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Olga A. Sobko*, Petr V. Fisenko, Irina V. Kim, Nathalia V. Matsishina

FSBSI “Federal Scientific Center of Agricultural Biotechnology of the Far East A.K. Chaiki”
30 B, Volozhenina st., Timiryazevsky stl., Ussuriysk, Primorsky Krai, 692539, Russia

*Correspondence Author: o.eyvazova@gmail.com

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О.А. Собко*, П.В. Фисенко,
И.В. Ким, Н.В. Мацишина

ФГБНУ «Федеральный научный центр агро-биотехнологий Дальнего Востока им. А.К. Чайки»
692539, Россия, Приморский край, ул. Воложенина, 30б

*Автор для переписки: o.eyvazova@gmail.com

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Potato viruses of 7 commercial cultivars grown in field Primorsky Krai of Russia

Abstract

Scientific relevance. Plant viruses cause a significant economic loss to potato production, especially if plants are infected at early growth stages and infections are mixed. Viral diseases reduce both yield and quality of harvested crops. Detection and identification of plant viruses are key important to prevent their spreading and to achieve potential yield predetermined by characteristics of varieties.

Research methods. Seven potato varieties, bred in Russia and overseas, were used in the field experiment: Smak, Avgustin, Yantar, Laperla, Labelba, Red Lady, Sante, Belmonda. Viral infection rate was measured by the percent of plants with symptoms to the total number of plants. In addition to infection frequency, a disease rate was described after visual estimation. Total RNA was isolated from the collected leaves according to Bekesiova I. et al. 1999 [13]. Qualitative and quantitative estimation of plant viruses in the samples were conducted by single-step real-time RT-PCR with fluorescent detection with the Applied Biosystems QuantStudio 5 and commercial kits “Potato Virus X, Y, M, L, S, A, PSTVd-RT” (Syntol Company) according to the official protocol of the kits.

Results. As a result of our research, symptoms of mixed viral infection were described for potato varieties depending on concentrations and proportions of these viruses in a plant. Mixed viral infection in the potato field in Primorsky Krai comprised PVY, PVX, PVA, PVS, PVM, also PLRV and PSTVd.

Keywords: plant viruses, insect vectors, mixed viral infections, Solanum tuberosum.

Зараженность 7 сортов картофеля вирусами в полевых условиях Приморского края РФ

Резюме

Актуальность. Фитовирусы приводят к большим экономическим потерям в производстве картофеля, особенно если растения инфицируются на ранних стадиях или при смешанных инфекциях. Вирусные инфекции не только снижают урожай, но и ухудшают его качество. Обнаружение и идентификация вирусов растений имеет первостепенное значение для предотвращения их распространения и обеспечения урожайности, заложенной характеристиками сортов.

Методика исследования. В полевом эксперименте, заложенном стационарно, использовалось 7 сортов картофеля российской и зарубежной селекции: Смак, Августин, Янтарь, Laperla, Labelba, Red Lady, Sante, Belmonda. Проявление фитовирусной инфекции оценивали по наличию и соотношению этих вирусов на растении. Тотальную РНК выделяли из зеленых частей растений и анализировали одношаговой ОТ-ПЦР с флуоресцентной детекцией в реальном времени в амплификаторе QuantStudio 5 (Applied Biosystems).

Результаты. В результате исследований были описаны симптомы проявления ассоциативной вирусной инфекции на сортах картофеля, в зависимости от концентрации и соотношения этих вирусов в растении. Смешанная фитовирусная инфекция на картофельном поле в Приморском крае состояла из PVY, PVX, PVA, PVS, PVM, PLRV, PSTVd.

Ключевые слова: фитовирусы, насекомые-векторы, mixed viral infections, Solanum tuberosum.
**Introduction**

Damage, inflicted on the agricultural sector by viral diseases, is enormous. Viruses reduce both yield and quality of harvested crops. Ways and means of virus distribution vary significantly and depend on host-organism characteristics. Thick cellulose cell membranes make flowering plants impenetrable for viruses, and invasion occurs only through wounds. Insects are the most important virus carriers and serve both as vectors and as hosts. Relations between viruses and host plants or vectors are very specific. Viruses can accumulate on the stylet while an insect feeds on infected plants. When a vector feeds on healthy plants, viruses penetrate damaged cells, vascular fluids and cause infection. Ability of an insect vector to infect plants with viruses depends not only on cell permeability in its alimentary canal and salivary glands but also on a possibility to infect the vector organism. Virulence of non-persistent or semi-persistent viruses predetermines their need for time (from a few minutes to several hours) and temporary attachment (usually) to the stylet or the foregut of an insect. On the contrary, persistent viruses, that move through the barrier of the midgut and accumulate in the salivary glands, can cause infection for a period from a few days to several months [1].

Potatoes are the main non-cereal food product. Potato production faces such obstacles as pests and diseases, including viral ones. Viral diseases play a significant role among factors that limit potato production [2]. Virus infection symptoms appear at the germination stage and predominantly at the stages of growth, flowering and fruiting [3]. Viruses of the following families are widespread in Primorsky Krai: Bromoviridae, Potyviridae, Flexiviridae, Luteoviridae, and Posiviridae. PVY (potato virus Y, fam. Potyviridae) poses the main problem for solanaceous crops. Apart from potato, it infects pepper and tomato. PVY reduces total yield and negatively affects the quality of harvested crops. Seeds infected by PVY can serve as an infection source and present a problem for certification because symptoms appear depending on potato varieties and sometimes cannot be estimated visually [4]. The following factors determine epidemiological importance of PVY:

1. non-persistent transmission by more than 50 species of aphids, potato ladybird *Hemosepalachna vigintioctomaculata*, and grass bug *Lygus pratensis* [5];
2. high genetic variability, conditioned by several strains;
3. a wide circle of host-plants comprising weeds that grow in potato fields and at field borders.

It all proves that effective virus transmission depends not only on specific traits of vectors and the virus but also on accessibility of the virus to a carrier [6]. Plant viruses can lead to significant economic loss to potato production, especially if plants are infected at early growth stages and infections are mixed [2]. Mixed infection implies that there are more than one virus coexisting in a plant. It causes appearance of different symptoms. Presence of more than one virus always hampers disease etiology understanding. Most viral diseases are not diagnosed due to their latency or weak symptomatic expression, or similarity of symptoms with fungal and bacterial infections. It can be true for a plant infected with one specific virus. In case of mixed infection, more severe symptoms usually appear [7]. Viruses can infect one host-plant simultaneously (co-infection) or subsequently (superinfection). Plants, attacked by viruses, activate complex protection mechanisms, which work on different levels and often require considerable inner resources. It decreases the yield. Due to all these factors, PVY can cause significant loss of potato yield with mixed infections with PVX, PVM, PVS and some other viruses [6]. Symptom appearance depends on a type of interactions among viruses in a host-organism. Unrelated viruses usually interact with each other in a synergistic way whereas interactions among related viruses are predominantly antagonistic [8]. Detection and identification of viruses, causing infection, are crucial for successful treatment of viral diseases, especially with mixed infections [7]. All these reasons determined the purpose of this study. The research purpose is to study viral infection load in the agricultural ecosystem of potato fields, estimate the influence of qualitative and quantitative interrelation among viruses in plants on symptom appearance, and determine the plant virus concentration, which causes expression of symptoms on potato plants in field.

**Methods**

Seven potato varieties (cvs. Smak, Avgustin, Yantar, Laperla, Labella, Red Lady, Sante, Belmonta) were used in the field experiment. Smak is a medium late variety (breeder – FSBSI “FSC of Agricultural Biotechnology of the Far East named after A.K. Chaika”, Russia), moderately resistant to late blight and Alternaria leaf spot, resistant to potato wart disease and susceptible to nematode *Globodera rostochiensis* Wollenweber. Cv. Avgustin is a medium variety (breeder – FSBSI “FSC of Agricultural Biotechnology of the Far East named after A.K. Chaika” Russia), susceptible to *Globodera rostochiensis*. Cv. Yantar is a medium late variety (breeder – “FSC of Agricultural Biotechnology of the Far East”, Russia), resistant to potato wart disease (Synchytrium endobioticum (Schilberszky) Percival) and susceptible to Globodera rostochiensis, with leaves, stems and tubers susceptible to late blight. Cvs. Laperla and Labella are early varieties (breeder – Den Hartigh BV, Netherlands), resistant to potato wart disease, pathotype I and *Globodera rostochiensis* (R01). According to the breeder, they are resistant to leaf curl. Cv. Red Lady is an early variety (breeder – SOLANA GMBH & CO KG, Germany), resistant to potato wart disease, pathotype I and *Globodera rostochiensis* (R01), with leaves and stems susceptible and roots moderately susceptible to late blight *Phytophthora infestans* Mont. de Bary. Cv. Sante is a medium early variety (breeder – AGRICO U.A., Netherlands, “FSC of Agricultural Biotechnology of the Far East”), resistant to potato wart disease, pathotype I and *Globodera rostochiensis* (R01), late blight and viruses. Cv. Belmonta is a medium early variety (breeder – SOLANA GMBH & CO KG, Germany), resistant to potato wart disease, pathotype I and *Globodera rostochiensis* (R01) [9]. The selected potato varieties are resistant forms in breeding programs.

Plants were planted with 50 tubers per plot. The site is located in the Southern taiga agricultural soil and climate zone (43.850516, 131.960421). The area of the plot is 40 sq. m. Potato is planted on ridges of 70x40 cm at the rate of 37 thousand plants per 1 ha. The size of a plot is 25 sq. m. The plot soil is meadow-brown podzolized. Soil preparation: underwater plowing to a depth of 22 cm, early spring harrowing, pre-sowing cultivation, and two cultivations for vegetation were used.

For virus identification, symptomatic potato leaves were collected in filter paper bags, folded in polyethylene packaging and frozen at – 20°C for the following PCR analysis of the viruses. The leaf surface was cleaned with non-woven materi-
Virus infection rate was measured by the percent of plants with symptoms to the total number of plants. In addition to infection frequency, disease rate was described after visual estimation. To estimate the degree of disease development on individual plants, we used 9-point scale of virus-resistance evaluation, described in “The broad unified CMEA classifier and international classifier of CMEA for potato varieties of section Tuberarium (Dun.) Buk. of genus Solanum L.” [11, 12]. Petunia sp. was used as a bio-indicator [13].

Total RNA was isolated from the collected leaves according to Bekesiova I. et al. 1999 [14]. The extracted RNA was additionally cleaned with the mixture of chloroform/ amyl alcohol (24/1) and/or chroloform/ phenol (1/1). Efficiency of isolation was measured by electrophoresis in 1 % agarose gel stained with ethidium bromide. The result of electrophoresis was documented by GelDoc XR+ (BioRad). RNA concentration in the preparation was determined with the usage of the Invitrogen Qubit 4 Fluorometer and the RNA BR Assay Kit with subsequent dilution to 50 nanograms/ microlitre. Qualitative and quantitative estimation of plant viruses in the samples were conducted by single-step real-time RT-PCR with fluorescent detection with the Applied Biosystems QuantStudio 5 and commercial kits “Potato Virus X, Y, M, L, S, A and PSTVd-RT” (Syntol Company, Russia) according to the official protocol of the kits. 250 ng of total RNA were used in each RT-PCR reaction. Qualitative estimation of infection level was conducted with the comparative Ct method (ΔΔCt) [15]. Inner control of the reaction served as the endogenous control. Positive control samples of the reagent kits were used as a reference sample.

The statistical data processing was conducted with the IBM SPSS Statistics software (Version).

Research results and discussion
To study the process of virus accumulation, we performed an experiment on artificial infection with petunias as indicator-plants. The plants were grown from seeds and divided in two groups – infected test and control. The juice from potato leaves with a high PVY concentration established by PCR analysis was applied to the leaves of the test indicator-plants. The plants of the control and infected groups were further grown separately. Necessary arrangements were made to prevent accidental infection. As a result of the experiment, when the concentration of PVY was increased by 1.8 *10^4 times compared to the asymptomatic Petunia sp. plants, the following symptoms were observed: red and purple strokes and spots appeared on the petals depending on their color, the leaf veins brightened up, the leaf blades remained green. A lower concentration of the plant virus did not lead to development of visible symptoms. It indicates the latent infection (fig. 1 a, b). Consequently, there is a clear correlation between quantitative plant virus load and symptoms of the infection.

To continue the laboratory experiment with the indicator-plants, we made field records and measurements of disease progress. The following symptoms could be observed on potato plants in experimental field plots with natural infection in 2021: mosaics and mottling, chlorosis, curled and rippled leaf edges, distorted and withered flower petals, unopened buds at the flowering stage, red border on leaves, and dwarf plants. The symptoms varied on plants of different varieties and on the plants of the same variety. After visual estimation, the average disease rate on cv. Smak (I) was about 2.4±0.12. The disease appeared as mosaics, leaf necrosis, chlorosis of leaf veins, unopened flower buds. The plants of this variety in the second and third repetitions had the disease rate equal 1.1±0.52 (14 out of 25 plants). Infected plants were characterized by the presence of mosaics on their leaves (fig. 2). This fact can be explained by special isolation and unequal distribution of vectors.

Virus infection on plants of cv. Laperla was the same in all three repetitions and included mosaics, leaf edge necrosis, leaf galls, short height of green parts (dwarf plants). The disease rate was from 3.0±0.12 to 4.0±0.12. Virus infection symptoms on plants of cv. Labella included chlorosis and mosaics ranging from barely discernible yellow spots to pronounced lesions. The disease rate was in average 2.0±0.25. After visual estimation, the number of damaged plants of cv.

Рис. 1. Проявления инфекции на Petunia sp.: а) здоровое растение; б) больное растение
Fig. 1. Symptoms of PVY infection on Petunia sp.: a) a healthy plant; b) a diseased plant
AVGUSTIN varied from 6 to 14 depending on a repetition. Appeared symptoms were mild (a slight leaf curl). Only few plants displayed leaf curl and mosaics. The average disease rate was 0.82±0.12. Only few individual plants of cv. Belmonda had curled leaves. The disease rate was zero (fig. 2).

Viral infection on plants of cv. Red Lady was shown as leaf curl, mosaics and dwarf plants. The disease rate was in average 0.37±0.02. Plants of cv. Sante had symptoms of: pink leaf borders, intercostal chlorosis, and leaf curl. The disease rate was 1.20±0.05 (fig. 2).

We selected plants with a minimal and a maximum degree of visible infection symptoms for every variety. Mixed infection was identified by PCR and included viruses of mosaic group: PVX, PVA, PVS, PVM, also PLRV and PSTVd. Viral load differed among varieties. We suppose that appearance of symptoms on potato plants depended both on qualitative and quantitative composition of plant viruses. Thus, symptoms on plants of cv. Smak differed depending on the presence or the absence of a particular virus, also on proportion of viruses (fig. 3, tab.).
High PVX concentrations in plants of variety Laperla with almost an equal proportion of other viruses led to appearance of chlorosis on leaf edges, necrosis and leaf galls, dwarf plants and mosaics.

Low concentrations of PVY, PVX, PVA, PVS, PVM and PLRV in plants of cv. Labella were shown only as chlorosis. Leaf chlorosis, mosaics and yellow leaves could be observed on plants when the PVY concentration was increased by \( 7 \times 10^3 \) times, the PVM concentration by 90 times, and the PVS concentration by 2 times, compared to the plants with chlorosis only (fig. 4, tab. 1).

A high PVM concentration was expressed as leaf curl on plants of cv. Avgustin compared to the plants without visible symptoms. Its infection load, being higher than the one of PVY by 10.5 times in coinfection, did not produce any changes in symptoms. Simultaneously the combination of high PVY and PVS concentrations caused leaf curl on plants of cvs. Belmonda and Red Lady compared to asymptomatic plants. The total viral load of PVY, PVX, PVA, PVS, PVM and PLRV (fig. 5) led to appearance of pink borders on the leaves of potato cv. Sante, mosaics and chlorosis on the leaves of cv. Avgustin compared to the plants, which were infected but did not have visible symptoms.

There are several types of interactions among viruses in an infected plant, which are usually called synergistic and antagonistic. They cause more severe symptoms than single viral infection does [7]. Propagation of one virus is supposed to be facilitated by propagation of the other in such systems. Mixed PVS and PVX infection can increase both the titer of PVS and enhance appearance of symptoms on potato leaves [16]. Synergy between potyvirus PVY and flexivirus PVX leads to an enhanced propagation rate of the latter increases its titers and consequently aggravates symptoms [17]. Mixed infection of calivirus PVA and luteovirus PLRV allows the later to infect all cell types in leaves, whereas the phloem limits its distribution when infection is single. It must occur due to the fact that movement proteins of PVA can complement PLRV movement deficiency [18]. After examining and collecting leaf samples, M.S. Kolychikhina et al. (2021) concluded that potato plants of cv. Ramos, grown in an experimental field in Lipetsk oblast, suffered from a multiple infection: PVY, PVM + PVS. They also identified a
mixed viral infection in potato plants of cv. Impala in Astrakhan oblast: PVM + PVS, PVM + PVS + PVY [19]. Mixed viral infection was more complex in our experiments and comprised not only plant viruses of mosaic group but also PLRV and PSTVd. Multiple viral infections, caused by heterologous viruses such as PVY and PLRV, are common for potato [20]. PVX when combined with PVY leads to development of potato rugose mosaic [21]. We suppose that single infection of potato plants should not to be studied in field conditions because viruses circulate around the agricultural ecosystem of potato fields and can infect various host-plants, including both wild species and cultivars. Coexistence of several plant viruses and occurrence of mixed infections in plants are conditioned by obligate parasitism. Also most of the vectors are polyphagous. Due to this fact, they can accumulate virus from one plant and transmit it to another that might be already infected. It creates conditions for superinfection [7]. Behavior and physiology of carriers can be affected by changes in plants, caused by viruses, and this factor, in its turn, facilitates distribution and epidemiology of infection. Attraction of carriers to infected plants increases risk of infection. Mixed PVY and PLRV infection enhanced fertility of aphid species Myzus persicae and Macrosiphum euphorbiae, feeding on potato plants, what determined their choice of these plants. According to Chatzivassiliou et al., such behavior can be explained by an increase in the content of sugars and amino acids in the phloem of potato plants which makes them more attractive for insects. Because mixed infections of these viruses often occur in potato plants, qualities of hosts and behavior of carriers can have important epidemiologic consequences in the course of this interaction [21]. Knowledge, acquired as the result of the study on mixed viral infections, can become a rich source of useful information, for example, for development of effective management techniques or even for creation of plants resistant to viruses.

Conclusions

1. As the result of the research, we identified mixed viral infection in potato fields in Primorsky krai. Mixed plant virus infection in the potato fields in Primorsky krai comprised PVY, PVX, PVA, PVS, PVM, also PLRV and PSTVd. The study on virus composition in the agricultural ecosystem of potato fields requires further research.
2. The symptoms varied on plants of different varieties and on the plants of the same variety. Disease symptoms depended on the concentration and proportion of viruses in plants.
3. PVY, PLRV, PVS and PVW were identified in plants of all potato varieties. Cvs. Yantar and Laperla I were not infected with PVA. Cvs. Belmonda, Laperla I and Smak I were not infected with PVX. PSTVd was identified only in plants of varieties Red Lady and Smak.
4. Mixed viral infection was expressed as mosaics and leaf curl.
5. Quantity of viruses in plants influenced appearance of symptoms on these plants. The concentration of PVY and PVS, higher by 5.86 and 1.87 times respectively than in asymptomatic plants, was expressed as mosaics on leaves of potato cv. Smak. In addition to mosaics, curl of leaf edges, unopened buds, distorted and withered flower petals were observed on individual plants when the PVM concentration was higher by 104 times than in asymptomatic plants and PSTVd was present.
6. The PVX concentrations, higher than in asymptomatic plants, were expressed as chlorosis of leaf edges, necrosis, dwarf plants and leaf galls on plants of cv. Laperla. A heavy viral load of PVY, PVM and PVS led to mosaics and yellow leaves on plants of cv. Laperla.
7. A high PVM concentration was expressed as leaf curl on plants of cvs. Avgustin and Smak. A high concentration of PVY and PVS led to leaf curl on plants of cv. Red Lady. Pink borders on leaf edges of potato cv. Sante could be observed when PVY, PVX, PVA, PVS, PVM, PLRV and PSTVd were present. These viruses were expressed as mosaics and chlorosis on potato plants of cv. Avgustin.
8. An increase in quantity of viruses – causative agents of potato diseases – and changes in geographical range of their distribution reflect the general process of interactions among plant viruses and their hosts in modern agricultural production. Understanding of global situation is necessary to optimize all steps of integrated protection, which will allow ensuring stable production of high quality potato in Russia.
About the authors:
Olga A. Sobko – Ph.D. student, Junior Researcher at the laboratory of breeding and genetic research of field crops, https://orcid.org/0000-0002-4383-3390, Correspondence Author, o.sobko2000@gmail.com
Petr V. Fisenko – Cand. Sci. (Biology), Leading Researcher, acting head of the laboratory of breeding and genetic research of field crops, https://orcid.org/0000-0003-1727-4641
Irina V. Kim – Cand. Sci. (Agriculture), Leading Researcher, acting head of the laboratory of potato disease diagnostics, https://orcid.org/0000-0002-0656-0645
Natalia V. Matishina – Cand. Sci. (Biology), Senior Researcher, the laboratory of breeding and genetic research of field crops, mnathalie134@gmail.com, https://orcid.org/0000-0002-0165-1716

Ob авторах:
Ольга Абдулхамидовна Собко – аспирант, молодой научный сотрудник лаборатории селекционно-генетических исследований полевых культур, https://orcid.org/0000-0002-4383-3390, автор для переписки, o.sobko2000@gmail.com
Петр Викторович Фисенко – кандидат биологических наук, ведущий научный сотрудник, и.о. заведующего лабораторией селекционно-генетических исследований полевых культур, https://orcid.org/0000-0003-1727-4641
Ирина Васильевна Ким – кандидат сельскохозяйственных наук, ведущий научный сотрудник, и.о. заведующего лабораторией диагностики болезней картофеля, https://orcid.org/0000-0002-0656-0645
Наталия Валериевна Матишина – кандидат биол. наук, ст.н.с. лаборатории селекционно-генетических исследований полевых культур, mnathalie134@gmail.com, https://orcid.org/0000-0002-0165-1716

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