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RESEARCH ARTICLE

Comparative transcriptome profiling of two maize near-isogenic lines differing in the allelic state for bacterial brown spot disease resistance

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Abstract

The bacterial brown spot disease (BBS), caused primarily by *Pseudomonas syringae* pv. *syringae* van Hall (*Pss*), reduces plant vigor, yield and quality in maize. To reveal the nature of the defense mechanisms and identify genes involved in the effective host resistance, the dynamic changes of defense transcriptome triggered by the infection of *Pss* were investigated and compared between two maize near-isogenic lines (NILs). We found that *Pss* infection resulted in a sophisticated transcriptional reprogramming of several biological processes and the resistant NIL employed much faster defense responses than the susceptible NIL. Numerous genes encoding essential components of plant basal resistance would be able to be activated in the susceptible NIL, such as *PEN1*, *PEN2*, *PEN3*, and *EDR1*, however, in a basic manner, such resistance might not be sufficient for suppressing *Pss* pathogenesis. In addition, the expressions of a large number of PTI-, ETI-, PR-, and WRKY-related genes were pronouncedly activated in the resistant NIL, suggesting that maize employ a multitude of defense pathways to defend *Pss* infection. Six R-gene homologs were identified to have significantly higher expression levels in the resistant NIL at early time point, indicating that a robust surveillance system (gene-to-gene model) might operate in maize during *Pss* attacks, and these homolog genes are likely to be potential candidate resistance genes involved in BBS disease resistance. Furthermore, a holistic group of novel pathogen-responsive genes were defined, providing the repertoire of candidate genes for further functional characterization and identification of their regulation patterns during pathogen infection.

Keywords: maize (*Zea mays* L.), bacterial brown spot disease, RNA-Seq analysis, disease resistance

1. Introduction

As a Gram-negative bacterium and one of the most adaptive plan pathogens, *Pseudomonas syringae* exists as over 50 races and can infect a wide range of plant species, including the model plant *Arabidopsis* and crops, and consequently leads to severe yield and quality losses (Höfte and de Vos 2006). A notable feature of the pathogenesis of *Pseudomonas syringae* is its implementing of the type III secretion system to direct the translocation of effectors into the host cell cytoplasm. Then these type III effectors will utilize different strategies to interfere with various aspects of plant defense pathways, such as altering host protein turnover and RNA metabolism, and/or inhibiting MAP kinase signaling (Collmer *et al.* 2000; Block *et al.* 2008).

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In maize, *Pseudomonas syringae* pv. *syringae* van Hall (*Pss*) causes bacterial brown spot disease (BBS), also known as holcus leaf spot (Gonzalez and Vidaver 1979; Nyvall 1979). The infection takes place on leaves, mainly makes use of wounding sites, and induces the formation of lesions with a water-soaked appearance. BBS in maize is a seed-borne disease, and tends to be favored during warm and wet weather (Nyvall 1979; Höfte and de Vos 2006). Although BBS appears somewhat less prevalent in maize, BBS may become severer once injuries are created by hail, blowing soil or high winds.

Our previous studies showed that *Psy*, a single dominant locus, conferred the BBS resistance in maize inbred line F349 and was delineated in a 300-kb interval flanked by markers AE11 and FG29-3 on chromosome 10 (Xu *et al.* 2009). After retrieving maize B73 RefGen_v2 genome, 10 genes were predicted to locate within this interval. Two of which, *Psy1* and *Psy2*, with an extracellular B-lectin domain and an intracellular serine-threonine kinase domain, were considered as the top candidate genes for the reason that multiple receptor-like kinase (RLK) genes have been identified to be associated with pathogen resistance in many crop species (Feuillet *et al.* 1997; Chen *et al.* 2006; Kawahigashi *et al.* 2011). Extensive studies aiming to molecularly characterize these two genes are on the way and the underlying functional mechanism awaits full exploitation.

Plants have evolved an armory of defense mechanisms against pathogen invasion, and the defense system can be generally divided into two types, i.e., PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI) (Jones and Dangl 2006). On the external face of host cell, elicitors termed pathogen-associated molecular patterns (PAMPs) such as bacterial flagellin or fungal chitin, could be recognized by a group of membrane proteins, termed as pattern recognition receptors (Jones and Dangl 2006). Perception of PAMPs is common in all multicellular organisms and will trigger a chain of signaling events and defense responses, collectively, called PAMP-triggered immunity (PTI). Therefore, PTI is considered as basal defense responses, preventing pathogen further spread. To antagonize the PTI, successful pathogens evolved a specialized type III secretion system to deliver effectors directly into host cell to interfere with plant defense (Collmer *et al.* 2000; Block *et al.* 2008). In turn, some plant species have evolved R-genes to recognize these effectors either directly or indirectly, activate ETI system and induce the formation of hypersensitive response (HR) (Jones and Dangl 2006).

Near-isogenic lines (NILs) have been shown to be the valuable materials for studying single gene effect by minimizing effects of genetic background and gene interactions, and widely used in the fields of gene mapping, molecular biology and marker-assisted breeding (Koester *et al.* 1993; Kim *et al.* 2011). In this study, two NILs carrying BBS-resistant and -susceptible alleles were developed by repeated backcrossing of the resistance line F349 as the donor parent and BBS-susceptible line P25 as the recurrent parent. To explore the complexity of transcriptome dynamic changes in response to BBS disease, a genome-wide transcriptional profiling analysis was performed at two time points after pathogen infection using RNA-Seq technique. A set of novel pathogen-responsive genes were identified, providing a repertoire of candidate genes for further functional characterization of the roles in pathogen resistance. These findings provide the first insight into the molecular aspect of BBS resistance in maize, and will increase our understanding of the interactions of plant-pathogen in crop.

2. Results

2.1. BBS symptoms in leaf

Our preliminary experiments showed that in contrast with the fully immunity to the BBS infection in resistant NIL plants, water-soaked disease spots were detected in the BBS-susceptible NIL plants in field. To monitor the progression of BBS disease more precisely, in the present study, we inoculated seeds with *Pss* culture and then grow plants under controlled condition.

Consistent to the observation in field, the susceptible NIL plants showed the characteristic circular water-soaked disease spots on the edge of first and/or second leaves initiated from 15 days post planting (dpp) (Fig. 1-A). At 20 dpp, the lesions continued to spread and appeared on the other parts of leaves, and occasionally merged to form a large necrotic area (Fig. 1-C). By contrast, no any lesions were readily detected on the leaves of resistant NIL plants at both 15 and 20 dpp (Fig. 1-B and D).

2.2. *Pss* infection leads to a transcriptional reprogramming

To investigate the specific responses involving in the resistant interaction, the levels of gene transcription in the resistant NIL were compared with the corresponding levels in the susceptible NIL across two different time points. Two housekeeping gene families, *ACTIN* and Elongation factor 1-alpha (*EF1*A), were used for the evaluation of gene expression (Manoli *et al*. 2012; Lin *et al*. 2014). In total, 12 *ACTIN* genes and 9 *EF1A* genes were used as reference genes. The value of the fold change (R/S) ranged from 0.61 (*EFIA* gene family gene GRMZM2G149768 at 20 days post planting (dpp)) to 1.37 (*EFIA* gene family gene GRMZM2G154218