Linkage map development and QTL mapping for leaf rust resistance in the model plant Brachypodium distachyon

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Aims of the work

Brachypodium distachyon (L) Beauv. has emerged as a new model plant for the functional genomics of the Triticeae as its biological attributes (e.g. a small diploid genome of 35M) would greatly facilitate the elucidation of complex traits of small grain cereals (Draper et al. 2001). In particular, the completely sequenced Brachypodium genome (http://www.brachypodium.org) could act as a "genomic bridge" to cereal species like wheat and barley and thereby considerably ease the task of mapping orthologous genes. In this work the model plant Brachypodium has been employed to analyze the genetics of quantitative resistance to leaf rust. Accordingly, our main goals were (i) to develop an acceptable number of molecular markers throughout Brachypodium genome, (ii) to develop a new linkage map in Brachypodium based on an F2 population segregating for leaf rust resistance, and (iii) mapping QTL involved in resistance to leaf rust in Brachypodium.

Plant material

Looking for a population that was segregating for more than one trait (at least one abiotic and one biotic stress tolerance), but also able to stand among others of the international initiatives, a cross between Brachypodium inbred lines was considered for the present study. An F2 mapping population of 110 individuals was thus generated at USDA-ARS between two diploid inbred lines showing different responses to leaf rust (Puccinia brachypodi) infections. B3-1 (partially resistant) and B3-1 (susceptible). The B3-1 x B3-1 F2 population was used for map construction and leafrust first resistance test, the derived F3 families used for the second resistance test, and a final germline resource of inbred recombinant inbreds (IRIs) is being developed at the University of Modena and Reggio Emilia. An additional set of B3-1 x B3-1 Recombinant Inbred Lines is available at D.G. laboratory in Minnesota.

Leaf rust incidence

Establishing a suitable infection scoring system for this species was a priority to carry out efficient evaluation of the plant material. In total, four different infection tests were conducted in the greenhouse of the Laboratory of Plant Breeding of the Wageningen University by means of artificial inoculation with a virulent isolate of Puccinia brachypodi (collected in R.N.). Two preliminary tests were performed on F2 plants for the choice of the best disease evaluation method: one at seedling stage - 21 days after sowing (here referred to as F2s), and one at adult plant stage - 37 days after sowing (referred to as F2s). Two different infection tests were then repeated in the next year on the F2-derived F3 families at seedling stage - 21 days after sowing (here referred to as F3t1 and F3t2).

In our experiments, The Area Under Disease Progress Curve (AUDPC) has been proved to be a valuable component of quantitative resistance to leaf rust and was successfully used to evaluate the infection response of the 110 F2 plants and the respectively derived F3 families. Disease evaluations showed continuous, quantitative and transgressive segregation (FIGURE 2 and 3).

QTLs for leaf rust resistance

Interval mapping and MOM mapping were performed on the data of the four different experiments by using the software package MapQTL 5.0 and then QTL positions were compared in our preliminary AFLP-based linkage map (FIGURE 1 and TABLE 2).

Two major genomic regions involved in partial resistance to leaf rust were detected. Together they accounted for about 40-50% of the observed phenotypic variation. Our results suggest that leaf rust resistance in B. distachyon is an oligogenic trait influenced by few major genes with large effect as observed in other Triticeae.

Conclusions and Future research

• This work demonstrates the potential of Brachypodium distachyon to study interaction between grasses and leaf rusts.
• The developed linkage map is a preliminary version based on AFLP markers, and as a first step, still needs to be referred to the 5 recognized Brachypodium chromosomes. For reaching this goal, besides mapping the conservative orthologous markers, a tight collaboration with the International initiative is under way.
• Conserved markers between Brachypodium and barley, such as EST-derived markers, are currently being developed and will provide anchor points for constructing a genetic linkage map of Brachypodium distachyon in the model plant Brachypodium distachyon.


TABLE 2. Initial set of Wagenignen Barley EST (WBE) that are currently being located on the Bd3-1 x Bd1-1 map. ESTs were blasted against the Brachypodium Draft Genome in order to identify homologous genes. New primers were then designed to look for sequence polymorphisms between the two parents and to develop new conserved orthologous sequence (COS) markers.

| Family | Locus | Length (bp) | Unique primers | Match to the reference population, our results will be easily compared with those coming from the Brachypodium research community and the newly developed genomic tools will be useful for the future improvement of the present AFLP-based map.

In addition to the initial AFLP framework, conserved markers between B. distachyon and barley, such as EST-derived markers, are currently being developed to be added to the map, thus providing anchor points for comparative genomics studies. In TABLE 1 are shown the ones currently developed.

AFLP molecular markers in Brachypodium distachyon

To develop molecular markers for linkage mapping studies in Brachypodium, the application of arbitrary primers fragmenting DNA (AFLP) was used to generate AFLP molecular markers in Brachypodium distachyon. The AFLP technique was chosen on the basis of good reproducibility and the simultaneous identification of a large number of marker loci. A suitable AFLP protocol was therefore successfully set up, based on PstI + Msel + three primer combinations.

A first genetic map of Bd3-1 x Bd1-1

A total of 244 AFLP markers were then available for linkage mapping. A preliminary linkage map of Brachypodium was developed (FIGURE 1). Composed by 192 loci, the map consists of ten linkage groups with at least 6 markers per group. In the beginning of the present research no genetic maps were reported, and still nowadays two reference maps are being developed by two complementary projects (Wright et al. 2008; Hoo et al. 2008). Both linkage maps will be based on F2 populations from Bd3-1 x Bd1-1. Since the Bd3-1 parent is in common with the reference population, our results will be easily compared with those coming from the Brachypodium research community and the newly developed genomic tools will be useful for the future improvement of the present AFLP-based map.

In conclusion, this new model system for functional genomics in grasses is showing great promise in the development of new strategies to study the complex interaction between grasses and leaf rust.