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Short Communication

Reduction of Potato Virus Y Damage on Pepper Plants through Systemic Acquired Resistance

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Abstract

Systemic Acquired Resistance (SAR) against Potato Virus Y (PVY) was induced in pepper plants using natural elicitors based on salicylic acid. Treatment of pepper plants with elicitors induced SAR against PVY infection, which resulted in a reduction in DAS-ELISA values and viral symptoms in plants caused by PVY. Treatment of pepper plants with a combination of two elicitors 3 days before virus inoculation induced strong PVY resistance in the experimental pepper plants.

Keywords: PVY, SAR, pepper, salicylic acid, treatment, resistance

Резюме

Индуцирахме Системна Придобита Устойчивост (SAR) срещу картофения вирус Y (PVY) в пиперни растения, използвайки естествени елиситори на базата на салицилова киселина. Третирането на пиперните растения с елиситори, индуцира SAR срещу PVY инфекция, което доведе до намаляване на стойностите на DAS ELISA и вирусните симптоми в растенията, причинени от PVY. Третирането на растения от пипер с комбинирана схема от два елиситора 3 дни преди инокулирането на вируса предизвика силна устойчивост към PVY в експерименталните пиперени растения.

Introduction

Potato virus Y (PVY) was first reported in 1931 as an aphid-borne virus belonging to a group of viruses causing damage to potatoes (Smith, 1931). PVY is the typical representative of the genus *Potyvirus* of the family *Potyviridae* (Shukla *et al.*, 1994). PVY was first reported in 1942 in Bulgaria by Kovachevski (Kovachevski, 1951), who found isolated cases of symptoms in pepper caused by this virus. Later, the author proved that the same disease is widespread in tobacco and it is also the cause of curling of leaves in potatoes. He was the first to report that PVY induced necrotic symptoms in tobacco and experimentally transmitted the virus by aphids *Myzus persicae* (Kovachevski, 1951).

Pepper isolates of PVY were classified into three pathotypes: PVY-0, PVY-1 and PVY-1.2, according to their ability to overcome the resistance genes (*vy1*, *vy2*) present in pepper cultivars (Selassie *et al.*, 1985). Within these three groups, pepper isolates were further defined as „common" (PVY^C) or „necrotic" (PVY^N) (D'Aquino *et al.*, 1995).

Several recessive genes for resistance to *Potyvirus* such as *pvr1* have been identified in pepper. The different alleles of these genes encode the translation initiation factor eIF4E. Homozygous potivirus resistance genes have point mutations that interfere with the interaction of plant initiation factor eIF4E with the viral protein VPg. In this way, stable phenotypes are obtained at different levels that prevent viral accumulation, movement of the virus from cell to cell or over long distances (Kang *et al.*, 2005).

To date, no effective means to control PVY virus infection have been identified. However, plants have built a kind of protection against phytopathogens. Systemic acquired resistance (SAR) is characterized by broad-spectrum resistance to disease and is activated by local damages caused by necrosis-inducing pathogens, such as viruses, bacteria or fungi (Kuc, 1982; Kessmann *et al.*, 1994). Chemicals capable of activating SAR in very low concentrations in various plant species (Metraux *et*

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al., 1991; Kessmann *et al.*, 1994) served as a basis for the discovery of local and systemic defense mechanisms against diseases (Ryals *et al.*, 1996; Sticher *et al.*, 1997). In dicotyledonous plants, the biological induction of SAR by local infections caused by necrogenic pathogens is associated with systemic accumulation of salicylic acid (SA) and pathogenicity-related proteins (PR). SA levels increase in both infected tissues and adjacent uninfected tissues (Kessmann *et al.*, 1994; Sticher *et al.*, 1997).

Material and Methods

Introduction of elicitors

Plants were divided into groups of 15 plants each and were treated according to several different schemes:

1) with 3 mM aqueous BION solution, pH = 7 only
2) with EXIN only
3) with EXIN + BION

For each treatment scheme, treatment group and cultivar, the following controls were used: K- (healthy plant treated with distilled water only), K+ (PVY infected plant treated with distilled water only), K- BION (healthy plant treated with BION for phytotoxicity), K- EXIN (healthy plant treated with EXIN for phytotoxicity), K- BION+EXIN (healthy plants treated with this combination).

Pepper plants of the cv. Kurtovska Kapiya were treated 3 days before inoculation with PVY virus in three groups:

Group I - with EXIN 4.5 HP (Phytoxin VS, with the active ingredient 4.5% salicylic acid) at a concentration of 1 packet (10 ml) in 1 L of water.

Group II – with BION (cytokinin derived from salicylic acid, BTH) at a concentration of 3 mM.

Group III was combination with EXIN and 2.35 mM BION.

Spraying was carried out in a greenhouse at a temperature range of 21-24°C and a relative humidity of 45% at a dose of 5-15 ml of solution of the compounds (depending on the size of the plant) in a 3-day interval and 6 treatments.

Inoculation of plants with virus

One gram of leaf mass of a plant with pronounced symptoms was homogenized in 1 ml of cooled to 4°C 0.1 M 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 *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, 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 triple washed 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

Benzothiadiazole (benzo-1,2,3-thiadiazole-7-carbothionic acid S-methyl ester) is a functional analogue of SA and has the trade name BION. Its resistance-inducing effect against many fungal plant pathogens has been shown (Hukkanen, 2008). In plant samples treated with BION (1-15, Fig. 1), half reduction of DAS-ELISA values for PVY was obtained and in plants treated with EXIN (19-33, Fig. 1) - only slight reduction of DAS-ELISA values. Complete reduction of PVY symptoms and DAS-ELISA values to the level of healthy plants was achieved in the plants treated with the combination BION+EXIN (37-51, Fig. 1).

BION, applied alone or in combination with potassium phosphonate as a foliar spray, reduces the spread of root rot caused by *Phytophthora* in coniferous *Pinus radiata* and other plants. This shows the future potential of these resistance inducers to displace toxic and dangerous fungicides. Commonly used chemical inducers are salicylic acid, methyl salicylate and benzothiadiazole, which affect the production of phenolic compounds in plants. Inducers of systemic resistance have a negative effect on insect pests, fungal and viral diseases

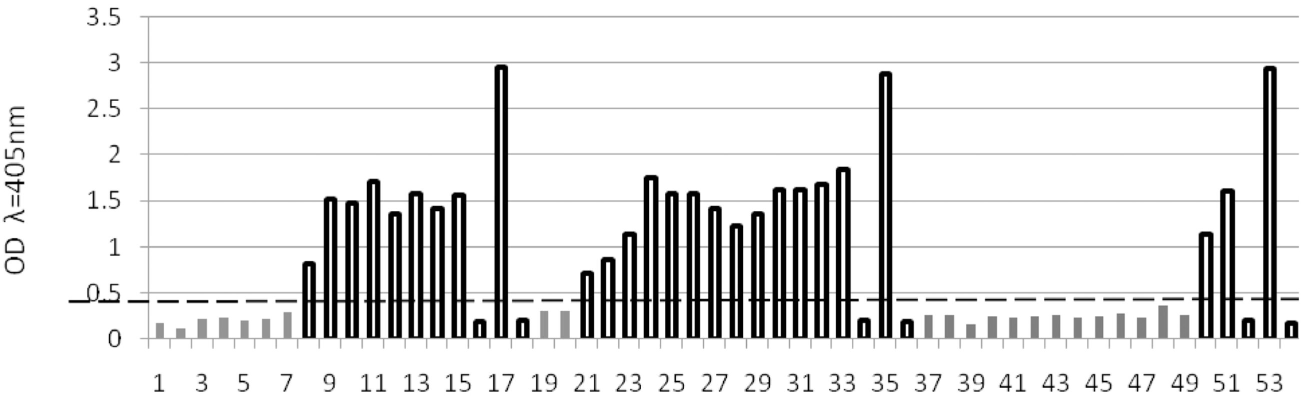

Fig. 1. Results from DAS -ELISA after treatment of pepper plants of cv. Kurtovska Kapiya with BION and EXIN and before inoculation with PVY. 1-15 - plants treated with BION and inoculated with PVY; 16 - plants treated with BION only (for toxicity); 17 - plants infected with PVY only (K+); 18 - untreated and non-inoculated plants (K-); 19-33 - plants treated with EXIN and inoculated with PVY; 34 - plants treated with EXIN only (for toxicity); 35 - plants infected with PVY only (K+); 36 - untreated and non-inoculated plants (K-); 37-51 - plants treated with BION + EXIN and inoculated with PVY; 52 - plants treated with BION + EXIN only (for toxicity); 53 - plants infected with PVY only (K+); 54 - untreated and non-inoculated plants (K-)

(Holopainen *et al.*, 2009). Salicylic acid is involved in the response to pathogenic attack in various plant species. Induction of salicylic acid synthesis is required in viral, fungal and bacterial resistance reactions (Murphy and Carr, 2002; Shah, 2003). When treating sensitive tobacco or tissue plants with SA or its derivatives such as aspirin, a reduction in Tobacco mosaic virus (TMV) accumulation is observed (White *et al.*, 1983).

Conclusion

The best result was obtained after treatment of plants with the combination BION + EXIN. Healthy plants were 86% (13 of 15 plants) 21 days after inoculation with PVY.

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