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Identification of genomic regions that affect grain-mould incidence and other traits of agronomic importance in sorghum

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Abstract Grain-mould is a major problem in grain sorghum utilization as mouldy grain has a reduced quality due to the deterioration of the endosperm and reduced embryo viability. Here, our objective was to use genome mapping to improve knowledge of genetic variation and co-variation for grain-mould incidence and other inter-related agronomic traits. Grain-mould incidence, kernel-milling hardness, grain density, plant height, panicle peduncle length, foliar-disease incidence, and plant color were measured on 125 F_5 genotypes derived from a cross of Sureño and RTx430. Quantitative trait loci were detected by means of 130 mapped markers (44 microsatellites, 85 AFLPs, one morphological-trait locus) distributed among ten linkage groups covering 970 cM. One to five QTLs affected each trait, with the exception of grain density for which no QTLs were detected. Grain-mould incidence was affected by five QTLs each accounting for between 10 and 23% of the phenotypic variance. The effects and relative positions of QTLs for grain-mould incidence were in accordance with the QTL distribution of several inter-related agronomic traits (e.g., plant height, peduncle length) and with the correlation between these phenotypic traits and grain-mould incidence. The detection of QTLs for grain-mould incidence was dependent on the environment, which is consistent with heritibility estimates that show strong environmental and genotype × environment effects. Several genomic regions affected multiple traits including one region that affected grain-mould incidence, plant height, panicle peduncle length, and grain-milling hardness, and a second region that influenced grain-mould (in four environments) and plant height. One genomic region, which harbors loci for plant color, influenced the severity of foliar disease symptoms and the incidence of grain-mould in one environment. Collectively QTLs detected in the present population explained between 10% and 55% of the phenotypic variance observed for a given trait.

Keywords Sorghum · Recombinant inbred line · Molecular markers · QTLs

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Introduction

Sorghum ranks among the world's most important cereal crops. Despite its importance, comprehensive genetic characterization of it has been limited. Sorghum is well-adapted to growth under semi-arid conditions, having numerous mechanisms to survive and to be productive in these harsh environments, but yield and quality are constrained by many factors including disease and insects. Sorghum serves as a host for over 100 pathogens, including fungi, bacteria, viruses and nematodes (Thakur et al. 1997). These pathogens, individually or in combination, lead to considerable losses in yield and grain quality. Disease management through host plant resistance has been an effective means of reducing losses in sorghum. Although detailed information on the genetics of resistance is still needed, it is generally recognized that, except for complex diseases such as stalk rot and grain-mould, resistance to most diseases is controlled by major genes (for a review see Thakur et al. 1997).

Grain-mould is defined as grain deterioration resulting from the infection of fungi on developing sorghum kernels. It is considered a major problem in grain sorghum utilization as mouldy grain has a reduced quality due to the deterioration of the endosperm and reduced embryo viability (Castor and Frederiksen 1980). Grain

infected with mould is also more likely to be contaminated with mycotoxins, and these metabolites can present hazards to consumers (Williams and Rao 1981). All of these factors result in reduced grain quality and yield, and hence a reduced market value of the crop (Rooney and Serna-Saldivar 1991).

The severity of grain-mould is dependent on the genotype and the environment in which it is grown. Warm and humid environments during grain maturation favor development of the disease. Losses of up to 100% in highly susceptible hybrids are not uncommon when environmental conditions favor mould development (e.g., heavy rains during grain maturation). Major efforts to breed for mould resistance have resulted in significant progress, but grain-mould remains a major constraint to sorghum production with global losses estimated at $130 million. A majority of the germplasm lines resistant to grain-mould have a pigmented testa and high tannin levels (Menkir et al. 1996). The presence of tannins in caryopses is an undesirable trait for food and feed processors, and therefore the use of these compounds as a source of grain-mould resistance is not acceptable. Consequently, recent research on grain-mould resistance has focused on mechanisms of resistance that are suitable for food quality and feed quality sorghums. Moderate levels of grain-mould resistance in white-grained, food quality sorghum have been identified and mechanisms of resistance have been suggested (for reviews see Glueck and Rooney 1980; Stenhouse et al. 1997). Grain characteristics that may contribute to mould resistance include grain hardness, a thin pericarp, a thick surface wax layer, grain density and grain integrity (Glueck and Rooney 1980; Esele et al. 1993). Panicle and glume traits that have been shown to affect grain-mould include open panicles and long glumes, both of which are reported to reduce grain-mould incidence. Plant height has also been implicated in grain-mould resistance due to differences in the micro-climate (humidity, air movement) at varying distances from the soil surface (Castor 1981; Rao and Rana 1989). Recently, anti-fungal proteins were identified in sorghum grains and these may play a role in protecting grain from fungal attack (Rodriguez-Herrera et al. 1999).

The genetics of grain-mould resistance is believed to be multigenic with recent estimates indicating a minimum of four genes controlling resistance in white-grained sorghums (Rodriguez-Herrera et al. 2000). A complex of fungi, including *Curvularia lunata*, *Fusarium* spp., *Alternaria* spp., *Phoma sorghina* and *Helminthosporium* spp., are known to cause grain-mould (Castor 1981). Most of these fungi are unspecialized or facultative parasites, and the predominant species vary depending on location, year and the environment. Grain-mould resistance is subject to large environmental and genotype by environment (G × E) interactions due to pathogen variability and variability in environmental conditions that are critical for grain-mould development (Rodriguez-Herrera 1999). Utilizing 19 inbred sorghum lines, Rodriguez-Herrera (1999) demonstrated that genotypic, environmental, and genotype × environment variation each accounted for approximately the same percentage of the phenotypic variability of grain-mould incidence.

The recent use of molecular markers in quantitative genetic studies has facilitated the study of complex, quantitatively inherited traits and made it possible to dissect polygenes for such traits into individual Mendelian factors (Paterson et al. 1991). Using molecular-linkage genetic maps and quantitative trait locus (QTL) mapping technology, it is possible to estimate the number of loci controlling genetic variation in a segregating population and to determine the map positions of these loci in the genome. Several qualitative traits and QTLs of agronomic importance in sorghum have been mapped with the help of molecular markers. Molecular markers for disease resistance, tolerance to environmental stress, leaf phenotypic traits, seed and panicle characteristics, and plant status (height, flowering, maturity, tillering) have been identified (for a review see Subudhi and Nguyen 2000). The identification of QTLs underlying traits of agronomic importance is a prerequisite for using molecular markers for sorghum genetic improvement.

In this study we analyzed a population of 125 F_5 recombinant inbred lines (RILs) derived from a cross between Sureño and RTx430 – one highly susceptible to grain-mould and the other moderately resistant. Our objectives were to map QTLs underlying grain-mould resistance in a series of environments and map other agronomic important trait loci that differentiate RTx430 and Sureño. Trait loci underlying grain-mould incidence, foliar disease severity, plant color (tan vs purple), plant height, panicle peduncle length, and seed quality traits (grain density, grain-milling hardness) were mapped in a series of environments across Texas. The association between grain-mould incidence and other phenotypic traits was examined to improve our understanding of the genetic variation and co-variation of grain-mould incidence and other inter-related agronomic traits.

Materials and methods

Development of a recombinant inbred population

The population used for mapping consisted of 125 F_5 RILs from a cross between RTx430 and Sureño. The pedigree of Sureño is [(SC423 × CS3541) × E35–1]-2 (Meckenstock et al. 1993). Sureño is a dual-purpose grain and forage variety with moderate resistance to grain-mould. Sureño is photoperiod-insensitive (*dw_1 Dw_2 Dw_3 dw_4*), has a tan plant color (*pp qq*) with tan glumes, and seed with a white translucent pericarp (genotype of pericarp traits; *RR yy ZZ ll b_1b_1 B_2B_2 SS*). RTx430 is a widely adapted inbred line with excellent combining ability, and is a common restorer line in many U.S. grain sorghum hybrids. RTx430 is highly susceptible to grain-mould and is susceptible to many foliar diseases. The pedigree of RTx430 is (Tx2536 × SC-170–6-5–1-E2)-10–4-4–1-4 (Miller 1984). The caryopsis is white with a yellow endosperm (pericarp genotype; *RR yy II ss b_1b_1 b_2b_2 ZZ TP TP PP QQ*). RTx430 is a three-dwarf *($dw1$ Dw_2 dw_3 dw_4)* line with a purple plant color.

The 125 F_5 lines were developed by single-seed descent from a cross between Sureño and RTx430. In the summer of 1993, at Lubbock, Tex., hand-emasculated panicles of Sureño and RTx430

were pollinated with pollen from RTx430 and Sureño, respectively. In the winter of 1993, parents and F_1 seed were planted in Puerto Rico and F_1 plants were self-pollinated. In the summer of 1994, a random sample of F_2 plants was self-pollinated to produce a set of $F_{2:3}$- derived families. To advance the population, three panicles in each F_3 line were self-pollinated with one panicle selected for generation advancement. This process was repeated in each F_4 line to produce a set of $F_{2:5}$ lines.

Field trial and phenotypic determination

A set 125 F_5 lines, along with the two parental lines, were evaluated in the field in a randomized complete block design with two replications at each environment. The experimental unit was one row 6.3-m in length, with a spacing of 0.76 m. RILs were evaluated under six environments across Texas. The environments in which grain-mould was rated included: College Station, Tex. in 1997 under two moisture regimes (sprinkler irrigated and non-irrigated), Corpus Christi, Tex. (1997), Beeville, Tex. (1997), and College Station, Tex. (1998, non-irrigated). RILs were also planted in replicated plots in Lubbock, Tex. (1998) representing an environment with low grain-mould pressure. The phenotypic traits measured included: grain-mould rating (rating 1 = grain free of mould damage; rating 5 = grain highly infested, embryo dead, endosperm deteriorated), foliar disease rating (rating 1 = disease free; rating 5 = highly susceptible), plant height (cm), peduncle length (rating 5, ≥20 cm; rating 4, 15–20 cm; rating 3, 10–15 cm; rating 2, 5–10 cm; rating 1, 0–5 cm), grain-milling hardness (T.A.D.D., % residual seed weight), grain density (g/cm^3) and plant color (tan vs purple). The foliar diseases evaluated included: anthracnose, zonate leaf spot and bacterial leaf stripe. Disease identification and disease-incidence rating scales were as previously described (Frederiksen et al. 1976). Grain-milling hardness was determined by de-corticating the sorghum kernels with an abrasive de-huller (PRL Mini-Dehuller, Nutana Machine Co., Saskatoon, Canada) until 10% of the pericarp was removed. Bulk density was determined in triplicate by a nitrogen gas-displacement pycnometer (Multipycnometer, Model MVP-1, QuantaChrome Corporation Power Instrumentation, Syosset, N.Y.). To minimize the influence of grain-mould on grain density and milling hardness, determination of grain density and hardness were obtained on seed grown in Lubbock, Tex. (a low mould-pressure environment). Those seed lots exhibiting weathering were treated as missing plots in grain density and milling hardness determinations. Means over replications, for each trait, were used in data analyses.

Genotype determination

For each of the 125 F_5 lines, leaves from approximately ten field-grown plants were bulked for DNA-extraction. Leaf tissue was lyophilized, macerated, and DNA extracted essentially as described (Klein et al. 2000). A set of 100 oligonucleotide primer pairs for sorghum microsatellites were obtained from Dr. Gary Hart (Texas A&M University), and sequences of an additional 15 sorghum microsatellites were obtained from Dr. Steven Kresovich (Cornell University). Microsatellites were examined for polymorphism between Sureño and RTx430 genomic DNA, and each microsatellite displaying polymorphism between Sureño and RTx430 was utilized in genotypic analysis of the F_5 lines. Heterozygous genotypes at a given marker/locus combination (theoretically 8.125%, in reality 13.2% averaged over 44 co-dominant markers) were treated as missing data. Amplification fragment length polymorphism (AFLP) probes were generated for each of the 125 F_5 lines and the parental lines. AFLP-DNA template preparation and the AFLP-PCR reaction conditions were as described (Klein et al. 2000). Thirty two +3/+3 unique primer combinations were examined to produce 157 informative AFLP markers (high image quality, easily scorable polymorphic bands). Detection of AFLP products was conducted using a LiCor 4200 IR gel detection system. AFLP data analysis (marker segregation) utilized the Bionumerics software package (Applied Mathematics, Belgium). AFLP markers mapping to the same linkage map coordinates were examined for potential co-dominance (the same AFLP +3/+3 primer combination, matching segregation patterns through the RI population).

Statistical analysis

Trait means and standard deviations were calculated using Microsoft Excel (Microsoft, Tacoma, Wash.). Phenotypic correlations were calculated using QGene software (Nelson 1997). The expected segregation ratios (1:1) of SSR and AFLP markers in the RIL population were tested for distortion by chi-square (QGene, $P \leq 0.05$). Data was transformed using Microsoft Excel (Microsoft, Tacoma, Wash.). Normality of untransformed and of transformed data was examined using Mapmaker QTL version 1.9 (M.J. Daly, Whitehead Insititute, Massachusetts Institute of Technology).

Linkage-map construction

Recombination fractions between pairs of linked markers were calculated using the software packages MAPMAKER Macintosh V2.0 and MAPMAKER/EXP version 3.0 on a Sun II workstation (Fremont, Calif.). The two estimates were in good agreement. All AFLP and SSR markers were assigned to linkage groups (LGs) by pairwise comparison (''group'' command) with an initial threshold LOD-score of 5.0. This unusually conservative LOD-score threshold for a genome of ten chromosomes and approximately 1500 cM was necessary to avoid spurious associations of genome regions due to a subset of AFLP markers showing severely distorted segregation. A framework linkage map consisting of highly informative and well-spaced SSR markers was established for each suspected LG by analysis with the commands ''group'' (a threshold LOD-score of 7.5 to 10.0), ''first order'', and ''ripple''. The relative order of these SSRs was consistent with the maps of Kong et al. (2000) and that of Bhattramakki et al. (2000), although several of the ten LGs of the previous map were represented here in two-to-three subsets. The remaining markers in a group were ordered with respect to the framework map using the commands ''near'', ''try'', and ''ripple''. The order of each marker on a LG was established at a LOD-score greater than 3.0. The command 'drop marker' was used on each LG to identify markers that added a significant length (e.g., greater than 3 cM) to the framework map and, hence, may represent markers with questionable data points (apparent high double-crossover frequencies). Data from each LG were re-examined at this point for scoring errors that resulted in apparent double-crossover events. Errors were corrected and the linkage map was re-examined for relative marker order and recombination frequency. At this point, the linkage map consisted of 15 LOD-score 3.0 groups with several groups easily recognizable as subsets of LG B, LG D and LG E of the map of Bhattramakki et al. (2000). To merge these subsets into ten LGs, the distal markers of each LG subset were ordered using the commands ''try'' and ''ripple'' (LOD-score greater than 3.0). Finally, the order of markers in each of the ten LGs was confirmed using the ''order'' command (MAPMAKER/EXP, LOD-score 3.0, and theta 0.2). The Kosambi mapping function was used to convert recombination frequencies to map distances in cM (Kosambi 1944).

QTL analyses

The analyses of QTLs linked to markers for each trait in the RIL population was performed using both single-point regression analyses (QGene, Nelson 1997) and interval mapping using both QGene and MAPMAKER/QTL version 1.9. In simple regression analysis, significant ($P \leq 0.001$) differences in marker-class means were interpreted to indicate potential linkage of the genes controlling the trait with the marker locus. The confirmation of a QTL required detection using interval mapping at a LOD-score threshold of 2.8**.** This relatively stringent LOD-score threshold was chosen

Table 1 Means, standard deviations, and ranges of quantitative traits for recombinant inbred lines (F_5) derived from Sureño and RTx430. Bv. = Beeville, Tex.; C.C. = Corpus Christi, Tex.; C.S. = College Station, Tex.; Lub. = Lubbock, Tex.

Item	Sureño Mean ± SD	RTx430 Mean ± SD	*t*-test [a]	MP [b]	Range	F_5 Mean ± pop. SD
Plant height (cm)						
Lub. '98	71 ± 8	41 ± 4	**	57	32–88	52 ± 10
C.S.'98	72 ± 12	45 ± 4	**	59	31–91	56 ± 12
Peduncle length (1–5)						
Bv. '97	4.9 ± 0.2	3.0 ± 1.2	**	4.0	1–5	3.5 ± 1.3
C.S. '97 irrig.	4.9 ± 0.2	3.4 ± 0.5	**	4.1	1–5	3.0 ± 1.4
C.S. '97 non-irrig.	4.9 ± 0.3	3.0 ± 0.4	**	4.0	1–5	3.0 ± 1.6
Grain mill hardness (T.A.D.D.)	12.7 ± 0.2	16.6 ± 1.3	**	14.6	9.2–25.4	15.2 ± 3.2
Grain density (g/cm^3)	1.37 ± 0.0002	1.365 ± 0.0004	*	1.368	1.335–1.386	1.362 ± 0.011
Grain-mould (1 – 5)						
Bv. '97	1.6 ± 0.2	4.8 ± 0.3	**	3.2	2.0–5	3.2 ± 0.6
C.C. '97	1.9 ± 0.2	4.3 ± 0.2	**	3.1	2.1–4.5	3.1 ± 0.5
C.S. '97 non-irrig.	1.8 ± 0.2	4.2 ± 0.3	**	3.0	2.1–4.5	3.0 ± 0.5
C.S. '97 irrig.	2.1 ± 0.1	4.5 ± 0.1	**	3.3	2.2–4.6	3.4 ± 0.5
C.S. '98	2.3 ± 0.2	4.3 ± 0.1	**	3.3	1.9–4.4	3.0 ± 0.5
Anthracnose (1–5) [c]	1.6 ± 0.2	2.9 ± 0.3	**	2.2	1.4–3.4	2.5 ± 0.5
Zonate leaf spot (1–5) [c]	1.1 ± 0.1	2.8 ± 0.3	**	2.2	1.4–3.4	1.75 ± 0.5
Bacterial leaf stripe (1—5)	1.3 ± 0.6	3.3 ± 0.4	**	2.5	1–5	2.75 ± 1.1

[a] Significance for *t*-test of parental means, * $P \leq 0.01$ and ** $P \leq 0.001$; [b] MP, mid-parent; [c] Anthracnose and zonate leaf spot represent the mean of three locations

based on the map density and population size (Lander and Botstein 1989) and the reduced variance associated with a RIL population (M.J. Daly, Whitehead Institute, Massachusetts Institute of Technology, personal communication). For those traits that showed consistent detection of QTLs across environments, a combined analysis (mean over environments) was conducted for QTL analyses. The percentage of phenotypic variation explained (PVE) was obtained by MapMaker/QTL (interval analysis using ''sequence'' and ''map'' commands). The total PVE by all QTLs was obtained in Mapmaker QTL by fitting a model including all putative QTLs for a given trait. In interval mapping, data transformation for each trait was tried to improve the normality of the distributions of the traits, and transformed data were also subjected to QTL analysis. For most traits, transformed and untransformed data gave similar results in QTL identification. For each trait, two-way interactions were tested between significant markers associated with QTLs and all other marker loci in the genome using the interaction function of QGene (Nelson 1997).

Results

Trait variation and distribution

The parental lines of the RIL population, Sureño and RTx430, were significantly different for most traits examined (Table 1). Sureño exhibited a consistently lower incidence of grain-mould in all environments tested. The grain of RTx430 showed extensive discoloration, and deterioration of the grain was prevalent, whereas the grain of Sureño was free of mould or showed only a slight discoloration. Sureño also exhibited less-severe symptoms for the foliar diseases of anthracnose, bacterial leaf stripe and zonate leaf spot, in all environments examined. Sureño was largely free of foliar disease or showed slight symptomatic development, whereas foliage of RTx430 clearly displayed symptoms indicating an intermediate-to-moderate level of susceptibility. Sureño was significantly taller than RTx430 with height differences averaging 27 to 30 cm, and panicle peduncle lengths ranged 5–10 cm longer for Sureño compared to RTx430. Differences in grain-milling hardness between the parental lines were detected, with Sureño grain being more resistant to abrasion. The parental lines showed minimal differences in grain density with the grain of each parental line being moderately dense.

Differences amongst the RILs were observed for all traits examined (Table 1). The distribution of the traits examined showed continuous variation, which is consistent with the suggestion of polygenic inheritance and/or environmental variance for these traits (data not shown). In general, the population average (mean and median) was similar to the mid-parent value for all traits quantified. Transgressive segregants were not observed for grain-mould incidence as the parental lines represented the phenotypic extremes for grain-mould severity. Transgressive segregation for plant height and foliar disease incidence was nominal as the range of scores of the RIL population was similar to the values of the parental extremes (although several RILs showed extreme symptoms of bacterial leaf blight). Transgressive segregants were detected for panicle peduncle length with RILs showing shorter peduncles than RTx430. The greatest transgressive segregation was detected for the kernel traits of grain density and grain-milling hardness. Despite minimal difference in the grain densities of the parental lines, the grain of numerous RILs exhibited densities that

Table 2 Correlation coefficients (*r*) among morphological traits for the 125 F_5 lines derived from the cross of Sureño and Rtx430. Bv. = Beeville, Tex.; C.C. = Corpus Christi, Tex.; C.S. = College Station, Tex.

Trait	1	2	3	4	5	6	7	8	9	10	11	12
Grain-mould												
Bv. '97 (1)												
C.C. '97 (2)	0.623**[a]											
C.S. '97 non-irrig. (3)	0.545**	0.847**										
C.S. '97 irrig. (4)	0.600**	0.771**	0.758**									
C.S. '98 (5)	0.465**	0.540**	0.542**	0.550**								
Plant height [b] (6)	–0.627**	–0.602**	–0.434**	–0.581**	–0.418**							
Peduncle length [b] (7)	–0.352**	–0.286**	–	–0.337**	–	0.356**						
Grain density (8)	–0.266*	–0.285*	–	–0.277*	–	–	–					
Milling hardness (9)	–	–	–	–	–	–	0.409**	–0.320**				
Plant color (10)	–	–	–0.278*	–	–	–	–	–	–			
Anthracnose [c] (11)	–	–	–	–	–	–	0.250*	–	0.286*	–0.697**		
Zonate leaf spot [c] (12)	0.294**	–	0.246*	–	–	–	–	–	–	–0.528**	0.499**	
Bacterial leaf stripe (13)	–	–	–	–	–	–	–	–	–	–0.546**	0.524**	0.351**

[a] Significance for correlation coefficients, * $P \leq 0.01$ and ** $P \leq 0.001$
[b] Plant height and peduncle length represent the mean of two and three locations, respectively
[c] Anthracnose and zonate leaf spot represent the mean of three locations

were more extreme than that of the parental lines. Similarly, the range of grain-milling hardness of the RILs was significantly greater than that of RTx430 and Sureño (Table 2).

Trait correlations

The correlation between phenotypic traits was evaluated by regressing phenotypic values of one trait on those of all other traits. A total of 34 phenotypic correlations were significant (27 correlations at $P \leq 0.001$, 7 at $P \leq 0.01$). Mould-incidence scores of the RIL population were positively correlated across all five test environments. The highest correlation coefficients were observed for the 1997 mould-incidence scores obtained at the two College Station environments (with and without sprinkler irrigation) and the Corpus Christi environment (*r*-values between 0.758 and 0.847). Mould susceptibility scores in 1997 at the Beeville environment were also positively correlated with 1997 environments at Corpus Christi and College Station (*r*-values between 0.545 and 0.623). The greatest difference in mould-susceptibility scores amongst the RILs was observed when comparing 1997 environments to the one environment in 1998 at College Station (*r*-values between 0.46 and 0.55). The differences observed in mould scores between the 1997 and 1998 environments most likely reflect significant variation in pathogen pressure and climatic conditions between the 2 years.

At all test environments, grain-mould severity was negatively associated with plant height (taller lines, less mould incidence). The strongest association between plant height and mould severity was observed under overhead sprinkler irrigation (College Station environment, *r*-value –0.627). A modest association was observed between the length of the panicle peduncle and grain-mould severity in three of the five environments examined (*r*-values 0.286 to 0.352). A weak association was observed between grain density and grain-mould severity (*r*-values 0.266 to 0.285) while the association between milling hardness and grain-mould incidence was not significant. The kernel traits of grain density and milling hardness showed a weak association as denser grain tended toward greater resistance to milling abrasion.

A positive correlation was observed amongst the foliar disease ratings for anthracnose, zonate leaf spot and bacterial leaf stripe (*r*-value range, 0.351 to 0.524). Additionally, variation in plant color was strongly associated with the severity of foliar disease symptoms. Plant color is a qualitatively inherited trait with Sureño exhibiting a tan plant color and RTx430 a purple plant color. In the cross between Sureño and RTx430, tan plant color was associated with less-severe symptom development for all foliar diseases examined (*r*-value range, 0.546 to 0.697). Grain-mould resistance and tan plant color were also associated in one environment (Beeville Tex., $P = 0.002$) though the correlation coefficient indicates a rather weak association between these two traits (*r*-value, 0.278).

Genetic marker segregation and linkage map construction

Of the 115 microsatellite (SSR) markers examined, 50% showed clear polymorphism between Sureño and RTx430. Due to the moderate degree of polymorphism between the parental genotypes, larger numbers of SSRs were not mapped in this RIL population. Never-

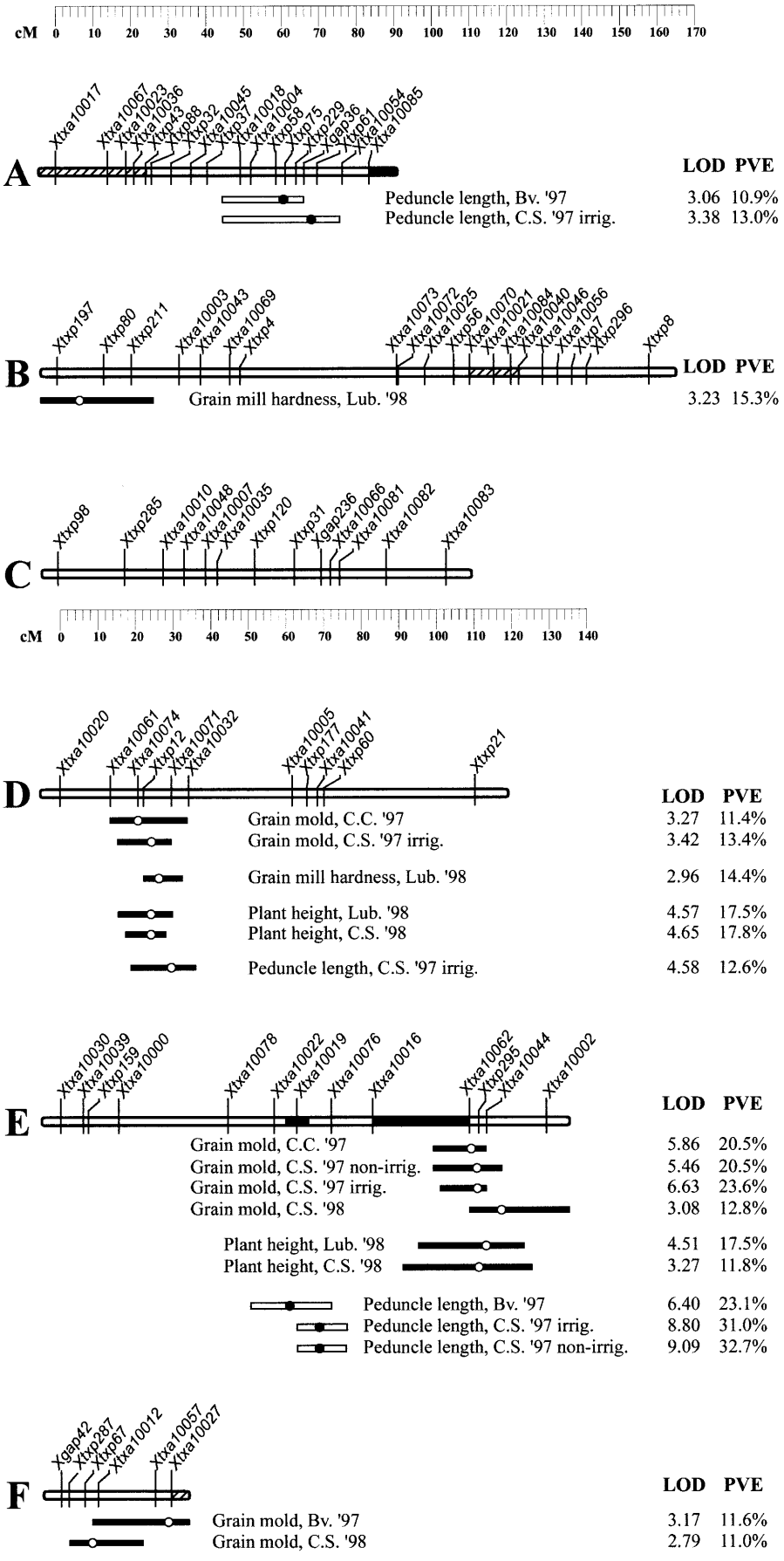
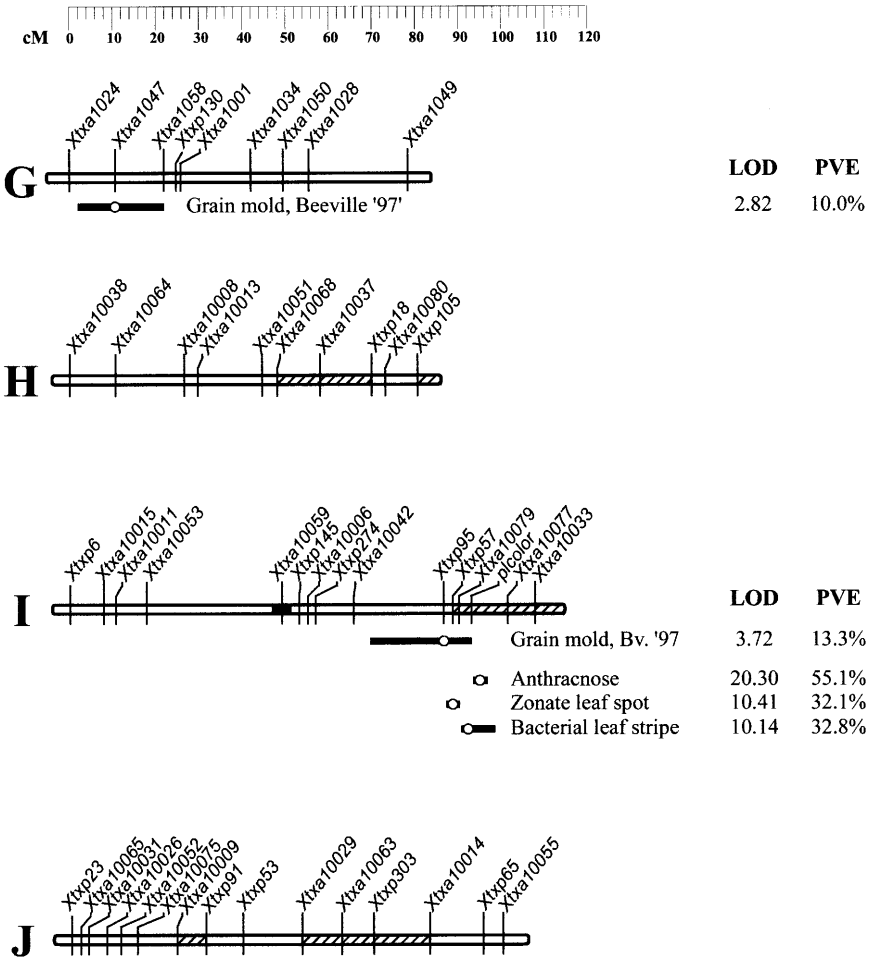


Fig. 1A–J Sorghum linkage map and locations of putative QTLs. The centimorgan scale is given at the top with distances in Kosambi centiMorgans. The letter designation of each LG (*A–J*) is based on the notation of Peng et al. (1999). *Shaded or hatched regions* of LGs indicate markers showing segregation distortion towards either the RTx430 (*hatched*) or Sureño (*shaded*) allele. Genetic markers with the prefix *Xtxp* or *Xgap* designate microsatellites while AFLP markers are designated by the prefix *Xtxa*. *Bars* show positions of QTLs with the peak LOD-score identified by the symbol 'o'. QTL bars represent a one-LOD-score confidence interval surrounding the peak score. *Darkened bars* represent trait QTLs affected by the Sureño allele, while *open bars* represent QTLs affected by the RTx430 allele

Fig. 1G–J



theless, 44 polymorphic SSRs provided a framework for the placement of AFLP markers. Utilizing 32 AFLP primer combinations (+3/+3 selectable primers) a total of 157 informative AFLP markers (polymorphic, unambiguous allelic determination, acceptable segregation distortion) were scored in the mapping population. The resulting LOD-score ≥ 3.0 linkage map placed 130 loci (44 SSRs, 85 AFLPs, one morphological-trait locus) on ten LGs spanning a genetic length of 970 cM with an average distance between markers of 7 cM (Fig. 1). There are seven gaps of greater than 25 cM between LOD-score ≥ 3.0 markers including gaps on LG B (32 cM), LG D (two gaps, 27 cM each), LG E (two gaps of 26 and 29 cM), LG G (23 cM) and LG I (32 cM). Deviation from the expected 1:1 segregation ratio of alleles ($P \leq 0.05$) was detected at 29 loci with the distortion favoring the RTx430 allele at 24 of these loci. Loci showing segregation distortion were largely clustered on LG A, LG B, LG E, LG H, LG I and LG J. The linear order of SSR markers for the present RI map were in good agreement with that of the high-density linkage map derived from a cross between BTx623 and IS3620 C (Bhattramakki et al. 2000). The map of Bhattramakki et al. (2000) contains 323 RFLPs and 147 SSRs covering ten LGs and 1406 cM. Several regions of the sorghum map presented here are not as well-represented as on the genetic linkage map of Bhattramakki et al. (2000). By comparing the coverage of SSRs between the two maps, under-represented map regions were detected in gaps (noted above) and on the distal ends of LG A, LG C, LG D and LG F. Residual heterozygosity in the mapping population was higher than expected for all co-dominant markers examined (actual 13.3%, theoretical 8.125%). Residual heterozygosity in the mapping population resulted in marker-scoring ambiguity since AFLPs are scored mostly as dominant markers. This ambiguity, and the modest level of polymorphism between the parental genotypes, reduced the number of markers that could be placed in the framework map (LOD-score ≥ 3.0) and most likely reduced the overall coverage of the genome. Nevertheless, many QTLs of interest should be linked to markers in this framework map, although some QTLs may go undetected as map-coverage is not complete.

Mapping QTLs underlying traits

For each trait, single-point analysis and interval mapping assessed the linkage of QTLs to molecular markers

mapped in the RIL population. Because single-point analysis ($P \leq 0.001$) and interval analysis (LOD-score ≥ 2.8) gave nearly identical results in identifying QTLs for each trait, graphical presentations only for interval analysis are presented here.

QTLs that influence grain-mould incidence and associated traits

Interval mapping and single-factor analysis of variance identified five QTLs with significant effects on mould-disease incidence. Grain-mould incidence QTLs were detected on LG D, LG E, LG F, LG G, and LG I, with each QTL accounting for 10 to 24% of the phenotypic variation (Fig. 1). At each locus, reduced mould incidence was associated with the Sureño allele. In agreement with an environmental interaction with grain-mould incidence, the detection of a given QTL was dependent on the location and year tested. In Corpus Christi or in College Station under sprinkler misting conditions (1997), QTLs on LG D and LG E were associated with reduced grain-mould incidence. In combination, the two QTLs accounted for between 29 and 32% of the phenotypic variation in these environments. In College Station environments without sprinkler irrigation, the mould-incidence QTL on LG E accounted for 20% (1997 environment) and 13% (1998 environment) of the phenotypic variance in grain-mould incidence. Without sprinkler irrigation at the College Station location, the grain-mould incidence QTL on LG D was not significant at the present LOD-score threshold (1998 environment, LOD-score 2.4). In the Beeville environment, the QTLs on LG D or LG E did not contribute to grain-mould incidence (LOD-score of 1.9) although three QTLs were detected for this environment on LG F, LG G and LG I. Individually, each QTL accounted for between 10 and 14% of the phenotypic variation and, collectively, the three loci accounted for 30% of the phenotypic variance at the Beeville location. While the QTLs on LG G, and LG I were unique to the Beeville environment, a grain-mould incidence QTL on LG F was detected at College Station (1998, non-irrigated) with a slightly reduced LOD-score threshold of 2.7. This College Station-mould QTL mapped to the same general location on LG F as one of the three QTLs affecting grain-mould incidence at the Beeville location.

As plant phenology has been implicated in contributing to grain-mould incidence, QTLs for plant stature (plant height, panicle peduncle length) and kernel characteristics (grain-milling hardness, grain density) were mapped to examine their relationship to QTLs for grain-mould incidence. QTLs for plant height were detected on LG D and LG E, and collectively accounted for 32% of the phenotypic variation. The QTLs for height on LG D corresponded closely with QTLs affecting grain-mould incidence in the Corpus Christi environment and under the sprinkler-irrigated environment at the College Station. The QTL for plant height on LG E corresponded closely to grain-mould incidence QTLs in four of the five environments examined. The association between QTLs for plant height and grain-mould incidence on LG D and LG E accounts for the strong correlation between these traits (see Table 2). In each case, the allele associated with greater plant height (Sureño allele) were associated with reduce grain-mould incidence.

Markers linked to QTLs influencing panicle peduncle length (panicle exsertion) were mapped in this RIL population. QTLs for peduncle length were detected on LG A, LG D and LG E. Collectively, the three QTLs for pancile peduncle length accounted for 51% of the phenotypic variance (under irrigated conditions at College Station). The QTL for panicle peduncle length on LG E was observed in all three test environments, accounting for 23 to 33% of the phenotypic variance. This genomic region for peduncle length did not coincide with the region of LG E that influenced grain-mould incidence and plant height. By contrast, the genomic region on LG D that influenced panicle peduncle length was coincident with QTLs for plant height and grain-mould incidence. The LG D QTL for peduncle length showed an interaction with the environment as the QTL was detected at the College Station only under supplemental irrigation. Similary, the peduncle length QTL on LG A was observed in the Beeville environment and at the College Station with supplemental irrigation, but not in the latter location without irrigation. The interaction of the QTLs for peduncle length with the environment is consistent with the observed effect of moisture on panicle exsertion (see discussion below). For both the peduncle length QTLs on LG E and LG A, the RTx430 allele conferred a longer peduncle and, hence, a greater panicle exsertion. For the region of LG D that influenced peduncle length, plant height and grain-mould incidence, the Sureño allele conferred longer panicle peduncles, greater plant height, and reduced grain-mould incidence.

The genome was also scanned for the kernel characteristics of grain-milling hardness and grain density. Grain-milling hardness QTLs were detected on LG B and LG D. Collectively, the QTLs of LG B and LG D accounted for 27% of the variance in grain-milling hardness. The Sureño allele conferred increased milling hardness for both genomic regions. Of the milling hardness QTLs detected, only the locus on LG D was associated with a region of the genome containing a grain-mould incidence QTL. This same region of LG D also encoded loci that controlled plant height and panicle exsertion. Transgressive segregants in grain-milling hardness were observed with RILs exhibiting extremes in hardness greater than those displayed by the parental lines. As no RTx430 alleles for milling hardness were detected, a significant portion of the observed transgressive segregation was most likely the result of environmental and $G \times E$ variance.

Scanning the genome for grain density did not reveal loci influencing this kernel trait. Grain density was obtained on seed stock from the two low-mould-incidence locations of Lubbock and Halfway, Tex. Using RIL seed stock from both locations, the genome was scanned for

regions that influenced grain density, but none were detected. In addition, grain-density ratings for individual RILs did not correlate well between locations (Halfway 1997, Lubbock 1998, *r*-value = 0.28). While the grain of the parental lines displayed very similar densities, the grain of the RILs showed a wide range of extreme densities when compared to the parental lines. Collectively, these observations suggest that, in the present RIL population, a large portion of the phenotypic variance resulted from the influence of environmental factors on grain density.

QTLs that influence foliar disease incidence and other agronomic traits

A genomic region of LG I harbored disease-response QTLs for a series of unrelated foliar pathogens. The detection of the QTLs for a given foliar disease was consistent across different environments (data not shown). The phenotypic variation explained by the LG I disease-response QTLs ranged from 32% (bacterial leaf blight, zonate leaf spot) to 55% (anthracnose). Disease-response QTLs for other foliar diseases (e.g., oval leaf spot) were also mapped to this location of LG I (data not shown). Upon fixing the genomic region of LG I and re-scanning the genome, no additional disease-response QTLs were detected (data not shown). The correlation of disease ratings with other phenotypic traits indicated a strong relationship with the severity of foliar disease symptoms and the morphological trait of plant color in this RIL population (see Table 1). Plant color was scored as a qualitative trait (tan vs purple) and the marker was inserted into the genetic map as a morphological-trait locus (*plcolor*). Plant color mapped to the same region of LG I that contained the linked QTLs for foliar disease-symptom severity. The insertion of this morphological marker into the linkage map did not inflate the map length (e.g., minimal drop marker score), indicating no extraneous variation in scoring plant color. Tan plant color was associated with reduced foliar disease symptoms with Sureño contributing the allele for both traits.

Epistasis

Markers associated with each of the significant QTLs were tested for possible two-way interactions with all other markers in the genome. Only 5.5%, 0.8%, and 0.09% of the pairwise tests were significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively, which are close to the frequencies that would be expected by chance. Markers with significant interactions rarely coincided with other markers associated with other QTLs for the same trait. The sole exception was a positive interaction between markers for mould incidence QTLs on LG D and LG E for the College Station environment in 1998.

Discussion

The parents of the RIL population were the inbred line RTx430 and the variety Sureño. RTx430 is a widely adapted inbred line with excellent combining ability, and is a common and popular pollinator parent in many U.S. grain sorghum hybrids. RTx430, however, is highly susceptible to grain-mould. Sureño is a dual-purpose grain and forage variety with moderate resistance to grain-mould. Sureño and RTx430 differ for several important agronomic and grain quality traits including grain-mould resistance, plant height, plant color, panicle peduncle length, grain-milling hardness, and the severity of foliar disease symptoms. From a cross between RTx430 and Sureño, the present RIL population of 125 $F_{2:5}$ derived-lines was created. All agronomic and grain quality traits examined (except plant color) showed quantitative variability in the RIL population, indicating the presence of multiple loci (QTLs) and/or environmental variance. Rodriguez-Herrera (1999) estimated a minimum of four genes segregating for grain-mould incidence in this RIL population. Because of the complex inheritance of grain-mould resistance and the large environmental effects in disease expression, obtaining genetic markers linked to QTLs influencing grain-mould incidence would facilitate breeding advances, particularly for white-grained cultivars. To this end, a genetic map of this RIL population was constructed and the genome was scanned for QTLs linked to grain-mould resistance and other important agronomic traits.

Genetic map construction

For QTLs mapping in the present RIL population, a genetic map was constructed *de novo*. The framework of the present genetic map was a set of sorghum microsatellites that were previously mapped in another RIL population of sorghum (Bhattramakki et al. 2000; Kong et. al 2000). The microsatellites permit the alignment and comparison of this low-density map with the dense map of Bhattramakki et al. (2000), which has 323 RFLPs and 147 SSRs covering ten LGs spanning 1406 cM at an average spacing of 3.1 cM between markers. In the RTx430 x Sureño RIL population, only 50% of the 115 microsatellites examined were polymorphic between the parental genotypes. Because of this moderate level of polymorphism and the resulting incomplete coverage of the genome by the SSRs, a genetic map consisting entirely of SSRs that gave adequate genome coverage could not be constructed. To ensure coverage of the sorghum genome with linked markers, AFLPs were used in conjunction with SSRs and a linkage map consisting entirely of PCR-based markers was constructed. AFLP genetic markers were chosen for genetic map closure as they represent a multiplex marker system that requires little DNA and, unlike RAPD markers, are both reproducible and robust markers in our laboratory. The resulting LOD-score ≥ 3.0 linkage map placed 130 mapped mark-

ers (44 microsatellites, 85 AFLPs, one morphological-trait locus) on ten LGs spanning a genetic length of 970 cM with an average distance between markers of 7 cM. The linear order of SSR markers for the present genetic map was in good agreement with that of the high-density linkage map of Bhattramakki et al. (2000). However, the genetic distances between SSRs in certain regions differed between the two maps. The differences in the total map distance between the present map and that of Bhattramakki et al. (2000) is most likely attributed to better coverage by the 570 markers utilized in the previous study and to differences in the populations (number of informative lines, level of polymorphism). Nevertheless, for exploratory mapping, the resolution and genome coverage of the present linkage map should be adequate to detect a significant portion of the major QTLs influencing grain-mould incidence and other agronomic traits.

Quantitative trait loci

The number of QTLs detected for a given trait examined ranged from zero (grain density) to five (grain-mould incidence). The percentage of phenotypic variance explained by a single QTL ranged from 11% (QTLs for several traits) to 55% (for anthracnose disease severity). Due to the relatively small size of the mapping population, only QTLs having relatively large phenotypic effects were most likely detected. QTLs affecting grain-mould (and other quantitative traits) with smaller effects probably went undetected at the LOD-score threshold for QTL detection. For many QTLs, effects were reasonably constant across environments, although some QTLs were specific for a given environment. For example, the environment influenced the detection of QTLs for grain-mould incidence, which is consistent with the variability across locations in climatic conditions and pathogen incidence. By contrast, QTLs for the severity of foliar disease symptoms mapped to the same genomic region in all environments examined. The trait levels in the parents, and the predominant directions of QTL effects, were consistent with what is generally known about the parental lines. Sureño was the primary source of QTL alleles for reduced grain-mould incidence, less-severe foliar disease symptoms, increased grain-milling hardness and increased plant height. In general, only small amounts of transgressive segregation were detected for most traits, which supports the observation that QTLs for many traits are attributable to only one parent. Transgressive segregation, however, was detected for panicle peduncle length, grain-milling hardness and grain density. Transgressive segregants in panicle peduncle length (panicle exsertion) resulted, in part, from Sureño and RTX430 each contributing alleles for this trait. In contrast, extreme phenotypes for grain-milling hardness and grain density appear to be due to the strong influence of environmental factors on the expression of these kernel traits rather than genetic contributions from each parental line (see below).

Quantitative trait loci for grain-mould incidence and related phenotypic traits

QTLs for grain-mould incidence were found on five LGs (D,E,F,G,I) and, in each case, the Sureño allele decreased grain-mould incidence. The relative position of QTLs on several LGs reflected the phenotypic correlation between grain-mould incidence and other phenotypic traits. The negative correlation between plant stature (plant height, panicle peduncle length) and grain-mould incidence was partially due to pleiotropic effects of a single locus and/or closely linked QTLs on LG D and LG E. LG E harbors a genomic region that influenced plant height and grain-mould incidence, with the Sureño allele controlling taller plants with a reduced incidence of grain-mould. Similarly, a genomic region of LG D contains QTLs for plant height, panicle peduncle length and grain-mould incidence, with the Sureño allele controlling taller plants with greater panicle exsertion and a reduced incidence of grain-mould. Whether these traits are controlled by a single locus on each LG or by several closely linked loci is unclear. It has been proposed that plant stature can influence the incidence of grain-mould in certain environments (Castor 1981; Rao and Rana 1989). In locations such as Corpus Christi and College Station, the microclimate nearer the soil surface (warm, high relative humidity, reduced air movement) is more conducive to grain-mould development. In support of this hypothesis, the strongest association between height and grain-mould incidence was under sprinkler-irrigated conditions at College Station. It is unclear why QTLs for grain-mould incidence did not correspond with height QTLs in the Beeville location (mould QTLs LG D and LG E, LOD scores 2.0). Using a larger population may reveal an association between plant stature and mould incidence across all environments. Unique climatic conditions at the Beeville location may also, in part, explain the lack of an association between plant stature and mould incidence (and the detection of three unique QTLs) at the Beeville location. Nevertheless, in four of the five test environments, QTLs for grain-mould incidence were coincident with at least one QTL for plant stature. Further studies are warranted to determine whether plant height and peduncle length have pleiotropic effects on grain-mould incidence or whether the association of these traits is due to linked loci. Mapping grain-mould resistance in a white grain sorghum population that does not segregate for plant height will provide a needed insight into the relationship of plant stature and grain-mould incidence.

As it is generally believed (Gueck and Rooney 1980; Esele et al. 1993) that certain kernel characteristics may act as structural defense mechanisms against grain-mould, the sorghum genome was scanned for QTLs influencing grain-milling hardness and grain density. Two loci were detected for grain-milling hardness; located on LG B was a QTL controlling about 16% of the phenotypic variance in milling hardness, while a second one on LG D controlled 15% of the phenotypic variance. Sureño contrib-

uted both alleles for increased grain-milling hardness, which is consistent with the phenotype of the parental lines. The QTL for grain hardness on LG D was associated with several other phenotypic traits including grain-mould, plant height and peduncle length. The only other QTL for milling hardness was not coincident with a locus influencing grain-mould incidence (LG B, LOD score 3.2). Scanning the genome for grain density did not reveal any QTLs influencing this trait in the present population. The low level of PVE for grain-milling hardness and the inability to detect loci influencing grain density may reflect the small phenotypic differences between the parental lines and the presence of large environmental determinants (see Table 1). As a consequence, the relationship between loci influencing grain-mould incidence and these kernel traits was inconclusive. In addition to kernel hardness and grain density, the kernel traits of grain-surface wax layer, thin pericarp, grain integrity, and antifungal proteins have been implicated in acting as defense mechanisms against grain-mould infestation. Mapping the QTLs influencing grain-kernel traits in a series of large populations may reveal a more accurate determination of the regions of the genome governing kernel traits and the role of kernel characteristics as structural defense mechanisms against grain-mould (Glueck and Rooney 1980). While a detailed understanding of the physiological basis of grain-mould resistance is not a prerequisite for marker-assisted breeding, selection of QTLs for grain-mould may adversely impact grain quality by altering linked kernel characteristics. Hence, elucidating the physiological basis for a genome region influencing grain-mould incidence will permit more informed decisions during the selection of QTLs for grain-mould and the impact of this selection on other kernel characteristics.

Quantitative trait loci for foliar-disease symptoms and plant color

Scanning the genome for QTLs influencing the incidence of foliar diseases revealed a region of LG I harboring disease-response QTLs for a series of pathogenic-unrelated foliar diseases. For each foliar disease examined, the Sureño allele decreased the severity of disease symptoms. Plant color mapped to LG I with tan color (Sureño allele) linked in coupling phase with QTLs influencing the symptom severity of some foliar diseases. Tan plant color marker was approximately 4.6 cM from the peak LOD score marker for QTLs influencing the severity of zonate leaf spot, while plant color was coincident with the peak LOD-score for anthracnose and bacterial leaf spot. Some breeders (Rana et al. 1976; Torres-Montalvo et al. 1992) have noted a relationship between tan plant color and apparent resistance to foliar and panicle disease. The association between the severity of leaf disease symptoms and tan plant color is suggestive of a relationship between plant pigmentation and the hypersensitive response in sorghum. Anthrocyanins produced in response to pathogen infection differ in the quantity and type of anthrocyanin elicited among tan, red and purple plants (R. Frederiksen, personal communication). The anthrocyanins elicited in red and purple sorghums can cause greater tissue necrosis in susceptible plants than the pigments elicited in tan plants. As a consequence, tan plants will exhibit an apparent general resistance to pathogen attack owing to an altered hypersensitive response when compared to red or purple sorghums. This observation would explain the linkage between the severity of symptoms for a set of pathogenic-unrelated diseases and the relationship between tan plant color and symptom development. Alternately, this region on LG I could harbor a cluster of disease-response loci as has been observed in other crop species (for review see McMullen and Simcox 1995).

Other agronomic traits and QTL × environment interactions

Panicle peduncle length (panicle exsertion) showed clear transgressive segregation as a result of both parental lines contributing alleles for greater length at different loci. Of the two parental lines, Sureño exhibited a greater peduncle length and was the source of the allele for this trait on LG D. Despite exhibiting a short peduncle, the inbred line RTx430 was the source of alleles for greater peduncle length on LG A and LG E. Collectively, the RTx430 alleles on LG A and LG E accounted for 36% of the phenotypic variance in peduncle length, while 51% of the phenotypic variance was accounted for by all three QTLs. We have noted that hybrid crosses involving RTx430 can exhibit greater panicle exsertion when compared to the inbred parents. The present quantitative trait loci data support this observation and provides a possible genetic explanation for these results.

We have also noted that, under conditions of limited moisture, panicle exsertion is suppressed. In support of this observation, the peduncle length QTLs on LG A and LG D were significant in the College Station environment with supplemental irrigation, but not at this location without irrigation. By contrast, the QTL influencing peduncle length on LG E was insensitive to moisture status, exhibiting the same level of significance at the College Station under both irrigated and dryland conditions. The reasons for these and other QTL × E interactions could range from direct influences of the environment on gene expression, to complex influences of the environment on plant growth and development. This study could not determine the direct cause of QTL × E interactions for peduncle length or other traits, including grain-mould incidence, but it demonstrates the importance of studying QTL effects in more than one environment. In a single environment, QTLs for grain-mould incidence and peduncle length would have gone undetected or the effects of primary QTLs could have been either over- or underestimated with respect to other locations or years. Hence, to give the breeder the ability to manipulate trait QTLs

during marker-assisted selection, it is necessary to quantify QTL interactions with the environment and to detail those environmental determinants that modulate the expression of trait loci.

Comparison to quantitative trait loci in other sorghum crosses

Previous studies have provided the basis for relating the plant-height QTLs reported herein and the *Dw* loci (Chittenden et al. 1994; Lin et al. 1995; Pereira and Lee 1995; Austin and Lee 1996; Peng et al. 1999). Based on these studies, the *Dw*3 gene corresponds to the QTL on linkage group D. This is consistent with the height genotypes of Sureño and RTx430; the lines have been reported to differ at their *Dw*3 loci with the RTx430 allele being recessive. According to the assumed parental genotypes, only the *Dw*3 locus is segregating in the present population although two QTLs for plant height were identified. Possible explanations are that the *Dw*2 locus is also segregating, or else that another unreported locus controlling plant height is segregating in this population. The second height QTL on LG E could correspond to *Dw*2 based on the correspondence between the LGs of Peng et al. (1999) and of Chittenden et al. (1994).

Rami et al. (1998) utilized two sorghum caudatum × guinea RIL populations to map QTLs influencing grain quality and grain-mould incidence. The authors suggested that the *B*2 allele (caudatum parent) conferring high tannin levels could be related to QTLs influencing grain-mould incidence and grain quality traits (e.g., hardness). Despite the use of a common caudatum parental line, the parents of only one population (population RIL 379) segregated for grain-mould incidence. Comparing the genomic regions influencing grain-mould incidence and grain hardness in RIL 379 to the RIL population of Sureño × RTx430, make it apparent that orthologous loci governing these traits were not identified. In the caudatum line, a genomic region on LG F harbored QTLs for numerous grain quality traits including grain-mould incidence, grain hardness/friability and the *B*2/*b*2 gene (presence of a high-tannin testa layer). No orthologous genomic region on the corresponding LG in the present population (LG B) was identified that controlled a major portion of the phenotypic variation for various kernel traits. This lack of orthologous loci is most likely due to differences in the genetic backgrounds utilized to map these traits in the present study and that of Rami et al (1998). In particular, the parental lines RTx430 and Sureño do not contain a pigmented testa and high tannin levels, and thus the physiological/morphological traits underlying resistance to grain-mouldiness may be unique to white-grained sorghums and those containing a pigmented testa (tannins). The influence of tannins and a pigmented testa partially explain the lack of orthologous kernel-hardness QTLs as tannins have also been associated with kernel quality traits. Unfortunately, few studies have attempted to identify loci governing grain-mould incidence and related grain quality traits in sorghum. As mouldy grains and high tannin caryopses are both major deterrents in grain sorghum utilization, further efforts are warranted to map genomic regions that reduce the incidence of grain-mould in genetic backgrounds lacking tannins and to utilize this knowledge to transfer mould resistance into elite sorghum germplasm.

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