Combating Crown Rot with Antimicrobial Peptides

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When attacked by a disease-causing microorganism, plants and animals respond in a similar way by producing small antimicrobial proteins or peptides slowing down or killing the pathogen. This ancient form of disease protection is gaining recognition as a means to battle antibiotic resistance and improve animal and plant health. Scientists with USDA-ARS in St. Paul, MN, and colleagues at the University of Minnesota and Donald Danforth Center in St. Louis, MO, investigated one type of antimicrobial peptide called plant defensins as a means to reduce the damage from crown rot disease in alfalfa.


Crown Rot

Crown rot is characterized by the persistent loss of crown buds and decay of crown and root tissues. The disease often results in asymmetric plant growth due to death of affected portions of the crown and development of secondary crown branches. Wounds caused by forage harvest, machinery, grazing animals, winter injury, and insects enable pathogens to gain entry into the crown. Pathogens decay the crowns during a period of months or years, making it more susceptible to winterkill and eventually killing the plant. Not only does crown rot reduce stand life, it also severely affects stand productivity, reducing forage yields and predisposing plants to abiotic stresses. The organisms causing crown rot of alfalfa are a complex of microbes differing by geographic location. Some of the most common fungal pathogenic species are *Phoma medicaginis*, *Colletotrichum trifolii*, and *Fusarium* species. No cultivars with resistance to crown rot are currently available and attempts at resistance breeding have been unsuccessful. Fungicides with the required persistent root and crown activity are not available.

Defensins are naturally found in many plant tissues including seeds, leaves, flowers, and fruit. Their antimicrobial activity was recognized early on, and due to their potent antifungal activity, defensins are being exploited in biotechnological applications to generate disease-resistant crops. There have been at least 14 reports of disease resistance in transgenic plants expressing defensins. To identify defensins with the required broad activity against alfalfa pathogens, core amino acid sequences required for activity were tested against fungal, bacterial, and oomycete pathogens of alfalfa. The peptides from barrel medic, an annual medic closely related to alfalfa, were the most active against crown rot pathogens, particularly the fungus *P. medicaginis*, causing foliar disease and is also involved in crown rot. Using the shorter core peptides was found to be a rapid means to identify the defensin peptides with desired activity. Importantly, they also reflected the activity of full-length defensin peptides. The barrel medic peptides had activity against bacterial plant pathogens, which was surprising because few defensins had been reported to be active against bacteria. Testing against bacterial pathogens of humans showed the barrel medic defensins were also potent inhibitors of these organisms, suggesting plants are an untapped resource for novel therapeutics against organisms causing disease in humans and animals.

The gene for the most active barrel medic defensin has now been used to generate transgenic alfalfa plants steadily expressing the defensin throughout the plant. In preliminary experiments, plants show increased resistance in leaves to *P. medicaginis* and *C. trifolii*. Experiments investigating the mode of action of the barrel medic defensins indicate they insert into fungal and bacterial membranes, likely causing the cells to lose integrity. This mode of action would be very difficult for the pathogens to overcome and become resistant to defensins. The next stage of the research will be to cross the transgenic plants to generate seeds for further testing and field experiments to measure resistance to crown rot. Additionally, expression of the defensin only when plants are attacked by a pathogen will be tested to determine the most efficient way of deploying this defense. Transgenic expression of defensins could be utilized to implement an eco-friendly, protein-based strategy providing alfalfa with enhanced resistance against crown rot and the correlative increases in forage yield and stand life.