Structure of the radial parenchyma of annual shoots of wild species of Tian-Shan mountain ash (Sorbus tianschanica Rupr.)

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Abstract. This article presents the results of a comparison of the anatomical structure of annual shoots of Tian Shan mountain ash growing in the highlands of the Dzhungarian Alatau. In the anatomical structure of the stem of the Tian Shan mountain ash, the ray parenchyma is represented mainly by single-row rays, two-, three- and four-row rays are few. This research provides as a theoretical basis for vegetative reproduction of Tian Shan mountain ash by green grafting.

1. Introduction
The species Tian Shan mountain Ash (Sorbus tianschanica Rupr.) belongs to the genus Sorbus L. of woody plants from the Rosaceae family. In total, there are more than 100 species of mountain ash, of which about one third grows on the territory of the former USSR, in the Republic of Kazakhstan - 3 species. Mountain ash is widely distributed throughout Europe, Asia and in North America. The main and natural distribution area of the species is in Central Asia, in the mountain systems of the Tian Shan and Dzungarian Alatau in Kyrgyzstan and Kazakhstan (Eastern Tian Shan, Terskey Alatau, spurs of mount Baskarkara) [1-3]. On the territory of the Trans-Ili Alatau and Dzungarian Alatau Tian Shan mountain ash grows at altitudes of 1300-3600 m above sea level in the undergrowth of sparse spruce, spruce-deciduous, deciduous forests, juniper forests.

Tian Shan mountain Ash is deciduous shrub, rarely a small tree 4-5 m high. Overwintering buds are pubescent. Crown is thick, oval. The shoots are first pubescent, then shiny, red-brown. Leaves 12-15 cm long, complex, non-pinnate, small leaves in the number of 9-15 pieces, 3-5 cm long, lanceolate, wedge-shaped narrowed on both sides, along the edge of the upper part of the plate sharply saw-toothed, dark green above, waxy, shiny, gray below, glabrous on both sides. Flowers 1.5 cm in diameter, white, collected in large numbers in pubescent, dense, corymbose inflorescences 8-12 cm in diameter. Fruits 0.8 cm in diameter, spherical, bright red, blooms in May. The species is frost-resistant to minus 23°C, light-and moisture-loving [2-3].

The conservation of plant species that form the basis of valuable agro-biodiversity is important for the ecological and food security of our country and the entire planet. Among the wild relatives of cultivated plants, special attention is paid to wild species of Apple, apricot, mountain ash, barberry, and hawthorn, which are widely distributed in the Dzungarian Alatau. However, as a result of excessive economic activity, their global significance as the progenitors of numerous cultural species and varieties
is underestimated. Therefore, the study and conservation of these species in natural conditions, in the natural cenotic environment of their distribution, is important in the future.

The authors of many studies indicate that there is a relationship between the root-forming ability and the anatomical structure of plants [4-7]. 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 green cuttings. 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 vegetative reproduction of this species.

2. Materials and methods
The object of the study was the annual shoots of Tian Shan mountain Ash collected in the territory of the Small Baskan cordon of the Dzhungar-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 [8]. 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 [8]. 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-116. 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 [9]. 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 [10].

3. Results and discussion
When studying the stem of the Tian Shan mountain ash with the naked eye, the core and sapwood are clearly distinguished on the cross section. In the core part there is a xylem consisting of vessels of different sizes. At the same time, the arrangement of vessels in the xylem is unsystematic (figure 1).
Transition from early wood of the annual layer to late wood occurs gradually. The annual layer of
mountain ash is slightly sinuous and uneven in width. The annual layer slightly narrows or expands,
forming a ring of slightly irregular shape.

The core is fully formed, and with further development of the annual shoot stem, it will not change
its size and occupies 25.7% in the radial direction from the center to the periphery. However, with the
increase of conducting complexes, the relative proportion of the core will decrease (table 1).

Conducting complexes are at the stage of intensive development and, in the future, their share in the
cross-section will significantly increase. At the time of sampling, the xylem radius was 46.2%, and the
phloem radius was 18.9%. There is a laying and formation of the secondary cover tissue of the cork.

Table 1. Dimensions of plant tissues and complexes on a cross-section of a year-old shoot of Tian
Shan mountain ash in microns (x 100).

| Core (1/2 radius) | Perimedullary zone | Xylem | Cambial zone | Primary cortex | Cork | Total length |
|------------------|--------------------|-------|--------------|----------------|------|--------------|
| 520              | 111                | 934   | 40           | 382            | 34   | 2021         |
| 25.7%            | 5.5%               | 46.2% | 2.0%         | 18.9%          | 1.7% | 100.0%       |

Tian Shan mountain ash is characterized by anatomical small irregularities of the perimedullary zone
associated with the laying and formation of the annual cambium ring. These irregularities vary within
the perimedullary zone, which averages 5.5% or 111 microns.

The ray parenchyma of the Tian Shan mountain ash on a cross-section anatomical section consists
of primary medullary rays. The primary rays at the lane are one-, two-, three - and four-row, while
single-row rays are 89.1%, double- row are 7.6%, three-row- 1.7%, four-row – of 1.6%. In the initial
period of formation of the annual shoot, all rays are formed by a single row rays, later 10.9% of these
rays become two-, three - or four-row (table 2).

In the xylem of the annual shoot of the Tian Shan mountain ash, single – row medullary rays
significantly predominate and make up 89.1%, and multi-row (2 or more) - 10.9%.
On the cross section, single-row medullary rays are divided into two types according to the addition of ray cells. The first type of single-row rays is heterocellular, the addition of which is dominated by square cells. The second type of single-row rays is homocellular, consisting of recumbent ray cells. Two-, three- and four-row rays are also composed of recumbent ray cells. However, in the perimedullary zone, all rays are formed by 5-8 square cells (figure 1).

The radial parenchyma of the Tian Shan mountain ash on a tangential anatomical section consists of single-row and multi-row medullary rays. In the field of view of the microscope on the tangential section, single-row rays predominate and make up 78.6%. The height of single-row rays is equal to 10-15 ray cells or 150-180 microns. Two- and three-row rays make up 21.4%, have a height of 12-22 ray cells or 120-150 microns.

Single-row rays consist of two morphological types of ray cells: square (isodiametric) and standing, i.e. elongated in the vertical direction. Standing cells form completely single-row rays or endings of rays 2-5 cells high. Square cells in single-row rays are located in the Central part. Thus, single-row rays are divided into two types: homocellular (only from standing cells) and heterocellular (from standing and square cells).

According to the histostructure, two- and three-row rays consist only of square ray cells, these rays will be homocellular medullary rays.

The radial cross section makes it possible to more accurately determine the morphological types of ray cells in the structure of the medullary rays. In the radial parenchyma, there are two types of ray cells: cells with the most elongated axis in the radial direction (recumbent ray cells) and cells that are elongated in the vertical direction (standing ray cells).

Isodiametric ray cells on a radial section of wood have the form of square ray cells and represent a modification of standing ray cells. The main parameters of the ray parenchyma cells - the height and length of the ray cells composing the studied rays - were determined on the radial section. It is on the radial crossing section of the medullary rays that it is possible to study the features of the histostructure of radial growth of wood.

On the radial section of the wood of the annual shoot of the Tian Shan mountain ash near the perimedullary zone, ray cells of primary rays of procambial origin are located, they are large, standing or square.

Later in the process of cambial growth of wood, the histostructure of the medullary rays changes to recumbent ray cells. Along the length of the medullary bundle, it is during this period that annual shoots are harvested for green cuttings, so when describing the ray parenchyma, it is necessary to consider in more detail the addition of rays in this zone.

Homocellular rays of Tian Shan ash are formed in the addition of medullary rays, consisting of recumbent cells that have a long radially elongated axis 2.0-2.5 times the height.

Square or standing ray cells are located in the pericore zone of the medullary rays, i.e. in the initial period of formation of the medullary ray. Therefore, such rays belong to homocellular medullary rays.

In general, the radial parenchyma of the wood of the annual shoot of Tian Shan mountain ash is represented by homocellular palisade, homocellular medullary rays, folded recumbent cells and heterocellular rays, folded by different types of ray cells.

### Table 2. The Number of medullary rays in the wood of the annual shoot of Tian Shan mountain ash (X400).

| Indicators of the radial parenchyma | Cross section | Tangential section |
|------------------------------------|---------------|-------------------|
| Total number of rays per slice      | 264±2.3       | 14.0±1.0          |
| Primary rays                       | 264±2.3       | 14.0±1.0          |
| Among them single row               | 236±3.4       | 11.0±0.3          |
| Double-row                         | 20±1.4        | 2.0±0.6           |
| Three-row                          | 4±0.4         | 1.0±0.6           |
| Four-row                           | 4±0.4         | 1.0±0.6           |

On the cross section, single-row medullary rays are divided into two types according to the addition of ray cells. The first type of single-row rays is heterocellular, the addition of which is dominated by square cells. The second type of single-row rays is homocellular, consisting of recumbent ray cells. Two-, three- and four-row rays are also composed of recumbent ray cells. However, in the perimedullary zone, all rays are formed by 5-8 square cells (figure 1).
The found connections between the features of the medullary rays of the stem and the formation of adventitious roots served as the basis for our research on the histostructure of the medullary rays in the Tian Shan mountain ash.

In the course of research, the average parameters of ray cells (width, length, height) were determined. To determine the aspect ratio of the average cell, the width indicator was taken as a unit and the relative length and height indicators were determined. As a result of statistical processing, the ratio of the size of the ray cells of the wood of the annual shoot of the Tian Shan mountain ash was 1:2.7:1.7. This ratio of the ray cells indicates the addition of the medullary rays mainly by recumbent ray cells (table 3).

Table 3. Dimensions of ray cells of the medullary rays of the wood of the Tian Shan mountain ash stalk on anatomical sections, microns.

| Directions of anatomical sections of the stem | Cross | Tangential | Longitudinal | Radial |
|---------------------------------------------|-------|------------|--------------|--------|
| Width | Lenth | Height | Breadth | Lenth | Height |
| 7.7   | 18.3  | 13.3   | 9.0 | 27.1  | 15.6   |

By structure, all multi-row (two-, three- and four-row) medullary rays are composed only of recumbent ray cells. Based on the number of two- and three-row medullary rays on the transverse (10.9%) and tangential sections (21.4%), it can be predicted that the potential rootability of green cuttings can average up to 20%. However, the formation of lateral roots depends on the presence of radially elongated cells in the structure of the rays in all rays. Part of the single-row medullary rays also consists of recumbent ray cells, so the potential rootability of green cuttings will be significantly higher than expected. The results obtained are confirmed by scientific data [11], according to which the rootability of green cuttings of Tian Shan mountain ash will be more than 30% (figure 2).

The length of the ray cells varies depending on the annual growth of the xylem of the annual shoot. In this case, the change in the length of the ray cells is determined by the intensity of activity of the cambial cells and environmental factors (soil, climate). The change in length occurs in waves during the growing season. If the harvesting period of green cuttings coincides with the period of intensive growth of recumbent radiation cells, the root-forming capacity of green cuttings may increase by up to 30%.

Figure 2. Length of ray cell on a cross section of Sorbus tianschanica Rupr.

On the tangential section the annual layers are not visible, since the section is only in one part of the annual layer — in early or late wood. It should be noted that the tracheids on the tangential walls are without edged pores; the edged pores are visible as thickenings on the dissected radial walls. Parallel to the long walls of the tracheids in almost all preparations, you can find vertical resin passages that have the same appearance as on the radial section, the medullary rays, cut across, have the form of strips. On this section of the bundle, you should count the number of cells by height and width (row). Most bundles
are single-row multi-layer (from 2 to 15 layers). There are also wide medullary bundles (two - or multi-row) with horizontal resin channels. The resin channel is lined with epithelial cells and surrounded by parenchymal cells of the medullary rays (figure 2).

4. Conclusions

The core and wood are clearly distinguished on the cross section of the stem of the annual shoots of the Tian Shan mountain ash. The wood contains a year-old layer of xylem, the radius of which is slightly uneven and has small protrusions in the perimedullary zone of the core.

In the structure of the annual shoot, the radius of the core is 25.7%, xylem- 46.2%, phloem-18.9%. Vessels of annual wood have an equalized diameter and their number decreases by the end of the growing season. Therefore, by the location and size of vessels, mountain ash belongs to the breeds with scattered vascular core wood. Green cuttings they consist of a section of a stem with several leaves, so the axial parts have a secondary anatomical structure.

On the cross-section, the stem of the Tian Shan mountain ash is characterized by very thin medullary rays. The radial parenchyma is represented by primary medullary rays. The cross section is dominated by single-row primary medullary rays (89.1%), multi-row rays are 10.9%.

The medullary rays consist of cells stretched in a radial direction, the length of which is 2.0-2.2 times the width (recumbent cells). Square ray cells are located next to the perimedullary zone. On the tangent section of the stem of the annual shoot of the Tian Shan mountain ash, the ray parenchyma is represented by one-, two -, three-ray rays. Single-row beams make up 78.6%, two - and three-row bundles-21.4%. Most rays are built in square cells, and standing cells are only found at the end ends. The radial section of wood is dominated by recumbent cells, square cells are located near the perimedullary zone of annual growth.

When averaging all parameters of the ray parenchyma of the Tian Shan mountain ash, an "average" cell is characteristic, having a ratio of width, length and height of 1:2.7:1.7, which indicates the addition of the medullary rays mainly by cells elongated in the radial direction. Based on the number of single - and multi-row rays and their addition by recumbent cells, the potential rootability of green cuttings will be about 20%.

References

[1] Baitenov M S 1985 High-Mountain flora of the Northern Tien Shan (Alma-ATA: The science) p 230
[2] Goloskokov V P 1984 Flora Of The Dzungarian Alatau (Alma-ATA: Nauka) p 224
[3] Dzhangaliev A D , Salova T N and Turekhanova R M 2001 Wild and fruit plants of Kazakhstan (Almaty) p 133
[4] Bakhtaulova A S and Orlov P N 1995 Cytomorphology of core rays of different rooting fruit plants Izvestiya TACA Magazine (Moscow) 2 118-36
[5] Bakhtaulova A S and Karipbayeva R K 2020 Cytostucture of the radial parenchyma of annual shoots of wild species of Meyer’s currant (Ribes Meyeri Maxim.) IOP Conf. Series: Earth and Environmental Science 548 072021 doi:10.1088/1755-1315/548/7/072021 1
[6] Bakhtaulova A S, Zhakupzhanova M F and Kambarova A 2017 The influence of growth regulators on root-forming ability of softwood cutting of ornamental plants Science Way 2(36) 32-5
[7] Bakhtaulova A S 2020 Technology of Malus sieversii softwood cutting IOP Conf. Series: Earth and Environmental Science 421 082023 doi:10.1088/1755-1315/421/8/082023 1
[8] Prosine M N 1960 Botanical microtechnology (Moscow: Higher school) p 207
[9] Yatsenko-Khmelevsky A A 1954 Fundamentals and Methods of Anatomical Research of Wood (Moscow: Publishing house of the USSR Academy of Sciences) p 338
[10] Zaitsev G N 1990 Mathematical Statistics in Experimental Botany (Moscow: Science) p 275
[11] Tarasenko M T 1991 Green cuttings of forest and garden crops (theory and practice) (Moscow: Moscow Timiryazev Agricultural Academy) p 272