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Induction of Ecological Resistance to Potato Virus Y in Potato Varieties through Silencing of Main Viral Genes

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Abstract

Outbreaks of potato virus Y (PVY) have a serious negative impact on agricultural production, leading to significant losses. The need for an effective PVY control technique is essential to reduce yield losses in agriculture. Plants use a defense mechanism called RNA silencing, which was used to control PVY. Post-transcriptional gene silencing (PTGS) of major viral genes was induced by specific siRNAs that trigger specific degradation of viral RNA by forming a multi-component RNA-induced silencing complex (RISC). By using PTGS, resistance of potato varieties to PVY was achieved.

Keywords: PVY, PTGS, RNA silencing, siRNAs, potatoes, resistance

Резюме

Епидемиите от картофения вирус Y (PVY) имат сериозно отрицателно въздействие върху селскостопанската продукция, което води до значителни загуби. Необходимостта от ефективна техника за контрол на PVY е от съществено значение за намаляване на загубата на добив в селското стопанство. Растенията използват защитен механизъм, наречен РНК заглушаване, който ние използвахме за контрол на PVY. Ние индуцирахме посттранскрипционно генно заглушаване (PTGS) на основни вирусни гени чрез специфични миРНКи, които индуцират специфична деградация на вирусната РНК чрез формиране на многокомпонентен РНК-индуциран заглушаващ комплекс (RISC). Чрез използването на PTGS постигнахме устойчивост на картофени сортове спрямо PVY.

Introduction

Potyviruses are the largest group of plant viral pathogens that are constantly expanding their territories and causing damage to their cultural hosts around the world. Outbreaks of PVY have a serious negative impact on agricultural production, leading to significant losses. The need for an effective PVY control technique is essential to reduce yield losses in agriculture.

Plants use a protective mechanism called RNA silencing, which can be used to target potyviruses. RNA gene silencing, of which post-transcriptional gene silencing (PTGS) is a part, is a general term describing the related gene regulatory mechanisms driven by RNA in plants (Vance and Vaucheret, 2001). The first cases of gene silencing were reported with transgenic plants carrying artificially introduced transgenes (Napoli *et al*., 1990). The process is initiated by double-stranded RNAs (dsRNAs) - molecules that are produced during viral replication. The double-stranded RNAs are recognized by the plant as „non-self“ and subsequently cleaved by cellular Dicer-like enzymes, forming small interfering RNAs (siRNAs) with a length of 21-25 bp (Hammond *et al*., 2000). These siRNAs are a major component of RNA gene silencing (Denli and Hannon, 2003). They initiate complement-specific RNA degradation by forming a multi-component cell complex (RISC), inducing RNA gene silencing, which destroys related mRNAs (Martinez *et al*., 2002).

A remarkable feature of RNA gene silencing is its ability to propagate both from cell to cell and over long distances by inducing systemic RNA silencing throughout the body. This happens through

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complement-specific signal silencing obtained after induction of RNA gene silencing in single cells. In response, plant viruses encode proteins capable of suppressing RNA gene silencing (Mlotshwa *et al.*, 2002).

Material and Methods

Virus for plant inoculation: PVY $^{N/NTN}$ virus strain

Host plants: 15 pots with potatoes cv. Marabel

Inoculation of plants with virus

One gram of leaf mass of a plant with clear symptoms was homogenized in 1 ml, cooled to 4°C 0.1M, potassium-sodium phosphate buffer, pH 8.0, containing 0.2% Na_2SO_3 and 0.2% ascorbic acid. Inoculations were performed by lightly rubbing the leaves with this homogenate. After 3-5 minutes the plants were washed with water. The next day they were imported into the phytostat. Symptoms were reported 7-25 days after inoculation, depending on the species (Petrov, 2015).

Total RNA extraction

RNEasy Plant Mini Kit (Qiagen, Germany) was used. Extraction was performed according to the instructions of the manufacturer.

In vitro system for production of double-stranded RNA (dsRNA)

dsRNA was synthesized by a combination of in vitro transcription and DNA template replication (according to the instructions of the Replicator RNAi Kit, Finnzymes, Finland). The DNA template for dsRNA synthesis was obtained by PCR reaction using Phusion High-Fidelity DNA polymerase. The PCR primers were designed so that the resulting PCR product contained our target sequence (PVY HC-Pro) surrounded by a T7 promoter sequence at its 5′ end and a phy6 qRdRP promoter sequence at its 3′ end. The PCR product was purified and transcribed into single-stranded RNA (ssRNA) by T7 viral RNA polymerase. This ssRNA was replicated in dsRNA from phi6 qRdRP viral replicase. The sequences of the constructed primers were: HC-Pro dsRNA 1 (5‘-TAA TAC GAC TCA CTA TAG GG TAG GAT TCT GTC GAA TGC CGA CAA TTT T -3‘), HC-Pro dsRNA 2 (5‘-GGA AAA AAA TAC TGC AGA CCA ACT CTA TAA TGT TT -3‘) (Petrov *et al.*, 2015).

Detection of viral infection by DAS-ELISA

A kit from LOEWE Biochemica GmbH, Sauerlach, Germany, was used. ELISA plates were loaded with antiserum (IgG) for PVY, previously diluted according to the manufacturer‘s instructions in 0.05M carbonate buffer. They were incubated for 4 hours at 37°C and the unbound components were washed three times with PBS-T buffer for 5 minutes. All samples were ground in extraction buffer containing 1% PVP (polyvinyl pyrrolidone) in a ratio of 1:10. The plaques were left for 16 hours at 4°C. After triple washing, alkaline phosphatase conjugate for PVY was added and the plates were incubated for 4 hours at 37°C. The substrate used was para-nitrophenyl phosphate (p-nitrophenyl phosphate, Sigma) in diethanolamine buffer (pH 9.8) at a ratio of 1 mg/1 ml. The reaction was carried out in the light and at room temperature, 3N NaOH was used to stop the reaction. Color adsorption was measured on a Multifunction Detector (DTX 880) at a wavelength of 405 nm. The samples for which the optical density (OD, absorption) was more than twice the value of the negative control (called cut-off value or Cut Off) were accepted as positive (Petrov *et al.*, 2015).

Results and Discussion

In the present study, post-transcriptional gene silencing (PTGS) of a major multifunctional viral gene such as HC-Pro was induced, using specific dsRNAs that initiate specific degradation of viral RNA by forming a multicomponent RNA-induced attenuation complex (RISC). By using PTGS, potato resistance to PVY was achieved (Fig. 1).

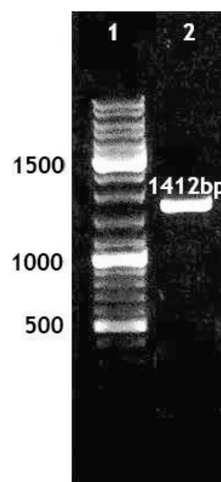

Fig. 1. PVY HC-Pro dsRNA

None of the potato plants treated with HC-Pro-dsRNAs and inoculated with PVY $^{N/NTN}$ developed visual symptoms of disease. The optical density (OD) values of the plant samples from these pots remained below the limit value and were close to healthy control plants. The DAS-ELISA value (0.4) of one of the treated plants was close to the limit value, but was negative. Symptoms of infection in this pot were not observed either.

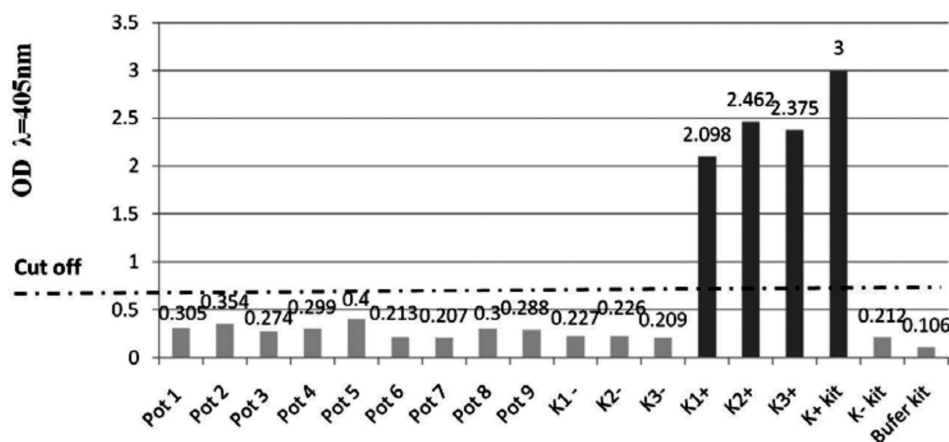

Fig. 2. DAS-ELISA assay of potato plants after treatment with dsRNA for HC-Pro of PVY $^{N/NTN}$ and after inoculation with PVY $^{N/NTN}$. Samples 1-9: pots of potato plants treated with HC Pro-dsRNAs and inoculated with PVY $^{N/NTN}$; K1-, K2-, K3-: healthy potato plants not treated and not inoculated with virus; K1+, K2+, K3+: potato plants inoculated with PVY $^{N/NTN}$ and not treated with HC-Pro-dsRNAs; K+ kit: positive control by ELISA kit; K- kit: negative control by ELISA kit)

High OD values were obtained for potato leaf samples inoculated with PVY $^{N/NTN}$ (K +) only, indicating successful viral infection with PVY $^{N/NTN}$ (Fig. 2).

Treatment of plants with dsRNAs specific for the HC-Pro gene region of PVY $^{N/NTN}$ resulted in the growth of healthy plants that were not prone to developing disease symptoms of PVY $^{N/NTN}$ (Fig. 2, pots 1-9). The strategy applied in this study to control viral infection was based on knowledge of the mechanisms of PVY replication and the protective responses of plants against pathogens, assuming that blocking a key viral protein for virus replication and suppressing PTGS will lead to blocking the development of the infection process itself. Gene silencing has been successfully induced against PVY also in tobacco plants and in potato plants of Arinda variety through the use of specific dsRNAs and siRNAs for the HC-Pro region of PVY, which effectively reduced the systemic spread of the virus (Petrov and Stoyanova, 2011; Petrov, 2012). Decreased expression of the PVY ^{N} HC-Pro gene was found in newly grown potato leaves and therefore, decreased viral replication in all virus-inoculated plants. Old PVY leaves inoculated with potato plants remained infected and later defoliated. All new leaves of potato plants inoculated with PVY of Arinda variety that sprouted after treatment with dsRNA and siRNA remained healthy and virus-free (Petrov *et al.*, 2015).

Conclusion

Epigenetic control of viral infection was achieved by induction of post-transcriptional gene attenuation in Marabel variety potato plants by specific siRNAs targeting the HC-Pro gene region of PVY $^{N/NTN}$. This strategy effectively blocks the systemic spread of the virus and inhibits viral replication in host plants.

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