

Breeding for resistance to bacterial spot: prospects for marker assisted selection**David M. Francis¹, Jay W. Scott², and Sally A. Miller³****¹Dept. of Horticulture and Crop Science and ³Dept. of Plant Pathology, The Ohio State University OARDC, 1680 Madison Ave., Wooster, OH 44691. ²Gulf Coast Research and Education Center, University of Florida, 5007 60 th Street East, Bradenton FL 34203-9324**

Xanthomonas campestris pv. *vesicatoria* (Xcv) causes bacterial spot on both pepper (*Capsicum* spp.) and tomato (*Lycopersicon* spp.). The target population in the Mid-west U.S.A. has been identified as 67% tomato race 1 (T1), 29% T2, and 4% T3. Resistance to copper and streptomycin in *X. campestris* pv. *vesicatoria* strains in Ohio were significantly increased during sampling between 1994 to 1996 (Sahin and Miller, 1995; Sahin, 1997). The target population for our breeding efforts is therefore diverse.

The objectives of this project were to characterize resistance to race T1 of bacterial spot in a *L. esculentum* x *L. esculentum* cross, to identify polymorphic molecular markers linked to genes conferring that resistance, and to use markers in the context of an inbred backcross breeding program to develop breeding lines with improved resistance. Resistance to race T1 involves more than one locus, and the hypersensitive response does not predict field performance. Our strategy was to treat resistance as a quantitative trait in early generation field evaluation. However, our working hypothesis was that resistance is controlled by Mendelian loci that are genetically linked to existing resistance genes in tomato. We set out to test this hypothesis by mapping partial-resistance to Race T1 strains in *L. esculentum* x *L. esculentum* crosses by using markers developed from conserved domains of previously cloned resistance genes. Previous studies suggested that three loci may be involved in a hypersensitive response to Race T1 of bacterial spot in tomato (Wang et al., 1994b; Yu et al., 1995). These studies were based on a wide cross between the resistant variety H7998 and *L. pennellii* LA716, and the genetic markers from that cross are not appropriate for transferring resistance from H7998 into *L. esculentum* breeding lines. The identification of polymorphic markers within *L. esculentum* is considered difficult due to restricted genetic variation (Miller and Tanksley, 1990; Williams and St. Clair, 1993). Our approach was to use conserved sequences from previously cloned resistance genes to identify polymorphisms. This strategy was based on the observation that resistance genes in plants tend to be clustered in a number of plant species, and the observation that high levels of polymorphism are associated with resistance genes. Using this approach, we have identified genetic markers that are polymorphic between H7998 and elite processing tomato lines adapted to the midwest. Two of these markers are linked to loci identified in the wide cross.

Data was collected from a greenhouse screen as bacterial populations present in leaf tissue 6 days post inoculation. This assay results in a population difference of over 10^2 between resistant and susceptible parents. The marker linked to the Rx1 locus explains 12% of the total variation in the backcross population and only 1% of the variation in the F₂ population. This PCR based marker is dominant and genetic information is therefore lost in the F₂ population, a fact that may explain some of the difference between the two populations. However, these results cannot be interpreted as evidence that Rx1 plays a role in resistance. This result contrasts with previous studies (Wang et al., 1994b; Yu et al., 1995) and may be due to differences between the bacterial strains used, differences in the procedure used to score resistance (hypersensitive reaction vs. bacterial populations), or differences in growth environment. In contrast, the chromosome 5 marker (linked to Rx3) explains as much as 45% of the variation in the greenhouse test and does appear to be linked to a locus that controls resistance ($P < 0.008$)

Greenhouse results were confirmed in the field using an advanced backcross (AB) population (O 88119 X H 7998 BC3 to 88119). 91 families were planted in plots consisting of 8 plants from each AB family. Families were completely randomized and replicated in two blocks and plants were inoculated with the race T1 isolate, 110C. Data were collected on a Horsfall-

Barrett scale on individual plants within a plot. Ratings of disease severity were done twice. Analysis of variance was performed using genotypic means of plot high score, plot low score, and plot average. Genotype differences were highly significant ($P < 0.002$) and block differences were not significant. As in the greenhouse studies, a locus on chromosome 5 is significantly associated with resistance to race T1 ($P < 0.05$) in both rating periods. The locus on chromosome 1 was not associated with resistance to Race T1.

The locus on chromosome five, Rx3, is the most important locus for field resistance against the T1 strain, 110C. It explains 45% of the variation in both greenhouse and field studies, and is thus a robust locus. Markers from chromosome 1 (Rx1) and chromosome 5 (Rx3) are being used in a backcross breeding program aimed at introducing resistance to bacterial spot into cultivars used in the Midwest. We have yet to develop markers linked to Rx2. Populations designed to facilitate the selection of recombination events that will incorporate resistance to both bacterial speck and bacterial spot on chromosome 5 have been developed. The DNA based markers developed for genetic mapping will facilitate selection of recombinant plants. Selections from 2 families, AB129 and AB147, have been advanced for breeding purposes based on horticultural characteristics and disease resistance.

A second source of resistance, PI 114490, confers resistance to Race T1, T2, and T3 of bacterial spot (Scott et al., 1997). Heritability for T2 resistance is moderate to high based on F_3 to F_4 parent-offspring regression. We have developed a large (approx. 160 lines) Inbred Backcross population based on PI114490 as a donor of resistance and the adapted high lycopene variety Ohio 9242 as a recurrent parent. This population has been evaluated for two years in our T2 resistance nursery. Estimates of gene number are in the range of 2-3. A better understanding of the genetic basis of resistance from PI114490 will allow us to combine resistance to T1 based on Rx3 and resistance to T2.

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