Differences in photosynthetic characteristics and accumulation of osmoprotectants in saplings of evergreen plants grown inside and outside a glasshouse during the winter season

S.C. JOSHI*, S. CHANDRA**, and L.M.S. PALNI***

*G.B. Pant Institute of Himalayan Environment and Development, Himachal Unit, Mohal-Kullu -175 126, H.P., India**
*National Centre for Natural Product Research, Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, MS 38677, USA***
*State Biotechnology Programme, Government of Uttarakhand, Biotech Bhavan, Haldi, U.S. Nagar - 263 146, Uttarakhand, India****

Abstract

Photochemical efficiency, photosynthetic capacity, osmoprotectants, and relative water content (RWC) were recorded in saplings of two evergreen plants (*Boehmeria rugulosa* Wedd. and *Olea glandulifera* Wall. *ex* G. Don) grown inside (GL) and outside (OP) a glasshouse during the winter season. The OP plants experienced 2.0–2.5 °C lower air temperature and dew formation in comparison to GL plants. Diurnal observations indicated no change in RWC in the leaves of GL and OP plants, while significant reduction in both transpiration and net photosynthetic (P_N) rates was observed in OP plants: the reduction in P_N was much more prominent as was also reflected by poor water use efficiency of these plants. Similarly, OP plants also showed decrease in the apparent quantum yield and irradiance-saturated CO_2 assimilation rate. The decrease in P_N was not associated with decreased stomatal conductance. However, a significant reduction in the ratio of variable to maximum chlorophyll (Chl) fluorescence (F_v/F_m) and Chl content was recorded in the OP plants which also contained more total soluble saccharides but less proline contents. The greater enhancement of P_N at 15 °C in comparison to measurements taken at 10 °C in OP plants over GL plants probably indicated an increase in mesophyll capacity of the OP plants' growth at increased temperature. Hence the enhanced growth and productivity of plants grown in sheltered environments could be associated to their higher photosynthetic activity that may have important bearing on their field establishment and productivity in the long run. The response varied with plant species; reduction in P_N was greater in *B. rugulosa* than in *O. glandulifera*. However, the recovery of OP plants in terms of F_v/F_m in the subsequent months revealed that photosynthetic system of these plants is revocable.

Additional key words: *Boehmeria rugulosa*; chilling temperature; *Olea glandulifera*; photochemical efficiency; photosynthesis; relative water content; transpiration rate; water use efficiency.

Introduction

Environmental factors greatly influence the growth and development of plants. Among various environmental factors, temperature is the most important one influencing the growth, survival, productivity, and natural distribution of plants. Tropical and sub-tropical plants generally experience chilling (0–12 °C) temperatures during winter. The inhibition of photosynthesis and growth has been reported in evergreen plants exposed to low (chilling) temperatures (Lyons 1973, Berry and Björkman 1980, Long *et al.* 1987). Plants of the Himalayan region generally experience chilling temperature during winter months from October to February. The occurrence of low temperature for such long periods adversely affects not only the growth and development of plants, but also timely availability of their quality seedlings and saplings. This has significant bearing on the success of afforestation and restoration of wasteland programmes, which depend on a timely and constant supply of quality seedlings (Vyas *et al.* 1999).

Some sheltered environment/microclimate modification techniques like glasshouses, polyhouses, polypits, shadecards, shelterwood, and Chhapar (thatch), which

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*Corresponding author; fax: +91-1902-226347, e-mail: joshisc@hotmail.com

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moderate extremes in environmental conditions, are useful to increase the growth, productivity, and quality of plants in many situations. These microclimate modification techniques are also used to protect plants from harsh environmental conditions such as high and low temperatures, irradiances, vapour pressure deficits, soil water content, and frost events, and to obtain enhanced growth and productivity in plants during winter (Childs and Flint 1987, Dalton and Messina 1995, Groot and Carlson 1996, Nuberg 1998). However, the underlying mechanisms for the increase are still unclear (Childs and Flint 1987). Nonetheless, the shelter-induced growth and productivity enhancement in plants over those of the field grown during winter have generally been attributed to improved microclimate, augmentation of soil moisture content, and improvement of plant water status, but less attention has been paid to differences in their photosynthetic parameters (Holly *et al.* 1994).

Photosynthesis has been successfully used as a valuable tool for the evaluation of responses of plants to various environmental stresses and for the rapid selection of plants most suitable for different habitats (Joshi and Palni 2005, Monclus *et al.* 2006). Further, since photosynthesis is one of the first physiological processes to be greatly affected by temperature (Berry and Björkman 1980), photosynthetic characteristics of plants grown in sheltered environments could also differ from those grown in the field during winter. Further, the positive effects of microclimatic techniques on the growth and productivity of plants vary with site conditions and plant species (Groot and Carlson 1996). This is why we studied the effect of sheltered environment (glasshouse – GL) on photosynthetic characteristics, relative water content (RWC), and accumulation of osmoprotectants in plants during winter using saplings of two multipurpose evergreen plants, namely *Boehmeria rugulosa* Wedd. (*Urticaceae*) and *Olea glandulifera* Wall. *ex* G. Don (*Oleaceae*), which are extensively used in plantation programmes.

Materials and methods

Plants: Saplings of *B. rugulosa* and *Q. glandulifera* were grown singly in earthen pots (28 cm diameter; 25 cm depth) containing a mixture of farmyard manure, sand, and garden soil (1 : 1 : 1). The plants were kept in the botanic garden of High Altitude Plant Physiology Research Centre, Srinagar-Garhwal (altitude 550 m a.s.l.; latitude 30°13', longitude 78°48'). During November approximately two-year-old healthy saplings of the same size were divided into two sets of 60 plants each. One set of plants of both species was shifted to a glasshouse and the other set was left in the botanic garden. Both sets of plants were watered regularly. The following observations were recorded on youngest fully expanded leaves during January and February.

Environmental parameters such as photosynthetic photon flux density (PPFD), air temperature, relative humidity, and wind speed on the day of observations were recorded using an automatic weather station and data logger (*Campbell Scientific*, Utah, USA).

CO_2 gas exchange parameters were measured using climate-controlled Compact Minicuvette System (*H. Walz*, Effeltrich, Germany) with cuvette air temperature of 10 °C (unless otherwise stated) and 65–70 % relative humidity. Cuvette humidity was maintained by regulating the dew point temperature of the air entering into the cuvette. PPFD was varied (0–2 000 µmol m^{-2} s^{-1}) by using an artificial light source (type *LA4*) with neutral density filters. Gas exchange was measured on 5 randomly selected plants in each case. The observations were taken for 30–45 min until steady-state CO_2 exchange values were obtained. Net photosynthetic rate (P_N), transpiration water loss (E), stomatal conductances for CO_2 (g_s) and water vapour, and intercellular CO_2 concentration (C_i) were calculated according to Caemmerer and Farquhar (1981). Water use efficiency (WUE) was computed as a ratio of P_N and E.

Chlorophyll (Chl) fluorescence kinetics was recorded on cloudless days with portable Plant Efficiency Analyzer (*Hansatech*, King's Lynn, U.K.) using 6–8 plants. A dark-adaptation period of 30 min was used before the Chl fluorescence characteristics (F_0 – constant fluorescence, F_m – maximum fluorescence, F_v – variable fluorescence, and F_v/F_m – photochemical efficiency of photosystem 2) were measured. Fluorescence was excited by red (actinic) radiation with 650 nm peak wavelength. A PPFD of 1 600 µmol(photon) m^{-2} s^{-1} for *O. glandulifera* and 2 000 µmol(photon) m^{-2} s^{-1} for *B. rugulosa* was used. In preliminary experiments these excitation irradiances were found to be sufficient to induce maximum fluorescence in respective plants. Diurnal recordings of Chl fluorescence characteristics were made at 4-h intervals. These observations were recorded on 5 different days during January and February. The relationships between photochemical efficiency (F_v/F_m) and environmental factors were determined by correlation analysis.

RWC and substance contents: Immediately after Chl fluorescence measurements, leaves were harvested and their middle portions excluding midribs were used for the estimation of RWC, total soluble saccharides, proline, and Chl. The RWC was recorded at 4 h intervals for 2 consecutive days following the method of Weatherley (1950).

Estimation of total soluble saccharides, proline, and

Chl contents: The methods of Dubois *et al.* (1956) for total soluble saccharides and of Bates *et al.* (1973) for proline were followed. These estimations were made at 4-h intervals for 2 consecutive days. Chl content was determined using dimethylsulfoxide (DMSO) extract following the method of Hiscox and Israelstam (1979).

Recovery of plants kept in the open was determined by using Chl *a* fluorescence technique in the subsequent months (March–April).

Results

Environmental parameters: Fig. 1*A,B* shows diurnal changes in environmental parameters namely, PPFD, air temperature, relative humidity, and wind speed. The air temperature was minimal during nights (*i.e.* 04:00–08:00 h), and maximum during the day time. A reverse trend was observed for relative humidity. The glasshouse ambient conditions in respect to air temperature and PPFD differed from those of outside ambient. The air temperature of glasshouse was 2.0–2.5 °C higher than outside air temperature while the PPFD was approximately 10 % less than that of outside ambient.

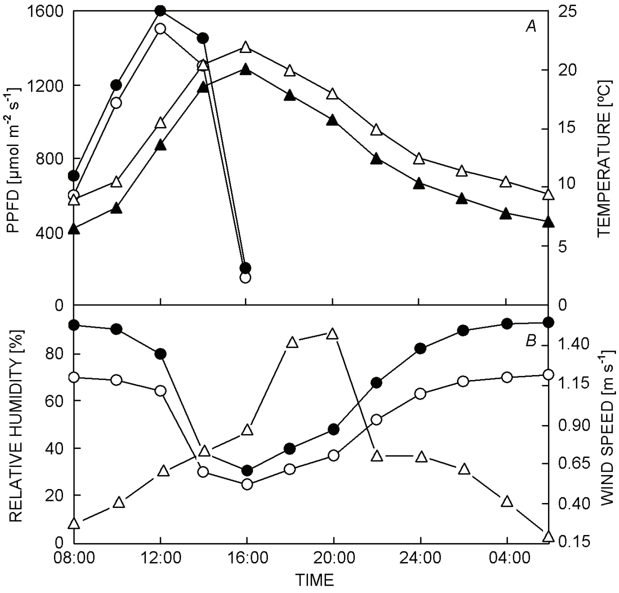

Fig.1. Diurnal changes in environmental parameters: *A*: photosynthetic photon flux density, PPFD (*circles*) and air temperature (*triangles*); *B*: relative humidity (*circles*) and wind speed (*triangles*) inside (*open symbols*) and outside (*closed symbols*) the glasshouse.

CO_2 gas exchange: In contrast to GL plants, OP plants invariably showed more than 3-fold decrease in P_N (Fig. 2*A*). The reduction of P_N was more in *B. rugulosa* than in *O. glandulifera*. The OP plants showed low irradiance (200 μmol m^{-2} s^{-1}) saturation and higher respiration rate. Respiration rate was 0.55±0.04 and 0.79±0.03 μmol(CO_2) m^{-2} s^{-1} in GL and OP plants of *B. rugulosa*, respectively, and likewise 0.66±0.02 and 0.72±0.03 μmol m^{-2} s^{-1} in *O. glandulifera*. There was a significant ($p<0.01$ and $p<0.05$ for *B. rugulosa* and *O. glandulifera*, respectively, according to *t*-test) decrease in *E* in OP plants, the differences being more apparent at high irradiance (Fig. 2*B*). A similar trend in g_s for CO_2 was also recorded (Fig. 2*C*). The WUE data showed that *B. rugulosa* was inferior to *O. glandulifera* under both conditions (Fig. 2*D*). Although the OP plants showed

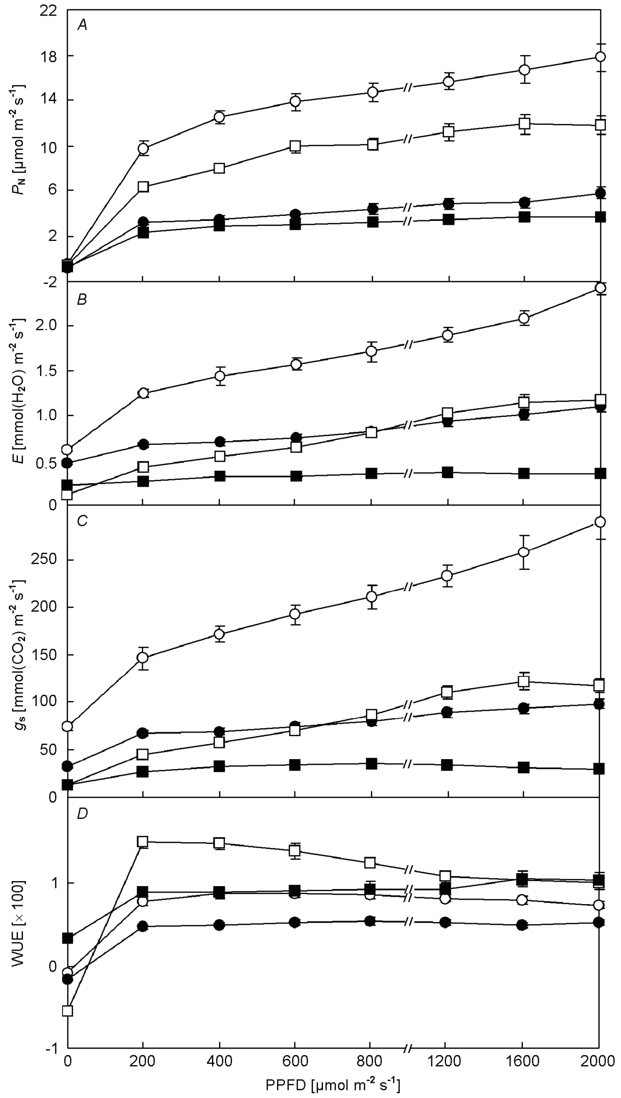

Fig. 2. Rates of net photosynthesis, P_N (*A*) and transpiration, *E* (B), stomatal conductance for CO_2, g_s (*C*), and water use efficiency, WUE (*D*) as functions of photosynthetic photon flux density (PPFD) in the leaves of *B. rugulosa* (*circles*) and *O. glandulifera* (*squares*) plants kept in the open (*closed symbols*) and inside the glasshouse (*open symbols*). Means (±SD) of 5 plants.

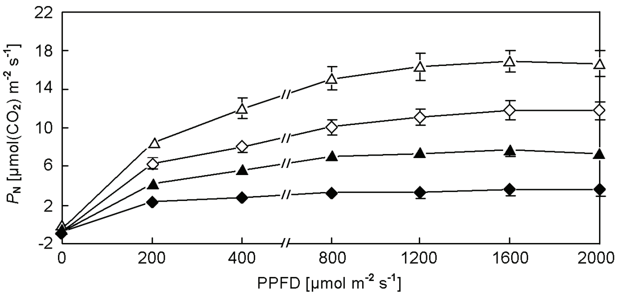

Fig. 3. Net photosynthetic rate (P_N) in the leaves of *O. glandulifera* plants kept in the open (*closed symbols*) and inside the glasshouse (*open symbols*). Measurements were made at 10 °C (◊) and 15 °C (Δ) cuvette temperature.

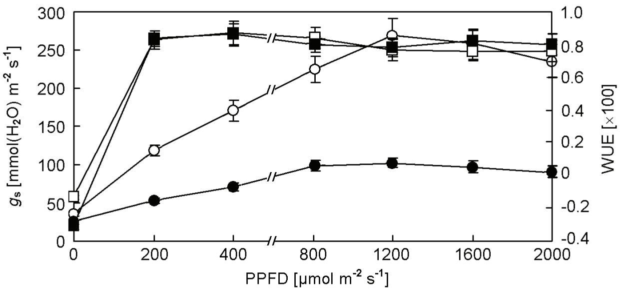

Fig. 4. Stomatal conductance to water vapour, g_s (*circles*) and water use efficiency, WUE (*squares*) at 15 °C cuvette temperature in the leaves of *O. glandulifera* plants kept in the open (*closed symbols*) and inside the glasshouse (*open symbols*).

significantly ($p<0.001$ and $p<0.01$ for *B. rugulosa* and *O. glandulifera*, respectively) lower g_s, their C_i values were either slightly higher or at par with those of GL plants.

Photosynthetic capacity of *O. glandulifera* GL and OP plants increased when measurements were made at 15 °C. However, in relation to data taken at 10 °C the increase was greater in OP plants compared to the GL ones (Fig. 3): the maximum P_N increased from 3.70 to 7.28 μmol(CO$_2$) m^{-2} s^{-1} in OP plants and from 11.79 to 16.50 μmol(CO$_2$) m^{-2} s^{-1} in GL plants. Likewise both plant species grown in OP and GL conditions showed increased g_s at 15 °C when compared with measurements made at 10 °C. Stomatal conductance to water vapour of OP plants was significantly ($p< 0.05$) less than that of GL plants, but no significant differences in their WUE were observed (Fig. 4). At 15 °C, there was little difference in C_i between the OP and GL plants (data not shown). This probably indicates an increase in mesophyll capacity of *O. glandulifera* plants which were kept in the open at increased temperature. Similar observations were also recorded for *B. rugulosa*.

Chl fluorescence: The photochemical efficiency of PS2 measured as F_v/F_m was also significantly ($p<0.01$) low in OP plants (Fig. 5*A*). In both species, the decrease in F_v/F_m resulted from decrease in F_m and an increase in F_0 (Fig. 5*B,C*). However, a decrease in F_0 was also observed in *O. glandulifera* (Fig. 5*C*). A significant ($p<0.001$) reduction in area over the curve between F_0 and F_m representing the pool size of electron acceptors on the reducing side of PS2 was also observed in OP plants (Fig. 5*D*). In general, the decline in fluorescence parameters was maximum at midday. The relationships between photochemical efficiency and environmental factors are shown in Table 1. In both species, photochemical efficiency was adversely affected by temperature and irradiance and the effect was more pronounced in *O. glandulifera*.

RWC: The RWC of *B. rugulosa* OP plants did not differ from that of GL plants. However, in the 24-h cycle, a slight but non-significant increase in RWC was observed in OP plants of *O. glandulifera* compared to the GL ones.

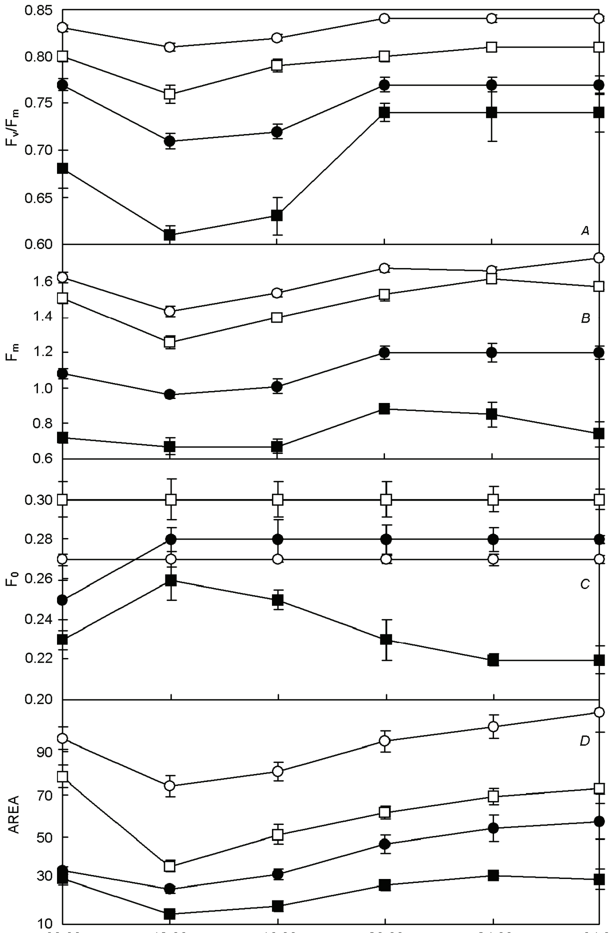

Fig. 5. Diurnal changes in chlorophyll fluorescence characteristics – *A*: F_v/F_m; *B*: F_m; *C*: F_0; *D*: Area (indicating the pool size of electron acceptors on the reducing side of PS2) in the leaves of *B. rugulosa* (*circles*) and *O. glandulifera* (*squares*) plants kept in the open (*closed symbols*) and inside the glasshouse (*open symbols*). *Each vertical bar* represents an average of 30–40 plants ±SD.

Table 1. Pearson's correlation coefficients between photochemical efficiency (F_v/F_m) and environmental parameters. Diurnal mean values of F_v/F_m obtained using 5–6 plants of each species for each treatment on five different days during winter were used for the correlation coefficients. *Significant at $p \leq 0.01$.

	Plant species	Photochemical efficiency	Temperature	Irradiance	Relative humidity	Wind speed
O. glandulifera	Photochemical efficiency	1.00	–0.43	–0.93*	0.23	0.21
	Temperature	–0.43	1.00	0.45	–0.95	0.68
	Irradiance	–0.93*	0.45	1.00	–0.17	–0.09
	Relative humidity	0.23	–0.95	–0.17	1.00	–0.74
	Wind speed	0.21	0.68	0.09	–0.74	1.00
B. rugulosa	Photochemical efficiency	1.00	–0.43	–0.74	0.11	0.37
	Temperature	–0.23	1.00	0.45	–0.95	0.68
	Irradiance	–0.74	0.45	1.00	–0.17	–0.09
	Relative humidity	0.11	–0.95	–0.17	1.00	–0.74
	Wind speed	0.37	0.68	–0.09	–0.74	1.00

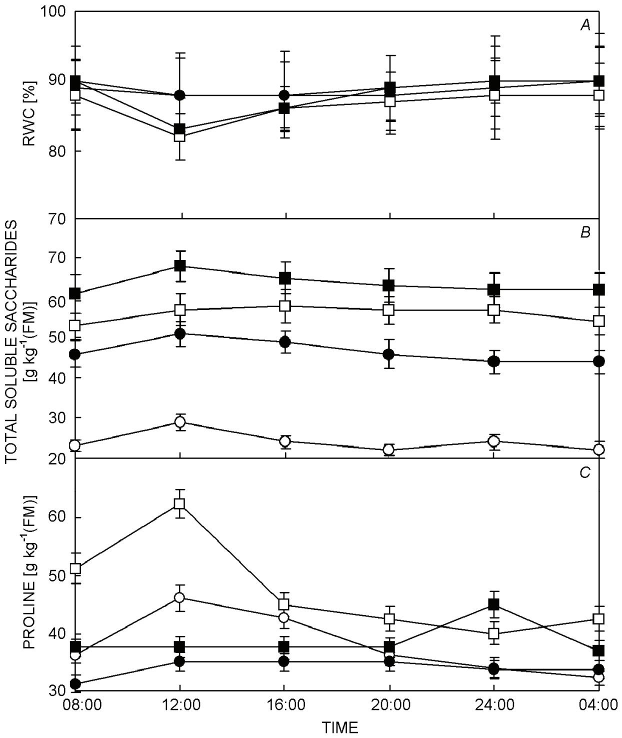

Fig. 6. Diurnal changes in relative water content, RWC (*A*) and contents of total soluble saccharides (*B*) and proline (*C*) in the leaves of *B. rugulosa* and *O. glandulifera* plants. For symbols and other details see Fig. 2.

A slight decline in RWC was also observed in *O. glandulifera* at midday (Fig. 6*A*).

Contents of total soluble saccharides, proline, and Chl: There was a significant ($p<0.001$) increase in content of total soluble saccharides in OP plants. In comparison to *O. glandulifera*, the increase in *B. rugulosa* was higher. An increase in soluble saccharides during day time was observed in plants grown under both the conditions (Fig. 6*B*). On the other hand, proline content was low in OP plants. In both *O. glandulifera* and *B. rugulosa* proline accumulation was maximal during day time in GL plants (Fig. 6*C*). However, no definite trend was observed in OP plants. Chl content (*a*, *b*, and *a/b* ratio) was also low in OP plants. Although the reduction in Chl *a* and *b* was more in *B. rugulosa* than in *O. glandulifera*, the Chl *a/b* was less affected in *B. rugulosa* compared to *O. glandulifera* (Table 2).

Table 2. Chlorophyll (Chl) *a* and *b* [g kg^{-1}(FM)] contents and *a/b* ratio in the leaves of plants grown inside (GL) and outside (OP) the glasshouse. Measurements were made at noon. Values (± SD) are means of 5 plants.

Plant species	Treatment	Chl *a*	Chl *b*	Chl *a/b*
O. glandulifera	GL	1.67±0.12	0.41±0.03	4.07
	OP	0.80±0.05	0.28±0.02	2.86
B. rugulosa	GL	1.91±0.15	0.50±0.04	3.82
	OP	0.67±0.04	0.21±0.03	3.19

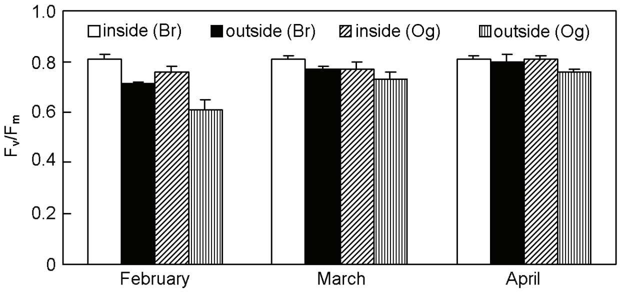

Fig. 7. Recovery of F_v/F_m measured during March to April in the leaves of *B. rugulosa* (Br) and *O. glandulifera* (Og) plants kept in the open and inside the glasshouse. *Each vertical bar* represents an average of 5 plants ±SD.

Recovery of plants: The recovery of OP plants during the subsequent months when air temperature became favourable is shown in Fig. 7. The average minimum and maximum temperatures during March and April were

10.06 and 23.86 °C and 15.18 and 32.38 °C, respectively. The F_v/F_m started increasing in OP plants during March and this ratio in *B. rugulosa* became almost at par with that of GL plants during April.

Discussion

The tropical and subtropical plants of the Himalayan region experience low (chilling) temperature during the winter months from October to February which results in inhibition of photosynthesis and growth in evergreen plants. Our OP plants experienced 2.0–2.5 °C lower air temperature than the GL plants. Though the temperature of leaves was not recorded, we assume that in the OP plants it might have been substantially lower than in GL plants as plants kept in the open also experienced dew formation during the winter months. In the present investigation, OP plants of both species showed drastic reduction of P_N and E compared to GL plants. While both the parameters were significantly reduced, the reduction in P_N was much more prominent as reflected also by the poor WUE of OP plants. The reduction in P_N was more significant in *B. rugulosa* than in *O. glandulifera*. The WUE of *B. rugulosa* was poor to *O. glandulifera* under both the conditions. At 15 °C, there were no significant differences in C_i between OP and GL plants. A greater enhancement of P_N at 15 °C in OP plants compared to GL plants probably indicated an increase in the mesophyll capacity of OP plants with increase in temperature.

Further analysis of photosynthetic data shows that both the apparent quantum yield (initial slope of the curve; Fig. 2*A*) and the photosynthetic capacity decreased in OP plants as evidenced by their irradiance response curves. A number of factors can be invoked to explain this reduction as photosynthesis is a complex of diffusive, photochemical, and biochemical processes (Graham and Patterson 1982, Walker *et al.* 1991, Holaday *et al.* 1992). Although we observed significant reduction in g_s in OP plants, a corresponding decrease in C_i was not observed: C_i values were either slightly higher or at par with those of GL plants. Hence stomatal closure was not the major cause of decreased P_N in OP plants. Differences among plants in stomatal and non-stomatal limitations to their photosynthesis were documented by Ni and Pallardy (1985).

A significant reduction in the photochemical efficiency of PS2 indicates that photochemical processes (energy conversion) were more affected in OP plants. An inhibition of photochemical efficiency of plants upon exposure to low (chilling) temperature was reported in spruce by Bolhàr-Nordenkampf and Lechner (1988) or in spinach by Somersalo and Krause (1990) when grown in the field during winter. The OP plants showed a considerable reduction in the F_v/F_m at midday probably on account of photoinhibition. The midday reduction was more prominent in *O. glandulifera*. The plants of *O. glandulifera* were more sensitive to irradiation than those of *B. rugulosa*. Existence of significant negative correlation between irradiation and photochemical efficiency of *O. glandulifera* supports this (Table 1). We observed partial recovery of F_v/F_m in *B. rugulosa* during night. Somersalo and Krause (1990) considered this kind of photoinhibition as a protective system for thermal dissipation of excess photon energy in plants during winter. Further, a considerable reduction in area over the curve between F_0 and F_m which represents the pool size of electron acceptors on the reducing side of PS2 was observed in OP plants. This finding is in conformity with the work of Öquist and Ögren (1985) with *P. sylvestris* during winter stress. Considerably low Chl *a* and *b* contents and *a/b* ratio were also observed in OP plants. There exists an inverse relationship between Chl content and fluorescence yield. Nevertheless, we observed the recovery of F_v/F_m of both plant species kept in the open during March–April.

In our investigation, the RWC of *B. rugulosa* OP plants did not differ from that of GL plants. On the other hand, in the 24 h cycle, a slight but non-significant increase in RWC was observed in OP plants of *O. glandulifera* compared to the GL plants. However, there were significant differences in the patterns of accumulation of various osmoprotectants (soluble saccharides and proline) in OP and GL plants. In many plants, exposure to low temperature has resulted in the accumulation of osmoprotectants (Sakai and Larcher 1987, Konstantinova *et al.* 2002). These osmoprotectants lower osmotic potential of plants, preventing perturbation of cell metabolism during stress, which potentially facilitates recovery of plants after stress alleviation (Konstantinova *et al.* 2002). According to Morgan (1984) they also protect plasma membranes and proteins from the deleterious effects of freezing and dehydration. More total soluble saccharides are associated with plants grown in the open. Soluble sugars may increase the low temperature tolerance capacity of plants (Sakai and Larcher 1987). We found that low photosynthetic activity was invariably associated with plants containing larger amounts of soluble saccharides. For example, *B. rugulosa* contained more soluble saccharides and showed larger reduction in P_N. This finding is supported by Huang *et al.* (1989) who reported high content of soluble sugars and inhibition of photosynthesis at low temperature in rice leaves. On the other hand, high photosynthetic activity was associated with high proline content (Fig. 2*A*). The GL plants contained more proline and showed higher P_N than the OP plants.

In summary, the enhanced growth and productivity of plants grown in sheltered environments during winter could be ascribed to their higher photosynthetic activity as GL plants invariably showed 3–4 fold increase in P_N compared to OP plants. This may have significant bearing on their field establishment and productivity in the long run. Further, the response varied with plant species; reduction in P_N was more expressed in *B. rugulosa* than in *O. glandulifera*. However, the recovery of OP plants in terms of F_v/F_m in the subsequent months reveal that photosynthetic system of these plants was revocable.

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