6 Quantitative Trait Loci for Clubroot Resistance in *Brassica oleracea*

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1 Introduction

Clubroot, caused by the fungus *Plasmodiophora brassicae* Wor., is one of the most damaging diseases of *Brassica oleracea* crops, including cauliflower, broccoli, Brussels sprouts, cabbage, and other cruciferous crops worldwide. The pathogen causes swelling of parts of the roots and sometimes of the stem base, giving rise to the characteristic "clubs" for which the disease is named (Fig. 1). These clubs inhibit nutrient and water transport, stunt the growth of the plant, and increase the susceptibility to wilting. Later on, the clubbed roots decay, weakening the support of the plant.

Clubroot is a widespread disease. Crête (1981) estimated that in northwestern Europe, Japan, North America, and Australia about 10% of a total of 660,000 ha of *B. oleracea* crops was infested with clubroot. For all cruciferous crops, the infested area in those regions amounted to 196,000 ha. The disease is not only widespread, but also very persistent in the soil. The spores can remain infectious for at least 15 years (Mattusch 1977). Therefore, crop rotation does not offer much promise, especially since most cruciferous crops and cruciferous weeds will maintain the disease presence. Cultural practices, especially the application of calcium and boron, and lime to decrease the pH of the soil, may reduce disease pressure but these measures are often not sufficient to keep the crop healthy. On the other hand, it is known that some soil types have a significant suppressive effect on the occurrence of clubroot (Murakami et al. 2000). Chemical control is limited, since the few effective treatments are either banned or too expensive.

The introduction of cultivars with resistance or tolerance to clubroot would therefore be desirable. However, only a very small number of resistant cultivars have been released. Breeding programs aimed at the introduction of resistance to clubroot have been few and the results often disappointing. This general lack of success is due to the relatively small number of known sources of resistance, the recessive and often apparently complex inheritance of resistance, and the genetic variability of the pathogen.

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Fig. 1. Roots of a cauliflower plant severely affected by clubroot

Tools now exist for analyzing genetic complexity and for introgressing the genes concerned that were not available only two decades ago. This chapter illustrates the advantages of using doubled haploids (DHs), molecular markers, and quantitative trait loci (QTL) mapping algorithms.

In *B. oleracea*, doubled haploids were first regenerated from haploid microspores through anther culture (Keller and Armstrong 1981). However, this procedure was laborious and the yield was low. The microspore culture technique, first developed for *B. napus* (Lichter 1982) and adapted for *B. oleracea* crops by Duijs et al (1992) was an improvement. Large numbers of microspores can be obtained by grinding complete flower buds, thus eliminating the isolation of individual anthers. Also, the average number of plants obtained per flower bud is larger with microspore culture. This made the generation of large numbers of doubled haploids a practical tool in both breeding and genetic studies. For genetic studies, the advantages of a doubled haploid population, compared with, e.g., an F2, are twofold: the individual DH

genotypes can be maintained indefinitely through selfing, as they are fully homozygous; and because all heterozygous genotypes are missing the segregation patterns are less complicated. In the case of quantitative characters, there is an additional advantage: the absence of intermediate heterozygous genotypes makes it easier to discriminate between the different genotypic classes.

Molecular markers, as aids in breeding and genetic studies, have been used for a long time. A case in point concerns the resistance to *Meloidogyne incognita* in tomato, which was shown in 1974 to be linked to the isozyme *Aps-1*[1] (Rick 1983). Nowadays, mostly genomic DNA markers are used to compile linkage maps. The first DNA markers to be used for genetic studies were restriction length fragment polymorphisms (RFLPs); subsequently, a multitude of different DNA markers techniques have been established. The essence of all molecular marker methods is that they yield information about the allelic composition at specific loci in different individual plants. The simultaneous segregation observed at many different loci in a population allows one to calculate recombination frequencies between loci. From these recombination frequencies, the most likely linkage map of these loci can be calculated.

The use of molecular markers is not restricted to the compilation of linkage maps. A very important application is the new possibility of locating chromosomal regions involved in the expression of quantitative traits, and the estimation of the effects of these regions. For these regions, the term quantitative trait loci (QTL) has been coined. Statistical methods have been developed to estimate the location, support intervals and effects of QTL for many different situations: different population types, dominant or codominant markers, dominant or additive action of QTL, mono-QTL or multiple-QTL models, etc.

For our study, we obtained a population of doubled haploid plants from the F1 of a cross between a clubroot-resistant cabbage and a susceptible broccoli parent. In this DH population, we scored two types of molecular markers: RFLPs and AFLPs (amplified fragment length polymorphisms). Although the principles of these two types of markers are very different, from a genetic viewpoint, they can be used in much the same way. One difference is that RFLPs can in general be scored codominantly, while AFLPs are scored dominantly, except when reliable quantitative scoring of band intensities on DNA gels is possible. For our study, the difference was not relevant since our DH population consisted solely of homozygotes. Finally, we developed a reliable quantitative scoring method for resistance with a distribution suitable for the application of a multiple QTL model. We then used this model to perform a QTL analysis of clubroot-resistance segregating in our DH population.

With the aid of these novel techniques, we were able to identify and characterize two previously unknown genes for clubroot resistance, as well as closely linked markers that can be used, directly or after conversion to more easily handled marker types, for marker-assisted selection (MAS).

2 Results

2.1 Resistance Tests

The severity of clubroot symptoms was measured by assigning symptom grades based on visual inspection, as well as by measurements of healthy and diseased root tissue, and calculating an R value (see Protocols, below). The mean symptom grade and the mean R value were calculated for all DH lines, parents and F1; the R values were corrected for test and block effects. A high proportion of the DH lines, as well as the F1 and the susceptible parent, scored the maximum disease symptom grade (Fig. 2, bottom), suggesting a recessive inheritance of resistance. In contrast, the distribution of the R values was much less skewed, and the F1 value was about halfway between the parental values (Fig. 2, top). Clearly, the grading scale is compressed at the susceptible side, and expanded at the resistant side compared with the R value scale.

2.2 Molecular Markers

Among the 107 DH lines of the mapping population, 92 polymorphic markers were scored: 66 RFLPs and 26 AFLPs.

RFLPs. Since almost all probe/enzyme combinations detected polymorphisms, no preliminary screening of parental blots was performed. Of 40 probes used, 37 detected a total of 66 polymorphisms using restriction endonuclease *Eco*R1. Three probes (1NH12, 2NA11, 3NG10) did not detect any RFLPs. Five RFLP groups without recombination were found (1NE1c/3ND12, 1NG9/2NA8a/3NB4b, 1NF2b/2NA1a, 1NF2a/1NH3a/2NF10, and 3NB4c/3NC3b). Since the *Eco*R1 restriction fragment sizes detected were unequal within all these groups, these markers detected different but closely linked loci.

AFLPs. With one set of selective primers 26 AFLPs were detected. Five AFLP pairs without recombination were found. In four pairs (A11/A12, A13/A14, A17/A18, and A22/A23), both amplified fragments were inherited from the same parent. Since the sizes of both fragments in these pairs were very similar, it is possible that they represent double bands as reported by Vos et al. (1995), and not different markers. In one pair (A04/A05), each of the two parents contributed one fragment.

2.3 Construction of the Linkage Map

Ninety-two markers were scored for 107 DH lines. We determined the grouping of the markers in linkage groups at a range of log of odds (LOD) scores and calculated preliminary maps for these linkage groups. If gaps larger than

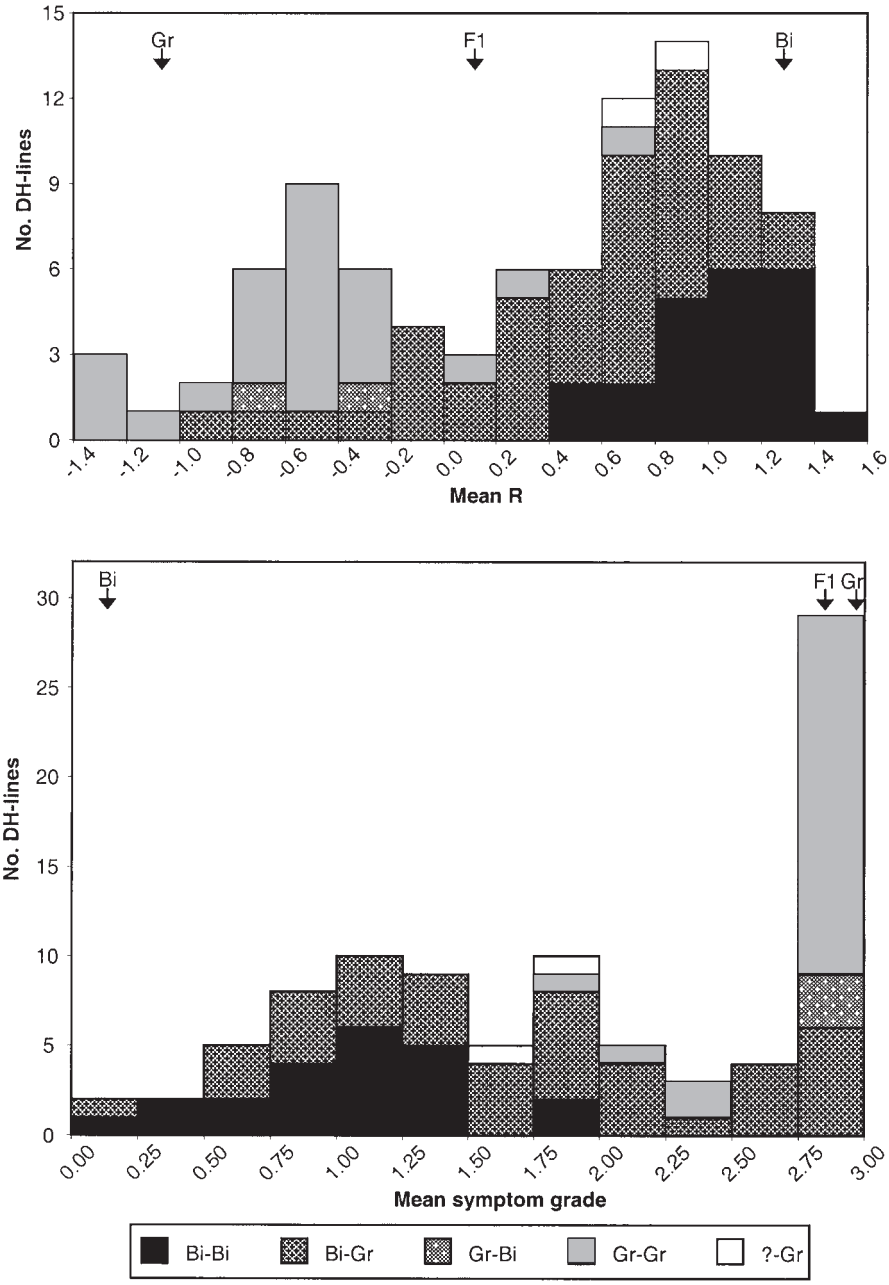

Fig. 2. Frequency distributions for the means of the measure of clubroot resistance R (*top*) and of the symptom grade (*bottom*) in a population of doubled haploid lines, in relation to the genotypic composition (Bi or Gr allele) for the two marker loci 4NE11a and 2NA8c most closely linked to clubroot resistance loci *pb-3* and *pb-4*, respectively