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Leaf anatomy and photosynthetic acclimation in *Valeriana jatamansi* L. grown under high and low irradiance

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Abstract

Relationship of leaf anatomy with photosynthetic acclimation of *Valeriana jatamansi* was studied under full irradiance [FI, 1 600 µmol(PPFD) m^{-2} s^{-1}] and net-shade [NS, 650 µmol(PPFD) m^{-2} s^{-1}]. FI plants had thicker leaves with higher respiration rate (R_D), nitrogen content per unit leaf area, chlorophyll *a/b* ratio, high leaf mass per leaf area unit (LMA), and surface area of mesophyll cell (S_{mes}) and chloroplasts (S_c) facing intercellular space than NS plants. The difference between leaf thickness of FI and NS leaves was about 28 % but difference in photon-saturated rate of photosynthesis per unit leaf area (P_{Nmax}) was 50 %. This indicates that P_{Nmax} can increase to a larger extent than the leaf thickness with increasing irradiance in *V. jatamansi*. Anatomical studies showed that the mesophyll cells of FI plants had no open spaces along the mesophyll cell walls (higher S_c), but in NS plants wide open spaces along the mesophyll cell wall (lower S_c) were found. Positive correlation between S_c and P_{Nmax} explained the higher P_{Nmax} in FI plants. Increase in mesophyll thickness increased the availability of space along the mesophyll cell wall for chloroplasts (increased S_c) and hence P_{Nmax} was higher in FI plants. Thus this Himalayan species can acclimate to full sunlight by altering leaf anatomy and therefore may be cultivated in open fields.

Additional key words: chlorophyll; chloroplast; leaf thickness; mesophyll; photosynthetic acclimation; respiration rate; shade.

Introduction

Sunlight is the energy source for all photosynthetic organisms, which are finely tuned to harvest it efficiently. Plants are able to respond differently to changing irradiance, including changes in leaf anatomy (Taiz and Zeiger 1998), morphology (Boardman 1977), and photosynthesis (Chazdon and Kaufmann 1993). There is a close relationship between the leaf characteristics and the mean irradiance experienced by the leaves. Hence, distribution of irradiance can be used as a predictor for spatial variation of leaf properties (Pons *et al.* 1993, Anten and Werger 1996). The empirical relationship between leaf irradiance and leaf characteristics are widely used to scale up photosynthesis from leaf to canopy level (Sinoquet *et al.* 2001). Leaf photosynthetic characteristics adapt remarkably to irradiance (Evans 1989) and generally, leaves developed at high irradiance exhibit higher photosynthetic capacity per unit leaf area as compared to shade leaves (Niinemets and Tenhunen 1997). However, maintaining high photosynthetic capacity is costly and advantageous only under high irradiance (Mooney and Gulmon 1979) because it requires construction of thick leaves with large investment of nitrogen in photosynthetic enzymes (Björkman 1981, Terashima *et al.* 2001). Since chloroplasts contain all photosynthetic enzymes, sun exposed leaves need to have large number of chloroplasts in the mesophyll cells. However, high photosynthetic capacity is not always accompanied by thick leaves. Leaf thickness is determined by the irradiance at leaf development, and changes little after they have matured (Sims and Pearcy 1992). When leaves are transferred to high irradiance after maturation, their photosynthetic capacity often increases (Yamashita *et al.* 2000) but does not achieve the rate of leaves

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Abbreviations: C_i = intercellular CO_2 concentration; Chl = chlorophyll; LMA = leaf mass per leaf area unit; P_{Nmax} = net photosynthetic rate per leaf area at saturating irradiance; PPFD = photosynthetic photon flux density; S_c = surface area of chloroplasts facing intercellular space; S_{mes} = surface area of mesophyll cell facing intercellular space.

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developed under high irradiance (Frak *et al.* 2001).

Valeriana jatamansi has traditionally been used in medicine practice in India (Anonymous 1976). The species is now cultivated for valepotriates in China and Germany (Foss and Houghton 1997) which are used as sedative and tranquiliser. However, in India the species is now endangered, due to intensive harvesting of the plant from its natural habitat to meet the ever growing demand (Kaul and Handa 2000). *V. jatamansi* is an under-storey herb in temperate areas of the Himalaya region. In an earlier study we reported its acclimation and ability to thrive under open conditions (Vats *et al.* 2002). In present study, we attempted to understand the mechanism that increases the photosynthetic capacity of mature leaves of *V. jatamansi* when grown under high irradiance. We hypothesized that the increase in the photosynthetic capacity under high irradiance is associated with alteration in leaf anatomy and chloroplast arrangement in mesophyll cells. Using this hypothesis we studied the changes in physiological and anatomical characteristics of *V. jatamansi* grown in full irradiance (FI) or net-shade (NS).

Materials and methods

Plants: *V. jatamansi* Jones, a perennial herb, was grown in plastic pots (15×20 cm) containing sand : soil : farm yard manure (1 : 1 : 1, m/m/m). Plants were transferred in full sunlight [1 600 μmol(PPFD) m^{-2} s^{-1}] and under nylon-net shade (placed at 2 m above ground), receiving about 650 μmol(PPFD) m^{-2} s^{-1} (recorded at midday under clear sky) during first week of September, 2003 in the Institute's Experimental Farm at Palampur (32°06'N, 76°33'E at 1 300 m a.s.l.). All the observations on these plants were recorded during February–March, 2004, when plants were acclimated for about six months.

During this period the experimental site had a mean max/min temperature 21.8/9.8 °C, relative humidity 65.12 %, wind speed of 5.14 km h^{-1}, bright sunshine of 7.51 h, and 5.57 rainy days with 83.27 mm rainfall.

Photosynthesis measurements: A portable computerized open gas system IRGA (*Li-6400*, *Li Cor,* Lincoln, NE, USA) was used for measuring photon saturated rate of CO_2 assimilation per unit area ($P_{\text{Nmax-area}}$) of fully expanded mature 2nd/3rd leaf of plants (20–25 cm high). Plants were exposed to PPFD of 2 000 μmol m^{-2} s^{-1} (at which photons saturated photosynthesis) for 30 min before recording observations. P_{Nmax} was determined at constant CO_2 concentration (360 μmol mol^{-1}) and cuvette temperature (25 °C). For generating P_N/C_i curves, the CO_2 concentration in leaf chamber was reduced using a *6400-01* CO_2 injector. PPFD was kept constant at 2 000 μmol m^{-2} s^{-1}. A time interval of 90 s was given for leaf to equilibrate to the new conditions in each measurement. Irradiance response curve was generated at PPFD of 0–2 500 μmol m^{-2} s^{-1} at constant CO_2 (360 μmol mol^{-1}) and cuvette temperature of 25 °C. A cool light source (*6400-02 LED*) fitted on top of the leaf chamber provided software adjustable PPFD of 0–3 000 μmol m^{-2} s^{-1}. The curves were generated using automatic logging system for each set of P_N/PPFD and P_N/C_i curves.

Respiration rate (R_D) measurements: Oxygen uptake of the leaf discs (6.0 mm diameter each) was measured polarographically using computerized *Hansatech* (UK) oxygen electrode as described by Pandey *et al.* (1998). Leaf discs were suspended in air saturated water and oxygen uptake was recorded after 10-min incubation in dark.

Chlorophyll (Chl), leaf mass per leaf area unit (LMA): Total Chl amount was estimated from 5 leaf discs (1.2 cm diameter each) for each replicate, obtained from randomly selected mature 2nd–3rd leaves from FI and ND plants, following the method of Pandey and Nagar (2002). Concentrations of Chl *a* and *b* in the extracts were determined from absorbances at 642.5 and 660.0 nm. LMA [g m^{-2}] was determined by drying 45 (15 per replication) leaf discs (obtained as stated for Chl estimation) at 70 ºC till constant mass.

Total nitrogen content [mmol m^{-2}] of leaves was estimated from 15 dried leaf discs (1.2 cm diameter each) for each replicate, obtained from mature randomly selected 2nd–3rd leaves from FI and NS plants using modified micro-Kjeldahl method (Concon and Soltess 1973).

Anatomical measurements: Leaf segments (3×7 mm) from the middle of the lamina were fixed in FAA (formaldehyde : acetic acid : 50 % ethanol, 5 : 5 : 90) and dehydrated in a *t*-butylalcohol series. Sections (10 μm thick) were stained with safranine-fast green, and the slides were mounted in DPX [80–10 g *Distrene* (British resin product), 5 cm^3 dibutylphthalate, and 35 cm^3 xylene]. The photographs were taken under a microscope *Nikon (Biophot)* No. *78508* (Japan) at 40× magnification using a digital camera (*Nikon DXM 1200*). Micrographs were analyzed with the NIH (National Institute of Health) image software to determine thickness of epidermis and palisade and mesophyll cells.

The area of the mesophyll cell surface (S_{mes}) and area of chloroplast surfaces facing intercellular space per unit leaf area (S_c) were calculated from photographs by the method of Thain (1983). A curvature factor (F) was determined assuming that the shape of palisade cells was a cylinder with flat ends and that of the spongy cells was a spheroid. The value of F was 1.38 for the palisade and 1.45 for the spongy cells.

Statistical analysis: Values were subjected to analysis of variance (ANOVA) employing a randomised complete block design (RBD) with three replications and differences among means were tested against critical difference at $p<0.01$.

Results

P_{Nmax} values at PPFD-saturation were 16 and 8 μmol(CO_2) m^{-2} s^{-1} in FI and NS leaves, respectively, and were significantly higher in FI than NS plants (Table 1). Significantly higher values for R_D, nitrogen content, carboxylation efficiency, and LMA were found in FI leaves than in NS ones (Table 1). Chl content on leaf area basis was significantly lower in FI than NS leaves but the ratio Chl *a*/*b* was significantly higher in FI than NS leaves. PPFD response curves of FI and NS plants showed that FI plants were photon saturated at PPFD of 2 000 μmol m^{-2} s^{-1} whereas NS plants at 1 000 μmol m^{-2} s^{-1} (Fig. 1).

Table 1. Anatomical and physiological characteristics of leaves of *V. jatamansi* grown under full irradiance (FI) and net shade (NS). *Different letters* in superscript following the values in rows showed significant differences at $p<0.01$.

Leaf characteristics	FI	NS
Upper epidermis thickness [μm]	15.22^{a}±0.46	11.99^{b}±0.59
Lower epidermis thickness [μm]	12.44^{a} ±0.29	8.89^{b} ±0.20
Mesophyll thickness [μm]	105.64^{a} ±1.30	77.36^{b} ±1.04
Palisade thickness [μm]	50.32^{a} ±1.56	32.54^{b}±0.22
Leaf thickness [μm]	134.12^{a} ±0.55	95.31^{b} ±1.92
S_{mes} [m^2 m^{-2}]	12.50^{a} ±0.80	5.50^{b} ±0.65
S_c [m^2 m^{-2}]	11.80^{a} ±0.77	4.30^{b} ±0.50
S_c/S_{mes}	0.94±0.05	0.78^{b} ±0.05
LMA [g m^{-2}]	77.83^{a} ±2.88	38.62^{b} ±2.31
Respiration [μmol(O_2) m^{-2} s^{-1}]	1.10±0.20	0.40^{b} ±0.12
Chl [mg m^{-2}]	348.00^{b} ±5.80	443.00^{a} ±5.90
Chl *a/b*	2.18^{a} ±0.50	2.02^{b} ±0.45
Leaf nitrogen [mmol m^{-2}]	81.13^{a} ±3.22	72.47^{b} ±2.45
Carboxylation efficiency [μmol(CO_2) m^{-2} s^{-1} kPa^{-1}]	0.074^{a} ±0.004	0.052^{b} ±0.003
$P_{\text{Nmax-area}}$ [μmol(CO_2) m^{-2} s^{-1}]	16.00^{a} ±1.54	8.00^{b} ±1.20

Anatomical observations of leaf cross sections (Fig. 2) showed that NS leaves had one layer of palisade whereas FI leaves had two layers. No other significant differences in arrangement of cells were observed but a remarkable difference in the arrangement of chloroplasts of mesophyll cells was noticed. In NS leaves chloroplasts were randomly dispersed throughout the mesophyll cell (Fig. 2*D*), whereas in FI leaves chloroplasts were aligned with the mesophyll cell wall (Fig. 2*C*). Plants in FI had thicker leaves, epidermis, mesophyll, and palisade cells than NS plants (Fig. 2*A,B*; Table 1). However, the thickness of leaves and mesophyll cells was 26–28 % greater in FI plants. The surface area of mesophyll cells (S_{mes}) and chloroplasts (S_c) facing intercellular spaces were two times greater in FI leaves than in NS ones. The ratio of S_c to S_{mes} was not so high but it was significantly higher in FI leaves. Correlation analysis showed a significant positive correlation between P_{Nmax} and S_c and the transfer of plants from NS to FI conditions increased S_c, which was accompanied by higher P_{Nmax} (Fig. 3).

Discussion

The difference between leaf thickness of FI and NS plants was about 28 % but the difference in P_{Nmax} reached 50 % (Table 1) indicating that P_{Nmax} can increase with increasing irradiance more than leaf thickness. S_{mes} and S_c facing intercellular space was significantly higher in FI leaves and P_{Nmax} was linearly related with S_c under both growing conditions (Table 1; Fig. 3) suggesting that a larger surface area of chloroplasts was crucial to support increased photosynthesis. However, an increase in S_c was only possible when all the chloroplasts were aligned with the mesophyll cell surface (Fig. 2*C*) and also due to the availability of open space along the mesophyll cell surface to accommodate additional chloroplasts (Terashima *et al.* 2001, Oguchi *et al.* 2003). The value of S_c/S_{mes} increased from 0.78 in the NS leaves to 0.94 in FI leaves which also attributed to the about two fold increase in P_{Nmax} (Table 1) because CO_2 conductance from the intercellular spaces to chloroplasts depends on S_c (Evans 1994), since diffusion of CO_2 in liquid phase is very slow (Nobel 1991).

In NS plants, open spaces at the mesophyll cell surface were due to the random distribution of chloroplasts throughout the cytoplasm and their absence along the mesophyll cell surface (Fig. 2*D*), which could also be related to a drastic decline in S_c/S_{mes} ratio in NS leaves. Such differences in values of S_c/S_{mes} were also observed among other species (Syvertsen *et al.* 1995). Further, Honda *et al.* (1971) reported that each species had its own S_c/S_{mes} determined by the number of chloroplasts per cell. Hence, increase in the number of chloroplasts in mesophyll cells (values not shown) of FI plants may be associated with increase in S_c values (Table 1). In FI plants, S_{mes} increased with increasing thickness of mesophyll (Table 1) which is considered to be beneficial for increasing the photosynthesis of FI plants since S_{mes} is the space where chloroplasts physically exist.

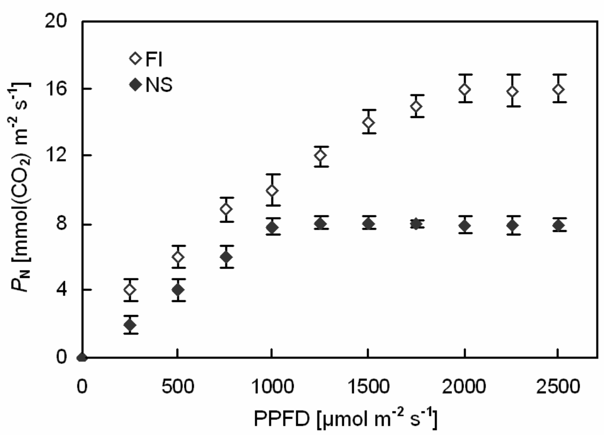

Fig. 1. Photosynthetic irradiance (PPFD) response curves of full irradiance (FI, ◊) and net shade (NS, ♦) grown *Valeriana jatamansi*. Vertical bars ± SE.

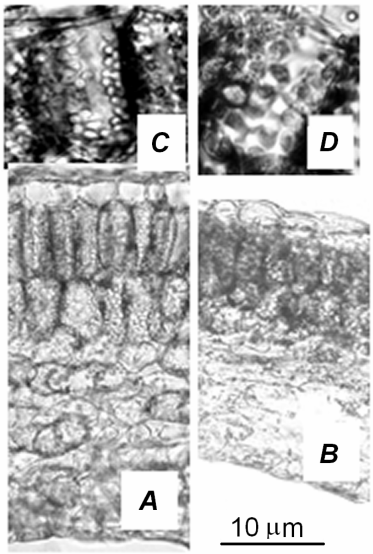

Fig. 2. The light micrographs of the sections of (*A*) full-irradiance, FI and (*B*) net-shade, NS grown leaves of *Valeriana jatamansi* and arrangement of chloroplasts in mesophyll cell under (*C*) FI (arrangement along the cell wall) and (*D*) NS (random dispersion in the cytoplasm).

In this study, reduction in Chl content in leaves of FI plants with significant increase in Chl *a/b* ratio (Table 1) appeared most likely due to changes in the organization of both light-harvesting and electron transport components (Schiefthaler *et al.* 1997) and such changes are widely reflected in decreased Chl content and increased Chl *a/b* (Pandey *et al.* 2003). At high irradiance, the amount of ribulose-1,5-bisphosphate carboxylase/oxygenase and the amount of reaction centres of photosystem 2 (PS2) increase at the expense of the light-harvesting complex 2 (LHC2) of PS2 (Nishio *et al.* 1994, Hikosaka and Terashima 1995). The PS2 core complex mainly contains Chl *a,* whereas LHC2 has both Chl *a* and *b*. Further, to increase the number of PS2 reaction centres for efficient electron transport capacity under high irradiance the LHC2 is reduced leading to increased Chl *a/b* of the leaf (Genty and Harbinson 1996).

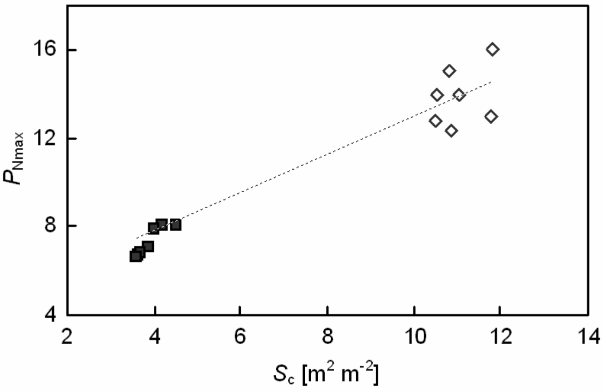

Fig. 3. Relationship between PPFD-saturated rate of photosynthesis per leaf area (P_{Nmax}) and surface area of chloroplasts facing intercellular space per unit leaf area (S_c). Data for the leaves grown in full irradiance (◊) and net shade (♦). Regression = 3.14x – 6.12, $r = 0.97$.

High P_{Nmax} along with high LMA and N content in FI than NS leaves of *V. jatamansi* (Table 1) showed the acclimation response of this under-storey herb under full sun (Vats *et al.* 2002). However, increase in leaf thickness (high LMA) provides greater open space along the mesophyll cell wall to accommodate additional chloroplasts in order to maximize S_c and hence CO_2 assimilation (Oguchi *et al.* 2003). FI leaves imply large investment of biomass in order to enhance their photosynthetic capacity under full sunlight, which may be too costly for shade leaves. Higher N content in FI than NS leaves (Table 1) is beneficial for the increased capacity of leaves to utilize energy under increased irradiance (Mohotti and Lawlor 2002), and this also agrees with the conclusion of Ramalho *et al.* (1997) that nitrogen availability is key factor in acclimation of coffee plants. Further, high carboxylation efficiency (0.074±0.004) of FI plants (Table 1) confirms the acclimation ability of *V. jatamansi* to full irradiance. Further, 2.75 times higher R_D in FI than NS plants also showed acclimation ability of *V. jatamansi* under high irradiance, since variations in respiration have been widely proposed as a component of acclimation to photon availability (Lusk and Reich 2000). Plants growing in shade have lower carbon losses *via* dark respiration than their counterparts exposed to full irradiance, because the associated advantages of high metabolic potential (high photosynthetic capacity) can not be realized in such habitats (Grime 1965, Givnish 1988).

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