УДК 578.85/86

DIAGNOSIS OF SEED VIRAL INFECTION OF VEGETABLE CROPS

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Встановлено показник ураженості насіння комерційних сортів томату їстівного (Lycopersicum esculentum Mill.), перцю овочевого (Capsicum annuus L.) та синього баклажана (Solanum melongena L.) вірусними патогенами. Найбільший уміст антигенів вірусу огіркової мозаїки детектувався у сортах томатів Де-Барао червоний, Яблунька Росії, перцю Анастасія, Солодкий пастух, баклажана Алмаз. У насінні томату сорту Де-Барао червоний та перцю сорту Ротунда виявлено антигени вірусу мозаїки томатів. Встановлено, що вірусна інфекція не впливала на лабораторну схожість, але знижувала енергію проростання та силу росту насіння.

***Ключові слова*:** *віруси, імуноферментний аналіз, посівні якості насіння, пасльонові, проросток, зародок.*

The yield losses and reduction of crop quality caused by plant pathogens have gained increasing recognition in recent years. The use of varieties resistant to pathogens appears to be one of the best control strategies, but is not always possible or effective [1]. Being obligate parasites phytoviruses capable of infecting new plants at intervals appropriate to the life cycle of the host [2]. There are some types of response by plants to inoculation with a virus.

Virus does not replicate in immune (extremely resistant) plants, doesn't spread in it and causes no symptoms. Pathogens spread through the plant and high titer of infectious agents in tissues in the absence of clear disease symptoms (often latent infection) are observed in tolerant plants [3]. In this case of plant response of viral infection visually is not identified, but may be spread in biocenosis and infect other plants. As a result crop yields are reduced and production quality is decreased.

An increasing number of data showing the expansion of the range of the most harmful pathogens of viral diseases, propagation of complex and latent infections, the emergence of new infections forms with altered pathogenicity are published in recent years [4, 5].

There are nearly 110 specific viruses of different taxonomic affiliation, which differ in their biological, serological, and environmental characteristics and mode of transmission in vegetable crops according to the taxonomic committee on viral disease [6].

Some pathogens have a wide range of plant hosts. For example, *Tobacco* rattle virus affects over 400 species of plants from 50 families. Many plants can be affected by several viral agents. In the case of mixed infections, drastic reductions in yield may occur due to synergistic effect.

About one fifth of the known plant viruses are transmitted through the seed of infected host plants [2]. The reasons why some pathogens are transferred with seeds and others are not are not still understood despite the progress in studying the issue of seed phytovirus distribution. The ability to invade generative tissues from vegetative is typical for many viruses, including the *Potyvirus*, *Nepovirus*, *Cucumovirus* genera [1].

In plants of the family *Solanaceae* pathogens are efficiently transmitted by seeds. Vi-

ruses are localized in the embryo, endosperm and testa [2, 7, 8]. The degree of virus transfer from a germ seed to plant depends on the ability of the pathogen to survive at a time of seed resting phase and on the ability to reactivate during or after germination.

Under favorable conditions, pathogen may be present in the seed for years until it loses its vitality. So it is possible to estrange the time of pathogens' spread on much longer period than while infection through vegetative parts of plants. Thus, *Bean common mosaic virus* (BSMV) was isolated from bean seeds after 30 years of storage [1, 2].

There are contradictory data regarding the impact of these pathogens on seed conditioning properties. According to Johansen E. [9] and other reports, e.g. on *Pea seed-borne mosaic virus* (PSbMV) in pea [10], *Soybean mosaic virus* (SMV) in soybean [11], *Pea early browning virus* (PEBV) in pea [12], *Turnip yellow mosaic virus* (TYMV) in *Arabidópsis thaliána* [13] did not have significant effect on seed germination of studied cultures. Johansen E. [9] considers that the decrease in seed germination, most likely caused by the effects of lesions of the parent plant than of an infectious agent presence in the seed. Kazinczi G. and Horvath J. [14] reported that *Sowbane mosaic sobemovirus* (SoMV), seed-transmitted in *Chenopodium* spp., caused a reduced viability of seeds from infected plants. The viability of seeds from *Solanum nigrum* L. plants infected with *Tobacco mosaic tobamovirus* (TMV) was also significantly reduced compared to that of seeds from healthy plants [15]. There are no reliable means of combating with viral infection nowadays. Therefore, the only effective way of containment is its early diagnostics [16].

The aim of our study was to establish the presence of viruses in the seeds of plants of the family *Solanaceae* and their impact on sowing qualities of seeds.

MATERIALS AND METHODS

Seeds of paprika (*Capsicum annuus* L.), tomato (*Solanum lycopersicum* L.) and eggplant (*Solanum melongena* L.) were used for the study.

Research was conducted using commercial cultivars of Solanaceae seeds of 3 firms: «Semena Ukrainy» (tomato cultivars «Grusha krasnaya», «De Barao krasnyy», «Yablonka Rossii», «Kobzar Tarasenko», paprika cultivars «Anastasia», «Zolotoy fazan», «Sladkiy pastuh», «Shorok-shary», «Veronika», eggplant cultivars «Long-pop», «Chornyy krasavets», «Almaz», «Aysberg»), «Flora market» (tomato cultivars «Kron prynz», «Dgyna», paprika cultivars «Orangevoe chudo», «Bogatyr», «Kolobok») and «Vesna» (tomato cultivars «Novichok», «Lahidnyy», «Efimer», «Lyana», paprika cultivars «Lumina», «Rotunda», «Krasnyy tsygan»).

Sowing qualities of seeds were carried in accordance with All Union State standard 12038–84 [17]. Seeds were analyzed for the presence of RNA viral antigens: *Alfalfa mosaic virus* (AMV, *Alfamovirus*), *Cucumber mosaic virus* (CMV, *Cucumovirus*), *Pepper mild mosaic virus* (PMMV, *Tobamovirus*), *Tobacco rattle virus* (TRV, *Tobravirus*), *Tomato mosaic virus* (ToMV, *Tobamovirus*), *Tobacco mosaic virus* (VTM, *Tobamovirus*), *Potato virus Y* (PVY, *Potyviridae*), *Tobacco ringspot virus* (TRSV, *Nepovirus*), *Tomatto ringspot virus* (ToRSV, *Nepovirus*) and *Pepino mosaic virus* (PepMV, *Potexvirus*), which are dominant on the plants of *Solanaceae* family.

Signification of viral antigens was conducted with «Indirect» and «sandwich» ELISA. Seeds were germinated for 7 days at room temperature (25°C) before carrying out serological tests. Sterile Petri dishes and water, which was filtered through a bacterial filter (25 microns) to avoid contamination by extraneous micro flora, were used for germination of seeds.

Germinated seeds were homogenized in mortars in phosphate buffer (0.1 M PBS, pH 7.4) at a ratio of 1:4 (m/v). Homogenate had been centrifuged at 5000 rpm for 20 min. at 4°C for cleaning material from plant components. Supernate was used for the determination of viral pathogens. Morphology of purified vibrions was assessed under electron microscope JEM–1400 using 2% uranyl acetate as contrasting agent.

In addition, the determination of viral pathogens was conducted in water samples

where seeds were soaked during the germination. This test was done to identify viral pathogens transmitted by seeds not only in the embryo, but also on surface structures (testa). When setting ELISA we used diagnostic antiviral serums (Aschersleben, Germany) and commercial polyclonal test systems (Loewe Biochemica, Germany) following the manufacturer's recommendations. The results were read at the wavelength of 405/630 nm using microplate reader Termo Labsystems Opsis MR (USA) with software Dynex Revelation Quicklink.

RESULTS AND DISCUSSION

Solanaceae seeds of 25 varieties were tested on a wide range of viruses. Research showed that among all seeds samples there were no viral antigens to AMV, PMMV, TRV, VTM, PVY, TRSV, ToRSV and PepM.

The presence of viral antigens to CMV in tomato cultivar «Yablonka Russii» and complex infection of CMV and ToMV in cultivar «De Barao Krasnyy» was revealed. 11 commercial varieties of paprika seeds were checked and 2 of them were found to be infected by CMV. Those were cultivars «Anastasia» and «Sladkiy pastuh». ToMV was identified in seeds of paprika cultivar «Rotunda». CMV was detected in one of 5 studied cultivars of eggplant. That was cultivar «Almaz».

Determination of viral pathogens was conducted in water samples where seeds were soaked during the germination. Antigens of CMV were identified in water samples of seeds of tomato cultivars «De Barao krasnyy», «Yablonka Rossii», paprika cultivars «Anastasia», «Sladkiy pastuh» and eggplant cultivar «Almaz». Overall, these results indicate that CMV was identified both in the embryo and on the seed surface structures.

The second set of analyses was to examine the impact of phytoviruses on sowing qualities of infected seeds of different cultivars, namely, laboratory germination, seed vigor, germinative power and humidity. Comparison of healthy and infected seeds sowing qualities was conducted to reveal the impact of viruses on sowing qualities of seeds.

It can be seen from the data in Table that the worst sowing qualities of tomato seeds revealed cultivar «Kron prynts». The best laboratory germination had cultivar «Dgyna». Infected and healthy seeds of tomato had the same laboratory germination of 83–84%. This indice of healthy paprika seeds was 61–98%, and of infected ones it was 71–84%. Laboratory germination of healthy eggplant seeds was 74–85%, and of infected ones (cultivar «Almaz») it was 83%. As we can see, viral infection had no impact on laboratory germination of tomato and eggplant seeds, but decreased it for paprika seeds.

Seed vigor of healthy tomato seeds, except of cultivar «Kron prynts», was 76–95%, while of infected ones it was 69–70%. This measure of healthy eggplant seeds was higher in comparison with infected. There were no significant differences found between results in seed vigor of healthy and of infected paprika seeds. Overall, this research has found that generally viral infection decreased seed vigor of *Solanaceae* family seeds.

Germinative power of healthy tomato seeds, except of cultivar «Kron prynts», was 75–86%. Infected tomato seeds had lower measure of germinative power, it was 65–67%. Germinative power of healthy paprika seeds was 56–94%, but of infected ones (cultivars «Anastasia», «Sladkiy pastuh», «Rotunda») it was 59–69%. Germinative power of healthy eggplant seeds was 76–81%, and of infected ones it was 66%. The deformation of leaves and their lag in growth were observed comparing infected and healthy seeds.

Duration of seed storage depends on its humidity. High humidity of seed increases its respiration. As a result it decreases its sowing qualities. There are different standards of seed humidity for different zones. For warm and dry regions, like Ukraine, seed humidity is 14%. It can be seen from the data in Table 1 that, humidity of examined seeds was 10.0–13.5%. Viral infection didn't have significant impact on this property.

CONCLUSIONS

The results of this investigation showed the presence of viral antigens in seeds of some

Sowing qualities of seeds

Crop	Cultivar	Germinative power, %	Seed vigor, %	Humidity, %	Laboratory germination, %
tomato	“De Barao Krasnyy”	65	69	12.0	84
tomato	“Yablonka Rossii”	67	70	10.0	83
tomato	“Kobzar Tarasenko”	83	84	11.0	91
tomato	“Grusha krasnaya”	81	78	13.0	85
tomato	“Novichok”	76	79	11.0	83
tomato	“Lahidnyy”	77	76	12.0	80
tomato	“Efimer”	86	83	13.0	88
tomato	“Lyana”	75	81	13.4	86
tomato	“Kron prynts”	56	59	12.3	61
tomato	“Dgyna”	90	95	13.0	98
paprika	“Anastasia”	59	64	11.0	71
paprika	“Sladkiy pastuh”	69	76%	12.0	84
paprika	“Rotunda”	68	79%	10.0	80
paprika	“Zolotoy fazan”	82	84	10.0	88
paprika	“Lumina”	70	69	10.0	79
paprika	“Shorok shary”	94	91%	13.5	96
paprika	“Veronika”	89	86%	11.5	92
paprika	“Krasnyy tsygan”	64	61%	10.8	66
paprika	“Orangevoe chudo”	56	69%	12.3	61
paprika	“Bogatyr”	90	93%	13.0	98
paprika	“Kolobok”	82	83%	12.0	78
eggplant	“Almaz”	66	68	9.7	83
eggplant	“Long-pop”	81	77	12.0	83
eggplant	“Chornyy krasavets”	76	81	12.6	85
eggplant	“Aysberg”	80	74	10.7	81

cultivars of paprika, tomato and eggplant. It was shown that the CMV had the highest dispersion. Antigens of CMV were identified in seeds of tomato cultivars «De Barao Krasnyy», «Yablonka Rossii», paprika cultivars «Anastasia», «Sladkiy pastuh» and eggplant cultivar «Almaz». CMV was identified both in the embryo, and on the seed surface structures. ToMV was revealed in tomato cultivar «De Barao Krasnyy» and paprika cultivar «Rotunda». This research has found the impact of pathogens on sowing qualities of seeds. These results suggest that viral infection had no impact on laboratory germination, but decreased seed vigor and germinative power.

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