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Physiological and Biochemical Variations among the Natural Populations of two Medicinal Plants, *Plantago major* L. and *Astilbe rivularis* D. Don. from Darjeeling Himalayas

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ABSTRACT

Studies on different populations of Plantago major *and* Astilbe rivularis *were found to have considerable differences in the length and diameter of different morphological parts. The content of biochemical constituent viz. free amino acids, soluble proteins, soluble & insoluble carbohydrates, DNA & RNA and photosynthetic pigments revealed considerable differences. The activity of peroxidase and catalase was more pronounced in the specimens from colder regions and the physiological growth was higher in specimens collected from warmer regions*

INTRODUCTION

A large number of people living in remote places are dependent on medicinal plants for their health care needs. With a doctor to people ratio of 1: 5163, Darjeeling Himalaya is the right candidate for flourishing traditional medicine systems based on local plants. The herb *Plantago major* L. is used in the traditional medicine for disorders of the epidermis, headache, toothache, diarrhea, piles cold and cough. The root possesses astringent and febrifuge properties (Kumar, 2002). *Astilbe rivularis* D. Don. is used locally in toothache, diarrhea and dysentery. A mixture of the roots *A. rivularis* with *Berginia ciliata* is used to ease body pain after childbirth (Rai and Sharma, 1994).

The present investigation reports the morphological and biochemical analysis of *P. major* and *A. rivularis* collected from three altitudinal zones of Darjeeling Himalayas. Similar study involving other medicinal plants has been done earlier by various workers (Bhat and Purohit, 1984; Krishnan *et al.*, 2000; Kuniyal *et al.*, 2002).

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MATERIALS AND METHODS

Fresh plant parts (leaves, shoot and root/rhizome) from the actively growing plants of *Plantago major* L. (Family Plantaginaceae) and *Astilbe rivularis* D. Don. (Family Saxifragaceae) were collected within 9 a. m. from the different altitudinal locations in Darjeeling Himalayas viz. Chamong (1538 m), Darjeeling (2134 m) and Senchal (2511 m). The collected plant parts were rinsed in teepol for 5 minutes and washed four times with sterile distilled water. All biochemical estimates were done the same day of collection.

For the estimation of free amino acids, 500 mg of plant part was homogenized in ethanol and centrifuged at 3,000 rpm for 10 minutes. The supernatant was collected in a watch glass and evaporated to dryness. The chlorophyll was washed with and drained carefully from the surface of the watch glass with the help of solvent

ether. The inner surface of the watch glass was then carefully washed with 80% ethanol and residual solution was collected and made up to 5 ml with 80% ethanol. From the non-photosynthetic parts the supernatant was collected directly after centrifugation following homogenization with 80% ethanol. These extracts served as the source of free amino acids (Sadashivam and Manickam, 1996). The estimation was done with ninhydrin reagent as per Moore and Stein (1948). Soluble protein was estimated from the samples by homogenizing 1 g of plant parts in 19 ml of 62.5 mM Tris-HCl buffer (pH 6.8) with neutral sand. The sample was centrifuged at 15000 rpm for 15 minutes and the soluble protein was estimated from the supernatant by the method of Lowry *et al.*, (1951) as modified by Bollag *et al.*, (1996).

Soluble carbohydrate was extracted from 100 mg of each type of tissue in the same manner as that of free amino acid. The extract served as the source of soluble carbohydrate. The residue from the above extraction was dissolved in 5 ml of 25 % H_2SO_4 at 80 °C for 30 minutes (Rai *et al.*, 1992) and centrifuged at 5, 000 rpm for 15 minutes. The supernatant was collected as a sample for insoluble carbohydrate. Estimation of both type of carbohydrate was done following the method of Mc. Cready *et al.*, (1950). Extraction of nucleic acids (RNA and DNA) were made from different plant parts following the method described by Cherry (1962) and the quantitative estimation of the same were done following the method of Markham (1955) as modified by Choudhury and Chatterjee (1970).

For the estimation of peroxidase activity 100 mg of tissue was homogenized with 5 ml of 300 µM Na-phosphate buffer (pH 6.8) and centrifuged at 1000 rpm for 10 minutes. The supernatant was used as the enzyme source and the enzyme activity was estimated following the method of Kar and Mishra (1976). To estimate the catalase activity 500 mg of plant sample was homogenized with 10 ml of 0.1 M chilled Na-phosphate buffer (pH – 7.0) and centrifuged at 5,000 rpm for 10 minutes. The enzyme activity was assayed from the supernatant according to the method of Snell and Snell (1971). For estimating protease activity the extraction was done by homogenizing 500 mg of tissue in 5 ml of chilled Na-phosphate buffer (pH- 6.5) and centrifugation of the homogenate for 10 minutes at 10,000 rpm. The supernatant was used as the enzyme source. The assay mixture contained 1 ml of the enzyme extract, 0.1 ml of $MgSO_4$, $7H_2O$ (0.1 M) and 1 ml of 500 µg / ml BSA (Chhetri *et al.*, 2003). The extent of activity of all these enzymes was calculated according to Fick and Qualset (1975).

Photosynthetic pigments (chlorophyll a, b and carotenoids) were extracted and estimated from fresh leaf samples collected from different altitudes. For the estimation of photosynthetic pigments 50 mg of leaf tissue was homogenized with 5 ml of 96% ethanol and centrifuged for 10 minutes at 5000 rpm. The estimate was done following the method of Lichtenthaler (1987).

To study the morphological variations, natural population of *P. major* and *A. rivularis* were selected from three different altitudinal habitats. Ten identical plants from each habitat were selected to study morphological variations in the last week of July, 2004. Shoot diameter was measured at the middle portion in case of *P. major* and 5 cm above ground level in case of *A. rivularis*, then shoot length of the plants were measured. After that root / rhizome length and diameter were measured upon carefully uprooting the plants. For measuring the dimension of the leaves 10 mature leaves/leaflets were selected from 10 different plants of each altitude. Fresh weight of leaves/leaflets, shoots, roots/rhizome were estimated. For dry weight estimation, plant material was oven dried at 80 °C and data were recorded until the weight remained unchanged (Kuniyal *et al.*, 2002).

RESULTS AND DISCUSSIONS

The free amino acid content in the leaves and stems of *P. major* was found to contain higher amino acid from the specimens collected from Darjeeling, while it was highest in the root samples in the specimens form Senchal. However, in case of *A. rivularis* the content of free amino acid was highest in all the morphological parts of leaflets, stems and rhizome in the specimens collected from Senchal pointing towards a higher level of both synthesis and degradation of proteins in the specimens of *A. rivularis* from the relatively colder region of Senchal. The content of soluble protein in different morphological parts may be an indication of the vigor of plants (Chhetri *et al.*, 1993). In the present study maximum leaf protein in case of *P. major* was found in the specimens collected from Darjeeling while the same was true in case of *A. rivularis* in the specimens collected from Senchal. The level of stem protein was highest in *P. major* from Chamong and *A. rivularis* from Senchal. Same trend was visible in case of root protein of *P. major* and rhizome protein of *A. rivularis* respectively (Table 1). Besides the simple indication of overall vigor, the higher level of protein in *A. rivularis* may be explained as a plant response towards meeting the stress condition caused by the relatively extreme climate (De and Mukherjee, 1996) of Senchal.

The carbohydrate level showed a general trend of content from high to low in the 3 relation: Root>Stem>Leaves and in terms of altitude from Senchal<Darjeeling<Chamong in both the plant species *P. major* and *A. rivularis*. The content of both the soluble and insoluble carbohydrate was the highest in roots / rhizome of both the plants and the relatively warmer location of Chamong showed its highest concentration pointing towards a high level of photosynthetic activity at a lower altitude and the root/rhizome being the sink for the photosynthesis (Table 2).

Table 1. Variations in free amino acid and soluble protein content (mg / g) in different morphological parts of *Plantago major* L. and *Astilbe rivularis* D. Don. collected from three geographical locations of the Darjeeling Himalaya

Parameters	Location & altitude	*Plantago major*			*Astilbe rivularis*		
		Leaf	Stem	Root	Leaflet	Stem	Rhizome
Free amino acid content (mg /g)							
	Chamong (1538 m)	17.0 ± 0.35	11.67 ± 0.76	12.12 ± 0.27	21. 35 ± 0.17	22.35 ± 0.57	21.31± 0.65
	Darjeeling (2134 m)	21.0 ± 1.42	23.1 ± 0.12	13.82 ± 0.16	22.01 ± 0.23	33.20 ± 0.70	43.00 ± 0.32
	Senchal (2511 m)	14.08 ± 0.18	12.46 ± 0.43	15.23 ± 0.69	31.84 ± 0.10	37.31 ± 1.26	52.01 ± 0.22
Soluble protein content (mg / g)							
	Chamong (1538 m)	20.29 ± 0.41	20.68 ± 0.77	20.33 ± 1.18	28.27 ± 1.64	26.35 ± 0.50	30.40 ± 1.53
	Darjeeling (2134 m)	16.64 ± 0.55	9.88 ± 0.82	14.82 ± 0.67	18.43 ± 1.09	25.00 ± 0.28	36.10 ± 0.38
	Senchal (2511 m)	12.16 ± 1.51	10.84 ±0.37	11.55 ± 0.14	40.35 ± 0.82	39.30 ± 0.15	62.70 ± 1.42

[Values are mean ± SE, n=3]

Table 2. Variations in soluble and insoluble carbohydrate content (mg / g) in different morphological parts of *Plantago major* L. and *Astilbe rivularis* D. Don. collected from three geographical locations of the Darjeeling Himalaya

Parameters	Location & altitude	*Plantago major*			*Astilbe rivularis*		
		Leaf	Stem	Root	Leaflet	Stem	Rhizome
Soluble carbohydrate (mg /g)							
	Chamong (1538 m)	14.6 ± 0.52	15.4 ± 0.24	32.1 ± 0.10	64.0 ± 1.09	73.5 ± 0.94	99.5 ± 0.42
	Darjeeling (2134 m)	14.0 ± 0.17	15.6 ± 0.61	21.7 ± 0.67	37.5 ± 0.41	40.5 ± 0.77	88.5 ± 0.39
	Senchal (2511 m)	12.4 ± 0.94	14.0 ± 0.20	25.9 ± 1.05	36.7 ± 0.33	42.1 ± 0.85	73.1 ± 1.31
Insoluble carbohydrate (mg / g)							
	Chamong (1538 m)	26.0 ± 0.45	20.5 ± 0.53	22.0 ± 0.32	33.5 ± 1.82	36.0 ± 0.66	53.0 ± 1.50
	Darjeeling (2134 m)	16.2 ± 0.30	11.8 ± 0.21	14.9 ± 0.24	23.4 ± 0.35	31.2 ± 0.34	51.1± 0.59
	Senchal (2511 m)	13.9 ± 0.20	12.9 ± 0.13	14.5 ± 0.28	24.2 ± 0.79	23.7 ± 0.32	43.8 ± 0.66

[Values are mean ± SE, n=3]

Table 3. Variations in DNA and RNA content (mg / g) in different morphological parts of *Plantago major* L. and *Astilbe rivularis* D. Don. collected from three geographical locations of the Darjeeling Himalaya

Parameters	Location & altitude	Plantago major			Astilbe rivularis		
		Leaf	Stem	Root	Leaflet	Stem	Rhizome
DNA content (mg /g)							
	Chamong (1538 m)	21.09 ± 0.32	17.40 ± 0.26	19.37 ± 0.23	21.32 ± 0.38	21.38 ± 0.62	21.47 ± 0.36
	Darjeeling (2134 m)	23.20 ± 0.50	22.8 ± 0.26	21.60 ± 0.49	16.46 ± 0.12	18.79 ± 0.20	25.10 ± 0.41
	Senchal (2511 m)	17.27 ± 1.59	25.04 ± 0.69	24.58 ± 0.18	20.34 ± 0.38	17.21 ± 0.61	21.67 ± 0.66
RNA content (mg / g)							
	Chamong (1538 m)	53.75 ± 0.72	49.5 ± 0.33	40.25 ± 0.54	55.75 ± 0.31	52.20 ± 1.01	39.50 ± 1.24
	Darjeeling (2134 m)	48.25 ± 0.70	45.00 ± 0.52	43.25 ± 0.53	45.68 ± 0.37	38.25 ± 0.19	52.5 ± 0.61
	Senchal (2511 m)	39.50 ± 0.84	35.60 ± 0.90	27.51 ± 0.14	68.59 ± 0.28	59.50 ± 0.90	45.34 ± 0.20

[Values are mean ± SE, n=3]

Table 4. Variations in Peroxidase and Catalase activity (units/hr/g/of fresh tissue) in different morphological parts of *Plantago major* L. and *Astilbe rivularis* D. Don. collected from three geographical locations of the Darjeeling Himalaya

Parameters	Location & altitude	*Plantago major*			*Astilbe rivularis*		
		Leaf	Stem	Root	Leaflet	Stem	Rhizome
Peroxidase activity							
	Chamong (1538 m)	28.5 ± 0.25	24.5 ± 0.15	23.8 ± 0.15	44.1 ± 0.15	36.3 ± 0.65	28.7 ± 0.11
	Darjeeling (2134 m)	27.4 ± 0.26	29.3 ± 0.06	25.3 ± 0.11	38.5 ± 0.35	29.6 ± 0.35	20.8 ± 0.24
	Senchal (2511 m)	35.6 ± 0.30	28.5 ± 0.43	18.4 ± 0.15	31.9 ± 0.11	29.1 ± 0.26	26.9 ± 0.35
Catalase activity							
	Chamong (1538 m)	6.1 ± 0.37	5.7 ± 0.12	4.0 ± 0.15	8.1 ± 0.40	7.8 ± 0.20	6.3 ± 0.15
	Darjeeling (2134 m)	10.2 ± 0.20	4.6 ± 0.17	6.3 ± 0.26	9.4 ± 0.05	7.8 ± 0.14	6.8 ± 0.26
	Senchal (2511 m)	12.4 ± 0.20	8.3 ± 0.27	9.3 ± 0.26	7.5 ± 0.15	6.0 ± 0.57	5.6 ± 0.20

[Values are mean ± SE, n=3]

The content of DNA showed more or less similar level with the leaves in *P. major* and rhizomes in *A. rivularis* showing its higher concentration. Of the three locations, the samples from Darjeeling showed the highest DNA concentration in both the species. This data does not agree with the findings of high content of DNA with the growth at low temperature (Patterson and Graham, 1987). The RNA content also showed a higher level in both the plants collected from Chamong except in case of root/ rhizome where the level of RNA was high in the specimens collected from Darjeeling, the midpoint in terms of altitude (Table 3). It again negated the theory of increased RNA content in cold acclimation (Bhattacharjee and Mukherjee, 1995). The level of activity of the free radical scavenging enzyme peroxidase and catalase may be an indication of the vigor of plants since it removes the toxic radicals from the plant system. In the present study, the activity of peroxidase was highest in the leaf tissues from Senchal and Chamong in case of *P. major* and *A. rivularis* respectively. The activity of Catalase was more pronounced in leaves of both the species and at the colder location (Table 4). The activity of protease was found to be most prominent in the leaf tissue and the activity was found to be more significant in the specimens collected from the relatively warmer region of Chamong (Fig. 1). The high protein content in the leaves despite a higher protease activity may be due to a higher level of protein synthesis as compared to proteolytic degradation. A higher level of RNA content in the leaf tissue also supports this view.

The content of photosynthetic pigment, chlorophyll-a was found to be highest in the leaf tissue from Darjeeling in case of *P. major* while that from Chamong in case of *A. rivularis*. Chlorophyll-b content was highest in Darjeeling specimens of both the species. Carotenoids was highest in the *P. major* specimens from Darjeeling and *A. rivularis* specimens from Chamong (Fig. 2). Findings of the studies on morphological variations among the population of *P. major* and *A. rivularis* is shown in Fig. 3 and 4. The length and width of leaf was more in plants collected from Chamong in both the species of medicinal plants. The shoot and root dimensions were also maximum in *P. major* from Chamong. Maximum fresh weight and dry weight of all the plant parts were also observed in plants collected from Darjeeling in case of *P. major*. In *A. rivularis* both the dry weight and fresh weight was seen to be highest in the stem and rhizome specimens collected from Darjeeling. In general, a gradual increase in shoot and root length

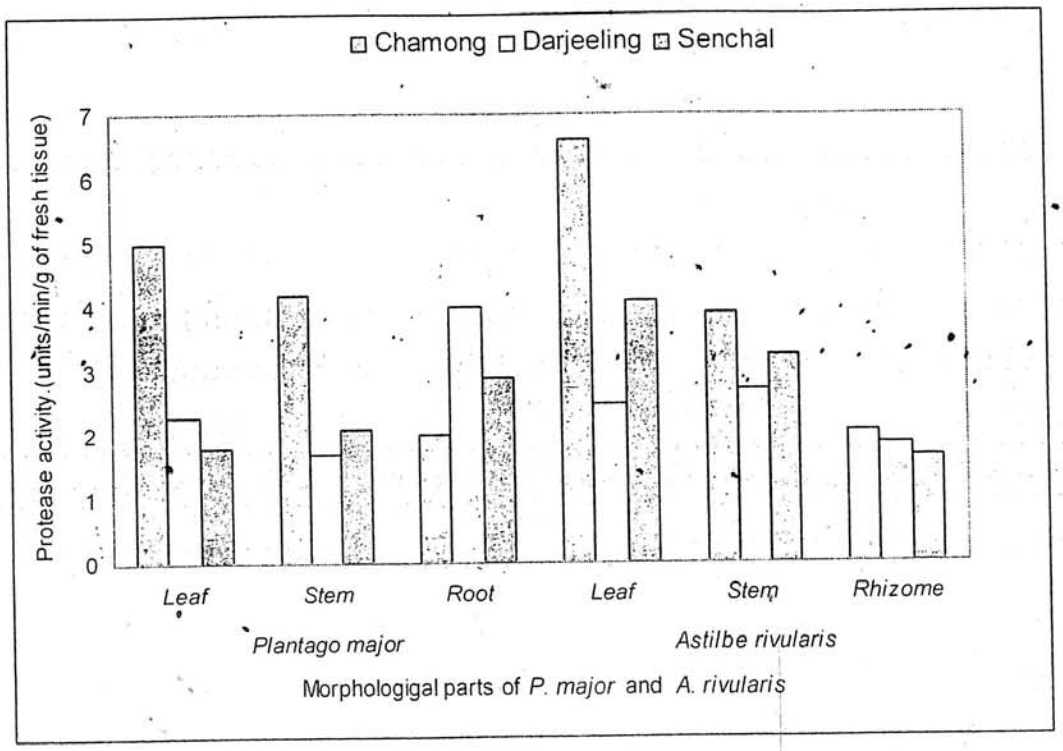

Fig. 1. Variations in Protease activity (units/min/g/of fresh tissue) in different morphological parts of *Plantago major* L. and *Astilbe rivularis* D. Don. collected from three geographical locations of the Darjeeling Himalaya

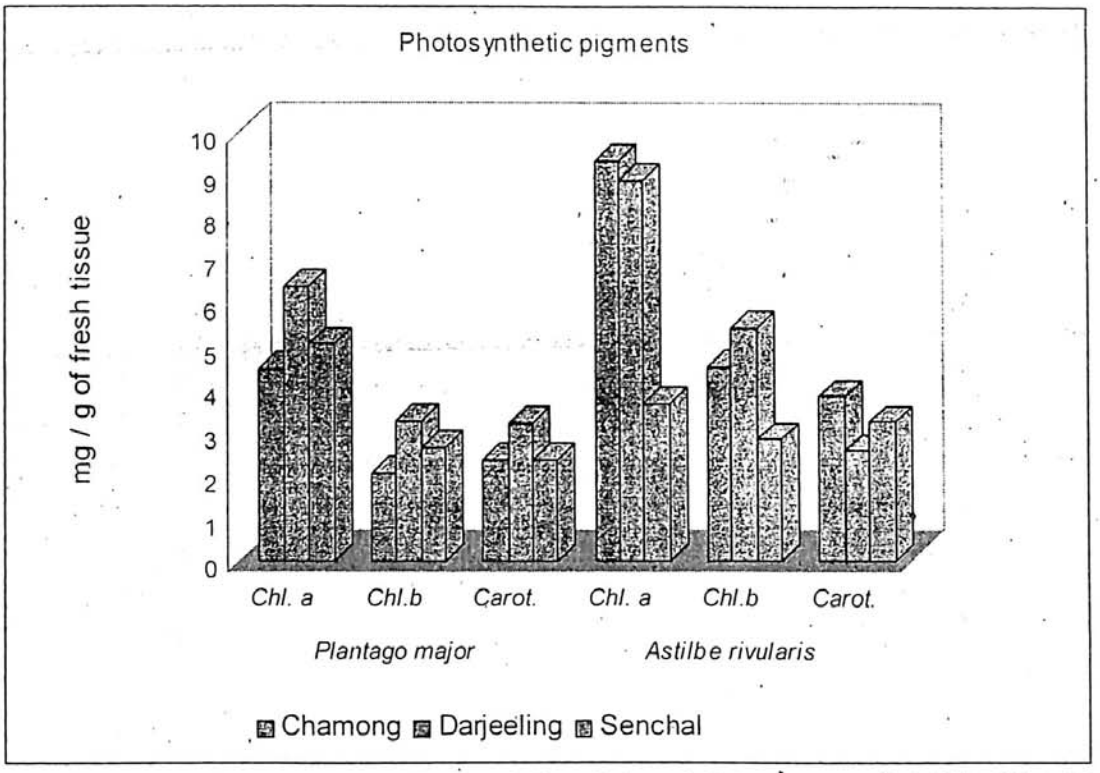

Fig. 2. Variations in Photosynthetic pigments (Chlorophyll-a, Chlorophyll-b and Carotenoids;mg/g fresh tissue) in *Plantago major* L. and *Astilbe rivularis* D. Don. collected from three geographical locations of the Darjeeling Himalaya.

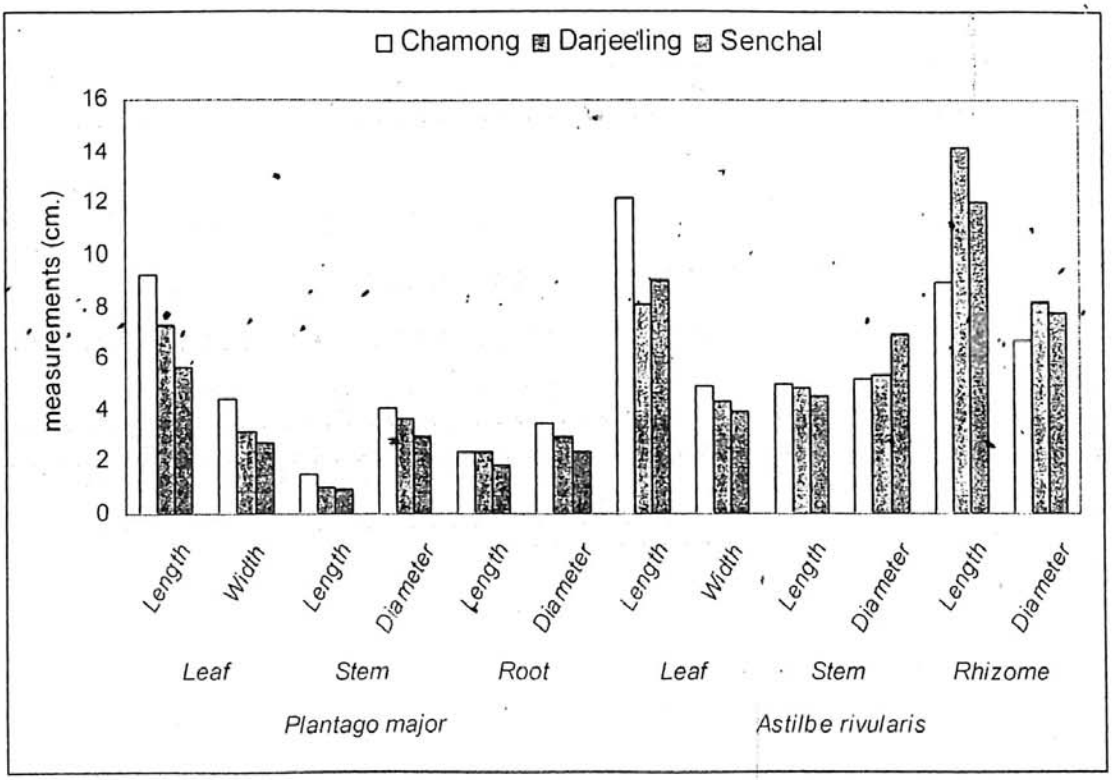

Fig. 3. Morphological variations in the length and width/diameter (cm) of *Plantago major* L. and *Astilbe rivularis* D. Don. leaf/leaflet, stem and root/rhizome collected from three geographical locations of the Darjeeling Himalaya.

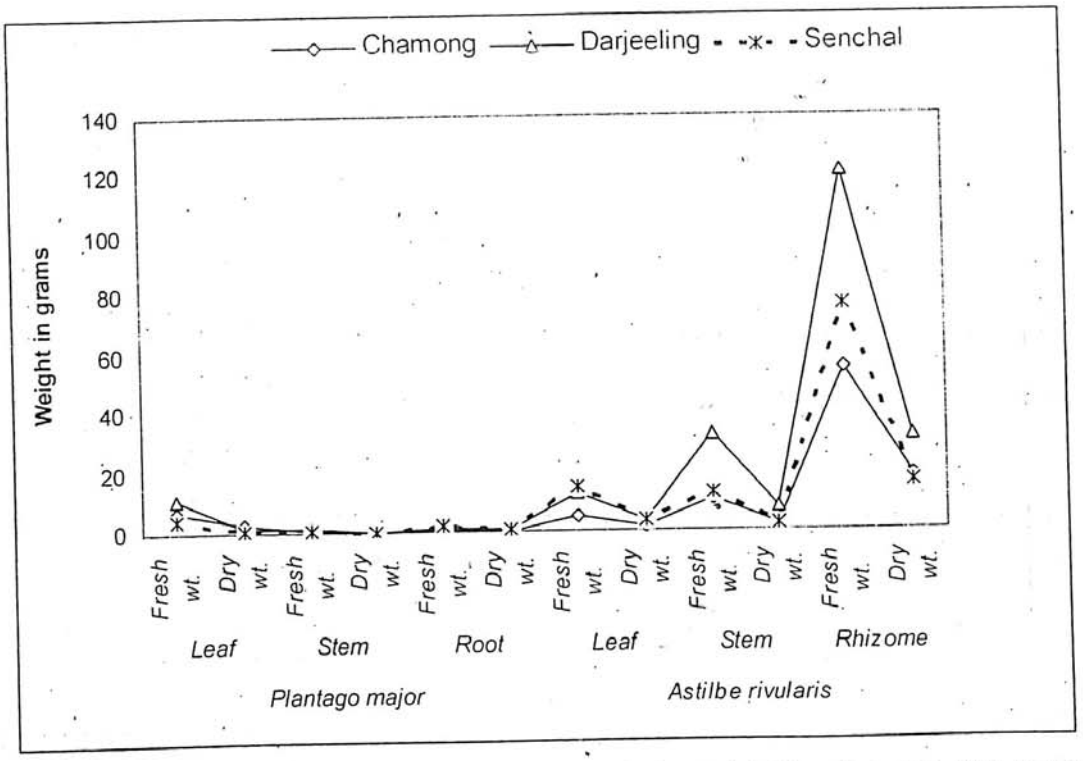

Fig. 4. Variations in the fresh wt. and dry wt. (g.) of *Plantago major* L. and *Astilbe rivularis* D. Don. leaf/leaflet, stem and root/rhizome collected from three geographical locations of the Darjeeling Himalaya.

with increasing altitude has been reported from the plants grown along an altitudinal gradient (Rajsekaran et al., 1998). The present study was in conformity with such findings. Belowground biomass was more in all the sites, which may be interpreted as an adaptive response to severe environmental conditions at the high altitude (Weber and May, 1997).

Studies on *P. major* and *A. rivularis* revealed that the morphological and biochemical variations in different population of these plants may be associated with varying climatic and soil conditions as well as plant responses towards such conditions. However, both the species growing in different locations shows the characteristics of different ecotypes with considerable phenotypic diversity.

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