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BRIEF COMMUNICATION

Photosynthetic response to irradiance in *Valeriana jatamansi* Jones, a threatened understorey medicinal herb of Western Himalaya

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Abstract

Net photosynthetic rate (P_N) of *Valeriana jatamansi* plants, grown under nylon net shade or under different tree canopies, was saturated with photons at 1 000 µmol m^{-2} s^{-1} photosynthetic photon-flux-density (PPFD), whereas open-grown plants were able to photosynthesise even at higher PPFD, *e.g.* of 2 000 µmol m^{-2} s^{-1}. Plants grown under net shade had higher total chlorophyll (Chl) content per unit area of leaf surface. However, Chl *a/b* ratio was maximal in open-grown plants, but remained unchanged in plants grown in nylon net shade and under different tree canopies. Sun-grown plants had thicker leaves (higher leaf mass per leaf area unit), higher wax content, and higher P_N than shade grown plants. Thus *V. jatamansi* is able to acclimate to high PPFD and therefore this Himalayan species may be cultivated in open habitat to meet the ever-increasing industrial demand.

Additional key words: acclimation; chlorophyll; photosynthetic photon flux density; shade.

In natural environment plants can adapt to extreme differences in irradiance at several integration levels. Plants can change the fraction of biomass invested in leaves, stems, and roots by modulating their leaf area per unit biomass, by altering their anatomy, and also by changing relative investment of nitrogen between photosynthetic components (Evans and Poorter 2001). Plants grown under shade have lower net photosynthetic rate (P_N) and leaf mass per leaf area unit (LMA) and these two parameters affect the productivity of a plant. In contrast to this, plants grown under high irradiance increase their LMA due to extra layers of palisade or longer palisade cells (Hanson 1917). Besides, they absorb a large amount of photons and sustain high P_N. Chloroplasts of these leaves contain less chlorophyll (Chl) in their antennae, but relatively more reaction centres and components of the electron transport chain and larger amounts of photosynthetic enzymes. Thus, these chloroplasts have relatively higher CO_2 assimilation capacity than shade chloroplasts (Anderson 1986). Plants under tree canopies can be exposed to sudden sunflecks which can occur several times in a day and their irradiance can be one or even two orders of magnitude larger than the irradiance experienced most of the time. For these plants it is extremely important to find a balance between radiation utilisation and protection against photodamage.

Valeriana jatamansi or the Indian valerian has traditionally been used in several indigenous systems of medicines practised 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. In India the industrial demand of this plant is met almost exclusively through collections made in the forest. Recently, *V. jatamansi* has been listed as critically endangered and threatened species in Western Himalaya (Kaul and Handa 2000). Therefore, in order to meet the ever-growing demand of pharmaceutical industries, cultivation of this plant is of commercial

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Abbreviations: Chl – chlorophyll; C_i – internal CO_2 concentration; LHC – light-harvesting complex; LMA – leaf mass per leaf area unit; P_N – net photosynthetic rate per unit leaf area; PS – photosystem.

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significance.The plant is an under-storey herb of Western Himalaya that thrives well under shaded and moist conditions. Large-scale cultivation of this plant under artificial shade is economically not feasible. Therefore, present investigation aims to study the adaptability of this plant under different irradiance, which may be of economic significance for successful cultivation.

Nursery seedlings of *V. jatamansi* Jones of five months age and 4-5 cm high were planted with a row spacing of 40×40 cm^2 during late October in 1998, in three different layouts. Plants in open field received full sunlight, but those under nylon-net shade (placed at 2 m above ground) received about one third of it (maximum PPFD of 650 $\mu mol\ m^{-2}\ s^{-1}$ recorded at midday under clear sky). The third layout was under the canopy shade of 7-8 year-old plantation of four different tree species (*Bauhinia variegata*, *Grevillea robusta*, *Jacaranda acutifolia*, and *Morus alba*) grown in the Institute's Experimental Farm at Palampur (32°06'N, 76°33'E at 1 300 m a.s.l.) for biomass production studies. The canopy shade was evaluated as an alternative shade condition to the nylon net structure.

The site has a mean annual temperature of 18 °C but the optimal growth season for most species extends from spring-summer (April-June; max./min. temperature 28/18 °C) to the end of rainy season (July-September; max./min. temperature 26/19 °C). Plants were watered regularly during the summer months but irrigation was not required after the onset of monsoon rains. Rainy season accounts for about 65 % of the total annual rainfall exceeding 2 500 mm, and is consequently associated with low sunshine hours (Rama Das and Vats 1993). Observations related to physiological response to irradiance were therefore recorded well before the onset of monsoon in fully-grown plants of about nine-month-old, during the month of June, 1999.

For measuring P_N response to varying PPFD and different CO_2 concentrations (C_i), fully expanded 2nd/3rd leaf of plants of 20-25 cm high was exposed to PPFD of 2 000 $\mu mol\ m^{-2}\ s^{-1}$ for 30 min before recording observations, using a portable computerised open system IRGA (*Li-6400*, *LiCor*, Lincoln, Nebraska, USA). P_N per unit area was determined at different PPFD of 50-2 000 $\mu mol\ m^{-2}\ s^{-1}$ at constant CO_2 concentration of 360 $\mu mol\ mol^{-1}$. CO_2 response curves were determined as ratio of P_N to C_i. These curves were generated using automatic logging system with a cuvette temperature set at 25 °C and set to match the sample and reference analysers before recording each new observation. A cool light source (*6400-02 LED*) fitted on top of the leaf chamber, capable of providing software adjustable PPFD of 0-2 000 $\mu mol\ m^{-2}\ s^{-1}$, served as the radiation source. 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 $\mu mol\ m^{-2}\ s^{-1}$. A time interval of 90 s was given for leaf to equilibrate to the new conditions in each measurement. Replicates were obtained using automatic logging for each set of P_N/PPFD and P_N/C_i curves.

Total Chl content was estimated in 10 leaf discs (0.785 cm^2 each) for each replication, obtained from randomly selected 2nd-3rd leaves from top of the three different plants of each habitat, following the method of AOAC (1990) using 80 % (v/v) acetone and expressing per leaf area. Concentrations of Chl *a* and *b* in extracts were determined from absorbances at 642.5 and 660.0 nm. Wax content was estimated according to Barnes *et al.* (1996) using procedure described by Pandey and Nagar (2002) for leaf-discs (0.785 cm^2 each). Both adaxial and abaxial surfaces of discs were washed with chloroform (HPLC grade) dispensed from a burette, taking care not to contaminate the waxes extracted from one surface with those from the another. Samples were filtered through pre-rinsed *Whatman* No. *1* filter paper, followed by 0.2 µm *Sartorius* filter into pre-weighed evaporation flask. The solvent was reduced below 40 °C under vacuum before drying to constant mass at room temperature. The amount of wax was expressed per unit leaf area and per unit leaf fresh mass. LMA was determined by drying 10 leaf discs (obtained as stated earlier in case of Chl estimation) at 70 °C till constant mass was achieved.

Values were subjected to analysis of variance (ANOVA) employing a randomised complete block design (RBD) with three replications. Duncan's multiple range test was used to compare the differences among means at $p<0.05$.

Plants grown under net shade and different tree canopies reached photon saturated photosynthesis at PPFD around 1 000 $\mu mol\ m^{-2}\ s^{-1}$, but those grown under full sunlight lacked saturation even at 2 000 $\mu mol\ m^{-2}\ s^{-1}$ (Fig. 1*A,B*). The sun grown plants also exhibited higher P_N at PPFD from 50 to 2 000 $\mu mol\ m^{-2}\ s^{-1}$. P_N was lowest in plants grown under net shade (Fig. 1*A*). P_N determined at different C_i was higher in plants grown in full sunlight but was lowest in plants grown under net shade (Fig. 1*C*). Similarly, the initial slope of P_N/C_i curve was higher in plants grown in full sunlight compared to those grown under net-shade [0.0726±0.0020 and 0.0545±0.0050 $\mu mol(photon)\ m^{-2}\ s^{-1}/\mu mol(CO_2)\ mol^{-1}$, respectively].

Leaves under net shade were softer, dark green, and contained relatively more Chl. The Chl *a*/*b* ratio in leaves under net shade and those under different canopies did not vary much but was significantly higher in sun leaves, which developed thicker texture and higher LMA (Table 1). Total leaf wax content, calculated on fresh mass basis, was higher in sun leaves compared to those under net shade. Within different canopy shades, leaves grown under the shade of *M. alba* developed high total wax content, much due to late development of leaves by late summer. Adaxial surface had significantly more wax than abaxial surfaces in all the cases (Table 1).

Those species, which withstand large changes in

PPFD, are able to do so by inducing suitable biochemical, physiological, and/or structural changes (Han *et al.* 1999, Higuchi *et al.* 1999, Singh and Singhal 2001). In the present study, the reduction in Chl in leaves grown under full sun, with significant change in Chl *a/b* ratio (Table 1), appeared most likely due to changes in both light-harvesting and electron transport components (Schiefthaler *et al.* 1999). Since Chl b is associated with light-harvesting complex 2 (LHC2) of photosystem 2 (PS2), PS2 is more vulnerable to photoinhibitory damage than PS1 (Nishio *et al.* 1994), and reduction in its antenna size may serve to protect under high PPFD. Also, to increase the number of PS2 reaction centres for efficient electron transport capacity under high irradiance the LHC2 is reduced and this results in increased Chl *a/b* of the leaf (Genty and Harbinson 1996). However, plants of *V. jatamansi* grown in full sunlight did not show visible symptoms of photooxidation, such as bleaching of chloroplast *etc.*, but exhibited higher P_N per unit leaf area.

Plants of *V. jatamansi* grown under full sunlight did not saturate in terms of P_N at 2 000 μmol m^{-2} s^{-1} (Fig. 1*A*), probably because of higher PPFD (more than 2 000 μmol m^{-2} s^{-1}) generally encountered in Himalayan mountain regions. Alpine plants of open habitat lack saturation of P_N at PPFD of 2 000 μmol m^{-2} s^{-1} (Körner and Diemer 1987). On the contrary, plants of *V. jatamansi* grown under different tree canopies and nylon net shade did not show photon saturation curves representative of obligate shade species, but saturated around 1 000 μmol m^{-2} s^{-1} (Fig. 1*B*), a characteristic response of spring geophytes and agricultural C_3 plants (Larcher 1995).

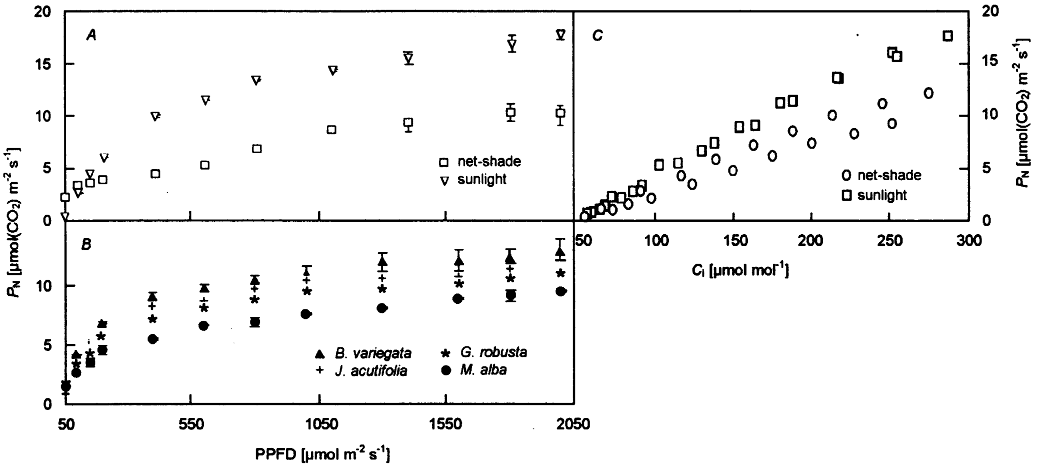

Fig. 1. Response of net photosynthetic rate (P_N) to irradiance (*A, B*) or internal CO_2 concentration (*C*). Plants of *Valeriana jatamansi* were grown under full sunlight or nylon-net shade of maximal 650 μmol m^{-2} s^{-1} (*A*) or under canopy shade of four different tree species, viz. *Bauhinia variegata, Jacaranda acutifolia, Grevillea robusta*, or *Morus alba* (*B*) or under an irradiance of 650 or 2 000 μmol m^{-2} s^{-1} (*C*). Initial slope of the P_N/C_i curve was 0.0726±0.0020 and 0.0545±0.0050 μmol m^{-2} s^{-1}/μmol mol^{-1}(CO$_2$) for plants grown in full sunlight or nylon net shade, respectively (*C*).

Table 1. Changes in epicuticular wax and chlorophyll (Chl) contents, and in leaf mass per area (LMA) of *Valeriana jatamansi* leaves grown under full sunlight, net shade, and canopy shade of three different tree species. Different letters in superscripts following the values in columns indicate statistically significant differences at $p<0.05$.

Plants grown under	Wax content [mg m^{-2}] adaxial surface	Wax content [mg m^{-2}] abaxial surface	Wax content [mg kg^{-1}(f.m.)]	Chl [mg m^{-2}]	Chl *a/b*	LMA [g m^{-2}]
Full sunlight	131^a	79^a	425.3^a	310^b	2.19^a	69.7^a
Net shade	68^f	46^e	367.3^e	360^a	2.13^b	35.7^d
Jacaranda acutifolia	96^c	66^c	344.7^f	210^e	2.12^b	41.7^{cd}
Bauhinia variegata	89^{cd}	71^b	401.8^c	260^c	2.10^b	62.9^a
Morus alba	109^b	70^b	420.6^b	240^{cd}	2.08^b	53.9^b
Grevillea robusta	81^e	62^d	396.7^d	230^d	2.13^b	45.7^c

V. jatamansi plants grown under full sunlight developed more wax on both surfaces of leaf (Table 1) to withstand high photon load. In shade and sun leaves of ivy (*Hedera helix* L.) the latter had the higher wax content than the former (Hauke and Schreiber 1998). Light scattering by epicuticular wax causes significant differences in reflectance and transmittance within leaves, which protects against the deleterious effects of excessive radiation (Grant *et al.* 1995). In *V. jatamansi* the adaxial surface of leaves is generally exposed to solar radiation. This situation may enhance susceptibility to photodamage

(Nishio *et al.* 1994), therefore greater deposition of wax on adaxial surface may protect the plant against external influences such as high irradiance, air pollutants, and attack by pest and pathogens (Percy *et al.* 1994).

Increase in P_N proportional to LMA has been observed in the plants of *V. jatamansi* grown in full sunlight, which could be the response of acclimation under high PPFD. This enhancement in P_N is most likely because of the increase in ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) activity and amount of electron carriers per unit area of leaf (Björkman 1981). Enhanced RuBPCO activity in sun leaves of *V. jatamansi* may be supposed from steeper initial slope of P_N/C_i response curve (Fig. 1*C*). The greater carboxylation efficiency recorded in the leaves of *V. jatamansi* grown under full sunlight also suggests its acclimation to high PPFD. Further, higher relative growth rate (values not shown) of plants grown under full sunlight indicates its better efficiency to thrive well under open condition.

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