IOP Conference Series: Earth and Environmental Science

PAPER • **OPEN ACCESS**

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To cite this article: N Kakareka *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **937** 032108

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Possibilities of Obtaining and Controlling Virus-Free Material in the Process of Selection and Seed Production of Main Crops

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Abstract. It is known that after being infected with phytoviruses plants do not recover. Breeding virus-resistant cultivars also does not give reliable results due to the high variability of pathogens and their diversity. And since it is impossible to obtain stable forms then one of the goals of protection a virus-free material and preventing its re-infection. To improve the health of vegetative propagated plant species, you can use the method of tissue culture of the apical meristem. In some cases true seeds can be healed by thermal or chemical treatment. The data on viruses infecting the main agricultural crops of the Russian Far East are presented; the features of their distribution and interaction with plants are described. Possible measures are given to prevent of phytoviruses spreading and re-infection of virus-free plants in the process of breeding and seed production, in gardening and landscape design.

Introduction

Food safety of the country is one of the most important requirements for agricultural production. Therefore the problem of creating high-yielding cultivars of agricultural crops adapted to local conditions and resistant to viral diseases is of great importance. Getting disease resistant plant varieties is challenging. The high variability of phytopathogens whether they are fungi or viruses overcomes resistance in a short time. But if the fight against fungi is possible with the help of pesticides then the fight against viruses is impossible. An infected plant does not recover. And since it is impossible to obtain stable forms, the task is to obtain a virus-free material. In the case of vegetative propagating plant species, the apical meristem tissue culture method can be used. As known if you cut out a upper part of the meristem with a growing point then you can use certain techniques and methods from this meristem to grow a virus-free plant [1-6]. In plants the apical meristem (0.1-0.3 mm in size) as a rule with two axile buds consists of actively dividing cells. Since it is devoid of vascular elements viruses cannot quickly penetrate the meristem. If the top is removed from the plant under aseptic conditions it can be grown in a specially selected medium [7]. Shoots, leaves and roots are formed over time [8, 9]. Formed plants are tested for the absence of viral infections, cuttings thus multiplying the healthy material.

This methodology has been worked out on potatoes, ornamental (carnation for example), fruit and berry crops. But it is not enough to heal the plant; it must be protected from re-infection [10]. In this

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regard it is especially important to identify dangerous phytopathogens local and introduced from other regions and countries to develop a methodology for their testing and to study the mechanisms of plant protection against phytoviruses. Currently in connection with the active import of new varieties and crops to the Russian Far East (The Netherlands, USA, Canada and especially from neighboring countries – China, Korea, Southeast Asia) new viral diseases have appeared and it became necessary to quickly and efficiently diagnostics [11-13].

A number of viruses are transmitted with true seeds. And in this case it is important to protect crops especially seed nurseries from vectors, since the level of seed infection depends on the time of infection. The later the virus enters the plant the less infected the seeds. There are also special ways to discard infected seeds of some crops. But in this case control and prevention of the phytoviruses spreading is an actual goal [14]. An important rule should be to check the original parental pairs for the presence of viral infection when breeding new cultivars by hybridization [15].

In this regard one of the main directions of research of the virology laboratory is the prevention of viral invasions in natural and artificial plant communities and the reduction of losses of agricultural production in the Far East of Russia. This aspect of research is relevant for ensuring of food safety.

Materials and Methods

Identification of phytoviruses includes the study of characteristics (morphology, composition, range of hosts and vectors) and involves obtaining purified preparations, immunizing of animals with these preparations in order to obtain specific antisera. In order to obtain the most sensitive antisera strains with the largest number of epitopes should be used [16]. For this studies the following methods were used:

1. Electron microscopy;
2. Biological testing;
3. Obtaining of purified virus preparations;
4. Electrophoresis of virus components;
5. Immunization of animals and polyclonal antisera obtaining;
6. Testing by immunochemical methods;
7. Testing by polymerase chain reaction (PCR).

Results and Discussion

It is known that in the Far East the main agricultural row crops are potatoes and soybeans and grain crops are barley, oats and corn. In small commercial farms, suburban gardens and plots a wide range of vegetables and industrial crops is cultivated: tomato, pepper, eggplant, cucumber, onion, garlic, beans, zucchini, pumpkin, watermelon, species of the Brassica genus, radishes, carrots, beets, sunflowers.

Recently, an emergency situation has developed in connection with the infection with viruses of products of small-scale commercial farms, which supply the bulk of commercial potatoes and vegetables to the market. These farms, for the most part, do not comply with phytosanitary norms and rules, and often do not have the opportunity to purchase virus-free planting material. As an example, we can cite the fact that most farmers use uncertified and even just marketable potatoes (including those imported from abroad) for planting, thereby contributing to an even more active spread of viruses. In these farms, the degree of potatoes infection according to immunochemical testing data can reach 50 percent or more [17].

In general, the following viruses were identified on potatoes in the Far Eastern Federal District:

– *Potato virus X* (PVX) (4 strains) (Tymovirales, Alphaflexiviridae, *Potexvirus*)
– *Potato aucuba mosaic virus* (PAMV) (Tymovirales, Alphaflexiviridae, *Potexvirus*)
– *Potato virus Y* (PVY) (more than 15 strains) (Patatavirales, Potyviridae, *Potyvirus*)
– *Potato virus A* (PAV)(12 strains) (Patatavirales, Potyviridae, *Potyvirus*)
– *Potato virus S* (PVS) (2 strains) (Tymovirales, Betaflexiviridae, *Carlavirus*)
– *Potato virus M* (PVM)(3 strains) (Tymovirales, Betaflexiviridae, *Carlavirus*)

– *Potato leaf roll virus* (PLRV) (Tolivirales, Luteoviridae, *Polerovirus*)
– *Tobacco mosaic virus* (TMV) (Martellivirales, Virgaviridae, *Tobamovirus)*
– *Tomato mosaic virus* (ToMV) (Martellivirales,Virgaviridae, *Tobamovirus*)
– *Cucumber mosaic virus* (CMV) (Martellivirales, Bromoviridae, *Cucumovirus)*
– *Alfalfa mosaic virus* (AMV) (Martellivirales, Bromoviridae, *Alfamovirus).*[17]

In recent years, we have identified especially dangerous necrotic strain of the potato virus Y – PVY_{NTN} which had not previously been identified in our region [18, 19] (Fig.1).

No less dangerous variable and contagious is the cucumber mosaic virus which was first identified on potatoes of the Anosta cv in the Primorsky Territory. Highly sensitive and universal immunodiagnosticums were prepared for these virus strains (PVYNTN, CMV).

All these viruses can be cleared by apical meristem tissue culture. It is important to select the most healthy material for isolating the meristem (especially without the potato spindle-tuber viroid from which it is almost impossible to free the meristem).

In general the system of healing and growing potatoes has existed for a long time and has been worked out to the smallest detail [20, 21]. New and improved methods of thermo-, cryo- and chemotherapy have been developed [22-24].

Figure 1. Potato tuber infected by NTN strain of potato virus Y and potato spindle tuber viroid.

Earlier in the Primorsky Territory large potato farms cultivated virus-free potatoes, which were rehabilitated from phytoviruses under local conditions and multiplied as a super elite and elite in a closed zone in the foothills of the Chuguevsky region [25]. The virus-free material has been tested previously and at present by immunochemical methods and PCR. Unfortunately now there are practically no specialized farms that are certified and capable of propagating virus-free material with protection against re-contamination and quality control.

Vegetable crops cultivated in the Far Eastern Federal District are also highly infected by viruses. Currently, the list of viruses detected and identified in vegetable crops is as follows:
– Cucumber mosaic virus
– Tomato aspermy virus (TAV) (Martellivirales, Bromoviridae, Cucumovirus)
– Radish mosaic virus (RMV) (Martellivirales, Bromoviridae, Comovirus)
– Tobacco mosaic virus
– Tomato mosaic virus
– Garlic mosaic virus (GMV) (Tymovirales, Betaflexiviridae, Carlavirus)
– Cauliflower mosaic virus (CaMV) (Ortervirales, Caulimoviridae, Caulimovirus)
– Turnip mosaic virus (TuMV) (Patatavirales, Potyviridae, Potyvirus)
– Onion yellow dwarf virus (OYDV) (Patatavirales, Potyviridae, Potyvirus)
– Watermelon mosaic virus 2 (WMV) (Patatavirales, Potyviridae, Potyvirus)
– Tobacco ringspot virus (TRSV) (Picornavirales, Secoviridae, Nepovirus)
– Tobacco etch virus (TEV) (Patatavirales, Potyviridae, Potyvirus) [26].

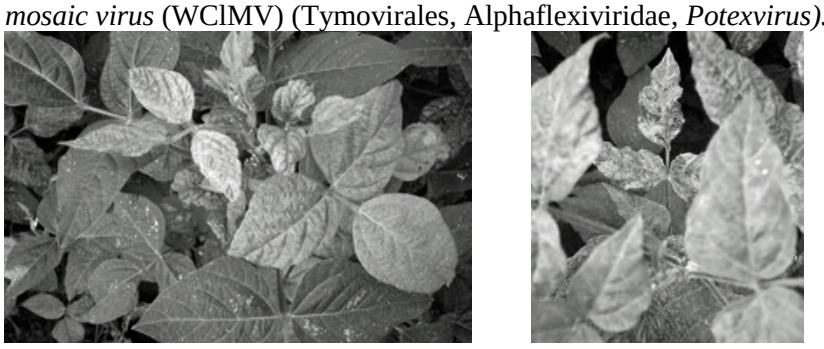
The most common and harmful is the cucumber mosaic virus (Fig. 2), which can infect almost all types of vegetables, except for Alliaceae, and has a wide range of aphid vectors. In natural plant communities, CMV often forms natural foci of infection which are a source of infection for cultivated species [26, 27]. At the same time, CMV is rarely transmitted by true seeds, except for leguminous strains. Therefore with competent protection of plantings from vectors, especially in the early stages of the growing season, it is possible to avoid the massive spread of CMV.

Figure 2. Cucumber fruit affected by cucumber mosaic virus.

In some cases, heat-sensitive viruses can be inactivated in seeds by chemical and heat treatment and thus prevent their spread in crops. [28].

Soybeans are the main legume crops in the Far East and throughout the world. On private farms, it is grown as food – peas, beans, rarely broad beans. From decorative plants lupine, a sweet pea is grown. The list of viruses infecting legumes includes the following species:

- *Soybean mosaic virus* (3 strains) (Patatavirales, Potyviridae, *Potyvirus*) (Fig. 3 a,b)
- *Bean yellow mosaic virus* (Patatavirales, Potyviridae, *Potyvirus*)
- *Bean common mosaic virus* (Patatavirales, Potyviridae, *Potyvirus*)
- *Cucumber mosaic virus*
- *Tobacco ringspot virus*
- *Alfalfa mosaic virus*
- *Pea enation mosaic virus* (PEMV) (Tolivirales, Luteoviridae, *Enamovirus*)
- *White clover mosaic virus* (WClMV) (Tymovirales, Alphaflexiviridae, *Potexvirus).*[29]



a b

Figure 3. (a) Soybean plant affected by a moderately pathogenic strain of soybean mosaic virus, (b) Soybean plant affected by a severe pathogenic strain of soybean mosaic virus.

Almost all viruses that infect legumes can be spread by both vectors and seeds. And the sooner a plant becomes infected, the more likely it is to infect the seeds. Therefore, an important aspect of preventing the spread of phytoviruses in this case is vector control and improving the quality of seed.

In the Far East of Russia, few cereal crops (mainly spring crops) are currently grown. Rice growing began to recover in the Khanka Valley, small areas are used for oats as a fodder crop and for corn, which is grown mostly for export. The following viruses have been identified and identified on cereals:
– *Brome mosaic virus* (BMV) (Martellivirales, Bromoviridae, *Bromovirus)*
– *Northern cereal mosaic virus* (NCMV) (Mononegavirales, *Rhabdoviridae*, *Cytorhabdovirus*) (Fig. 4)
– Oat russian mosaic virus (ORMV) (*Bunyavirales*, *Phenuiviridae*, *Tenuivirus*)
– *Barley stripe mosaic virus* (BSMV) (Martellivirales, Virgaviridae, *Hordeivirus*)
– *Rice stripe virus* (RSV) (*Bunyavirales*, *Phenuiviridae*, *Tenuivirus*)
– *Poa semilatent virus (*PSlV) (Martellivirales, Virgaviridae, *Hordeivirus*)
– *Wheat streak mosaic virus* (WSMV) (Patatavirales, *Potyviridae*, *Tritimovirus*)
– *Maize dwarf mosaic virus* (MDMV) (Patatavirales, *Potyviridae*, *Potyvirus*)
– *Barley yellow dwarf virus* (BYDV) (Tolivirales, *Luteoviridae*, *Luteovirus*) [30]

Figure 4. Wheat infected by northern cereal mosaic virus.

Several more diseases have been revealed including those on wild-growing cereals that can become a natural reservoir of infection but their causative agents have not yet been identified. It should be noted that most viruses in cereals are not transmitted by seeds. An exception is the barley stripe mosaic virus and wheat streak mosaic virus the level of infection of seeds of some varieties with it can reach 100% [30, 31]. Therefore the improvement of barley crops can be carried out by selecting varieties resistant to this virus and using resistance donors in breeding [32].

As noted above, the method of tissue culture of the apical meristem can improve vegetative propagated crops, and there are not so many of them - potatoes, garden and ornamental crops. Improvement of crops propagated by seeds implies mandatory prevention of the spread of the virus, especially in specialized seed nurseries [33, 34, 10]. The list of protective and preventive measures includes:

In breeding and seed production:
– Control over the introduction of seeds, tubers, bulbs, seedlings, flowers, cuttings, *etc*.;
– Selection of healthy and resistant lines for reproduction and hybridization work;
– Cultivation of certified healthy plant material in specialized farms;
– Compliance with the rules of seed production and plant breeding on virus-free basis;
– Control of sowing material in seed farms by methods of immunodiagnostics and PCR;
– Compliance with crop rotation;
– Weed control;

– Control of virus vectors.
In landscape design:
– Elimination of diseased plants from plantings;
– Selection of healthy plants and seedlings for reproduction.
In gardening:
Selection of healthy plants for rootstocks and scions

Conclusion

Thus, there are all the prerequisites for improving seed production and selection of agricultural crops on a virus-free basis using, for control, antisera and immunodiagnostics, developed and produced at the Federal Research Center of Biodiversity FEB RAS [16]. The center has the ability to conduct control tests of virus-free potato and vegetable material (bank of immunodiagnostics for ELISA and other methods, the necessary equipment and high-class specialists).

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