BIOTECHNOLOGY FOR THE CONTROL OF SOYBEAN DISEASES

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Introduction

Approximately 80% of the total soybean production in the United States ocurrs in the North Central States. Ten of the 12 most productive states are located in this region (Doupnik 1993). During a three-year period (1989 - 1991) soybean production and disease loss for the North Central States was estimated at 13.17% or 236,730,000 bu (Doupnik 1993). At the current price of $7.00/bu that level of loss corresponds to a loss to the North Central States soybean growers of $552,370,000 per year!.

Soybean cyst nematode and Phytophthora root and stem rot are two major diseases of soybeans and often cause the greatest loss in production. Brown stem rot and the seed disease caused by Soybean Mosaic Virus also cause profit losses to the growers. Some soybean plants contain natural resistance or tolerance to these diseases which breeders have taken advantage of in developing new varieties.

Biotechnology offers an array of tools that may help to control soybean diseases. Biotechnology can help us to clone the genes that provide natural resistance or tolerance and can help us to understand how these genes work. Once we understand how the resistance mechanisms work we can use these new technologies to engineer novel forms of resistance into the soybean. A team of researchers at Iowa State University have joined together, with collaborators from across the United States, to use molecular biolological techniques to develop methods by soybean diseases can be controlled. This team studies the diseases caused by Soybean cyst nematode, Phytopthora root and stem rot, Brown stem rot, and Soybean Mosaic Virus, and attacks the problems of disease control by studying the pathogen as well as the plant. This paper will discuss some approaches used in the efforts to clone the genes conferring resistance to the fungal pathogen Phytophthora sojae, the pathogen causing Phytophthora root and stem rot.

Experimental Approaches and Rationales

A number of different approaches are being used to clone the disease resistance genes associated with Phytophthora root and stem rot. Some of these (two-hybrid yeast system, differential display, etc.) are very experimental and are too preliminary to report. Other approaches include chromosome landing and map-based cloning, as well as a candidate gene approach using what has been learned from other cloned resistance genes. It is the latter approaches that will be addressed in this paper.

Genetic Mapping of Disease Resistance Genes

Phytophthora root and stem rot is one of the most serious diseases of soybean (Athow 1987). At least six genes are known to confer resistance to various races of the fungal pathogen, and at some loci more than one form of the resistance gene has been reported (Athow 1987). The resistance genes are all 'dominant'. This means that a plant carrying one 'resistance' allele and one 'sensitive' allele, will appear to be resistant. That is, resistance is dominant over susceptibility.

In the early 1990's, we made crosses between soybean lines susceptible to Phytophthora root and stem rot and soybean lines resistant to the disease. From these crosses we obtained populations that were segregating for resistance or susceptibility. We used molecular markers (RFLPs) to identify linkages between the markers and the segregation of the resistance genes. Tight linkage of molecular markers to genes is called 'tagging' and co-segregation or near-co-segregation of the marker and the resistance trait told us that the gene for resistance resided very close to the marker on the soybean chromosome. By integrating this information with genetic maps of each chromosome we were able to place the location of the resistance genes onto the soybean genetic map. This resulted in the mapping of all but two of the known resistance genes (Diers et al. 1992). In a related experiment, we later showed that one of the resistance genes, Rps2, was tightly linked to a resistance gene for powdery mildew (Rmd), and to a gene controlling nodulation and nitrogen fixation by rhizobium (Rj2). This cluster of genes was also placed into the soybean genetic map (Polzin et al. 1994).

Tagging of resistance genes with molecular markers allows us to follow the resistance gene through a breeding program simply by monitoring for the presence or absence of the molecular marker, rather than by determining by extensive testing whether or not the plant is resistant or susceptible. This, in some cases, is a much cheaper, faster, and more efficient method of selecting for resistant plants and could speed the recovery of improved soybean lines in breeding programs.

Map-Based Cloning and 'Really Big DNA' Libraries

Map-based cloning approaches involves placing molecular markers very close to the resistance gene and using them to clone the resistance gene. The process of placing many markers within a confined region of a chromosome is called 'chromosome landing' and it entails the application of several different molecular marker systems. In short, the system that costs the least and which can place the most markers on a map in the shortest amount of time, is the marker system of choice. We normally use a system that is called AFLP and which can identify approximately 30 - 60 chromosomal positions in each experiment.

Map-based cloning requires 'living libraries' of soybean DNA. Even though the genetic distance between a marker and a gene may seem to be small, the physical distance between the marker and the gene may represent hundreds of thousands of genetic units (nucleotides). Because we need cloned pieces of soybean DNA that can span this oftentimes vast physical distance, special libraries of cloned DNAs are required. Because of reported ease of construction and ease of use, the library system of choice is the Bacterial Artificial Chromosome system (BACs) (Woo et al 1994).

We developed a BAC library of soybean DNA that contains approximately 40,000 individual clones that are hand-picked and stored in individual containers at -70 degrees C. The average size of each clone is 150,000 nucleotides. By considering the total size of all the soybean chromosomes, this library has a 5 X genome equivalent. That means that there is a 95 % chance that any gene on any chromosome will be present within our library in about 5 copies.

This library will be used in conjunction with our chromosome landing and gene tagging experiments to 'walk' along the chromosome until we reach the disease resistance gene. Once we reach the gene, we can clone it and analyze it to determine how it functions. Once we understand how it functions, we can begin to design experiments to engineer disease resistance in the soybean.

Resistance Gene Analogs (Candidate Resistance Genes)

A variety of plant disease resistance genes have been cloned and analyzed. These have been isolated from dicots and monocots and confer resistance to diseases caused by fungal, viral and bacterial pathogens. Regardless of the sources of the gene, and regardless of the type of pathogen to which the gene confers resistance, one striking observation is that the genes contain similar genetic sequences and conserved amino acid domains (Staskawicz et al. 1995). This suggests that plants may respond to pathogens in a limited number of ways. It also suggested to us that the conserved regions of disease resistance genes could be used to advantage to clone disease resistance genes from soybean.

We designed some molecular 'hooks' based upon the conserved regions of different genes from Arabidopsis and tobacco. Using these hooks we were able to clone many hundreds of genetic sequences from soybean that contained similar conserved regions (Kanazin et al. 1996). We found at least nine classes of disease resistance gene analogs that showed striking similarities to cloned resistance genes from other species. We mapped over 50 of these onto the soybean genetic map and found that they were distributed throughout the chromosomes. They exist in tight clusters (as do some plant disease resistance genes), and some mapped extremely close to one of our targeted genes; Rps2 (Figure 1).

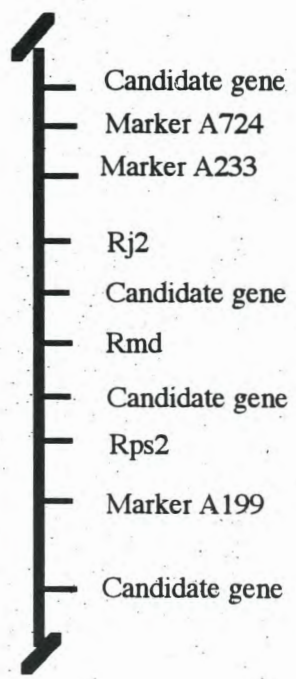

Figure 1. Candidate disease resistance genes map very close to known disease genes on soybean linkage group J. These candidate genes may be seful for cloning disease resistance genes or for 'tagging' disease resistance genes. In this example, the closest candidate genes will be inherited with resistance genes 99 times out of 100.

We are currently discovering many more candidate genes and are adding to the number of candidate genes that we are mapping. Hopefully this will help us to 'tag' disease resistance genes much more closely than would be possible using traditional methods. This approach may also allow us to directly clone specific resistance genes. As we learn more about the function of resistance genes, these candidate (analog) genes may be useful in engineering super resistant soybean plants.

Disclaimer

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