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 355 Mb) 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.brachybase.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 brachypodii*) infections, Bd3-1 (partially resistant) and Bd1-1 (susceptible). The Bd3-1 x Bd1-1 F2 population was used for map construction and leafrust first resistance test, the derived F3 families used for the second resistancetest, and a final germplasm resource of immortalized recombinant inbreds (RILs) is being developed at the University of Modena and Reggio Emilia. An additional set of Bd1-1 x Bd3-1 Recombinant Inbred Lines is available at D.G. laboratory in Minnesota.

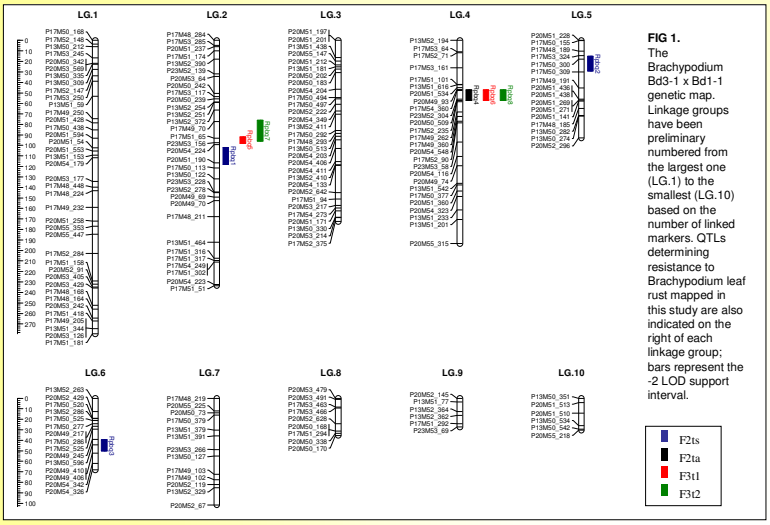

FIG 1. The Brachypodium Bd3-1 x Bd1-1 genetic map. Linkage groups have been preliminary numbered from the largest one (LG.1) to the smallest (LG.10) based on the number of linked markers. QTLs determining resistance to Brachypodium leaf rust mapped in this study are also indicated on the right of each linkage group; bars represent the -2 LOD support interval.

AFLP molecular markers in Brachypodium distachyon

To develop molecular markers for linkage mapping studies in Brachypodium, the application of amplified fragment length polymorphism (AFLP) markers was investigated. 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 *Pst*I +2 /*Mse*I +3 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; Hou et al. 2008). Both linkage maps will be based on F2 populations from Bd21 x Bd3-1. 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 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.

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 brachypodii* (collected by 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 (referred to as F2ts), and one at adult plant stage - 37 days after sowing (referred to as F2ta). 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).

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

Barley [a] probe	Barley chrom	Tentative annotation (TIGR)	BLAST to (Bd_draft_seq)	BLAST to (Bd_8x_release)	Fwd primer	Rev primer	Ta(°C)	Product length	#SNPs (Bd1-1 vs Bd3-1)	Mapped as
WBE102	4HS(bin_06.2)	similar to UPIO22632 (O22632) Nitrate-induced NOI protein, complete	super_11.547 plus	Bradi1g43430	5'-gaggcgggaattggagatgtag-3'	5'-gagaaaaaggctgggaatggac-3'	55	709 bp	7	CAPS(*Apo*I)
WBE104	4HL(bin_12.2)	UPIQ43487 (Q43487) Oxalate oxidase-like or germin-like protein (Germin-like 8), complete	super_3.1423	Bradi3g15220	5'-tctttctcctgctgctctactcg-3'	5'-tgccttggccaaaacatcatc-3'	58	759 bp	3	CAPS(*Fok*I)
WBE106	7HL(bin_11.2)	homologue to UPIQ8W428 (Q8W428) Chitinase 2, complete	super_4.2916	Bradi2g47190	5'-gtgcccaactgcctctgct-3'	5'-cgcgttgatgatgttggtga-3'	64	740 bp	1	CAPS(*Eag*I)
WBE111	2HL(bin_15.2)	similar to UPIO49866 (O49866) Peroxidase precursor , partial (96%)	super_0.76	Bradi5g27130	5'-gccctcgtcggaaccctacc-3'	5'-ccatggacgccgcgaaactg-3'	61	424 bp	0	-
WBE122	7HS(bin_07.1)	weakly similar to UPIQ9ZNZ5 (Q9ZNZ5) Peroxidase precursor, partial (50%)	super_12.816	Bradi1g77460	5'-gctgctccgcctctcttcc-3'	5'-taattacgcgcatgcttgtcca-3'	55	581 bp	1	CAPS(*Taq*I)
WBE214	4HL(bin_11.2)	weakly similar to UPIQ84VY8 (Q84VY8) At2g36290, partial (30%)	super_1.5473	Bradi1g77460	5'-cgatggtgtgaggatgacgag-3'	5'-cgcaaagtgaatggagaaaacc-3'	55	491 bp	1	CAPS(*Tru*1I)
WBE215	6HS(bin_06.1)	similar to UPIQ9M7S1 (Q9M7S1) 4-coumarate-CoA ligase 4CL3 , partial (95%)	super_6.2326	Bradi3g05750	5'-cacacggcgatcgacaggttac-3'	5'-ttgggaatggagtcggtgaaga-3'	58	648 bp	6	CAPS(*Acc*I)
WBE217	7HS(bin_03.1)	similar to GB|AAP88295.1|31711878|BT008856 At5g37370, partial (52%)	super_1.1621	Bradi1g51190	5'-cggtgcgggtacaggtgcta-3'	5'-tccgctcagtatgccaagtcaa-3'	55	555 bp	1	CAPS(*Bse*DI)
WBE218	3HL(bin_15.1)	similar to UPIQ6TXT3 (Q6TXT3) NADK1, partial (30%)	super_13.535	Bradi2g61240	5'-taaggtcgatcccgtgaagttg-3'	5'-gccgtcgagggattgagtct-3'	58	410 bp	1	CAPS(*Hin*fI)
WBE221	1HL(bin_14.1)	Barley mRNA for aspartic proteinase .	super_2.2772	Bradi2g16160	5'-ttgtccgcatcctaacttccta-3'	5'-acctgccaatatcccttctgag-3'	55	533 bp	1	Allele-specific PCR
WBE223	1HL(bin_09.2)	UPIQ94IN0 (Q94IN0) P-type ATPase (Fragment), complete	super_2.2052	Bradi2g21180	5'-caatggcgaaggaaatagagtg-3'	5'-tgctttcaggcttgttaccag-3'	55	543 bp	1	Allele-specific PCR
WBE226	2HS(bin_05.2)	similar to UPIQ9LLR3 (Q9LLR3) Vacuolar targeting receptor bp-80, partial (61%)	super_0.4002	Bradi1g17670	5'-catggggaagctacgagtgtg-3'	5'-ggccgaagaagataaccagag-3'	55	546 bp	1	CAPS(*Ase*I)
WBE228	5HL(bin_10.5)	Hordeum vulgare lipoxygenase 2 (LoxC) mRNA, complete cds	super_0.5153	Bradi1g09270	5'-tggccgtgtggacggctatt-3'	5'-gctgttggggatgcctctgg-3'	61	680 bp	1	CAPS(*Bsr*I)
WBE231	2HL(bin_08.1)	Hordeum vulgare mRNA for apoplastic invertase 1 (cwinw1 gene)	super_10.248	Bradi5g09420	5'-actgggtggcactgaagacg-3'	5'-aggtgccgacggtgtagtaatc-3'	61	561 bp	1	Allele-specific PCR
HvSAD	6HL(Bin4)	Hordeum vulgare gene for Scutellum and Aleurone DOF transcription factor	super_5.1288	Bradi3g51510	5'-aagacgtgccgccgctactg-3'	5'-ctcatggccacgcgtgttgtta-3'	64	748 bp	2	Allele-specific PCR

[a] Marcel TC, RK Varshney, M Barbieri, H Jafary,MJD de Kock, A Graner, RE Niks (2007). A high-density consensus map of barley to compare the distribution of QTLs for partial resistance to Puccinia hordei and of defence gene homologues. Theor Appl Genet 114:487-500.

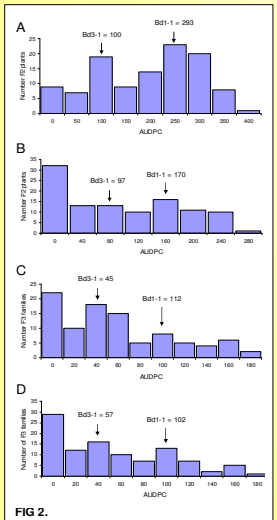

FIG 2. Frequency distribution of AUDPC values for F2ts (**A**), F2ta (**B**), F3t1 (**C**) and F3t2 (**D**). In C and D each score was based on the average of five plants derived from a single F2. Bd3-1 and Bd1-1 are indicated by arrows.

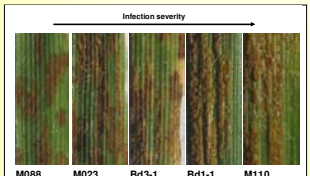

FIG 3. Examples of different reactions to leaf rust infection in Bd3-1, Bd1-1, and in some F3 plant taken as example. The name of the F3 is referred to the original F2 genotype.

QTLs for leaf rust resistance

Interval mapping and MQM mapping were performed on the data of the four different experiments by using the software 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*.

TABLE 2. Summary of QTLs detected in the Bd3-1 x Bd1-1 population using Multiple QTL Mapping (MQM) and a LOD threshold = 3.0

QTLs	Linkage group	Peak marker	LOD	R²(%) [a]	mu_a [b]	mu_c [b]	mu_b [b]	mu_d [b]
F2ts								
Rphq1	LG.2	P20M51_190	9.50	43.0	-	-	297.3	205.2
Rphq2	LG.5	P17M50_300	3.72	14.8	243.3	211.0	-	-
Rphq3	LG.6	P13M50_596	4.40	17.0	123.4	229.6	-	-
F2ta								
Rphq4	LG.4	P23M52_304	6.86	27.2	186.1	96.3	-	-
F3t1								
Rphq5	LG.2	P23M53_156	3.93	12.9	40.6	74.3	-	-
Rphq6	LG.4	P23M52_304	7.66	26.9	108.2	56.7	-	-
F3t2								
Rphq7	LG.2	P17M51_65	4.09	18.3	-	-	77.4	55.0
Rphq8	LG.4	P23M52_304	8.25	32.6	101.4	49.9	-	-

[a] R2(%) is the proportion of phenotypic variance explained by the QTLs
[b] "a" and "d" indicate the Bd3-1 alleles whereas "b" and "c" indicate the Bd1-1 alleles

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 conserved 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 they will provide anchor points for studies in comparative genomics. The newly developed map offers the opportunity to future identification of genomic regions underlying complex traits. This will allow to compare possibly the genetic architecture of already known leaf rust partial resistance QTL, between the model plant Brachypodium and the barley crop.

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