Quantitative Trait Loci for Head Bug Resistance in Sorghum

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Introduction

The mirid panicle-feeding bug (= head bug) *Eurystylus oldi* Poppius has recently become a key pest of sorghum *[Sorghum bicolor* (L.) Moench] in the savanna areas of the West and Central Africa (WCA) region, where this cereal is the most important food crop. Diallel analyses showed that additive gene effects could be very important in the inheritance of resistance to this pest, and suggested high heritability (Ratnadass et al. in press). A QTL mapping project aimed at completing these earlier inheritance studies was undertaken by CIRAD in Mali and France. This report presents its preliminary results.

Materials and Methods

A F_2 progeny derived from a cross between head-bug resistant sorghum cultivar Malisor 84-7 and head-bug susceptible S 34, was selected for mapping studies. The mapping population consisted of 217 plants. The F_2 phenotypic evaluation trial was sown at the Samanko research station of the ICRISAT-CIRAD Joint Sorghum Program, Mali, during the 1997 rainy season, in a plot consisting of ten 6-m rows, with an inter-row spacing of 0.75 m. In order to avoid selection, it was sown in continuous lines, and thinned two weeks after sowing, so as to have an inter-plant spacing of 0.20 m, with one plant per hill. The F_2 plot was bordered with one row of each of the parents on each side.

The head-cage technique used in earlier inheritance studies (Ratnadass et al. in press) was slightly modified so as to allow the artificial infestation of the upper part of the panicle with 10 head bug pairs, the protected bottom part serving as a control for parameters measured at grain maturity, namely thousand kernel weight (TKW) and germination rate (GER); head-bug damage was assessed visually on a 1-9 scale (where 1 = all grains fully developed with only a few head bug feeding punctures, and 9 = most grains undeveloped and barely visible outside the glumes due to head bug feeding and oviposition) (Ratnadass et al. in press) on the infested part of the panicle (NOTF2). The following criteria were used to account for head-bug damage:

- %TKW: relative difference in TKW between the protected and the infested parts of the panicle [100 x $(TKW_p - TKW_1)/ TKW_p$] calculated over the plants on which the parameter could be measured on several replications of 1000 grains, namely 136 plants out of 217.
- DGER: difference in germination rate between the protected and the infested parts of the panicle [$GER_p - GER_1$].

Seeds of the protected (and self-pollinated) bottom part of each of the 217 plants were sown in the greenhouse and the DNA was extracted from a bulk of five F_3 seedlings, representing each F_2 plant.

During the 1999 cropping season, seeds of F_4 plants derived from the remnant seeds of the protected (and self-pollinated) bottom part of 110 F_2 panicles of the 1997 trial, representing the F_3 families, were sown at Samanko in a randomized complete block design trial with two replications and one 5-m row per plot, with one row of each of the two parents every 10 rows. At grain maturity, panicles of the F_5 plants representing F_3 families were scored for head bug damage under natural infestation, using the 1-9 scale (NOTF3).

For building the sorghum genetic map, 345 RFLP probes, selected according to their localization on our reference map (Dufour et al. 1997, Boivin et al. 1999, Ventelon et al. 2001), were screened in combination with six restriction enzymes *(Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, *Hind*III and *Sst*I) for their ability to reveal polymorphism. Probes were obtained from various sources: rice (RZ prefix), oat (CDO prefix) and barley probes (BCD prefix) from Cornell University; rice probes (R and C prefixes) from the Rice Genomic Project; maize probes (UMC prefix from the University of Missouri, BNL prefix from the Brookhaven National Laboratory, CSU from California State University); pearl millet probes (PSM prefix) from the John Innes Centre; sugarcane probes (SSIR prefix) from CIRAD and sorghum probes (SbRPG prefix) produced in collaboration with RUSTICA PROGRAIN GENETIQUE and CIRAD. Forty-nine microsatellite markers developed by Brown et al. (1996) and Taramino et al. (1997) were also screened (m prefix on the map). The computer software Mapmaker 3.0 (Lander et al. 1987) was used for map construction. A LOD threshold of 5.0 and a maximum distance of 50 centiMorgans (cM) were used to establish linkage groups. Markers were ordered by multipoint analyses. Genetic distances were

estimated with the Haldane mapping faction. Linkage groups (LGs) were named on the basis of their homology with the LGs of our reference map.

QTLs were detected using the PlabQTL software package (Utz and Melchinger 1995). The analysis was performed using composite interval mapping (C1M) with a LOD value of 2.0, and the marker the closest to the QTL was used as a co-factor. A QTL was declared significant when the LOD value was above 3.0. This threshold was determined by the permutation method implemented in the QTL Cartographer software (Basten et al. 1997) with a global type-I error of 5%. A QTL was declared putative when the LOD value was between 2.0 and 3.0.

Results and Discussion

Among the 345 RFLP probes tested, 81 could reveal polymorphism between the two parents. In addition, 14 microsatellite markers gave usable amplification products. The genetic map based on the Malisor 84-7 x S 34 cross includes 92 distributed over 13 LGs, covering a total distance of 1160 cM. Three markers remained independent. Composition and order of markers in this map are globally consistent with those of the most recent composite map (which includes 416 RFLP loci distributed over 11 linkage groups, covering a genetic distance of 1495 cM: Ventelon et al. 2001; and unpublished data). However, genome coverage remains low in some regions, particularly for LGs A, B and J (Fig. 1).

Three significant and seven putative QTLs were detected (Table 1). The significant QTLs, which explained an important part of the phenotypic variation (R^2), were placed on the genetic map (Fig. 1). Concerning reduction in TKW, one QTL that accounted for 13% of the phenotypic variation was detected in the interval between markers SbRPG943 and RZ630 on LG C2. For this QTL, resistance is conditioned by me Malisor 84-7 allele and is dominant. A QTL for TKW was also found in the same region of LG C by Rami et al. (1998).

Two QTLs were detected for visual damage score under natural head bug infestation (NOTF3). These were on LG D, in the interval between markers RZ476 and SbRPG872, and on LG E, between markers SbRPG667 and CDO580. They explained 16 and 26% of the phenotypic variation for this trait, respectively. Resistance from the QTL on LG D is conditioned by me S 34 allele, whereas resistance from the QTL on LG E is provided by the Malisor 84-7 allele; in both cases, resistance is recessive. No significant QTLs were detected for NOTF2 and DGER but co-localization of two putative QTLs for these traits was observed in the interval between markers BNL 5.37 and SbRPG749 on LG G2 and in both cases, resistance is conditioned by the S 34 allele.

These results are partly in line with the recessive nature of head bug resistance suggested by earlier results based on visual damage assessment on the one hand, and me existence of resistance genes in me susceptible parent, suggested by transgressive segregations, on the other hand. Since mere was no correlation between NOTF2 and

Table 1. Genetic characteristics of significant and putative QTLs detected for the traits measured under natural and artificial infestation of sorghum progenies with head bugs.

	Cofactors	N	LG	Markers interval	Position	LOD	R^2	a	d	Direction
F_2 (natural infestation)										
NOTF2	*BNL5.37*	*1*	*G2*	*BNL5.37-SbRPG749*	*16.5*	2.9	*6.5*	*-0.44*	*0.64*	*PB*
%TKW	RZ630, BNL5.37	1	C2	SbRPG943-RZ630	132	4.19	13.2	10.31	-7.31	PA
DGER	*BNL5.37. RZ123. UMC29*	*2*	*G2*	*BNL5.37-SbRPG749*	*18.5*	*2.15*	*4.9*	*-6.62*	*6.28*	*PB*
			I	UMC29-SbRPG931	14	2.45	5.4	7.13	6.02	PA
F, (artificial infestation)										
NOTF3	SbRPG826. RZ476, CDO580,	6	*C2*	*CDO20-C223*	*16*	2.08	*10.4*	*-0.09*	*0.19*	*PB*
	UMC 139		*C2*	*RZ630-SbRPG826*	*144*	*2.5*	*11.9*	*-0.19*	*0.15*	*PB*
			D	RZ476-SbRPG872	36	3.65	16.2	-0.09	0.30	PB
			E	SbRPG667-CDO580	5.9	5.91	26.1	0.24	0.20	PA
			E	*RZ244b-SbRPG852*	*55.9*	*2.49*	*11.5*	*-0.19*	*0.13*	*PB*
			F	mAGB03-UMC139	76	2.44	11.2	0.13	0.18	PA

Italic lines indicate that the QTL was detected at a non significant level (LOD<3)
N: number of QTLs detected for each trait
LG: linkage group
Position: cumulative distance in cM from the first marker of the LG to the position of me LOD peak
R^2: percentage of me phenotypic variation explained by the QTL
a and d: additive and dominance effects as estimated by the program
Direction: origin of the allele contributing to the resistance: Parent A (Malisor 84-7) or Parent B (S 34)

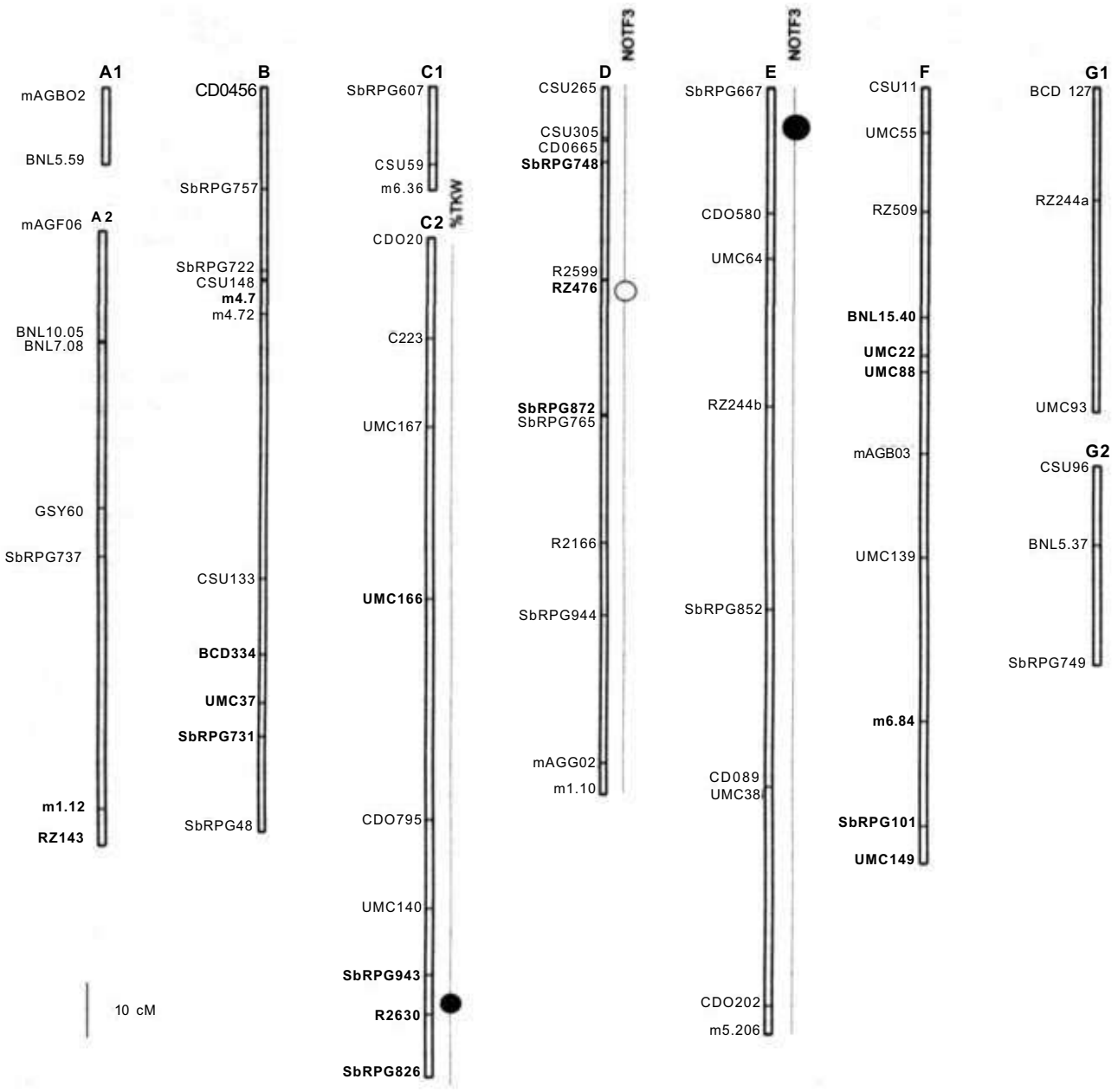

Figure 1. Genetic map and localization of significant QTLs for head-bug resistance in sorghum.
Each QTL detected at LOD score >3.0 is represented by circle located on its LOD peak (white circle resistance conditioned by the allele of the susceptible parent S 34; grey circle resistance conditioned by the allele of the resistant parent Malisor 84-7).

N0TF3, they also suggest the possible existence of different mechanisms of resistance under natural infestation on the one hand (namely under multiple-choice conditions and possibility of no coincidence between pest population peak and plant susceptible stage), and under artificial infestation on the other hand (under no-choice conditions, and no possibility of "escape", time- or space-wise).

Much remains to be done before application of marker-assisted selection for head-bug resistance can be envisaged. As a first step, a new phenotyping of families derived from this cross should be considered, with multilocational testing. However, the number of progenies that are still available for this testing (less than a hundred) might not suffice, and it could be relevant to start all over with a cross with parents more distant genetically so as to have more polymorphic markers, a more saturated map, and higher probability for QTL detection. Based on the pattern of segregation for head-bug resistance observed for some parameters in the F_2 progeny (e.g., TKW), the artificial infestation technique could be refined by reducing head-bug pressure on the infested part of the panicle. Other parameters usually highly correlated with damage score and considered as translating sorghum grain reaction to head bug attacks, could also be evaluated (e.g., percent flotation in a sodium nitrate solution).

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References

Hasten CJ, Weir BS and Zeng ZB. 1997. QTL cartographer: a reference manual and tutorial for QTL mapping. Raleigh, NC, USA: North Carolina State University, Department of Statistics.

Boivin K, Deu M, Rami JF, Trouche G and Hamon P. 1999. Towards a saturated sorghum map using RFLP and AFLP markers. Theoretical and Applied Genetics 98:320-328.

Brown SM, Hopkins MS, Mitchell SE, Senior ML, Wang TV, Duncan RR, Gonzalez-Candelas F and Kresovich S. 1996. Multiple methods for the identification of polymorphic simple sequence repeats (SSRs) in sorghum (*Sorghum bicolor* (L.) Moench). Theoretical and Applied Genetics 93:190-198.

Dufour P, Deu M, Grivet L, D'Hont A, Paulet F, Bouet A, Lanaud C, Glaszmann JC and Hamon P. 1997. Construction of a composite sorghum genotype map and comparison with sugarcane, a related complex polyploid. Theoretical and Applied Genetics 94:409-418.

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE and Newburg L. 1987. MAPMAKER, an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174-181.

Rami JF, Dufour P, Trouche G, Fliedel G, Mestres C, Davrieux F, Blanchard P and Hamon P. 1998. Quantitative trait loci for grain quality, productivity, morphological and agronomical triats in sorghum (*Sorghum bicolor* L. Moench). Theoretical and Applied Genetics 97:605-616.

Ratnadass A, Chantereau J, Coulibaly MF and Cilas C. (in press). Inheritance of resistance to the panicle-feeding pests *Eurystylus oldi* and the sorghum midge *Stenodiplosis sorghicola* in sorghum. Euphytica.

Taramino G, Tarchini R, Ferrario S, Lee M and Pe ME. 1997. Characterization of simple sequences repeats (SSRs) in *Sorghum bicolor.* Theoretical and Applied Genetics 95:66-72.

Utz HF and Melchinger A. 1995. PLABQTL A computer program to map QTL. Stuttgart, Germany: Institute of Plant Breeding, Seed Science, and Population Genetics, University of Hohenheim. ftp://ftpuni-hohenheimde/anonymou/pub/plabqu7

Ventelon M, Deu M, Garsmeur O, Doligez A, Ghesquiere A, Lorieux M, Rami JF, Glaszmann JC and Grivet L. 2001. A direct comparison between the genetic maps of sorghum and rice. Theoretical and Applied Genetics 102:379-386.