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Short Communication

Quantitative trait loci for head-bug resistance in sorghum

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QTLs were mapped in F2 progeny derived from a cross between the head-bug resistant sorghum cultivar Malisor 84- 7 and susceptible S 34. The phenotypic evaluation was conducted in Mali. The mapped population consisted of 217 F2 plants, with 345 homologous and heterologous RFLP probes and 49 microsatellite markers tested. Eighty-one RFLP markers revealed polymorphism between the two parents, and 14 microsatellite markers gave usable amplification products. A genetic map including 92 loci distributed over 13 linkage groups, and covering a total distance of 1160 cM was built. Three significant and seven putative QTLs were detected and placed on the map.

Key words: Head-bug, *Eurystylus oldi,* sorghum, resistance, RFLP, microsatellite, QTL.

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is the most important food crop in savanna areas of West and Central Africa (WCA). Mirid panicle-feeding bugs (= head-bugs), particularly *Eurystylus oldi* Poppius, have recently become major pests of sorghum (Ajayi et al., 2001) in the region. This is seriously threatening sorghum production because of the recent adoption of improved compact-headed cultivars of the caudatum race, which are better yielding but more susceptible to head-bug feeding and oviposition punctures than local loose-headed guinea landraces. These punctures result in severe quantitative and qualitative losses, including a higher incidence of grain mold (Ratnadass et al., 2003; Showemimo, 2003).

The use of resistant cultivars is often the most cost-effective means of controlling crop pests, particularly for small-scale farmers with limited access to inputs, so sorghum improvement programs in WCA have thus focused on the resistance breeding option. Earlier efforts by ICRISAT, CIRAD and NARS in the region led to the development of reliable screening techniques, which confirmed the high and stable resistance in compact-panicled sorghum cultivar Malisor 84-7. Diallel analyses revealed that additive gene effects could be very important in the inheritance of resistance to this pest, and suggested high heritability (Ratnadass et al., 2002). A QTL mapping project was undertaken by CIRAD in Mali and France from 1997-2000 to complement these earlier inheritance studies, particularly by identifying useful molecular markers linked to resistance genes.

MATERIALS AND METHODS

F2 progeny derived from a cross between the head-bug resistant sorghum cultivar Malisor 84-7 and head-bug susceptible S 34 was selected for mapping studies. The mapped population consisted of 217 plants. An F2 phenotypic evaluation trial was planted during the 1997 rainy season at the Samanko research station within the framework of the ICRISAT- CIRAD Joint Sorghum Program, Mali (Lat.8°25'N; Long.12°32'W) in a plot consisting of ten 6 m-rows with 0.75 m inter-row spacing. The sorghum was sown in

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continuous lines in order to avoid selection, and thinned 2 weeks after planting to achieve 0.20 inter-plant spacing, with one plant per hill. The F2 plot was bordered with a row of parent plants, i.e. a row of resistant plants on one side and a row of susceptible plants on the other side.

The head-cage technique used in earlier inheritance studies (Sharma et al., 1992; Ratnadass et al., 2002) was slightly modified to allow artificial infestation of the upper part of the panicle by 10 head-bug pairs, with 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., 2002) 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 infested parts of the panicle [100(TKW_P - TKW_I / TKW_P)] calculated for plants in which the parameter could be measured via several replications with 1000 grains, namely 136 plants out of 217.
DGER: difference in germination rate between the protected and infested parts of the panicle [GER_P - GER_I]

Seeds of the protected (and self -pollinated) bottom part of each of the 217 plants were planted in the glasshouse and DNA was extracted from a bulk of five F3 seedlings, representing each F2 plant.

During the 1999 cropping season, seeds of F4 plants derived from remnant seeds of the protected (and self-pollinated) bottom part of 110 F2 panicles from the 1997 trial representing the F3 families were planted in a randomized complete block design 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 F5 plants representing F3 families were scored for head-bug damage under natural infestation using the 1-9 scale (NOTF3).

To build 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 (Xpsm prefix) from the John Innes Centre; sugarcane probes (SSCIR 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 Mapmaker 3.0 software package (Lander et al. 1987) was used for map construction**.** An 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 function. 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 (CIM) (Zeng, 1994; Jansen and Stam, 1994) with an LOD value of 2.0, and the marker closest to the QTL was used as a co-factor. A QTL was considered significant when the LOD value was above 3.0. This threshold was determined by the permutation method implemented in the QTL Cartographer software program with a global type-I error of 5%. A QTL was considered putative when the LOD value was between 2.0 and 3.0.

RESULTS AND DISCUSSION

Among the 345 RFLP probes tested, 81 revealed 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 markers distributed over 13 LGs, covering a total distance of 1160 cM. Three markers remained independent. The composition and order of markers in this map are generally 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, the genome coverage is low in some regions, particularly for LGs A, B and J (Figure 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 (Figure 1). Concerning the reduction in TKW, one QTL which 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 determined by the Malisor 84-7 allele and is dominant. Interestingly, a QTL for TKW was also found in the same region of LG C by Rami et al. (1998).

Two QTLs were detected for 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 determined by the 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 two putative QTLs for these traits were co-localized in the interval between markers BNL 5.37 and SbRPG749 on LG G2 and resistance is determined by the S34 allele in both cases. These results are partly in line with the recessive nature of head-bug resistance suggested by earlier studies (Ratnadass et al., 2002; Aladele and Ezeaku, 2003), and by the existence of resistance genes in the susceptible parent, as indicated by transgressive segregations. Since there was no correlation between NOTF2 and NOTF3, the results also suggest the possible existence of different mechanisms of resistance under natural or artificial infestation conditions, as discussed elsewhere (Ratnadass et al., 2002).

However, much remains to be done before an application with respect to marker-assisted selection for head-bug resistance can be envisaged. As a first step, new phenotyping of families derived from this cross should be considered, with multilocational testing. Other

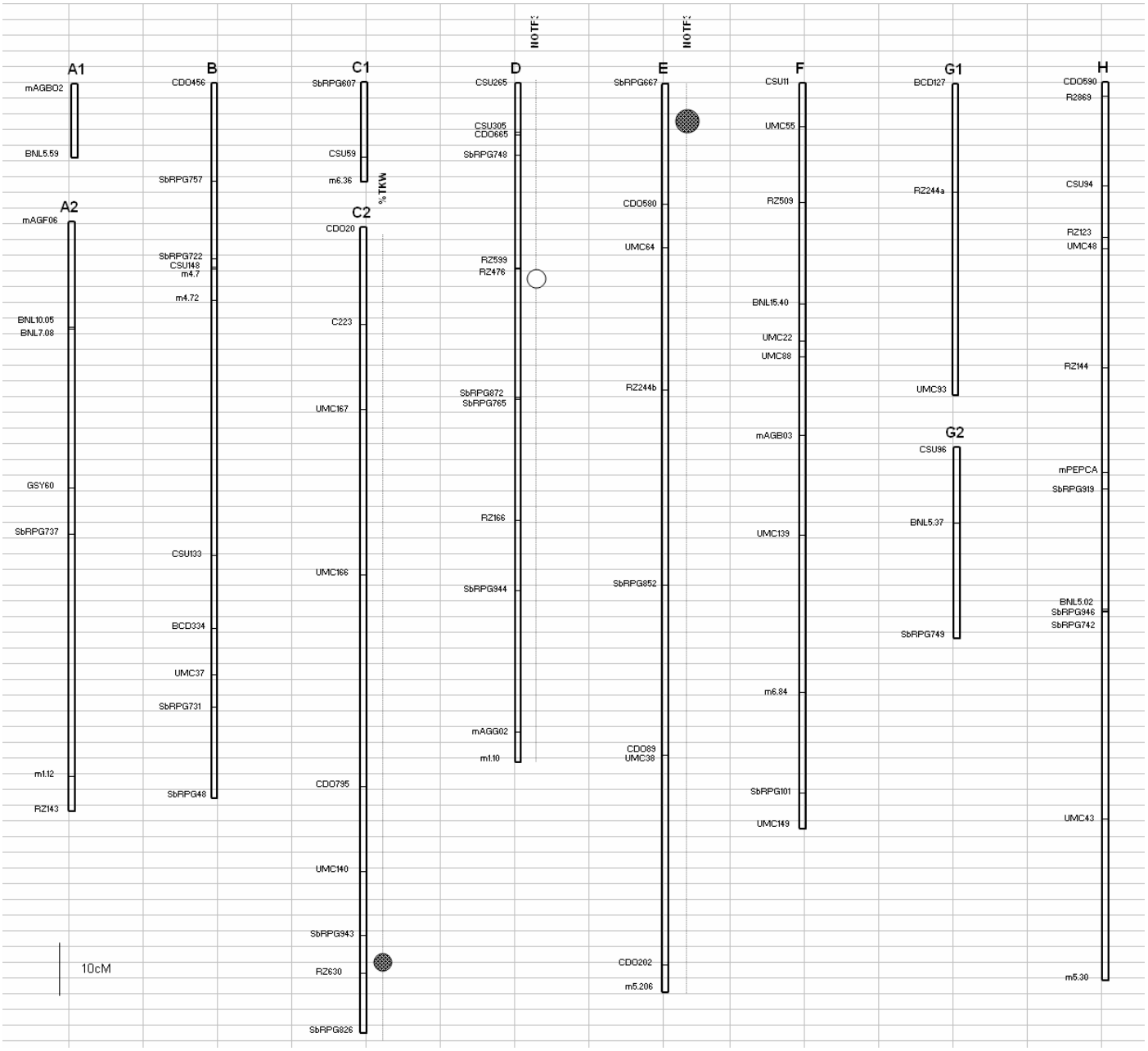

Figure 1. Genetic map and localization of significant QTLs for head-bug resistance in sorghum. Each QTL detected at LOD score >3.0 is represented by a circle located on its LOD peak. The colour of the circle indicates the origin of the parental allele contributing to the resistance for this QTL (white circle: resistance determined by the allele of the susceptible parent S34; grey circle: resistance determined by the allele of the resistant parent Malisor 84-7).

Table 1. Genetic characteristics of significant and putative QTLs detected for the parameters measured under natural and artificial infestation.

Parameters	**Cofactors**	**N**	**LG**	**Markers interval**	**Position**	**LOD**	**R^2**	**a**	**D**	**Direction**
F2 (natural infestation)										
NOT	*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*

Table 1.contd.

F3 (artificial infestation)										
NOT	SbRPG826, RZ476, CDO580,	6	*C2*	*CDO20-C223*	*16*	*2,08*	*10,4*	*-0,09*	*0,19*	*PB*
	UMC139		*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 the LOD peak
R^2: percentage of the phenotypic variation explained by the QTL
a and d: additive and dominance effects as estimated by the programme
Direction: origin of the allele contributing to the resistance: Parent A (Malisor 84-7) or Parent B (S34).

parameters usually highly correlated with damage score and considered as translating sorghum grain reaction to head-bug attacks, could also be evaluated (e.g. per cent flottation in a sodium nitrate solution).

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