Cytostructure of the radial parenchyma of annual shoots of wild species of Meyer’s currant (Ribes Meyeri Maxim.)

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Abstract. This article presents the results of the anatomical structure of the radial parenchyma of the annual shoots of the Meyer’s currant, grown in conditions of high Jungar Alatau. In the anatomical structure of the stem Meyer’s currants ray parenchyma is represented mainly by single rays, two- and three-row rays are few. This study provides a theoretical basis for microclonal propagation of wild currants.

1. Introduction
Ribes Meyeri is a rare plant that grows in the Altai, Tien Shan, Tarbagatai Mountains and in Western China in deciduous and mixed forests, along river banks, on rocks and in thickets of shrubs. The species forms many kilometers of bushy thickets on the slopes of mountains. On the territory of the Dzungarian Alatau, Meyer's currant is distributed in the forest belt of the mountain range and forms significant populations in the gorges of Arganakty, small Baskan and Kokzhot [1-3].

Ribes meyeri belongs to the genus currant (Ribes L.), gooseberries family (Grossulariaceae). According to literary sources, Meyer's currant is classified as a variety of black currant [4]. Ribes Meyeri Maxim. is a rare plant that grows in the mountains. It is found mainly on the slopes and gorges of mountains, in thickets of shrubs, in the undergrowth of maple, walnut, spruce-fir forests, in humid areas along the Pamir-Alai, Tien-Shan, Tarbagatai mountains, Western China; it rises in the mountains up to 3500, and the plant is also protected in nature reserves [5].

Currant is propagated by traditional methods of propagation, most often by cuttings, but due to the difficult rooting of the plant (according to literary sources, depending on the variety, about 30-80%, depending on climatic conditions), traditional methods such as propagation by cuttings, layering, etc., remain unprofitable for rapid and successful propagation of currants [6]. Compared with traditional biotechnological methods, it allows to quickly getting planting material that is healthy from fungal and bacterial pathogens, viral and nematode infections. In addition, cellular technologies allow to work throughout the year, regardless of climatic conditions, and save space needed for growing planting material. In addition, the plant material required for breeding is reduced, which is relevant for the propagation of rare plant species, including Meyer’s currant.

The authors of many studies indicate that there is a correlation between the root-forming ability and the anatomical structure of plants. This correlation is mainly studied using traditional methods of vegetative reproduction, but the analysis of the radial parenchyma can be successfully used for preliminary diagnostics of the success of microclonal reproduction. Many vital abilities of plants are determined by their anatomical structure, such as nutrition, accumulation and storage of nutrients, etc. The established correlation between the structure of medullary rays and the degree of rooting of fruit
varieties of plants allows us to give a preliminary assessment of the effectiveness of microclonal reproduction. The study of the features of the anatomical structure of annual shoots allowed us to determine the features of the cytological structure of the stem of the annual shoot, to gain knowledge on the structure of the radial parenchyma, which is of great practical importance in the microclonal reproduction of this species.

2. Materials and methods
The object of the study was the annual shoots of Meyer’s currant collected in the territory of the Small Baskan cordon of the Zhongar-Alatau state natural Park.

The typical anatomical structure of annual shoots is formed by the end of the growing season and does not undergo significant changes until spring. To work annual shoots from wild plants were harvested in the autumn-winter period after leaf fall, fixing the lower, middle and upper parts of the stem. At least 20 anatomical sections were performed on the lower third of the stem. For storage, they were enclosed in 70% alcohol with the addition of 1:4 glycerol by volume [7]. The formation of adventitious roots in green cuttings can occur in both the nodes and internodes of the shoot, so, given the complexity of the anatomical structure in the nodal region of the stem, the work was limited to studying the histostructure of the medullary rays only in the internodes.

Cytohistological studies were carried out on equipment and with the help of chemicals from the bioresources laboratory of the research Institute of biotechnology problems. Anatomical sections were made on a sledge microtome with a thickness of 25 to 50 microns. The color of the slices was carried out blue water and chrysoidine for the division M.N. Prozina [7]. The preparations were encased in glycerine and viewed with a Nikon binocular microscope. In total, about 100 cross-sections were viewed, and about 40 of them were studied in detail. Measurements of the width and height of the medullary rays and measurement of ray cells were carried out using an eyepiece-micrometer OSM-1-16. Anatomical drawings and photos of the microslides were made using a Nikon microscope connected to a computer and software.

The cellular organization of the medullary rays was studied on the transverse and longitudinal tangential and radial sections of the internodes of the stem, which allowed us to study in detail the addition of the radial parenchyma and measure both the rays themselves (row, layer and length) and their constituent parenchymal cells (width, height and length). Calculation, measurements and description of medullary rays were performed using the method of A. A. Yatsenko-Khmelevsky [8]. The parameters of the medullary rays were determined by the number of ray cells and in micrometers, and the size of the ray cells – in micrometers.

To determine the number of medullary rays on transverse and tangential sections, the number of rays and their ratio in series were calculated.

Calculations and measurements of the medullary rays in all planes of the cut of the internodes of the stem, as well as measurements of different types of ray cells are made in 3-5-fold repetition with the measurement of one parameter at least 5-20 times in each repetition.

Statistical processing of the obtained data was carried out using generally accepted methods in biometrics [9].

3. Results and discussion
An important indicator of the plant's ability to reproduce, including conditions in vitro, is the anatomical structure of the stem wood. The efficiency of functioning of this complex tissue, which performs conducting, storing and mechanical functions, largely determines the life processes occurring in the plant body (movement of water and mineral salts, the supply of nutrients, the strength of the stem, etc.). The anatomical structure of the trunk corresponds to its main functions. The stem has a developed complex system of conducting tissues that connects all the organs of the plant into a single whole; the presence of mechanical fabrics ensures the performance of the support function.

The radial parenchyma, as well as the axial parenchyma, performs a double function-the supply of plastic substances and their conduct in the radial direction. Medullary rays differ in the structure and
shape of ray cells, row (width), ply (number of cells in the height of the ray), the presence and size of single-row endings, the presence or absence of contacts with conducting elements. Standing, square, and recumbent cells also differ in function: standing and square cells are better suited for contact with water supply elements and the transfer of nutrients from the radiation cells to the vessels. Recumbent cells are usually devoid of contact with water supply elements and are better specialized in storing nutrients and transporting them in a horizontal direction [10].

The width of the rays is measured by the number of cell layers in the tangential direction and is designated as a series. This feature has significant stability in many types of plants. In width (row), the rays are single-row, double-row, three-row and multi-row. Primary single-row rays significantly predominate, there are no secondary rays. It is important to note the presence of more specialized wide two- and three-row rays among the single-row rays characteristic of the currant species under study. This is due to the fact that as the annual diameter of the cambial zone increases, some of the single - row rays become two-and three-row. This occurs as a result of converting fusiform cambial initials into ray cells (table 1).

There are several morphological types of ray cells: recumbent, elongated along the ray; standing, elongated in the axial direction; square, belonging to the intermediate type.

In the cross section, the radial parenchyma is represented by single-row, two-row, and three-row rays. The number of single-row rays in the parenchyma is 23.0±5.0 PCs. or 67.6 % of the total number of rays. Further growth of wood increases the number of rows in single-row rays and they become double-row. In this case, the radial parenchyma of Meyer’s currant is represented mainly by single-row medullary rays.

### Table 1. The number of medullary rays of the stem of the annual shoot of Meyer’s currant on the transverse (at the magnification of 10x10) and tangential sections (n the field of view of the microscope at the magnification of x150).

|                      | All of the rays at the section | Single-row rays | Double-row rays | Three-row rays |
|----------------------|-------------------------------|-----------------|-----------------|---------------|
|                      | PCs. %                        | PCs. %          | PCs. %          | PCs. %        |
| The cross section    | 34±4.0 100                    | 23±5.0 67.6     | 9±2.5 26.5      | 2±2.0 5.9     |
| The tangential section| 14±1.1 100                    | 8.3±0.8 59.3    | 5.3±0.7 37.9    | 0.4±0.2 2.8   |

Two-row medullary rays make up 9±2.5 PCs. or 26.5% of the total number of rays. The length of two-row rays varies significantly from 28 to 44 cells, which is determined by the intensity of growth of the annual wood of the stem during the growing season and the exposure of the shoot in space. The parenchyma also contains three-row rays (5.9%).

On tangential sections, the height of the rays is determined by the number of cell layers in the axial direction, as well as in absolute units of length – micrometers. In height (ply), the rays can be low (up to 20 cells, rarely higher), medium (up to 50, rarely higher) and high (50-100 cells or more). In Meyer's currant, the number of ray cells (height) was determined by 20-fold repetition from different sections of the tangential section. The wood of the studied species is characterized by rays of only average height, the number of which amounted to an average of 33.3 ray cells (table 2). The predominance of medium and high ply rays in wood contributes to the vitalization of the xylem and possibly compensates for the storage function and movement of plastic substances in the vertical direction in species with poor axial parenchyma. The presence or absence of single-row endings in (two-, three-and multi-row) rays and their size are one of the characteristics that characterize the level of structural organization of this tissue. In the course of evolution, when heterogeneous two-row rays become specialized, along with other structural changes (reducing the height of cells and increasing their length), their single-row endings are shortened and eliminated.
Single-row medullary rays with an average height of 42 ray cells (59.3%) dominate the tangential section. The number of double-row rays is 5.3 pieces or 37.9% and three-row only 2.8%. At the same time, two-row and three-row rays are low, with a height of only 15-24 ray cells.

**Table 2.** Dimensions of the medullary rays of the stem of the Meyer’s currant shoot on a cross section (magnification 10x10), tangential section (in the field of view of the microscope with magnification x150) PCs.

| Parameters of rays | Single-row rays | Double-row rays | Three-row rays |
|--------------------|-----------------|-----------------|-----------------|
|                    | By number of cells | On μm | By number of cells | On μm | By number of cells | On μm |
| The cross section  |                 |       |                 |       |                 |       |
| Length             | 35±2.1          | 509.7±4.3 | 36±2.5          | 503.4±4.9 | 39±1.4          | 497.5±5.1 |
| Width              | 1               | 10.1±1.8 | 2               | 19.8±1.3 | 3               | 27.3±0.5  |
| The tangential section |             |       |                 |       |                 |       |
| Height             | 42±3.5          | 634.2±3.9 | 15±1.2          | 162±2.5 | 24±1.1          | 256.8±5.3 |
| Width              | 1               | 15.1±2.1 | 2               | 21.6±1.1 | 3               | 32.1±1.8  |

On the cross section of the stems, single-row medullary rays consist of two types of cells: elongated along the radius of the stem – recumbent (the length of the cells significantly exceeds their width) and having approximately flat dimensions of length and width – square (table 2). Both types of ray cells have a width of 8.9-10.1 microns, the length of recumbent cells is 35.7 microns, square -14.3 microns. The length of these rays is equal to 35 ray cells or 509.7 microns (figure 1).

![Figure 1. Structure of the medullary rays of Meyer’s currant on cross sections a – 40X, b – 200X.](image)

On the tangential section, the medullary rays vary in height (ply). According to the number of ray cells, fluctuations in the height of the ray range from 15 to 42 cells. The radial parenchyma on the tangential section is composed mainly of square cells.

**Table 3.** Dimensions of ray cells of the stem of the annual shoot of Meyer’s currant on transverse and longitudinal sections (μm).

| The number of rows of rays | The length of the cell | The width of the cell | Height |
|---------------------------|-----------------------|----------------------|--------|
| The cross section         |                       |                      |        |
| Single-row                | 29.4                  | 10.1                 | -      |
| Double-row                | 27.2                  | 8.9                  | -      |
Three-row 26.9 9.1
The tangential section
Single-row - 9.1 15.1
Double-row - 11.7 10.8
Three-row - 10.9 10.7
Radial cross section
44.2 - 16.9
Size of the middle cell 31.9 9.9 13.4

The ratio of width, length, and height of the middle cell of the medullary rays was 1:3.4:1.35. This ratio allows us to conclude about the predominance of recumbent cells in the medullary rays of Meyer’s currant. The potential root-forming ability of Meyer’s currant is determined by the size of the "average" ray cell. Given that the radial parenchyma is composed of elongated cells in the radial direction, contributing to the formation of adventitious roots, so the species has potential data for easy rooting in microclonal reproduction.

4. Conclusions
As a result of studying the medullary rays of the stem of annual shoots of Meyer’s currant on longitudinal and cross sections, the following conclusions are made:

The Radial parenchyma of the Meyer’s currant is represented by substantially predominant primary single-row rays, secondary rays are absent in the xylem. On a cross-section of the stem wood, the ray parenchyma of the Meyer’s currant is represented by single-row, two-row, and three-row rays. Single-row rays make up 67.6% of the total number of rays. The number of two-row and three-row rays is 26.5% and 5.9%, respectively.

Among the single-row rays, there are more specialized wide two-and three-row rays, composed mainly of recumbent cells. Knowledge of the features of the anatomical structure of the core rays in the studied species can serve as a theoretical basis for vegetative propagation of Meyer’s currant for further improvement of the technological process of green and microclonal propagation with the use of various growth regulators.

Based on the assumption of a correlation between the anatomical structure and rootability of plants, it can be assumed that Meyer's currant belongs to the group of easily rooting plants. The main diagnostic indicators were the size of the average radial cell, the ratio of the parameters of width, length and height of which is 1:3.4:1.35 and indicates the addition of mainly recumbent radial cells.

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