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Methods of Combating Tomato Fruit Brown Rot Virus

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Abstract: This article discusses issues such as biological purification and reproduction of viruses, preparation of infectious juice and mechanical infection of plants, detection of disease symptoms using indicator plants, and determination of final liquefaction level of the virus.

Keywords: viruses, plants, molecular-genetic methods, biological purification, pure preparation.

In recent years, more than 1,000 plant-infecting phytoviruses have been identified, and these viruses infect wild plants as well as important agricultural plants, reduce productivity and product quality, and cause great damage to the national economy.

Tomatoes, cucumbers, cotton, wheat, potatoes, bell peppers, eggplants, alfalfa and beans can be mentioned among such plants. Among them, tomato (Lycopersicum esculentum) plant has been found to be infected by more than 20 plant viruses. Examples of these include: tomato mosaic virus, tomato strain of tobacco mosaic virus, tomato spotted wilt disease, and cucumber mosaic virus. The decrease in productivity of tomato plants infected with viral diseases, the decrease in the quality of tomato fruits, as well as the shortening of the growing season of tomato plants have been studied. This has a negative impact on agriculture and the country's economy. Therefore, their identification is important in the fight against viral diseases and in order to determine their spread and develop countermeasures.

Preparation of infectious aphid and mechanical infection of plants. Mechanically, the virus is introduced into the cell through micro-injuries in the leaf epidermis. Microinjuries in the leaf epidermis are created with the help of fine powder of abrasives such as diatom algae (celite) or carborundum (silicon carbide) or corundum (aluminum oxide). The size of fine powder of abrasives is 400-700 mesh (the number of pores (holes) in 2.5 cm mesh). Thoroughly sterilized carborundum in an autoclave or drying cabinet, corundum is dusted on the surface of the leaf before inoculation. When abrasive is used, the inoculation effect increases 20-50 times.

In order to separate the viral sap from the viral sample, a sample of plant parts infected with the virus (root, stem and mostly leaves) is taken and crushed in a porcelain mortar with buffer added (1:1). A homogenizer is used to grind a large amount of sample. Then it is passed through a four-layer gauze. The filtered juice is centrifuged at a speed of 6000-8000 revolutions per minute. In order to infect the plants with the prepared viral sap, the surface of the leaf is dusted with corundum or carborundum, 2-3 drops of sap are dropped on the leaf of a healthy plant, and it is thoroughly washed and gently rubbed with an air-dried finger. The strength of rubbing depends on the condition of the leaf, its age, and the quality of the abrasives. After 10-15 minutes, excess virus preparation and abrasives are washed off with distilled water. Labels with the name of the virus, date, etc. are attached to the infected plant. The plant is kept in a cool place for 1-2 hours and monitored.

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Labels with the name of the virus, date, etc. are attached to the infected plant. The plant is kept in a cool place for 1-2 hours and monitored. As a result of observations, mosaic from systemic diseases in various indicator plants - simple, yellow, dark green, intervein mosaic, leaf plate deformations - downward or upward twisting of leaves, swelling, coarsening, threading, fern-like, anthocyanosis, enation , smallness, lengthening or shortening of joints (puchkovidnaya verkhushka), focal symptoms - necrosis and chlorotic spots are observed. Symptoms of the disease are more clearly observed at the growing point of the plant, because regardless of which part of the plant the virus enters, it first moves to the top and goes to the growing point. Symptoms of viral diseases in various plants can be observed with the naked eye, i.e. visually.

Biological purification and propagation of ToBRFV. Different strains of the same virus can cause different symptoms in indicator plants. 50 g of tomato plant varieties with disease symptoms. samples were collected separately and ground in a porcelain mortar by adding phosphate buffer (0.1 M, pH 7.2). The obtained homogenate was spun in a centrifuge for 20 minutes at a speed of 6000 rpm in order to clean it from plant residues, bacteria and fungi. The supernatant was isolated and inoculated into Nicotiana glutinosa L. plant under laboratory conditions. After 5 days, large red necrosis was formed on the leaves of Nicotiana glutinosa L. One of the necroses was isolated and re-homogenized and mechanically infected with Nicotiana tabacum plant. 4-5 days after mechanical infection, it was observed that small black necrosis and then yellow mosaic symptoms appeared on Nicotiana tabacum leaves. Biologically pure virus purified from the mixed infection was obtained and inoculated into Nicotiana tabacum by repeated infection three times in the same manner. 1 kg of mosaic leaves formed in Nicotiana tabacum was stored at -20°C. Obtaining a purified preparation of ToBRFV and determining the degree of purity. 1 kg of leaves of Nicotiana tabacum plant infected with ToBRFV stored in a refrigerator were taken. To this virus sample, 0.1 M phosphate buffer (FB) (pH 7.2) was added (1:1 buffer ratio equal to the weight of the virus leaf obtained) and ground in a homogenizer for 15 minutes, and the resulting mass was passed through four layers of cheesecloth. after this, the homogenate was centrifuged at 6,000 rpm for 20 minutes, the supernatant (ChUS) was obtained, and chloroform was added to the supernatant at a ratio of 8:1, and after shaking for 20 minutes, it was centrifuged at 6,000 rpm for 20 minutes. was centrifuged at . Then 25% ammonium sulfate salt (NH4)2SO4 was added to ChUS, kept at room temperature for 1 day and centrifuged at 8000 rpm for 20 minutes. The viral pellet was separated, and the ChUS was discarded. The precipitate was dissolved in 0.01 M (pH 7.2) FB, the virus was transferred to the solution, and a partially purified preparation of the virus was obtained by centrifugation for another 15 minutes at 3000 rpm. The gel filtration method was used to obtain a homogeneous virus preparation.

TSK NW-65 gel was mixed with FB in a homogenizer for 1 minute. The crushed gel was shaken with 2-3 times more buffer than the main volume and allowed to stand for 5 minutes, the upper part was carefully poured off, and the precipitated gel pieces were placed in a column. When the gel particles were placed in the column, the water in it was reduced to the maximum, gently mixed with a glass rod (so that air did not enter between them) and the chromatographic column was filled. After the filled column was washed with 0.1 M FB (pH 7.2) for 1 day, a very small amount of sucrose was added to the buffer, and 2 ml of the partially purified preparation of ToMV was placed under the buffer in the column. After the sample was placed in the column, the eluents separated from it were collected in separate test tubes of 3 ml, the elution rate was one drop in 4-5 seconds. The degree of purity and the amount of the purified virus were determined by the UV absorption of the obtained fractions at a wavelength of 260-280 nm in a "Cary 60 UV" spectrophotometer.

Determination of final liquefaction level (OSD) of the virus. In order to distinguish between viral diseases of tomato, 50-60 grams of plant leaves infected with this virus were taken and crushed in a porcelain mortar to determine the final dilution level (OSD). The crushed mass was passed through a 4-layer gauze and 1 ml was left for control. Then, 1 ml of viral sap was added to the first of 10 test tubes containing 9 ml of buffer, and after mixing thoroughly, 1 ml of this mixture was taken, put into the next test tube and mixed. Thus dilution 10-1, 1 0 "" 2 ,1 0 "" 3 ,1 0 "" 4,1 0 "" 5,1 0 "" 6,1 0 "" 7, 10-8 ,10-9 was carried out and the leaves of Nicotiana glutinosa L were infested with the control sap on the left side and the diluted sap on the right side, and labels were placed on each of them and

observed every 2-3 days for 15 days until the disease symptoms appeared. The results were determined based on the necrotic spots that appeared.

Conclusion

In performing the work, the preparation of infectious aphids and mechanical infection of plants, identification of disease symptoms using indicator plants, determination of the level of spread of viruses, biological purification, obtaining a clean drug and determining its degree of purity, determination of the final liquefaction level of the virus, phytovirus From virological methods such as determining the point of loss of activity under the influence of temperature (HTFY) and PCR detection of viral diseases affecting tomato plants, and the effect of viruses on plant physiological processes such as the amount of pigments (chlorophyll a, b and caratinoids) was studied. The spread of ToBRFV in some tomato fields in Tashkent region was determined by QT-PCR analysis.

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