A gene influencing tolerance to common root rot is located on linkage group IV

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Breeding for tolerance to common root rot, caused by the pathogen *Aphanomyces euteiches*, has been difficult because of the polygenic nature of the tolerance and the strong influence of the environment. Crosses between tolerant and susceptible lines or between different tolerant lines often produce progeny with little or no tolerance (J. Kraft, personal communication). In the last few years we have concentrated on investigating the genetic basis of tolerance in MN313, a line developed by Dr. D. Davis at the University of Minnesota. Further selection of this release was performed by one of the current authors (C.R.G.). This line displays good tolerance (scores of 1.5 to 2.0 on a 5 point scale, where 1.0 indicates no evidence of the disease and 5.0 indicates plant death before pod set) in fields infested with common root rot in Minnesota and Wisconsin.

Materials and Methods

Our approach has been to make crosses between MN313 and lines possessing markers or other traits of interest and to produce populations of recombinant inbred lines (RILs), with each RIL derived from a separate F_2 plant through single seed descent. One such cross was made with OSU1026, a release from the breeding program of Dr. Jim Baggett at Oregon State University. OSU1026 was a dwarf, white-flowered, multiple disease resistant release, containing *sbm1*, *En*, *er1*, *mo*, and *Fw*. To our knowledge OSU1026 was never selected for tolerance to common root rot. MN313 lacked resistance to powdery mildew, and the goal of the cross was to introgress powdery mildew resistance (*er1*) into a line with good tolerance to common root rot. However, the RIL population proved to be very useful for identifying a gene in MN313 contributing to tolerance to common root rot.

The MN313 x OSU1026 RIL population consisted of 45 RILs. We grew the population in the common root rot nursery at Le Sueur, MN as an F_5 in 1999 and an F_6 in 2000. We also grew the F_6 in the common root rot nursery at the Spillman Farm, Pullman, WA in 2000. In each case at least 10 seeds per RIL were planted. The plants were scored twice during the growing season for general vigor and symptoms of common root rot. Plants were scored for disease severity on a scale of 1 to 5 as described above.

A linkage map was constructed for the population using classical markers, isozymes and DNA polymorphisms (STS markers and RAPDs). Possible relationships between markers and disease response were tested by one way ANOVA calculations.

Results

In 1999 at Le Sueur the MN313 parent was given a 1.5 rating and all susceptible controls (Sparkle, A1078-239, Badger) were scored as 5.0. OSU1026 was not tested due to insufficient seed available. All except one of the MN313 x OSU1026 RILs displayed some tolerance (score of 3.5 or better), with 22 of the 45 lines scoring 2.0 or better (Fig. 1). The one line that was scored as susceptible (5.0) was located at the end of a row and may have been subjected to other environmental effects.

In 2000 at LeSueur, a similar pattern was observed in that approximately half the lines were rated as good or better than the MN313 parent. The other half of the lines ranged from moderately tolerant to susceptible (2.5 to 4.0). The results for individual lines were highly consistent between years, although those lines rated slightly tolerant in 1999 often displayed more severe symptoms in 2000. Again the susceptible controls all were rated between 4.0 and 5.0. The distribution of two-year averages at Le Sueur for each line is given in Fig. 2. The distribution is clearly bimodal, suggesting the influence of a single gene segregating in a 1:1 ratio.

Joint segregation analysis of genes or markers segregating in the RILs and common root rot resistance at Le Sueur failed to reveal a correlation with *er1*, *sbm1*, or DNA markers on linkage groups II, VI or VII. However, a significant correlation ($p<0.0001$) was found between the root rot resistance phenotype and markers on linkage group IV (P393 and $PgmF_{390}$). The mean score for lines with the MN313 allele was 1.85±0.11, while that for lines with OSU1026 allele was 3.27+ 0.13.

The results from Pullman were significantly different. Both parents expressed modest tolerance (2.5 to 3.5). The RILs appeared to segregate for tolerance, but few lines were highly tolerant or highly susceptible (Fig. 3). There was little correlation between the results at Pullman and those at Le Sueur. No correlation was observed between linkage group IV markers and root rot tolerance at Pullman. More generally, we could not find an association between the segregation of plant phenotype (disease rating score) at Pullman and that for any of the markers we have scored in this population. However, significant gaps still exist in our linkage map for this population, and a lack of correlation at this point does not necessarily indicate that the variation in disease response at Pullman lacks a genetic component.

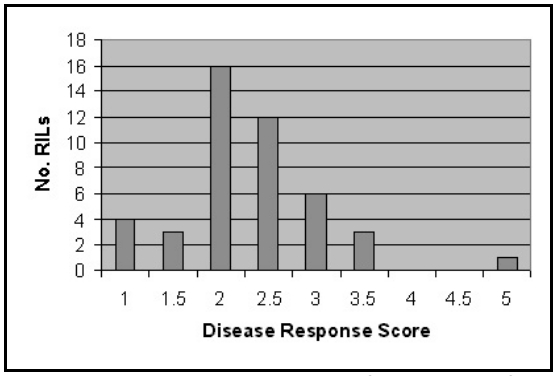

Fig. 1. Disease response scores of the 45 RILs from the cross MN313 x OSU1026 planted in the Aphanomyces nursery at Le Sueur, MN in the summer of 1999. The resistant parent, MN313 showed a response score of 1.5. Susceptible lines in the nursery had a response score of 5.0.

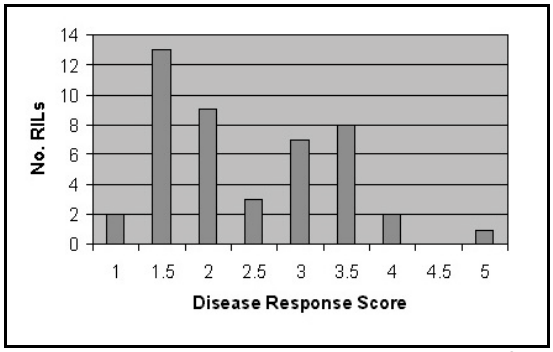

Fig. 2. Disease response scores at Le Sueur of the 45 RILs from the cross MN313 x OSU1026 averaged over two years. The resistant parent, MN313 showed a response score of 1.5. Susceptible lines in the nursery had a response score of 5.0.

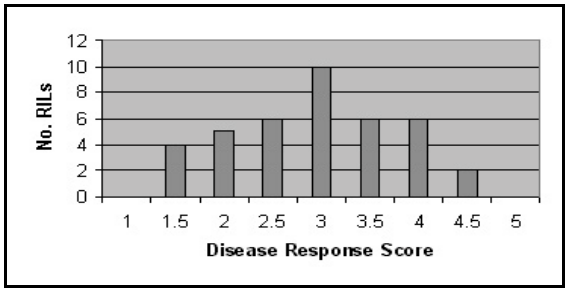

Fig. 3. Disease response scores of the 45 RILs from the cross MN313 x OSU1026 planted in the Aphanomyces nursery at Pullman, WA in the summer of 2000. The resistant parent, MN313 showed a response score of 2.5. The susceptible parent, OSU1026, gave a response score of 3.5.

Discussion

Our results reveal an important locus affecting the susceptibility of a pea plant to common root rot in the Aphanomyces nursery at Le Sueur, MN. The effect was consistent over two years although in the first year the bimodal pattern was not as obvious. Apparently the greater severity of the disease in summer 2000 caused the more susceptible genotype to display more prominent symptoms and thus become a distinct group in the analysis (Fig. 2).

Two major conclusions can be made from the results presented. One is that a gene in MN313 located on linkage group IV near P393 has a significant influence on the expression of tolerance to common root rot in the field at Le Sueur. This gene represents the major genetic difference between MN313 and OSU1026 in their respective responses to the pathogen. In a different RIL population, derived from the cross MN313 x A1078-239, we have obtained evidence that this same gene is segregating and influencing Aphanomyces tolerance in the field at Le Sueur (data not presented). However, the effect is less clear because A1078-239 is susceptible (response score 5.0), and we have yet to tag and locate the other gene(s) affecting tolerance that appears to segregate in this population. MN313 and OSU1026 must share these other genes bestowing tolerance to common root rot.

The second conclusion is that the pathogen or set of pathogens in the Le Sueur field is different from that at Pullman. Mn313 exhibited high levels of tolerance both years at Le Sueur and was clearly superior to

Sparkle or A1078-239. At Pullman MN313 displayed little tolerance and was not rated above either of the susceptible controls. The reverse situation has been observed in experiments on RILs derived from crosses using common root rot tolerant lines developed by John Kraft. In this case Kraft's lines were definitely superior at Pullman but not at Le Sueur (Clare Coyne, personal communication). This conclusion is supported by the work of Malvick and Percich (1) who demonstrated significant phenotypic differences between strains of *A. euteiches* sampled from Minnesota and Oregon. We are investigating the nature of the pathogens in both fields.

Small quantities of seed are available for several of the MN313 x OSU1026 RILs that combine resistance to common root rot at Le Sueur with powdery mildew resistance. Breeders and researchers interested such seed should contact the senior author.

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1. Malvick, D.K. and Percich, J.A. 1998. Phytopathology 88: 915-921.