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Cambial activity, annual rhythm of xylem production in relation to phenology and climatic factors and lignification pattern during xylogenesis in drum-stick tree (*Moringa oleifera*)

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ABSTRACT

The interrelationship among seasonality of cambium, wood formation, cell size variation, lignification, tree phenology and climatic factors has been examined in *Moringa oleifera*, a tropical evergreen tree. The vascular cambium in *Moringa* is a storied with a distinct seasonal variation in its structure due to dimensional changes in rays. Though cambium remains active throughout the year it is sensitive to water availability. Peak cambial cell division and rate of xylem differentiation are influenced by average rainfall during the monsoon period. Cambial cell division reaches higher up in the tree trunk when it is supporting a high number of branches and leaves. Statistical analysis of cell size variation and climate factors revealed that xylem cell development is greatly influenced by rainfall and rarely by temperature. Lengths of fusiform initials and vessel elements are positively correlated. The pattern of lignification during xylogenesis shows that the vessels are the first element to develop lignified walls and ray cells are the last elements to become lignified. Fiber cell walls show more syringyl lignin, while the cell walls of other xylem elements are characterized by relatively more guaiacyl lignin units.

Introduction

Studies on seasonal cambial activity and xylem development (xylogenesis) in relation to internal and external regulatory factors are important to understand the biology of tree growth and development, which is important in predicting timber and biomass yield, and in determining forest dynamics (Eckstein et al., 1995; Larson, 1994; Priya and Bhat, 1999). Xylogenesis has a great importance not only because of the function of xylem is essential in sustaining the existence of the vascular plants, but also because xylem formation appears to be a good model system for the analysis of differentiation processes in higher plants (Fukuda, 1996). The sequential developmental process in the cambium which leads to its differentiation into specialized xylem, forming finally the wood, is known as xylogenesis. Xylogenesis represents an example of cell differentiation in an exceptionally complex form.

Lignification constitutes one of the most important biochemical events that accompany the formation of the secondary plant cell wall during xylogenesis. Lignin is a polyphenolic polymer which is deposited in the secondary wall within the template of cellulose and hemicelluloses and also occurs in junction areas of the middle lamella and cell corners. Lignin in the dicotyledonous wood consists of two monolignols, namely guaiacyl and syringyl lignins. The distribution of these two units shows high variability among various xylem elements of different species. The quantity and composition of lignin influences strongly the wood properties, which in turn make wood suitable for different commercial purposes.

The activation, duration, and cessation of cambial activity as well as the rate of xylogenesis and the pattern of the lignification process are controlled by a wide variety of factors, both exogenous (extrinsic) and endogenous (intrinsic) ones, and by interaction between them (Christophe et al., 2001). The genetic constitution and the physiological circumstances act as intrinsic signals which regulate the cambial growth and development, while extrinsic factors such as temperature, water (rainfall), relative humidity, and photoperiod, i.e., the peculiar growing season climate, regulate the cambial activity indirectly by controlling the timing and extent of metabolite production and hence primary and secondary growth of the plants (Savidge, 1996).

Our current understanding about the biology of cambial seasonality and wood formation is greatly based on many studies with

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the relatively less diversified tree flora in the temperate regions (Antonova, 1996; Antonova and Stasova, 1997; Deslauriers and Morin, 2005; Rensing and Samuel, 2004) and on a few studies only with tropical trees (Pumijumnong and Wanyaphet, 2006; Pumijumnong and Buajan, 2013; Rao and Rajput, 2001; Singh and Venugopal, 2011) and lianas (e.g., Rajput et al., 2012). Under tropical climates tree diversity is distinctly higher and the species show quite a wide variation in their secondary growth pattern and xylem structure. This has previously received only little attention with respect to seasonality of cambium and wood formation. Therefore, the present work focuses on short-term monitoring of seasonal anatomical changes in the cambium and wood development in the evergreen tree species *Moringa oleifera* Lam. in relation to environmental factors and tree phenology and on the pattern of lignin deposition during xylogenesis.

Materials and methods

Collection of samples

Samples of cambial tissue along with the outer sapwood and inner phloem were collected at monthly intervals for 1 year (2009) from three trees of *Moringa oleifera* growing near the Department of Biosciences, Sardar Patel University, Gujarat, India. The samples, measuring 60 mm × 20 mm × 20 mm in length, width, and depth respectively, were collected at the chest height of the main straight trunk using chisel and hammer and fixed immediately in formaldehyde-acetic acid-alcohol fixative (FAA; Berlyn and Miksche, 1976) and air was removed from tissues, applying low pressure to the storage vessels after arriving in laboratory. After a week in FAA, samples were transferred to 70 percent ethanol for preservation. Phenological details of the trees, such as sprouting of new leaves, leaf maturation, yellowing of leaf, defoliation, development of flower buds, flowering, fruit set, fruit maturation, and dispersal, were recorded at the time of each collection. Meteorological data such as total rainfall, humidity, minimum, maximum, and average temperature were obtained from the Meteorological Station of Anand Agriculture University, Anand, Gujarat.

Sectioning

For light microscopy, suitably trimmed small pieces of cambial samples were sectioned on a sliding microtome (Leica SM 2000R) in transverse, tangential, and radial longitudinal sections at a thickness of 12–14 μm. Sections were arranged in series on slides and fixed with cotton thread for the staining process. When the cambium is active, it is difficult to get good sections on a sliding microtome due to separation of bark from sapwood. Such samples were subjected to paraffin embedding. For this, small pieces of samples were dehydrated in *Tert*-butyl alcohol (TBA) series and infiltrated in paraffin wax at 58–60 °C in a heating oven (Berlyn and Miksche, 1976). Samples infiltrated with paraffin wax were sectioned on a rotary microtome (Zeus LS-2055) at a thickness of 10–12 μm.

Staining

To study the anatomical details of cambium and its derivatives, sections were stained in toluidine blue 'O' (Johansen, 1940) and tannic acid-ferric-chloride-lacmoid solution (Cheadle et al., 1953). Sections taken on sliding microtome and rotary microtome were stained with toluidine blue 'O' (Sigma). After washing several times in water, sliding microtome sections were passed through an alcohol–xylene series and mounted in DPX, while rotary microtome sections after washing in water were air dried, deparaffinized, and mounted in DPX. For Lacmoid staining, sections tied with cotton thread were treated with 1% tannic acid solution for 2 min, washed several times in water and then subjected to 2% ferric chloride solution for 2–3 min. After treating with 0.5% sodium bicarbonate solution for 1 h, sections were kept in Lacmoid stain for three to four days, and then passed across a graded series of alcohol–xylene before mounting in DPX. Paraffin sections were first deparaffinized and then processed for staining.

Histochemical methods

Weisner reaction (Dean, 1997) was used to study the pattern of lignin deposition in various xylem elements during active cambial cell differentiation. Transverse sections of samples including the cambial zone and phloem were submerged in 1% phloroglucinol solution prepared in ethanol for 1 min, then were transferred on a glass slide, treated with a drop of concentrated HCl, and then covered with a cover glass. Lignin gives dark pink to reddish color after staining. Maule's reaction was used (Meshitsuka and Nakano, 1979) for localizing different lignin monomers. For this, transverse sections of freshly collected samples were stained in 1% $KMnO_4$ for 2–3 min, washed in distiled water, immersed in 3–5% HCl, washed in distiled water (DW) and then treated with a few drops of ammonium hydroxide. S-lignin gives a reddish color, while G-lignin shows a yellowish-brown color. Sections were observed and photographed immediately after the staining.

Fluorescence microscopy for lignin localization

Unstained transverse sections of cambium along with xylem and phloem were used for fluorescence microscopy. Lignin autoflorescence was measured at green excitation (λ_{exc} = 510–560 nm) and images were taken using a Leica DM 2000 binocular epifluorescence microscope attached with Cannon DC 150 digital camera.

Maceration of wood samples

A 1 mm thick xylem tissue close to cambium was macerated in Jeffery's fluid (Berlyn and Miksche, 1976). After boiling in Jeffery's fluid for 2–3 min samples were washed in 1% sodium bicarbonate to remove excess acids and then washed in DW. Samples were then kept on a glass slide, a drop of Toluidine blue 'O' was put on them and spread using a needle, and finally they were mounted in 50% glycerin.

Measurement of cells

Dimensional details of cambial cells and wood elements were recorded by using ocular micrometer. Cambial and differentiating xylem zone were measured from transverse sections while length and width of fusiform initials as well as height and width of rays were measured from tangential longitudinal sections. Frequency of fusiform and ray initials were also measured with the help of micrometer scale (1 cm^2). The length and width of fibers and vessel elements were measured from macerated xylem samples. One hundred random readings were taken for each parameter.

Statistical analysis

Pearson correlation (r) analysis, linear multiple regression (R^2), and t-value calculations were carried out to determine relations between rate of cambial activity and xylem formation, dimensions of xylem elements and environmental factors, using Sigma State 3.5 version software. Significance difference (95% confidential level) between two parameters was also determined by Student's *t-test* using Sigma State software.