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ALTITUDINAL VARIATION IN THE CONTENT OF PROTEIN, PROLINE, SUGAR AND ABSCISIC ACID (ABA) IN THE ALPINE HERBS FROM HUNZA VALLEY, PAKISTAN

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Abstract

Leaves of four herbaceous alpine plant species were collected during summer, 2002 from two different altitudes viz., 3,000–3,500 m a.s.l. from the east-facing slope of Ultar pasture of Hunza valley, Pakistan to determine the protein, proline, sugar and abscisic acid (ABA) contents of leaves. The average maximum temperature recorded during the months of July and August varied from 22ºC to 24ºC and minimum temperatures varied between 11ºC to 13ºC, whereas average maximum humidity varied from 39-35% and minimum varied from 19 to 21% respectively. Soils of Ultar are acidic.

At the altitude of 3,000 m a.s.l. *Galium aparine* L. showed the maximum endogenous ABA; *Onobrychis dealbata* Stocks., showed the highest sugar and protein content, whereas *Polygonum alpinum* All., exhibited maximum proline. All the plant species showed a general trend for increased accumulation of protein, sugar, proline and free endogenous ABA in leaves at high altitude. The extent of increase appears to be determined by the basal level of the above mentioned biochemical contents and is necessary for survival of that species at high altitude.

Introduction

Cold acclimation is associated with several physiological and biochemical alterations in the plants. The best-characterized changes include alterations in gene expression, changes in hormone levels, increases in soluble sugars, amino acids and organic acids, accumulation of osmoprotectants and protective proteins as well as modification of membrane lipid composition (Hughes & Dunn, 1996; Palva & Heino, 1998; Thomashow, 1999). Both genetic and biochemical studies established that ABA plays an essential role in stress responses serving as a signal, which triggers processes leading to increased frost tolerance (Abromeit *et al*., 1992; Sarnighausen, 1994; Leung & Giraudat, 1998; Mahajan & Tuteja, 2005).

The exposure of chilling sensitive plants to low temperatures raises the endogenous level of ABA (Rikin, 1976; Daie & Campbell, 1981; Eamus & Wilson, 1983; Doerffling, 1998). The soluble carbohydrates increase from fall to winter and decrease in spring as they deharden (Levitt, 1980; Alden & Hermann, 1971; Tumanov, 1979; Siminovitch, 1981). The study of localization of sugar in cells of hardy plants is of great importance in order to explain their role at low temperature and to understand the mechanism of their protective effect (Heber, 1959). Deharden proteins are induced in plants by dehydration related environmental stresses such as low temperature, drought or high salinity (Close, 1996; Borovskii *et al*., 2000).

The objective of the present investigation was to investigate the changes in endogenous hormone, abscisic acid, sugar and proline in plants with respect to variation in altitude in Hunza valley and constitute the first report of that area.

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Materials and Methods

Study area: The research was conducted in Hunza Valley. The area is localized (36.38 North, 73.34 East) in the northern part of Pakistan. Ultar is a multi species grazing area, where the inhabitants of Karimabad and adjoining villages take their cattle for grazing by mid of May till mid of September. This pasture area is located north of Karimabad, Hunza. There is practically only one slope that is the eastern slope where vascular plants grow. The other slopes are very steep and rocky.

Collection of plant samples: Plant leaves were collected during summer, 2002 from the Ultar pastures, Hunza Valley from two different altitudes viz., 3,000 m a.s.l. to 3,500 m a.s.l. for the analyses of protein, proline, sugar and ABA content of leaves. The fully expanded actively synthesizing green leaves of each species were collected randomly from 3-5 plants and stored at –80°C till further analysis.

Extraction of leaf tissue for protein: The leaves were analyzed for protein content following the method of Lowry *et al*., (1951).

Extraction of leaf tissue for proline: Proline content was measured according to the method of Bates *et al*., (1973).

Extraction of leaf tissue for sugar: The leaves were analyzed for sugar content following the method of Dubo *et al*., (1956) as modified by Johnson *et al*., (1966) using glucose as standard.

Extraction of leaf tissue for ABA: The leaves were extracted and purified for endogenous ABA following the method of Kettner & Doerffling (1995).

The freeze-dried plant leaves (0.50 g) were homogenized in 80% methanol supplemented with 10 mg L^{-1} butylated hydroxytoluene. The plant extract was left for 72 h at 4ºC in 80% methanol with concomitant change in the solvent at each 24 h. The extract was centrifuged at 3,000 g for 10 min., and the supernatant was evaporated to dryness on RFE at 35ºC.

The extract was concentrated to an aqueous residue (30 mL) and adjusted to pH 2.5 with 2 N H_2SO_4, and extracted with EtOAc (3×10 mL). The aqueous residue was discarded, and the EtOAc extracts were combined and evaporated to dryness on RFE at 35ºC and dissolved in methanol. The samples were passed through a 0.45-µm polytetrafluoroethylene disposable filter. The sample was injected onto a C_{18} column and eluted with a linear gradient of methanol (10–70%), containing 0.01% acetic acid, at a flow rate of 4 ml/min. The retention time of ABA was determined by using authentic standards (Sigma Chemical Company), at 210 nm.

Physico-chemical characteristics of soil: At each elevation, a soil sample from 20 cm depth was taken with a stainless steel auger. The samples collected randomly from 8 sites, were pooled together, stored in plastic bags, sealed and labeled. Soil samples were dried in an air-forced oven at 40° C. The dried samples were sieved out to remove stones and plant residues and were ground in a stainless steel mill and passed through a 2-mm sieve. The sieved soils were collected and stored till further analyses.

Table 1. Analysis of soil samples of Ultar pasture, Hunza valley collected during summer, 2002.

Altitude (m) a.s.l	pH	EC μS/cm	Macro & micro nutrients (ppm)							
			Na	NO_3^--N	P	K	Ca	Mg	Mn	Fe
2500 ↓ 3500	6.3	179	4	1.3	7.07	129	274	81	6	6

Results and Discussion

The average maximum temperature recorded during the months of July and August varied from 22ºC to 24ºC and minimum temperatures varied between 11ºC to 13ºC, whereas average maximum humidity varied from 39-35% and minimum varied from 19 to 21% respectively. Table 1 shows the variation in physico-chemical characteristics of soil in the study area. Soils of Ultar are acidic. Values for NO^-_3 – N, K, P and Mn were observed to be considerably higher at Ultar.

Cold acclimation is a complex process involving a number of biochemical and physiological changes, associated with the accumulation of sugars, several types of proteins, lipids, abscisic acid and other products of altered metabolism (Pinedo *et al.,* 2000; Szalai *et al*., 2000; Atici *et al*., 2003; Nagao *et al*., 2005). A trend of increasing freezing resistance with increasing altitude was found in seeds of *Eucalyptus pauciflora* (Pryor, 1956). Similar results were obtained by Sakai & Wardle (1978) and Alberti *et al*., (1985). Hardiness increased with increasing altitude in *Abies sachalinensis* (Eiga & Sakai, 1984). The species specific variation in protein, proline, sugar and ABA content is represented at 3000 m a.s.l. taking as control. Considering the measurement at 3000 m a.s.l. the leaf protein content (Fig. 2) was found to be maximum in *Onobrychis dealbata* while *Thymus serpyllum* showed the minimum protein content.

The proline content (Fig. 3) was found to be maximum in leaves of *Polygonum alpinum*, while *Galium aparine* exhibited minimum proline content. The sugar content (Fig. 4) was higher in leaves of *Onobrychis dealbata*, while *Galium aparine* showed minimum sugar content. *Galium aparine* showed the highest endogenous level of ABA (Fig. 5) whereas; *Onobrychis dealbata* had lowest level of endogenous ABA. With the increase in altitude from 3000 m a.s.l. to 3500 m a.s.l. all the plant species showed increase in protein, proline sugar and for endogenous ABA content. However the magnitude of increase for each biochemical content differs substantially with species.

With increase in altitude for 500 from previous, *Thymus* sp., having minimum protein showed maximum increase, whereas, *Onobrychis* sp., having maximum protein at 3000 m a.s.l. showed least increase. Similarly, the *Polygonum* sp., which did not show maximum sugar content in leaves exhibited maximum increase in sugar content as compared to *Thymus* sp., which showed maximum increase in leaf sugar with the increase in altitude.

In case of proline and ABA the accumulation was further augmented with the increase in altitude for 500 m in those species which had high basal level of proline and ABA; for example *Polygonum* sp., having maximum proline at low altitude of 300 m showed further increase in proline content whereas, *Gallium* sp., having minimum leaf proline at low altitude showed least increase in leaf proline with the increase in altitude. *Gallium* sp., with maximum ABA content among the species at low altitude showed further increase in ABA and *Onobrychis* sp., having minimum ABA showed least increase.

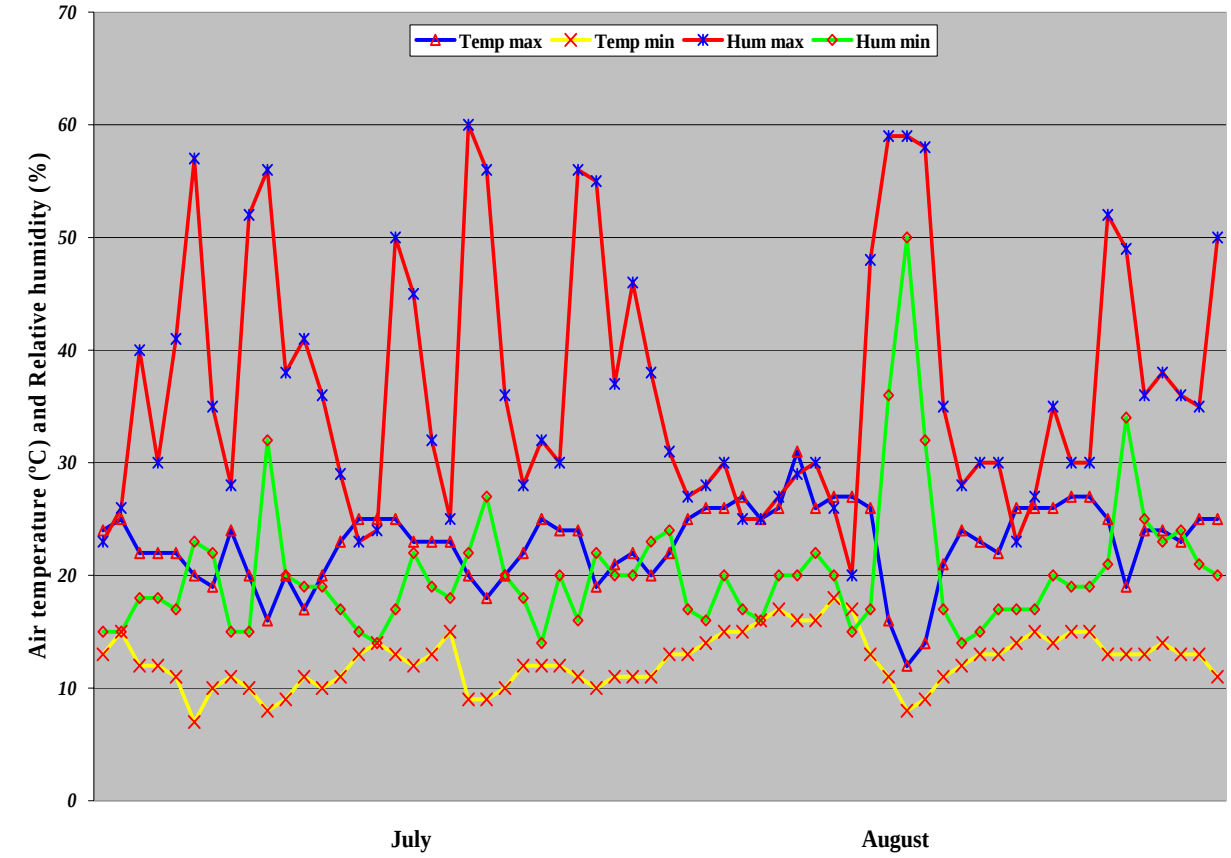

Fig. 1. Data showing the records of Relative humidity (%) and Air temperature (ºC) at Ultar during July- August, 2002.

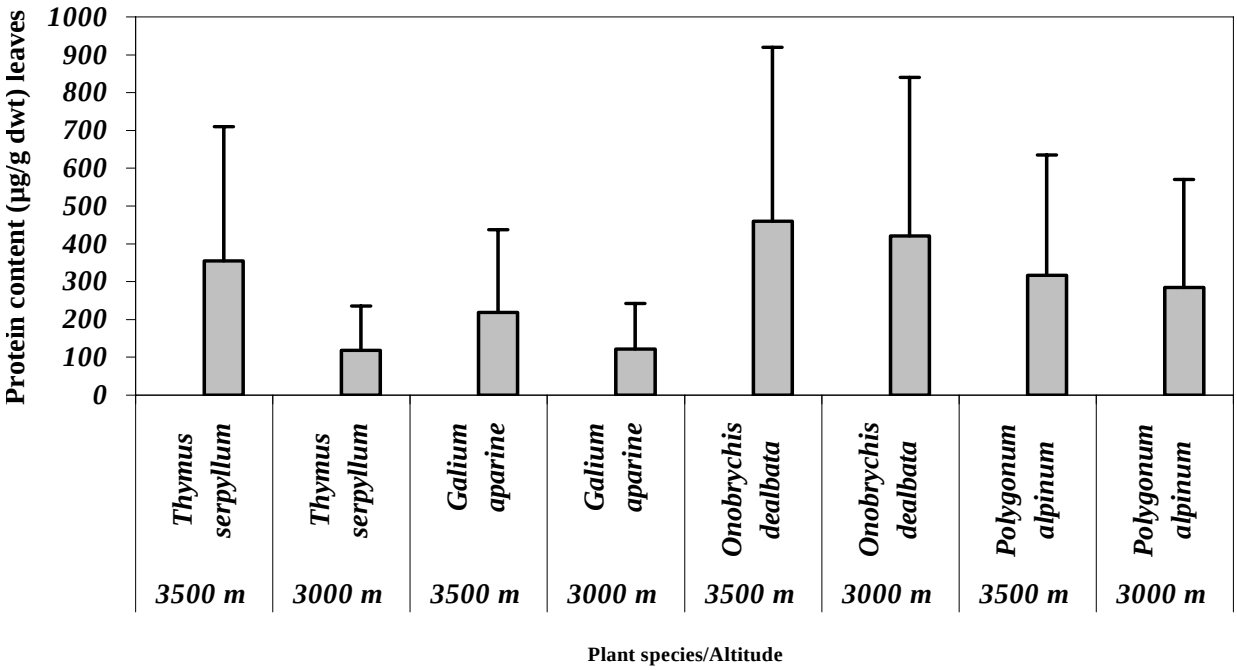

Fig. 2. Protein content (µg/g dwt) in leaves of plant species collected from two different altitudes of Ultar pastures. The plant samples were collected at flowering stage at altitudes ranging from 3,000 m a.s.l. to 3,500 m a.s.l. during summer season. Bars indicate SE.

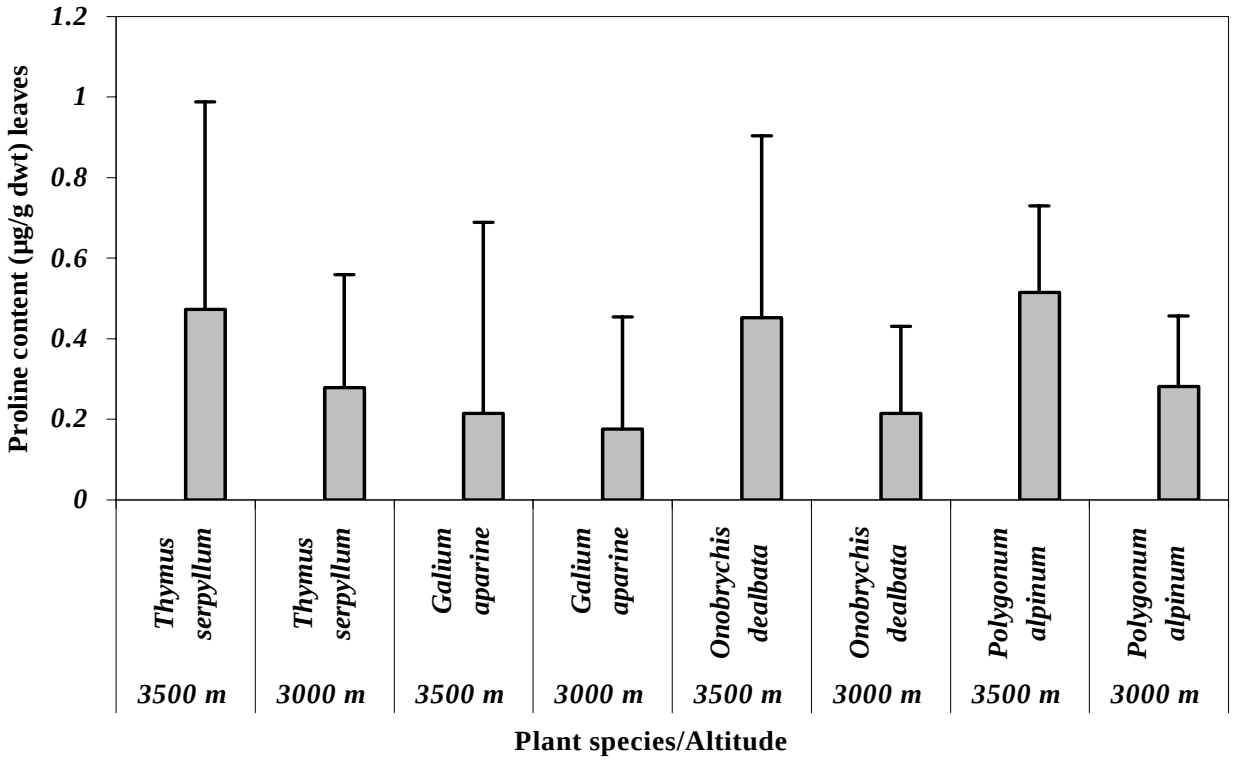

Fig. 3. Proline content (µg/g dwt) in leaves of plant species collected from two different altitudes of Ultar pastures. The plant samples were collected at flowering stage at altitudes ranging from 3,000 m a.s.l. to 3,500 m a.s.l. during summer season. Bars indicate SE.

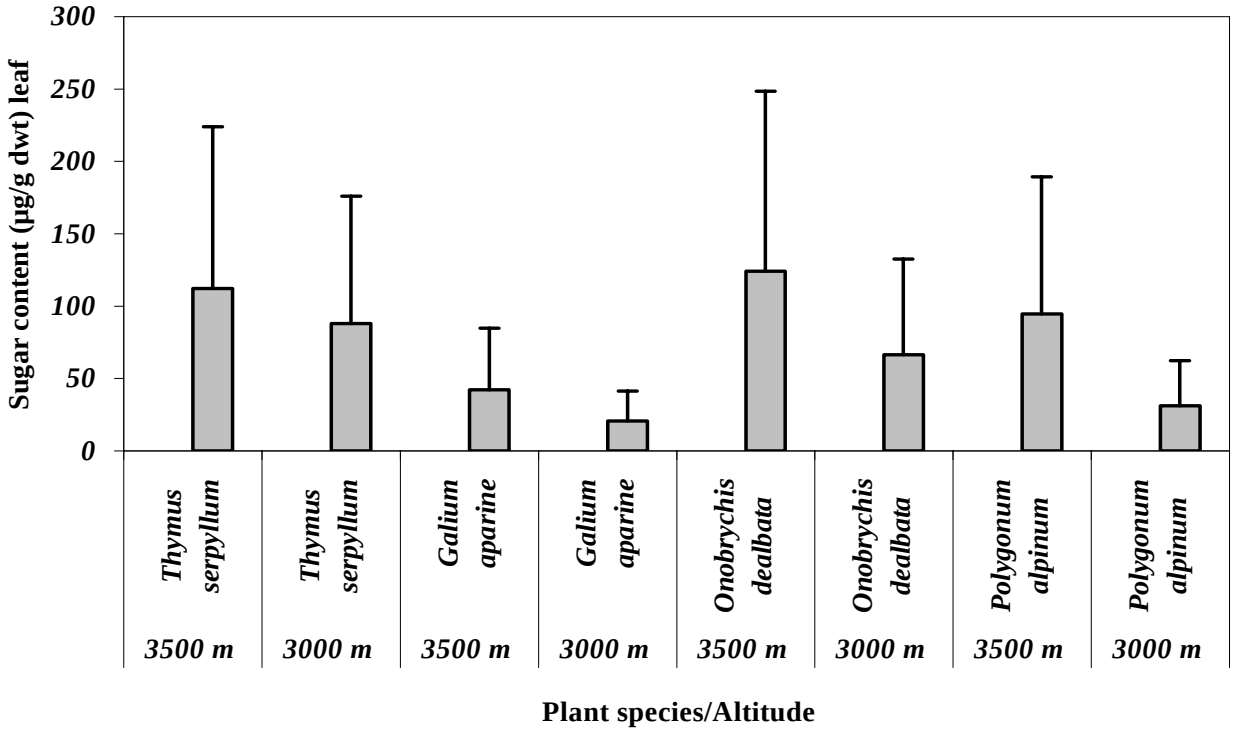

Fig. 4. Sugar content (µg/g dwt) in leaves of plant species collected from two different altitudes of Ultar pastures. The plant samples were collected at flowering stage at altitudes ranging from 3,000 m a.s.l. to 3,500 m a.s.l. during summer season. Bars indicate SE.

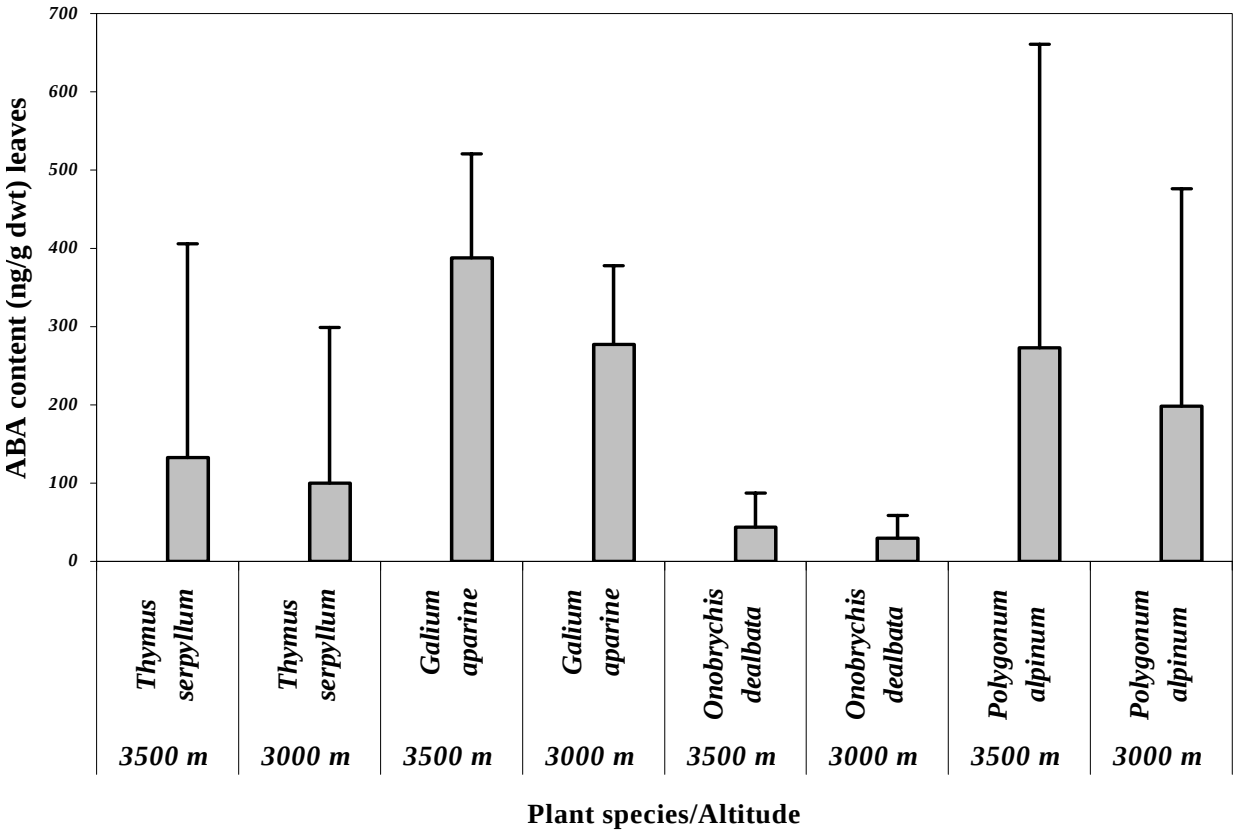

Fig. 5. Endogenous ABA content (ng/g dwt) in leaves of plant species collected from two different altitudes of Ultar pastures. The plants were collected at flowering stage at altitudes ranging from 3,000 m a.s.l. to 3,500 m a.s.l. during summer season. Bars indicate SE.

The presence of anti-freeze proteins is one of the mechanisms of plants to tolerate low temperatures. They have been found in wheat, barley, rye, alfalfa (Hon *et al*., 1994; Antikainen, *et al*., 1996; Antikainen & Griffith, 1997) and carrots (Worralld, *et al.,* 1998, Smallwood, *et al*., 1999). Seasonal variation in protein quantity and quality has also been studied in several woody plant species. Soluble protein concentrations of pine needles increase during cold acclimation (Pomeroy & Siminovitch, 1970; Nasholm & Ericsson, 1990; Nozzolillo, *et al*., 1990). Transgenic tobacco plants accumulating high levels of proline, or glycine betaine exhibited tolerance to cold temperature (Konstantinova *et al*., 2002; Parvanova *et al*., 2004) and a peak in proline content of a winter wheat was observed after 3-week hardening period (Doerffling *et al*., 1990). In winter wheat the hydroxyproline-resistant lines were found significantly more frost tolerant than wild-type (Doerffling *et al*., 1993). Significant positive correlations between proline level and frost tolerance have been found in a broad spectrum of genotypes (Galiba, 1994).

Among the several protection mechanisms against low temperatures adaptability at high altitude increased sugar content acting as osmoregulant have been reported (Korner, 1999). Sucrose and other sugars play a central role as signalling molecules that modulate the physiology, metabolism and development of plants (Koch 1996, Coruzzi & Zhou, 2001; Arroyo *et al*., 2003). A combination of both stress associated proteins and sucrose are reported to confer stress protection (Robertson, 1994). It has also been extensively reported by research workers that many plant species exhibit an increase in endogenous ABA concentrations when exposed to low temperature (Irving, 1969; Chen & Gusta, 1983) and that exogenous ABA can increase cold tolerance by 6 to 23ºC (Keith & Mckersie, 1986; Xin & Li, 1992) and increase in ABA levels as an early response to cold

hardening temperatures (Cowan *et al*., 1997). ABA content under chilling conditions is probably directly affected by the temperature (Zhang & Li, 1986).

Chilling-sensitive species including maize have been shown to exhibit increased levels of ABA when exposed to low temperature (Capell & Doerffling, 1993; Janowiak & Doerffling, 1996; Janowiak, 2003). ABA level has been shown to increase under conditions that lead to increased freezing tolerance both in woody plants (Li, 2002) and in herbaceous species (Chen *et al*., 1983; Lang *et al*., 1994; Faltusova *et al*., 2002; Li *et al*., 2005).

It is possible that proline and ABA have more intimate association with survival adaptability of plants at high altitude. Whereas, sugar and protein content have species specific variation, determined by the plasticity of individual, the critical basal level of sugar and protein at low altitude and the sensitivity of a plant to respond to cold stress. From the results it appears that there is a certain critical level of sugar and protein to be attained at high altitude. Profile of saturated and unsaturated lipids and the electrolyte leakage data are required for future investigations.

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