known about its plasticity to light intensity. The aim of the present work is to throw light on cypress adaptation to different light conditions by studying the photosynthetic characteristics of sun and shade leaves grown under field conditions at the northern edge of the species distribution.

2. Materials and methods

2.1. Plant materials

Sun and shade leaves were collected from a 20-year-old cypress tree (*C. sempervirens*), var. *pyramidalis*, grown under field conditions at the Istituto Agrario di San Michele all' Adige, Italy (46°12′ North, 11°08′ East).

In order to simplify the experimental procedure, we classified leaf samples of the same age (2nd year) into two groups according to the intensity of light received on the leaf surface and canopy position: shade leaves were collected from the inner part of the tree at a PAR of less than 50 µmol m^{-2} s^{-1}, whereas sun leaves were collected from a well-irradiated outer canopy position with a maximum PAR of 1700 µmol m^{-2} s^{-1}. Leaves were sampled early in the morning before they had received direct sunlight. The average hours of sun per day (18th September 2009) to leaf sample was 8 h (SD was 2 h 50′). Average maximum and minimum air temperatures for this period were 25.6 °C (SD 3.7 °C) and 12.0 °C (SD 2.9 °C), respectively. The highest air temperature recorded was 32.5 °C, the lowest 4.2 °C. Shade influenced not only the light microclimate but also air and leaf temperatures; the maximum leaf temperature on sun plants was 33 °C (18th September).

2.2. Pigment determination

Chl was extracted with 100% acetone from leaves frozen with liquid N_2 and stored at −20 °C. Chl and Car were analysed spectrophotometrically according to Lichtenthaler's [34] method.

2.3. Chl fluorescence in leaves and thylakoid membranes

Chl fluorescence was measured on leaves using a PAM 2000 fluorometer (H. Walz, Effeltrich, Germany). Fo was measured by

switching on the modulated light at 0.6 kHz; PPFD was less than 0.1 μmol m^{-2} s^{-1} at the leaf surface. F_m was measured at 20 kHz with a 1s pulse of 6000 μmol m^{-2} s^{-1} of white light.

Modulated Chl fluorescence on isolated thylakoid membranes at room temperature was also measured with a PAM 2000 fluorometer. Measurements were carried out in a 1 mL reaction mixture containing 50 mmol/L Tris–HCl (pH 7.5), 2 mmol/L MgCl$_2$, 10 mmol/L NaCl, 100 mmol/L sucrose and 10 μg Chl equivalent thylakoid membranes. The integrated intensity of measuring light (480 nm) was 0.15 μmol m^{-2} s^{-1}, the intensity of red actinic light (650 nm) 100 μmol m^{-2} s^{-1}.

2.4. Photosynthetic electron transport activities

Thylakoid membranes were isolated from the leaves as described by Berthhold et al. [35]. Oxygen evolution (H$_2$O → DCBQ; H$_2$O → SiMo (PSII activity)] or uptake [DCPIPH$_2$ → MV (PSI activity)) was measured according to Nedunchezhian et al.'s [36] method using a Clark-type O$_2$ electrode (Hansatech, UK) fitted with a circulating water jacket at 27 °C. Actinic light from a slide projector was applied to the side of the electrode chamber filtered through 9.5 cm of water. Light intensity at the surface of the water bath cell was 1100 μmol m^{-2} s^{-1}. Thylakoid membranes were suspended at 10 μg Chl mL^{-1} in the assay medium containing 20 mmol/L Tris–HCl (pH 7.5), 10 mmol/L NaCl, 5 mmol/L MgCl$_2$, 5 mmol/L NH$_4$Cl and 100 mmol/L sucrose supplemented with 500 μmol/L DCBQ and 200 μmol/L SiMo for oxygen evolution (PSII), and 1 mmol/L MV, 2 mM ascorbate, 5 mmol/L DCMU, 1 mmol/L sodium azide and 100 μmol/L DCPIP for uptake (PSI). The rate of whole-chain electron transport (H$_2$O → MV) in isolated thylakoid membranes was measured as described by Armond et al. [37]. Thylakoid membranes were suspended at 10 μg Chl mL^{-1} in the assay medium containing 20 mmol/L Tris–HCl (pH 7.5), 10 mmol/L NaCl, 5 mmol/L MgCl$_2$, 5 mmol/L NH$_4$Cl and 100 mmol/L sucrose supplemented with1 mmol/L MV and 1 mmol/L sodium azide.

2.5. DCPIP photoreduction measurements

DCPIP reduction was determined as the decrease in absorbance at 590 nm using a Hitachi 557 spectrophotometer. The reaction mixture (3 cm^3) contained 20 mmol/L Tris–HCl, pH 7.5, 5 mmol/L MgCl$_2$, 10 mmol/L NaCl, 100 mmol/L sucrose, 100 μmol/L DCPIP and thylakoid membranes equivalent to 20 μg of Chl. Where mentioned, the concentrations of MnCl$_2$, DPC and NH$_2$OH were 5, 0.5 and 5 mmol/L, respectively.

2.6. SDS–PAGE

Thylakoid membranes were separated using Laemmli's [38] polyacrylamide gel system with the following modifications. Gels consisted of a 12–18% gradient of polyacrylamide containing 4 M urea. Samples of thylakoid membrane preparation were solubilised at 20 °C for 5 min in 2% (w/v) SDS and 60 mmol/L DTT and 8% sucrose using an SDS–Chl ratio of 20:1. Electrophoresis was performed at 20 °C with a constant current of 5 mA. Gels were stained in methanol/acetic acid/water (4:1:5, v/v/v) containing 0.1% (w/v) Coomassie brilliant blue R and destained in methanol/acetic acid/water (4:1:5, v/v/v). Thylakoid membrane proteins were estimated according to Lowry et al.'s [39] method.

2.7. Immunological determination of thylakoid membrane proteins

The relative contents of certain thylakoid membrane proteins per mg chlorophyll were determined immunologically by western blotting. Thylakoids were solubilised in 5% SDS, 15% glycerine, 50 mmol/L Tris–HCl (pH 6.8) and 2% mercaptoethanol at room temperature for 30 min. The polypeptides were separated by SDS–PAGE as described above and proteins were then transferred to nitrocellulose by electroblotting for 3 h at 0.4 A after saturation with 10% milk powder in TBS buffer (pH 7.5). The first antibody in 1% gelatine was allowed to react overnight at room temperature. After washing with TBS containing 0.05% Tween-20, the secondary antibody (Anti-Rabbit IgG (whole molecule) Biotin Conjugate, Sigma) was allowed to react in 1% gelatine for 2 h. A polyclonal antiserum against spinach D1 protein (kindly provided by Prof. I. Ohad, Jerusalem, Israel) was used to detect D1 protein; the antibody against the 33 kDa protein of the water-splitting system was a gift from Dr. Barbato, Padua, Italy. Densitometry analysis of western blots was performed with a Bio-Image analyser (Millipore Corporation, Michigan, USA).

2.8. Determination of soluble proteins

Soluble protein was extracted by grinding leaves (0.3–0.5 g fresh weight) in a mortar with 6 mL of 100 mmol/L Tris–HCl, pH 7.8 containing 15 mmol/L MgCl$_2$, 1 mmol/L EDTA, 10 mmol/L 2-mercaptoethanol, 10 mmol/L PMSF in the presence of liquid nitrogen. Homogenates were filtered through nylon cloth. After centrifugation at 11,000g for 10 min, the concentration of soluble proteins was determined in the supernatant by the Bradford method [40].

2.9. Extracts and assay of Rubisco activity

Leaves were cut into small pieces and homogenised in a grinding medium of 50 mmol/L Tris–HCl, pH 7.8, 10 mmol/L MgCl$_2$, 5 mmol/L DTT and 0.25 mmol/L EDTA. The extract was clarified by centrifugation at 10,000g for 10 min. The clear supernatant was decanted slowly and used as the Rubisco. The assay of Rubisco activity was measured as described by Nedunchezhian and Kulandaivelu [41].

2.10. Nitrate reductase activity

For this analysis, 100 mg of leaves were suspended in a glass vial containing 5 cm^3 of the assay medium consisting of 100 mmol/L KH$_2$PO$_4$–KOH, pH 7.0, 100 mmol/L KNO$_3$, and 1% (v/v) n-propanol. The vial was sealed and incubated in the dark at 27 °C for 60 min. Suitable aliquots of the assay medium were removed for nitrite analysis. The amount of nitrite formed was expressed as μmol NO$_2^-$ formed/g fresh mass/h [42].

3. Results

3.1. Leaf pigments

To evaluate possible differences in the photosynthetic behaviour of leaves growing in environments with different light availability, photosynthetic pigments, soluble proteins, Rubisco and in vivo nitrate reductase activity were measured in sun and shade leaves (Table 1). When measured on a fresh mass basis, shade leaves showed higher Chl and Car values than sun leaves. Levels of both Chl a and Chl b were higher in leaves collected from the inner part of the crown (PAR < 50 μmol m^{-2} s^{-1}) compared with leaves from well-irradiated portions of the crown (PAR = 1700 μmol m^{-2} s^{-1}), but the difference was more pronounced for Chl b with sun leaves presenting less than 60% of the amount of this pigment found in shade leaves while for Chl a the figure was 74% (Table 1). Total Car was only slightly higher in shade leaves, just 11% more than in sun leaves.

Table 1
Differences in photosynthetic pigments, soluble proteins, Rubisco and *in vivo* nitrate reductase activities (in relation to various reference units, and with and without KNO_3 fertilisation) between cypress leaves collected from sun and from shade positions. Means (±SD) of five experiments.

Parameters	Sun	Shade
Chl a [mg g^{-1} (f.m).]	0.94 ± 0.07	1.27 ± 0.06[*]
Chl b [mg g^{-1} (f.m).]	0.28 ± 0.02	0.48 ± 0.02[*]
Chl (a + b) [mg g^{-1} (f.m).]	1.22 ± 0.10	1.76 ± 0.18[*]
Chl a/b	3.29 ± 0.16	2.63 ± 0.12[*]
Car ($x + c$) [mg g^{-1} (f.m).]	0.28 ± 0.01	0.31 ± 0.03
Chl (a + b)/Car ($x + c$)	4.38 ± 0.21	5.64 ± 0.32[*]
Soluble proteins [mg g^{-1} (f.m).]	43.61 ± 1.85	27.32 ± 1.51[*]
Soluble protein/Chl ratio	13.25 ± 1.44	10.38 ± 1.87
Rubisco [μmol (CO_2) mg^{-1} (protein) h^{-1}]	48.2 ± 2.12	29.8 ± 1.23[*]
Nitrate reductase activity (μmol (NO_2) g^{-1} (f.m) h^{-1})	0.18 ± 0.01	0.12 ± 0.01[*]
Nitrate reductase activity (μmol (NO_2) g^{-1} (f.m) h^{-1})[a]	0.32 ± 0.01	0.12 ± 0.01[*]
Nitrate reductase activity (μmol (NO_2) g^{-1} (chl) h^{-1})[a]	0.26 ± 0.01	0.07 ± 0.01[*]
Nitrate reductase activity (μmol (NO_2) g^{-1} (protein) h^{-1})[a]	7.34 ± 0.34	4.69 ± 0.21[*]

[a] 15 mmol/L of KNO_3 applied externally.
[*] Statistically different from sun values ($p < 0.01$).

3.2. Soluble proteins and enzyme activity

A marked difference in the total content of soluble proteins was found between the two conditions, the sun leaves presenting a protein content almost 60% higher than shade leaves. Similarly, the Rubisco activity detected in sun leaves was 61% higher than in shade leaves (Table 1). As nitrogen metabolism is considered crucial in regulating photosynthetic acclimation to different light environments [43], the activity of nitrate reductase, a key enzyme in nitrogen assimilation, was measured in sun and shade leaves in relation to several reference units and with or without nitrogen supply (Table 1). Nitrate reductase activity was found to be 50% higher in sun leaves than in shade leaves even without an artificial supply of nitrogen When a source of nitrogen was added nitrate reductase activity increased by 78% in sun leaves, while little or no variation was observed in shade leaves (Table 1). When enzymatic activity was measured using different reference units, the observed trend remained the same although the actual figures varied.

3.3. Chl fluorescence and photosynthetic electron transport activity

PSII activity was investigated by measuring chlorophyll fluorescence [44] *in vivo* using leaves from sun and shade portions of the crown which had been dark-adapted for 30 min. Clear differences were noticed, with shade leaves showing reduced PSII efficiency. Statistically significant differences were found between sun and shade leaves for the parameters F_m and F_v/F_o ($p < 0.01$) and F_v/F_m ($p < 0.05$) (Fig. 1). Both F_v/F_m and F_v/F_o indicate differences in photosynthetic performance, although the latter has the advantage of being more sensitive.

In order to study the whole electron transport chain and also obtain information on PSI activity, photosynthetic electron transport was measured using isolated thylakoid membranes from sun and shade leaves (Fig. 2). The general trend for the whole chain ($H_2O \rightarrow MV$) confirmed the results obtained by chlorophyll fluorescence measurements, with shade leaves presenting a highly statistically significant reduction in efficiency ($p < 0.01$). With respect to PSI activity ($DCPIPH_2 \rightarrow MV$), a slight but significant ($p < 0.05$) decrease in efficiency (10%) was found in shade leaves. Finally, oxygen evolution was followed in order to investigate PSII activity using two different parameters ($H_2O \rightarrow DCBQ$ and $H_2O \rightarrow SiMo$). When DCBQ was the electron acceptor, little or no variation in PSII efficiency was found, while a highly statistically significant difference ($p < 0.01$) was measured (33%) with SiMo as electron acceptor (Fig. 2).

To throw light on the mechanisms involved in controlling photosynthetic efficiency of sun and shade leaves, variations in Chl fluorescence in the presence of exogenous electron donors ($MnCl_2$, DPC and NH_2OH) [45] in isolated thylakoid membranes was investigated (Table 2). No variation in Chl fluorescence was observed in thylakoid membranes extracted from sun leaves, with or without
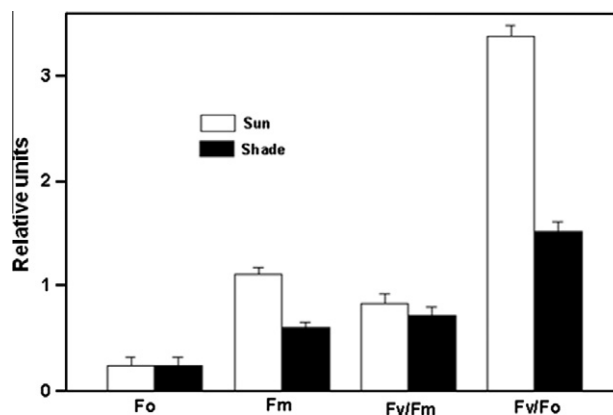

Fig. 1. The relative levels of Chl fluorescence denoted as F_o, F_m, F_v/F_m and F_v/F_o in sun and shade leaves. Means (±SD) of five experiments.
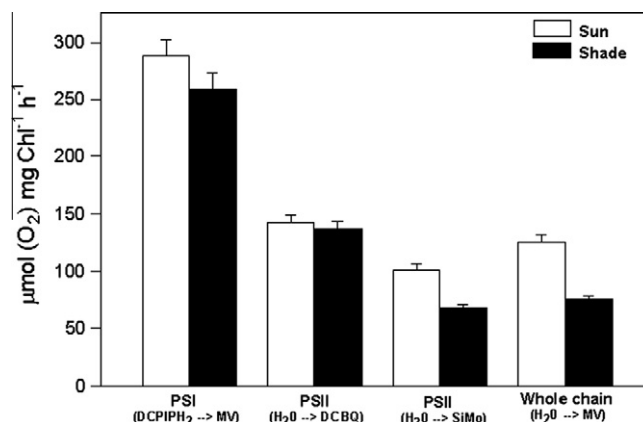

Fig. 2. The rates of whole-chain electron transport ($H_2O \rightarrow MV$), PSII ($H_2O \rightarrow DCBQ$; $H_2O \rightarrow SiMo$) and PSI ($DCPIPH_2 \rightarrow MV$) in thylakoids isolated from sun and shade leaves measured as O_2 evolution (PSII) or O_2 uptake (PSI and whole chain) by Clark-type O_2 electrode. Means (±SD) of five experiments.

Table 2

Differences in the relative levels of Chl fluorescence denoted as minimum fluorescence (F_o), maximum fluorescence (F_m) and the ratio of variable to maximum fluorescence (F_v/F_m) and F_v/F_o in thylakoids isolated from sun and shade leaves with or without exogenous electron donors. Concentrations of $MnCl_2$, DPC and NH_2OH were 5, 0.5 and 5 mmol/L, respectively. Means (±SD) of five experiments.

Addition	F_o	F_m	F_v/F_m	F_v/F_o
Sun leaves				
None	1.12 ± 0.07	7.10 ± 0.19	0.84 ± 0.03	5.45 ± 0.25
DPC	1.11 ± 0.08	7.51 ± 0.20	0.85 ± 0.04	5.82 ± 0.29
NH_2OH	1.13 ± 0.07	7.40 ± 0.18	0.85 ± 0.04	5.73 ± 0.23
$MnCl_2$	1.12 ± 0.06	7.12 ± 0.20	0.84 ± 0.02	5.45 ± 0.27
Shade leaves				
None	1.11 ± 0.07	2.91 ± 0.08	0.62 ± 0.02	1.64 ± 0.09
DPC	1.10 ± 0.05	4.50 ± 0.14	0.75 ± 0.03[*]	3.09 ± 0.12[*]
NH_2OH	1.10 ± 0.06	4.32 ± 0.10	0.74 ± 0.04[*]	2.91 ± 0.11[*]
$MnCl_2$	1.13 ± 0.06	3.12 ± 0.06	0.64 ± 0.02	1.82 ± 0.08

[*] Statistically different from the values measured without exogenous electron donors ($p < 0.01$).

Fig. 4. Polypeptide profiles of thylakoid membranes isolated from sun and shade leaves stained with Coomassie brilliant blue. Lane A, sun; lane B, shade. Gel lanes were loaded with equal amounts of protein (100 μg).
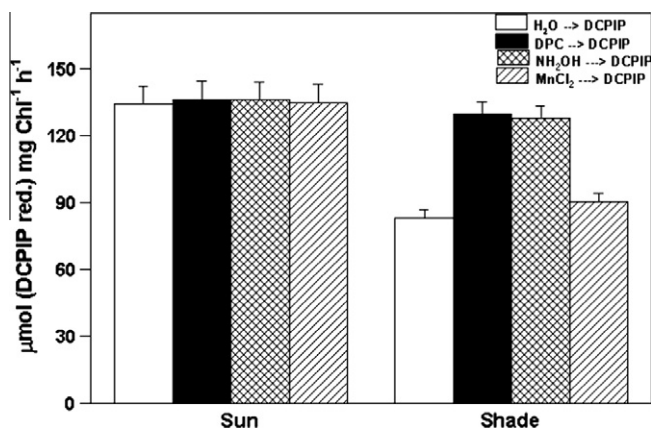

Fig. 3. Effects of various exogenous electron donors on PSII activity ($H_2O \rightarrow DCPIP$) measured as DCPIP reduction in thylakoids isolated from sun and shade leaves. Means (±SD) of five experiments.

Fig. 5. Amounts of D1 and 33 kDa proteins in shade leaves compared with sun leaves. Lane a, sun; lane b, shade. Each lane was loaded with equal amounts of Chl (5 μg). Protein amounts were obtained from densitometrical evaluation of the western blots.

application of an electron donor. However, while there was no significant variation in thylakoid membranes extracted from shade leaves in the presence of $MnCl_2$, there was a clear increase in Chl fluorescence with DPC and NH_2OH (Table 2). Again, both F_v/F_m and F_v/F_o were used to evidence differences in PSII performance.

To shed further light on the effect of exogenous electron donors on PSII activity, PSII-mediated reduction of DCPIP was measured in isolated thylakoid membranes after external application of $MnCl_2$, DPC and NH_2OH (Fig. 3). As with Chl fluorescence, no significant differences were measured in thylakoid membranes extracted from sun leaves, while a highly statistically significant ($p < 0.01$) increase in the DCPIP reduction rate was observed in thylakoid membranes isolated from shade leaves when DPC or NH_2OH were applied, but little or no effect was found with $MnCl_2$ (Fig. 3).

3.4. Thylakoid protein analysis

Since the differences in photosynthetic electron transport activities may be attributable to differences in the assembly and reorganisation of thylakoid components, the polypeptide profiles of thylakoid membranes extracted from sun and shade leaves were analysed and compared using SDS–PAGE (Fig. 4). The results seem to indicate that some of the components are present at lower levels, as the bands corresponding to 23 kDa, 28–25 kDa and 33 kDa polypeptides appeared less intense after Coomassie blue staining
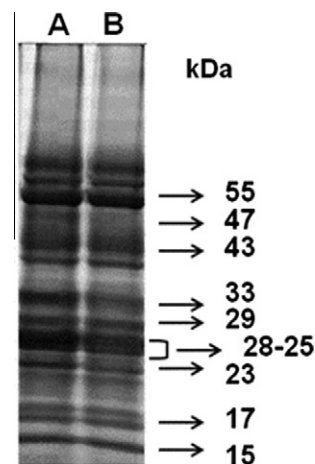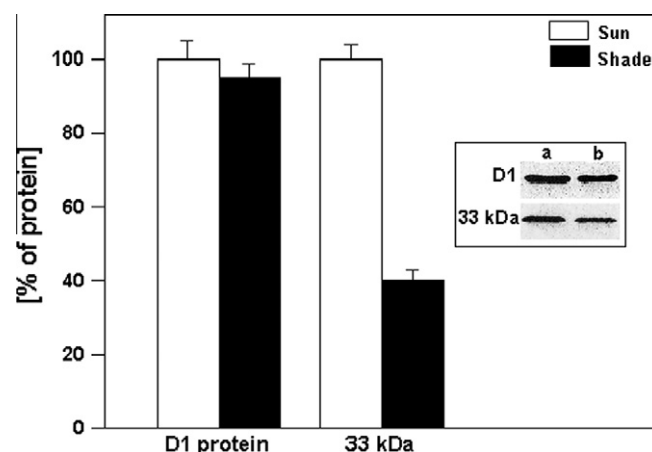
(Fig. 4). In order to confirm these results and to carry out a more sensitive analysis, a polyclonal antibody against the oxygen-evolving extrinsic protein PsbO (33 kDa) was obtained and used in a western blot to assess differences between sun and shade thylakoids (Fig. 5). A polyclonal antibody against the D1 protein of PSII was used for comparison. The results indicate that while only a slight decrease (5%) was detectable in the D1 protein content of shade leaves, a larger difference was noted for PsbO. In fact, the amount of PsbO in thylakoid membranes extracted from shade leaves was equal to only 40% of that in thylakoid membranes extracted from sun leaves (Fig. 5).

4. Discussion

Photosynthesis in cypress is significantly inhibited by low [46] and high temperatures [47], ageing [48], and canker infection [49], even though canker resistant and susceptible clones have different molecular mechanisms [50].

The results presented in this study show that cypress leaves collected from different portions of the crown with contrasting light availability differed in pigment content, enzyme activity, photosynthetic performance and nitrogen response. Differences in

thylakoid membrane composition were also found. Cypress leaves harvested from the inner part of the crown, and therefore growing in low light conditions, had a higher content of both Chl a and Chl b pigments compared with leaves collected from the parts exposed to direct sunlight. A similar result was obtained when total Car content was measured. These findings are consistent with other studies on various woody species, whether deciduous, such as beech (Fagus sylvatica), maple (Acer pseudoplatanus L.) and small-leaved lime (Tilia cordata Mill.), or coniferous, such as silver fir (Abies alba Mill.) [8]. Nevertheless, another study found a significant decrease in chlorophyll content when Bougainvillea glabra plants were exposed to more than 50% shade. However, the use of plastic screening for shading in this study may account for the different results [51]. Griffin et al. [52] suggest that a decrease in Chl b content may be an indication of chlorophyll destruction through excess light. Although the mechanisms differ in young and mature leaves, high irradiance induces photoinhibition in cypress, which largely protects photosynthetic pigments and the electron transport apparatus from destruction [53].

In the present study, sun leaves were found to have a higher Chl a/b ratio than shade leaves, while at the same time they had a lower Chl/Car ratio. In a previous study on gingko (Gingko biloba L.) and beech (Fagus sylvatica) [54], not only was the same trend observed, but the measured values were also very similar to those presented here for cypress, suggesting similar mechanisms for controlling plant adaptation to different light conditions. Differences in Chl a/b and Chl/Car are thought to be due to differences in the adaptation response of the photosynthetic apparatus to high light irradiance when there are fewer light-harvesting Chl a/b proteins (LHCII) and a greater number of reaction centres [55].

Plants grown under shade conditions have relatively low levels of water-soluble proteins and soluble protein/chlorophyll ratios. This has been reported for soybean leaves [56], while a recent study found that different shading conditions influenced the water-soluble protein content in Cedrela sinensis [43]. Most water-soluble proteins are thought to function as physiologically active enzymes, while the role of insoluble proteins is structural [57]. Given that Rubisco is one of the most extensively studied water-soluble proteins, we investigated the activity of this enzyme in cypress and found it to be far greater in sun leaves than in shade leaves. In Quercus glauca, nitrogen remobilized through Rubisco degradation was found to translocate from old to new leaves, causing increased crown-level photosynthesis [58]. We might speculate that a similar mechanism could remobilize nitrogen from low light to high light portions of the crown, although the existence of such a mechanism is yet to be proven. Nevertheless, the results presented here show that the activity of nitrate reductase, a key enzyme in nitrogen metabolism, was found to be higher in sun leaves than in shade leaves. Moreover, when an external source of nitrogen was provided, shade leaves failed to respond with increased nitrate reductase activity, while sun leaves responded promptly. Nitrate reductase activity is known to be regulated by light and ultraviolet-B [59,60], although the signalling mechanism is not yet known. In a recent work on Scots pine (Pinus sylvestris), UV-B/UV-A exclusion was found to cause a decrease in the level of nitrate reductase activity in one-year-old leaves [61].

Another factor that was found to vary between sun and shade cypress leaves was PSII photosynthetic efficiency measured in terms of relative fluorescence. Again, PSII activity was higher in sun leaves, mainly due to increased F_m, while there was no significant variation in F_o. The F_v/F_m ratio or the equivalent F_v/F_o are widely used to measure the intrinsic efficiency of PSII [62] and as indicators of photoinhibition [63]. Experiments conducted on trees transferred from low to high light have revealed a rapid decrease in F_v/F_m [46,64–66].

Electron acceptors in thylakoid membranes isolated from sun and shade leaves were used to measure various electron transport activities; the results showed lower activity of the whole electron transport chain in shade leaves compared with sun leaves. Only a marginal effect on PSI-mediated reactions was observed, suggesting that shade could have a main action site in the PSII. It is well known that when plants grown under low light conditions are exposed to excess light, the whole photosynthetic apparatus is impaired [67]. This phenomenon, known as photoinibition, affects PSII to a greater extent than PSI [68]. The degree of photo-induced damage is thought to be determined mainly by the rates of degradation and synthesis of the D1 protein, one of the key components of PSII [69]. Similar lower rates of PSII activity have been reported in overwintering evergreen broad-leaved trees [70]. It has been shown that high light treatment can cause a marked decrease in the whole electron transport chain in cypress [46]. Analyses of electron transport in thylakoid membranes isolated from shade leaves showed that O_2 evolution was significantly lower when the electron acceptor was SiMo, but not when the electron acceptor was DCBQ. Since DCBQ is known to accept electrons directly from Q_A [71,72], the rates measured represent the true rate of photochemistry by PSII, without the influence of the PQ pool. This seems to suggest that the donor side of PSII in shade leaves is functional. In order to locate the possible site of shade-induced inhibition, we measured PSII-mediated DCPIP reduction in the presence of various artificial exogenous electron donors acting at the oxidising side of PSII. Among the artificial electron donors tested, DPC and NH_2OH were found to be more effective in increasing PSII activity in shade leaves. Similar results have also been found in papaya leaves (Carica papaya L.) infected by the papaya mosaic virus (PMV), where a pathogen-induced loss in photosynthetic electron transport was observed [73]. It should, however, be noted that a single concentration of each electron donor was used in the present work, in accordance with Wydrzynski and Govindjee [45], and we cannot, therefore, rule out the possibility that the concentration of $MnCl_2$ used was not optimal to recover the activity of PSII. As a matter of fact, the manganese-containing cluster in the Oxygen Evolving Complex (OEC) in PSII is considered the primary target of photodamage [74]. A further experiment in which modulated Chl fluorescence was measured seemed to support the results described so far. After adding DPC and NH_2OH to thylakoid membranes isolated from shade leaves, a marked increase in the level of variable fluorescence occurred. These results strongly suggest that shade acts on the donor side of PSII in cypress leaves. It has been suggested that in low light conditions oxidative damage may be caused by generation of active oxygen species when charge recombination between Q_A^{2-} or Q_B^{2-} and the oxidised state on the donor side of PSII is coupled with generation of the triplet state of Chl [75].

As with all photosynthetic organisms, higher plants have the ability to synthesise carbohydrates from carbon dioxide and water using light as an energy source. This process occurs in the thylakoid membrane of the chloroplasts. To assess whether the lower PSII activity detected could be correlated with a corresponding change in the polypeptide composition of the thylakoid membranes, the polypeptide profiles of thylakoid membranes extracted from sun and shade leaves were analysed using SDS–PAGE. The results revealed differences in at least three distinct proteins of 23 kDa, 28–25 kDa and 33 kDa. The 28–25 kDa polypeptides are thought to be part of the pigment-protein complex for the outer light-harvesting antenna of PSII (LHCII) [76]. We know from studies on rice [77] and bean [78] that impairment of Chl b synthesis or plants grown in intermittent light conditions can lead to a reduction in LHCII proteins. A recent work investigating two Arabidopsis Chl b-less mutants with reduced amounts of LHCII proteins, showed that all six LHCII proteins play a role in balanced excitation

of PSII and PSI, and in stabilising the structure of PSII super-complexes and stimulating grana formation [79].

The 23 kDa and 33 kDa polypeptides are the two main components of thylakoid lumenal proteins. The thylakoid lumen is a space of 40–100 Å enclosed by the thylakoid membrane [80]. At present, several hundred proteins are thought to be located in the lumenal space [81,82], the most abundant of these being the extrinsic PSII proteins PsbO (33 kDa), PsbP (23 kDa) and PsbQ (16 kDa) [81]. Several studies seem to indicate that PSII activity is strictly correlated with the contents of some Psb proteins. Transgenic tobacco plants showing reduced PSII activity have been found to have a correspondingly lower level of PsbO protein [83]. PsbO is thought to play a central role in the assembly and function of PSII [84]. The results of a recent study in which proteomes of dark-adapted and light-adapted *Arabidopsis* thylakoids were analysed, indicate that plants exposed to eight hours of light have increased abundances of several extrinsic PSII proteins, such as PSbP and PsbQ [85]. PsbP is an essential component for regulating and stabilising PSII in higher plants [86]. Moreover, photoinhibition has been shown to be a consequence of imbalance between damage and repair of PSII [87]. Photosynthetically-generated Reactive Oxygen Species (ROS) can cause direct damage to a variety of important photosystem components, and they have also been shown to play a role in inhibiting the recovery of photodamaged PSII [88]. Taken all together, these studies corroborate the data we obtained from Italian cypress, which show lower PSII activity to be correlated with differences in the polypeptide composition of thylakoids, with at least three important components involved: PSbP, PsbQ and LHCII.

Cypress was planted in many sites around the big pre-alpine lakes many centuries ago [89,25]. Cypress seedlings from natural regeneration often survive and grow slowly in the shaded understory, creating a seedling bank capable of re-establishing the canopy following disturbances. More effective management of this kind of cypress forest requires an understanding of cypress plasticity to changes in the environment. Our objective was to shed light on the photosynthetic plasticity of *C. sempervirens* to light intensity in order to obtain knowledge that would provide the basis on which to implement appropriate silvicultural practises. Such practises must take into account changes in both light and temperature in order to maximise growth potential in natural and managed regeneration (i.e. optimising planting density and selecting appropriate aspects and cypress varieties).

We studied only var. *pyramidalis* of *C. sempervirens*, as it is the most widespread variety in Europe and North America, planted mainly for its ornamental and aesthetic attributes. However, it should be pointed out that Schiller et al. found the average daily transpiration rate of var. *horizontalis* to be nearly 50% greater than that of var. *pyramidalis* [90]. An interesting topic for future research would be to investigate the photosynthetic differences between these two varieties.

5. Conclusions

The results presented in the present work clearly suggest that the photosynthetic apparatus of cypress leaves has indeed considerable plasticity. All the techniques used produced similar results showing that shade leaves have lower photosynthetic efficiency and seem to be particularly impaired on the donor side of PSII. The photosynthetic plasticity of the leaves enables them to acclimate to a wide range of light levels, from deep shade to full sunlight, and will prove especially beneficial in allowing this species a wider distribution beyond the bottom and lower slopes of some valleys of the Alps to which it is currently restricted. However, an integrated analysis that takes into account the overall energetic balance under light stress conditions encompassing such mechanisms as respiration and cyclic photophosphorylation, along with analysis of dynamic responses to sun flecks, would yield a more complete overview of adaptation to the light environment in the inner crown of this species. The considerable leaf plasticity demonstrated by cypress is a clearly advantageous trait in enabling it to respond to the environmental conditions of new planting sites and to global climate change.

References

[1] F. Valladares, U. Niinemets, Shade tolerance, a key plant feature of complex nature and consequences, Annu. Rev. Ecol. Evol. Syst. 39 (2008) 237–257.
[2] U. Niinemets, Photosynthesis and resource distribution through plant canopies, Plant Cell. Environ. 30 (2007) 1052–1071.
[3] F. Valladares, E. Martınez-Ferri, L. Balaguer, E. Perez-Corona, E. Manrique, Low leaf-level response to light in Mediterranean evergreen oaks: a conservative resource-use strategy?, New Phytol 148 (2000) 79–91.
[4] U. Niinemets, Responses of forest trees to single and multiple environmental stresses from seedlings to mature plants: past stress history, stress interactions, tolerance and acclimation, For. Ecol. Manage. 260 (2010) 1623–1639.
[5] A. Calatayud, D. Roca, E. Gorbe, P.F. Martynez, Light acclimation in rose (*Rosa hybrida* cv. Grand Gala) leaves after pruning: effects on chlorophyll a fluorescence, nitrate reductase, ammonium and carbohydrates, Scientia Horticulture 111 (2007) 152–159.
[6] L. Dietzel, K. Brautigam, T. Pfannschmidt, Photosynthetic acclimation: state transitions and adjustment of photosystem stoichiometry – functional relationships between short-term and long-term light quality acclimation in plants, FEBS J. 275 (2008) 1080–1088.
[7] H.K. Lichtenthaler, F. Babani, Light adaptation and senescence of the photosynthetic apparatus, changes in pigment composition chlorophyll fluorescence parameters, and photosynthetic activity, in: G.C. Papageorgiou, Govindjee (Eds.), Chlorophyll Fluorescence: A Signature of Photosynthesis, Springer, Dordrecht, 2004, pp. 713–736.
[8] H.K. Lichtenthaler, A. Ac, M.V. Marek, J. Kalina, O. Urban, Differences in pigment composition, photosynthetic rates and chlorophyll fluorescence images of sun and shade leaves of four tree species, Plant Physiol. Biochem. 45 (2007) 577–588.
[9] O. Bjorkman, J. Troughton, M.A. Nobs, Photosynthesis in relation to leaf structure, Brook Haven Symp. Biol. 25 (1973) 206–226.
[10] J.H. McClenden, G.G. McMillen, The control of leaf morphology and the tolerance to shade by woody plants, Bot. Gaz. 143 (1982) 79–83.
[11] J.D. Rochaix, Regulation of photosynthetic electron transport, Biochim. Biophys. Acta 2011 (1807) 375–383.
[12] J.M. Anderson, Photoregulation of the composition, function and structure of thylakoid membranes, Annu. Rev. Plant Physiol. 37 (1986) 93–136.
[13] W.S. Chow, E.H. Kim, P. Horton, J.M. Anderson, Granal stacking of thylakoid membranes in higher plant chloroplasts: the physicochemical forces at work and the functional consequences that ensue, Photochem. Photobiol. Sci. 4 (2005) 1081–1090.
[14] J.M. Anderson, W.S. Chow, J. De Las Rivas, Dynamic flexibility in the structure and function of photosystem II in higher plant thylakoid membranes: the grana enigma, Photosynth. Res. 98 (2008) 575–587.
[15] S.I. Allakhverdiev, Recent progress in the studies of structure and function of photosystem II, J. Photochem. Photobiol. B 104 (2011) 1–8.
[16] B. Demming-Adams, W.W. Adams, Photoprotection and other responses of plants to high light stress, Annu. Rev. Plant Physio. Plant Mol. Biol. 43 (1992) 599–626.
[17] C. Kulheim, J. Agren, S. Jansson, Rapid regulation of light harvesting and plant fitness in the field, Sci. 297 (2002) 91–93.
[18] T. Pfannschmidt, Chloroplast redox signals: low photosynthesis controls its own genes, Trends Plant Sci. 8 (2003) 33–41.

[19] Z. Li, S. Wakao, B.B. Fischer, K.K. Niyogi, Sensing and responding to excess light, Annu. Rev. Plant Biol. 60 (2009) 239–260.

[20] N.K. Boardman, Comparing photosynthesis of sun and shade plants, Annu. Rev. Plant Physiol. 28 (1977) 355–377.

[21] O. Kull, Acclimation of photosynthesis in canopies: models and limitations, Oecologia 133 (2002) 267–279.

[22] G. Brofas, G. Karetsos, P. Dimopoulos, C. Tsagari, The natural environment of Cupressus sempervirens in Greece as a basis for its use in the Mediterranean region, Land Degrad. Develop. 17 (2006) 645–659.

[23] C.W. Woodall, C.M. Oswalt, J.A. Westfall, C.H. Perry, M.D. Nelson, A.O. Finley, Selecting tree species for testing climate change migration hypotheses using forest inventory data, For. Ecol. Manage. 259 (2010) 778–785.

[24] C.J. Linares, A.P. Tiscar, Climate change impacts and vulnerability of the southern populations of Pinus nigra subsp. salzmannii, Tree Physiol. 30 (2010) 795–806.

[25] F. Bagnoli, G.G. Vendramin, A. Buonamici, A.G. Doulis, S.C. Gonzalez-Martinez, N. La Porta, D. Magri, P. Raddi, F. Sebastiani, S. Fineschi, Is Cupressus sempervirens native in Italy? An answer from genetic and palaeobotanical data, Mol. Ecol. 18 (2009) 2276–2286.

[26] A. Zocca, C. Zanini, A. Aimi, G. Frigimelica, N. La Porta, A. Battisti, Spread of a plant pathogen and associated insect vectors at the northern range margin of cypress (Cupressus sempervirens), Acta Oecol. 33 (2008) 307–313.

[27] L. Pedron, P. Baldi, A.M. Hietala, N. La Porta, Genotype-specific regulation of cold-responsive genes in cypress Cupressus sempervirens L., Gene 437 (2009) 45–53.

[28] P. Baldi, L. Pedron, A.M. Hietala, N. La Porta, Cold tolerance in cypress (Cupressus sempervirens L.): a physiological molecular study, Tree Genet. Genomes 7 (2011) 79–90.

[29] N. La Porta, P. Capretti, I.M. Thomsen, R. Kasanen, A.M. Hietala, K. Von Weissenberg, Forest pathogens with higher damage potential due to climate change in Europe, Can. J. Plant Pathol. 30 (2008) 177–195.

[30] G. Maracchi, F. Benincasa, G. Zipoli, Elements of Agrometerology, Centro 2P, Firenze, 1984 (Italian) CNR-IATA.

[31] F. Barbaliscia, P. Masullo, G. Ravaioli, Spatial statistical correlation of solar energy and heliophany over Italy, in: IEEE Conferences, Telecommunications Energy Conference 1986, INTELEC '86, International, 1986, pp. 311–315.

[32] N. Matzinger, M. Andretta, E.V. Gorsel, R. Vogt, A. Ohmura, M.W. Rotachi, Surface radiation budget in alpine valley, Quart. J. Roy. Met. Soc. 129 (2003) 877–895.

[33] P. Kostopoulou, K. Radoglou, O. Dini-Papanastasi, Performance and quality of Cupressus sempervirens L. mini-plug seedlings under reduced photoperiod, Eur. J. For. Res. 130 (2011) 579–588.

[34] H.K. Lichtenthaler, Chlorophylls and carotenoids, the pigments of photosynthetic theromembranes, Meth. Enzymol. 148 (1987) 350–382.

[35] D.A. Berthold, G.T. Babcock, C.A. Yocum, Highly resolved O_2 evolving photosystem II preparation from spinach thylakoid membranes, FEBS Lett. 134 (1981) 231–234.

[36] N. Nedunchezhian, F. Morales, A. Abadia, J. Abadia, Decline in photosynthetic electron transport activity changes in thylakoid protein pattern in field grown iron deficient Peach (Prunus persica L.), Plant Sci. 129 (1997) 29–38.

[37] P.A. Armond, U. Schreiber, O. Bjorkman, Photosynthetic acclimation to temperature in the desert shrub Larrea divaricata. II. Light-harvesting efficiency and electron transport, Plant Physiol. 61 (1978) 411–415.

[38] U.K. Laemmli, Clevage of structural proteins during the assembly of the head bacteriophage T_4, Nature 227 (1970) 680–685.

[39] O.H. Lowry, N.J. Rosenbrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin Phenol Reagent, J. Biol. Chem. 193 (1951) 265–275.

[40] M.M. Bradford, A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding, Anal. Biochem. 72 (1976) 248–254.

[41] N. Nedunchezhian, G. Kulandaivelu, Effect of enhanced radiation on ribulose-1,5 bisphosphate carboxylase in leaves of Vigna sinensis L, Photosynthetica 25 (1991) 231–435.

[42] E.G. Jaworski, Nitrate reductase assay in intact plant tissues, Biochem. Biophys. Res. Commun. 43 (1971) 1274–1279.

[43] K. Yoshimura, Irradiance heterogeneity within crown affects photosynthetic capacity and nitrogen distribution of leaves in Cedrela sinensis, Plant Cell Environ. 33 (2010) 750–758.

[44] G.H. Krause, E. Weis, Chlorophyll fluorescence and photosynthesis: the basics, Annu. Rev. Plant Physiol. Plant Mol. Biol. 42 (1991) 313–349.

[45] T. Wydrzynski, Govindjee, A new site of bicarbonate effect in photosystem II of photosynthesis: Evidence from chlorophyll fluorescence transients in spinach chloroplasts, Biochem. Biophys. Acta 387 (1975) 403–408.

[46] N. La Porta, M. Bertamini, N. Nedunchezhian, P. Raddi, K. Muthuchelian, Photoinhibition of photosynthesis in needles of two cypress (Cupressus sempervirens L.) clones, Tree Physiol. 25 (2005) 1033–1039.

[47] F. Frouxa, M. Ducrey, D. Epronc, E. Dreyera, Seasonal variations and acclimation potential of the thermostability of photochemistry in four Mediterranean conifers, Ann. For. Sci. 61 (2004) 235–241.

[48] N. La Porta, M. Bertamini, N. Nedunchezhian, K. Muthuchelian, High irradiance induced changes on photosystem 2 in young and mature needles of cypress Cupressus sempervirens L., Photosynthetica 42 (2004) 263–271.

[49] K. Muthuchelian, N. La Porta, M. Bertamini, N. Nedunchezhian, Cypress canker induced inhibition of photosynthesis in field grown cypress (Cupressus sempervirens L.) needles, Physiol. Mol. Plant Pathol. 67 (2005) 33–39.

[50] K. Muthuchelian, M. Bertamini, N. La Porta, N. Nedunchezhian, Photoinhibition and recovery of photosynthesis in canker-susceptible and resistant needles of cypress (Cupressus sempervirens L.), J. Phytopathol 153 (2005) 337–343.

[51] M. Saifuddin, A.B.M.S. Hossain, O. Normaniza, Impacts of shading on flower formation and longevity, leaf chlorophyll and growth of Bougainville glabra, Asian J. Plant Sci. 9 (2010) 20–27.

[52] J.G. Griffin, G.R. Thomas, D.M. Pharr, Photosynthesis, chlorophyll florescence, and carbohydrate content of Illicium taxa grown under varied irradiance, J. Am. Soc. Hort. Sci. 129 (2004) 46–53.

[53] N. La Porta, M. Bertamini, N. Nedunchezhian, K. Muthuchelian, Photosynthetic changes that occur during aging of cypress (Cupressus sempervirens L.) needles, Photosynthetica 44 (2006) 555–560.

[54] G. Sarijeva, M. Knapp, H.K. Lichtenthaler, Differences in photosynthetic activity, chlorophyll and carotenoid levels and in chlorophyll fluorescence parameters in green sun and shade leaves of Ginkgo and Fagus, J. Plant Physiol. 164 (2007) 950–955.

[55] H.K. Lichtenthaler, G. Kuhn, U. Prenzel, D. Meier, Chlorophyll-protein levels and degree of thylakoid stacking in radish chloroplasts from high-light, low-light and bentazon-treated plants, Plant 56 (1982) 183–188.

[56] K.O. Burkey, R.F. Wilson, R. Wells, Effects of canopy on the lipid composition of soybean leaves, Physiol. Plant 101 (1997) 591–598.

[57] T. Takashima, K. Hikosaka, T. Hirose, Photosynthesis or persistence. Nitrogen allocation in leaves of evergreen and deciduous Quercus species, Plant Cell Environ. 27 (2004) 1047–1054.

[58] S.I. Miyazawa, A.A. Suzuki, K. Sone, I. Terashima, Relationships between light, leaf nitrogen and nitrogen remobilization in the crowns of mature evergreen Quercus glauca trees, Tree Physiol. 24 (2004) 1157–1164.

[59] C. Lillo, Signalling cascades integrating light-enhanced nitrate metabolism, Biochem. J. 415 (2008) 11–19.

[60] G.I. Jenkins, Signal transduction in responses to UV-B radiation, Annu. Rev. Plant Biol. 60 (2009) 407–431.

[61] M. Krywult, J. Smykla, H. Kinnunen, F. Martz, M.L. Sutinen, K. Lakkala, M. Turunen, Influence of solar UV radiation on the nitrogen metabolism in needles of Scots pine Pinus sylvestris L., Environ. Pollut. 156 (2008) 1105–1111.

[62] K. Maxwell, G.N. Johnson, Chlorophyll fluorescence – a practical guide, J. Exp. Bot. 51 (2000) 659–668.

[63] N.R. Baker, Chlorophyll fluorescence. A probe of photosynthesis in vivo, Annu. Rev. Plant Biol. 59 (2008) 89–113.

[64] N.C. Houter, T.L. Pons, Gap size effects on photoinhibition in understorey saplings in tropical rainforest, Plant Ecol. 179 (2005) 43–51.

[65] M. Naramoto, S-i. Katahata, Y. Mukai, Y. Kakubari, Photosynthetic acclimation and photoinhibition on exposure to high light in shade-developed leaves of Fagus crenata seedlings, Flora 201 (2006) 120–126.

[66] M.S. Mielke, B. Schaffer, Leaf gas exchange, chlorophyll fluorescence and pigment indexes of Eugenia uniflora L. in response to changes in light intensity and soil flooding, Tree Physiol. 30 (2009) 45–55.

[67] S.B. Powles, Photoinhibition of photosynthesis induced by visible light, Annu. Rev. Plant Physiol. 35 (1984) 15–44.

[68] J. Barber, B. Andersson, Too much of a good thing: light can be bad for photosynthesis, Trends Biochem. Sci. 17 (1993) 61–66.

[69] B. Andersson, E.M. Aro, Photodamage and D1 protein turnover in photosystem II, in: E.M. Aro, B. Andersson (Eds.), Regulation of Photosynthesis, Kluwer Acad. Publ., Dordrecht, 2001, pp. 377–393.

[70] J. Yamazaki, K. Kamata, E. Maruta, Seasonal changes in the excess energy dissipation from Photosystem II antennae in overwintering evergreen broad-leaved trees Quercus myrsinaefolia and Machilus thunbergii, J. Photochem. Photobiol. B 104 (2011) 348–356.

[71] J. Cao Govindjee, Chlorophyll transient as an indicator of active and inactive photosystem II in thylakoid membranes, Biochem. Biophys. Acta 1015 (1990) 180–188.

[72] S. Dudekula, M. Fragata, Investigation of the electron transfer site of p-benzoquinone in isolated photosystem II particles and thylakoid membranes using alpha- and beta-cyclodextrins, J. Photochem. Photobiol. B. 85 (2006) 177–183.

[73] R.M. Nanda, B. Biswal, Biotic stress induced demolition of thylakoid structure and loss in photoelectron transport of chloroplasts in papaya leaves, Plant Physiol. Biochem. 46 (2008) 461–468.

[74] V.D. Kreslavski, R. Carpentier, V.V. Klimov, N. Murata, S.I. Allakhverdiev, Molecular mechanisms of stress resistance of the photosynthetic apparatus, Biol. Membr. 24 (2007) 195–217.

[75] N. Keren, A. Berg, P.J.M. van Kan, H. Levanon, I. Ohad, Mechanism of photosystem II photoinactivation and D1 protein degradation at low light: the role of back electron flow, Proc. Natl. Acad. Sci. USA 94 (1997) 1579–1584.

[76] S. Jansson, The light-harvesting chlorophyll a/b-binding proteins, Biochim. Biophys. Acta 1184 (1994) 1–19.

[77] T. Terao, A. Yamashita, S. Katoh, Chlorophyll b-deficient mutants of rice II. Antenna chlorophyll a/b-proteins of photosystem I and II, Plant Cell Physiol. 26 (1985) 1369–1377.

[78] W.S. Chow, C. Funk, A.B. Hope, Govindjee, Greening of intermittent-light-grown bean plants in continuous light; thylakoids components in relation to photosynthetic performance and capacity for photoprotection, Indian J. Biochem. Biophys. 3 (2000) 395–404.

[79] E.H. Kim, X.P. Li, R. Razeghifard, J.M. Anderson, K.K. Niyogi, B.J. Pogson, W.S. Chow, The multiple roles of light-harvesting chlorophyll a/b-protein

complexes define structure and optimize function of *Arabidopsis* chloroplasts: a study using two chlorophyll b-less mutants, Biochim. Biophys. Acta 1787 (2009) 973–984.

[80] I.B. Kovalenko, A.M. Abaturova, P.A. Gromov, D.M. Ustinin, E.A. Grachev, G.Y. Riznichenko, A.B. Rubin, Direct simulation of plastocyanin and cytochrome f interactions in solution, Phys. Biol. 3 (2006) 121–129.

[81] M. Schubert, U.A. Petersson, B.J. Haas, C. Funk, W.P. Schröder, T. Kieselbach, Proteome map of the chloroplast lumen of *Arabidopsis thaliana*, J. Biol. Chem. 277 (2002) 8354–8365.

[82] J.B. Peltier, O. Emanuelsson, D.E. Kalume, J. Ytterberg, G. Friso, A. Rudella, D.A. Liberles, L. Söderberg, P. Roepstorff, G. von Heijne, K.J. van Wijk, Central functions of the lumenal and peripheral thylakoid proteome of *Arabidopsis* determined by experimentation and genome-wide prediction, Plant Cell 14 (2002) 211–236.

[83] B. Cai, A. Zhang, Z. Yang, Q. Lu, X. Wen, C. Lu, Characterization of photosystem II photochemistry in transgenic tobacco plants with lowered Rubisco activase content, J. Plant Physiol. 167 (2010) 1457–1465.

[84] P.K. Jain, A. Kochhar, J.P. Khurana, A.K. Tyagi, The psbO Gene for 33-kDa precursor polypeptide of the oxygen-evolving complex in *Arabidopsis thaliana* – nucleotide sequence and control of its expression, DNA Res. 5 (1998) 221–228.

[85] I. Granlund, M. Hall, T. Kieselbach, W.P. Schröder, Light induced changes in protein expression and uniform regulation of transcription in the thylakoid lumen of *Arabidopsis thaliana*, PLoS One. 4 (2009) e5649.

[86] K. Ifuku, Y. Yamamoto, T.A. Ono, S. Ishihara, F. Sato, PsbP protein, but not PsbQ protein, is essential for the regulation and stabilization of photosystem II in higher plants, Plant Physiol. 139 (2005) 1175–1184.

[87] Y. Nishiyama, H. Yamamoto, S.I. Allakhverdiev, M. Inaba, A. Yokota, N. Murata, Oxidative stress inhibits the repair of photodamage to the photosynthetic machinery, Embo J. 20 (2001) 5587–5594.

[88] Y. Nishiyama, S.I. Allakhverdiev, H. Yamamoto, H. Hayashi, N. Murata, Singlet oxygen inhibits the repair of photosystem II by suppressing the transport elongation of the D1 protein in *Synechocystis* spp. PCC 6803, Biochem. (USA) 43 (2004) 11321–11330.

[89] R. Cormio, The Historic Trees – The Cypress of Somma Lombardo from a Botanical and Historical Point of View. Contribution to the Study to Determine the Age of Standing Trees [Italian] Ed. Tipografia del Popolo d'Italia, Milano, 1935.

[90] G. Schiller, Y. Moshe, E.D. Ungar, Transpiration of *Cupressus sempervirens* L. as influenced by canopy structure, Israel J. Plant Sci. 52 (2004) 9–19.