Effect of CO_2 Enrichment on Photosynthetic Behavior of *Podophyllum Hexandrum* Royle, an Endangered Medicinal Herb.

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Abstract: The effect of a doubling in the atmospheric CO_2 concentration on the morpho-physiology of an endangered medicinal herb, *Podophyllum hexandrum* Royle was investigated. Open top chambers (OTCs, 3.0 m diameter, 2.4 m in height) were used to expose plants to ambient and elevated CO_2 concentration (650±50 µmol mol^{-1}) from June to September at an alpine expanse of Garhwal Himalaya. Photosynthetic rate (A, µmol m^{-2} s^{-1}) expressed per unit leaf area was stimulated during the first 30 days thereafter a significant decrease in its rate was recorded. Transpiration rate (E, mmol m^{-2} s^{-1}) was found decreased significantly throughout the CO_2 enrichment wherein stomatal conductance (g_s, mol m^{-2} s^{-1}) have shown a significant reduction initially. At the end of the study, SLA was reduced by 29.45 %, which can partly be explained by an increased dry matter content of the leaves. Total dry matter production significantly increased by 96.40 % after 90 days of CO_2 exposure. This significant increase may be explained by increased RWR. Organic Carbon in aboveground and belowground compartment has shown a significant increment whereas in case of total nitrogen results were found non-significant. Overall study concludes that the medicinally used part of this plant *i.e*. rhizome/root have shown a significant increment. Thus, further studies on impact of elevated CO_2 on principle active component of this endangered plant may be observed.
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Keywords: Elevated CO_2, photosynthetic rate (A), transpiration rate (E), stomatal conductance (g_s), specific leaf area (SLA) and root weight ratio (RWR)

1. Introduction

Doubling of present CO_2 concentrations with rising of global air temperature (1.8-4.0°C) over this century are predicted (IPCC, 2007). Increased atmospheric carbon dioxide concentrations associated with increasing temperatures are predicted to have profound impacts on terrestrial ecosystems (Ward and Strain, 1999). The effects of simulated environmental changes, such as temperature, soil water content, nutrient availability, UV-B radiation and CO_2 concentration, on plant growth and productivity have already been studied in arctic, subarctic and alpine regions (Chapin and Shaver, 1996; Havström et al., 1993; Körner et al., 1997; Wada et al., 2002). But, till date no such study have been initiated in alpine and sub alpine regions of India.

Different species respond differently to elevated CO_2. Bazzaz et al., (1995) showed that even individual families might respond differently to elevated atmospheric CO_2, and suggested that future CO_2 levels would lead to increased intensity of natural selection. Likewise, there is a choice to be made whether to represent variation within a species, or to minimize genetic noise by moving from species → provenance → population → half-sib family → full sib family, i.e. clone. Gaining knowledge from each genetic level is relevant to the question of up scaling results, and selection under elevated CO_2 of genotypes for future forests. Plant age and the use of single leaves, single branches, single trees, stands or full ecosystems are of great importance to results and possible interpretations of effects of elevated CO_2 (Saxe et al.,1998).

In various studies, the photosynthetic enhancement that occurred at elevated CO_2 either persisted indefinitely (Ziska et al.,1990) or was partly to fully reversed after days to weeks of CO_2 enrichment (DeLucia et al.,1985; Yelle et al.,1989). Elevated CO_2 directly increases leaf-level net photosynthesis in many plant species (Dahlman 1993), whereas stomatal conductance in usually decreased (Tyree and Alexander 1993).

Podophyllum hexandrum is an endangered medicinal herb of Western Himalaya, belongs to the family podophyllaceae, locally known as Van kakri. In Garhwal, it is found in restricted pockets between 2700-3600 m asl altitudes in sub-alpine and alpine regions between Quercus scrub or between boulders in shady areas. To the best of our knowledge effect of elevated CO_2 on the physiology of this plant in near natural condition has not been assessed. The present study has been conducted with the objectives as how the morphology of selected medicinal plant species will be affected under elevated CO_2? Whether photosynthetic acclimation will contribute to increased productivity?

2. Materials and methods

2.1 Site description

The selected site for the present work was the alpine field station of High Altitude Plant Physiology Research Centre of H.N.B. Garhwal University, Srinagar, Garhwal, situated at Tungnath (30^0 14'N Latitude and 79^0 13'E longitude at an altitude of 3600 m asl.).

2.2 CO_2 enrichment

Open top chambers (OTCs, 3.0 m diameter, 2.4 m in height) were used to expose plants to ambient and elevated CO_2 concentrations from June through September 2008. One of the Open Top Chambers (OTC) was treated as Control Open Top Chamber (COTC) in which ambient air was circulated and monitored whereas in other Open Top Chamber (EOTC) elevated CO_2 (650±50 µmolmol^{-1}) was maintained. The pure carbon dioxide was supplied through five (20 Kg gas capacity) carbon dioxide cylinders fitted in a row. Air circulation was done to the chamber via air blowers located near the base of the chamber and CO_2 was added to the incoming air maintaining a positive air pressure and flow within the chambers {design modified from (Uprety 1998)}.

Twenty seedlings of *Podophyllum hexandrum* were transplanted inside both COTC (where ambient CO_2 was provided) treated as control and in EOTC (where elevated CO_2 was provided). In selection of seedling transplantation, most homogeneous seedlings of same age were selected for exposure. CO_2 exposure/ CO_2 enrichment was initiated after two weeks of acclimatization inside Open Top Chambers. Morphological and photosynthetic measurements were performed at a three time interval as 30, 60 and 90 days after CO_2 treatment (DAT). Changes in dry matter content of above ground and belowground compartment were done at final harvest (90 DAT).

2.3 Morphological variations

Morphological variations were recorded in marked seedlings for different morphological observations *viz.*, plant height, and number of leaves and leaf area.

2.4 Measurement of photosynthetic parameters

Leaves of each marked plant were randomly selected for leaf gas exchange measurements. The leaf chamber was oriented to obtain maximum light interception, to get light saturated readings by means of a Portable Photosynthetic System (LCPro+, ADC Ltd, UK). The following parameters were assessed per unit leaf area: Photosynthetic rate (A), stomatal conductance (g_s) and transpiration water loss (E). Measurements were performed for cloudless days.

2.5 Shoot-Root dry weight, Specific leaf area (SLA), Leaf area ratio (LAR), Shoot weight Ratio (SWR) and Root weight ratio (RWR)

After 90 days of CO_2 exposure, marked 10 plants were harvested from both condition i.e. control (ambient CO_2) and elevated CO_2 (650±50 µmolmol^{-1}). Plants were brought to laboratory and separated into above ground and belowground parts. Dry weight (total, leaf, root, shoot dry weight) were recorded after keeping plants inside oven (80°C) for 24 hrs. Leaf area ratio (LAR), Specific leaf area (SLA), Shoot weight ratio (SWR) and Root weight ratio (RWR) were calculated as per Hunt (1982).

2.6 Nutrient analysis (organic carbon and Total nitrogen)

Dried powdered samples of above ground and belowground parts were used for Organic Carbon content as per Okalebo et al. (1993) and Total Nitrogen (%) according to method of Allen, 1974.

3. Results

3.1 Morphological features

Plants exposed to elevated level of CO_2 have shown increase in plant height throughout the experiment (ns, $P>0.05$). In case of leaf area initially after 30 days of CO_2 enrichment, there was a slight increase in leaf area of plants grown in elevated CO_2 (ns, $P>0.05$); thereafter a decrease in leaf area of plants grown in elevated CO_2 was found ($P<0.01$). Leaf numbers were not found significantly differed throughout the experiment. Variations in plant height, leaf area, and leaf number of *P. hexandrum* grown at ambient and elevated CO_2 conditions are shown in Figure 1, 2 and 3 respectively.

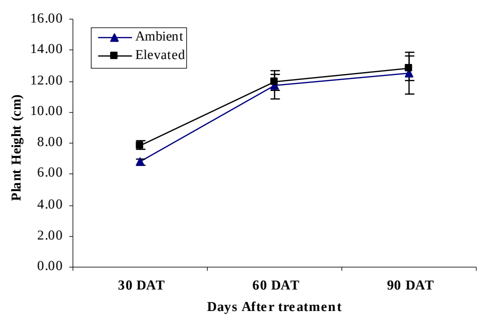

Figure 1. Variation in plant height of *P. hexandrum* exposed to CO_2 enrichment

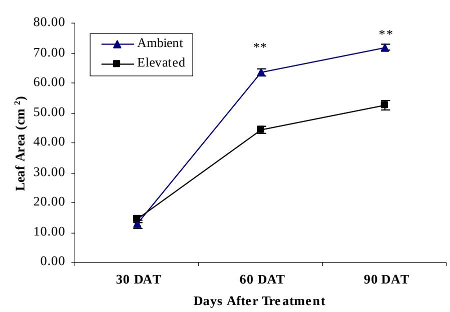

Figure 2. Variation in leaf area of *P. hexandrum* exposed to CO_2 enrichment

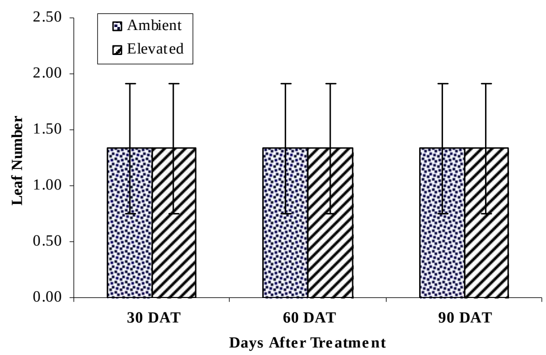

Figure 3. Variation in leaf number of *P. hexandrum* exposed to CO_2 enrichment

3.2 Photosynthetic characteristics

Net photosynthetic rate (A) of the leaves of elevated CO_2-grown plants were found increased initially during 30 DAT as compared to those of ambient CO_2-grown plants (P<0.01, Figure 4), thereafter during 60 DAT and 90 DAT, the A of elevated CO_2-grown plants was found significantly decreased (P<0.01) as compared to those of ambient CO_2-grown plants respectively. Transpiration water loss (E) was found significantly decreased in plants grown in elevated CO_2 (P<0.01) compared to ambient grown plants throughout the CO_2 exposure (Figure 5). Stomatal conductance (g_s) found decreased initially during 30 DAT as compared to those of ambient CO_2-grown plants (P<0.05, Figure 6), thereafter during 60 DAT and 90 DAT, the A of elevated CO_2-grown plants was found increased (ns; P>0.05) as compared to those of ambient CO_2-grown plants respectively.

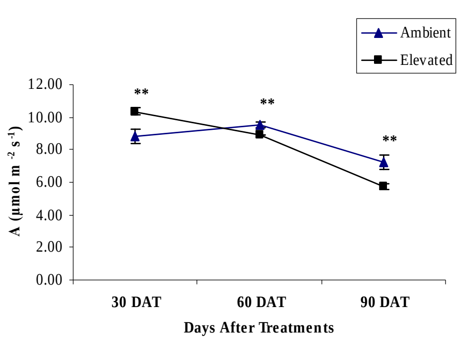

Figure 4. Changes of photosynthetic rates (A) of *P. hexandrum* during CO_2 enrichment.

Values are Mean+ SD (n = 5). Significant differences at the 5% (*) and 1% (**) level were determined with Fisher's protected LSD test.

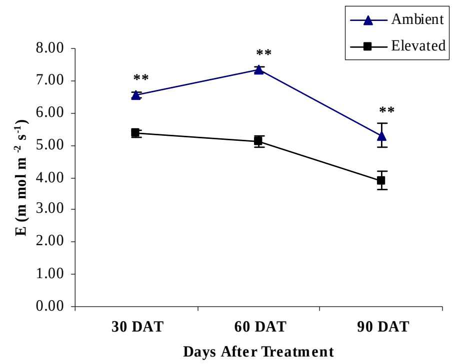

Figure 5. Effect on Transpiration rate (E) of *P. hexandrum* to CO_2 enrichment.

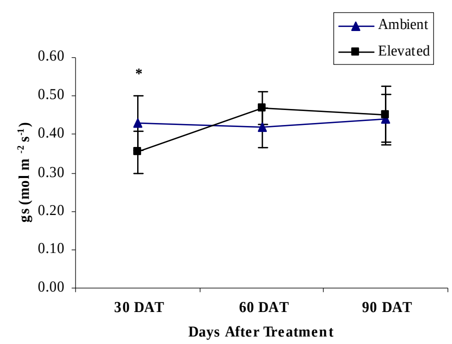

Figure 6. Stomatal conductance (g_s) of *P. hexandrum* in ambient and elevated CO_2 conditions.

3.3 Dry matter distribution, Leaf area ratio (LAR), Specific leaf area (SLA), Leaf weight ratio (LWR), Shoot weight ratio (SWR) and Root weight ratio (RWR)

Total biomass and dry matter partitioning to roots of ambient (330±50µmolmol^{-1}) and elevated (650±50µmolmol^{-1}) CO_2-grown plants were measured at 90 DAT. Total biomass at final harvest was 96.40% greater in elevated than in ambient CO_2-grown plants. Root biomass significantly increased in elevated CO_2-grown plants (P<0.01), whereas shoot biomass did not (P>0.05). CO_2 treatment effect on the allocation of dry matter from stem to roots was seen (Table 1). However, the investment of dry matter in relation to the acquired leaf area was altered, leading to a reduction of the SLA (P<0.01; Table 1). As a consequence of the reduction of the SLA and LWR, the LAR, the product of SLA and LWR, was also reduced at high CO_2. Whereas increased RWR (P<0.05) was found in plants grown in elevated CO_2 (Table 1).

Table 1. Variation in dry matter content, LAR, SLA, SWR, and RWR of *P. hexandrum* exposed to elevated CO_2

Parameters	Ambient	Elevated	Level of Significance
Leaf dry weight (g)	0.27±0.02	0.29±0.03	ns
Shoot dry weight (g)	0.37±0.01	0.40±0.01	ns
Root dry weight (g)	1.29±0.61	2.88±0.18	**
Total dry weight (g)	1.67±0.60	3.28±0.22	**
LAR (m^2 g^{-1})	46.48±14.22	16.04±0.61	*
SLA (m^2 g^{-1})	260.83±14.17	183.99±15.55	**
LWR (g g^{-1})	0.18±0.05	0.09±0.01	*
SWR (g g^{-1})	0.24±0.08	0.12±0.03	*
RWR (g g^{-1})	0.76±0.08	0.88±0.03	*

Values are Mean+ SD. Significant differences at the 5% (*) and 1% (**) level were determined with Fisher's protected LSD test.
ns non- significant
* significant at LSD<0.05
** significant at LSD<0.01

3.4 %Organic carbon and % total nitrogen in aboveground and belowground compartments

Organic carbon was found significantly increased in above and belowground compartments (P<0.05 and P<0.01 respectively), whereas for % total nitrogen results were found non-significant (Table 2).

Table 2. Variation in % organic carbon and % total nitrogen of aboveground and belowground compartments of *Podophyllum hexandru*m exposed to elevated CO_2

Nutrients	Compartments	Ambient	Elevated	LSD
Organic carbon %	Aboveground	1.60±0.43	2.33±0.13	0.55*
	Belowground	3.45±0.21	5.55±0.37	0.91**
Total Nitrogen %	Aboveground	0.51±0.16	0.37±0.08	ns
	Belowground	0.42±0.14	0.56±0.14	ns

Values are Mean+ SD. Significant differences at the 5% (*) and 1% (**) level were determined with Fisher's protected LSD test.

4. Discussion

The results of elevated CO_2 on *P. hexandrum* Royle indicates that this species responded similarly to elevated levels of atmospheric CO_2 compared to other species previously tested (Kimball 1983, Cure & Acock 1986). Total dry weight was increased by 96.40%, which may be due to a stimulation of the growth during the first thirty days of the experiment. Such a transitory stimulation of the growth rate has been reported for many other species (Bazzaz 1990). The positive response to elevated CO_2 was due to the stimulation of the rate of photosynthesis initially. However constant reduction of the SLA as a transient effect of the CO_2 level on photosynthesis is indicated by the drop of the photosynthesis rate at high CO_2 as duration of exposure increased i.e. 60 DAT and 90 DAT. This time course has been found for many species, although for some the positive effect is maintained for a much longer period (Bazzaz 1990).

The increase in photosynthesis is partly offset by a decrease in SLA Similar to other studies previously done (Poorter et al., 1988). This decrease in SLA is at least partly may be due to accumulation of starch (Wong 1990). Thus, one of the causes of the relatively minor stimulation of high CO_2 concentrations is that the increased photosynthetic supply is not used for investment in new actively growing material (e.g. leaf area) but accumulated as starch in the chloroplasts. The decrease in SLA is a phenomenon which occurs in many plants exposed to elevated CO_2 levels (Bazzaz 1990). This effect is partly explained by the higher dry matter concentration in the leaves.

The product of the SLA and the LWR *i.e.* LAR was affected similarly as the SLA. This indicates that under high CO_2 conditions these *P. hexandrum* plants acquire a smaller total leaf area relative to the total plant weight. Whereas RWR was found increased that may be partly due to allocation of dry matter to roots. To date many conflicting information on effects of CO_2 level on dry matter allocation have been published.

Frequently observed response of elevated CO_2 is the production of leaves with a higher C: N ratio than at ambient CO_2 (Luo et al., 1994; Curtis, 1996). Our results correlates with these studies as C: N ratio was increased in elevated CO_2 grown plants as compared to ambient grown plants.

In several experiments where leaf N decreases in plants under elevated CO_2. This is true even when N-Contents are corrected for starch and soluble sugar Accumulation in elevated CO_2 (Curtis et al., 1995), suggesting that C-dilution does not account entirely for this effect. Changes in tissue composition induced by elevated CO_2 can influence forest processes biomass allocation and growth (Luo et al., 1994) and nutrient dynamics (Pregitzer et al., 1995). It might be said that leaf N concentrations are reduced as a result of increased N-use efficiency. However, as species differ in their uptake and allocation of nutrients like nitrogen (Den Hertog & Stulen 1990). In P. hexandrum, decrease of the leaf nitrogen concentration due to a doubling of the CO_2 concentration as in other species may therefore show a shift in the balance between root respiration for growth and that for ion uptake, leading to a zero net change (Den Hertog & Stulen 1990).The C-economy of this species under the given growth conditions is alike with that of other species (Den Hertog et al., 1993). This points out that the physiology of the plant is still affected by the higher atmospheric CO_2 level.

Although the elevated CO_2 resulted in increased dry matter, increased root dry weight contributed mainly to this increase, but the effect of elevated CO_2 on medicinal property of the plant is not assessed. Nautiyal and Purohit (2000), in case of aconites found 8 to 11- fold higher production of tubers in plants grown inside polyhouse (4-5^{0}C higher temperature than open) compared to open field grown plants. They also concluded that percent alkaloids were also higher in plants grown in polyhouse. Accordingly, the role of elevated CO_2 on the medicinal property of this plant requires further attention. It is a challenging goal to try and relate the effects of CO_2 concentration to active constituents of the plant for future mitigation processes under changing climate with increased temperature and CO_2 enriched environment.

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