Research Journal of

Medicinal Plant

ISSN 1819-3455

Academic
Journals Inc.

www.academicjournals.com

Research Journal of Medicinal Plant 5 (3): 302-311, 2011
ISSN 1819-3455 / DOI: 10.3923/rjmp.2011.302.311
© 2011 Academic Journals Inc.

Altitudinal Variation in Morpho-physiological Attributes in *Plantago major* : Selection of Suitable Cultivation Site

[1]Vinay Prakash, [2]Hemlata Bisht and [3]P. Prasad
[1]Department of Seed Science and Technology, Chauras Campus,
[2]Department of Botany and Microbiology, Chauras Campus,
[3]High Altitude Plant Physiology Research Centre, HNB Garhwal University, Srinagar Garhwal, Uttarakhand 246 174, India

Corresponding Author: Dr. Vinay Prakash, Department of Seed Science and Technology, Chauras Campus, HNB Garhwal University, Srinagar Garhwal, Uttarakhand 246 174, India

ABSTRACT

Plantago major is an important medicinal herb of temperate Himalayan region and having high market demand, which is fulfilled by the exploitation of the plant from nature. To restrict the exploitation from the nature, there is need to cultivate the plant in suitable and accessible sites. The studies were conducted to identify the most suitable cultivation sites with an aim to produce high yield and potency of the plants. For this purpose three altitudinal (550, 1800 and 2500 m asl) sites were selected and the morphological and biochemical variations were analyzed to find out the best suited cultivation sites of *P. major* without loosing the medicinal potential of the plant. Morphological and biochemical variation, showed a definite trend and adaptive feature along with altitudinal gradient. Percent dry matter and morphological attributes, i.e., plant height, root length, leaf length, petiole length, spike length were performed well at 1800 m asl., followed by 550 m and lowest at 2500 m altitude. Chlorophyll and carotenoid showed decreasing trend as altitude increased, the amount of soluble protein and FAA were high at 1800 and 2500 m altitude, respectively. Peroxidase and ascorbate peroxidase enzymes which were required for adaptation found in high concentration at 1800 m during acclimatization. These findings reflect that the 1800 m is the best suited altitude for commercial cultivation of this species and could not be economically viable at much higher and much lower altitude.

Key words: Altitude, stress, acclimatization, adaptation, antioxidant

INTRODUCTION

Out of the ca. 17,500 flowering plant species found in India, over 1600 are used in traditional medicinal system (BSI, 1993)) and 1500 have already come under the various categories of threatened plants (Rao, 1994). Majority of the plants used by the drug industries are harvested from the wild especially from high altitudes, this has led to the depletion of resources and extinction of some of the species. Collection from the wild also creates problems like possibility of adulteration, incorrect identification and non-availability of material throughout the year (Nautiyal and Purohit, 2000). These unwanted happenings can only be restricted through the conservation of medicinally important plant which is necessary to the continued existence of particular species. In the present scenario the cultivation practices of these highly demandable species is the most promising approach to conserve the species as well as to meet the demand of the day.

Plantago major (family Plantaginaceae) is one of the valuable medicinal herbs of temperate alpine Himalayan region of market demand but yet not be taken under consideration for conservation. *Plantago major* is perennial herb with erect, stout root stock, leaves are radial, ovate or oblong entire or toothed, 8-20 cm long and 4-7 cm broad, small flowers, green, crowded or scattered in long slender rather lax spikes. The plant is considered homeostatic and wound healing in burns and inflammation of tissues. In homeopathy, it is used in disorders of the epidermis and in headache, earache and toothache. The leaves are considered cooling alternative, febrifuge, diuretic, astringent and vulnerary. An infusion of the leaves is useful in diarrhea and piles. The roots are saline and sweetish to taste. They are considered astringent and febrifuge and their decoction is used for coughs (Kritikar and Basu, 1988). The seeds are considered demulcent, stimulant, diuretic and tonic and are used as a remedy for dysentery and diarrhea.

Keeping these medicinal characteristics, market value and conservation concern of *P. major* in view, the studies were conducted to develop cultivation strategies of this species to conserve its natural status and meet the demand. Natural habitat of this species belongs to approximately 2500 m exposed with the harsh climate conditions i.e. cold weather, low temperature, high wind, water deficit and short growing season and also far from the human settlements so the cultivation practices in the nearby areas seems irrelevant. The most important characteristic for the survival of species is basically the ability of its physiological functions to synchronize with the environmental periodicity at a particular altitude. Acclimatization studies are important in understanding the adaptive modifications in relation to changes in one or more environmental factors. Each species has different habitat requirements performs different ecological functions in different ecosystems. So the present study was an attempt to identify the suitable cultivation sites for the *P. major*. For the same, three altitudes (550, 1800 and 2500 m asl) were examined and on the basis of variation in morphological and biochemical attributes the performance of the plant was analyzed which help to recognize the cultivation site with high potency and yield. Many other reports also support the fact that the high altitude is not too suitable for cultivation even for the species of alpine origin. Nautiyal *et al*. (2005) suggested the cultivation of two aconitum species of alpine habitat at lower altitude.

Antioxidant enzymes viz., peroxidase, catalase and ascorbate peroxidase were analyzed in the plants cultivated under different altitudes with an aim to identify the tolerance capacity during adaptation in new environment. In the present study cultivation trials were carried out in two lower altitudes sites (1800 and 550 m) where proper sunlight and temperature was there because sunlight is the driving force for photosynthesis and the balance between light absorption and utilization is important for plant's normal life. Cold temperatures during winter can inhibit the enzymatic reactions of photosynthesis but high temperature provokes antioxidant enzyme responses (Wang *et al*., 2009). The role of antioxidant in stress tolerance and acclimatization in different species were also recorded by Sairam *et al*. (2002), Meloni *et al*. (2003), Pasternak *et al*. (2005) and Cicek and Cakirlar (2008). Pigmentation (chlorophyll and carotenoid content) and protein content also be considered as the indicator for the adaptation and stress tolerant capacity (Adamska, 2001; Wang *et al*., 2009) so these parameters were also analyzed in *P. major* during cultivation to establish the plant in planting sites with proper potential.

MATERIALS AND METHODS

Plant material: Present investigations were carried out with *Plantago major* Linn. The seeds of this species were collected from its natural habitat (2500 m altitude) in the month of September

2008 and kept under proper storage conditions in the laboratory situated at Srinagar (550 m), Uttarakhand, India. Dried seeds were sown in styrofoam seedling trays filled with soil, sand and farmyard manure (2:1:1) to raise the seedlings in the month of April, 2009. After one month when the seedlings reached two to four leaf stage, these were transplanted to selected cultivation sites located at Tala (1800 m asl) and Dugalbita (2500 m asl) situated in Rudraprayag district of Uttrakhand and a set of seedlings kept at Srinagar (550 m asl). Observations were made for growth analysis and biochemical characterization.

Recording of data: After three months of acclimatization of plants, randomly fifty plants were uprooted from each of the experimental site. The uprooted plants were washed carefully in tap water followed by distilled water to remove any dirt and blotted off moisture with the help of blotting paper. Fresh weight of individual plant parts were recorded and then dried in an electronic oven at 60°C for 48 h after which the dry weights of individual plant parts were recorded. Different biochemical estimations were analyzed/assayed in the laboratory.

Data analysis: The growth parameters were calculated on the basis of formulae described by Evans (1972). Absolute leaf water content was calculated with the help of formulae given by Hughes *et al*. (1970). The details of the formulations followed are given below.

$$\text{Leaf Weight Ratio (LWR) mg mg}^{-1} = \frac{\text{Leaf dry weight}}{\text{Whole plant dry weight}}$$

$$\text{Root Shoot Ratio (R:S Ratio) mg mg}^{-1} = \frac{\text{Root dry weight}}{\text{Shoot dry weight}}$$

$$\text{Absolute Leaf Water Content (LAWC) mg} = \text{Fresh weight-Dry weight}$$

$$\text{Specific Leaf Water Content (SLWC) mg mg}^{-1} = \frac{\text{Leaf water content}}{\text{Leaf dry weight}}$$

$$\text{Percent Dry Matter Content (PDMC)} = \frac{\text{Dry weight}}{\text{Fresh weight}} \times 100$$

Biochemical estimations

Pigmentation (chlorophyll and carotenoids): Young leaves of *Plantago major* were collected after rearing the plants at different altitudes for 90 days and homogenized with the help of mortar and pestle in 100% acetone. The homogenates were centrifuged at 1500 rpm for 15 min in a desktop centrifuge. The clear supernatants were used for estimation of chlorophyll and carotenoids by the method of Holm (1954). A suitable volume of supernatant was diluted properly with pure acetone and the absorbance was measured at 662, 644 and 440.5 nm using a Beckman DU-640 spectrophotometer. The pigment contents were calculated by substituting absorbance values in the following equations (Holm, 1954).

$$\text{Chl.a (mg g}^{-1}\text{ fresh weight)} = \frac{(9.78A_{662})\text{-}(0.99A_{644}) \times \text{volume}}{1000 \times \text{fresh weight (g)}}$$

$$\text{Chl.b (mg g}^{-1}\text{ fresh weight)} = \frac{(21.4A_{644})\text{-}(4.65A_{662}) \times \text{volume}}{1000 \times \text{fresh weight (g)}}$$

$$\text{Total Chlorophyll} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

$$\text{Total carotenoids (mg g}^{-1}\text{ fresh weight)} = \frac{(4.69A_{440.5}) - (\text{Chl.a+chl.b}) \times 0.267 \times \text{volume}}{1000 \times \text{fresh weight (g)}}$$

Soluble protein: Plant leaf was homogenized with 0.1 M Tris HCl buffer (pH-7.5) in the presence of a pinch of PMSF (phenyl methane sulphonyl fluoride) in a porcelain mortar and pestle. The homogenates were centrifuged at 10,000 rpm for 20 min in refrigerated centrifuge at 4°C. The clear supernatants were used for the estimation of soluble protein by the method of Lowry *et al.* (1951). In suitable volume of supernatant 5 mL reagent C (Reagent A (2% Na_2CO_3 in 0.1 N NaOH) + Reagent B (0.5 % $CuSO_4.5H_2O$ in 1 % Potassium Sodium Tartrate) (50:1)) was added with constant mixing and left for 5 min at room temperature and then added 0.5 mL reagent D (Folin ciocolteu's reagent (diluted with distilled water in 1:1)) with instant mixing. After 20 min at room temperature, the absorbance was measured at 660 nm in a spectrophotometer (Beckman DU-640). Bovine serum albumin (BSA) was used as standard.

Free Amino Acids (FAA): Total free amino acid contents in samples were determined by the method of Moore and Stein (1954). Known weight of leaf material was homogenized in known volume of 80% cold ethanol using a mortar and pestle. The homogenates were centrifuged at 3000 rpm for 20 min in a refrigerated centrifuge at 4°C. The clear supernatant was used for free amino acid estimation. The 0.1 mL supernatant was made to 1 mL with 80% ethanol and then finally 2 mL of ninhydrin reagent (2 g Ninhydrin + 70 mL ethylene glycol + 30 mL 0.2 M acetic buffer pH -5.0) was added. The contents were mixed well and placed in a boiling water bath for 30 min. After cooling the contents absorbance was measured at 570 nm in Beckman DU-640 spectrophotometer against a suitable blank (1 + 2 mL of 80% alcohol and ninhydrin reagent respectively). Glycine was used as standard for the calibration curve.

Antioxidant enzyme activities

Catalase (CAT) activity: Two hundred millilgrams plant material was homogenized with 10 mL of ice-cold extraction buffer (0.1 M potassium phosphate, 2 mM EDTA [Ethyline-diaminetetra-acetic acid] and 1% PVP [Poly Vinyl Pyrollidone], pH-7.5 in the presence of 2 mM PMSF [Phenylmethylsulphonyl fluoride]. The homogenates were centrifuged at 10,000 rpm for 15 min at 4°C in a refrigerated centrifuge. The clear supernatant was used for determination of catalase activity as described by Rao *et al.* (1996). CAT activity was determined by following the consumption of H_2O_2 at 240 nm for 5 min by using a Beckman DU-640 spectrophotometer. The 3 mL of a reaction mixture contains 100 mM potassium phosphate buffer, pH-7 and 10 µL of 30% H_2O_2 and suitable volume of supernatant.

Peroxidase (POX) activity: Plant material (200 mg) was homogenized with 10 mL of ice cold extraction buffer (0.1 m potassium phosphate, 2 mM EDTA and 1% PVP, pH-7.5) in the presence of 1 mM PMSF. The homogenates were centrifuged at 10,000 rpm for 15 min at 4°C in a refrigerated centrifuge. The clear supernatant was used for determination of POX activity as described in the method of Rao *et al.* (1996). POX activity was determined at 470 nm for 5 min in a 3 mL reaction mixture containing 100 mM Potassium phosphate buffer pH 6.5, pyrogallol and 10 µL of 10% H_2O_2 and suitable amount of supernatant using a Beckman DU-640 spectrophotometer.

Ascorbate Peroxidase (APX) activity: Two hundred millilgrams plant material was homogenized with 10 mL of ice-cold extraction buffer (0.1 M potassium phosphate, 2 mM EDTA and 1% PVP, pH-7.5) in the presence of 1 mM PMSF. The homogenates were centrifuged at 10,000 rpm for 15 min at 4°C in a refrigerated centrifuge. The clear supernatant was used for determination of APX activity as described by Rao *et al.* (1996). APX activity was determined by observing the decrease in absorbance at 290 nm for 3 min in a 2 mL of a reaction mixture containing 100 mM Potassium phosphate buffer pH 7.5, 0.5 mM ascorbate (sodium ascorbate) and 0.2 mM H_2O_2 and suitable volume of supernatant using a Beckman DU-640 spectrophotometer.

RESULTS

Since, this investigation was aimed at finding out the extent of stability and flexibility in character during acclimatization or adaptation of the species under different altitudes, it was considered essential to have an idea of general behavior of species in its natural habitat. So, the seedlings were experimented at 2500 m in its natural habitat as well as comparatively lower altitudes 1800 and 550 m). As described under materials and methods, seedlings of *P. major* species raised in styrofoam trays at Srinagar were planted at three different altitudes and after three months of acclimatization of plants at all the experimental site, some morphological and biochemical attributes were studied. The general trends of the results are described below:

Growth analysis: Variations in morphological characters of *Plantago major* are summarized in Table 1 and 2.

Plant height: It was observed that the plant height was maximum (17.66 cm) at 1800 m followed by lower altitude at 550 m and minimum (10.58 cm) at high altitude (2500 m).

Root length: Root length found maximum (22.6 cm) and minimum (13.24 cm) at middle and high altitude, respectively.

Leaf number: Number of leaves was highest (8.0) at high altitude and lowest (5.2) at lower altitude.

Leaf Length (LL): Spike Length (SL) and Leaf Width (LW): Leaf Length, spike length and Leaf Width found maximum at middle altitude in comparison to other altitudes.

Table 1: Growth behavior pattern of *P. major* after acclimatization at different altitudes

Growth period	Altitude (m asl)	Plant height (cm)	Root length (cm)	No. of leaves	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	Spike length (cm)
3 months after	550	14.66±1.51	15.14±1.035	5.2±0.837	5.34±0.67	4.34±0.24	8.22±0.85	4.48±0.39
acclimation	1800	17.66±1.37	22.6±1.706	7.4±0.894	5.98±0.78	5.12±0.28	11.3±1.11	6.14±0.45
	2500	10.58±1.02	13.24±1.383	8.0±1.581	4.1±0.42	3.48±0.31	5.74±0.48	4.04±0.28

Table 2: Growth behavior pattern of *P. major* after acclimatization at different

Growth period	Altitude (m asl)	Leaf weight ratio (mg mg^{-1})	Root shoot ratio (mg mg^{-1})	Percent dry matter	Absolute leaf water content (mg)	Specific leaf water content (mg mg^{-1})
3 months after	550	0.27±0.003	0.55±0.0280	19.7±1.380	1.44±0.0178	5.1±0.601
acclimation	1800	0.18±0.002	0.96±0.0901	28.6±1.20	0.64±0.020	3.8±0.350
	2500	0.10±0.003	1.11±0.0981	20.1±1.570	0.61±0.018	4.7±0.255

Table 3: Variation in total chlorophyll and Carotenoid contents in *Plantago major* at different altitudes after acclimatization

	Total chlorophyll (mg g^{-1} fresh weight)		Carotenoid (mg g^{-1} fresh weight)	
Altitude (m asl)	Transplanting time	3 months after acclimation	Transplanting time	3 months after acclimation
550		1.63±0.023		0.62±0.018
1800	1.98±0.012	0.94±0.15	0.95±0.11	0.49±0.015
2500		0.65±0.13		0.31±0.010

Table 4: Variation in Soluble protein and free amino acid contents in *P. major* at different altitudes after acclimatization

	Total chlorophyll (mg g^{-1} fresh weight)		Carotenoid (mg g^{-1} fresh weight)	
Altitude (m asl)	Transplanting time	3 months after acclimation	Transplanting time	3 months after acclimation
500		20.16±1.91		9.02±1.0
1800	17.52±2.61	27.86±2.21	7.86±0.86	10.57±0.94
2500		19.84±1.94		12.78±1.24

Petiole length: The petiole length observed maximum at middle altitude (1800 m) and minimum at high altitude (2500 m) (Table 1).

Leaf Weight Ratio (LWR): The leaf weight ratio progressively decreased with increasing altitude. The minimum leaf weight ratio was recorded at high altitude and maximum LWR at low altitude.

Root Shoot Ratio (R/S): R/S ratio progressively increase with increasing altitude, R/S ratio was minimum (0.55) at 550 m and maximum (1.11) at 2500 m altitude.

Percent Dry Matter (%DM): The percent dry matter shown in Table 2, indicated that percent dry matter was found highest at 1800 m asl. Followed by 2500 m and lowest at 550 m altitude.

Absolute Leaf Water Contents (ALWC) and Specific Leaf Water Content (SLWC): ALWC increasing with decreasing altitude, while SLWC was recorded minimum at middle and maximum at lower altitude (Table 2).

Biochemical estimations

Total chlorophyll and carotenoid contents: Chlorophyll and carotenoid content in *P. major* decreased gradually with increasing altitude during acclimatization (Table 3).

Protein and Free Amino Acids (FAA): Variation was observed in case of protein and free amino acid contents after acclimatization of *P. major* at three different altitudes. The leaf of selected species showed high quantity of protein at Tala (1800 m) and subsequently followed by Srinagar (550 m) and Dugalbita (2500 m). After Three months of acclimation, FAA was observed high at Dugalbita (2500 m), which was followed by Tala (1800 m) and Srinagar (550 m) (Table 4).

Antioxidant enzymes activities: Figure 1 shows the variation in Catalase activity of *P. major* which were grown at different altitudes. Catalase (CAT) activity was higher at Srinagar (550 m) in comparison to rest of the experimental altitudes. Catalase activity showed decreasing trend with increasing altitude and higher activity was observed after 3 months acclimation in comparison to initial stage at Srinagar and Tala except at Dugalbita (2500 m).

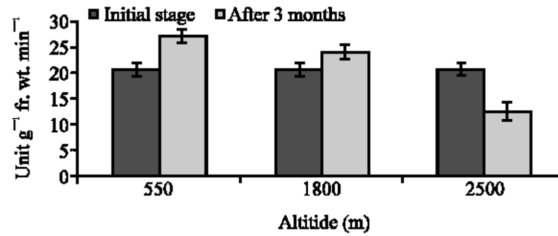

Fig. 1: Changes in Catalase activity in *Plantago major* after three months of acclimatization at different altitudes

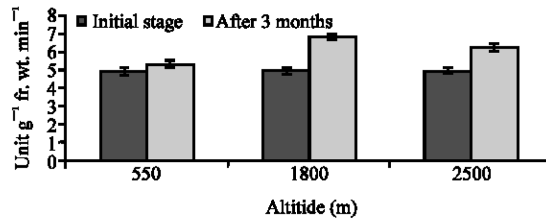

Fig. 2: Changes in Peroxidase activity in *Plantago major* after three months of acclimatization at different altitudes

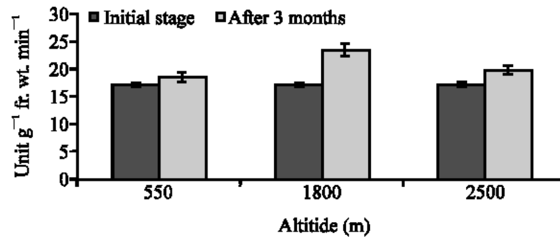

Fig. 3: Changes in Ascorbate Peroxidase activity in *Plantago major* after three months of acclimatization at different altitudes

Peroxidase (POX) activity was higher at middle altitude (1800 m) in contrast to other altitude. The higher activity was observed after three months acclimation in comparison to initial stage at all the three experimental altitudes. At the altitude of 550 m, POX activity was low in comparison to other altitudes after three months acclimatization (Fig. 2).

Ascorbate peroxidase (APX) followed a trend similar to that of POX, showed higher activity at 1800 m in comparison to other altitudes (Fig. 3) after acclimatization.

DISCUSSION

Altitudinal variations in growth parameters, biochemical characteristics and antioxidant enzymes activity were noticed during acclimatization under three selected altitudes. Remarkable altitudinal variation were observed in morphological parameters viz. plant height, leaf width, leaf length, petiole length and spike length in *P. major*. At middle altitude (1800 m) highest range of these parameters showed a direct and adaptive response to the climate associated with changing altitude (Table 1, 2). Reduced growth noticed at higher altitudes (2500 m) must be due to the stressful climatic conditions which showed incompatibility of the species in respect of commercial

cultivation at this altitude. Stressful factors are known to influence growth and development of plant species but only limited information is available regarding the behavior of plants to these factors under natural conditions. Environmental factors affect plant growth through their effects on assimilatory apparatus and the resulting effect is expressed in the magnitude of changes in the relative growth rate.

The other attributes, which control the growth behavior of plants along an altitudinal gradient are root shoot ratio (R/S) and leaf weight ratio (LWR). The LWR in *P. major* decreases with increasing altitude indicate advanced growth of underground parts due to faster translocation towards it which is a characteristic of high altitude species to overcome the effect of stresses especially in winter season when the area was covered with snow. The percent dry matter was found high at middle altitude in contrast to very high and low altitude (Table 2). Saxena (1980) also observed a decline in dry matter accumulation with progressive increase in altitudes. The changes in SLWC and R/S ratio bring out the fact that demand of underground parts to assimilate and rate of translocation increases progressively with growing season and with increasing altitude. The low leaf weight ratio at higher altitude (Table 2) could be due to the relatively lower photosynthetic pigment contents (chlorophyll) at higher altitude.

In the present study, *P. major* at higher altitude characterized by low pigments content than lower altitudes (Table 3). The fall in chlorophyll and carotenoid contents at higher altitudes has been considered to be due to the photo-oxidation of pigments in presence of high irradiance and low temperature at higher altitude.

The high soluble protein content at middle (1800 m) altitude in *P. major* clearly indicates the higher metabolism at this altitude. The reduction in soluble protein levels at higher altitudes may be due to low enzyme activity which is considered as a consequence of the developmental state of the leaves. Middle altitude temperature is comparatively very high if compared to high altitude (2500 m) and increase in the soluble protein concentration under high temperature as compared to natural habitat could be related to an increase in the protein synthesis related to acclimation to new conditions as well as to cell protection against these stresses. Soluble protein acts as osmotic agent or osmoprotectors that play a major role in the osmotic adjustment of water deficit (Yang and Miao, 2010). In contrast to protein, the amino acid content in the leaves of *P. major* was recorded high at high altitudes and may correlate with the less soluble protein present in these organs. Prakash *et al.* (2001) has also reported the similar results in *Polygonum* spp. On the basis of pigmentation, protein and amino acid analysis it was investigated that the plants acclimatized and survived well at 1800 m altitude.

A common feature of different stress factors is their potential to increase the production of Reactive Oxygen Species (ROS) in plant tissues (Arora *et al.*, 2002). In plant cells CAT, POX, APX exist as multiple isoforms and the spectrophotometric analysis indicates only the combined activity of different isoforms. Leaf antioxidant system can prevent or alleviate the damage caused by ROS under stress condition and include enzymes such as SOD, CAT, POX APX and metabolites including ascorbate and glutathione (Niyogi, 1999; Xu *et al.*, 2008). These findings support the observations made by the authors in *P. major*. Present results indicate the increase in the activity of CAT and POX in *P. major* at middle altitude (1800 m) where the conditions are different than the natural habitat of this species but the species adapted and cultivated well (Fig. 1). This may offer protection from the active oxygen species that are generated during the acclimatization. Taehyun *et al.* (2002) reported that the maintenance of these enzyme activities during the ripening stages may potentially help in the detoxification of active oxygen species that are generated during

catabolic activities. Like CAT, POX and APX activity was also recorded minimum at 550 m (Srinagar) altitude and higher at 1800 m altitude in *P. major* (Fig. 2, 3). Wang *et al.* (2009) also found that antioxidant metabolites and enzymes, CAT and POX were significantly up regulated during acclimation in the two Rhododendron species. Cold temperatures during winter can inhibit the enzymatic reactions of photosynthesis but high temperature provokes antioxidant enzyme responses (Wang *et al.*, 2009). Higher plants have supposedly evolved several mechanisms to avoid photo inhibition and activation of antioxidants system is one of them (Verhoeven *et al.*, 2005). Much of the injury to plants exposed to stress is connected with oxidative damage at the cellular level (Foyer and Noctor, 2003) but the plants have developed the scavenging mechanism of ROS (Reddy *et al.*, 2004; Demiral and Turkan, 2005) because of which despite of introduction of the species in new environment, *P. major* grows well at 1800 m which clearly indicates that the increase in antioxidant enzyme activity protect the plant against the stress conditions during adaptation and the region may be suitable for the commercial cultivation of the plant.

The overall studies revealed that altitudinal variation differentially influenced morphological and biochemical responses of *P. major*. Altitudinal variations in growth performance as well as all the biochemical attributes including antioxidant enzyme activity of this species indicates their adaptive potential and suitability of cultivation practices at middle altitude (Tala, 1800 m) in comparison to much higher (2500 m) or lower altitudes (550 m). Conclusions also support the fact that the plants will change their morphology and physiology with changing environmental conditions in some extent to adapt them and also the production of antioxidant enzymes plays a great role for the same.

REFERENCES

Adamska, I., 2001. The ELIP Family of Stress Proteins in the Thylakoid Membranes of Pro and Eukaryota. In: Advances in Photosynthesis and Respiration-Regulation of Photosynthesis, Aro, E.M. and B. Andersson, (Eds.). Kluwer Academic Publishers, Dordrecht, Netherlands, pp: 487-505.

Arora, A., R.K. Sairam and G.C. Srivastava, 2002. Oxidative stress and antioxidative system in plants. Curr. Sci., 82: 1227-1238.

BSI., 1993. Conservation of medicinal plant of India. Ministry of Environment and Forest, India, Botanical Survey of India.

Cicek, N. and H. Cakirlar, 2008. Changes in some antioxidant enzyme ectivities in six soybean cultivars in response to long-term salinity at two different temperatures. Gen. Applied Plant Physiol., 34: 267-280.

Demiral, T. and I. Turkan, 2005. Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. Environ. Exp. Bot., 53: 247-257.

Evans, G.C., 1972. The Quantitative Analysis of Plant Growth. Blackwell Science Publication, Oxford.

Foyer, C.H. and G. Noctor, 2003. Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroisomes and mitochondria. Physiol. Plant., 119: 355-364.

Holm, G., 1954. Chlorophyll mutation is barely. Acta Agric. Scand., 4: 457-471.

Hughes, A.P., K.E. Cockshull and O.V.S. Heath, 1970. Leaf area and absolute leaf water content. Ann. Bot., 34: 259-266.

Kritikar, K.R. and B.D. Basu, 1988. Indian Medicinal Plants. Leading Book Publishers, Dehradun, Uttarakhand, India.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with folin phenol reagent. J. Biol. Chem., 193: 265-275.

Meloni, D.A., M.A. Oliva, C.A. Martinez and J. Cambraia, 2003. Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. Environ. Exp. Bot., 49: 69-76.

Moore, S. and W.H. Stein, 1954. A modified ninhydrin method for use in the chromatography of amino acids. J. Biol. Chem., 176: 367-388.

Nautiyal, M.C. and A.N. Purohit, 2000. Cultivation of himalayan aconites under polyhouse conditions. Curr. Sci., 78: 1062-1063.

Nautiyal, B.P., V. Prakash, R.S. Chauhan, H. Purohit, R. Vashistha and M.C. Nautiyal, 2005. Ciltivation of *Aconitum* species. J. Trop. Med. Plants, 6: 193-201.

Niyogi, K.K., 1999. Photoprotection revisited: Genetic and molecular approaches. Annu. Rev. Plant Physiol. Plant Mol. Biol., 50: 333-359.

Pasternak, T., V. Rudas, G. Potters and M.A.K. Jansen, 2005. Morphogenic effects of abiotic stress reorientation of growth in *Arabidopsis thaliana* seedlings. Environ. Exp. Bot., 53: 299-314.

Prakash, V., P. Prasad and M.C. Nautiyal, 2001. Changes in biochemical attributes during acclimatization of *Polygonum* species at different altitudes in Garhwal Himalaya. Indian J. Plant Physiol., 6: 244-248.

Rao, R.R., 1994. Biodiversity in India (Floristic Aspects). 1st Edn., Bishen Singh Mahendrapal Singh, Dehra Dun, India.

Rao, M.V., G. Paliyath and D.P. Ormrod, 1996. Ultraviolet-B and ozone-induced biochemcial changes in the antioxidant enzymes of *Arabidopsis thaliana*. Plant Physiol., 110: 125-136.

Reddy, A.R., K.V. Chaitanya and M. Vivekanandan, 2004. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. J. Plant Physiol., 161: 1189-1202.

Sairam, R.K., K.V. Roa and G.C. Srivastava, 2002. Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. Plant Sci., 163: 1037-1046.

Saxena, P.K., 1980. Physiological behavior of two graminaceous crops at different altitudes in garhwal himalayas. D.Phil Thesis, Garhwal University, Uttarakhand India.

Taehyun, A., A. Schofield and P. Gopinadhan, 2002. Changes in antioxidant enzyme activities during tomato fruit development. Physiol. Mol. Biol. Plants, 8: 241-249.

Verhoeven, A.S., A. Swanberg, M. Thao and J. Whiteman, 2005. Seasonal changes in leaf antioxidant systems and xanthophyll cycle characteristics in Taxus x media growing in sun and shade environments. Physiol. Plant, 123: 428-434.

Wang, X., Y. Peng, J.W. Singer, A. Fessehaie, S.L. Krebs and R. Arora, 2009. Seasonal changes in photosynthesis, antioxidant systems and ELIP expression in a thermonastic and non-thermonastic Rhododendron species: A comparison of photoprotective strategies in overwintering plants. Plant Sci., 177: 607-617.

Xu, P., Y. Guo, J. Bai, L. Shang and X. Wang, 2008. Effects of long-term chilling on ultrastructure and antioxidant activity in leaves of two cucumber cultivars under low light. Physiol. Plant, 132: 467-478.

Yang, F. and L.F. Miao, 2010. Adaptive responses to progressive drought stress in two poplar species originating from different altitudes. Silva Fennica, 44: 23-37.