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ИНДУЦИРАНЕ НА СИСТЕМНА УСТОЙЧИВОСТ (SAR) В ПИПЕР КЪМ *X. EUVESICATORIA* ПАТОТИП *P* СЛЕД ТРЕТИРАНЕ С АВИРУЛЕНТНИ ЩАМОВЕ ПАТОТИП *T* НА *X. GARDNERI* И *X. VESICATORIA*

INDUCTION OF SYSTEMIC ACQUIRED RESISTANCE (SAR) IN PEPPER TO *X. EUVESICATORIA* PATHOTYPE *P* AFTER TREATMENT WITH AVIRULENT PATHOTYPE *T* STRAINS OF *X. GARDNERI* AND *X. VESICATORIA*

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Abstract

Bacterial spot caused by *Xanthomonas euvesicatoria* is an economically important disease of pepper which is the reason for yearly losses in vegetable production. The control of the pathogen is hard and is mainly relied on the extensive use of copper-based chemicals. In the present study we successfully induced systemic resistance in pepper plants of the susceptible cultivar *Californian miracle* towards the pepper pathotype *X. euvesicatoria*. We used avirulent for pepper strains of the tomato pathotype of *X. gardneri* race T1 and *X. vesicatoria* race T1, T2, and T3 to achieve that effect. The protection was very well expressed in the young leaves which grew after the treatment. After a competitive infection with the pepper pathotype of the closely related pathogen *X. euvesicatoria* the plants grew and developed without symptoms of bacterial spot. The plants were inspected until the first fruit formation.

Key words: *X. euvesicatoria,* systemic resistance, SAR, avirulent strain, pepper.

INTRODUCTION

Bacterial spot of pepper caused by *X. euvesicatoria* is a devastating disease which causes losses yearly. Control is mainly based on copper-based chemicals, however, some strains are resistant to the most used in practice concentrations (Garton, 2009) or only weakly sensitive (Kizheva et al., 2013). Therefore, other approaches of control have been investigated.

Systemic acquired resistance (SAR), or also known as induced systemic resistance (ISR), is a plant resistant response to a microbial challenge as a result

of induced signal transduction pathway which is innate and essential for the plant defence against pathogens. SAR activation results in the development of a broad-spectrum, systemic resistance (Ryals et al., 1996). SAR can be triggered by certain chemicals like acibenzolar-*S*-methyl (ABM). This substance was able to induce systemic response in pepper plants against subsequent infections with *Xanthomonas euvesicatoria* (Romero et al., 2001). Elicitation of SAR was also achieved by the use of plant-associated bacteria (Kloepper et al., 2004) or by inoculation of avirulent strain of the same species (Lee and Hwang, 2005).

In the present study we demonstrate the protective effect of inoculation of avirulent strains of related pathogens *X. gardneri* and *X. vesicatoria* against challenge with X*. euvesicatoria*.

MATHERIALS AND METHODS

Plant material: healthy pepper plants of the sensitive cultivar California wonder in phase 2-3 leaf.

Pathogenic bacterial test strain: *X. euvesicatoria* pepper pathotype strain from the region of Plovdiv isolated in 2010 (Vancheva et al., 2014).

Bacterial strains for treatments: *X. gardneri*, *X. vesicatoria* race T1, T2 and T3 strains from the region of Plovdiv (all tomato pathotype, avirulent for pepper) (Kizheva et al., 2013).

Treatments were carried out with *X. gardneri* and *X. vesicatoria* avirulent strains individually. Pepper plants were inoculated with bacterial suspension in concentration 10^8 cfu/ml from 36 h culture by the vacuum infiltration method (Bogatzevska, 1988). After 24 hours the plants were infiltrated with *X. euvesicatoria* pathogenic strain as bacterial suspension in concentration 10^8 cfu/ml from 36 h culture by the same method. The plants were kept in nutrient solution in laboratory conditions at room temperature, indirect sunlight for 5-6 days until emergence of symptoms. Disease incidence was evaluated by calculation of average attack rate (Ar) according to the modified 5th grade scale (Bogatzevska et al., 2006), index of infection (Ii) and index of defoliation (Di) (Pesti et al., 1985). After observations the plants were moved to pots in vegetation house and bred until the first fruit formation. Periodical observations for symptoms of bacterial spot were held. The experiment was carried out with 20 pepper plants for each treatment in three repeats. Inoculated with *X. euvesicatoria* non-treated plants served as controls.

RESULTS AND DISCUSSION

The bacteria used for treatment in the present study are tomato pathotype strains, avirulent for pepper. On the 24^{th} hour after infiltration with these strains, the plants exhibited hypersensitive reaction (HR) (fig. 1) or did not show any symptoms. Five-six days after inoculation with *X. euvesicatoria* strain, the plants developed varying reactions to the pathogen according to the treatment strain used. Symptoms of bacterial spot mainly from rate 1 emerged on 11-21% of the leaves while more than $2/3^{rd}$ of the leaves of the non-treated control plants exhibited spots from rate 1 to rate 5 (fig. 2, tabl. 1). About ½ of the leaves of the treated plants remained healthy and between 21-37% with HR which did not develop further symptoms.

Фиг. 1. *HR в листа от пипер 24 часа след вакуумна инфилтрация с X. vesicatoria раса T3*
Fig. 1. *HR in pepper leaf 24 hours after infiltration with X. vesicatoria race T3*

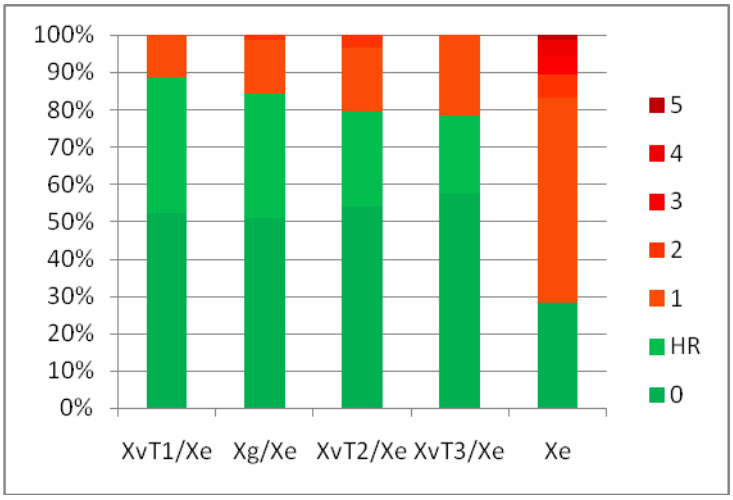
Фиг. 2. *Симптоми на бактерийно струпясване по пипер след инокулация с X. euvesicatoria при нетретирани растения*
Fig. 2. *Symptoms of bacterial spot of non-treated pepper plants after inoculation with X. euvesicatoria*

HR is a reaction of the plant which prevents the further spread and development of a microorganism within the plant by trapping it in a small site of dead tissue. Since these sites remain located and result from the activation of suicide processes encoded by the plant genome, hypersensitive reaction is generally considered as a variation of plant immune response (Pontier et al., 1998). The pepper leaves with HR did not become visually infected with *X. euvesicatoria* and did not develop symptoms of bacterial spot like the healthy leaves. On the basis of the proportion of leaves without symptoms of bacterial spot (including healthy leaves and leaves with HR) and leaves with symptoms of disease we achieved from 2.7 to 3.2 times reduction of disease emergence (fig. 3).

Таблица 1. Процент здрави и болни листа след третиране на растения от пипер с *X. vesicatoria* раси T1, T2 и T3 и *X. gardneri* преди изкуствено заразяване с причинителя на бактерийното струпясване по пипера *X. euvesicatoria*

Table 1. Percent of symptomless and diseased leaves after treatment of pepper plants with *X. vesicatoria* races T1, T2 and T3 and *X. gardneri* prior to inoculation with the causal agent of bacterial spot of pepper *X. euvesicatoria*

Щам за третиране/ Патоген/ Strain for treatment/ pathogen	Среден общ брой листа/ Average total number of leaves	Устойчиви листа, в % Resistant leaves, in %		Болни листа от степен 1-5, в % Diseased leaves of rate 1-5, in %				
		Здрави Healthy (symptom-less)	Листа с HR Leaves with HR	1	2	3	4	5
XvT1/Xe	101±6	53±3	37±3	11±2	0	0	0	0
XvT2/Xe	94±5	51±2	24±3	16±1	3±1	0	0	0
XvT3/Xe	101±4	57±2	21±2	21±2	0	0	0	0
Xg/Xe	95±3	49±3	32±2	14±2	1±1	0	0	0
-/Xe	97±8	28±3	0	54±5	6±2	5±1	4±2	1±1



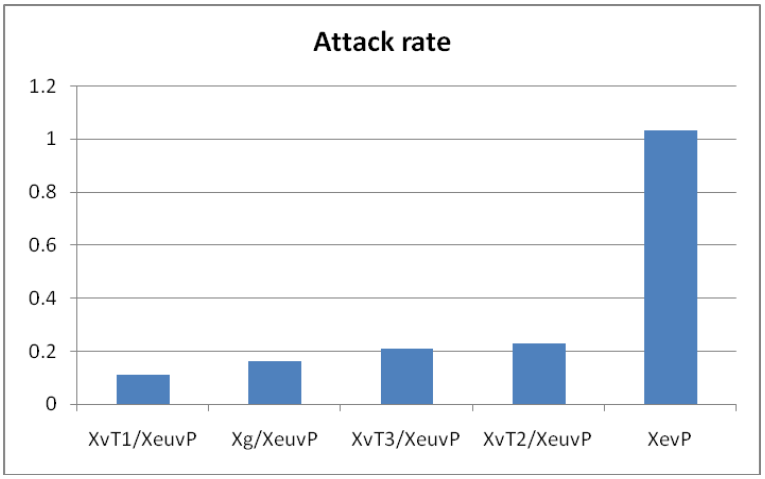
***Фиг. 3**. Процент устойчиви листа (здрави и листа с HR в зелено) срещу болни листа (в червено)*

***Fig. 3.** Percent of resistant leaves (healthy leaves and leaves with HR in green) against diseased leaves (in red)*

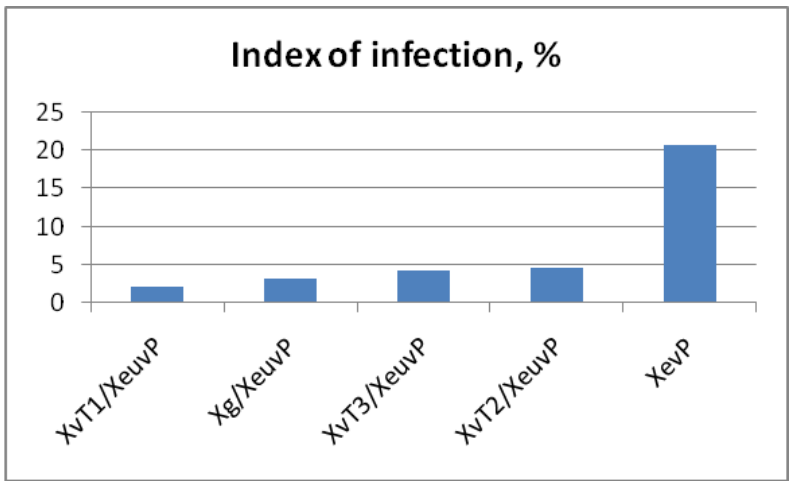
For further evaluation of disease incidence attack rates (Ar), index of infection (Ii) and index of defoliation (Di) were calculated (tabl. 2). Attack rates of the four groups of treated pepper plants varied between 0,11±0,02 to 0,23±0,01 which is 4,5-9,4 times lower compared to 1,03±0,11 for the non-treated controls. Indices of infection of treated plants also varied between 4,4-9,6 times lower than the non-treated control plants (tabl. 2). Lower attack rates and indices of infection were achieved after treatments with the strains of race T1 of *X. vesicatoria* and *X. gardneri* (tabl. 2, fig. 4, 5) although the average percent of leaves with HR were higher. Indices of defoliation were commensurate for all of the treated and non-treated plants.

Таблица 2. Оценка на болестта при третирани растения и нетретирани контроли
Table 2. Evaluation of disease incidence of the treated and the non-treated control plants

Щам за третиране/патоген Strain for treatment/Pathogen	Ar	Ii%	Di%
XvT1/XeuvP	0,11±0,02	2,14±0,32	20±8
XvT2/XeuvP	0,23±0,01	4,63±0,15	21±4
XvT3/XeuvP	0,21±0,01	4,17±0,24	24±3
Xg/XeuvP	0,16±0,02	3,18±0,31	31±3
-/XevP	1,03±0,11	20,57±2,29	24±3



Фиг. 4. *Средна степен на нападение на третирани растения и нетретирани контроли*
Fig. 4. *Average attack rate of the treated and of the non-treated control plants*



Фиг. 5. *Среден индекс на инфекция на третирани растения и нетретирани контроли*
Fig. 5. *Average index of infection of the treated and of the non-treated control plants*

The development of the plants was continuously observed until the first fruit formation. The newly grown young leaves were symptomless, the diseased leaves defoliated and the plants developed normally indicating the lack of further disease development.

Our results are similar to the results of Lee and Hwang (2005) who reported induction of SAR in the non-inoculated (secondary) pepper leaves after inoculation with an avirulent strain of *Xanthomonas vesicatoria* against a virulent strain of the same species.

The experiments showed that after infiltration in phase 2-3 leaf the races of tomato pathotype of *X. vesicatoria* and *X. gardneri* induce systemic responce in pepper which provides defence in plants against the related causal agent of bacterial spot *X. euvesicatoria*.

The avirulent for pepper strains of *X. vesicatoria* and *X. gardneri* induce SAR against *X. euvesicatoria* which is best observed in the young leaves growing after inoculation with the pathogen.

CONCLUSIONS

Tomato pathotype strains of *X. vesicatoria* and *X. gardneri* induced SAR against the causal agent of bacterial spot *X. euvesicatoria.* Treatments of young pepper plants resulted in 2,7 to 3,2 times reduction of disease emergence, up to 9,5 times lower attack rates and indices of infection, subsequent liberation of the plant from the diseased leaves and normal further growth. Best results were achieved with treatment with *X. vesicatoria* T1 strain.

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