Identification of genetic markers of resistance to Potato virus Y and Golden potato cyst nematode in promising potato varieties by means of PCR

T N Chekushkina, P V Fisenko, I V Kim and T R Raifegerst

Federal State Scientific Institution "Federal Scientific Center of Agricultural Biotechnology of the Far East named after A. K. Chaika"

E-mail: kimira-80@mail.ru

Abstract. The study used genetic material of 13 field-grown potato varieties: Avgustin, Belmonda, Dachniy, Kazachok, Koroleva Anna, Labella, Laperla, Lilly, Red Lady, Sante, Smak, Yubilyar, Yantar and 6 varieties grown in vitro – Adretta, Zhukovskyi rannyi, Khazachiok, Sante, Smak, Yantar. The development of molecular genetic methods for identification of genetic markers of resistance to Potato virus Y and Golden potato cyst nematode was carried out. Meteor variety was used as a positive control due to having the desired genes in its genome. After screening testing of 16 promising potato varieties for resistance to Potato virus Y YES 3A marker of Ry sto gene have not been identified. Golden potato cyst nematode resistance HI genes markers 57R and N195 have been identified in Belmonda, Zhukovskyi rannyi, Koroleva Anna, Labella, Laperla, Lilly, Red Lady, Sante, Smak, and Yubilyar varieties.

Potato is the most economically significant crop in Russia, however, viral diseases are a permanent factor that reduces potato yield and leads to its degeneration. The development of phytopathogen-resistant varieties is the most reliable way to reduce potato yield losses.

At the present stage plant breeders have data bases that include both results of phenotyping of breeding material and information about the presence of DNA markers associated with QTL genes that determine economically valuable traits. For the past two decades numerous DNA markers for potato have been developed. They allow conducting marker-assisted selection (MAS) of promising genotypes [1-3] that are widely used for molecular screening tests in different countries [4-11]. DNA markers associated with Ry sto and Ry-f sto genes are used in breeding programs for marker-assisted selection and to study the genetic diversity of breeding gene pool. Most frequently used markers of the genes stated above are SCAR-marker YES 3A and CAPS-marker GP 122-406/ EcoRV. Polymorphic SSR marker STM003 previously developed by foreign colleagues have not been involved in domestic breeding programs, although, it can be used for molecular screening and genotyping [12].

A number of breeding-valuable traits, such as resistance to plant pathogens and crop pests, are of a monogenic nature. Among them are the following phytopathogens: late blight (caused by Phytophthora infestans Mont. de Bary), Potato virus X (PVX), Potato virus Y (PVY), Potato virus S (PVS), Potato Leaf Roll Virus (PLRV), Potato cyst nematodes (PCN), and Potato canker, caused by Synchytrium endobioticum (Schilb.) Pers., n. Molecular markers linked to various genes, such as: Rpi genes, Ry sto, Ry adg, and Ry cve genes that control PVY immunity, Rvl gene that controls PVX immunity, HI and Gro
1-4 Globodera rostochiensis (Wollenweber) Behrens resistance genes, and Sen1 potato canker resistance gene can become an effective tool for breeding stimulation [13]. Parental lines selection for breeding is more reasonable and simple due to molecular genetic characteristics of potato hybrids and varieties used by breeders as sources of resistance to harmful pathogens. Involvement of genetically diverse varieties in hybridization allows combining high and stable resistance to the main phytopathogens with a complex of economically valuable traits in hybrid progeny.

In this regard, the purpose of the work was to develop a method of screening samples of the potato variety collection of FSBSI "FSC of Agricultural Biotechnology of the Far East named after A. K. Chaika" by means of PCR for the resistance genes to Golden potato cyst nematode and Potato virus Y using 57R and N195 markers of the H1 gene, and YES 3A marker of the Ry sto gene, respectively.

Genetic material of the following 13 potato varieties grown under field conditions was used in the study: Avgustin, Belmonda, Dachniy, Kazachok, Koroleva Anna, Labella, Laperla, Lilly, Red Lady, Sante, Smak, Yubilyar, Yantar, and 6 varieties grown in vitro – Adretta, Zhukovskyi rannyi, Khazachiok, Sante, Smak, Yantar.

Total DNA was isolated by the salt method with an additional step of purification of the extract with a mixture of chloroform/isoamyl alcohol-24/1 both from green parts of the plant and from tubers. The quality and quantity of DNA were evaluated by gel electrophoresis on 1% agarose gel with lambda phage DNA of known concentration as a standard.

PCR was performed in a T100 amplifier (Bio-Rad) using BioMaster HS-Taq PCR-Color (2×) reaction mix (Biolabmix); the reaction was optimized by setting reactions of a control sample with addition of different amounts of magnesium chloride. To assess the quality of PCR and detect non-specific hybridization of primers, a check test containing a complete reaction mixture without adding DNA was conducted.

Primer for identification of genetic markers of resistance to PVY and Golden nematode, as well as the thermal amplification profile, were selected from literature sources [8, 13]. YES 3A marker amplified by the F – TAACTCAAGCGGAATACCC and R – AATTCACCTGTTCATGCTTCTTGTG primers was used to identify the PVY resistance gene Ry sto; estimated product length – 341 bp [3]. 57 R marker was amplified using F – TGCCTGCCTTCCGATTITCT and R – GTTTCAGCAAAAGCAAGGACGTG primers; estimated fragment length – 452 bp [5]. N195 marker was amplified using F – TGGAAAATGGCCACCTA and R – CATCATGTTCTCATTGTGCAC primers; estimated fragment length – 337 bp [13]. To select the efficient concentration of magnesium chloride, the Meteor variety DNA was used, as it has the desired markers in its genome. The PCR conditions for each marker were selected individually. PCR products were separated by electrophoresis in a 1.4% agarose gel stained with a 1% solution of ethidium bromide; visualization was performed by irradiating the gel with ultraviolet light using the gel doc XR+ (BioRad) gel documenting system.

To identify the PVY and Golden potato cyst nematode resistance genes, methods of DNA isolation and PCR were worked through. For definitive interpretation of amplified fragments the Meteor potato variety was used as a positive control, due to having the desired genes markers in its genome according to the literature sources. The variety has 5 resistance genes: Ry sto (PVY resistant), Rx (PVX resistant), Sen1 (potato canker resistant, pathotype 1), Gpa2 (White potato cyst nematode resistant, pathotype Pa2), and H1 (Golden nematode resistant, pathotypes Ro1 and Ro4) [11].

As a result of screening testing of the selected variety samples for the presence of PVY resistance Ry sto gene YES 3A marker was not identified, while stable amplification of the fragment marker was observed in plants of the Meteor variety, which suggests the absence of the desired gene in the studied samples.

The PCR markers 57R and N195 were used to identify the Globodera rostochiensis resistant gene H1. The Meteor potato variety was used as a positive control. Screening tests for the Golden nematode resistance gene H1 fragment markers were found in the following varieties: Belmonda, Zhukovskyi rannyi, Koroleva Anna, Labella, Laperla, Lilly, Red Lady, Sante, Smak, Yubilyar (figure 1).
Figure 1. Electrophoregram of amplification products of 57R marker of six potato varieties grown in vitro.
M – fragments length marker 100 bp ladder, 1-5 – Smak, 6-9 – Zhukovskyi rannyi, 10-14 – Kazachok, 15-19 – Sante, 20-24 – Yantar, 25-29 – Adretta, 30, 31 – positive control, Meteor variety, N – negative control.

It should be noted that some varieties were polymorphic according to the markers used. Specifically, the Smak variety grown in test tubes showed presence of 57R and N195, while in plants grown in the field conditions, the markers were found in the form of very faintly luminous fragments. The rest of the test tube plants also revealed polymorphism. Both markers were identified in four out of five plants of Smak and Zhukovskyi rannyi varieties.

Further research with the involvement of more plants of each variety (not fewer than 10) is required. Additionally, it is planned to expand the number of detected genes.

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