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Adding genotypic differences in reproductive partitioning and grain set efficiency for estimating sorghum grain number

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Abstract. Current models of sorghum crop growth predict grain number using a calculated plant growth rate around flowering and a genotype-dependent parameter that describes the relationship between both traits. Few values for this parameter have been reported, being similar within triple-dwarf or single-dwarf sorghum genotypes. This approach narrows genotypic differences in grain number determination mostly to differences in traits affecting biomass production. Relevant traits such as biomass partitioning to reproductive structures and grain-set efficiency are not specifically considered, but both vary across genotypes and could improve grain number estimations. We first explored variation for these traits (CGR, crop growth rate around flowering; P_R, biomass partitioning to reproductive structures during this period; E_G, grain set per unit of accumulated reproductive biomass) for a set a sorghum commercial hybrids and inbred lines growing under different conditions. Later, we used a second set of experiments to test whether considering genotype-specific P_R and E_G improved estimates of grain number compared with the current approach used in crop simulation models.

Grain number variations (14–63 $\times$ 10^3 grains m^{-2}) due to genotype and environment were a consequence of significant differences ($P < 0.05$) in all analysed traits (CGR, P_R, E_G). Biomass partitioning and grain set per unit of accumulated reproductive biomass showed consistent genotypic differences ($P < 0.001$); however, they also showed significant environment or genotype $\times$ environment effects. When these specific genotypic parameters dealing with biomass partitioning and grain-set efficiency were used for estimating grain number in other non-related experiments, the predicted accuracy improved ($r^2 = 0.47$, $P < 0.05$, RMSE = 7029 grains m^{-2}) relative to the general approach using a constant parameter for most genotypes ($r^2 = 0.14$, $P < 0.28$, RMSE = 12 630 grains m^{-2}) or a calculated parameter for each genotype ($r^2 = 0.38$, $P < 0.10$, RMSE = 8919 grains m^{-2}). We propose that these traits (P_R and E_G) need to be considered and included in sorghum crop growth models, as they help predict grain number performance of different genotypes in different growth environments.

Additional keywords: *Sorghum bicolor* (L. Moench), grain sorghum, grain size, yield, genotypic variation, modelling.

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Introduction

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 multi-genic control and have low heritability and a high genotype $\times$ management $\times$ environmental interaction, which complicates selection for these traits in breeding programs (Chapman *et al*. 2000). The identification of secondary traits that have an impact on grain number and yield, showing diversity across genotypes and more environmental stability, becomes an alternative approach for yield improvement (Egli 1998; Slafer 2003; Araus *et al*. 2008).

Studying several eco-physiological models accounting for differences in grain number, Egli (1998) concluded that the Charles-Edwards' concept, applied to reproductive plant parts, integrates most requirements of a mechanistic framework for a crop species. The concept (*i*) includes all environmental factors that influence grain number, (*ii*) relates plants parts involved in its determination, and (*iii*) accounts for genetic differences in the determination of grain number (Charles-Edwards 1984):

$$GN = CGR\ P_R/A_G$$

where GN is the number of grains established per unit of land area, CGR is the crop growth rate around the time when grain number is determined, P_R is the proportion of current assimilates partitioned to reproductive plant parts during this period, and A_G is the minimum assimilate flux required by an individual flower

Abbreviations: P_R, reproductive biomass partitioning; E_G, grain-set efficiency.

primordium to continue development and establish a grain (Charles-Edwards 1984).

Much evidence in different crops has demonstrated the importance of canopy photosynthesis or crop growth during a particular developmental period on the definition of grain number (Egli and Zhen-wen 1991; Andrade *et al*. 1999). Reproductive biomass partitioning (P_R) and the minimum assimilate flux requirement per grain (A_G) have received less attention, probably because both traits are more difficult to measure directly. These two traits, however, provide a means for incorporating genetic differences in grain number that are not explained by the capacity of the canopy to fix carbon and grow.

The trait P_R is dynamic; it is estimated as the ratio between the reproductive growth rate and the crop growth rate around the grain determination period, assuming that reproductive biomass is zero at the beginning of this period (Vega *et al*. 2001). Another simpler estimate considers the ratio between the reproductive biomass at the end of this period and the crop growth rate (Borrás *et al*. 2009). Vega *et al*. (2001) showed that, for a specific genotype, this trait is quite stable across different plant growth rates above the threshold required to sustain reproductive growth in contrasting crop species. In sorghum, differences in biomass partitioning to reproductive structures explain most differences in grain number across genotypes contrasting in plant height (Blum *et al*. 1997; van Oosterom and Hammer 2008).

The inclusion of A_G reflects that grain number is not only dependent upon the ability of the canopy to fix carbon and the allocation of this carbon in reproductive organs, but also upon the ability of the sink to utilise that carbon (Egli 1998). This parameter is also difficult to measure directly, and is estimated relating the reproductive biomass at the end of the grain number determination period with the number of grains set. Using its inverse or 'grain-set efficiency', i.e. the number of grains set per unit of accumulated reproductive biomass (E_G), Vega *et al*. (2001) showed that for a specific genotype, this parameter is stable across different plant growth rates above the threshold required to sustain reproductive growth. For sorghum, this minimum assimilate requirement per seed is small, in accordance with the reduced grain size (van Oosterom and Hammer 2008; Gambín and Borrás 2010). Although variation among commercial genotypes is evident in other species (Echarte *et al*. 2006; Acreche *et al*. 2008; Rotundo *et al*. 2012), genotypic differences have not been described for this trait in sorghum.

Crop simulation models can estimate with reasonable accuracy crop phenology, leaf area, and biomass production, but they are often less accurate for estimating yield components (grain number and individual grain size) and final yield (Heiniger *et al*. 1997; Hammer and Broad 2003; Setiyono *et al*. 2010). In models such as SORKAM (Gerik *et al*. 2004) or ASPIM (Hammer *et al*. 2010), grain number in sorghum is simulated from an estimated plant growth rate around flowering and an empirical, cultivar-dependent parameter for relating the daily increase in aboveground dry weight to grain number. Few values for this parameter have been reported, being similar within triple-dwarf or single-dwarf sorghum genotypes (Heiniger *et al*. 1997; Gerik *et al*. 2004; Hammer *et al*. 2010). Differences between short and tall types are consistent with differences in grain set per unit of crop growth (van Oosterom and Hammer 2008; Hammer *et al*. 2010). This approach simplifies grain number simulations to a few specific parameters for different genotypes, making grain number definition mostly dependent upon variations in aboveground dry matter accumulation. Including genotype-specific P_R or E_G parameters might help to predict genotype × environment differences in grain number with more accuracy if differences in these parameters are evident.

The objectives of the present study were: (*i*) to study grain number determination in a set of sorghum genotypes focusing on the traits defined by the Charles-Edwards' model (CGR, P_R, and E_G); and (*ii*) to evaluate whether grain number predictions improve when these traits (P_R and E_G) are considered compared with the current, more general approach. We conducted five experiments: Expts I and II for testing genotype and environmental differences in parameter estimations; and Expts III–V for evaluating grain number estimations when considering these genotype-specific parameters in other growth environments.

Materials and methods

Experimental design

Experiments were conducted during five experimental seasons (Table 1): 2005–06 (Expt I), 2009–10 (Expt II), 2007–08 (Expt III), 2010–11 (Expt IV), and 2011–12 (Expt V). Experiments I and III were located at the Campo Experimental, Departamento de Producción Vegetal, Universidad de Buenos Aires (35°35S, 59°29W). Experiments II, IV, and V were located at the Campo Experimental Parque Villarino, Facultad de Ciencias Agrarias, Universidad Nacional de Rosario (33°0S, 60°8W). Soils at both sites were of the silty clay loam type, Vertic Argiudoll (Soil Taxonomy, Soil Survey Staff 2010).

Experiments I, II, and III included commercial hybrids (DK68T, X7761, and X9946 from Monsanto Co., Argentina,

Table 1. Details of field experiments with sorghum for reproductive partitioning and grain-set efficiency parameterisation and testing

Year	Expt	Site	Water regime	Genotype	Type	Stand density (plants m^{-2})
2005–06	I	FA-UBA	Irrigated	DK68T	Hybrid	20–10
				X7761	Hybrid	
				X9946	Hybrid	
2009–10	II	FCA-UNR	Rainfed	A9721R	Hybrid	20–10
				A9758M	Hybrid	
				IA28	Line	
				IA71	Line	
				IA80	Line	
				P89008	Line	
2007–08	III	FA-UBA	Irrigated	A9721R	Hybrid	20
				A9758M	Hybrid	
2010–11	IV	FCA-UNR	Rainfed	IA28	Line	20
				IA71	Line	
				IA80	Line	
				P89008	Line	
2011–12	V	FCA-UNR	Rainfed	IA28	Line	20
				IA71	Line	
				IA80	Line	
				P89008	Line	

in Expt I; and A9721R and A9758M from Nidera S.A., Argentina, in Expts II and III). Experiments II, IV, and V included four public inbred lines provided by the USDA (three small-stature inbred lines, IA28, IA71, and IA80; and one tall inbred line, P89008; www.ars-grin.gov). Planting was on 19 October (Expt I), 28 October (Expt II), 30 November (Expt III), 21 October (Expt IV), and 26 October (Expt V). Nitrogen was applied at a rate of 120 kg ha^{-1} at sowing in all cases. Experiments were conducted under irrigated (Expts I and III) and rainfed (Expts II, IV, and V) conditions.

Stand density was 20 plants m^{-2} in all experiments. In Expts I and II, an additional lower stand density treatment (10 plants m^{-2}) was included, and treatments (genotype and stand density) were applied in a randomised block factorial design with three replicates. In Expts III–V, treatments were arranged in a randomised complete block design with three replicates. Each replicate involved five (Expts I and III) or four rows (Expts II, IV, and V) 0.52 m apart and 5.5 m long. Plots were oversown and thinned at the eight-leaf stage (ligulate leaves) in Expt I and at the three-leaf stage in all other experiments.

Phenotypic measurements

Data from Expts I and II were used to explore variations in crop growth rate around flowering, reproductive partitioning, and grain-set efficiency in a set of different genotypes, in order to obtain P_R and E_G genotypic parameters. Non-destructive allometric models (Vega *et al*. 2001; Gambín *et al*. 2008) were used for the estimation of plant biomass at two growth stages: ~20 days pre-anthesis and 10 days post-anthesis. Anthesis was recorded when 50% of the plants in each plot had at least one visible anther. The allometric approach was used to ensure the closest representation of plant biomass, corresponding to tagged plants that remained in the field until final harvest, from which grain number data were obtained, following Gambín *et al*. (2008). Models were developed from three or four additional tagged plants per replicate, harvested for each hybrid at pre- and post-anthesis stages.

The pre-flowering allometric biomass model was based on the linear regression between shoot biomass and stem volume. The stem volume was calculated from plant height (from ground level to the uppermost collar) and stem diameter at the base of the stalk. Number of tillers was also included in the regression when present. The post-flowering model involved stem volume and number of fertile tillers for estimating the vegetative biomass; and panicle length or diameter at the base of the peduncle for estimating the reproductive biomass. In Expt I, non-grain panicle dry weight at physiological maturity was used to estimate the reproductive biomass at the post-flowering stage. As shown by van Oosterom and Hammer (2008), non-grain panicle dry mass achieves a maximum value close to anthesis and remains constant during the entire grain-filling period.

Models were used to estimate the biomass of 30 plants per replicate ($\geq$1.5 m^2 harvested area) that remained in the field until physiological maturity. At this stage, plants were harvested and individual grain number per plant was determined by manual counting a 10-g subsample. Shoot biomass was always obtained after drying plants in an air-forced oven at 65°C for at least 1 week.

Data were analysed at the canopy level (m^2). Crop growth rate around flowering was calculated as the ratio between the accumulated aboveground biomass (in g m^{-2}) from pre- to post-anthesis biomass samples and the thermal time (base temperature 11°C; Hammer *et al*. 1993) between stages. Reproductive biomass partitioning (i.e. P_R) was calculated as follows:

$$P_R = \text{reproductive biomass}/\text{CGR} \quad (1)$$

where reproductive biomass represents the panicle dry weight 10 days after anthesis (g m^{-2}), and CGR is the crop growth rate around anthesis (g m^{-2} degree-day^{-1}). Panicle dry weight at 10 days after anthesis was used instead of panicle growth rate to avoid errors associated with the duration of the period of panicle growth, as the pre-flowering sample was not done at the same panicle growth stage for the different genotypes and replicates. Panicles were always cut 1 cm below the first primary branch (being fertile or not). Grain-set efficiency (i.e. E_G) was calculated as follows (Acreche *et al*. 2008):

$$E_G = \text{grain number}/\text{reproductive biomass} \quad (2)$$

where grain number is the final number of grains m^{-2} and reproductive biomass is the panicle dry weight at 10 days after anthesis (g m^{-2}).

Final plant height (from ground level to the top of the panicle) and grain yield m^{-2} were also measured in all experiments. Mean grain weight was estimated as the ratio between grain yield m^{-2} and the number of grains m^{-2} at maturity.

Data analyses

Each experiment was analysed separately. An analysis of variance (ANOVA) was done for each trait using PROC GLM of SAS® (SAS Institute 1999). Genotype, stand density, and genotype × stand density interaction effects were evaluated using the following linear model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \beta_j * \gamma_k + \varepsilon_{ij} \quad (3)$$

where Y_{ijk} is the observed trait value of the kth genotype at the jth density in the ith block, μ is the overall mean, α_i the block effect, β_j the stand density effect, γ_k the genotype effect, $\beta_j * \gamma_k$ the stand density × genotype interaction effect, and ε_{ij} the residual effect. Linear regression analysis was applied to the relationships among variables.

Observed v. estimated grain number

We compared the performance for simulating grain number using three approaches:

Approach 1: Grain number per plant (GNP) was estimated as a function of average plant growth rate (PGR) from 7 days after panicle initiation to 10 days after anthesis:

$$GNP = PGR/K \quad (4)$$

This is the approach taken in current simulations models (Rosenthal *et al*. 1989; Gerik *et al*. 2004; Hammer *et al*. 2010), where parameter K is taken as constant. Based on plant height, a common parameter value of 0.00083 g $grains^{-1}$ was assigned to most genotypes (Rosenthal *et al*. 1989; Hammer *et al*. 2010). For the tall genotype P89008, this parameter value was replaced by 0.001 g $grains^{-1}$ (Hammer *et al*. 2010). Grain number

per area was calculated from the estimated GNP and stand density.

Approach 2: Eqn 4 was included, but considering genotype-dependent parameter K obtained using data from Expt II, as the inverse of a linear regression relating grain number per plant and plant growth rate around flowering, and forcing the regression through the origin (Table 2). Grain number was estimated in Expts III–V from measured plant growth rate and K. Grain number per area was calculated from the estimated GNP and stand density.

Approach 3: Specific genotypic parameters were included describing reproductive partitioning and grain-set efficiency for each genotype. Parameters obtained for each genotype in Expt II were used for estimating grain number in Expts III–V. From measured CGR, reproductive biomass was estimated considering the parameter P_R (Eqn 5). From reproductive biomass, grain number per area was estimated considering the parameter E_G (Eqn 6):

$$\text{Reproductive biomass} = \text{CRG} \times P_R \quad (5)$$

$$\text{Grain number m}^{-2} = \text{reproductive biomass} \times E_G \quad (6)$$

The accuracy of simulated values was tested against observed values using the r^2 statistic as described by Vleeshouwers and Kropff (2000) for biological model testing, and by the root mean square error (RMSE). Details of these equations can be found in Borrás *et al*. (2009).

Table 2. Calculated values of parameter K, representing the inverse of the slope of the relationship between grain number plant^{-1} and plant growth rate around flowering for each genotype, using data from Expt II

Genotype	K (g grain^{-1})	r^2
A9721R	0.0012	0.91 ($P<0.001$, $n=6$)
A9758M	0.0017	0.71 ($P<0.001$, $n=6$)
IA28	0.0012	0.75 ($P<0.001$, $n=6$)
IA71	0.0014	0.88 ($P<0.001$, $n=6$)
IA80	0.0011	0.95 ($P<0.001$, $n=6$)
P89008	0.0031	0.53 ($P<0.01$, $n=6$)

Results

Phenology and plant height in Experiments I and II

Within each experiment, genotypes showed small (3–4 days) but statistically significant ($P<0.05$) differences in time to anthesis (Table 3). Stand density treatments created small differences in Expt II, where anthesis was delayed ~1.6 days at the low density in all genotypes (Table 3).

Genotypes differed significantly ($P<0.05$) with respect to final plant height in both experiments (range 98–202 cm; Table 3). In both experiments, however, plant height showed a significant ($P<0.01$) genotype × density interaction. For some genotypes, the higher stand density resulted in taller plants. The difference was 6–16 cm in the responsive genotypes (Table 3).

Table 3. Thermal time (TT, degree-days) to anthesis, plant height, final yield, grain number per square meter, mean grain weight, crop growth rate around flowering (CGR, g m^{-2} degree-day^{-1}), reproductive biomass partitioning (P_R), and grain set efficiency (E_G) for different genotypes growing during two years at two stand densities

P_R was calculated as the ratio between panicle dry weight 10 days after anthesis (g m^{-2}) and CGR. E_G was calculated as the ratio between grain number m^{-2} and panicle dry weight 10 days after anthesis. *$P<0.05$; **$P<0.01$; ***$P<0.001$; n.s., not significant. Values within parentheses indicate l.s.d. at $P=0.05$

Expt	Genotype	Stand density (plants m^{-2})	TT to anthesis	Plant height (cm)	Final yield (kg ha^{-1})	Grain no, m^{-2}	Mean grain weight (mg)	CGR	P_R	E_G
I	DK68T	20	853	140	13 639	62 461	21.9	1.9	114	296
		10	844	143	10 963	34 297	32.0	1.4	110	215
	X7761	20	870	131	13 328	62 956	21.2	1.9	149	237
		10	859	140	11 414	38 587	29.6	1.4	134	207
	X9946	20	830	150	12 272	56 023	21.9	2.0	123	238
		10	835	142	10 600	39 528	26.8	1.4	139	207
		Gen (G)	**(14)	**(4)	*(770)	n.s.	**(1.0)	n.s.	***(8)	***(7)
		Density (D)	n.s.	n.s.	***(628)	***(2546)	***(0.8)	*(0.1)	n.s.	***(6)
		G × D	n.s.	**(6)	n.s.	**(4411)	**(1.4)	n.s.	**(12)	***(11)
II	A9721R	20	814	153	7510	26 848	28.1	2.0	84	159
		10	825	137	7710	26 690	28.9	1.8	110	135
	A9758M	20	828	163	7786	26 822	29.0	3.2	51	177
		10	853	148	7427	24 870	29.9	2.2	76	147
	IA28	20	821	97	5345	24 077	22.2	1.6	115	128
		10	813	98	4104	18 524	22.5	1.2	124	122
	IA71	20	779	120	6639	23 342	28.3	2.0	71	157
		10	810	110	5339	20 530	26.0	1.6	100	127
	IA80	20	816	113	7185	29 881	24.1	1.8	84	194
		10	816	107	6711	24 965	27.0	1.6	96	155
	P89008	20	810	202	6423	19 087	33.5	4.1	71	66
		10	849	202	4803	14 706	32.6	2.4	68	87
		G	**(20)	***(4)	*(1390)	***(4782)	***(2.2)	***(0.4)	***(8)	***(26)
		D	**(11)	***(2)	*(802)	*(2761)	n.s.	***(0.2)	***(4)	*(15)
		G × D	n.s.	**(6)	n.s.	n.s.	n.s.	**(0.5)	**(11)	n.s.

Differences in height were not associated with differences in time to flowering within or across experiments ($P > 0.05$).

Yield and yield components in Experiments I and II

Final yield differed across genotypes in both experiments ($P < 0.05$), and ranged from 11.5 and 12.4 t ha^{-1} under irrigated conditions in Expt I, and from 4.7 to 7.6 t ha^{-1} under rainfed conditions in Expt II when averaged across densities (Table 3). Plant density also modified final yield per unit land area (Table 3); no genotype × stand density interactions were evident ($P > 0.05$). In both experiments, the highest yields were obtained at the highest plant density ($P < 0.05$; Table 3). Yield differences between density treatments were ~1 and 2 t ha^{-1} in Expts I and II, respectively. This response to stand density was in agreement with the very low number of tillers observed for all genotypes. Fertile tillers plant^{-1} at maturity ranged from 0 to 0.01 at the high stand density of 20 plants m^{-2} and from 0 to 0.2 at the low stand density of 10 plants m^{-2} across genotypes.

Density treatments modified grain number m^{-2} in both experiments ($P < 0.05$), being higher at the higher stand density (Table 3). In Expt I, there was a genotype × plant density interaction for grain number ($P < 0.05$; Table 3). The lower stand density always showed reductions in grain number per unit area compared with the high stand density, but the magnitude of the reduction differed depending on genotype. Grain number m^{-2} differed ($P < 0.01$) across genotypes in Expt II, ranging from 14 706 to 26 848 grains m^{-2}, but genotypes responded similarly, i.e. there was no significant ($P > 0.05$) genotype × stand density interaction.

Average grain weight differed across genotypes in both experiments ($P < 0.05$), ranging from 24 to 27 mg grain^{-1} in Expt I, and from 22 to 33 mg grain^{-1} in Expt II (Table 3). Stand density treatments modified average grain weight in Expt I ($P < 0.05$), and a significant ($P < 0.05$) genotype × plant density interaction was detected (Table 3), whereby the lower stand density increased grain weight to different magnitudes depending on the genotype.

Final yield was closely correlated with variations in grain number m^{-2} within and across experiments ($r^2 = 0.85$, $n = 18$, $P < 0.001$), whereas no significant correlation could be established between yield and average grain weight ($r^2 = 0.06$, $n = 18$, $P = 0.30$).

Crop growth rate around flowering, reproductive partitioning, and grain set efficiency in Experiments I and II

Stand density treatments modified CGR in both experiments ($P < 0.05$; Table 3), being higher at the higher stand density. Crop growth rate around flowering differed across genotypes only in Expt II ($P < 0.05$; Table 3), and genotype × density interaction was found in this trial ($P < 0.05$; Table 3), indicating that the lower stand density reduced CGR to a different magnitude depending on the genotype. For any specific genotype, higher growth rates achieved at the highest density enhanced grain number per area, but no linear relationship was found between CGR and grain number per unit area when data from both experiments were pooled ($r^2 = 0.05$, $n = 18$, $P = 0.38$; Fig. 1*a*).

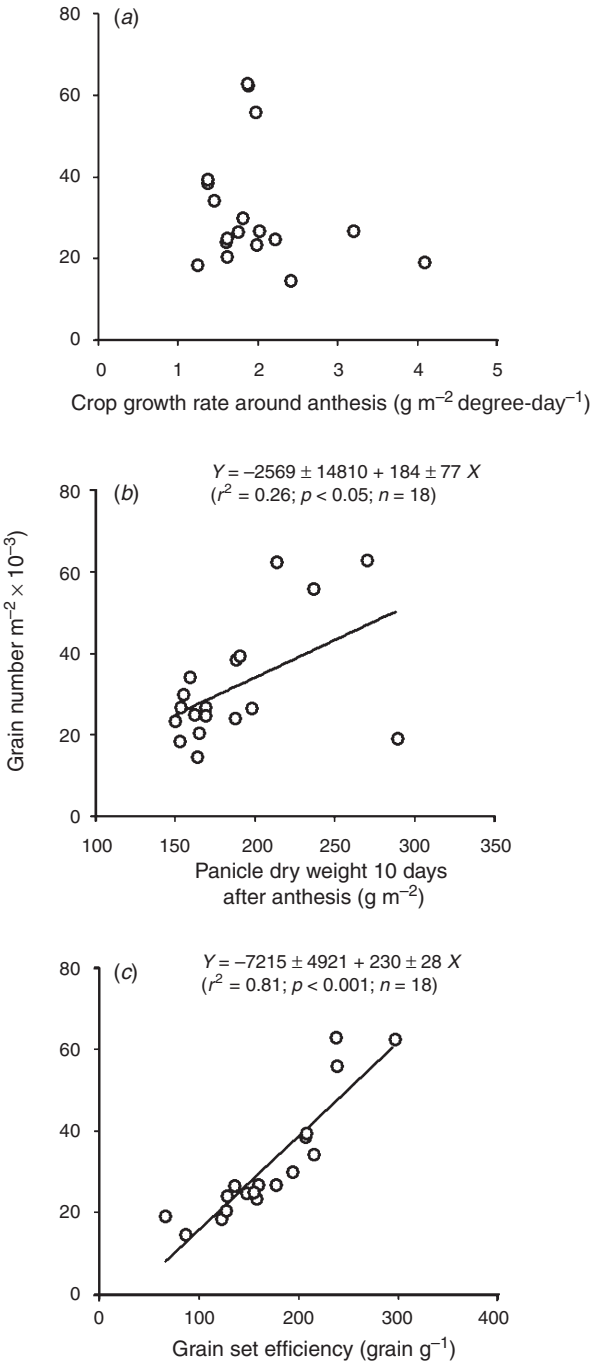

Fig. 1. Grain number as a function of (*a*) crop growth rate around anthesis, (*b*) panicle dry weight 10 days after anthesis, and (*c*) grain-set efficiency per unit of accumulated panicle biomass for Expts I and II.

Reproductive biomass partitioning (P_R, ratio between panicle dry weight 10 days after anthesis and crop growth rate around flowering) varied significantly across genotypes in both experiments ($P < 0.001$; Table 3). Differences were of relatively low magnitude across hybrids in Expt I compared with the more diverse set of genotypes tested in Expt II (Table 3). Stand density treatments modified reproductive partitioning in

Expt II ($P < 0.001$), and a genotype × stand density interaction was found in both experiments ($P < 0.01$; Table 3). Reproductive partitioning was stable across stand densities for some genotypes, and was slightly lower at the higher stand density for others (with the exception of hybrid X7761, in which P_R was higher at the higher stand density; Table 3). Variations in P_R associated with stand density, however, were always of low magnitude (Table 3).

Final plant height was positively correlated with the CGR ($r^2 = 0.54$, $n = 18$, $P < 0.001$), where taller genotypes grew at higher rates than shorter ones. No significant association was found between height and P_R ($r^2 = 0.18$; $n = 18$, $P = 0.07$), and significant genotypic differences in P_R for genotypes of similar plant height were evident (Table 3).

Grain set efficiency (E_G) varied significantly across genotypes in both experiments ($P < 0.001$; Table 3), ranging from 222 to 255 grains g^{-1} of reproductive biomass for hybrids in Expt I, and from 66 to 194 grains g^{-1} of reproductive biomass across genotypes in Expt II (Table 3). Stand density treatments also significantly ($P < 0.05$) modified E_G in both experiments, being higher at the higher stand density (Table 3). A genotype × density interaction was found in Expt I, in which E_G was reduced at the lower stand density to a different magnitude depending on the genotype.

Final grain number showed a significant linear relationship with panicle dry weight 10 days after anthesis ($r^2 = 0.26$, $n = 18$, $P < 0.05$; Fig. 1*b*) and with E_G ($r^2 = 0.81$, $n = 18$, $P < 0.001$; Fig. 1*c*). There was a trend showing P_R and E_G to be positively correlated ($r^2 = 0.26$, $n = 18$, $P < 0.05$).

Observed v. *estimated grain number*

We first estimated grain number from independent experiments (Expts III–V; Table 1) using the approach currently used in crop simulation models (Fig. 2). As an input we used the crop growth rate from each specific genotype × environment case for calculating the grain number outcome based on literature-reported parameters according to plant height (Fig. 2*a*) or a specific parameter calculated for each genotype (Fig. 2*b*). In the first case, the estimation was not significantly correlated with the observed value ($r^2 = 0.14$, $n = 10$, $P = 0.28$; Fig. 2*a*), and the accuracy of the estimation was not high (RMSE = 12 630 grains m^{-2}). When considering a specific parameter for each genotype (Table 2), the overall estimation improved (RMSE = 8918 grains m^{-2}) but the correlation was still not accurate ($r^2 = 0.38$, $n = 10$, $P < 0.10$; Fig. 2*b*).

The third observed *v.* estimated test was done considering the specific genotypic parameters P_R and E_G calculated for each genotype in Expt II. However, because these genotypic traits showed significant environment and genotype × environment effects in Expts I and II, we first tested how the average genotypic value (P_R and E_G) from Expt II correlated with observed values from the same genotypes when grown in Expts III–V. Observed values for these independent experiments are shown in Table 4, and correlations are depicted in Fig. 3. Reproductive partitioning (P_R) showed a significant correlation ($r^2 = 0.72$, $n = 10$, $P < 0.01$; Fig. 3*a*), and E_G showed a correlation that was significant at $P < 0.10$ ($r^2 = 0.33$, $n = 10$, $P = 0.08$; Fig. 3*b*). As such, although the two genotype-specific parameters showed a different accuracy when predicted and observed values were correlated, mostly related to environment and genotype × environment effects described in Table 3, the calculated values described genotypes with reasonable accuracy. The genotype ranking was maintained.

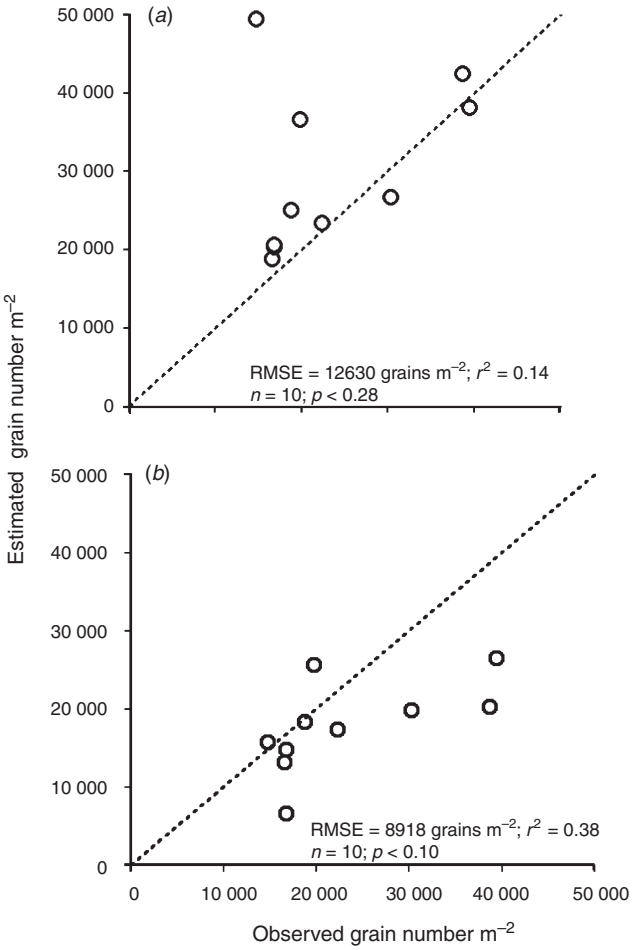

Fig. 2. Observed *v.* estimated grain number m^{-2} for an independent set of experiments (Expts III–V). Grain number was estimated considering measured crop growth rate around flowering and: (*a*) values of parameter K reported for sorghum (Rosenthal *et al.* 1989; Hammer *et al.* 2010), and (*b*) calculated parameter K for each genotype. See Table 2 and *Materials and methods* for further details. Dotted line represents the 1 : 1 relationship.

Table 4. Values of reproductive biomass partitioning (P_R) and grain set efficiency (E_G) for genotypes used in testing experiments (Expts III–V)
Values in parentheses indicate the coefficient of variation (CV%). See *Materials and methods* for further details

Expt	Genotype	P_R	E_G
III	A9721R	92 (3)	176 (5)
	A9758M	74 (11)	189 (11)
IV	IA28	117 (1)	79 (48)
	IA71	102 (8)	124 (14)
	IA80	118 (7)	163 (3)
	P89008	78 (9)	77 (56)
V	IA28	122 (23)	126 (32)
	IA71	87 (28)	146 (61)
	IA80	109 (29)	142 (29)
	P89008	70 (23)	146 (28)

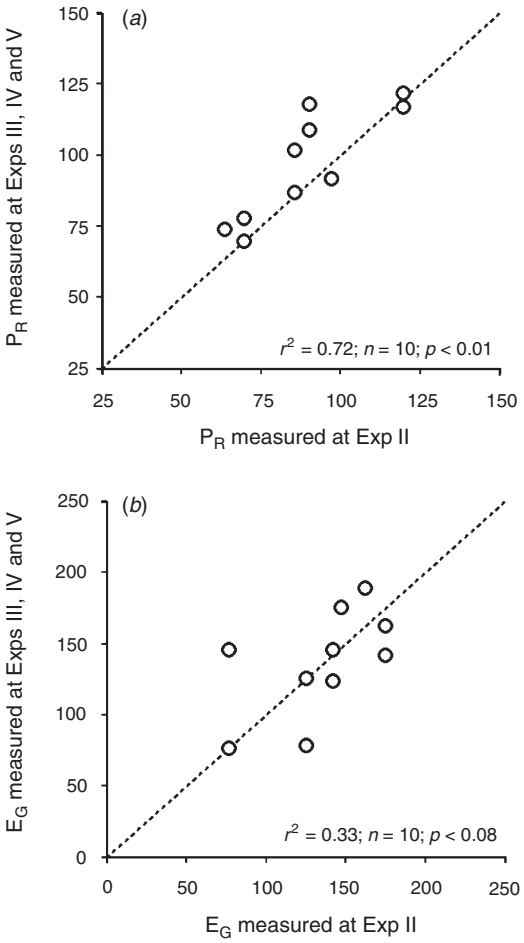

Fig. 3. Relationship between measured parameter values of (*a*) reproductive biomass partitioning, P_R, and (*b*) grain-set efficiency, E_G, in Expt II with values measured in Expts III–V for the same genotypes. In Expt II, values were averaged across densities for each genotype. Values are shown in Tables 2 and 3. Dotted line represents the 1 : 1 relationship.

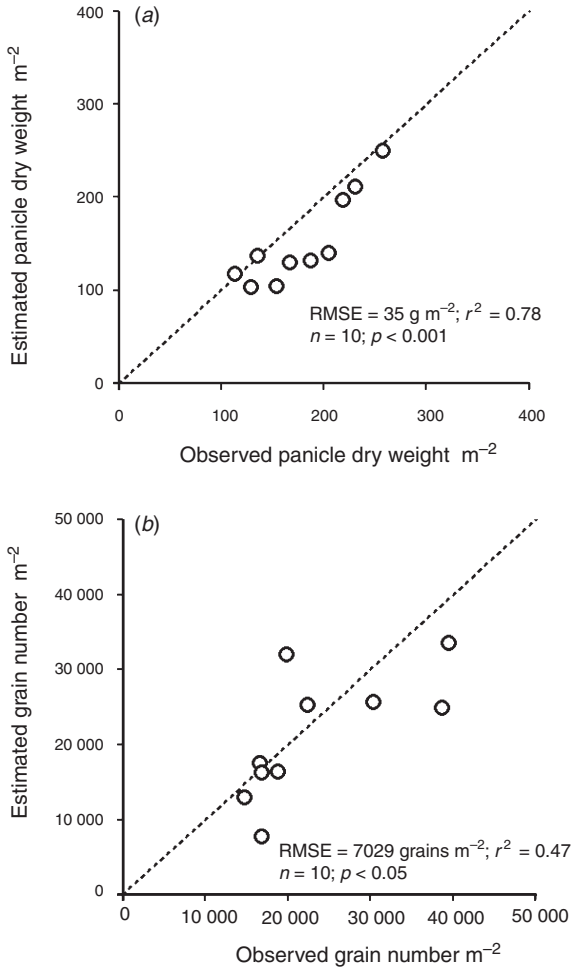

Fig. 4. Observed *v.* estimated (*a*) panicle dry weight m^{-2} and (*b*) grain number m^{-2} for an independent set of experiments (Expts III–V). Panicle dry weight m^{-2} was estimated using a genotype-specific reproductive partitioning parameter (P_R) and the crop growth rate around flowering. Final grain number was further estimated using a genotype-specific grain-set efficiency parameter (E_G) and the estimated panicle dry weight. See *Materials and methods* for further details. Dotted line represents the 1 : 1 relationship.

When estimating the accumulated panicle mass at the end of the grain-set period with the specific P_R genotypic parameter and the specific CGR, panicle dry weight was significantly estimated ($r^2 = 0.78$, $n = 10$, $P < 0.001$, RMSE = 35 g m^{-2}; Fig. 4*a*). Final grain number was then calculated using the estimated panicle mass and the specific E_G genotypic parameter. The overall accuracy of the grain number prediction using this approach was also significant ($r^2 = 0.47$, $n = 10$, $P < 0.05$) and more accurate than the general approach shown in Fig. 2 (RMSE = 7029 grains m^{-2}; Fig. 4*b*). Differences in estimation accuracy between hybrids and lines were not evident.

Discussion

Dealing with yield definition in grain crops, Egli (1998) discussed that it is important to consider the components of yield, and not yield itself, for understanding mechanisms behind this complex trait. A fundamental division would be to consider grain number and grain size. Following the Charles-Edwards' concept (Charles-Edwards 1984), grain number is dependent upon the crop growth rate around a specific period (CGR), upon the proportion of assimilates partitioned to reproductive plant parts (P_R), and upon the minimum assimilate flux required by an individual flower primordia to continue development (or its inverse, the grain set efficiency, E_G; Vega *et al*. 2001). All traits showed variations across the sorghum genotypes tested in the present study. The statistically significant variations reported in E_G add new information to the already described variations in CGR and P_R across sorghum genotypes (Blum *et al*. 1997; van Oosterom and Hammer 2008; Hammer *et al*. 2010).

We support the use of these simple traits despite the predictable association that is expected between them (e.g. between grain number and grain-set efficiency; Fig. 1*c*). This predictable association has been considered by Donald and Hamblin (1976) when discussing the value of correlations between yield, biological yield, and harvest index (i.e. correlations between variables that share common terms).

Although those authors concluded the need for caution in the use of these correlations in agronomic studies, it was also emphasised that they do not invalidate direct comparisons among genotypes. The existence of genotypic diversity for all traits is important as they could be potentially combined for increasing yield by trait pyramidation (Donald and Hamblin 1976).

Current sorghum crop simulation models predict grain number considering an average plant growth rate around anthesis and a cultivar-dependent parameter that describes the relationship between both traits (Rosenthal *et al*. 1989; Gerik *et al*. 2004). However, there is a need for a more detailed simulation of grain number through the specific consideration of reproductive sinks (Hammer *et al*. 2010). This is important for predicting genotypic differences in grain number when models are intended to be used to assist crop genetic improvement (Hammer *et al*. 2006). We showed that the use of specific parameters dealing with reproductive biomass partitioning and grain-set efficiency for each genotype improved the overall accuracy of grain number estimates, and accurately estimated panicle mass at the end of the flowering period. Although validations considering a larger set of genotypes and environments might be needed to determine the advantages of this approach, it is clear that a more detailed approximation of grain number simulations by considering reproductive sinks is needed.

Variations in E_G across genotypes were of significant magnitude, demonstrating the importance of considering this trait at future studies. Assimilate partitioning within the panicle might differ across genotypes, explaining, at least partially, these differences. While some genotypes might favour the development of growing florets, other genotypes might favour structural components of the panicle, as suggested by Acreche *et al*. (2008), who found differences in E_G in a set of old and modern wheat varieties. Considering the large variations in sorghum panicle architecture (Doggett 1988; Brown *et al*. 2006), contrasting allocation within the panicle is possibly behind genotype differences. Whether the final number of fertile florets is higher or flower abortion lower in genotypes with high grain-set efficiency is not known.

Differences in E_G might also involve differences in grain size, as large developing ovaries require more assimilates per reproductive structure to progress (Egli 1998). This is supported by the accumulated evidence of simultaneous definition around flowering of grain number and potential grain size (Sadras 2007; Gambín and Borrás 2010; Sadras and Lawson 2011). In accordance with this concept, results from the present study showed a negative correlation between average grain size and grain-set efficiency ($r = –0.51$, $n = 18$, $P < 0.05$). As previously suggested for maize (Gambín *et al*. 2006), this simultaneous definition could help improve estimations of both yield components (grain number and individual size) in crop simulation models. This approach was considered by Messina *et al*. (2009) for maize.

Conclusions

Sorghum genotypes differed in biomass production around the flowering period, in reproductive biomass partitioning during this period, and in grain-set efficiency (number of grains set per unit of allocated reproductive biomass). This is the first study to document differences across genotypes in grain-set efficiency per unit of biomass allocated to the panicle for sorghum, a trait that has shown genotypic differences in other crops.

Consideration of specific parameters dealing with reproductive biomass partitioning and with grain-set efficiency for each genotype improved the overall estimates of grain number per unit land area compared with grain number estimates based on plant growth rate and a single parameter relating crop growth rate and grain number.

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References

Acreche MM, Briceno-Félix G, Sánchez JAM, Slafer GA (2008) Physiological bases of genetic gains in Mediterranean bread wheat yield in Spain. *European Journal of Agronomy* **28**, 162–170. doi:10.1016/j.eja.2007.07.001

Andrade FH, Vega CRC, Uhart SA, Cirilo AG, Cantarero M, Valentinuz OR (1999) Kernel number determination in maize. *Crop Science* **39**, 453–459. doi:10.2135/cropsci1999.0011183X0039000200026x

Araus JL, Slafer GA, Royo C, Serret MD (2008) Breeding for yield potential and stress adaptation in cereals. *Critical Reviews in Plant Sciences* **27**, 377–412. doi:10.1080/07352680802467736

Blum A, Golan G, Mayer J, Sinmena B (1997) The effect of dwarfing genes on sorghum grain filling from remobilized stem reserves, under stress. *Field Crops Research* **52**, 43–54. doi:10.1016/S0378-4290(96)03462-4

Borrás L, Astini JP, Westgate ME, Severini AD (2009) Modeling anthesis to silking in maize using a plant biomass framework. *Crop Science* **49**, 937–948. doi:10.2135/cropsci2008.05.0286

Brown PJ, Klein PE, Bortiri E, Acharya CB, Rooney WL, Kresovich S (2006) Inheritance of inflorescence architecture in sorghum. *Theoretical and Applied Genetics* **113**, 931–942. doi:10.1007/s00122-006-0352-9

Chapman SC, Cooper M, Hammer GL, Butler DG (2000) Genotype by environment interactions affecting grain sorghum. II. Frequencies of different seasonal patterns of drought stress are related to location effects on hybrids yields. *Australian Journal of Agricultural Research* **51**, 209–221. doi:10.1071/AR99021

Charles-Edwards DA (1984) On the ordered development of plants. 1. An hypothesis. *Annals of Botany* **53**, 699–707.

Doggett H (1988) 'Sorghum.' (Longman Group UK Ltd: London)

Donald CM, Hamblin J (1976) The biological yield and harvest index of cereals as agronomic and plant breeding criteria. *Advances in Agronomy* **28**, 361–405. doi:10.1016/S0065-2113(08)60559-3

Echarte L, Andrade FH, Vega CRC, Tollenaar M (2006) Kernel number determination in Argentinean maize hybrids released between 165 and 1993. *Crop Science* **44**, 1654–1661.

Egli DB (1998) 'Seed biology and the yield of grain crops.' (CAB International: Wallingford, UK)

Egli DB, Zhen-wen Y (1991) Crop growth rate and seed number per unit area in soybean. *Crop Science* **31**, 439–442. doi:10.2135/cropsci1991.0011183X003100020043x

Gambín BL, Borrás L (2010) Resource distribution and the trade-off between seed number and seed weight: a comparison across crop species. *Annals of Applied Biology* **156**, 91–102. doi:10.1111/j.1744-7348.2009.00367.x

Gambín BL, Borrás L, Otegui ME (2006) Source–sink relations and kernel weight differences in maize temperate hybrids. *Field Crops Research* **95**, 316–326. doi:10.1016/j.fcr.2005.04.002

Gambín BL, Borrás L, Otegui ME (2008) Kernel weight dependence upon plant growth at different grain-filling stages in maize and sorghum. *Australian Journal of Agricultural Research* **59**, 280–290. doi:10.1071/AR07275

Gerik TJ, Rosenthal WD, Vanderlip RL, Wade LJ (2004) Simulating seed number in grain sorghum from increases in plant dry weight. *Agronomy Journal* **96**, 1222–1230. doi:10.2134/agronj2004.1222

Hammer GL, Broad IJ (2003) Genotype and environmental effects on dynamics of harvest index during grain filling in sorghum. *Agronomy Journal* **95**, 199–206. doi:10.2134/agronj2003.0199

Hammer GL, Carberry PS, Muchow RC (1993) Modelling genotypic and environmental control of leaf area dynamics in grain sorghum. I. Whole plant level. *Field Crops Research* **33**, 293–310. doi:10.1016/0378-4290(93)90087-4

Hammer G, Cooper M, Tardieu F, Welch S, Walsh B, van Eeuwijk F, Chapman S, Podlich D (2006) Models for navigating biological complexity in breeding improved crop plants. *Trends in Plant Science* **11**, 587–593.

Hammer GL, van Oosterom E, McLean G, Chapman SC, Broad I, Harland P, Muchow RC (2010) Adapting APSIM to model the physiology and genetics of complex adaptive traits in field crops. *Journal of Experimental Botany* **61**, 2185–2202. doi:10.1093/jxb/erq095

Heiniger RW, Vanderlip RL, Welch SM (1997) Developing guidelines for replanting grain sorghum: I. Validation and sensitivity analysis of the SORKAM sorghum growth model. *Agronomy Journal* **89**, 75–83.

Messina C, Hammer G, Dong Z, Podlich D, Cooper M (2009) Modelling crop improvement in a GXEXM framework via gene-trail-phenotype relationships. In 'Crop physiology: Applications for genetic improvement and agronomy'. (Eds VO Sadras, D Calderini) pp. 235–265. (Elsevier: Amsterdam)

Rosenthal WD, Vanderlip RL, Jackson BS, Arkin GF (1989) 'SORKAM: A grain sorghum crop growth model.' Miscellaneous Publication MP-1669. (Texas Agricultural Experiment Station: College Station, TX)

Rotundo JL, Borrás L, De Bruin J, Pedersen P (2012) Physiological strategies for seed number determination in soybean: biomass accumulation, partitioning and seed set efficiency. *Field Crops Research* **135**, 58–66. doi:10.1016/j.fcr.2012.06.012

Sadras VO (2007) Evolutionary aspects of the trade-off between seed size and number in crops. *Field Crops Research* **100**, 125–138. doi:10.1016/j.fcr.2006.07.004

Sadras VO, Lawson C (2011) Genetic gain in yield and associated changes in phenotype, trait plasticity and competitive ability of South Australian wheat varieties released between 1958 and 2007. *Crop & Pasture Science* **62**, 533–549. doi:10.1071/CP11060

Saeed M, Francis CA, Clegg MD (1986) Yield components analysis in grain sorghum. *Crop Science* **26**, 346–351. doi:10.2135/cropsci1986.0011183X002600020028x

SAS Institute (1999) 'The SAS Online Doc v.8.' (SAS Institute: Cary, NC)

Setiyono TD, Cassman KG, Specht JE, Dobermann A, Weiss A, Yang H, Conley SP, Robinson AP, Pedersen P, De Bruin JL (2010) Simulation of soybean growth and yield in near-optimal growth conditions. *Field Crops Research* **119**, 161–174. doi:10.1016/j.fcr.2010.07.007

Slafer GA (2003) Genetic basis of yield as viewed from a crop physiologist's perspective. *Annals of Applied Biology* **142**, 117–128. doi:10.1111/j.1744-7348.2003.tb00237.x

Soil Survey Staff (2010) 'Keys to soil taxonomy.' 11th edn (USDA/NRCS: Washington, DC)

Stickler FC, Pauli AW (1961) Influence of data of planting on yield and yield components in grain sorghum. *Agronomy Journal* **31**, 21–22.

van Oosterom EJ, Hammer GL (2008) Determination of grain number in sorghum. *Field Crops Research* **108**, 259–268. doi:10.1016/j.fcr.2008.06.001

Vega CRC, Andrade FH, Sadras VO (2001) Reproductive partitioning and seed set efficiency in soybean, sunflower and maize. *Field Crops Research* **72**, 163–175. doi:10.1016/S0378-4290(01)00172-1

Vleeshouwers LM, Kropff MJ (2000) Modelling field emergence patterns in arable soils. *New Phytologist* **148**, 445–457. doi:10.1046/j.1469-8137.2000.00773.x