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Development of distinct cell wall layers both in primary and secondary phloem fibers of hemp (*Cannabis sativa* L.)

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ABSTRACT

Formation of thickened cell wall allows plant fibers to obtain the strength necessary to the realization of their function as mechanical tissue. It is due to such cell wall that fibers of textile crops, like flax, hemp, and ramie, acquire characteristics that make possible to use these fibers in textile and technical applications. In the hemp stem, primary and secondary phloem fibers originating from the different type of meristems are formed. Analysis of ultrastructure coupled with immunolabelling demonstrated distinct layers within thickened cell wall in the fiber of both types: the outer layer is built as typical secondary cell wall of xylan-type, while the major portion was identified as the layers of tertiary (gelatinous or G-layer) cell wall. A newly deposited layer of the tertiary cell wall (Gn) is transformed into mature G-layer by the post-synthetic modification. The general design of cell wall in primary and secondary phloem fibers is similar, but with xylan layer being considerably thicker in secondary fibers than in primary ones. The formation of the tertiary cell wall in primary and secondary hemp fibers was associated with the synthesis of pectin component – rhamnogalacturonan I together with β-(1,4)-D-galactan, which has been detected and characterized both in the buffer-extractable fraction and among the strongly retained within cell wall polysaccharides. Comparison of the obtained results with data on the flax fiber cell wall development permitted to find similarities, as well as some differences of G-fiber cell wall organization in different plant species.

1. Introduction

Hemp is one of the oldest textile crops and has probably been grown for at least 6000 years (Small, 2015). In today's world, hemp fibers have great prospects for their use in various innovative applications as the ecological, biodegradable and renewable resource with unique properties (Ashik and Sharma, 2015; Pickering et al., 2016). Understanding of the development of the cell wall – the major component of plant fiber – is important to form the basis for further improvement of hemp fiber yield and quality.

From the viewpoint of plant biology, fiber is an element of sclerenchyma, an individual cell with a thickened cell wall, reaching a length of several tens of millimeters, with a diameter of not more than a few tens of micrometers (Gorshkova et al., 2012). In the hemp stem, there are the primary phloem fibers formed from procambium and secondary phloem fibers, the result of cambium activity. Since the primary fibers are formed from the primary meristem, they appear earlier during plant biogenesis and are present from bottom to the top of the stem (Hernandez et al., 2006; Snegireva et al., 2015). Secondary phloem fibers do not occur at the top part of the stem, they emerge closer to the stem middle, and the largest number of secondary fibers is located in the lower part of the stem. The number and occurrence of the secondary fiber along the stem length depend on the stem diameter and growth conditions of plants (Fernandez-Tendero et al., 2017). Secondary phloem fibers are known to have lower quality parameters than primary ones (Pickering et al., 2007; Placet et al., 2014). Comparison of the development of two types of phloem fibers that have the same genetic background may add understanding of the factors that determine the quality of fibers.

Primary phloem fibers of hemp stem during their formation pass through the following stages of development: initiation, coordinated growth, intrusive growth and formation of the thick cell wall (Snegireva et al., 2015). The scenario for the development of secondary phloem fibers of hemp stem is the same, with the exception of the absence of coordinated elongation stage, since the formation of such fibers occurs in the stem region which has ceased to grow in length. Fibers that ceased their growth begin to thicken cell wall.

Thickened cell walls of hemp fibers are often considered as

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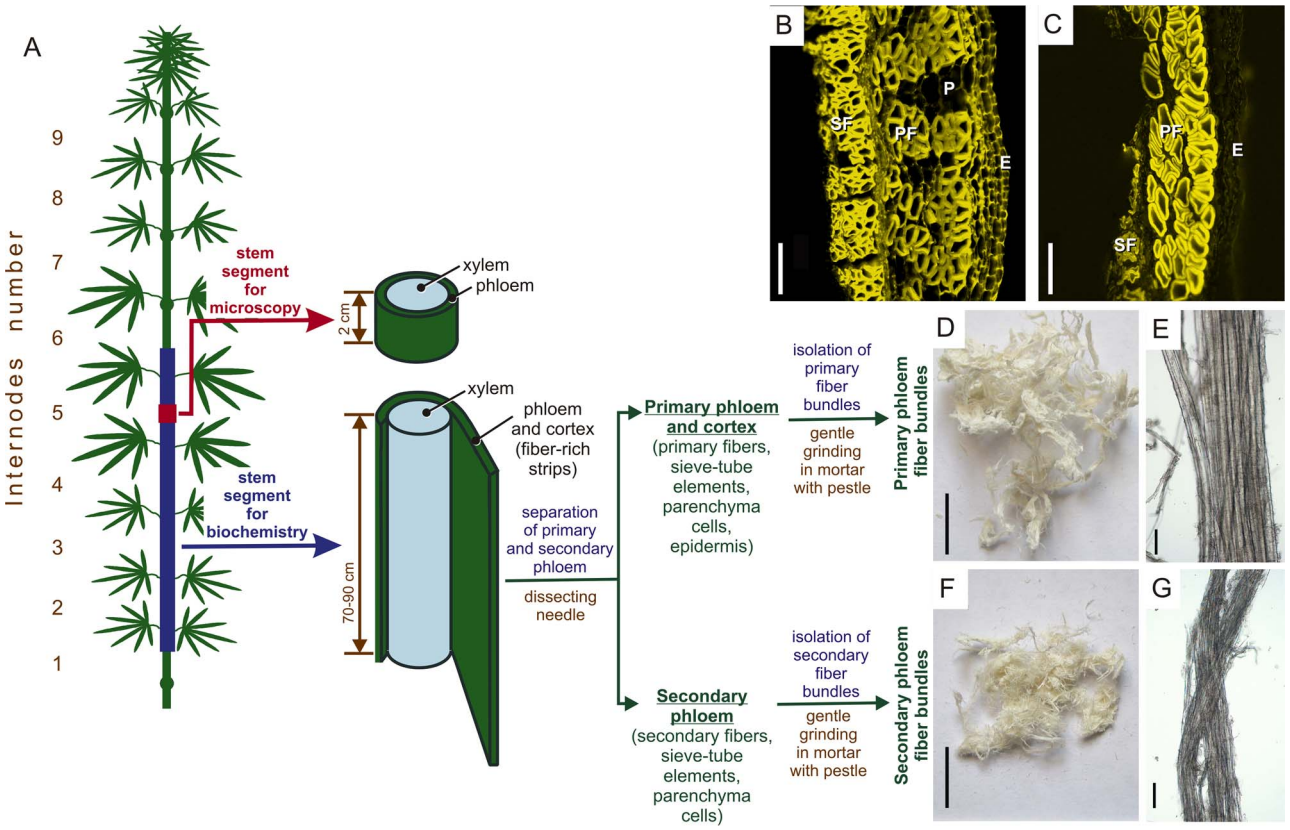

Fig. 1. Scheme of sample collection from hemp stem for microscopy and biochemical analysis. (A) Phloem fiber-rich strips from lower half of the stem were used for subsequent isolation of primary and secondary phloem fiber bundles for biochemistry research; samples for microscopy were collected from the 5th internode. (B) Cross-section of fiber-rich strips of hemp stem. (C) Cross-section of fiber-rich strips after detachment of secondary phloem tissue by dissecting needle. (D-G) Isolated primary and secondary phloem fiber bundles. Confocal microscopy, stained with 0.5% toluidine blue (B, C). E – epidermis, P – parenchyma cells, PF – primary fibers, SF – secondary fibers; scale bar 100 μm (B, C, E, G); 1 cm (D, F). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

secondary cell walls (Crônier et al., 2005; Blake et al., 2008; Dai and Fan, 2010; George et al., 2014). The characteristic features of secondary cell walls in angiosperms are the considerable proportions of xylan and lignin (Albersheim et al., 2010). Secondary cell walls often have several layers (S1-S3) that are rather similar in composition but differ in cellulose microfibril orientation. The total thickness of secondary cell walls is around 1–2 μm. Cell walls of phloem fibers in hemp seem to differ from such description. The cell wall of hemp fibers is lignified no more than 3–5.5% (McDougall et al., 1993; Garcia-Jaldon et al., 1998; Crônier et al., 2005; Fernandez-Tendero et al., 2017), with lignification extending only to the outer layers of the cell wall (Bonatti et al., 2004; Crônier et al., 2005; Fernandez-Tendero et al., 2017). The same cell wall pattern of distribution, i.e. through outer layers, was observed by immunofluorescence labeling with the antibody specific for xylan (Blake et al., 2008). The total cell wall width of hemp fibers may reach 14 μm (Crônier et al., 2005) – much higher value that is observed for secondary cell wall. Cellulose microfibrils in the main layer of hemp fiber cell wall are located almost parallel to the longitudinal axis of the cell (Dai and Fan, 2010). All those features resemble G-layer (tertiary cell wall) described for flax phloem fibers and tension wood (Mikshina et al., 2013; Gorshkova et al., 2015, Guedes et al., 2017). However, epitopes for the antibody specific for β-(1,4)-D-galactan, which labeling is characteristic for the gelatinous cell walls of fibers in flax and several other plant species (Arend, 2008; Gorshkova et al., 2015; Gritsch et al., 2015; Guedes et al., 2017), were not detected in the cell wall of hemp fibers (Blake et al., 2008), despite the high content of galactose observed in cell wall fractions biochemically extracted from phloem fiber-enriched material (Crônier et al., 2005). In such samples, galactose can be the component of the parenchyma cell walls, which effectively bind antibody specific for β-(1,4)-D-galactan (Blake et al., 2008).

The important polymer of the tertiary cell wall is stage-specific rhamnogalacturonan I (RG-I) (Mikshina et al., 2013; Gorshkova et al., 2015; Gritsch et al., 2015; Guedes et al., 2017), the presence of which was not assayed in hemp fibers. The intensive post-synthetic modification of RG-I by tissue-specific galactosidase leads to transformation of cell wall structure (Roach et al., 2011) and considerable maturation of cell wall mechanical properties (Arnould et al., 2017). In flax, the nascent RG-I can be extracted from buffer-soluble fraction (Gorshkova et al., 1996). In the cell wall, the large portion of this polymer is entrapped by cellulose microfibrils and can be obtained only after their dissolution (Gurjanov et al., 2008). Both buffer-soluble polymers and those strongly retained by cellulose microfibrils were not analyzed in hemp fibers, same as the presence of post-synthetic cell wall modifications.

This work aimed at revealing the ultrastructural and biochemical features of the cell wall development in primary and secondary phloem fibers in hemp stem. For this, the deposition and remodeling of G-layers of fiber cell walls in developing stems were compared for both types of fibers, the main groups of cell wall polysaccharides and β-galactosidase were immunolocalized, as well as the biochemical and structural analysis of cell wall polysaccharide fractions from isolated primary and secondary phloem fibers were carried out. The obtained results permit to designate the major part of hemp fiber cell wall as tertiary (gelatinous) cell wall and to describe its similarities and differences with other plant species.