Advances In Anthracnose Stalk Rot Resistance

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Abstract

Anthracnose stalk rot, caused by *Colletotrichum graminicola*, is the most significant stalk rot pathogen in North America corn. Early plant death and deterioration of stalks by anthracnose leads to loss in yield and increased risk of stalk lodging. Researchers at Pioneer have identified, mapped, fine mapped, and cloned a rare maize gene, which provides improved resistance to *C. graminicola (Cgr)*. Through the use of molecular breeding techniques, Pioneer is working to incorporate this valuable trait into North American hybrids which should be available commercially in the future.

Introduction

Anthracnose stalk rot (ASR) caused by the fungus *Colletotrichum graminicola* (Ces.) G.W. Wils., is one of the major stalk rot diseases of corn. Identification of plant genes involved in stalk rot resistance will enable us to better understand the host-pathogen interaction at the molecular level. Furthermore, such genes can be used for developing resistant maize hybrids after introgression into susceptible germplasm either by a transgenic approach or by marker assisted selection, using the markers identified in the map-based cloning process.

Disease Symptoms

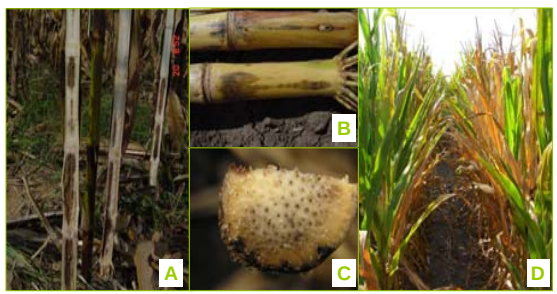

Fig. 1
A. The internal stalk tissue or pith becomes discolored, turning dark brown and, eventually, disintegrates.

B. Late in the season shiny black, linear streaks and blotches appear on the surface of the lower stalk above the brace roots.

C. The fungus travels up the vascular bundles; discolored vascular bundles are often the first symptoms of the advancing edge of disease.

D. ASR often causes premature death of plants. Premature death occurs above the ear with the plant tissue below the ear remaining green.

QTL Mapping of a Resistance Locus from MP305

An RFLP study (Jung et al., 1994) conducted in the early 1990's indicated that a major quantitative trait locus (QTL) conferring resistance to ASR is located on the long arm of chromosome 4 (chr-4L). Near isogenic lines (NILs) were advanced to develop a segregating population for map-based cloning strategy. A large BC7 segregating population derived from a cross between the introgressed resistant line, DE811ASR, and the susceptible recurrent parent, DE811 was studied both for additional DNA markers and disease phenotype in order to complete the fine mapping of the region on chr-4L. QTL interval mapping indicated the position of the resistance locus (*Rcg1*) between markers FLP8 and FLP27 (Fig 2). From the integrated physical and genetic map it was possible to identify a region on the physical map between these markers that consisted of three contiguous bacterial artificial chromosomes (BACs) on the Mo17/B73 physical maps (Fig 2).

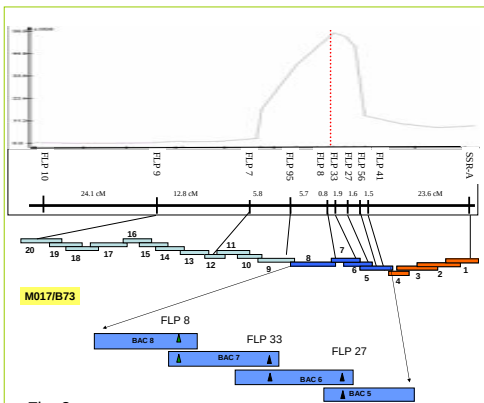

Fig. 2
Linkage between physical and genetic map around the Rcg1 locus.

Fig. 3.
Isolation of ASR susceptible mutants from Mu-tagged materials.

Map-Based Cloning

In order to isolate the gene responsible for the phenotype conferred by the *Rcg1* locus, BACs between the FLP8 and FLP27 markers were isolated from a BAC library prepared from the resistant line, DE811ASR. The library was probed with overlapping oligonucleotide probes designed on the basis of unique sequences found in the BAC sequences derived from the B73 and Mo17 BAC sequence between FLP8 and FLP27. Four BACs that spanned the entire region were sequenced.

One candidate gene with homology to a putative disease resistance gene in rice, similar to the nucleotide-binding site, leucine rich repeat (NBS-LRR) class of resistance genes, was found. No allelic version of this gene was found in the B73 and Mo17 BAC sequences.

Validation of Gene Candidate

Validation of the *Rcg1* gene candidate was done using a knockout strategy of Mutator. The strategy to achieve loss of resistance in maize to ASR fungal pathogen involves introducing active mutator materials into a maize line carrying given resistance-genes (i.e. MP305). By selfing or inter-mating, the F2s are made homozygous for the resistance-gene and homozygous resistant families are identified by DNA markers. These are then propagated by test cross and screened for the loss of resistance. This loss of resistance can be the result of Mu-induced modifications in the resistance-gene itself or its regulators, or modifications in the signal transduction pathway invoked as a part of the resistance response.

A number of putative susceptible mutants were identified (Fig 3) from the test crossed progenies inoculated with *C. graminicola*. Four independent knockouts were identified in the putative *Rcg1* gene candidate, indicating that we have cloned the right gene.

Rcg1, the Phenotype

Introgression confirmed that the *Rcg1* gene could be successfully backcrossed into inbreds, and that hybrids produced with the *Rcg1* inbred line have enhanced *C. graminicola* resistance.

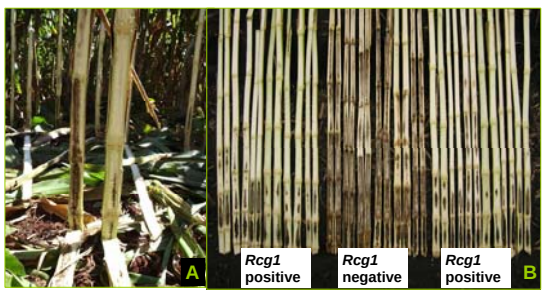

Fig. 4
A. Segregating lines containing *Rcg1* (plant on right) and without *Rcg1* (plant on left). Plants were jab-inoculated in the first elongated internode with a spore suspension of *C. graminicola.*

B. Backcross lines (dose 4) showing the resistance phenotype conferred by *Rcg1*. Plants were jab-inoculated in the first elongated internode with a spore suspension of *C. graminicola.*

Conclusions

1. A major QTL for resistance to ASR derived from MP305 was successfully cloned, using a map-based cloning approach.

2. The QTL was introgressed into several inbred lines and the disease resistance phenotype was recovered in *Rcg1*-containing lines.