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A Possible Involvement of *ACR4,* a Receptor Like Kinase, in Plant Defence Mechanism

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Abstract

Many developmentally important Receptor Like Kinases (RLKs), also known as receptor kinases have been shown to play independent roles in plant defence. In order to investigate the role of *Arabidopsis CRINKLY4* (*ACR4*) in plant defence mechanism, pathogen challenge experiments were carried out. It was found that *ACR4* knockout leaves show reduced susceptibility to the necrotrophic pathogen, *Botrytis cinerea.* It is therefore possible that the *ACR4* receptor might interact with other proteins that regulate specific defence responses. Reduced susceptibility of *ACR4* mutant to *B. cinerea* could also be due to the possible epidermal defect of *acr4* leaves. A detailed study of the cuticular lipid composition of *acr4* leaves may help ascertain whether epidermal defects in *acr4* leaves are responsible for resistance against *B. cinerea.*

Key words: ACR4, *Botrytis cinerea*, pathogen and defence mechanism.

Introduction

Precise activation of Receptor Like Kinases (RLKs), also known as receptor kinases plays a vital role in both development and the response to pathogens. Research in the last few years revealed that some developmentally important receptors also play roles in defence or disease resistance in both plant and animal systems (Aderem *et al*., 2000; Chinchilla *et al.,* 2007).

ACR4 (Arabidopsis CRINKLY4) is a membrane bound receptor which plays diverse roles in plant development, including maintenance of root stem cell populations (Stahl *et al.,* 2009), formation of lateral roots (De Smet *et al*., 2008), and development of the ovule integuments (Gifford *et al.,* 2003). Thus, our current understanding of ACR4 receptor is limited mainly for its role in plant development. Although no published data are available on the role of *ACR4* in plant defence, in the last few years microarray data (Zimmermann *et al*., 2004; Winter *et al.,* 2007) have revealed that *ACR4* is differentially expressed in response to different pathogens. In response to *Botrytis cinerea,* a necrotrophic pathogen, for example, *ACR4* expression is down regulated significantly (Zimmermann *et al*., 2004). This suggests that *ACR4,* which is known to be involved in development, could also have a role in plant defence.

In order to clarify the potential involvement of *ACR4* in plant immunity *acr4* null mutants were challenged with *B. cinerea,* a necrotrophic fungal pathogen to identify how this mutant behaves in response to the pathogen.

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Materials and Methods

Plant material and growth condition: Dry seeds were sterilized in 70% ethanol + 0.5% Triton-X-100 for 15 minutes. Seeds were then washed twice in 95% ethanol for 5 minutes, dried on sterile Whatman filter paper in a sterile hood and scattered onto Murashige and Skoog (MS) agar plates [0.5x MS salts, 0.6% sucrose, 1% plant micro agar, pH 5.7 (adjusted with KOH) and any appropriate antibiotics]. The plates were stratified for 3-days at 4ºC before being transferred to growth room (22ºC and constant light) for 10 days. Seedlings were then transferred to soil (3 parts soil, 1 part sand, 1 part perlite with Intercept fungicide) and grown at 22ºC, 50% humidity with 8hrs light/16 hrs dark (short day). Trays were covered with clear plastic covers for 3-4 days and then covers removed.

Arabidopsis thaliana ecotype *Columbia* was used as wild type. T-DNA inserted homozygous knockout line *acr4-2* was available in the lab and was screened using primers 5' GTCGACTTTGATAAGCTCCATGTCTC 3' and 5' GCT TCCTATTATATCTTCCCAAATTACCAA TACA 3'.

Inoculation with B. cinerea: The glycerol stock of *B. cinerea* (Nurmberg *et al.,* 2007) was kindly provided by

Loake Lab, IMPS, University of Edinburgh, UK. The pathogen was grown on half strength Potato Dextrose Broth (PDB) and plates were incubated under continuous light for 10-12 days. To prepare fungal spore suspensions, colonies were washed with sterile water (1 plate with 15 ml of water) and filtered twice using Minar cloth to remove mycelium and centrifuged at 4000g for 10 minutes to pellet the spores. The pellet was resuspended in 10 ml of ½ PDB and 5 µl droplet of the 1/10 dilution of the spore suspension was inoculated onto 6-week old short day grown leaves. Control plants were inoculated with just ½ PDB. The plants that were kept covered with clear plastic cover to ensure high humidity for 4 days.

To assess disease symptom development, plants were scored as follows, 0=no necrotic lesions, 1=plants showing small dry lesions, 2=plants showing a mix of small & medium size lesions, 3=plants showing medium size and spreading lesions, 4=plants showing predominantly spreading lesions, 5=plants showing predominantly wide necrotic lesions. Scoring was adapted from You *et al.* (2009).

Results

A mutation in ACR4 shows reduced susceptibility to B. cinerea: As mentioned earlier, the expression of *ACR4* shows a decrease in response to *B. cinerea,* a necrotrophic pathogen (Zimmermann *et al*., 2004). Interestingly, a similar down regulation of *ACR4* was observed in an unpublished transcriptome data provided by Dr. Katherine Denby, University of Warwick, UK (personal communication). This suggested a possible role for *ACR4* in defence against *B. cinerea.* To understand if there is any potential link between the function of *ACR4* and susceptibility to *B. cinerea*, it was decided to inoculate both wild type and *acr4* null mutants with *B. cinerea* spores.

The six-week old attached leaves of wild type *Col-0* and *acr4* mutant were inoculated with two droplets of the 5µl fungal suspension. The progression of disease was monitored and photographs were taken 5 days after inoculation. It was found that disease symptoms were less severe on *acr4* mutant leaves than on the wild type leaves (Figure 1).

To assess disease symptom development, plants were scored for their disease severity three days after inoculation, using a scale of 0 to 5. Scoring was adapted from You *et al.* (2009). Scoring overall symptom development with a disease index showed that *acr4* mutants were less susceptible to *B. cinerea* than wild-type (Figure 2).

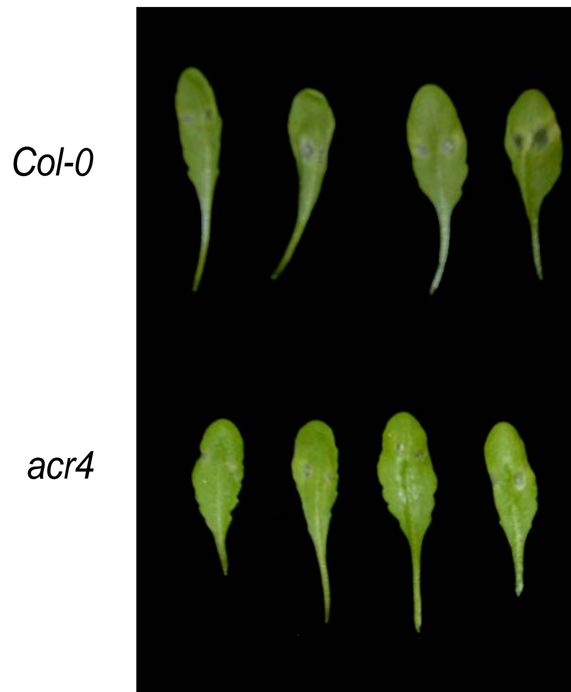

Figure 1. The *acr4* mutant exhibits decreases susceptibility to *B. cinerea*

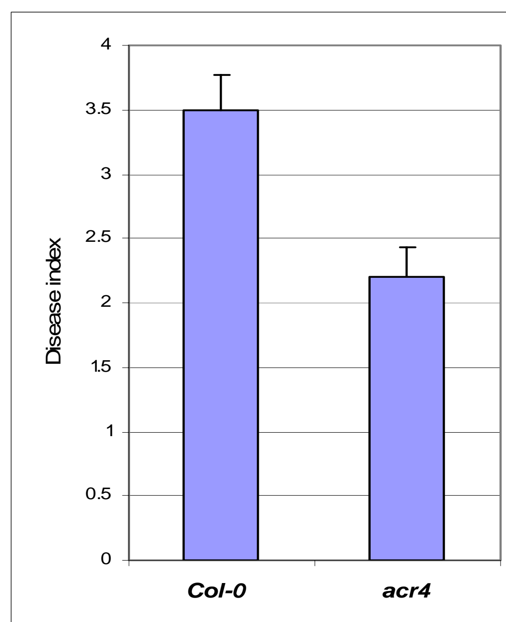

Figure 2. Scoring overall symptom development with a disease index indicated that *acr4* mutants show reduced susceptibility to *B. cinerea*

Discussion

Our result uncovered an unexpected role of a developmental regulator, *ACR4*, in plant–pathogen interactions. This resistance phenotype of *acr4* mutant is quite striking, given the conventional view that RLKs are normally involved in mediating resistance responses to pathogens. The mechanism underlying this resistance phenotype is still unclear.

In recent years, research on cuticular defective mutants has revealed that several distinct *Arabidopsis* lines with defective cuticle are resistant to *B. cinerea* (Bessire *et al.,* 2007; Tang *et al.,* 2007; Chassot *et al.,* 2007; Voisin *et al.,* 2009). It has been shown that permeable cuticle allows diffusion of antifungal compounds that interfere with fungal growth and hence the mutants with increased permeability are resistant to *B. cinerea.*

The *acr4* leaves look much like wild type (Gifford *et al.,* 2003). Watanabe *et al.* (2004) have shown that young *acr4* mutant leaves (18 days old) are permeable to the hydrophilic dye, toluidine blue. It also provides a potential explanation for the *Botrytis* resistance phenotype of *acr4* mutants and supports the notion that cuticle defects could, themselves, activate defence responses. To further understand the nature of cuticular defect of *acr4* leaves, a detailed study of the cuticular lipid composition of *acr4* leaves is important.

An alternative hypothesis is that *acr4* mutants show constitutive up regulation of some pathogen response pathways, leading to decreased susceptibility to some pathogens. It could also be that ACR4, being a receptor, could interact with other proteins that are normally involved in repressing defence responses in the absence of attack, preventing a premature increase of immune responses and associated costs. In either case, it is logical to propose that *ACR4* expression could be down-regulated as part of the response to pathogen attack.

The microarray analysis produced by the Ingram Lab in 2004 indicated that in *acr4* mutant floral meristem tissues the expression of *LIPOXYGENASE2* (*LOX2*), a gene encoding an essential enzyme in jasmonic acid biosynthesis, is upregulated (Bell *et al.,* 1995). Jasmonic acid signalling is required for resistance against *Botrytis* (Thomma *et al.,* 1998). This suggested that the high level of expression of *LOX2* in *acr4* mutant could potentially have a role in resistance against *Botrytis*. It would be interesting to determine the expression level of *LOX2* in *acr4* leaves prior to pathogen infection by quantitative RT-PCR.

Conclusion

The reduced susceptibility of the *ACR4* mutant plants to *B. cinerea* indicated a possible role of a developmental regulator in plant defence. At present, it is not known whether the defective cuticle of *acr4* leaves allows enhanced diffusion of antifungal compounds or ACR4 receptor itself is involved in plant-pathogen interaction. Future research could help us to determine the precise role of this kinase in plant defence mechanism.

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