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RESEARCH ARTICLE

MORPHO-ANATOMICAL CHARACTERISTICS OF LEAVES OF *TAXUS WALLICHIANA* ZUCC. FROM NORTH EASTERN AND NORTH WESTERN HIMALAYAS

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ABSTRACT

Leaves of *Taxus wallichiana* Zucc. from NE and NW Himalayas were studied to determine the variation in anatomical characteristics of both regions. The present study revealed that the leaves of NE Himalayas were larger in size than that of NW Himalayas. Anatomically, the spongy parenchyma of selected sites (except Morsing) of NE Himalaya did not contain pigmented cells. The number of palisade layers, stomatal bands and stomatal density were greater in leaves of NE Himalayas. Also, palisade cell size and vascular bundle size were larger in leaves of NE Himalayas as compare to NW Himayalas. Statistically, there were significant differences in palisade cell size, stomatal density and diameter of vascular bundle among selected sites of both regions.

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INTRODUCTION

Taxus wallichiana Zucc. is a large slow growing evergreen tree of moderate height. It has great economic and medicinal values among gymnosperms and widely distributed in Northern Hemisphere. In India, it is found along the Himalayas between 2300m-3400m (Purkayastha, 1992) and also in Meghalaya, Manipur, Nagaland and Arunachal Pradesh at high elevation. The bark and leaves of *T. wallichiana* are the prime source of taxol, a diterpene with exceptional anticancerous activity. It has also been reported that *Taxus wallichiana* also contain 10-deacytyle baccatin III, a precursor for the synthesis of taxol (Chattopadhayay *et al*, 1994). Since, a lot of work has been done on taxonomical, medicinal and conservational aspects of this species (Purohit *et al*., 1999, Khan *et al*., 2006, Spjut, 2007) but limited information is available on anatomical characteristics of leaves of Taxus. Spjut (2007) presented phytogeographic analysis of Taxus specimens on the basis of number of stomata rows in a stomata band and number of epidermal cells without papillae between leaf margin and stomatal band. It was debated over the years that *Taxus wallichiana* of NE states is better in quality and yield of taxol than the one found in NW Himalayas.

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Since, anatomical characters are well established criteria for identification of plants and herbal drugs. Therefore, the aim of present study is to study morpho-anatomical characteristics and variation in anatomical characteristics of leavesfrom both NE and NW Himalayas.

MATERIALS AND METHODS

Plant material

The leaves of *T. Wallichiana* Zucc. were collected from wild growing trees from NE and NW Himalayas. The selected sites along with geographical coordinates are listed in Table 1.

Table 1. Location of sites

Determination of stomatal band and stomatal density

15 fully mature leaves were selected from each site. The epidermis on abaxial side was separated from mesophyll by following the method of Locosselli and Ceccantini (2012). The permanent slides were prepared. Stomatal bands were determined by counting the rows of stomata between mid rib and leaf margin. While, stomatal density was determined by using graph eye piece.

Table 2. Leaf anatomical characteristics of *Taxus wallichiana* from selected sites of North Eastern and North Western Himalayas

Parameter	Shimla	Mandi	Morsing	Myodia	Bomdila
Leaf length (mm) (Mean ± SD)	27.27± 1.89	26.28± 1.77	32.81±2.32	33.16± 3.18	29.95±3.69
Leaf width (mm) (Mean ± SD)	2.37± 0.19	2.19± 0.22	2.81± 0.26	3.4±0.16	2.55±0.13
Palisade layer	1-2	1-2	1-2	1-3	1-3
Stomata band	9-10	8-11	13-17	15-17	15-16
Stomata Density (Mean ± SD)	30.80±1.99	31.60±2.55	39.00±2.94	37.50±3.14	31.30± 2.11
Palisade cell size (µm) (Mean ± SD)	34.84±5.99	33.93±7.04	58.50±10.14	68.77± 15.07	66.82±12.86
Diameter of vascular bundle(µm) (Mean ± SD)	244.20±12.67	256.83±9.41	425.25±13.10	309.46±10.12	393.67±6.86

Table 3. ANOVA of leaf anatomical characteristics of *Taxus wallichiana* from different sites of North Eastern and North Western Himalayas

Parameter		Sum of Squares	df	Mean Square	F	Sig.
Leaf length	Between Sites	983.227	4	245.807	34.292**	.000
	Within Sites	860.172	120	7.168		
	Total	1843.399	124			
Leaf width	Between Sites	22.114	4	5.528	136.462**	.000
	Within Sites	4.862	120	.041		
	Total	26.975	124			
Stomata density	Between Sites	605.320	4	151.330	22.654**	.000
	Within Sites	300.600	45	6.680		
	Total	905.920	49			
Palisade cell size	Between Sites	23249.398	4	5812.349	50.008**	.000
	Within Sites	11041.784	95	116.229		
	Total	34291.182	99			
Diameter of Vascular bundle	Between Sites	130890.761	4	32722.690	286.74**	.000
	Within Sites	2282.412	20	114.121		
	Total	133173.173	24			

Note: **= 1% at P < 0.01 level i.e. highly significant

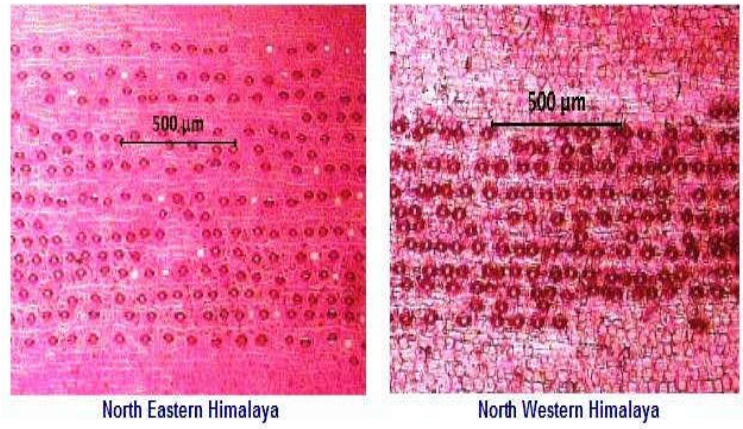

Fig. 1. Stomatal bands between mid-rib and leaf margin

Morpho-anatomical Characteristics

For anatomical investigations, 25 fully mature leaves were selected from each site to measure their length and width. Some of leaves were preserved in 70% alcohol for anatomical investigations. The middle portion of the selected leaves were cut and processed for rotary microtomy. Sections of 8-10 µm thickness were cut and permanent slides were prepared by following standard laboratory protocol. A total of 50 leaves (10 leaves from each site) were taken for this parameter. Anatomical characteristics of leaves, palisade cell size and vascular bundle size were studied from the selected slides.

Statistical analysis

Statistical analysis was performed by using SPSS 18.0 software.

Photomicrography

Photomicrographs were taken with the help of image analysis system.

RESULTS AND DISCUSSION

Leaves play an important role in survival and growth of the plant.

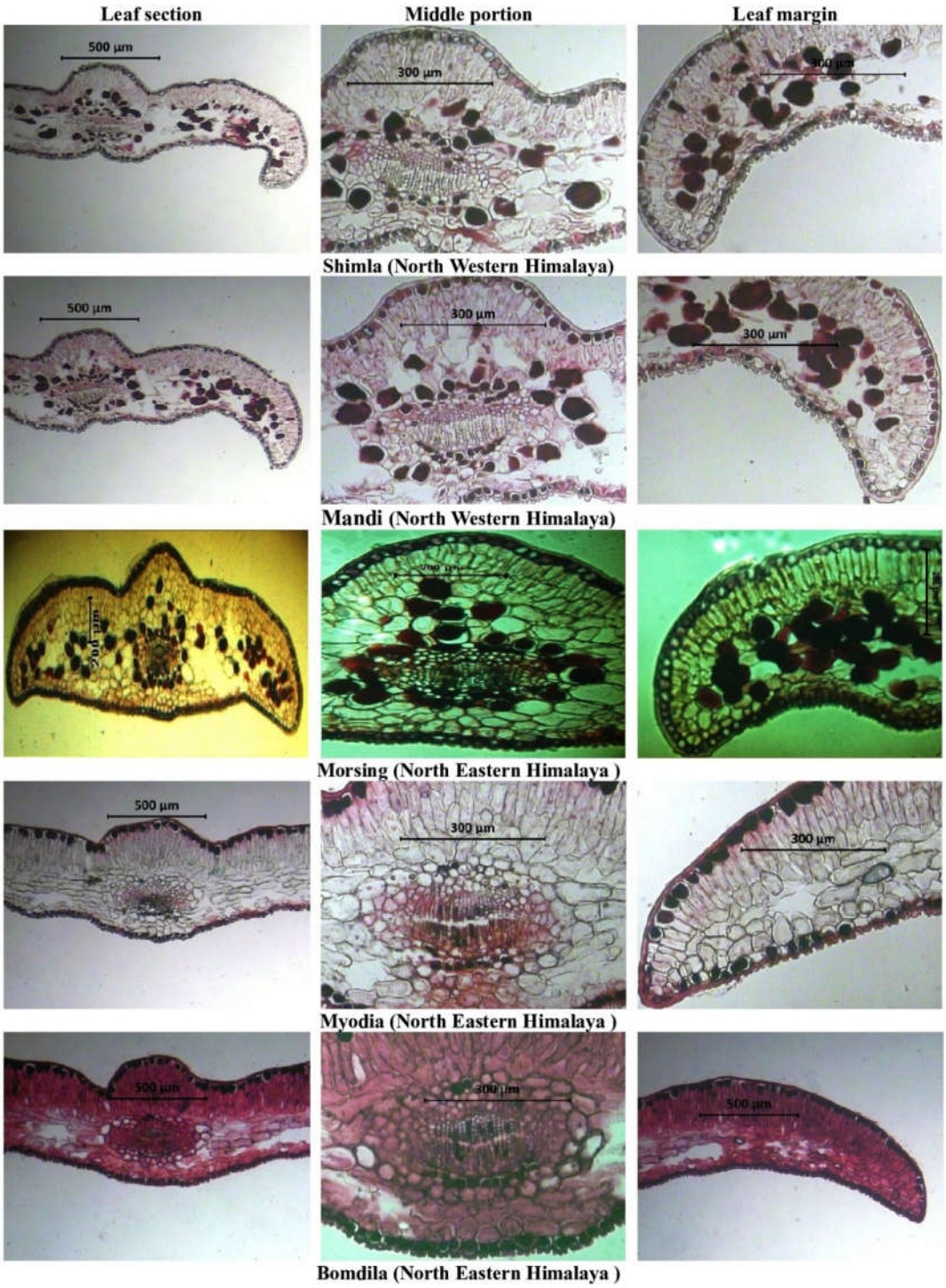

Fig. 2. T. S. of leaves collected from different sites of North East and North West Himalayas

The leaves of both NE and NW Himalayas were needle in shape. The leaves of NE Himalayas were larger in size than NW Himalayas (Table 2). According to Givnish (1984), the plants in the drier climate have smaller leaves to reduce evaporation and in more humid climates they have larger leaves because of available water. In the present study, the leaves of NE Himalayas were larger than NW Himalayas and is in agreement with the findings of Givnish (1984).

Anatomically, *Taxus wallichiana* Zucc. leaf was covered by epidermis on both adaxial and abaxial surfaces. The epidermal cells were smaller and without papillose on adaxial surface whereas the epidermal cells were larger and papillose on the abaxial surface. Some of the leaves of NW Himalayas were revolute along the margin and no differentiation in the cell size of upper and lower epidermal was observed .The stomata were hypostomatic. Below epidermis, mesophyll was differentiated into palisade and spongy parenchyma.

Palisade parenchyma cells were elongated and arranged in 1-3 rows beneath adaxial epidermis. The spongy parenchyma cells were more or less oval in shape and loosely arranged. There was only one vascular bundle in the mid rib region and surrounded by vascular bundle sheath. The vascular bundle was collateral type having xylem on the upper side and phloem on lower side. A few cells of transfusion tissue were also present on the side of xylem and phloem.

The anatomical study of leaves is in agreement with the previous anatomical studies (Hamidipour *et al.,* 2011). In the present study, the parenchyma cells were filled with dark red or yellowish brown deposits in both epidermal layers, vascular bundle sheath and cells of spongy parenchyma in the leaves of both sites of NW Himalayas and in one site (Morsing) of NE Himalayas (Fig. 2.), While, these pigmented cells were found absent in vascular bundle sheath and spongy parenchyma of other selected sites of NE Himalayas. The stomata were arranged in 8-11 rows between leaf margin and mid rib in leaves of NW Himalayas while these were 13-17 rows in leaves of NE Himalayas (Fig. 1). The available literature reveals that plants growing in more stressful environment conditions have more stomatal density (Dinis *et al*., 2011).

On contrary to this, Locosseli and Ceccantini (2012) report higher stomatal density and stomatal index in *Podocarpus lambertii* growing in warmer and drier climates than individuals growing in colder and rainier site. The results given in Table 2 shows that stomatal density in selected sites of NW Himalayas were more or less same. On the other hand, the maximum stomatal density was observed in Morsing (39.00±2.94) among selected sites of NE Himalayas. There was significant difference in stomatal density of both region (Table 3). The difference in stomatal density may be due to larger size of leaves of NE Himalayas. There was not much variation in number of palisade layers of both regions. However, the palisade cell size varied from 33.93 ± 7.04µm to 34.84 ± 5.99 µm in selected sites of NW Himalayas and from 58.50 ± 1014µm to 68.77 ± 15.07µm in NE Himalayas (Table 2). Likewise, the size of vascular bundles varied from 244.20±12.67µm to 256.83 ± 9.41µm in NW Himalayas and 309.46 ± 10.12µm to 425.13.10µm in NE Himalayas (Table 2). There was significant differences in palisade cell size and vascular bundle size of both the regions (Table 3). The significant differences in these parameters may be due to ecological adaptation of the plant to different climatic conditions.

Conclusions

The present study revealed uniform anatomical structure of leaves of both NE and NW Himalayas. Morphologically the leaves are of same shape.

The leaves of NE Himalaya are larger in size. Anatomically, the number of palisade layer, stomatal bands, stomatal density, size of palisade cells and vascular bundles are greater in NE Himalayas than NW Himalayas, which might be due to differences in ecological conditions.

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