

Comparative morphological, epidermal, and anatomical studies of *Pinus roxburghii* needles at different altitudes in the North-West Indian Himalayas

Satyendra Prakash TIWARI*, Pradeep KUMAR, Deepika YADAV, Devendra Kumar CHAUHAN

Sahni Palaeobotany Laboratory, Department of Botany, University of Allahabad, Allahabad, India

Received: 01.10.2011 • Accepted: 11.09.2012 • Published Online: 26.12.2012 • Printed: 22.01.2013

Abstract: The aim of the present study was to understand the ecological adaptation of *Pinus roxburghii* Sarg. in the North-West Himalayan region. *P. roxburghii* needles showed morphological, epidermal, and anatomical variation at different altitudes. Needle length was negatively correlated with altitude. Stomatal characters like stomatal density, stomatal index, and guard cell lengths were found to be affected by environmental factors and showed a direct correlation with altitude. The results showed that potential conductance index was dependent on the climatic conditions of the habitat. The anatomical properties of needles exhibited variation from lower to higher elevation, especially in the number and position of resin ducts.

Key words: Ecological adaptation, *Pinus*, resin duct, stomatal density, stomatal index

1. Introduction

Chir pine (*Pinus roxburghii* Sarg.) is the dominant tree species in the North-West region of the Indian Himalayas. It is a hard pine of lower elevation, occurring between altitudes of 500 and 2500 m and is extensively distributed from Bhutan to Afghanistan. Chir pine grows gregariously, often forming pure formation in xerophytic and well-lit environments (Mehra, 1988). *P. roxburghii* is an economically valuable species, balancing the ecosystem of the Indian mountains. The plants show microhabitat-related morphological and anatomical features at different altitudes.

Körner (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 & Körner, 2004), plant physiology and morphology (Hoch & Körner, 2003), gene ecology (Reisch et al., 2005), and life history characteristics (Klimes, 2003).

Leaf traits are often affected by the ecosystem's characteristics, as they are directly exposed to the environment. In vascular plants the stomata of leaves are the most important physiological apparatus for photosynthesis and transpiration. The development of stomata (about 400 million years ago) is therefore considered a key event in the evolution of advanced land plants (Hetherington & Woodward, 2003). Stomatal

differentiation and development are determined by genetic factors (He et al., 1998). Stomatal initiation is controlled by the CO₂ HIC gene, setting stomatal number during leaf formation (Gray et al., 2000). Stomatal parameters are specific for a particular species but are affected by multiple ecological factors, including altitude gradient (Beerling & Kelly, 1996), atmospheric CO₂ concentration (Van de Water et al., 1994), temperature, light, and irradiance (Lockheart et al., 1998). Environmental effects on stomatal density, stomatal conductance, and stomatal index have been widely studied in living as well as in fossil plants (Woodward & Bazzaz, 1988; Royer et al., 2001; Kouwenberg et al., 2003). $\delta^{13}\text{C}$ and stomatal density are popular tools for determining palaeoatmospheric CO₂ level (Beerling et al., 1995). Morphological and anatomical features of *Pinus* needles also depend on abiotic factors (Fahn & Bemayoun, 1976; Schoettle & Rochelle, 2000). Physical factors like growth, altitude, decrease in air temperature, atmospheric pressure, increasing precipitation, and wind velocity affect plant growth (Friend & Woodward, 1990; Körner, 2007). At very high elevation sites severe environmental conditions become severe for plant development and growth. In the present study we describe morphological, epidermal, and anatomical variations observed in the needles of *P. roxburghii* growing at different altitudes. The paper also mentions ecological adaptation adopted by *P. roxburghii* plants in response to stressed environmental conditions.

* Correspondence: sptiwariau@yahoo.co.in

2. Materials and methods

Needles of *P. roxburghii* were collected from 3 different altitudes (1215, 1350, and 1775 m) in the Kumaun region. The Kumaun Mountains occupy the central sector of the Himalayas from lat 28°44' to 30°49'N and long 78°45' to 81°1'E (Figure 1). This vast region has variable topography, climate, soil, and vegetation. Besides the mountainous forms, needles of the same species were collected from a plant growing in the Department of Botany, University of Allahabad, Allahabad, Uttar Pradesh, India. Allahabad is situated in the upper Gangetic Plain of India. All the climatic datasets of different altitudes were obtained from

the Indian Meteorological Department, New Delhi (Table 1). For the purpose of this study 5 trees were selected at each site (98, 1215, 1350, and 1775 m). Thirty needles were selected from well-grown shoots of each tree.

For morphological observations 20 needles were randomly selected from each tree. The length was measured by the conventional method. Micro-slides were prepared by traditional methods and the technique proposed by Eo (2012). Transverse sections were stained with a combination of Safranin and Fastgreen (Johansen, 1940). All microscopic slides were examined under a light microscope (Olympus CH20i) and electronic image

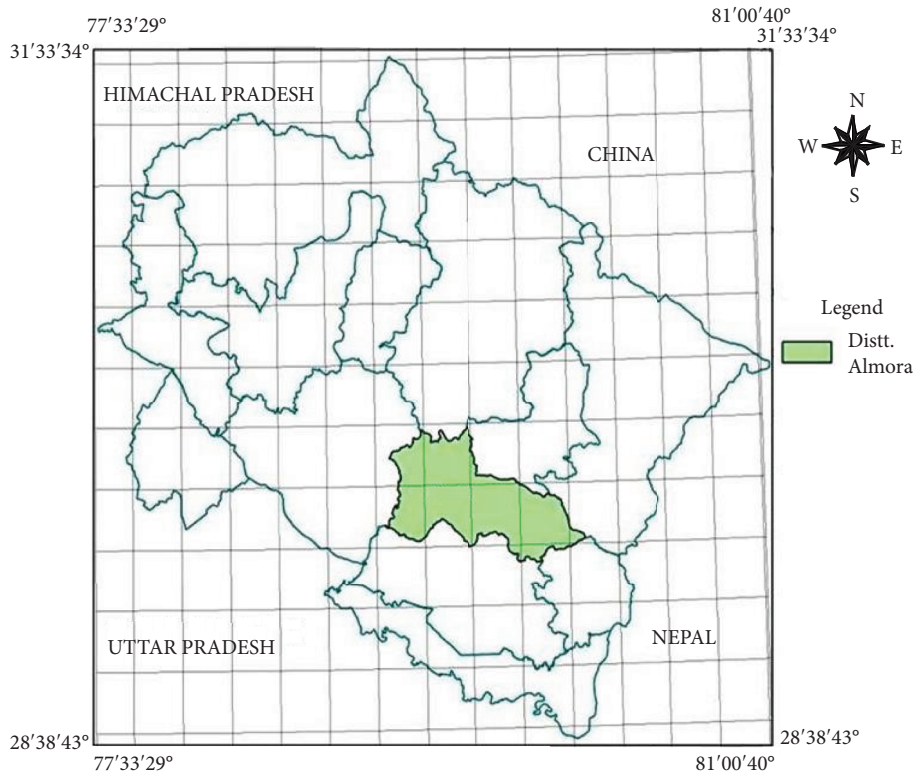


Figure 1. Location map showing plant collection site.

Table 1. Climatic dataset from all 4 elevations showing temperature, average rainfall, and relative humidity.

Altitude (m)	Temperature (°C)		Rainfall average (mm)	Relative humidity (%)
	Max (mean)	Min (mean)		
98	32.55	21.25	80.42	70
1215	28.27	15.22	648	85
1350	28.55	15.22	659	85
1775	25.62	12.35	1152	90

Temperature (maximum and minimum means of centigrade), average rainfall (millimetres), relative humidity (percentage).

analysis equipment (Leica DM 2500 and Motic 2.0 Image Plus).

Stomatal parameters, like guard cell lengths of 15 stomata, were measured at 40× (resolution 648 × 486, 1296 × 97) from each of the needles from all 4 elevations. Stomatal density was determined by the method of Hultine and Marshall (2001). Thirty needles were selected randomly for the stomatal count. The epidermis of the leaf was separated by maceration and stomatal count made from the middle part of the needles at 10× and 20× with the help of a Motic 2.0 Image Plus camera. The stomatal density, stomatal index, and potential conductance index were calculated with the help of the formulae given below as equations 1–3:

$$SD = SC/N_L \times N_w \dots\dots\dots (1)$$

$$SI = SC \times 100/SC + nEC \dots\dots\dots (2)$$

$$PCI = (\text{guard cell length})^2 \times SD \times 10^{-4} \dots\dots\dots (3)$$

PCI = Potential conductance index, SD = Stomatal density, SI = Stomatal index SC = Stomatal counts, N_L = needle length, N_w = needle width, nEC = number of epidermal cells.

All the statistical analysis was performed with the help of SPSS v. 10.0 and STATISTICA 11 software; graphs were prepared using Origin 6.1.

3. Results

3.1. Morphological analysis

In this study, the needle morphology of *Pinus roxburghii* was affected by the altitude gradient. Length of needles from different elevations was measured and the needle length was negatively correlated (r = 0.9635, P = 0.0364) with altitude. Needle length at 98 m (N_L = 29.98 cm) was 2 times greater than that at 1775 m (N_L = 15.14 cm) (Table 2). Needle length was less variable at medium elevation, showing about 15% decrease from 1215 m to 1350 m compared to that of lower to higher elevation (20% needle

length decrease from 98 m to 1215 m, 24% decrease from 1350 m to 1775 m).

3.2. Epidermal analysis

Epidermis of *Pinus roxburghii* needles showed highly contrasting characters at different elevations. The distance between 2 stomatal rows significantly decreased in needles of plants from lower to high altitudes. At an elevation of 98 m the needles showed 13.61 rows of nonstomatiferous cells, which decreased to 4.80 rows of nonstomatiferous cells at an elevation of 1775 m. It was highly correlated (r = 0.9815) with altitude. Stomatal density increased due to decreases in the number of nonstomatiferous rows, as they are inversely correlated to each other. Stomatal density showed a positive correlation (r = 0.8284, P = 0.1716) with altitude (Figure 2). Stomatal density increased 59.46% from lower altitude to higher altitude. Stomatal index also showed a positive correlation (r = 0.8689, P = 0.1310) with altitude. The length of guard cells in needles of *P. roxburghii* was also affected by a change in elevation and it increased with altitude (Figure 3). Stomatal conductance depended on both stomatal density and size of stomatal aperture (Holland & Richardson, 2009). Potential conductance index was also measured and found to be significantly correlated (r = 0.8637, P = 0.1365) with altitude.

3.3. Anatomical analysis

Anatomical characters of *Pinus roxburghii* needles varied with altitude. Transverse sections of needles from all 4 sites were studied. They showed a thick cuticle and well-developed mesophyll tissue. The hypodermis was commonly 2–4 layered, showing less variation at 98, 1215, and 1350 m altitudes but showing much variation at higher elevations. The layer of hypodermis decreased and became single layered (Figure 4). As *P. roxburghii* is a hard pine, the vascular bundles numbered 2 and were situated close to each other, but needles from higher elevations showed vascular bundles located opposite each other.

Table 2. Variations in stomatal density, stomatal index, nonstomatiferous cells, needle length, potential conduct index, guard cell length, position of resin duct, and number of resin ducts.

Altitude (m)	Stomatal density Pore/mm ² (mean ± SD)	Stomatal index	NSCs between stomata (mean)	N _i (cm) mean ± SD	PCI	Guard cell length (µm) mean ± SD	Position of resin duct	Number of resin ducts
98	29.0 ± 2.127	4.208	13.614	29.98 ± 3.511	4.30	38.51 ± 2.444	EX	3
1215	37.75 ± 4.632	6.404	9.266	23.70 ± 2.101	7.71	45.22 ± 3.234	EX, SM	0, 1, 2
1350	43.40 ± 6.707	7.451	7.466	20.15 ± 1.889	9.57	46.96 ± 2.593	EX, M	2
1775	71.55 ± 8.140	12.451	4.800	15.14 ± 0.900	17.71	49.76 ± 2.351	M, ED	3

Stomatal density and guard cell length (data are mean of 15 replicates and standard deviation); NSC: Nonstomatiferous cells; N_i: Needle length; PCI: Potential conduct index.

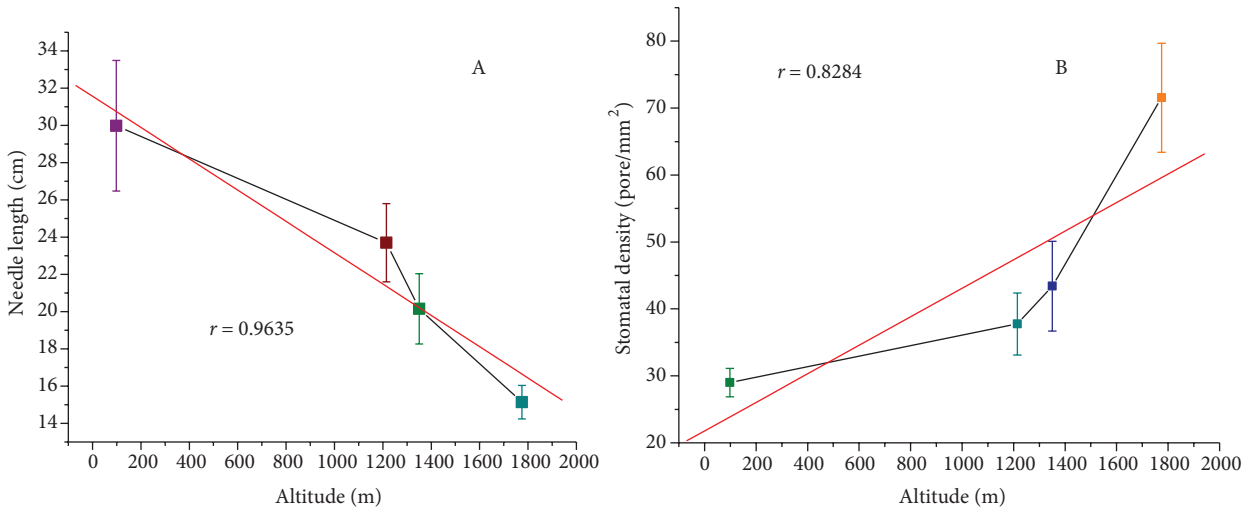


Figure 2. A- Negative correlation between needle length and altitude. B- Correlation between stomatal density (SD) and altitude.

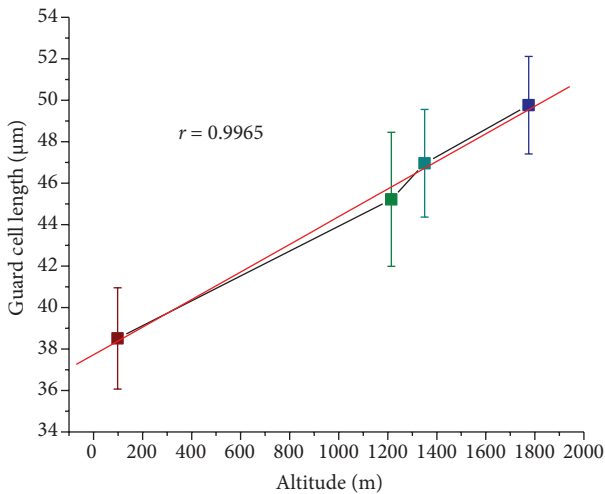


Figure 3. Correlation between guard cell length of stomata and altitude.

Pinus roxburghii had 2–3 resin ducts per needle, situated medianly and externally. At lower elevation (98 m) needles showed 3 resin ducts placed externally (Figure 4). At higher altitude (1215 m) the number of resin ducts varied from 0 to 2. These were situated slightly medianly and externally. Needles of plants at 1350 m altitude had 2 resin ducts, placed medianly and slightly externally. At higher elevation (1775 m), resin ducts numbered 3 but were endonal in position (Figure 4), being just the opposite of those at the lower elevation.

4. Discussion

There are complex ecological factors affecting plant growth at different altitudes, especially conifers, which are present over a range of elevated regions in the world. Altitude

plays an important role in changing the physical factors, decreasing total atmospheric pressure, and reducing atmospheric temperature with implications for ambient humidity (annual temperature decreases with elevation by about 6.5 °C per km), increasing radiation under a cloudy sky because of reduction in turbidity and a high fraction of radiation at any given total radiation (Körner, 2007).

Changes in epidermis structure in needles are an eco-physiochemical process. Environmental factors show a direct response on stomatal pattern in the epidermis of needles in the Kumaun Mountains. Epidermal features such as stomatal density, stomatal index, guard cell length, and potential conductance index (PCI) vary with altitudes. The present study shows that stomatal parameters play a significant role in the adjustment of plants at different altitudes. Stomatal arrangement responds to light intensity; an increase in light intensity results in an increase in stomatal index (Coupe et al., 2006). *P. roxburghii*, which grows on the eastward slopes of the Kumaun Mountains, receives more light intensity and light period than plants growing on the westward slopes. The stomata of *P. roxburghii* are more affected by the light intensity. Körner (1988, 1999) suggested that the elevation changes the stomatal density due to the effect of foliar light interruption. In the case of the first type of mountains, insulation increases at higher elevation because shorter atmospheric path length reduces scattering and absorption. In the case of the second type of mountains, the frequency of cloud immersion increases with elevation and insulation decreases with elevation. The first described mountains show stomatal density that typically increases with elevation due to less scattering of light or a higher transpiration rate. The North-West Himalayan mountainous region has diverse topography; it is considered the first type of mountain system because

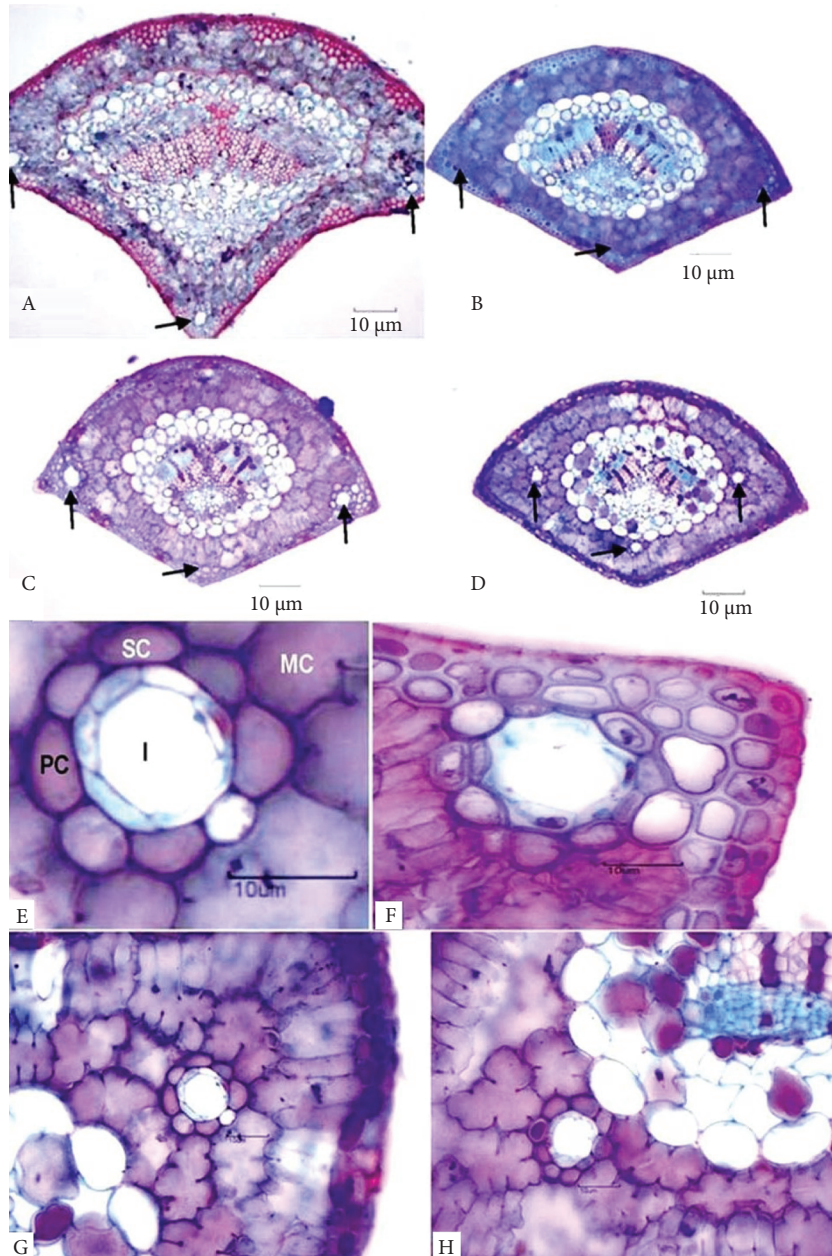


Figure 4. Transverse section of *Pinus roxburghii* needles. A- Needle section at 98 m, showing 3 external resin ducts, B- Needle section at 1215 m, resin duct absent, C- Needle section at 1350 m, 2 external resin ducts, D- Needle section at 1775 m, 3 resin ducts 1 medial and 2 endonal in position, E- Structure of a resin duct, F- External resin duct, G- Medial resin duct, H- Endonal resin duct. Abbreviations: EC = epithelial cell, SC = sheath cell, MC = mesophyll cell, L = lumen of resin duct, scale bar = 10 μ m.

the stomatal density of *P. roxburghii* increases with altitude. Stomatal density is the major measure that indicates gaseous changes. According to Woodward (1987), stomatal density and stomatal index decrease with increasing atmospheric CO_2 level both in geological time and under laboratory conditions. It is reported that CO_2 can directly affect stomatal differentiation (Lockheart et al., 1998) and that stomatal density is negatively correlated

with atmospheric CO_2 below 3000 m (Qiang et al., 2003) because CO_2 level decreases with increasing elevation.

Previous studies have shown increases in stomatal density, with elevation acting as a limiting factor in photosynthesis. Increases in stomatal density resulting in increasing stomatal conductance should offset the decreases in pCO_2 , but it is reported that such CO_2 availability is not a limiting factor (McElvain, 2004). Stomatal density

and size are considered as eco-physiological parameters because they conjugately influence stomatal conductance. According to Körner and Cochrane (1985), stomatal density did not reflect variations in stomatal conductance under an integrated influence of specific environment at higher altitude. Guard cell length in needles of *P. roxburghii* increases with altitude. Potential conductance index is significantly correlated from all elevation sites because stomata at higher altitude were not open entirely under severe environmental conditions such as low temperature and irradiation. Enhanced UV-B radiation at higher altitude limits stomatal opening normally and leads to decreases in stomatal conductance, but in the present study potential conductance index increased with altitude. Stomatal conductance might be constrained at higher altitude by low air and soil temperatures because they inhibit stem sap flow, which increases the water potential gradient and induces partial stomatal closure, leading to decreased stomatal conductance. Water availability is an important factor from lower altitudes to higher altitudes that affects stomatal size and stomatal conductance. Stomatal traits change with elevation due to response of water availability rather than CO₂ significance (Beerling & Kelly, 1996). Stomatal characters are modified by climatic changes, which is also a significant finding in the present study.

In the present study, *Pinus roxburghii* showed morphological traits negatively correlated with altitude. Length of needle decreased with higher elevations. Effect of altitude on morphology has been studied in *Pinus sylvestris* L. (James et al., 1994), *Pinus pumila* Regel. (Kajimoto, 1993), and *Pinus contorta* Douglas ex Loudon (Schoettle, 1990). They all have shown reduced leaf length, shoot growth, and leaf production per year with increasing elevation. Leaf structure is modified according to need in nature. Leaf morphology is affected by amount of $\delta^{13}\text{C}$ and drought. Generally air becomes drier with increasing elevation, but the diffusion coefficient of water vapour in air also increases at higher elevation; both phenomena affect the needle's morphology. A sharp increase in needles' $\delta^{13}\text{C}$ suggested a strong capacity for CO₂ assimilation, resulting in rapid plant growth. Length of needles also varies due to other environmental gradients like seasonal variation (Armstrong et al., 1988) and temperature. The present study reveals that the length of *P. roxburghii* needles is sensitive to the limiting factors in any given environment and shows morphological changes.

Anatomical properties like leaf structure, leaf shape, and cell distribution also change together with leaf function for adaptation to severe ecological conditions. Chir pine needles from all 4 elevation sites showed interesting anatomical variation. Epidermal cells were

smaller and narrower in plants at higher elevation sites than in those at lower elevation. Number of hypodermal cell layers decreased from lower to higher altitudes. The mesophyll cells showed a similar arrangement but differentiation in size because CO₂ concentration affected mesophyll cell structure and development (Lin et al., 2001). Leaf thickness increased due to well-developed mesophyll tissue. Elevation also affected light intensity, because the photosynthetic rate changed. Needle anatomy is also influenced by soil resource enrichment or nutrient availability in soil.

Transverse sections of *Pinus* needles were cut to study the structure and position of resin ducts. Resin ducts are a characteristic feature of conifers, occurring in vascular tissues and ground tissues of all plant organs. Resin ducts are also useful in the identification of species. The role of resin ducts in classification of *Pinus* is more appreciated compared to other polyphyletic traits, which are often treated as ambiguous. In the resin duct of *Pinus*, the duct cavity is surrounded by a thin layer of unligified cells, which are termed epithelial cells. Outside these are one or more layers of cells with relatively thick unligified walls, termed sheath cells. In *P. roxburghii*, resin ducts have a wide cavity, surrounding a layer of 6–7 thin and delicate epithelial cells. The sheath cells may vary in number from 8 to 12 and have thick walls (Figure 4). According to Napp-Zinn (1966) and Biswas and Johri (1997), 4 types of resin ducts are present in the needles of *Pinus*: 1) ducts in contact with the endodermis (i.e. endonal); 2) ducts in contact with the hypodermis (i.e. external); 3) ducts present in the middle of mesophyll layers (i.e. median); 4) ducts inside the bundle sheath (i.e. septal) (Figure 4). Most *Pinus* species contain 1 or 2 types of resin ducts in the needle. Needles of *P. roxburghii* generally show 2–3 external or medial resin ducts (septal resin duct is not seen) (Figure 4). Variation in the arrangement pattern of resin ducts in *P. roxburghii* at different altitudes was studied here for the first time. The number of resin ducts, however, does not vary from lower to higher elevation sites. The middle elevation (1215, 1350 m) sites show much variation (0, 1, 2). The most significant feature is the position of the resin duct, which varied from external (98, 1215 m) to medial (1215, 1350 m) to endonal (1775 m) at different elevations (Figure 4). The number of resin canals in *P. roxburghii* is controlled genetically because it does not vary in number but variation is seen in the position of the mesophyll. The change in position may be attributed to the change in altitude gradient and climatic factors.

Variations in resin duct patterns and changes in the number and size of mesophyll cells are reportedly affected by altitude (Sheue et al., 2003). Variations in the distribution and number of resin ducts in the plant body

are also affected by several genetic and environmental factors, such as height and age of the tree, nutrition, sunlight, radiation, temperature, wind, freezing, fire, insect attack, and phytohormones (Helmers, 1943; Fahn & Bemaoun, 1976). There is no significant change in the number of resin ducts with increasing elevation. Matziris (1984) reported that the number of resin ducts increases with increasing latitude. The evolutionary significance of the number of resin ducts is not well understood, but the relationship between them and the resistance of genotypes to specific insect attack has been reported (Overhulsen & Cara, 1981). Thus it is clear that altitude affects the position and number of resin ducts. Physiochemical changes adapted by plants may also have an effect but this requires detailed study.

5. Conclusion

Pinus roxburghii, a common wild species of conifer in the North-West Himalayan region, grows at a wide range of altitudes. It is the principle resin-producing species in India. The present study reveals that *P. roxburghii* is mainly affected by the altitude gradient or climatic factors. Trees native of lower elevations can be distinguished from trees of higher elevations on the basis of epidermal, morphological, and anatomical characters of needles. Stomatal density, stomatal index, and potential conductance index show significant variation at different altitudes due to environmental factors such as CO₂ concentration, light intensity, and water availability (Figure 5). Needle length is also affected by ecological factors; they are significantly negatively correlated with altitudes. The most interesting

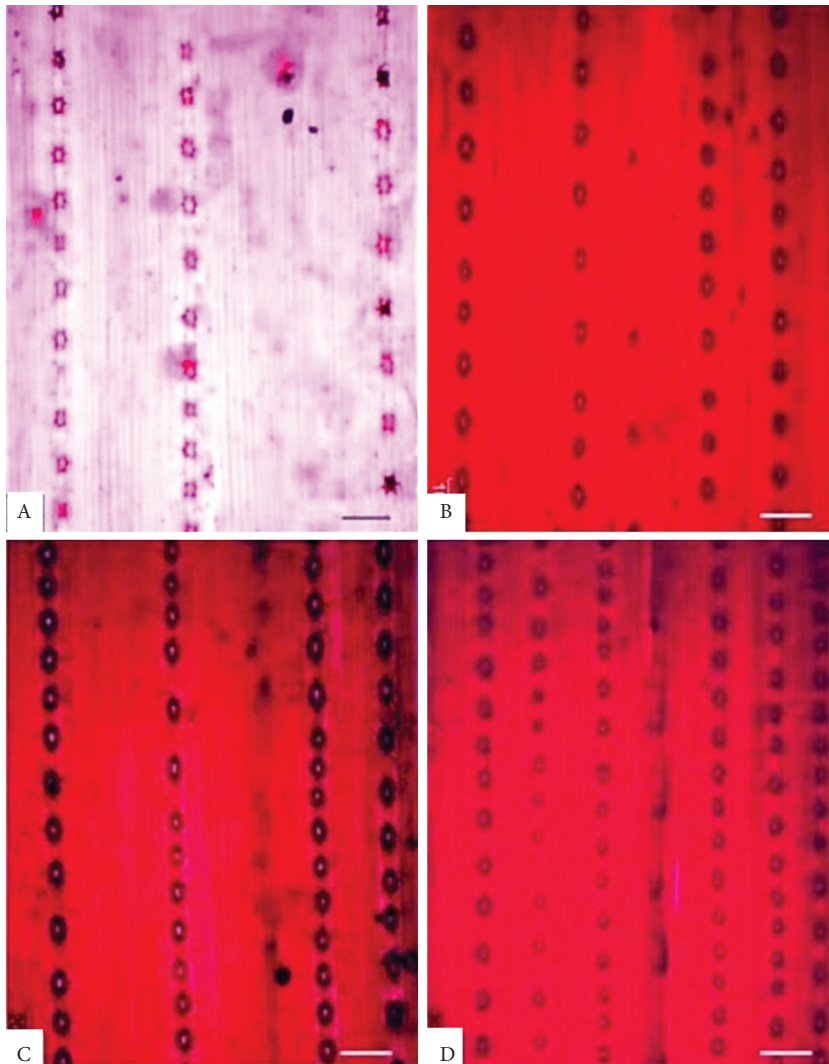


Figure 5. Array and distribution of stomata on epidermis of *Pinus roxburghii* needles. A- at 98 m height, B- at 1215 m height, C- at 1350 m height, D- at 1775 m height. Scale bar = 20 μ m.

feature shown, however, is the variation in the position of the resin duct (external, medial, and endonal) at different elevations, which is a genetically controlled characteristic in plant parts. Trees at lower elevation, with external resin ducts, can be distinguished from higher elevation trees, having medial and endonal resin ducts. Thus, we can conclude that altitude and environmental factors can affect the physiochemical process of *P. roxburghii* in the Indian Himalayan region.

References

- Armstrong JK, Williams K, Huenneke LF & Mooney HA (1988). Topographic position effects on growth depression of California (USA) Sierra Nevada pines during the 1982–1983 El Niño. *Arctic and Alpine Research* 20: 352–357.
- Beerling DJ, Birks HH & Woodward FI (1995). Rapid late-glacial atmospheric CO₂ changes reconstructed from the stomatal density record of fossil leaves. *Journal of Quaternary Sciences* 10: 379–384.
- Beerling DJ & Kelly CK (1996). Evolutionary comparative analysis of the relationship between leaf structure and function. *New Phytologist* 134: 35–51.
- Biswas C & Johri BM (1997). *The Gymnosperms*. New York: Springer-Verlag.
- Coupe SA, Palmer BG, Lake JA, Overy SA, Oxborough K, Woodward FI, Gray JE & Quick WP (2006). Systemic signalling of environmental cues in *Arabidopsis* leaves. *Journal of Experimental Botany* 57: 329–341.
- Eo JK (2012). A simple technique for cross-sectioning Gymnosperm needle leaves using microtome. *Turkish Journal of Botany* 36: 213–216.
- Fabbro T & Körner Ch (2004). Altitudinal differences in flower traits and reproductive allocation. *Flora* 199: 70–81.
- Fahn A & Benayoun J (1976). Ultrastructure of resin ducts in *Pinus halepensis* development, possible sites of resin synthesis, and mode of its elimination from the protoplast. *Annals of Botany* 40: 857–863.
- Friend AD & Woodward FI (1990). Evolutionary and ecophysiological responses of mountain plants to the growing season environment. *Advances in Ecological Research* 20: 59–124.
- Gray JE, Holroyd GH, van der Lee F, Sijmons PC, Woodward FI, Schuch W & Hetherington AM (2000). HIC: a gene involved in controlling stomatal development in responses to changes in atmospheric CO₂ concentration. *Nature* 408: 713–715.
- Helmers AE (1943). Ecological anatomy of ponderosa pine needles. *The American Midland Naturalist* 29: 55–71.
- Hetherington AM & Woodward FI (2003). The role of stomata in sensing and driving environmental change. *Nature* 424: 901–908.
- Hoch G & Körner Ch (2003). The carbon charging of pines at the climatic treeline: a global comparison. *Oecologia* 135: 10–21.
- Holland N & Richardson AD (2009). Stomatal length correlates with elevation of growth in four temperate species. *Journal of Sustainable Forestry* 28: 63–73.
- He X-Q, Lin Y-H & Lin J-X (1998). Research on correlation between stomatal density and variation of atmospheric carbon dioxide during a century. *Chinese Science Bulletin* 43: 860–862 (in Chinese).
- Hultine KR & Marshall JD (2001). A comparison of three methods for determining the stomatal density of pine needles. *Journal of Experimental Botany* 52: 369–373.
- James JC, Grace J & Hoad SP (1994). Growth and photosynthesis of *Pinus sylvestris* at its altitudinal limit in Scotland. *Journal of Ecology* 82: 297–306.
- Johansen DA (1940). *Plant Microtechnique*. New York: McGraw-Hill Book Co.
- Kajimoto T (1993). Shoot dynamics of *Pinus pumila* in relation to altitudinal and wind exposure gradients on the Kiso mountain range, central Japan. *Tree Physiology* 13: 41–53.
- Klimes L (2003). Life-forms and clonality of vascular plants along an altitudinal gradient in East Ladakh (NW Himalayas). *Basic and Applied Ecology* 4: 317–328.
- Körner C (1988). Does global increase of CO₂ alter stomatal density? *Flora* 181: 253–257.
- Körner C (1999). *Alpine Plant Life*. Berlin: Springer-Verlag.
- Körner C (2007). The use of 'altitude' in ecological research. *Trends in Ecology and Evolution* 22: 11.
- Körner C & Cochrane O (1985). Stomatal responses and water relations of *Eucalyptus pauciflora* in summer along an elevational gradient. *Oecologia* 74: 443–445.
- Kouwenberg LLR, McElwain JC, Kürschner WM, Wagner F, Beerling DJ, Mayle FE & Visscher H (2003). Stomatal frequency adjustment of four conifer species to historical changes in atmospheric CO₂. *American Journal of Botany* 90: 610–619.
- Lin J, Jach ME & Ceulemans R (2001). Stomatal density and needle anatomy of Scots pine (*Pinus sylvestris*) are affected by elevated CO₂. *New Phytologist* 150: 665–674.

Acknowledgements

The authors are highly grateful to Prof Nupur Bhowmik for going through the manuscript and giving her advice about completing this paper. We are indebted to the Head of Department for providing laboratory facilities. The authors are also thankful to Prof Manju Sahney for her valuable suggestions and all other members of the Palaeobotany and Morphology laboratory.

- Lockheart MJ, Poole I, Van Bergen PF & Evershed RP (1998). Leaf carbon isotope composition and stomatal characters: important consideration for palaeoclimate reconstructions. *Organic Geochemistry* 29: 1003–1008.
- Luo TX, Pan Y, Ouyang H, Shi P, Luo J, Yu Z & Lu Q (2004). Leaf area index and net primary productivity along subtropical to alpine gradients in the Tibetan Plateau. *Global Ecology and Biogeography* 13: 345–358.
- McElwain JC (2004). Climate-independent paleoaltimetry using stomatal density in fossil leaves as a proxy for CO₂ partial pressure. *Geology* 32: 1017–1020.
- Matziris DI (1984). Genetic variation in morphological and anatomical needle characteristic in the Black pine of Peloponnesos. *Silvae Genetica* 33: 4–5.
- Mehra PN (1988). *Indian Conifer, Gnetophytes and Phylogeny of Gymnosperms*. New Delhi: Rajbandhu.
- Nagy L, Grabherr G & Körner C (2003). Alpine biodiversity in space and time: a synthesis. *Alpine Biodiversity in Europe* 167: 453–464.
- Napp-Zinn K (1966). *Anatomie des Blattes. I. Blattanatomie der Gymnospermen*. Berlin-Nikolassee: Gebrüder Borntraeger.
- Overhulsen D & Cara RI (1981). Occluded resin canals associated with egg cavities made by shoot infecting *Pissodes*. *Forest Science* 27: 297–298.
- Qiang W, Wang X, Chen T, Feng H, An L, He Y & Wang G (2003). Variations of stomatal density and carbon isotope values of *Picea crassifolia* at different altitudes in the Qilian Mountains. *Trees* 17: 258–262.
- Reisch C, Anke A & Rohl M (2005). Molecular variation within and between ten populations of *Primula farinosa* (Primulaceae) along an altitudinal gradient in the northern Alps. *Basic of Applied Ecology* 6: 35–45.
- Royer DL, Wing SL, Beerling DJ, Jolley DW, Koch PL, Hickey LJ & Berner RA (2001). Paleobotanical evidence for near-present-day levels of atmospheric CO₂ during part of the Tertiary. *Science* 292: 2310–2313.
- Schoettle AW (1990). The interaction between leaf longevity and shoot growth and foliar biomass per shoot in *Pinus contorta* at two elevations. *Tree Physiology* 7: 209–214.
- Schoettle AW & Rochelle SG (2000). Morphological variation of *Pinus flexilis* (Pinaceae), a bird-dispersed pine, across a range of elevations. *American Journal of Botany* 87: 1797–1806.
- Sheue CR, Yang YP & Huang LLK (2003). Altitudinal variation of resin ducts in *Pinus taiwanensis* Hayata (Pinaceae) needles. *Botanical Bulletin Academia Sinica* 44: 305–313.
- Van de Water PK, Leavitt SW & Betancourt JL (1994). Trends in stomatal density and ¹³C/¹²C ratio of *Pinus flexilis* needles during last glacial-interglacial cycle. *Science* 264: 239–243.
- Woodward FI (1987). Stomatal numbers are sensitive to increases in CO₂ from pre-industrial levels. *Nature* 327: 617–618.
- Woodward FI & Bazzaz FA (1988). The response of stomatal density to CO₂ partial pressure. *Journal of Experimental Botany* 39: 1771–1781.