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SHORT COMMUNICATION

Verticillium Ave1 effector induces tomato defense gene expression independent of Ve1 protein

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ABSTRACT

Verticillium resistance is thought to be mediated by Ve1 protein, which presumably follows a "gene-for-gene" relationship with the *V. dahliae* Ave1 effector. Because *in planta* analyses of Ave1 have relied so far on transient expression of the gene in tobacco, this study investigated gene function using stably expressing 35S:*Ave1* transgenic tomato. Transgenic *Ave1* expression was shown to induce various defense genes including those coding for PR-1 (P6), PR-2 (βbeta-1,3-glucanase) and peroxidases (anionic peroxidase 2, Cevi16 peroxidase). Since a *Ve1*$^{-}$ tomato cultivar served as germplasm, these results indicate that Ave1 induces these defense genes independently of Ve1.

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One of the most costly plant diseases is vascular wilt, caused by fungi of the genus *Verticillium*.[1-3] By far, the most prevalent are *V. dahliae* and *V. albo-atrum*.[4,5] These species infect many economically significant crops grown in Canada and throughout the world, including alfalfa, cotton, cucurbits, eggplant, mint, olive, potato, sunflower, strawberry and tomato, as well as many weeds.[6-9]

Resistance typically results from plant R-proteins recognizing pathogen effector molecules called avirulence factors.[10] R-protein activation then leads to a cascade of signaling events, culminating in appropriate defense responses.[11-13] In tomato the *Ve* locus, which consists of two homologous genes (*Ve1* and *Ve2*) that encode putative transmembrane resistance receptor proteins, has been associated with resistance against virulent *Verticillium* spp.[14] However, there is ongoing debate whether only one or both are functional R-proteins.[14,15]

Kawchuk et al.[14] suggested that *Ve1* and *Ve2* independently confer resistance to virulent *Verticillium* in potato. On the other hand, *Ve1* but not *Ve2* was shown to provide Vd1 resistance in MoneyMaker tomato and *Arabidopsis*.[15,16] For the tomato Ve R-proteins, a candidate fungal effector is the Ave1 protein isolated from *V. dahliae* race 1 strains by high-throughput population genome sequencing.[17] Ave1 has been hypothesized to interact with the Ve1 protein, thereby activating downstream defense responses,[18] and apparently contributes to fungal virulence.[17] Additionally, it can activate a hypersensitive response (HR) by co-expression with *Ve1* in tobacco but not in Arabidopsis.[18,19] This tobacco HR was exploited recently for mutational analyses of the Ve proteins. The leucine-rich repeat (LRR) domain was revealed to be important for Ve protein function, specifically the eLRR1-eLRR8, eLRR20-eLRR23 and eLRR32-eLRR37 regions.[20] The cytoplasmic tail of the Ve1 protein is required to activate immune signaling while its counterpart in Ve2 did not have this function.[21] Similar studies utilizing stably expressing tomato transgenics are still lacking and have not been pursued.

To better understand the function of the Ave1 effector *in planta*, the pFAST-R02-Ave1 construct[18] containing the *Verticillium Ave1* gene downstream of the 35S CaMV promoter was used to transform tomato plants cv. Craigella GCR26 (Ve1^{-}) using *Agrobacterium*-mediated transformation modified from an original procedure by McCormick et al.[22] The 35S: *Ave1* transformants did not look morphologically different from the untransformed controls. Total nucleic acid was extracted from 0.5 g of fresh plant material as previously described[23,24] and mRNA levels were determined by RT-PCR analyses as also previously described.[25,26] Transgene presence was assessed by PCR amplification of *Ave1* (Fig. 1, right panel) with primers IDT510 (5′-GAGCGGATCCTTATATCTGTCTAAATTCG) and IDT5 11 (5′-GATACAGAATAAAATGCC). RT-PCR amplification of the *Ave1* mRNA with the same primers was used to identify a positive line that was used for subsequent analyses of defense-related transcript levels. Gene expression values were normalized against a reference housekeeping gene actin. Statistically significant values were determined based on the Student's *t*-test ($P < 0.05$).

As shown in Fig. 1 and in contrast to *Ve2* mRNA, *Ave1* mRNA is present only in the transformed plant and only traces of mutant *Ve1* mRNA were observed. The latter observation is consistent with a premature termination codon,[15] which is known to result in nonsense mediated mRNA decay.[27] Nevertheless, in the absence of Ve1 protein, the 35S:*Ave1* transgenic plant showed upregulation of key defense-related and pathogenesis-related (PR) genes such as anionic peroxidase 2 (AP2), the PR-1 protein P6, βbeta-glucanase and Cevi16 peroxidase (Fig. 1). These genes were chosen because they were representative of

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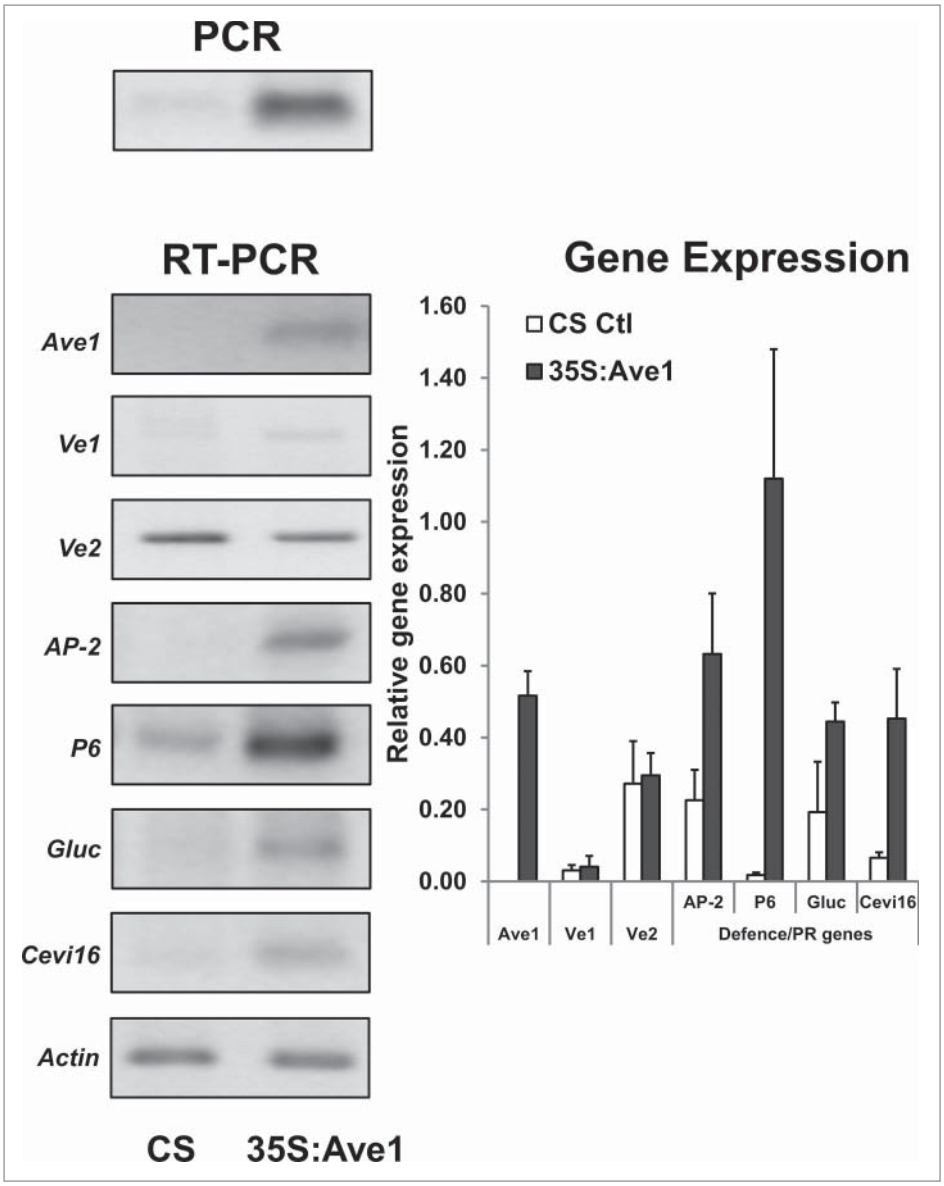

Figure 1. Gene expression in a tomato plant expressing the *Verticillium* Ave1. The Ave1-pFAST-02 binary vector construct was used to transform Craigella GCR26 tomato plants, as described in the text. Total nucleic acid was prepared from the 35S:*Ave1* transgenic line 002 and used as a template for PCR amplification with *Ave1*-specific primers (upper left panel). Subsequently, the total nucleic acid was used as a template for RT-PCR amplification with primers specific to *Ave1*, *Ve1*, *Ve2* and key defense genes (lower left panel). PCR and RT-PCR products were fractionated on 2% agarose gels and images were captured using Molecular Analyst software (Bio-Rad). Untransformed plants were used as negative control (CS). Actin was used as the internal control for gene expression. The chart (right panel) summarizes the average transcript levels (±SD) relative to actin for at least three 3 RT-PCR replicates.

genes induced most significantly in Vd1-infected tomato plants.[28] PR proteins typically comprise the majority of soluble protein change during the plant defense response.[29,30]

Overall, our results indicate that the Ave1 effector protein is being perceived by the CS tomato plant resulting in changes in defense gene expression. Since the CS isoline possesses a full-length Ve2 receptor but no full length Ve1 protein,[15] these observations emphasize the fact that, at least in tomato, Ave1-induced defense gene expression is independent of Ve1 and raises the possibility that the signal is transduced by another receptor, possibly the functional Ve2 protein.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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