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Original Research Article

Variations in Tracheid Characteristics of *Pinus kesiya* Royle ex Gordon

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Abstract: *Pinus kesiya* Royle ex Gordon is a fast growing and tropical pine species of Eastern Himalaya.Its natural stands are widely distributed in Khasi and Jaintia hills of Meghalaya.The present investigations were conducted (a) to see radial variation in tracheid characteristics (tracheid number, tracheid diameter and tracheid wall thickness) in different sampling strata i.e. annual ring, early wood and latewood widths of *P. kesiya* separately and (b) to examine the relationship of tracheid characteristics in different classes of rings with these sampling strata. The study was carried out in cross-sectional discs at breast height of the trees. Radial variation in tracheid characteristics was studied in single radial direction. Large, medium and narrow rings were selected to see relationship of tracheid characteristics with these selected strata. Tracheid characteristics were measured in five radial rows of tracheids per annual ring with an image analysis system. In the study, there was an irregular decrease in tracheid number and increase in wall thickness from pith to bark in all sampling strata. Tracheid diameter did not show any distinct pattern of variation in latewood. Most of the tracheid characteristics had greater coefficient of variations in medium rings than larger and narrow rings. Among the tracheid characteristics, tracheid number was the most important contributor for all classes of annual rings in determining the width of selected sampling strata. Since earlywood and latewood are formed during different seasons and constitute the total ring width, therefore, ring width of medium rings may be the most suitable stratum for dendroclimatological studies in this pine species.
Key words: Annual ring, earlywood, latewood, tracheid characteristics, radial variation

Introduction

Pinus kesiya Royle ex Gordon is one of the most important fast growing, three needle pine. It occurs in India, Myanmar, Xizang and Yunnan in China, Laos, Vietnam, Thailand and Northern Philippines (Orwa *et al*., 2009). It is a promising species as an exotic and has been introduced in many countries of the world like Africa, South America, Central America, Australia, Malaysia, New Guinea, Zambia, Brazil, Zimbabwe, Madagascar, Malawi, Kenya, Uganda, Nigeria, Tanzania etc. (Armitage and Burley, 1980; Srivastava and Bahar, 2007; Orwa *et al*., 2009). It is the only tropical pine restricted to Eastern Himalayas and its natural stands are widely distributed in Khasi and Jaintia Hills of Meghalaya at an altitude of 800-1800 m. Each and every part of the tree is used by local people of Meghalaya. The needles are used for stuffing mattresses, chair cushions and pillows. These are also used as cementing fibres in mud plastered walls. The branches are used in destructive distillation of resin (Srivastava and Bahar, 2007). Besides these, it produces an excellent timber which is stable after seasoning. Its wood is straight grained, moderately fine in texture, fairly heavy, relatively strong, hard, easy to saw and work with hand held or machine tools. The heartwood is yellowish to reddish brown and sapwood is creamy white to white in colour

(Purkayastha, 1989). Locally, the timber is used for house constructions, flooring, ceiling, panelling, furniture, telephone poles etc. (Purkayastha, 1989; Srivastava and Bahar, 2007). Nowadays, *Pinus kesiya* is being cultivated under plantation programmes by the forest departments in North eastern states to reclaim forest areas after shifting cultivation. The plantation is also under progress in other states of India namely Andhra Pradesh, Kerala, Orissa and Tamil Nadu.

Wood provides mechanical support to the plant body, conducts water and store biochemicals. It is a product of cambium and formed by four phases namely cell division, cell expansion,cell wall thickening and finally programmed cell death (Scheutz *et al*., 2013) Thus, wood is a complex and heterogeneous biological material. In softwoods, tracheids are the main cellular elements and constitute 90% by volume of wood (Panshin and deZeeuw, 1980). Formation of wood takes place in the form of annual rings. Both earlywood and latewood of an annual ring formed during different seasons represent the total ring width. Ring width depends on cambial activity and is also governed by both genetic and environmental conditions (Dufour and Morin, 2010; Rossi *et al*., 2012).

Tracheid characteristics i.e. number and size of tracheids, their wall thickness and wall to lumen ratio are highly variable like other wood characteristics. They vary from pith to bark radially as well as from earlywood to latewood within an annual ring (De Soto *et al*., 2011). Though these variations cause difficulty to assess the wood performance accurately but they also provide us with an opportunity for selection of superior or plus trees with wood of superior quality in tree improvement programmes. In addition, they are an important source of required information to understand the phylogenetic adaptations and the response of trees with changing environmental conditions (Chave *et al*., 2009; Eilmann *et al*., 2011).

A perusal of literature reveals that studies on tracheid characteristics were carried out in conifers by many researchers. Deslauriers *et al*. (2008) reported cell size as an important contributor to ring width and is positively related with large and narrow rings in *Pinus leucodermis*. Xu *et al*. (2014) observed strong influence of tracheid number on ring width in *Picea crassifolia*. Irbe *et al*. (2015) did not find any significant increase in earlywood thickness with age in *Picea glauca*. However, Olano *et al*. (2012) reported decrease in tracheid number, tracheid size and wall thickness from earlywood to latewood in *Juniperus thurifera*. Variation in wood properties of *Pinus kesiya* Royle ex Gordon of Assam, Burma and Philippines provenances grown in Zambia were investigated to draw up guidelines for plantation sampling (Burley, 1969, 1970; Burley and Andrew, 1970) and in Malawi (Missanjo and Matsumura, 2016 a, b).Though the authors have studied ring width variation and intra-ring variation in tracheid length in *Pinus kesiya* (Gogoi *et al*., 2014, 2017), but no study has been carried out on its tracheid characteristics so far. Therefore, the main objectives of the present investigation are (a) to study the radial variation in tracheid characteristics (tracheid number, tracheid diameter and tracheid wall thickness) in different sampling strata i.e. annual ring, earlywood and latewood widths separately and (b) to see the relationship of tracheid characteristics in different classes of annual rings with selected sampling strata

Material and methods

Sample Collection

Five trees of *Pinus kesiya* with straight bole, uniform crown having no major visible defects were randomly selected from the Pine forest of Jaintia Hills district of Meghalaya. The geographical co- ordinates of the selected sites recorded with GPS were N 25°29'628" and E 92°10'304". The average height and average diameter of trees were 23±2.45 m and 31.07±1.36 cm, respectively (Table1). The cross-sectional discs of about 10 cm thickness were taken at breast height (1.37 m above the ground) from each tree.

Table 1. Height and diameter of selected trees of *P.kesiya*.

Tree number	Age (Yrs)	Height (m)	D.B.H. (cm)
Tree 1	41	25	30.88
Tree 2	44	26	30.56
Tree 3	41	22	33.42
Tree 4	41	20	30.56
Tree 5	46	22	29.92

Each cross- sectional disc was marked into eight cardinal directions namely North, South, East, West, North East, North West, South East and South West. Some easily identifiable rings were marked for equal counting of rings in all directions around the circumference. All radial wedges were sawn out from bark to pith and were smoothened with an electric planer and a sliding microtome.

The wedges were soaked in water till they became fully saturated and water was replaced regularly to prevent contamination of wood samples. The mixture of 0.15 gm methylene blue and 0.15 gm malachite green in 25 cc of 100 % ethyl alcohol was applied on the radial wedges to make the boundary distinct between the earlywood and latewood. The widths of annual rings, earlywood and latewood were measured with an ocular micrometre fitted in one of the eye pieces of a stereomicroscope at 20 X.

Annual rings were classified into large, medium and narrow rings by the formulae of Xu *et al.* (2014). A total of 280 annual rings were selected randomly from 40 wedges of five selected trees of *P. kesiya*.

Cross-sections of 20-25µ thickness were cut from all radial wedges with the help of a sliding microtome (Leica SM2000R) and stained with the standard procedure (Johansen, 1940).

A single radial direction (North) was selected to study its tracheid characteristics (tracheid number, diameter and wall thickness) variation from pith to bark in annual ring, earlywood and latewood widths.The annual rings of different classes (large, medium and narrow) were also selected from permanent slides. The tracheid characteristics were measured with the help of an image analysis system. The five radial rows in each annual ring were selected to study these parameters (Fig.1).

Statistical analyses like mean, coefficient of variation, multiple correlation and regression were carried out using SPSS 18.0 software package. Graphs were plotted by using Origin 8.0 software package.

Results

Present study showed that the annual ring width, earlywood and latewood widths decreased irregularly from pith to bark

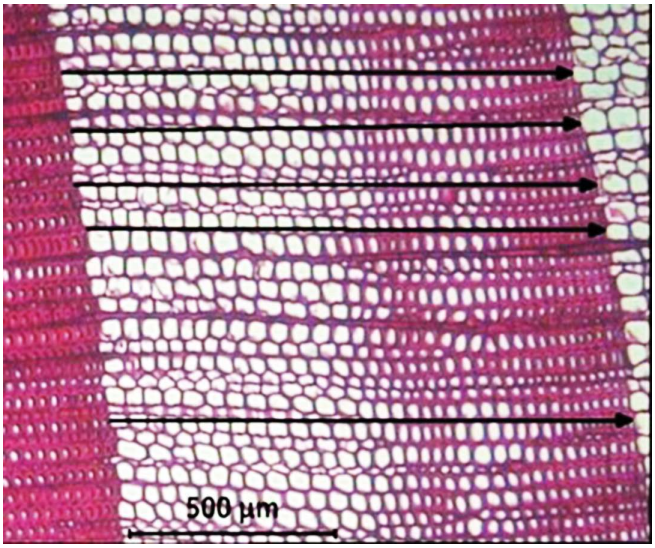

Fig.1. Cross-section of an annual ring showing radial rows of tracheids in *P.kesiya.* Figure shows characteristics measurement in large, medium and narrow annual rings. The annual rings were classified on the basis of their width (More than 5mm-Large rings; 2-4 mm-medium rings and 1-2mm-narrow rings). The five radial rows in each annual ring as shown in figure were selected from permanent slides. The tracheid numbers were counted in both early wood and late wood. Tracheid diameter and wall thickness of tracheids were also measured with the help of an image analysis system.

radially. Similar patterns were observed in tracheid number of annual ring, earlywood and latewood (Fig. 2a - b). On the contrary to it, tracheid wall thickness showed irregular increase in the annual ring, earlywood and latewood radially. Tracheid diameter gradually increased up to 10 years and then remained more or less constant in annual ring and earlywood whereas latewood did not show any distinct pattern of variation radially in this parameter (Fig. 2c - d).

The ring width and tracheid characteristics of large, medium and narrow rings revealed that larger rings had maximum tracheid number as compared to the medium and narrow rings. On the contrary to these, wall thickness was maximum in all sampling strata of medium rings. Maximum tracheid diameter was observed in earlywood stratum of narrow rings. The coefficient of variation for ring width and most of tracheid characteristics was maximum in the medium rings for all strata (Table 2).

Multiple linear correlation and regression analyses were carried out to see the relationship of tracheid

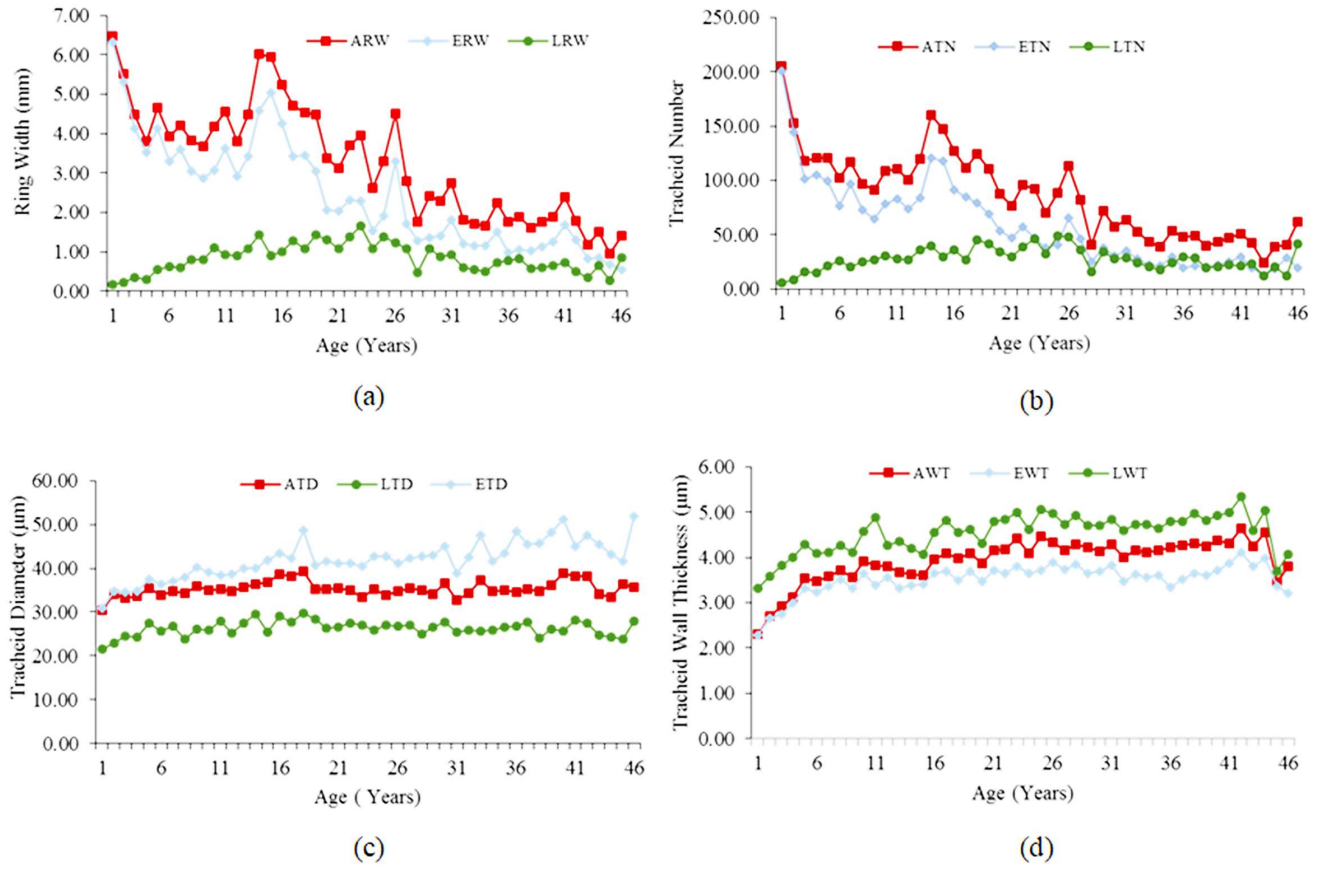

Fig. 2(a-d). Ring width and tracheid characteristics variations in different sampling strata. A-Annual ring; E-Early wood and L- Late wood)

Table 2. Tracheid characteristics in different types of annual rings along with their ring width in *P. kesiya*

Sampling strata	Ring classification	Ring width (mm) Mean (CV)	Tracheid number Mean (CV)	Tracheid diameter (µm) Mean (CV)	Wall thickness (µm) Mean (CV)
Annual ring	Large ring	6.85 (0.23)	175.38(0.26)	37.55(0.10)	3.75(0.20)
	Medium ring	2.97(0.36)	78.02(0.38)	34.96(0.10)	3.98(0.17)
	Narrow ring	1.05(0.21)	29.12(0.27)	33.92(0.11)	3.89(0.14)
Earlywood	Large ring	5.78(0.33)	143.10(0.38)	39.91(0.12)	3.54(0.18)
	Medium ring	2.08(0.50)	49.03(0.60)	41.44(0.13)	3.57(0.18)
	Narrow ring	0.65(0.34)	13.89(0.38)	44.67(0.15)	3.27(0.15)
Latewood	Large ring	1.07(0.65)	32.29(0.55)	27.83(0.14)	4.41(0.17)
	Medium ring	0.89(0.53)	28.99 (0.46)	26.39(0.13)	4.60(0.16)
	Narrow ring	0.40(0.43)	15.22(0.41)	24.03(0.09)	4.44(0.14)

(CV = coefficient of variation)

characteristics (tracheid number, tracheid diameter and tracheid wall thickness) with different sampling strata. The multiple correlation coefficients of tracheid characteristics were statistically highly significant in all the sampling strata except earlywood of narrow rings. All the classes of annual rings were more influenced by tracheid number than tracheid diameter and tracheid wall thickness in selected sampling strata (Table 3).

Table 3. Relationship of tracheid characteristics with selected sampling strata in *Pinus kesiya*

				Regression coefficients (Standard error)			Standardized coefficients (Beta)		
	Multiple correlation coefficient	Multiple coefficient of determination	Regression constant	Tracheid Number	Tracheid Diameter	Tracheid Wall Thickness	Tracheid Number	Tracheid Diameter	Tracheid Wall Thickness
	r	r2	B0	B1 (SB1)	B2 (SB2)	B3 (SB3)	β 1	β 2	β 3
Annual ring									
Large ring	0.920**	0.847	-3.454	0.035**(0.003)	0.082* (0.031)	0.273 ns (0.157)	1.044	0.196	0.129
Medium ring	0.891**	0.794	-1.372	0.032**(0.001)	0.043* (0.012)	0.078 ns(0.065)	0.883	0.144	0.051
Narrow ring	0.625**	0.390	0.104	0.018** (0.004)	0.005 ns (0.009)	0.063 ns (0.061)	0.644	0.091	0.161
Earlywood									
Large ring	0.899**	0.808	-3.569	0.037** (0.004)	0.082*(0.036)	0.233 ns (0.269)	1.041	0.201	0.080
Medium ring	0.866**	0.750	-0.413	0.033** (0.002)	0.021* (0.009)	0.001 ns(0.072)	0.916	0.001	0.112
Narrow ring	0.557*	0.310	0.014	0.021** (0.007)	-0.001ns (0.006)	0.123 ns(0.078)	0.492	-0.039	0.265
Latewood									
Large ring	0.759**	0.576	-0.636	0.024** (0.006)	0.004 ns(0.023)	0.190 ns (0.134)	0.602	0.020	0.202
Medium ring	0.849**	0.720	-0.147	0.029** (0.002)	-0.006 ns (0.006)	0.084** (0.029)	0.826	-0.043	0.133
Narrow ring	0.946**	0.895	-00.121	0.026** (0.002)	0.004 ns (0.006)	0.006 ns (0.018)	0.923	0.056	0.021

The levels of significance used are ns = non- significant, * = significant at P <0.05 level, **= significant at P <0.01 level

Discussion

Relationship of tracheid characteristics with ring width provides valuable eco-physiological information (Dufour and Morin, 2010; Krause *et al*., 2010). Tracheid characteristics are also important to detect intra-annual climatic events responsible for sharp changes in cellular features and to resolve the effects of many controlling factors on wood formation (Olano *et al*., 2012). In *P. kesiya*, the boundary between juvenile and mature woods is demarcated at 10 years (Gogoi *et al*., 2014). Juvenile wood present near the pith is characterised by wider annual rings, shorter tracheids with thin cell wall and large lumen than the mature wood (Pinto *et al*., 2004). Decrease in annual ring width, earlywood and latewood widths variation along the radial direction is due to presence of juvenile wood near the pith. The response of cambial activity to environmental and physiological factors may responsible for irregular variation in widths of annual ring, earlywood and latewood (De Slauriers et al., 2008; Rathgeber *et al*., 2011)

Variations in tracheid dimensions (tracheid number, tracheid diameter and wall thickness) from pith to bark may be associated with the process of cambium maturity with age and assessment of development of growth of trees (Zobel and van Buijtenen, 1989). There was a great difference in tracheid number and wall thickness between early wood and latewood as latewood is formed due to less cambial activity at the end of the growing season. More number of anticlinal divisions than periclinal divisions lead to lesser number of latewood tracheids with small size and more wall thickness. Also, maximum tracheid diameter in earlywood of narrow rings and maximum wall thickness in latewood of medium rings were observed. Present results collaborates with the findings of Hannrup *et al*., 2001; Rathgeber *et al*., 2006.

Since latewood did not show any distinct pattern of variation in wall thickness. *P. kesiya* is a fast growing conifer and has less amount of latewood, it could be a probable reason for showing not any distinct pattern of variation in the wall thickness. The present study is in confirmation with the findings of Briand *et al*. (1993) who reported similar results in the fast growing conifers.

Ring width in conifers is a function of tracheid number and tracheid size (Vaganov *et al*., 2006). The number of

tracheids is more closely related to annual ring width than tracheid diameter (Camarero *et al.*, 1998; Mäkinen *et al.*, 2003). In the present study, the multiple correlation coefficient was statistically positive and highly significant for all ring classes in the selected strata except earlywood width of narrow rings. Among all the tracheid characteristics, tracheid number contributed more than other parameters in large, medium and narrow rings. Hence, tracheid number is the best predictor for widths of annual ring, early wood and latewood. The present study is in confirmty with the findings of other workers (Briand *et al.*, 1993; Mäkinen *et al.*, 2003; Deslauriers and Morin, 2005; Xu *et al.*, 2014).

Conclusions

In *P. kesiya*, tracheid numbers showed large variation than other tracheid characteristics from pith to bark. Among the tracheid characteristics studied, tracheid number decreased but tracheid diameter and wall thickness increased irregularly from pith to bark. More number of earlywood cells are formed in large rings but earlywood cells are large in size in narrow rings. Medium rings have maximum coefficient of variation for most of the selected tracheid characteristics than the other rings. Present finding suggests their possible role in dendroclimatological studies and the tracheid number is the best predictor among the selected tracheid characteristics for determining the width of selected sampling strata.

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