# CELL WALL STRUCTURE AND MECHANICAL PROPERTIES OF *SALIX PSAMMOPHILA*

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> > (Received August 2016)

# ABSTRACT

Salix psammophila can grow rapidly in desert and grassland areas. As an abundant bioresource, it is useful to understand its cell morphology, chemical compositions and mechanical properties. In this study, Anatomical properties of Salix psammophila in different annual rings were measured and compared. Fourier transform infrared spectroscopy and chemical titration were used to estimate cell wall chemical compositions. Moreover, mechanical properties in different annual rings were measured through nanoindentation. Fiber cell lumen diameter, fiber cell wall thickness, vessel cell lumen diameter and vessel cell wall thickness of Salix psammophila were measured to be 7.371, 2.285, 32.541 and 1.926  $\mu$ m, respectively. Fiber cell lumen diameter, fiber cell wall thickness, vessel cell lumen diameter differs among the annual rings. The cellulose, hemicellulose and lignin contents of Salix psammophila were 44.43, 34.99 and 17.93 %, respectively. Both hemicellulose and lignin contents varied among the annual rings with more hemicellulose but less lignin at the annual ring closer to the pith. The modulus of elasticity (MOE) of fiber cell wall of Salix psammophila decreases from pith to bark.

KEYWORDS: Fiber morphology, vessel morphology, chemical compositions, mechanical properties.

## **INTRODUCTION**

Salix psammophila is a xerophytic deciduous shrub growing in Inner Mongolia's Kubuqi Desert that can survive in water-deficit environment (Kubo et al. 2013). It grows fast and can reach five meters tall in the first three years. It is a good practice to trim Salix psammophila above ground every 3 years to allow it to regenerate. The research of 3-year-old Salix psammophila is practical for the use of the trimmed salix materials (Xu et al. 2006a). According to the data, harvestable biomass can also reach 5.2 t/ha in 3 years in Inner Mongolia's Kubuqi Desert (Kubo et al. 2013). Thus, it can be a promising bioresource to ease short supply of wood. However, most of the abundant wood resource has traditionally been used as fuel and animal fodder. Only a few of them has been used as raw material in fiberboards, wood pulp and the potential uses in the bioethanol (Sun et al. 2006; Jiang et al. 2011; Xue et al. 2011).

Cell morphology, chemical composition and mechanical properties of fiber impact wood properties and pulp quality. In previous studies, vessel length, vessel diameter, and fiber cell width of 3-year-old Salix psammophila have been measured, and found fiber width of earlywood was larger than latewood (Feng et al. 1996; He et al. 2000). Xu et al. (2006b) measured the fiber length and width of Salix psammophila, and found the ratio of fiber length to width (L/W) was 35 and the ratio of fiber double cell wall thickness to lumen diameter (2M/L) was 0.47. This has proved that *Salix psammophila* can be papermaking raw material (L/W>33 and 2M/L<1). Vessel element length (341  $\mu$ m) and fiber length (641  $\mu$ m) gradually increased from pith to bark (Yang et al. 2010). Chemical composition of Salix psammophila has also been determined (Xue et al. 2011). Though mechanical properties of Salix psammophila have been investigated in macro scale (Sun et al. 2012), it is hard to analyze it in detail for its small diameter, such as in different annual rings. For most above studies, the whole plant was chosen as experimental object. It was unclear whether the annual ring has effect on Salix psammophila fiber and vessel cell lumen diameter, cell wall thickness, chemical composition and mechanical properties, but studies have shown that wood age has influence on cell morphology, chemical composition and mechanical properties (Wang et al. 2005; Shi et al. 2006; Ishiguri et al. 2004; He et al. 2009; Niklas 1997).

This study was to explore the cell morphology, chemical compositions and micromechanical properties of 3-year-old *Salix psammophila*. The anatomical parameters of earlywood and latewood and radial variation within the annual rings were compared. The Fourier Transform infrared (FTIR) spectroscopy was used to compare relative content of its woody chemical compositions at different annual rings. Meanwhile, mechanical properties of *Salix psammophila* on a cell structure level were first analyzed by nanoindentation (NI). Overall, this work extended the knowledge of the structure and properties of *Salix psammophila*, which were important to the utilization of it.

# MATERIAL AND METHODS

#### Sample preparation

*Salix psammophila* were felled in Inner Mongolia's Kubuqi Desert, 600 kilometres (370 miles) west of Beijing. In Inner Mongolia of China, in winter, usually from November to March, is cold, and dry with frequent blizzards. Other seasons are short, hot, and have heavy sandstorms. Most of this region is classified as a cold arid regime.

Three 3-year-old *Salix psammophila* plants were cut in May. Plant height was 2.6-2.8 m and the diameter at breast height was 12 mm. From each plant, sample with a length of 370 mm was prepared. Two 10 mm thick discs and a 30 mm thick disc were cut from the sample. One

10 mm thick disc was further processed into strips 6 ×8 ×10 mm (R × T ×L) for the anatomical measurement, the other for Fourier



Fig. 1: Salix sample preparation.

Transform infrared spectroscopy (FTIR) analysis. And the 30 mm thick disc was prepared for NI testing. A stem with length of 350 mm was prepared for chemical titration analysis (Fig. 1).

## Anatomical explorations

The strips for anatomical measurement were heated in water at 100°C, and then soften for 7 h with 1:1 alcohol (95 %)/glycerin. To measure cell wall thickness and cell lumen diameter at different annual rings and different locations within a growth ring, samples were sectioned to the 15  $\mu$ m thickness using a sliding microtome. The sections were dehydrated through alcohol series (30 %, 5 min; 50 %, 5 min; 80 %, 5 min; 95 %, 5 min; 100 %, 10 min) and stained with safranine for 2 minutes. The cell structure was observed and studied with Leica DMLB light microscope and an image analysis system (Japan, Q570). The function of arbitrary line in the system was used to measure tangential cell lumen diameter and double radial cell wall thickness at more than 100× magnification. Three categories were chosen within every annual ring from pith to bark, and 100 fiber cells and 100 vessel cells for each plant were measured.

## **Chemical titration**

For the chemical titration, air dried samples were cut into size of matchsticks  $3 \times 3 \times 20$  mm (R × T ×L), and then milled into fine powder. They were used to determine the content of cellulose, lignin, and holocellulose in *Salix psammophila* according to the ASTM standard (ASTM D1103-60:1960). Each sample was measured two times, and the result was averaged. The error between two measured values has been limited less than 0.4 %.

#### Fourier transform infrared spectroscopy (FTIR)

In FTIR, the experimental samples were selected from three plants (Fig. 1). The discs were processed from three annual rings in the log. The epidermis, phloem and pith were removed. The discs were dried and grinded to wood flour. KBr flour and wood flour were oven-dried for 24 hours under the conditions of  $103 \pm 2^{\circ}$ C, and then cooled in a desiccator. The flour was compressed into small pellets (the diameter was 10 mm). The prepared sample was placed in an infrared light (Nicolet Impact 410, USA) to acquire the spectroscopy. The heights of characteristic peak were obtained by OMNIC software. The ratio of characteristic peaks height of corresponding chemical composition was used to compare the content of chemical components (Colom et al. 2003; Stevanic and Lennart 2008; Pandey and Pitman 2003).

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#### NI testing

Three strips in the size of  $1 \times 1 \times 30$  mm (R  $\times$  T  $\times$ L) for NI testing were prepared from the latewood in three annual rings. The epoxy resin was molded (Wang et al. 2013) and they were placed in a vacuum desiccator for 12 h and cured in an oven at 60°C for 36 h. The wood strips were then mounted into the embedding mold parallel to the l-axis of the cell wall. The top surface was smoothed with a diamond knife in an ultramicrotome. The specimens were conditioned at 20°C and a relative humidity of 65 % for at least 24 h before NI test. The conditioned samples then were placed on the sample table of the nanoindenter (Triboindenter, Hysitron, USA). The diamond Berkovich indenter was used with the radius of indenter tip less than 100 nm and the taper angle of 135°. Quasi-static constant speed loading and unloading mode was set with the maximum load of 250 µN, the loading rate of 50 µN.s<sup>-1</sup>, and the loading time of 6 s.

## Statistical analysis

A one-way analysis of variance (ANOVA) was used for comparative analyses. A statistical analysis of the differences among all the positions was made using *t-test*. The correlation coefficients between chemical composition and the relative peak intensity ratio of the spectrum were calculated to determine their relationship.

# **RESULTS AND DISCUSSION**

## Cell morphology

Fig. 2 depicted the cross sections of three annual rings of *Salix psammophila*, which was a diffuse porous hardwood. The vessel pores were solitary with a few of multiple pores.



Fig. 2: Typical cross-sectional images of three rings of Salix psammophila- A) Ring 1. –B) Ring 2. –C) Ring 3.

Cell morphologic characters of *Salix psammophila* including fiber cell lumen diameter, fiber cell wall thickness, vessel cell lumen diameter and vessel cell wall thickness were measured. They were presented in Tab. 1.

Plants	Fiber cell lumen diameter (µm)	Fiber cell wall thickness (µm)	Vessel cell lumen diameter (µm)	Vessel cell wall thickness (µm)
1	7.758±1.897	2.630±0.430	35.678±4.276	2.079±0.317
2	6.697±0.924	2.487±0.563	30.538±4.765	1.889±0.360
3	7.657±1.094	1.737±0.361	31.407±4.874	1.809±0.317
Average	7.371±1.449	2.287±0.601	32.541±5.146	1.925±0.349

Tab. 1:	Cell	size	of	<sup>c</sup> different	three	plants	of	Salix	psammo	phila
			./	././		/	./		/	

Fiber cell lumen diameter, fiber cell wall thickness, vessel cell lumen diameter and vessel cell wall thickness of *Salix psammophila* at 3 years old were 7.371, 2.285, 32.541 and 1.926  $\mu$ m on average. The ratio of double cell wall thickness to lumen diameter (2M/L) was 0.62 for fiber cells and 0.12 for vessel cells. These ratios combined with other fiber morphology made Salix to be a good papermaking raw material (Xu et al. 2006a, b).

## Cell morphology at different annual rings

The cell lumen diameters and wall thicknesses of wood fiber and vessel in each annual ring were measured and presented in Tab. 2.

Annual	Fiber cell lumen		Fiber cell wall		Vessel cell lui	men	Vessel cell wall	
rings	diameter (µ	m)	thickness (µm)		diameter (µm)		thickness (µm)	
1	8.374±1.617	А	2.015±0.519	Α	29.936±4.521	А	1.837±0.335	А
2	7.268±1.050	В	2.288±0.552	В	32.935±4.794	В	1.993±0.353	В
3	6.469±0.881	C	2.556±0.608	C	34.752±4.967	С	1.946±0.345	В
P-value	1.01E-20	1.01E-20 3.63E-09		4.10E-10		0.0087		

Tab. 2: Cell size and its variance analysis of Salix psammophila at different annual rings.

Notes: Different letters after values in the same column indicates that there is a significant difference between annual rings at p < 0.05 (Student Newman Keuls test), same in the following tables.

Morphological parameters of the three trees were organized and divided into 3 groups according to annual ring. The difference significance of cell morphology in three growth rings was analyzed based on *t-test*. The sizes of fiber cell lumen diameter, fiber cell wall thickness, vessel cell lumen diameter were significantly different at the each annual ring. Fiber cell diameter and lumen diameter decreased from the first to the third annual rings. The fiber cell wall thickness and vessel cell lumen diameter increased gradually. All the ratio of double cell wall thickness to lumen diameter (2M/L) of fiber and vessel were less than 1, and 2M/L of fiber increased from annual ring 1 to ring 3.

The fiber diameter and fiber cell lumen diameter were affected by the growth hormone. In the early stage of growth, more hormones increase cell division in trees. The apical growing point was more active, and the fiber diameter and fiber cell lumen diameter produced during this time were relatively larger (Zha et al. 2005). The radial variation of fiber cell wall thickness in the salix and 2M/L were consistent with those in poplar tree (Gu et al. 2007; Pan et al. 2010; Zha et al. 2005) and willow (Dai et al. 2001). Paper made from thinner cell wall and larger cell lumen fiber generally has better burst strength and folding endurance (Anjos et al. 2011; Lien et al. 2004). *Salix psammophila* fibers in annual ring 1 were suitable than other two annual rings in papermaking.

## Cell morphology in different positions within an annual ring

Tab. 3 shows the cell lumen diameter, wall thickness of wood fiber and vessel at three different positions within an annual ring.

Tab. 3: Cell size and its variance analysis of Salix psammophila in different positions within an annual ring.

Position	Fiber cell lu diameter (µ	men 1m)	Fiber cell wall thickness (µm)		Vessel cell lum diameter (µn	ien 1)	Vessel cell wall thickness (µm)	
Inside	7.667±1.692	Α	2.194±0.566	Α	34.833±4.840	А	1.876±0.353	A
Middle	7.431±1.469	AB	2.262±0.591	Α	33.333±4.616	В	1.920±0.372	A
Outside	7.013±1.049	В	2.398±0.635	Α	29.457±4.444	С	1.981±0.317	A
P-value	0.0086 0.0682		2.89E-13		0.1279			

The results of *t-test* to analyze the difference significance of cell morphology in three positions revealed that cell morphology differs much at the different positions even within the same annual ring. From early wood to late wood, fiber cell lumen diameter and vessel cell lumen diameter significantly decreased. However, fiber cell wall thickness and vessel cell wall thickness did not show any statistically difference (Fig. 2).

#### Variation of cell morphology from pith to bark

The fiber lumen diameter decreased although fiber cell wall thickness increased, but the vessel cell lumen diameter and cell wall thickness had no marked trend from pith to bark (Fig. 3).



Fig. 3: Radial variation of cell size in different position from pith to bark of Salix psammophila. A) The radial variation of fiber cell lumen diameter from pith to bark. B) The radial variation of fiber cell wall thickness from pith to bark. C) The radial variation of vessel cell lumen diameter from pith to bark. D) The radial variation of vessel cell wall thickness from pith to bark.

Zhao et al. (2014) reported the anatomical properties of juvenile wood of fast growing *Populus×euramericana* cv., which was also a typical diffuse-porous wood. The fiber cell wall thickness of the samples varied from 2.5 to 3.3 µm, which was larger than *Salix psammophila*. Vessels were almost uniform in size and distribution, which was differs from *Salix psammophila*. And variations in poplar fiber lumen diameter and thickness were also significant at different annual rings, which was the same as in *Salix psammophila*. Furthermore, by comparing the sections, the number of *Salix psammophila* vessel in unit area was more than poplar and the vessel lumen diameter was much smaller (Zhao et al. 2014). These features sustained the water transport

so that Salix can adapt to the high temperature and low precipitation in desert (Cao et al. 1991, Huang et al. 2005a, b).

## Comparison of chemical titration and FTIR spectroscopy methods

Chemical titration can accurately measure the chemical composition contents in the wood (ASTM D1103-60:1960). The cellulose content of *Salix psammophila* was measured to be 44.43 %. Hemicellulose content 34.99 % and the lignin content 17.93 % (Tab. 4).

Plants	Lignin content (%)	ignin content (%) Hemicellulose content (%)		Holocellulose content (%)	
1	17.48	35.74	43.12	78.86	
2	18.97	34.97	45.41	80.38	
3	17.33	34.26	44.76	79.02	
Average	17.93	34.99	44.43	79.42	

Tab. 4: Chemical composition content of Salix psammophila by means of chemical titration.

The production of bioethanol extracted from lignocellulose depends on the holocellulose content and lignin is not contribute much (Sticklen et al. 2008). By comparing with fast-growing *Populus×euramericana* cv., the holocellulose content of *Salix psammophila* was higher than that of poplar about 67.7 %, but the lignin content of *Salix psammophila* was less than that of poplar about 25.4 % (Zhao et al. 2014). The fact suggested that *Salix psammophila* could be a better material than poplar in producing bioethanol.

The assignment of bands in the FTIR spectrum of cell wall polymers was listed in Tab. 5. The FTIR spectra of the three trees were shown in Fig. 4.

Tab. 5: Assignment of bands in the FTIR spectrum of cell wall polymers.

	Wavenumber (cm <sup>-1</sup> )	Band assignment
Lignin	1510	Aromatic skeletal vibration
Xylan	1730	C=O stretch
Cellulose	895	Antisymmetric C-O-C bridge stretching
Holocellulose	1158	C–O–C vibration in cellulose and hemicellulose
Holocellulose	1375	C–H deformation in cellulose and hemicellulose



Fig. 4: FTIR spectra of three different trees.

Bands at 1510, 1730 and 895 cm<sup>-1</sup> were corresponding to the aromatic skeletal vibration in lignin, the C=O stretch in xylan and the antisymmetric C-O-C bridge stretch in cellulose respectively. Bands at 1158 and 1375 cm<sup>-1</sup> are corresponding to the C–O–C vibration and C–H

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deformation in cellulose and hemicellulose. By contrast, the intensity of the band at 1510 and 895 cm<sup>-1</sup> observed in the spectrum was the strongest in plant 2 as that occurred in the lignin and cellulose content, the intensity of the band at 1730 and 1510 cm<sup>-1</sup> was the weakest in plant 3 as that occurred in the hemicellulose and lignin content in Tab. 2. Bands ratio of *Salix psammophila* was presented in Tab. 6.

Plants	I <sub>1510</sub> /I <sub>1158</sub>	I <sub>1510</sub> /I <sub>1375</sub>	I <sub>1510</sub> /I <sub>895</sub>	I <sub>1730</sub> /I <sub>895</sub>	I <sub>1730</sub> /I <sub>1158</sub>
1	1.675	0.958	2.570	4.362	2.846
2	1.316	0.900	2.542	4.128	2.140
3	1.457	0.738	2.581	3.719	2.170

Tab. 6: The ratio of characteristic peaks height of three plants of Salix psammophila.

The correlation analysis among three main chemical compositions and characteristic bands ratio was used to characterize the relationship between chemical and FITR spectroscopy results (Schwanninger et al. 2004; Åkerholm and Salmén 2003). The correlation coefficient,  $R \leq -0.8$ , indicated a closely negative relationship between lignin content and  $I_{1510}/I_{895}$ , between cellulose content and  $I_{1510}/I_{1375}$  or  $I_{1730}/I_{895}$ . In addition, the correlation coefficient,  $R \geq 0.8$ , indicated a closely positive relationship between hemicellulose and  $I_{1510}/I_{1158}$  or  $I_{1730}/I_{158}$ .

Tab. 7: Correlation coefficients between chemical composition and ratio of characteristic peak height.

	I <sub>1510</sub> /I <sub>1158</sub>	I <sub>1510</sub> /I <sub>1375</sub>	I <sub>1510</sub> /I <sub>895</sub>	I <sub>1730</sub> /I <sub>895</sub>	I <sub>1730</sub> /I <sub>1158</sub>
Lignin	-0.745	0.342	-0.981	0.236	-0.460
Cellulose	-0.778	-0.872	0.032	-0.921	-0.950
Hemicellulose	0.995	0.277	0.679	0.381	0.895

The content of chemical composition can be measured with FTIR spectroscopy method through these correlations by assigning the bands.  $I_{1510}/I_{895}$  was used to determine lignin content,  $I_{1730}/I_{895}$  to determine cellulose content and  $I_{1730}/I_{1158}$  to determine hemicellulose content (Tab. 7).

#### Chemical compositions at different annual rings

Fig. 5 depicted the FITR spectra of three annual rings of *Salix psammophila*. The intensity of the 1730 cm<sup>-1</sup> peak in annual ring 3 was weaker than that of annual ring 2 and stronger than that of annual ring 1.



Fig. 5: FTIR spectra of three annual rings of Salix psammophila.

These phenomena indicated that the hemicellulose content was the lowest in annual ring 1 and the highest in annual ring 2. In the same way, the intensity of the 1510 cm<sup>-1</sup> indicated that the lignin content increased from annual ring 1 to 3. And the intensity of the 895 cm<sup>-1</sup> revealed

that the cellulose content at annual ring 2 was the highest, while the lowest in annual ring 3. The relative peak intensity ratio in different growth rings (Tab. 8) was calculated as the order of hemicellulose content. The highest hemicellulose content was found to be at annual ring 2, the lowest at annual ring 1.

Annual rings	I <sub>1510</sub> /I <sub>895</sub>		I <sub>1730</sub>	/I <sub>895</sub>	$I_{1730}/I_{1158}$		
1	2.706	А	3.678	A	1.220	А	
2	2.185	В	3.633	A	1.683	В	
3	2.111	В	3.754	A	1.661	В	
P-value	3.271	E-05	0.6085		1.27E-14		

Tab. 8: The ratio of characteristic peaks height and its variance analysis at different annual rings.

The lowest lignin content was found at annual ring 1. While, the highest lignin content was at ring 3. Data showed that there was no significant difference at the lignin content and hemicellulose content between ring 2 and ring 3, but they had significant variation between ring 2 or ring 3 and ring 1. This is the same as the structure in the poplar wood (Pu et al. 2002, Zhou et al. 2010). The small variation ranges of three ratios of characteristic peaks in Tab. 8 indicated that *Salix psammophila* chemical compositions varied in a small range. Thus, the variation of chemical compositions in different annual rings can be ignored during the processing and utilization of *Salix psammophila*.

#### NI testing

The longitudinal modulus of elasticity (MOE) and hardness of *Salix psammophila* were measured with NI testing. Fig. 6 shows the smooth tested surface. MOEs and hardnesses of *Salix psammophila* in three annual rings were obtained from the valid indentations and presented in Tab. 9.



Fig. 6: An image of NI test on Salix psammophila cell.

Annual rings	MOE (GPa)		Hardness (GPa)		
1	15.93(1.31)	А	0.535(0.059)	А	
2	15.84(1.82)	А	0.516(0.050)	А	
3	13.54(1.27)	В	0.528(0.038)	А	
Mean	15.10		0.526		
Р	2.87E-9		0.324		

Tab. 9: MOE and hardness of Salix psammophila and their variance analysis at different annual rings.

MOE of *Salix psammophila* was 6.3 % higher than that of poplar (Wu et al. 2009). MOE of wood near bark was lower than that of other two annual rings. Wood in the first ring has highest

hardness values. The hardness of *Salix psammophila* was greater than that of fast grown poplar about 0.490 GPa (Wu et al. 2009).

## CONCLUSIONS

Salix psammophila cell morphology at various annual rings differs significantly. The fiber cells near pith have larger fiber cell lumen diameter with less fiber cell wall thickness and smaller vessel cell lumen diameter. From pith to bark, fiber cell lumen diameter decreases from 8.374 to 6.469  $\mu$ m, fiber cell wall thickness increases from 2.015 to 2.556  $\mu$ m, and vessel cell lumen diameter varies from 29.9 to 34.8  $\mu$ m. The ratio of double cell wall thickness to lumen diameter (2M/L) of fiber and vessel in three annual rings are less than one.

The cellulose content has not shown significant differences among three annual rings. However, both hemicellulose and lignin contents vary among the annual rings with more hemicellulose and less lignin contents at the annual ring near the pith.

MOE in the third annual ring is significantly lower than in the inner two annual rings. Hardness values were the same among three annual rings. For the stronger micromechanical properties, the thinner cell wall and larger cell lumen, fibers near the pith could be a good raw material in papermaking.

#### ACKNOWLEDGMENT

The authors thank the State Key Laboratory of the Chinese Academy of Forestry Foundation (CAFYBB2012044), the National "973" Project Foundation of China (2012CB114506) and National natural science foundation (31260158, 31370562) for financial support.

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