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SHORT COMMUNICATION

Allele-specific CAPS marker in a *Ve1* homolog of *Capsicum annuum* for improved selection of *Verticillium dahliae* resistance

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Abstract Verticillium wilt (*Verticillium dahliae*) is an economically important disease for many high-value crops. The pathogen is difficult to manage due to the long viability of its resting structures, wide host range, and the inability of fungicides to affect the pathogen once in the plant vascular system. In chile pepper (*Capsicum annuum*), breeding for resistance to Verticillium wilt is especially challenging due to the limited resistance sources. The dominant *Ve* locus in tomato (*Solanum lycopersicum*) contains two closely linked and inversely oriented genes, *Ve1* and *Ve2*. Homologs of *Ve1* have been characterized in diverse plant species, and interfamily transfer of *Ve1* confers race-specific resistance. Queries in the chile pepper WGS database in NCBI with *Ve1* and *Ve2* sequences identified one open reading frame (ORF) with homology to the tomato *Ve* genes. Comparison of the candidate *CaVe* (*Capsicum annuum Ve*) gene sequences from susceptible and resistant accessions revealed 16 single nucleotide polymorphisms (SNPs) and several haplotypes. A homozygous haplotype was identified for the susceptible accessions and for resistant accessions. We developed a cleaved amplified polymorphic sequence (CAPS) molecular marker within the coding region of *CaVe* and screened diverse germplasm that has been previously reported as being resistant to Verticillium wilt in other regions. Based on our phenotyping using the New Mexico *V. dahliae* isolate, the marker could select resistance accessions with 48% accuracy. This molecular marker is a promising tool towards marker-assisted selection for Verticillium wilt resistance and has the potential to improve the efficacy of chile pepper breeding programs, but does not eliminate the need for a bioassay. Furthermore, this work provides a basis for future research in this important pathosystem.

Keywords *CaVe* · Cleaved amplified polymorphic sequence · Chile pepper · Marker-assisted selection · Resistance breeding · Single nucleotide polymorphism

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Results and discussion

The *Ve* gene is a cell surface-localized receptor that confers resistance to race 1 of the vascular wilt fungus *Verticillium dahliae* through (Fradin et al. 2009; Kawchuk et al. 2001) recognition of the *Ave1* effector (Song et al. 2017). Homologous *Ve* genes have been

Table 1 Single nucleotide polymorphism designation, location, nucleotide, and predicted amino acid of the putative *CaVe* gene identified between resistant and susceptible chile pepper accessions as well as the four *TaqαI* restriction sites used for CAPS marker development

Polymorphism designation	Location (bp)	Nucleotide polymorphism	*TaqαI* restriction site	Amino acid polymorphism
1	304	C/T	–	L/F
2	308	G/A	+	R/Q
	404		+	
3	852	A/T	–	P
4	916	C/G	+	R/G
5	1006	A/G	–	K/T/E/R/A
6	1007	A/C/G	–	K/T/E/R/A
7	1008	G/A	–	K/T/E/R/A
8	1127	A/G	–	R/Q
9	1350	A/T	–	A
	1403		+	
10	1508	G/A	–	S/N
11	1894	A/G	–	N/D
12	3015	G/C	–	L/F
13	3056	C/G	–	S/C
14	3064	C/A	–	P/T
15	3141	A/G	–	T
16	3305	A/G	–	E/G

identified in many land plants from diverse plant families (Song et al. 2017). These homologs have been found to mediate resistance in other solanaceous species like diploid tomato (*Solanum lycopersicoides*) (Chai et al. 2003), polyploidy potato (*S. tuberosum*) (Bae et al. 2008; Uribe et al. 2014), eggplant (*S. melongena*) (Liu et al. 2015), wild eggplant (*S. torvum*), tobacco (*Nicotiana glutinosa*) (Fei et al. 2004; Song et al. 2017), and in a non-solanaceous species such as *Mentha longifolia* (Vining and Davis 2009), grape (*Vitis vinifera*) (Tang et al. 2016), lettuce (*Lactuca sativa*) (Hayes et al. 2011), and cotton (*Gossypium barbadense*) (Chen et al. 2016). Additionally, transformation of individual tomato *Ve* genes into susceptible potato plants conferred resistance against *Verticillium*, demonstrating that the tomato *Ve*-resistant genes are effective in other Solanaceae species (Kawchuk et al. 2001).

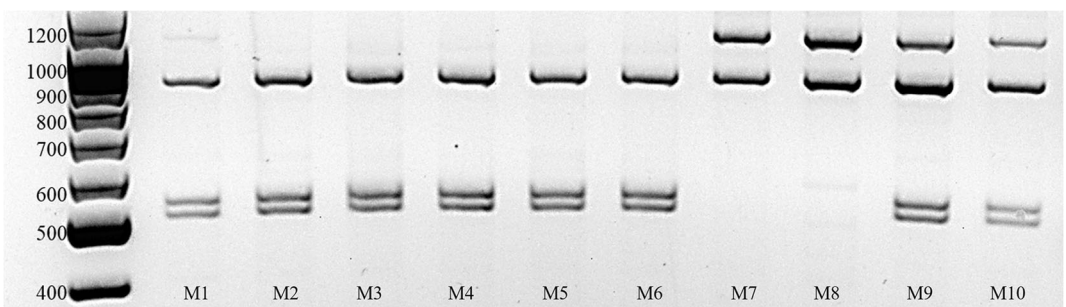

Fig. 1 Segregation of co-dominant CAPS marker in resistant and susceptible chile pepper accessions. Total DNA was isolated from individual chile plants and amplified with *CaVe* primers. Amplicons were digested with *TaqαI*, run on a 1.5% gel, and visualized with EtBr staining. Lane 1 is a size standard 100 bp ladder marker (GeneRuler 100 bp plus), lanes M1–M6 are resistant accession samples, lanes M7 and M8 are susceptible accession samples, and lanes M9 and M10 are heterozygotes

The five previously reported primer sets for *Ve*-associated markers based on homology to tomato and potato were evaluated with chile pepper genomic DNA (Bae et al. 2008; Park et al. 2008; Uribe et al. 2014). None of these *Ve* primer sets amplified PCR products of the expected size. However, the primers amplified expected amplicons from control tomato or potato DNA, confirming that the primers and reaction conditions were suitable for amplification of *Ve* homologs. Based on these results, it appeared that the chile pepper *Ve* orthologs are sufficiently divergent from the tomato and potato genes to preclude amplification using these primers.

Using the tomato *Ve1* (accession: FJ464557.1) tomato *Ve2* (accession FJ464562.1), we queried the draft chile pepper genome (GCA_000710875.1 v1.0) and identified a 3423 bp ORF that was 97 and 88% identical to the tomato *Ve1* and *Ve2* genes, respectively. Similar to *Ve* in tomato, the chile pepper *Ve* homolog was located on chromosome 9 between 110,090,054 and 110,087,826 bp. Based on the sequence of this ORF, primers were designed to amplify the putative *Ve* homologs from VW resistant and susceptible accessions of *C. annuum*. These primers produced amplicons from both VW susceptible and VW resistant accessions that were cloned and sequenced. These sequences were submitted to the National Center for Biotechnology Information (accessions MG062689-MG062698). The *CaVe* ORF encodes a putative polypeptide of 1140 amino acids. The *Ve* genes of tomato encode extracellular leucine-rich repeat receptor-like proteins (eLRR-RLPs) (Kawchuk et al. 2001; Wang et al. 2008, 2010). Similarly, the predicted *CaVe* is leucine-rich (15.5%) as is expected for a resistance gene with many of the L residues occurring in canonical L rich repeats (LRR) that align well with tomato *Ve* genes. Between the known resistant and susceptible plants, 16 nucleotide polymorphisms were identified, 13 of which were nonsynonymous mutations (Table 1).

For CAPS marker development, the *CaVe* primers (CaVeFw19: 5′-CTATGGCTTTTCTTGATACCCTT-3′ CaVeRv2223: 5′-GTTGACAAGAGA TTTTGGCA GCT -3′) were developed and the restriction enzyme *Taqα1* was used for digestion. There were four recognition sites in the resistant accessions but only two in the susceptible accessions (Table 1 and Fig. 1). The resistant lines had digestion bands at ~ 530, 570, and 1000 bp, while the susceptible lines had digested bands at ~ 1000 and 1200 bp (Fig. 1). However, despite using reported resistant and susceptible lines for co-dominant marker development, when screened using diverse *Capsicum* germplasm, the *CaVe* marker had 48% selection accuracy (Table 2). There are several possible reasons for the low level of matching between the marker and the phenotype. Firstly, the number of resistance sources in

Table 2 Genotypic and phenotypic disease rating of diverse germplasm evaluated in testing the efficacy of the CaVe-CAPS marker developed in known resistant and susceptible lines

Accession origin[z]	Accession	Genotype	Phenotype	Match
	Resistant check	R[y]	4.8[x]	+[w]
	Susceptible check	S	9.0	+
	NMCA 10690	S	4.0	–
	15c 1590-1	R	6.7	–
	16c 686	R	5.8	–
GRIF 9073	16c 644	R	5.0	+
GRIF 9073	16c 648	R	4.5	+
PI 439297	15c 1580-2	R	8.0	–
PI 439297	15c 1580-8	R	7.8	–
PI 594125	16c 664	R	6.1	–
PI 594125	16c 665	R	4.9	+
PI 594125	15c 1581-11	R	8.5	–
PI 281396	16c 667	R	3.2	+
PI 281396	16c 673	R	4.0	+
PI 281396	16c 678	R	4.5	+
	16c 681	R	6.3	–
	15c 1584-3	R	6.3	–
	15c 1585-4	R	4.5	+
	16c 682	R	5.4	–
	16c 683	R	5.5	–
	16c 684	S	6.5	+
	15c 1588-2	H	5.6	–
	Joe E. Parker	R	2.2	+

[z] Parental material from which the accessions used in this study were derived. These lines were previously published as being resistant to Verticillium wilt in California (Gurung et al. 2015)

[y] Digested *CaVe* fragment of either R (resistant), S (susceptible), or H (heterozygous)

[x] Phenotypic response of the diverse *Capsicum* germplasm inoculated with 4 million spores of the New Mexico Isolate of *Verticillium dahlia* (PWB 190). Plants with scores of 1–5 were considered resistant because these could flower and produce a harvestable crop. Plants with scores > 5 were considered susceptible because they would eventually die without producing fruit

[w] + = match of phenotype and genotype; − = mismatch between phenotype and genotype

Capsicum is limited (Gurung et al. 2015), which is due in no small part to the overall lack of an unequivocal disease screen. It is also possible that the *V. dahliae* isolate collected in New Mexico used in this study is different from other isolates use in previous studies. These two points are highlighted by the difference in phenotypic results in our study and those reported by Gurung et al. (2015).

This molecular marker is a promising tool that can be used in marker-assisted select for Verticillium wilt resistance. The marker developed here has the potential to improve the efficacy of chile pepper breeding programs, but does not eliminate the need for a bioassay. Therefore, we suggest that this work be used to provide a basis for future research in plant breeding and plant pathology. Research needs to be conducted to better understand the epidemiology of *V. dahliae* and to characterize the isolates collected from diverse production regions. Furthermore, the identification of highly resistant accessions of chile pepper is necessary before we can better understand the function of *Ve* homologs in chile pepper.

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