Survey for the Four Major Viruses in Potato 'Poluranka' Local Cultivar in Herzegovina

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Summary

Potato production is directly influenced by various biological factors among which the most important viruses take the place. There are more than 20 known viruses that affect potato causing malformation in growth and yield reduction. In order to preserve the native potato cultivar 'Poluranka', which is historically dominant cultivar in the mountainous region of Herzegovina (western part of BiH), it is necessary to take phytosanitary measures. Many other indigenous plant genetic resources are gone forever from this region and this cultivar should not share the same fate. A main aim of this research was identification of some viruses that cause infections on this native potato tuber since there are no such official results. The first step in achieving this was testing the existing plant material for the presence of four economically most important potato viruses. The paper presents the results of DAS ELISA on samples taken from two locations in Herzegovina (Jasenica and Rakitno) that showed 100% infection with viruses PLRV and PVY on all 180 collected samples of seed tubers of 'Poluranka'. Until this research, health quality of 'Poluranka' was not tested and adequate measures to preserve this useful breeding material were not taken. Propagation *in vitro* should be used to gain healthier propagation material for better yield, preservation and maintenance of Herzegovina native potato 'Poluranka'.

Key words

Herzegovinian native potato cultivar, 'Poluranka', viruses, ELISA

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Introduction

Potato is widely spread crop in the world and has important place in agriculture of Bosnia and Herzegovina. Modern cultivars are mainly used today in production and processing. Before the introduction of modern cultivars local Herzegovinian potato 'Poluranka' was predominant in potato production of the hilly and mountainous region. Introduction of modern high productive varieties supplanted this variety from the production. Herzegovina native potato cultivar 'Poluranka' has good nutritive value with higher content of dry matter and starch in comparison with mostly cultivated commercial varieties in Herzegovina (western part of BiH) (Beljo et al., 2006). Currently, 'Poluranka' is rarely grown and it is almost extinct cultivar due to its high susceptibility to the pests and diseases.

Potato production is directly influenced by various biological factors among which the most important viruses take the place. There are more than 20 known viruses (Van der Zaag, 1996., Gavran, 1997, van Baarlen et al., 2005) on potatoes that influence growth and reduce yield up to 50 % (Milošević, 2009). As potato is a vegetative propagated crop, degeneration due to the viruses is more likely to occur and infection increases through subsequent generations (Milošević, 1989).

Earlier studies (Mujković et al.. 2009) have found that PVY (*Potato Y potyvirus*), followed by PLRV (*Potato leafroll virus*) and PVX (*Potato X potexvirus*) have been most common viruses (detected by ELISA technique) on tubers from second reproduction of potato seed in central part of Federation of Bosnia and Herzegovina. Vukojević et al. (2015) found four economically harmful viruses (PLRV, PVX, PVY and PVA (*Potato A potyvirus*)) on 69 tested samples of seed potatoes of cultivar 'Desire' collected in 2014 in area of Travnik and Bugojno (central part of BiH). Results of infection showed different presence of investigated viruses, where the most abundant was PVY (98.55%), followed by PLRV (10.14%) and PVA (1.44%), while PVX was not detected. These are the only official data about virus presence on potato seed cultivar obtained in BiH.

Symptoms, as a result of the virus presence in potato plant depend on the virus strain, potato cultivar, inoculation time (primary and secondary infection), and environmental conditions (for example temperature) and other factors (Panjan, 1967). PLRV symptoms mainly appear on the leaves, rarely on tubers. The mature leaves curl inwards and young one wraps on the leaf top (primary symptoms). Necrosis could appear on phloem system or on the tube intersection as internal "net" necrosis (primary and secondary infection) (Douglas and Pavek, 1972). Generally, typical symptoms of PVY show leave lesions, abnormal colors and leaf formations, external discoloration of the stems and vegetative organs, and the whole plant has reduced growth. The PVY symptoms on *Solanum tuberosum* are weak to mild leaf mosaic, or strip type necrosis, or necrosis 'leaf-drop streak' (Beemster and Rozendaal, 1972). PVX symptoms on affected plant cause mild mosaic necrosis and leaf wrinkling, or acute leaf tip necrosis usually followed by plant death. Infection on potato and tomato is especially visible when PVX is present together with other viruses (Smith, 1931; Murphy and McKay, 1932); the presence of PVX is associated with a significant increase in the replication of potato Y potyvirus (Stouffer and Ross, 1961). Symptoms of PVA show weaker and rarely stronger mosaics chlorosis alternately replaced with light yellow and dark green surfaces. Appearance of leaf wrinkling with leaf curling of margins is visually noticeable. In a case of mixed infections by X and Y viruses, there is a synergy and the symptoms are more expressed (Van der Zaag, 1996; Baarlen et al., 2005). Virus infection is sometimes hard to recognize by visual identification so it is not reliable method regardless on some typical marks, and the best way of detection is serological analysis as a confirmation method.

In this study, the testing of 'Poluranka' seed tubers for the presence of economically most important viruses PLRV, PVY, PVX and PVA was done by ELISA. This will allow identification and preservation of virus free planting material required for further reproduction and preservation of potato 'Poluranka'.

Materials and methods

Sample collections and field inspections were done during 2009, in two main potato growing areas of Herzegovina, Jasenica (plain area) and Rakitno (mountain area). Hundred seed tubers were collected from Jasenica and 80 tubers from Rakitno. Preparation of tubers for the analysis included washing and tuber germination. Germination was carried out under controlled conditions in dry oven (SANYO MLR 351) at temperature of 25°C, air humidity 70%, in dark for two weeks. After two weeks the sprouts emerged on tubers in appropriate size for the analysis. Seed tubers were analyzed on the presence of PLRV, PVY, PVX, and PVA by DAS-ELISA (Clark and Adams, 1977). Serological reactants were provided by commercially purchased kits (BIOREBA, Reinach, Switzerland), and tests were conducted according to the manufacturer's instructions. A portion of seed tuber (0.5 g) was grounded in extraction buffer at a 1:20 ration. We have analyzed samples according to NAK tables (Conversion table Testing Protocol Duch General Inspektion Service - NAK - Nederlandse Algemene Keuringsdienst, the Dutch General Inspection Service for Agricultural Seed and Seed Potatoes) (Toussaint et al., 2005). Well on microtiter plate had samples from sprouts of four different tubers. Absorption value for samples were measured using a Tecan Sunrise spectrophotometer (software MAGELLAN™ 6, Männedorf, Switzerland) at 405 nm wavelength 30 and 120 min after adding the substrate (*p*-nitrophenilphosphate). Reactions three times superior to healthy control were considered positive. The NAK tables were used for calculation the percentage of infected seed tubers (Toussaint et al., 2005).

Results and disscusion

In our study on 'Poluranka' we found the same viruses as was previously reported for central BiH (Vukojević et al., 2015). ELISA test of all 180 seed tubers of 'Poluranka' taken from two locations in Herzegovina (Jasenica and Rakitno) showed 100% infection with viruses PLRV and PVY. PVX was found in 2% of samples from location Jasenica, whereas no presence of PVA was found for both locations. Severe virus infection found in 'Poluranka' was expected since this cultivar have been produced and maintained in extensive production and there has not been any organized production of healthy potato seed tuber. Use of virus free tuber is of great importance for production (Radcliffe and Ragsdale, 2002).

Results of our analysis of local cultivar 'Poluranka' are in agreement with previous reports on PLRV and PVY infection of modern commercial cultivars cultivated in Federation of Bosnia and Herzegovina (Mujković et al., 2009; Vukojević et al., 2015). Native potato seed has been grown in high mountain area in an organic oriented system production that enables its good quality and preservation for longer period. The forested belts and biodiversity of growing area have been serving as a buffer from climatic changes and as suppressor of pests and diseases impact. Landscape diversity or complexity, and proximity to semi-natural habitats tend to produce a greater abundance and species richness of natural enemies (Bianchi, et al., 2006). According to Milošević (2009) only uninhabited areas mainly at higher altitudes may be considered as locations of low virus infection pressure. Production of 'Poluranka' today is at a neglected level so this cultivar is endangered with tendency to complete extinction. As we have not found the healthy seed material on today's growing area we suggest *in vitro* production for getting healthy material. Healthy plant material is needed for commercial production of basic and certified 'Poluranka's' tuber seed. When it is not possible to find even one healthy plant of particular genotype or potato variety, we should try to "cure it" with *in vitro* method (meristem culture), followed by multiplication with nodal explants (cuttings) (Milošević, 2009). By *in vitro* micropropagation of healthy plant material in a short time on the small area (such as growth chambers, screen house, ect.) a large number of healthy, virus-free plants can be produced (Milošević, 2009). Preservation of local potato 'Poluranka' is interesting due to its nutritive characteristics and good quality. Potato seed tuber of 'Poluranka' is highly affected by viruses and it is difficult to find virus free material in production in Herzegovina.

EPPO certification scheme for seed potatoes PM 4/28(1) (EPPO/OEPP, 1999) suggests testing of potato tuber on 17 viruses: PVA, *Potato M carlavirus* (PVM), *Potato S carlavirus* (PVS), PVX, PVY, PLRV, *Alfalfa mosaic alfamovirus* (AMV), *Cucumber mosaic cucumovirus* (CMV), *Potato aucuba mosaic potexvirus* (PAMV), *Potato mop-top pomovirus* (PMTV), *Potato V potyvirus* (PVV), *Tobacco mosaic tobamovirus* (TMV), *Tobacco necrosis necrovirus* (TNV), *Tobacco rattle tobravirus* (TRV), *Tomato black ring nepovirus* (TBRV), *Tomato mosaic tobamovirus* (ToMV), *Tomato spotted wilt tospovirus* (TSWV) and one viroid *Potato spindle tuber viroid* (PSTVd). However, these analyses are conducted in accordance with the Regulations on mandatory health examination of crops and objects, seeds and planting material of agricultural and forest plant ("Official journal SFRJ" 52/86 & 3/87), which is taken over by decree with law force ("Official journal BiH" 2/92 & 13/94), and by which there is the obligation of testing potato tuber on four viruses: PLRV, PVY, PVX and PVA.

Conclusions

Presented results are the first official finding of PLRV and PVY infection of Herzegovina native potato cultivar 'Poluranka'. Further analysis and detection of potato viruses should be done subsequently. Testing of potato seed (tuber) in production is necessary to gain healthy propagation material of good quality. Introduction of phytosanitary measures is the first step to prevent virus spreading mechanically during seed manipulation, cutting and preparation for germination. Use of insecticides against vectors is also useful. *In vitro* propagation, i.e. meristem cultivation, and regeneration should be used to gain healthier propagation material for better yield, preservation and maintenance of local potato cultivar 'Poluranka'.

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