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Ecological Wood Anatomy of *Pinus roxburghii* in Central Nepal

Lajmina Joshi[1*] & Pratikshya Chalise[2]
[1] Tahachalmarg, Kathmandu, Nepal
[2] National Herbarium and Plant Laboratories, Godawari, Lalitpur, Nepal
[*] **Email:** lajmina@gmail.com

Abstract

Pinus roxburghii Sarg. is a gymnosperm tree belonging to the family Pinaceae having extensive distribution in Bangladesh, Bhutan, China (Tibet), India, Nepal and Pakistan. In Nepal, it is found in subtropical region at an elevational range from 500 to 2700 m asl. The aim of the present study is to determine the ecological variation in wood characters and non-anatomical characters such as diameter at breast height (DBH), tree height and altitude. Nine wood block samples were collected from the outermost part of the trunk of three matured canopy trees at three different localities between 1100 and 1350 m above sea level from central Nepal. Correlation and regression analysis were carried out to study variation in wood anatomical characters. Multiple regression analysis was done using non-anatomical factors as independent variables and anatomical features as dependent variables. Quantitative wood characters were found to be affected by change in ecological factors but not on its qualitative wood characters. Tracheids length as well as tracheid's pit significantly varies with altitude. A weak correlation was found between wood anatomical characters and non-anatomical parameters. Tracheids length exhibited negative correlation whereas tracheids pit exhibits positive correlation with altitude. Since tracheids are the main conducting tissues in xylem of pines, decreasing length and increasing pit size of tracheids is of ecological importance. This study will help in identifying how wood characters are adapted in response to change in ecological factors as well as help to predict ecological factor disturbances.

Keywords: Ecological adaption, Ecological parameter, Wood structure

Introduction

Pinus roxburghii Sarg. (Local name - Khote Salla, Common name - Chir pine), a very tall, 50 m in height and over 1m in diameter at breast height is a coniferous evergreen softwood tree. It has an extensive distribution in Bangladesh, Bhutan, China (Tibet), India, Nepal and Pakistan (Rajbhandari et al., 2020). In Nepal, it is distributed in the subtropical region at an altitude ranging from 500 to 2700 m above sea level (asl). *P. roxburghii* also has a rich history of utilization in folk remedies by various ethnic groups to treat diverse ailments (Kaushik et al., 2013).

Wood anatomy of *P. roxburghii* has been described by many scientists (Greguss, 1955; Person & Brown, 1988; Rajbhandari et al., 2020; Suzuki & Noshiro, 1988) in different aspects. Wood structural features are of great use in ecology. Schweingruber (1996, 2001) has added a vast amount of knowledge in this field by publishing a book entitled "Dendroecological wood anatomy" and also suggested the impact of lightning on tree growth. According to Kucera et al. (1985), lightning causes rapid water loss in wood, which causes the cells in the most recently formed tree-ring to collapse. Hartig (1897) has already shown that lightning cause callus formation, scars, traumatic resin duct formation and collapsed cells in the trees. Burkhalter (1988) has reconstructed the dynamics of leaning trees through cross-dating compression wood in tree-rings to determine the impact of extreme winds.

Very narrow or absent tree-rings were used to identify forest fires due to crown damages that have interrupted radial growth (Ortloff, 1996). Several studies have been conducted on these pine species from different parts of the Himalayas. Dendrochronological studies have also been carried out to determine the impact of climate change on growth of *P. wallichiana* (Bajwa et al., 2015). Similar work was conducted on *P. roxburghii* to understand age, structure, soil erosion, disturbance history and tree health (Speer et al., 2016). Similarly, studies have been carried out to study the growth performance and tree ring structure of *P. roxburghii* with the changing climate (Bhattacharya et al., 1992; Bhuju & Gaire, 2012; Tiwari et al., 2020). But very few studies haves been done in ecological aspects with regard to gymnosperms. Variation in wood anatomical features have been analyzed in angiosperms with several ecological factors such as macroclimatic divisions, moisture availability, habit or phenology in genera, families or woody floras: for example, *Ilex* (Baas, 1973a), *Symplocos* (Oever et al., 1981), Oleaceae (Baas et al., 1988), and Rosaceae (Zhang et al., 1992). Similarly, woody floras of south California (Carlquist & Hoekman, 1985), Europe (Baas & Schweingruber, 1987) and Israel and adjacent regions (Baas et al., 1983; Fahn et al., 1985) has been published. All these studies have explained the dependence of wood anatomical features upon several ecological factors and so are not often useful phylogenetically.

Nepal, the Himalayan country of Asia is very rich in woody flora and has a wide altitudinal range within the same monsoon climatic zone. Therefore, Nepal could be an ideal site for the study of ecological wood anatomy in relation to altitude. Ecological wood anatomy of angiospermic plants like *Alnus nepalensis* (Joshi, 2000; Noshiro et al., 1994), ecological wood anatomy of *Rhododendron* (Noshiro et al., 1995), wood anatomical variation in *Rhododendron* (Pandey et al., 2020; Pathak et al., 2018) along altitudinal gradient have been carried out. But, studies on ecological wood anatomy of gymnospermic plants like *Pinus* have not been carried out yet. Since *P. roxburghii* is a commercially important species in the Himalaya and is extensively used for timber, turpentine and several medicinal and cultural purposes (Siddique et al., 2009; Tiwari, 1994), it becomes very crucial to study the ecological adaptation as well as the underlying features of this species. Therefore, the present study was carried out to highlight the relationship of wood structure with ecological factors such as altitude, height, diameter at breast height (DBH) and also to examine its effect on qualitative and quantitative wood characters.

Materials and Methods

Study area

The study area lies in Dhading district, a part of Bagmati province of Central Nepal (Figure 1). The area is situated within 27°40' N and 28°17'N latitude and 80°17' E and 84°35' E longitude within an altitude ranging from 300-1450 m above the sea level and it has sub-tropical climate. Dhading is the only district of Nepal which ranges from high mountains like Ganesh Himal to the Chure-Bhawar pradesh of Terai. Ganesh Himal is the predominant mountain range located within Dhading district.

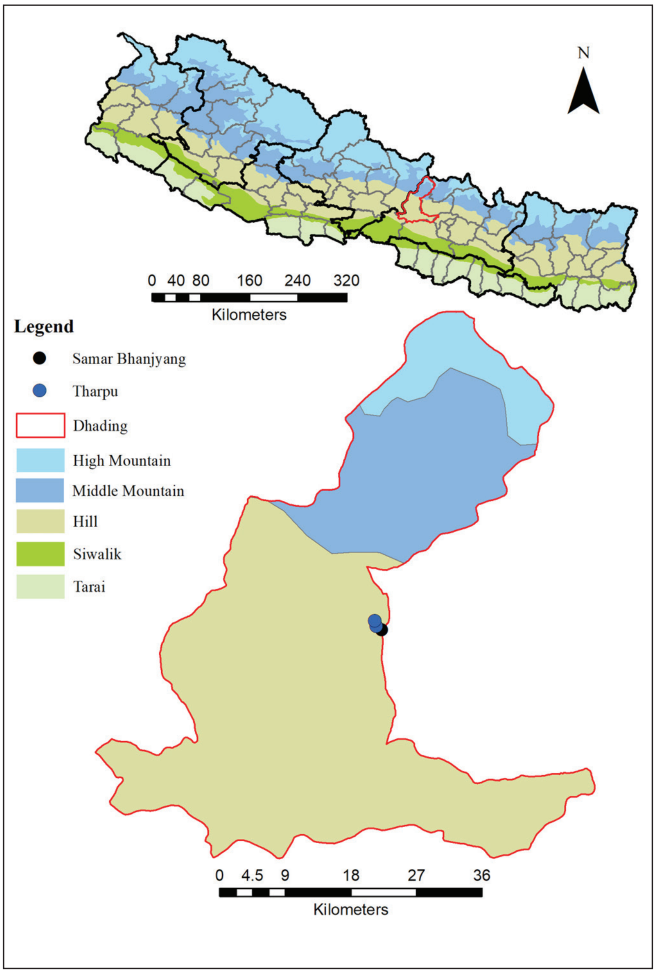

Figure 1: Map of study area showing sampling localities of *P. roxburghii*

Study design

The sampling was done in July, 1994. Within the study area three different natural forest of *P. roxburghii* were selected in different altitudes between 1100-1350 m of two localities, one in Samar

Bhanjyang (1100 m) and other two in Tharpu (1200 and 1350 m) altitude. Three well grown canopy individuals were selected at each locality and cubic wooden blocks were cut off from the outermost part of the trunk at breast height. Tree height (H), diameter at breast height (DBH) and altitude (ALT) were noted.

The anatomical work was carried out in National Herbarium and Plant Laboratories in April, 2022. Six characters of tracheid, radial and tangential diameter of early wood tracheid (ERD and ETD) and late wood tracheid (LRD and LTD), inter-tracheid pit diameter (Pit), tracheid length (TL), two characters of uniseriate rays, uniseriate ray height (URH), and uniseriate ray density (URD), three characters of axial resin canal, radial and tangential diameter of axial resin canal (ARCRD and ARCTD), axial resin canal density (ARCD), and three characters of radial resin canals, radial resin canals height (RRCH), radial resin canal width (RRCW) and radial resin canal density (RRCD) were measured. Tracheids and resin canals were measured in cross-section. Tracheid lengths were measured by macerating the piece of woods. Maceration was done by boiling the wood pieces in a mixture of 10% nitric acid and 19% chromic acid (Jeffrey, 1917). All the measurements were taken with an optical microscope. The mean of an average of 25 measurements were taken in each anatomical feature.

Altogether, 14 wood anatomical characters were analyzed. Correlation and multiple regression analyses were conducted to examine the relationship between wood anatomical characters and three non-anatomical characters. Correlation among these 14 wood anatomical characters and 3 non-wood anatomical characters, DBH, tree height and altitude were assessed, and then multiple regression analysis was carried out to examine wood anatomical characters against the three non-anatomical factors as independent variables. The general linear hypothesis in SYSTAT-5 was applied for multiple regression analysis.

The correlation coefficient helps in measuring the extent of the relationship between two variables and also in locating the critically important variables on which others depend. Similarly, multiple regression analysis helps in estimating the relationships between dependent variables and one or more independent variables and also to know how strong the relationship is between two or more independent variables and one dependent variables. While studying the ecological wood anatomy, these two analysis has a great significance to know in what degree the ecological parameters such as altitude, tree height, temperature, moisture, rainfall etc. affect the wood characters as well as to know how strong the relationship is between them.

Results and Discussion

General description of wood is not given in the present study as it has been already described by Suzuki et al. (1991) and Rajbhandari et al. (2020). There was no exceptional variation in the wood structure of the studied material, therefore only the quantitative characters have been studied. Three photomicrographs of cross-section (TS), tangential (TLS) and radial longitudinal section (RLS) of *P. roxburghii* are shown to visualize the wood structure which consists of earlywood tracheids, latewood tracheid, resin canal and rays (Figure 2). The quantitative data of all those 17 parameters considered are given in Table 1 and Table 2. Correlation among these parameters is given in Table 3 and the result of multiple regression analysis between wood anatomical characters and the three non-anatomical factors studied is presented in Table 4.

In the studied area DBH varied very much in some localities. The maximum DBH was found to be two times the minimum at the altitude of 1100 m and 1350 m varying between 40 to 90 and 36 to 70 cm and in the rest it was almost constant. Tree height varied very little within the localities and ranged from 2 to 6 m (Table 1). Tree height as well as DBH did not exhibit significant correlation with altitude. Similarly, no correlation between altitude and DBH was observed. But there was a significant positive correlation between DBH and tree height (Table 3). Altitude showed significant positive correlation only with tracheid length and axial resin canal tangential diameter at 5% level of significance. Similarly, DBH showed significant positive correlation with uniseriate ray and radial ray canal characters at 5% level of significance. However, it did not show significant correlation with other wood anatomical characters. Tree height had strong positive correlation with radial resin canal characters at 1% level of significance.

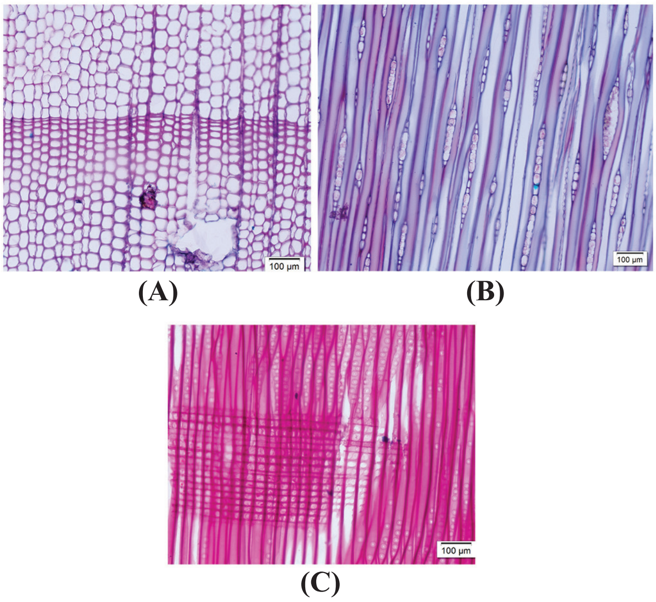

Figure 2: *Pinus roxburghii* Sarg., **A.** Cross section showing growth ring boundary and resin canal, **B.** Tangential section showing uniseriate rays, tracheids and a fusiform ray with a broken horizontal resin canal, **C.** Radial section showing tracheids with bordered pits and pinoid cross field pits. Photographs taken under Olympus CX43 via Olympus LC30 camera under magnification (10x+2x)

Correlation between non-anatomical factors and wood anatomical characters were mostly weak (Table 3). DBH had significant and strong correlation with uniseriate ray height, uniseriate ray density, radial resin canal height, redial resin canal width but it did not show a significant correlation with other wood anatomical characters. Tree height is positively correlated with radial resin canal width. Similarly, altitude shows a positive correlation with tracheid length only. Thus, it showed that DBH had a strong correlation than tree height and altitude. It might be due to positive correlation of DBH with tree height.

Tracheids character did not show correlation with each other and with other uniseriate ray character, axial resin canal characters and radial resin canal characters. Similarly, the uniseriate ray character and radial resin canal character also did not show correlation within and among each other. Axial resin canal character showed a correlation with tracheid length. Since the wood anatomical characters are not correlated with each other, they were independent from other characters. The coefficient of variance is low, which ranges from 0.12- 0.46 (Table 3), the wood anatomical characters are constant and their variations are not affected by the other anatomical characters within the studied samples.

To assess dependency on the three non-anatomical factors, wood anatomical characters were examined by multiple regression analysis, using non-anatomical factors as independent variables (Table 4). Significant correlations at 5% level were obtained for two anatomical characters, i.e., uniseriate ray density and radial ray canal width. Coefficients of determination were 0.841 and 0.850 for the two characters at 5% level of significance, where the non-anatomical factors explain 84.1-85.0% of the variation for these characters. Here, at 5% level of significance, the regression coefficients were significant for latewood tracheid with DBH, and for latewood tracheid, pit and tracheid length with altitude and for uniseriate ray density and radial resin canal width with tree height. Together, at 1% level of significance, standard regression coefficients of DBH with uniseriate ray density were highest while at 5% level of significance, it was highest with latewood tracheid diameter.

However, at 5% level of significance, regression coefficients were significant for altitude with latewood tracheid diameter, pit, tracheid length and radial resin canal diameter. Similarly, the regression coefficients for tree height were significant with uniseriate ray density and radial resin canal width. Thus, two characters of the wood anatomical characters are significantly affected by DBH in the first place and by altitude secondarily and lastly by tree height.

Table 1: Mean values of wood anatomical characters related to earlywood and late wood tracheids and non-anatomical characters of *P. roxburghii*

Specimen No.	Altitude (m)	DBH (cm)	Height (m)	Early wood tracheid				Late wood tracheid				Tracheid pit (µm)	Tracheid length	
				RD		TD		RD		TD				
				µm	sd	µm	sd	µm	sd	µm	sd		(µm)	sd
9495027	1100	40	11	53	1.8	40	1.3	28	0.35	32	0.72	44	3	32.11
9495028	1100	90	14	56	1.31	45	1.2	10	1.5	14	2	40	3.8	27.55
9495029	1100	70	16	46	0.69	51	0.63	35	0.43	34	0.38	42	3.2	35.44
9495032	1200	85	18	63.3	1.4	35	1.1	41	0.94	26.1	1.2	40	4.5	39.8
9495033	1200	90	18	47	1.21	40	1.66	17	0.57	26	1.5	31	3.5	30.62
9495034	1200	90	18	49	1.8	34	1.18	16	0.52	21	0.84	37	3.6	21.28
9495035	1350	70	18	40	2	38	1.2	20	0.79	16	0.53	36	4.5	46.6
9495036	1350	70	18	44.3	1.6	25	0.83	29	0.7	26	1.6	36	4.5	42.71
9495037	1350	36	10	52	0.9	48	1.05	30	0.9	24	1.01	30	4	38.86
Total	10950	641		450.6	12.71	356	10.15	226	6.7	219.1	9.78	336	34.6	314.97
Mean	1216.7	71.2	15.7	50.07		39.56		25.11		24.34		37.33	3.84	
SD	109	20.8	3.24	6.9		7.89		10.01		6.59		4.71	0.57	
CV	0.089	0.292	0.206	0.137		0.199		0.39		0.27		0.126	0.148	

Note: sd = standard deviation; CV = coefficient of variation; DBH = Diameter at Breast Height; RD = radial diameter; TD = tangential diameter

Table 2: Mean values of wood anatomical characters related to rays, resin canals, ray parenchyma of *P. roxburghii*

Specimen No.	Uniseriate ray (mm)			Axial resin canal (µm)					Radial resin canal (µm)			Ray parenchyma cells (µm)	
	Height	sd	D/mm	RD	sd	TD	sd	D/mm	Height	Width	D/mm	TD	VD
9495027	265	18.11	4.3	169	2.98	161	1.11	2	286	40	2	27	49
9495028	374	15.9	6.6	206	2.1	161	2.8	1.9	413	43	1.3	20	48
9495029	384	12.52	5.3	230	1.3	161	1.41	4.5	400	43	1.3	35	60
9495032	381	28.9	6.2	268	2.7	161	3.28	1.3	480	55	1.3	15	30
9495033	307	19.35	6.9	200	1.4	161	2.96	2.2	641	47	1.3	15	24
9495034	384	19.69	6.5	225	4.08	161	5.3	1.3	660	46	1.3	15	28
9495035	312	25.48	5.1	212	5.35	161	3.78	1.4	774	46	1.3	17	31
9495036	325	11.9	5.8	266	4.2	161	4.1	2	503	44	1.2	12	23
9495037	300	8.17	5.6	227	2.9	161	3.2	2.2	209	23	1	10	15.3
Total	3032	160.02	52.3	2003	27.01	1449	27.94	18.8	4366	387	12	166	
Mean	336.89		5.81	223		161		2.09	485	43	1.33	1.33	
SD	44.64		0.831	31.21		48.19		0.97	182.6	9.086	0.26		
CV	0.132		0.14	0.139		0.206		0.46	0.376	0.201	0.2		

Note: D/mm = density per millimeter; VD = vertical diameter; sd = standard deviation; RD = radial diameter; TD = tangential diameter

Table 3: Correlation table of non-anatomical data and wood anatomical characters of *P. roxburghii*

	ALT	DBH	Ht	ERD	ETD	LRD	LTD	Pit	TL	URH	URD	ARC(RD)	ARC(TD)	ARCD	RRCH	RRCW	RRCD
ALT	1																
DBH	0.16	1															
Ht	0.16	**0.792***	1														
ERD	-0.42	0.070	-0.287	1													
ETD	-0.42	-0.275	-0.564	0.108	1												
LRD	0.21	-0.536	-0.245	0.295	0.109	1											
LTD	-0.30	-0.393	-0.168	0.047	0.132	0.628	1										
Pit	-0.71	0.012	-0.065	0.300	0.058	0.052	0.302	1									
TL	**0.75***	0.180	0.413	-0.007	-0.526	0.172	-0.505	-0.353	1								
URH	-0.705	**0.694***	0.480	0.279	0.047	-0.056	-0.173	0.267	0.130	1							
URD	-0.023	**0.792***	0.470	0.222	-0.150	-0.406	-0.421	-0.444	0.204	0.527	1						
ARC(RD)	0.436	0.270	0.483	0.142	-0.425	0.488	0.016	-0.153	**0.702***	0.535	0.313	1					
ARC(TD)	0.357	0.327	0.271	0.012	-0.362	-0.135	-0.561	-0.082	**0.709***	0.396	0.338	0.608	1				
ARCD	0.607	0.156	0.549	-0.365	-0.215	0.377	0.131	-0.556	0.432	0.207	0.231	0.630	0.022	1			
RRCH	0.253	**0.672***	**0.858****	-0.488	-0.466	-0.531	-0.438	-0.197	0.340	0.211	0.353	0.097	0.110	0.417	1		
RRCW	-0.281	**0.763***	**0.814****	0.126	-0.479	-0.222	-0.048	0.393	0.199	0.463	0.310	0.255	0.108	0.097	0.646	1	
RRCD	-0.641	-0.227	-0.302	0.200	0.117	-0.135	0.325	0.666	-0.630	-0.346	-0.515	-0.718	-0.641	-0.651	-0.146	0.182	1

Note: ERD = Earlywood tracheid radial diameter; ETD = Earlywood tracheid tangential diameter; LRD = Latewood tracheid radial diameter; LTD = Latewood tracheid tangential diameter; TL = Tracheid length; URH = Uniseriate ray height; URD = Uniseriate ray density; ARC(RD) = Axial resin canal radial diameter; ARC(TD) = Axial resin canal tangential diameter; ARCD = Axial resin canal density; RRCH = Radial ray cell height; RRCW = Radial ray cell width; RRCD = Radial ray cell density; ALT = Altitude; Ht = Height; DBH = Diameter of breast height
*significant at 5% level of significance
**significant at 1% level of significance

Table 4: Multiple Regression analysis of wood anatomical characters of *P. roxburghii*

Characters	MCC	CD	F-ratio	Significance (p- value)	Partial regression coefficient				Standardized regression coefficient		
					Constant	ALT	DBH	HT	ALT	DBH	HT
ERD	o.577	0.33	0.833	0.531	71.888	-0.009	0.22	-1.685	-0.1439	0.6632	-0.7911
ETD	0.663	0.439	1.305	0.37	83.175	0.078	0.078	1.648	1.0772	0.2058	0.6767
LRD	0.621	0.386	1.046	0.443	83.175	-0.014	-0.554	2.066	-0.1512	-1.1524	0.6688
LTD	0.814	0.663	3.282	0.117	84.115	-0.052*	-0.485*	2.403	-0.8547	-1.532	1.1817
Pit	0.812	0.659	3.22	0.12	87.122	-0.044 *	-0.186	1.086	-1.0188	-0.8204	0.7469
TL	0.812	0.659	3.212	0.12	87.122	-0.044 *	-0.186	1.086	-8.4189	-6.7788	6.1713
URH	0.712	0.507	1.712	0.288	311.1	-0.057	1.514	-0.786	-0.1399	0.7061	-0.0571
URD	**0.921**	0.85	**9.379***	0.017	0.157	0.004	0.064	-0.228*	0.4994	1.6141	-0.8882
ARC(RD)	0.606	0.367	0.967	0.477	20.612	0.115	0.132	3.362	0.4014	0.0882	0.349
ARC(TD)	0.628	0.395	1.088	0.435	-151.658	0.301	2.228	-8.925	0.6802	0.9627	-6001
ARCD	0.53	0.281	0.653	0.615	8.962	-0.006	-0.035	0.157	0.6312	-0.7512	0.5234
RRCH	0.869	0.755	5.126	0.551	-593.774	0.294	1.327	40.021	0.1753	3.04	0.0472
RRCW	**0.917**	0.841	**8.843***	0.019	51.117	-0.037	-0.06	2.661 *	-0.4488	-0.1377	0.9491
RRCD	0.796	0.634	2.887	0.142	4.436	-0.002*	-0.012	0.048	-1.0383	-0.9724	0.5997

Note: MCC = multiple correlation coefficient; CD = coefficient of determination; ERD = Earlywood tracheid radial diameter; ETD = Earlywood tracheid tangential diameter; LRD = Latewood tracheid radial diameter; LTD = Latewood tracheid tangential diameter; TL = Tracheid length; URH = Uniseriate ray height; URD = Uniseriate ray density; ARC(RD) = Axial resin canal radial diameter; ARC(TD) = Axial resin canal tangential diameter;

Wood structure of *P. roxburghii* in central Nepal was constant in general. But there was a variation in its quantitative characters. This is because of the influence of the three non-anatomical factors, altitude, DBH and tree height. Though there is a variation within each local population, general trends are manifested in the tracheid length, uniseriate ray height, uniseriate ray density, radial ray canal height, radial ray canal width. Among the characters found significant by the multiple regression analysis, increase of tracheid length, uniseriate ray height, uniseriate ray density, radial ray canal height, radial ray canal width increase with increase in DBH, which is similar to the trends found within several genera as well as families in angiosperms by Baas (1973b, 1982), Baas et al. (1988) and Oever et al. (1981) but differ from trends in *Syringa oblata* var. *giraldii* (Zhang et al., 1988) as he used stem wood samples in his study.

Among the three non-anatomical factors, DBH had the greatest effect on the wood structure of *P. roxburghii* followed by altitude. However, DBH cannot exert a direct influence on wood structure or growth of individuals. There should be other external factors such as changes in precipitation, transpiration and soil moisture which affects indirectly on the three non-anatomical factors, so, these should also be considered on the growth of individual trees.

Correlation between tracheid length and axial resin canal radial diameter and axial resin canal tangential diameter and non-correlation between other wood anatomical characters showed that there was no interdependency in *P. roxburghii*. Multivariate analysis between all of these characters showed any relationship between all of them except between tracheid length and axial resin canal radial diameter and axial resin canal tangential diameter. The study shows some independent variation from ecological factors and seems to be related to other factors than the studied ones or vary by chance. Among them, axial resin canal density varies greatly between individuals with the largest coefficient of variance 46% (Table 2). Variance of the tracheid pits was less than that of the other tracheid characters and its coefficient of variance is 12.6% (Table 1). It is nearly equal to early-wood tracheid diameter. Independent variance of the tracheid pit diameter indicates the absence of a relationship between tracheid conductivity and non-anatomical factors, which is highly doubtful. The Hagen-Poisseuille equation does not seem to apply for the tracheids of *P. roxburghii*. Thus, tracheids having various sizes represent hydraulic conductivity of *P. roxburghii* and are directly related with non-anatomical or ecological factors.

Conclusion

The basic structure of woods is determined by the genetic factors. Therefore, the overall wood anatomical structure of tree species is constant, and is utilized in wood identification. However, there is a certain variability of structures that are subject to modification through the environment. Size and shape of cells may change, the number of cell types and features, or specific feature occur that are normally not seen to change. Hence, wood characters are changed quantitatively but not qualitatively with respect to change in ecological factors such as drought, fire, moisture availability, temperature etc. The present study helps to understand adaptive strategies and mechanisms as well as has also added substantial knowledge to our understanding of wood anatomical diversity.

Wood characters are not influenced directly by the three non-anatomical factors, altitude, DBH and tree height. The general trend is maintained only in the tangential diameter of axial resin canals and radial resin canal width though there is a variance within each population of a locality. This could be attributed to age related anatomical features. With the increase in DBH there is an increase in uniseriate ray height, uniseriate ray density, radial resin canal height and radial resin canal width. Similarly, with the increase in altitude there is an increase in tracheid length. Very little interdependency is shown in *P. roxburghii* as the correlation between all tracheids character and axial resin canal characters are not low.

Author Contributions

L. Joshi carried out the field work for sample collection. L. Joshi and P. Chalise carried out the anatomical study, analyzed and wrote the manuscript. L. Joshi as a corresponding author, is the guarantor for this article.

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