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STUDY OF ANATOMICAL STRUCTURE PECULIARITIES OF ANNUAL SHOOTS OF SIEVERS APPLE (*MÁLUS SIEVÉRSII*) AND APORT DOMESTIC APPLE (*MALUS DOMESTICA*) CULTIVARS

Abstract. Formation of Zhongar-Alatau State National Natural Park has a universal value in conservation of biodiversity and renewal of natural mountain landscapes with special genetic, ecological, aesthetic and historical significance. It also provides the possibility to conduct research and various types of recreational work as well as implementation of educational work. The main purpose of the park is to preserve the gene pool of wild fruit forests of global importance.

There are currently urgent issues in the field of conservation and development of genetic resources in Zhongar-Alatau SNNP in the Republic of Kazakhstan. Threat of plant diversity extinction in forests has dramatically increased because of rapid development of scientific and technological progress. In this regard, we sought new opportunities for improving the productivity of genetic reserves and preserving the biological diversity of Sievers apple wild fruit plantations.

The goal of the study was to examine and compare anatomical structure of Sievers apple and Aport domestic apple annual shoots, grown in the conditions of Zhongar Alatau highland. When conducting research, we used the following methods for microscopic studies of botanical objects: making micro-specimens, staining, defining the biometric parameters of cells, statistical data processing, anatomical description, comparison and analysis of obtained results.

Key words: Malus sieversii, Aport apple, annual shoots, cuttings.

Introduction. Many scientists argue that the main geographical centre of origin for cultivated apple tree is extensive areas of the largest mountain systems in Asia-Kopetdag, Alai range, Zailiysky and Dzhungarian Alatau, Ferghana and Zeravshan ranges, Talas Alatau, Tien Shan. This geographic region concentrates the main species diversity of wild apple trees of more ancient origin than other types of apple trees known to science (*Malus* genus). The Russian botanist Johann Sievers first described wild apple trees of Kazakhstan. Sievers apple (latin *Malus Sieversii*) is widespread in the foothills of Central Asia and Kazakhstan. According to DNA research, it is a true ancestor of many modern varieties of cultivated apple trees. On the territory of Kazakhstan, about 75% of Sievers apple groves are concentrated mainly in Zailiysky and Dzhungarian Alatau [1].

Domestic apple (*Malus domestica*) does not grow in the wild form, it is an artificially created species. In 2010, a group of scientists from different countries deciphered the complete genome of domestic apple (Golden Delicious variety). It contains about 57 thousands of genes [2-6]. According to DNA analysis, we also defined that known 2500 varieties of domestic apple are derived from Sievers apple tree [7-15]. One of the famous varieties of domestic apple is Aport (first mention in 1175 monastic records). It was brought to Kazakhstan (to the city of Verny, now Almaty) by the migrant Yegor Redko in 1865. He inculcated a few cuttings of Aport on the Sievers apple tree and grew large, tasty, beautiful fruits weighing up to 250 grams [16-19]. Many clones were derived from Aport apple in various zones of the country, some of which received varietal and other local names as a result of selection within centuries.

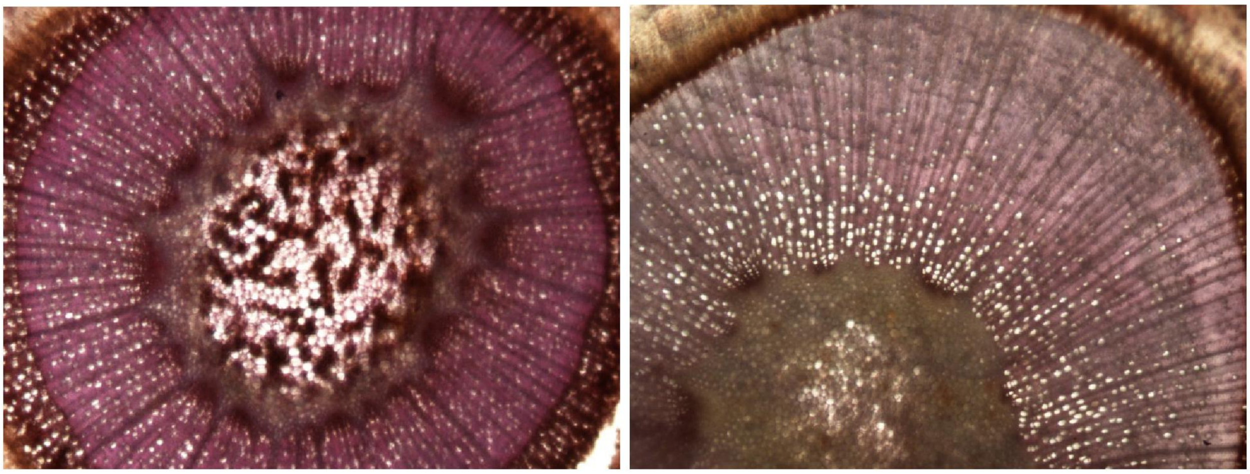
Considering the fact that Sievers Apple played a large role as rootstock in formation and origin for Aport variety, it is possible that anatomical characteristics of annual shoot structure of these plants have the same structural characteristics acquired as a result of selection process. If study of anatomical structure peculiarities of Sievers apple and Aport domestic apple annual shoots allows us to find identity in stem anatomy, knowing the ray parenchyma structure will provide practical application of vegetative reproduction for these species. Besides, this will serve as the basis for creating technology of softwood cutting and confirm the origin of Sievers Apple.

The goal of this study is to study and compare anatomical structure of Sievers apple and Aport domestic apple annual shoots, grown in the conditions of Dzhungarian Alatau highland.

Methods: to conduct research we used methods of microscopic studies of botanical objects: preparation of micro-specimens, staining, determination of cell biometric parameters, statistical data processing, anatomical description, comparison and analysis of obtained results.

Results and discussion. An apple tree shoot formed from buds in the process of spring growth is covered with epidermis. On the cross-section we can see the pith with adjoining primary xylem and early-emerging continuous layers of secondary xylem, cambium, phloem and primary cortex. However, cork cambium - phellogen - is laid under epidermis in summer, and periderm is formed. Lenticels form under certain stomata. Epidermis cells die after periderm formation at the end of autumn.

When examining the apple stem, we can clearly distinguish between the pith and sapwood with the naked eye on the cross-section (figure 1).



A B

Figure 1 – Cross-section: A – Sievers apple stem, B – Aport domestic apple stem (x40)

In the pith, there is a xylem, consisting of vessels of different sizes. Meanwhile, vessel composition in the xylem has unsystematic character. Transition from early wood of annual layer to late wood happens gradually. Apple annual layer is winding slightly and uneven by width. Annual layer either narrows or widens, forming a ring of wrong form.

We prepared objects for research at the time of intensive stem growth in thickness due to lateral meristem. Therefore, cambial zone with young undifferentiated cells occupies 9.4-13.0% on the cross-section of apples (table 1).

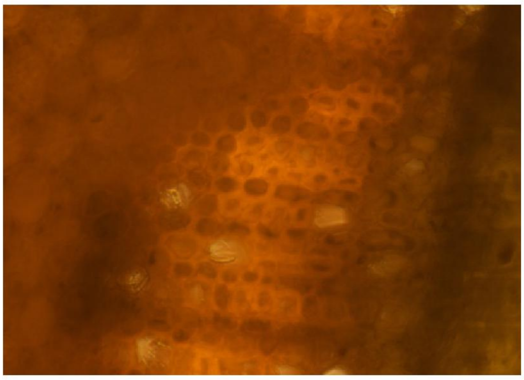
Table 1 – Measurements of plant tissues and complexes and their correlation on the cross-section (x40)

Type	Pith	Xylem	Cambium	Bark	Cork	Total length
Sievers Apple	217	141.5	83	144.5	43.5	629.5
	34.5%	22.5%	13.0%	23.0%	7.0%	100.0%
Aport Apple	204	322	73	141	34	774
	26.4%	41.6%	9.4%	18.2%	4.4%	100.0%

For study, we selected stems of annual shoots of nearly same diameter. Comparison of absolute and relative indicators of plant tissues and complexes contributed to identification of similarities and differences in the anatomical stem structure of studied apple tree species. In general, it has been established that indicators of relative values of main tissue complexes are slightly different in both types of apple. There is a small difference in the pith diameter and conductive components. Domestic apple of Aport variety forms a thicker layer of xylem (41.6%) than phloem layer (20.3%), while Sievers apple - pith (34.5%) and equally xylem (22.5%) and phloem (23.0%) in the result of cambium activity.

Sievers apple is characterized by more marked anatomical unevenness of perimedullary zone, associated with founding and formation of annual cambium ring. This unevenness varies in Sievers apple in the range of 30-100 microns, Aport apple variety is less marked and amounts to 20-40 microns.

The studied species of apple trees have very narrow medullary rays, which are almost invisible to the naked eye. Ray parenchyma of annual shoot consists of primary medullary rays. Primary medullary rays, composed of cells of procambium and cambium origin, connect the pith of stem with its bark (figure 2).



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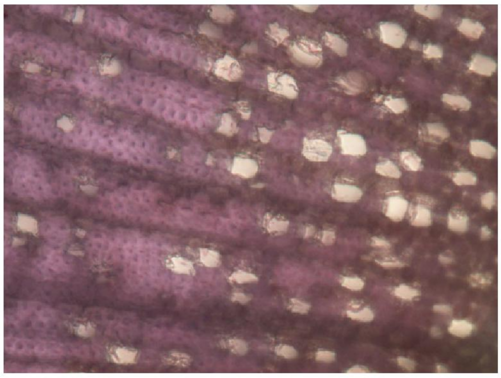


B

Figure 2 – Structure of annual shoot on the cross-section:
A – Sievers apple (x400); B – domestic apple of Aport variety on the cross-section (x400)

Anatomical specimens show that while a primary medullary ray develops, its histological structure changes in composition of ray cells. In the field of procambium formation, medullary rays are composed of square ray cells mainly. Then shape of cells often changes on the ray axis, becoming elongated along the ray axis (lying cells).

Single-row rays predominate in the structure of ray parenchyma both in Sievers apple and Aport domestic apple. The number of double-row rays is much less (8-12.9% on the cross-section, 21.1-23.8% on the tangential section).

By histological structure, there are homocellular palisade medullary rays, composed of standing cells, and heterocellular medullary rays, composed of both standing and square cells in the tangential section. On the cross-section, these square cells can be lying, especially near the cambial zone. In the case of Aport variety, heterocellular double-row rays have lying cells in the middle part of rays.

Table 2 – The number of medullary rays in the wood of the stem of the Apple Sivers on the anatomical cross-section (x40)

Indicators of the radial parenchyma	Sievers apple		Aport variety	
	PCs.	%	PCs.	%
Total number of medullary rays on the cut	116.6±3.5	100.0	150.0±2.4	100.0
Number of primary rays including:	116.6±3.5	100.0	150.0±2.4	100.0
single-row	101.2±2.1	87.1	138.1±1.9	92.0
double-row	13.6±1.4	12.9	11.9±1.3	8.0

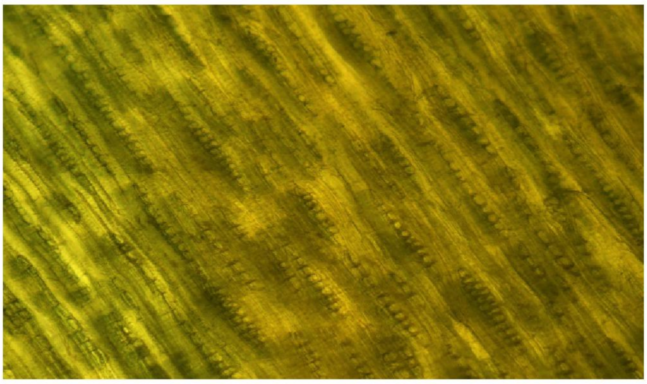
Single- and double-row rays on the longitudinal tangential sections of Sievers apple stem wood also represent ray parenchyma. Single-row medullary rays, layer of which is equal to 5-25 ray cells or 120-420 microns, dominate the composition of ray parenchyma by rows. Single-row rays are composed of cells of two morphological types: square (isodiametric) and standing, i.e. elongated along the axis of stem. Standing cells are located in single-row rays as single boundary or form the ending of 2-5 ray cells. Square cells also take place the middle part of single-row rays. Thus, single-row medullary rays according to the type of cell composition are heterocellular.

Double-row rays on the tangential sections are 21.1% of the total number of rays visible in the microscope (table 3). Average layer of double-row rays is 10-20 ray cells or 150-200 microns. According to histological structure, double-row rays are composed of only square ray cells (homocellular ray type) or square and standing, where square cells are dominant, i.e. ray type - heterocellular.

Table 3 – Number of medullary rays in the tangential section of Sievers Apple stem wood (in the of view of microscope x100)

Indicators	Sievers Apple		Aport Apple	
	piece	%	piece	%
Total medullary rays	19.6±2.05	100.0	21.1±2.0	100.0
Including single-row	15.3±1.2	78.9	16.1±1.3	76.2
double-row	4.3±0.8	21.1	5.0±0.9	23.8

Standing cells with height 1.5 times greater than width are mostly located in single boundary rays, which can be characterized as heterocellular rays with regular type of ray cell composition (figure 3).



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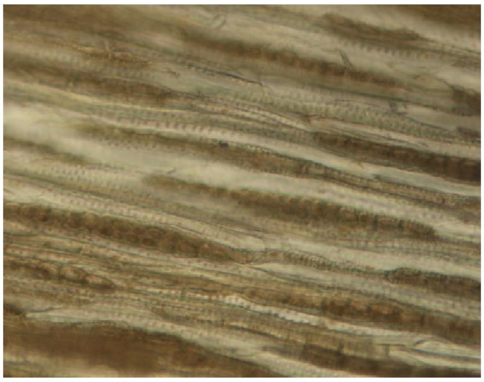


B

Figure 3 – Longitudinal tangential section: A – Sievers apple stem (x100); B – Aport domestic apple stem (x100)

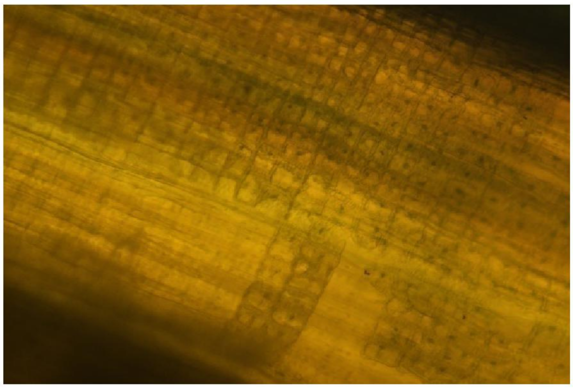
Single- and double-row rays on the longitudinal tangential sections of Aport apple stem wood also represent ray parenchyma. Single-row medullary rays, layer of which is equal to 15-30 ray cells or 420-611microns, dominate the composition of ray parenchyma by rows. Single-row rays are composed of cells of two morphological types: square (isodiametric) and standing, i.e. elongated along the axis of stem. Single-row rays are of two types. The predominant first type of rays consists of standing cells only. The second type has standing cells. Standing cells are located in single-row rays as single boundary or form the ending of 2-5 ray cells. Square cells also take place the middle part of single-row rays. Thus, single-row medullary rays according to the type of cell composition are homocellular and heterocellular.

Double-row rays on the tangential sections are 23.8% of the total number of rays visible in the microscope (table 3). Average layer of double-row rays is 18-26 ray cells or 190-260 microns. According to histological structure, double-row rays are composed of only square ray cells (homocellular ray type) or square and standing, which are dominated by square cells, i.e. ray type - heterocellular.

The study of structure of medullary rays in the longitudinal radial sections allows us to determine morphological type of ray cells accurately. There are two main types of ray parenchyma cells: cells, the long axis of which is oriented radially (lying ray cells), and cells, in which this axis is oriented vertically (standing ray cells). Cells, which look isodiametric on the radial sections of wood, are called square ray cells and represent modification of standing type cells. Main defining parameters of ray parenchyma in the radial section are layer (height) and length of medullary rays and, respectively, height and length of ray cells. We can see characteristic changes in their histological structure in the process of ray growth of wood on radial sections of rays.

Areas of primary medullary rays, located near perimedullar zone, are composed of square and standing cells of procambial origin on the longitudinal radial section of Sievers apple stem wood. As cambium grows, wood structure of ray parenchyma changes, and lying ray cells appear in its structure.

These areas along the ray length form in the period of harvesting and planting to root softwood cuttings, so when we characterize ray parenchyma, first of all, we pay attention to this part of medullary rays (figure 4). Standing cells, whose height is only 1.5 times greater than their width, are located in such rays mostly as single boundary, which allows us to characterize these rays as heterocellular (figure 4).



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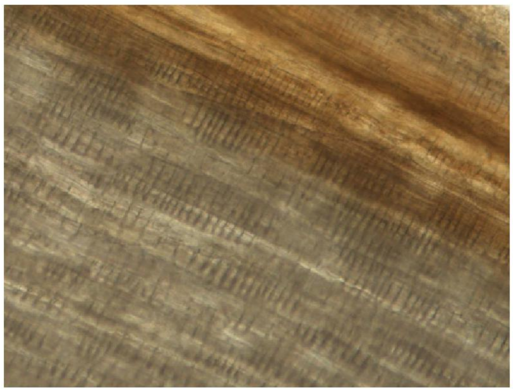

Б

Figure 4 – Longitudinal radial section of Sievers apple stem wood (x200) and Aport domestic apple stem wood (x200)

Areas of primary medullary rays, located near the perimedullar zone, are composed of square and standing cells of procambial origin on the longitudinal radial section of Aport apple stem wood. As cambium grows, wood structure of ray parenchyma changes, and lying ray cells appear in its structure. Lying ray cells are located in even and parallel rows.

In order to determine ratios of ray cell values (width, height, length), we took width as 1, and length and height are calculated in proportion to its value.

Thus, value ratio of medium ray cell in Sievers apple medullary rays was 1:1.3:1.1 and in Aport apple - 1:1.4:1.2. This ratio allows us to make a conclusion about predominance of square cells in the structure of medullary rays for both types of apple trees.

Parameters of width, length and height of "mid" cell of Sievers Apple ray parenchyma have the ratio equal to 1:1.3:1.2, which indicates that medullary rays are mostly composed of square ray cells (table 5).

Table 5 – Dimensions of apple ray cells (meas. 400)

Type	Cross-section		Longitudinal section			
			Tangential		Radial	
	length	width	height	width	length	height
Sievers apple	58.1	47.1	56.1	55.8	75.8	54.2
Aport apple	77.3	53.6	70	49.1	64.7	53.1

The established connection between peculiarities of composition of stem medullary rays and formation of adventitious roots opens real possibilities to forecast rooting ability of plants when selecting them for softwood cutting [20]. Therefore, study of anatomical features of structure of stem annual shoot and ray parenchyma of Sievers apple and Aport domestic apple allows us to compare and determine the level of similarities and differences, and to determine potential of plant propagation by softwood cutting.

Based on the results obtained in number of double-row medullary rays on the cross-section and tangential sections, we can assume that the potential rooting ability of Sievers Apple softwood cuttings will not be more than 17% on average, for Aport domestic apple - not more than 16%. Considering that composition of double-row rays include lying cells found in the middle part only of certain rays, and rooting ability of softwood cuttings will be significantly lower than their number. The results obtained are confirmed by literary data, according to which the rooting of Sievers apple softwood cuttings was 12% [21-22].

Conclusions. On the basis of obtained data on anatomy of annual shoots and literature data analysis of studied apple varieties, we can make the following conclusions:

1. The share of pith (34.5%) is dominant in the anatomical structure of Sievers Apple annual shoot stem, xylem and phloem are in equal proportions (22.5% and 23.0% respectively). Medullary rays are very narrow and divided into single- and double-row primary rays. Single-row rays dominate in the ray parenchyma of Sievers Apple wood (87.1%), whereas double-row rays make up only 12.9% on the cross anatomical section.

Single- and double-row rays on longitudinal tangential sections of Sievers apple stem wood also represent ray parenchyma. Single-row medullary rays dominate (78.9%), double-row rays are up to 21.1%. Square ray cells dominate on the radial section of medullary rays.

2. The share of xylem (41.6%) is dominant in Aport domestic apple while the share of pith (26.4%) and phloem (18.2%) is significantly less. Medullary rays are also very narrow and divided into single- and double-row primary rays. Single-row rays dominate in the ray parenchyma of Aport Apple wood (92.0%), whereas double-row rays make up only 8.0% on the cross anatomical section.

Single- and double-row rays on the longitudinal tangential sections of Sievers apple stem wood also represent ray parenchyma. Single-row medullary rays dominate (76.2%), double-row rays are up to 23.8%. Square ray cells dominate on the radial section of medullary rays, but there are areas of lying cells, arranged in parallel rows.

3. Comparative analysis of anatomical structure of stem in Sievers apple and Aport domestic apple established that studied plants differ in intensity of forming secondary cambium tissue. Conductive complexes (phloem and xylem) develop evenly in Sievers apple, while Aport apple variety is dominated by development of xylem. Single-row rays mainly represent ray parenchyma of both apple types.

Based on the statement on existing dependence of rooting abilities of softwood cuttings from the structure of stem ray parenchyma, we can assume that investigated species belong to the group of hard-rooted plants by softwood cuttings. Thus, main diagnostic parameters, indicating hard rooting of Sievers apple and Aport domestic apple, are a small number of double-row rays and composition of medullary rays of square cells mostly. However, presence of heterocellular medullary rays with lying cells in the middle part in the wood of annual shoots during procurement of softwood cuttings may increase rooting abiltity up to 20%.

Knowledge of anatomical structure of medullary rays in the studied plants may be used as the theoretical basis for Sievers apple and Aport domestic apple vegetative reproduction by softwood cuttings and further improvement of technological process of softwood cutting by using different terms of cutting harvesting and growth regulators.

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СИВЕРС (MÁLUS SIEVÉRSII) АЛМАСЫНЫҢ ЖӘНЕ АПОРТ СОРТЫ МӘДЕНИ АЛМАСЫНЫҢ (MALUS DOMESTICA) ЖЫЛДЫҚ ӨРКЕНДЕРІНІҢ АНАТОМИЯЛЫҚ ҚҰРЫЛЫСЫН ЗЕРТТЕУ

Аннотация. Жоңғар-Алатау мемлекеттік ұлттық табиғи паркін құру әмбебап маңызға ие – ерекше генетикалық, экологиялық эстетикалық және тарихи құндылыққа ие табиғи тау ланшафтарының биоалуантүрлілігін сақтау және жаңарту, сонымен қатар ғылыми зерттеулер және түрлі рекреациялық жұмыстар жүргізу, сонымен қатар білім арттыруды жүзеге асыруға мүмкіндік береді. Парктің негізгі мақсаты өзекті маңызға ие жабайы жемісті орман генофондын сақтау болып табылады.

Қазіргі уақытта Қазақстан Республикасында Жоңғар-Алатау МҰТП генетикалық ресурстарын сақтау және дамыту саласындағы өзекті мәселелер байқалады. Ғылыми-техникалық прогрестің қарқынды дамуына байланысты орман өсімдіктерінің алуантүрлілігінің жойылу қауіпі кенет өсті. Осыған байланысты, генетикалық резервтердің өнімділігін арттыру және Сиверс алмасының жабайы жемісті ағаштарының биологиялық алуантүрлілігін сақтаудың барлық жаңа мүмкіндіктері іздестілуде.

Зерттеу мақсаты: Жоңғар-Алатау биік таулы жағдайларында өсетін Сиверс алмасының және апорт сорты мәдени алмасының жылдық өркендерінің анатомиялық құрылысын зерттеу және салыстыру.

Зерттеу жүргізу барысында ботаникалық нысандарды микроскопиялық зерттеу әдістері, микропрепараттар дайындау, бояу, клеткалардың биометриялық көрсеткіштерін анықтау, мәндерді статистикалық өңдеу, анатомиялық сипаттау, алынған нәтижелерді салыстыру және сараптау әдістері қолданылды.

Түйін сөздер: Сиверс алмасы, апорт, жылдық өркендер, қалемшелер.

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ИЗУЧЕНИЕ ОСОБЕННОСТЕЙ АНАТОМИЧЕСКОГО СТРОЕНИЯ ГОДИЧНЫХ ПОБЕГОВ ЯБЛОНИ СИВЕРСА (MÁLUS SIEVÉRSII) И ЯБЛОНИ ДОМАШНЕЙ (MALUS DOMESTICA) СОРТА АПОРТ

Аннотация. Формирование Жонгар-Алатауского государственного национального природного парка обладает универсальным значением – это сохранения биоразнообразия и возобновление естественных горных ландшафтов, обладающих особенной генетической, экологической, эстетической и исторической ценностью; кроме того, обеспечивает возможность проведения научных исследований и различных видов рекреационных работ, а также реализацию просветительской работы. Основной же целью парка считается сохранение генофонда дикоплодовых лесов имеющих глобальное значение.

В настоящее время в Республике Казахстан наблюдаются острые проблемы в области сохранения и развития генетических ресурсов в Жонгар-Алатауском ГНПП. Из-за бурного развития научно-технического прогресса резко возросла угроза исчезновения растительного разнообразия лесов. В связи с этим изыскиваются все новые возможности для повышения продуктивности генетических резерватов и сохранения биологического разнообразия дикоплодовых насаждений яблони Сиверса.

Целью исследования было изучить и сравнить анатомическое строение годичных побегов яблони Сиверса и яблони домашней сорта Апорт, произрастающих в условиях высокогорья Джунгарского Алатау. При проведении исследований использованы методы проведения микроскопического изучения ботанических объектов: изготовление микропрепаратов, окрашивание, определение биометрических показателей клеток, статистическая обработка данных, анатомическое описание, сравнение и анализ полученных результатов.

Ключевые слова: яблоня Сиверса, апорт, годичные побеги, черенки.

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