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Secondary traits explaining sorghum genotype by environment interactions for grain yield

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Abstract. Effective plant improvement depends on understanding grain yield genotype by environment (G × E) interactions. Studies focusing on more heritable (secondary) traits provide a way for interpreting the nature of these interactions and assist selection by adapting hybrids to specific adaptation patterns. The objective of our study was to explore some specific traits to help describe G × E interactions for yield in grain sorghum. A set of 22 representative hybrids were grown at eight different environments varying mainly in water and nitrogen availability. Studied traits were yield, phenology (time to anthesis and grain-filling duration), numerical yield components (grain number and individual grain weight) and physiological components (biomass at maturity and harvest index).

The G × E interaction to G component variance represented 3.48 for grain yield, 1.03 for grain-filling duration, 0.87 for biomass at maturity, 0.71 for time to anthesis, and less than 0.5 for the rest of the traits. Although the G × E interaction for yield was large, the relative genotypic contribution of most studied traits suggests that G × E interaction is not a major impediment for attaining high selection responses to these traits. Pattern analysis applied to G × E best linear unbiased predictors defined three genotype and three environmental groups. Environments were grouped suggesting different water stress levels during early or pre-flowering stages, whereas genotype groups depicted different yield responses across environmental groups. Phenology differences among genotypes explained a large portion of the G × E interaction throughout its influence on grain weight. Late flowering genotypes performed poorly in terms of grain weight and yield across all environments, showing that these materials are not the best option for our production system. Longer grain filling contributed to grain weight and yield at environments with low stress levels, particularly when combined with intermediate or short maturity. Early materials contributed to grain weight and yield at the highest stressful environments. We provide useful information to sorghum breeders at temperate environments, and described secondary traits that could assist selection at particular environments.

Additional keywords: breeding, indirect selection, *Sorghum bicolor* (L.) Moench.

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Introduction

Large genotype by environment (G × E) interaction is common in plant breeding. It represents a problem generating uncertainty when selecting genotypes. This uncertainty is the result of reducing genotypic correlations among environments, and, consequently, the way in which these environments discriminate across genotypes (Cooper and DeLacy 1994). A description of the physiological basis behind G × E interactions is relevant for improving breeding programs efficiency by accommodating or exploiting them to improve selection gains (Ivory *et al*. 1991; Cooper and DeLacy 1994).

There are several ways to analyse G × E interactions (Cockerham 1963; Muir *et al*. 1992; Cooper and DeLacy 1994; DeLacy *et al*. 1996; Yan *et al*. 2007). Analysis of variance has been traditionally used to quantify the G × E interaction by measuring the relative variance components size. If the ratio of G × E interaction to genotypic variation is high, the interaction is traditionally considered a breeding problem (Cooper and DeLacy 1994). Another way to analyse G × E interactions is to distinguish between interactions due to heterogeneity of genotypic variance among environments (Shorter and Mungomery 1981; Lefkovitch 1985), or due to the lack of correlation among environments (Dickerson 1962). Interactions due to lack of correlation represents a problem as it could lead to genotype ranking changes, modifying the ideal genotype depending on the environment (Eisemann *et al*. 1990). More recently, the advantages afforded by statistical tools allow incorporating genotype, environmental and management covariates if available into models to explore particular G × E, genotype × management (G × M) and G × E × M interactions (Gambin *et al*. 2016).

A better understanding of the physiological basis of differential hybrid responses to specific environments can

contribute to the overall breeding program efficiency by adapting hybrids to specific adaptation patterns (de la Vega and Hall 2002*a*, 2002*b*). Although the magnitude of the G × E interaction has been studied in different crops including rice (Liang *et al*. 2015), maize (Abakemal *et al*. 2016), wheat (Bassi *et al*. 2016) and soybean (Qin *et al*. 2015), less attention has been given to unravel the physiological basis of these interactions. In sorghum, G × E interactions have been extensively analysed in tropical and subtropical environments of Australia based on classifying environmental types (Chapman *et al*. 2000*a*, 2000*b*). In this analysis, emphasis was given on the environmental component of the G × E interaction rather than on the genotypic one. An analysis of the physiological basis of sorghum G × E interactions can provide information to increase the genetic progress in temperate environments, which seems to be limited (Unger and Baumhardt 1999; Mason *et al*. 2008; Assefa and Staggenborg 2010; Gizzi and Gambin 2016).

Grain sorghum breeding programs in Argentina are mostly based on yield selection. Recommended maturity in the central temperate production area is intermediate, although seed companies offer hybrids with large variations in time to anthesis (~60–90 days). Recommended sowing date to reduce the risk of low temperature at sowing is late October to early November, but this sowing usually implies high probability of water stress during pre-flowering stages. Commercial hybrids also vary in other traits like grain number m^{-2}, individual grain weight, total biomass, and harvest index (Gizzi and Gambin 2016), and it is not clear if this variability could be exploited. If G × E interactions are explained by one or more secondary traits, it will provide valuable opportunities to assist breeding selection and exploit these interactions (de la Vega and Hall 2002*a*, 2002*b*).

The objective of our study was to describe several traits that could help explain G × E interactions for grain yield in sorghum. For this, a reference set of 22 representative commercial hybrids were grown during 4 years under different managed environments. Environments varied mainly in water and nitrogen (N) availability from early stages. Yield was analysed based on phenology (time to anthesis and grain-filling duration), numerical components (grain number per square metre and individual grain weight) and physiological components (biomass at maturity and harvest index).

Materials and methods

Plant material

The hybrids composing the reference genotype set were selected from the Advanta Argentina testing program. A total of 22 hybrids were tested. Thirteen are representative commercial hybrids from six different seed companies (ADV123, ADV114, ADV1200, ADV2499, VDH305, VDH314 from Advanta; 81G29, 83G29 and P84G62 from Pioneer, DK64T from Monsanto; ACA550 from ACA Semillas; MAXIMO from El Sorgal and MS102 from Dow), and were selected based on their contrasting grain yield relative performance across environments (P. A. Pardo, unpubl. data). The other nine hybrids are advanced stage experimental hybrids (ADV-1 to 9) from the Advanta Argentina breeding program, included to explore a wide genetic diversity range. These hybrids are originated from female and male inbred lines developed for Argentina, Australia, and the USA.

All hybrids are tested and released as grain sorghum, although two (ADV2499 and Exp ADV-9) are also commercialised as dual propose hybrids due to their high biomass production.

Managed environments

Field experiments were conducted during 2011–2012, 2012–2013, 2013–2014 and 2014–2015 growing seasons at the Campo Experimental Villarino, Facultad de Ciencias Agrarias, Universidad Nacional de Rosario, at Zavalla (33°1′S, 60°53′W, 130-m altitude), Santa Fe Province, Argentina. The soil type was a silty clay loam Vertic Argiudoll (Soil Taxonomy, Soil Survey Staff 2014).

Genotypes were tested under eight different managed environments (Env.). Environments (identified from I to VIII) are a combination of year × management, and differ in N availability, water availability, and plant density (Table 1). These managed environments follow the concept defined by Cooper *et al*. (1995, 1997) to identify superior lines for a broader set of target environments. Some of these environments are representative of the recommended management for the area (stand density of 20 plants m^{-2} and intermediate levels of N application; Envs II and VIII), or represent the common management done by farmers (including no N application; Envs I and V). At some years, these experiments were

Table 1. General description of tested environments

Env.	Year of sown	Planting date	N at sowing[A] (kg ha^{-1})	Plant density (plant m^{-2})	SAWC[B] at sowing (mm)	Pre-anthesis rainfall (mm)	Post-anthesis rainfall (mm)	Rainfall during critical period (mm)
I	2011	1 Nov.	33	20	–[C]	213	294	104
II	2012	3 Nov.	116	20	264	355	117	14
III	2013	13 Nov.	224	20	264	444	118	132
IV	2013	12 Nov.	16	30	264	344	118	32
V	2013	12 Nov.	16	20	264	344	118	32
VI	2014	13 Nov.	28	30	134	262	144	65
VII[D]	2014	13 Nov.	28	30	134	262	144	65
VIII	2014	13 Nov.	178	20	134	262	144	65

[A]Soil (0–60-cm depth) plus fertiliser.
[B]SAWC, soil available water content.
[C]Not available.
[D]Defoliation of ~75% of the leaf area ~15 days pre-anthesis.

complemented by additional treatments for attaining higher or lower yields. Higher yield was obtained through irrigation and high N application levels (Env. III), and lower yielding environments were obtained through treatments increasing the level of stress from early or pre-flowering stages. A stress from early vegetative was done by increasing the stand density to 30 plants m^{-2} (Envs IV, VI and VII). In one of these environments (Env. VII), an additional stress from the pre-flowering stage was done through defoliation (Table 1). The defoliation treatment consisted in reducing leaf area by ~75% relative to the untreated control at the beginning of the seed set period (~20 days before anthesis; Pepper and Prine 1972; van Oosterom and Hammer 2008). For this, the upper four leaves of each plant were kept untouched, and all leaves below these were hand removed. To quantify the treatment magnitude, radiation interception was measured around anthesis. Percentage of intercepted radiation was significantly reduced ($P < 0.01$) from 95% to 74%, and no genotype differences were evident ($P > 0.05$). Environments II, III and IV were previously described in Gizzi and Gambin (2016), where 10 genotypes are also used in this study.

Not all the genotypes were tested at every environment. Genotypes 81G29, 83G19, ACA550, ADV1200, DK64T, MAXIMO and MS102 were not tested at Envs I and V, DK64T at Env. II, 81G29, ADV123, Exp ADV-1, Exp ADV-2 and Exp ADV-4 at Env. III, and ADV123, P84G62 and Exp ADV-1 at Env. IV.

The experimental design at each environment was a randomised complete block with three replicates. Each replicate plot was four rows 5.5 m long with 0.52 m row spacing. Plots were over-sown and thinned after emergence to the target stand density (Table 1).

Soil samples (0–60 cm) were taken before sowing and analysed for phosphorus (P) (0–20 cm) and N-NO_3 (0–60 cm). Nitrogen (UREA) was applied at V3–V5 stage (Vanderlip and Reeves 1972) to reach different fertilisation levels depending on the particular environment (Table 1). Additionally, MAP was applied at sowing at a rate of 150 kg ha^{-1}, except Envs IV and V where P was not applied.

Diseases were controlled using fungicide applications during the flowering period using recommended commercial products. Irrigation was applied with a sprinkler irrigation system based on visual plant water status observations and weather forecast.

Phenotypic traits

Anthesis was recorded when 50% of the plants within each plot had at least one visible anther. Physiological maturity was recorded after visual black layer observations in 5 of 10 consecutive plants at basal panicle positions (van Oosterom and Hammer 2008). Time to anthesis and grain-filling duration (from anthesis to physiological maturity) was computed in days and thermal time. For thermal time, a base temperature of 11ºC was used before anthesis and 5.7ºC after anthesis (Hammer *et al.* 1993; Heiniger *et al.* 1993). Analysis using days or thermal time produced similar results, so we decided to present results in chronological days for easier interpretation.

Grain yield was determined harvesting the panicles of 2 m^2 per plot in centre rows at physiological maturity. Aboveground biomass samples were determining cutting 0.5 m^{-2} within this area. Biomass and panicles were dried and panicles threshed and weighted. Grain yield was reported with 14% moisture. Individual grain weight was estimated from using 200 grains sample per plot, and grain number was calculated as the ratio between grain yield and individual grain weight. Harvest index was determined as the quotient between grain yield and total plant biomass at physiological maturity (stover + grain).

Statistical analyses

Data were analysed using linear mixed effects models in R statistical package (R Core Team 2014, version 3.0.2, lme4 package, lmer function) (Bates *et al.* 2014). In this model, environment was considered a fixed effect whereas block within environment, hybrid, and hybrid × environment interaction were considered random effects. The best linear unbiased predictors (BLUP) (Robinson 1991) for the random terms (i.e. predictors that were adjusted for the unbalanced nature of the data) were computed from REML analysis.

Following similar procedures described in Curti *et al.* (2014), BLUP of G × E interaction effects for grain yield were used in pattern analysis. Pattern analysis was used for the classification of genotypes and environments (Cooper and DeLacy 1994). For this analysis, grain yield BLUP were standardised within environments (Fox and Rosielle 1982), giving units in standard deviations. Classification employed a hierarchical agglomerative clustering procedure (Williams 1976) based on dissimilarity measure squared Euclidean distance, and the grouping strategy was incremental sum of squares (Ward 1963). Two dendograms were constructed. One dendogram to investigate similarities in performance among genotypes in terms of their relative responses at particular environments. The second dendogram to investigate similarities in performance among environments in terms of the way they influence the relative genotype performance. The principal components of the Euclidean distance matrix of grain yield were estimated using singular value decomposition, and an AMMI biplot of the first two principal components was constructed from this analysis. This analysis was chosen for effectively explaining G × E interactions by integrating additive and multiplicative components into an integrated least-squares analysis (Zobel *et al.* 1988). Pattern analyses were done using R (agricolae package, de Mendiburu 2014; cluster package, Maechler *et al.* 2013).

Performance plots were used to analyse the physiological basis of G × E interactions (DeLacy *et al.* 1996). It represents the traits response of different genotype groups against different environmental groups. Performance plots were based on standardised BLUP within environments for the studied traits (Curti *et al.* 2014). Standardised BLUP were grouped according to their belonging group, and an average value and standard deviation were calculated. Matrix correlations following Pearson method were done to explore the relationship between BLUP traits.

For grain yield, G × E interaction was partitioned into components due to heterogeneity of variance (V) and lack of correlation among environments (L) (Cockerham 1963) as described in Chapman *et al.* (2000*b*) (Eqns 1 and 2):

$$V = \frac{\sum_{j<j'} (\sigma_g(j) - \sigma_g(j'))^2}{e(e-1)} \quad (1)$$

$$L = \sigma^2_{ge} - V \quad (2)$$

for comparisons among environments j to j′, where σ_g is the standard deviation for genotypes, σ^2_{ge} is the variance component for G × E interaction, and e is the number of environments.

Results

Description of managed environments

Environments simulated common growing conditions in the area, and included additional treatments at different timings during the crop cycle to reduce (through irrigation and N application) or increase (through increasing the stand density and defoliation) the level of stress (Table 1).

Nitrogen at sowing varied from 16 to 224 kg ha^{-1} (Table 1). Water availability differed considerably across years. Soil available water content at sowing was close to field capacity in 2012 and 2013, and was 50% of field capacity in 2014 (Table 1). Rainfall during pre-flowering was higher in 2012 and 2013 when compared with 2011 and 2014 (mean of 370 vs 250 mm, respectively), being the historical average ~300 mm (mean of 42 years). Rainfall during the seed set period ranged from 14 to 132 mm and post-anthesis rainfall varied from 117 to 294 mm, across environments (Table 1).

Variance components

Across environments, mean grain yield varied from 7703 to 11 293 kg ha^{-1} (Table 2). The highest yield was reached under irrigation and high N (Env. III), and the lowest under rain-fed, low N and defoliation (Env. VII). Yield at the defoliated treatment was reduced ~20%, similar to the reduction in intercepted radiation when comparing to the untreated control (Env. VI). Almost half of yield variation (48.1%) was related to variation among environments, 3.9% was due to genotype variation, and 13.6% was associated with the G × E interaction (Table 2).

Grain number and individual grain weight varied similarly to grain yield (Table 2). As expected, the environmental component for grain number variation was higher than the one for grain weight (26.7 vs 7.2%). Variation among genotypes explained 22.6% and 50.3% for grain number and grain weight variation for the entire dataset, respectively (Table 2).

Genotype-to-genotype variation in time to anthesis represented 39.7% of the total variance. Variation among environments and G × E interaction for time to anthesis also showed to be relevant (24.0% and 28.2%, respectively; Table 2). Interestingly, the grain-filling duration variability was mostly explained by environmental differences (54.4% of the total variance), in spite of the significant genotype differences. Variation among genotypes and G × E interaction represented 10% of the total variance each (Table 2).

Crop biomass at maturity varied similarly to grain yield. It was higher under irrigation and high N condition (Table 2). As expected, the environmental component for biomass variation was higher than for harvest index (42.3% and 10.3%, respectively). Variation among genotypes explained 9.3% and 16.6% of biomass and harvest index variation for the entire dataset (Table 2).

Grain yield G × E interaction represented 3.5 times the genotypic variance, representing the largest ratio among the studied traits (Table 2). It was followed by grain-filling duration (1.03), biomass at maturity (0.87) and time to anthesis (0.71). The G × E to G ratio was lower than 0.5 for the rest of the traits (Table 2).

Pattern analyses

Genotypes and environments were grouped into three clusters based on grain yield BLUP. The genotype and the environmental clustering had a truncation level that retained ~50.8 and 60.4% of the G × E interaction sum of squares (see Supplementary materials figures S1 and S2, as available at

Table 2. Average ± standard deviation for phenotypic traits analysed at eight environments (Env.)

Env.	Grain yield (kg ha^{-1})	Time to anthesis (days)	Grain-filling duration (days)	Grain number (#m^{-2})	Grain weight (mg)	Biomass at maturity (g)	Harvest index
I	8107 ± 1471	90 ± 9	39 ± 3	28 527 ± 5230	22 ± 2	1922 ± 269	0.33 ± 0.06
II	10 358 ± 1093	79 ± 5	49 ± 5	37 600 ± 5008	23 ± 3	2356 ± 161	0.38 ± 0.04
III	11 293 ± 1715	81 ± 8	60 ± 5	39 743 ± 6630	27 ± 3	2767 ± 342	0.39 ± 0.04
IV	8093 ± 581	79 ± 10	57 ± 6	33 121 ± 4830	23 ± 3	2222 ± 216	0.35 ± 0.04
V	8210 ± 643	76 ± 10	57 ± 5	32 345 ± 3528	24 ± 3	2126 ± 226	0.36 ± 0.04
VI	9169 ± 672	75 ± 5	54 ± 3	36 947 ± 4444	24 ± 4	2235 ± 223	0.38 ± 0.04
VII	7703 ± 625	76 ± 5	53 ± 6	31 471 ± 3436	23 ± 3	1850 ± 158	0.39 ± 0.04
VIII	9718 ± 731	80 ± 3	53 ± 3	39 223 ± 5192	24 ± 4	2347 ± 274	0.40 ± 0.04
% Variance[A]							
E	48.1	24.0	54.4	26.7	7.2	42.3	10.3
G	3.9	39.7	10.1	22.6	50.3	9.3	16.6
G × E	13.6	28.2	10.4	10.7	16.8	8.0	7.1
Block (E)	1.5	2.9	0.3	0.1	0.6	1.6	0.0
Residual	32.8	5.2	24.8	39.9	25.2	38.8	65.9
G × E/G	3.48	0.71	1.03	0.47	0.33	0.87	0.43

[A]Variance components (in percentage) associated with environment (E), genotype (G), G × E interaction, block nested within E and residual are described.

journal's website). Groups of genotypes G1, G2 and G3 were composed by 6, 6, and 10 genotypes, respectively (Supplementary materials figure S1). Groups of environments E1, E2, and E3 were composed by 4, 3, and 1 environments, respectively (Supplementary materials figure S2).

Environmental group E3 enclosed the irrigated and high N environment (Env. III), group E2 included Envs I, II, and V, and group E1 Envs IV, VI, VII, and VIII (Supplementary materials figure S2; Table 1). E1 enclosed environments that combined reduced water content at sowing, higher stand densities and lower pre-flowering precipitations, particularly during the seed set period, when compared with environments grouped in E2 (Table 1). Globally, this suggests that these environments experienced higher levels of water stress from early or pre-flowering stages. Environmental group E3 showed the highest average grain yield (11 293 kg ha^{-1}), followed by E2 (8771 kg ha^{-1}), and E1 (8395 kg ha^{-1}) (Table 2), confirming a reduced environmental quality from E3 to E1. Results from the ordination analysis for grain yield confirmed this cluster analysis (Fig. 1). The environments that grouped together were located near each other. The first and second principal components together accounted for 68.3% of the G × E interaction variability.

A small angle between environmental vectors that belong to the same cluster reflects the strong positive correlation between environments. Environments grouped in E3 (Env. III) and E2 (Envs I, II and V) showed an almost a 90-degree angle, indicating no correlation in genotype performance among groups. Groups E3 and E1 tented to be negatively correlated, as the angle formed between vectors was higher than 90 degrees (Fig. 1). A negative correlation was found between E2 and E1 (Fig. 1). This negative correlation was more evident for Env. IV versus Envs II and V than for Env. I versus Envs VI, VII, and VIII.

Genotypes grouped in G2 and G3 were located near E2 and E1 environmental vectors, respectively. This indicted a relative higher performance of genotypes at these particular environments. Genotypes corresponding to G1 were located close to the origin, showing similar performance across tested environments (Fig. 1).

Physiological basis of G × E interaction

G1 had the lowest grain yield, whereas G2 and G3 were similar (Table 3). Performance plot indicated different patterns of genotype specific performance across environments (Fig. 2). G1 evidenced the lowest grain yield in all environments (Fig. 2*a*). In accordance to the AMMI biplot (Fig. 1), G2 evidenced a better performance at environments with lower stress levels (E3 and E2), whereas G3 showed highest yield in the more stressful environments (E1) (Fig. 2*a*). In agreement with results described in Fig. 2*a*, partitioning the G × E interaction indicated both types of interaction were present (59% of the interaction was due to heterogeneity of variance, whereas 41% was due to lack of genotypic correlation among environments).

Genotypes within G1 showed short grain-filling duration, small grain weight and high grain number m^{-2} (Table 3; Fig. 2*c*, *e*, *d*). They also tended to have longer time to anthesis and reduced biomass at maturity, although a large variation among genotypes within this group for both traits was evident

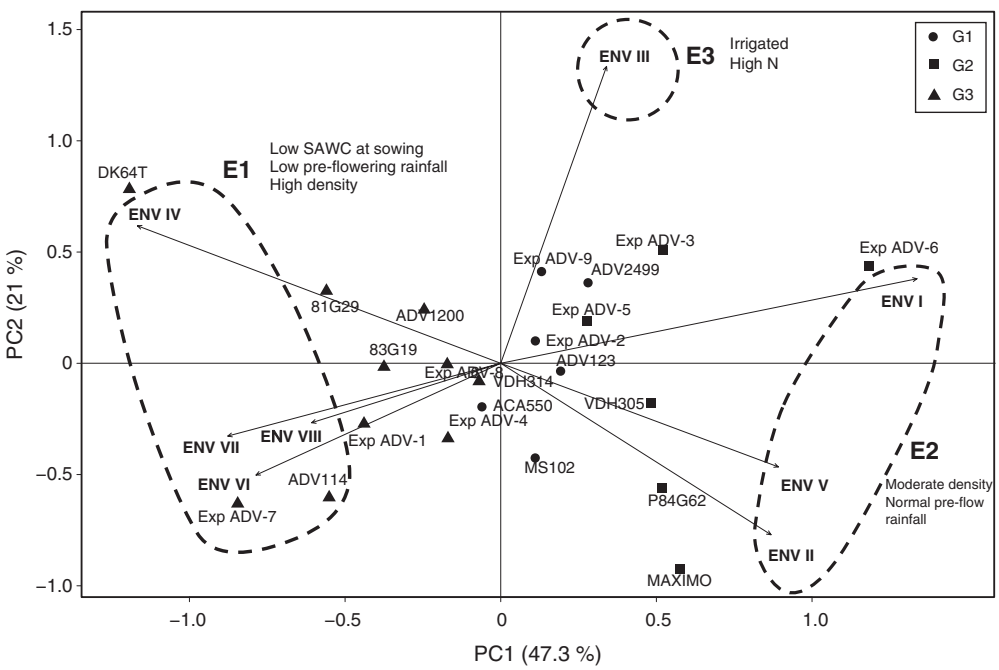

Fig. 1. AMMI biplot of the first and second principal components for grain yield of 22 genotypes grown in eight environments. Genotypes are represented by symbols and environments by vectors. Similar symbols indicate genotypes belonging to the same cluster (G1, G2 and G3) based on similarities in performance at particular environments. Environments belonging to cluster E1, E2 or E3 are enclosed in dashed lines, and the main environmental or management variable defining each group indicated beside. SAWC, soil available water content.

Table 3. Mean phenotypic traits (±standard deviation) of the three genotypic groups resulting from the hierarchical agglomerative clustering method for best linear unbiased predictors of grain yield

Group	Grain yield (kg ha^{-1})	Time to anthesis (days)	Grain-filling duration (days)	Grain number (#m^{-2})	Grain weight (mg)	Biomass at maturity (g)	Harvest index
G1	8233 ± 1736	80 ± 15	49 ± 11	36 277 ± 8079	20 ± 4	2099 ± 455	0.35 ± 0.07
G2	9266 ± 1743	79 ± 8	54 ± 7	33 550 ± 5731	25 ± 2	2253 ± 320	0.37 ± 0.05
G3	9287 ± 1335	77 ± 7	53 ± 7	34 682 ± 5702	25 ± 3	2182 ± 301	0.38 ± 0.04

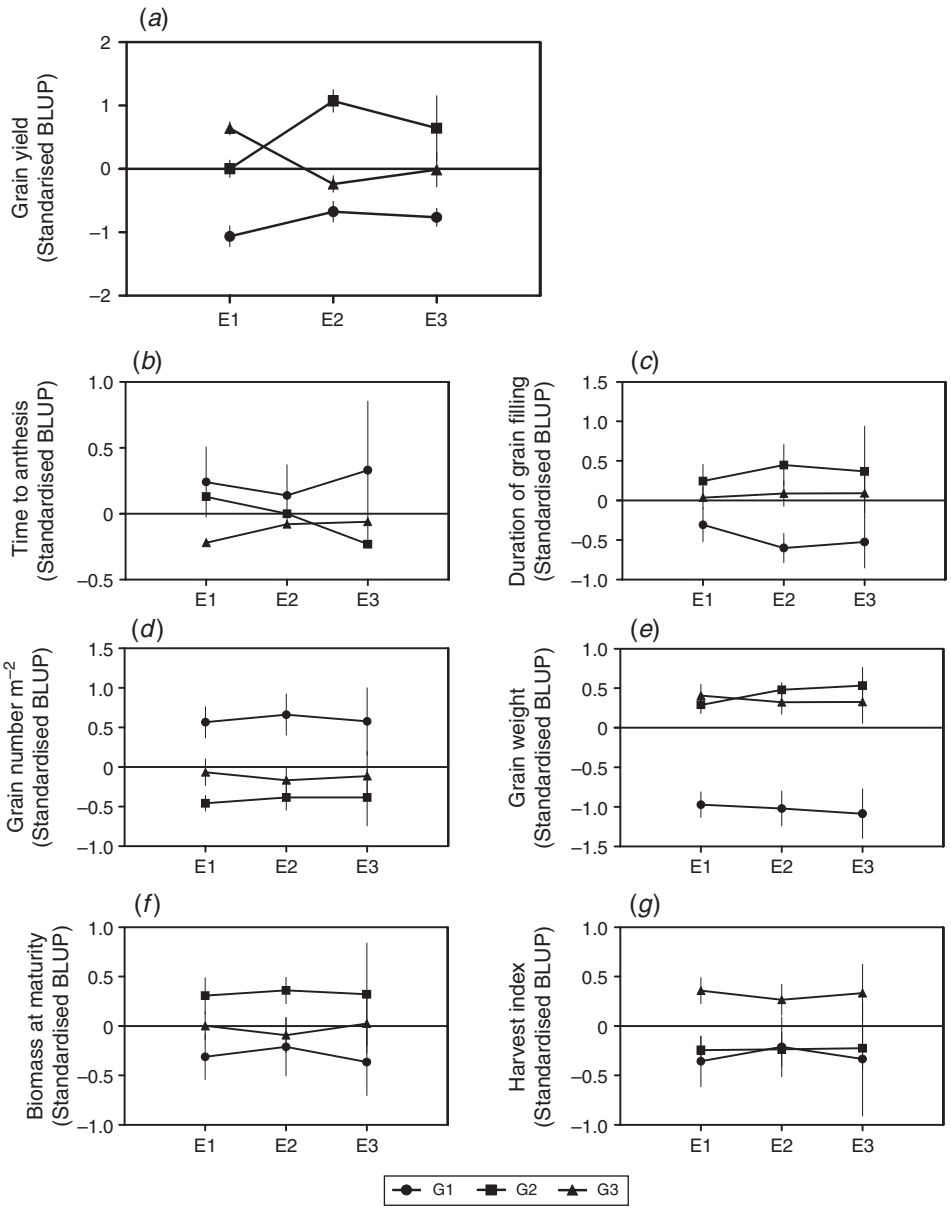

Fig. 2. Plot responses of (*a*) grain yield, (*b*) time to anthesis, (*c*) duration of grain filling, (*d*) grain number m^{-2}, (*e*) grain weight, (*f*) biomass at maturity and (*g*) harvest index, for the three groups of grain sorghum genotypes identified by cluster analysis plotted against three environment groups. Vertical bars indicate standard deviation.

(Fig. 2*b*). All these traits were stable across environments. No differences were evident among groups for the rest of the studied traits (Table 3; Fig. 2).

Contrary to G1, genotypes within G2 showed long grain-filling duration, larger grain weight, and lower grain number m^{-2} (Table 3; Fig. 2*c*, *e*, *d*). They also tended to produce more biomass

at maturity (Fig. 2*f*). These four traits were also stable across environments. This genotype group showed a reduced time to anthesis at the fertilised and irrigated environment E3, and a clear delay in flowering time at more stressful environments (Fig. 2*b*).

Group G3 exhibited average values for most traits (Fig. 2). They showed a shorter time to anthesis, and a larger individual grain weight and harvest index (Fig. 2*b*, *e*, *g*). These traits were also stable across environments.

The association between BLUP for grain yield and the six traits differed depending of the environment. Grain yield BLUP were significantly correlated ($P < 0.05$) with grain weight in all environmental groups (Table 4; Fig. 3*a*, *b*, *d*). In E2, grain yield BLUP were also positively correlated with grain-filling duration ($P < 0.05$; Table 4; Fig. 3*c*). In the more stressful environment E1, grain yield was negatively associated with time to anthesis ($P < 0.05$; Table 4; Fig. 3*e*). Grain yield BLUP showed no significant correlation with the rest of the traits (Table 4).

Grain number m^{-2} and grain weight were negatively correlated in all environmental groups (Table 4). The same was found between time to anthesis and harvest index, and between harvest index and biomass at maturity. Grain weight was also negatively associated with time to anthesis, although this was only significant in E3 (Table 4). Biomass at maturity was positively correlated with time to anthesis in E2 (Table 4).

Discussion

Genotype and G × E interaction relative contributions for grain yield, phenology, numerical and physiological components found in current commercial materials in this study are in general agreement to previous sorghum results (Chapman *et al.* 2000*b*; Rakshit *et al.* 2012; Gizzi and Gambin 2016). Large G × E to G ratio for grain yield complicates identification of superior genotypes, and could partially explain the low genetic gain found for sorghum in this production region (Gizzi and Gambin 2016). It also suggests that indirect response to selection for target environments from selection using a few managed environments based only on yield would be difficult. However, large relative contribution of G effects for most secondary traits suggests that G × E interaction would not be a major impediment for attaining high selection response for these traits. Stability across environments for different traits also provides opportunities for selection in different environments.

Partitioning the G × E interaction for grain yield into variance heterogeneity and lack of correlation among environments provides a better understanding of the interaction (Cooper and DeLacy 1994). Variance heterogeneity was not minor, and represented 59% of G × E interaction. A group of genotypes showing relative low performance across environments were identified, indicating that these genotypes do not express desirable traits for high grain yields at the growing conditions explored in this study. This group has distinctive and stable characteristics in terms of phenology (late flowering, reduced grain-filling duration) and yield components (small grain weight) (Fig. 2). These traits could be used as undesirable secondary ones during selection. These genotypes set a high grain number m^{-2}, which does not compensate for the reduction in grain weight. Later flowering genotypes reached anthesis early-mid February, exposing the pre-anthesis period to more favourable conditions in terms of water availability compared with shorter materials. This could explain their high grain number. However, the post-anthesis period for these materials is placed to progressive unfavourable conditions in terms of radiation and temperature, explaining the reduction in grain-filling duration and grain weight. This is also supported by the negative correlation between time to anthesis and harvest index in all environments (Table 4). Late flowering genotypes are usually recommended for earlier sowings, confirming present results.

Table 4. Pearson's correlation coefficients among traits for each environmental group
Significant correlations ($P < 0.05$) are shown in bold

Environmental group	Trait	Time to anthesis	Grain-filling duration	Grain number m^{-2}	Grain weight	Biomass at maturity	Harvest index
E3	Yield	–0.19	0.31	0.02	**0.45**	0.26	0.10
	Time to anthesis	–	–0.12	0.31	**–0.48**	0.29	**–0.77**
	Grain-filling duration	–	–	–0.03	0.24	–0.09	0.26
	Grain number m^{-2}	–	–	–	**–0.67**	0.15	0.01
	Grain weight	–	–	–	–	0.32	0.28
	Biomass at maturity	–	–	–	–	–	**–0.39**
E2	Yield	–0.23	**0.58**	–0.38	**0.49**	0.17	0.00
	Time to anthesis	–	–0.12	0.16	–0.33	**0.58**	**–0.80**
	Grain-filling duration	–	–	–0.23	0.38	0.08	0.04
	Grain number m^{-2}	–	–	–	**–0.80**	–0.03	0.14
	Grain weight	–	–	–	–	0.16	0.22
	Biomass at maturity	–	–	–	–	–	**–0.59**
E1	Yield	**–0.51**	0.08	–0.23	**0.67**	0.25	0.40
	Time to anthesis	–	0.17	0.11	–0.42	0.34	**–0.84**
	Grain-filling duration	–	–	0.06	–0.10	–0.18	0.00
	Grain number m^{-2}	–	–	–	**–0.83**	–0.21	0.19
	Grain weight	–	–	–	–	0.33	0.13
	Biomass at maturity	–	–	–	–	–	**–0.58**

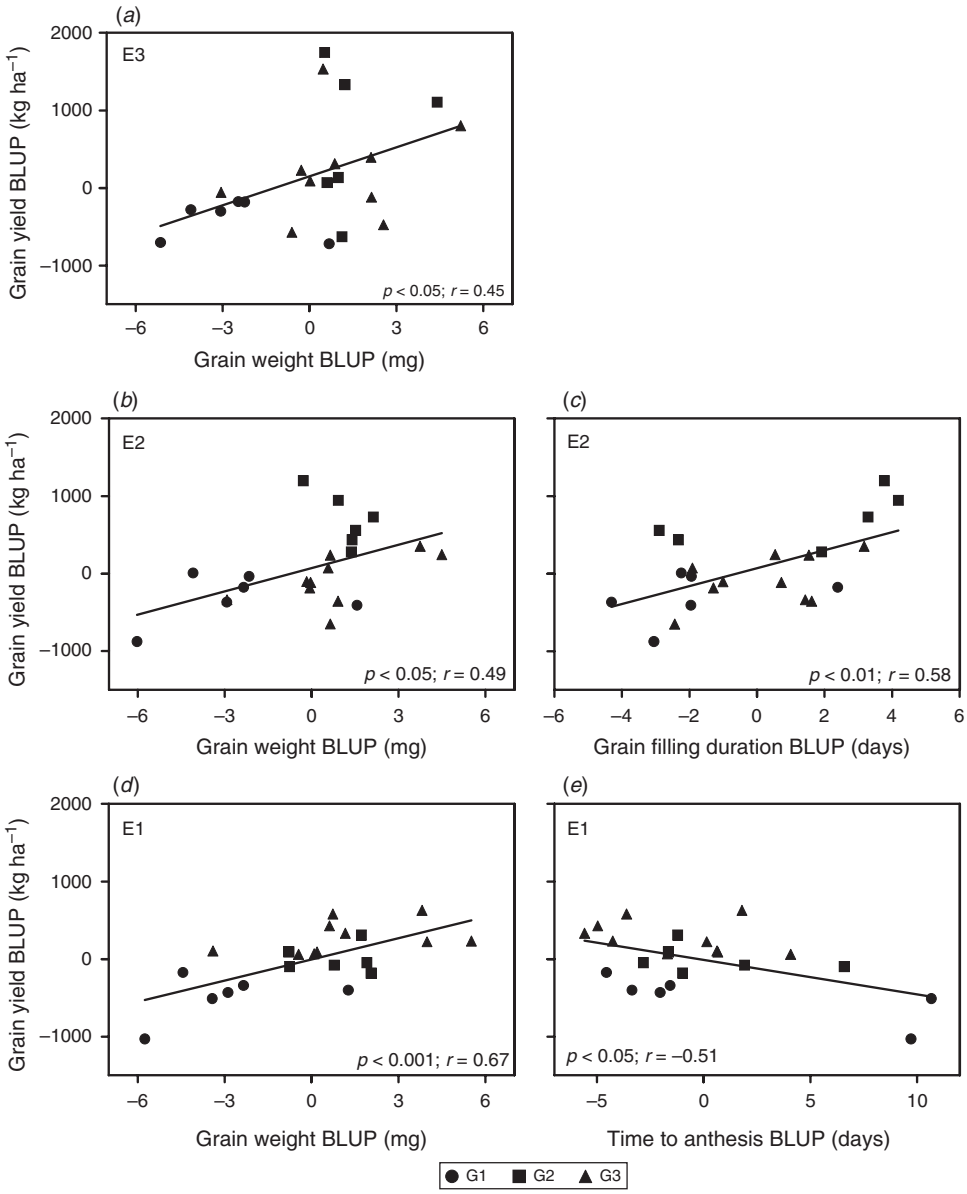

Fig. 3. Scatter diagrams of association between the best linear unbiased predictors (BLUP) for grain yield and (*a*) grain weight at environmental group E3, (*b*) grain weight and (*c*) grain-filling duration at environmental group E2, and (*d*) grain weight and (*e*) time to anthesis for environmental group E1. Each point represents an individual genotype. Same symbol indicate genotype belonging to the same cluster based on similarities in performance at particular environments.

Results suggested that selection strategies to increase grain yield across the population of environments should be focussed on short or intermediate maturity genotypes.

However, interaction due to lack of correlation was 41%. Lack of correlation represents a problem for breeding due to re-ranking of genotypes (Chapman *et al*. 2000*b*). Environments clustered following water availability and use during early or pre-flowering stages, suggesting a gradient of lower level of stress from E1, E2 and E3, respectively. Lack of correlation indicated that genotypes responded differently to stress around early or pre-flowering stages, and provides evidences that selection based on yield under low stress might not imply improvement under stress. This is similar to previous wheat studies (Cooper *et al*. 1995) but different to maize, in which positive genetic progress was evident under both well-watered and drought conditions (Cooper *et al*. 2014).

Correlation between genotypic effects for yield and other traits across different environments help define traits that can be used as secondary ones for indirect selection to improve performance at each environmental type (Curti *et al*. 2014). We found that genotypic effects on grain-filling duration contributed positively to grain weight and grain yield at

environments with low stress levels (E3 and E2), although the contribution was significant only at E2 (Fig. 3; Table 4). Sorghum grain weight is highly dependent on source availability during the effective grain-filling period (Gambín and Borrás 2007), suggesting that these environments show favourable conditions during the post-anthesis period, and that genotypes that could exploit it produce higher yield. Working with inbred lines at similar environments, Gambín and Borrás (2011) described large variations in grain-filling duration and concluded that selecting for longer grain filling can increase grain weight and yield without negative trade-off correlations with grain number. The lack of correlation between time to anthesis and duration of grain filling suggests that both traits could be independently combined.

However, genotypic effects on time to anthesis made a significant contribution on grain yield only at the most stressful environments (Fig. 3), showing that early materials (G3) are recommended for environments showing reduced water availability at early or pre-flowering stages. Interestingly, a shorter time to anthesis impacted positively on yield by increasing grain weight. Genotype group G2 evidenced a delay in flowering time under these conditions, behaving similarly to late flowering genotypes G1 (Fig. 2*b*). Sorghum is known for its capacity to delay flowering time when exposed to limited water conditions during pre-flowering stages (Donatelli *et al*. 1992; Craufurd *et al*. 1993). Our results suggest this delay is not desirable due to its negative consequences on grain weight. A delay in flowering time implies exposing the post-flowering period to poorer growing conditions, similar to late flowering genotypes. We also confirm the existence of genotypic differences for this trait that were not previously reported.

Interestingly, yield was consistently and positively correlated with grain weight in all environments and was not correlated with grain number m^{-2} (Table 4), meaning that genotypic variations in grain weight were more important than grain number variations. Grain number and weight were negatively correlated (Table 4). Based on our results, grain yield selection should indirectly increase grain weight and reduce grain number across environments.

The use of managed trials for identifying superior genotypes for a broader set of target environments was proposed to assist breeding selection (Zavala-García *et al*. 1992; Cooper *et al*. 1997). In this context, the definition of which specific conditions are representative of the target population of environments is critical. In our study we are not able to clearly define which environments are representative of the target population of environments unless more managed environments are tested together with correlations in target environments. A better understanding of the types of stress defining environmental types is evidently important.

Conclusions

Large G × E interaction for grain yield in sorghum was evident. Although this, large relative contribution of genotypic effects for most secondary traits suggests that G × E interaction would not be a major impediment for attaining high selection response for these traits.

We concluded that selection strategies to increase grain yield across temperate environments should be focussed on early or intermediate flowering hybrids with long grain-filling duration that allow maximising grain weight. Late flowering hybrids locate the grain-filling period under unfavourable growing conditions affecting grain size.

Under environments showing limited water conditions at pre-flowering stages, early flowering materials are desirable. Hybrids expressing a delay in flowering time under this situation are not desirable due to its negative consequences in reducing grain weight.

Conflicts of interest

Authors declare no conflicts of interest.

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