The Study of Plant Immunity as a Route to Practical Applications

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At the time I started studying potato plant pathology, genes in potato that conferred resistance to potato late blight (R genes) were well known and used by plant breeders. After a 10-year period these R genes were overcome by the pathogen that had only to instill in the population one mutation to overcome each if the R genes' defense. The same is true for the R genes used by breeders of wheat, rice, barley, etc. All of these genes are now being studied by breeders. They are mapped, sequenced, and tagged in an attempt to find similar genes hidden away in the plants' genomes. A typical R gene that was once active in potato against Late Blight was called "W". In 1952 it was functional for the grower.

In nature, if the "Aw" gene for virulence in the pathogen, which matches with the corresponding W gene in the plant, is mutated to an inactive state, the fungus can infect the plant. The mutant will also out compete all of the other non-mutated fungal spores and it will take over as the prominent race. This then makes useless the W gene (an R gene), the single dominant gene that once controlled late blight resistance in potato.

The information below compares disease resistance induced by a single dominant gene with resistance made up of many genes called "nonhost resistance". Nonhost resistance is harder to analyze genetically, but it does not break down easily.

Single Dominant Gene Resistance Against a True Pathogen	**Non-Host Resistance (wrong pathogen)**
W matched with Aw = Resistance If Aw is mutated = Susceptibility	**Many Immunity Host Genes Activated by Many Elicitors = Stable Resistance (One mutation Does Not Influence Resistance)**

Most plant pathogens have a range of host plants that they can infect, but plant hosts outside that range resist such pathogens very vigorously. Most of my research centered on a system in pea (pea pod) that vigorously resists a bean pathogen but eventually succumbs to a related pea pathogen. Nonhost resistance is a response induced by this mismatch between plant and fungus. This response is related to the basic immunity of our own bodies.

Surprisingly, the individual genes of this response have cousins in all plants. It is a matter of getting a bunch of these response genes to rapidly become active enough to ward off the true pathogens of potato.

So this nonhost resistance is not necessarily a matter of the inherited genetics so much as it is the plant responding to the fungal signals it is receiving. It is the mechanisms for responding that differ from one plant species to another and this is undoubtedly related to the inherent genetics of the responding genes. We have used molecular biological techniques to identify and clone these response genes. We cloned them first in peas and then found there were similar genes in potatoes.

The pea pod is a good place to understand the mechanisms of this signaling because interactions between the bean pathogen and the pea resulting in total resistance are complete in 6 hours.

Here is what we know:

1. The fungus releases multiple signals. The major ones are:
   Chitosan –released from the cell wall of many fungi
   DNase - a DNA clipping enzyme that is released from fungi

2. Both of these signals get inside the plant cell and can go to the cell's nucleus.

3. The nucleus is the place where gene expression starts. A lot of the plant genes are wrapped tightly in a nuclear material called chromatin. Both the chitosan and DNase signals cause chromatin to relax. As a result some defense genes become active.

4. We know which genes are the major genes that make up the defense response. The functions of these genes range from enzymes that degrade fungal walls to toxic peptides similar to those in scorpion toxins.

5. These gene products cause fungal growth to slow. This is bad for the fungus since it is a type of constipation that allows its own digestive juices to accumulate. The same fungal DNase that becomes a signal in the plant can accumulate in the fungus growing tip and cause the nucleus to degrade and block fungal growth.

How does this information lead to a practical application?

1. Clone the defense genes and transfer them to other plants. We did this to develop resistance in potatoes, and created a Shepody potato containing a pea defense gene that resisted early blight in the field. Unfortunately, current perspectives against GMO technology will not allow commercialization of this potato.

2. Use the fungal DNase gene. We connected the fungus DNase gene that induced resistance in pea to a switch that responded to a pathogen challenge and transferred them to tobacco. We showed that it could induce the entire defense response in tobacco against a tobacco

pathogen. We have also found that Wild Yeast can be used to distribute both cell wall signals and DNase signals to potato. We have several lines of data that indicate that DNase released by the yeast can make small changes in the DNA of the plant nucleus. When DNase cuts one strand of the double stranded DNA of the plant, there is a surge in the production of defense proteins. This increase is usually associated with increased disease resistance in the plant.

3. Chitosan: the other signaling compound. There are now practical uses of chitosan world-wide. My lab was the first to recognize its ability to induce defense genes and also directly slow the growth of many fungi. Chitosan can be obtained in large commercial quantities from crab and shrimp shell wastes. It now has many horticultural and greenhouse uses. We found that it could be beneficial along with copper sulfate pentahydrate for late blight control and could be certified as organic.

The defense genes associated with non-host resistance have also been useful for those engaged in mapping plant chromosomes. These defense genes are used to locate very similar genes in most plants and subsequently be enriched in plants by plant breeders. There are regions of the plant DNA that the breeders show can contribute to disease resistance called QTLs (regions of qualitative resistance traits). "Qualitative" indicates that by themselves they cannot develop total immunity, but can be impactful in combination with other QTLs.