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Different photosynthetic responses of wild and cultivated plants to high irradiance

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Abstract

In addition to other factors, high altitude (HA) environment is characterized by high photosynthetic photon flux density (PPFD). Photosynthetic characteristics of wild and cultivated plants were studied at different irradiances at Losar, India (altitude 4 200 m). Wild plants were tolerant to high PPFDs. Slopes of curve between net photosynthetic rate (P_N) and intercellular CO_2 concentration (C_i) or stomatal conductance (g_s) increased with increase in irradiance suggesting insensitivity or tolerance of these plants to higher PPFD. Cultivated plants, however, were sensitive to higher PPFD, their slopes of curves between P_N and C_i or g_s decreased with increased PPFD. Tolerance or insensitivity to higher PPFD was an important parameter affecting plant performance at HA.

Additional key words: *Aquilegia*; *Hordeum*; intercellular CO_2 concentration; irradiance; photosynthetic photon flux density; *Pisum*; *Rumex*; species differences; stomatal conductance.

Introduction

High altitude (HA) environment is characterized by low partial pressure of air (P_a), low mean temperature, and high solar radiations, which affect plant performance (Streb *et al.* 1998). Several studies have discussed the impact of partial pressure of CO_2 (pCO_2) on photosynthesis (Körner and Diemer 1987, Körner *et al.* 1988, Kumar *et al.* 2004, 2005, 2006), but only a few studies have been carried out in relation to irradiance (Körner and Diemer 1994, Streb *et al.* 1998).

Favourable growth period of plants at HA is very short and hence an efficient photosynthesis system facilitates optimal plant performace (Körner and Larcher 1988). HA plants are well adapted to all kinds of HA environmental factors (Körner 1982, Körner and Diemer 1987, Körner and Larcher 1988). Higher solar irradiance, one of the most noticeable environmental factors prevailing at HA, could cause oxidative stress to the plants leading to photoinhibition (Foyer *et al.* 1994). High irradiances activate oxygen in the photosynthetic tissue through Mehler reaction (Asada 1994, Foyer *et al.* 1994). Plants have developed a variety of protective mechanisms to either scavenge toxic activated oxygen species or avoid their production. For example, carotenoids act as scavengers of activated oxygen and xanthophyll cycle pigment zeaxanthin dissipates excess excitation energy (Demmig-Adams and Adams 1993). Leaves of HA-plants are resistant to photoinhibition (Streb *et al.* 1998).

We compared gas exchange characteristics of wild plants growing naturally at HA with the cultivated plants grown at HA.

Materials and methods

Location: Studies were conducted at HA location “Losar” located at 32°26'N latitude, 77°45'E longitude, and altitude of 4 200 m [atmospheric pressure 62.8 kPa and photosynthetic photon flux density (PPFD) of 2 500–2 900 μmol m^{-2} s^{-1} (district Lahaul and Spiti, Himachal Pradesh, India)].

Wild plants: Studies were carried out on *Aquilegia fragrans* Benth. and *Rumex nepalensis* Spreng. growing naturally at HA. Measurements were carried out between

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Abbreviations: C_a – ambient CO_2 concentration; C_i – intercellular CO_2 concentration; ECU – efficiency of carbon uptake; g_s – stomatal conductance; HA – high altitude; I – irradiance; P_a – partial pressure of air; pCO_2 – partial pressure of CO_2; P_N – net photosynthetic rate; P_{Nmax} – maximum P_N; PPFD – photosynthetic photon flux density; PS2 – photosystem 2; SOC – slope of the curve.

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the last week of July to the 2nd week of August. Mean monthly day temperatures during the month of data recording was 15.5±1.7 ºC.

Cultivated plants: *Hordeum vulgare* L. and *Pisum sativum* L. were sown at Losar during first week of May and the various physiological parameters were determined on flag leaves of *H. vulgare* and 3rd leaf of *P. sativum* between the last week of July to the 2nd week of August.

Gas exchange parameters were measured on 10 plants from each plant species. Net photosynthetic rate (P_N) was measured using a portable open gas exchange system, *LI-6400* (*Li-COR*, Lincoln, NE, USA). The instrument was calibrated using a calibrated gas (505 cm^3 m^{-3}) and a portable dew point generator (*LI-610*; *Li-COR*, USA). A "cool light" source (*6400-02 LED*) fitted on top of the leaf chamber, capable of providing software adjustable from 0–3 000 µmol m^{-2} s^{-1}, was used to determine P_N at different PPFD ranging 0–3 000 µmol m^{-2} s^{-1} at ambient CO_2 concentration (C_a). The same instrument was used to construct P_N *versus* C_i (intercellular CO_2 concentration) curve, wherein the C_a of desired level was generated using a *6400-01* CO_2 injector and a CO_2 mixer supplied along the instrument. Temperature of the chamber was maintained at 25 °C through a Peltier cooling and heating system and the PPFD was maintained at 2 000 µmol m^{-2} s^{-1} while constructing the curve. Leaves were allowed to equilibrate for 90 s at each PPFD/C_a.

Statistical analysis: Data sets were analyzed using one-way analysis of variance (ANOVA) as described by Gomez and Gomez (1984). Differences between means were tested against critical difference at $p<0.01$.

Results

P_N was not significantly different at PPFD between 1 500–3 000 µmol m^{-2} s^{-1} (I_{1500}–I_{3000}) in all the plants tested (Table 1). The slope of curve (SOC) drawn between P_N at different C_i at the above PPFD showed no significant change in *A. fragrans* (Fig. 1, Table 1). In *R. nepalensis*, SOC showed no significant change between I_{1500} and I_{2000}, but increased significantly by 12 % at I_{2500} and by 24 % at I_{3000} compared to I_{1500} µmol (Fig. 1, Table 1). SOC showed no significant change in *H. vulgare* at I_{1500} and I_{2000}, but significant increase by 37 % at I_{2500} and then decrease by 18 % at I_{3000} compared to I_{1500} (Fig. 1, Table 1) was recorded. In *P. sativum*, no significant difference in SOC was observed at I_{1500} and I_{3000}, but an increase by 22 % at I_{2000} and by 19 % at I_{2500} compared to I_{1500} was recorded (Fig. 1, Table 1).

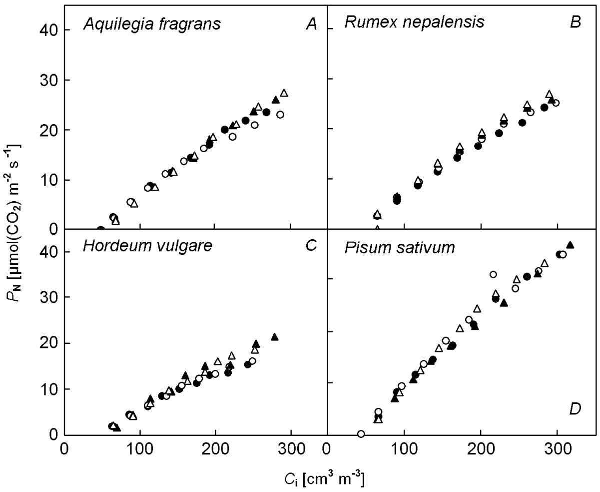

Fig. 1. Net photosynthetic rate *versus* intercellular CO_2 concentration (P_N/C_i) curves at 1 500 (●), 2 000 (○), 2 500 (▲), and 3 000 µmol m^{-2} s^{-1} (∆) of photosynthetic photon flux density (PPFD) for *Aquilegia fragrans* (*A*), *Rumex nepalensis* (*B*), *Hordeum vulgare* (*C*), and *Pisum sativum* (*D*). Data were pooled from measurements of 6–8 different individuals per plant species. All differences within the species are highly significant ($p<0.01$, by ANOVA).

At ambient C_a no significant change in g_s was observed at I_{1500}, I_{2000}, and I_{2500} in *A. fragrans*, but a significant increase was recorded at I_{3000} compared to I_{1500} (Table 1). In *R. nepalensis*, g_s increased significantly by 20 and 24 % at I_{2000} and I_{2500}, respectively, as compared to I_{1500} values (Table 1). No significant change in g_s was observed at I_{1500} and I_{2000} in *H. vulgare*, but an increase by 65 and 34 % at I_{2500} and I_{3000}, respectively, compared to I_{1500} was recorded (Table 1). However, g_s decreased by 19 % at I_{3000} compared to I_{2500} (Table 1). No significant change in g_s was observed at I_{1500}, I_{2000}, and I_{3000} in *P. sativum*, but it decreased by 42 % at I_{3000} compared to I_{1500} (Table 1). A curve plotted between g_s at different C_i clearly demarcated the impact of PPFD on g_s, the later being higher in *A. fragrans* and *R. nepalensis* (Fig. 2*A*,*B*) but lower in *H. vulgare* and *P. sativum* at higher PPFD (Fig. 2*C*,*D*).

A. fragrans and *R. nepalensis* evidently exhibited increase in g_s with increase in PPFD beyond I_{1500} (Fig. 3*A*,*B*), whereas *H. vulgare* and *P. sativum* showed decline (Fig. 3*C*,*D*; all values obtained from P_N/C_i plotted at different PPFD). A plot of P_N at different g_s (value obtained from light curve) clearly showed the impact of PPFD on stomatal opening (Fig. 4).

Discussion

The slope values in *A. fragrans* and *R. nepalensis* were either unchanged or increased with increase in irradiance (Fig. 1, Table 1), whereas in *H. vulgare* and *P. sativum* the slope value first increased and decreased thereafter. Slope of the curves of P_N and C_i is the measure of ECU. The higher the slope value, the higher the ECU. Higher ECU would suggest a decrease in mesophyll limitation to carbon uptake in HA plants. Further, g_s increased with increase in irradiance in wild plants (Figs. 2 and 3) suggesting ECU was not limited by the mesophyll component. Earlier papers have also demonstrated higher ECU at HA (Körner and Diemer 1987, 1994, Körner *et al.* 1988). The relationship between photosynthesis and altitude in wild species with large altitudinal ranges (Caberra *et al.* 1998) was not limited by mesophyll parameters.

Growing season of alpine herbaceous plants is very short. In order to complete their annual life cycle within 2–3 months, they have to rely on efficient carbon assimilation system (Körner and Larcher 1988). Several studies have shown that photosynthesis of alpine plants is well adapted to the climate through a very broad temperature range and the utilization of the warmest and brightest periods without any decline of P_N under photon energy saturation (Körner 1982, Körner and Diemer

Table 1. Gas exchange parameters of *A. fragrans*, *R. nepalensis*, *H. vulgare*, and *P. sativum* measured at HA location at Losar. Means ± SE ($n = 4$). Values indicated by different letters in the superscript, to be compared with corresponding column values, show significant difference at $p<0.01$. Values in parentheses show % increase as compared to the values obtained at 1 500 μmol m^{-2} s^{-1} PPFD. P_{Nmax} values are taken from the P_N/C_i curves constructed at different PPFD.

	PPFD [μmol m^{-2} s^{-1}]	P_N [μmol(CO$_2$) m^{-2} s^{-1}]	P_{Nmax} [μmol(CO$_2$) m^{-2} s^{-1}]	g_s [mmol(CO$_2$) m^{-2} s^{-1}]	Slope
Aquilegia fragrans	1 500	14.30^a ± 1.45	21.90^a± 0.56	212^a ± 14	0.115^a ± 0.014
	2 000	14.80^a ± 1.34	23.10^a± 0.78	215^a ± 17 (1.4)	0.122^a ± 0.009 (6.0)
	2 500	15.80^a ± 1.11	26.20^b± 1.10 (19.6)	232^a ± 17 (9.4)	0.124^a ± 0.011 (7.8)
	3 000	16.00^a ± 1.76	27.40^b± 0.41 (25.1)	264^b ± 15 (24.5)	0.127^a±± 0.010 (10.4)
Rumex nepalensis	1 500	21.30^a ± 2.45	24.30^a± 1.17	402^a ± 17	0.104^a ± 0.008
	2 000	23.00^a ± 1.67	25.60^a± 0.59	483^b ± 21 (20.1)	0.112^a ± 0.009 (7.6)
	2 500	23.20^a ± 2.47	27.10^b± 0.47 (11.5)	500^b ± 11 (24.3)	0.117^b ± 0.011 (12.5)
	3 000	24.90^a ± 2.08	28.40^b± 0.69 (16.8)	517^b ± 14 (28.6)	0.129^c ± 0.008 (24.0)
Hordeum vulgare	1 500	13.70 ^{a} ± 1.47	15.50^a± 1.67	182^a ± 9	0.083^a ± 0.012
	2 000	14.80 ^{a} ± 1.67	16.30^a± 1.46	199^a ± 11 (9.3)	0.091^a ± 0.009 (9.6)
	2 500	15.40 ^{a} ± 0.48	21.60^b± 1.09 (39.3)	301^b ± 14 (65.3)	0.114^b ± 0.012 (37.3)
	3 000	13.90 ^{a} ± 1.67	18.70^a± 1.56	244^c ± 12 (34.1)	0.098^c±± 0.012 (18.0)
Pisum sativum	1 500	26.10^a ± 1.40	34.60^a± 1.47	632^a ± 21	0.136^a ± 0.009
	2 000	25.10^a ± 1.48	34.70^a± 1.67	688^a ± 19 (8.8)	0.167^b ± 0.011 (22.7)
	2 500	25.70^a ± 1.88	36.60^a± 1.21	589^a ± 17 (–6.8)	0.163^b ± 0.012 (19.8)
	3 000	22.40^a ± 0.67 (–14.1)	33.00^a± 2.58	367^b±± 21 (–41.9)	0.140^a ± 0.007 (2.9)

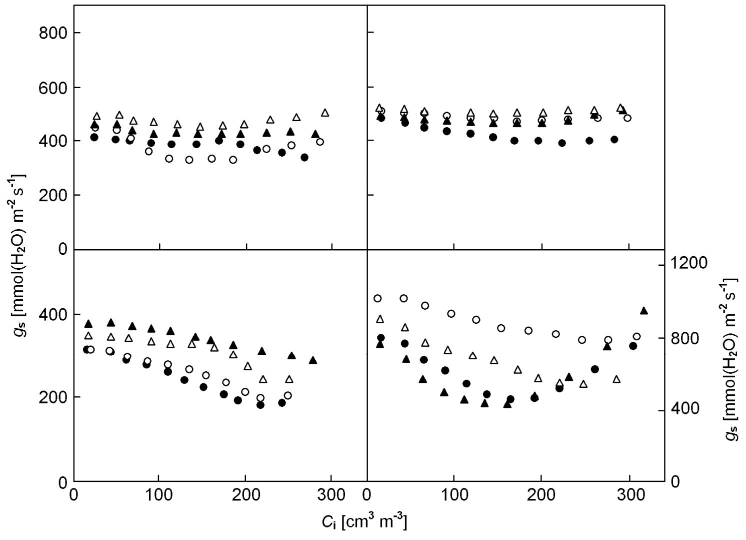

Fig. 2. Stomatal conductances (g_s) at 1 500 (●), 2 000 (○), 2 500 (▲), and 3 000 μmol m^{-2} s^{-1} (Δ) of photosynthetic photon flux density (PPFD) for *Aquilegia fragrans* (*A*), *Rumex nepalensis* (*B*), *Hordeum vulgare* (*C*), and *Pisum sativum* (*D*). Data were pooled from measurements of 6–8 different individuals per plant species. All differences within the species are highly significant ($p<0.01$, by ANOVA).

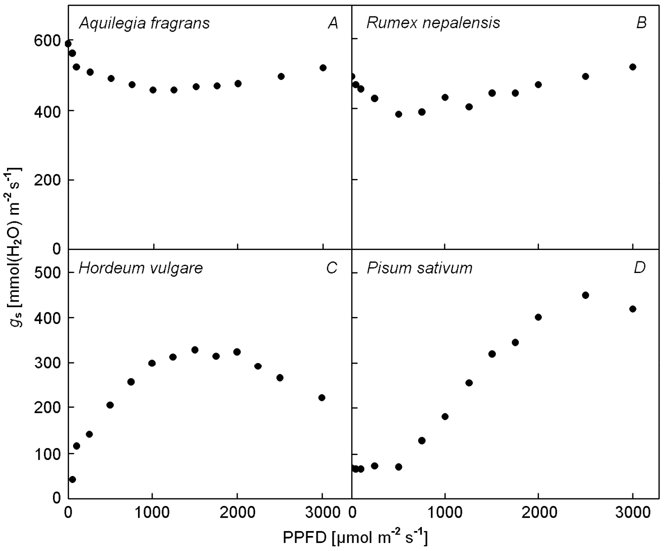

Fig. 3. Relationship of stomatal conductance (g_s) with photosynthetic photon flux density (PPFD) in *Aquilegia fragrans* (*A*; $r = -0.285$, $p<0.01$), *Rumex nepalensis* (*B*; $r = 0.600$, $p<0.01$), *Hordeum vulgare* (*C*; $r = 0.778$, $p<0.01$), and *Pisum sativum* (*D*; $r = 0.960$, $p<0.01$). Data were pooled from measurements of 6–8 different individuals per plant species.

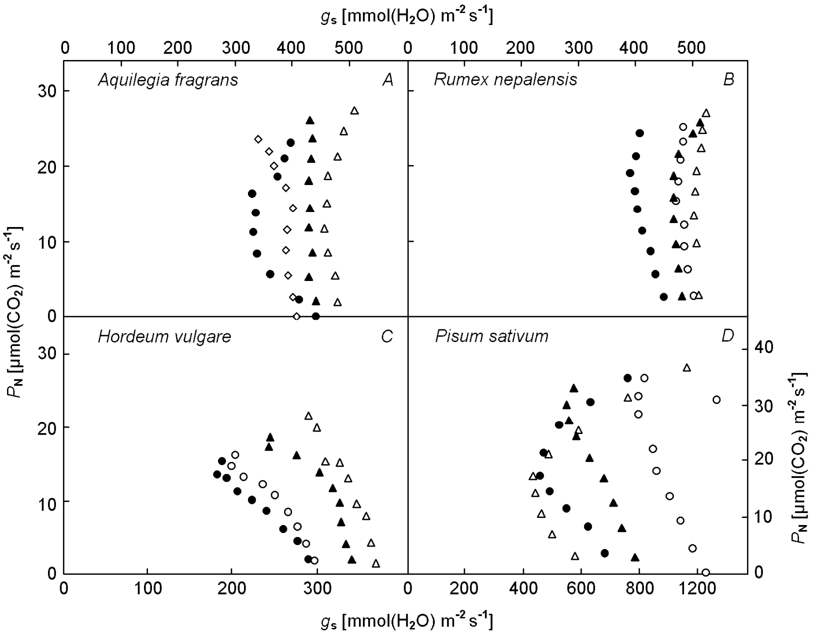

Fig. 4. Relationship of net photosynthetic rate (P_N) with stomatal conductance (g_s) at 1 500 (●), 2 000 (○), 2 500 (▲), and 3 000 µmol m^{-2} s^{-1} (Δ) of PPFD for *Aquilegia fragrans* (*A*), *Rumex nepalensis* (*B*), *Hordeum vulgare* (*C*), and *Pisum sativum* (*D*). Data were pooled from measurements of 6–8 different individuals per plant species. All differences within the species are highly significant ($p<0.01$, by ANOVA).

1987, Körner and Larcher 1988). We found that g_s and P_N decreased at high irradiances in cultivated plants but not in wild plants (Figs. 3 and 4) suggesting a decrease in ECU in cultivated plants as compared to the wild ones. Alpine plants have higher rate of transpiration (Tranquillini 1964), which is beneficial for plants to protect photosystem 2 (PS2) from high energy damage. Stomata of wild plants were insensitive to irradiance, whereas these were sensitive in cultivated species (Fig. 4). P_{Nmax} of wild plants increased with PPFD, whereas cultivated plants behaved differently (Table 1).

High mountain plants are resistant to photoinhibition of PS2, even if their leaves are incubated on ice or when new synthesis of the D1 protein in the chloroplasts is prevented by the application of the translation inhibitor chloramphenicol (Streb *et al.* 1997). These observations strongly suggest that the light-dependent turnover of the D1 protein in leaves of HA plants must have been very slow relative to lowland plants. The D1 protein turnover was compared in three species of alpine plants, *Homogyne alpina*, *Ranunculus glacialis*, *Soldanella alpina*, and the lowland plant *Taraxacum officinale* by radioactive labelling in light and subsequent chase experiment (Shang and Feierabend 1998). The turnover of the D1 protein was considerably lower in alpine plants as compared to *T. officinale*. Further, alpine plants adopt other adaptive mechanisms such as deposition of cuticular waxes that protect leaves from water loss due to high visible irradiance (Robinson *et al.* 1993) or high UV-B radiation *via* increased reflection and absorptive screening of the incoming radiation (Caldwell *et al.* 1980, Caldwell 1981). Some HA plants have highly reflective waxy layers (Pilon *et al.* 1999). Alpine plants possess constitutively higher contents of UV-B absorbing compounds than lowland species (Ziska *et al.* 1992). Exposure of leaves to excessive irradiances is a well-known cause of photoinhibition, which decreases the capacity for photosynthesis in many plants (Baker and Bowyer 1994). Photoinhibition is caused by damage to the photosynthetic components and it may be short-term and reversible or long term and irreversible, and is related to leaf temperature and to high irradiance when electron transport to acceptors is limited (Melis 1999).

The g_s of cultivated plants decreases at high PPFD (Küppers *et al.* 1986). Plotting the graph between P_N and g_s from the light curve showed an obvious differentiation between wild and cultivated plants (Fig. 4). Such a plot, if tested with a large number of plants, could serve as a tool to distinguish the plants from different altitudes that receive different PPFD. In fact, while working with *Podophyllum hexandrum* such trends were also found (Vats and Kumar 2006).

We confirmed that HA wild plants are adapted to wide range of PPFD and stomata are resistant to high irradiances. Further molecular and biochemical characterization of leaf and stomata of HA plants would help in understanding such behaviour. Our data suggest that tolerance or insensitivity to higher PPFD is important for the adaptation of plants to HA.

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