

## ABSTRACT

**of the PhD thesis by Sagi Soltanbekov on the topic " The revival of the Aport apple based microcloning, selection of rootstocks, and assessment the resistance of the variety to the main diseases ", submitted for the degree of Doctor of Philosophy (PhD) in the specialty - 6D081100 - "Plant Protection and Quarantine"**

### **Relevance of the research topic**

To date, a huge part of orchards is planted with foreign commercial varieties and their imported seedlings, which are not always adapted to the conditions of the Republic. Although the State Register of breeding achievements recommended for use in the Republic of Kazakhstan includes 23 varieties and 1 clonal scion of Kazakhstani selection, two forms of wild apple as a seed scion, as well as 23 clonal varieties adapted to our conditions and having an important value both for the breeding process and for practical horticulture. Varieties, rootstocks and clone varieties presented in the register have a huge genetic and practical potential for producers, growers, nurserymen, breeders, even of interest to the world community of scientists. The list includes old varieties such as 'Aport', 'Reinette kazakhstanskiy', 'Zarya Alatau', 'Kazakhskoye Yubileinoe', wild forms of *Malus niedzwetzkyana* and *Malus sieversii*, clone varieties selected from the population of *Malus sieversii*.

In all countries of the world, local old varieties of crops, including apple, are valuable assets and natural heritage that have not been given adequate attention for promotion and processing. This is due to the fact that old apple varieties were mostly grown in numerous small orchards and were not involved in scientific breeding programs. However, the 'Aport' became not only a natural heritage for Kazakhstanis, but also an object of research of the national scientific community, a state important cultural heritage, which explained the allocated funding for research and production.

The main reasons that led to the degradation of the variety are related to the placement of 'Aport' orchards in an unfavorable zone for it, high virus infection, violation of agrotechnology, displacement of it from the ecological zone, as well as, uncontrolled multiplication of clones with negative traits and the use of genetically incompatible rootstocks.

Despite the noted problems, reduction of the zone suitable for cultivation of the variety, the south-eastern and southern regions of the republic have the potential for growing 'Aport' orchards. To date, there are about 10 orchards and producers of 'Aport' variety in Almaty and Zhetysu regions. The area of the gardens with Aport reached 2000 hectares; the number of seedlings is 416 thousand; the gross harvest is 24 thousand tons. However, the available orchards have not been planted with properly selected varietal and scion combinations, which may lead to further variety degradation, land depletion and high costs. Unfortunately, such a valuable variety as 'Almaty Aport' has almost disappeared with its special form in Kazakhstan and nowadays the quality of Aport is much lower and the manifestation of the variety is weaker than its ancestors. The most

suitable rootstock for 'Aport' is *M. sieversii*, which is very polymorphic and each form is ambiguous as a rootstock for 'Aport'.

Modern biotechnology uses molecular techniques to identify, isolate important genes, determine genotype compatibility and modify DNA sequences. And cloning biotechnology is an effective tool for pathogen recovery, rejuvenation and multiplication of genotypes. Application of modern methods of biotechnology, molecular biology, pomology and phytopathology can solve the problems of restoration, rejuvenation and revival of valuable genotypes of fruit crops.

The above-mentioned problems of the industry, variety recovery and the advantages of biotechnological methods are the determining factors for the choice of research topics.

### **Purpose and objectives of the research**

*The purpose of the research is to* - to study scion-grafting combinations of 'Aport' selected for economically valuable traits, including those obtained *in vitro* and *M. sieversii* in terms of physiological and biological indicators of growth, photosynthetic potential, productivity potential, water homeostasis, early fruitfulness, adaptive potential and disease resistance in experimental garden conditions during the period of the beginning of its entry into fruiting and to identify the best combinations for realization of their biological potential.

#### *Objectives:*

1. Study the biometric growth performance of 11 cultivar-rootstock combinations of 'Aport' and *M. sieversii*.

2. Improve the technology of 'Aport' recovery and clonal micropropagation under *in vitro* conditions.

3. Study water regime (SCC, water-holding capacity, leaf water content, transpiration rate, leaf hydration, PPP, photosynthetic potential, productivity potential, adaptive potential, ecological status).

4. To give molecular-genetic and phytopathological assessment of 'Aport' infestation and resistance on different rootstocks by the main diseases (scab, powdery mildew, fire blight) in the period of the beginning of the orchard entry into fruiting.

### **Description of the main results of the study**

For health improvement, re-juvenilization (rejuvenation) and propagation, explants from lateral and terminal buds were introduced into tissue culture according to three vegetation phases: dormancy period (March), bud opening (April) and beginning of active growth (May). Samples for microcloning were taken from Issyk (old and young orchard), from Pomological garden (three forms of 'Aport').

To carry out genotyping of 'Aport' and *M. sieversii* clones, samples from mother trees of 5 'Aport' forms and 30 from *M. sieversii* were taken and fixed for DNA isolation. Nuclear DNA was isolated and amplification with 6 ISSR molecular markers, electrophoresis and gel documentation were carried out. As a result of molecular genetic analysis, one form of 'Aport' and 11 forms of *M. sieversii* were selected for seedling production.

In the experimental garden, studies of physiological growth processes of 11 varieties of *M. sieversii* and 'Aport' were carried out. Out of 11 varieties of 'Aport', forms No. 5, No. 6, No. 18, No. 1, No. 2, and No. 10 were distinguished in terms of stem diameter, height, crown width, and growth. During the vegetation period, the productivity potential of 11 'Aport' varietal substitution combinations was studied, 50% of the studied forms showed high productivity potential: Nos. 1, 5, 6, 8, 10 and 18. Photosynthetic productivity was shown on forms No. 1, 2 and 18 during three years of research. The study of earliness showed that all selected forms of *M. sieversii* accelerate the entry of 'Aport' into fruiting. It was found that the greatest adaptation resistance and stress tolerance was possessed by variety-rootstock combinations No. 1,2,3,5,6,10 and 18.

The prevalence and development of diseases on average for three years as scab and powdery mildew were weak and leaf lesions of scab amounted to 1.0%, powdery mildew 1.4%. Fire blight incidence was 2.8%. Molecular genetic analysis of apple for resistance to Fire blight showed that resistant was variety- rootstock combination No. 5, in which both scion and scion had a gene for resistance to *E.amylovora* and which is recommended for use in the production of 'Aport' seedlings resistant to bacterial blight. Molecular genetic analysis of apple for resistance to apple scab showed that out of 11 forms of varietal- rootstock combinations - 8 forms showed resistance to disease: No. 1,2,3,4,5,6,9 and 10. Thus, variety- rootstock combination No. 5 has stress tolerance and resistance to *E.amylovora* and *V.inaequalis*. In the seventh year, when the orchard started to enter fruiting, fruits weighing from 298.1 to 443.0 grams were obtained.

#### **Justification of novelty and significance of the results obtained**

For the first time, grafting and scion combinations of apple trees of Aport variety and *M. sieversii* were selected on the basis of molecular studies of genetic compatibility. A utility model patent "Method of early diagnosis of physiological and biological compatibility of apple tree scion and scion" was obtained.

The regulations of clonal micropropagation of 'Aport' and obtaining container culture with optimization of hormonal and salt composition of nutrient medium were worked out.

An experimental 'Aport' orchard was planted on the area of 5 ha with 'Aport' and *M. sieversii* combinations selected according to qualitative indicators.

Resistant varietal- rootstock combinations to major fungal and bacterial diseases were determined by molecular analyses.

New information has been obtained on the influence of wild apple forms *M. Sieversii* as a scion on growth, development, productivity, adaptive potential, ecological status and resistance to major diseases of apple cultivar 'Aport'.

#### **Practical relevance**

The developed method of creating a varietal-rootstock combination of 'Aport' and *M. sieversii* on the basis of DNA technology, as well as biotechnology of health improvement, clonal micropropagation and rejuvenation (rejuvenilization) of 'Aport' *in vitro* are an effective, science-based stage in the organization of production of elite planting material characterized by good affinity,

high adaptive potential and productivity, early fruitfulness and resistance to bacterial infection.

The selected 'Aport' and *M. sieversii* varietal and rootstock combinations of the experimental garden will serve as initial mother and cuttings for variety restoration and revitalization, as well as for seedling production.

Scientifically-based approaches and agro-technological methods of cultivation of 'Aport' will be an effective tool for restoration, revival of the variety. This will preserve the cultural and natural heritage of Kazakhstan, which has become a "brand" of Almaty city and a geographical indicator of the Republic.

The isolated forms of 'Aport' and *M. sieversii* resistant to major fungal and bacterial diseases may be targets for commercialization.

Introduction of biotechnological and molecular methods in practical horticulture, in particular clonal micropropagation, evaluation of genetic compatibility of variety and rootstock, planting material of new best combinations of 'Aport' and *M. sieversii* will allow to increase the area of orchards, increase their yield, rationally use land resources, increase export potential, thus strengthening food security and economy of the country.

#### **Relevance to science development trends and government programs**

Research work on the topic of the dissertation was carried out from 2015 to 2023 within the framework of scientific projects and programs: 0115PK02205, program code O.0724 (2015-2018); BR06249308, program code O.0887; Project registration number 0118PK01334 (2018-2020); BR10765032, program code O.0987; Project registration number 0121PK00793 (2021-2023).

#### **Description of the doctoral student's contribution**

Directly the applicant carried out all research works on revival of 'Aport' apple: selection of 'Aport' forms, expeditions to wild apple populations and selection of genotypes, procurement of seedlings from wild apple tree seeds, tissue culture, cultivation of seedlings, planting of the orchard, agrotechnical measures, physiological observations, evaluation of its resistance to major diseases. The text of the thesis is written personally by the applicant. The selection of research objects, mastering of methods, development of the structure of the dissertation work, interpretation of scientific articles, planning of experiments and statistical processing of data were carried out jointly with scientific supervisors.

#### **The following main points are put forward for defense:**

1. Methodological bases of selection of rootstock for 'Aport' apple.
2. Improvement of regulations for clonal micropropagation, in vitro rooting and container culture of 'Aport'.
3. Use of molecular-genetic, phytopathological and physiological, pomological methods in the study of compatibility of 'Aport' and *M. sieversii* varietal- rootstock combinations.
4. Science-based approaches of restoration and revitalization of apple trees of 'Aport' and their applied significance.

#### **Approbation of the results of the thesis**

The main principles of the dissertation work were considered and discussed at scientific and technical councils of the Faculty of "Agrobiological" of Kazakh

National Agrarian Research University, extended meeting of the Department of "Horticulture, Plant Protection and Quarantine". The main results of the dissertation on the topic were voiced at international scientific-practical conferences.

The results of the dissertation work are included in interim and final reports on scientific research work (BR06249308, BR10765032, 0115RK02209)

*Publications.* On the theme of dissertation work 8 scientific articles were published, including 3 articles - in the editions recommended by the Committee on quality assurance in the sphere of education and science of the Ministry of Science and Education of the Republic of Kazakhstan, 2 articles - in the edition included in the SCOPUS base, 3 articles - in the collections of International conferences, a monograph was published in co-authorship, a patent for a useful model was received.

**The scope and structure of the thesis.** The total volume of the dissertation is 140 pages. The list of used literature includes 181 titles, the thesis contains 26 figures, 22 tables and 7 appendices.