Emerging devastating diseases, such as Huanglongbing (HLB) and citrus canker, have caused tremendous losses to the citrus industry worldwide. Genetic engineering is a powerful approach that could allow us to increase citrus resistance against these diseases. The key to the success of this approach relies on a thorough understanding of defense mechanisms of citrus. Studies of Arabidopsis and other plants have provided a framework for us to better understand defense mechanisms of citrus. Salicylic acid (SA) is a key signaling molecule involved in basal defense and resistance (R) gene-mediated defense against broad-spectrum pathogens. The Arabidopsis gene NDR1 (NON-RACE-SPECIFIC DISEASE RESISTANCE 1) is a positive regulator of SA accumulation and is specifically required for signaling mediated by a subset of R genes upon recognition of their cognate pathogen effectors. Our bioinformatic analysis identified an ortholog of NDR1 from citrus, CsNDR1. Overexpression of CsNDR1 complemented susceptibility conferred by the Arabidopsis ndr1-1 mutant to Pseudomonas syringae strains and also led to enhanced resistance to an oomycete pathogen, Hyaloperonospora arabidopsidis. Such heightened resistance is associated with increased SA production and expression of the defense marker gene PATHOGENESIS RELATED 1 (PR1). In addition, we found that expression of PR1 and accumulation of SA were induced to modest levels in citrus infected with Candidatus Liberibacter asiaticus, the bacterial pathogen associated with HLB disease. Thus, our data suggest that CsNDR1 is a functional ortholog of Arabidopsis NDR1. Since Ca. L. asiaticus infection only activates modest levels of defense responses in citrus, we propose that genetically increasing SA/NDR1-mediated pathways could potentially lead to enhanced resistance against HLB, citrus canker, and other destructive diseases challenging global citrus production.

**Keywords:** Pseudomonas syringae, salicylic acid, citrus canker, Huanglongbing, greening disease, Candidatus Liberibacter asiaticus, genetic engineering

**INTRODUCTION**

Huanglongbing (HLB, also called citrus greening disease), citrus canker, and other emerging diseases have imposed serious threats to the citrus industry worldwide (Bove, 2006; Gottwald, 2007). Citrus canker is caused by the gram negative bacterium Xanthomonas axonopodis pv. citri. Wind and rain facilitate the dispersal of the pathogen and spreading of the disease. More than 16 million citrus trees have been destroyed in Florida in an effort to restrict the disease (Gaskalla, 2006). HLB is even more devastating than citrus canker since it is highly contagious and lethal to afflicted plants (Bove, 2006; Brlansky and Rogers, 2007, Gottwald, 2007). The parasitic bacterium Candidatus Liberibacter that lives in the phloem tissue of a citrus tree is believed to be the associating agent of HLB. The disease is transmitted by a small insect vector of the family Psyllidae. The growth of psyllids cannot yet be reliably controlled by conventional insecticide application (Ichinose et al., 2010). Without successful measures to control the causal pathogen and its transmission vector, HLB is endemic to a variety of citrus species and related plants. Therefore, it is imperative to develop strategies to contain and eventually eradicate HLB and other diseases challenging the production of citrus worldwide. Successful manipulation of citrus disease resistance relies on a thorough understanding of defense mechanisms of the plant. Although not well understood yet in citrus, defense mechanisms are well studied in other plants, in particular the model plant Arabidopsis thaliana, and have been shown to be relatively conserved among plants (Nishimura and Dangl, 2010). Therefore, prior knowledge of defense mechanisms from other plants should help us to better understand citrus defense and ultimately develop effective strategies to combat devastating diseases challenging the citrus industry.

Plant defense can be preformed or induced. The preformed defense includes some existing physical structures and chemical compounds produced by plants before infection that can restrict pathogen invasion. The induced defense can be activated upon pathogen invasion, involving sophisticated surveillance systems to recognize general elicitors from pathogens and subsequently to activate basal defense. Much stronger defense can be induced
when host resistance (R) proteins specifically recognize their cognate pathogen effectors; thus this defense is also termed as R protein-mediated defense. The largest class of R proteins is represented by a family of proteins that have two conserved domains, nucleotide binding site (NBS) and leucine-rich repeat (LRR; Martin et al., 2003; McDowell and Simon, 2006). This class of R proteins can be further divided into two groups according to the N-terminal sequence, coiled-coil (CC)–NBS–LRR and Toll-interleukin-1 receptor (TIR)–NBS–LRR. Some CC–NBS–LRR type proteins are found to signal through NON-RACE–SPECIFIC DISEASE RESISTANCE 1 (NDR1). For instance, an ndr1 mutation compromises resistance conferred by the CC–NBS–LRR proteins RPS2, RPM1, or RP5 to Pseudomonas syringae expressing the avirulence effectors avrPto2, avrRpt1 and avrRpt6, or avrPph3, respectively (Century et al., 1995; Aarts et al., 1998). On the other hand, some TIR–NBS–LRR type proteins functionally require ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1). For instance, an eds1 mutant is immune-compromised to P. syringae avrRpt4, resistance to which is conferred by the TIR–NBS–LRR protein RP5 but not by TIR–NBS–LRR type R proteins (Aarts et al., 1998; Falk et al., 1999). These observations suggest a general rule that these two subgroups of R proteins can activate distinct downstream signaling pathways. However, exceptions to this rule also exist for some other NBS–LRR R proteins (McDowell et al., 2000; Bittner-Eddy and Beynon, 2001; Xiao et al., 2001).

The small phenolic molecule salicylic acid (SA) plays a key role in signaling both basal and R protein-mediated defense (Hammond-Kosack and Jones, 1996; Tsuda et al., 2008) and is involved in resistance against diverse pathogens and in response to various stress conditions (Malamy et al., 1990; Raussmann et al., 1991; Sharma and Davis, 1997; Tsuda et al., 2008). While increased SA accumulation and/or signaling lead to enhanced disease resistance, disrupting these processes by gene mutations or transgene expression result in compromised defense against pathogens (Durant and Dong, 2004; Liu, 2009). Genes involved in SA-mediated defense can affect SA biosynthesis, accumulation, and/or signaling (Liu, 2009). For instance, SA INDUCTION–DEFICIENT 2 (EDS1) encodes a protein with similarity to the majority of SA biosynthesis (Wildermuth et al., 2001). Both NDR1 and EDS1 are known to act upstream of SA to regulate SA accumulation (Falk et al., 1999; Shapiro and Zhang, 2001). Downstream of SA signaling, NONEXPRESSOR OF PR GENES 1 (NPR1) acts as a signal transducer that regulates systemic acquired resistance, a long-lasting defense against broad-spectrum pathogens at the whole plant level (Cao et al., 1997; Byls et al., 1997; Shah et al., 1997; Dong, 2004). Overexpression of NDR1, EDS1, NPR1, or several other SA regulators confers enhanced disease resistance to a range of pathogens in Arabidopsis and/or in other plants (Chorn et al., 2001; Fitzgerald et al., 2004; Lin et al., 2004; Makandar et al., 2006; Malnoy et al., 2007; Pegdaeraju et al., 2007; Sandhu et al., 2009; Gao et al., 2010). Therefore, manipulation of SA-mediated defense has the potential to introduce broad-spectrum disease resistance in plants.

NDR1 encodes a glycosyl-phosphatidyl inositol-anchored plasma membrane protein that belongs to a large protein family (Dormann et al., 1995; Varett et al., 2002; Coppinger et al., 2004; Zheng et al., 2004). A recent study implicates the function of NDR1 in mediating plasma membrane–cell wall adhesion (Knepper et al., 2011). NDR1-like genes widely exist in different plants (Lee et al., 2006; Cheong et al., 2008; Cacas et al., 2011). Besides NDR1, some Arabidopsis homologs of NDR1 were shown to be highly induced by pathogen infection and/or to confer enhanced disease resistance to P. syringae when overexpressed (Varett et al., 2002, 2003; Coppinger et al., 2004; Zheng et al., 2004). Thus NDR1 and some members in the family are critical components of plant defense.

In this study, we report the isolation and characterization of a functional ortholog of NDR1 in citrus, named CsNDR1. We found that overexpression of CsNDR1 complements the susceptibility of Arabidopsis ndr1-1 mutant to P. syringae avrRpt2 and further confers enhanced disease resistance to P. syringae avrRpt4, which normally is not affected by the endogenous NDR1. Overexpression of CsNDR1 also led to increased resistance to the oomycete pathogen Hyaloperonospora arabidopsidis (Hpa) isolate Noco2. CsNDR1-induced disease resistance is associated with increased SA accumulation and expression of the defense marker gene PATHOGENESIS RELATED 1 (PR1) in the transgenic Arabidopsis plants. In addition, we found that citrus infected with Candidatus Liberibacter asiaticus, a pathogen associated with the HLB disease, expressed modestly increased CsNDR1 and SA levels, compared with mock-treated plants. We propose that genetic engineering to enhance SA/CsNDR1 signaling pathway in citrus could potentially enhance its resistance to HLB, citrus canker, and other emerging diseases.

**MATERIALS AND METHODS**

**PLANT MATERIALS**

Arabidopsis plants were grown in growth chambers with a 12 h light/12 h dark cycle, light intensity at 200 μmol m−2s−1, 60% humidity, and 22°C. The ndr1-1 mutant was previously described (Century et al., 1995, 1997). Citrus plants, "Valencia" (Citrus sinensis [L.] Osbeck), were grown on the greenhouse bench and kept at 24°C under natural light conditions. Plants were irrigated as needed and fertilized every 3 weeks using a water-soluble fertilizer mix, 20–10–20P-20K (Peters Professional, The Scotts Company, Marysville, OH, USA).

**BIODINFORMATIC ANALYSIS**

Basic Local Alignment Search Tools (BLAST) was used to search protein sequence databases for Arabidopsis and Citrus sinensis, using appropriate query sequences. Sequence alignment and phylogenetic analysis were performed with the MEGA program (version 5.0). To construct the phylogenetic tree, the neighbor-joining method with 1000 bootstrap replications was used.

**PATHOGEN INFECTIONS**

Pseudomonas syringae strains used in this study were previously described (Lee et al., 2006; Wang et al., 2011b). Bacteria were cultured at 28°C with King's B medium (10 g proteose peptone, 1.5 g NaH2PO4, 3.2 ml 1 M MgSO4, and 5 g glucose/l) containing the appropriate antibiotics. Freshly cultured bacteria at the optimal
Valencia plants were used as controls. Plants were pruned immediately after graft-inoculation to promote new leaf growth and disease-free tissue pieces obtained from healthy greenhouse-grown strains. Sixty nanograms of DNase-treated RNA were used in a total volume of 20 μl. For detection of CaNDR1 transcripts, forward primer 5'-TCTCTCCAGCCCTCAGTC-3' and reverse primer 5'-TCATGAGGAGATCGAGGAA-3' were used. For detection of CaNDR1 rRNA transcripts, forward primer 5'-GCTGAGGAGATCGAGGAA-3' and reverse primer 5'-TCATGAGGAGATCGAGGAA-3' were used. Melting curve analysis was performed to ensure amplification of a single product and the absence of primer-dimers. For relative quantification of gene expression, the 2-ΔΔCt method was applied as previously described (Livak and Schmittgen, 2001), using cycle threshold (Ct) values of 18S rRNA for normalization.

DNA AMPLIFICATION, DNA CONSTRUCTION, AND PLANT TRANSFORMATION

To obtain the full-length CaNDR1 cDNA sequence, we used the SMARTer® RACE cDNA Amplification Kit (Clontech) to make a cDNA library from RNA extracted from Ca l. asiaticus-infected Valencia plants. Nested primers, NaR1-5R-P1 (CACCTTCTGATCGTACCGAGGAC) and NaR1-5R-P2 (CAATGCGTACCGAGTACGAT) were used to amplify the 5' end missing sequence while NaR1-5R-P1 (CATCGCCTGATCGTACCGAG) and NaR1-3R-P2 (CATCGCCTGATCGTACCGAG) were used to amplify the 3' end missing sequence. The amplified fragments were cloned in the pJET cloning vector, using the CloneJET PCR Cloning Kit (Thermo Scientific).
Scientific), and sequenced to obtain the full-length cDNA sequence. The full-length CsNDR1 cDNA was further amplified from the library with NDR1-F1 (ATGTCAGAAAACGCCGGTG) and NDR1-R1 (TTAACGAAAATAACGACGACAAAAAXATCG) and cloned into the pBIET vector. At least 10 individual colonies were prepared for DNA and analyzed by sequencing. The sequence with few mismatches, compared with the reference sequence, and a correct open reading frame was used as the template for further cloning into the binary vector pBINplusARS under the control of the CAMV 35s promoter. The construct was confirmed by sequencing and transferred to Agrobacterium tumefaciens for ndr1-1 transformation, using the floral dipping method (Clough and Bent, 1998). T4 seeds were selected for T1 plants on MS plates containing kanamycin, resistance to which was conferred by the binary vector. T1 transgenic plants were collected for seeds, which were further selected for homozygous T2 plants.

ION LEAKAGE MEASUREMENT

Leaves of 25-day-old Arabidopsis plants were infiltrated with a bacterial suspension Pma avrRpt2 (OD600 = 0.1) with a blunt-end syringe, using 10 mM MgSO4 treatment as a control. At 0, 4, and 7 h post-inoculation, five leaf discs, cut with a 6-mm core borer, were collected, washed in de-ionized water, and placed in a 15-ml tube with 5 ml of de-ionized water. Duplicate tubes for each sample were gently shaken for 15 min followed by the measurement of solution conductivity, using an electrical conductivity meter (The London Company, Welwyn International Inc. Cleveland, OH, USA). Each tube was measured three times to derive average conductivity.

SA MEASUREMENT

Free and total SA (glucosylated SA) were extracted from 25-day-old Arabidopsis plants (Ng et al., 2011; Wang et al., 2011a). The same protocol was used to extract SA from leaves of Citrus sinensis that demonstrated HLB symptoms. SA separation and detection were conducted with a high-performance liquid chromatography (HPLC) instrument as previously described (Ng et al., 2011; Wang et al., 2011a).

RESULTS

IDENTIFICATION OF A CITRUS NDR1 ORTHOLOG

Since NDR1 plays a critical role in Arabidopsis defense, we set out to identify the citrus ortholog and investigate its role in defense regulation. In Arabidopsis, NDR1 belongs to a large protein family with over 40 members, named NDR1/HIN1-like (NHL) proteins (Dörmanna et al., 2000). BLAST searching of the sequence database of Citrus sinensis revealed a citrus protein (Citrus sinensis 1.1.g028712m) with the highest similarity to NDR1 (the E-value is 2.6e−53) and three other top hits with E-values below 1.0 e−5. We further used CsNDR1 as the query to search the Arabidopsis protein database and retrieved sequences of NDR1 and 14 NHL proteins as the top hits. To determine the extent of similarity among these proteins, phylogenetic analysis was conducted, using the MEGA program (version 5.0, Tamura et al., 2011). Figure 1 shows that CsNDR1 is in the same cluster with NDR1 with 99% bootstrap support. Two other citrus NHL proteins (orang1.1.g041808m and orang1.1.g08713m) are also in the same cluster with NDR1 but with lower confidence levels in bootstrap support. Thus, bioinformatic analysis suggests that CsNDR1 is an ortholog of NDR1.

ECTOPIC EXPRESSION OF CsNDR1 COMPLEMENTS Arabidopsis ndr1-1 MUTANT

To test if CsNDR1 shares conserved function with its Arabidopsis correspondence, we used a genetic complementation approach. The full-length CsNDR1 cDNA was amplified via RT-PCR from a cDNA library made from Citrus sinensis and cloned initially to the pET vector and then to the binary vector pBINplusARS under the control of the CAMV 35s promoter. The CsNDR1/pBINplusARS construct was used to transform ndr1-1 via the standard floral dipping method (Clough and Bent, 1998). The presence of the transgene was confirmed by PCR with gene-specific primers. Initial infection of the T1 transgenic plants with a virulent strain P. syringae pv. maculicola ES4326 (Pma) indicated that some of the transgenic plants were more resistant than ndr1-1 (data not shown). We further isolated homozygous plants for eight independently transformed lines (ndr1-1 + CsNDR1). Infection of these plants with Pma showed that all lines were more resistant than ndr1-1 and some were even more resistant than Col-0 (Figure 2A). Total RNA was isolated from these plants and northern blotting indicated that the level of disease resistance in some transgenic plants was correlated with the degree of transgene expression (Figure 2B). Thus, our results suggest that CsNDR1 positively regulates Arabidopsis defense.

The Arabidopsis RPS2 is a CC-NBS–LRR type R protein. When recognizing the avirulent strain Pma avrRpt2, RPS2 activates strong defense responses. Such defense activation requires the function of NDR1 and sometimes leads to HR, a rapid programmed cell death in the infected region (Aarts et al., 1998). We found that all transgenic plants showed enhanced disease resistance to Pma avrRpt2 (OD600 = 0.0084), compared with ndr1-1 (Figure 3A). In addition, the ndr1-1 mutant showed compromised HR in response to a high dose of Pma avrRpt2 (OD600 = 0.1), as indicated by the lack of leaf collapse (Figure 3B, top panel). We found that all transgenic plants showed partial to full rescue of HR-defect of ndr1-1 (Figure 3B, bottom panel).

Interestingly, line 15 that showed the highest level of CsNDR1 expression, had a low frequency of leaf collapse in the HR assay, albeit still more than the ndr1-1 mutant. We also noticed that this line is smaller than other lines (Figure 4A). Thus we suspected that the small leaf size might obscure the HR scoring. To better quantify the HR cell death, we performed ion leakage measurement with this line and another line (99) that showed medium CsNDR1 expression (Figure 2B). When challenged with Pma avrRpt2 (OD600 = 0.1), ndr1-1 had the lowest level of ion leakage (Figure 4B, Zhang et al., 2004), consistent with its HR-deficit. The ion leakage level was highest in line 15 and medium in line 9, compared with Col-0. Together disease resistance and HR assays suggest that CsNDR1 functions similarly as in Arabidopsis.
FIGURE 1 | Neighbor-joining phylogenetic tree to show relationship among NDR family proteins from Arabidopsis thaliana (Walter et al., 2002) and citrus (Lu et al., 2013). The protein sequences were retrieved from BLAST search of Citrus sinensis protein database with NDR1 sequence as a query or from BLAST search of Arabidopsis protein database with CsNDR1 sequence as a query, using an E-value cutoff of $e^{-4}$. The MEGA program (version 5.05) was used to construct the tree. Protein sequences of the indicated genes were aligned with the ClustalW method and the tree was generated with the neighbor-joining method, using 1000 bootstrap replications. Numbers on the tree indicate bootstrap support (values $< 50\%$ not shown). Branch lengths were drawn to scale; size bar represents number of amino acid substitutions per site. RIN4 protein sequence was used as an outgroup to root the tree.

Arabidopsis NDR1 in both basal and resistance protein-mediated defense.

ECTOPIC EXPRESSION OF CsNDR1 LEADS TO ACTIVATION OF SA-MEDIATED DEFENSE AND BROAD-SPECTRUM DISEASE RESISTANCE

Salicylic acid is a key signaling molecule regulating defense pathways including basal defense, R gene-mediated resistance, and systemic acquired resistance (Durrant and Dong, 2004; Lu, 2009). To test whether SA-mediated defense is activated in the transgenic plants, we quantified SA levels. We found that line 15 but not line 9 accumulated much higher levels of both free and total SA (glucosylated SA; Figure 4C). Consistent with its high SA levels, line 15 also showed higher expression of the SA marker gene PR1 (Figure 4D). These results suggest that overexpression of CsNDR1 to a certain level activates SA signaling.

To further investigate how overexpressing CsNDR1 affects disease resistance, we challenged line 9 and 15 with additional $P$. syringae strains. Ectopic expression of CsNDR1 complemented susceptibility conferred by $ndr1-1$ to the virulent strain $P$. syringae pv. tomato DC3000 ($Pto$, Figure 5A, left). Line 15 is also more resistant to the isogenic avirulent strain $Pto$ avrRps4 (Figure 5A, right), which is recognized by RPS4 (a TIR–NBS–LRR type of R protein) independently of NDR1 (Aarts et al., 1998). Thus, these results indicate that ectopic expression of CsNDR1 leads to activation of resistance to a pathogen that the endogenous gene otherwise does not have an effect on. In addition, we found that line 9 and 15 showed increased resistance to the virulent oomycete
FIGURE 2 | CsNDR1 rescues enhanced disease susceptibility conferred by Arabidopsis ndr1-1 to a virulent P. syringae strain. (A) Bacterial growth assay. Twenty-five-day-old plants were infected with the virulent strain P. syringae pv. maculicola ES4326 (Pma; OD600 = 0.0001). Bacterial growth was assessed 3 days after infection. Data represent the average of bacterial numbers in six samples ± standard error. Statistical analysis was performed with log transformed data, using one-way analysis of variance (ANOVA) Fisher’s protected least significant difference (PLSD) tests (StatView 5.0.1). Different letters indicate significant difference among the samples (P < 0.05).

(B) Expression analysis of transgenic plants carrying CsNDR1. Total RNA was extracted from 25-day-old plants and analyzed by northern blotting. A probe specific to CsNDR1 was used to detect expression of the transgene. The 18S rRNA probe was used to indicate equal loading in the samples. These experiments were repeated two times with similar results.

pathogen Hpa Noco2, compared with ndr1-1 (Figure 5B). Thus, overexpressing CsNDR1 could lead to broad-spectrum disease resistance.

SA ACCUMULATION AND EXPRESSION OF CsNDR1 ARE MODESTLY INDUCED BY Ca. L. asiaticus INFECTION

To see how SA signaling is affected by HLB in citrus, we infected 15-month-old ‘Valencia’ plants with Ca. L. asiaticus, using mock-treated plants as a control. We began to observe at 11 wai HLB symptoms, chlorosis and/or blotchy mottling of leaves, which increased in severity by 16 wai (Figure 6A). We did qPCR with the symptomatic and control leaves, using primers specific to Ca. L. asiaticus. The average Ct values of the symptomatic leaves were 21.8 at 11 wai and 20.7 at 16 wai. No Ca. L. asiaticus was detected in the control. Thus these symptomatic leaves were confirmed to be Ca. L. asiaticus positive. The symptomatic leaves were further collected for SA measurement and RNA analysis. Compared with the mock control, the symptomatic leaves from infected plants had about twofold more total SA levels (Figure 6B). Similarly, expression of CsNDR1 was also induced about twofold more in the infected leaves (Figure 6C). These data suggest that SA and/or
CSNDR1 overexpression leads to increased ion leakage, SA accumulation, and PR1 expression. (A) Picture of 25-day-old plants. Other transgenic lines resemble Col-0 (not shown). (B) Ion leakage measurement. The fourth to seventh leaves of 25-day-old plants were infiltrated with Pma avrRpt2 (OD600 = 0.1) and collected at the indicated times post-infection for ion leakage measurement. The data represent average of triplicate samples, each of which contained five individual leaf disks of 6 mm in diameter. (C) SA quantitation. Twenty-five-day-old plants were harvested for SA extraction followed by HPLC analysis. Asterisks indicate significant difference between the samples and Col-0 (P < 0.05; n = 3). (D) Northern blotting. DNA fragments specific to PR1 and 18s rRNA, respectively, were used to probe blots containing RNA samples from Col-0, ndr1-1, and transgenic lines 9 and 15. These experiments were repeated two times with similar results.

DISCUSSION
In this study, we presented bioinformatic and experimental evidence suggesting that the citrus gene CSNDR1 is an Arabidopsis NDR1 ortholog. Overexpression of CSNDR1 in Arabidopsis rescues ndr1-1 conferred susceptibility to P. syringae infection and leads to broad-spectrum disease resistance to the oomycete pathogen Hpa Noco2. We also found that both SA accumulation and CSNDR1 expression are induced to modest levels in citrus upon infection with Ca. L. asiaticus, the agent associated with HLB. Our data suggest a possibility that manipulation of SA/CsNDR1-mediated defense may lead to enhanced resistance to HLB and other devastating diseases in citrus.

SA plays a critical role in regulating plant resistance against various pathogens. Broad-spectrum disease resistance has been successfully introduced into several economically important plants via manipulation of the SA pathway. Much of the previous studies have been focused on NPR1, a key SA signal transducer. For instance, overexpression of Arabidopsis NPR1 and/or its homologs from other plants confers resistance against diverse bacterial and fungal pathogens in Arabidopsis, apple, citrus, soybean, tomato,
proteins (Aarts et al., 1998; Coppinger et al., 2004). Although not to be specifically required for defense activated by some CC–imidacloprid (Imid, Admire®), a range of pathogens (Campbell et al., 2002). Consistent with this, the relatively low level of host defense in response to L. asiaticus is quite small (Figure 6B). In addition, our gene expression data (Figure 6C) and microarray analyses (Albrecht and Bowman, 2008, 2012) indicate that the spectrum and intensity of defense genes induced by Ca. asiaticus are also quite limited. These observations suggest that when infected by Ca. asiaticus, citrus plants do not activate considerable host defense. This can be explained with at least two possible reasons: (1) a lack of recognition of effector proteins from Ca. asiaticus by citrus; and/or (2) a suppression of host defense by Ca. asiaticus. Thus, the interaction between citrus and Ca. asiaticus can be viewed as a compatible interaction, leading to disease symptom development in the host. The relatively low level of host defense in response to Ca. asiaticus also suggests a possibility that HLB resistance can be achieved if we could manipulate the host to enhance its defense levels.

Genetic engineering is a particularly attractive approach to introduce disease resistance traits into citrus because citrus has long juvenile growth – it typically takes 5–15 years for a citrus plant to flower. In addition, most commercial citrus cultivars produce polyembryonic seeds asexually, which complicates the process of introducing novel traits into citrus via traditional breeding (Koltunow et al., 1996). Recently the Arabidopsis NPR1 gene was shown to increase resistance to the canker disease when overexpressed in citrus (Zhang et al., 2010). Thus, CsNDR1, citrus NPR1, and other citrus homologs of SA regulatory genes are ideal candidates that can be genetically manipulated to increase their expression in order to test if these genes confer resistance to HLB in citrus. Engineering such genes could yield citrus plants with enhanced disease resistance.
that are also more acceptable to the consumers than those engi-
neered with similar genes from other plants. Moreover, the newly
released citrus genome sequence has greatly facilitated the iden-
tification of additional citrus defense genes. We anticipate that
large-scale functional genomic analysis could uncover defense
genes that play critical roles in resistance to HLB, citrus
canker, and/or emerging diseases challenging the citrus
industry worldwide.

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