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RESEARCH

Quantitative Trait Loci of Plant Attributes Related to Sorghum Grain Number Determination

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ABSTRACT

The genetic basis of grain number determination in sorghum [*Sorghum bicolor* (L.) Moench] was studied based on canopy growth traits. Traits were crop growth rate (CGR) around flowering, plant reproductive biomass partitioning (P_R) to the panicle, and grain-set efficiency (E_G) per unit of accumulated panicle biomass. Previous evidence has shown that these traits vary across commercial germplasm and that P_R and E_G are genotype-specific traits with low environmental effects. Our hypothesis was that P_R and E_G are highly heritable traits correlated to grain number (and yield) for which environmentally consistent quantitative trait loci (QTL) could be detected. Studied recombinant inbred lines (RILs) showed important variation in yield, grain number per square meter, time to anthesis, plant height, CGR, P_R and E_G, and growth environments created significant genotype × environment interactions for most. Variability in grain number per square meter was significantly correlated with P_R ($p < 0.001$) and E_G ($p < 0.001$) but not with CGR ($p > 0.05$). Heritability estimates for P_R and E_G were larger than estimates for CGR, grain number per square meter, or yield. A multitrait, multienvironment approach over CGR, P_R, and E_G identified 12 QTL (LOD ≥ 2.5), explaining 21 to 36% of observed trait variability. No QTL were detected for CGR, while two and one environmentally consistent QTL were found for P_R and E_G, respectively. Results highlighted relevant information that could be potentially exploited in breeding programs.

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Abbreviations: BLUE, best linear unbiased estimator; CGR, crop growth rate; DArT, diversity arrays technology; E_G, grain-set efficiency; LOD, logarithm of odds; MT-MIM, multitrait multiple-interval mapping; P_R, reproductive biomass partitioning; QTL, quantitative trait loci; RIL, recombinant inbred line; SNP, single-nucleotide polymorphism.

Final yield in grain sorghum is highly correlated with the number of grains set per unit of land area (Stickler and Pauli, 1961; Saeed et al., 1986). Both grain number and yield are quantitative traits under multigenic control. They show low heritability and large genotype × environmental interactions, complicating selection for these traits in breeding programs (Chapman et al., 2000). Studies attempting to detect genomic loci or QTL associated with sorghum grain number have been limited (e.g., Rami et al., 1998; Brown et al., 2006; Boyles et al., 2016), and evidence from other crops show they will be prone to common environmental inconsistencies (e.g., Ribaut et al., 1997; Peng et al., 2011). A trait dissection approach has been proposed (Tardieu, 2003; Reymond et al., 2003) in which a complex trait is dissected into simpler and more heritable component traits.

Simpler traits need to be associated with the trait of interest, express considerable variation among genotypes, show environmental stability, and evidence a higher heritability than the targeted, more complex metatrait. We consider that the traits

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within the model proposed by Charles-Edwards (1984) could provide such dissection for grain number. The model describes genotype and environmental effects over grain number within a mechanistic framework:

$$GN = \frac{CGR\ P_R}{A_G}$$

where GN is the number of grains set per unit of land area, CGR is the crop growth rate around the period when grain number is determined (~20 d preanthesis and 10 d postanthesis for sorghum; Pepper and Prine, 1972; van Oosterom and Hammer, 2008; Gambín et al., 2008), P_R is the proportion of crop growth that is partitioned to reproductive plant parts during this period, and A_G is the minimum assimilate flux required by an individual flower primordium to continue development and establish a grain. Because of the difficulty in measuring A_G, its inverse (E_G), is commonly used (Vega et al., 2001). Grain-set efficiency can be estimated as the number of grains set per unit of accumulated reproductive biomass (Vega et al., 2001; Gambín and Borrás, 2013; Slafer et al., 2015). In brief, the model states that grain number depends on the biomass accumulation at the reproductive structure bearing grains during flowering and on the efficiency this biomass is used for setting grains.

In sorghum, panicle biomass accumulation is dependent on CGR and on the partitioning of this biomass to the panicle (Blum et al., 1997; van Oosterom and Hammer, 2008). As such, the combination of three traits are responsible for sorghum differences in grain number as a result of genotypes or growing conditions: CGR, P_R, and E_G. Canopy growth rate represents the increase rate of total crop aboveground biomass. We have recently shown that all these traits vary among commercial sorghum genotypes (Gambín and Borrás, 2013).

While canopy growth is highly influenced by the environment, P_R and E_G are known to be genotype specific (van Oosterom and Hammer, 2008; Gambín and Borrás, 2013). There are large genotypic differences in biomass partitioning to reproductive structures across sorghum genotypes, and this variability is negatively correlated with plant height (Blum et al., 1997). Grain-set efficiency is also variable among genotypes and is identified as an environmentally stable trait mostly governed by genotypic effects (Gambín and Borrás, 2013). To date, the traits described by Charles-Edwards (1984) have not been the subject of genetic analysis or QTL mapping studies in sorghum, while two QTL studies in maize (*Zea mays* L.) following a similar approach have been previously reported (Messina et al., 2011; Amelong et al., 2015).

The objective of our study was to investigate the genetic architecture of selected canopy traits that are known to be associated with grain number and yield in sorghum (Egli, 1998; Andrade et al., 1999; Vega et al., 2001; van Oosterom and Hammer, 2008; Gambín and Borrás, 2010). There is general consensus about which canopy traits are important for sorghum grain number determination, but to date, no genetic studies have been undertaken on these traits. Our working hypothesis is that some of the traits contributing to grain number variation described in the Charles-Edwards (1984) model, such as P_R and E_G, are less influenced by environment than grain number per se and are therefore better targets for exploitation by plant breeders.

MATERIALS AND METHODS

Plant Material

We used a population of 250 F_5 lines and their parental lines. The RILs were derived by single-seed descent from a cross between sorghum inbred lines IS8525 and R931945-2-2 (Parh et al., 2006). Genotype R931945-2-2 is an elite line developed by the Department of Agriculture and Fisheries (Queensland, Australia). The line has high levels of the stay-green drought adaptation trait and has been the subject of numerous genetic and physiological studies. The line IS8525 is derived from an African landrace of the kafir racial type and has been used extensively as a source of variation for resistance to sorghum ergot (Dahlberg et al., 2001). Panicle architecture is similar for both parents, showing a semicompact overall appearance. Both lines have recently been resequenced (Mace et al., 2013).

Field Experimental Design

Field experiments were conducted during the 2010–2011 and 2011–2012 growing seasons (hereafter referred to as 2011, 2012) at the Campo Experimental Villarino, Universidad Nacional de Rosario, at Zavalla, Santa Fe, Argentina (33° S, 60°8′ W). Soils were silty-clay loam type defined as Vertic Argiudoll. Each experiment was arranged in a randomized complete block design with three replicates. Individual plots in each replicate consisted of two rows 0.52 m apart and 5.5 m long. Planting dates were 21 Oct. 2011 and 26 Oct. 2012. Plots were overplanted and thinned after emergence to reach a uniform final stand density of 20 plants m^{-2}.

Experiments were conducted under rainfed conditions. Nitrogen was applied at a rate of 16 kg N ha^{-1} as monoammonium phosphate (10–50–00, N-P-K) at planting during both years. At V4, we also applied 70 kg N ha^{-1} as urea (46–0–0, N-P-K) in 2011. Nitrogen application was based on available soil nitrate analysis until 60 cm depth taken before planting, higher in 2012, and followed common recommended rates for the region. Predecessor crop was soybean [*Glycine max* (L.) Merr.] both years. Starting at preflowering, pests were controlled every 15 d with insecticide applications (cipermethrin) using standard agronomic practices for the region. Diseases were controlled by fungicide application weekly during the flowering period.

Phenotypic Measurements

Anthesis was determined when 50% of the plants in each plot had at least one visible anther. Biomass samples were obtained by cutting one square meter of total aboveground biomass at the beginning and end of the critical period of grain number

and yield determination (~20 d preanthesis and 10 d postanthesis; Pepper and Prine, 1972; van Oosterom and Hammer, 2008; Gambín et al., 2008). Shoot biomass was always obtained after drying plants in an air-forced oven at 65°C for at least 1 wk. Panicle biomass at the preflowering sampling stage (~20 d preanthesis) can be considered negligible (van Oosterom and Hammer, 2008) and was set to zero. Panicle biomass at the postflowering sampling was weighed separately. Panicles were always cut 1 cm below the first primary branch (being fertile or not). Tillers were treated as any other main stem. At physiological maturity, two square meters of panicles were harvested per plot. Panicles were dried and threshed and the number of grains was estimated from the weight of a sample of 200 grains. Plant height at maturity (from ground level to the top of the panicle) was measured on five consecutive plants per plot.

Crop growth rate around anthesis was calculated as the ratio between the accumulated aboveground biomass (g m^{-2}) from pre- to postanthesis biomass samples and the thermal time (base temperature 11°C; Hammer et al., 1993) between samples. Partitioning to the reproductive structure during flowering was calculated as follows:

$$P_R = \text{reproductive biomass/CGR}$$

where reproductive biomass represents the panicle dry weight 10 d after anthesis (g m^{-2}), and CGR is the crop growth rate around anthesis (g m^{-2} degree day [°Cd]$^{-1}$). Panicle dry weight at 10 d after anthesis was used instead of panicle growth rate to avoid errors associated with the duration of the period of panicle growth, as the preflowering sample was not done at the same panicle growth stage for the different genotypes and replicates (Gambín and Borrás, 2013). Grain-set efficiency was calculated as follows:

$$E_G = 1/\text{individual mean grain weight}$$

where individual mean grain weight (g) was estimated as the ratio between yield per square meter and the number of harvested grains per square meter. Individual mean grain weight is here used as an estimate of the minimum requirement to set one grain (Egli, 1998) and is correlated to E_G measured as the quotient between grain number and panicle mass (Gambín and Borrás, 2013).

Statistical Analysis

Genetic variation among RILs for each trait was assessed using linear mixed effects models in R (R Development Core Team, 2014). The model included environment (year), block nested within environment, genotype, and genotype × environment interaction. Genotype, environment, and genotype × environment interaction were considered fixed effects, while blocks within environment were considered random effects. For each trait, best linear unbiased estimators (BLUEs) of the line effect were computed. Models were fitted using the restricted maximum likelihood method.

Broad-sense heritability (H^2) of each trait was calculated on a mean basis as follows:

$$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \frac{\sigma_{GE}^2}{\eta} + \frac{\sigma_e^2}{r\eta}}$$

where σ_G^2 is the genotypic variance, σ_{GE}^2 is the genotype × environment variance, σ_e^2 is the plot residual variance, and η and r are the number of environments and replicate plots, respectively (Hallauer and Miranda, 1988).

Pearson correlation coefficient was used to measure the degree of association between attributes.

Genetic Map Construction and Quantitative Trait Loci Analysis

Total genomic DNA of the RILs was extracted from 2-wk-old plant seedlings using a modified CTAB-based extraction protocol (Saghai-Maroof et al., 1984; Doyle and Doyle, 1987). The samples were genotyped with diversity arrays technology (DArT) sequencing, which represents a combination of DArT complexity reduction methods based on methyl filtration and next-generation sequencing platforms (http://www.diversityarrays.com/). Using this platform, ~30,000 single-nucleotide polymorphisms (SNPs) were genotyped for each sample. From the total data set, a subset of 700 SNPs showing expected Mendelian segregation were selected to construct the genetic linkage map. The Chi-square test was used to verify 1:1 Mendelian segregation for each molecular marker (Kearsey and Pooni, 1996). The physical base pair locations of the SNP markers were used to assign marker order along each chromosome. Linkage analysis was performed using Map Manager QTX (Manly et al., 2001) to determine the genetic linkage distance in centimorgans (cM) (Kosambi, 1943).

Quantitative trait loci mapping was performed using WinQTL Cartographer v2.5 (Wang et al., 2011). First the whole genome was scanned using a one-QTL model, using the multitrait mapping procedure, to analyze multiple traits simultaneously by considering trait and environment correlations through an unstructured residual variance–covariance matrix (Jiang and Zeng, 1995). The multitrait mapping procedure implements composite interval mapping. A stepwise regression analysis for cofactor detection was implemented by using a threshold of 0.05 for input and output of the putative QTL to be used as cofactors. The threshold for declaring the presence of a significant joint QTL was a logarithm of odds (LOD) equal to 6 (empirical testing), with scanning intervals of 1 cM between markers and a putative QTL. Quantitative trait loci positions were assigned to relevant regions at the point of maximum LOD. Quantitative trait loci positions detected in the analysis were regarded as candidate QTL and constituted an initial multi-QTL model, in which we tested the effects and significance of each QTL into a unique model and possible interactions between them. For this, we used the multitrait multiple-interval mapping (MT-MIM) procedure. With the initial model provided, the MT-MIM procedure estimated the model parameters, refined the estimates of QTL positions within intervals, tested the significance of all parameters, searched for more QTL and epistatic interactions, and finally calculated the genetic variance explained by the model (Basten et al., 2004). The threshold for declaring the presence of a significant QTL for an individual trait under a specific environment within the multi-QTL model was LOD = 2.5 (Van Ooijen, 1999). We used the likelihood ratio test to compare the significance of each model refinement (Kao et al., 1999).

Table 1. Time to anthesis, plant height, grain yield, grain number, crop growth rate around anthesis (CGR), partitioning to reproductive biomass (P_R), and grain-set efficiency (E_G) among parental lines (R931945-2-2 and IS8525) and mean, minimum, and maximum of 250 recombinant inbred lines (RILs) from the population under two environmental conditions (Year 2011 and 2012). The coefficient of variation (CV), broad-sense heritability (H^2) estimates, and variance components are described for each trait.

Year	Genotype or parent	RIL	Time to anthesis	Plant height	Grain yield	No. grains	CGR	P_R	E_G
			°Cd†	cm	kg ha^{-1}	grain m^{-2}	g m^{-2} °Cd^{-1}	°Cd^{-1}	grain g^{-1}
2011	R931945-2-2		796	83	2474	10,354	1.57	92	42
	IS8525		883	147	3115	12,081	2.17	77	39
		Mean	749	107	3168	13,256	1.59	92	42
		Min.	627	62	547	2347	0.71	21	29
		Max.	1073	169	6719	26,480	4.20	156	54
2012	R931945-2-2		975	81	1911	8,004	1.32	99	42
	IS8525		966	163	3934	16,038	1.74	61	41
		Mean	838	112	4252	18,655	1.56	106	44
		Min.	735	67	1112	4,998	0.93	42	35
		Max.	1054	168	7563	35,583	2.32	165	57
Mean	R931945-2-2		886	82	2193	9,179	1.45	96	42
	IS8525		925	155	3525	14,060	1.96	69	40
		Mean	794	110	3710	15,956	1.58	99	43
		Min.	681	64	830	3,673	0.82	32	32
		Max.	1064	169	7141	31,032	3.26	161	56
CV (%)‡			3	12	29	29	22	21	8
H^2			0.92	0.88	0.42	0.48	0.57	0.78	0.73
Genetic (G) variance (%)§			35	55	11	13	19	38	33
Environmental (E) variance (%)			53	4	27	32	3	10	11
G × E variance (%)			4	3	12	12	3	4	9
Residual variance (%)			8	38	50	43	75	48	47

† °Cd, degree days.

‡ Coefficient of variation from the combined ANOVA is defined as the 100 times the RMSE (which estimates the standard deviation of each trait) divided by the mean value of the trait.

§ Proportion of phenotypic variation explained by genetic variance, environmental variance, genotype × environment interaction variance, and residual variance for the combined analysis across years.

Because target traits for QTL analysis are known to correlate with plant height (Blum et al., 1997; George-Jaeggli et al., 2011), an alternative QTL analysis was done based on phenotypic data adjusted for plant height. Best linear unbiased estimators adjusted for height were obtained fitting linear models that included genotype nested within environment (year) as fixed effects since trait × height interaction varied at each environment. Fixed effects were environment and height × environment and genotype × environment interactions. The QTL analysis based on BLUEs adjusted for height was compared with the previous QTL analysis.

RESULTS

Time to Anthesis, Plant Height, and Grain Yield

Parental lines showed similar time to anthesis ($p > 0.05$), while phenology of the 250 RILs varied from 627 to 1073 °Cd in 2011 and from 735 to 1054 °Cd in 2012 ($p < 0.001$; Table 1). Flowering duration of RILs was 33 and 23 d in 2011 and 2012, respectively. While this flowering range was ~1 mo, >95% of the lines flowered within a period of 2 wk during both growing seasons. More than 35% of the phenotypic variation in phenology was explained by genotype-to-genotype variation (Table 1).

The parental line R931945-2-2 was shorter in height than IS8525 in both growing seasons ($p < 0.001$; Table 1). The RILs varied in plant height ($p < 0.001$), ranging from 62 to 169 cm in 2011 and from 67 to 168 cm in 2012. More than 50% of the phenotypic variation in plant height was explained by genotypic variation (Table 1).

Parental line IS8525 showed higher grain yield at maturity than parental line R931945-2-2 under both environmental conditions ($p < 0.01$; Table 1). Recombinant inbred line average yield was 3168 and 4252 kg ha^{-1} in 2011 and 2012, respectively, and significant genotype differences were evident across the RIL population ($p < 0.001$; Table 1). Recombinant inbred line yield ranged from 547 to 6719 kg ha^{-1} in 2011 and from 1112 to 7563 kg ha^{-1} in 2012. Variance components indicated that 11% of the phenotypic variation was due to variation among genotypes, 27% was due to environment, and 12% was due to genotype × environmental interaction.

Inbred line IS8525 produced more grains per square meter than R931945-2-2 in both growing seasons ($p < 0.01$; Table 1). The RIL population showed significant genotypic variation in harvested grains, ranging from 2347 to 26480 grains m^{-2} in 2011 and from 4998 to 35583

Table 2. Pearson correlations and significance level between traits (time to anthesis, plant height, grain yield, grain number per square meter, crop growth rate around flowering [CGR], reproductive partitioning [P_R], and grain-set efficiency [E_G]) for 250 recombinant inbred lines (RILs) and the two parental lines (R931945-2-2 and IS8525) grown at two environmental conditions (Years 2011 and 2012).

	Year	Time to anthesis	Plant height	Grain yield	Grain no. per square meter	CGR	P_R
Plant height	2011	0.22 **					
	2012	0.24 **					
Grain yield	2011	−0.07	−0.01				
	2012	−0.19 **	0.08				
Grain no. per square meter	2011	−0.14 *	−0.06	0.95 ***			
	2012	−0.17 *	0.13	0.94 ***			
CGR	2011	0.29 ***	0.52 ***	0.08	0.04		
	2012	0.37 ***	0.55 ***	0.09	0.10		
P_R	2011	−0.34 ***	−0.47 ***	0.37 ***	0.43 ***	−0.41 ***	
	2012	−0.47 ***	−0.49 ***	0.37 ***	0.37 ***	−0.38 ***	
E_G	2011	−0.15 *	−0.07	−0.12	0.12	−0.09	0.12
	2012	−0.07	0.15	0.10	0.38 ***	0.05	0.10

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

grains m^{-2} in 2012 (Table 1). The partition of phenotypic variance was comparable with grain yield: 13% was explained by genotype, 32% by environment, and 12% by genotype × environment interaction.

Crop Growth Rate around Anthesis, Reproductive Partitioning, and Grain-Set Efficiency

Crop growth rate around anthesis was different for both parental lines ($p < 0.01$; Table 1), where IS8525 showed a higher CGR than R931945-2-2 in both years. There were large differences between genotypes within the RIL population: CGR around anthesis ranged from 0.71 to 4.20 g m^{-2} °Cd^{-1} and from 0.93 to 2.32 g m^{-2} °Cd^{-1} for 2011 and 2012, respectively ($p < 0.001$; Table 1).

Contrary to CGR, the parental line R931945-2-2 showed higher P_R than IS8525 ($p < 0.05$; Table 1). Genotypes from the RIL population also showed large genotypic differences in P_R ($p < 0.001$; Table 1), ranging from 21 to 156 °Cd^{-1} in 2011 and from 42 to 165 °Cd^{-1} in 2012.

Parental lines showed similar values for E_G, but the RIL population showed significant genotype differences (Table 1). The RILs varied from 29 to 54 grains g^{-1} in 2011 and from 35 to 57 grains g^{-1} in 2012 ($p < 0.001$; Table 1).

The partition of variance components for P_R and E_G was mostly similar (Table 1), where the phenotypic variation explained by genotype was higher for both P_R and E_G than CGR (38, 33, and 19%, respectively).

Residual variance varied across measured traits and were higher for those traits largely affected by growing conditions or expected higher experimental error such as grain yield, grain number, and CGR (Table 1). As expected, residual variance increased as correlation among replicates reduced ($r = -0.90$; $p < 0.01$; data not shown). Mean correlation across replicates varied from 0.23 ($p < 0.001$) to 0.82 ($p < 0.001$) for CGR and thermal time to anthesis, respectively.

Heritability Estimates

Coefficient of variation for all traits showed values ranging from 3 to 29% (Table 1), allowing precise heritability estimations. Broad-sense heritability was highest for time to anthesis (0.92; Table 1), final plant height (0.88), P_R (0.78), and E_G (0.73). Crop growth rate around flowering had an intermediate heritability estimation (0.57). Traits showing the lowest heritability values were yield (0.42) and grain number (0.48).

Traits Correlations

We tested the correlation among all phenotyped traits at both years (Table 2). Pearson correlation indicated that grain yield was highly correlated with grain number ($r = 0.95$ and $p < 0.001$ for 2011; $r = 0.94$ and $p < 0.001$ for 2012), and that grain yield was also positively correlated with P_R ($r = 0.37$ and $p < 0.001$ for 2011; $r = 0.37$ and $p < 0.001$ for 2012) but showed no association with CGR or E_G ($p > 0.05$; Table 2). In turn, grain number was also correlated with P_R ($r = 0.43$ and $p < 0.001$ for 2011; $r = 0.37$ and $p < 0.001$ for 2012) and to a lesser extent with E_G ($r = 0.12$ and $p > 0.05$; $r = 0.37$ and $p < 0.001$ for 2012), while it was not associated with CGR ($p > 0.05$). As such, grain yield was strongly associated with the number of grains set around flowering, and grain number differences were related mostly to the proportion of plant biomass that was partitioned to the reproductive structure.

For the crop physiology traits of interest (CGR, P_R, and E_G), CGR and P_R were negatively correlated in both years, indicating that genotypes with higher growth rates

Table 3. Quantitative Trait Loci (QTL) detected for the parameters of the ecophysiological model at each growth environment (2011 and 2012) for crop growth rate around flowering (CGR), reproductive partitioning (P_R), and grain-set efficiency (E_G).

Trait	LG†	Peak‡	Peak§	CI¶	Additive effect#		LOD††		R^2‡‡	
					2011	2012	2011	2012	2011	2012
		bp	cM							
CGR	–§§	–	–	–	–	–	–	–	–	–
P_R	2	6,484,071	56.01	54.5–60.6	–8.52	–	3.35	–	0.33	0.36
	2	74,148,200	212.01	208.4–213.2	–	–8.36	–	3.56		
	4	5,892,271	11.01	8.5–11.4	–	10.51	–	5.19		
	6	47,215,969	93.01	92–95.9	–17.53	–6.55	11.90	2.50		
	7	7,744,178	87.01	84.3–90.3	–	10.78	–	5.83		
	7	60,224,166	142.01	141–145.4	–14.44	–15.02	9.33	9.57		
	8	2,338,235	27.01	26–36.6	–	7.09	–	2.59		
E_G	1	7,808,004	34.61	29.6–39	2.13	2.15	5.33	5.04	0.21	0.34
	2	74,148,200	210.01	209–216.6	–	–2.08	–	3.19		
	4	50,375,326	54.21	53–55	–	–2.10	–	4.03		
	7	7,744,178	87.11	81.3–89.8	–	1.72	–	2.50		
	8	2,228,235	27.01	26–36.6	1.57	–	3.00	–		

† LG, linkage group.

‡ Physical position of the peak QTL location based on the Sbi1.4 genome assembly.

§ Genetic distance of the peak QTL location.

¶ CI, confidence intervals based on 1-LOD support interval.

Additive effect for the IS8525 allele for P_R (°Cd^{-1}) and E_G (grain g^{-1}).

†† LOD, logarithm of odds.

‡‡ Proportion of the phenotypic variance explained by the genetic model.

§§ No significant QTL detected.

partitioned proportionally less to panicle growth around anthesis (Table 2). Absolute panicle biomass was positively correlated with CGR ($r = 0.44$ and $p < 0.001$ for 2011; $r = 0.39$, $p < 0.001$ for 2012). There was no clear correlation between P_R and E_G (Table 2). Crop growth rate and E_G were not correlated.

Taller lines were found to be partly associated with a longer time to anthesis ($r = 0.22$ and $p < 0.01$ for 2011; $r = 0.24$ and $p < 0.01$ for 2012; Table 2). Plant height was positively correlated with CGR ($r = 0.52$ and $p < 0.001$ for 2011; $r = 0.55$ and $p < 0.001$ for 2012) and negatively correlated with P_R ($r = -0.47$ and $p < 0.001$ for 2011; $r = -0.49$ and $p < 0.001$ for 2012; Table 2), but significant variations in CGR and P_R were found for similar plant height (data not shown).

Quantitative Trait Loci Detection

In total, 625 SNPs were included in the final genetic linkage map. The total map length was 1879.3 cM with an average density of one marker every 2.68 cM (Supplemental Table S1). The initial multitrait mapping, using CIM analysis, identified 10 highly significant putative joint QTL, which were used in the subsequent MT-MIM analysis. All 10 candidate QTL were retained in the final model. Additionally, a new significant QTL was detected. No significant epistatic interactions were identified in the final multi-QTL model.

The final multi-QTL model consisted of 12 QTL located on chromosomes 1, 2, 4, 6, 7, and 8. Table 3 describes the position and additive effects for the IS8525 allele of detected QTL for each trait of interest (CGR, P_R, and E_G). No QTL were detected for CGR. The additive effect for the IS8525 allele ranged from −17.53 to 10.78 °Cd^{-1} for P_R and from −2.10 to 2.15 grain g^{-1} for E_G (Table 3). The percentage of explained genetic variation by the final MT-MIM model ranged from 21 to 36% across traits and environments (Table 3). For P_R, the percentage of explained phenotypic variance by detected QTL was 33 and 36% in 2011 and 2012, respectively. For E_G, 20 and 34% of the total phenotypic variance was explained by detected QTL in 2011 and 2012, respectively (Table 3).

Several QTL with important and consistent additive effects across years were identified for P_R on chromosomes 6 and 7 and for E_G on chromosome 1 (Table 3). There was no evidence of linked or pleiotropic QTL with consistent effects across P_R and E_G in agreement with the lack of correlation between these traits (Table 2).

Quantitative trait loci analysis based on BLUEs for each trait adjusted for height (data not shown) was similar to QTL analysis based on BLUEs without height adjustment, indicating detected QTL are specific to target traits.

DISCUSSION

Determining the genetic control of complex quantitative traits is often complicated by genetic × environmental interactions. One approach to deal with this challenge is to dissect the trait of interest into simpler, component traits (Egli, 1998; Araus et al., 2002; Reynolds and Tuberosa, 2008). The present study used a modified version of the

model developed by Charles-Edwards (1984) to explore the genetic basis of grain number determination in sorghum. This framework has been used in a number of previous studies to describe genotype and environmental differences in grain number in many species (Egli, 1998; Andrade et al., 1999; Vega et al., 2001). The Charles-Edwards (1984) model describes grain number depending on CGR, P_R, and E_G. A relevant feature is that the ecophysiological model takes into account the common trade-off between grain number and individual grain weight (Sadras, 2007; Gambín and Borrás, 2011) by explicitly considering an individual assimilate availability per grain (Egli, 1998).

We showed clear genotypic differences among the RIL population for CGR, P_R, and E_G. We also demonstrated that grain number was largely related to changes in P_R and E_G. While a number of studies have investigated variation in plant or canopy attributes linked to resource capture and growth and their relation to grain number (Blum et al., 1997; van Oosterom and Hammer, 2008; Hammer et al., 2010; George-Jaeggli et al., 2013), studies associated with P_R and E_G are limited (Ferrante et al., 2015; Aisawi et al., 2015; Slafer et al., 2015). Sorghum grain yield improvement in Argentinean commercial hybrids has been associated with increased E_G (Gizzi and Gambin, 2016), demonstrating the trait value.

Reproductive partitioning and E_G showed higher heritability (0.78 and 0.73, respectively) than grain number (0.48) (Table 1) in agreement with our working hypothesis. These results are consistent with those found by van Oosterom and Hammer (2008) and Gambín and Borrás (2013) in studies involving a reduced number of genotypes. A trait that has high heritability and is physiologically correlated to grain number determination is very relevant for genetic improvement. Studying these simpler component traits can help researchers understand the causes of environmental and genotype × environment interaction effects (Tardieu and Tuberosa, 2010). In some circumstances, it may be more efficient to select for genes associated with the component traits than to indirectly select for the more complex trait. One key finding of this study is that the component traits P_R and E_G were not negatively correlated, implying there are opportunities for improving both traits simultaneously.

Our study is a first step toward identifying genomic regions that include potentially important genes involved in the determination of sorghum grain number. We found individual and consistent QTL for P_R and E_G with significant additive effects (Table 3) with the QTL for P_R on chromosomes 4 and 6 colocating with grain number QTL described previously in sorghum (Nagaraja Reddy et al., 2013; Phuong et al., 2014). Detected QTL for P_R on chromosome 7 colocated with one major gene for height described previously and represents a new evidence of the association between a major gene and a quantitative trait with important consequences for breeding (Mace and Jordan, 2010). Quantitative trait loci analysis with traits adjusted for height produced similar results, suggesting that it is possible to increase P_R without modifying plant height. In agreement with the lack of negative association between P_R and E_G, three QTL colocated for both traits on chromosomes 2, 7, and 8 (Table 3). Additive effects were consistent for both phenotypes. However, before such information can be used, it will be necessary to determine the importance and consistency of these QTL across a wider range of genetic backgrounds and environments (Stuber et al., 1999; Alvarez Prado et al., 2014). No significant QTL were detected for CGR, in agreement with the low trait heritability.

We have previously shown that traits such as P_R and E_G can help improve sorghum grain number predictions under different conditions in a limited number of genotypes (Gambín and Borrás, 2013). The detection of relevant genomic regions (Table 3) provide opportunities to predict grain number by estimating P_R and E_G with genetic information. This approach has shown to help predict the phenotype for other quantitative traits with more (Reymond et al., 2003) or less (Amelong et al., 2015) accuracy.

Although height reduction in sorghum enabled mechanical harvesting and avoided lodging problems, it was never associated with the yield increases as was the case in other crops like wheat (*Triticum aestivum* L.) (Austin et al., 1980; Gale and Youssefian, 1985; Borner et al., 1993; Flintham et al., 1997). In sorghum, reduced height is frequently associated with reduced yield (Hadley et al., 1965; Casady, 1967; Campbell and Casady, 1969; Campbell et al., 1975; Jordan et al., 2003; George-Jaeggli et al., 2011). Results from the present study do not support this statement. Our results are in general agreement with George-Jaeggli et al. (2011), showing that height reduction is associated with less canopy growth (or CGR).

In summary, the current study has increased our knowledge about the genetic basis of sorghum grain number and yield determination through the identification of QTL associated with reproductive traits. Results highlighted relevant information related to P_R and E_G that could be potentially exploited in breeding programs.

Conflict of Interest

The authors declare that there is no conflict of interest.

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