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RELATIONSHIP BETWEEN THICKNESS OF THE CELL WALL AND AMOUNT OF HOLOCELLULOSE AND ALPHACELLULOSE IN ELEVEN SPECIES OF CONIFERS GROWING IN PAKISTAN

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ABSTRACT

The total thickness of cell wall as well as its component layers was determined in eleven coniferous species viz, *Abies pindrow* Royle, *Cedrus deodara* (Rox. ex. Lamb), *Cupressus arizonica* Endl, *C.. funebris* Endl, *C.sempervirens*, L.*C. torulosa* D.Don. *Picea Smithiana* (wall). Boiss. *Pinus halepensis* Miller, *P. roxburgii* Sargent, *P. wallichiana* A.B.Jackson and *Thuja orientalis* L. The thickness of the secondary wall varied between 4.69 $\pm$ 0.41μm in *A. pindrow* to 2.56 $\pm$ 0.19μm in *C. arizonica*.
The amounts of α (alpha) cellulose and holocellulose in the cell walls were determined from extractive free wood. The correlation coefficients between thickness of cell wall and its component layers were correlated with holocellulose and alpha-cellulose contents of cell walls. It was found that compound middle lamella (CML) was weakly correlated with holocellulose and alphacellulose. S_1 (outer layer of the secondary wall) was positively correlated with holocellulose. S_2 (middle layer of secondary wall) was negatively correlated with holocellulose while positively correlated with alphacellulose. S_3 (inner layer of the secondary wall) was strongly correlated with alphacellulose. S_w (secondary wall) was uncorrelated with holocellulose but significantly correlated with alphacellulose.

Key-words: Cell wall materials, holocellulose, alphacellulose, conifers, Pakistan,

INTRODUCTION

The distinct feature of coniferous wood is the presence of growth rings and within the growth rings the zones of earlywood (E.W) and latewood (L.W). The cells of earlywood, are larger in size and their cell walls are thinner than that of late wood cells which are smaller in size than early wood cells (Desch and Dinwoodie, 1981). The early wood and latewood zones are mainly composed of tracheids. The structure and formation of the cell wall of a typical softwood (conifer wood) tracheid has been described by Wardrop and Bland (1959). On the basis of development and structure, three parts are commonly recognized in cell walls of tracheids viz the intercellular substance or middle lamella, the primary wall and the secondary wall (Fig.**1**). The intercellular substance occurs between the primary walls of two contiguous cells and the secondary wall is laid down over the primary that is next to lumen. The secondary wall consists of three layers which are designated as S_1 (secondary wall outer layer), S_2 (secondary wall middle layer) and S_3 (secondary wall inner layer). The thickness of secondary wall and its S_2 (middle layer) is much greater than S_1 and S_3 layers. (Desch and Dinwoodie, 1981). The bulk of a full grown cell wall consists of S_w. It has received close technological attention because it is responsible for the special characterstics of the raw materials such as wood, textile fibers, paper fibers, cellulose, straw and cork. Secondary wall has been the object of diversified studies such as morphology, chemical nature, biosynthesis, ultrastructure and physical characterstics (Desch and Dinwoodie, 1981)

The chemistry of cell wall is mainly based on the carbohydrate cellulose. The common constituents of the cell wall other than cellulose are hemicelluloses and pectic compounds. Many woody cell walls are impregnated with lignin (Brett and Waldron, 1990; Rose, 2000). Cellulose is of special interest because it is used as a raw material for manufacturing various kinds of paper (Desch and Dinwoodie, 1981). Cellulose can be extracted from the cell walls in the form of two fractions, known as holocellulose and α-cellulose. Holocellulose consists of a complex consisting of cellulose and the "hemicelluloses" The extraction of holocellulose with strong alkali gives "alpha cellulose" (Wenzl, 1970). A number of chemical studies of the cell walls of coniferous woods have shown that the amount of holocellulose and α-cellulose show different values in different cell walls (Larson, 1968; Siddique, 1976; Sene *et al.*, 1994).

In the present paper estimation of holocellulose and α-cellulose contents from wood (earlywood + latewood) of eleven coniferous species have been correlated with the total thickness of secondary wall of tracheids as well as with the component layers of the secondary wall, and correlation coefficients between variables of interest have been calculated.

MATERIALS AND METHODS

The total thickness pf cell wall as well as its component layers was determined in eleven coniferous species viz, *Abies pindrow.* Royle, *Cedrus deodara* (Rox. ex. Lamb), *Cupressus arizonica*, Endl, *C. funebris* Endl, *C. sempervirens*, L. *C .torulosa* D.Don. *Picea Smithiana* (Wall). Boiss. *Pinus halepensis* Miller, *P. roxburgii* Sargent, *P. wallichiana* A.B.Jackson and *Thuja orientalis* L. Selected wood material (from the 3rd annual ring for each species) were converted into cubes of 2.5cm for sectioning (Larson, 1959). Each cube contained a portion of earlywood (E.W) and Latewood (L.W) tracheids. The cubes of dry wood were processed for sectioning following Giebel and Dickson (1976). Generally the wood samples were boiled in water or in 2% KOH before microtomy. Transverse, radial and tangential sections were cut with a sliding microtome at 15μm by freezing the wood blocks with bursts of frozen $C0_2$ (Steedman, 1960). Sections were stained with safranin-fast green following Jensen (1962). Total thickness of the cell wall and its component layers from the earlywood and latewood tracheids was measured under a polarization microscope using a calibrated eye piece micrometer. Each reading was an average of 3 replicates (Table 1). The average thickness of cell walls was determined by adding values of early wood + latewood for each species and dividing by 2.

For the estimation of holocellulose and α-cellulose wood samples comprising of equal proportions of earlywood and latewood were chipped and grounded in an Apex Knife mill. The fractions of wood meal which passed a 40 mesh British standard sieve (420μm) was used for analysis. Estimation of holocellulose and α-cellulose contents was carried out from extractive free wood. Alcohol benzene extractives were removed by TAPPI standard method T6m.59 (Anon., 1975) and hot water soluble by TAPPI standard method T207-05-75 (Anon., 1975). For the extraction of holocellulose modified chlorite method of Wise *et al.* (1946) developed by Erickson (1962) was adopted. Six one-hour sodium chlorite treatments were required to fully delignify the wood. Analysis of alpha-cellulose content was carried out as described by Siddiqui (1976). Staistical analysis was performed following Zar (1995).

RESULTS AND DISCUSSION

Thickness of the cell wall

The total thickness of the cell wall and its component layers is shown in Table-1. These values were obtained by taking the average values of earlywood and latewood for each species. The thickness of the secondary wall (S_w) varied between 4.69 $\pm$ 0.41μm in Abies pindrow to 2.56 $\pm$ 0.19 in Cupressus arizonica. It can be seen from the table that the thickness values for ML (middle lamella), S_1 (outer layer of the secondary wall) and S_3 (inner layer) of the secondary wall are much smaller than those of S_2 (middle layer of secondary wall) and S_w (Secondary wall). It has been stated that S_1 forms 10%, S_2,85% and S_3 about 1% of the total thickness of the S_w (secondary wall) (Desch and Dinwoodie, 1981).

Timell (1965) has generalized the thickness of soft wood tracheids/hard wood fibers and according to him the thickness of primary wall, S_1, S_2 and S_3 were 0.1-1.2 μm, 0.1-0.3μm, 1.0-5.0μm and 0.1μm, respectively. Total cell wall thickness of conifers presented in the present studies (Table-1) is not at much variance from values reported by Timell (1965). Siddiqui (1976) has investigated the thickness of S_2 and Sw in Douglas fir and Red Pine. The thickness of S_2 and S_w were 4.49μm and 6.55μm respectively in the case of Douglas fir and 4.5μm and 5.02μm in the case of Red pine. However, values for thickness of S_2 and secondary walls of tracheids given by Siddiqui are at variance from the present results. There can be two reasons, nainely variation due to species and method of measuring cell wall thickness. Siddiqui measured thickness of cell wall layers from electron micrographs which gave a fairly enlarged image whereas during present studies the measurements were made with the help of polarization microscope, using a micrometer. Moreover the climatic factors and the provenance also pay a major role (Siddiqui, 1976).

Holocellulose and alphacellulose contents of the cell wall:

Results of estimation of holocellulose and α-cellolose obtained during present investigations are in agreement with findings of Escolano and Bawagan (1975), Siddiqui (1976), and Pollisco and Eusebio (1978). Escolano and Bawagan reported 65.20% holocellulose in Mindoropine, whereas Siddiqui found holocellulose content of Douglasfir and Redpine ranging between 69.62-73.35%. Pollisco and Eusebio reported 65.60% holocellulose in wood of Pinus insularis. The amount of holocellulose extracted from cell walls of eleven conifers (Table 1) was found highest in Cupressus torulosa (75.06$\pm$ 0.78%) and the lowest value was recorded for Cupressus funebris (62.38 $\pm$ 0.69%). Similarly the highest value for the amount of alphacellulose was recorded for Pinus roxburgii (49.33 $\pm$ 17%) and the lowest value was recorded in Cupressus sempervirens (31.53 $\pm$ .52%).

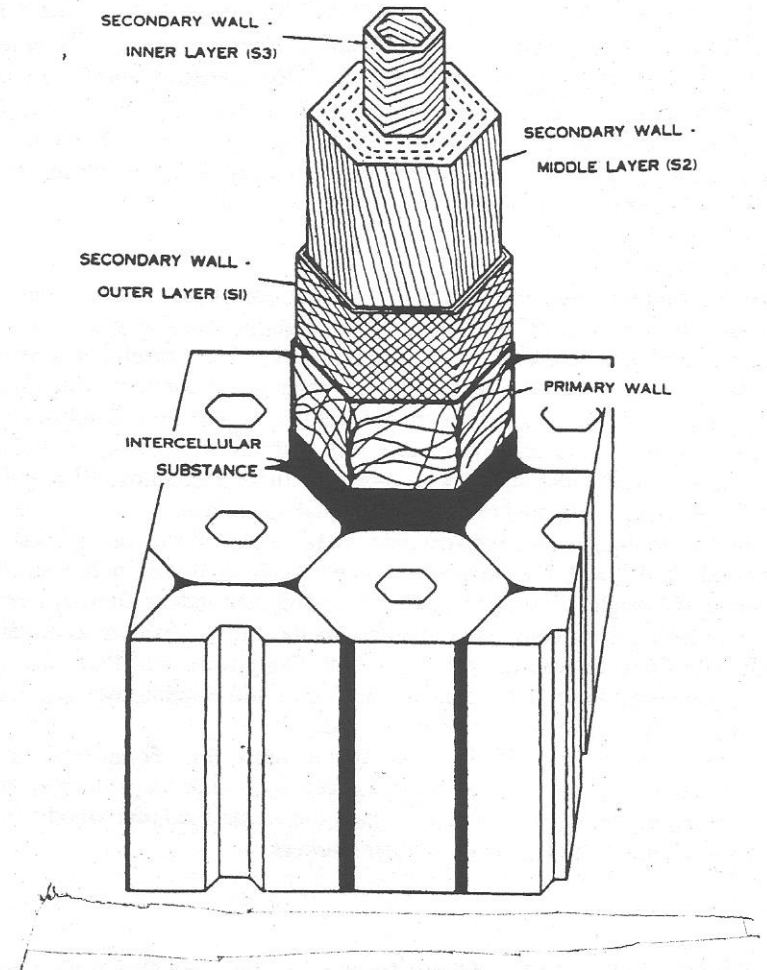

Fig.1. Simplified structure of the cell wall of a softwood tracheid (Wardrop and Bland, 1959).

Table 1. Thickness estimates of cell wall layers of tracheids and amount of holocellulose and alphacellulose estimated from them in eleven conifers.

S.NO.	Species	Average thickness of cell wall layers (EW + LW)μm					Amount of Holocellulose and ∝-cellulose	
		(1) ML	(2) S_1	(3) S_2	(4) S_3	(5) $S_w(S_1\text{-}S_2\text{-}S_3)$	(6) Holocellulose %	(7) ∝-cellulose (%)
1	Abies pindraw Royle.	0.72 ± .07	0.43 ± .06	3.96 ± .30	0.30 ± .05	4.69 ± 0.41	64.67 ± 0.36	45.41 ± 0.20
2	Cedrus deodara (Rox.ex.Lamb).	0.66 ± 0.04	0.36 ± .02	3.86 ± 1.0	0.27 ± .01	4.31 ± 0.13	66.32 ± 0.82	44.78 ± 0.23
3	Cupressus arizonica. Endl.	0.53 ± 0.11	0.58 ± .01	1.82 ± .09	0.17 ± .01	2.57 ± 0.11	66.86 ± 0.86	31.53 ± 0.52
4	Cupressus funebris.Endl	0.68 ± .03	0.27 ± 0.2	2.25 ± 0.16	0.14 ± 0.1	2.56 ± 0.19	62.38 ± 0.69	37.36 ± .48
5	Cupressus sempervirens L.	0.69 ± .05	0.19 ± 0.01	2.64 ± 0.10	0.14 ± 0.1	2.97 ± 0.21	66.86 ± 0.81	31.53 ± .52
6	Cupressus torulosa D Don.	0.58 ± .03	26.0 ± .04	2.91 ± 0.13	0.15 ± 00	3.32 ± 0.17	75.06 ± 0.78	41.33 ± 0.53
7	Picea smithiana (wall).Boiss.	0.49 ± .05	0.36 ± 0.04	3.89 ± .16	0.25 ± .03	4.50 ± 0.23	64.10 ± 0.23	46.15 ± 0.13
8	Pinus halepensis Miller.	0.57 ± 0.12	0.45 ± 0.11	2.85 ± 0.22	0.26 ± 0.04	3.56 ± 0.37	68.94 ± 0.23	48.0 ± 0.25
9	Pinus roxburgii Sargent.	0.62 ± 0.05	0.62 ± 0.11	2.82 ± 0.14	0.36 ± .07	3.80 ± 0.32	64.85 ± 0.37	49.33 ± .17
10	Pinus wallichiania A.B Jackson.	0.45 ± 1.0	0.34 ± 0.17	3.14 ± 0.20	0.28 ± .06	3.66 ± 0.43	70.07 ± 0.22	48.12 ± .05
11	Thuja oriental L.	0.69 ± .04	0.30 ± 0.0	2.73 ± .05	0.13 ± 0.0	3.16 ± 0.05	67.93 ± 0.18	44.09 ± .08

± = Standard error of the mean. E.W = Earlywood, L.W = Latewood

In the present study amount of ∝-cellulose in coniferous wood varied between 31.53-49.33% (*Cupressus arizonica*, *Pinus roxburghii*). Conifers containing high ∝-cellulose content were *Pinus wallichiana* (48.12%), *P. halepensis* (48.00%), *Abies pindrow* (47.57%), *Picea smithiana* (46.15%), *Cedrus deodara* (44.78%), *Taxus baccata* (44.08) and *Thuja orientalis* (42.73%). Wise *et al.* (1946) estimated ∝-cellulose contents of *Douglasfir,*

Blackspruse, redwood and Ponderosa pine to be 50.09, 49.70, 35.75 and 51.90%, respectively. Timell (1965) reported ∝-cellulose content in three conifers viz. *Abies balsamea, Picea glauca* and *Pinus strobus*. The amount of ∝-cellulose in these conifers was 42.0, 41.0 and 41.0%, respectively. Siddiqui (1976) compared ∝-cellulose content in early and latewood (comined) of two conifers viz. Douglas fir and Red pine. In Douglas fir ∝-cellulose content was estimated to be 45.88% whereas for Red Pine alphacellulose content was found to be 46.52%. Beigelman *et al.* (1979) analyzed woods of *Larix siberica* and reported 51.7 ∝-cellulose in them. Values for ∝-cellulose content of coniferous woods analyzed during present studies are close to values given by other workers.

Correlation coefficients between cell wall characters:

The amount of holocellulose and alpha cellulose were correlated with the thickness of ML, S_1,S_2,S_3 and S_w. But since S_2 and S_w form the major bulk of cell wall (Desch and Dinwoodie, 1981), attention was focused on correlation between amount of holocellulose and alphacellulose contents of S_2 layer and S_w. The correlation coefficients between thickness of cell wall layers and holocellulose and alphacellulose contents of the cell wall show that primary wall + middle lamella (CML) was weakly correlated with holocellulose and alphacellulose. S_1 was positively correlated with holocellulose ($P < 0, 01$).S_2 was negatively correlated with holocellulose ($P < 0.01$) while positively correlated ($P < 0.001$) with alphacellulose.S_3 was strongly correlated with alphacellulose ($P < 0.001$). S_w was uncorrelated with holocellulose but significantly correlated ($P < 0.01$) with alphacellulose.

Siddiqui (1976) computed simple correlation coefficients between percentage areas of various cell wall layers taken from earlywood (E.W) and latewood (L.W) with chemical constituents such as lignin, holocellulose and alphacellulose extracted from the cell walls of Douglas fir and red pine. The computed values showed that lignin was significantly correlated with the relative thickness of compound middle lamella (ML) as well as secondary wall (S_w). Lignin was significantly correlated with CML as well as with S_w in both Douglas fir and Red pine. On the other hand correlation coefficients between holocellulose and secondary wall (S_w) and middle layer of secondary wall (S_2) were significant in the case of Douglas fir while non-significant for red pine.

On the basis of Siddiqui's (1976) findings and our own results it can be concluded that for establishing closer correlation between cell wall thickness and major chemical constituents of the cell wall larger data in terms of wood samples from a number of conifer trees will be required. This may not be possible because conifer wood is in great demand for its multiferous uses in Pakistan and attracts high prices in timber markets.

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