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Research article

Arabidopsis cysteine-rich receptor-like kinase 45 positively regulates disease resistance to *Pseudomonas syringae*

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ABSTRACT

Arabidopsis cysteine-rich receptor-like protein kinase 45 (CRK45) was found to be involved in ABA signaling in *Arabidopsis thaliana* previously. Here, we reported that it also positively regulates disease resistance. The *CRK45* overexpression plants increased expression of the defense genes, and enhanced resistance to *Pseudomonas syringae* whereas the *crk45* mutant were more sensitive to *P. syringae* and weakened expression of the defense genes, compared to the wild type. We also found that treatment with *P. syringae* leads to a declined expression of *CRK45* in the *npr1* mutant and the *NahG* transgenic plants. At the same time, significantly decreased expression of *CRK45* transcript in the *wrky70* mutant than that in the wild type was also detected. Our results suggested that CRK45 acted as a positive regulator in Arabidopsis disease resistance, and was regulated downstream of NPR1 and WRKY70 at the transcriptional level.

1. Introduction

Plants often suffer from various attacks from pathogens and they have evolved an array of complicated defense mechanisms that are activated by multiple defense signaling pathways. During the defense responses, plants have a common feature: activating a large number of genes after pathogen infection or treatment with pathogen elicitors [1]. In this process, salicylic acid (SA) plays a prominent role as a signal molecule. When treated with exogenous SA or encountering pathogen infection, SA accumulated in local and systemic tissues, and the systematic acquired resistance (SAR) usually established, leading to the expression of a set of PATHOGENESIS-RELATED (*PR*) genes and production of defense proteins [2]. NONEXPRESSOR OF PR GENES 1 (NPR1), a cytosolic protein, is considered to be the crucial component of the SA signaling pathway [3]. In response to increased SA levels, NPR1 interacting with TGA transcription factors are necessary for *PR1* gene expression [3,4]. At the same time, several members of the WRKY family also act as mediators of pathogen-associated transcriptional reprogramming in plants [5].

The WRKY transcription factor family is specific to plants and appears to be involved in the regulation of plant defense reaction [6,7]. The Arabidopsis genome encodes totally 74 WRKYs, and members of this family contain at least one conserved DNA-binding region, designated as the WRKY domain, which contains a conserved WRKYGQK sequence followed by a Cys_2His_2 or $Cys_2HisCys$ zinc-binding motif, and specifically recognize the W-box sequences (TTGAC) located in the promoter region of most defense-related genes [6,8,9]. Many WRKYs encoding genes are rapidly induced after treatment with elicitors or after infection by pathogens [10]. One member of the WRKY family, WRKY70, has been functionally characterized as a positive regulator in the SA-mediated gene expression and disease resistance of plants and as a negative regulator in SA biosynthesis [11].

Abbreviations: AIG1, AvrRpt2-induced gene 1; CRKs, cysteine-rich receptor-like kinases; DIG, digoxigenin; JA, jasmonic acid; LAR, local acquired resistance; MeJA, methyl jasmonic acid; NPR1, non-expressor of PR genes 1; PR1, pathogenesis-related gene 1; PR2, pathogenesis-related gene 2; *Pst* DC3000, *Pseudomonas syringae* pv. *tomato* DC3000; RLKs, receptor-like kinases; SA, salicylic acid; SAR, systemic acquired resistance; TF, transcription factor; TGA, TGACG sequence-specific binding protein; WRKYs, WRKY DNA binding proteins.

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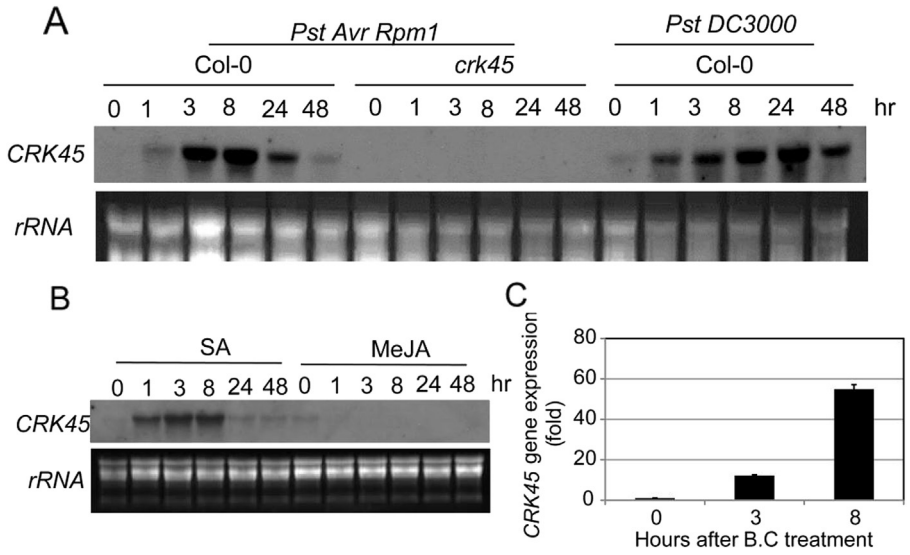

Fig. 1. The expression pattern of *CRK45* after treated by *P. syringae*, SA, MeJA and *B. cinerea*. (A) RNA gel blot analysis showing the expression of *CRK45* in wild type and the *crk45* mutant after *Pst* DC3000 and *Pst AvrRpm1* treatment. (B) RNA gel blot analysis showing the expression of *CRK45* in wild type Arabidopsis after SA or MeJA treatment. After pathogen inoculation or spraying with 1 mM SA or MeJA, total RNA was extracted from leaves of four-week-old plants grown in pots containing soil mixture and samples were collected at the indicated times. Ten micrograms of RNA was loaded per lane, and the *CRK45* gene-specific probe was used for hybridization. (C) The expression of *CRK45* in wild type after *B. cinerea* treatment. Leaves of two-week-old seedlings growing in GC vials were inoculated with 4×10^5 spores per vial. Samples were harvested 3 or 8 h after inoculation and total RNA was extracted for qRT-PCR analysis. *EF1α* was chosen as an internal control. Expression level for each gene was normalized to that of the control. For all experiments, three independent experiments were performed, and each data point represents the average of three technical replicates ± SD.

Receptor-like kinases (RLKs) belong to one of the largest gene families in plants. For instance, there are more than 610 members in Arabidopsis. RLKs are well known as conserved signaling components that regulate development, disease resistance, hormones perception, and self-incompatibility in plants [12,13]. Cysteine-rich receptor-like kinases (CRKs) are one of the largest RLK groups with 44 members, which have been suggested to play important roles in regulation of defense responses and programmed cell death [14]. Most of the CRKs are induced by pathogen attack and application of SA at the transcriptional level [15,16]. Accordingly, several CRKs are involved in regulation of defense and cell death in Arabidopsis. For example, constitutive over-expression of *CRK5* (At4g23130) led to increased resistance to the virulent bacterial pathogen *Pseudomonas syringae* pv. tomato DC3000 (*Pst* DC3000) [16]. Over-expression of *CRK13* (At4g23210) resulted in enhanced resistance to *P. syringae* and increased SA content [17], while CRK11 was reported to take part in the interaction between pathogen and Arabidopsis and in defense responses [15].

In this study, we found that one member of the CRKs family, At4g11890, named as CRK45 [14] or ARCK1 [18], is involved in disease resistance. Overexpression of *CRK45* resulted in resistance to the virulent bacterial pathogen strain *Pst* DC3000 and elevated expression of the pathogenesis-related (*PR*) genes, while the *crk45* mutant conferred the contrary phenotypes with slightly declined expression of the *PR* genes, indicating that CRK45 positively regulates Arabidopsis disease resistance.

2. Results

2.1. CRK45 is induced by P. syringae and salicylic acid

In a microarray analysis aimed to identify Arabidopsis genes that are induced in response to infection by *P. syringae* [19,20], *CRK45* was identified as one of the early pathogen-responsive genes. To confirm that *CRK45* was indeed one of the pathogen-responsive genes, we detected the *CRK45* transcript after *P. syringae* pv. *tomato* DC3000 (*Pst* DC3000) and *Pst AvrRpm1* inoculation. The results showed that *CRK45* were induced by both Pseudomonas strains (Fig. 1A). At the same time we also detected the *CRK45* transcript after *Botrytis cinerea* (*B.c.*), a necrotroph fungi, infection, and similar results were obtained (Fig. 1C). This suggested that CRK45 might have functions during interaction of plants with a broad-spectrum of pathogens. In addition, *CRK45* was also responsive to SA treatment (Fig. 1B). While after MeJA treatment, the transcript induction of *CRK45* has no obvious changes compared with the *PDF1.2* gene expression as a control (Fig. 1B and S1), indicating that this gene responded only to pathogens.

In our previous studies, we had obtained a *CRK45* T-DNA insertion line (Salk-057538) from the Arabidopsis Biological Research Center, and the *CRK45* overexpression plants were also generated by putting the *CRK45* genomic DNA sequence under the control of the *35S* promoter from cauliflower mosaic virus [21]. To further determine the biological function of CRK45 in plant defense response, we generated the *CRK45* complementary plants as well by putting the *CRK45* genomic DNA sequence under the control of the *35S* promoter and transformed it into the *crk45* mutant. As shown in Fig. 1A, the transcript of *CRK45* was undetectable by Northern blotting in the *crk45* mutant, indicating that the T-DNA insertion resulted in completely knock-out of *CRK45*. The expression level of *CRK45* showed different level of increase in both the overexpression and complementary lines (OE-15, OE-37 and com-17), and these transgenic lines were used in the following experiments unless stated otherwise (Fig. 2S).

The expression of *CRK45* in different tissues including siliques, leaves, stem and flowers were detected, and the results showed that the *CRK45* transcript expressed at a higher level in leaves than in other tissues (Fig. 3S).

2.2. Knock out of CRK45 resulted in sensitivity to P. syringae

To determine the contribution of CRK45 to disease resistance in Arabidopsis, we sprayed *Pst* DC3000 (10^8 cfu mL^{-1} ($OD_{600} = 0.1$)) to Col-0 (wild type), the *crk45* mutant, *CRK45* overexpressing plants,