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Victoria Blight, defense turned upside down☆

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ABSTRACT

Genes that confer disease resistance to biotrophic pathogens typically encode nucleotide-binding, leucine-rich-repeat proteins (NB-LRRs). These proteins confer resistance by detecting the presence of virulence effectors secreted by biotrophic pathogens. Recognition triggers NB-LRR activation and subsequently, the defense response which often includes localized host cell death. The fungus, *Cochliobolus victoriae*, is a necrotrophic pathogen that causes a disease called Victoria Blight. Virulence of this fungus is dependent on its production of a peptide called "victorin" that has been traditionally described as a toxin. Only plants that respond to victorin are susceptible to *Cochliobolus victoriae* whereas those that do not are resistant to the fungus. Genetic and molecular analyses have revealed that victorin functions like a biotrophic effector recognized by a NB-LRR resistance protein in Arabidopsis. Further, numerous plant species express victorin sensitivity suggesting there are numerous NB-LRRs that recognize victorin. Thus, through expression of victorin, *C. victoriae* is able to exploit plant defense to cause disease and is capable of evoking this response in an array of different plants.

1. Introduction

Victoria Blight is a disease originally described on oats and is caused by the necrotrophic pathogen, *Cochliobolus victoriae*. The fungus is pathogenic by virtue of its production of a family of unusual peptides called victorin. Victorin has historically been referred to as a host-specific toxin and is absolutely required for virulence. Isolates that make victorin are fully pathogenic whereas isolates or outcrosses that do not are non-pathogenic [1]. On the host side, sensitivity to victorin is conditioned by a single dominant gene called *Vb*. Only oats that are sensitive to victorin are susceptible to the pathogen while oats that are insensitive, (homozygous recessive for *Vb*) are completely resistant to the pathogen.

The molecular features of Victoria Blight are reminiscent of classically described gene-for-gene interactions except that the predominant phenotypes are reversed. In Victoria Blight, virulence (i.e. victorin production) and host susceptibility are the dominant phenotypes as contrasted with classic gene-for-gene interactions where avirulence and disease resistance are genetically dominant [6]. This association is particularly interesting when considering the origin of Victoria Blight.

Victoria Blight arose as a consequence of the widespread introduction of the *Pc2* gene for resistance to the crown rust pathogen, *Puccinia coronata* [2]. Crown rust conforms to a classic gene-for-gene interaction where *Pc2* confers genetically dominant disease resistance presumably through recognition of the effector, AvrPc2. Because of its utility for controlling crown rust, cultivars containing *Pc2*, derived from the oat cultivar "Victoria", were widely planted throughout North America [2]. Soon after, a new disease arose on oats [3]. Because of its exclusive association with cultivars containing Victoria-derived resistance, this disease was called "Victoria Blight". Efforts to retain Victoria-type resistance to crown rust while removing susceptibility to Victoria Blight have failed, suggesting that *Vb* and *Pc2* could be the same gene [4,5]. Further, various physiological studies suggest that victorin induces a defense-like response in sensitive oats [6] and thus reinforce a potential connection between *Vb* and *Pc2*. The association of Victoria Blight susceptibility with crown rust resistance implies that a defense gene to one disease can confer susceptibility to another and suggest that some pathogens can exploit defense for susceptibility.

Thus Victoria blight presents a nexus between two seemingly opposite host responses and suggests that pathogens can evoke susceptibility by "intentionally" activating defense. This has serious implications because it means that at least some genes deployed to limit disease can actually lead to increased disease with potentially devastating consequences. Recent molecular characterizations of this disease interaction provide compelling support for this possibility.

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2. Victorin structure

Structural analyses revealed that victorin activity is not due to a single compound but actually derives from a group of closely-related, cyclized pentapeptides all of which contain glyoxylic acid and a cyclic combination of five unusual amino acids [7–9]. Structure-activity studies of the predominant form, victorin C, revealed that the aldehyde moiety of the glyoxylate residue is essential to victorin's mode-of-action. In addition, it was found that the free ε-amino group of the hydroxylysine residue can be derivatized while retaining host-selective activity [10]. This allows the production of a variety of derivatives that can be used for "tracking" victorin both *in vivo* and *in vitro*. *In vivo* binding studies with labeled victorin reveal that it binds covalently to a low molecular weight protein of about 13–15 kDa in both sensitive and insensitive oat genotypes and to the 100 kDa P-protein of the glycine decarboxylase complex (GDC) only in sensitive genotypes [11]. However, numerous studies confirmed that the GDC is not the primary site of action and that binding to the P-protein likely occurs due to genotype-specific changes in mitochondrial permeability following victorin treatment [12,13].

3. Identification of a genetic model for Victoria Blight

Molecular characterization of the *Vb* gene would undoubtedly clarify whether *Vb* and *Pc2* are the same gene. However, due to the large genome size and paucity of molecular characterization, a molecular genetic approach in *Avena sativa* (oats) is only recently becoming a possibility. Therefore, to initiate a molecular genetic analysis of victorin sensitivity, a screen of *Arabidopsis thaliana* ecotypes was conducted [14]. This screen originally identified six ecotypes that clearly display victorin sensitivity. Genetic analyses of individuals selected from these ecotypes demonstrated that victorin sensitivity in Arabidopsis, as in oats, is conferred by a single dominant gene which was allelic in all the sensitive ecotypes tested [14]. We named this locus "*LOV*" for locus orchestrating victorin effects. Thus, the genetics of the response to victorin in Arabidopsis is similar to oats. Also similar to oats, it was found that the victorin response is exclusive to genotypes carrying *LOV1*. Concentrations at least 1000 times higher (the highest concentrations tested) than required for a response in sensitive Arabidopsis do not affect insensitive (*lov1/lov1*) Arabidopsis genotypes. Also like oats, victorin induces electrolyte leakage only in sensitive Arabidopsis and the Arabidopsis victorin response involves Rubisco cleavage, DNA degradation and victorin binding to the mitochondrial P-protein of the glycine decarboxylase complex [15]. This latter response is associated with a mitochondrial permeability transition in oats and indicates a significant role for mitochondria in the regulation of victorin-induced cell death in both oats and Arabidopsis. Also similar to oats, where ethylene inhibitors reduce victorin sensitivity [16], it was found that the ethylene response mutant *ein2* attenuates victorin sensitivity in Arabidopsis [17]. Additionally, victorin induces rapid expression of *PR1*, salicylic acid accumulation, and camalexin production in Arabidopsis [17]. All of these responses are consistent with the resistance-like responses associated with victorin treatment of oats.

Because *LOV* clearly mediates victorin sensitivity, *LOV* was tested for its ability to confer susceptibility to *C. victoriae* [14]. Plants were spray-inoculated with *C. victoriae* spores that had been washed to remove residual victorin and evaluated for disease symptoms. Toxin-insensitive Columbia plants do not develop symptoms. However, victorin-sensitive plants show leaf chlorosis and necrosis. Microscopic examination revealed that while conidia germinate and form appressoria on insensitive Columbia leaves, little or no penetration by the fungus is evident. In contrast, on leaves of victorin-sensitive plants, hyphae penetrate tissue and proliferate in the intercellular spaces of the mesophyll. In completion of Koch's postulates, infected tissue, following surface sterilization and plating on water agar, displayed extensive sporulation of *C. victoriae* from tissue of victorin-sensitive Arabidopsis.

These analyses demonstrate that victorin-sensitive Arabidopsis display a susceptible phenotype whereas insensitive Arabidopsis are resistant to *C. victoriae*. Thus, Arabidopsis can be a host of *C. victoriae* and victorin remains, by definition, a "host-selective" compound. Consequently, the Arabidopsis *LOV* gene, as is the case with the *Vb* gene in oats, appears to be a true disease susceptibility gene and apparently functions by conditioning the victorin response.

A map-based cloning effort [17] identified *LOV1* as At1g10920. *LOV1* encodes a CC-NB-LRR protein homologous to members of the RPP8 resistance gene family in Arabidopsis. This gene family includes *RPP8*, *RCY1* and *HRT* [18–20]. *LOV* shows approximately 70% identity and greater than 86% similarity to *RPP8* and the other family members. *RPP8* confers gene-for-gene type resistance to the oomycete, *Hyaloperonospora arabidopsidis*, whereas *RCY1* and *HRT* confer resistance to the yellow strain of cucumber mosaic virus and contribute to resistance to turnip crinkle virus, respectively [19,20]. Thus an NB-LRR gene, a type of gene normally associated with disease resistance and belonging to a family of known resistance genes, confers victorin sensitivity and disease susceptibility in Arabidopsis. In addition, evaluation of victorin sensitivity in a survey of 30 ecotypes [21] revealed that sensitivity and therefore functional *LOV* is the predominant phenotype in Arabidopsis with sensitivity occurring in 28 of the 30 ecotypes. Sequence analyses of the *LOV* locus among the ecotypes revealed very limited allelic variation and suggested the possibility of a recent selective sweep. Given that positive selection would not likely occur for a role of LOV in conferring victorin sensitivity and disease susceptibility, these data suggest that *LOV* very likely functions as a disease resistance gene to a widespread, common pathogen of Arabidopsis. Thus, victorin sensitivity and disease susceptibility in Arabidopsis is conditioned by a NB-LRR-encoding gene closely related to genes known to confer disease resistance and possibly has a role in conferring disease resistance to an unknown pathogen. Collectively, this set of conditions is highly reminiscent of what has been described in oats and suggests that Arabidopsis is a relevant model for examining the Victoria Blight disease interaction.

4. Defining the nature of LOV

Even though *LOV* encodes a NB-LRR and data suggest a role in conferring resistance to a presently unidentified pathogen, in the absence of the identity of the pathogen, an unambiguous role in defense cannot be assigned to *LOV*. In other words, it is currently impossible to define *LOV* as a resistance gene. Thus, the identification of *LOV* in Arabidopsis does not unequivocally prove that a resistance gene can confer disease susceptibility and that pathogens can activate defense as a form of virulence. This is a critical implication of Victoria Blight that needs clarification. In the absence of the identification of the resistance specificity of *LOV*, it remains possible that LOV is an atypical NB-LRR that confers disease susceptibility through a mechanism distinct from that of typical NB-LRRs in conferring defense. To resolve this possibility, efforts were directed at structural, mechanistic and functional evaluations of LOV to determine if LOV displays properties shared by other defense proteins or is an atypical NB-LRR.

4.1. Structural analysis of LOV

Ethyl methanesulfate (EMS)-mutagenesis of victorin-sensitive