Transcript analysis of some pathogenicity-related elements in an Iranian A* isolate of Xanthomonas citri subsp. citri

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Abstract: Asiatic citrus canker is a devastating disease resulting in drastic economic losses in citrus culture worldwide. Amongst three different types of the disease, i.e. A, A* and A*, the A* type is genetically less known. In order to comprehend the behavior of the Asiatic citrus canker A* -type strain (Xanthomonas citri subsp. citri) in the vicinity of the host cells, a targeted semi-quantitative transcript analysis approach via RT-PCR was carried out. A subset of sixteen genes, as representative of different steps involved in phytopathogenicity, was analyzed on the culture medium (as uninduced) and compared with the subset isolated from the infected Mexican lime (Citrus aurantifolia L.) plants (as induced). The results showed that certain genes were up-regulated in induced condition, suggesting a putative role in bacteria-host interaction. Furthermore, the transcripts in induced condition could be classified into constitutive, early- and late-responsive genes, demonstrating their functional relevance during the host-pathogen interaction.

Keywords: RT-PCR, Xcc, citrus canker, Mexican lime

Introduction

Xanthomonas spp. are Gram-negative bacteria belonging to Gammaproteobacteria in Xanthomonadaceae family, with 27 species, causing serious diseases in nearly 400 plant species in a host and/or even tissue specific manner (Qian et al., 2008; Ryan et al., 2011). Amongst others, some pathotypes (A, A* and A*) of Xanthomonas citri namely asiatic citrus canker (ACC) affect a wide range of hosts including most commercial citrus species and many close relatives of Rutaceae (Verniere et al., 1998; Cubero and Graham, 2002; Qian et al., 2008; Vojnov et al., 2010). Consequently and as a result of quarantine restrictions, citrus market and trade are affected enormously endangering the zest for its production (Brunings and Gabriel, 2003; Schaad et al., 2005; Guo et al., 2011). Hence, control of the disease poses a continuous challenge to the citrus orchardists (Traore et al., 2009; Bui Thi Ngoc et al., 2010). As in the war field, understanding the enemy and its tactics helps to better change the course and improve the controlling strategies. Here, a transcript analysis was performed to better appreciate the pathogenicity-related elements of ACC A*-type strain.

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Among different pathotypes of ACC, most scientific findings were focused on A with a wide range of hosts (Shiotani et al., 2009; Bock et al., 2010; Vojnov et al., 2010; Ryan et al., 2011), while little is known about A* and A w with narrow range of host range specificity (Verniere et al., 1998; Bui Thi Ngoc et al., 2010). The main plant that gets affected by A* is Mexican lime (Citrus aurantifolia L.), whilst A w causes canker in Mexican lime and aleom (Citrus macrophylla L.). Recently, the genetic diversity of a fairly large collection of Iranian Xanthomonas citri subsp. citri (Xcc-A*) strains was evaluated (Soltaninejad et al., 2010a, b; Rezaei et al., 2012) and it was noted that A* is genetically more divergent than A as previously reported by Bui Thi Ngoc et al., (2010). Interestingly, the intra-population diversity was also remarkable among the A* strains. Here, temporal expression of 16 virulence and/or pathogenicity-related genes (Table 1) in an Xcc-A* strain was monitored semi-quantitatively. The relative importance of the investigated genes at different time points during establishment of the pathogen in the host plant is discussed.

Materials and method

Plant materials

Citrus aurantifolia young seedlings were prepared from Hormozgan province, Iran. The seedlings were kept in greenhouse at 30 °C under natural light and constant humidity for three months.

Bacterial strains and inoculation

NIGEB-088, an isolate of Xanthomonas citri subsp. citri (Xcc), was provided by the Bacterial Citrus Canker Collection of National Institute of Genetic Engineering and Biotechnology (NIGEB). Bacterium was cultured on YPGA (yeast extract 3 g.l⁻¹, pepton 5 g.l⁻¹, glucose 7 g.l⁻¹ and agar 15 g.l⁻¹) at 28 °C for 48 h. A single colony was sub-cultured in YP medium (yeast extract 3 g.l⁻¹ and pepton 5 g.l⁻¹) on a rotary shaker at 180 rpm at 28 °C till OD600 reached up to 0.3, i.e., 5 × 10⁸ cfu/ml. To prepare the inoculum, the culture was centrifuged 10 min at 5000 rpm and the pellet was resuspended and diluted with dH₂O to reach a concentration of 1 × 10⁸ cfu/ml and 50 µl of which was considered as the uninduced bacterium. For inoculation, host plant leaves (4 leaves per plant in a total of 10 plants) were surface-sterilized with ethanol and the inoculum (50 µl of 1 × 10⁸ cfu/ml) was injected into the parenchymal space on the abaxial leaf side.

Bacterial recovery from the inoculated leaves

According to Mehta and Rosato (2003), bacteria were recovered in different time points (1, 2, 3, 4, and 7 days post inoculation) for RNA isolation. The harvested leaves were sterilized with 70% (v/v) ethanol for 3 min and washed with dH₂O. The leaves were cut into small pieces and placed in 20 ml dH₂O for 20 min to allow realease of bacterial cells into the solution. The resuspended bacterial solution was centrifuged at 13,000 rpm and the bacterial cell pellet was stored at -80 °C.

RNA extraction and first strand cDNA synthesis

Bacterial total RNA was isolated using RNX-Plus kit (CinnaGen Inc., Tehran, Iran) according to the manufacturer’s instructions. The RNA was treated with DNase I, RNase-free (Fermentas GmbH., Opelstrasse, Germany) and its integrity and quantity was evaluated via 1.6% (w/v) agarose gel electrophoresis and spectrophotometry, respectively. To check for DNA contamination, melting curve was generated by real-time PCR using RNA as template. First strand cDNA was synthesized according to Gonzalez and Robb (2007) using 1 µg RNA. First strand cDNA was made with four separate hexamers (ATTTTT, GTTTTT, TGTGTTT, and TTTTTT). The synthesized cDNA for each hexamer was mixed with others at the same ratio and called mixed-primer cDNA which was used in PCR as template.

Polymerase chain reaction

The PCR reaction contained 1 × PCR buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs, 200 pmol mixed forward and reverse primers, 1.25 U Taq DNA polymerase (CinnaGen Inc., Tehran, Iran) and 2.5
μl cDNA as template. The primer pairs (100 nmoles each) used for each amplicon are listed in Table 1 and designed using Primer3 (Rozen and Skaletsky, 2000, available at http//frodo.wi.mit.edu). PCR was performed with an initial denaturation for 5 min at 95 °C followed by 30 cycles of [95 °C for 30 sec; 58 °C for 30 sec; 72 °C for 1 min], followed by 72 °C for 10 min. PCR products were separated on 1% (w/v) agarose gels containing 0.1 mg/ml Ethidium bromide.

Table 1 General characteristics of gene specific primers with their names and TMs, designed for transcript analysis of candidate genes involved in pathogenicity of *Xanthomonas citri* subsp. *citri*. Candidate genes are categorized based on their *in vivo* expression into the host plant cells.

<table>
<thead>
<tr>
<th>Candidate gene Accession No.</th>
<th>Organism</th>
<th>Molecular Function</th>
<th>Specific primers (5’-3’)</th>
<th>Expression pattern</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>hh</strong></td>
<td>XAC3673</td>
<td><em>Xanthomonas axonopodis</em> pv. <em>citri</em> strain 306</td>
<td>Signal transduction</td>
<td>hrp-F: CCTACCTGGCCCTACTCTG GG ACCAGCCAGTACCTCGA hrpB4-F: GGGATGTCGGGATTGTCG hrpB4-R: CAGTTCAGGACCTTGCAAGG hrpB-R: CAGTTCGACGGATTGACG</td>
<td>Early</td>
</tr>
<tr>
<td><strong>hrpB4</strong></td>
<td>XAC0410</td>
<td><em>Xanthomonas axonopodis</em> pv. <em>citri</em> strain 306</td>
<td>PAI-virulence protein</td>
<td>HrpB4: CAGTTCAGGACCTTGCAAGG hrpB-R: CAGTTCGACGGATTGACG</td>
<td>Early</td>
</tr>
<tr>
<td><strong>rpfB</strong></td>
<td>XAC1355</td>
<td><em>Xanthomonas axonopodis</em> pv. <em>citri</em> strain 306</td>
<td>long-chain fatty acyl CoA ligase</td>
<td>hrp-F: CAGTTCGACGGATTGACG</td>
<td>Early</td>
</tr>
<tr>
<td><strong>btuB</strong></td>
<td>XAC1310</td>
<td><em>Xanthomonas axonopodis</em> pv. <em>citri</em> strain 306</td>
<td>TonB-dependent receptor</td>
<td>btuB-F: CAGTTCAGGACCTTGCAAGG btuB-R: CAGTTCGACGGATTGACG</td>
<td>Late</td>
</tr>
<tr>
<td><strong>hrpF</strong></td>
<td>XAC394</td>
<td><em>Xanthomonas axonopodis</em> pv. <em>citri</em> strain 306</td>
<td>Translocator of effector proteins</td>
<td>hrp-F: CAGTTCGACGGATTGACG</td>
<td>Late</td>
</tr>
<tr>
<td><strong>phpA</strong></td>
<td>EF473086</td>
<td><em>Xanthomonas citri</em> pv. <em>citri</em> Strain: Xc270</td>
<td>Avirulence protein</td>
<td>PhpA-F: CAGTTCGACGGATTGACG</td>
<td>No amplification</td>
</tr>
<tr>
<td><strong>avrBs2</strong></td>
<td>XAC0076</td>
<td><em>Xanthomonas axonopodis</em> pv. <em>citri</em> strain 306</td>
<td>Avirulence protein</td>
<td>avrBs2-F: CAGTTCGACGGATTGACG avrBs2-R: CAGTTCAGGACCTTGCAAGG</td>
<td>Constant</td>
</tr>
<tr>
<td><strong>hrpC</strong></td>
<td>XAC0450</td>
<td><em>Xanthomonas axonopodis</em> pv. <em>citri</em> strain 306</td>
<td>TSSS Membrane protein</td>
<td>HrpC: CAGTTCGACGGATTGACG</td>
<td>Constant</td>
</tr>
<tr>
<td><strong>clp</strong></td>
<td>XAC0485</td>
<td><em>Xanthomonas axonopodis</em> pv. <em>citri</em> strain 306</td>
<td>cAMP-Regulatory protein</td>
<td>clp-F: CAGTTCGACGGATTGACG</td>
<td>Constant</td>
</tr>
<tr>
<td><strong>fleE</strong></td>
<td>XAC1983</td>
<td><em>Xanthomonas axonopodis</em> pv. <em>citri</em> strain 306</td>
<td>Flagellar biosynthesis hook protein/motor activity</td>
<td>fleE-F: CAGTTCGACGGATTGACG</td>
<td>Constant</td>
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<tr>
<td><strong>hrpC</strong></td>
<td>XAC0415</td>
<td><em>Xanthomonas axonopodis</em> pv. <em>citri</em> strain 306</td>
<td>HrcC protein/transporter activity</td>
<td>hrcC-F: CAGTTCGACGGATTGACG hrcC-R: CAGTTCGACGGATTGACG</td>
<td>Constant</td>
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<tr>
<td><strong>hrpE</strong></td>
<td>XAC0397</td>
<td><em>Xanthomonas axonopodis</em> pv. <em>citri</em> strain 306</td>
<td>HrpE protein</td>
<td>hrpE-F: CAGTTCGACGGATTGACG hrpE-R: CAGTTCGACGGATTGACG</td>
<td>Constant</td>
</tr>
<tr>
<td><strong>hrpG</strong></td>
<td>XAC1265</td>
<td><em>Xanthomonas axonopodis</em> pv. <em>citri</em> strain 306</td>
<td>two-component response regulator activity</td>
<td>hrgP-F: CAGTTCGACGGATTGACG hrgP-R: CAGTTCGACGGATTGACG</td>
<td>Constant</td>
</tr>
<tr>
<td><strong>hrpXCT</strong></td>
<td>XAC1260</td>
<td><em>Xanthomonas axonopodis</em> pv. <em>citri</em> strain 306</td>
<td>Transcription factor activity</td>
<td>hrgP-F: CAGTTCGACGGATTGACG hrgP-R: CAGTTCGACGGATTGACG</td>
<td>Constant</td>
</tr>
<tr>
<td><strong>tlyC</strong></td>
<td>XAC1709</td>
<td><em>Xanthomonas axonopodis</em> pv. <em>citri</em> strain 306</td>
<td>hemolysin C</td>
<td>tlyC-F: CAGTTCGACGGATTGACG tlyC-R: CAGTTCGACGGATTGACG</td>
<td>Constant</td>
</tr>
<tr>
<td><strong>16S rRNA</strong></td>
<td>DQ66089</td>
<td><em>Xanthomonas citri</em> strain FB-1314</td>
<td>housekeeping gene</td>
<td>16S-F: CAGTTCGACGGATTGACG 16S-R: CAGTTCGACGGATTGACG</td>
<td>Constant</td>
</tr>
</tbody>
</table>
Transcript analysis of some pathogenicity-related elements in Xcc  

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Results

A number of pathogenicity-related candidate genes were expressed in non-inducible culture medium

Amongst sixteen reported pathogenicity-related genes that were analyzed here, three were constitutively expressed in non-inducible medium (Fig. 1A). Surprisingly, two of these genes (hk and rpfB) were those involved in quorum sensing and cell to cell communication pathway. The other in-vitro responding gene was btuB, a gene involved in iron uptake in bacteria. The rest of the genes appeared not to be expressed in non-inducible condition. The 16S rRNA gene was used as reference gene for data normalization.

Disease-related candidate genes were expressed in a time course-dependent manner

Except for one gene, pthA* whose expression was not detected in this experiment, all other genes appeared to be expressed in different time points after inoculation (Fig. 1B-D). Different patterns of gene expression were noticed. Three candidate genes, hk, hrpB4 and rpfB showed an early-responsive pattern of expression. A relatively strong expression immediately after inoculation with no or very weak expression thereafter was demonstrated for these genes. The late-responsive genes to inoculation were hrpF and btuB. The rest of the candidate genes were constitutively expressed in the course of time including avrBs2, clp, flgE, hrcJ, hrpB2, hrpE, hrpG, hrpXCT, and tlyC. The 16S rRNA expression level was considered to serve as the reference gene.

Discussion

A relatively precise comprehension of chronological events involved in establishment, pathogenicity, quorum sensing and the regulation of all the steps required for a pathogenic system to function properly, can be achieved via transcript analysis of bacterial key genes. This data may lead to targeted approaches of how, where and when to enforce plant cells against the invasion of bacterial cells with devastating effects on yield and the quality of produce. A candidate gene approach including 16 hrp-related genes, previously reported to be involved in different steps of Xanthomonas citri subsp. citri (strain 306) pathogenicity (daSilva et al., 2002), was undertaken for the pathotype A’. The transcript analysis was performed for bacterial cells grown in culture media (uninduced) and cells isolated from leaves of Mexican lime (induced).

In Xanthomonas citri subsp. citri, pthA and its homologs are recognized as genes indispensable for pathogenicity and elicitation of specific symptoms of asiatic citrus canker disease (Swarup et al., 1992; Al-Saadi et al., 2007). However, using gene specific primers designed based on the A*-type strain (Xc270) sequence failed to produce any amplicons with either cDNAs in both uninduced and induced conditions (Fig. 1A and 1C) or genomic DNA (data not shown). The pthA and its homologs, pthA*, pthA*, pthB and pthC are known to be involved in pathogenicity response of Xcc (Al-Saadi et al., 2007). These genes are members of avrBs2 gene family responsible for host specificity and avirulence of plant bacterial pathogens (Gurlebeck et al., 2006). It has been demonstrated that alteration in the nucleotide sequence of the members of this gene family causes failure in specific host recognition (Yang et al., 2005). Thus, sequence conversions are probable between and within pathotypes as we have reported earlier (Soltaninejad, et al., 2010).

Bacterial cells use quorum sensing, a way of communicating between cells for the regulation of diverse arrays of physiological activities, such as biofilm formation, competence, conjugation, antibiotic production, motility, sporulation, symbiosis, virulence, and pathogenicity (Miller and Bassler, 2001). Although for such cooperative actions a local threshold cell density is required (Diggle et al., 2007). Similarly, Xcc utilizes the system for the regulation of xanthan (EPS) biosynthesis, gene expression, motility, adaptation, and bacterial virulence (Laia et al., 2009). Amongst many genes involved in this biological process, two
genes were transcriptionally analyzed; \( hk \), histidine kinase sensor which is a member of two-component signal transduction systems, and \( rpfB \), involved in the production of a diffusible signal factor.

Prokaryotes and *Xanthomonas* use HK and a response regulator (RR) to sense and respond to the surrounding stimuli (Qian et al., 2008; Laia et al., 2009), suggesting a possible role in quorum sensing (Neiditch and Hughson, 2007). In our study, \( hk \) was expressed in uninduced medium and further analysis on Mexican lime demonstrated its expression in the first few days of inoculation and its disappearance in the following days (Fig. 1B). Our findings were in agreement with the previously reported role of HK to be a bacterial sensor, sensing the neighboring milieu during quorum sensing until bacterial cells reach a certain population density.

\( rpfB \), a member of gene cluster known as \( rpf \) family, is part of the diffusible signal factor type quorum sensing with a critical role in regulating the production of virulence factor (Barber et al., 1997; He et al., 2007). Similar to \( hk \), \( rpfB \) was expressed in the first day post inoculation, but stronger than \( HK \) and disappeared in the following days (Fig. 1B). The early expression pattern of \( rpfB \) gene in the first day post inoculation may reflect its pivotal role in pathogenicity process of \( Xcc-A^{*} \) and its necessity for expression of the other genes involved in a successful compatible interaction of the bacterium with its proper host plant. Similarly, \( rpfB \) was expressed in uninduced condition (Fig. 1A). It can be concluded that this gene amongst many others is involved in bacterial colonization and host infection, if the environmental conditions are in favor of the bacterial cells.

Within the late expressing genes, \( hrpF \) showed an expression near to nil in first two days and a blast in expression in the following days (Fig. 1C). This data is in agreement with the reported function of \( hrpF \) as being protein terminator of the injectisome and translocator of effector proteins into the host plant cytoplasm (Rossier et al., 2000).

In addition to the effector proteins that are directly involved in pathogenicity, other gene products are necessary to weaken host defense mechanisms. These may induce signaling molecules and specifically reactive oxygen species (ROS) (Fones and Preston, 2011). One of such genes is \( btuB \) that may indirectly suppress the ROS action in host cell. Bacterial cells use superoxide dismutases (SODs) for ROS detoxification. Some of which are iron dependent dismutases (FeSOD); failing to act in iron deficient environments (Kim et al., 1999). This highlights the critical role of iron in pathogenicity (Pandey and Sonti, 2010), and the need of bacterial cells to have a rather sophisticated machinery for iron uptake. A gene with a pivotal role in this system is \( btuB \) that through insertional mutation analysis, an iron uptake deficiency in *Xanthomonas campestris* pv. campestris was demonstrated (Qian et al., 2005). Here, analysis of \( btuB \) expression pattern revealed that its expression starts at day 3 post inoculation and increases between days 4 to 7 (Fig. 1C). The surge in later days may infer the time that the bacterial cell density has passed a certain limit that has turned on the ROS metabolic pathways in the host that is needed to be detoxified by iron-dependent dismutases such as BtuB. Furthermore, it has been noted that \( btuB \) is expressed in uninduced media (Fig. 1A). Nutritional deficiency is an inevitable consequence of bacterial growth in the medium. In these situations, iron uptake system is activated to overcome the iron deficiency in the media. The next step in the hierarchy of pathogenesis, would be to introduce the regulatory genes for both providing bacterial survival and enforcing proper infection by the bacteria. The controlling genes can be divided into transcription factors, genes involved in plant-bacteria interactions and quorum sensing.

Some of the structural genes, involved in this study, including \( hrcJ \), \( hrpB2 \) (Schulz and Buttner, 2011), and \( hrpE \) (Weber and Koebnik, 2005) demonstrated constant low expression, while \( hrcC \) expression was high during the first week of induction (Fig. 1D). It is apparent that the gene products that are being used as the
core components of injectisome (Schulz and Buttner, 2011) are expressed all the way through the establishment of the structure. Therefore, a low continuous expression suffices the bacterial need. In contrast, HrcC is an oligomeric protein complex, comprising the outer part of the core (Deng and Huang, 1999; Lin et al., 2006), and due to its polymeric nature a constant high expression is required by bacterial cells (Fig. 1D). Interestingly in contrast, hrpB4 was expressed only one day after inoculation (Fig. 1D). This different expression pattern may be due to its role in the formation of injectisome. HrpB4 is one of the main building blocks of the core that perhaps is needed early in the structural formation of the basal parts of the injectisome. Nevertheless, this hypothesis needs to be tested via in situ localization studies. Eventually, by finalizing the injectisome structure it is time for some of the effector proteins to be biosynthesized and be translocated into the host cytoplasm via the tube-like structure. It is worth stating that some of the effectors keep synthesizing all the way through, even before the injectisome construction is completed. The constant low expression of these effector proteins seems to be significant in signaling to the virulence apparatus that the bacterial cells have approached the host and further might have a vital role in keeping virulence machinery turned on. These hypotheses need to be further confirmed by mutant analyses of the genes followed by determining any behavioral changes in bacterial pathogenicity.

Clp (cAMP receptor-like protein) as a global transcription factor, controls the expression of a subset of genes involved in pathogenesis in xanthomonads (Leduc and Roberts, 2009) and its regulatory function is apparent from its constitutive expression (Fig. 1D).

The secretion of effector proteins need to be tightly regulated by regulatory elements known as HrpG and HrpX regulons (Yamazaki et al., 2008; Guo et al., 2011). A recent global analysis of transcripts revealed that these two regulons are vital for the pathogenicity of Xcc (Guo et al., 2011). HrpG and HrpX regulons control all 24 T3SS secretion system genes, 23 T3SS effector genes, and 29 type II secretion system substrate genes. Furthermore, these two regulons adjust the bacterial behavior in response to the host environment by altering a range of metabolic pathways including amino acid biosynthesis; oxidative phosphorylation; pentose-phosphate pathway; transport of sugar, iron, and potassium; and phenolic catabolism (Guo et al., 2011). Our data about the constant low expression pattern of hrpG and hrpXCT conforms to their global regulatory and signaling roles.

Among the constitutively expressed genes, avrBs2 and tlyC (Astua-Monge et al., 2005) showed low expressions from day 1 inferring that these genes belong to the second class with a signaling role (Fig. 1D).

The last gene was flgE (hook) that is involved in flagellum motility and biofilm formation. In the absence of flagellin the pathogenicity of Xanthomonas is reduced (Malamud et al., 2011). flgE had a constant expression pattern (Fig. 1D) indicating that the studied Xcc-A* strain may require its flagellum to cause disease. In conclusion, in this study we took advantage of RT-PCR technology to find elements of regulatory interconnections between genes involved in pathogenicity of Xanthomonas citri subsp. citri type A*. The dynamics of injectisome formation was also elucidated. This research demonstrated that pathogenicity system in the NIGEB-088 A*-type strain of Xcc, is activated upon bacterial infiltration into the host plant leaf cells in the first day after inoculation. The expression of hrpF gene and translocon synthesis on the fourth day post inoculation shows eventually that injectisome is completed after four days and that the T3SS of the given strain is apparently ready to secrete protein effectors into the host plant cells to trigger pathogenicity reaction. By including more candidate genes and using a more sensitive method of gene expression analysis such as real time PCR, it would be possible to find more regulatory elements in the pathogenicity network.
Figure 1  *In vitro* and *in vivo* transcript analysis of sixteen candidate genes involved in pathogenicity of *Xanthomonas citri* subsp. *citri*. Uninduced condition: A) Candidate genes that expressed in uninduced condition. Amongst 16 candidate genes, RT-PCR demonstrated the expression of three genes in vitro condition, namely *rpfB*, *btuB*, and *hk*. Induced condition: B) The expression pattern of early responsive genes C) The expression pattern of late responsive genes D) The expression pattern of candidate genes with constant expression from day 1-7 after inoculation. The PCR product of reverse transcription was separated on 1% agarose gel. 16S rRNA was used as standard. dai: days after inoculation.

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آنالیز ترانسکرپتیه بعضی از عناصر دخیل در بیماری زایی یک جدایی ایرانی از تیب بیماریزای A باکتری Xanthomonas citri subsp. citri

روعنه روحانی 1، مسعود سلطنی نجف آبادی 2، سید مهدی علوی 3، ناصر فرجی 4 و مسعود شمسی بهش 5

چکیده: شاکر آسیایی مركبات از جمله بیماری‌های مخربی است که زبان‌های ستگنی بل اقتصاد کشاورزی کلیه کشورهای تولیدکننده مركبات در جهان وارد می‌سازد. در بین سه نوع مختلف بیماری A، A* و Aw از نظر زننده کمتر مطالعه شده‌اند. به منظور اندازه‌گیری این بیماری باکتری (Xanthomonas citri subsp. citri) A*، از نمونه‌های کشاورزی گیاه گیاهی در مرکز آهلی شورا و در شرایط حیاتی و حیاتی در بیماری‌های مختلف داشت. روش RT-PCR در مواجه با گیاه میزان، از روی شرایط (نظامی) با یکدیگر مقایسه شدند. نتایج حاکی از بیش بانی بخاری زنها در شرایط این نسبت به حالت غیرقابلی بود که به دلیل بالا در میزان بهترکنش باکتری و کاهش این میزان مورد مطالعه در پاسخ به پیشنهاد حاصل از جدایی مذکور را می‌توان در سه کلاس دانمارک، روحنجی و دیرپاش طبقه‌بندی نمود.

واژگان کلیدی: Xcc, RT-PCR, شاکر مركبات, لیموی شیراز.