Detection of the crucial potato viruses in different potato tissues by immunological and molecular methods

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INTRODUCTION

To keep cultivars identity, humans propagate the potato crop vegetatively. Therefore, once a virus enters the plant, it accumulates in subsequent generations. Frequent replacement of seed potatoes is the best way to reduce viral infections. Production of healthy seed tubers involves obligatory testing for most critical viral pathogens including Potato virus Y (PVY, Y), potato leaf virus (PLRV, L) and potato virus M (PVM, M). For this purpose, Plant Health Inspectorates use a grow-out test. It consists of cutting out eye plugs from tubers, breaking dormancy, sprouting, planting the sprouted eye plugs in the greenhouse and examining the leaves collected from offspring plants bye the ELISA test. The procedure is beneficial, but requires a lot of time and is expensive. These pitfalls can be withdrawn by detecting viruses directly in tubers (Treder et al. 2009). Unfortunately, Hill and Jackson (1984) showed that the sensitivity of the ELISA test is not sufficient for this purpose. An alternative to the grow-out test may be the sprout test developed for PLRV by Syller (1988). Another option is RT-PCR assay (Singh et al. 1995, Boonham et al. 2008). This approach is sensitive but costly. Therefore, the low-cost variant of RT-PCR has been proposed (Zacharzewska et al. 2014). Isothermal RT-LAMP assay offers another molecular approach for sensitive virus detection (Treder et al. 2018). However, the efficacy and reliability of both molecular assays have to be compared to routine tuber evaluation by the grow-out test.

MATERIAL AND METHODS

Three potato families selected for this purpose by independent contractors on the basis of different susceptibility to viral infections were subjected to the study comparing the detection of viruses in sprouts with detection in tubers. For comparison, the same potato families were tested by means of an eye test. Healthy seed potatoes were planted in the field in the presence of PVY, PVM, PLRV virus infectors. After 4 weeks of storage, a fragment of the hebone tissue was removed. The extracts obtained were tested by means of a cocktail-ELISA for the presence of potato viruses. Eyelets for sprouts and eyelets for eyelet plants were cut out. The presence of viruses in the extracts obtained from germs and from plants from eye samples was assessed by DAS ELISA. The results were compared with the results of the ELISA cocktail test performed directly on the heel end of the tubers. The compared results compare the effectiveness of virus detection in sprouts using DAS ELISA with the efficiency of detection in tubers using cocktail-ELISA and the efficiency of the eye test. Testing experience was also performed, whether real-time RT-PCR (RT-qPCR) and RT-LAMP can be adapted to detect Y potato potato directly in tubers and sprouts. From 30 tubers of three moderately susceptible potato varieties grown as above, RNA was isolated and RT-qPCR and RT-LAMP tests were performed. Sprouts obtained from the same tubers were tested with DAS-ELISA and with both molecular tests. The reference method to compare efficacy was the eye test.

RESULTS AND DISCUSSION

Table1. Comparison of virus detection by grow-out test to direct detection in tubers and sprouts.

2018	The Contractor I			The Contractor II			The Contractor III		
	Y	M	L	Y	M	L	Y	M	L
Tubers Number	146	146	146	90	90	90	90	90	90
Grow-out test	**96**	**59**	**0**	**60**	**2**	**0**	**62**	**27**	**1**
%	*65,8*	*40,4*	*0,0*	*66,7*	*2,2*	*0*	*68,9*	*30,0*	*1,1*
Tubers Cocktail ELISA	**31**	**98**	**0**	**35**	**0**	**0**	**28**	**26**	**1**
%	*21,2*	*67,1*	*0,0*	*38,9*	*0,0*	*0*	*31,1*	*28,9*	*1,1*
Compatibility	**23**	**57**	**0**	**35**	**0**	**0**	**27**	**19**	**1**
Sprouts Cocktail-ELISA	**125**	**85**	**0**	**61**	**0**	**1**	**62**	**17**	**0**
%	*85,6*	*58,2*	*0,0*	*67,8*	*0,0*	*1,1*	*68,9*	*18,9*	*0,0*
Compatibility	**88**	**52**	**0**	**60**	**0**	**0**	**62**	**15**	**0**
Sprouts DAS-ELISA	**116**	**79**	**0**	**60**	**0**	**0**	**62**	**22**	**0**
%	*79,5*	*54,1*	*0,0*	*66,7*	*0,0*	*0,0*	*68,9*	*24,4*	*0,0*
Compatibility	**85**	**52**	**0**	**60**	**0**	**0**	**62**	**17**	**0**

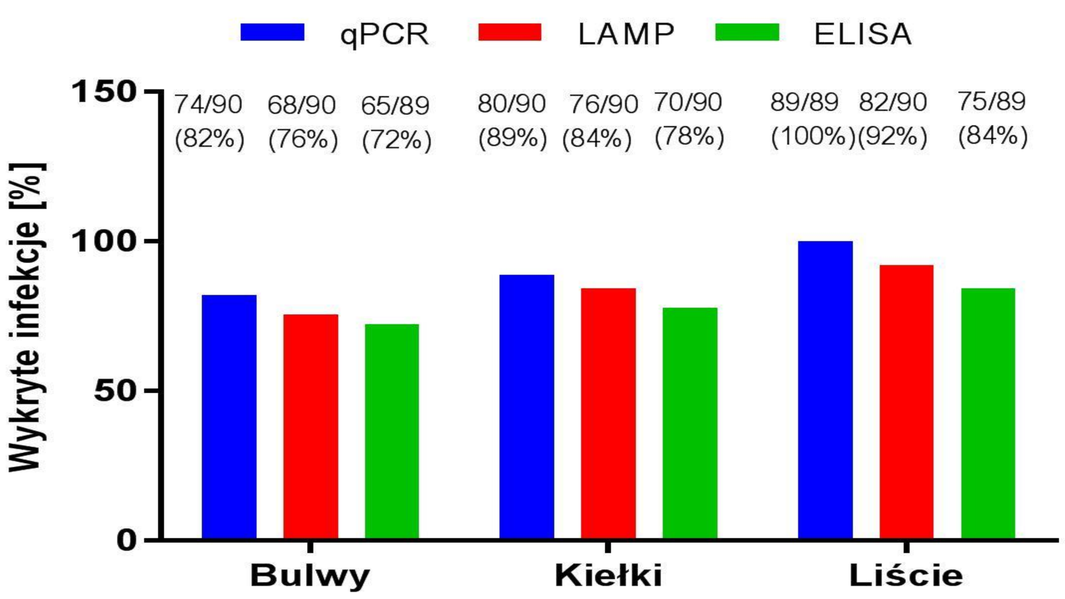

Fig. 1. Comparison of the effectiveness of potato Y virus detection in tubers, sprouts and plants obtained in the eye test (leaves) by ELISA, real-time RT-PCR (qPCR) and RT LAMP. Above the bars illustrating the percentage of plants positively diagnosed by a given test, the number of detections is given in relation to the total number of plants tested and the percentage of patients in brackets.

In 2018, all contractors detected high PVY infection in an eye test, over 60%. Contractor I detected 3 times more PVY paralysis in the eye test than in the tubers. On 31 tubers with the PVY 23 virus they gave the result identical to the eye test. Contractors II and III also found significantly less infection in tubers than in the eye test, with almost all of them compatible with the eye test. This is due to the dormancy of the tubers and the multiplication of the virus only in the leaves of daughter plants. All contractors detected a cocktail test on sprouts with more or the same amount of PVY infection than with an eye test. The test gave very good compliance and level of detection of PVY paralysis with the eye test at all contractors. As in 2017, the number of PVY detections and their agreement between the cocktail test and the eye test were very similar for contractors II and III. The DAS-ELISA on the sprouts also gave higher results than the eye test, but to a slightly lesser extent than the cocktail-ELISA. A lower PVM paralysis was found than in 2017. Moderately high PVM paralysis was noted by contractors I and III (respectively 40.4 and 30%) and very low by contractor II, only 2.2%. Contractor I has detected more PVM paralysis in tubers and sprouts than in the eye test. PLRV occurred in the experience of Contractor III in only 1 plant out of 90 tested. The results of the tuber and ocular test were consistent (the plant detected in the tuber test was also detected by the tuber test). Contractor II detected it only in sprouts of one plant. In 2018, RT-qPCR and RT-LAMP were tested for detecting viruses directly in tubers and sprouts (Fig. 1). Potato viruses and 30 tubers of three moderately susceptible potato varieties (Quincy, Fresco and Zeus) were selected as the model. This year, RNA was isolated from tubers using a 3-zone RNA isolation kit from Novazym Polska. The tuber tissue was ground in liquid nitrogen and suspended in 3-zone factor, further steps were carried out according to the manufacturer's instructions. RNA obtained from tubers was of high purity and quality, as assessed spectrophotometrically. The results obtained indicate that the most effective method of detecting PVY was RT-qPCR in both tubers, sprouts and leaves (Fig. 1). 100% infection detection efficiency was recorded in leaves, 89% in sprouts, and 82% in tubers using the RT-qPCR test.

CONCLUSION

By testing the detection of viruses in potato tubers and sprouts using a cocktail and the DAS ELISA test, good compliance of virus detection in sprouts using an eye test was confirmed. The cocktail ELISA was more effective than the DAS-ELISA for sprouts. Higher efficiency of PVM detection directly in tubers than in leaves by eye test was found. The RT-qPCR test was more effective in assessing leaf, embryo and tuber infections than RT-LAMP and DAS-ELISA. This leaf test showed the highest performance.

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