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Effect of Altitude on Biochemical and Photosynthetic Characteristics of *Aconitum balfourii* and *Podophyllum hexandrum:* High Value Endangered Medicinal Herbs from Himalayas

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Abstract: *Aconitum balfourii* and *Podophyllum hexandrum* are two high value endangered medicinal plant species from the alpines of Central Himalaya. To understand their adaptability potential at lower altitude, leaf morphology, biochemical characteristics and temperature response of photosynthesis was studied in their natural habitat (3600 m) and at a comparably lower altitude (550 m) above mean sea level (amsl). In both the species, leaf length, width, area, accumulation of Chlorophyll a (Chl a), Chl b, total Chl, Carotenoids (Car) and phosphoenolpyruvate carboxylase (PEPC) activity were lower whereas, leaf thickness, Chl a/b ratio and ribulose-1, 5-bisphosphate carboxylase/oxygenase (RuBPCO) activities was higher in high altitude grown plants as compared to their lower altitude grown counterparts. Altitudinal variation in temperature (T) response of photosynthesis varied with species. Optimum temperature (T_{opt}) for photosynthesis (P_N) was at 20 °C in high and at 25 °C in the plants grown at lower altitude in both the species. The level of P_N, however, varied with species and altitude. Mesophyll efficiency (ME) increased with T and observed higher at 550 m altitude in both the species. Similar to P_N, water use efficiency (WUE) increased with temperature up to a highest level and decreased at higher temperatures at both the altitudes, higher WUE was however, recorded in lower altitude grown plants of these species. Our study suggests that the shift in T_{opt} towards higher temperatures, higher ME together with higher WUE in these plants when grown at lower altitude, indicates the adaptability and cultivation potential of these species at comparatively lower altitudes.

Key Words: *Aconitum balfourii; Podophyllum hexandrum*; Altitude; Photosynthesis; Transpiration; Water use efficiency.

INTRODUCTION

Environmental factors like low and high temperature extremes, atmospheric pressure, precipitation, soil composition, wind speed, snow-cover, vegetation period, and the intensity of radiation under clear sky conditions greatly differ between low and high altitude sites. These factors play an important role in the distribution and adaptation of plant species to a particular altitude. It has been reported that high altitude plants fix more CO_2 per unit leaf area than the comparable plants from low altitude at any given partial pressure of CO_2 (Korner and Diemer, 1987). Further, a global comparison of carbon isotope discrimination in high mountains and low land plants indicates that this high efficiency of CO_2 utilization in high altitude plants is a general phenomenon (Korner *et al.*, 1988). In high altitude species $P_{N\ max}$ reach at lower temperatures as compared to the low

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elevation species when grown under uniform conditions (Korner and Diemer, 1987). Further, alpine vegetation has been shown to exhibit a higher amplitude of adaptation. The pattern of vegetation in alpine environment is a result of adaptations such as the ability of certain plant species to acclimate to a wide range of light and temperature conditions, combined with rapid metabolic changes. A shift in enzymes systems and T_{opt} for photosynthesis in high altitude plants grown at lower elevation has been reported (Mooney and West, 1964; Billings and Mooney, 1968).

In mountainous environment, vegetation and their plasticity to survive under changing environmental conditions change in short distance along with altitudinal gradient. The influence of altitude on plant growth and distribution has been studied for quite some time. Several morpho-physiological modifications have been found in plant species along with altitudinal gradient (Korner and Larcher, 1988; Lutz, 2011). Some of these physiological and morphological changes (growth form, plant hight, leaf area, stratification of phytomass, and phynological cycles) have been directly associated with altitudinal variation of microclimate. However, such modification by environmental factors associated with altitudinal gradient varies from species to species.

Aconitum balfourii (distribution 2200 – 4000 m altitude) and *Podophyllum hexandrum* (distribution 2000 – 4000 m altitude) are two high value endangered medicinal plant species from alpines of Central Himalaya (Khossoo, 1993). In recent past, due to ecological disturbances, unscientific and uncontrolled extraction for commercial purposes and increasing grazing pressure, various important herbs of high medicinal and other economic values growing at the high reaches of Central Himalayas are under immense pressure (Lata, 1997). As a result, many of them have already been declared threatened, rare or endangered (Nayar and Sastri, 1987, 1988 and 1990). *Aconitum balfourii* and *Podophyllum hexandrum* are two of them known for high medicinal values and included in a list of species requiring priority attention for conservation (Khossoo, 1993). Therefore, in order to meet the ever-growing demand of pharmaceutical industry, conservation of these species through cultivation is of commercial significance. Present investigation is aimed to study the adaptability potential of these two species to survive under higher temperature at lower elevation.

MATERIAL AND METHODS

Environmental Parameters

Environmental parameters such as photosynthetic Photon flux density (PPFD), air temperature and relative humidity concentration on the days of observations were recorded using an automatic weather station and data logger (*Campbell Scientific, Utah, USA*). Ambient CO_2 concentration was recorded using portable Photosynthesis system (*Model LI-6400, LI-COR Inc., Lincoln, NE, USA*)

Plants and Experimental Sites

Uniform seedlings of *Aconitum balfourii* and *Podophyllum hexandrum* were collected from their natural habitat at 3600 m altitude of Central Himalaya. These were transplanted in poly-bags of equal volume containing 1:2 mixture of farm yard manure and garden soil at alpine field station. After fifteen days, thirty bags of each species were placed to the experimental stations located at 3600 m amsl (30°14′N, 79°13′E) and 550 m amsl, (30°31′N, 78°48′E) in Central Himalayas.

Altitudinal Variations in Leaf Morphological Traits

Fully mature healthy leaves of each species at high and low altitudes were used for morphological studies *i.e.* leaf length (cm), width (cm), leaf area (cm^2) and leaf thickness (μM).

Gas and Water Vapour Exchange Studies at Two Different Altitudes

Gas exchange and water vapour exchange parameters in *Aconitum balfourii* and *Podophyllum hexandrum* were recorded next year during summer, after acclimatization of these species at different altitudes for one complete life cycle. As leaf emergence and active growth period of alpine plants occurs little earlier at lower altitude, recordings of data were first made on

the plants at lower altitude (550 m) and then at higher altitude (3600 m). At both the altitudes, observations were recorded on selected top four fully mature healthy leaves for each species in nine plants separately. Mean values were used in computation.

All the observations were made during morning hours using a closed portable photosynthetic system (Model LI-6400, LI-COR Inc., Lincoln, NE, USA) equipped with light, temperature, humidity and CO_2 controls. Fully expanded healthy leaves of these species were exposed to 10, 15, 20, 25 and 30 °C temperatures at photosynthetic photon flux densities (PPFD) of 2000 –mol $m^{-2}s^{-1}$ (this light intensity was found to be optimum) with the help of artificial light source (Model LI-6400-02; light emitting silicon diode; LI-COR), fixed on the top of the leaf chamber. Photosynthetically active radiation (PAR) was recorded with the help of quantum sensor kept in range of 660-675 nm wave radiations, fixed at the leaf level. Temperature of the cuvette was controlled by the integrated peltier coolers, which is commanded by the microprocessor. All the measurements were first recorded at lowest temperature (i.e. 10 °C) and subsequently at increasing temperatures. Relative humidity was kept nearly constant (60 ± 5%) throughout the experiment. Four gas exchange parameters viz., photosynthetic carbon dioxide assimilation rate (P_N), transpirational water loss (E), stomatal conductance for CO_2 (g_s) and intercellular CO_2 concentration (C_i) were measured simultaneously at steady state condition under various temperature conditions. Water use efficiency was worked out based on the data of transpiration and net assimilation rate. Water use efficiency (WUE) was calculated as the ratio of rate of P_N and E and a ratio of C_i and g_s (C_i/g_s) was used as an indicator of mesophyll efficiency (ME).

Altitudinal Variations in Leaf Biochemical Traits

After one-year acclimatization of these species at two different (3600 m and 550 m amsl) altitudes, fully mature top healthy four leaves from five separate plants of same age (55 ± 5 days old) as used for gas exchange measurements of each species were harvested for the comparison in foliar pigment contents (Chl a, Chl b, total Chl and Carotenoids), and enzyme assay of Ribulose-1, 5-bisphosphate Carboxylase/ Oxygenase (RuBPCO) and Phosphoenolpyruvate Carboxylase (PEPC).

Leaf Pigment Contents at Different Altitudes

Chlorophyll a, b and Carotenoids of leaves from four individual plants for each species were determined according to the method described by Hiscox and Israelstam Hiscox, and Israelstam (1979). Fifty mg leaf tissue was extracted in 10 ml of dimethyl sulfoxide solvent incubated at 55-60 °C for 4-5 h or till tissue became colorless. Absorbance was recorded at 663, 645 and 440.5 nm using Beckman Spectrophotometer (model DU-40). Pigment contents are expressed as mg g^{-1} fresh weight of tissue.

Leaf Enzymes at Different Altitudes

Enzyme assay of RuBPC and PEPC activities were monitored using the method of Seeman et al. (1985), modified by Dann and Pell (1989). After the gas exchange measurements, leaf samples harvested and frozen immediately in liquid nitrogen were used for the determination of the RuBPCO and PEPC activity. Leaf tissue (0.04g fresh weight) was ground in 4 mL of freshly prepared extraction buffer (CO_2-free distilled water was used for the preparation of extraction buffer) containing 0.1 M Tris-HCl (pH 7.8), 0.1 mM ethylenediamine tetraacetic acid (EDTA), 1.5% (W/V) polyvinylpyrrolidone (PVP), 5 mM β-mercaptoethanol and 1mM phenylmethylsulfonyl fluoride (PMSF) at 0 – 4 °C. The extract was centrifuged at 15,850 g for 20 min at 4 °C. The supernatant was used for rubisco and PEP carboxylase activities.

Total RuBPCO activity (fully activated) was determined by mixing 900 mmL supernatant with 100 mmL of activation buffer (100 mM $NaHCO_3$ and 200 mM $MgCl_2$) and allowed to incubate at room temperature for 10 min. Fifty mmL of incubation mixture was mixed with 450 mmL of assay buffer containing 0.1 M Tris-HCl (pH 8.0), 20 mM $MgCl_2$, 1m M EDTA and 13 m M $NaHC^{14}O_3$ (2.6 μ Ci per vial) in a vial. The reaction was initiated by adding 0.5 mM RUBP and was terminated after 30s by adding

100 mmL of 2 N HCl. The entire solution was evaporated to dryness in an oven at 70-80^{O}C. The residue was resuspended in 10 mL of a cocktail solution containing 2 g of 2, 5-diphenyl oxazole (PPO), 50 mg (1, 4 - bis- [2-(5-phenyloxazolyl)]-benzene (POPOP) and 10 mL toluene. The acid-stable ^{14}C radioactivity was determined by liquid scintillation counter (Beckman LS-6500, USA). Reaction buffers were degassed prior to use with the help of a vacuum pump.

PEPC Activity was determined by using 50 µL of incubated mixture, mixed with 450 µL of assay buffer containing 0.1 M Tris-HCl (pH 8.0), 20 mM $MgCl_2$, 1 mM EDTA, 13 mM $NaHC^{14}O3$ (2.6 mm Ci per vial) in a vial. The reaction was started by adding 0.5 mM PEP and was terminated after 30s by adding 200 mmL of 2N HCl. The entire solution was evaporated to dryness in an oven at 70-80^{O}C. The residue was re-suspended in 10 mL of a cocktail solution containing 2 g PPO, 50 mg POPOP and 10 mL toluene. The radioactivity (^{14}C activity) was measured with a liquid scintillation counter (Beckman LS-6500, USA).

Foliar Nutrients Content at Two Different Altitude

For the determination of leaf nutrients *i.e*. nitrogen (N), phosphorus (P), potassium (K) and carbon (C), leaves were collected and oven dried at 80^{O} for 48 h. The dried leaves were ground to yield a fine powder, which was treated with a digestion mixture (0.42 g selenium powder, 14 g lithium sulfate, 350 ml of 30% hydrogen peroxide, and all were mixed well). To this mixture, 420 ml of concentrated sulfuric acid was added slowly while cooling in an ice bath. The mixture was stored at 2 ^{O}C and remained stable for four weeks.

Five ml of digestion mixture was added to each 50 mg leaf powder and the samples were digested in a fumehood chamber. The digestion in fumehood chamber was continued till the solution became colorless and that remained sandwhite. After digestion, samples were allowed to cool for half an hour, and 25 ml distilled water was then added and mixed well till all the sediments dissolved in water. Clear digested solution was used for the estimation of nitrogen, phosphorus, potassium and organic carbon **(**Okalebo *et al*., 1993).

Statistical Analysis

Pearson's correlation and regression analysis were performed to assess the relation between studied traits using SYSTAT software package (SYSTAT Inc. Evanston, IL). The source of variation was low and high altitude location. The differences for all comparable morphological, biochemical and gas exchange parameters were calculated at the significance level of $p<0.05$.

RESULTS AND DISSCUSSION

The major aim of the study was to find out the effect of altitude on the photosynthetic characteristics of these species in a distinct range of temperature at their natural habitat (3600 m) with plants grown at comparatively lower altitude (550 m). Diurnal variations in environmental parameters at two different altitudes during the course of study are shown in Figure 1. Leaf length, leaf width and leaf area in both of the species were found higher at lower altitude whereas, leaf thickness was higher at higher altitude. Except leaf width of *A. balfourii* and leaf area of *P. hexandrum* the variation in morphological parameters (leaf length, width, area and thickness) in these two species at two different altitudes was observed statistically significant (Table 1). Billings and Mooney (1968) have documented the small structure of alpine species as compared to plants from lower altitudes. In a transplantation experiment, Korner et al. (1989) have also reported larger and thinner leaves of *Arbis alpina*, *Geum montanum*, *Geum reptans*, *Linaria alpina* and *Oxirria digyna* at lower altitude (600 m) than their natural habitat (2000 to 3200 m).

Except N, all other nutrient contents (P, K and C) were recorded lower at high altitude grown plants of both the species (Table 2). Similar to Korner 1989, our data shows comparative higher nitrogen (%) content in the leaves at higher altitude grown plants as compared to plants grown at lower altitude however, these differences were not found statistically significant. Overall, in both the species, Chl a, Chl b, total Chl and carotenoids contents were

Table 1: Average values of leaf length (cm ± SD), leaf width (cm ± SD), leaf area (cm^2 ± SD) and leaf thickness (µm ± SD) in the species at grown at natural habitat (3600 m AMSL) and lower altitude (550 m AMSL) in Central Himalayas (*n* = 20).

Parameters	*Species*	*Altitude (m)*		
		3600	*550*	*LSD, p < 0.05*
Leaf length	*A. balfourii*	4.88 ± 0.22	6.82 ± 0.42	1.10
	P. hexandrum	10.29 ± 0.26	12.98 ± 0.91	1.21
	LSD, p < 0.05	2.58	1.24	
Leaf width	*A. balfourii*	2. 67 ± 0.12	2.76 ± 0.3	0.94
	A.hexandrum	8.98 ± 0.27	12.12 ± 0.8	1.31
	LSD, p < 0.05	2.33	1.85	
Leaf area	A.balfourii	23.22 ± 3.78	29.44 ± 3.25	5.43
	P.hexandrum	57.32 ± 6.25	69.24 ± 6.98	9.00
	LSD, p < 0.05	2.83	2.02	
Leaf thickness	*A.balfourii*	397.45 ± 21.22.	352.41 ± 23.29	22.87
	P.hexandrum	423.76 ± 18.32	377.23 ± 14.89	23.37
	LSD, p < 0.05	38.21	32.76	

Table 2: Variations in Chl a (mg g^{-1} fresh weight), Chl b (mg g^{-1} fresh weight), total Chl (mg g^{-1} fresh weight), Chl a/b ratio, carotenoids (mg g^{-1} fresh weight), Total Rubisco activity (nmol CO_2 mg^{-1} fresh weight min^{-1}) and PEP Carboxylase activity (nmol CO_2 mg^{-1} fresh weight min^{-1}) in the species grown at natural habitat (3600 m elevation) and lower altitude (550 m elevation) in central Himalayas (*n*=15)

Parameters	*Species*	*Altitude (m)*		*LSD, p < 0.05*
		3600	*550*	
Chl a	A.balfourii	1.03 ± 0.21	1.55 ± 0.14	0.65
	P.hexandrum	1.24 ± 0.34	1.70 ± 0.36	0.53
	LSD, p < 0.05	0.34	0.55	
Chl b	A.balfourii	0.21 ± 0.05	0.47 ± 0.06	0.11
	P.hexandrum	0.29 ± 0.07	0.52 ± 0.08	0.49
	LSD, p < 0.05	0.13	0.28.	
Total Chl	A.balfourii	1.34 ± 0.41	2.11 ± 0.20	0.68
	P.hexandrum	1.55 ± 0.28	2.37 ± 0.34	0.65
	LSD, p < 0.05	0.43	0.52	
Chl a/b ratio	A.balfourii	4.27 ± 0.53	3.48 ± 0.36	0.95
	P.hexandrum	4.13 ± 0.49	3.34 ± 0.42	0.96
	LSD, p < 0.05	0.67	0.49	
Carotenoids	A. balfourii	0.12 ± 0.04	0.18 ± 0.04	0.09
	P. hexandrum	0.13 ± 0.06	0.16 ± 0.07	0.14
	LSD, p < 0.05	0.07	0.12	
Total Rubisco	A. balfourii	8.23 ± 0.98	7.04 ± 0.79	1.24
	P. hexandrum	12.62 ± 1.24	10.84 ± 0.95	1.39
	LSD, p < 0.05	1.22	1.69	
PEP Carboxylase	A. balfourii	6.36 ± 0.32	9.50 ± 0.27	1.42
	P. hexandrum	8.48 ± 0.87	12.42 ± 0.70	1.16
	LSD, p < 0.05	1.54	1.67	

lower and Chl a/b ratio was higher at higher altitude (Table 2). Our results are in agreement with the Todaria (1990), who has reported a decrease in Chlorophyll and carotenoids and an increases in Chl a/b ratio with increasing elevation. However, these variations were not observed statistically significant except, for Chl b in *A. balfourii* and total Chl in both the species. An increase in soluble protein and RuBP carboxylase and a decrease in PEP carboxylase activity was observed with altitude however, the altitudinal differences were only significant for RuBP (LSD = 1.24 at $p < 0.05$ for *A balfourii*, $p < 1.39$ for *P. hexandrum*) and PEP carboxylase (LSD = 1.42 at $p < 0.05$ *A. balfourii*, and LSD = 0.14 at $p < 0.05$ for *P. hexandrum*) activity in both the species.

Altitudinal variations in P_N, E, g_S, C_i/g_S and WUE of these species at two different altitudes are shown in figures 2, 3,4 and 5, respectively. The temperature optima for photosynthesis in *A. balfourii* was observed around 20 ^{O}C at both the altitudes. Whereas, it was observed at 20 ^{O}C in alpine plants of *P. hexandrum* and around 25 ^{O}C in the plants grown at lower altitude. Similar results have been reported by Rawat (1989) and Rawat and Purohit (1991) in *Rheum moorcroftianum* from Central Himalayas. In a publication Korner and Dimmer (1987) have reported variability in optimum temperatures for photosynthesis in some alpine plants from Austrian Alps. The average optimum temperature for these plants comes around 21 ^{O}C which is close to the values we obtained in alpine species reported here. On other hand, relatively high temperature optima for photosynthesis in some alpine plants from Europe and North America have been reported (Mooney and Billings, 1961 and Korner 1982). The differences could be due to the original habitats of the plants or different genetic makeup of the species in these areas. There are reports indicating shifting of the temperature optima for the photosynthesis towards higher temperature when plants of same species were grown at lower altitude (Mooney and West, 964) and also with respect to prevailing growth temperatures under controlled conditions (Billings and Mooney, 1968 and Chabot and Chabot, 1977). Overall, according to statistical analysis the variations in P_N in *A. balfourii* and *P. hexandrum* at different temperature (10 to 30 ^{O}C) at two different altitude were not found significant (F-ratio = 0.014, df = 1, $p < 0.09$). These results agree with Rawat and Purohit (1991), who reported virtually no differences in the rate of photosynthesis in *R. emodi* at similar altitudinal difference.

Conductance and therefore transpiration in both the species (*A. balfourii* and *P. hexandrum*) was higher at high altitude (3600m) as compared to lower altitude (550m) under the different temperature. In the young leaves of adult trees of *Eucalyptus pauciflora* Kornar and Cchrane (1985) have also reported higher leaf diffusive conductance for water vapour and therefore, higher transpiration in leaves of plants from higher elevation as compared to those from lower elevation. However, it was shown that sharply decreasing humidity deficit with increasing altitude lessened the expected large differences in transpiration rates per unit leaf area along the altitudinal transect. Our results further confirm earlier reports in which plants from higher altitude exhibit higher stomatal conductance as well as higher rate of transpiration (Rawat and Purohit, 1991 and Singh and Purohit, 1997). However, the effect of altitude on the stomatal conductance of both the species (*A. balfourii*, $p < 0.98$ and *P. hexandrum*, $p < 0.16$) were statistically insignificant. Effect of altitude on transpiration was significant in *A. balfourii* ($p < 0.03$) whereas, it was statistically insignificant in *P. hexandrum* ($p < 0.06$). Rawat and Purohit (1991) have also reported no specific changes in conductance as well as in transpiration of *Rheum moorcroftianum* grown at two altitudes of Central Himalaya.

According to our results, effect of altitude on Ci, Ci/Ca and WUE of these two species was also observed statistically insignificant. However, the effect on Ci/gs ratio, which reflects mesophyll efficiency of plants was statistically significant for both the species *A. balfourii* (F-ratio = 44.34, df = 1, $p < 0.001$) and *P. hexandrum* (F-ratio = 23.60, df = 1, $p < 0.001$). The mesophyll efficiency was observed higher in lower altitude grown plants for both the species.

Overall, the results from a physiological point of view, suggest that cultivation of these herbs as a crop can be easily done at lower altitude. However, as roots and rhizomes are

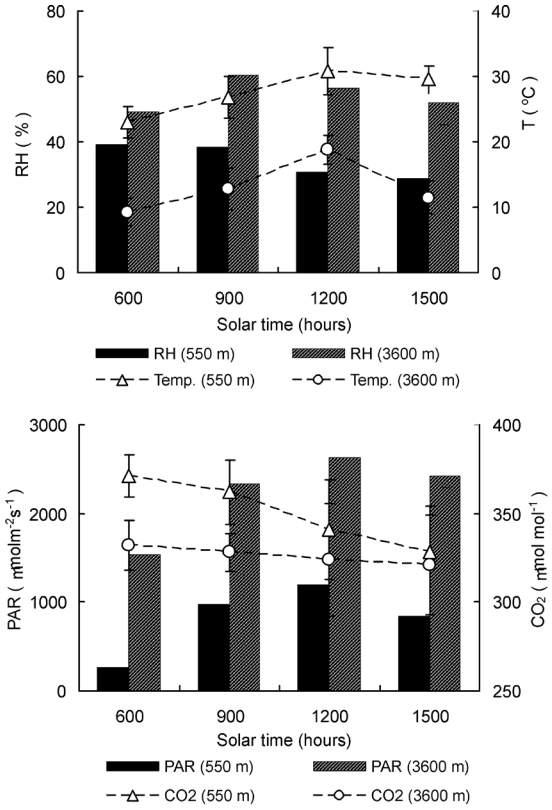

Figure 1: Diurnal variations in environmental parameters under field conditions at 3600 m and 550 m altitudes. RH: Relative Humidity, Temp.: Temperature, PAR: Photosynthetically Active Radiation, CO_2: Ambient CO_2 Concentration.

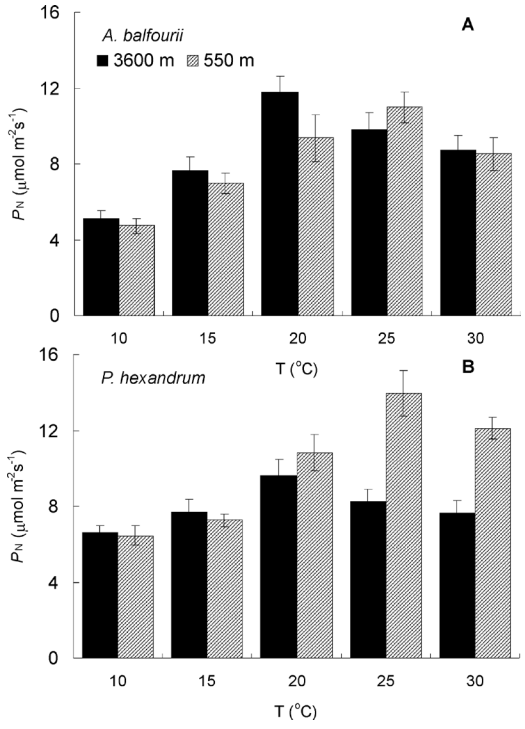

Figure 2: Temperature (T) response of photosynthesis (P_N) in *A. balfourii* (A) and *P. hexandrum* (B) at two different altitudes

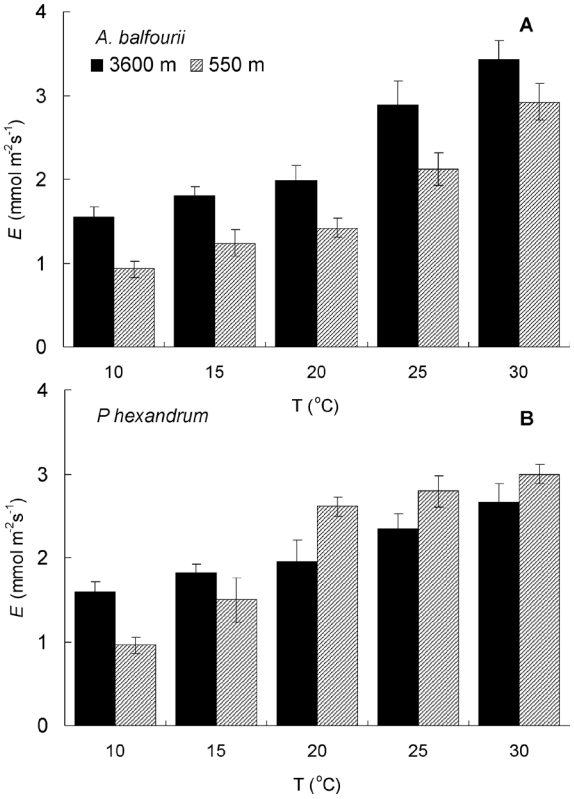

Figure 3: Variations in temperature (T) response of Transpiration (*E*) in *A. balfourii* (A) and *P. hexandrum* (B) at high and low altitudes

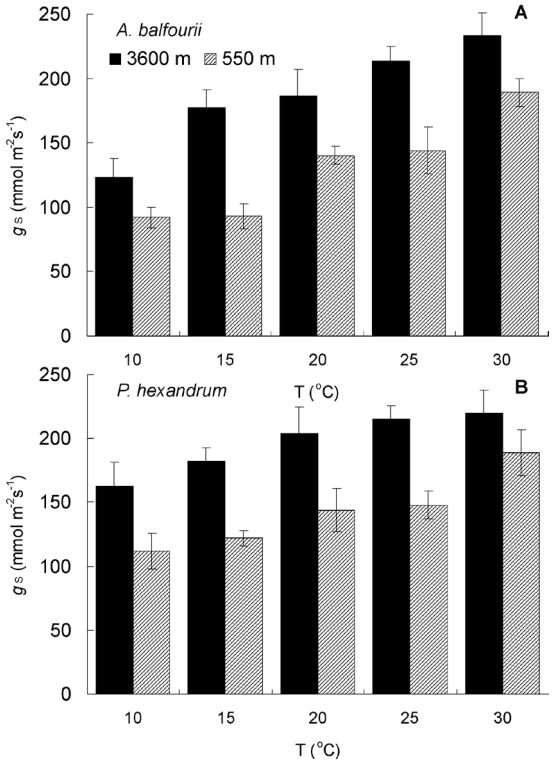

Figure 4: Variations in stomatal conductance (g_s) in *A. balfourii* (A) and *P. hexandrum* (B) at high and low altitudes under different temperature (T) conditions.

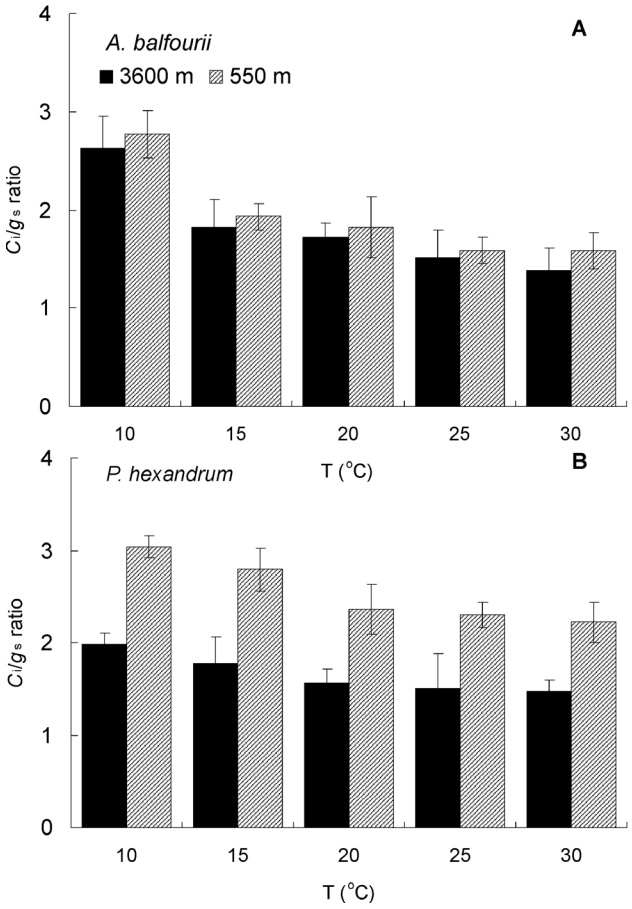

Figure 5: C_i/g_s ratio in *A. balfourii* (A) and *P. hexandrum* (B) at high and low altitudes

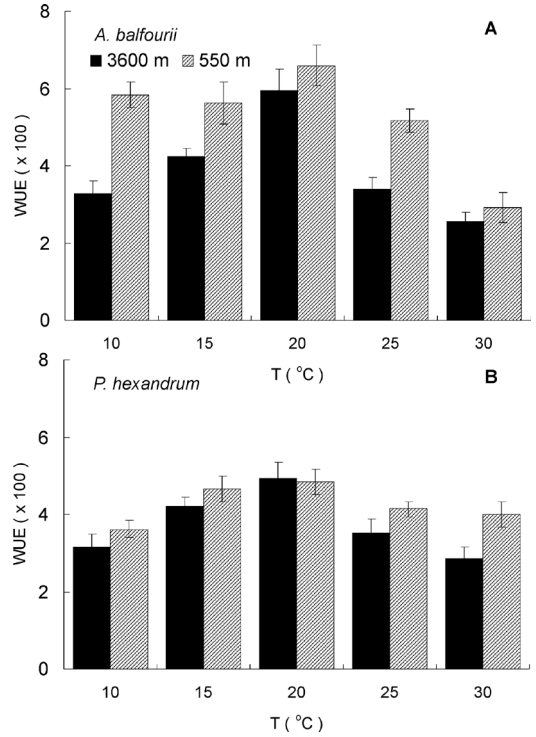

Figure 6: Variations in water use efficiency (WUE) in *A. balfourii* (A) and *P. hexandrum* (B) at two different altitudes under variable temperature (T) conditions

more exploitable parts in these species, the studies on translocation of useful metabolites to the underground part should also be taken in consideration before making the final recommendations in this regard.

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