BION AND EXIN AS SAR ELICITORS AGAINST POTATO VIRUS Y INFECTION IN TOMATO

Nikolay Petrov*, Rayna Andonova**
** Nikola Poushkarov Institute of Soil Science, Agrotechnologies and Plant Protection, 7 Shosse Bankya str., 1080 Sofia, Bulgaria*
***University of forestry, 10 Kliment Ohridski Blvd., 1756 Sofia, Bulgaria*
m_niki@abv.bg

ABSTRACT

Potato virus Y - PVY infects different species of family *Chenopodiaceae, Commelinaceae*, and *Solanaceae*. It causes significant losses and reduces product quality in economically important crops of potatoes, tomatoes, pepper and tobacco. Control strategies are extremely difficult and don't directly affect viral replication. In the present study effective control of viral infection has been achieved by induced systemic acquired resistance (SAR) in tomato cultivar Ideal. BION and EXIN, preparations based on salicylic acid were used in various schemes of treatment alone and in combination. Treatment with BION alone resulted in 72% to 86% protection against PVY and 46% -92% when combining elicitors.

Key words: *PVY, BION, EXIN, SAR, tomato*

Introduction

Potato Virus Y (PVY) is the type species of the Potyvirus genus, which, together with other genera, Rymovirus, Bymovirus, Macluravirus, Tritimovirus and Ipomovirus forme the family *Potyviridae* (Brunt, 1992; Salm et al., 1996; Badge et al., 1997; Pringle, 1999). Potyvirus include more than 200 members which makes *Potyviridae* the largest family of plant viruses representing the 25% of all known plant viruses. PVY was first recognized in 1931 as an aphid-transmitted member within a group of viruses associated with potato degeneration, a disorder known since the eighteenth century (Smith, 1931). Its experimental host range comprises plants in 495 species in 72 genera of 31 families. PVY has a wide host range, including 287 species in the family Solanaceae (among which 141 Solanum species and 70 Nicotiana species), 28 species of Amaranthaceae, 25 species of Fabaceae, 20 species of Chenopodiaceae, and 11 species of Asteraceae. Aphids in 70 species, all in the family Aphidinae, were demonstrated to be able to transmit PVY, most of them with very low efficiencies compared to that of Myzus persicae. Apterae and Alatae are vectors (Kerlan, 2006).

PVY cause significant losses in four main crops around the world: potato, pepper, tomato, and tobacco. The most frequent encountered disease symptoms were mosaic, leaf mottling and deformation, leaf spotting, chlorosis, necrotic ring spots on the tubers.

The first report of PVY in Bulgaria was in 1942 (Ковачевски, 1942), who found single cases of necrotic symptoms caused by PVY on pepper. Latter he proved that the virus was wide spread disease on tobacco and the causal agent of leaf curling on potatoes.

Till now there are no effective control measures against PVY infection. However plants have defense mechanisms against plant pathogens. Generally, plant defense mechanisms are based on prevention, tolerance or resistance (Thakur, 2007). These mechanisms are activated in different ways, depending on the type of pathogens attacking the plant (or biotrophes appearing necrotrophic) (Jalali et al., 2002). Such mechanisms may be constitutive or induced. (Karban, 1997). This is the first provided protection of initial invasion of a pathogenic agent by physical barriers or preformed biochemical. Whereas induced resistance is only activated as a response to pathogen attack (Cruz-Borruel, et al., 2006).

This is achieved through the activation signals induced by non-specific elicitors and specific compounds that are first recognized by the plant to activate signaling cascade and therefore defense response (Ebel and Cosio, 1994).

Systemic acquired resistance (SAR) is characterized by broad-spectrum disease resistance that is activated systemically in induced plants following localized inoculations with necrogenic pathogens which can be viruses, bacteria or fungi (Kuc, 1982; Kessmann et al., 1994).

Salicylic acid (SA) is not a systemic signal, but is necessary for activation SAR in tobaco (Vernooij, 1994). Exogenic appliance of SA on tobacco leaded to resistance to diseases and was related with the expression of pathogen related (PR) proteins (White, 1979), which were specific for the plant. Benzothidiazole (S-methyl benzo[1,2,3]thiadiazole-7-carbothiate (acibenzolar-S methyl)) is a functional analog of SA marketed under the trade name BION. It induced resistance against many fungi plant pathogens (Hukkanen, 2008).

Material and methods:

Tomato variety Ideal was grown at 22-25C°, 75-85% relative humidity, constant photo-period of 16/8 hours, light intensity 3000 lux. The reporting of the symptoms was made 7-25 days after virus inoculation.

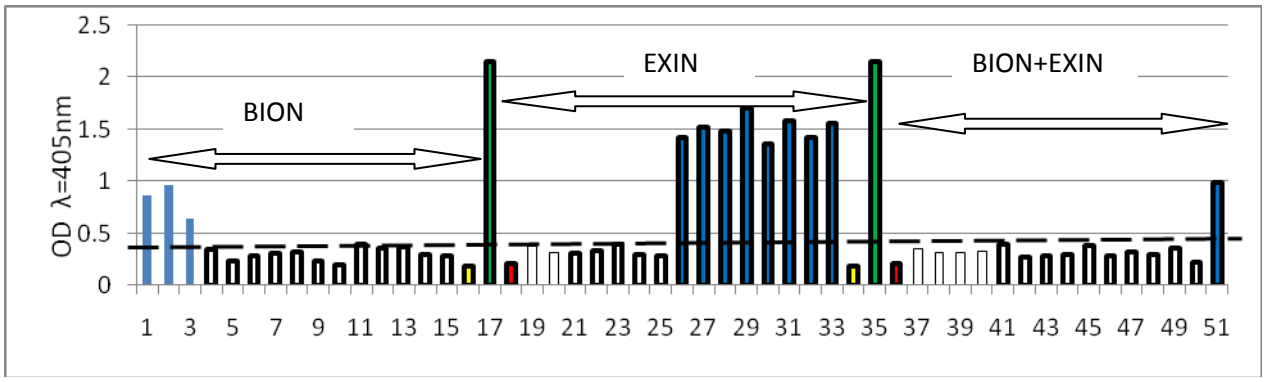
Plants were treated three days before artificial infection with strain PVY^{Wilga} by Exin 4.5 HP (Phytoxin VS, with 4.5% active ingredient salicylic acid) at a concentration of 1 packet (10 ml) in 1 L of water; BION at a concentration of 3mM and BION 2.35 mM in combination with EXIN. Sprays were conducted in a greenhouse at a temperature of 21°C to 24°C and a relative humidity of 45% with a dose of 5-15 ml solution of compounds in the 7-day interval and 7 treatments. Tomatoes were inoculated with PVY according to Noordam (1973).

The plants were divided into groups of 15 plants each and were treated in several schemes: 1) with an aqueous solution of 3mM BION, pH =7, 2) EXIN and 3) EXIN+BION. For each treatment were used controls for comparison: K (healthy plant treated with water only), K (PVY infected plant not treated with chemicals, only with water), K-BION (healthy plant treated with BION only, measuring for citotoxicity), K-EXIN, (healthy plants treated with EXIN only, measuring for citotoxicity). Infection and treatment plant was in a phase $9\text{-}10^{th}$ leaf of the variety Ideal. Homogenization of tissue infected with PVY was 1:10 w/v buffer potassium sodium phosphate buffer pH 7, and then was carried inoculation of plants with PVY. About 25 days after inoculation, samples were taken for DAS-ELISA (Clark and Adams, 1977) with kits of LOEWE Biochemica GmbH Sauerlach, Germany. Reading of the reaction was made with Multifunctional detector type DTX 880, at a wavelength of 405 nm. For infected plants have been adopted extinction values exceeding twice the value of healthy controls.

Results and discussion:

For induction of systemic acquired resistance by exogenous inducers against PVY were used two preparation based on salicylic acid to activate a cascade of metabolic effects of salicylic acid. BION contained 2.35mM benzotiadiazol derivative of salicylic acid. Salicylic acid was the active substance in the preparation EXIN in concentrations 4.5%. Fig. 1 (1-15) represented the values of the tomato variety Ideal treated with BION, 3 days before viral inoculation. Only three plants were infected with PVY (1-3), the other 12 plants remained negative. In seven plants of the variety Ideal (Fig. 1, 19-25) was induced resistance by EXIN, and they remained uninfected with PVY.

Oostendorp et al. (2001) administered BION at a dose of 1.2-3.7g/da and managed to reduce 60% of the symptoms induced by PVY on tobacco. Nie (2005) used SA in concentration 0.1mM to 5mM and sprayed 2 times a day, 3 days before viral inoculation and was able to reduce the symptoms of necrotic yellow vein induced by $PVY^{N:O}$ on tobacco from 41% to 14.1%.



Фиг. 1. DAS-ELISA values of plant variety Ideal treated with BION, EXIN and BION + EXIN 3 days before viral inoculation with PVY
Legend: 1-18: treated with BION; 19-36: with EXIN; 37-51: EXIN + BION; Controls - 16 34: uninfected and untreated plants, 17 and 35: untreated infected plants 18, 36: uninfected and treated plants

Our results indicate full protection against PVY of the tomatoes. Combined treatment of plants with EXIN and 2.35mM BION showed very good results (Fig. 1, 37-51) of protection the plants from infection of PVY. All plants except for one were not infected with PVY.

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