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Quantitative trait loci for grain quality, productivity, morphological and agronomical traits in sorghum (*Sorghum bicolor* L. Moench)

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Abstract Quantitative trait loci (QTLs) for grain quality, yield components and other traits were investigated in two Sorghum caudatum × guinea recombinant inbred line (RIL) populations. A total of 16 traits were evaluated (plant height, panicle length, panicle compactness, number of kernels/panicle, thousand-kernel weight, kernel weight/panicle, threshing percentage, dehulling yield, kernel flouriness, kernel friability, kernel hardness, amylose content, protein content, lipid content, germination rate and molds during germination and after harvest) and related to two 113- and 100-point base genetic maps using simple (SIM) and composite (CIM) interval mapping. The number, effects and relative position of QTLs detected in both populations were generally in agreement with the distributions, heritabilities and correlations among traits. Several chromosomal segments markedly affected multiple traits and were suspected of harbouring major genes. The positions of these QTLs are discussed in relation to previously reported studies on sorghum and other grasses. Many QTLs, depending on their relative effects and position, could be used as targets for marker-assisted selection and provide an opportunity for accelerating breeding programmes.

Key words Sorghum · Grain quality · Yield · Quantitative trait loci · Marker-assisted selection

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Introduction

Sorghum (*Sorghum bicolor* L. Moench) is a major cereal for human consumption in Asian and African countries, being well adapted to areas with insufficient rainfall for growing other grasses such as maize, rice or wheat. In Africa, sorghum has been cultivated for more than five thousand years and represents the main carbohydrate source for at least 20 million people.

In Africa, most sorghum breeding programs have been focused on agronomic performance in order to insure food security. However, in African traditional agricultural systems, grain quality is also an essential requirement for the development and use of improved cultivars. Many grain quality criteria can be identified because of the wide range of sorghum culinary dishes prepared by different African ethnic groups. This multiplicity and the difficulty of designing rapid simple methods to evaluate these factors have delayed the development of improved cultivars with acceptable grain quality. Most types of cultivated sorghum in West Africa are therefore landraces with adequate grain technological characteristics and regular productivity that are associated with a tolerance of low soil fertility, environmental instability and biotic stress.

The influence of different physical and biochemical sorghum grain characteristics on the quality of traditional food has been established (Bello et al. 1990; Fliedel 1995; Taylor et al. 1997). Endosperm texture, i.e. the relative proportion of corneous to floury endosperm, has been described as being one of the most important characteristics affecting sorghum food quality (Rooney and Murty 1982; Rooney et al. 1986). Corneous grains should be suitable for thick porridges, intermediate ones for unfermented breads, boiled rice-like products, malting and brewing and floury ones for fermented breads. Bello et al. (1990) stated that corneous endosperm sorghum generally produced good quality tô (a West African traditional thick porridge) with a firm texture, and softer endosperm sorghum produced poor quality tô with a softer texture. However, Fliedel (1995),

who developed a laboratory test to screen advanced breeding material for tô quality, found no correlation between tô firmness and grain vitreousness; instead, she showed that varieties with high amylose content, high starch solubility and good dehulling properties gave a good quality tô. High-molecular-weight (HMW) polymers of amylose and amylopectin have been observed in the starch of corneous endosperm (Cagampamg et al. 1985). These HMW polymers enhance the solubilization and retrogradation of starch and thus provide firmer food gel textures (Bello et al. 1995). The dehulling behaviour of sorghum grains depends on grain hardness or vitreousness (Reichert et al. 1981; Fliedel et al. 1989). Hard and corneous grains give a higher dehulling yield and produce flours with lower lipid, ash and fiber contents and thus better quality tô (Bello et al. 1990; Fliedel 1995). However, little is known about the genetic control of grain quality parameters and their relationships with the main components of sorghum productivity.

The development of DNA markers and statistical treatments for linkage mapping and quantitative trait loci (QTLs) analysis represent powerful tools for quantitative trait breeding. Breeders have access to new strategies through the dissection of complex traits into simple genetic factors tagged by molecular markers. Marker-assisted selection (MAS) is based upon the localization and estimation of the effects of QTLs in segregating populations. Several breeding strategies can be used to further exploit these simple Mendelian factors (Tanksley and Nelson 1996). In sorghum, recent results illustrate the usefulness of genome mapping in dissecting complex traits (Pereira and Lee 1995; Pereira et al. 1995; Lin et al. 1995).

Following upon our recent progress in the generation of a composite sorghum genome map using two sorghum recombinant inbred line (RIL) populations (Dufour et al. 1996), in the study presented here we highlight the localization of QTLs involved in sorghum grain quality and in several morphological and agronomical traits. and This should enhance opportunities for applying marker-assisted selection strategies for sorghum breeding in African countries

Materials and methods

Plant material

Two populations of recombinant inbred lines (RILs) were obtained at the Saria experimental station (Burkina Faso) by a modified single-seed descent method from the IS 2807 genotype (from the ICRISAT collection) used as female crossed with the 379 and 249 genotypes (from the CIRAD collection) used as males. The two populations comprised of 110 and 90 individuals, respectively, are hereafter called RIL379 and RIL249.

IS2807 is a caudatum landrace from Zimbabwe with medium productivity under Saria conditions and poor technological characteristics for West African traditional preparations. 249 is a guinea landrace from Burkina Faso with good technological characteristics and well-adapted to Sudanian cropping systems. 379 is a guinea landrace belonging to the South African group (Deu et al. 1994). It is earlier than 249 but has a lower productivity and a poorer quality performance.

The RIL379 population was grown in 1993 at the F_5 generation, in a Fisher block experimental design with three replications, while the RIL249 population was grown in 1995 at the F_7 generation, in a 9×10-lattice design with three replications.

Trait evaluation

The following morphological traits were monitored in the field before harvest: plant height (PH) measured in centimeters from the soil surface to the top of the panicle, panicle compactness (PC) evaluated on a scale of 1 (loose) to 4 (compact) and panicle length (PL) measured in centimeters from the base to the top of the panicle.

Yield components such as number of kernels per panicle (NK), kernel weight per panicle (KW), thousand-kernel weight (TK) and threshing percentage (TP, weight of grain as a percentage of total panicle weight) were measured after harvest.

For technological trait evaluation, several panicles of each RIL were selfed and bulked to represent the current generation. Grain samples were cleaned to remove glumes, dust and broken grains using a densimetric column. Flouriness (FL) was assessed by visual observation of the proportion of vitreous and floury endosperm on grain cross-sections. The samples were scored from 1 to 5, with 1 corresponding to a totally vitreous endosperm and 5 to a totally floury endosperm. Dehulling yield (DY) was measured using a tangential abrasive dehulling device (Reichert et al. 1981). Thirty grams of grains was dehulled for 5 min. Dehulling yield is the percentage of dehulled grain to whole-grain weight.

For the RIL379 population, 20 g of dehulled grain was milled in a cyclotec using a 0.5-mm screen. Amylose content (AM) was measured by differential scanning calorimetry (Mestres et al. 1996) using 10 mg of flour. Protein content (PR) was evaluated by total nitrogen determination using the Kjeldahl method with 1 g of flour.

For the RIL249 population, grain technological characteristics were evaluated by near-infrared reflectance spectroscopy (NIRS). The whole grain was milled using a cyclotec with a 0.5-mm screen, and spectral acquisition was performed on whole flour using spin cells and a NIRSystem 6500 (Pertsorp Analytical). NIR calibrations were developed for chemical characteristics based on differential scanning calorimetry, Soxhlet ether extraction (Osagie and Kates 1984) from 5 g of flour and Kjeldahl analysis were the referential methods for amylose, lipid (LI) and protein contents, respectively. Kernel friability (KF) was determined by NIRS calibration based on the particle size index method (PSI) (Fliedel et al. 1989). Twenty grams of grain was milled in a Falling Number KT 30 mill and sifted for 1 min through a 250-μm sieve using an air jet sifter (Alpine 200LS). PSI was determined as the proportion of milled grain passing through the sieve. It is inversely proportional to grain hardness. Kernel hardness (KH) was also estimated by the method of the American Association of Cereal Chemists (1989) developed for wheat according to the formula:

$$KH = 1475 \times \log(1/R_{2230}) - 1099 \times \log(1/R_{1680})$$

where R_{1680} and R_{2230} are the reflectance at 1680 and 2230 nm, respectively.

Three additional traits were also recorded: the germination rate (GR), measured as the percentage of germinated grains in a petri dish, and mold sensitivity, evaluated both (1) directly on threshed grains after harvest (MR) and ranked from 1 (no mold) to 5 (more than 50% of molded grain area) and (2) after 6 days of germination in a petri dish (MG) as the percentage of molded grains. Some of these traits were evaluated for only one population. For example, PC, GR and MG were only evaluated for RIL379 and KF, KH, LI and TP only for RIL249. To facilitate further notation, we include with each trait abbreviation the population number on which it was evaluated (e.g. LI_{249} means lipid content evaluated on RIL249).

Molecular data

The genotypic data used for the QTL detection were those recently obtained for the RIL249 and RIL379 populations (Dufour et al. 1997). The first map (RIL249) was built using 115 maize probes, 8 sugarcane probes, four cloned genes (ADH1, PEPC3, PEPC4 and PTA71) and one morphological marker (B2/b2) covering a total map distance of 878 cM. The second map (RIL379) was constructed using 126 maize probes, 19 sugarcane probes, four cloned genes (ADH1, PEPC3, PEPC4 and PTA71) and two morphological markers (P/p, B2/b2) for a total map distance of 977 cM. These sorghum maps correspond to about 90% of the saturated map of Pereira et al. (1995) and represent a large part of the sorghum genome. The average genetic distances between markers are 8 and 7 cM respectively, for RIL249 and RIL379. Comparisons between these maps and those of Pereira et al. (1995) and Lin et al. (1995) show that linkage group K is a part of linkage group C (Dufour 1996). In this paper, these two linkage groups are thus considered as parts of one linkage group named K/C.

Statistical analysis

Distribution analyses were performed for each trait using the univariate procedure of the SAS program (SAS institute 1988). Normality of distributions was tested ($P < 5.10^{-3}$) with the W test described by Shapiro and Wilk (1965). Phenotypic correlations were estimated and tested for significance ($P < 5.10^{-4}$) using the Corr procedure.

Analysis of variance (ANOVA) was performed for each population to estimate genetic and environmental effects using the GLM procedure according to two models:

$P_{ij} = \mu + G_i + r_j + e_{ij}$
for the Fisher block design (RIL379 population)

$P_{ijk} = \mu + G_i + r_j + b_{jk} + e_{ijk}$
for the lattice design (RIL249 population)

where $P_{ij(k)}$ is the phenotypic value of the i^{th} line, μ the population mean, G_i the genetic effect for the i^{th} line, r_j the replication effect, b_{jk} the block effect in each replication (for lattice design) and $e_{ij(k)}$ the residual error.

For each trait, heritabilities were calculated as:

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2} \quad \text{with} \quad \sigma_g^2 = \frac{(MSg - MSe)}{k} \quad \text{and} \quad \sigma_e^2 = MSe$$

where k is the number of replications, MSg and MSe the genotypic and residual mean squares.

QTL detection

QTL detection was performed for the two populations using adjusted means for the three replications. A first analysis was conducted by simple interval mapping (SIM) (Lander and Botstein 1989) with the Plabqtl software package (Utz and Melchinger 1995) that uses the regression approach as described by Haley and Knott (1992). The threshold for QTL declaration was fixed to a LOD value of 2.6 for RIL379 and 2.7 for RIL249. These values were determined by the permutation method described by Churchill and Doerge (1994) implemented in the Mqtl software package (Tinker et al. 1996) with a global type-I error rate of 5%. A second analysis was performed using composite interval mapping (CIM) (Zeng 1994; Jansen and Stam 1994). CIM cofactors were chosen as the nearest marker of each QTL detected with SIM. The cofactor set used for each trait in each progeny is given in Table 1. CIM scans were performed using the Plabqtl programme with the same LOD threshold value as in SIM. No CIM analysis was performed when SIM did not detect a QTL.

The confidence interval for each QTL was set at a one-LOD support interval, as described by Lander and Botstein (1989). The phenotypic variance explained by a single QTL was calculated as the square of the partial correlation coefficient with the observed variable, adjusted for cofactors in case of CIM (Utz and Melchinger 1995). The additive effect of a putative QTL was estimated by half the difference between the two homozygous classes. It was assumed that the positive effect was carried by the guinea allele. The total phenotypic variance explained by all QTLs was obtained by fitting a model including all putative QTLs for a given trait.

Results and discussion

Statistical analyses

The results obtained for the three parental lines were in line with the typical characteristics of the guinea and caudatum races (Table 1). The caudatum genotype IS2807 was short with a small compact panicle, relatively few grains per panicle and good productivity due to the medium kernel size (TK ranged from 20 to 22 g). The technological traits showed a floury endosperm (FL = 4.5–5), medium hardness (KF < 19) and a slight sensitivity to abrasion. Both guinea genotypes were taller than IS2807 (whereas 249 was twofold higher than 379) and had a long loose panicle with many grains. It is noteworthy that while 379 had small grains, 249 had a thousand-kernel weight equivalent to that of the caudatum cultivar. Consequently, 249 had higher productivity than caudatum IS2807. Concerning technological traits, both guineas had a semi-vitreous (FL = 3–3.5) and very hard (KF < 13) grain that was more resistant to abrasion than that of the caudatum cultivar. A few differences were noted with respet to biochemical grain components. The three parental lines had intermediate results for amylose, protein and lipid contents. The results also indicated that the caudatum line was more sensitive to mold after harvest and during germination.

Although few differences were found between the parental lines for certain traits, the two populations showed a wide range of variation for all traits (Table 1), indicating that transgressive segregations have occurred. Eleven distributions showed a significant deviation from normality. However, such deviations would not disturb QTL detection since the method used is based on the regression approach (B. Goffinet, personal communication).

ANOVA revealed significant differences between RI lines for each trait measured. Heritability of the different traits ranged from 28 to 94, but most of the values were between 60 and 90 (Table 1). Low heritabilities for MR_{379}, MR_{249}, GR_{379} and TP_{249} indicated a marked influence of environment on these traits. NK presented low heritability for RIL379 but high heritability for RIL249. A comparison of KW_{379} and KW_{249}

Table 1 Statistical analysis and QTL detection of the different traits measured in RIL379 and RIL249 populations

			Parents				Progenies				SIM detection			CIM detection		
			Caudatum	Guinéa	Mean	SD	Min	Max	W	P > W	h^2	*n*	R^2	Cofactor set	*n*	R^2
PH	Plant height (cm)	RIL379	106.20	222.80	196.03	66.07	91.60	343.80	0.91****	0.0001	93.30	2	67.3	UMC23; UMC76	2	67.3
		RIL249	176.53	492.00	314.61	81.20	162.00	513.20	0.94***	0.0008	92.9	2	45.2	UMC23	2	45.7
PL	Panicle length (cm)	RIL379	20.20	40.50	27.69	4.75	17.50	43.20	0.96	0.0125	83.60	4	54.1	UMC23; UMC76; UMC136; BNL15.21	6	66.2
		RIL249	21.20	39.67	27.52	4.88	17.60	40.60	0.98	0.5524	86.0	2	28.3	UMC146; UMC122	1	17.7
PC	Panicle compatness (1–5)	RIL379	4.00	1.00	1.87	0.80	1.00	4.00	0.89***	0.0001	74.50	3	40.3	UMC23; UMC76; UMC136; UMC4	2	36.8
		RIL249	–	–	–	–	–	–		–	–	–	–		–	–
NK	Number of kernels/panicle	RIL379	1140.00	1560.00	1578.48	446.57	472.00	3336.00	0.99	0.8020	45.80	1	12.3	UMC23	2	23.3
		RIL249	1064.33	1506.67	1044.75	521.11	147.00	2951.00	0.96	0.0411	75.7	2	30.4	BNL6.25; BNL14.07	2	30.8
TK	Thousand-kernel weight (g)	RIL379	20.30	12.60	17.49	2.78	10.20	24.40	0.98	0.4010	76.00	1	35.2	UMC23	3	46.3
		RIL249	22.30	21.80	20.05	2.91	12.20	33.40	0.99	0.8382	85.0	0	–		–	–
KW	Kernel weight/ panicle (g)	RIL379	23.20	19.70	27.80	9.37	9.00	60.90	0.97	0.1807	55.00	1	31.3	UMC23	1	31.3
		RIL249	23.67	32.53	20.87	10.49	2.00	51.40	0.96	0.0322	74.7	1	19.4	BNL14.07	1	19.4
TP	Threshing percentage (%)	RIL379	–	–	–	–	–	–		–	–	–	–		–	–
		RIL249	76.73	62.37	65.91	10.13	14.30	80.40	0.90***	0.0001	55.9	1	15.3	BNL8.01	1	15.3
DY	Dehulling yield (%)	RIL379	58.60	77.30	61.31	11.17	29.70	84.20	0.96	0.0201	61.00	3	43	BNL7.50; B2/b2; UMC22	4	49.4
		RIL249	65.38	88.96	71.91	9.28	37.70	89.47	0.97	0.1029	65.7	2	25.5	UMC58; UMC115	–	–
FL	Kernel flouriness (1–5)	RIL379	4.50	3.50	4.40	0.64	3.00	5.00	0.87***	0.0001	63.70	2	57.1	B2/b2; UMC55	2	59.1
		RIL249	5.00	3.17	4.04	0.72	2.50	5.00	0.94***	0.0004	50.6	1	17.6	B2/b2	2	29.7
KF	Kernel friability (PSI)	RIL379	–	–	–	–	–	–		–	–	–	–		–	–
		RIL249	16.65	10.14	15.08	2.28	9.62	22.60	0.97	0.2474	81.8	1	13.7	UMC58	2	24.5
KH	Kernel hardness (AACC)	RIL379	–	–	–	–	–	–	–	–	–	–	–		–	–
		RIL249	297.49	353.46	317.98	17.76	286.84	361.15	0.96			4	44.8	UMC58; BNL15.40; BNL8.01	2	37
AM	Amylose content (%)	RIL379	23.82	24.65	23.69	1.48	17.62	26.59	0.95**	0.0025	71.90	2	38.9	B2/b2	2	48.4
		RIL249	22.36	24.60	22.60	1.27	19.19	26.27	0.97	0.1996	72.1	2	23.2	BNL15.40	1	22
PR	Protein content (%)	RIL379	11.81	12.18	12.35	1.15	9.42	16.78	0.97	0.0699	68.10	2	26	UMC84	1	19.1
		RIL249	11.44	14.21	12.98	1.35	9.56	16.44	0.97	0.1118	70.4	1	16.5	BNL7.25	3	40.2
LI	Lipid content (%)	RIL379	–	–	–	–	–	–		–	–	–	–		–	–
		RIL249	3.00	3.96	3.40	0.38	2.39	4.46	0.99	0.8712	64.2	1	14.7	UMC115	1	14.7
GR	Germination rate (%)	RIL379	66.00	82.00	66.39	17.13	8.00	98.00	0.94***	0.0001	50.10	2	43	UMC23; UMC76	3	51.4
		RIL249	–	–	–	–	–	–		–	–	–	–		–	–
MG	Molds during germination (%)	RIL379	95.00	27.00	74.58	28.93	6.00	100.00	0.83***	0.0001	74.60	3	33.8	UMC167; B2/b2	4	48
		RIL249	–	–	–	–	–	–		–	–	–	–		–	–
MR	Molds after harvest (1–5)	RIL379	4.00	2.00	3.39	0.89	2.00	5.00	0.94***	0.0001	55.10	1	28.3	RDNA	1	28.3
		RIL249	2.50	2.33	2.09	0.28	2.00	3.00	0.53***	0.0001	27.9	0	–		–	–

highlighted the same situation, which could be explained by the close positive correlation of this trait with NK. This suggests that the residual increase for NK_{379} was due to environmental effects rather than measurement errors.

A total of 41 and 35 phenotypic correlations, respectively, were found to be significant in the RIL249 and RIL379 populations (Table 2). Sixteen correlations were significant in the two crosses, 38 correlations were significant in one population but were not evaluated in the other population; 9 correlations were significant for only one of the two crosses. The highest correlation coefficients were between KW and NK (0.944 for RIL379 and 0.854 for RIL249). A positive correlation between KW and TK was detected for RIL379, but this correlation was not significant for RIL249, which indicates that plant productivity could mainly be explained by the number of kernels in the RIL249 population. Plant height and panicle length were positively correlated, and both were positively correlated with yield components. NK was negatively correlated with PC and positively correlated with PL (which was negatively correlated with PC). This tends to discriminate between long, loose panicles with many grains (guinea type) and short, compact panicles with relatively few grains (caudatum type). With respect to technological traits, AM, DY, KF, KH and FL were closely correlated. These correlations indicate that high amylose content grains are more vitreous, harder, and less sensitive to abrasion. AM was also negatively correlated with PR and positively correlated with yield components (NK and KW). Considering that starch and protein are the two major grain components, the negative correlation between protein and amylose content indicates that variations in amylose content closely match variations in starch content, and thus the amylose to amylopectin ratio should be constant in the two populations. No significant correlations between protein content and grain physical properties were found. Mold at germination and mold after harvest were positively correlated (0.510). Both were negatively correlated with germination rate. All significant correlations between mold susceptibility and other traits were poor. It is noteworthy that phenotypic correlations indicated that in both populations grain quality, as defined by a vitreous hard endosperm that is resistant to abrasion and has a high amylose content, was not negatively correlated with high productivity.

QTL detection and localization

For QTL detection and localization, both simple interval mapping (SIM) and composite interval mapping (CIM) were performed with the two populations (Fig. 1 and Table 1).

QTL detection with RIL249 gave a total of 18 QTLs with SIM and 17 QTLs with CIM. For RIL379, 29 QTLs were detected with SIM and 35 with CIM. Differences in the number of QTLs detected in the two populations may mainly be due to the lower size of the RIL249 population (90 individuals instead of 110). No QTLs were found on linkage groups E, I and J. Total phenotypic variance, as explained by the QTLs detected in relation to heritabilities, can provide interesting information on the genetic control of the analysed traits. For example, 2 QTLs were found for PH_{379}, explaining 67.3% of the phenotypic variance. This suggests that few major genes affect the variations in this trait. On the contrary, no QTLs were found for TK_{249} despite the strong heritability calculated for this trait. In this case, many genetic factors with minor effects are probably involved which cannot be detected in this population owing to its small size.

The results obtained with CIM were quite close to those obtained with SIM. However, in some cases new QTLs were detected with CIM. The use of cofactor markers reduces the variance of each genotypic class at positions other than those being tested and increases the efficiency of QTL mapping. A total of 15 additional QTLs were thus recorded with both populations. In other cases, QTLs detected by SIM were not found using CIM. For example, 2 QTLs were detected with SIM for DY_{249} on linkage groups (l.g.) A and K, and no QTLs were detected with CIM. The same situation was observed for PL_{249} on l.g. B, PR_{379} on l.g. D and KH_{249} on l.g. G. A possible explanation could be the correlation between one of the QTL flanking markers and one of the cofactors used for CIM (cofactors for DY_{249} were UMC58 and UMC115 linked at a LOD of 2.14). Such correlations occur by chance in small populations. CIM analysis was also of interest in terms of the efficiency of discrimination between 2 closely linked QTLs. Cofactors chosen at each QTL reduced the extent of variation due to 1 QTL when scanning the other. For example, CIM analysis for AM_{249}, AM_{379}, PC_{379} MG_{379} on l.g. F and DF_{379} on l.g. H resulted in the loss of one of the two closely linked QTLs detected with SIM. In contrast, the 2 QTLs detected with SIM for DY_{379}on l.g.F were also both detected with CIM.

Grain quality traits

Quantitative trait loci for grain components were found on seven linkage groups (l.g. A, D, K, C, F, G and H).

A chromosomal segment located on linkage group F was found to play a major role in grain quality. For RIL379, 4 QTLs for flouriness, dehulling yield, amylose content and mold during germination were detected at the top of the linkage group, very closely linked with each other. These 4 QTLs explained an important part of the phenotypic variance (from 15% for MG_{379} to 54% for FL_{379}). The additivity effects indicated that the presence of guinea alleles at these loci resulted in an

Table 2 Pearson phenotypic correlations in RIL379 population (top number) and RIL249 (bottom number)

	PH	PL	PC	NK	TK	KW	TP	DY	FL	KF	KH	AM	PR	LI	GR	MG
PL	0.657															
	0.791															
PC	−0.567	−0.720														
	–	–														
NK	0.463	0.437	−0.408													
	ns	0.405	–													
TK	0.627	0.377	ns	ns												
	0.440	0.368	–	ns												
KW	0.676	0.538	−0.451	0.854	0.656											
	0.446	0.507	–	0.944	ns											
TP	–	–	–	–	–	–										
	ns	ns	–	0.549	0.371	0.628										
DY	ns	0.375	−0.353	ns	ns	ns	–									
	0.414	0.358	–	ns	0.560	ns	ns									
FL	ns	ns	ns	ns	ns	ns	–	−0.638								
	−0.393	−0.414	–	ns	ns	−0.374	ns	−0.602								
KF	–	–	–	–	–	–	–	–	–							
	−0.358	ns	–	ns	−0.437	ns	ns	−0.812	0.675							
KH	–	–	–	–	–	–	–	–	–	–						
	0.476	–	–	ns	0.388	0.373	–	0.693	−0.604	−0.672						
AM	ns	ns	ns	0.340	ns	0.332	–	0.595	−0.629	–	–					
	ns	ns	–	0.364	ns	0.414	0.391	0.524	−0.502	−0.662	0.449					
PR	ns	ns	ns	−0.500	ns	−0.398	–	ns	ns	–	–	−0.353				
	ns	ns	–	−0.548	ns	−0.455	−0.423	ns	ns	ns	ns	−0.528				
LI	–	–	–	–	–	–	–	–	–	–	–	–	–			
	0.566	0.448	–	ns	ns	ns	ns	ns	−0.448	−0.350	0.527	ns	ns			
GR	0.561	0.509	−0.486	0.367	0.351	0.456	–	0.421	ns	–	–	ns	ns	–		
	–	–	–	–	–	–	–	–	–	–	–	–	–	–		
MG	ns	ns	ns	ns	ns	ns	–	ns	ns	–	–	ns	ns	–	−0.477	
	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
MR	ns	−0.328	ns	ns	ns	ns	–	−0.423	ns	–	–	ns	ns	–	−0.416	0.510
	ns	ns	–	ns	ns	ns	ns	ns		ns	ns	ns	ns	−0.353	–	–

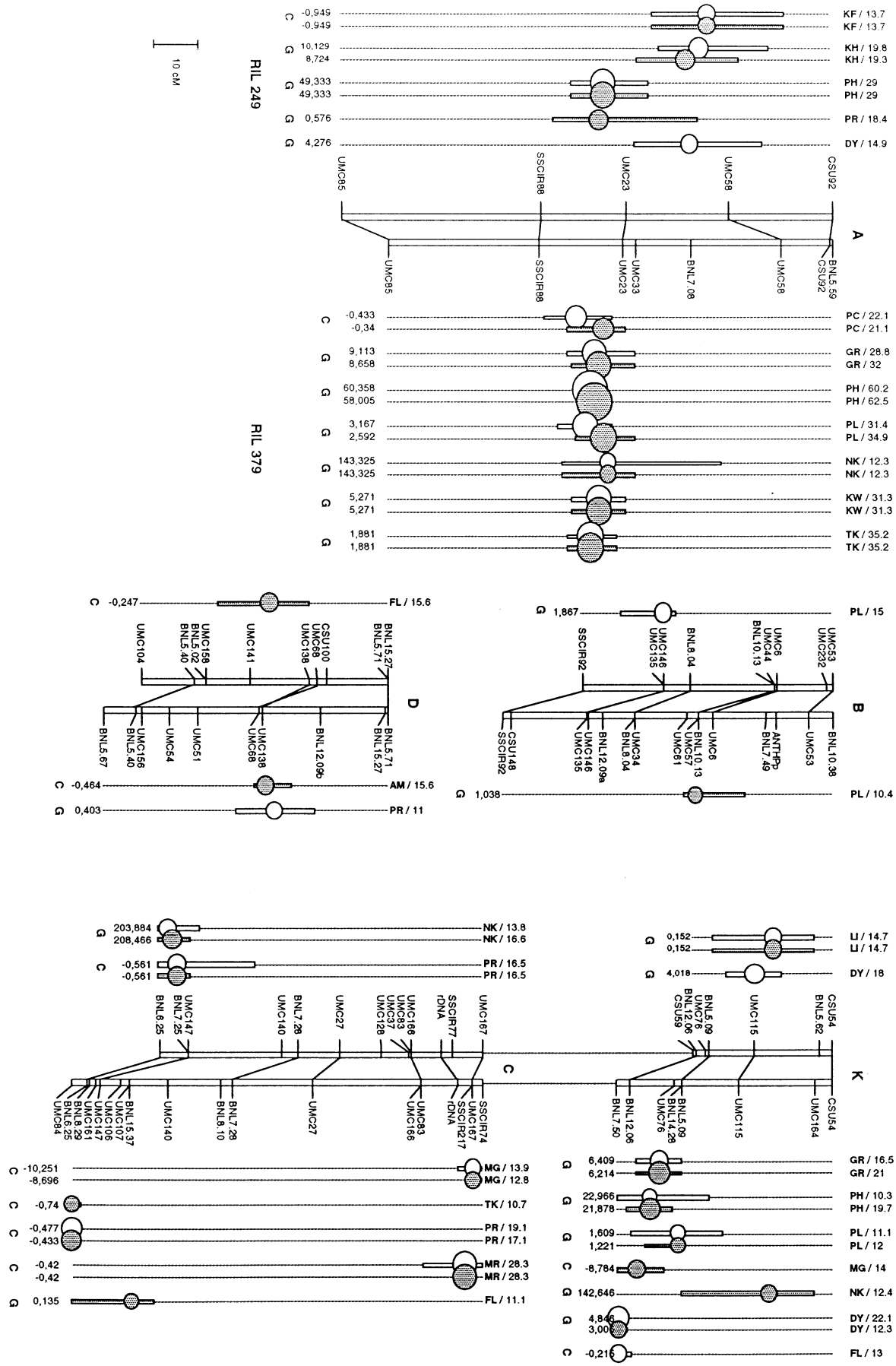

Fig. 1 See page 612 for legend

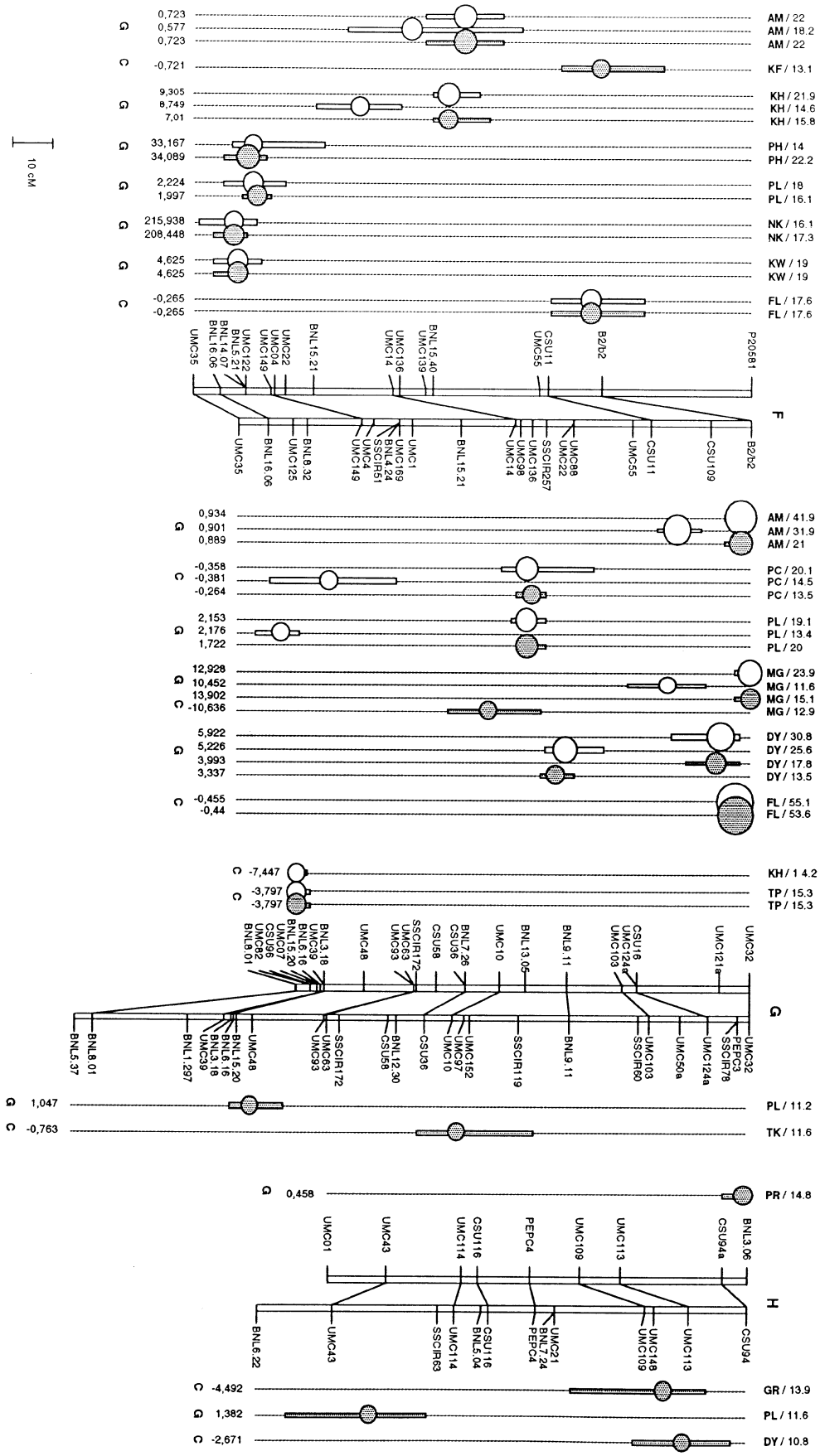

Fig. 1 Genetic maps and QTLs detected for RIL249 (*left*) and RIL379 (*right*) populations. Each QTL is represented with a *circle* located on the LOD peak and with a *box* representing confidence interval. *White* QTLs were detected with *SIM*; *gray* QTLs were detected with CIM. The *upper number* is the percentage of phenotypic variance explained by the QTL and the *lower number* is the additivity effect. *G* or *C* at the *bottom* of each QTL represents the racial (guinea or caudatum) origin of positive QTL effect

amylose-rich endosperm that was more vitreous, resistant to abrasion but more sensitive to mold during germination. On the same linkage group, 4 important QTLs were detected on RIL249 for flouriness, kernel friability, kernel hardness and amylose content (13–22% of the phenotypic variance). In this population, guinea alleles conferred a hard, vitreous endosperm with high amylose content. These results are consistent with the close correlation found between amylose content and kernel physical properties. However, little is known about the relations between endosperm texture and amylose content. In three semi-vitreous sorghum cultivars, Cagampang et al. (1985) observed that floury endosperm starches contained between 0.14% and 0.54% more amylose than corneous starches. Otherwise, starch content is reported to decrease from the floury to the vitreous part of sorghum grain. This suggests that the co-localization of QTLs for amylose content and endosperm texture on l.g. F is due to two different, but linked genes rather than to a unique gene with pleiotropic effects. This could explain part of the relations already described between endosperm texture and thick porridge quality. The *B2/b2* gene controlling the presence of the high-tannin testa layer in the sorghum grain has been phenotypically mapped in this region. In our crosses, the caudatum genotype had the *B2* allele (presence of tannins), whereas both guinea genotypes had the *b2* allele (without tannins). This gene could be involved in the QTL for mold sensitivity found with RIL379. Indeed, high tannin content in the sorghum grain has been reported to be associated with grain mold resistance (Harris and Burns 1973; Esele et al. 1993; Melake-Berhan et al. 1996). A relation between the presence of a testa layer and grain hardness has also been reported (G. Fliedel, personal communication). Tannins could play a role in endosperm texture by affecting the grain dehydration rate during maturation. In addition, this chromosomal segment, which seems to play a key role in sorghum grain quality, is homoeologous to the 7s chromosome arm of maize. The maize gene *opaque2* has been mapped between the Npi400a and CSU13 loci which also flanks the *b2* gene in sorghum (Dufour 1996). The *opaque2* mutation results in modification of the grain amino acid composition and in floury endosperm. Changes in amino acid composition have been attributed to a substantial reduction in the 22-kDa zein family. However, the relation between this zein family and textural alteration of the kernel endosperm is not clearly understood. Modifier genes of *o2* mutations which restore endosperm vitreousness in *o2* quality protein maize (QPM) have also been localized on maize chromosome 7 (Lopes et al. 1995). Our results suggest that an orthologous locus of one of these genes could be the source of the QTLs for FL, KF and KH detected in each population.

A relation between grain mold resistance and grain hardness has also been described (Jambunathan et al. 1992). We found a QTL association consistent with these results on l.g. K/C. Two QTLs for MR_{379} and MG_{379} were found at the top of linkage group C, while 3 QTLs for MG_{379}, DY_{379} and FL_{379} were found at the corresponding extremity of l.g. K. No clear conclusions could be drawn because of the lack of genetic markers mapped in this region. However, these 5 QTLs could be due to the expression of a unique QTL with marked effects, with the caudatum allele conferring a floury endosperm sensitive to abrasion and mold. This QTL association may result from a physical relationship between grain hardness and mold resistance. Finally, a QTL for GR_{379} was found in the same region, which could be the consequence of mold resistance during germination.

Four QTLs for grain quality were detected on l.g. A for RIL249 (KF, KH, DY and PR), explaining 14%, 19%, 15% and 18% of the the phenotypic variance, respectively. No QTL was found in the same region with the other population. The confidence intervals for KF, KH, and DY were wide (approx. 30 cM) and overlapped, which indicates that these QTLs could be due to the expression of a unique gene. This was consistent with the close correlations between these traits (Table 2); the guinea allele conferred higher mechanical resistance to the kernel. In addition, in the same region the guinea allele had a positive effect on grain protein content. Associations between protein content and kernel physical properties were found in other segments of the genome. Two QTLs for PR_{249} and PR_{379} were found at one end of l.g. C, explaining approximately 17% of the phenotypic variance, with a positive effect of the caudatum allele. These QTLs were associated with a QTL for FL_{379}, explaining 11% of the phenotypic variance, with a positive effect of guinea. Another association was found at the top of l.g. H between a QTL for PR_{249} ($r^2 = 15\%$) and a QTL for DY_{379} ($r^2 = 11\%$). At this locus the allele carried by 249 had a positive effect on protein content, while the allele carried by 379 had a negative effect on dehulling yield. Finally, a QTL was found for PR on linkage group D for RIL379, while a QTL for flouriness was found in a nearby region for RIL249. These co-localizations are not in line with the fact that all correlations observed between whole protein content and physical grain properties were not significant, suggesting that only a few specific grain proteins could play a role in endosperm texture. Associations between endosperm texture and storage proteins have been described in sorghum (Watterson et al. 1993; Mazhar and Chandrashekar 1995). Watterson et al. found that vitreous endosperm contained from 1.5- to 2-fold more total nitrogen than floury endosperm and that this difference was mainly explained by the kafirin level. Mazhar and Chandrashekar (1995) noted that the level of γ-kafirin, a cysteine-rich body-surface protein, was higher in harder endosperm and assumed that this protein could confer rigidity to the endosperm by forming disulphide link-

ages with itself or other peripheral proteins. Such observations have also been described in maize (Lopes and Larkins 1991; Mestres and Matencio 1996). These authors noted the involvement of the zein protein family in kernel vitreousness and friability, especially the 27-kDa γ-zein. Many molecular and functional homologies have been found between sorghum kafirin and maize zein (DeRose et al. 1989).

A QTL for AM was found on l.g. D for RIL379. It was closely linked with a QTL for PR with an opposite additivity effect, which was consistent with the negative correlation between these 2 traits. Comparisons with other grasses could provide some guidelines for interpreting the QTL for amylose content found on l.g. D. The gene coding for the starch branching enzyme (SBEIII) has been mapped in rice on chromosome 2 (Harrington et al. 1997). Rice chromosome 2 is homoeologous to sorghum l.g. D. Furthermore, the CDO1302 and CDO1380 probes which flank the SBEIII gene in rice have been mapped in sorghum near the UMC138 probe (data not shown). This suggests that the QTL for AM_{379} found on l.g. D could be due to the expression of an orthologous gene of rice SBEIII.

Linkage group K was also found to be involved in grain quality. Two QTLs for lipid content and dehulling yield were found in RIL249 (15% and 18% of phenotypic variance). However, the validity of the QTL for DY is uncertain. Indeed, this could be a "ghost" QTL due to the correlation obtained between UMC58 and UMC115 (see above). This was supported by the fact that no QTL was detected by CIM analysis using UMC58 as cofactor. Moreover, there was no significant correlation between LI and DY, as there was no functional explanation for the co-localization of QTLs for lipid content and dehulling yield.

Yield components and morphological traits

A major chromosomal region involved in yield components and morphological traits was found on l.g. A for the RIL379 population. On this linkage group, 4 QTLs with marked effects on GR, NK, KW and TK were detected in association with 3 QTLs for PC, PH and PL. At this locus, the guinea allele resulted in a high plant with a long, loose panicle, many large grains and thus good productivity. In the other population, only the QTL for PH was detected in the same region. The QTL for NK_{379} is consistent with the positive correlation obtained with PL_{379}, and its sign effect is in agreement with the superiority of the 379 genotype for this trait. The QTL for TK_{379} was slightly more unexpected. Indeed, this is the biggest QTL found for this trait on this population with a positive effect derived from the guinea genotype, while this parent has very small grains (TK = 12.6). A possible explanation could be a pleiotropic effect on the thousand-kernel weight of the QTL involved in morphological components. Blum et al. (1997) assumed that this effect could be due to the fact that tall genotypes have greater stem reserve storage than short genotypes, which could become an important source for grain filling under environmental stress. It could be assumed that the guinea allele, owing to its effects on plant morphology, enable full expression of other QTLs for TK coming from the caudatum parent. We found a positive correlation ($r = 0.3$) between TK and the percentage of caudatum genome (estimated as the percentage of caudatum alleles for all mapped markers weighted by the genetic distance covered by each marker) for individuals carrying the guinea allele at the UMC23 locus (Fig. 2). Moreover, on the other population, the 249 genotype, as already noted, had a high TK for a guinea. The fact that no QTL for TK was detected in RIL249 could indicate that the 249 genotype contains parts of the caudatum positive alleles and could explain why no pleiotropic effect of PH on TK was found on l.g. A. Pleiotropic effects on yield components for genes involved in plant height have already been described. Plant height is controlled by four independent genes (Quinby and Karper 1954), *Dw1*, *Dw2*, *Dw3* and *Dw4*. The dwarf gene *Dw3* has pleiotropic effects on the number of kernels per panicle, kernel weight as well as on tiller number and panicle size (Casady 1965). *Dw2* may have pleiotropic effects on panicle length, main head yield, seed weight and leaf area (Graham and Lessman 1966), while no pleiotropic effect was identified for the two other loci. Pereira and Lee (1995) found 4 QTLs for plant height in sorghum. Considering the co-localizations with other QTLs involved in morphological and yield components, these authors related these QTLs to *Dw* loci. In particular, a QTL on l.g. A also identified by Lin et al. (1995) was assigned to *Dw3*. Our results are in agreement with this hypothesis.

Two other QTLs for plant height were detected on l.g. K and F, thus explaining 19% and 22% of the phenotypic variance. None of these were previously

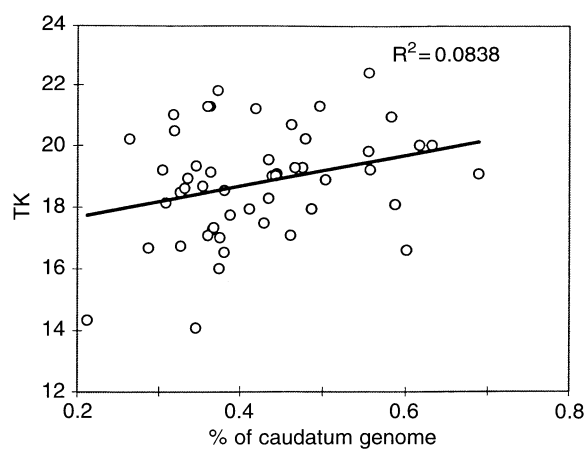

Fig. 2 Thousand-kernel weight of individuals carrying guinea alleles on UMC23 versus percentage of caudatum genome

reported by Pereira and Lee (1995), whereas the QTL on l.g. K was also clearly observed by Lin et al. (1995). These 2 QTLs are associated with QTLs for panicle length, suggesting that the genes involved in these QTLs could contribute to stem elongation. The QTL for PH_{249} on l.g. F was also associated with QTLs for NK and KW ($r^2 = 17\%$ and 19%). It could thus be tentatively attributed to *Dw2*. However, this gene has been described as being tightly linked to the *Ma1* gene controlling flowering time. On this basis, Lin et al. positioned *Dw2* on l.g. B, while Pereira and Lee (1995) located it on l.g. H.

Many additional QTLs with lesser effects were detected for morphological and yield traits. A QTL for PL was detected for both populations on l.g. B. Two QTLs for PL_{379} and PC_{379} were found on l.g. F. These QTLs could be related to the QTL for mold sensitivity during germination found in the same region. At these loci, caudatum alleles contribute to a short, compact panicle sensitive to mold. Two other QTLs for PL_{379} were found on l.g. G ($r^2 = 11\%$) and H ($r^2 = 12\%$). A QTL for TK_{379} with a positive allele from the caudatum genome was found on l.g. G, and a QTL for NK_{379} was found on l.g. K with a positive effect attributed to the guinea parent. Finally, a QTL involved in threshing yield was found on l.g. G for the RIL249 population. This QTL was associated with a QTL for total yield, explaining 15% of the phenotypic variance (data not shown).

Conclusion

The identification of QTLs affecting important traits is a key step in the use of molecular markers for plant improvement and in understanding the genetic factors that determine these traits. Here we used two recombinant inbred line sorghum populations to identify QTLs controlling grain quality traits and yield components. Our results are in agreement with many previous studies concerning biochemical components of technological grain quality. In some cases, genetic dissection of those complex traits explained their technological relations in terms of functional relation or genetic linkage. One extremity of l.g. F was thus found to be involved in amylose content, kernel texture and tannin content, but no causal relation between these traits was found. On the contrary, co-localizations of QTLs involved in protein content and grain hardness or vitreousness suggest the involvement of certain storage grain proteins in kernel physical properties. Further analyses focusing on different grain protein families concerning the same material should be performed to confirm these results.

Major known genes were suspected to be involved in some major QTLs detected in this study. For example, the *B2* gene controlling the presence of the high-tannin testa layer in sorghum could affect grain mold resistance as well as kernel vitreousness. Moreover, tannins strongly affect the technological and nutritional quality of sorghum flours. Likewise, the *Dw3* gene, hypothetically mapped on l.g. A, could have pleiotropic effects on the main morphological and productivity traits. This suggests that in some cases key genes are involved in major QTLs and affect more than one trait. These QTLs with strong effects are thus less suitable for molecular breeding of focused traits. Attention should be focused on QTLs with smaller but specific effects in material with fixed alleles at some identified key loci. The detection ability of minor genetic factors depends on the size of the populations and the efficiency of the detection methods used for such studies.

Finally, the results obtained on both populations show that there were no genetic obstacles for recombination of genetic components of both productivity and grain quality in guinea x caudatum crosses. Suitable breeding schemes combined with efficient QTL detection methods, such as composite interval mapping, could therefore provide a good tool for creating new productive sorghum cultivars with appropiate technological grain quality characteristics.

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