The similarities and differences in microstructure of phloem and xylem of *Betula nana* and *Betula pubescens* species

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Abstract. The formation of woody plants of the Arctic zone occurs with a certain direction of morphological and microstructural changes. An advanced approach to sample preparation associated with preliminary cryomechanical destruction and freeze-drying was used for microscopy of *Betula nana* and *Betula pubescens* sections. The sample images were obtained using an SEM Sigma VP Zeiss. Significant differences were found in two positions: the width of the secondary xylem fibers and the thickness of their cell walls. There is an anisotropy of the secondary phloem ray cells stretching: *Betula nana* cells are elongated vertically, and *Betula pubescens* – horizontally, and the ratio of cell height to width differ by more than 2 times. Thus, all morphological differences of the microstructure are concentrated only in the secondary xylem. Extreme temperatures of the Arctic territories are the cause of abnormal structural changes in the structure and affect the direction of morphogenesis of anatomical elements, which is especially evident in the development of xylem fibers.

1. Introduction

*Betula nana* grows in the North of the European part of Russia, Western Siberia and Yakutia, Chukotka, Kamchatka and the mountains. *Betula nana* belongs to the same genus as the *Betula pubescens*, although it does not reach 1 m in height and is a shrub. The formation of woody plants of the Arctic zone occurs with a certain direction of morphological and microstructural changes. Xylem of such plants as *Betula nana* has its own specific features: small cell size and thinness of all anatomic elements, variety of tracheal structures [1].

Studies of the biochemical transformation of cambial cells and the formation and structural organization of xylem and phloem anatomic elements are of vital importance for contemporary plant physiology. The fundamental principles of the biosynthesis theory of higher plant biomass are given in Evert (2006). The biosynthesis of xylem and phloem anatomic elements is initiated by the vascular cambium [2]. The aim of the work is to compare the morphogenesis of the main anatomical elements of the phloem and xylem of birch *Betula nana* and *Betula pubescens* species.

2. Materials and methods

For this study, we harvested the stems and branches of birch *Betula pubescens* trees growing in the University Northern (Arctic) Federal University Arboretum (Russia, Arkhangelsk region, 64°32′N, 40°33′E). Parts of the branch and tree stems were sectioned for tissue collection. A birch branch from a *Betula pubescens* tree was cut at diameter 15 mm (August). The sample of birch *Betula nana* were harvested on an expedition to the North of the East European plain of Russia in the tundra zone.
(Russia, Nenets Autonomous Area, 68°03′N, 61°38′E) in August. An advanced approach to sample preparation associated with preliminary cryomechanical destruction and freeze-drying was used. The samples were treated with liquid nitrogen (-196 °C) in a plastic vessel until boiling no longer occurred. Freeze-drying was performed using a Labconco freeze dryer (FreeZone 2.5 L, Labconco Corporation, USA). The sample images were obtained using an SEM Sigma VP Zeiss (Carl Zeiss Microscopy GmbH, Germany). To increase the image contrast of the samples, a gold-palladium coating with a thickness of 5 nm at a ratio of 80:20 was applied to the split surface. For this treatment, we used a Q150T ES (Quorum Technologies Ltd, UK). The exosome sizes, morphological characteristic of fibers and vessels were analyzed using SEM images. The SmartTiff («Zeiss») semiautomatic measuring method was applied in the analysis.

3. Results and discussion

The general scheme of morphogenesis of anatomical elements of the studied species of birch is the same. However, the species Betula nana is characterized by a relatively large volume of rays and axial parenchyma as a storage tissue. But the structure of the axial parenchyma is identical in both species (Fig. 1 A, B). It is known that exosomes are extremely excreted by plants when attacked by pathogens, but the direct participation of exosomes in plant development (exocytosis) has not been previously discussed and taken into account. There is every reason to believe that exosomes transport cellulases, which are involved in the destruction of the cell walls of the anatomical elements of the phloem and xylem. Exosome structures were identified for both species (Fig. 1 C, D). The appearance of exosomes in the phloem is due to internal physiological processes. The erosion occurs in localization sites of exosomes on the inner surfaces of the plant cell walls. The hydrolases action leads to changes and reorganization of plant wall polysaccharides. The septa destruction is necessary for the formation of sieve elements involved in assimilation transport.

Primary xylem fibers practically do not differ in two species (Fig. 1 E, F). It is noted that the Betula nana has a large proportion of primary xylem relative to the total weight of wood.

Ultra-microstructure of the cell wall of secondary xylem fibers (diameter of cellulose microfibrils, angle to the fiber axis in S1 and S2 layers, pit size and structure, size and localization of exosomes on the cell wall) in Betula pubescens and Betula nana are almost identical (approx. 100 nm). The Betula nana is characterized by smaller sizes of the xylary fibers (Fig. 1 G, H). In xylem fiber weakly expands in the radial direction and as a result formed cells of small size, the width of which is about 2 times less in the Betula nana, than in Betula pubescens. It is important to note that Betula pubescens has more developed conductive system, represented by a large number of large vessels (Fig. 1 G). Features of the structure of anatomical elements of two species of birch are given in table 1.

| Birch species | Size of parenchymal phloem cells (µm) | Size of ray cells (µm) | Pit sizes in vessels (µm) | Fiber length of secondary xylem (µm) | Cell wall thickness of secondary xylem fiber (µm) | Diameter of primary xylem fiber (µm) |
|---------------|--------------------------------------|------------------------|--------------------------|--------------------------------------|---------------------------------------------|---------------------------------|
| Betula pubescens | 21.68±3.41 | 26.66±6.01 | 2.95±0.24 | 17.58±2.18 | 2.10±0.34 | 8.05±1.10 |
| Betula nana | 24.88±6.55 | 20.89±2.92 | 2.52±0.40 | 8.56±1.35 | 1.33±0.25 | 7.45±1.80 |

Significant differences were found only in two positions. This is the width of the secondary xylem fibers and the thickness of their cell walls. In addition, the observed anisotropy of the cells stretching of the secondary phloem rays.
Betula pubescens (A, C, E, G images) and Betula nana (B, D, F, H images) phloem and xylem. (A, B) Axial parenchyma (radial section); (C, D) Exosomes on internal surface of phloem cell wall; (E, F) Primary xylem fiber (radial section); (G, H) Secondary xylem (cross section). AP – axial parenchyma, B – beads, CW – cell wall, EXs – exosomes, P – primary cell wall, Pr – protoplast, S – septa, S1 – internal layer of secondary cell wall, R – ray, V – vessels.

Bars: A, B, G, H – 10 µm; C, D – 1 µm; E – 2 µm

The cells are elongated vertically in Betula nana, and horizontally – in Betula pubescens. The ratio of cell height to width differ by more than 2 times (Figure 2). Thus, all morphological differences of the microstructure are concentrated only in the secondary xylem.
The main stage of plant biosynthesis is the production of glucose from solar energy, CO₂ and H₂O. In addition, the rate of this biochemical reaction is determined by the temperature (Van-Goff rule). Limitation of this process can occur on any of these factors.

4. Conclusion
In the transition from a temperate climate to a subarctic zone, sunlight limitation switches to temperature limitation. The polar day lasts during the entire vegetative period at low temperatures. The resulting sucrose moves to the pathway: phloem - rays - xylem.Sucrose deficiency leads to the fact that phloem and rays receive normal nutrition, and nutrition does not reach xylem. The geometric dimensions of the anatomical elements of the xylem are determined by the internal turgor pressure in the period before the formation of the secondary envelope of the cell wall. Turgor pressure in plant cells can reach 1 MPa and “straighten” fibers up to 50 µm in width and several millimetres in length. The lack of sugars that determine it leads to a smaller volume occupied by the fiber and a smaller thickness of the secondary cell wall. This leads to small fibers sizes and small thickness of their cell walls.

There are also vessels with a width of less than 10 µm. The underdevelopment of the conduction system makes difficult the supply of outlying shoots. Dwarfism is manifested in slow axial growth, the transition to sympodialis and a preferential elongation of the lower branches, often outrunning the main axis on the growth. The transition of high-stem life forms to dwarf and shrubby is observed not only in the North, but also in the highlands, wetlands and aridity[3].

Acknowledgements
The reported study was funded by RFBR according to the research project № 18-33-00855 “Supramolecular organization of cellulosic microfibrils derived from plant and bacterial cellulose”, Northern (Arctic) Federal University, 2018. The research involved scientific equipment from the Shared Use of Equipment Center “Arktika” (NArFU) (RFMEFI59417X0013).

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