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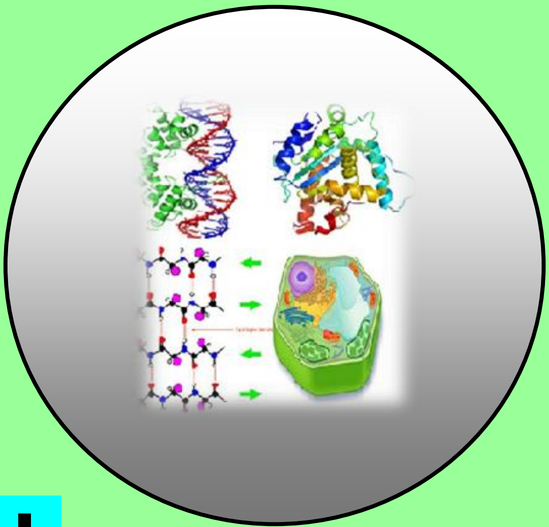

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RESEARCH PAPER

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Altitudinal Influences on Leaf and Wood Anatomy and its Ecological Implications in *Cephalotaxus griffithii* of Indian Himalayas

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ABSTRACT

A comparative study was conducted on leaf and stem anatomy of Cephalotaxus griffithii Hook. Collected from different regions of Himalayas. Several morphological and anatomical characters were investigated for both leaf and stem and it was found that these characters varied with altitudinal gradient, all probably xerophytic adaptations due to low temperature as we move to higher altitudes.

The important findings include difference in leaf shape, leaf margins, leaf venation, leaf width, leaf thickness, cuticle thickness, epidermis width and thickness, thickness of palisade parenchyma and spongy parenchyma, vascular bundle length and width, resin canal width and length and wood characters like number of leaf bases, presence or absence of resin duct, difference in the structure of axial tracheids, axial parenchyma etc.

These differences in leaf and stem anatomy strongly point out to the fact that altitude has a strong role in determining changes or alterations in morphological as well as anatomical characters since gradient in altitude leads to temperature variations and other related climatic and edaphic conditions. So the present paper aims to elucidate ecological adaptations by C. griffithii Hook. In response to stressed environmental conditions.

Keyword: Cephalotaxus, Himalayas, Xerophytic Adaptations, Parenchyma, Resin Canal, and Altitudinal Effect.

INTRODUCTION

Gymnosperms represent an ancient seed-plant group that originated approximately 300 Ma in the late Carboniferous (Kramer and Green 1990; Bowe et al. 2000). It is accepted that in terms of diversity, gymnosperms have manifestly been outdistanced by angiosperms (Brodribb et al. 2012). They are confined almost exclusively to high latitudes and high elevations and have been virtually eliminated from tropical regions (Bond 1989; Enright and Hill 1995; Coomes et al. 2005; Farjon 2008; Lusk 2011). Recent analysis of molecular data show that all living gymnosperms are monophyletic (Bowe et al. 2000; Chaw et al. 2000; Ran et al. 2010) and are significantly diverse and can be divided into four groups recognizable at divisional or subclass levels: conifers, cycads, ginkgophytes and gnetophytes.

Conifers of the Northern Hemisphere comprise mainly cone-bearing, long-living trees and shrubs, typical of mountainous and boreal environments (Farjon 2010). Tropical and southern conifers, in contrast, show a much larger morphological and ecological diversity (Enright and Hill 1995; Farjon 2008). Conifers are by far the largest gymnosperm group comprises 70 accepted genera, 6 families with 615 species and show a cosmopolitan distribution (Farjon 2008, 2010; Eckenwalder 2009; Debreczy and Racz 2011).The family Cephalotaxaceae of Coniferales has medicinal importance and is represented by a single genus *Cephalotaxus* with 20 species. It is restricted to southern and eastern Asia, distributed in Japan, Korea, south, central and eastern China, Hainan, Taiwan, India, Myanmar, Laos and parts of Vietnam.

For any floral diversity altitudinal gradients are among the most powerful natural experiments for testing ecological and evolutionary responses of biota to geophysical influences, such as low temperature. However, there are two categories of environmental changes with altitude: those physically tied to meters above sea level, such as atmospheric pressure, temperature and clear-sky turbidity; and those that are not generally altitude specific such as moisture, hours of sunshine, wind, season length, geology and even human land use. Korner (2007) proposed the altitude-related theory of biological phenomenon, which adversely affected the plant communities like reduction in plant species number (Nagy 2003), plant productivity (Luo et al. 2004), body or organ size trends (Fabbro and Korner 2004), plant physiology and morphology (Hoch and Korner 2003), gene ecology (Reisch et al. 2005) and life history characteristics (Klimes 2003).

Cephalotaxus has been an intriguing plant right from the beginning, one because of its taxonomic ambiguity and secondly because of its uniqueness as far as anatomy and embryology is concerned. The plant is also economically important because it yields number of alkaloids that can be used as alternative source of Taxol, an anticancerous drug obtained from *Taxus*. The survey indicated that the plant occurs in very few numbers in Northern and North-eastern Himalayas and therefore there is need to increase its area of plantation. Further, conservation strategies can only be planned and executed if one is well aware of extent of diversity within and between the populations of a species. It has already been seen that in certain conifers such as in *Pinus* (Sinha et al. 2015) the genetic variations may be due to altitudinal gradient related to climatic change.

In the present study we described morphological and anatomical variations observed in the leaf and stem of *Cephalotaxus griffithii* Hook. Growing at different altitudes in various regions of Himalayas. The paper also aims to elucidate ecological adaptations by *C. griffithii* Hook. in response to stressed environmental conditions.

MATERIALS AND METHODS

Sample collection

The plant material from different plant samples were collected from three different altitudes. Darjeeling (altitude- 6982 feet, lat-27.0500 º N, long-88.2667º E), West Bengal, Shillong (altitude- 4908 feet, lat-25.5667 º N, long-91.8833º E), Meghalaya in the North-eastern Himalayan region. Beside the North-eastern Himalaya leaves and stem of same species were also collected from Forest Research Institute, Dehradun (altitude- 2200 feet, lat-25.5667 º N, long-91.8833º E), Uttarakhand, India (Fig.1-Map). This vast region has variable topography, climate, soil and vegetation. All the climatic datasets of different altitudes were obtained from the Indian Meterological Department, New Delhi (Table-1). For the purpose of study number of tree were selected. Species was identified with the help of available literature and existing herbaria in National Botanical Research Institute (NBRI), Lucknow.

Preparation of slides

For morphological observations 20 needles were randomly selected from each tree. The length and width of leaves were measured by the conventional method. Freshly collected materials were fixed in formalin/acetic acid/alcohol (FAA) fixative for one week and then preserved in 50% alcohol. Sections of leaf and wood were then prepared (by hand as well as microtome), dehydrated in an alcohol series, and then stained with safranin and fast green (Johansen 1940) and ultimately mounted in Canada balsam. Measuring of the dimensions of anatomical characters was performed by micrometre scale. For each anatomical character, number of preparation was made and different anatomical characters were analyzed.

Analysis

All microscopic slides were examined under a light microscope (Magnus-MLX-X) under 10X and 40X magnification and electronic image analysis equipment (Leica DM 1000).

Descriptive statistical analysis was made for all samples using SPSS and Statistica 11.0 statistical software. For the statistical analysis of leaf and stem, 10-15 specimens were studied. Unused plant materials, as well as microscopic preparations, are preserved in the herbarium of the Botany Department, Lucknow University, Lucknow.

RESULT

The present work attempted morphotaxonomical studies on stem and leaf of *Cephalotaxus griffithii* collected from various regions in Northern and Eastern Himalayas in order to see ecological implications on them.

Leaf

Leaves were dorsiventral, linear, flat, pointed, more or less of same size, falcate, subfulcate with rounded base, truncately rotundate or partly sub-cordately incised, shining green and whitish at abaxial surface, arranged in decussate and distichous fashion, margins were slightly revolute towards inside. In the mid rib region a keel like structure called hump was present.

The cross sections of the leaf revealed three layers: adaxial epidermis, mesophyll and abaxial epidermis (Fig. 2 a, b, c, d, e, f). Both upper and lower epidermises of the leaf were well developed and covered with cuticle. Cuticle was thick and penetrated toward the border between the cells. Upper epidermal cells were usually square to rectangular shaped and single layered with thick walls and usually with small lumen.

Inside the epidermis stomata were present that were retracted in comparison with the surface of the epidermis. Layers of mesophyll were well developed and made of spongy and palisade tissue. Palisade parenchyma cells were cylindrical in shape present in a single layer below the adaxial epidermis of the leaf. Spongy parenchyma on the other hand was made of round cells, and the intercellular space could be observed in between. Vascular bundle circumfused with bundle sheath that is compact, xylem on the adaxial surface, phloem on the abaxial surface. The resin canal was well developed and was located on the abaxial side of the vascular bundle. (Fig. 2 b, d, f).

Apparently not many variations were observed among the leaves of different accessions. However certain discrepancy in morphological and anatomical characters was observed.

Stem

The young branchlets were green, prominently grooved while the old ones were brown and smooth having decussate and distichously arranged leaves. In the anatomical layout of stems in a cross section collected from three different altitudes included outermost single layered epidermis with number of folding due to long leaf bases, covered with cuticle. Hypodermis with thick wall, cortex with large parenchymatous cells and small tanninferous cells. Stone cells were in group of 2 or 3 in cortical region (Fig. 3 a). In wounded stem resin patches had been found in cortex. Resin ducts were present in centre in the pith region in younger stem. In old stem the growth rings were distinct (Fig. 3 a) with polygonal cells. Rays were distinct, broad, uniseriate with unpitted horizontal wall. Axial parenchyma was squarish or polygonal in shape. Axial parenchyma present with smooth transverse wall and show less pitting or may be absent (less visible) and diffused around the parenchyma rays, rays were 1-15 cells high (generally 2–4 cells are visible in abundance) (Fig. 4 a, c, e) in tangential longitudinal section. They also contained some living material. Ray cells were more elliptical as compared to that of other species, whereas ray tracheids were absent. Pitting was present on radial walls. Axial tracheids were long and broad, distantly placed, cupressoid type and uniseriate in nature. In radial longitudinal section, rays were more elongated and indenture was absent. Cross-field pitting varied from 1 to 3 (mainly 1–2, rarely three) (Fig. 4b, d, f). Cupressoid and taxodioid both type of cross-field pits were present. Spiral thickenings were present on radial as well as on tangential walls (Fig. 4a, b, c, d, f).

In cross section, sieve cells, phloem parenchyma cells form continuous tangential rows of one cell in width respectively, which occur alternately. Sclerenchyma cells also form continuous tangential rows, each with a radial width of 1-4 cells. The interval between the rows was rather wide. The type of phloem fiber and the quantity of sclereids may serve as the characters for taxonomic identification of species. The secondary phloem of this genus contained more or less crystalliferous parenchyma cells, in the inner tangential walls of which calcium oxalate crystals are embedded. This character has not been reported in the other families and the genera of conifers.

DISCUSSION

A general observation suggested uniformity throughout the morphological and anatomical structure of leaves and stem belonging to trees growing at different altitudes of the Himalayan region; Dehradun (2200 feet), Shillong (4908 feet) and Darjeeling (6982 feet).

However what was interesting was the variations in morphological and anatomical characters of stem and leaves like leaf shape, leaf margins, leaf venation, leaf width, leaf thickness, cuticle thickness, thickness of epidermis, epidermis width, thickness of palisade parenchyma and spongy parenchyma, vascular bundle length and width, resin canal width and length and wood characters like number of leaf bases, presence or absence of resin duct, difference in the structure of axial tracheids, axial parenchyma, that were studied in detail to assess structural changes with respect to altitude. Though gymnosperms are considered to be conservative as far as their structure or reproductive morphology is considered but fascinatingly certain changes had been observed.

The tree of *C. griffithii*, which inhabits a range of altitudes seen to get acclimatized to the ecological conditions of the habitat and therefore represent structural variations within its accessions. At low altitudes it had been observed that leaf became broader while at high altitude the leaf area reduced for control of transpiration. Comparing leaf anatomy of *C. griffithii* from different altitudes it was observed that the epidermis width was approximately similar in all the three altitudes whereas the significant difference in thickness of the epidermis and cuticle had been observed as both were increased with increasing altitude, an adaptation to reduce transpiration. (Fig. 2 a, b, c, d, e, f) (Table-2).

Stomatal position and number, size of resin ducts in leaves show a direct response to environmental factors. Stomata at low altitude were more clearly visible than at higher altitudes (Fig. 2 e, f) (Table-2) It was observed that the area of resin duct decreased with increasing altitude both in leaf and stem of almost same year plants probably because at higher altitude these plants encounter less pathogenic threats and resin ducts may be important surrogate measures of tree investment in defense since they are responsible for the production and storage of oleoresin (Hodges et al. 1985 ; Franceshi et al. 2005). At lower altitudes, there were few mechanical cells around the vascular bundle (Fig. 1a, b) while with higher altitude the mechanical cells became thicker and more in number (Fig. 2e, f). The thickness of palisade tissue decreased with increasing altitude because of high intensity of sun and dorsiventrality. Spongy tissue thickness and vascular bundle width was similar in all the three altitude but significant difference had been observed in thickness of vascular bundle as thickness increased with increasing altitude (Fig. 2a, b, c, d, e, f) (Table-2).

It has been earlier reported that leaf traits are often affected by the ecosystem's characteristics, as they are directly exposed to the sunlight however their morphological and anatomical features also depend on abiotic factors. Physical factors like growth, altitude and decrease in air, temperature, atmospheric pressure, increasing precipitation and wind velocity affect plant growth (Friend and Woodward 1990; Körner 2007) while P'yankov et al. (2001) suggested that leaf characteristics, such as area, thickness, number of cell layers or specific leaf weight may increase, decrease and even not change with altitude. At very high elevation sites severe environmental conditions also became severe for plant development and growth.

While comparing the stem of all the three regions it was found that almost same year wood show difference in thickness with increasing altitude. Stem thickness, stone cells and tannin cells decrease with increasing altitude while intercellular spaces increase when we move to higher altitude (Fig. 3a, b, c, d, e, f).

Tangential longitudinal section of stem of three altitudes showed that although in all three regions medullary rays were uniseriate but the number of cells increase with increasing altitude and in radial longitudinal section of stem cross field width decreases with increasing altitude (Figs. 3a, b, c, d, e, 4a, b, c) (Table-2). In contrast no significant changes in stem anatomy had been observed by Pande et al. (2005) with change in altitude in eastern Himalayan region.

The results of descriptive statistics of anatomical characters of leaf and stem of *C. griffithii* have shown that mean values change with a change in altitude; however, there is no obvious regularity related to these changes. The present work on comparative leaf and stem anatomy of *C. griffithii* inhabiting the three different altitudes of Himalaya is being reported for the first time.

Environmental conditions such as temperature and altitude have a considerable effect on morphological and anatomical characters of leaf and stem since they directly affect the physiological functioning of the plant. The variations observed in present study may be due to the diverse distribution area with varied climatic conditions or ecological changes or may be due to edaphic changes which still need to be proved and can be justified by looking at the stress level to which plants are exposed as they grow at different altitudes.

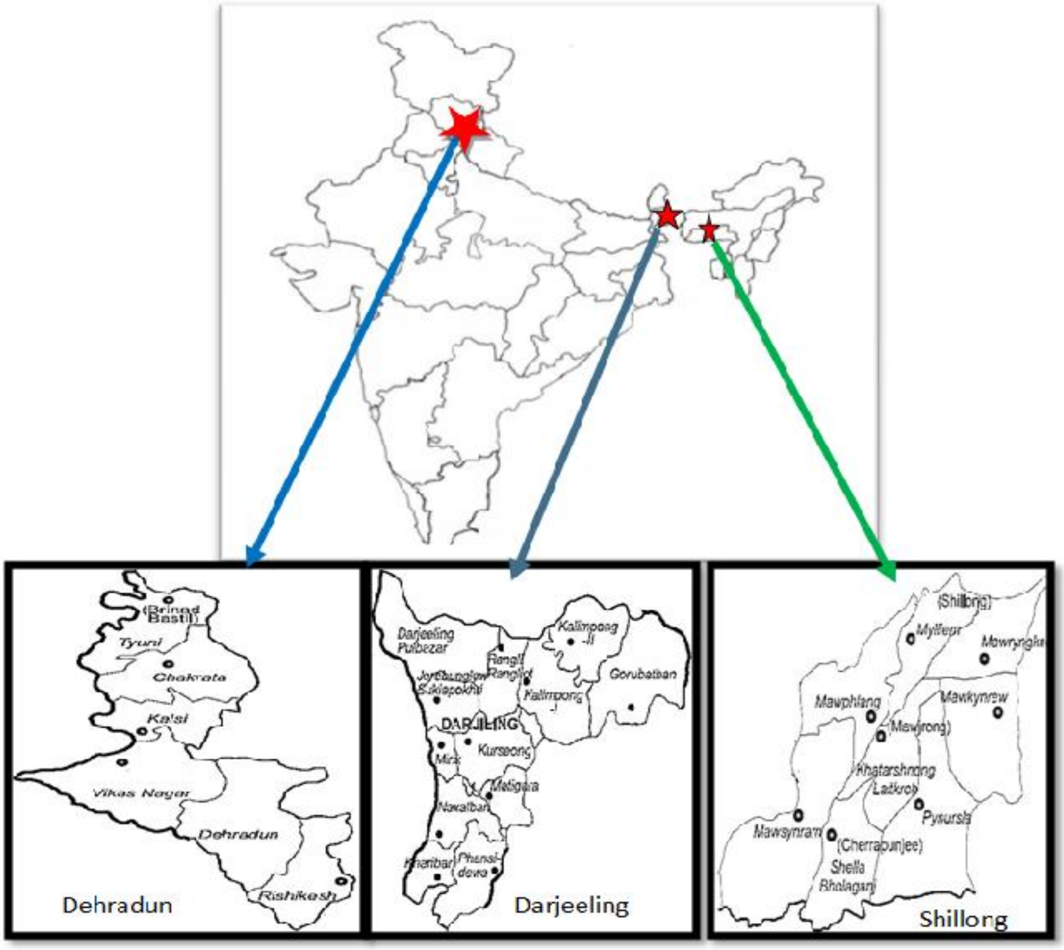


Fig. 1 Map showing the collection localities.

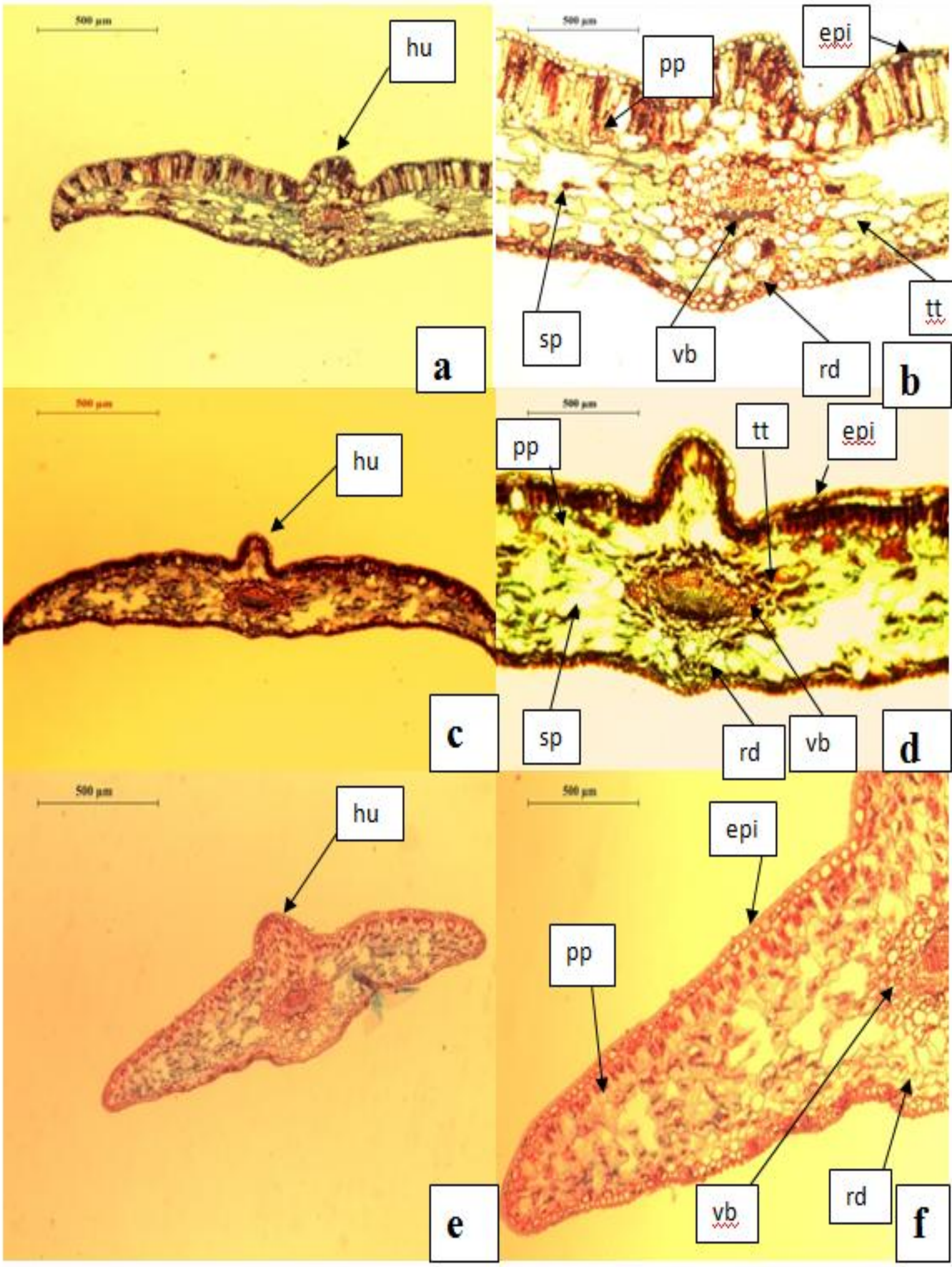
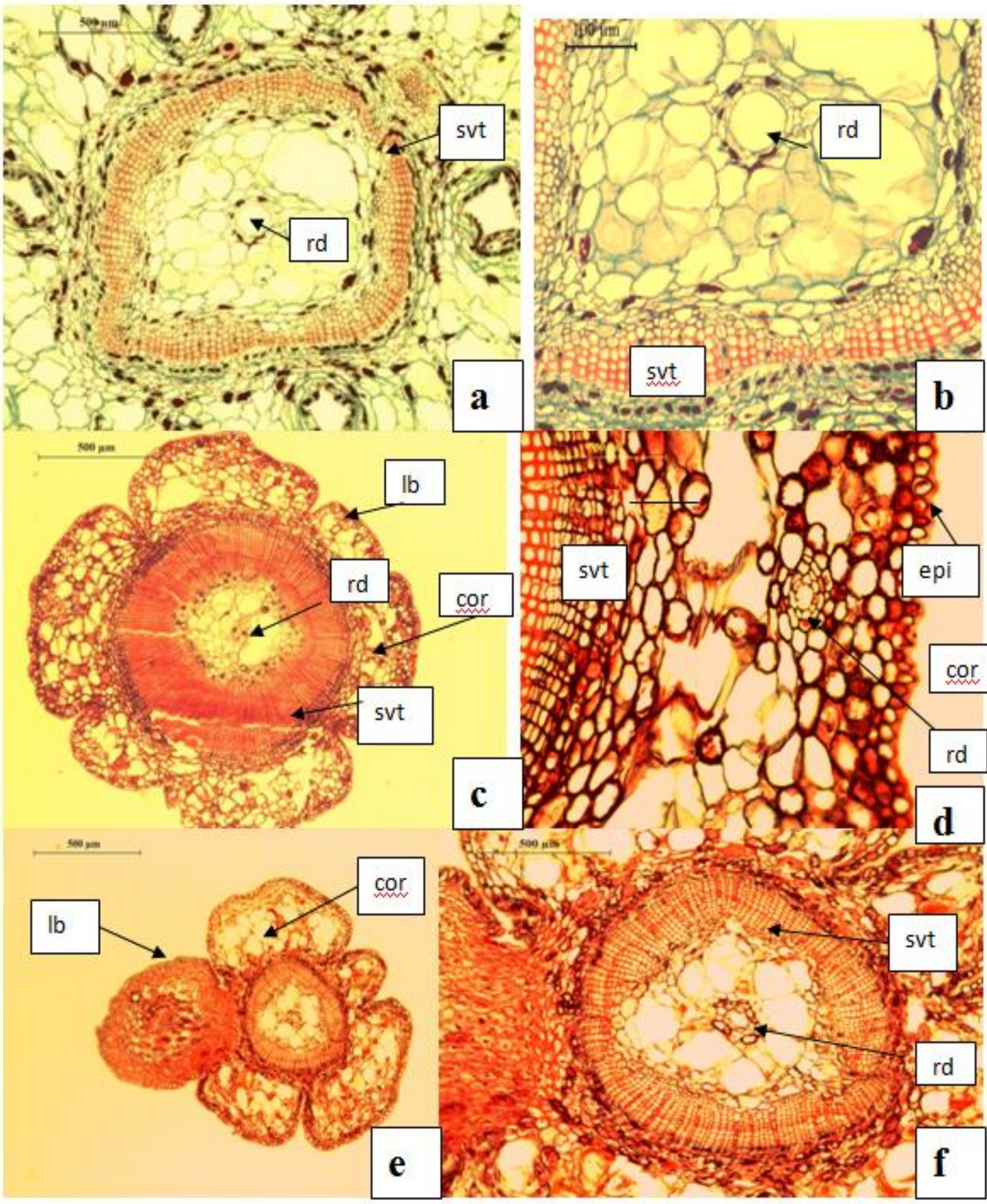


Fig. 2 Light microscopic photographs of leaves of *Cephalotaxus griffithii*. a , b *Transverse section* of leaf from Dehradun (altitude-2200 feet). c , d *Transverse section* of leaf from Shillong (altitude- 4908 feet). e , f *Transverse section* of leaf from Darjeeling (altitude- 6982 feet).



svt, secondary vascular tissue; rd, resin duct; lb, leaf base; epi, epidermis; cor, cortex

Fig. 3 Light microscopic photographs of *Cephalotaxus griffithii* wood. a , b *Transverse section* of stem at 2200 feet. c , d *Transverse section* of wood at 4908 feet. e , f *Transverse section* of wood at 6982 feet.

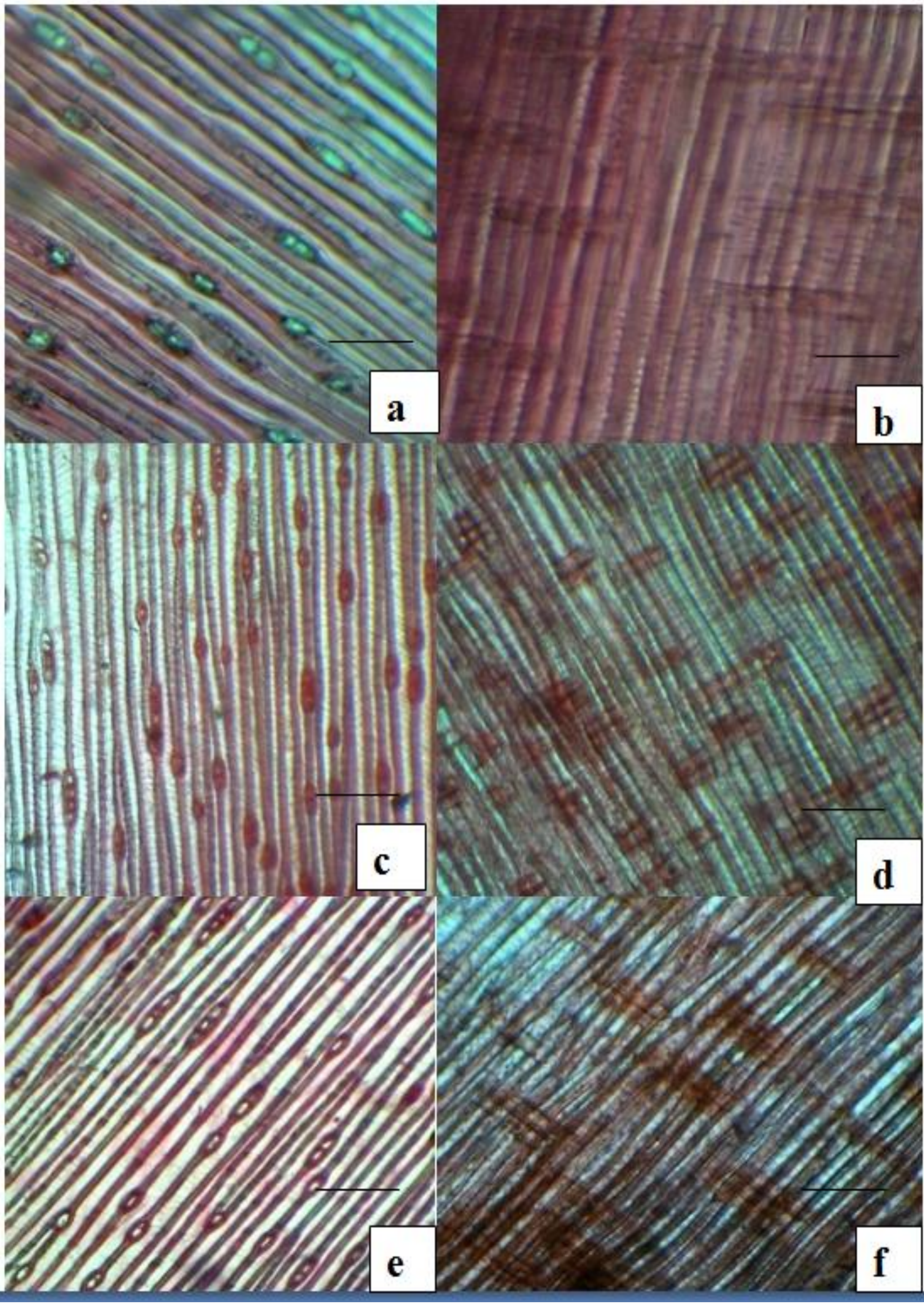


Fig. 4 Light microscopic photographs of *Cephalotaxus griffithii* wood. a, b at 2200 feet height (Scale bar 100 µm). c , d *Tangential longitudinal section* and *Radial longitudinal section* of wood at 4908 feet height. e, f *Tangential longitudinal section* and *Radial longitudinal section* of wood at 6982 feet height.

Table 1. Climatic dataset from all three sites showing temperature and average rainfall for the year 2015.

	Dehradun (Site-1)	Shillong (Site-2)	Darjeeling (Site-3)
Height(from sea level)	2200 feet	4908 feet	6982 feet
Summer Temperature	Max: 36 ^oC Min: 16 ^oC	Max: 23 ^oC Min:14 ^oC	Max: 20^oC Min: 8.5^oC
Winter Temperature	Max: 23 ^oC Min: 5 ^oC	Max: 22 ^oC Min: 4 ^oC	Max:6.0^oC Min:1.5 ^oC
Rainfall average	207 cm	295 cm	320 cm

Table 2. Variations in different anatomical traits of leaves from trees of *Cephalotaxus griffithii,* collected from various regions of North and East Himalayas.

Trait (unit)	Dehradun	Shillong	Darjeeling
CT (mm)	0.14±0.02	0.14±0.07	0.52±0.05
LW (mm)	13.14±0.20	11.60±0.25	8.40±0.08
LT (mm)	2.60±0.15	2.00±0.15	1.80±.08
EL (mm)	0.14±0.04	0.14±0.04	0.18±0.74
EW (mm)	0.25±0.11	0.27±0.08	0.30±0.08
PT T(mm)	0.58±0.25	0.28±0.08	0.12±0.04
VBW (mm)	1.54±0.05	1.56±0.07	1.62±0.10
VBL (mm)	0.70±0.07	0.51±0.08	1.08±0.08
STT (mm)	1.25±0.58	1.22±0.17	1.02±0.51
RDA(mm2)	0.20±0.05	0.18±0.07	0.12±0.02

Abbreviations of Characters

CT (mm) - cuticle thickness
LW (mm) - leaf width
LT (mm) - leaf thickness
EL (mm) - epidermis length
EW (mm) - epidermis width
PTT (mm) - palisade tissue thickness
VBW (mm) - vascular bundle width
VBL (mm) - vascular bundle length
STT (mm) - spongy tissue thickness
RDA (mm^2)-resin duct area

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