MAPPING OF RESISTANCE GENES TO POWDERY MILDEW (*ERYSIPHE BETAE*) IN SUGAR BEET.

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ABSTRACT

Powdery mildew (*Erysiphe betae*) is a fungal foliar disease on sugar beet which can cause yield losses up to 30%. Genetic resistance is a powerful tool to control the disease and limit any economic damage. Within the germplasm of cultivated sugar beet considerable variation is present which is oligogenic based. Subsequently, various sources of resistance have been identified in wild *Beta vulgaris* and *Beta* spp., of which one has been identified as a monogenic source of resistance.

Syngenta seeds developed mapping populations in which either the oligogenic or the monogenic based resistance is segregating. Phenotypic evaluations were carried out in greenhouse as well as under field conditions with natural exposure to the disease. Numerous QTL's were identified for the oligogenic based resistance which explained up to 35% of the phenotypic variation. The monogenic resistance was mapped to a single locus confirming that a single gene is responsible for the resistance. Fine mapping of the gene is ongoing. The relation of the two types of resistance is discussed.

INTRODUCTION

Powdery mildew (*Erysiphe betae*) is the most widespread disease on sugar beet. It occurs in all sugar beet producing areas, but its damage is often limited or can be controlled by chemicals. However, in arid climates, e.g parts of Turkey, Spain and California, sugar yield losses of up to 30% have been reported (Weltzien and Ahrens, 1977). Genetic resistance is a powerful tool to control the disease and limit any ecomic damage. Within the germplasm of sugar beet considerable variation has been identified. High levels of resistance have been detected in *Beta vulgaris* subsp. *maritima* and other wild *Beta* species (Asher et al. 2001). A marker analysis on *maritima* accession PI504236 revealed the presence of 2 QTL's (Francis, 2002). A monogenic inheritance has been identified in *maritima* accessions WB97 and WB242 and the gene has been designated as *Pm* (Lewellen and Schrandt, 2001). This gene has shown a high level of resistance, but its durability has been questioned. The resistance level in currently used sugar beet cultivars can be described as susceptible up to moderate. If present, the resistance appears to be quantitatively based, but little is known about the inheritance and relationship with the previous described resistance factors.

Selection for resistance to powdery mildew has been done through phenotypic selection under natural infection in field trials as well as in greenhouse tests by artificial inoculation. The greenhouse test is more labourious, but enables to

standardize infection level and produce reliable results. No major diffences have been observed between field observations and greenhouse test results.

We have studied and compared the inheritance of different resistance sources with the aim to develop molecular markers as a selection tool in sugar beet breeding.

MATERIALS AND METHODS

1.- PLANT MATERIAL

For QTL identification, mapping populations were developed from paircrosses between sugar beet inbred lines, which have been used as components in commercial hybrids. Single F1 plants were subsequently inbred twice through single seed descent to produce up to 300 S2 inbred lines per mapping population.

As source for the monogenic resistance, CP02, has been used. This accession has been derived from repeated backcrossing of WB242 into conventional sugar beet germplasm (Lewellen, 2000). The high level of resistance has been confirmed in greenhouse tests. Individual plants from CP02 were selected and backcrossed to 4 different inbred lines to produce BC1 populations.

2.- *ERYSIPHE BETAE*, GREENHOUSE SCREENINGS

For the quantitative resistance, the plants were sown in boxes with 10 plants per row for each replication. All S2 lines were tested in 4 replications. In the screening of monogenic resistance the BC1 plants were tested individually. Powdery mildew infected source plants were placed evenly distributed among the 4 weeks old plants to be tested, with one source plant every 6th row or 24 individual plants, respectively. The infected plants were placed in a higher position and the leaves of the infection sources were tapped with a bamboo stick every day to spread the conidia. The temperature in the greenhouse was 15-16°C at nights and 20-23°C during day time.

The scoring was done 3-4 weeks after infection. In the test of quantitative resistance the 10 plants from one replication were given a mean score. The plants for monogenic resistance were scored individually. The scoring was in a 1-9 scale. 1 represents plants with all leaves totally covered with powdery mildew, whereas 9 are totally healthy plants (Picture 1)

3.- MARKER ANALYSES

DNA was isolated from freeze-dried leaves according to Hjerdin et al. (1994) but with minor adjustments to compensate for volume losses in the 96 format. AFLP analyses were performed as described in Hansen et al. (1999). SSR analyses were performed using fluorescently labeled primers analysed on Base Station sequencer from MJ Research. Marker mapping was done with Joinmap 2.0 and QTL analyses with PlabQTL.

RESULTS AND DISCUSSION

MAPPING OF MONOGENIC RESISTANCE

92 BC1 individuals were analyzed with six AFLP primer combinations. All polymorphic bands which were present in the donor parent and absent in the recurrent parent were scored. This resulted in 124 markers that were used for mapping. 77 markers were assigned to nine linkage groups and 68 of these were successfully mapped. *Erysiphe* resistance data were converted to genotypic scoring by considering all scores between 2 and 4 as susceptible (i.e. homozygous for the recurrent parent allele) and all scores of 9 as resistant (i.e. heterozygous). The resistance locus was then successfully assigned to a linkage group and mapped to an interval of 6.4 cM between the flanking AFLP markers. The markers on this linkage group are known to map to chromosome II in the terminology of Butterfass (1964). Four individuals were recombinant between the resistance locus and the closest AFLP marker (Fig. 1). However, two of these individuals were also recombinant between the resistance locus and the closest marker on the other side. Thus, the latter two individuals had two recombination events within 6.4 cM and are candidates for being misclassified in the phenotypic test. Together with other individuals, which are recombinant in this genomic region they have been selfed and the progeny will be tested to get a more reliable phenotypic score. This step is important for the development of tightly linked markers. Mapping was also done in the three other BC1 populations, all using the same donor parent but different recurrent parents. Results for all populations were quite similar, but in several cases the resistance locus mapped to a terminal position. Most likely this indicates that the error frequency of the phenotypic scoring was somewhat higher for these populations resulting in inflated recombination frequency estimates for the resistance locus. Selfing of recombinant individuals and phenotyping the offspring should resolve this problem.

MAPPING OF QUANTITATIVE RESISTANCE

Two different mapping populations each representing 288 S1 genotypes were used. In both populations, the variation for *Erysiphe* resistance was clearly quantitative and the distribution approached a normal distribution (Fig. 2). In population A, 45 SSR markers were mapped and used in the QTL mapping. The analysis revealed 5 QTL on 4 different linkage groups (Table 1). The two linked QTL on chromosome VI were separated by 40 cM. One parent contributed the resistant allele at four of the QTL, whereas the resistant allele for the 5th QTL originated from the other parent. Together the QTL explained 27% of the phenotypic variation. One QTL mapped to the same chromosome as the monogenic resistance (Table 1). It is difficult to compare the exact positions, but the monogenic resistance gene maps to the same region of the chromosome as the QTL. They could thus represent the same gene, but might just as well represent different, linked resistance genes. 45 SSR markers were mapped and used in QTL mapping of population B. Two QTL on different linkage groups together explained 19% of the phenotypic variation (Table 1). The resistance

allele came from the same parent for both QTL. The number of QTL found in the two different populations correlates with the differences between the resistance levels of the parents.

Figure 1. Distribution of resistance score for the mapping of monogenic resistance. The population was divided according to the genotype at the SSR marker closest to the resistance gene. Black represents individuals homozygous for the allele from the recurrent parent, and grey represents heterozygous individuals.

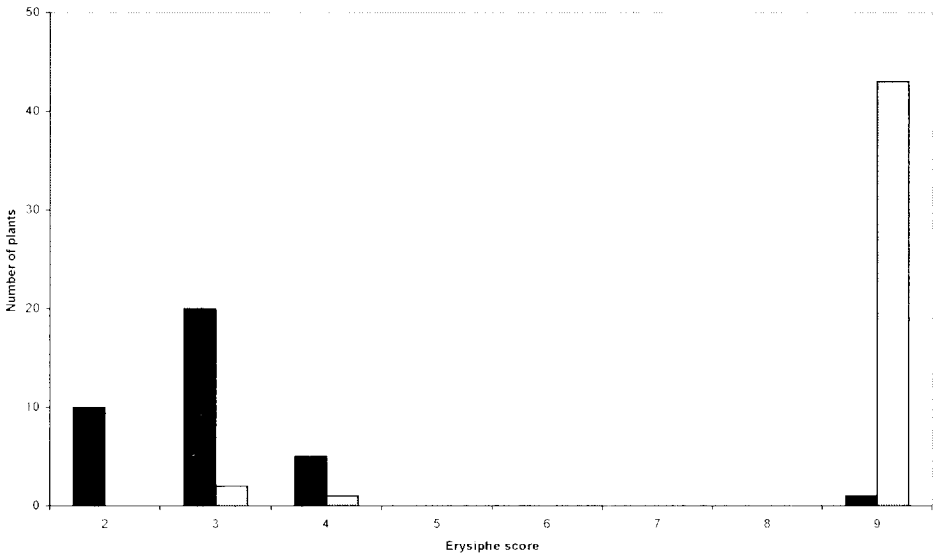
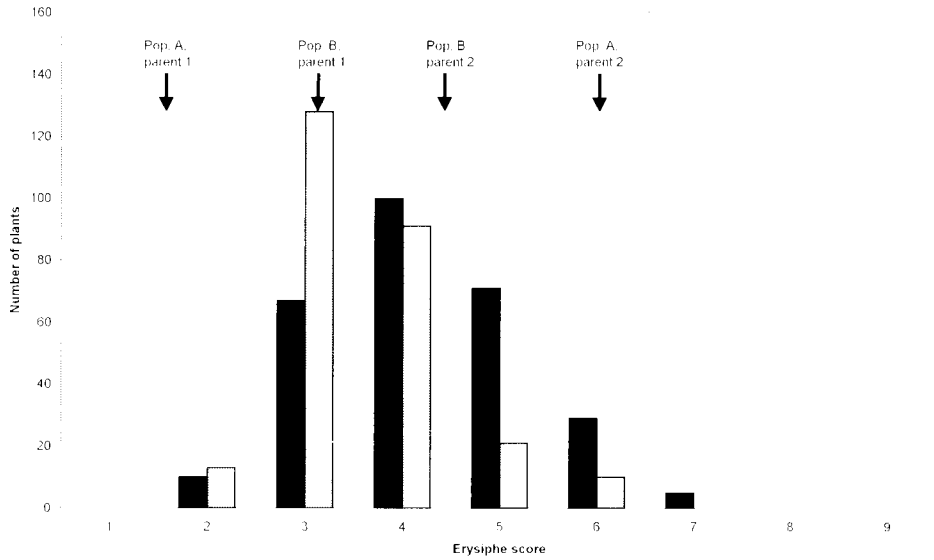


Table 1. Additive effects for the QTL detected. For the single gene, the additive effect was not calculated, but the heterozygous individuals showed nearly complete resistance. Chromosome numbers are according to the terminology of Butterfass (1964).

Chromosome	Monogenic	Population A	Population B
I			
II	X	0.35	
III		0.56	
IV		0.35	
V			
VI		0.32; -0.41	
VII			0.39
VIII			0.31
IX			

Figure 2. Distribution of resistance score for the mapping of the quantitative resistances. Black represents population A and grey population B.



CONCLUDING REMARKS

The powdery mildew resistance originating from WB242 has been confirmed to be monogenic and was mapped on chromosome II. The moderate resistance level, present in conventional breeding lines, was clearly quantitative. QTL mapping revealed a polygenic inheritance in the two mapping populations investigated. The development of tightly linked markers as a breeding selection tool for the monogenic resistance is fairly straightforward. However, developing markers for the QTL identied for the quantitative resistance is much more difficult and will require the development of fine mapping populations. The easiest strategy to combine both types of resistance would therefore be the use of markers to introgress the monogenic resistance into a line with polygenic resistance. This combination will hopefully result in longer durability of the powdery mildew resistance.

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