Theor Appl Genet (2006) 112: 1295–1305
DOI 10.1007/s00122-006-0232-3

ORIGINAL PAPER

F. A. Feltus · G. E. Hart · K. F. Schertz
A. M. Casa · S. Kresovich · S. Abraham
P. E. Klein · P. J. Brown · A. H. Paterson

Alignment of genetic maps and QTLs between inter- and intra-specific sorghum populations

Received: 2 September 2005 / Accepted: 15 January 2006 / Published online: 21 February 2006

Abstract To increase the value of associated molecular tools and also to begin to explore the degree to which interspecific and intraspecific genetic variation in *Sorghum* is attributable to corresponding genetic loci, we have aligned genetic maps derived from two sorghum populations that share one common parent (*Sorghum bicolor* L. Moench accession BTx623) but differ in morphological and evolutionarily distant alternate parents (*S. propinquum* or *S. bicolor* accession IS3620C). A total of 106 well-distributed DNA markers provide for map alignment, revealing only six nominal differences in marker order that are readily explained by sampling variation or mapping of paralogous loci. We also report a total of 61 new QTLs detected from 17 traits in these crosses. Among eight corresponding traits (some new, some previously published) that could be directly compared between the two maps, QTLs for two (tiller height and tiller number) were found to correspond in a non-random manner ($P < 0.05$). For several other traits, correspondence of subsets of QTLs narrowly missed statistical significance. In particular, several QTLs for leaf senescence were near loci previously mapped for 'stay-green' that have been implicated by others in drought tolerance. These data provide strong validation for the value of molecular tools developed in the interspecific cross for utilization in cultivated sorghum, and begin to separate QTLs that distinguish among *Sorghum* species from those that are informative within the cultigen (*S. bicolor*).

Electronic Supplementary Material Supplementary material is available for this article at http://dx.doi.org/10.1007/s00122-006-0232-3 and is accessible for authorized users.

Communicated by F. Salamini

K.F. Schertz: deceased

F. A. Feltus · A. H. Paterson (✉)
Plant Genome Mapping Laboratory, University of Georgia,
111 Riverbend Road, Athens, GA 30602, USA
E-mail: feltus@uga.edu

G. E. Hart
Department of Soil and Crop Sciences, Texas A & M University,
TX 77843, USA

K. F. Schertz
United States Department of Agriculture, TX 77843, USA

A. M. Casa · S. Kresovich · P. J. Brown
Institute for Genomic Diversity and Department of Plant Breeding,
Cornell University, Ithaca, NY 14853, USA

S. Abraham
Rubber Research Institute of India,
686009, Kottayam, Kerala, India

P. E. Klein
Institute for Plant Genomics and Biotechnology,
Texas A & M University, Texas 77843, USA

Introduction

Sorghum (*Sorghum bicolor* L. Moench) is a staple cereal in arid and semi-arid regions of the world, and typically ranks fifth in importance among the world's cereals in annual tonnage (http://www.fao.org). Two high-density genetic maps have been completed for sorghum, including one derived from an interspecific cross between *S. bicolor* and *S. propinquum* consisting of 2,512 sequence-tagged loci spaced at 0.4 cM intervals on average (Bowers et al. 2003); and a *S. bicolor* intraspecific map with 470 RFLP and SSR markers spaced at 3 cM on average (Bhattramakki et al. 2000) and more than 2,500 AFLP loci (Menz et al. 2002). These represent the most detailed STS-based (RFLP) map (Bowers et al. 2003) and the most detailed SSR-based map (Bhattramakki et al. 2000; Menz et al. 2002) available for this important crop.

It has long been a concern for applied geneticists in many crop communities that many primary maps are based on wide crosses. In sorghum, many raise questions about the degree to which the *S. bicolor* × *S. propinquum* cross, in particular, is representative of the genome organization and gene function of the

cultivated gene pool. Even the intraspecific cross, although within the cultivated species (*S. bicolor*) is wider than most crosses routinely made in mainstream breeding programs.

A critical issue in the use of QTLs in breeding programs is that they can only be assumed a priori to be relevant to the cross and environment in which they were mapped. Comparison of QTL positions across mapping populations, species boundaries, and environments permits one to investigate the degree to which the underlying genes contribute to variation in the phenotype under different genetic backgrounds and environmental conditions. For example, a comparative mapping study of height and maturity QTLs across the Poaceae family revealed corresponding QTLs between sorghum, rice, and maize (Lin et al. 1995). A similar study identified corresponding flowering time QTLs between sorghum and sugarcane (Ming et al. 2002). Common genomic regions that frequently account for phenotypic variation in many different crosses are more probable targets for marker-assisted selection, and higher priorities for positional cloning.

Herein, we address these questions at two levels. First, to explore the transferability of molecular tools across the inter- versus intraspecific maps, we have mapped 106 common markers at well-spaced intervals in each of the two maps. This provides for the alignment of these two detailed sorghum maps, and the establishment of common nomenclature for chromosomes/linkage groups that is needed to organize sorghum genomics efforts generally.

Second, we explore similarities and differences in the QTL repertoires detected in these crosses. These populations each share one common parent, BTx623 (BT; *S. bicolor*; $2n = 2x = 20$), but differ by morphologically and evolutionarily-distant alternative parents, specifically *S. bicolor* accession IS3620C (IS) and *S. propinquum* (SP; $2n = 2x = 20$). This invites comparisons of factors that differentiate the two *Sorghum* species from one another. Many genetic loci controlling important agronomical quantitative traits have been mapped in sorghum as quantitative trait loci (QTL), including tillering (Paterson et al. 1995b; Hart et al. 2001), 'stay-green' (Tao et al. 2000; Xu et al. 2000; Haussmann et al. 2002; Sanchez et al. 2002), rhizomatousness (Paterson et al. 1995b), insect resistance (Agrama et al. 2002; Katsar et al. 2002), disease resistance (George et al. 2003) and plant height/maturity (Lin et al. 1995; Hart et al. 2001; Ming et al. 2002). By engaging both previously-published and new data (which reveals 68 new QTLs), a total of eight traits could be directly compared. Several statistically-significant correspondences, and additional suggestive correspondences are found, beginning the process of separating QTLs that contribute to morphological and physiological divergence among *Sorghum* species from those that contribute to diversity within the cultigen (*S. bicolor*).

Materials and methods

Mapping population and genetic map construction

The BT × IS population consisted of 137 F_{6-8} RILs mapped with a subset of 145 SSR/RFLP markers as previously described (Hart et al. 2001). The BT × SP population consisted of 370 F_2 progeny and was mapped with a set of 96 RFLP markers as previously described (Paterson et al. 1995b). Genetic maps were reconstructed using MAPMAKER/EXP v3.0b (Lander et al. 1987). Recombination frequencies were converted to centiMorgans using the Kosambi function (Kosambi 1944). The 145 BT × IS markers were a subset of a 2,926 marker high-density map (Bhattramakki et al. 2000; Menz et al. 2002). The 96 BT × SP markers were a subset of a 2,512 marker high-density map (Bowers et al. 2003). Fifty-three common markers from the two high-density maps were identified. In addition, 36 new SSR markers were mapped in the BT × SP population, and 15 SSR and 17 new RFLP markers were mapped in the BT × IS population as previously described (Bhattramakki et al. 2000). These markers were integrated into their respective high-density maps and provided an additional 53 common markers. In total, 106 markers common to both BT × IS and BT × SP were used to construct 'bridge maps' with the centiMorgans positions taken from the high-density maps. Both QTL maps and both bridge maps were visualized using CMAP software (http://www.gmod.org/cmap/index.shtml). This integration will also be deposited in Gramene.

Phenotype measurements

Trait means and standard deviations were calculated using Microsoft Excel (Microsoft, Tacoma, WA, USA). Not all traits were measured in replicate plots, so the absence of a replicate QTL is not indicative of a failure to replicate the QTL. Explanations of trait measurements are as follows:

BT × IS trait definitions

Parental lines and RILs were evaluated in 1994 at College Station (CS94) or Lubbock (LB94), Texas as previously described (Hart et al. 2001). *Culm height (CUH)*: Average of ten plants/plot in centimeter. Data are from 2 reps at LB94 and 2 reps at CS94. *Glume cover (GCV)*: Percentage of glume cover over the caryopsis, scored on a plot basis of ~50 plants. Data are from one rep at CS94 and one rep at LB94. *Glume persistence (GPE)*: Glume persistence after threshing. Seed from three plants/plot were rated on a 0–5 scale, with 0 = 0% kernels with glumes, 1 = 1–5%, 2 = 6–30%, 3 = 31–60%, 4 = 61–80%, and 5 = 81–90%. Data are from 1 rep at LB94. *Grain weight (GWT)*: Weight in lbs of threshed

heads from 10.5 feet of each plot (~40 plants). Data are from 2 reps at LB94. *Head exsertion (HEX)*: The extension of the head in relation to the position of the lowermost whorl of branches located above the flag leaf (+ value) or below the flag leaf (− value) was recorded in centimeter. Approximately 50 plants were measured and an average value recorded for each plot. Data are from 1 rep at LB94. *Head weight (HWT)*: Weight in lbs of unthreshed heads from 10.5 feet of each plot (~40 plants). Data are from 2 reps at LB94. *Height uniformity (HTU)*: Visual estimate of the average height in centimeter of ~50 plants in each plot. Data are from 2 reps at LB94. *Kernel weight (KWT)*: Weight in grams of 1,000 seed collected from each plot. Data are from one rep at LB94. *Leaf curve (LCV)*: Distance in centimeter, measured on one unobstructed plant/plot, from the tip of the third leaf to the junction of that leaf and the main culm. Data are from two reps at CS94. *Leaf Length (LLN)*: Average length in centimeter of the third leaf, measured from the tip to the main-culm junction, of three plants/plot. Data are from two reps at CS94. *Leaf pitch (LPT)*: Length in centimeter, for the same leaf as was measured for LCV, of the leaf blade from the apex of the naturally-curved leaf to the junction of the main culm. Data are from two reps at CS94. *Leaf scorch (LSC)*: Leaf surface area affected by insecticides as evidenced by chlorotic and/or necrotic tissue, scored on a plot basis (~50 plants) on a 1–5 scale with 1 = least burned and 5 = most burned. Data are from two reps at CS94. *Leaf senescence (LSN)*: Scored on a plot basis (~50 plants) at 134 days after planting, at or near full grain maturity on a 1–5 scale with 1 = least amount of green (and many leaves necrotic) and 5 = largest amount of green. Data are from two reps at CS94. *Leaf width (LWD)*: Average width in centimeter at the widest point of the third leaf of three plants/plot. Data are from two reps at CS94. *Maturity50 (MA50)*: Number of days from planting until 50% of plants flower. One rating/plot. Data are from 2 reps at LB94 and 2 reps at CS94. *Panicle width (PAW)*: Average of three plants/plot in centimeter. Data are from 2 reps at LB94 and 2 reps at CS94. *Tiller height (TIH)*: Height in centimeter of tallest basal tiller on basal-tillered plants. Average of ten plants/plot. Data are from 2 reps at LB94 and 2 reps at CS94. *Tiller number (TINB)*: Number of basal tillers per basal-tillered plant. Average of up to ten plants/plot. Data are from 2 reps at LB94 and 2 reps at CS94.

BT × SP trait definitions

Parental lines and F2 progeny were evaluated in 1992 near College Station, Texas (CS92) as previously described (Lin et al. 1995; Paterson et al. 1995b). *Culm height (CUH)*: as previously described (Lin et al. 1995; Paterson et al. 1995b). *Height uniformity (HTU)*: The standard deviation of main stem height, tallest tiller height, and shortest tiller height. *Kernel weight (KWT)*: Average of 1,000 seeds. *Leaf length (LLN) and leaf width (LWD)*: The fourth leaf below the flag leaf was measured at flowering, in centimeter. *Maturity*: Days to flowering (pollen shed) was scored for the main culm (*MA1*) and up to the first four flowering tillers (*MA5*) as described (Lin et al. 1995; Paterson et al. 1995b). *Tiller height (TIH)*: Height at flowering was scored for the first four flowering tillers as described (Lin et al. 1995; Paterson et al. 1995b). *Tiller number (TINB)*: Number of basal tillers per plant as described (Paterson et al. 1995a, b).

QTL analysis

Quantitative trait loci were detected using the interval mapping method implemented in MAPMAKER/QTL v1.1b (Lander and Botstein 1989). The genomes were scanned at 1 cM intervals using a significance threshold of LOD 2.5. 1-LOD and 2-LOD support intervals were determined.

QTL correspondence between the mapping populations

The hypergeometric probability function (sampling without replacement) allows for the formal determination of correspondence between QTLs (Larsen and Marx 1985). QTLs were assumed to be orthologous if they explained a significant portion of phenotypic variation for a directly comparable trait measured in both populations and the 1-LOD confidence intervals overlapped. The equation is as follows:

$$p = \frac{\binom{1}{m}\binom{n-1}{s-m}}{\binom{n}{s}}$$

n the number of intervals which can be compared (defined as 30 cM, approximating a QTL likelihood interval); m the number of 'matches' declared between QTLs (when 1-LOD likelihood intervals for two taxa overlapped); 1 = the total number of QTLs found in the larger sample; s the number of QTLs found in the smaller sample.

Results

Genetic map alignment

Linkage maps were reconstructed for both the BT × IS (Hart et al. 2001) and BT × SP (Paterson et al. 1995b) crosses. Due to the paucity of common markers between the QTL maps obtained from these two populations, we used marker information from high-density genetic maps (Menz et al. 2002; Bowers et al. 2003). By interleaving markers from the high-density maps with the subset of markers used in QTL

mapping, we identified 53 common markers in silico. In addition, 36 new SSR markers were mapped in the BT × SP population, and 15 SSR/17 RFLP markers were mapped in the BT × IS population resulting in 53 additional common markers. Thus, a total of 106 common markers were shared by the two maps, with 4–18 per linkage group, spaced at average intervals of 12.9 cM based on Bowers et al. (2003). Genetic map alignments are shown in Fig. 1 and detailed alignment with marker labels can be found in Supplemental Fig. 2. For each map, LG nomenclature was preserved from the original studies (e.g., BT × IS LG A = BT × SP LG C), and chromosome numbers are also shown as recently determined (Kim et al. 2005).

QTL scanning

Each population was scanned for QTLs using interval mapping as implemented in Mapmaker/QTL (Lander and Botstein 1989). Seventeen quantitative traits were analyzed in the BT × IS population, and eight quantitative traits were tested in the BT × SP population. Traits for which QTLs have not been previously published are listed in Table 1, and several traits were reanalyzed from previous studies (Lin et al. 1995; Paterson et al. 1995b; Hart et al. 2001). The relative QTL locations between the aligned genetic maps are presented in Fig. 1. Sixty-one novel QTLs (LOD > 2.5; includes replicates/dual environment QTLs) representing seventeen traits were detected (Table 2 and below).

Newly-reported QTLs

Glume morphology

A total of seven QTLs for glume cover and glume persistence were detected in the BT × IS cross. For glume cover, five QTLs (*QGcv.txs-Ba/Bb, QGcv.txs-C, QGcv.txs-Ia/Ib*) explained 35.9/24.2% of phenotypic variance (V_a) in two environments, with two QTLs corresponding in both environments. For glume persistence, two QTLs (*QGpe.txs-A, QGpe.txs-E*) explained 34.4% V_a. Glume morphology was not analyzed in the BT × SP cross.

Head exsertion

One QTL was detected for head exsertion in the BT × IS cross, *QHex.txs-C*, explaining 12.9% V_a. This trait was not analyzed in the BT × SP cross.

Seed size and morphology

Twelve QTLs (five in the BT × IS cross; seven in the BT × SP cross) were detected that contributed to phenotypic variance in grain weight/head weight and kernel weight. Ten kernel weight QTLs were detected. In the BT × IS cross, three QTLs (*QKwt.txs-D, QKwt.txs-G, QKwt.txs-I*) explained 35.2% V_a. In the BT × SP cross, seven QTLs (*QKwt.uga-A, QKwt.uga-B, QKwt.uga-C, QKwt.uga-D, QKwt.uga-E, QKwt.uga-F, QKwt.uga-J*) explained 48.6% V_a. For grain weight, one QTL *QGwt.txs-G*, explained 14.3% of phenotypic variance for the BT × IS cross—the same interval also accounts for a (presumably pleiotropic) head-weight QTL, *QHwt.txs-G*, explaining 13.9% V_a. Two pairs of kernel weight QTLs (*QKwt.txs-I, QKwt.uga-D; QKwt.txs-D, QKwt.uga-F*) showed correspondence between the two crosses (see below).

Leaf morphology

Thirty-two QTLs (24 in the BT × IS cross; 8 in the BT × SP cross) contributing to phenotypic variance in leaf length, pitch, width, scorch, senescence, and curve phenotypes were detected.

Three unique leaf length QTLs were detected in the BT × IS cross (*QLln.txs-F, QLln.txs-Ga/Gb, QLln.txs-Ha/Hb*), collectively explaining 26.3/17.8% V_a (two replicates). Two of the QTLs (*QLln.txs-Ga/Gb, QLln.txs-Ha/Hb*) were supported by data from each of two replicates. Two leaf length QTLs (*QLln.uga-F, QLln.uga-D*) were detected in the BT × SP cross, and together they explained 23.9% V_a. None of these QTLs corresponded between the two crosses (see below).

A total of ten leaf width QTLs were detected. Three loci (*QLwd.txs-Ea/Eb, QLwd.txs-F, QLwd.txs-H*) collectively explained 29.0/18.8% V_a (two replicates) in the BT × IS cross, with one QTL (*QLwd.txs-Ea/Eb*) supported by data from two replicates. For the same trait, six QTLs (*QLwd.uga-A1, QLwd.uga-A2, QLwd.uga-B1, QLwd.uga-B2, QLwd.uga-J, QLwd.uga-D*) were found in the BT × SP cross that collectively explained 52.2% of V_a. One QTL pair (*QLwd.txs-E*, *QLwd.uga-J*) showed correspondence between the two populations (see below).

Several additional leaf traits were measured only in the BT × IS cross. Two leaf-pitch QTLs (*QLpt.txs-D, QLpt.txs-G*) explained 20.7% of V_a. When the leaf pitch measurements were normalized to leaf length and reanalyzed, *QLpt.txs-G* no longer passed the significance threshold. One leaf scorch QTL, *QLsc.txs-B*, explained 8.5% V_a. Four unique leaf-senescence QTLs (*QLsn.txs-B, QLsn.txs-C, QLsn.txs-Ea/Eb, QLsn.txs-Fa/Fb*) explained 58.4/56.2% V_a (two replicates) with two QTLs corresponding between replicates. The replicated QTL on CHR07 (*QLsn.txs-Ea/Eb*) itself explained 31.3% V_a on average between replicated QTLs. Finally, six leaf-curve QTLs (*QLcv.txs-D1, QLcv.txs-D2, QLcv.txs-G, QLcv.txs-Ha/Hb, QLcv.txs-I*) explained 36.6/16.8% V_a (two replicates), with one (*QLcv.txs-Ha/Hb*) found in both replicates. However, only one QTL (*QLcv.txs-I*) was retained after correcting for total leaf length.

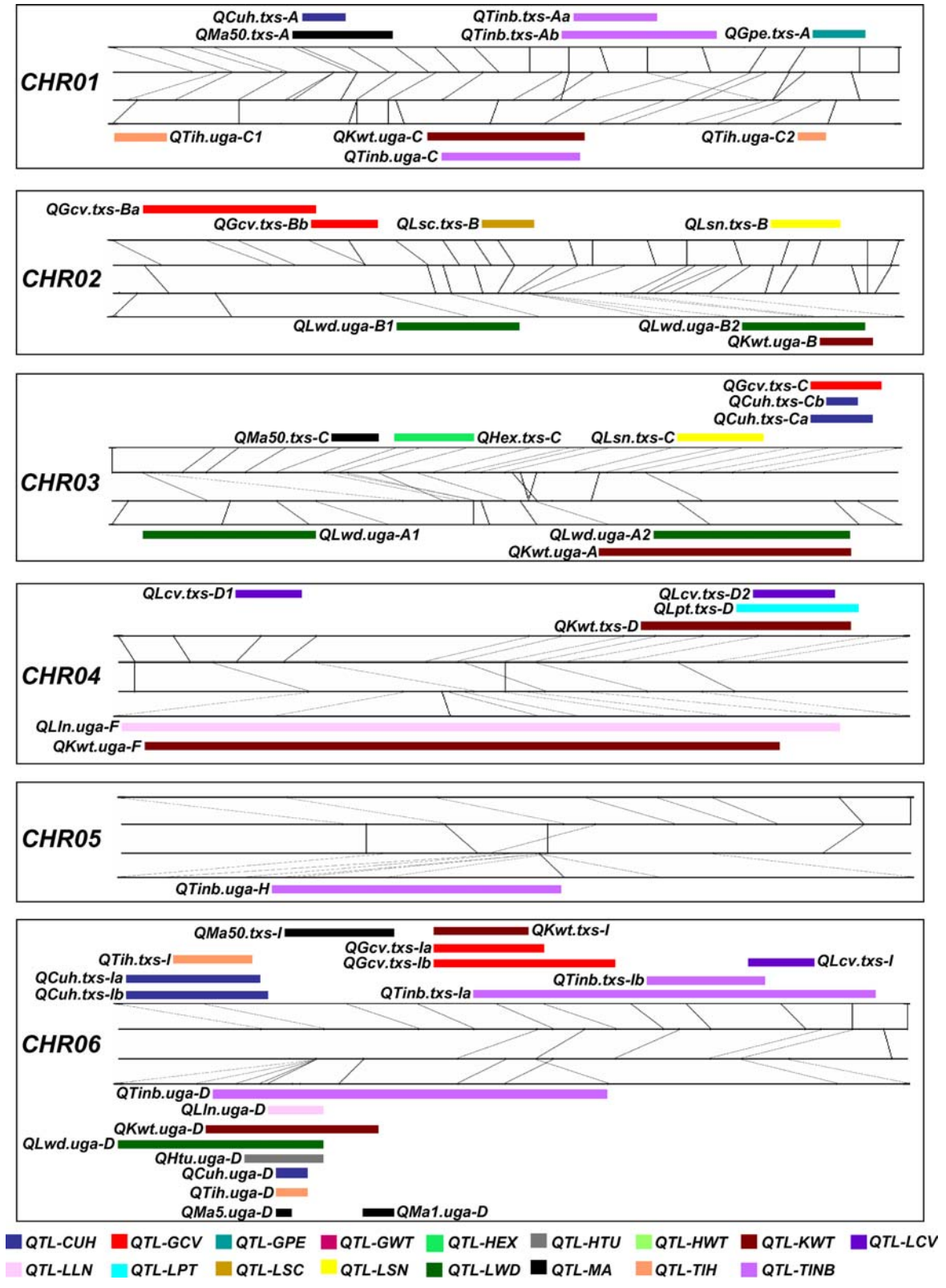

Fig. 1 Alignment of two sorghum genetic maps and locations of putative QTLs. Ten chromosomal genetic maps for BTx623/IS3620C (*top line*) and BTx623/*S. propinquum* (*bottom line*) populations are shown as *horizontal thin grey lines* connected by common markers. The *middle two horizontal lines* represent bridge maps derived from common markers and centimorgan positions from high-density genetic maps (see Materials and methods). QTLs are shown as *thick horizontal lines* with BTx623/IS3620C QTLs above the chromosome (labeled with *txs*) and BTx623/*S. propinquum* and shown below the chromosome (labeled as *uga*). Alignments for the entire genome are available from Gramene

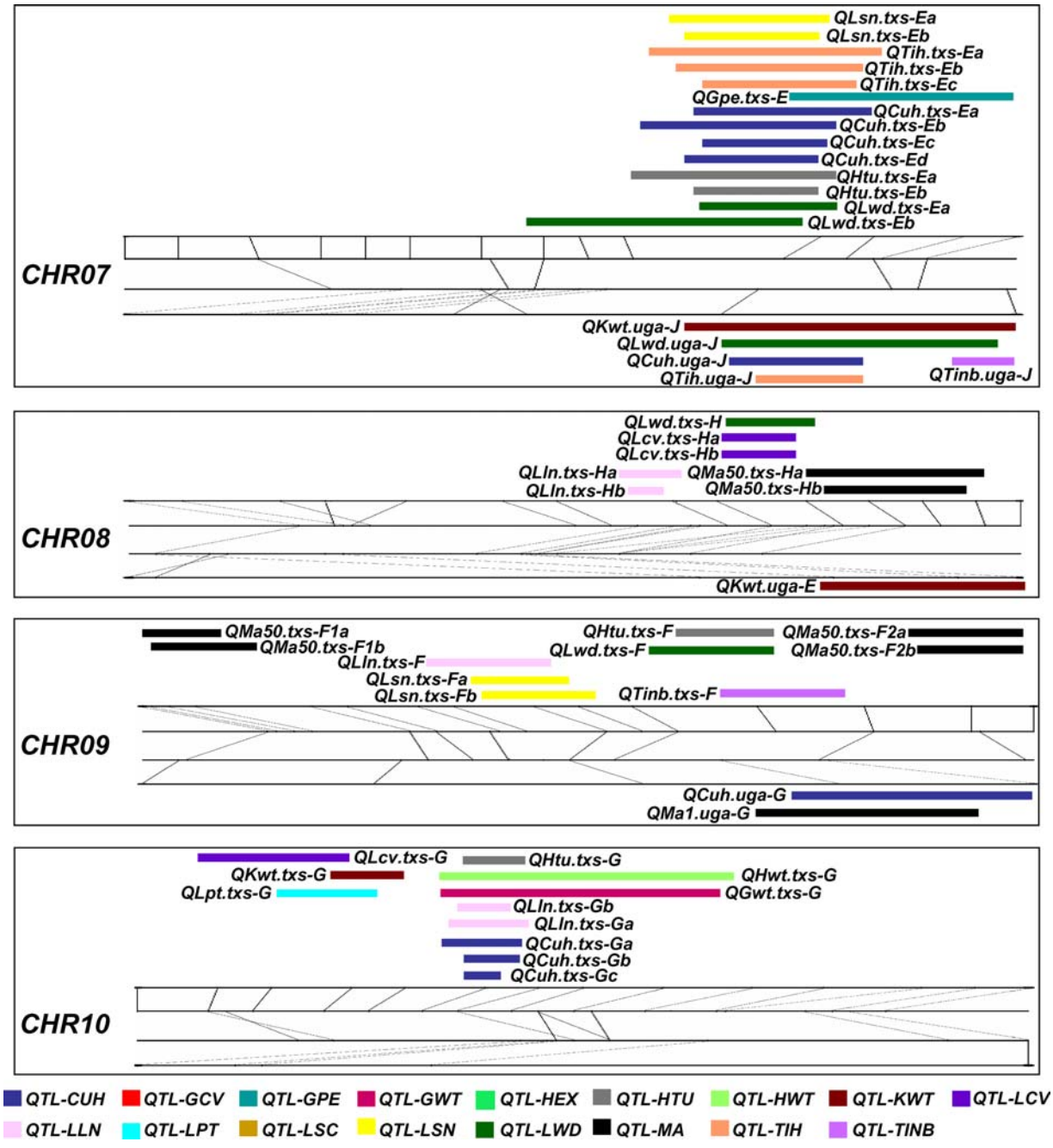

Fig. 1 (Contd.)

Tiller height and uniformity

Four QTLs were detected for tiller height in the BT × SP cross. *QTih.uga-C, QTih.uga-C2, QTih.uga-D, QTih.uga-J* explained 20.4% V_a. For height uniformity, four QTLs in the BT × IS cross (*QHtu.txs-Ea/Eb, QHtu.txs-F, QHtu.txs-G*) explained a total of 62.4/25.9% V_a (two replicates) with one QTL found in both replicates. The height-uniformity QTL with the largest effect (*QHtu.txs-Ea/Eb*) was replicated on CHR07 and by itself explained 30.0% V_a (based on the average of the two replications). A single height uniformity QTL (*QHtu.uga-D*) was detected in the BT × SP cross.

Correspondence among QTLs

Eight traits were comparable in both studies, and the overlap of comparable QTLs derived from these traits was determined (BT × IS = 28 QTLs; BT × SP = 30 QTLs). The hypergeometric probability distribution (Larsen and Marx 1985) was employed to generate a

Table 1 New traits examined in two sorghum crosses

Trait	Site[a]	μ[b]	σ[c]	Range	QTLs[d]	V_a^e
Glume cover	LB94/CS94	63.5	16.3	20–100	3/2	35.9/24.2
Glume persistence	CS94	2.7	1.0	1–6	2	34.4
Grain weight	LB94/Lb94	2.8	0.9	0.51–5.19	1/0	14.3
Head exsertion	LB94	2.6	8.6	−20–28	1	12.9
Head weight	LB94/Lb94	3.5	1.1	0.65–6.52	1/0	13.9
Height uniformity	LB94/Lb94	135.6	40.9	67–255	3/1	62.4/25.9
Kernel weight	CS94	17.2	2.7	9.73–25.26	3	35.2
Leaf curve	CS94/CS94	18.0	5.3	4–29.5	4/2	36.6/16.8
Leaf length	CS94/CS94	67.0	8.5	41–89.3	3/2	26.3/17.8
Leaf pitch	CS94/CS94	31.7	6.6	16–52.5	2/0	20.7
Leaf scorch	CS94/CS94	2.2	1.0	1–5	1/0	8.5
Leaf senescence	CS94/CS94	2.5	1.3	1–5	3/3	58.4/56.2
Leaf width	CS94/CS94	6.4	0.8	4.3–8.9	2/2	29.0/18.8
Tiller height	CS92	310.2	92.4	105–480	4	20.4

[a]Experimental location (see Materials and methods). Values separated by a forward-slash represent multiple replications/environments
[b]Average trait value
[c]Standard deviation
[d]Number of QTLs identified (LOD >2.5)
[e]Percent variance explained by full sets of QTLs found in respective environments (as determined using Mapmarker-QTL)

formal statistical test of the likelihood that overlap of the QTL-likelihood intervals could be explained by chance as described (Paterson et al. 1995a). Of 11 QTL pairs found to overlap, 4 pairs representing 2 traits corresponded more frequently than would be expected by chance (Table 3; $P<0.05$). These traits include tiller height (2 QTLs; $P=0.014$) and tiller number (2 QTLs; $P=0.038$). Seven overlapping QTL pairs explaining partial phenotypic variance for five other traits were found. Correspondence among six QTLs for culm height (2), maturity (2), and kernel weight (2) among the total of 18 detected for these traits narrowly missed statistical significance (0.062–0.119), while only one of 13 QTLs for leaf width, leaf length and height uniformity matched, clearly explicable by chance. It should be noted that two QTLs (*QTih.txs-A*, *QMa.txs-G*) that were detected in a previous study (Hart et al. 2001) were not detected in our re-analysis.

Discussion

The very close correspondence of the genetic maps derived from the BTx623 × IS3620C and BTx623 × *S. propinquum* crosses provides strong validation for the value of molecular tools developed in the interspecific cross for utilization in cultivated sorghum. The interspecific and intraspecific maps show a very high degree of colinearity. Of the 106 common markers found, only 6 pairs of consecutive markers show rearrangements in the most likely gene order: *Xumc152.1-Xumc10* (CHR03); *Xtxp343-Xcdo1380* (CHR04); *Xcdo475-Xtxp141* (CHR10); *Xrz476-Xcdo17* (CHR10); *Xtxp354-Xcdo459* (CHR08); *Xisu36.2-Xtxp225* (CHR05), most involving closely-spaced markers that could easily be due to error in one of the small mapping populations. In only one case did a marker, *Xumc167* (CHR01), map to a truly incongruous location on the corresponding linkage group. Due to multiple bands on the autoradiograph, the most likely explanation is that paralogous loci were mapped in the two populations. Clearly, there is a high degree of transferability of molecular tools between the interspecific and intraspecific sorghum maps. Moreover, the alignment of these maps, together with the recent publication of a system of chromosome numbers for sorghum (Kim et al. 2005), provides for a common system of nomenclature for use in the sorghum community and for comparison of the sorghum chromosomes to those of other taxa.

This colinearity also is a platform for the analysis of QTLs that differentiate *S. propinquum* from IS3620C, morphologically and evolutionarily-distant genotypes that represent wild and cultivated sorghums, respectively. We scanned each genome for QTLs representing 17 quantitative traits of which 14 traits had not been previously analyzed. This resulted in the discovery of 61 new QTLs. In addition, 58 QTLs representing eight corresponding traits were compared. These resulted in the identification of overlapping QTLs for two traits whose correspondence was greater than could be attributed to chance, and three more traits that narrowly missed significance.

The correspondence of QTLs for tiller height ($P=0.014$) and tiller number ($P=0.038$) suggests that the genetic basis of tillering is partly-overlapping in the divergent species. However, the lack of correspondence of QTLs for other traits in the two crosses has several possible interpretations. Genetic differentiation of IS3620C from *S. propinquum* may cause different sets of QTLs to segregate (or at least to produce most of the measured variation) in the two crosses. For example, the absence of QTL correspondence for leaf traits such as width ($P=0.408$) and length ($P=0.807$) implies that leaf development controls which differentiate between *S. bicolor* and *S. propinquum*, may be invariant (or largely so) between the two *S. bicolor* genotypes and

Table 2 QTLs identified in two sorghum crosses. Sixty-five QTLs (LOD > 2.5) identified in the BT×623/IS3620C cross and thirty QTLs in the BT×623/S. propinquum cross are listed by trait. [a]Measured trait. [b]Chromosome. [c]Quantitative trait locus. QTL symbols for the BT×623/IS3620C cross contain *txs* and BT×623/S. propinquum contain *uga*. The letter after the hyphen indicates linkage group location. [d]Highest LOD score between replicate QTLs. [e]Average percent variance explained by the QTL (*indicates average Va for replicate QTLs). [f]Number of replicated QTL. [g]Number of environments in which a QTL was found. [h]Marker interval where LOD score was highest. [†]Published QTL. [ψ]Comparable trait. QTLs connected with lines are correspondent

[a]Trait	[b]CHR	[c]QTL	[d]LOD	[e]V_a	[f]R	[g]E	[h]Interval
[ψ]Culm Height	1	*QCuh.txs-A*[†]	2.5	12.6	1	1	*Xumc104-Xumc167*
	3	*QCuh.txs-C*[†]	3.0	10.7*	2	1	*Xumc124.1-Xumc121*
	7	*QCuh.txs-E*[†]	8.1	24.3*	4	2	*Xtxp92-Xtxs1579*
	10	*QCuh.txs-G*[†]	3.6	10.4*	3	2	*Xumc109.2-Xumc21*
	6	*QCuh.txs-I*	3.3	12.2*	2	1	*Xcdo718-Xtxp6*
	7	*QCuh.uga-J*[†]	7.2	10.5	1	1	*pSB164-pSB815*
	9	*QCuh.uga-G*	4.5	7.3	1	1	*pSB416-pSB445*
	6	*QCuh.uga-D*[†]	47.4	50.4	1	1	*pSB189-pSB188x*
Glume Cover	2	*QGcv.txs-Ba*	2.5	10.1	1	1	*Xisu151-Xisu71*
	2	*QGcv.txs-Bb*	3.6	14.1	1	1	*Xtxp211-Xtxp25*
	3	*QGcv.txs-C*	2.6	9.2	1	1	*Xumc124.1.-Xumc121*
	6	*QGcv.txs-Ia*	3.0	10.2	1	1	*Xtxp105-Xtxs1030*
	6	*QGcv.txs-Ib*	3.0	9.8	1	1	*Xtxp105-Xtxs1030*
Glume Persistence	1	*QGpe.txs-A*	3.4	15.7	1	1	*Xcdo457-Xumc90*
	7	*QGpe.txs-E*	5.0	18.7	1	1	*Xtxp295-Xtxs1554*
Grain Weight	10	*QGwt.txs-G*	4.2	14.3	1	1	*Xtxs1106-Xbnl5.04*
Head Exsertion	3	*QHex.txs-C*	3.2	12.9	1	1	*Xtxs1175-Xcdo1160*
Head Weight	10	*QHwt.txs-G*	4.0	13.9	1	1	*Xtxs1106-Xbnl5.04*
[ψ]Height Uniformity	7	*QHtu.txs-Ea*	7.7	30.0*	2	1	*Xtxp92-Xtxs1579*
	9	*QHtu.txs-F*	3.0	14.7	1	1	*Xumc128-Xumc167*
	10	*QHtu.txs-G*	3.0	10.7	1	1	*Xumc109-Xumc21*
	6	*QHtu.uga-D*	5.9	10.3	1	1	*pSB188x-pSB580*
[ψ]Kernel Weight	4	*QKwt.txs-D*	4.2	16.2	1	1	*Xtxp51-Xcdo516.1*
	10	*QKwt.txs-G*	2.5	8.7	1	1	*Xisu136.2-Xcdo516.2*
	6	*QKwt.txs-I*	2.8	9.2	1	1	*Xtxs1030-Xisu138*
	1	*QKwt.uga-C*	4.1	7.3	1	1	*SHO68-pSB062*
	2	*QKwt.uga-B*	3.3	4.8	1	1	*pSB077-pSB643x*
	3	*QKwt.uga-A*	5.8	10.3	1	1	*pSB443c-pSB109*
	4	*QKwt.uga-F*	3.2	5.5	1	1	*pSB341ab-pSB637b*
	7	*QKwt.uga-J*	3.8	6.9	1	1	*pSB637a-pSB164*
	8	*QKwt.uga-E*	4.7	9.0	1	1	*pSB504-pSB200*
	6	*QKwt.uga-D*	6.7	10.1	1	1	*pSB521a-pSB428a*

primarily due to introduction of *S. propinquum* alleles. The detection of QTL correspondence for these traits in additional *S. bicolor* × *S. bicolor* intraspecific crosses would lend support to this notion. Although the two crosses shared one common parent (BTx623) and phenotypic measurements were overseen by the same individual (K. Schertz), the experimental conditions differed in several ways. The BT × IS population consisted of $F_{6\text{-}8}$ RILs and the BT × SP population consisted of F_2 individuals; in the former case, heterotic QTLs should be absent and in the latter case, the lack of replication reduces power to detect QTLs with small effects. The somewhat greater degree of linkage disequilibrium in the F_2 (BT × SP) than the RILs (BT × IS) may cause occasional failure to detect relatively small-effect QTLs in the latter. This could be tested by via the creation of

Table 2 (Contd.)

[a]Trait	[b]CHR	[c]QTL	[d]LOD	[e]V_a	[f]R	[g]E	[h]Interval
Leaf Curve	4	*QLcv.txs-D1*	2.6	8.6	1	1	*Xumc40.2-Xisu56*
	4	*QLcv.txs-D2*	3.0	12.0	1	1	*Xtxp51-Xcdo516.1*
	10	*QLcv.txs-G*	3.6	13.7	1	1	*Xtxs1163-Xisu136.2*
	8	*QLcv.txs-Ha*	2.6	10.2	1	1	*Xtxs1220-Xcdo459*
	8	*QLcv.txs-Hb*	2.7	11.3	1	1	*Xtxs1220-Xcdo459*
	6	*QLcv.txs-I*	2.6	8.8	1	1	*Xcdo244.2-Xtxs1868*
[ψ]Leaf Length	9	*QLln.txs-F*	2.7	11.0	1	1	*Xtxs3000-Xtxp67*
	10	*QLln.txs-G*	3.4	10.6*	2	1	*Xumc109.2-Xumc21*
	8	*QLln.txs-H*	2.8	8.9*	2	1	*Xcdo459-Xtxs645.2*
	4	*QLln.uga-F*	3.9	5.2	1	1	*pSB201-pSB193*
	6	*QLln.uga-D*	17.2	20.6	1	1	*pSB189-pSB188x*
Leaf Pitch	4	*QLpt.txs-D*	3.6	13.7	1	1	*Xtxp51-Xcdo516.1*
	10	*QLpt.txs-G*	2.5	9.6	1	1	*Xtxs1163-Xisu136.2*
Leaf Scorch	2	*QLsc.txs-B*	2.6	8.5	1	1	*Xisu86-Xumc139*
Leaf Senescence	2	*QLsn.txs-B*	2.9	11.3	1	1	*Xtxs283-Xumc125*
	3	*QLsn.txs-C*	3.1	10.4	1	1	*Xtxs578-Xtxs1092*
	7	*QLsn.txs-Ea*	4.9	27.1	1	1	*Xtxp92-Xtxs1579*
	7	*QLsn.txs-Eb*	6.6	35.4	1	1	*Xtxp92-Xtxs1579*
	9	*QLsn.txs-Fa*	2.9	12.8	1	1	*Xtxs3000-Xtxp67*
	9	*QLsn.txs-Fb*	3.3	12.1	1	1	*Xtxs943-Xtxs3000*
[ψ]Leaf Width	7	*QLwd.txs-E*	3.4	14.0*	2	1	*Xtxp92-Xtxs1579*
	9	*QLwd.txs-F*	2.7	12.8	1	1	*Xtxp339-Xbcd454.2*
	8	*QLwd.txs-H*	2.6	9.4	1	1	*Xtxs1220-Xcdo459*
	3	*QLwd.uga-A1*	3.7	9.4	1	1	*pSB443a-pSB379*
	3	*QLwd.uga-A2*	4.2	5.5	1	1	*pSB109-pSB243*
	2	*QLwd.uga-B1*	9.7	15.8	1	1	*pSB075-pSB500*
	2	*QLwd.uga-B2*	14.7	22.0	1	1	*pSB080-pSB495*
	7	*QLwd.uga-J*	8.2	11.1	1	1	*pSB164-pSB815*
	6	*QLwd.uga-D*	7.3	9.4	1	1	*pSB188x-pSB580*
[ψ]Maturity	1	*QMa50.txs-A*	3.6	16.9	1	1	*Xumc128-Xumc167*
	3	*QMa50.txs-C*	2.5	10.7	1	1	*Xtxs422-Xumc16*
	9	*QMa50.txs-F1*†	7.7	19.5*	2	1	*Xisu140-Xcdo393*
	9	*QMa50.txs-F2*†	4.6	11.8*	2	1	*Xtxp358-Xrz390*
	8	*QMa50.txs-H*	3.4	11.2*	2	1	*Xtxs1294-Xtxp105*
	6	*QMa50.txs-I*	3.1	10.0	1	1	*Xumc119-Xcdo718*
	9	*QMa1.uga-G*†	2.6	4.0	1	1	*pSB416-pSB445*
	6	*QMa1.uga-D*†	81.4	88.1	1	1	*SHO74-pSB643a*
	6	*QMa5.uga-D*†	72.6	70.0	1	1	*pSB188x-pSB580*

BT × SP RILs and verifying the absence of QTL correspondence. Finally, the experiments were grown in two different years, and a subset was in different locations. A small fraction of non-corresponding QTLs may be false positives, but the stringent statistical thresholds used should make this a rare event.

Based upon this analysis, an interesting overlap between culm- and tiller-height QTLs is revealed. Two pairs of culm-height QTLs overlapped between the two crosses: *QCuh.txs-E*↔*QCuh.uga-J* and *QCuh.txs-I*↔*QCuh.uga-D*, but because of the relatively large number of culm height QTLs this degree of correspondence marginally missed statistical significance ($P = 0.062$). These regions corresponded with two pairs of tiller height QTLs ($P = 0.014$): *QTih.txs-I*↔*QTih.uga-D* and *QTih.txs-E*↔*QTih.uga-J*, for which correspondence

Table 2 (Contd.)

[a]Trait	[b]CHR	[c]QTL	[d]LOD	[e]V_a	[f]R	[g]E	[h]Interval
[ψ]Tiller Height	7	*QTih.txs-E*[†]	3.4	15.6[*]	3	2	*Xtxp92-Xtxs1579*
	6	*QTih.txs-I*	2.5	9.1[*]	1	1	*Xcdo718-Xtxp6*
	1	*QTih.uga-C1*	2.5	3.8	1	1	*pSB041-pSB102*
	1	*QTih.uga-C2*	3.0	6.6	1	1	*pSB088-pSB508*
	6	*QTih.uga-D*	6.6	7.9	1	1	*pSB314-pSB189*
	7	*QTih.uga-J*	9.5	12.7	1	1	*pSB164-pSB815*
[ψ]Tiller Number	1	*QTinb.txs-A*[†]	3.9	11.9[*]	2	1	*Xtxs1129-Xtxp58*
	9	*QTinb.txs-F*	2.9	11.8	1	1	*Xumc132-Xtxp339*
	6	*QTinb.txs-I*[†]	4.6	13.4[*]	3	2	*Xcdo244.2-Xtxs1868*
	1	*QTinb.uga-C*[†]	3.6	5.1	1	1	*SHO68-pSB062*
	6	*QTinb.uga-D*[†]	6.6	7.9	1	1	*pSB189-pSB188x*
	7	*QTinb.uga-J*[†]	5.5	6.9	1	1	*pSB637a-pSB164*
	5	*QTinB.uga-H*[†]	5.5	7.4	1	1	*pSB510-pSB419c*

was statistically significant. Interestingly, a large-effect, replicated height uniformity QTL, *QHtu.txs-Ea/Eb*, was localized to the tiller/culm height correspondence region on CHR07 (BT × IS). The co-localization of all of these height-related QTLs to two linkage groups suggests that these regions play an especially critical role in the control of height variation in sorghum. Also to be noted is that the region on CHR06 (BT × SP) corresponds to a region of plant height control in maize (Lin et al. 1995).

The identification of corresponding QTLs for kernel weight and tiller number suggests that these loci may be especially promising targets for marker-assisted selection strategies. Two sets of overlapping QTL pairs for kernel weight were found: *QKwt.txs-D*↔*QKwt.uga-F* and *QKwt.txs-I*↔*QKwt.uga-D*. Although the overlap of the kernel-weight QTLs did not reach our statistical threshold for non-random correspondence ($P = 0.12$), they do represent loci that should be considered in future QTL alignments. Two corresponding tiller-number QTLs ($P = 0.038$) were found: *QTinb.txs-A*↔*QTinb.uga-C* and *QTinb.txs-I*↔*QTinb.uga-D*. Interestingly, these loci do not overlap with the sorghum ortholog of rice MOC1 that was recently shown to control tillering in rice (Li et al. 2003).

Table 3 QTL Correspondence Probability

Trait	Symbol	l[a]	s[b]	m[c]	P-value
Tiller height	*QTih*	4	2	2	0.014
Tiller number	*QTinb*	4	3	2	0.038
Culm height	*QCuh*	5	3	2	0.062
Maturity	*QMa*	6	3	2	0.089
Kernel weight	*QKwt*	7	3	2	0.119
Leaf width	*QLwd*	6	3	1	0.408
Leaf length	*QLln*	3	2	0	0.807
Height uniformity	*QHtu*	4	1	0	0.867

Hypergeometric probability distribution
[a]Total number of QTLs found in the larger sample
[b]Number of QTLs found in the smaller sample
[c]Number of matches declared between QTLs (when 1-LOD likelihood intervals overlapped)

'Stay-green' is an important aspect of drought tolerance in sorghum for which several QTLs have been identified (Crasta et al. 1999; Tao et al. 2000; Xu et al. 2000; Haussmann et al. 2002). In the present study, four leaf-senescence QTLs (BT × IS) were mapped: *QLsn.txs-B*, *QLsn.txs-C*, *QLsn.txs-Ea/Eb*, *QLsn.txs-Fa/Fb* explaining a high degree of phenotypic variance (65.4%). Interestingly, *QLsn.txs-C* and *QLsn.txs-F* overlap with 'stay-green' QTLs previously identified on CHR03 and CHR09, respectively, among five such QTLs found (Tao et al. 2000). Although the overlap does not reach our statistical threshold for non-random correspondence ($P = 0.11$), it may be of interest to take the BT × IS leaf senescence QTLs into account in breeding for drought tolerance in sorghum.

In total we have detected overlap of eleven QTL pairs, and at least four of these QTL correspondences are unlikely to be due to chance. However, it should be noted that there could be an increased tendency to correspondence of QTLs if the underlying chromatin is located in a region of low recombination or high gene-density (Noor et al. 2001). Low recombination rates have a tendency to combine the effects of a number of small-effect genetic factors into apparent large-effect QTL. This effect can be offset by low gene density, but it can be enhanced by high gene density. Low recombination rates are especially common in centromeric and telomeric regions. The hypergeometric probability function does not take these biases into account, and corrections must wait until a sorghum recombination/gene-density map is developed.

Collectively, these data provide strong support for the transferability of molecular tools between interspecific and intraspecific crosses, and begin the process of separating QTLs that contribute to morphological and

physiological divergence among *Sorghum* species from those that contribute to diversity within the cultigen (*S. bicolor*). Such a 'categorization' of QTLs is of value for setting priorities in ongoing studies of botanical diversity, and crop improvement, respectively.

Acknowledgments USDA National Research Initiative (A.H.P., K.F.S.), US National Science Foundation (A.H.P., P.E.K.), and USDA-ARS (K.F.S.).

References

Agrama A, Widle E, Reese C, Campbell R, Tuinstra R (2002) Genetic mapping of QTLs associated with greenbug resistance and tolerance in *Sorghum bicolor*. Theor Appl Genet 104:1373–1378

Bhattramakki D, Dong J, Chhabra AK, Hart GE (2000) An integrated SSR and RFLP linkage map of Sorghum bicolor (L.) Moench. Genome 43:988–1002

Bowers JE, Abbey C, Anderson S, Chang C, Draye X, Hoppe AH, Jessup R, Lemke C, Lennington J, Li Z, Lin YR, Liu SC, Luo L, Marler BS, Ming R, Mitchell SE, Qiang D, Reischmann K, Schulze SR, Skinner DN, Wang YW, Kresovich S, Schertz KF, Paterson AH (2003) A high-density genetic recombination map of sequence-tagged sites for sorghum, as a framework for comparative structural and evolutionary genomics of tropical grains and grasses. Genetics 165:367–386

Crasta OR, Xu WW, Rosenow DT, Mullet J, Nguyen HT (1999) Mapping of post-flowering drought resistance traits in grain sorghum: association between QTLs influencing premature senescence and maturity. Mol Gen Genet 262:579–588

George ML, Prasanna BM, Rathore RS, Setty TA, Kasim F, Azrai M, Vasal S, Balla O, Hautea D, Canama A, Regalado E, Vargas M, Khairallah M, Jeffers D, Hoisington D (2003) Identification of QTLs conferring resistance to downy mildews of maize in Asia. Theor Appl Genet 107:544–551

Hart GE, Schertz KF, Peng Y, Syed NH (2001) Genetic mapping of *Sorghum bicolor* (L.) Moench QTLs that control variation in tillering and other morphological characters. Theor Appl Genet 103:1232–1242

Haussmann BI, Mahalakshmi V, Reddy BV, Seetharama N, Hash CT, Geiger HH (2002) QTL mapping of stay-green in two sorghum recombinant inbred populations. Theor Appl Genet 106:133–142

Katsar CS, Paterson RH, Teetes GL, Peterson GC (2002) Molecular analysis of sorghum resistance to the greenbug (Homoptera: Aphididae). J Econ Entomol 95:448–457

Kim JS, Klein PE, Klein RR, Price HJ, Mullet JE, Stelly DM (2005) Chromosome identification and nomenclature of *Sorghum bicolor*. Genetics 169:955–965

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

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

Larsen RJ, Marx ML (1985) An introduction to probability and its applications. Prentice-Hall, Inc, Englewood Cliffs

Li X, Qian Q, Fu Z, Wang Y, Xiong G, Zeng D, Wang X, Liu X, Teng S, Hiroshi F, Yuan M, Luo D, Han B, Li J (2003) Control of tillering in rice. Nature 422:618–621

Lin YR, Schertz KF, Paterson AH (1995) Comparative analysis of QTLs affecting plant height and maturity across the Poaceae, in reference to an interspecific sorghum population. Genetics 141:391–411

Menz MA, Klein RR, Mullet JE, Obert JA, Unruh NC, Klein PE (2002) A high-density genetic map of *Sorghum bicolor* (L.) Moench based on 2926 AFLP, RFLP and SSR markers. Plant Mol Biol 48:483–499

Ming R, Del Monte TA, Hernandez E, Moore PH, Irvine JE, Paterson AH (2002) Comparative analysis of QTLs affecting plant height and flowering among closely-related diploid and polyploid genomes. Genome 45:794–803

Noor MA, Cunningham AL, Larkin JC (2001) Consequences of recombination rate variation on quantitative trait locus mapping studies. Simulations based on the *Drosophila melanogaster* genome. Genetics 159:581–588

Paterson AH, Lin YR, Li Z, Schertz KF, Doebley JF, Pinson SRM, Liu SC, Stansel JW, Irvine JE (1995a) Convergent domestication of cereal crops by independent mutations at corresponding genetic loci. Science 269(5231):1712–1718

Paterson AH, Schertz KF, Lin YR, Liu SC, Chang YL (1995b) The weediness of wild plants: molecular analysis of genes influencing dispersal and persistence of johnsongrass, *Sorghum halepense* (L.). Proc Natl Acad Sci USA 92:6127–6131

Sanchez AC, Subudhi PK, Rosenow DT, Nguyen HT (2002) Mapping QTLs associated with drought resistance in sorghum (*Sorghum bicolor* L. Moench). Plant Mol Biol 48:713–726

Tao YZ, Henzell RG, Jordan DR, Butler DG, Kelly AM, McIntyre CL (2000) Identification of genomic regions associated with stay green in sorghum by testing RILs in multiple environments. Theor Appl Genet 100:1225–1232

Xu W, Subudhi PK, Crasta OR, Rosenow DT, Mullet JE, Nguyen HT (2000) Molecular mapping of QTLs conferring stay-green in grain sorghum (*Sorghum bicolor* L. Moench). Genome 43:461–469