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Adaptation and plasticity of yield in hybrid and inbred sorghum

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Abstract

Local adaptation and genotype by environment (G×E) interactions affect the expression of phenotypes in crop species. An investigation on the interplay between adaptation and G×E on sorghum heterosis phenotypes is lacking. To address this question, a set of 112 diverse grain sorghum hybrids and their 22 inbred parents of local and exotic origins, representing the primary female and male heterotic groups, were tested in five contrasting sorghum-growing environments across 2 years in Queensland, Australia. Plant height, days to flowering, seed yield, grain weight, and grain number were measured and used in the estimation of heterosis. Midparent heterosis for yield ranged from −25% to 217% and was highly influenced by grain number. In contrast to observations in maize, the magnitude of heterosis for yield was not associated with site mean. Striking differences were observed in heterosis in hybrids from locally developed inbred parents compared with hybrids that were developed from exotic inbred parents developed in other countries. Heterosis in the latter combination was higher on average across all the test environments for the majority of traits. We hypothesize maladaptive phenotypic plasticity in the exotic parents contributed to the observed differences in heterosis estimates. These data confirm that heterosis estimates in sorghum must be obtained and interpreted in relevant genetic and environmental contexts. Breeders in developing countries with low sorghum hybrid seed uptake will find these insights useful when selecting hybrids for broader adaptation, improving efficiency of their breeding programs.

1 | INTRODUCTION

Heterosis or hybrid vigor is the superior phenotypic performance of a hybrid over its inbred parents (East, 1936; Shull, 1952). This phenomenon has been exploited for over a century to improve productivity in several major cereal and oilseed crops (Duvick, 1999), driving multibillion-dollar hybrid seed

production businesses (Phillips, 1999). The most important contributor to heterosis is thought to be directional dominance for favorable alleles (Mackay et al., 2021). Exploitation of heterosis is likely to expand to new crops and regions as new hybrid production systems are developed.

The degree of observed heterosis varies widely between plant species, with outcrossing types having generally higher levels relative to self-pollinating species (Hochholdinger & Baldauf, 2018). Fixation of alleles due to inbreeding, reduction of genetic load, and the purging of deleterious alleles over

Abbreviations: BLUEs, best linear unbiased estimates; BLUPs, best linear unbiased predictions; G×E, genotype by environment; MET, multi-environment trial.

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time in selfing species (Barrett & Charlesworth, 1991) could explain the lower heterosis levels observed in selfing species. The degree of heterosis varies widely across measured traits within a species, the genetic background (Tracy & Chandler, 2006), and test environments (Flint-Garcia et al., 2009; Lippman & Zamir, 2007; Mindaye et al., 2016). In the absence of excessive genetic drift or sufficient gene flow, plant populations tend to adapt to artificial or natural selective forces from biotic and abiotic challenges resulting in local adaptation to an environment (Janzen et al., 2022; Leimu & Fischer, 2008). The distinction between local and exotic plant performance can be tested in such environments where the local plant types are deemed adapted relative to exotic introductions (Kawecki & Ebert, 2004). Given the general association between genetic divergence and heterosis (Jordan et al., 2003; Moll et al., 1965; Zhang et al., 2010), the interplay of the genetic and phenotypic divergence in different environments is critical for understanding and exploiting heterosis in crop breeding programs that make use of material of multiple origins.

Recent studies in maize have reported higher fitness in locally adapted maize landraces in comparison to exotic introductions that can display reduced fitness (Janzen et al., 2022). Phenotypic plasticity, which is the distinct performance of genotypes across varied environments, has been described in plants (Bradshaw, 1965; Marais et al., 2013), with a recent study in maize (Liu, Du, et al., 2021) reporting its contribution to adaptation and heterosis. Hybrids and their inbred parents may respond differently to the same environment, with the latter often being more susceptible to biotic and abiotic stresses (Troyer & Wellin, 2009). These differences coupled with the adaptation of materials used in a study may influence the observed estimate of heterosis in different contexts.

Heterosis in sorghum is widely exploited by commercial breeders; however, despite its importance, there is little published research on the phenomenon. Commercial sorghum hybrids are almost exclusively based on the A1 cytoplasmic male sterility (CMS) system (Jordan et al., 2011; Reddy et al., 2007), which was first identified by Stephens et al. (1952). The initial hybrids were the result of crosses between the Caudatum and Kafir races. These two races currently dominate the male (Caudatum dominant) and female (Kafir dominant) heterotic groups in sorghum globally. The features of CMS have particularly constrained the diversity of female parents in commercial breeding programs (Jordan et al., 2011). Subsequently, both the male and female heterotic groups have remained relatively consistent across breeding programs in multiple countries.

Early studies reported heterosis in sorghum (Bartel, 1949; Conner, 1927), and subsequent investigations provided the initial platform for commercial hybrids (Blum, 1970; Conner, 1927; Kambal & Abu-El-Gasim, 1976; Kirby & Atkins, 1968; Quinby, 1963). Most of these studies, while pivotal, are now dated. These analyses of heterosis utilized a small number of genotypes tested typically in a single environment (Kam-

Core Ideas

- Adaptation of sorghum inbred parents to test environment impacts the estimates of heterosis.
- Heterosis for yield in sorghum was not associated with mean site yields.
- Grain number in sorghum drives heterosis for yield.

bal & Abu-El-Gasim, 1976; Quinby, 1963). More recently, Mindaye et al. (2016) examined heterosis across three different environments for nine locally adapted genotypes and eight exotic seed parents in combinations between 2-dwarf and 3-dwarf sorghum lines, reporting locational differences in heterosis. However, a study of the performance of local and exotic pollen and seed parent combinations across environments in sorghum is still lacking. Further understanding of the importance of the underlying hybrid genotype and the influence of environment on the expression of heterosis for seed yield in hybrid sorghum is required to obtain the maximum benefit from deploying hybrid cultivars.

In this study, we investigated heterosis for yield, yield components, height, and flowering time in a factorial combination of 112 hybrids produced by crossing eight females and 14 male parents grown in five different environments across the Australian sorghum growing region. We specifically investigated the interplay between local adaptation and genotype by environment (G×E) interaction on the expression of sorghum heterosis phenotypes.

2 | MATERIALS AND METHODS

2.1 | Description of plant material

Sorghum parental inbred lines of two distinct origins representing the primary female and male heterotic groups were used in the generation of hybrids. The first group, the local inbred lines, is a product of the Queensland Government and Grains Research and Development Corporation sorghum prebreeding program. They consist of lines adapted to the sorghum-growing conditions in Queensland and northern New South Wales and are of preferred height (3 dwarf), flowering time, and maturity for these Australian growing conditions. The second group is of exotic origin and consists of inbred parents from various international programs. They were selected for height (3 dwarf), flowering, and maturity attributes considered suitable for Australian growing conditions and for good general combining ability in the target production environment in their originating country (Table S1). A total of 18 female and 32 male parents were used

to generate a total of 192 unique hybrids that were planted across five test locations, depending on seed availability. From this broader set, a balanced subset of 112 common hybrids planted across the five test growing locations were selected for further study. These 112 hybrids were generated from eight females (three local and five exotic) and 14 males (six local and eight exotic). The 112 hybrids consisted of 18 hybrids derived from local-by-local crosses, hereafter referred to as local hybrids; 54 mixed hybrids derived from local by exotic parental crosses, hereafter referred to as mixed hybrids; and 40 exotic hybrids derived from exotic-by-exotic crosses, hereafter referred to as exotic hybrids.

2.2 | Experimental design and phenotypic measurements

Five locations in the Australian sorghum-growing region were used as test environments for hybrid performance. These were the Hermitage Research Facility, Warwick, Australia (Lat. 28° 12'S Long. 152.06 °E, with an elevation of 470 m above sea level), Biloela (Lat. 24.38 °S Long. 150.52 °E with an elevation of 175 m above sea level), Dalby (Lat. 27.18 °S Long. 151.26 °E with an elevation of 344 m above sea level), Dalby Box (a location in Dalby but with contrasting soil type), and Springsure (Lat. 24.12 °S Long. 148.09 °E with an elevation of 345 m above sea level). The experiments were planted during the Australian summer sorghum-cropping seasons of 2001/2002 and 2002/2003 between November and February. A row–column design (Piepho et al., 2015) with two replications was used at all five trial locations. Four 6-m rows with a row spacing of 0.75 m were used across the locations with measurements and harvests made from the two middle datum rows using a small plot combine harvester. Days from planting to 50% flowering, plant height, and yield data were collected following the sorghum trait descriptors (House, 1985). Standard agronomic and pest management was applied across all the test locations.

2.3 | Statistical analysis

Linear mixed models were fitted as a multi-environment trial (MET) analysis to estimate best linear unbiased estimates (BLUEs). BLUEs were used to calculate estimates of heterosis. The MET model was also used to calculate genetic variances, genetic correlations, and best linear unbiased predictions (BLUPs).

The standard representation of a linear mixed model is given by

$$\mathbf{y} = \mathbf{X}\boldsymbol{\tau} + \mathbf{Z}\mathbf{u} + \mathbf{e}, \quad (1)$$

where \mathbf{y} is the vector of observations with the sites stacked, \mathbf{X} is the design matrix for fixed effects, $\boldsymbol{\tau}$ is the vector of fixed effects, \mathbf{Z} is the design matrix for random effects, \mathbf{u} is the vector of random effects that has a normal distribution with mean 0 and variance \mathbf{G} ($\mathbf{u} \sim N[0, \mathbf{G}]$), with fixed and random spatial effects included as necessary (see Table S2) (Gilmour et al., 1997), and \mathbf{e} is the vector of residuals $\mathbf{e} \sim N(0, \mathbf{R})$.

BLUEs were estimated by including the genotypes as fixed effects in model (1) (contains a main effect for site and for genotypes nested in sites), while BLUPs were predicted from model (1) where site by genotype was included as a random effect. A factor analytic structure (FA k) with $k = 2$ was used in the analysis (Tolhurst et al., 2019). The model was fitted separately for each trait; random and fixed terms were included as necessary per site (see Table S1). A pooled two-independent-sample t -test was used for mean separation. All analyses were conducted in the R package (R Core Team, 2021) environment version 4.04; the package ASReml-R (Butler et al., 2021) was used to fit all models, and the package ggplot2 (Wickham, 2016) was used in the visualization of all figures.

2.4 | Heterosis calculation

The BLUEs generated for all the measured traits from each MET were used to calculate heterosis. Estimation of percent heterosis was calculated by finding the ratio of the deviation of the midparental trait value from the hybrid trait value, computed as below

$$\%MPH = \frac{F_1 - MP}{MP} \times 100,$$

where %MPH is the percent midparent heterosis, F_1 is the hybrid trait value, and MP is the midparent trait value computed as the average trait value for the two inbred parents. If the two inbred parents are P_1 and P_2 , midparent trait value is $\frac{P_1 + P_2}{2}$.

2.5 | Molecular marker data

Genotypic data generated previously were available for 19 of the 22 parents of the balanced subset of 112 hybrids. The genotypic data were prepared as follows. DNA samples were extracted from young leaves using the CTAB method (Doyle & Doyle, 1987). DArTseq technology was used in the genotyping of the inbred parents by Diversity Arrays Technology Pty Ltd (www.diversityarrays.com). The procedure involves complexity reduction of genomic DNA removing repetitive sequences by use of methylation-sensitive restriction enzymes (HpaII, MseI) and generates high-density

TABLE 1 Mean plant height, flowering time, and yield for the 122 hybrids and their 22 parental inbreds across the five test locations (sites).

Site	Plant height (cm)			Flowering time (days)			Yield t/ha		
	Inbred	Hybrid	%MPH	Inbred	Hybrid	%MPH	Inbred	Hybrid	%MPH
Springure	91	106	15.32	55	55	−2.06	1.52	2.15	43.5 ^{bc}
Dalby Box	91	105	14.12	72	66	−7.26	2.37	3.23	35.7 ^c
Dalby	94	110	15.95	76	70	−6.93	2.95	4.34	52.9 ^{ab}
Hermitage	91	106	19.56	74	71	−3.58	3.08	4.84	62.6 ^a
Bileola	95	108	13.35	59	57	−3.84	3.96	5.03	28.1 ^d

Note: Percent midparent heterosis (%MPH) for each of the measured traits in the five locations. Superscripts (a–d) indicate significant differences ($p \leq 0.05$) between the heterosis values for yield between the locations.

genome-wide single-nucleotide polymorphisms (Edet et al., 2018).

2.6 | Genetic distance measurements

A total of 7852 DArTseq markers were used to estimate the genetic distances between the parental lines used in the crosses. The Sokal–Michener dissimilarity measure (Sokal & Michener, 1958) was employed using simple matching to estimate the extent of dissimilarity between the parental inbred lines used in the hybrids. The dissimilarity was calculated using the expression below

$$d_{ij} = \frac{u}{m + u},$$

where d_{ij} is the dissimilarity between units i and j , u is the number of unmatching variables, and m is the number of matching variables.

3 | RESULTS

3.1 | Analyses of flowering time, plant height, and seed yield in sorghum inbreds and hybrids

Flowering time ranged from 50 to 71 days in the early and late flowering hybrids, respectively, with a mean of 64 days. Local inbreds flowered between 54 and 79 days, while the exotic inbreds flowered between 50 and 84 days. However, the mean flowering times were similar for local and exotic inbred parental classes at 69 days. Plant height ranged from 103 to 107 cm on average in all the hybrid classes across the locations, which is a small range consistent with 3 dwarf sorghum with height selected for machine harvesting suitability. The local inbreds ranged in height from 77 to 110 cm and the exotic inbreds from 66 to 113 cm, yet both classes of inbreds had a similar mean height of 92 cm (Table 1).

Mean seed yields obtained from the 112 hybrids in the five test locations ranged from 2 to 5 tonnes per hectare (t/ha) in the low- and high-yielding locations, respectively (Figure 1).

The mean seed yields of local hybrids were greater than the exotic hybrids in all the locations, except for Dalby (Figure S1). Mixed hybrids had mean seed yield values comparable to the local hybrids but higher seed yields than the exotic hybrids across all the locations except for Dalby Box (Figure S1). The local inbred parent seed yields ranged from 1.3 to 4.9 t/ha across the test locations, while the exotic inbreds had seed yields ranging from 0.6 to 4.5 t/ha. The mean seed yield from local inbreds was higher than that of the exotic inbred across all the test locations (Figure S2).

The genetic correlation for flowering time between locations ranged from 0.217 to 0.938 (mean 0.588) for hybrids and from 0.117 to 0.917 (mean 0.543) for inbreds. The genetic correlations for plant height were consistently higher for hybrids, ranging from 0.746 to 0.918 (mean 0.823) in contrast to values ranging from 0.670 to 0.995 (mean 0.879) for inbreds, indicating the reduced impact of G×E interactions on plant height for both inbreds and hybrids (Table S3A–C).

Estimates of genetic correlations for seed yields between the five locations varied from −0.403 to 0.713, with a mean of 0.224 when only the hybrids were included in the analysis. The genetic correlation ranged from −0.283 to 0.964, with a mean of 0.264 when only inbred parents were analyzed. This is consistent with the high levels of G×E interactions commonly observed in Australian sorghum-growing environments for seed yield. The genetic variances for the inbred parents in all the measured traits across all the locations were higher than the hybrids except for flowering time at Springure (Table S4).

A wide range of midparent heterosis was observed in all the measured traits across all the environments. Flowering time midparent heterosis ranged from −14% to 3% with a mean of −5%, while plant height heterosis ranged from −6% to 39% with a mean of 15%. Midparent heterosis for seed yield ranged from −25% to 217%, with a mean of 42.9% (Figure 1). The mean midparent heterosis (%) for yield was significantly different between all the locations except for between Hermitage and Dalby, Dalby and Springure, and Dalby Box and Springure (Table 1). Analysis of grain traits from a single location (Hermitage) revealed variation of heterosis for grain size ranging from −14% to 21% with a mean of 1.8%, while grain

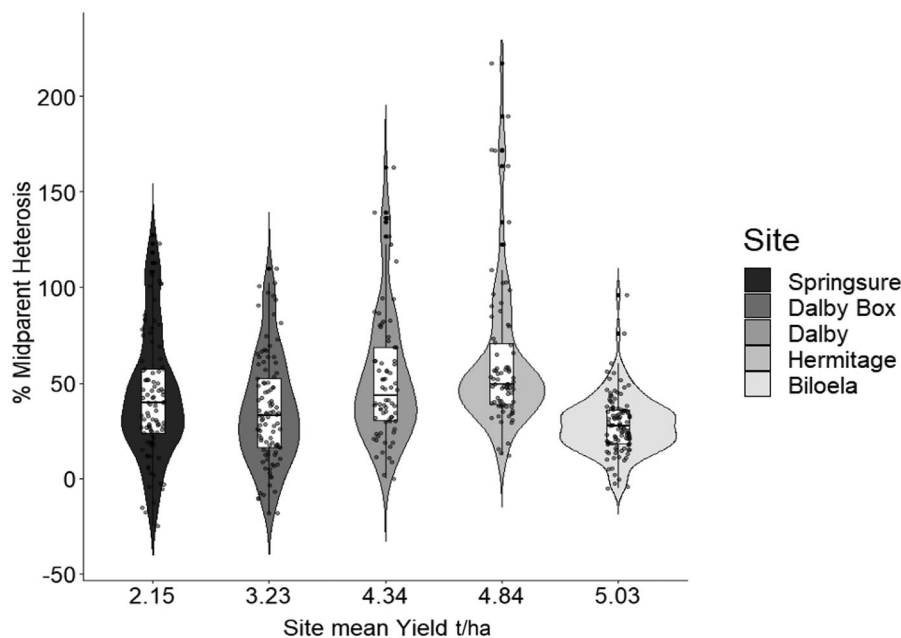


FIGURE 1 Percent midparent heterosis for yield and site mean yield in tonnes per hectare across five test locations. Site mean yield is arranged in an ascending order on the *x*-axis from left to right.

number heterosis ranged from 2% to 247% with a mean of 68%.

3.2 | Parental origin and heterosis

Genetic distances calculated from dissimilarity values of the inbred parents indicated the extent of divergence of the parents in the respective hybrid classes (Table S5; Figure S3). The greatest genetic distance between parents was evident in mixed hybrids (0.431), followed by exotic hybrids (0.397), then local hybrids (0.378). The dissimilarity values showed no association with reported heterosis (Figure S4).

Exotic hybrids showed significantly higher average midparent heterosis for yield at the higher yielding locations of Hermitage and Biloela when compared with local hybrids. No significant differences were observed between the hybrid classes in all other remaining locations for yield heterosis. Average midparent heterosis for height was not significantly different across the hybrid classes in all locations except for Dalby. Average heterosis for flowering time was significantly different at Dalby Box, Dalby, and Biloela, where exotic hybrids flowered earlier than the local hybrids relative to their inbred parents. No significant differences were observed for flowering time at Springsure and hermitage across the hybrid classes (Figure 2A–C). The mean percent midparent heterosis for plant height was 13%, 15%, and 16% for the local, mixed, and exotic hybrids, respectively. For flowering time, it was –4%, –5%, and –6%. Heterosis for seed yield ranged from –3% to 103% in local hybrids with a mean of 33%. In exotic hybrids, seed yield heterosis ranged from –25% to

217% with a mean of 50%. Yield heterosis estimates for the mixed hybrids ranged from –18% to 189% with a mean of 40% and appeared to be intermediate between the local and exotic classes. A detailed investigation of the inbred parent seed yields also showed the exotic parents were on average lower yielding across all the planting environments relative to the yield of the local inbred parents, but only significantly so at the lowest and highest yielding locations. These observations may indicate their less successful adaptation to Australian growing conditions in conducted plantings (Figure S2) especially in the extreme environments. The results indicate that crosses with other exotics and local parents can produce hybrids that yield better than the local hybrids in the different test growing regions.

3.3 | Genetic control of grain number and size

Detailed analysis of seed yield in the 112 hybrids grown at Hermitage revealed that grain number contributed to 31% of the observed variation in grain yield (Figure 3A). Grain size was found to have a nonsignificant negative trend with grain yield (Figure 3B). A comparison of hybrid grain number and midparent grain number on a one-to-one scale using a line with a zero intercept and a slope of one revealed a significantly different intercept and slope, with hybrid grain number shifted upward above the one-to-one line (Figure 3C), indicating that hybrid grain number was significantly higher than midparent grain number. The difference was highest for lower grain numbers, then reduced as the grain number increased,

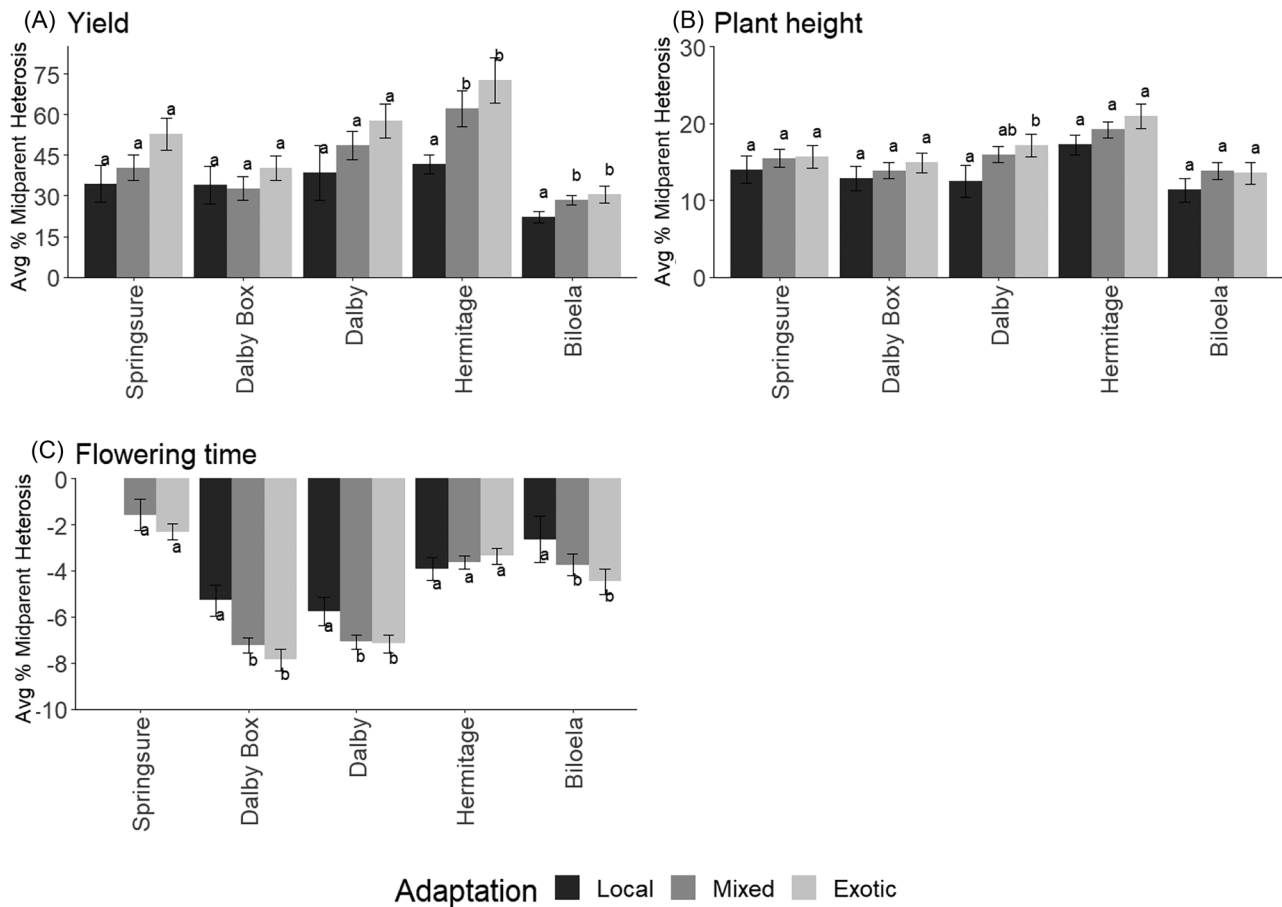


FIGURE 2 Average percent midparent heterosis for yield (A), plant height (B), and flowering time (C) across five locations in Local, Mixed, and Exotic hybrids. Lowercase letters indicate significant differences between treatments ($p \leq 0.05$), from a pooled sample t -test. Bars with same letters within a location are not significantly different. The error bars represent the standard error of the mean.

indicative of a ceiling for the increase in grain number after hybridization. These observations suggest that both additive and nonadditive gene action is responsible for the control of grain number in these hybrids.

A similar analysis for grain size also showed a significant deviation from the one-to-one line, with the hybrid grain size showing a slight upward shift above the one-to-one line, followed by a decrease and intersection with the one-to-one line as the grain size increased, indicative of a ceiling for the grain size trait (Figure 3D). Overall, the midparent grain number could explain $\sim 38\%$ of the observed variation in hybrid grain number (Figure 3C), while midparent grain size explained $\sim 54\%$ of the observed variation in hybrid grain size (Figure 3D), suggesting the potential of midparent grain size as a predictor of hybrid grain size in sorghum breeding.

4 | DISCUSSION

This study sought to elucidate the magnitude of heterosis in grain sorghum in specific genetic and environmental contexts

to enhance its exploitation in breeding programs. Heterosis estimates varied across measured traits in the test environments, with G \times E effects observed for both the hybrids and inbred parental lines.

4.1 | Magnitude of heterosis varies across environments but is not associated with mean yield

The average contribution of heterosis to yield varied between locations in this study, consistent with previous studies across crop species (Makumbi et al., 2011; Manjarrez-Sandoval et al., 1997). Further, the different hybrid classes reported different levels of heterosis for yield. Heterosis has been reported to make a larger contribution to yield in lower yielding locations in previous maize (Araus et al., 2010; Betrán et al., 2003; Duvick et al., 2004; Makumbi et al., 2011) and rice studies (Ereful et al., 2021). We hypothesize that similar observations will hold true for sorghum. Drought is the primary driver of variation in yield in Australian crop production environments

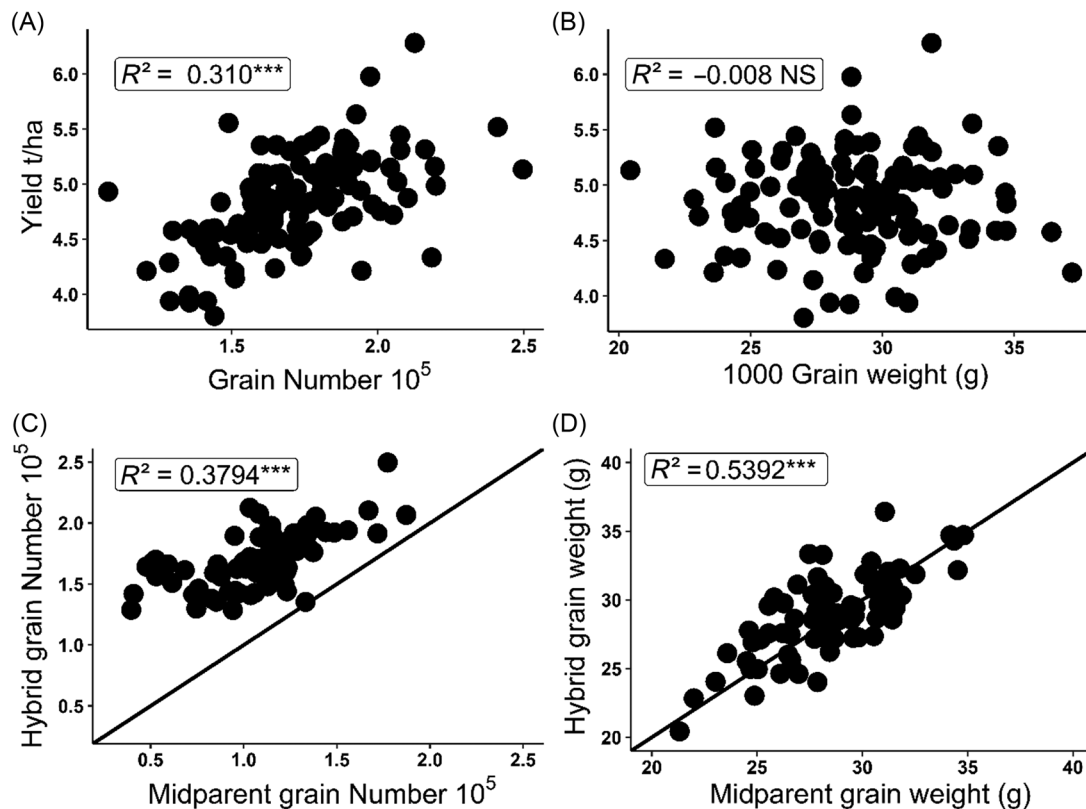


FIGURE 3 Yield against grain number (A) and grain weight (B). Hybrid grain number and midparent grain number (C), and hybrid grain weight and midparent grain weight (D) for data from Hermitage site. The diagonal line is a one-to-one line with its origin at zero for comparison of the relationship between hybrid values and midparent values. (***) $p \leq 0.0001$; NS, not significantly different).

(Ababaei & Chenu, 2019; Passioura, 1994). Variation in trial mean yield of crops can be a proxy for stress conditions in the production environment (Finlay & Wilkinson, 1963; Fischer & Edmeades, 2010). Taken as an indicator of water stress in this study, the variation in mean yield across the test environments showed no association with the observed level of heterosis in contrast to studies in maize and rice, suggesting that other factors or stresses are driving the differences in heterosis between environments.

When heterosis is expressed as a ratio of the hybrid value over midparental value, poor parental performance will tend to inflate the reported heterosis. Maize inbred parents show substantial inbreeding depression and are generally of poor phenotypic value relative to their hybrids, a scenario that may be magnified in stress environments. By contrast, hybrids having a combination of favorable alleles from both parents will have a better performance in stress environments than the average of the parents leading to the high reported percent heterosis value observed in the previous maize studies. While heterosis varies across measured traits, crop species, and production environments (Hochholdinger & Baldauf, 2018), the average heterosis for plant height and flowering time in the current study was comparable across all the test environments reflecting the high heritability and lower G×E observed for these traits.

The contribution to G×E interaction for the inbred parents and hybrids was evidenced by low correlation across the locations for yield. The hybrids displayed a broader adaptation to the environmental variation than their inbred parents. The differences in adaptation to the environment could potentially affect the estimated heterosis. These results indicate that beyond the yield advantage, sorghum hybrids provide a broad adaptation advantage, similar to observations in historical maize studies (Troyer & Wellin, 2009). This finding is in line with observations in maize where phenotypic plasticity has been shown to contribute to heterosis and adaptation (Liu, Du, et al., 2021; Marais et al., 2013).

4.2 | Adaptation of parent lines has an impact on the magnitude of heterosis

Ancestry and geographical separation of parental material have been used previously to estimate genetic differentiation in the study of heterosis in maize (Moll et al., 1965) and sorghum (Jordan et al., 2003). Locally adapted maize lines have been reported to have better fitness than exotic lines in a recent transplant experiment (Janzen et al., 2022). Heterosis estimates in the current study were on average consistently higher in hybrids derived from exotic parents than

from local parents, while the hybrids from mixed parents showed estimates for heterosis comparable with hybrids from local parents. Heterosis estimates from the exotic hybrids were likely inflated because the exotic parents performed poorly across all the environments compared to the local parents. Local inbred parents are adapted to the local climatic conditions and will be affected by the season in a like manner as their hybrids, consistent with reports in maize (Janzen et al., 2022; Troyer, 2006). The exotic hybrids may have been affected by the season differently from the exotic inbreds since the hybrids have a combination of alleles from two exotic inbreds and could have generated enhanced adaptation and reduced sensitivity to the environment in the hybrid combination. The mixed hybrids performed comparably to the local hybrids, suggesting that mixed hybrids may be deployed for a yield advantage and stability across environments. Similar mixed maize hybrids have previously been reported to show high mean yields with intermediate heterosis (Cossa et al., 1987).

The lower G×E effect observed in the sorghum hybrids has potential utility for breeders in developing countries that currently develop inbred cultivars (Axtell et al., 1999). The potential use of hybrids developed from crosses between local and exotic parents as demonstrated by Mindaye et al. (2016) would have two benefits. First, farmers would benefit from the enhanced yield of hybrids while exploiting the adaptation of local parents suited to local conditions and farmer preferences (Axtell et al., 1999; Mindaye et al., 2016). Similar observations have been reported in sorghum hybrids under extreme stress conditions (Blum et al., 1990; Gangadhar Rao et al., 1999) where crop phenology is vital to secure yields. Second, since these hybrids would likely exhibit broader adaptation, individual breeding programs could service a larger region enabling efficiencies in cultivar development and the logistics and economics of hybrid seed distribution and sale.

While it is important to select inbred lines that would give desirable general combining ability, the adaptation of the inbred line to the target population of environments is an additional crucial consideration for long-term gains in the beneficial exploitation of heterosis in sorghum. The calculated genetic distances between the parental lines were not correlated with heterosis, reaffirming the unreliability of genetic distance measurements as a predictor of heterosis in sorghum, consistent with previous reports in cereals (Conner, 1927; Crozier et al., 2020; Jordan et al., 2003; Stuber et al., 1992; Yan et al., 2010).

4.3 | Grain number drives yield heterosis in sorghum

Grain number in cereal crops is strongly correlated to grain yield and has been exploited by breeders for yield

improvement (Peltonen-Sainio et al., 2007; Sadras, 2007; Van Oosterom & Hammer, 2008). The grain number in the test hybrids in this study was highly correlated to grain yield, consistent with previous studies (Blum, 1970; Heinrich et al., 1983; Peltonen-Sainio et al., 2007). This indicates that grain number in sorghum contributes substantially to observed variation in grain yield. Grain number in cereals is determined during inflorescence development (Liu, Lindsay, et al., 2021). In sorghum hybrids, increased cell division and cell numbers have been proposed as mechanisms for the observed differences with inbreds (Quinby, 1963). Consequently, grain number in these hybrids is a major contributor to heterosis for yield in sorghum. By contrast, the insignificant negative correlation of grain weight with yield suggests that grain weight is constrained by genetic potential and grain weight in sorghum may have been fixed by genetic drift during selection for domestication traits (Purugganan & Fuller, 2009).

Potential grain weight in sorghum is limited more by the genetic potential than assimilate availability (Tao et al., 2021), indicating an opportunity to breed for improved grain weight for yield improvement. This additionally reaffirms that heterosis contributes to yield via increases in grain number. Further, the heritability for grain weight-related traits has been reported to be medium to high, indicating its potential as a selection target in breeding (Tao et al., 2021, 2020). Comparisons of grain number in a hybrid with midparental grain number indicated that both additive and nonadditive gene actions were potentially involved in the control of grain number in these sets of hybrids. Additive gene effects were more important for the determination of grain weight. These observations provide insights for breeders for the potential to exploit the available variation in grain size and number for yield improvement in sorghum, especially for production systems in water-limited environments.

5 | CONCLUSION

This is one of the largest multi-environment studies investigating the magnitude of heterosis in 3-dwarf sorghum and clearly demonstrates the substantial contribution of heterosis to flowering time and to grain yield through grain number. In contrast to studies in maize and rice, no specific advantage of heterosis for yield was observed in stress environments; however, this study identified an underappreciated benefit of heterosis for yield, which is the reduced magnitude of G×E interactions. In the context of a practical breeding program, this reduction in G×E has the benefit of increasing heritability and selection accuracy and enables a breeding program to efficiently target a large target population of environments. With the challenges of climate change and the fact that many farmers in developing countries grow inbred varieties in highly variable environments, there is potential for hybrid cultivars to enhance yield

stability and enable greater rates of productivity gains if the challenges of seed production and seed distribution can be addressed.

AUTHOR CONTRIBUTIONS

Daniel Ajaku Otwani: Formal analysis; methodology; software; validation; visualization; writing—original draft; writing—review and editing. **Emma Mace:** Conceptualization; project administration; supervision; validation; writing—review and editing. **Colleen Helen Hunt:** Data curation; formal analysis; software. **Owen M Powell:** Writing—review and editing. **Anna Koltunow:** Supervision; writing—review and editing. **Alan William Cruickshank:** Resources; writing—review and editing. **David Jordan:** Conceptualization; funding acquisition; investigation; project administration; resources; supervision; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the current study are available on the dryad website <https://doi.org/10.5061/dryad.69p8cz984>.

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