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ABSTRACT
Bacterial spot caused by Xanthomonas vesicatoria is an economically important disease of pepper which is the reason for yearly losses in vegetable production. Control of the pathogen is difficult and is mainly relied on sanitation measures, crop rotation, use of disease-free seeds and copper-based chemicals. In the present study we induced systemic resistance in pepper plants from the susceptible cultivar Yellow kapia towards the pepper pathotype of X. vesicatoria. We used avirulent for pepper strains of tomato pathotype of X. vesicatoria races T1, T2, and T3 and Pseudomonas syringae pv. tomato race R0 and R1 to achieve this effect. Resistance is expressed as lack of visual symptoms or hypersensitive reaction in leaves after which the plants develop without symptoms.

Key words: X. vesicatoria, systemic resistance, SAR, avirulent strain, Pseudomonas syringae pv. tomato

Introduction
Bacterial spot caused by X. vesicatoria is a common disease of pepper which causes early defoliation and losses due to reduced number of fruits and yield of unmarketable fruits. Control is mainly based on sanitation measures, crop rotation, use of disease-free seeds and copper-based chemicals (Schwartz & Gent, 2007; Li, 2012). However, their effectiveness is limited by rainfall, dew formation and resistant strains (Zitter, 1985) and other approaches have been investigated.

Plants possess innate defensive mechanisms which ensure their resistance or tolerance to certain pathogens. It was found possible to trigger these mechanisms to provide protection against pathogens to which the plants are normally susceptible. Systemic acquired resistance (SAR) is a plant resistant response to a microbial challenge as a result of induced signal transduction pathway. SAR results in broad-spectrum resistance (Ryals et al., 1996) and can be induced by chemicals like acibenzolar-S-methyl (ABM) (Romero et al., 2001), by the use of plant-associated bacteria (Kloeper et al., 2004) or by inoculation of avirulent strain of the same species (Lee & Hwang, 2005).

We have investigated the protective effect of inoculation of young pepper plants with the avirulent for this species strains of a related pathogen – X. vesicatoria tomato pathotype, and of an unrelated pathogen – P. syringae pv. tomato against the pepper pathotype X. vesicatoria.

Materials and Methods

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

Pathogenic bacterial strain for inoculations: X. vesicatoria pepper pathotype (P) strain.

Bacterial test strains for treatments: X. vesicatoria race T1, T2 and T3 strains (all tomato pathotype, avirulent for pepper), P. syringae pv. tomato race 0 (R0) and race 1 (R1), avirulent for pepper.

Design of experiment

Treatments were carried out with X. vesicatoria and P. syringae pv. tomato strains individually. The experiment was
carried out with 20 pepper plants for each treatment in three repeats. Non-treated, inoculated with *X. vesicatoria* pathotype P plants served as positive controls and non-treated, non-inoculated plants served as negative controls.

Pepper plants were inoculated with bacterial suspensions from the test strains in concentration $10^8$ cfu/ml from 36 h culture. After 24 hours the appearance of hypersensitive reaction (HR) was recorded and the plants were infiltrated with *X. vesicatoria* pathogenic strain as bacterial suspension in concentration $10^8$ cfu/ml from 36 h culture. The plants were kept in nutrient solution in laboratory conditions for 5–6 days until emergence of symptoms. 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.

**Analysis of data**

Reactions as HR or symptoms of disease (spots or defoliation) were observed for each leaf individually. Symptoms were recorded according to the modified 5th grade scale (Bogatzevska et al., 2006). The average values from the three repeats and the standard deviation were estimated. Disease incidence was further evaluated by estimation of rates of attack (ms) (Bogatzevska et al., 2006), indices of infection (Ii) and indices of defoliation (Di) (Pesti et al., 1985). Results were presented as average values from the three trials and the standard deviation was calculated.

**Results**

On the 24th hour after infiltration with the test strains, lack of any symptoms or HR could be observed on the pepper leaves (Figure 1, Figure 2).

Five-six days after inoculation with the test pathogen – *X. vesicatoria* pathotype P, the plants were inspected for symptoms. Between 48–61% of the treated plants remained healthy and between 25–39% – with HR which did not develop further symptoms. Bacterial spot from rate 1 was observed on 10–20% of the leaves of the treated plants compared to more than half of the leaves of the non-treated control plants. Overall more than 2/3rd of the leaves of the control non-treated plants exhibited spots from rate 1 to rate 5 (Figure 1, Table 1). Only 2% of the plants treated with *X. vesicatoria* race T2 developed symptoms of the disease of rate 2 (Table 1).

The largest number of healthy leaves was achieved by treatment with *P. syringae* pv. *tomato* R0 – ~61% but the same strain also invoked the least number of leaves with HR – ~24% while the percent of leaves with bacterial spot was average (~16%). *X. vesicatoria* race T3 was the second to invoke defenses in most leaves – ~56% but it was also the strain to allow most diseased leaves – ~20%. Less percent of diseased leaves was achieved by *X. vesicatoria* race T1 while the number of healthy leaves and leaves with HR was average or above average (Table 1).

**Figure 1.** Pepper leaves after infiltration: 1 – with *P. syringae* pv. *tomato* R1 (treated leaf with HR), 2 – with water (negative control, non-treated, non-inoculated), 3 to 5 – with *X. vesicatoria* pathotype P (positive controls, diseased leaves with symptoms of bacterial spot).

**Figure 2.** Symptomless pepper plant after treatment with *X. vesicatoria* race T3 and inoculation with *X. vesicatoria* pathotype P.
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X. vesicatoria 

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pathotype P. syringae pv. tomato R0 and R1 prior to inoculation with the causal agent of bacterial spot of pepper X. vesicatoria pathotype P. 

Differences between the races of P. syringae pv. tomato and X. vesicatoria were not observed. The treatments with two races of P. syringae pv. tomato allowed similar number of diseased leaves (14–16%) but showed a large variety in the number of healthy leaves (48–61%). The treatments with three races of X. vesicatoria allowed similar number of healthy leaves (53–56%) but showed a large variety in the number of diseased leaves (10–20%) (Table 1). 

The pepper leaves which reacted with HR to our treatments did not develop symptoms of bacterial spot after inoculations with X. vesicatoria pathotype P. Based on the proportion of the numbers of symptomless leaves, including the healthy leaves and the leaves with HR, and the leaves with bacterial spot we achieved from 2.7 to 5.4 times reduction of disease emergence (Figure 3). 

Rates of attack of the treated pepper plants varied between 0.10±0.01 to 0.23±0.02 which is 4.7–10.8 times lower compared to ms values of 1.08±0.09 for the non-treated controls (Table 2). Indices of infection of treated plants varied between 4.7–10.7 times lower than the non-treated control plants (Table 2). Lowest rates of attack and indices of infection were achieved after treatments with the strain of race T1 of X. vesicatoria and highest rates – with race T3 of X. vesicatoria (Table 2, Figure 4, Figure 5) although both of these strains provoked similar indices of defoliation. Overall indices of defoliation did not show large variances in values for the treated and non-treated plants. 

Best results in disease incidence were achieved with treatment with X. vesicatoria T1 strain. The development of the plants was continuously observed until the first fruit formation in a vegetation house. The newly grown young leaves were symptomless, the diseased leaves defoliated and the plants developed normally indicating the lack of further disease development. The control non-treated diseased plants continued to develop single leaf spots.

<table>
<thead>
<tr>
<th>Strain for treatment/pathogen</th>
<th>Average total number of leaves</th>
<th>Resistant leaves, in %</th>
<th>Diseased leaves of rate 1–5, in %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Healthy (symptomless)</td>
<td>Leaves with HR</td>
</tr>
<tr>
<td>PstR0/ XvP</td>
<td>97±8</td>
<td>61±3</td>
<td>24±5</td>
</tr>
<tr>
<td>PstR1/ XvP</td>
<td>107±2</td>
<td>48±2</td>
<td>39±3</td>
</tr>
<tr>
<td>XvT1/ XvP</td>
<td>102±4</td>
<td>54±1</td>
<td>36±1</td>
</tr>
<tr>
<td>XvT2/ XvP</td>
<td>92±5</td>
<td>53±2</td>
<td>27±3</td>
</tr>
<tr>
<td>XvT3/ XvP</td>
<td>103±2</td>
<td>56±6</td>
<td>25±8</td>
</tr>
<tr>
<td>XvP</td>
<td>106±2</td>
<td>28±2</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 1. Percent of resistant and diseased leaves after treatment of pepper plants with X. vesicatoria races T1, T2 and T3 and P. syringae pv. tomato R0 and R1 prior to inoculation with the causal agent of bacterial spot of pepper X. vesicatoria pathotype P.

<table>
<thead>
<tr>
<th>Strain for treatment/pathogen</th>
<th>ms</th>
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<th>ll%</th>
</tr>
</thead>
<tbody>
<tr>
<td>PstR0/ XvP</td>
<td>0.15±0.02</td>
<td>2.52±0.98</td>
<td>16±3</td>
</tr>
<tr>
<td>PstR1/ XvP</td>
<td>0.13±0.03</td>
<td>2.67±0.50</td>
<td>24±3</td>
</tr>
<tr>
<td>XvT1/ XvP</td>
<td>0.10±0.01</td>
<td>2.02±0.24</td>
<td>23±3</td>
</tr>
<tr>
<td>XvT2/ XvP</td>
<td>0.23±0.02</td>
<td>4.53±0.31</td>
<td>19±3</td>
</tr>
<tr>
<td>XvT3/ XvP</td>
<td>0.19±0.03</td>
<td>3.80±0.53</td>
<td>22±1</td>
</tr>
<tr>
<td>XvP</td>
<td>1.08±0.09</td>
<td>21.53±1.86</td>
<td>17±9</td>
</tr>
</tbody>
</table>

Table 2. Evaluation of disease incidence of the treated and the non-treated control plants.

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

The present study was based on the supposition that related or unrelated pathogenic bacteria can invoke the plant defensive mechanisms in non-hosts. We used the closely related to the pepper pathotype of *X. vesicatoria* pathogen – *X. vesicatoria* tomato pathotype races and the unrelated tomato pathogen *P. syringae* pv. *tomato*. Some of the treated plants did not show any visual reaction to this challenge but other reacted with HR. HR is connected both with the bacteria’s factors of pathogenicity and the plant’s genome. Since the rapid necrosis at site of inoculation prevents further spread and development of the microorganism, HR is generally considered as a variation of plant immune response (Pontier et al., 1998). The pepper leaves which reacted with HR to our treatments did not develop symptoms of bacterial spot after the second challenge with *X. vesicatoria* and only a small number of the symptomless plants developed the mildest symptoms of the disease.

In the experiments of Block et al. (2005) challenge with avirulent strain of *X. vesicatoria* prior to inoculation with a virulent strain resulted in reduced symptom development in tomatoes though it did not affect bacterial growth in plant tissues. Salicylic acid (SA) is known to be involved in the mechanisms ensuring resistance to pathogens and generation of SAR in many species (Gaffney et al., 1993; Ryals et al., 1996). Treatments with SA-based products reduced both bacterial growth and plant disease symptoms following challenge and are related to induction of pathogenesis-related (PR) genes. Both local and systemic induction of PR genes takes place during SAR (Ward et al., 1991). Block et al. (2005) demonstrated SA accumulation-mediated systemic PR gene expression in tomatoes after first challenge with avirulent strain of *X. vesicatoria*. Based on their results the authors supported the hypothesis that inoculation with avirulent *X. vesicatoria* sensitized systemic defenses and caused their faster induction upon challenge, leading to disease reduction. However, in certain cases pathogen can bypass the induced defense responses. Treatment with avirulent *X. vesicatoria* was unable to induce resistance to *P. syringae* pv. *tomato* (Block et al., 2005).

Our experiments demonstrated that treatments with all tomato races of *X. vesicatoria* and *P. syringae* pv. *tomato* induce systemic response in young pepper plants which provided defence against the pepper pathotype of *X. vesicatoria*. Treatments of young pepper plants resulted in up to 5.4 times reduction of disease emergence, up to 10.7 times lower rates of attack and indices of infection, subsequent liberation of the plant from the diseased leaves and normal further growth. Induction of SAR was best observed in the young leaves growing after inoculation with the pathogen. Our results support Lee & Hwang (2005) who reported induction of SAR in the non-inoculated (secondary) pepper leaves after inoculation with an avirulent strain of *X. vesicatoria* against a virulent strain of the same species. The findings in the present study contribute to the information in literature of induction of SAR in different plant species which is essential for development of new strategies for disease control.
Acknowledgement

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References
