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Genomic regions influencing resistance to the parasitic weed *Striga hermonthica* in two recombinant inbred populations of sorghum

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Abstract Molecular markers for resistance of sorghum to the hemi-parasitic weed *Striga hermonthica* were mapped in two recombinant inbred populations (RIP-1, -2) of $F_{3:5}$ lines developed from the crosses IS9830 × E36-1 (1) and N13 × E36-1 (2). The resistant parental lines were IS9830 and N13; the former is characterized by a low stimulation of striga seed germination, the latter by “mechanical” resistance. The genetic maps of RIP-1 and RIP-2 spanned

1,498 cM and 1,599 cM, respectively, with 137 and 157 markers distributed over 11 linkage groups. To evaluate striga resistance, we divided each RIP into set 1 (116 lines tested in 1997) and set 2 (110 lines evaluated in 1998). Field trials were conducted in five environments per year in Mali and Kenya. Heritability estimates for area under the striga number progress curve (ASNPC) in sets 1 and 2 were respectively 0.66 and 0.74 in RIP-1 and 0.81 and 0.82 in RIP-2. Across sites, composite interval mapping detected 11 QTL (quantitative trait loci) and nine QTL in sets 1 and 2 of RIP-1, explaining 77% and 80% of the genetic variance for ASNPC, respectively. The most significant RIP-1 QTL corresponded to the major-gene locus *lgs* (low stimulation of striga seed germination) in linkage group I. In RIP-2, 11 QTL and nine QTL explained 79% and 82% of the genetic variance for ASNPC in sets 1 and 2, respectively. Five QTL were common to both sets of each RIP, with the resistance alleles deriving from IS9830 or N13. Since their effects were validated across environments, years and independent RIP samples, these QTL are excellent candidates for marker-assisted selection.

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Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the staple food for millions of people in the semi-arid tropics. Mean grain yield ranges from 800 kg ha⁻¹ in Africa to 3,400 kg ha⁻¹ in the Americas (FAO 2000). Major growing constraints to sorghum production in sub-Saharan Africa are the hemi-parasitic weeds *Striga hermonthica* (Del.) Benth. and *S. asiatica* (L.) Kuntze (Scrophulariaceae). These weeds attach to and penetrate host roots, extracting water and nutrients. Grain yield losses due to infestation of cereals in West Africa average 24% (Sauerborn 1991), but crop loss can be total when striga attack is compounded by drought. The two species also parasitize maize (*Zea mays*), pearl millet (*Pennisetum glaucum*), rice (*Oryza sativa*), fonio (*Digitaria exilis*) and teff (*Eragrostis tef*). A third species, *S. gesnerioides*

(Willd.) Vatke, attacks legumes. The shifting of cultivation to continuous cropping, concomitant loss in soil fertility and the frequent cultivation of susceptible crops are responsible for increased striga infestation (Kroschel 1999).

Reducing the striga seed bank in the soil is fundamental to striga control. This may be achieved by destroying seed in the soil, reducing striga reproduction and preventing striga seed dissemination from infested to uninfested fields (Berner et al. 1994; Hess and Haussmann 1999). Striga-resistant sorghum varieties may reduce striga reproduction and the number of viable seeds in the soil, but they are often not locally adapted and agronomically inferior. Resistant, farmer-preferred varieties could be an important component of integrated striga management, but progress in breeding for striga resistance has been slow due to a limited knowledge of the genetics of striga resistance and the difficulty in evaluating resistance in the field. Field screening is hampered by heterogeneous natural infestations, high variability of soils and large environmental effects on striga emergence. However, improved field testing methodologies have recently been developed (Haussmann et al. 2000a) and validated (Omanya et al. 2004).

Several mechanisms of striga resistance exist in sorghum and its wild relatives (Ejeta et al. 2000; Gurney et al. 2002; Heller and Wegmann 2000). A current research program at Purdue University is designed to dissect the infection cycle into key events (for example, germination, haustorium formation, striga penetration and development), develop physiological assays for each event, identify specific resistance sources in breeding materials or wild relatives using these assays and, finally, to combine the resistance factors in productive genetic backgrounds (Ejeta and Butler 1993; Ejeta et al. 2000; Kapran et al. 2002; Mohamed et al. 2003). However, these are long-term research goals, and the successful development of the program requires a great deal of time and energy.

The approach of quantitative trait locus (QTL) mapping has the appealing advantage that genomic regions affecting a complex, quantitative trait like striga field resistance can be identified without prior knowledge of their function. Resistance QTL, once identified, can be transferred into adapted cultivars using marker-assisted selection. This could greatly accelerate breeding progress, also because striga seed is quarantined, confining tests to areas where striga is endemic, and because some striga resistance genes are recessive (Ramaiah et al. 1990; Vogler et al. 1996; Haussmann et al. 2001a) and difficult to select in conventional backcross schemes. A major objective of our collaborative project was therefore to identify DNA markers for striga field resistance in sorghum.

Materials and methods

Genetic materials

Two sorghum [*Sorghum bicolor* (L.) Moench] recombinant inbred populations (RIP-1, -2) of 226 F_{3:5} lines each were developed from crosses: IS9830 × E36-1 (1) and N13 × E36-1 (2). Line IS9830 is a Sudanese feterita belonging to race caudatum and striga-resistant due to its production of low amounts of striga germination stimulants, resulting in the germination of fewer striga seeds (Haussmann et al. 2000c). In contrast, line N13, an Indian durra sorghum, stimulates abundant striga seed germination but forms a mechanical barrier to parasite penetration (Maiti et al. 1984). Line E36-1, a guinea/caudatum hybrid of Ethiopian origin, possesses drought-tolerance and high yield potential but is striga-susceptible. The crosses were selfed, and 226 F₂ plants per population were advanced by single-seed descent to the F₄ generation. The F₄ lines were multiplied by selfing 40 panicles per line, and the resulting F₅ seed was bulked. These F₃ -plant derived bulks in F₅ are designated F_{3:5} lines since each F_{3:5} line represents the genetic makeup of a single F₃ plant. One F_{3:5} line in RIP-1 proved to be an off-type and was removed from the final dataset.

Marker analysis

Marker analyses of the two mapping populations were performed with bulked DNA from 20 plants per F_{3:5} line. RIP-1 was genotyped for 235 marker loci [131 codominant and 20 dominant amplified fragment length polymorphisms (AFLPs), 67 simple sequence repeats (SSRs), 17 restriction (R)FLPs] and RIP-2 for 297 marker loci (122 codominant and 75 dominant AFLPs, 80 SSRs, 20 RFLPs). The AFLP analyses were carried out by Keygene (The Netherlands) using ten *EcoRI/MseI* primer combinations and the procedure described by Vos et al. (1995). RFLP data were produced by Biogenetic Services (Brookings, S.D.) according to standard procedures. The *bnl* (Burr and Burr 1991), *csu* (Gardiner et al. 1993), *php* (Beavis and Grant 1991), *umc* (Coe et al. 1990) and *isu* (cDNA) maize probes were selected from sorghum genetic maps published by Boivin et al. (1999), Dufour et al. (1997) and Pereira et al. (1994). The majority of SSR analyses were done by Celera AgGen (Davis, Calif.) using SSRs developed by Bhattaramakki et al. (2000), Brown et al. (1996), Kong et al. (2000) and Taramino et al. (1997). PCR conditions followed recommendations from the aforementioned authors. Data were generated on an ABI PRISM 377 DNA sequencer (Perkin Elmer Biosystems, Foster City, Calif.). Additional SSR analyses were performed in the ICRISAT MS Swaminathan Applied Genomics Laboratory in India using SSR markers (*Xcup*) developed by Schloss et al. (2002) and SSR markers (*Xisp*) elaborated specifically for the project by Celera AgGen. The latter markers are not publicly available. SSR markers were amplified using PCR, and the amplified

products were multiplexed post-PCR using fluorescently labeled primers. Data was generated on an ABI 3700 DNA sequencer (Perkin Elmer Biosystems). The raw data were extracted using GENESCAN and GENOTYPER software (Perkin Elmer Biosystems).

Phenotypic marker scores for the major low-germination stimulant (*Igs*) locus were obtained from the evaluation of RIP-1 for stimulation of *Striga hermonthica* seed germination using three geographic sources of striga (Hausmann et al. 2001a) and the agar-gel assay developed by Hess et al. (1992). In this assay about 4,500 preconditioned striga seeds were dispersed in 0.7% water-agar in a petri dish. The root of a 24-h-old sorghum seedling was inserted into the solidifying agar near one edge of the plate with the root tip pointing across the plate. After 5 days of incubation at 28°C in the dark, the maximal germination distance (distance between the host root and the most distantly germinated striga seed) was recorded under a dissecting microscope. Maximal germination distances above 10 mm refer to high-stimulant genotypes, while distances below 5 mm clearly point to low-stimulant sorghum entries. Striga sources from Samanko (Mali), Bengou (Niger) and Kibos (Kenya) were employed in different trials with six replications per trial—i.e., six F₅ plants of each F_{3:5} line were evaluated per striga source. Bimodal frequency distributions and a chi-square test of the ratio of low- to high-stimulant plants indicated the presence of one major recessive gene for low stimulation of striga seed germination (Hausmann et al. 2001a). On the basis of this distinction between low- and high-stimulant classes across the two West African striga sources—where the difference was clearer than with Kenyan striga—we developed a dominant genotypic score (*Igs_dom*) with “A” lines representing the low-

stimulant class and “C” lines the moderate- to high-stimulant class. In addition, a detailed analysis of the individual reactions of the six plants tested using striga seed from Samanko and Bengou resulted in two codominant scores (*Igs_Bgu* and *Igs_Sko*) where “A” denoted non-segregating, presumably homozygous low-stimulant F_{3:5} lines, “H” segregating F_{3:5} lines (i.e. the original F₃ plant was heterozygous for the *Igs* locus) and “B” non-segregating, presumably homozygous high-stimulant F_{3:5} lines.

Map construction

Genetic maps were constructed using JOINMAP 3.0 (Van Ooijen and Voorrips 2001). The allocation of markers to linkage groups was mostly stable for a wide range of LOD grouping thresholds (from ≤ 4.0 to ≥ 7.0) in both RIPs. Markers that were attributed to a linkage group at a LOD grouping threshold of less than 4.5 were only included when it was known from other published maps that they belong to this group. In final map construction, data points with a LOD score below 0.1 or a recombination fraction (REC) above 0.49 were ignored. These non-stringent thresholds were used to retain distantly linked markers in the dataset. Some linkage groups reacted to the parameter settings (LOD and REC) with changes in marker order. More stringent parameter settings were not applied because they resulted in marker orders inconsistent with the order of “top-linked markers” determined by JOINMAP or with the map of Bhatramakki et al. (2000). Recombination frequencies were converted to centiMorgans (cM) with Haldane’s mapping function (Haldane 1919). Linkage groups were named according to common SSR

Table 1 Details of field experiments

Site characteristics and experimental details	Kenya			Mali	
	Kibos LR ^a	Alupe LR	Alupe SR ^a	Samanko R ^a	Cinzana R
Altitude (m)	1,214	1,189		358	285
Latitude	00°04’S	00°29’N		12°31’N	13°15’N
Longitude	34°48’E	34°08’E		08°04’W	05°57’W
Soil type	Retroentric planosol; sandy loam	Orthic ferrosol, part. petroferic phase with orthic acrisols		Tropical ferruginous soil, leached; sandy loam	Ferrug. soil, leached, rich in sesquioxid; sandy loam
Set 1 of each RIP (1997) ^b					
Sowing dates	29 March	28 March	27 August	17 July	17 July
Plot size/striga counting area (m ²)	3.15	2.94	2.94	4.48	4.48
Total rainfall (mm)	541	671	1285	750	633
Set 2 of each RIP (1998)					
Sowing dates	08 April	08 April	10/21 September ^c	01 July	16 July
Plot size/striga counting area (m ²)	3.825	4.275	4.275	4.48	4.48
Total rainfall (mm)	657	784	584	982	683

^aLR, Long rains (March to July); SR, short rains (September to January); R, rains (June to September)

^bSet 1, 116 F_{3:5} lines tested in 1997; set 2, independent sample of 110 F_{3:5} lines tested in 1998

^cSowing dates for RIP-1/RIP-2

markers selected from the map of Bhatramakki et al. (2000). Following map construction using all marker data, clustering markers (mainly AFLPs) were removed from the datasets and new maps computed for each RIP in order to obtain a more uniform marker distribution on each linkage group. These maps are presented here and were used in QTL analysis. The goodness-of-fit of the constructed maps, reflecting the discrepancy between final recombination frequencies in the map and those apparent from individual marker data pairs, was expressed as a chi-square value and computed according to Stam and van Ooijen (1995).

Evaluation of striga resistance

To evaluate striga resistance we divided both RIPs into two sets. Sets 1 and 2 of each RIP consisted of 116 $F_{3:5}$ lines tested in 1997 and 110 lines evaluated in 1998, respectively. The $F_{3:5}$ lines were evaluated together with checks in a 11×11 hexa-lattice design. Field trials were conducted at Samanko and Cinzana (Mali) during the rainy season. In Kenya, where rainfall is bimodal, trials were conducted at Kibos and Alupe in the Long Rains and at Alupe in the Short Rains (Table 1). Additional details on the experimental sites, sowing dates and rainfall data have been summarized by Omany et al. (2004). The improved field testing methodology proposed by Haussmann et al. (2000a) was used in all trials. It consisted of: artificial infestation of on-station trials; six replicates; a lattice design; a two-row plot separated from the neighboring plot by one empty row; the use of novel resistance indices (Omany et al. 2004). Plot size ranged from 2.94 m² to 4.48 m², depending on the location. The striga resistance trait reported here is area under the striga number progress curve (ASNPC). It was computed from four or five counts of emerged striga plants performed at 2-week intervals during the growing season through adapting the formula for calculating area under the disease progress curve (AUDPC; Shaner and Finney 1977). The ASNPC accounts for both intensity and speed of the epidemic (Haussmann et al. 2000a). It was selected for QTL mapping because of its good differentiation at all test sites and high heritability estimates in all four sets of material.

Statistical analysis

Phenotypic data of the two sets per RIP were analyzed using the computer program PLABSTAT (Utz 1998), as described by Omany et al. (2004). Combined within-year across-location analyses were computed with lattice-adjusted entry means from each individual environment. Test locations were regarded as fixed effects, while all other effects were considered as normally distributed random effects. Because of heterogeneity of error variances, a conservative *F*-test of the entry × environment interaction variance was made with $t-1$ and n' degrees of freedom, where t designates the

number of entries and n' the number of error degrees of freedom in the experiment with the highest error variance (Cochran and Cox 1957). Broad-sense heritabilities were estimated on an entry mean basis (Hallauer and Miranda 1981) with 90% confidence intervals (CI) (Knapp et al. 1985).

QTL were detected by composite interval mapping (Jansen and Stam 1994; Zeng 1994) using the software PLABQTL ver. 1.1 (Utz and Melchinger 2000). A purely additive model was employed. Individual cofactor sets were selected via stepwise regression for each trait and data set by PLABQTL and subsequently extended or reduced by maximizing the R^2 value. Final selection was for the model that minimized Akaike's information criterion (AIC), a measure of the goodness-of-fit of the regression model (Jansen 1993). Empirical threshold values for the LOD scores were determined by computing 2,500 permutations (Churchill and Dirge 1994), using the "permute" command of the PLABQTL software. This was repeated twice, first with the cofactor set that was finally used for QTL identification in each set of material, and secondly with the automatic cofactor selection of the PLABQTL software. Following a recommendation of HF Utz (personal communication), the average threshold values from the two permutation runs (fixed vs. automatic cofactor selection) are used here. For the experiment-wise error rate of $\alpha=0.25$, the critical LOD scores to indicate QTL significance were therefore 2.78 and 2.90 for sets 1 and 2 of RIP-1 and 3.10 for both sets of RIP-2. QTL with a LOD score between 2.5 and these thresholds are also reported and may be regarded as suggestive. QTL positions were determined at the local maxima of the LOD-curve plot in the region under consideration. Adjacent QTL on the same chromosome were considered to be different when the curve had a minimum between peaks of at least 1 LOD unit below either peak and when the support intervals were non-overlapping. The proportion of phenotypic variance explained by a single QTL was obtained by the square of the partial correlation coefficient (R^2). Estimates of the additive effects of the QTL were computed by fitting a model including all putative QTL for a given trait. QTL × environment interaction was analyzed in each set of material as described by Utz and Melchinger (2000), and the proportion of genetic variance explained by the QTL was adjusted for QTL × environment interactions to avoid overestimation.

In the present study, QTL estimated in set 1 of each RIP (phenotypic data from 1997) were validated with set 2 of the same RIP (independent samples of $F_{3:5}$ lines tested in 1998). The validation therefore refers to genotype sampling and year effects. In addition, a cross validation was performed within each dataset. In this type of validation, QTL positions and effects are estimated with 80% of the data, and a validation is performed with the remaining 20%. This can be done five times such that each 20% of the data is used in validation (Utz and Melchinger 2000). In the cross-validation summary, both the list of QTL detected in the calibration runs and the percentage ranges

of phenotypic and genetic variance explained by the QTL in the calibration and validation runs reflect genotype sampling effects in the materials and provide additional information with respect to the reliability of the QTL.

Results

Genetic maps

The genetic map of RIP-1 consisted of 137 markers distributed over 11 linkage groups and spanning 1,498 cM (Fig. 1a). Linkage group B was split into two. The following markers were allocated to their linkage groups at a LOD grouping threshold below 4.5: *isp230* (grouped to linkage group D up to LOD=3.0) and *isp258* (mapped to linkage group J up to LOD=4.0). Because *isp230* equally mapped to linkage group D in RIP-2 and *isp258* to linkage group J in a different population (unpublished data), they were included in the RIP-1 map. The *lgs* loci were mapped to linkage group I only up to a LOD grouping threshold of 4.5. In addition, linkage group I reacted to the “LOD” and “REC” parameter settings during map computation with changes in marker order. The positioning of the *lgs* loci should therefore be regarded as preliminary. Markers *txp63*, *txp96* and *isp200* were unlinked in RIP-1. Markers *php20626* and *txp273* were mapped to linkage group H at LOD 3.0 but caused a gap of more than 60 cM. Because this would have made QTL mapping problematic, these markers were excluded from the final map. Large gaps (>25 cM) occurred in linkage groups B (two gaps on B1, one gap on B2 plus missing links between B1 and B2), D (1 gap), E (2), F (1), G (2), I (2) and J (1). A high goodness-of-fit (mean $\chi^2 < 1.7$, frequently <1.0, in the map construction of all linkage groups) indicated high reliability of the linkage map.

The genetic map of RIP-2 consisted of 157 markers distributed over 11 linkage groups and spanning a total of 1,599 cM (Fig. 1b). Linkage group D was split in two. The upper part of linkage group B was linked only up to a LOD grouping threshold of 4.5. On the other hand, the remainder of linkage group B joined linkage group J, and the two groups could only be separated at a LOD grouping threshold of 10.5. Large gaps (>25 cM) occurred in

linkage groups B (1 gap), C (1), D1 (1), E (4), G (1) and H (2). A high goodness-of-fit (mean $\chi^2 < 1.7$ in the map construction of all linkage groups) indicated high reliability of the linkage map.

The two maps had 54 markers in common (Fig. 1). The conserved order of common markers also reflected the high reliability of the two maps. The only exceptions were *isp344* and *txp295* whose closely linked positions were interchanged on linkage group E in RIP-1 compared to RIP-2.

Phenotypic data for area under the striga number progress curve

Striga infestation was medium to high at all sites, with environmental means for the number of emerged striga plants per square meter at 90 days after sowing ranging from 16 to 91 in RIP-1 and from 15 to 56 in RIP-2 (for details see Omany et al. 2004). Location means for ASNPC ranged from 7 to 32 in RIP-1 and from 9 to 22 in RIP-2 (Omany et al. 2004). Repeatabilities at the individual test sites for ASNPC were moderate or high and highly significant in both RIPs, ranging from 45% to 82% in RIP-1 and from 72% to 93% in RIP-2 (Omany et al. 2004). The combined analyses of variance across locations within years indicated highly significant genetic variances ($P=0.01$) among the tested $F_{3;5}$ lines for ASNPC in both RIPs (Table 2). $F_{3;5}$ line \times location interaction variances were also highly significant ($P=0.01$) in both RIPs and years. Estimated heritabilities for ASNPC were moderate to high, with RIP-2 generally revealing slightly higher heritability estimates than RIP-1. Frequency distributions of the $F_{3;5}$ lines for mean ASNPC were unimodal, indicating quantitative variation for striga resistance in both RIPs (Fig. 2). Transgressive segregation was apparent for susceptibility to striga, but none of the $F_{3;5}$ lines was more resistant than the resistant parental lines IS9830 or N13 [the difference between the most resistant $F_{3;5}$ line of set 2 in RIP-1 and parental line IS9830 (Fig. 2) was not significant at $P=0.05$].

Table 2 Estimated variance components (\pm standard error) due to $F_{3;5}$ lines (G, genotype), $F_{3;5}$ line \times location interaction [G \times E (environment)] and error and corresponding heritabilities (h^2) with

their 95% confidence interval (CI) of sets 1 and 2 of RIP-1 and -2 for area under the striga number progress curve (ASNPC), combined across five field experiments in Kenya and Mali

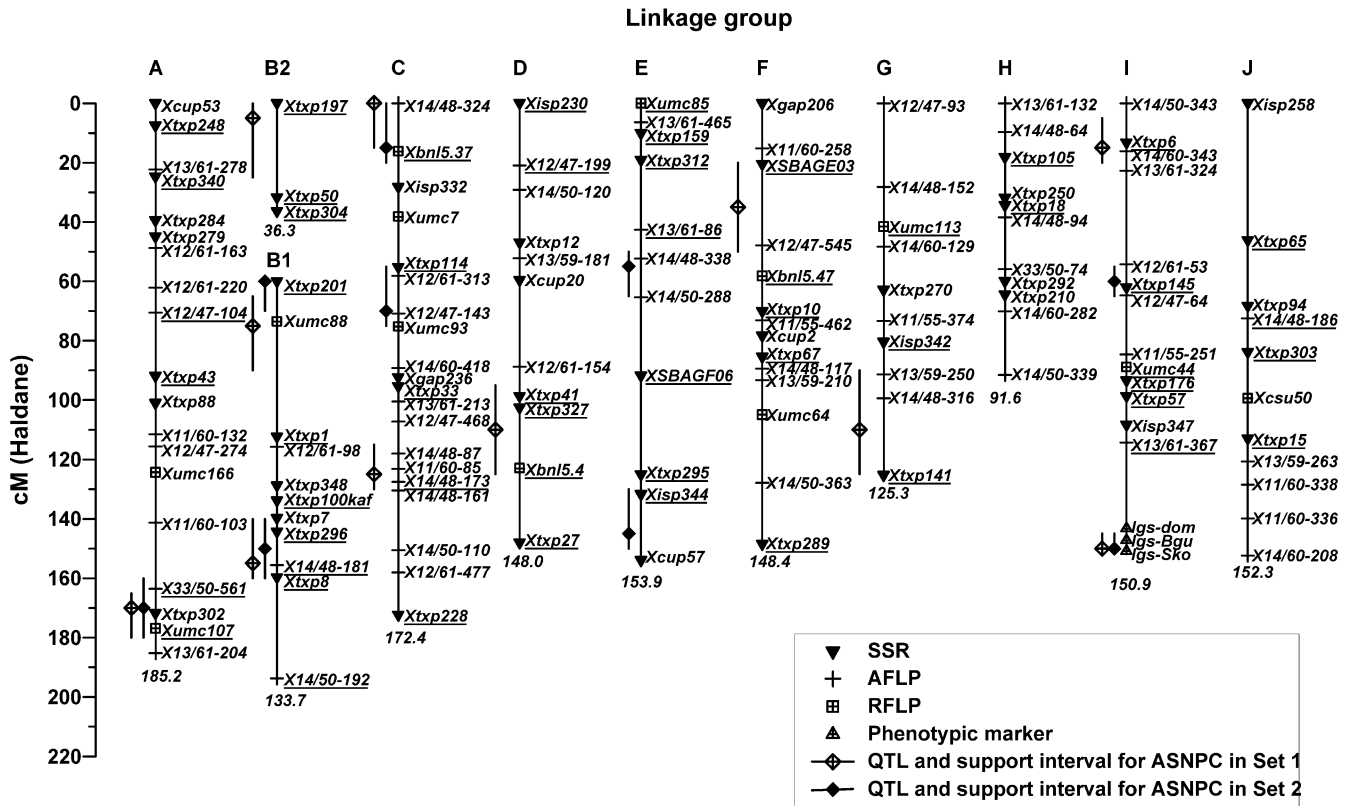
RIP ^a	Set ^b	Variance component for ASNPC due to:			h^2	95% CI
		G	G \times E	Error		
1	1	4.35** \pm 0.88	2.36** \pm 0.80	9.11 \pm 0.25	0.66	(0.53; 0.74)
	2	11.63** \pm 2.12	8.33** \pm 1.41	11.90 \pm 0.33	0.74	(0.65; 0.81)
2	1	26.70** \pm 4.32	16.49** \pm 2.07	14.39 \pm 0.40	0.81	(0.75; 0.86)
	2	22.70** \pm 3.71	9.46** \pm 1.70	14.88 \pm 0.41	0.82	(0.76; 0.87)

^aRIP-1 was derived from the cross IS9830 \times E36-1; RIP-2 was derived from the cross N13 \times E36-1

^bSet 1, 116 $F_{3;5}$ lines tested in 1997; set 2, independent sample of 110 $F_{3;5}$ lines tested in 1998

**Significant at the 0.01 probability level

a) RIP 1 (IS9830 x E36-1)



b) RIP 2 (N13 x E36-1)

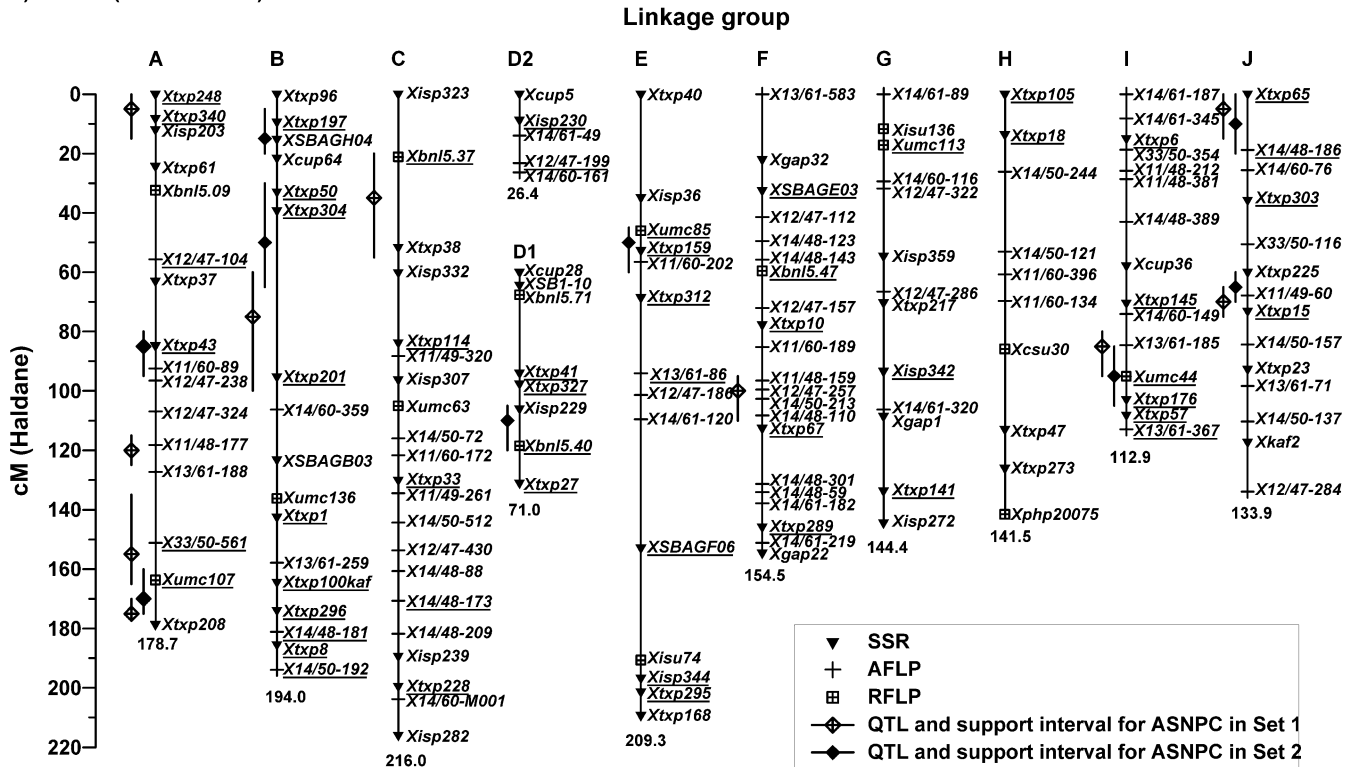


Fig. 1a, b Genetic linkage maps of RIP-1 (a) and RIP-2 (b) including the positions of putative QTL for area under the striga number progress curve (ASNPC). AFLP, RFLP and SSR marker names are indicated at the right of each linkage group. Underlined markers are common to both RIPs. Linkage groups are named according to Bhattaramakki et al. (2000)

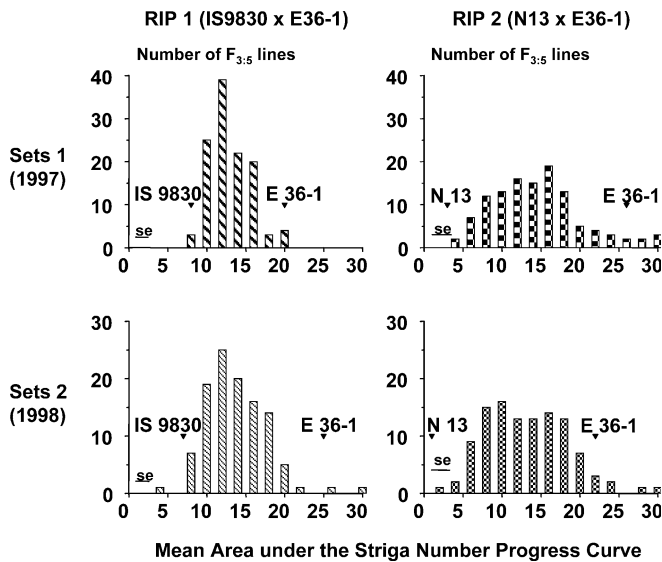


Fig. 2 Frequency distributions of the $F_{3:5}$ lines of RIP-1 (IS9830 \times E36-1) and RIP-2 (N13 \times E36-1) for mean area under the striga number progress curve (ASNPC), averaged over five test

QTL for area under the striga number progress curve

In the QTL analysis across locations with RIP-1 (IS9830 \times E36-1), composite interval mapping detected 11 and 9 putative QTL in sets 1 and 2, explaining 77% and 86% of the genetic variance, respectively (Fig. 1a, Table 3). Five QTL on linkage groups A, B1 (two QTL), C and I were common to both sets, with the resistance alleles being contributed by the resistant parent IS9830. The shared QTL on linkage group I at 150 cM corresponded to the *lgs* locus and explained the largest proportion of phenotypic variance (highest R^2 values) in both sets. None of the resistance QTL alleles that were expressed in both sets was derived from the susceptible parent line E36-1. Cross-validation results reflected some genotype sampling effects in RIP-1 since several QTL appeared only in one to three of the calibration runs. The mean percentages of genetic variance explained in the calibration and validation runs of the cross-validation procedure were slightly higher in set 2 (71.6% and 58.4%, respectively) than in set 1 (56.5% and 21.0%, respectively) of RIP-1.

Table 3 Linkage group (LG), position and support interval for a LOD decrease of 1.0 (sup. int.), flanking marker interval, LOD score, partial coefficient of determination (R^2) and estimated additive effect (a_i) of the QTL detected in the two sets of RIP-1

(IS9830 \times E36-1) for area under the striga number progress curve across five locations in Kenya and Mali. The values in bold indicate those QTL that are common to the two sets of one RIP

Set ^a	QTL	LG ^b	Position in centiMorgans (sup. int)	Flanking marker interval	LOD ^c	R^2	a^d	C^e
1	1	A	170 (165–180)	<i>33/50-561; tpx 302</i>	2.9	10.7	0.7	1
	2	B1	15 (5–30)	<i>umc88; tpx 1</i>	2.7	10.3	0.7	2
	3	B1	95 (80–100)	<i>tpx296; 14/48-181</i>	2.5	9.5	0.6	1
	4	B2	5 (0–25)	<i>tpx197; tpx 050</i>	3.0	11.6	0.8	3
	5	C	0 (0–15)	<i>14/48-324; bnl 5.37</i>	3.4	12.7	0.7	3
	6	C	125 (115–130)	<i>11/60-85; 14/48-173</i>	3.0	11.2	0.7	4
	7	D	110 (95–125)	<i>tpx327; bnl5.40</i>	2.7	10.2	0.8	3
	8	F	35 (20–50)	<i>sbase03; 12/47-545</i>	3.1	11.7	0.9	4
	9	G	110 (90–125)	<i>14/48-316; tpx141</i>	2.9	10.9	–0.8	2
	10	I	15 (5–20)	<i>tpx6; 14/60-343</i>	4.4	16.0	0.9	4
	11	I	150 (145–150)	<i>lgs_Bgu; lgs_Sko</i>	6.4	22.5	1.1	5
Total percentage genetic variance explained ^f						76.5		
2	1	A	170 (160–180)	<i>33/50-561; tpx302</i>	4.9	18.8	1.4	4
	2	B1	0 (0–10)	<i>tpx201; umc88</i>	5.8	21.9	1.3	5
	3	B1	90 (80–100)	<i>tpx296; 14/48-181</i>	5.0	18.9	1.4	5
	4	C	15 (0–20)	<i>14/48-324; bnl5.37</i>	3.5	14.1	1.1	3
	5	C	70 (55–75)	<i>12/61-313; 12/47-143</i>	2.9	11.3	1.0	3
	6	E	55 (50–65)	<i>14/48-338; 14/50-288</i>	2.8	11.1	1.1	2
	7	E	145 (130–150)	<i>isp 344; cup057</i>	3.6	15.7	–1.4	4
	8	I	60 (55–65)	<i>12/61-53; tpx145</i>	4.2	16.2	–1.2	5
	9	I	150 (145–150)	<i>lgs_Bgu; lgs_Sko</i>	12.7	41.5	2.4	5
	Total percentage genetic variance explained ^f						86.1	

^aSet 1, 116 $F_{3:5}$ lines tested in 1997; set 2, independent sample of 110 $F_{3:5}$ lines tested in 1998

^bLinkage groups are named according to Bhatramakki et al. (2000)

^cEmpirical LOD threshold values for QTL significance were 2.78 and 2.90 in sets 1 and 2, respectively ($\alpha=0.25$); QTL with LOD scores below these thresholds are suggestive

^dAdditive effect: half of the difference between the two homozygotes. Positive values, resistance allele was contributed by resistance donor IS9830; negative values, resistance allele was derived from striga-susceptible parent E36-1

^eNumber of calibration runs in which the respective QTL was detected during the fivefold cross-validation

^fValue corrected for QTL \times environment interaction

In RIP-2 (N13 × E36-1), 11 and 9 QTL were detected for ASNPC in sets 1 and 2 that explained 79% and 82% of the genetic variance, respectively (Fig. 1b, Table 4). Five QTL, on linkage groups A, B, I and J (two QTL) were common to both sets. For each of these stably expressed QTL, the resistance allele came from the resistant parent N13. The QTL on linkage group B fell in a large—larger than 50 cM—gap of the genetic map of RIP 2; its position could therefore not be determined precisely and should be regarded as preliminary. The majority of QTL were detected in four or even all five calibration runs of the cross-validation. In addition, the high mean percentages of genetic variance explained in the calibration (76.6% and 78.6% in sets 1 and 2, respectively) and validation runs (61.6% and 71.5% in the two sets) indicated good reliability of the detected QTL.

The QTL × location interaction within years was highly significant ($P=0.01$) in both sets of both RIPs and was reflected by deviating QTL effects at the different locations within each set of $F_{3;5}$ lines (Table 5). In RIP-1, some QTL effects even ranged from negative to positive values—i.e. the same QTL allele contributed to resistance

at one location but to susceptibility at another. Among the QTL showing strong QTL × location interaction was the QTL on linkage group I at 150 cM—i.e. the *lgs* locus—in set 1 of RIP-1. In RIP-2, where resistance is not due to the low-stimulant character, none of the resistance QTL alleles showed such a strong interaction with the environment.

Discussion

Genetic maps of the two populations

Powerful QTL mapping requires a genetic map with good genome coverage. Due to the low genetic polymorphism between the parental lines used in this investigation, it took several years to develop the present genetic maps of the two mapping populations. The recent addition of selected *txp*, *cup* and *isp* SSR markers and the phenotypic marker score for the low-stimulant character (in RIP-1 only) extended the maps of RIP-1 and RIP-2 by 207 cM and 145 cM, respectively, compared to an earlier version (Hausmann et al. 2002a, b). However, compared to the

Table 4 Linkage group (LG), position and support interval for a LOD decrease of 1.0 (sup. int.), flanking marker interval, LOD score, partial coefficient of determination (R^2) and estimated additive effect (a_i) of the QTL detected in the two sets of RIP-2

Set ^a	QTL	LG ^b	Position in centiMorgans (sup. int)	Flanking marker interval	LOD ^c	R^2	a^d	C^e
1	1	A	5 (0–10)	<i>txp248</i> ; <i>txp340</i>	3.3	12.5	–1.4	4
	2	A	120 (110–125)	<i>11/48-177</i> ; <i>13/61-188</i>	4.0	14.6	–1.7	5
	3	A	165 (160–175)	<i>33/50-561</i> ; <i>umc107</i>	4.5	16.4	2.0	4
	4	A	185 (180–185)	<i>umc107</i> ; <i>txp208</i>	6.7	24.2	3.0	5
	5	B	80 (60–110)	<i>txp304</i> ; <i>txp201</i>	4.5	16.5	2.1	5
	6	C	30 (10–55)	<i>bn15.37</i> ; <i>txp38</i>	2.5	9.5	1.2	2
	7	F	100 (95–110)	<i>12/47-257</i> ; <i>14/50-213</i>	3.8	14.0	1.3	4
	8	H	125 (110–135)	<i>txp47</i> ; <i>txp273</i>	2.6	9.9	–1.1	2
	9	I	90 (85–100)	<i>13/61-185</i> ; <i>umc44</i>	9.0	30.0	2.3	5
	10	J	5 (0–15)	<i>txp65</i> ; <i>14/48-186</i>	5.3	18.9	1.7	5
	11	J	70 (65–75)	<i>11/49-60</i> ; <i>txp15</i>	4.2	15.4	1.7	5
	Total percentage genetic variance explained ^f					78.7		
2	1	A	85 (80–95)	<i>txp43</i> ; <i>11/60-89</i>	7.4	26.5	–2.0	5
	2	A	180 (170–185)	<i>umc107</i> ; <i>txp208</i>	5.1	21.0	2.6	5
	3	B	15 (5–20)	<i>txp197</i> ; <i>sbagh04</i>	2.9	11.2	–1.6	2
	4	B	50 (30–65)	<i>txp304</i> ; <i>txp201</i>	6.0	22.1	3.2	3
	5	D1	50 (45–60)	<i>isp229</i> ; <i>bn15.40</i>	12.4	40.5	2.9	5
	6	E	50 (45–60)	<i>umc85</i> ; <i>txp159</i>	4.5	17.3	1.6	5
	7	I	100 (85–110)	<i>umc44</i> ; <i>txp176</i>	3.8	14.6	1.3	4
	8	J	10 (0–20)	<i>txp65</i> ; <i>14/48-186</i>	3.1	12.2	1.5	3
	9	J	65 (60–70)	<i>txp225</i> ; <i>11/49-60</i>	8.0	28.5	2.3	5
	Total percentage genetic variance explained ^f					81.5		

^aSet 1, 116 $F_{3;5}$ lines tested in 1997; set 2, independent sample of 110 $F_{3;5}$ lines tested in 1998

^bLinkage groups are named according to Bhatramakki et al. (2000)

^cEmpirical LOD threshold value for QTL significance was 3.1 for both sets ($\alpha=0.25$); QTL with LOD scores below this threshold are suggestive

^dAdditive effect: half of the difference between the two homozygotes. Positive values, resistance allele contributed by resistance donor N13; negative values, resistance allele derived from striga-susceptible parent E36-1

^eNumber of calibration runs in which the respective QTL was detected during the fivefold cross-validation

^fValue corrected for QTL × environment interaction

(N13 × E36-1) for area under the striga number progress curve across five locations in Kenya and Mali. The values in bold indicate those QTL that are common to the two sets of one RIP

Table 5 Estimated QTL effects at the individual locations for those QTL that were consistently identified in both sets of F_{3.5} lines of each RIP

RIP ^a	Set ^b	QTL		Additive effect ^d at test location:					
		LG ^c	Position in centiMorgans	Samanko	Cinzana	Alupe LR	Kibos LR	Alupe SR	
1	1	A	165–180	–0.2	0.2	1.9	0.5	1.3	
		B1	5–35	0.4	0.4	1.3	0.6	0.6	
		B1	80–100	0.6	0.5	0.5	0.9	1.1	
		C	0–25	0.2	0.4	0.9	0.6	1.8	
		I	150–155	1.4	2.3	1.3	0.7	–0.2	
	2	A	160–180	0.1	2.7	1.5	0.8	1.2	
		B1	0–10	0.3	1.4	2.9	1.0	0.7	
		B1	80–100	0.6	1.1	1.3	1.2	0.6	
		C	0–20	0.6	3.3	0.6	0.5	1.5	
		I	150–155	0.5	6.1	3.1	1.5	0.8	
	2	1	A	180–185	1.3	1.5	6.6	1.1	3.2
			B	60–110	0.9	2.0	5.5	2.0	3.1
			I	85–100	2.0	2.7	3.8	1.7	1.7
			J	0–15	0.6	1.6	2.8	1.1	1.4
			J	65–75	1.1	1.9	5.0	1.5	2.9
2	2	A	170–185	0.5	2.6	4.4	4.0	1.4	
		B	30–65	0.8	4.2	3.9	4.8	2.1	
		I	85–110	1.0	2.7	1.1	1.1	0.5	
		J	0–20	0.4	2.0	0.9	1.5	0.6	
		J	60–70	0.8	2.8	2.4	2.2	1.3	

^aRIPs-1 and -2 were derived from the crosses IS9830 × E36-1 and N13 × E36-1, respectively

^bSets 1 and 2 were tested in 1997 and 1998, respectively

^cLinkage groups are named according to Bhatramakki et al. (2000)

^dAdditive effect: half of the difference between the two homozygotes. Positive values, Resistance allele contributed by the resistance donor (IS9830 or N13); negative values, resistance allele derived from the striga-susceptible parent E36-1

map of Bhatramakki et al. (2000), about 130 cM in RIP-1 and 95 cM in RIP-2 are still not represented. In RIP-1 the missing regions include 10 cM each at the lower ends of linkage groups A and C, a 30-cM section linking the two parts of linkage group B, 25 cM at the top of linkage group E, 15 cM at the lower end of linkage group G and an approximately 40-cM link to *txp273* in linkage group H. In RIP-2 the missing regions include 25 cM at the lower end of linkage group A, a 35-cM missing link between the two groups of linkage group D, 20 cM at the upper end of linkage group H and 15 cM at the lower end of linkage group I.

Position of the low-germination stimulant locus in RIP-1

Positioning of the *lgs* locus in linkage group I should be regarded as preliminary since phenotypic marker scores for the *lgs* locus in RIP-1 were linked to the linkage group only up to a LOD grouping threshold of 4.5. In addition, this linkage group reacted with marker order changes to the LOD and REC parameter settings during the computation of the map. The positioning of the *lgs* locus therefore needs to be confirmed through the addition of more markers to the genetic map of RIP-1, especially to linkage group I. However, positioning the *lgs* locus on linkage group I was supported by (unpublished data) QTL analysis of the maximal germination distance data obtained in the agar-gel assay: when the phenotypic marker score from the RIP-1 map was excluded, there was only one QTL which consistently affected maximal germination distance in both sets of material, and this QTL equally mapped to linkage group I.

Genomic regions affecting striga resistance under field conditions

The present study clearly demonstrates that striga field resistance is a complex, quantitative trait influenced by multiple genomic regions, depending on test site and year. In fact, all of the linkage groups were involved in the expression of the trait when both RIPs, sets/years and all test environments were considered simultaneously. This is not surprising since a variety of interactions between striga and its host determine the reproductive success of the parasite. Following seed shed and a period of dormancy, striga seed will germinate only when exposed to moisture and a favorable temperature for several days (preconditioning). A germination stimulant is required, which is usually exuded by the roots of the host. Subsequent development (haustoria formation, attachment, penetration and growth) of the parasite also requires signals and resource commitment from the host plant (Ejeta et al. 2000; Haussmann et al. 2000a). These interactions provide opportunities to the host to resist the parasite. The following resistance mechanisms have been proposed in addition to low production of the germination stimulant (Ejeta and Butler 1993; Haussmann et al. 2000a; Heller and Wegmann 2000; Mohamed et al. 2003):

- mechanical barriers (e.g. lignification of cell walls);
- inhibition of germ-tube exoenzymes by root exudates;
- phytoalexin synthesis;
- post-attachment hypersensitive reactions or incompatibility;
- antibiosis (e.g. reduced striga growth through unfavorable phytohormone supply by the host);

- insensitivity to striga toxin (e.g., maintenance of stomatal opening and photosynthetic efficiency);
- avoidance through root growth habit (e.g., fewer roots in the upper 15–20 cm).

In each of the two RIPs, five QTL were stably expressed across test sites, years and independent mapping population samples (sets). These QTL may affect key events in the striga infection cycle. A proven example is the QTL on linkage group I at 150 cM in RIP-1. This QTL corresponds to the *lgs* locus for low stimulation of striga seed germination and explained 23% and 40% of the phenotypic variance for ASNPC in sets 1 and 2 of RIP-1. The fact that four additional stable QTL were identified in both sets of RIP-1 indicates that the resistant parent of this population, line IS9830, possesses other resistance mechanisms in addition to the low stimulation of striga seed germination. Line N13, the resistant parent of RIP-2, appears also to possess several resistance mechanisms as multiple QTL were identified in both sets of this population as well. A detailed physiological study using near-isogenic lines carrying different QTL alleles in a homozygous stage would be required to identify the precise resistance mechanisms encoded by resistance QTL alleles of the two parental lines.

The main reason for our success in mapping striga resistance QTL lies in the careful conduct of field evaluations. The employment of six replications, lattice design, specific plot layout, a comprehensive resistance measure (ASNPC) and multilocational tests produced the high heritabilities essential for QTL mapping. In addition, the testing of the two independent mapping population samples (the two sets per RIP) in different years allowed for comprehensive QTL validation and identification of the most reliable QTL. Those QTL that were stable across ten test environments (five sites \times 2 years) and the two independent mapping population sets should therefore be biological realities. Flanking markers to these stable QTL could be candidates for marker-assisted transfer of striga resistance alleles into adapted, farmer-preferred local cultivars.

QTL \times environment interaction

The highly significant QTL \times environment interaction in both RIPs was reflected by the variable effects of QTL alleles at individual test locations. While in RIP-1 (IS9830 \times E36-1) two of the five QTL identified in both sets showed changes in the sign of their effects, all five QTL identified in both sets of RIP-2 (N13 \times E36-1) contributed to resistance at all sites in both years. Resistance derived from N13 therefore may be more stable than resistance derived from IS9830. In field trials across diverse geographic regions, the total QTL \times environment interaction variance comprises interaction effects between QTL alleles and locations (soil, climate), between QTL and putative striga biotypes as well as the three-way interaction. *Striga hermonthica*, due to its outcrossing behavior, possesses high genetic variability

and can adapt to new hosts (Ejeta and Butler 1993; Koyama 2000). Striga populations at different locations could differ in virulence level or specificity as a result of adaptation to different host plant resistance mechanisms. Thus, the factor striga population most likely contributed to the QTL \times environment interaction. In order to determine the contribution of a geographical striga population to the total QTL \times environment interaction, sorghum materials would have to be evaluated at each location against striga populations of different geographic origin. For reasons of quarantine, this clearly cannot be done in Africa.

The *lgs* locus contributed the most to resistance at Cinzana (Mali) and had a slightly negative effect at Alupe (Kenya) in the 1997 Short Rains. These observations correspond to the coefficients of phenotypic correlation between maximal germination distance in the agar-gel assay and ASNPC at the individual test sites observed in the same genetic materials. The correlation coefficients were highest at Cinzana (0.63 and 0.51 in sets 1 and 2, respectively) and close to zero (and insignificant) for Alupe in the Short Rains (Omanya et al. 2004). Variable correlations between maximal germination distance and resistance under field conditions were also observed in diallel crosses of sorghum (Hausmann et al. 2000b). It therefore appears that the contribution of the *lgs* locus to striga field resistance depends on the materials under investigation as well as on the test environments, as previously suggested by Vasudeva Rao (1984). K. Wegmann (personal communication) hypothesized that abiotic stress followed by ethylene production by microorganisms in the soil could be one possible mechanism rendering the low-stimulant character less effective in certain site/season combinations. Ethylene has been shown to efficiently stimulate striga seed germination (Bebawi and Eplee 1986; Eplee 1975).

The reported QTL \times environment interactions confirm the pertinence of recommendations that the testing of breeding materials should occur at various locations with different striga populations in order to identify genotypes with stable resistance (Hausmann et al. 2000a, b, 2001b; Ramaiah 1987).

Mapping population size, power of QTL detection and importance of QTL validation

Theoretically, the simultaneous evaluation of all 226 F_{3:5} lines per mapping population should have resulted in a greater likelihood of QTL detection due to larger population size (Beavis 1998). However, from a practical standpoint it would have been extremely difficult, if not impossible, to simultaneously evaluate 226 genotypes for striga resistance under field conditions with the same precision as was achieved with half that number. In addition, our experimental design permitted QTL validation across genotype samples (sets of each RIP) and years. The limited number of common QTL across locations, years and independent mapping population sets for ASNPC under field conditions (five in each RIP) under-

lines the tremendous importance of QTL validation across genotype samples and environments. QTL studies based on a small mapping population and only one test environment should therefore be interpreted with great caution. QTL validation can consist of:

- QTL analysis in an independent mapping population sample as done in the present study and by Bohn et al. (2001);
- QTL analysis in a second mapping population derived from the same donor parent but a different recipient (e.g. Haussmann et al. 2002b);
- Statistical cross validation procedures as offered by the PLABQTL software, most useful in large mapping populations ($n > 200$) (Bohn et al. 2001; Utz and Melchinger 2000; Utz et al. 2000); or
- Verification of QTL effects in near isogenic lines.

Utz et al. (2000) conducted extensive maize experiments investigating different agronomic traits using cross-validation and validation with independent genotypic samples. They observed highly variable numbers of QTL in calibration and validation runs as well as high bias and sampling error of the estimated proportion of genotypic variance explained by QTL. The authors concluded that it is extremely difficult to obtain reliable, unbiased QTL estimates for complex quantitative traits. Huge population sizes ($n > 500$) and large numbers of environments would have to be employed, which would surpass the financial and logistic capacities of a breeding program (Bohn et al. 2001). Our use of QTL validation techniques addresses these concerns and should help realize gains from future marker-assisted selection.

Conclusion and outlook

The present experiments have yielded reliable QTL for striga field resistance. In a follow-up project, markers flanking the stable resistance QTL of N13 will be used in a marker-assisted foreground selection to improve local sorghum cultivars from Eritrea, Kenya, Mali and the Sudan. The present results may also facilitate the detection of striga resistance genes in syntenic genomic regions of maize, pearl millet, or rice.

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