ANNOTATION

Daulet Nurzhan's dissertation "Screening of promising apple varieties for resistance to *Erwinia amylovora* (bacterial blight causative agent) using molecular markers", submitted for the degree of Doctor of Philosophy (PhD) on the educational program 8D08104 - Plant Protection and Quarantine

Relevance of the research topic.

Fruits are an important and integral part of quality, rational nutrition, and ensure human health and longevity. In Kazakhstan, as in many countries of the world, the most important of fruit crops is apple. It is the leader in increasing fruit production.

According to the data of the Statistics Agency of the Republic of Kazakhstan for 2022, out of 47.18 thousand hectares of orchards covered by seed and stone fruit crops, 35.73 thousand hectares are apple orchards, which is 76% of the area (https://stat.gov.kz/official/industry/14/statistic/7). The State Register of selection achievements authorized for use in the Republic of Kazakhstan for 2023 includes 73 varieties of apple trees of different ripening dates, of which 29% are varieties of local selection, created in the Kazakh Research Institute of Fruit and Viticulture and 38% of varieties have no registered originators.

One of the most dangerous diseases of fruit crops is bacterial blight and it is a quarantine object for Kazakhstan. The disease is caused by bacterium *Erwinia amylovora (Burrill) Winslow et al* and, according to literature sources, affects more than 180 species of fruit and tree and shrub species of the family Rosaceae. Bacterial blight mainly affects apple (*Malus domestica*) and pear (*Pyrus communis*). The disease affects all organs of apple trees: flowers, buds, fruits, leaves, shoots, branches of different orders, and bark of the trunk.

The first manifestation of bacterial blight in Kazakhstan was noted in 2008, and by 2010 it began to cause significant damage to apple and pear orchards of the republic. Bacterial blight is unrivaled among known diseases of fruit crops in terms of harmfulness. The disease causes large yield losses and death of trees.

In the current situation, in order to avoid further spread of bacterial blight in Kazakhstan, it is very important to grow varieties of fruit trees resistant to bacterial blight. However, the problem is that today the data on resistance of domestic apple varieties to bacterial blight are very fragmentary, which does not allow recommending a suitable variety for industrial and private orchards in regions with increased risk of bacterial blight infection. A separate problem at present is the search for disease-resistant rootstocks of fruit crops.

Currently, a special problem is the search for varieties resistant to diseases of fruit crops. In the world breeding, the main attention is paid to the production of high quality pathogen-resistant varieties and rootstocks of fruit crops. Disease resistance of fruit crop varieties is an important indicator determining their value.

Selection of varieties resistant to the most harmful plant diseases always remains one of the main tasks. Therefore, optimal methods of evaluation of genetically determined resistance to pathogens will always be in demand. Due to the need to strengthen the breeding process, optimal methods for assessing genetically determined resistance will always be in demand.

Successes in the development of genetic breeding research are due to the availability of informative genetic markers. So far, morphological (phenotypic) traits are mainly used as genetic markers in fruit breeding programs in Kazakhstan. However, the number of introduced markers of this type is limited. In addition, morphological traits can have a complex pattern of inheritance and often depend on environmental conditions. This indicates the need to evaluate the molecular genetic diversity of cultivated and promising apple varieties and rootstocks and to search for new germplasm resources. In this regard, it is required to determine genetically resistant varieties and rootstocks of apple trees to the causative agent of bacterial blight using DNA marker analysis and to develop recommendations for improving the breeding process and increasing the productivity of plantations. This will make it possible to bring Kazakhstani fruit growing to the modern level.

Despite numerous studies conducted in different countries, a number of issues of resistance of fruit crop varieties to bacterial blight have been insufficiently studied. Increased resistance to diseases is one of the most important requirements of modern varieties of agricultural plants, including fruit crops. Therefore, optimal methods for assessing genetically determined resistance to pathogens will always be in demand.

Currently, the priority task is to develop fruit crop varieties with genetic resistance to the most harmful diseases, in particular bacterial blight. Cultivation of such varieties will reduce pesticide load, energy inputs, improve the environmental situation and obtain environmentally safe products based on organic production.

The solution of the studied problem is relevant not only in Kazakhstan, but also in a number of countries of the world. The results obtained will make it possible to identify genes encoding resistance of apple varieties and rootstocks to bacterial blight pathogen using modern molecular genetic methods based on DNA markers for intensification of fruit crops breeding process.

The purpose of the dissertation research.

Ensuring sustainable fruit production on the basis of identification of genetically resistant varieties and rootstocks of apple trees to harmful disease - bacterial blight using molecular markers and development of recommendations for breeding works.

Research objectives.

1. Screening of domestic and foreign varieties and rootstocks of apple trees to bacterial blight in the main industrial zone of horticulture (south and south-east of Kazakhstan).

2. Detection of bacterial blight pathogen by microbiological and molecular-biological methods in domestic and foreign apple varieties.

3 Determination of genetically resistant apple varieties to bacterial blight pathogen using molecular markers.

4. evaluation of genetic resistance of apple rootstocks to bacterial blight pathogen (Erwinia amylovora) using molecular markers.

Research methods.

For timely detection of bacterial scald, regular surveys of apple-tree plantations in the south and southeast of Kazakhstan during the growing season were conducted according to the methods of detection and identification of fruit-tree scald pathogen

SNP genotyping

Total DNA was isolated using a silica gel membrane and resuspended in TE buffer (10 mM Tris; 0.1 mM EDTA). DNA concentration was determined using a nanospectrophotometer and normalized to 20 ng/μL. Quantitative trait loci (QTLs) for resistance to bacterial blight caused by Erwinia amylovora were identified using 10 molecular markers: FBE-1_Y320, FBE-2_Y192, FBE-2_Y495, FBE-2_Y551, FB-MR5-NZsnEH034548_K35, FB-MR5-NZsnEH034548_R240, FB-MR5-NZsnEH034548_R249, FB-MR5-rp16k15_M106, RLP1a, RLP1b in the FBE, MR5, and RLP1 genes (Chagné et al). All 10 markers were true SNPs previously successfully evaluated using direct sequencing technologies (Jansch et al., Gardiner et al.). All 10 Taqman® e primer and probe pairs were designed using the Custom Taqman® Assay Design Tool (Chagné et al.).

Results were analyzed using Quantstudio® Design and Analysis software and Taqman® Genotyper (Thermo Fisher Scientific). Because the assays were newly designed, each genotyping result was manually verified by reviewing the real-time trace and fluorescence endpoint. Any manual changes were saved using Taqman® Genotyper software and exported as a genotype matrix for each individual sample.

Amplification of SCAR markers

Two markers associated with resistance to bacterial blight, AE10-375 and GE-8019 (Khan et al., 2007), were used in the present work. For each DNA sample, 60 ng of DNA was amplified in a 25 μL reaction mixture containing 1× Taq buffer (750 μm Tris HCl, pH 8.8, 200 μm (NH4)2SO4, 0.1% of 20), 2.5 μm MgCl2, 0.2 μm dNTP, 0.2 μm of each of the respective primers, and 1 unit of Tag polymerase (Thermo Scientific, USA). Amplification results were analyzed by electrophoresis in 1.5% agarose gel in TAE buffer.

The main provisions submitted for defense (proven scientific hypotheses and other conclusions that are new knowledge).

- As a result of monitoring surveys of apple-tree plantations in the south and south-east of Kazakhstan, the distribution and development of bacterial blight on the studied varieties and apple-tree rootstocks were revealed.

- In the course of scientific research using microbiological and molecular-biological methods, the causative agent of bacterial blight was identified in domestic and foreign varieties of apple trees.
- The main ideas on resistance to bacterial blight taking into account genetic origin and climatic conditions of apple tree varieties and rootstocks with new and innovative elements are put forward for defense.
- The expediency of introduction of apple-tree varieties and rootstocks resistant to bacterial blight into practical horticulture has been determined, which will improve the breeding process and increase fruit yield.

Description of the main findings of the study.

As a result of the study, the causative agent of bacterial blight was identified on the examined apple varieties of the main industrial zone of horticulture, the prevalence and development of the disease were established. Certain zonal focality in the manifestation of the disease was noted. As a result of microcopying under a light microscope it was confirmed that bacterial colonies obtained from pure culture are similar to E. amylovora in morphology. Testing of bacterial pathogenicity by White's method on immature apple fruits showed the presence of E. amylovora pathogen - the causative agent of bacterial blight.

Molecular identification of the causative agent of bacterial blight by PCR based on genomic DNA on apple cultivars identified the phytopathogenic bacterium Erwinia amylovora.

The results of genotyping 59 apple cultivars for 10 SNP markers showed that 10 out of 10 markers were polymorphic and successfully distinguished phenotypes susceptible to bacterial blight.

Among the 59 apple cultivars studied using SCAR markers AE-375 and GE-8019, apple cultivars Samouret, Honey Crisp, Pinova and Red Topaz were found to be resistant to bacterial blight and can be used as sources of resistance in breeding programs.

Evaluation of genetic resistance of foreign and local rootstocks to bacterial blight showed that apple trees Geneva 41, Geneva 16 and 62-396 were resistant to bacterial blight among other 11 rootstocks grown in the southern region of Kazakhstan.

The best varieties and rootstocks of apple trees with high resistance to bacterial blight have been selected and recommended for cultivation. Recommendations for improving the breeding process and increasing the productivity of plantations were developed.

Justification of the novelty and importance of the results obtained.

For the first time in Kazakhstan, genetically resistant promising apple varieties to bacterial blight pathogen were identified using the most effective SNP-markers. For the first time in the main fruit-growing regions of Kazakhstan as a result of molecular genetic identification, promising apple rootstocks resistant to bacterial blight pathogen were identified.

Based on the results of the analysis, recommendations on the use of apple-tree varieties and rootstocks resistant to bacterial scald for improvement of breeding process and production of high quality fruits were developed.

Compliance with scientific development directions or government programs.

Dissertation work was carried out in NAO "Kazakh National Agrarian Research University" on the budget program 217 "development of science", subprogram 102 "grant funding of scientific research", AR 09259636 "Study of genetic resistance of promising varieties and rootstocks of apple trees to dangerous disease-bacterial blight using SNP - markers".

Description of the doctoral student's contribution to the preparation of each publication.

In total on the final data of research work of doctoral student published 8 scientific papers, of which in scientific journals recommended by the Committee for control in the field of education and science of the Ministry of Education and Science of the Republic of Kazakhstan - 2 articles; in the materials of international scientific conferences - 2 articles; 2 articles in the journals included in the database Scopus with an index of 74 and 79 percentile and included in the quartile Q1 in the bases Web of Science and Scopus; published recommendations for production and received a patent for utility model "Set of synthetic oligonucleotides for the determination of SNP-markers associated with resistance to bacterial blight on the site of apple trees" №7973 from 21.04.2023 г.

Volume and structure of the dissertation.

The total volume of the thesis is 110 pages, consists of introduction, main part divided into 3 chapters, conclusion, suggestions for production and appendix. It contains 13 tables and 19 figures. The list of used literature is 247, including 232 in foreign editions.