Addition of Blue Mold Resistance To KTTII Burley Tobacco Varieties

Robert Miller
University of Kentucky Plant and Soil Sciences Department

Interim Report Submitted to the Council for Burley Tobacco November 4, 2015

Overview of Research Project:

Blue mold is generally a sporadic disease of tobacco grown in the United States, but it can cause substantial economic damages when epidemics occur under ideal weather conditions. Even in seasons when a blue mold epidemic does not occur, the use of preventative fungicide sprays can cost tobacco growers thousands of dollars. Although the sporadic nature of the disease makes the release of a blue mold resistant variety that is inferior in terms of yield or other desirable disease resistance impractical, the addition of blue mold resistance to otherwise outstanding varieties that have high resistance to black shank and fusarium wilt is highly desirable.

The objective of the current research project is to add blue mold resistance to several burley tobacco varieties previously released by KTTII. Breeding for blue mold resistance using traditional techniques is particularly difficult due to the interaction between the causal organism, *P. tabacina,* and the tobacco host plant. Because *P. tabacina* is an obligate parasite, selection for resistance is typically done under naturally occurring field epidemics; artificial inoculation cannot be implemented because of the possibility of initiating or worsening an epidemic in surrounding commercial tobacco crops. Disease reactions due to natural infestations are greatly dependent on weather conditions and the physiological status and age of tobacco plants. This variability in disease pressure and the incomplete nature of genetic resistance often make field experiments highly variable and unpredictable, resulting in inadequate or inconsistent disease pressure to allow selection of plants having high resistance to blue mold. By using molecular markers, this environmental variability can be eliminated. DNA from a single plant can be analyzed for the presence of markers linked to various disease resistance and other desirable genes, greatly enhancing and expediting the breeding process.

Because blue mold resistance appears to be largely controlled by a single incompletely dominant gene, the resistance allele must be contained by both parental lines of a hybrid variety in order to achieve moderate to high resistance to the disease. TKF 2002LC is a very high yielding KTTII breeding line that has exceptional general combining ability; a male sterile version of TKF 2002LC is the female parent for KTTII hybrid varieties KT 204LC, KT 206LC, KT 209LC, KT 210LC, and KT 212LC. KTTII has developed FT 2002e3, an elite early flowering (FT) strain of

parental line TKF 2002, that is also homozygous for the 3 "e" alleles that minimize the conversion of nicotine to nornicotine (varieties that have these 3 alleles are now referred to as "Zyvert" or "Z" varieties). The project to develop FT versions of KTTII breeding lines was partially funded by a grant from the Council for Burley Tobacco. The early flowering trait can effectively shorten the "seed to seed" cycle in a breeding program from the normal 150 to 165 days to 70 to 75 days, greatly decreasing the time required for backcross introgression of desirable traits into breeding lines. The goal of the current research project is to use marker assisted selection techniques and the FT early flowering trait to add blue mold resistance to existing KTTII burley tobacco parental lines and varieties.

Results to Date:

January – June, 2015:

Because TKS 2002 is the female parent for all KTTII hybrid varieties, the first step in this project was to introduce the blue mold resistance gene into the FT TKF 2002e3 and TKS 2002e3 parental lines. To initiate this process TKF 1112, an elite advanced breeding line that has high resistance to race 0 black shank and moderate to high resistance to race 1 black shank and PUTATIVE (believed to be) resistance to blue mold, was crossed onto FT TKF 2002e3, the male fertile version of FT 2002e3. The F_1 progeny were grown in a growth chamber and early flowering plants were self-pollinated to produce F_1S_1 seed. F_1S_1 progeny were then grown and early flowering plants were selected. Using molecular markers, individual plants that were homozygous for the genetic marker normally associated with blue mold resistance were identified and backcrossed to FT TKF 2002e3 and the e3 male parental lines for previously released KT hybrid varieties KT 204, KT 206, and KT 209, KT 210, and KT 212 respectively.

July, 2015 – Present:

During July, 2015, a significant outbreak of blue mold occurred in our primary race 1 black shank nursery in Greeneville, TN. This represented the first chance in over five years to visually evaluate breeding materials that had putative blue mold resistance, based on the presence of neighboring genetic markers that are normally linked to the actual gene conferring resistance to blue mold, to confirm that they indeed possess genetic resistance to the disease. The blue mold incidence was severe enough to clearly differentiate plants that had genetic resistance to blue mold versus those that were susceptible (Photo 1). This was the first time in the history of the KTTII breeding program that it was actually possible to visually select individual plants that had high resistance to both race 1 black shank and blue mold. In past seasons when blue mold occurred, the blue mold response was very light and extremely variable in KTTII black shank nurseries; this was because loss of plants from black shank resulted in very uneven stands that eliminated shading and resulted in an environment that was not conducive to uniform development of blue mold across the field. Under those conditions it is impossible to differentiate plants that have true genetic resistance versus those that fail to develop symptoms simply due to escape from infection.

The 2015 outbreak of blue mold in the Tennessee black shank nursery provided bad news and good news. The bad news was that TKF 1112, which had consistently been homozygous for the presence of the blue mold marker in numerous previous DNA analyses, displayed no resistance to blue mold in the black shank nursery in 2015. In addition, no breeding lines involving crosses or backcrosses to TKF 1112 that were presumed to be segregating for resistance to blue mold showed any plants with resistance. Leaf samples of these lines were collected and tested for the presence of the blue mold marker. All of the TKF 1112 samples and several plants from the segregating populations again tested positive for the blue mold marker, but had no visible resistance in the field. Although the accuracy of the blue mold marker is reported in research literature as being very reliable, these results make it obvious that the marker is not foolproof. Sometime since blue mold resistance was visually verified in TKF 1112 in the field five years ago, a chromosome crossover, breakage, and repair event evidently has occurred that has separated the blue mold marker from the actual gene conferring blue mold resistance, thereby rendering the marker unreliable in current TKF 1112 selections.

The good news from the 2015 blue mold outbreak was that several breeding lines that were genetically stable for blue mold resistance, verified to be homozygous both visually and through DNA analyses, were identified in 2015. These lines not only displayed good visual blue mold resistance, but moderate to high resistance to race 1 black shank. One of these lines, TKF 4028Z, is also homozygous for the three alleles that minimize the conversion of nicotine to nornicotine.

As a result of the events described above, the initial research described in the **January-June, 2015** section must be repeated, using visibly resistant TKF 4028Z as the blue mold donor parent rather than TKF 1112. The initial cross between FT TKF 2002e3 and TKF 4028Z was made in the greenhouse in August 2015. F_1 progeny seed have been collected and reseeded in a growth chamber. From this point onward, the breeding protocol described in the original research proposal, presented to and funded by the Council for 2015, will be followed to reach the original goals of incorporating blue mold resistance into existing KTTII breeding lines and varieties.

Photo 1. A comparison of a burley tobacco plant that has genetic resistance to blue mold versus one that is susceptible.