# Activity of Vascular Cambium in *Salix alba* L. from Temperate Climate of Kashmir Himalaya

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## ABSTRACT

Vascular cambium, a meristem responsible for the formation of wood by way of growth and divisions of its components i.e. ray and fusiform initials were analyzed during different months over two consecutive years in order to assess their impact on growth in *Salix alba* from temperate climate of Kashmir Himalaya. The maximum and minimum dimensions of fusiform initials and ray initials were in winter and summer seasons respectively while the horizontal and vertical diameters of ray initials were maximum and minimum in summer and winter seasons respectively.

***Key words:*** Vascular cambium, fusiform initial, ray initial, *Salix alba*

## INTRODUCTION

Woody trees possess a power of secondary growth by which they increase in girth. This activity of secondary growth results in the formation of wood which by nature is secondary xylem constituting vessels, fibers, rays, parenchyma etc. All these structures are formed by a mersitem called vascular cambium which is made up of two types of cells, called as fusiform and ray initials. The fusiform initials give rise to vertically oriented structures while ray initials give rise to horizontally oriented structures. *Salix* is a fast-growing species able to grow on various types of soils, even compacted, swampy, acidic or alkaline, provided roots have sufficient moisture. It is suitable for the biological control of soil erosion, siltation, nutrient cycling, phytoremedation, carbon sequestration and filtering of sewage and polluted water. White willow and several closely related species have been used for thousands of years to relieve joint pain and manage fevers. The bark is anodyne, anti-inflammatory, anti-periodic, antiseptic, astringent, diaphoretic, diuretic, febrifuge, hypnotic, sedative and tonic. The wood of *Salix* is suitable for veneer, pulp, plywood, laminated wood, reconstituted wood products, artificial limbs, fruit boxes, agriculture implements, furniture; tool handles and sports goods like cricket bat, polo balls.

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## MATERIAL AND METHODS

Kashmir, administratively one of the provinces of the Jammu and Kashmir State, is situated on the northernmost edge of India. The Valley of Kashmir, a great elliptical bowl, extends from 33°20' to 34°54' N latitudes and 73°55' to 75°35' E longitudes. It extends roughly 187 km in length and about 116 km in breadth along the latitudes of Srinagar. The Vale covers an area of 15,948 sq.km.

The territories forming Kashmir cover a wide area, mostly mountains with an outer fringe of alluvial plains bordering the outer hill region to the south. The entire territories of the Kashmir Valley form two distinct topographic divisions, the mountain ranges and the Valley proper. With lofty, snow-covered Himalayan Mountains girdling the Valley, its altitude ranges from 1,600m (Srinagar) to 5,420m (Kolahoi). Kashmir falls in the lesser Himalayan zone of the great Himalayas. The Himalayan ranges play the major and pivotal role in determining the climate of the Kashmir Valley. The southern flank of the Pir Panjal mountains certainly acts as an effective barrier to the summer monsoon, the bearer of moisture in the sub-continent. The summer rainfall of the valley clearly reflects this shadow effect. However, the Greater Himalayas exercise little obstruction influence on the influx of the westerly troughs, which frequent the valley from the west and the northwest during winter.

On an average, the climate of the Valley is sub-Mediterranean, with bixeric regimes, having two dry spells in June and September, and high precipitation during the cool season. The Kashmir valley enjoys an enchanting climate for the major part of the year. It has continental climate, marked by four well-defined seasons a year – (1) winter (December– February), (2) spring (March–May), (3) summer (June-August) and (4) September -November). The data on the climatic factors were collected from the meterological department Rambagh, Srinagar (Fig. 1-3).

The geographical location along with other characteristics of the selected site and material are summarised in Table 1.

**Table 1. Characteristics of selected site and material along with geographical location**

| Factor | Site (Khrew) |
|---|---|
| North Latitude | 30° 12' |
| East Longitude | 75° 35' |
| Elevation (m) | 2000 |
| Soil | Sandy clay |
| Age in years | 10-13 |

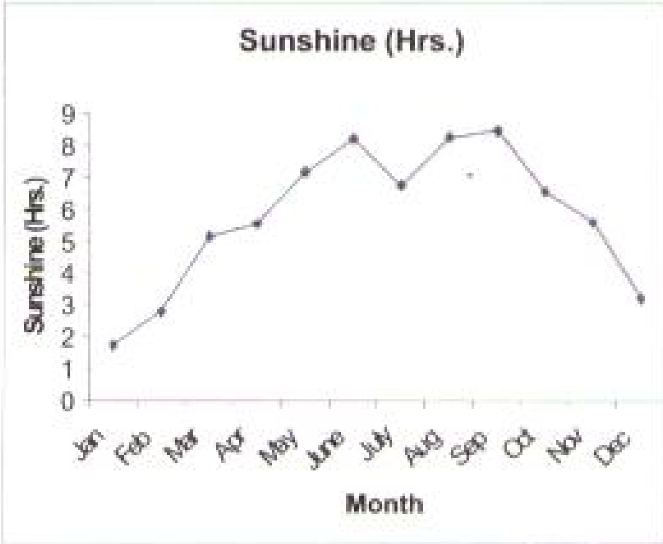



**Fig. 1.** Sunshine Hours during different months 2005-2006

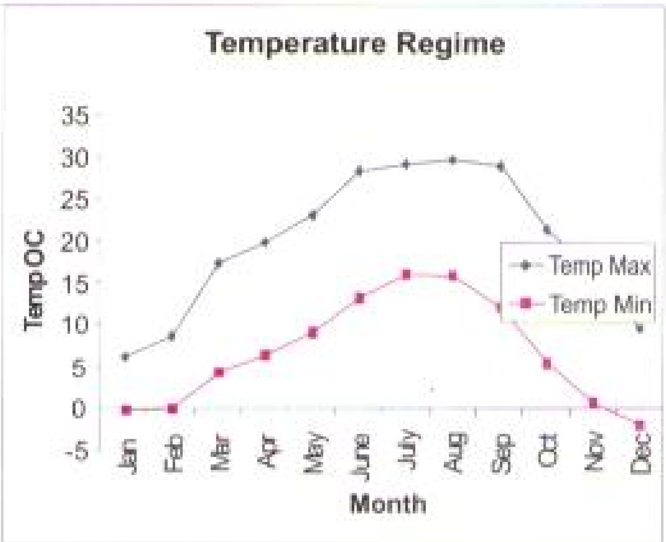



**Fig. 2.** Monthly Minimum and Maximum temperature during 2005-2006

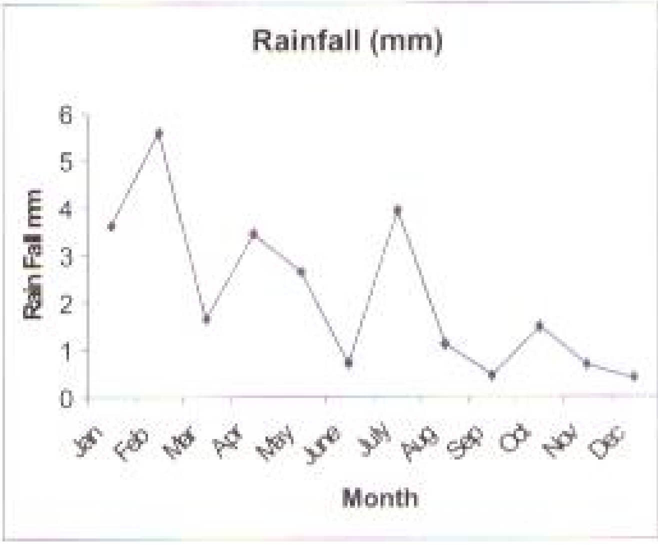



**Fig. 3.** Rainfall in mm during the different months 2005-2006

## Cambial Characteristics

### Field Work

To study the cambial characteristics (i.e. cambial activity in relation to season, twelve trees of *Salix alba* L., were selected from the natural habitats. Periodical monthly observations of cambial activity were made during two consecutive years (2005 and 2006). Cubical samples (2x2x2 cm) comprising tissues along with outer bark and inner sapwood were chiselled out at breast height (1.3m) from selected trees. Two trees were used on each occasion and no samples were collected from the same tree within three months. The samples were fixed in formalin Aceto-alcohol (F.A.A.) (Johansen, 1940). After three days the material was transferred to 70% ethyl alcohol for preservation.

### Laboratory Work

To assess the cambial activity 10-15 mm thick tangential longitudinal sections were cut with Reichert sliding microtome and also manually with the help of sharp razor.

### Staining procedure

For staining the section Foster (1934) and Cheadle *et al.* (1953) method was followed. The section were first put in 1% tannic acid for 5-10 minutes and then washed 3-5 times with distilled water. After washing with distilled water, sections were transferred to ferric chloride for 5-10 minutes. Again sections were washed with distilled water 3 to 5 times. After that the section were transferred to sodium bicarbonate solution for 30 minutes, then sections were put in lacomoid blue for 12 to 18 hours and after then washed with sodium hydrogen carbonate for few seconds. Now sections were transferred to clove oil for 10-15 minutes. After that they were transferred to different grades of alcohol and finally to xylene in order to achieve complete dehydration and finally mounted on glass slides with Diphenyl Pthalte Xylene (DPX) as mountant.

### Microscopy

To determine cell size, micrometry was done. The stages and Ocular micrometers were calibrated. A minimum of 100 randomly selected elements per block of each species were measured. The cell size was determined for each month after pooling all readings taken from relevant samples obtained during the two collection years as per Siddiqui (1983). The relative proportion of fusiform and ray initials was determined from Camera lucida drawings as per Ghouse and Yunus (1973). The ray height was classified into different categories viz. short (up to 300 mm) medium (301-600 mm) and tall (601 mm onwards), while those of varying width as uni-bi-tri and multiseriate.

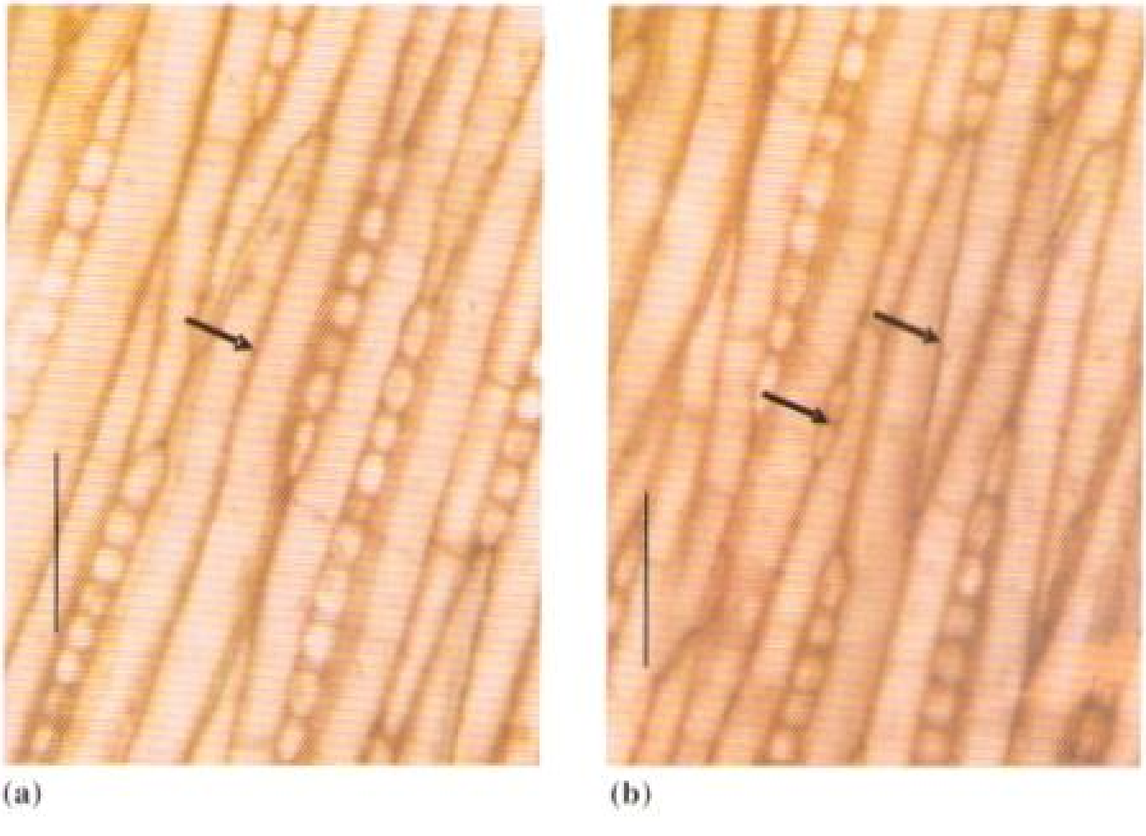
### Statistical analysis

The data collected for present study was statistically analyzed by using Sigma Plot 12.0 statistical software (SPSS, Chicago, IL, USA) and Minitab 11.0 for windows.

## RESULTS

The vascular cambium of *Salix alba* consists of two types of initials. Of these two types of initials one is comprised of spindle shaped cells called as fusiform initials. The other being almost iso-diametric to rectangular in shape, called as ray initials. The tapering ends of vertically aligned fusiform initials overlap one another irregularly rendering the cambium non-storied or non-stratified in structure. The cambial initials vary in dimensions with changing climatic conditions. Fusiform initial walls exhibit primary pit fields which become particularly prominent during the dormant phase of the cambium.

The average length and width of fusiform initials vary from 475µm (July) to 622 (January) and 16µm (July) to 21µm in (December) respectively. On whole, the fusiform initials are shorter during summer than during the rest period of the year Table 2. The average vertical and horizontal diameters of ray initials vary from 28-38 µm and 18-33 µm respectively. The fusiform initials divide pseudo transversely resulting into two initials Fig 4. The ray initials also divide to increase their number Fig 4. All this happens in order to cope with the expansion of the stem axis. New ray initials are also added by conversion of fusiform initials into ray initials through transverse, terminal or lateral segmentation Fig1. Depending upon the cambial makeup, the fusiform and ray initials in a tangential plane constituted 73 percent and 27 percent respectively. On the basis of height the cambial rays classified into three groups viz. – short (300 µm or below), medium (301-600 µm) and tall (above 601 µm). The rays of different height and width occur but tall cambial rays were predominating over the others with average height and width of 919 and 75 µm respectively.



(a) (b)

Scale bar = 40µm for a and b

**Fig. 4.** (a) T.L.S. of cambium of *Salix alba* showing dormant cambium with beaded appearance on radial walls of the fusiform initials (b) T.L.S. of *Salix alba* showing initiation of ray initial formation and fusiform initials showing pseudo transverse divisions

Table 2. Dimensions of fusiform and ray initials (μm) in *Salix alba* during different months

| Months | Length of fusiform initials | Width of fusiform initials | Vertical diameter of ray initials | Horizontal diameter of ray initials |
|---|---|---|---|---|
| January | 622 | 20 | 28 | 18 |
| February | 618 | 20 | 29 | 25 |
| March | 610 | 19 | 29 | 27 |
| April | 515 | 18 | 35 | 28 |
| May | 510 | 17 | 34 | 31 |
| June | 490 | 17 | 38 | 33 |
| July | 475 | 16 | 33 | 26 |
| August | 502 | 16 | 29 | 25 |
| September | 513 | 18 | 31 | 26 |
| October | 565 | 19 | 32 | 28 |
| November | 601 | 20 | 28 | 29 |
| December | 615 | 21 | 29 | 27 |
| LSD (≤ 0.05) | 33.46 | 1.96 | 1.13 | 2.22 |
| (≤ 0.01) | 45.51 | 2.65 | 1.53 | 3.01 |

## DISCUSSION

It was Grew (1682) who first introduced the term cambium and later Sanio (1863) stated that it is lateral meristem. It was De Bary (1884) who give the modern concept that it is a single layer of initials. Later the concept of cambial zone has been extended in order to incorporate undifferentiated derivatives in addition to the true initials. The same concept has been followed by Cockerham (1930), Artschwager (1950), Newman (1956), Kozlowski (1971), Ghouse and Iqbal (1975), Iqbal (1990), Fahn (1997), Paliwal and Yadav (1999) and Paliwal *et al.* (2002).

The basic composition of vascular cambium is elongated fusiform initials and roughly isodiametric ray initials. Bailey (1923) has recognized two basic patterns of cambial structure storied or stratified and non storied or non stratified. In former the fusiform initials occur in horizontal tiers with end of cells appearing approximately at the same level in a given tier, and in the latter the end walls of the adjacent initials overlap to a considerable extent. Bailey (1923) stated that the stratified type of cambium is phylogenetically advanced. Similar opinions were given by Metcalfe and Chalk (1950), Barghoom (1964), Fahn (1974), Esau

(1977), Iqbal (1979), Khan (1980), Siddiqui (1983), Ajmal (1985), Kafeel (1986), Khan (2001) and Mahmood (2001). In the presently investigated species of *Salix alba*, arrangement of cambial initials depicts a clear non-stratified structure. The non stratified structure of the cambium has been reported earlier by Khan (1977), Iqbal (1979), Khan (1980), Siddiqui (1983), Ajmal (1985), Kafeel (1986), Khan (2001), Khan *et al.* (2005), Khan and Siddiqui (2007a), Venugopal and Liangkuwang (2007) and Wani and Khan (2008, 2009).

Bailey (1923) while working on wide variety of temperate and tropical trees and concluded that cambial initials vary in length in non- stratified type from 460-4400µm and are generally short in stratified type of cambium. The observations regarding this aspect indicate that in the present investigated species of *Salix alba*, the mean length of fusiform initials vary from 475- 622µm, thus goes in agreement with result of Bailey (1923) but contradicts with some of workers like Ghouse and Iqbal (1975), Ghouse *et al* . (1980), Khan (1980), Cumbie (1983), Khan (2001), Mahmood (2001), Khan and Siddiqui (2007c) who have found fusiform initials length fall shorter than Bailey's reported limit for non-stratified cambium.

The anatomical variations which the cambium experiences during different seasons of the year have been analyzed by a number of workers like Eames and MacDaniels (1947), Esau (1965), Srivastava and O'Brien (1966), Robards and Kidwai (1969), Murmanis (1970,71), Khan (1977), Iqbal (1979), Khan (1980), Siddiqui (1983), Kafeel (1986), Rao and Dave (1986), Venugopal and Krishnamurthy (1989), Antonova (1996), Antonova and Stasova (1997), Khan (2001), Mahmood (2001), Rensing and Samuel (2004) and Venugopal and Liangkuwang (2007). The radial walls of the fusiform initials have been reported to be usually thicker than the tangential walls, especially during dormancy and the primary pit fields appear deeply depressed in tangential longitudinal view giving a beaded appearance to the radial walls. Similar observations have been made in the present investigated species.

Cambial initials periodically undergo anticlinal and periclinal divisions Bailey (1923), Eames and MacDaniels (1947), Bannan (1956), Rao and Dave (1986), Han and Woong (1991), Fahn (1997), Mahmood (2001), Khan (2001) and Esau (2002). The anticlinal divisions add to the cambial population while the periclinal ones increase the number of cambial derivatives emanating new phloem and xylem elements. Two fundamental types of anticlinal divisions have been recognized by Bailey (1923) in the cambium of vascular plants. In one type, the anticlinal division occurs in a radial longitudinal plane and in the other pseudo-transverse wall formation takes place running a skew intersecting the two radial walls at two different levels Philipson *et al.* (1971), Khan (1980), Khan and Siddiqui (1980), Zargoska-Marek (1984), Khan *et al.* (1988), Iqbal (1990),Han and Woong (1991), Venugopal and Krishnamurthy (1989), Khan (2001) and Mahmood (2001). In the present study only pseudo-transverse type of division were found as non stratified type of cambium structure were present. The anticlinal divisions in the cambium have been noted to be pseudo-transverse, as it has been found in the majority of forms having non-stratified cambium (Bailey, 1923; Esau, 1965; Fahn, 1974, Iqbal, 1990, and Khan, 2001). The pseudotransverse wall formation observed in the present study varies in length from short to long. Sometimes the dividing wall almost extending from one end of the cell to the other, as it has been reported by Khan (1977), Iqbal (1979), Khan (1980) and Siddiqui (1983).

Barghoorn (1940 a, b; 1941 a, b) and Braun (1955) carried out detailed work on ray initials in conifer and dicotyledons, while developmental studies were worked by Bannan (1950, 1951, 1953, 1956), Evert (1959,1961), Cumbie (1963, 1969 a,b); Ghouse and Yunus (1973); Ghouse and Iqbal (1977); Khan (1980); Khan *et al.* (1983); Siddiqui (1983); Ajmal (1985); Kafeel (1986); Ajmal and Iqbal (1987); Khan (2001); Mahmood (2001). Earlier works on ray initial formation indicate that the ray initials may originate in more than one way. Sometimes, they arise as a single cell which may be cut ends of fusiform initials as terminal segments (Bannan 1951, 1956; Braun 1955; Khan, 1980; Siddiqui, 1983). They may also arise either by transverse fragmentation of fusiform initials (Whalley, 1950; Bannan, 1951; Srivastava, 1996; Khan, 1980, Siddiqui,1983; Mahmood, 2001; Khan, 2001) or a declining fusiform initial may reduce to a single ray initial (Barghoorn, 1940, 1941a, Fahn, 1982).

As for as the presently investigated species is concerned, it show the first two types of ray formation. After their development. They continue to increase in number to a considerable extent mainly through multiplication of the existing initials as has been reported by Barghoorn (1941a,b), Braun (1955), Evert (1961, 1963), Ghouse and Yunus (1973), Khan (1977), Khan (1980), Khan *et al.* (1983), Mahmood (2001), Khan (2001). In the presently investigated species, rays also showed increase in width and height by fusion of two or more vertically and radially aligned rays. Such fusions result from intervening fusiform initials or by multiplication of already existing ray initials of the adjacent panel of rays (Barghoorn, 1941b; Philipson *et al.*, 1971, Bartwal *et al.*, 1983; Khan *et al.*, 1983, Rao, 1988). Splitting of rays also occurs as a result of intrusive growth of fusiform initials in all the species investigated as it has been reported by Khan (1980) in *C. citrinus, E. maculata and E. jambolana* and Khan *et al.* (1983) in *C. sinensis.*

Earlier workers have also recorded similar observations in various species (Barghoorn, 1940a, b, Esau, 1965; Evert, 1961; Cheadle and Esau, 1964, Khan 1977, Bartwal *et al.*, 1983; Siddiqui, 1983; Iqbal, 1990; Khan, 2001; Esau, 2002)

Bailey (1923) while studying the structure of *Pinus strobus* reported that the fusiform initials constitute about 87.5% of the total area of the cambial zone. Wilson (1963) calculated the surface area of the cambial zone of *Abies concolor* and found that the fusiform cells constitute more than 90% by volume of the cambium and its derivatives. Similarly, Kozlowski (1971), Butterfield (1972), Margaris and Papadogianni (1977), Ghouse and Jamal (1979), Ajmal (1985) and Kafeel (1986) had also recorded similar high percentage of fusiform initials. In the present study the fusiform initials constitute 73% in Salix alba, of the tangential area of the cambial cylinder which is much lower as compared to that of Bailey (1923) and Wilson (1963) observations, but are almost in accordance with the workers like Ghouse and Yunus (1973,1974a,b), Khan (1977), Khan and Siddiqui (1980, 1983), Venugopal and Krishnamurthy (1989), Mahmood (2001), Khan (2001), Khan *et al.* (2005), Khan *et al.* (2007), Khan and Siddiqui (2007a,b) and Wani and Khan (2008,2009).

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