



## Age-related Resistance and the Defense Signaling Pathway of *Ph-3* Gene Against *Phytophthora infestans* in Tomatoes

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### Abstract

Resistance (R) genes against plant pathogens often have age-related resistance (ARR) effects. However, the mechanism involved in this phenomenon remains unknown. In this paper, *Solanum lycopersicum* 'CLN2037B' and *S. pimpinellifolium* 'L3708' harboring the *Ph-3* gene, as well as *S. habrochaites* 'LA2099', 'LA1777' and 'LA1033' harboring quantitative trait loci (QTLs), were tested to investigate age-related resistance against late blight (LB; caused by *Phytophthora infestans*) in the three-leaf stage of the plants. The results demonstrated that the QTL-related LB resistance showed the same age-related resistance as the *Ph-3*-mediated resistance at the six- and nine-leaf stages compared with the three-leaf stage. This indicated that there is a common defense mechanism in tomatoes against *P. infestans* via ARR. In addition, we combined ethylene (ET), salicylic acid (SA) and jasmonic acid (JA) mutants with virus-induced gene silencing (VIGS) to study the *Ph-3*-dependent resistance signaling pathway. The results showed that ethylene and salicylic acid, but not jasmonic acid, are involved in the LB resistance mediated by the *Ph-3* gene.

**Keywords:** tomato; *Phytophthora infestans*; age-related resistance; QTL; pathway mutant; VIGS

### 1. Introduction

Late blight disease, caused by the oomycete pathogen *Phytophthora infestans* (Mont.) de Bary, is considered a major threat to potato and tomato production (Feng et al., 2004). To date, five LB resistance genes have been reported in tomatoes, namely *Ph-1* to *Ph-5*. Interestingly, all these genes were discovered from the wild tomato relative *Solanum pimpinellifolium* (Gallegly and Marvel, 1955; Peirce, 1971; Moreau et al., 1998; Chunwongse et al., 2002; Merk et al., 2012). Pyramiding of the *Ph-2* and *Ph-3* genes produced higher levels of resistance and thus the genes have been introduced

into several varieties of fresh and processed tomatoes (Li, 2010; Robbins et al., 2010). Meanwhile, quantitative trait loci (QTLs) against LB were also identified in wild tomato relatives, such as *S. habrochaites* 'LA2099', 'LA1777', 'LA1033', *S. pennellii* 'LA716', and *S. lycopersicoides* 'LA2051' (Lough, 2003; Robbins et al., 2010; Li et al., 2011). However, the drawbacks of QTLs, such as poorer effectiveness, sensitivity to environmental factors and strong linkage drag, have restricted their use in agricultural production.

Age-related resistance (ARR) is the phenomenon by

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which plants present increasing or decreasing resistance to pathogens during plant growth and development. ARR has been described in several plant-microbe interactions (Panter and Jones, 2002; Develey-Rivière and Galiana, 2007). For example, compared with adult tobacco (*Nicotiana benthamiana*) plants that showed full resistance to LB, young seedlings displayed lower resistance levels. Gourd (*Lagenaria siceraria*) plants were also reported to show ARR to certain diseases (Meyer and Hausbeck, 2013). For LB resistance in tomatoes, it has been reported that both the *Ph-2* and *Ph-3* genes have ARR effects (Chunwongse et al., 2002; Li et al., 2007; Li, 2010). However, the ARR effects on LB resistance that are controlled by QTLs remain unclear.

Plant hormones, such as ethylene (ET), salicylic acid (SA) and jasmonic acid (JA), are involved in the defense response against plant pathogens (Jones and Dangl, 2006; Dodds and Rathjen, 2010). Generally, the resistance mechanisms against pathogens vary with the life styles of the different pathogens. For example, SA is involved in the regulation of defense against biotrophic pathogens, whereas JA or ET have more important roles against necrotrophic pathogens and insects. *P. infestans* has a hemi-biotrophic nature; Therefore, there is controversy regarding which defense signals are involved in the interaction between tomatoes and LB pathogens. Resistance against LB disease requires the participation of SA, JA and SA-mediated basal defense in potatoes (Halim et al., 2009; Manosalva et al., 2010). However, studies on LB in tomatoes found that the participation of JA in R gene-mediated resistance was not necessary (Eschen-Lippold et al., 2010b). Shibata et al. (2010) reported that LB resistance in tobacco was attributed to a low concentration of SA in young plants, and the fully expressed resistance in adult plants resulted from programmed cell death induced by SA, as well as by phytoalexins induced by ET. To the best of our knowledge, in tomatoes, there have been no reports of *Ph-3*-mediated resistance mechanisms against LB, or mechanisms of ARR mediated by QTLs or other LB-resistant genes.

In this study, *P. infestans* isolate T<sub>1, 2, 4</sub> was selected to challenge plants at the three-, six- and nine-leaf stages. Our results indicated that the resistance regulated by QTLs was the same as that conferred by the single gene (*Ph-3*), showing an apparent correlation with plant age. In particular, the resistant levels of plants in the six- and nine-leaf stages were significantly higher than those in the three-leaf stage. In addition, mutants *Never ripe* (*Nr*), *defenseless* (*Def-1*) and *NahG*, which are involved in defense signaling pathways, together with virus-induced gene silencing (VIGS), were used to investigate the *Ph-3*-dependent resistant pathway. Our results showed that the resistance against LB was mediated by the *Ph-3* gene and required the participation of ET and SA, but not JA.

## 2. Materials and methods

### 2.1. Plant material

All experiments were performed in autumn 2011 and spring 2012 at the greenhouse of Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing. *S. lycopersicum* 'CLN2037B' and *S. pimpinellifolium* 'L3708' *Ph-3*-containing lines or the donor of the *Ph-3* gene, respectively, were used as resistant controls (kindly provided by the Asian Vegetable Research Center). *S. lycopersicum* 'LA2818' was used as the susceptible control (kindly provided by the Tomato Genetics Resources Center). Resistant tomato materials containing QTLs associated with LB resistance, including *S. habrochaites* 'LA1033', 'LA1777' and 'LA2099' (also provided by the Tomato Genetics Resources Center), were tested. To perform the age-related resistance test, a sowing interval of 15 days was used to obtain plants at different ages, including the three-leaf stage (20 days after sowing), six-leaf stage (35 days after sowing) and nine-leaf stage (50 days after sowing). All experiments contained three replicates with 15 plants in each block.

To investigate the downstream signaling pathway of *Ph-3*-mediated resistance, tomato hormone mutants, *def-1*, *Nr* and overexpression of SA-deficient mutant, *NahG* (provided by Dr. Yuling Bai, Wageningen UR Plant breeding), were crossed with 'CLN2037B'. The mutant *def-1* has a mutation in the *Defenseless-1* gene that participates in the JA signal transduction pathway. The *NahG* overexpression mutant expresses an SA hydroxylase that degrades SA. The mutant *Nr* (never ripening) is blocked for ET perception. The genetic backgrounds of these mutants were 'Moneymaker' (*def-1*), 'Pearson' (*NahG*), and 'VFN8' (*Nr*), all of which are tomato varieties susceptible to the tested isolate of LB. The *Nr* and *NahG* showed dominant inheritance, and *def-1* was recessive (Barry et al., 2001); Therefore, the F<sub>1</sub> (for *Nr* and *NahG*) or F<sub>2</sub> (for *def-1*) plants were selected using markers linked to *Ph-3* and identifying the phenotypes of the mutants, respectively. In total, 200 individuals from the F<sub>2</sub> population for each of the hormone pathway mutants and 'CLN2037B' were screened for homozygous progeny with the *Ph-3* gene using the genetically linked marker RGA2M1 (Zhang et al., 2013).

### 2.2. VIGS vectors

The *Tobacco rattle virus* (TRV)-based VIGS vectors pTRV2-*ACO1* for the ET pathway, and pTRV2-*NPRI*, pTRV2-*TGA1a* and pTRV2-*TGA2.2* for the SA pathway used in this study, were provided by Cheng Chiu-Ping from the National Taiwan University (Chen et al., 2009b). Empty vector pTRV200 was used as a control and the tomato *PDS* gene was used as the reporter.

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