Technology of malus sieversii softwood cutting

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Abstract. Sievers Apple tree is listed in the Red book of Kazakhstan and is protected by The International Union for Conservation Nature. The total area of wild forests in the territory of Zhongar – Alatau Natural Park is 8,573 thousand ha (1.05% of the park area). In order to preserve Apple forests, studies on Malus sieversiisoftwood cuttings have been carried out, taking into account their genetic purity. Genetic analysis of Malus sieversii diversity, using DNA markers, showed that only inaccessible and most remote reserves (Kokzhota 1, 2, Chernova river) are considered the best crops on the territory of natural Park, that are unaffected by the processes of genetic erosion and do not contain DNA Malus domestica. They served as mother plants for harvesting green cuttings for reproduction by green cuttings technology. On the basis of the obtained data, recommendations for the reproduction of Malus sieversii in the territory of the Zhongar-Alatau natural Park to strengthen the weakened populations of the species are developed. It is recommended to create temporary collections by green cuttings and transplanting into natural habitats.

1. Introduction
When propagating plants in a massive, industrial scale for recovery of degraded Apple forests of Kazakhstan, it is primarily necessary to study biological characteristics of plant. The gene pool of apple forest is of great value for planting as a material for restoring cultural varieties. Conservation of economically valuable species and varietal characteristics in perennials in full is possible in the process of growing planting material using vegetative reproduction. Softwood cutting is of considerable interest among vegetative reproduction methods. This is largely due to the fact that in this case it is possible to obtain genetically homogeneous vegetatively propagated plants on their own roots. Softwood cuttings with leaves have high meristematic activity; they are more responsive on impact to promote regeneration of adventitious roots. This gives the possibility to propagate so many hard-rooted species and varieties of plants by softwood cuttings [1].

Restoration of Sievers Apple by creating forest crops, when planting material is grown from seed, leads to spread of genetic erosion in areas of genetic reserves. When we use reproduction by growing roots, we observed damage to root system of wild apple. Even sanitary thinning is forbidden, excavation of root system is invalid in apple tree forests. In the greenhouse forestry “Orman” of Ile-Alatau National Natural Park survival rate of Sievers Apple root cuttings is only 2 percent.

Thus, analysis of special literature showed that currently rooting technology for wild Apple softwood cuttings is, unfortunately, poorly researched.

Softwood cutting as one of the effective propagation methods of fruit plants is based on the ability of softwood cuttings to form adventitious roots. Studies of rooting ability in vegetatively propagated plants were carried out by many researchers, which revealed different rooting ability of plants by
softwood cuttings. During research, attempts were made to explain different rooting ability of plants based on their phylogeny, life forms, genealogy links, taxonomies, etc. [2-3].

There are species and varieties with high and low rooting ability among all life forms, including trees. Therefore, it is impossible to define rooting degree of softwood cuttings for each specific plant, having based on life form or position in the taxonomic system.

Adventitious roots of trees and shrubs are formed by softwood cutting stem endogenously. According to most researchers, formation of root primordia in dicotyledonous trees and shrubs occurs in the bark of stem opposite the multi-row wide of medullary rays [4-6]. Taking onto account specified topography of shallow root sprouts, a number of authors associate degree of shoot stem ray parenchymatization with easy rooting of softwood cuttings. Thus, it is noted that forming adventitious roots depends on morphological structure of medullary rays. In plants, having homogeneous rays consisting of lying cells, formation of primary tubercle of adventitious root is faster than in plants with palisade and heterogeneous medullary rays, the structure of which is dominated by standing cells [7].

The established connection between features of stem medullary ray composition and formation of adventitious roots opens the real possibility of forecasting rooting ability of plants in their selection for softwood cutting [8]. Therefore, study of stem ray parenchyma of Sievers Apple will determine the potential of plant propagation by softwood cuttings and receive real data on degree of rooting ability of softwood cuttings.

2. Methods of softwood cutting

For vegetative propagation of Sievers Apple softwood cuttings, research team used annual shoots with five to seven leaves, harvested in the genetic reserves of ZhASNNP Kokzhota 1,2, Steep Tract, Chernova river. Anatomical structure of ray parenchyma of year shoot is characterized by hard rooting ability (10%). Different concentrations and combinations of physiologically active substances allowed us to determine the best option and develop the method of softwood cutting for Apple Sievers. Each variant used 15 softwood cuttings with 2-3 healthy buds and leaves. Harvesting of cuttings was conducted in the morning in the first week of June. Cuttings of annual shoots were sorted, bundled for easy handling in solutions of physiologically active substances. Stimulation of cuttings was carried out according to the scheme of experience, which includes 9 options, including two control. For this purpose we prepared in advance mother solutions of individual components of medium (table 1).

Table 1. Stock and working solutions of specimens used for softwood cutting treatment by the scheme of technological process of softwood cutting.

| No | Components of stock solutions | Concentration of mother solutions | The amount of stock solution to prepare 1 liter of working solution |
|----|--------------------------------|---------------------------------|---------------------------------------------------------------|
| 1  | 50 % alcohol solution          | 90 % alcohol dissolves in 1000 ml with bidistilled water | 1000 ml                                                   |
| 2  | Rutin, 25 mg/l                 | 0.025 g of drug dissolves in 100 ml with bidistilled water | 100 ml                                                   |
| 3  | HA, 1.5 g/100 ml               | In 100 ml 50 % alcohol solution dissolving 1.5 grams of drug | 100 ml                                                   |
| 4  | HA, 2 g/100 mg                 | 2.0 grams of drug dissolves in 100 ml 50 % alcohol solution | 100 ml                                                   |
| 5  | IBA, 25 mg/l                   | 0.025 grams of drug dissolves in 100 ml 50 % alcohol solution dissolve | 100 ml                                                   |
| 6  | IBA, 50 mg/l                   | 0.05 gram of drug dissolves in 100 ml 50 % alcohol solution | 100 ml                                                   |
| 7  | NAA, 25 mg/l                   | 0.025 grams of drug dissolves in 100 ml 50 % alcohol solution | 100 ml                                                   |
| 8  | NAA, 50 mg/l                   | 0.05 gram of drug dissolves in 100 ml 50 % alcohol solution | 100 ml                                                   |
| 9  | NAA, 100 mg/l                  | 0.1 gram of drug dissolves in 100 ml 50 % alcohol solution dissolve | 100 ml                                                   |
All prepared mother solutions are contained in glass containers and stored at +2°-4° temperature for 30 days. From mother solutions we prepared solutions based on 1.0 liter of distilled water. The solutions were poured into glasses with a wide mouth for dipping the cuttings and to stimulate rooting abilities. After processing of cuttings, they were removed from the solutions and covered with a damp cloth.

Treatment of alcoholic solution was carried out by one of growth regulators (drug). For preparation of alcoholic solution we take linkage from calculation of 1-30 mg and dissolved in a small volume of 96% ethyl alcohol, then add water to volume to obtain a 50% alcohol solution.

In option No. 1-2 cuttings were treated with 1.5% to 2% solution of IBA. In option No. 3-4 cuttings were treated in an alcohol solution of indole-3-butyric acid. In the No. 5-6 cuttings were processed in ethanol NAA solutions. Some phenolic compounds are cofactors in rooting. Their use in conjunction with auxin increases rooting ability of cuttings, especially hard-rooted. Strengthening stimulating action of auxin on cuttings of some fruit crops (mainly pome), is manifested when you add a routine. Based on these works, in our studies, relevant options were included at 25 mg/l routine and have introduced the second version of control.

As substrate for rooting of softwood cuttings used universal soil with trace elements with high content of organic substances. Soil filled volume of 0.4 m³ containers. A smooth flat plate to straighten the surface, then wooden tranbon compacted so that the thickness of the layer was 12-14 cm Cuttings are planted according to the scheme 5×5×10 cm and a planting depth of 1.5-2.0 cm, giving them a slight slope. The humidity of the rooting environment was maintained at 70% -80%, the dispersion with water spray. Temperature of root formation was in the range of +22°-25°. The technological sequence, given the parameters and modes of rooting, has a positive effect on the growth and formation of adventitious roots of softwood cuttings of Sievers Apple.

In the procurement of the annual shoots was marked damage of plants by pests, diseases and the presence of fire blight on Apple-tree leaves. Therefore, harvested annual shoots with damaged fallopian plants were treated with a fungicide Ditan M45, which significantly influenced the process softwood cuttings reduced rooting rate, the quality and the yield of standard seedlings. The effectiveness of softwood cutting largely depends on the timing of grafting that affect the rooting of cuttings. Therefore, harvesting of cuttings were conducted in two phases: during the period of cessation of growth of the leaves (27 June), during the period of cessation of growth of the annual shoot (July 11).

Rooted softwood cuttings from the climate chamber were planted for rearing in the experimental area. However, not all rooted cuttings successfully undergo a period of acclimatization.

Terms of harvesting cuttings also affect their rooting ability. In accordance with developed scheme of technological process of softwood cutting, experiments on rooting of softwood cuttings were carried out in three different timeframes, depending on the status of annual shoots: cessation of annual shoot growing, beginning of lignification of lower part in an annual shoot, lignification of up to half of an annual shoot [9].

3. Research results
Based on the results obtained by the number of double row medullary rays in cross cut (double row 10.9 per cent) and tangential sections (double row – 19.2%), potential rooting ability of Sievers Apple green cuttings will not be more than 15%. In this view, that in addition double row rays lying cells are found in the middle part only of certain rays, and rooting ability of green cuttings will be significantly lower than their number. The obtained results confirmed literature data, in accordance with which the rooting of green cuttings of Sievers apple destabilise 10%.

Using data from the genetic analysis of the diversity of the wild apple tree with the help of DNA markers, annual shoots were prepared for softwood cuttings from mother plants of the Sievers apple tree located in the genetic reserves "Kokzhota 1,2", "Chernova River" and "Steep Tract" ZhASNNP within different times.
In accordance with the developed scheme of softwood propagation process, experiments were carried out to root green cuttings in three different periods, depending on the condition of annual shoots: cessation of annual shoot growth, beginning of lignification of lower part of annual shoot, and lignification to half a year's shoot (table 2-4).

**Table 2.** Results of rooting of green cuttings of Sievers apple harvested during the period of cessation of growth of annual shoot (June 16, 2017).

| No | According to the variants of the experiment | Number of planted cuttings (pcs.) | Number of entrenched cuttings (pcs) | %% to the number of planted cuttings | Number of cuttings on growing (pcs) |
|----|--------------------------------------------|----------------------------------|------------------------------------|-----------------------------------|-----------------------------------|
| 1  | St - control (dist. water)                 | 15                               | 1                                  | 6.7                               | 0                                 |
| 2  | St1 - control- rutinpermeability - 25 mg/l | 15                               | 2                                  | 13.3                              | 0                                 |
| 3  | I - Heteroauxin 1.5% medium (HA)           | 15                               | 2                                  | 13.3                              | 0                                 |
| 4  | II - Heteroauxin 2% medium(HA)             | 15                               | 4                                  | 26.7                              | 2                                 |
| 5  | III - Indole-3-butyric acid (IBA) 25 mg/l  | 15                               | 4                                  | 26.7                              | 2                                 |
| 6  | IV - Indole-3-butyric acid (IBA) 50 mg/l   | 15                               | 5                                  | 33.3                              | 3                                 |
| 7  | V –g - naphthylactic acid (NAA) 25mg/l     | 15                               | 4                                  | 26.7                              | 3                                 |
| 8  | VI –g-naphthylactic acid (NAA) 50mg/l      | 15                               | 3                                  | 20.0                              | 1                                 |
| 9  | VII-g - naphthylactic acid (NAA) 100mg/l   | 15                               | 3                                  | 20.0                              | 1                                 |

**Table 3.** Results of rooting of green cuttings of Sievers apple-tree harvested at the beginning of the lignification of the lower part of the annual shoot (June 29, 2017).

| No | According to the variants of the experiment | Number of planted cuttings (pcs.) | Number of entrenched cuttings (pcs) | %% to the number of planted cuttings | Number of cuttings on growing (pcs) |
|----|--------------------------------------------|----------------------------------|------------------------------------|-----------------------------------|-----------------------------------|
| 1  | St - control (dist. water)                 | 15                               | 1                                  | 6.7                               | 0                                 |
| 2  | St1 - control- rutinpermeability - 25 mg/l | 15                               | 1                                  | 6.7                               | 0                                 |
| 3  | I - Heteroauxin 1.5% medium (HA)           | 15                               | 2                                  | 13.3                              | 1                                 |
| 4  | II - Heteroauxin 2% medium(HA)             | 15                               | 4                                  | 26.7                              | 2                                 |
| 5  | III - Indole-3-butyric acid (IBA) 25 mg/l  | 15                               | 4                                  | 26.7                              | 2                                 |
| 6  | IV - Indole-3-butyric acid (IBA) 50 mg/l   | 15                               | 6                                  | 40.0                              | 4                                 |
| 7  | V –g - naphthylactic acid (NAA) 25mg/l     | 15                               | 4                                  | 26.7                              | 3                                 |
| 8  | VI –g-naphthylactic acid (NAA) 50mg/l      | 15                               | 5                                  | 33.3                              | 2                                 |
| 9  | VII-g - naphthylactic acid (NAA) 100mg/l   | 15                               | 3                                  | 20.0                              | 1                                 |

**Table 4.** Results of rooting of Sievers apple softwood cuttings harvested during lignification period to half the annual shoot (July 13, 2017).

| No | According to the variants of the experiment | Number of planted cuttings (pcs.) | Number of entrenched cuttings (pcs) | %% to the number of planted cuttings | Number of cuttings on growing (pcs) |
|----|--------------------------------------------|----------------------------------|------------------------------------|-----------------------------------|-----------------------------------|
| 1  | St - control (dist. water)                 | 15                               | 1                                  | 6.7                               | 0                                 |
| 2  | St1 - control- rutinpermeability - 25 mg/l | 15                               | 2                                  | 16.3                              | 0                                 |
| 3  | I - Heteroauxin 1.5% medium (HA)           | 15                               | 3                                  | 20.0                              | 2                                 |
| 4  | II - Heteroauxin 2% medium(HA)             | 15                               | 5                                  | 33.3                              | 3                                 |
| 5  | III - Indole-3-butyric acid (IBA) 25 mg/l  | 15                               | 5                                  | 33.3                              | 3                                 |
| 6  | IV - Indole-3-butyric acid (IBA) 50 mg/l   | 15                               | 7                                  | 46.7                              | 5                                 |
| 7  | V –g - naphthylactic acid (NAA) 25mg/l     | 15                               | 5                                  | 33.3                              | 3                                 |
| 8  | VI –g-naphthylactic acid (NAA) 50mg/l      | 15                               | 6                                  | 40.0                              | 4                                 |
| 9  | VII-g - naphthylactic acid (NAA) 100mg/l   | 15                               | 4                                  | 26.7                              | 2                                 |

According to the results of the experiment, the highest rooting capacity of green apple cuttings was observed in variant No. 6 when treated with indolyl-3-butyric acid (IAA) at a concentration of 50 mg /
1. When harvesting during the period of cessation of growth of the annual shoot, the highest result of rooting of Sievers apple softwood cuttings apples was treated with indolyl-3-butyric acid (IAA) at a concentration of 50 mg/l. When harvesting during the beginning of lignification of the lower part of the annual shoot, the high rooting of green cuttings of Sievers apple tree is also characteristic of the variant when treated with indolyl-3-butyric acid (IAA) at a concentration of 50 mg/l (40%) and a variant when treating naphthyloxy acid (NA) at a concentration of 50 mg/l (33.3%). period lignification to half a year also the higher rooting of green cuttings of Sievers apple-tree was observed in variants No.6 and 8.

On the basis of experimental data obtained, it is necessary to consider the variant with the use of biological stimulation of the root-forming ability of cuttings indolyl-3-butyric acid (IAA) at a concentration of 50 mg/l and the lignification prepared for a period of lignification to half a yearlong shoot in the optimal variant of the technological process of green cuttings of Sievers apples.

Rooted green cuttings from the climate chamber were planted for rearing in the experimental area. However, not all rooted cuttings successfully completed the period of acclimatization.

Given that cuttings harvested from young plants have a higher rooting ability and survival, it is necessary to rejuvenate old queen cells. Old-aged specimens of Sievers apple-tree are especially protected on the territory of genetic reserves, therefore, rejuvenating cuts are prohibited. At the same time, the best material for cuttings is shoots formed on last year's growth in the lower but well-illuminated part of the crown, which have large developed buds and show no signs of disease. Therefore, for carrying out green propagation, it is necessary to obtain a young healthy starting material, genetically identical to the old-age specimens of the Sievers apple tree. Such material can be obtained by microclonal propagation.

4. Discussion

The data obtained showed that the radial parenchyma of Sievers' apple tree is represented mainly by single-row homocellular palisade and heterocellular core rays. Double-row rays are characterized as heterocellular, in the structure of which the recumbent cells are found only in the middle part. The presence of a regular type among heterocellular rays determines the possibility of the formation of meristematic tubercles of accessory roots with green cuttings.

Applying the available data that plants with medullary rays put by lying cages take roots green shanks easily, a with irregular heterocellular, standing or lying, but in a form of a brick laying, always so-so or difficult, it is possible to define that Sievers apple-tree belongs to the look which is hardly implanted by green cuttings.

Being based on the received results on quantity double-row medullary rays on a cross cut (double-row 10,9%), on a tangential cut (double-row – 19,2%), it is possible to assume that potential the rooting ability of Sievers apple-tree at softwood cuttings will be not more than 15%. Considering that in addition of double-row rays lying cages meet in an average part only at separate rays, and rooting ability of green cuttings will be much below than their quantity. The received results are confirmed by literary data with which in compliance rooting of green cuttings of Sievers Apple is 10% [4].

So, will serve as the key diagnostic parameters indicating a difficult rooting of green cuttings of an apple-tree of Sievers existence in wood of year shoots during the period of preparation of green shanks homoallylic rays from standing cages and heterocerus irregular medullary rays.

The knowledge of the anatomical deformation of the sacrificial rays of the Sievers apple tree served as a theoretical basis for the development of technological receptions for greenery.

As a result of the experiments, the technology of green cuttings of the Sievers apple tree was developed with the use of biological stimulation of the root-forming ability of cuttings with various concentrations and the ratio of physiologically active substances of auxin and cytokinin origin, which promote active growth and development of accessory roots.

View of the anatomical structure of the radial parenchyma of the annual shoot is characterized by a difficult rooting (no more than 10%). Different concentrations and combinations of physiologically active substances: indole-acetic acid (IAA, heteroauxin), indolyl-3-butyric acid (IMC), g-
naphthylacetic acid (NAA) allowed to determine the optimal variant and to develop a method of green cuttings of apple trees.

In accordance with the developed scheme of the green propagation process, experiments were carried out to root green cuttings in three different periods, depending on the condition of annual shoots: cessation of annual shoot growth, beginning of lignification of the lower part of the annual shoot, lignification to half a yearlong shoot. The highest rooting was characteristic of cuttings harvested in the period of lignification to half a year's shoot.

5. Conclusion
On the basis of obtained data on anatomy of a year shoot within technology of softwood cutting and analysis of literature data of studied species of Sievers Apple we concluded:

As a result of experiments, research team members defined optimum technological process of Sievers Apple green cuttings, characterized by indole-3-butyric acid (IBA) at the concentration of 50 mg/l within different times of harvesting cuttings. Maximum percentage of rooting of Sievers Apple softwood cuttings amounted to 46.7%. The optimal variant of technological process of Sievers Apple softwood cuttings, we can to consider the option of using biological stimulation rooting ability of cuttings indole-3-butyric acid (IBA) at the concentration of 50 mg/l preparation during lignification to half a year shoot.

In 10-15 years it is possible to seed areas with Sievers Apple by creating plantations with "natural" genes. Seed plots of apple shall be composed of apple varieties-clones, derived from genetic material in natural populations of Kazakhstan. Artificial seed plots must be created on the territories of genetic reserves "Kokzhota 1,2", "Fir and Soldier cracks", "Chernova river." Formation of such artificial seed plots will be sufficient for conducting large-scale reforestation works in apple forests, and accelerated forest designing and creation of artificial seed plots of apple trees will accelerate mass recovery-fruit forests in Dzhongar Alatau.

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