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SEASONAL VARIATION IN CAMBIAL ANATOMY AND XYLEM CELL DIFFERENTIATION IN *POPULUS×EURAMERICANA* CV. '74 /76'

Enhua Xi, Guangjie Zhao
Beijing Forestry University, College of Material Science and Technology
Beijing, China

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ABSTRACT

This study describes the variation of cambium and differentiated xylem cell of the fast-growing *Populus×euramericana* cv. '74 /76' during the active phase by the methods of quantitative anatomy, microscopic image analysis and statistics analysis. The results showed that the seasonal changes of cambium and xylem cell varied significantly. The proportion of wood fiber had a gradual increasing trend. However, the proportion of vessel elements decreased slightly. The proportion of ray increased then decreased and that of parenchyma was increasing overall. Length, width and wall to lumen of wood fiber showed a gradual increasing trend. The trends of the vessel element length, width and distribution frequency decreased then increased. In addition, the morphological relationships between cambium and xylem cell were discussed. The results supported that the morphological correlations of cambium cell and wood fiber were stronger than that of cambium cell and vessel elements during the active phase.

KEYWORDS: *Populus×euramericana* cv. '74 /76', active phase, cambium, xylem cell, morphological relationship.

INTRODUCTION

The vascular cambium of woody plants in the temperate zone presents a seasonal cyclical pattern of activity and dormancy (Chen et al. 2010). During this periodicity, cambial cells display changes in structure (Barnett 1973, Catesson 1994). The quality and quantity of the wood of trees reflect the periodicity. Therefore, many researchers have been interested in the events associated with cambial activity and have studied the anatomical, biochemical, cytological and histochemical changes that occur in the cambium (Catesson 1994, Larson 1994). Many reports dealt with the cellular changes associated with cambium periodicity, especially changes in the cytoplasm (Larson 1994, Iqbal 1995, Farrar and Evert 1997, Rensing and Samuels 2004). Studies on the cambial activity, mainly of xylem formation, provide not only data about the age of trees but also clues about the possible factors that control tree growth. This type of data is also helpful in dendrochronology, in predicting timber and biomass yield, and in determining forest dynamics (Jacoby 1989, Kozlowski et al. 1991, Eckstein et al. 1995, Priya and Bhat 1999). Numerous experiments have also demonstrated that the application of auxin to cambial tissues stimulates xylem production, i.e. cambial cell division (Sundberg et al. 2000). Studies about the seasonal formation of secondary xylem and phloem in tropical trees mainly Indian species have been published (Ghouse and Hashmi 1979, 1980a, 1980b, Dave and Rao 1982, Deshpande and Rajendrababu 1985, Ajmal and Iqbal 1987, Iqbal and Ghouse 1987, Venugopal and Krishnamurthy 1987, Rajput and Rao 1998, Rao and Rajput 1999, 2000, 2001, Rajput and Rao 2001, Venugopal and Liangkuwang 2007). Study about the relationship between seasonal cambial activity, xylem and phloem development, and phenology in *Schizolobium parahyba*, a fast growing semi-deciduous seasonal forest tree from southeastern Brazil has been discussed by Marcati et al. (2008). Marcati et al. (2006) studied the cambial activity and annual rhythm of xylem production of *Cedrela fissilis* (Meliaceae) from the standpoint of phenology and climatic factors.

Populus is presented as a model system for the study of wood formation (xylogenesis). The formation of wood (secondary xylem) is an ordered developmental process involving cell division, cell expansion, secondary wall deposition, lignification and programmed cell death. Because wood is formed in a variable environment and subject to developmental control, xylem cells are produced that differ in size, shape, cell wall structure, texture and composition (Mellerowicz et al. 2001). It is one of the fastest growing tree species, which is why it is used extensively in reforestation projects.

It is evident that the ability to improve wood properties depends on a fundamental understanding of the processes of xylem cell production and differentiation (Whetten and Sederoff 1991). The morphology of secondary xylem cells is determined in part by the dimensions of the cambial initials from which they are derived and in part by the developmental changes that occur during differentiation (Ridoutt and Sands 1993). Changes in cambial anatomy are reported to occur with stem diameter, height and season (Iqbal and Ghouse 1990). Variability in the rate and duration of the phases of differentiation is also known to occur (Denne and Dodd 1981). Previous studies have focused mainly on mature wood (secondary xylem) variation in cell morphology (Fahn and Werker 1990), not involved with the quantity and shape changes during the differentiation process. The purpose of this study was to describe the changes of cambial anatomy and xylem cell differentiation during the different stages based on the method of quantitative anatomy. Meanwhile, the morphological correlation between cambium cells and xylem cells was analyzed.

MATERIAL AND METHODS

Healthy plants of fast-growing two year-old *Populus×euramericana* cv.'74/76' grown in a plantation of Xiaotangshan in Beijing's Changping district (40°17″N, 116°39″E; Beijing, China) with the same diameter at breast height were chosen and marked. Plant materials were collected 1.3 m above the trunk and sampled once a week from April to October in 2009. On each occasion, blocks of about 10 mm^3 including phloem, cambium and xylem cells were immediately immersed in fixative formalin–acetic acid–alcohol. Upon returning to the laboratory, they were placed in the same fresh fixative under a slight vacuum for 30 min. Following vacuum, these pieces were fixed in fresh fixative and preserved at 4°C.

According to the existing research results, cambium active stage of *Populus×euramericana* cv. '74/76' is from April 22 to September 22 in 2009 (sampling time in Tab. 1). During the sampling period, until the third time, sampled materials exhibited thickening secondary wall in xylem cells. For convenience, the third to twenty-third batches of test-pieces were selected for measurements.

Tab. 1: Relationship of cambium activity with phenology of Populus×euramericana cv. '74/76'.

No.	Sampling time	Tissue differentiation	Phenology	Phase of cambium activity
1	Apr. 22, 2009	Phloem+Xylem	Leaf growing	Active phase
2	May 15, 2009	Phloem+Xylem	Full foliage	Active phase
3	May 22, 2009	Phloem+Xylem	Full foliage	Active phase
4	May 30, 2009	Phloem+Xylem	Full foliage	Active phase
5	Jun. 7, 2009	Phloem+Xylem	Full foliage	Active phase
6	Jun. 15, 2009	Phloem+Xylem	Full foliage	Active phase
7	Jun. 22, 2009	Phloem+Xylem	Full foliage	Active phase
8	Jun. 30, 2009	Phloem+Xylem	Full foliage	Active phase
9	Jul. 7, 2009	Phloem+Xylem	Full foliage	Active phase
10	Jul. 15, 2009	Phloem+Xylem	Full foliage	Active phase
11	Jul. 22, 2009	Phloem+Xylem	Full foliage	Active phase
12	Jul. 30, 2009	Phloem+Xylem	Full foliage	Active phase
13	Aug. 7, 2009	Phloem+Xylem	Full foliage	Active phase
14	Aug. 15, 2009	Phloem+Xylem	Full foliage	Active phase
15	Aug. 22, 2009	Phloem+Xylem	Full foliage	Active phase
16	Aug. 30, 2009	Phloem+Xylem	Full foliage	Active phase
17	Sep. 7, 2009	Phloem+Xylem	Full foliage	Active phase
18	Sep. 15, 2009	Xylem	Full foliage	Active phase
19	Sep. 22, 2009	Xylem	Full foliage	Active phase
20	Sep. 30, 2009	Xylem	Full foliage	Active phase
21	Oct. 7, 2009	-	Yellow leaf	Dormant phase
22	Oct. 15, 2009	-	Partial defoliation	Dormant phase
23	Oct. 22, 2009	-	Defoliation	Dormant phase

Cross slices with a thickness of 10 μm including phloem, cambium, and xylem were cut on a sliding microtome, then stained with 1 % safranine of alcohol solution for 2 hours. In the next stage, the slices were dehydrated with gradient alcohol, from 50 %, 70 %, 85 %, 95 %, 100 % into xylene, and then cemented with neutral gum. After that, an image analysis system and a research microscope (Olympus BH-2) were used to make measurements of tissue ratios, wall to lumen of wood fiber, distributing frequency of vessel element according to quantitative anatomy conventional methods of wood. Little sticks like matches with the length of 1 cm were cut around the xylem area, which was just differentiated. Then, the sticks were put into the segregation fluid with volume ratio of 1 to 1 hydrogen peroxide and glacial acetic acid for separating tissues, at 60°C through 24 hours in thermostat. After that, they were rinsed and stored in 50 % alcohol in order to observe momentarily for measuring the length and width of wood fiber and vessel element.

RESULTS AND DISCUSSION

Anatomical characteristics of cambial zone

Number of cambium cell, radial and tangential diameter of cambium cell were evaluated during the active phase of *Populus×euramericana* cv. '74/76' as is shown in Tab. 2. It can be seen from the Tab. 2 that over the period May to July the trend of cambium cell number was towards a sharp decrease in the monthly mean cambium cell number, followed by a slight decline from August to the end of the active phase. Average cambium cell number was greatest at 6.33 on June 7 and least at 2.67 on September 22. As can be seen in the Tab. 2, the average radial diameter (R-diameter) of cambium cell decreased from 6.71 μm on May 30 to 4.04 μm on October 22. Average tangential diameter (T-diameter) of cambium cell varied less regularly but was greatest at 23.58 μm on August 22 and least at 17.54 μm on May 22.

Tab. 2: The changes of cambium cell during the differentiation process.

Sampling time	R-diameter of cambium cell (μm)	T-diameter of cambium cell (μm)	Number of cambium cell
May 22, 2009	6.45±0.57	17.54±0.49	6.20±0.21 (4~9)
May 30, 2009	6.71±0.25	18.37±1.05	6.20±0.34 (4~9)
Jun. 7, 2009	5.47±0.34	19.06±1.08	6.33±0.47 (4~8)
Jun. 15, 2009	4.96±0.41	21.99±0.88	4.93±0.52 (4~6)
Jun. 22, 2009	5.34±0.37	21.19±0.31	4.27±0.35 (3~6)
Jun. 30, 2009	5.25±0.38	22.30±0.78	4.00±0.40 (2~6)
Jul. 7, 2009	4.97±0.34	20.32±0.64	3.93±0.26 (2~5)
Jul. 15, 2009	4.67±0.32	22.38±0.73	3.33±0.46 (2~5)
Jul. 22, 2009	5.26±0.41	20.31±1.24	4.20±0.31 (3~6)
Jul. 30, 2009	5.15±0.43	20.21±0.82	3.13±0.46 (2~4)
Aug. 7, 2009	4.42±0.28	20.16±0.73	3.00±0.52 (2~5)
Aug. 15, 2009	4.33±0.42	20.43±1.14	3.40±0.34 (2~5)
Aug. 22, 2009	5.07±0.42	23.58±0.97	4.93±0.54 (2~7)
Aug. 30, 2009	5.72±0.52	21.11±0.88	4.87±0.33 (3~7)
Sep. 7, 2009	5.25±0.33	20.97±1.04	4.07±0.43 (3~6)
Sep. 15, 2009	5.17±0.46	19.97±1.10	3.20±0.37 (2~5)
Sep. 22, 2009	4.83±0.32	18.64±1.35	2.67±0.42 (2~4)
Sep. 30, 2009	4.19±0.19	18.46±1.04	3.60±0.43 (2~5)
Oct. 7, 2009	4.35±0.23	18.41±0.77	3.47±0.32 (2~5)
Oct. 15, 2009	4.20±0.30	18.24±0.75	3.13±0.54 (2~5)
Oct. 22, 2009	4.04±0.44	18.21±0.76	3.27±0.45 (2~6)

The results of one-way analysis of variance (ANOVA) of cambium cell number, radial and tangential diameter of cambium cell are shown in Tab. 2. According to the ANOVA, the data of them were significant variation at the 0.01 level. This indicates that the characteristics of cambial zone are largely impacted by growth time during the active phase.

Tab. 3: Variance analysis of cambial features with time.

		Sum of Squares	df	Mean Square	F	Sig.
Number of cambium cell	Between Groups	460.552	20	23.028	22.029	P<0.01
	Within Groups	307.333	294	1.045		
	Total	767.886	314			
R-diameter of cambium cell	Between Groups	74.570	20	3.728	3.071	P<0.01
	Within Groups	101.970	84	1.214		
	Total	176.540	104			
T-diameter of cambium cell	Between Groups	449.505	20	22.475	6.253	P<0.01
	Within Groups	301.920	84	3.594		
	Total	751.425	104			

The change of xylem cells during the differentiation process

Tissue proportions of xylem cells during the differentiation process were measured. In order to better analyze the data, curves of wood fiber and vessel element proportions were plotted, and results are shown in Figs. 1 to 4. According to Fig. 1, the average proportion of wood fiber increased gradually throughout the whole process of differentiation. To be more exact, the maximum rate of increase was 12.1 % from May to June. In contrast, the proportion in June and July stayed unchanged. In addition, from July to August there was another marked rise, and the growth rate was 9.50 %. However, from August to October the ratio rose slightly, and the rise in rate was 3.73 % and 4.46 %, respectively. It was observed that the average proportion of vessel elements decreased gradually throughout the whole differentiation process. To be more precise, the maximum rate of decrease was 19.46 % from May to June. By contrast, the proportion in June and July remained stable. Furthermore, from July to August it also had a significant reduction, and the rate was 18.97 %. The rate of decline was 7.90 % and 16.14 % respectively from August to October.

It also can be seen form Fig. 1 and Fig. 2 that the proportion change was one to one correspondence between wood fiber and vessel element in each period. Wood fiber proportion increased, the opposite was that of vessel element to decrease accordingly. In the prophase of exuberant active stage from May to June, wood fiber proportion increased with the reduction of vessel element, which may be the mechanism to ensure supply ability of nutrients throughout the tree to meet the needs of cambium division activity and formation of a large number of different xylem cells simultaneously at this stage. On the contrary, in the anaphase of active phase, with the weakening of cambium division activity, although the produced xylem cells reduced, the cell wall material in per unit volume increased significantly. With the slowness of increase rate of wood fiber proportion, reduction rate of vessel element proportion slowed correspondingly. These results showed that trees might mainly through diverse differentiation ways of xylem cells to maintain the dynamic balance of the whole growth process of the trees.

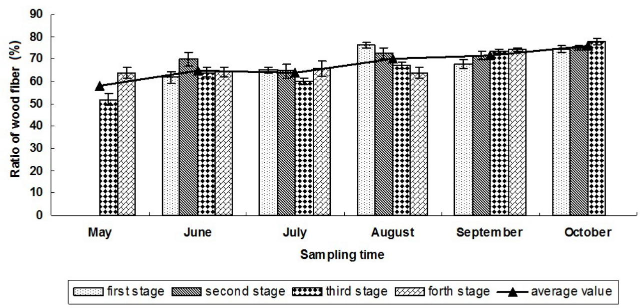

Fig. 1: The change of wood fiber proportion during the differentiation process.

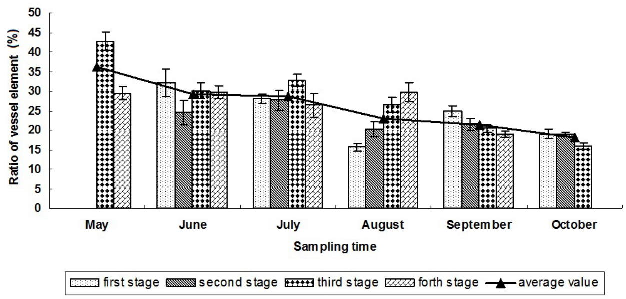

Fig. 2: The change of vessel element proportion during the differentiation process.

Fig. 3 illustrates the change of ray proportion during the differentiation process. It is known from the Fig. 3 that the ray proportion presented a trend which first increased then decreased. Among them, the maximum growth rate was 26.48 % from June to July; however, the largest decline rate was 10.72 % from July to August. Fig. 4 demonstrates the variation of parenchyma ratio during the differentiation process. As can be seen in the figure that there was an upwards trend in the proportion of parenchyma. To be specific, the greatest increase rate was 29.35 % from May to June and that was 16.81 % from June to July. It initially increased starting from July and then decreased in September and the rate was 8.39 %.

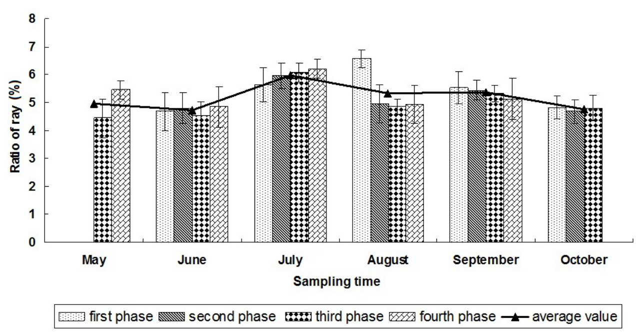

Fig. 3: The change of ray proportion during the differentiation process.

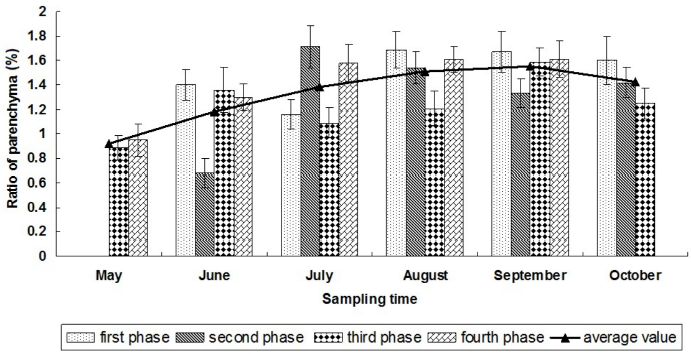

Fig. 4: The change of parenchyma proportion during the differentiation process.

The results of one-way ANOVA of tissue proportions of xylem cells are shown in Tab. 4. It was observed that the ratio of vessel element, wood fiber, ray and parenchyma were significant variation at the 0.01 level. This demonstrates that there is significant difference in wood fiber and vessel element with time during the active phase.

Tab. 4: Variance analysis of tissue proportion with time.

		Sum of Squares	df	Mean Square	F	Sig.
Ratio of vessel element	Between Groups	2773.675	19	145.983	21.720	P<0.01
	Within Groups	537.680	80	6.721		
	Total	3311.355	99			
Ratio of wood fiber	Between Groups	2737.286	19	144.068	18.127	P<0.01
	Within Groups	635.817	80	7.948		
	Total	3373.103	99			
Ratio of ray	Between Groups	33.632	19	1.770	2.741	P<0.01
	Within Groups	51.669	80	0.646		
	Total	85.301	99			
Ratio of parenchyma	Between Groups	11.245	19	0.592	4.584	P<0.01
	Within Groups	10.329	80	0.129		
	Total	21.575	99			

Fig. 5 shows the change of wood fiber length during the differentiation process. According to the figure, the trend was generally towards an increase in the whole differentiation process. More specially, it reduced slightly with a rate of 0.68 % from May to June. The smallest increase rate was 1.68 % from June to July and the greatest one was 15.67 % from August to September. The change of wood fiber width is indicated in Fig. 6. It can be seen in the figure that the width remained stable in the prophase of the differentiation process, but in the later stage it had a slight increase. Specially, there was a straight reduction from May to August and the decline rate was 2.68 %, 1.80 % and 1.24 % respectively, whereas from August to September the largest rate of increase was 14.68 %. According to the Fig. 7, the change of wall to lumen of wood fiber was a gradual increase trend in the whole differentiation process. The maximum rise was found between August and September with the rate of 46.34 %.

Most researches about morphological changes of wood fiber within the growth ring for the hardwood have focused on the wood fiber length. For the trees which do not have clear growth rings, the wood fiber length did not change significantly, while the ones with obvious growth rings, the variation trends of wood fiber was roughly similar to tracheid of softwood. Although wood fiber length decreased in mid-August, the trend of the whole active phase was gradually increasing. The variation trends of length to width, wall to lumen and proportion of wood fiber also were gradually increasing during the active phase. The increase of wood fiber proportion also can be reference to the reduction of tangential diameter and distribution frequency of vessel elements and the increase of wall to lumen of wood fibers.

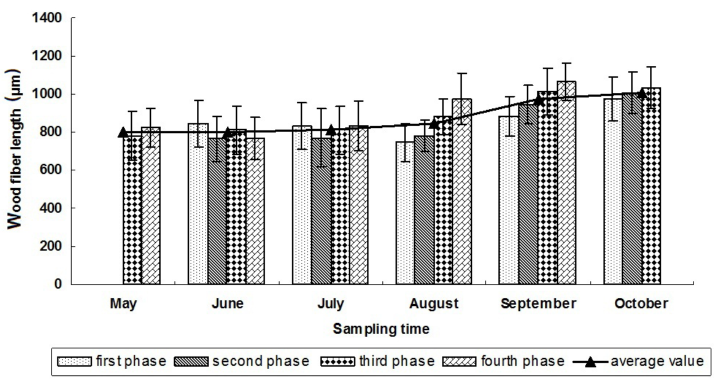

Fig. 5: The change of wood fiber length during the differentiation process.

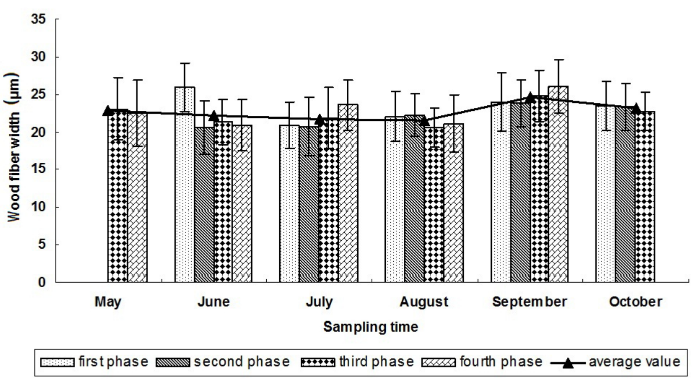

Fig. 6: The change of wood fiber width during the differentiation process.

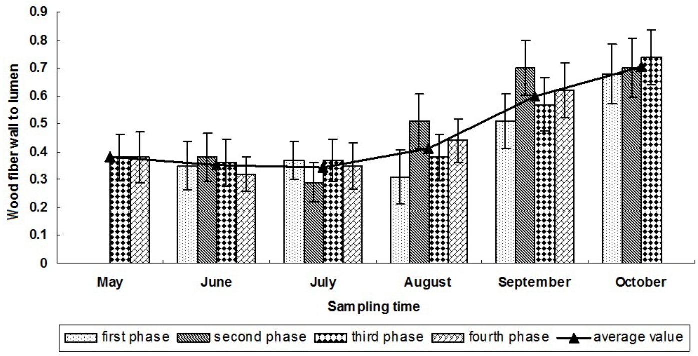

Fig. 7: The change of wood fiber wall to lumen during the differentiation process.

Fig. 8 shows the change of vessel element length during the differentiation process. According to the figure, the length change presented a trend which first decreased then increased in the whole process of differentiation.

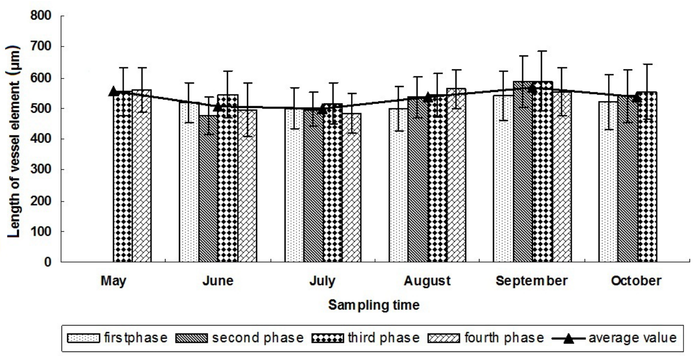

Fig. 8: The change of vessel element length during the differentiation process.

More precisely, the maximum rate of decrease was 8.69 % from May to June. The decrease rate was 1.83 % from June to July. After that, from July to August there was a marked rise, the growth rate was 7.31 %, however, form August to September the ratio rose slightly, and the rise rate was 5.95 %. Fig. 9 indicates the changes of vessel element width. It can be seen that the width reduced slightly then increased and stayed unchanged finally. To be more specific, there was a marked decrease from May to June and the decrease rate was 8.81 %, by contract, it had a significant increase from July to August and the increase rate was 9.68 %. Fig. 10 lists the changes of distribution frequency of vessel element during the differentiation process. The whole trend was first decreased then increased. To be precise, from May to June the rate of decrease was 10.48 %. In addition, from June to July the largest rate of decrease was up to 19.64 % and the rate of reduce was 19.43 % from July to August, however, the rate of increase was up to 25.06 % from August to September.

Change way of vessel element of hardwood is more complicated than that of softwood because of many types of xylem cell in hardwood and different distribution way of vessel element in xylem. Through the dynamic observation and analysis of the formation process of xylem cell, it was found that from early phase to late phase by the active process, despite the length change of a single vessel element was not significant, but the distribution frequency of vessel elements decreased gradually, and the later phase was clearly less than that in the early stage, and vessel element proportion also decreased significantly.

Tab. 5 shows the results of one-way ANOVA of tissue proportions of xylem cells. It was observed that the data of wood fiber and vessel element were significant variation at the 0.01 level. This demonstrates that there is significant difference in tissue proportions during the active phase.

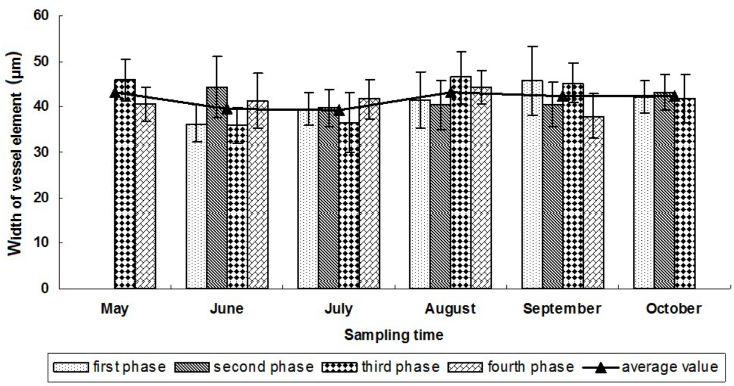

Fig. 9: The change of width of vessel element during the differentiation process.

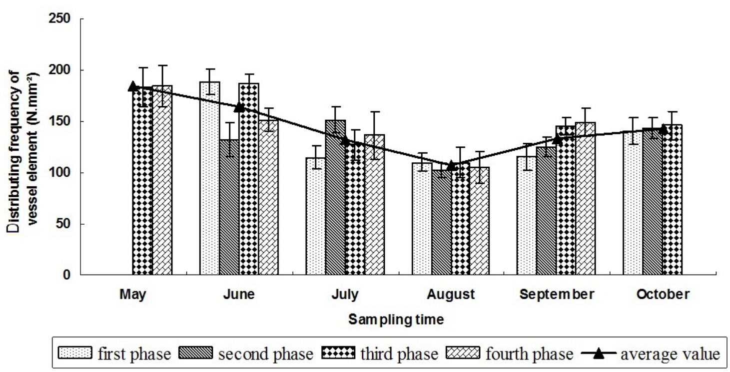

Fig. 10: The change of distribution frequency of vessel element during the differentiation process.

Tab. 5: Variance analysis of wood fiber and vessel element with time.

		Sum of Squares	df	Mean Square	F	Sig.
Wood fiber length	Between Groups	1.250E+07	20	625058.610	30.612	P<0.01
	Within Groups	2.528E+07	1238	20418.749		
	Total	3.778E+07	1258			
Wood fiber width	Between Groups	3760.724	20	188.036	9.933	P<0.01
	Within Groups	23455.930	1239	18.931		
	Total	27216.654	1259			
Wood fiber wall to lumen	Between Groups	12.993	20	.650	38.173	P<0.01
	Within Groups	10.365	609	.017		
	Total	23.358	629			
Vessel element length	Between Groups	1.553E+06	20	77635.992	8.322	P<0.01
	Within Groups	1.156E+07	1239	9329.146		
	Total	1.311E+07	1259			
Vessel element width	Between Groups	20152.384	20	1007.619	10.041	P<0.01
	Within Groups	208624.751	2079	100.349		
	Total	228777.135	2099			
Distribution frequency of vessel element	Between Groups	182110.347	20	9105.517	29.785	P<0.01
	Within Groups	57778.280	189	305.705		
	Total	239888.627	209			

The morphological correlation between the cambium cell and the xylem cell

For the tropical hardwood species, many studies have compared the morphological characteristics relationship between cambium fusiform initial cells and xylem cells derived from them. Most studies showed that there was positive correlation between length of cambium fusiform cell and length of wood fiber at different heights with time (Ridoutt and Sands 1993, Venugopal and Krishnamurthy 1987, Antonova et al. 1995). However, for the temperate ones, past researches made a great deal of analysis mainly on the variation of wood properties in different growth ring, even within the same growth ring of xylem cells, it was only mentioned that it had relationship with the change of cambium (Rao and Rajput 1999), what relationship existed on earth had not been further instructed.

Linear relationship analysis was made between cambium cell and xylem cells in morphological characteristics. The measured data were taken in comparison. The most significant relations are highlighted in Tab. 6. According to the Pearson's analysis, the number of Cambium cells had significant negative correlations with wood fiber length and wall to lumen and a significant positive correlation with vessel element length. The T-diameter of cambium cell presented significant negative correlations with wood fiber width, wood fiber wall to lumen and vessel element length. Additionally, the R-diameter of cambium cell presented a significant negative correlation with t wood fiber wall to lumen and a negative correlation with wood fiber length.

The results of this study give quantitative support to the notion that variation in cambium cell tangential width is an important mechanism influencing wood fiber width in *Populus×euramericana* cv.'74/76'. In addition, in the prophase of active stage, despite a large amount of wood fiber cells were generated, the cell wall material formed in per unit volume was not much, which may be the mechanism to ensure supply ability of nutrients throughout the tree to meet the needs of cambium division activity and formation of a large number of different xylem cells simultaneously at this stage (Yin et al. 2004). On the contrary, in the anaphase of active phase, with the weakening of cambium division activity, although the produced xylem cells reduced, the cell wall material in per unit volume increased significantly with the increase of proportion and wall to lumen of wood fiber. These results showed that trees might mainly through diverse differentiation ways of xylem cells to maintain the dynamic balance of the whole growth process of the trees (Khan et al. 1981).

Tab. 6: Correlation coefficient of anatomical properties of cambium cells and differentiation xylem cells.

	Length of wood fiber	**Width of wood fiber**	**Wood fiber wall to lumen**	**Length of vessel element**	**Width of vessel element**
Cambium cell number	-0.195**	0.014	-0.289**	-0.065	0.223**
Cambium cell R-diameter	-0.209*	-0.068	-0.295**	-0.002	0.44
Cambium cell T-diameter	-0.234	-0.257*	-0.389**	-0.281**	-0.103

** Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed).

A great deal of studies (Fan 1999) has proved that when the fusiform initial cells and their derived cells grow to be the tubular cells, they would be necessarily subject to certain factors. The environment near or within a cell determines its differentiation and development, and this internal environment is mainly composed of plant hormones with different capacity and proportion (Savidge 1994). In addition, seasonal external factors such as sunlight, moisture, nutrients and temperature will be significantly modified the xylem phenotype (Walsel and Fahn 1965). Therefore, the morphology of xylem cell is a unified reflection of its own genetic characteristics and variable regularity influenced by the environment. Therefore, if the future studies can comprehensively consider the various factors, they will truly reflect the differentiation of xylem cells.

CONCLUSIONS

The results reveal that the anatomical characteristics of cambium cell were varied significantly during the active phase. The changes of cambium cell number and radial diameter of cambium cell presented decreasing trends. However, the tangential diameter varied less regularly.

Furthermore, the change of tissue proportion of xylem cell was large during the differentiation process. The proportion of wood fiber and parenchyma presented gradual increasing trends and the greatest growth rate was 12.1 % and 29.35 % respectively. However, the proportion of vessel element presented a gradual decreasing trend and the greatest decline rate was 12.1 %. The proportion of ray first increased then decreased, and the maximum growth rate was 26.48 %, the maximum decline rate was 10.72 %. Trends of length, width and wall to lumen of wood fiber were gradually increasing from prophase to anaphase and the maximum increase rate was 15.67 %, 14.68 % and 46.34 %, whereas the trends of length, width and distribution frequency of vessel element were first decreasing then increasing and the largest decline and growth rate was 8.69 % and 7.31 %, 8.81 % and 9.68 %, 19.64 % and 25.06 % correspondingly.

It also can be pointed out that the morphological correlation of cambium cells with wood fiber was stronger than that with vessel elements during the differentiation process. Firstly, the number of Cambium cells had significant negative correlations with wood fiber length and wall to lumen and a significant positive correlation with vessel element length. Secondly, the T-diameter of cambium cell presented significant negative correlations with wood fiber width, wood fiber wall to lumen and vessel element length. Additionally, the R-diameter of cambium cell presented a significant negative correlation with t wood fiber wall to lumen and a negative correlation with wood fiber length.

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REFERENCES

1. Ajmal, S., Iqbal, M., 1987: Annual rhythm of cambial activity in *Streblus asper*. IAWA Bull. 8: 275–283.
2. Antonova, G.F., Cherkashin, V.P., Stasova, V.V., Varaksina, T.N., 1995: Daily dynamics in xylem cell radial growth of Scotch Pine (*Pinus sylvestris*). Trees 10(1): 24–30.
3. Barnett, J.R., 1973: Seasonal variation in the ultrastructure of the cambium in New Zealand grown *Pinus radiata* D. Don. Annals of Botany 37(5): 1005–1011.
4. Catesson, A.M., 1994: Cambial ultrastructure and biochemistry: Changes in relation to vascular tissue differentiation and the seasonal cycle. Int. J. Plant Sci. 155(3): 251–261.
5. Chen, H.M., Han, J.J., Cui, K.M., He, X.Q., 2010: Modification of cambial cell wall architecture during cambium periodicity in *Populus tomentosa* Carr. Trees 24(3): 533-540.
6. Dave, Y.S., Rao, K.S., 1982: Seasonal activity of the vascular cambium in *Gmelina arborea* Roxb. IAWA Bull. 3(1): 59–65.
7. Denne, M.P., Dodd, R.S., 1981: The environmental control of xylem differentiation. In: Barnett J.R. (ed.) Xylem cell development. Pp 236-255, Castle House, Kent.
8. Deshpande, B.P., Rajendrababu, T., 1985: Seasonal changes in the structure of the secondary phloem of *Grewia tiliaefolia* a deciduous tree from India. Annals of Botany 56(1): 61–71.
9. Eckstein, D., Sass, U., Baas, P., (eds.) 1995: Growth periodicity in tropical trees. In: Proceedings of internal meeting, Kuala Lumpur, Malaysia, November 1994. IAWA J. 16(4): 323-442.

10. Fahn, A., Werker, E., 1990: Seasonal cambial activity. In: The vascular cambium. Edited by M. Iqbal. John Wiley and Sons Ltd., New-York. Pp 139-154.
11. Fan, R.W., 1999: A review of the study on the developmental biology of xylem. Chinese Bulletin of Botany 16(4): 387–397.
12. Farrar, J.J., Evert, R.F., 1997: Seasonal changes in the ultrastructure of the vascular cambium of *Robinia pseudoacacia*. Trees 11(4): 191–202.
13. Ghouse, A.K.M., Hashmi, S., 1979: Cambium periodicity in *Polyalthia longifolia*. Phytomorphology 29(1): 64–67.
14. Ghouse, A.K.M., Hashmi, S., 1980a: Seasonal production of secondary phloem and its longevity in *Mimusops elengi* L.. Flora 170: 175–179.
15. Ghouse, A.K.M., Hashmi, S., 1980b: Longevity of secondary phloem in *Delonix regia* Rafin. Proc. Indian Acad. Sci. 89(1): 67–72.
16. Iqbal, M., Ghouse, A.K.M., 1987: Anatomy of the vascular cambium of *Acacia nilotica* (L.) Del. var. telia Troup (Mimosaceae) in relation to age and season. Bot. J. Linn. Soc. 94(3): 385–397.
17. Iqbal, M., Ghouse, A.K.M., 1990: Cambial concept and organisation. In: The vascular cambium. Edited by M. Iqbal. Research Studies Press/John Wiley and Sons, Inc., Taunton, UK. Pp 1–36.
18. Iqbal, M., 1995: The cambial derivatives. Gebruder Borntraeger, Berlin, 363 pp.
19. Jacoby, G.C., 1989: Overview of tree-ring analysis in tropical regions. In: Baas P., Vetter R.E. (eds.) Increment zones in tropical woods. IAWA Bull. 10(2): 99–108.
20. Khan, K.K., Ahmad, Z., Iqbal, M., 1981: Treads of ontogenetic size variation on cambial initials and their derivatives in the stem of *Bauhinia parvifiora* Vah1. Bull. Soc. Bot. Ft. 128: 165–175.
21. Kozlowski, T.T., Kramer, P.J., Pallardy, S.G., 1991: The physiological ecology of woody plants. Harcourt Brace Jovanovich, London, 411 pp.
22. Larson, P.R., 1994: The vascular cambium development and structure. Springer-Verlag, Berlin, Germany, 725 pp.
23. Marcati, C.R., Angyalossy, V., Evert, R.F., 2006: Seasonal variation in wood formation of *Cedrela fissilis* (Meliaceae). IAWA J. 27(2): 199–211.
24. Marcati, C.R., Milanez, C.R.D., Machado, S.R., 2008: Seasonal development of secondary xylem and phloem in *Schizolobium parahyba* (Vell.) Blake (Leguminosae: Caesalpinioideae). Trees 22(1): 3-12.
25. Mellerowicz, E.J., Baucher, M., Sundberg, B., Boerjanet, W., 2001: Unravelling cell wall formation in the woody dicot stem. Plant Molecular Biology 47(1-2): 239-274.
26. Priya, P.B., Bhat, K.M., 1999: Influence of rainfall, irrigation and age on the growth periodicity and wood structure in teak (*Tectona grandis*). IAWA J. 20(2): 181–192.
27. Rajput, K.S., Rao, K.S., 1998: Seasonal anatomy of secondary phloem of teak (*Tectona grandis* L., Verbenaceae) growing in dry and moist deciduous forests. Phyton 38(2): 251–258.
28. Rajput, K.S., Rao, K.S., 2001: Cambial activity and development of xylem in *Tamarindus indica* L. growing in different forests of Gujarat State. Acta Botanica Hungarica 43(3-4): 379–390.
29. Rao, K.S., Rajput, K.S., 1999: Seasonal behavior of vascular cambium in teak (*Tectona grandis*) growing in moist deciduous and dry deciduous forests. IAWA J. 20(1): 85–93.
30. Rao, K.S., Rajput, K.S., 2000: Cambial activity and development of wood in *Acacia nilotica* (L.) Del. growing in different forest of Gujarat State. Flora 195: 165–171.

31. Rao, K.S., Rajput, K.S., 2001: Relationship between seasonal cambial activity, development of xylem and phenology in *Azadirachta indica* growing in different forests of Gujarat State. Ann. For. Sci. 58(6): 691–698.
32. Rensing, K.H., Samuels, A.L., 2004: Cellular changes associated with rest and quiescence in winter-dormant vascular cambium of *Pinus contorta*. Trees 18(4): 373–380.
33. Ridoutt, B.G., Sands, R., 1993: Within-tree variation in cambial anatomy and xylem cell differentiation in *Eucalyptus globulus*. Trees 8(1): 18-22.
34. Savidge, R.A., 1994: The tracheid-differenciation factor of conifer needles. Journal of Plant Science 155(3): 272–290.
35. Sundberg, B., Uggla, C., Tuominen, H., 2000: Auxin gradients and cambial growth. In: R. Savidge, J. Barnett and R. Napier (Eds.) Cell and Molecular Biology of Wood Formation (SEB Experimental Biology Reviews). Pp 169–188, BIOS, Oxford.
36. Venugopal, N., Krishnamurthy, K.V., 1987: Seasonal production of secondary xylem in the twigs of certain tropical trees. IAWA Bull. 8(1): 31–40.
37. Venugopal, N., Liangkuwang, M.G., 2007: Cambial activity and annual rhythm of xylem production of elephant apple tree (*Dillenia indica* Lin.) in relation to phenology and climatic factor growing in sub-tropical wet forest of northeast India. Trees 21(1): 101–110.
38. Walsel, Y., Fahn, A., 1965: The effects of environment on wood formation and cambial activity in *Robinia pseudoacacia* . The new phytologist 64(3): 436–442.
39. Whetten, R., Sederoff, R., 1991: Genetic engineering of wood. For. Ecol. Manage 43(3): 301-316.
40. Yin, Y.F., Jiang, X., Liu, X., 2004: Dynamic changes in cambial anatomy and xylem cell differentiation of shoots in *Populus tomentosa*. Scientia Silvae Sinicae 40(2): 119–125.

Enhua Xi, Guangjie Zhao
Beijing Forestry University
College of Material Science and Technology
Qinghua Eastroad 35
Haidian
Beijing
China 100083
Phone: 86 010 62337751
Corresponding author: zhaows@bjfu.edu.cn