Assessment of resistance of potato hybrids from the Russian Potato Research Center’s collection to *Globodera rostochiensis*

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Abstract. The potato cyst nematodes (PCN) *Globodera rostochiensis* and *Globodera pallida* are the most common pests feeding on potato roots. These pests have an aggressive distribution dynamic and bring a lot of damage to yield. The study presents results of comprehensively test new potato genotypes for resistance to the golden potato nematode (*Globodera rostochiensis*), for its further use in breeding and the creation of economically valuable resistant varieties. Almost half of the samples showed laboratory resistance to golden potato nematode, the rest were susceptible. 26 samples have shown the presence of two molecular markers (N146, N19), indicating the presence of H1 gene. Almost all samples showed a correspondence between laboratory resistance and the presence of H1 gene markers.

1. Introduction

Potatoes (*Solanum tuberosum* L.) are one of the main crops and are cultivated on more than 19 million hectares of agricultural land worldwide with an annual production of more than 390 million tons [1].

Parasitic nematodes are causing great damage to potato production everywhere, with annual global crop losses of 10-15% of yield and an estimated loss of $78 billion [2].

Cyst-forming potato nematodes are obligate specialized sedentary parasites, which are characterized by the presence in the life cycle of cysts containing eggs and invasive larvae [3].

Among them, the most common in all areas of potato cultivation are the golden potato nematode *Globodera rostochiensis* (Woll.) and the pale potato nematode *Globodera pallida* (Stone). These species are considered objects of external and internal quarantine and are widespread worldwide: in North and South America, Africa, Oceania, Australia, Asia, Europe, including the territory of the Russian Federation [4, 5, 6].

Fighting a nematode is a very difficult and complex task. This parasite is practically not affected by chemical and traditional means of control. PCN can be controlled biologically or by crop rotation and trap cropping. However, growing resistant cultivars is believed to be economically the most effective and environmentally safe method of protecting potato crops against PCN [3].

It was shown, that resistance to the the most common *G. rostochiensis* pathotypes Ro1 and Ro4 is controlled by a single dominant gene H1, introduced from *Solanum tuberosum* L. ssp. *andigena* Hawkes [7]. The majority of cultivars resistant to PCN are protected by this gene [8]. The inheritance of Ro1 resistance from *S. pegazzinii* and *S. vernei* is complex and based on several loci [9]. In 1990 H1 gene was mapped to potato chromosome 5 [10].
Molecular markers linked to the loci of interest can be used in potato breeding for the selection of resistant genotypes. The phenotypic evaluation of resistance *Globodera* *spp.* is costly and time consuming. DNA markers can reduce costs, which additionally can be optimized by consecutive screening or by multiplex PCR assays [11]. A set of tightly linked markers, N146 and N195, were developed by Japanese researchers in 2009. These markers sandwich H1 gene with the recombination frequencies of 0.109% and 0.207%, respectively [12] and showed a high efficiency in detecting nematode-resistant potato lines in multiple studies [13, 14, 15, 16]. A major aim of our research is comprehensively test new potato genotypes for resistance to the golden potato nematode (*Globodera rostochiensis*), for their further use in breeding and the creation of economically valuable resistant varieties.

2. Materials and methods

2.1. Plant material

The 50 hybrid samples of the 2nd year of selection from the collection of the Russian Potato Research Center (Department of Genetics) have different origins: Velikan (Russia) x Newton (USA), Sante (Hollandia) x Gala (Germany), 27.75-10 x Mountain Rose (USA, Colorado), TerraRosa (USA) x Mayak (Russia), 541 x Newton, Matushka (Russia) x Bellarosa (Germany), Lekar’ (Russia) x Mountain Rose, 2686-3 x Krok (Belarus), Russian souvenir (Russia) x Gala, Matushka x 8 x 2 (Plamya) (Russ.), 4421-7 x Mountain Rose, 541 x Newton, Matushka x Bellarosa, Lekar’ x Mountain Rose, 625 x Gala, Orlan (Poland) x Krok, Vasilek (Russia) x Newton, 4421-16 x Mountain Rose, Vitelotte (France) x Piroshka (Russia), TerraRosa x Newton, 2678-1 x AmaRosa (USA), Vasilek x Gala, Severnoe siyanie (Russia) x Gala, Pigmey x Mountain Rose, Severnoe siyanie x Newton, 709 x Gala, Vasilek x Mountain Rose, Kumach (Russia) x 33(100), Mountain Rose x Newton, 2670-22 x Matushka, 2747-15 x Mountain Rose, Sante x Krok.

Additionally, cv. Lorch (Russia) was used as susceptible standard for tests with *G. rostochiensis*.

2.2. Laboratory contamination

The test was carried out in the quarantine laboratory of the All-Russian point for testing the resistance of potato varieties and hybrids to the potato nematode (on the basis of the Russian Potato Research Center) according to Guidelines for the assessment of potato cultivars for resistance to golden potato cyst nematode in laboratory (preliminary) tests [17] from November to April. The tubers were planted in plastic pots with a volume of 250-300 cm³. The pots were filled with a mixture of peat and soil (1:1) infected with golden potato nematode cysts propagated on the roots of susceptible potato varieties. The content of viable larvae and eggs in 100 cm³ of soil is not less than 3000-3500 pieces. One tuber is planted in each pot.

Pots with tubers were dropped into cuvettes with sand to a depth of 3-4 cm, and placed on racks. The control susceptible potato cultivar was planted in each cuvette.

During the growing season, the soil was loosened in pots, mineral fertilizers were applied. The temperature of the air and soil was maintained within the range of 20-26 °C, the moisture content of the soil was 70-80% of the total moisture capacity. The light conditions for the plants were maintained with fluorescent lamps for 10-12 hours.

Two months after germination, when white or golden yellow cysts appeared on the roots of the control plants, resistance was assessed. To do this, the pots with plants were watered abundantly, turned over and pulled out a lump of soil with roots. Visually examined the entire root system and counted the number of cysts.

Depending on the number of cysts, samples are assigned to different resistance groups, in accordance with the following unified international scale: 0-5 cysts on the roots - the sample is resistant; 6 or more cysts on the roots – the sample is susceptible. The test is considered valid if the root system of all control plants is severely affected.
2.3. **DNA extraction**

Total DNA was isolated from leaves with DNA Plant MiniKit (Qiagen) according to manufacturer protocol, using 1 g of leaf tissue for each sample.

2.4. **PCR amplification**

A set of DNA markers closely linked to H1 gene, N146 and N195, have been used for genotyping of potato cyst nematode resistant hybrids. A granule bound starch synthase (GBSS) gene has been used as a positive control in each multiplex PCR. PCR reaction was set in the volume of 25 μl consisting of 5 μl of template DNA (approximately 10-20 ng/μl), 0.5 units of BioTaq DNA polymerase and 0.3 μM primers (Table 1). All multiplex PCR amplifications were performed under the following conditions: 1 cycle of 5 min at 95°C, followed by 35 cycles of 30 s at 94°C, 30 s at 55°C and 1.5 min at 72°C, terminated with 1 cycle of 5 min at 72°C. PCR products were visualized by electrophoresis on a 2% agarose gel in TBE buffer, stained in Ethidium Bromide.

| Target gene | Marker | Primer sequence (5'-3') | Size, b.p. | Source |
|-------------|--------|-------------------------|------------|--------|
| GBSS        | GBSS   | GBSS-01 (ATGGCAAGCATCACAG) | 981        | Mori et al., 2012 |
|             |        | GBSS-02 (CAAACTTTAGGTGCCTC) |            |        |
| H1          | N146   | N146-17 (AAGCTCTTGCTAGTGCTC) | 506        | Takeuchi et al., 2009 |
|             |        | N146-22 (AGGCAGAATCAGCATG) |            |        |
| H1          | N195   | N195-09 (TGGAAATGGCCACCCGACTA) | 337        | Takeuchi et al., 2009 |
|             |        | N195-06 (CATCATGGTTCAGCTGA) |            |        |

3. **Results**

3.1. **Nematode Resistance Test**

If the number of nematode cysts on the roots were from 0 to 5, the sample is considered resistant to nematode, and if the number of cysts was higher - the sample considered susceptible. Accordingly to this system 26 out of 50 samples showed nematode resistance in laboratory test and 24 samples have been marked susceptible (Table 2).

3.2. **Genotyping**

After performing all multiplex PCR amplifications, the positive control GBSS band appeared in 50 out 50 samples, confirming successful reactions. Although in some samples (which showed the presence of all 3 used molecular markers) GBSS-band became more faded. In 26 samples N146-band has shown on gel electrophoresis, and N195-band appeared in 26 samples too. 26 samples have shown the presence of all three molecular markers, indicating the presence of H1 gene (Table 2).

Eventually 24 samples showed laboratory resistance, which was confirmed by the presence of resistance H1 gene markers (Table 2).
4. Discussion
All samples were examined for the relation between phenotypically expressed resistance and the presence of molecular markers linked to the resistance gene. This relation was confirmed in 46 out of 50 samples. Two of the four remaining samples showed laboratory susceptibility in the presence of
resistance gene markers, which may indicate a probabilistic error of the experiment. The other 2 samples were laboratory resistant in the absence of resistance gene markers. This might result of probabilistic error too, or different factors and events, which occurred during breeding process.

5. Conclusion
Potato varieties with resistance to a spectrum of pathogens are more competitive in the market and provide environmentally friendly products, that allows producers to safely grow potato on his farms. Therefore, creation of new genotypes with nematode-resistance genes and the creation of valuable resistant potato cultivars is among the major aims of potato breeding program.

In the course of the research, 24 genotypes were identified, laboratory resistance of which was confirmed by the presence of H1 gene markers.

This experience means that marker-assisted selection shows a fairly reliable result, but there are still mismatches in the experiment. The sources of resistance have a complex reason, and the use of DNA markers has shown that genetic and phenotypic data are not always consistent. Therefore, to obtain the most reliable result, it is better to use a comprehensive analysis for resistance, including both phenotypic evaluation and the DNA markers presents.

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