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Study of histostructure of ray parenchyma and rooting ability of Sievers apple (*Malus sieversii*) endemic species in green cutting

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Abstract. Wild apple forests represent a unique gene pool of not only our country but the entire planet. Therefore, conservation of genetic resources and restoration of wild fruit forests is an important task, and to solve it without help of research and financial support is impossible. In the absence of urgent and effective steps to study the biological features of wild Sievers apple structure and obtaining productive technologies of reproduction, the area of apple trees in the Dzhungar Alatau mountain range may be cut down by more than a third in 2030. One of the effective ways of vegetative propagation of fruit crops is green cutting. The established correlation between the structure of medullary rays and the degree of rooting with green cuttings from different rooting ability of fruit plant sorts allows identifying the potential plant rooting with green cuttings. Therefore, the study of radial parenchyma of Sievers apple trees and rooting degree of green cuttings of this endemic species is an urgent problem, requiring a timely solution. The research aim is to study the structure of medullary rays of annual shoots of the wild Sievers apple tree stem and to determine the potential degree of rooting with green cuttings. Raw materials, storage, annual shoots, preparation of anatomical slices, staining and examination of slides of medullary rays' histostructure are conducted with proven conventional methods in anatomy by means of modern microscopic equipment. Counting, measuring and description of medullary rays are made with A. A. Yatsenko-Khmelevsky's method. Statistical data processing was carried out with conventional biometry methods. As a result of the conducted research, the medullary rays of annual shoot stems on transverse and longitudinal sections were examined on tangential and radial cuts. We also calculated the number of rays, measurements of rays and ray cells. The description of radial parenchyma, which is typical for the stem of Sievers apple annual shoots, is conducted. Anatomical photos of rays on the transverse and longitudinal sections are taken. The analysis of obtained results is carried out and the potential level of rooting of Sievers apple green cuttings by the histostructure of radial parenchyma is identified.

K*ey words*: Histologic structure (histostructure), medullary rays, green cuttings, Sievers apple

Introduction

Sievers apple is listed in the Red Book of Kazakhstan and protected by the International Union for Conservation of Nature. It takes a special place among the valuable plant species of the Zhongar-Alatau State National Natural Park (NSNP) as forming significant arrays of wild-fruit forests (1.05% of park area).

Sievers apple plantings are located at the altitude of 900 - 1800 meters and represented by separate arrays, often disconnected from each other, ranging from a few hundred square meters to several tens of hectares. The total area of wild apple trees on the territory of the Zhongar-Alatau State National Natural Park is 8,573 hectares.

The Department of scientific works and mountain agro-biodiversity of the Institute of Botany and Phytointroduction (IBP) of Ministry of Education and Science conducted a survey and confirmed the natural purity of apple tree genotypes under the Project of Kazakhstan Government, the Global Environment Facility and the UN Development Program in Kazakhstan "Conservation insitu of mountain agrobiodiversity in Kazakhstan". The proportion of mature and maturing trees is up to 67%, indicating a high disturbance degree of the age structure of forests.

70 - 80% of these most productive plantations are not provided with natural regeneration; therefore, the ecological condition of wild fruit forests is estimated as unsatisfactory. It is a very real danger of losing unique forests that cover the foothills of Dzungar Alatau since the Cretaceous period. Currently the study of natural regeneration processes of Sievers apple and development of facilitating methods is particularly relevant. Sievers apple propagation is by seeds (20%) and vegetative way, shoot rooting, (80%) in vivo. The formation of root shoots does not absolutely guarantee high rooting of cuttings and its shoot excitability. Currently the restoration of Sievers apple is just a method of creating forest crops, planting material is grown

from seeds, collected at the sites of genetic wildlife reserves. Researchers of this project did not reach effective results. In the apple forests sanitary territory cutting is even forbidden, excavation of root system is invalid. In the greenhouse of "Orman" forestry of the Ile-Alatau National Natural Park, the survival rate of Sievers apple root shoots was only 2 percent. When propagating plants in mass and industrial scale for the recovery of degraded apple forests of Kazakhstan, it is necessary first to study biological characteristics of the plant itself.

Currently the technology for the rooting of wild apple green cuttings is, unfortunately, poorly studied. Green cuttings, as one of the most effective methods of fruit plant propagation, are based on the ability of green cuttings to form adventitious roots. The study of root-formation ability in vegetatively propagated plants were carried out by a number of researchers (Komissarov 1968; Tarasenko 1991 etc.), which revealed different rooting ability of plants with green cuttings (Tarasenko 1991). During these researches attempts to explain the different rooting ability of plants on the basis of their phylogeny, life forms, genealogical bonds, taxonomy, etc. were made. Among all life forms, including trees, there are species and varieties with high and low root-formation ability. Therefore, for each individual plant it is impossible to predetermine the degree of rooting of green cuttings, based on a life form or position in the taxonomic system.

Adventitious roots of trees and shrubs are formed with a stem of green cutting endogenously. According to the majority of researchers, the formation of root primordia in woody-shrubby dicotyledonous occurs in the bark of stem opposite the multi-row wide medullary rays (Tarasenko 1991; Faustov and Orlov 1985; Orlov and Bakhtaulova 1995). Considering the specified topography of the initiation of root primordia, many authors associate the degree of radial parenchymatization of shoot stem with the light rooting of green cuttings. Thus, it is noted that the growth of adventitious roots depends on the morphological structure of medullary rays. The plants with homogeneous rays, consisting of recumbent cells, form the primary tubercle of adventitious root more rapidly than the plants with palisade and heterogeneous medullary rays, the structure of which is dominated by upright cells (Orlov 1995; Faustov and Orlov 1985).

The established relation between the specific structure of stem medullary rays and formation of adventitious roots opens up the real possibility of forecasting the rooting of plants in their selection for green grafting (Orlov and Bakhtaulova 1995). Therefore, the study of the anatomical structure of annual shoot stem and radial parenchyma of Sivers apple will allow determining the potential of plant propagation with green cuttings.

Research object is the stems of Sievers apple annual shoots from old age trees in the fruit forests of the Zhongar-Alatau Natural Park.

Material and Methods

Raw materials, storage, annual shoots, preparation of anatomical slices, staining and examination of slides of medullary ray histostructure are conducted by proven conventional methods in anatomy by means of modern microscopic equipment.

Counting, measuring and description of medullary rays are made by A. A. Yatsenko-Khmelevsky's method. Statistical data processing was carried out by conventional biometry methods.

Results

Annual shoots develop from buds within one growing season. They form the shoot system of perennial plants and serve as sites for green grafting. Optimal time for green cuttings of most fruit plants is a phase of the intensive growth of shoots in length, including the fading phase of their linear growth. During this period, the stems of shoots have a secondary anatomical structure, cambium actively functions. Green cuttings are a leafy part of stem with one or more buds, so that the axial part of green cuttings has a secondary anatomical structure.

Studying Sievers apple annual shoot stems, one can clearly see with the naked eye the difference between the core and sapwood on the cross-section. There is a xylem, consisting of vessels of different sizes, in the heartwood. The arrangement of vessels in the xylem is sporadic. The early wood of annual ring gradually transits to the late wood of annual ring. The apple tree year layer is slightly sinuous and uneven in width, then it narrows, then widens, forming a ring of irregular shape. Apple has very narrow medullary rays, which are invisible with the naked eye.

In general, the radial parenchyma of annual shoot consists of primary and secondary medullary rays. Primary medullary rays are composed of cells of pre-cambial and cambial origins; they connect the stem core with the bark. Secondary medullary rays occur in the subsequent secondary growth of stem wood, they are of cambial origin and do not reach the stem core.

On the anatomical specimens, it is seen that while the primary medullary ray is developing, its histostructure is changing in the composition of radial cells. In the field of pre-cambial formation, the medullary rays are mainly composed of ray cells, elongated along the axis of stem (upright cells), and then along the ray axis the shape of cell often changes, becoming elongated along the ray axis (recumbent cells).

As a result of dividing isodiametrical radial initials of cambium in the addition of medullary rays, less high upright and isodiametric (square) or radially elongated (recumbent) radial cells appear. Thus, the addition of medullary rays in the one-year ontogeny of shoot appears as a result of various rearrangements of radial initials of cambium.

On the transverse anatomical slice of radial parenchyma of Sievers apple wood consists of primary and secondary medullary rays. On row arrangement (width) of primary rays these are single-, double-row, which are dominated by single-row (89.1%) and double-row. All of them, however, are formed by the pre-cambium as single-row rays in the initial stages of shoot growth. Further growth of the wood increases the amount of rows in 10.9 per cent of primary rays, and they become double-row. The formation and growth of secondary medullary rays occur in the summer-autumn period of vegetation; there-

fore, they occur only in the secondary wood and are short and single-row by structure (Table 1).

Indicators of medullary rays	Transverse section		Tangential section	
	Unit	%	Unit	%
Total number of medullary rays on the cut	170.6 ± 3.5	100.0	27.6 ± 2.05	100.0
Number of primary rays	170.6 ± 3.5	100.0		
Including: Single row	152 ± 2.1	89.1	22.3 ± 1.2	80.8
Double raw	18.6 ± 1.4	10.9	5.3 ± 0.8	19.2

Table 1. The number of medullary rays in the stem wood of the apple tree on transverse (on the cut) and tangential (in the view of microscope at x200 magnification) anatomical slices.

The radial parenchyma of Sievers apple is predominantly presented by single-row homocellular medullary rays of upright or square cells. There are also double-row medullary rays, in which the structure of boundary layers (top and bottom) is formed by square and upright cells. Recumbent radial cells are arranged in the middle part. Recumbent radial cells include cells, the radial axis of which is bigger in 2.0 - 2.2 times more than the vertical one. Such rays are called heterocellular. Recumbent cells of heterocellular rays form the layers of two types. In the first type the cells are arranged with lying offset rows as in "bricking", and the second type of recumbent cells lie in the parallel rows without displacement. Therefore, these heterocellular rays are divided into heterocellular irregular and heterocellular regular (Table 2).

1995). Obtained data showed that the radial parenchyma of Sievers apple is predominantly presented by single-row homocellular palisade medullary rays. Double-row rays are characterized as heterocellular, the structure of which has recumbent cells, found only in the middle part. The presence of heterocellular rays of a regular type determines the possibility of forming meristematic tubercles of adventitious roots in green cuttings.

Using the available data, the plants with medullary rays, folded by recumbent cells, easily root with green cuttings. The plants with heterocellular irregular, upright or recumbent, but in the form of "bricking", have always a medium or hard rooting ability with green cuttings. It is possible to determine that the Sievers apple refers to the species of the difficult rooting ability with green cuttings.

Being based on the obtained findings of medullary rays in the cross section (double-row – 10.9%),

Raw of a ray	Direction of anatomical stem sections					
	Transverse		Longitudinal			
			Tangential		Radial	
	width	length	height	width	length	height
Single raw	35.4 ± 0.5	24.1 ± 0.4	45.5 ± 0.6	25.5 ± 0.3	45.5 ± 0.8	34.5 ± 0.6
Double raw	14.5 ± 0.3	20.1 ± 0.5	23.6 ± 0.5	21.1 ± 0.4	-	-

Table 2. The size of cells of medullary rays in the wood of Sievers apple stem, in microns.

To determine the ratio of magnitudes of radial cells (width, height, length), width was taken as 1 and length and width are calculated to its value. So, the ratio of magnitudes of radial cells of Sievers apple medullary rays was 1 : 1.3 : 1.2. This ratio allows to make a conclusion about the predominance of medullary rays of square cells in the structure.

Discussion

The position about the existence of dependence in the root-formation ability of green cuttings from the structure of stem radial parenchyma allows using the testing of medullary rays as a preliminary diagnostic tool, aimed at searching new plants, suitable for green grafting (Orlov and Bakhtaulova

in the tangential section (double-row – 19.2%), we can assume that the potential root-forming ability of the Sievers apple tree with green cuttings will be no more than 15%. Taking into account that in the addition of double-row rays, recumbent cells are found in the middle part only in certain rays, and the root-formation ability of green cuttings will be significantly lower than their number. The obtained results are confirmed by the data, whereby the rooting of Sievers apple green cuttings was 12%.

Conclusions

So, the main diagnostic parameters, pointing to the difficult rooting of Sievers apple, are the ex-

istence of annual shoots in the wood during storing the green cuttings of homocellular rays from stagnant cells and heterocellular medullary rays.

Knowing the anatomical structure of Sievers apple radial parenchyma, the annual shoot stem can serve as a theoretical basis for improving technological methods of green grafting.

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