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Short communication

Application of nucleic acid aptamers for detection of Apple stem pitting virus isolates

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ABSTRACT

DNA aptamers (PSA-H and MT32) were applied for the detection of *Apple stem pitting virus* (ASPV) isolates using an Enzyme-Linked Oligonucleotide Assay (ELONA) and Western blot analysis. The specificity and effectiveness of aptamers were verified in comparison to a conventional Enzyme Linked Immunosorbent Assay (ELISA). A genetically diverse group of ASPV isolates was tested. The results showed that aptamer MT32 detected a wider range of ASPV isolates than an aptamer PSA-H and proved to be superior to commercially available monoclonal antibodies. Aptamer MT32 produced higher signal intensity in ELONA with a virus-infected plant extracts than antibodies in ELISA. Moreover, the ELISA method failed to detect ASPV in six samples. The results presented in this study indicated that aptamer MT32 can be used as a receptor molecule of various immunoassay protocols for ASPV detection.

Apple stem pitting virus (ASPV), a member of the genus *Foveavirus* in the family Betaflexiviridae [1], is one of the most common viruses of apple and pear trees worldwide. ASPV causes xylem pits in the stem of *Malus pumila* Virginia Crab as well as epinasty and decline of *Malus domestica* Spy 227, but remains symptomless in apple cultivars [2]. Sequence analysis of ASPV isolates revealed a significant level of genome heterogeneity among the isolates, which makes the reliable virus detection challenging [3–5]. Although PCR-related methods have become widely accepted molecular tools for virus detection [6–10], their application might be limited by a complicated procedure, the need for expensive sophisticated instruments and high reagent costs. Considering these drawbacks, detection of viruses with various immunoassay protocols seems to be preferred for screening a large number of samples [11]. Monoclonal antibodies have been produced for serological identification of ASPV isolates, and an Enzyme-Linked Immunosorbent Assay (ELISA) protocol has also been defined [12]. However, generation of highly selective antibodies remains one of the major bottlenecks of method development. In practice, antibody generation is laborious, time-consuming, and most important, could not circumvent the inherent limitations of antibody production. In the past decade, aptamers have been demonstrated to be viable alternatives to antibodies in many applications [13]. Aptamers are short (20–80 mer) single-stranded DNA or RNA sequences that interact with the target through their three-dimensional structures. They offer advantages over antibody-based affinity molecules due to their ease of production, purification, modification, physical stability, and lower cost [14,15]. Nucleic acid aptamers are selected in *in vitro* based on affinity for a target molecule, like proteins, bacteria, viruses, or cells using a molecular-based iterative enrichment method called systematic evolution of ligands by exponential enrichment (SELEX) [16]. So far, several high affinity DNA and RNA aptamers have been successfully selected against viral proteins or whole virus [17–22]. The first aptamers named MT32 and PSA-H against the virus coat protein of two ASPV isolates (MT32 and PSA-H), whose amino acid sequences show 81% identity, were selected previously. The aptamers decorated both native and denatured proteins and recognized specifically ASPV in crude plant extracts [23].

In this study, the application of aptamers PSA-H and MT32 for ASPV detection by Enzyme-Linked Oligonucleotide Assay (ELONA) and western blotting method is described.

The ASPV isolates originated from an apple and pear cultivar collection maintained at the Research Institute of Horticulture and from commercial orchards in Poland (Table 1). They were selected on the basis of the symptoms (only for pear), the results of biological indexing, and RT-PCR. The isolates representing three groups showing different CP gene length [24,25] have been used in the study. Additionally, two virus isolates MT32 (AF438521) and PSA-H (D21828), the targets for aptamer selection, were used in all experiments. In all cases, healthy plants (apple and pear) and ASPV positive control (Bioreba AG, Switzerland) were included as negative and positive controls, respectively. Although, most of the selected trees were co-infected with other viruses

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Table 1
Binding of aptamers PSA-H and MT32 to ASPV isolates from plant extracts in comparison to the conventional enzyme linked immunosorbent assay (ELISA).

Isolate	Source	Number of deletions in CP gene	Co-infection with other viruses	ELISA (T/N)[a]	ELONA PSA-H (T/N)[a]	ELONA MT32 (T/N)[a]
641	apple	0	ACLSV ASGV	4.8 ± 0.8	4.5 ± 0.3	5.4 ± 0.6
673	apple	0	ACLSV ASGV ApMV	2.9 ± 0.3	2.9 ± 0.3	9.9 ± 0.4
633	apple	0	ACLSV ASGV ApMV	3.0 ± 0.6	3.0 ± 0.3	7.4 ± 0.2
1178	apple	0	ACLSV ASGV	2,7 ± 0.2	2.5 ± 0.4	7.3 ± 0.5
676	apple	0	ASGV	2,6 ± 1.7	2.4 ± 5.1	9.4 ± 0.4
637	apple	0	ACLSV ASGV	3,0 ± 1.3	1.9 ± 0.2	2.5 ± 0.2
631	apple	1	ACLSV ASGV ApMV	2.4 ± 0.9	4.3 ± 0.2	11.3 ± 1.1
626	apple	1	ACLSV ASGV ApMV	4.2 ± 1.0	2.6 ± 0.2	6.1 ± 0.4
632	apple	1	ACLSV	2.7 ± 0.4	4.1 ± 0.8	4.7 ± 0.6
671	apple	1	ACLSV ASGV	3.1 ± 0.1	12.1 ± 0.3	15.1 ± 1.4
672	apple	1	ACLSV ASGV	0.9 ± 0.2	2.6 ± 0.3	9.4 ± 0.3
609	apple	1	ACLSV ASGV	3.2 ± 0.1	3.1 ± 0.3	7.2 ± 0.5
1179	apple	1	ACLSV ASGV	3.1 ± 0.1	2.5 ± 0.2	4.9 ± 0.2
MT32	apple	1	ACLSV ASGV	2.3 ± 0.3	2.7 ± 0.4	10.4 ± 0.8
PSA-H	pear	0	–	2.9 ± 0.3	5.7 ± 0.2	10.1 ± 0.7
2458	pear	0	–	3.4 ± 1.5	4.2 ± 0.2	15.1 ± 1.4
IV_51	pear	0	ACLSV	5.2 ± 1.2	4.9 ± 0.2	11.4 ± 0.8
VII_15	pear	0	–	3.6 ± 0.2	2.5 ± 0.1	11.7 ± 0.6
II_41	pear	0	ACLSV	6.2 ± 1.4	4.1 ± 0.7	3.0 ± 0.2
II_14	pear	0	–	1.6 ± 0.3	3.8 ± 0.3	10.9 ± 0.9
VIII_25	pear	0	–	4.4 ± 0.4	4.3 ± 0.2	10.4 ± 0.3
VI_46	pear	0	–	6.4 ± 0.2	2.1 ± 0.6	8.8 ± 0.6
VIII_49	pear	0	ACLSV	1.7 ± 0.1	3.2 ± 0.2	5.9 ± 0.2
VIII_15	pear	0	–	2.7 ± 0.4	4.3 ± 0.5	4.9 ± 0.3
III_82	pear	0	ACLSV	3.8 ± 0.1	2.1 ± 0.5	7.8 ± 0.9
VI_52	pear	0	ACLSV	1.5 ± 0.1	2.9 ± 1.4	6.3 ± 1.0
VIII/19	pear	0		2.1 ± 0.3	9.5 ± 0.3	10.1 ± 0.4
II_61	pear	0	–	5.2 ± 1.1	2.7 ± 0.4	9.8 ± 0.1
E_17	pear	0	–	4.2 ± 0.1	4.3 ± 0.3	12.2 ± 0.2
IV_28	pear	0/2	ACLSV	2.4 ± 0.8	5.3 ± 0.2	8.9 ± 0.1
V_47	pear	0/2	–	2.8 ± 0.7	3.0 ± 0.4	9.0 ± 0.1
VI_30	pear	0/2	–	4.1 ± 0.1	2.7 ± 0.3	6.6 ± 0.3
V_20	pear	1	ACLSV	2.1 ± 0.1	2.4 ± 0.2	7.4 ± 0.3
VIII_26	pear	1	–	1.8 ± 0.3	5.2 ± 0.4	13.7 ± 1.5
II_52	pear	1	ACLSV	5.1 ± 0.2	2.4 ± 0.3	9.3 ± 0.3
VII_55	pear	1	–	3.4 ± 0.4	3.3 ± 0.2	10.1 ± 0.4
III_100	pear	1	ACLSV	4.1 ± 0.8	2.4 ± 0.2	6.9 ± 0.2
I_15	pear	1	–	2.3 ± 0.1	3.2 ± 0.3	8.5 ± 0.1
VI_28	pear	1	–	3.0 ± 0.3	2.8 ± 0.2	6.1 ± 0.4
VIII_99	pear	1	–	3.5 ± 0.8	3.2 ± 0.5	10.5 ± 1.0
II_21	pear	1/2	ACLSV	1.6 ± 0.5	2.5 ± 0.6	9.5 ± 0.1
I_16	pear	2	–	2.7 ± 0.3	4.5 ± 0.4	7.5 ± 0.8
IV_23	pear	2	–	4.7 ± 0.4	8.9 ± 0.5	8.2 ± 0.7
I_63	pear	2	–	5.0 ± 0.3	5.7 ± 0.1	8.1 ± 0.5
V_2	pear	2	–	4.1 ± 0.8	4.1 ± 0.4	8.5 ± 0.2
V_24	pear	2	–	6.6 ± 0.5	2.5 ± 0.2	6.3 ± 0.4
V_74	pear	2	–	3.9 ± 0.1	2.7 ± 0.3	5.7 ± 0.1
K+ Bioreba Kit	–	–	–	5.1 ± 0.4	2.5 ± 0.4	2.7 ± 0.5
K-	apple	–	–	1.0 ± 0.2	1.3 ± 0.3	0.9 ± 0.3
K-	pear	–	–	1.2 ± 0.2	1.4 ± 0.6	1.1 ± 0.4
ACLSV	apple	–	–	1.4 ± 0.4	1.2 ± 0.4	1.5 ± 0.3
ApMV	apple	–	–	1.4 ± 0.2	1.3 ± 0.3	1.2 ± 0.5
ASGV	apple			1.3 ± 0.2	1.1 ± 0.3	1.1 ± 0.1

[a] Values indicate the ratio of absorbance readings for the test sample (T) versus negative control (N) using ELONA and ELISA. All data shown were calculated as the mean ± SD and data were from three independent experiments.