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Studies on anatomical properties of *Salix* hybrids wood

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Abstract
The anatomical properties of *Salix* hybrids' wood or clones basically determine their suitability for various end-uses. The main objective of this study was to investigate the inter-clonal variation of anatomical properties in 3 year old 37 *Salix* hybrids' clones grown in Naganji experimental area, Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan (H.P). Wood samples of three individual clones per *Salix* hybrid were randomly collected and prepared according to the standard anatomical techniques. All anatomical properties of *Salix* hybrids' wood showed significant inter-clonal variation, indicating the possibility of identifying clones with superior wood properties. The mean values of vessel frequency, pore (vessel) diameter, ray height, ray width, uniseriate ray frequency, fiber length and fiber diameter were varied from 117.067 (UHFS260/11)-279.663/mm^2 (UHFS412), 30.49 (UHFS208)-60.62 µm (UHFS111), 0.097 (J194)-0.426 mm (UHFS296), 7.91 (UHFS208)-9.36 µm (UHFS333), 36.534 (UHFS111)-88.672/mm^2 (UHFS113), 0.677 (NZ1002)-1.142 mm (UHFS260/11) and 18.33 (UHFS412)-21.53 µm (UHFS248), respectively. Overall, the results of this study indicated that all these *Salix* hybrids' clones have therefore good potential to be used in the willow wood industry, particularly, as raw material for pulp and paper yields. This information can also be used for comparative studies with other *Salix* species as well as other tree species being evaluated for wood quality improvement and other applications. However, further continuous study is needed to assess any change of anatomical characteristics associated with age increment and environmental factors.

Keywords: Anatomical properties, *Salix* species, *Salix* hybrids' wood, clones

Introduction

Salix species are eco-friendly, multipurpose, fast growing and are widely used for plantation throughout the world. The genus *Salix* is a member of the family Salicaceae, commonly known as willow and consists of 450-520 species worldwide (Wu *et al*., 2015) [22]. The *Salix* species show all forms of development from tall trees to shrubs and to creeping and dwarf shrubs. The genus *Salix* is distributed over wide ecological and climatic zones, largely in the Northern Hemisphere with a centre of abundance in China and the former Soviet Union (Argus, 1997) [1]. Due to its wide geographic adaptation and fast growth, the genus *Salix* species has a significant economic value and some of the *Salix* species have been cultivated for a variety of end- uses such as baskets, cricket bats, hurdles, furniture, plywood, paper and pulp, rope making, firewood, poles, wicker products, wood for utensils etc. (Sharma *et al*., 2014) [19].

The world demand for the wood products and global solid wood material source is being insufficient (Huda, 2014) [7]. As a result, shortage in supply of raw material became serious issue and far behind the demand for the pulp and paper, plywood, furniture industries, etc. (Saravanan *et al*, 2014) [17]. It is, therefore, necessary to find alternative fast-growing species which are suitable to fill the gap between wood production and its consumption by using short rotation tree plantations. Therefore, *Salix* hybrids' clones have received considerable attention for their high productivity due to their fast growth rate and easily hybridization of two or more species within the genus *Salix*.

Wood property information is essential to wood processors and end-users. The processing and utilization of woods for different products can be strongly influenced by their anatomical properties. Hence, the aim of this study was to investigate the anatomical properties of *Salix* hybrids' wood in Dr. Y S University of Horticulture and Forestry, Nauni, Solan (H.P), India.

Materials and methods

Three-year old 37 *Salix* hybrids were used for the study. Three clones per *Salix* hybrid and one clone per block, totally 111clones were randomly selected from Naganji experimental area, Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan (H.P), India. The elevation of the experimental area is 1200 m above mean sea level and located at 30°51'N latitude and 76°11'E longitude.

Wood samples from each clone, with the dimension of 25 x 10 x 10 mm^3 were sliced out separately and thin microscopic sections of size 5-15 µm were taken by using the Sliding Microtome. The permanent slides of transverse, radial and tangential sections were stained using 2% saffranine for 30 minutes as per the procedure outlined by Johansen (1940) [9]. These sections were washed with water for 5 minutes. Later, the sections were dehydrated through a series of alcohol solutions at different concentrations (30%, 50%, 70 %, 95% and 100% for 5 minutes each) and then dehydrated with Alcohol: Xylene solutions (3:1, 1:1 and 1:3 ratios) to ensure complete dehydration. These sections were subsequently dipped in pure Xylene for 5 minutes and finally mounted in DPX (a mixture of distyrene, plasticizer and Xylene) mountant to prepare permanent slides. Permanent slides were subjected to measurements and photography using Image analysis system consisting of a microscope, digital camera and Personal Computer (PC). The measurement of vessel frequency, pore (vessel) diameter, ray height, ray width and uniseriate ray frequency was done by using an ocular micrometer fitted in the eyepiece of a microscope at 10X magnification, standardized with the help of stage micrometer. The permanent slides were prepared and the sectioned images were studied using an image analyzer. Vessel frequency was determined by counting the number of vessels per mm^2 per field of view on the cross- sections and diameter (µm) of the pore (vessel) was measured on the cross-sections at the widest part of the opening. Ray height (mm) and width (µm) were measured on the tangential longitudinal sections (TLS) for all the clones. Frequency of uniseriate rays per sq. mm was calculated by counting the number of uniseriate rays from the randomly selected area per field on the tangential sections of the samples. Ten observations per sample were taken for all the characters.

Table 1: The details of clonal material

S. N	Clone name	Hybrid details	Sn	Clone name	Hybrid details
1	UHFS061	*Salix matsudana* x *S. babylonica x S. alba*	20	UHFS296	*S. matsudana x S. alba*
2	UHFS075	*Salix matsudana* x *S. babylonica x S. alba*	21	UHFS297	*S. matsudana x S. alba*
3	UHFS087	*Salix matsudana* x *S. alba x S. matsudana*	22	UHFS298	*S. matsudana x S. alba*
4	UHFS111	*Salix matsudana* x *S. alba x S. matsudana*	23	UHFS299	*S. matsudana x S. alba*
5	UHFS112	*Salix matsudana* x *S. alba x S. matsudana*	24	UHFS309	*S. matsudana x S. alba*
6	UHFS113	*Salix matsudana* x *S. alba x S. matsudana*	25	UHFS333	*S. matsudana*
7	UHFS121	*Salix matsudana* x *S. babylonica* x *S. alba* x *S. matsudana*	26	UHFS340	*S. matsudana*
8	UHFS144	*Salix matsudana* x *S. matsudana* x *S. arbutifolia* x *S. matsudana*	27	UHFS335	*S. matsudana*
9	UHFS165	*Salix matsudana* x *S. matsudana x S. alba*	28	UHFS412	*S. tetrasperma* X *S. tetrasperma*
10	UHFS187	*Salix matsudana* x *S. matsudana x S. alba*	29	UHFS242	*Salix matsudana* x *S.alba*
11	UHFS202	*Salix matsudana* x *S. matsudana x S. alba*	30	UHFS336	*S. matsudana*
12	UHFS208	*Salix matsudana* x *S. matsudana x S. alba*	31	UHFS370/12	*S. matsudana*
13	UHFS221	*Salix matsudana* x *S. tetrasperma*	32	NZ1002	*S. matsudana x S. alba*
14	UHFS240/11	*Salix matsudana* x *S. tetrasperma*	33	J795	*S. matsudana x S. alba*
15	UHFS248	*Salix matsudana* x *S. alba*	34	SI-64-017	*S. alba*
16	UHFS260/11	*Salix matsudana* x *S. alba*	35	Kashmiri Willow	*S. alba*
17	UHFS267	*S. matsudana* X *S. tetrasperma*	36	Austree	*S. alba x S. matsudana*
18	UHFS289	*S. matsudana x S. alba*	37	J194	*S. matsudana* x *S. arbutifolia* x *S. matsudana*
19	UHFS282	*S. matsudana x S. alba*			

Fibre length and diameter were determined by macerating the wood shavings in Jeffery's fluid, i.e. 10 per cent chromic acid and 10 per cent nitric acid (Pandey *et al*., 1968) [13]. Thereafter, the samples were thoroughly washed, stained with safranine, mildly tapped for separation of fibres and mounted with 10 per cent glycerin. The measurements were made with the help of Ocular Micrometer fitted in the eyepiece of a microscope at 40X magnification standardized with the help of Stage Micrometer. Ten observations per sample were taken for both characters.

ANOVA was used to analyse the obtained data, by testing the level of significance of all the studied anatomical traits, among the different *Salix* hybrids' wood. All the mean data obtained were statistically analysed using SPSS16.

Results and discussion

Table 2 presents the mean vessel frequency, pore (vessel) diameter, ray height, ray width, uniseriate ray frequency and fiber length and diameter of *Salix* hybrids` wood. The analysis of variance showed significant inter-clonal variation in all the studied anatomic traits among the different *Salix* hybrids' clones ($p < 0.05$).

The maximum vessel frequency of 279.66/mm^2 was recorded in clone UHFS412, whereas minimum value of 117.067/mm^2 was noticed in clone UHFS260/11. The results from this study showed that *Salix* hybrids' wood have very high vessel frequency. The result of this study was significantly higher than the vessel frequency (per mm^2) of different wood species as reported by Carrillo *et al*. (2015) [4] in 15-year-old *Eucalyptus globulus* trees (8), Quartey (2015) [14] in *Blighia sapida* (12), Lima *et al*. (2014) [10] in 26-year-old *Eucalyptus resinifera* wood (9 or 8-12), Yaman (2014) [23] in *Ficus carica subsp. carica* (8.5), Wagner *et al.* (2009) [21] in 1-year old *Salix × rubens* (121 or 96-135) and 10 years-old *Salix × rubens* (76 or 53-98). Moreover, Saravanan *et al*. (2013) have reported the values of vessel frequency 4.00, 4.00, 4.00, 5.00 and 5.00 mm^2 for one, two, three, four and five year- old *Melia dubia* wood, respectively and they have concluded that the vessel frequency haven shown significant increment with respect to increase in age.

The maximum pore (vessel) diameter of 60.62 µm was found in clone UHFS111 and minimum value of 30.49 µm was observed in clone UHFS208. Vessels are the main axial elements of the hardwoods. Their diameter and porosity are very changeable from one genus to another, and therefore this feature is one of the main characters for identification of wood. The lower end range values of this study were agree with that observed in previous studies of different clones and wood species as reported by Zhou *et al*. (2017) [24] in 3-year-old *Salix psammophila* (32.541µm or 30.538 - 35.678 µm). However, this study showed lower values as compared to the findings for different wood species as reported by Quartey (2015) [14] in *Albizia ferruginea* (121-310 µm), *Blighia sapida* (91-281 µm), *Sterculia rhinopetala* (85-257 µm), but higher than the values that reported by Carrillo *et al.* (2015) [4] in 15-year-old *Eucalyptus globulus* trees (9.27-10.24 µm). Saravanan *et al*. (2013) have also observed that the mean values of vessel diameter were 192.57, 207.11, 231.10, 247.54 and 276.96 µm for one, two, three, four and five year old *M. dubia* wood, respectively. This showed significant increment of vessel diameter with respect to increase in age.

The maximum ray height (mm) was recorded in clone UHFS296 (0.426) and minimum value in clone J194 (0.097). Ray height is a typical feature and is different in different species/genotypes and useful in identification. Lima *et al*. (2014) [10] have observed 0.225 mm ray height in 26-year-old *Eucalyptus resinifera* wood and Saravanan *et al*. (2013) have reported the values of ray height 0.336, 0.356, 0.377, 0.399 and 0.438 mm for one, two, three, four and five year old *Melia dubia* wood, respectively. They have stated that ray height showed significant increment with respect to increase in age. The values of the present are lower than that has been reported by Ayaz and Nasir (1992) for *Pinus eldarica* (8.192 mm), *Pinus geradiana* (6.169 mm), *Pinus roxburghii* (7.310 mm) and *Pinus wallichiana* (6.162 mm). This may be due to the variability in softwood and hard woods.

The highest ray width of 9.36 was observed in UHFS333 and lowest value of 7.91 was recorded in UHFS208. Rays are generally composed of only ray cells which are parenchymatous in nature. Ray cellular composition is one of the important features to identify and differentiate angiosperm woods. The ray widths of this study have beene uniseriate. Saravanan *et al.* (2013) have reported ray widths of 71.73, 77.00, 84.53, 91.73 and 98.66 µm for one, two, three, four and five year old *Melia dubia* wood, respectively. They have stated that the ray widths show significant increment with respect to increase in age. Lima *et al.* (2014) [10] have also observed ray Width of 23 µm in 26-year-old *Eucalyptus resinifera* wood.

Table 2: Variation in anatomical dimensions of *Salix* hybrids` wood

Clone name	Parameters						
	Vessel frequency (per mm^2)	**Pore (vessel) diameter (µm)**	**Ray height (mm)**	**Ray width (µm)**	**Uniseriate ray frequency (per mm^2)**	**Fiber length (mm)**	**Fiber diameter (µm)**
UHFS061	224.342	39.57	0.136	8.33	78.697	0.873	19.80
UHFS075	227.157	36.79	0.129	7.94	66.642	0.864	19.53
UHFS087	211.859	39.01	0.170	8.03	59.910	0.824	21.33
UHFS111	134.202	60.62	0.160	8.50	36.534	0.833	19.93
UHFS112	218.101	35.68	0.163	8.08	58.748	0.783	18.53
UHFS113	175.447	46.54	0.168	8.83	88.672	0.815	19.60
UHFS121	203.842	39.57	0.166	8.39	63.704	0.869	19.33
UHFS144	178.997	51.79	0.103	8.03	46.876	0.874	20.13
UHFS165	195.336	38.04	0.107	8.03	75.270	0.723	18.67
UHFS187	196.499	41.40	0.117	8.33	44.673	0.734	18.60
UHFS202	198.151	39.29	0.118	8.08	75.821	0.862	19.07
UHFS208	169.083	30.49	0.163	7.91	66.764	0.752	19.27
UHFS221	148.889	43.32	0.278	8.33	61.134	0.753	19.53
UHFS240/11	163.881	41.93	0.252	8.14	75.270	0.721	19.33
UHFS248	167.002	43.87	0.171	8.44	46.141	0.950	21.53
UHFS260/11	117.067	56.23	0.255	8.27	60.767	1.142	19.67
UHFS267	190.869	36.37	0.138	8.19	70.987	0.728	19.60
UHFS289	203.964	39.01	0.176	8.14	75.638	0.864	19.60
UHFS282	193.255	45.32	0.192	9.00	86.530	0.683	20.60
UHFS296	177.467	49.15	0.426	8.39	67.193	0.847	19.73
UHFS297	199.803	45.95	0.224	8.03	78.820	0.722	19.60
UHFS298	206.657	41.23	0.198	8.03	55.627	0.716	19.00
UHFS299	211.430	42.07	0.159	8.11	75.576	0.969	20.27
UHFS309	224.648	30.82	0.108	8.08	67.805	0.863	19.13
UHFS333	229.911	42.43	0.227	9.36	75.882	0.746	20.67
UHFS340	186.463	53.17	0.139	8.00	51.588	0.724	18.60
UHFS335	239.764	45.12	0.230	9.14	53.179	0.813	20.00
UHFS412	279.663	33.04	0.131	7.94	60.155	0.930	18.33
UHFS242	181.934	39.98	0.185	8.11	48.406	0.844	19.07
UHFS336	275.441	31.38	0.149	8.08	59.482	0.773	19.27
UHFS370/12	195.703	46.76	0.147	8.11	75.087	0.733	19.60
NZ1002	175.570	39.93	0.200	8.16	67.193	0.677	19.20
J795	259.530	40.82	0.151	8.11	68.784	0.736	19.93
SI-64-017	140.321	49.98	0.216	8.33	60.094	0.755	19.00
Kashmiri Willow	193.255	34.99	0.207	8.11	64.561	0.765	18.47
Austree	146.074	47.07	0.177	8.16	73.557	0.785	19.53
J194	191.419	38.60	0.097	8.33	69.702	0.815	19.27
SE	2.968	3.82	0.025	0.31	2.231	0.027	0.71

The maximum uniseriate ray frequency (per/mm^2) of 88.672 was recorded in clone UHFS113 and minimum value of 36.534 was observed in clone UHFS111. Similar to the current study, numerous uniseriate rays *i.e*. one cell row wide in natural stands of *Salix caprea* L. and *Salix pentandra* L have been reported by Sennerby-Forsse (1988) [18]. Rowell (2012) [12] has also added that the rays in willow (*Salix* species) are exclusively uniseriate. The variation in ray frequency (8.8/ mm^2) in *Ficus carica subsp. carica* has been reported by Yaman (2014) [23]. Lima *et al.* (2014) [10] while working on *Eucalyptus resinifera* wood have observed the ray frequency of 6 /mm^2 and Mahmood and Athar (1997) have found variation in ray frequency of 15 coniferous species. However, the ray frequency of this study is very high compared to other reports. According to some reports, the frequencies of ray number have shown a gradual change with age. For instance, the mean values of ray frequency (7.00, 8.00, 8.00, 10.00 and 11.00/ mm^2) for one, two, three, four and five years old *Melia dubia* wood were recorded, respectively (Saravanan *et al.*, 2013).

The highest fiber length of 1.142 mm was observed in clone 9_31 and minimum value of 0.677 mm was found in clone NZ1002. These results are in good agreement with results reported by Carrillo *et al.* (2015) [4] in 15-year-old *Eucalyptus globulus* trees (0.85 mm), Salem and Mohamed (2013) [16] for *Maclura pomifera* (0.84 -0.90 mm)), Wagner *et al*. (2009) [21] in 1-year old young *Salix* × *rubens* Schrank (0.711 mm or 0.650-0.987 mm) and 10-years adult stem of *Salix* × *rubens* Schrank (0.799 mm or 0.627-1.134 mm), Sennerby-Forsse (1988) [18] in *S. caprea* and *S. pentandra* wood (0.8mm or 0.6-1.0 mm). The fiber length of some clones are also more close to the studies carried out by Sharma *et al.* (2014) [19] in 4 years old willow clones (1.14 mm or 0.96 - 1.93 mm), Okon (2014) [12] in 25-year-old *Gmelina arborea* wood (1.24 mm), Lima *et al*. (2014) [10] in 26-year-old *Eucalyptus resinifera* wood (1.016 mm), Monteoliva and Senisterra (2005) [11] in six 13-year-old willow clones (0.837-1.142 mm). However, the values of this study were higher than values in 1 year-old 36 clones of *Salix alba* where it ranged between 0.45 and 0.65 mm (Gupta *et al*., 2014) [6].

As reported by many authors juvenile woods have shorter fibres or tracheid length than the mature wood (Bendtsen, 1978; Thomas, 1984; Zobel and Sprague, 1998) [3, 20, 25]. This suggests that average fibre length or tracheid length of trees depends highly on their juvenile wood contents, or their rotation ages. Izekor and Fuwape (2011) [8] have found the fibre length values ranged from 1.32 to 1.55, 1.58 to 1.85 and 1.80 to 2.11 mm for 15, 20 and 25- year old *Tectona grandis* wood, respectively. The authors have recognized the increase in fibre length due to increase in the length of cambial initial with increasing cambial age. Similarly, the mean fibre length values of 0.647, 0.825, 0.892, 1.093 and 1.159 mm for one, two, three, four and five year old *Melia dubia* wood have been observed, respectively (Saravanan *et al*., 2013).

The maximum fiber diameter (µm) of 21.53 was recorded in clone UHFS248 and minimum value of 18.33 was found in clone UHFS412. Similar findings have been found by Okon (2014) [12] in 25-year-old *Gmelina arborea* wood (21.21 µm) and Yaman (2014) [23] in *Ficus carica subsp. carica* (21.4 µm). However, the results of this research work have observed higher fibre diameter as compared to the values reported by Carrillo *et al*. (2015) [4] in 15-year-old *Eucalyptus globulus* trees (15.96 µm) and lower than *Castanopsis acuminatissima* (30.1), *Castanopsis tungurrut* (32.7), *Cinnamomum inners* (38.3), *Ficus nervosa* (40.6) and *Horsfielda glabra* (35.8) by Damayanti and Rulliaty (2010) [5].

The mean values of fibre diameter ranged from 29.20 to 24.52, 31.89 to 26.92 and 35.47 to 30.19 µm, for 15, 20 and 25-year-old *Tectona grandis* wood, respectively (Izekor and Fuwape, 2011) [8]. Similar results have also been reported by Saravanan *et al.* (2013) where the mean fibre values 24.00, 24.90, 26.01, 26.75 and 27.52 µm for one, two, three, four and five year old *Melia dubia* wood have been obtained, respectively. They have stated that the increase in fibre diameter is associated with the increasing age of the trees and it may be due to the many molecular and physiological changes that occur in the vascular cambium as well as the increase in the wood cell wall thickness during the tree aging process.

Conclusions

This article presents the results of anatomical properties of 3 years old 37 *Salix* hybrids' clones. The aim of the study was to investigate the variation in anatomical properties among the different *Salix* hybrids clones. The analysis of anatomical wood properties in *Salix* hybrids' clones exhibited significant difference among clones, indicating the possibility of identifying clones with superior wood properties. The results showed that all the *Salix* hybrids' clones have therefore good potential to be used in the willow wood industry, particularly as a raw material for pulp and paper yields. This information can also be used for comparative studies with other *Salix* species as well as other tree species being evaluated for wood quality improvement and other applications. However, further continuous study is needed to assess any change of anatomical characteristics associated with age increment and environmental factors.

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