Extended grain filling has potential to improve yield in grain sorghum

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Highlight

Exploitable variation for grain filling duration remains untapped in sorghum breeding. Genotypes with extended grain filling duration offer a yield advantage for the simulated Australian environments with non-limiting water post anthesis.

Abstract

Yield increase in sorghum has been achieved primarily by increasing grain number. Scope exists to increase yield by increasing grain size, however this has been limited by the negative correlation between grain size and grain number. Extending the duration of the grain filling period has potential to enable increased grain size without the trade-off with grain number. This study explored grain filling duration (GFD) in a diverse panel of 904 sorghum genotypes in three environments across two years. Significant variation in GFD observed, ranging from 400 to 680 degree-days, included entries with significantly longer GFD than current commercial hybrids. Longer GFD was shown to result in larger grain size. Additionally, only low associations between GFD and grain number per panicle, flowering time or plant height were observed, indicating that GFD could be manipulated without adverse penalty to these traits. A simulation study to estimate the benefit of an increased GFD across Australian sorghum growing environments over 60 years revealed positive impacts on yield when GFD was increased by either 10% or 20% in environments with low and mild post anthesis water stress but not in environments with sustained terminal water stress. However, maintaining overall crop duration by shortening time to flowering while extending GFD led to neutral or negative effects on yield. These results reveal opportunities to exploit GFD for improved genetic gains for yield in sorghum especially in environments or seasons where water does not become more limiting post anthesis.

Abbreviations

GFD; Grain filling duration, APSIM; The Agricultural Production Systems sIMulator, GxExM; Genetics by Environment by Management.

Key words

Physiological maturity, degree days, crop simulation modelling

1 Introduction

Productivity increases in major cereal crops have been attained mainly through increases in grain number per unit area (Boyles *et al.*, 2016; Peltonen-Sainio *et al.*, 2007), as observed in rice and wheat in the green revolution era (Khush, 1999; Reynolds *et al.*, 1999). While grain size also contributes to potential yield, a compensating trade-off between grain size and grain number (Boyles *et al.*, 2016; Khan *et al.*, 2022; Sadras, 2007), together with a limited genetic variation for grain size in cereals, has limited its exploitation for yield improvement. Grain filling duration, which is the time period from flowering to the formation of the abscission layer on the grain, and the rate of grain filling, which is the amount of assimilates partitioned to the grain per unit of time, both contribute to the final grain size in crops (Egli, 2006; Gambín *et al.*, 2008; Sadras and Egli, 2008; Xie *et al.*, 2015). A recent study on commercial sorghum hybrids reported no changes in grain filling duration, filling rate and grain size over the last six decades of breeding in the USA (Demarco *et al.*, 2023). In contrast, kernel weight has made a significant contribution to the genetic gains in maize hybrid yield in the same region, predominantly due to an extended kernel filling duration in new maize hybrids relative to old hybrids (Fernández *et al.*, 2022). Similar observations have been reported in rice (Yang *et al.*, 2008) and wheat (Chapman *et al.*, 2021), and a preliminary detailed study in sorghum (Yang *et al.*, 2010) suggested that opportunities exist to explore available variation in grain filling duration for yield in breeding programmes (Gambín and Borrás, 2012; Sadras and Egli, 2008).

The interplay between grain filling rate and duration and their contribution to yield in sorghum have been studied with inconclusive results (Demarco *et al.*, 2023; Woldesemayat *et al.*, 2015). There have been limited studies on the physiological and genetic control of grain filling duration in sorghum. Yang *et al.* (2010) reported a detailed study on kernel growth in a limited set of sorghum germplasm varying in grain size. They found that the long kernel filling duration of the large-seeded KS115-based germplasm (Tuinstra *et al.*, 2001) was the main mechanism associated with its increased grain yield. Variability exists in the rate of grain filling in cereals and legumes, however, this has been mostly yield neutral (Egli, 2006, 2017) as a consequence of the trade-offs with grain number. Since potential seed size in sorghum is more limited by genetic potential rather than assimilate availability (Tao *et al.*, 2017; Tao *et al.*, 2021), we hypothesise that with non-limiting assimilates, accounting for environmental variations in temperature, longer grain filling genotypes could provide yield advantage by enabling more assimilates partitioned to the grain (Eastin *et al.*, 1971). Understanding the physiological and genetic control of the nexus of grain traits that contribute to a yield advantage in sorghum could provide new avenues to improve and sustain yields in an increasingly unpredictable production environment. Here we aim to investigate the variation in grain filling duration of diverse sorghum lines across multiple environments and simulate the impact on sorghum yield and yield components of extending the grain filling duration.

Determining the potential value of variation in target traits to breeders and ultimately to producers is difficult without many years of empirical testing. Crop growth modelling (Hammer *et al.*, 2010; Kiniry and Bockholt, 1998) has been used for simulations of complex adaptive traits across target environments to understand the potential importance to breeding and consider their possible value against resource investment (Hammer, 2020; Kholová *et al.*, 2014). The APSIM model has been used to model photosynthesis and other traits in sorghum along with agronomic interventions to assess possible benefits to growers in developed and developing regions of the world (Dimes *et al.*, 2003; Wu *et al.*, 2019). Modelling sorghum with an extended grain filling period remains an open question in research and the potential benefits/losses have not been quantified.

Hence, the objectives of this study were to

(i) establish the extent of genetic variation for grain filling duration in a diverse set of sorghum genotypes,

(ii) establish the association of grain filling duration with other yield determinants in sorghum, and

(iii) examine the putative value of an extended grain filling duration to sorghum yield across environments through simulation modelling.

2 Materials and methods

2.1 Plant materials and experiments

The sorghum diversity panel (DP) (n = 904) previously described by (Tao *et al.*, 2020), was used in the current study. Three experiments were planted, two at the Hermitage Research Facility (HRF), Warwick, Queensland, Australia (28˚ 12′ S, 152˚ 5′ E, 470 m above sea level) in November 2020 and December 2021. 881 DP genotypes were planted in a row column design with partial replication where 30% of the genotypes were replicated two or more times while the remaining 70% were in single plots in 2020/21 season (HRF1) and a fully replicated trial in 2021/22 season (HRF2). The third experiment was planted at Gatton Research Facility (GAT), Gatton, Queensland, Australia, (27˚ 33′ S, 152˚ 20′ E, 94 m above sea level) in February 2021(Supplementary Fig. S1). A total of 609 DP genotypes were planted in a fully replicated trial of two replications in a row column design. All the trials were planted during the Australian summer growing season in single row plots 4 metres long with 0.76m spacing between rows, using an ALMACO GPS guided and spaced vacuum planter to achieve a planting density of 80,000 plants per hectare. Fertiliser was applied at the rate of 150 kg nitrogen per hectare. Supplemental irrigation was provided when required to avoid water stress. Standard agronomic practices were employed in the trial management to ensure timely pest and weed control. Overall, the experiments had 598 genotypes in common (Table 1). The DP lines were classified in to four racial groups based on population structure analysis as described in (Tao *et al.*, 2020) as Guinea, Caudatum, Kafir and Durras (Asian and East African origin), with admixtures types designated as mixed.

2.2 Phenotypic evaluation

Single plants of each genotype were tagged in each plot at the time of head exsertion prior to onset of flowering (Fig. 1A). All measurements for timing of flowering and maturity were recorded on the tagged plant. Flowering time was recorded as the date when the first anthers become visible at the tip of the panicle (Fig. 1B). The tagged plant was monitored throughout the season and the date of physiological maturity was recorded as the date when a sampled grain from the tip of the panicle first showed the abscission layer (black layer) at the point of connection of the grain (Fig. 1D, far right grain image). Plant height was measured at HRF2 by selecting one plant at random from the plot and measuring the distance from the base of the plant to the tip of the panicle at physiological maturity. Single panicles were harvested at HRF2, threshed, and cleaned before grains per panicle and thousand kernel weight (TKW) were measured using an automatic seed counter and weighing machine (Ball Coleman Gen3 seed counter). Daily weather data was recorded using a portable weather station placed within the trial to record daily maximum and minimum air temperatures for the duration of the experiment. Overall, the trial at Gatton experienced lower temperatures during anthesis and post-anthesis in the grain filling period.

2.3 Thermal time calculation

The observations for time (days) to flowering and physiological maturity for each entry were used to calculate the thermal time for grain fill duration using the method described in (Hammer and Muchow, 1994) to compute degree days for each day during the grain filling period as:

$\Delta TT = 0$ for $T < T_b$

$\Delta TT = T - T_b$ for $T_b < T < T_{opt}$

$\Delta TT = T_{opt} - T_b$ for $T > T_{opt}$

Where T_b = Base temperature for development (°C)

T_{opt} = Optimum temperature for development (°C)

T = Average daily temperature (°C)

The base temperature for grain filling duration and vegetative growth up to flowering were set at 5.7 °C and 11°C, respectively (Hammer and Muchow, 1994) and the optimum temperature for grain filling was taken as 23.5 °C (Hammer and Muchow, 1994), and 30 °C for vegetative growth. In addition, the maximum temperature for vegetative growth was taken as 42 °C (Kumar *et al.*, 2009). Throughout the duration of the experiment, the mean daily temperatures were within the optimum range for all the growth stages. This approach was used because grain filling duration is more associated with accumulated thermal time than with time in days and given the variation in daily temperature and the variation in flowering dates it is likely that the use of days to measure GFD would result in over and underestimation of the GFD for individual lines.

2.4 Modelling study

A simulation study was conducted using the APSIM NEXT GEN platform (Holzworth *et al.*, 2018), which incorporates the sorghum crop model detailed by Hammer *et al.* (2019); (2010). Three locations (Supplementary Fig. S1) representing the main sorghum production regions in Australia were used: Emerald in Central Queensland with a black vertosol soil of 1000 mm depth and 160 mm maximum plant available water content, Dalby in South Queensland with a black vertosol soil of 1800 mm depth and 306 mm maximum plant available water content, and Tamworth in northern New South Wales with a black vertosol soil of 1800 mm depth and 236mm maximum plant available water content as per Hammer *et al.* (2014). Sixty-years of historical daily weather data (1960-2020) was used for all the simulations. All simulations were conducted assuming 100mm available soil water at sowing and non-limiting nitrogen (N) availability. Three sowing dates were simulated for each season: one each in October , November, and December. Planting density was maintained at 5 plants per square metre with a solid row configuration at a row spacing of 1000 mm. Sowing depth was set at 30mm. Genotypic coefficients were set for the standard commercial hybrid ‘Buster’ at the values reported by Hammer *et al.* (2010) with tillering as reported by Hammer *et al.* (2014). The output included yield, yield components, leaf area index (LAI) at flowering and maturity, leaf number, fertile tiller number, leaf, stem, and total biomass at flowering and maturity, extractable soil water at flowering and maturity, and days to flowering and maturity. Each simulation was characterised by its environment type (ET1-ET5) depending on the dynamic of the plant water status through the season and its proximity to ETs as described in (Hammer *et al.*, 2014). Briefly ET1 on average experienced low levels of water limitation throughout the crop season (Low stress), ET2 on average experienced an increasing level of water limitation post flowering (Mild stress), ET3 on average experienced an early onset of water limitation that was relieved during the grain filling period post anthesis (Relieved stress), ET4 on average experienced an early water limitation pre anthesis that progressed post anthesis with no relief (Sustained stress), and ET5 on average had a gradual water limitation that started pre flowering and progressed post flowering (Terminal stress).

Three simulation treatments were imposed: i) Standard, where a normal time to flowering and maturity was maintained, ii) Extended, where normal time to flowering was maintained with the flowering to maturity phase being extended by 10 or 20% (~5 and 10 days respectively), and iii) Revised, where normal overall crop duration (time to maturity) was maintained but with a reduced time to flowering and grain filling extended by 10 or 20% (~5 and 10 days respectively).

2.5 Statistical analyses

Data from the three sites were combined in a multi-environment trial (MET) and analysed using a linear mixed model. This model was used to estimate the correlations between the sites and to calculate heritabilities for each site.

The standard representation of a linear mixed model is given by:

$$\mathbf{y = X\tau + Zu + e} \qquad (1)$$

Where y is the vector of observations with the sites stacked, **X** is the design matrix for fixed effects, **τ** is the vector of fixed effects, **Z** is the design matrix for random effects, **u** is the vector of random effects, which has a normal distribution with mean 0 and variance- covariance matrix **G (u~N(0, G)),** with fixed and random spatial effects included for each site as necessary (Gilmour *et al.*, 1997) and **e** is the vector of residuals **e~N (0, R).**

The variance-covariance matrix for the site by genotype interaction (GxE) was fitted using a correlation structure (corgh). This structure allows for a different genetic variance for each site and different correlations for each pair of sites. Different models were fitted separately for each trait, with random and fixed terms included as necessary per site (Supplementary Table S1). The simulation data was analysed using a linear mixed model with yield as response and simulation type, and environment type interaction included as fixed effects. The residual term has a variance-covariance matrix that allowed for separate residual variances per environment type (Supplementary Table S1). All analyses were conducted in R (RCoreTeam, 2024)) environment version 4.04, the package ASReml-R (TheVSNiTeam,

2023) was used to fit all models and the package ggplot2 (Wickham, 2016) was used in visualising all figures.

3 Results

3.1 Climatic conditions

Daily temperatures were recorded at each experimental site (Fig. 2) for the duration of the experiments. At HRF1 and HRF2, average daily temperature throughout the grain filling period was always above the base temperature for development (5.7 °C), so no adjustments were necessary in calculating thermal time. However, mean daily temperature exceeded 23.7 °C on a few instances and adjustments were made accordingly to account for thermal time accumulated. At GAT, the experiment experienced cooler temperatures around flowering time with instances where the average daily temperature was below the base temperature, so adjustments were made accordingly. Rainfall was adequate and well distributed throughout the season (data not shown) so that water was not limiting.

3.2 Variation in GFD

Across genotypes, GFD ranged from 20 to 60 days with means of 46 (28-60), 30 (22-38) and 34 (20-44) days at GAT, HRF1 and HRF2 respectively. When converted to thermal time, the GFD ranged from 400 to 680-degree days (Fig. 3). The means across the entries for each experiment were 510, 506 and 521-degree days for GAT, HRF1 and HRF2 respectively (Table 2). This indicates the major effect of temperature in generating duration differences across the experiments. The consistency of long GFD genotypes across the three experiments by observing the top 5% of genotypes indicated that more than 77% were consistent (Supplementary Table S2), similar to the observation in the genetic correlations (Table 2).

A comparison of GFD across the sorghum genotypes defined by races showed that race guinea had a different GFD on average from all the other races (Fig. 4).

3.3 Genetic variances, heritability, and correlation of GFD across experiments

Appreciable genetic variation for GFD was observed, with moderate broad sense heritability estimates ranging between 41% and 61%. Genetic correlations between sites were strengthened or unaltered when GFD was estimated in thermal time rather than in days (Table 2) especially where large temperature variations were observed, vis HRF1 and GAT.

3.4 Association of GFD with yield components

Across experiments, flowering time ranged from 523-degree days in the earlier flowering genotype to 914-degree days in the late flowering types. The overall mean flowering time was 734-degree days. No significant association was observed between GFD and flowering time at HRF1, while at HRF2 and GAT, GFD had a significant association with flowering time explaining 9% and 1.5% respectively of the observed variation (Fig. 5A). Plant height, when measured from the base of the plant to the tip of the panicle, ranged from 58.31 cm to 217.13 cm with a mean of 101.98 cm at HRF2. Variation in plant height explained between 0.8% - 2.8% of observed variation in GFD across the test locations (Fig. 5B). Grain number per panicle ranged from 572 to 3678 with a mean of 1615 grains. Significant variation in grain number was observed at HRF1 and HRF2 but not at GAT (Fig. 5C). The weight of a thousand grains (TKW) ranged from 9.47g to 45.34g with a mean of 22.39g. The commercial check had a TKW of 27.82g. TKW was significantly positively associated with GFD, explaining between 6% and 25% of the observed variation in GFD across the locations (Fig. 5D).

3.5 Simulation of GFD

The average sorghum yield in the simulation study was 4423 (2316-6663) kg/ha at Tamworth, 4231(2430-5480) kg/ha at Emerald and 4438 (2436-7154) kg/ha at Dalby. Extending the GFD of the simulated standard hybrid by 10% resulted in increases in yield of between 5.6% and 7.5% relative to the standard hybrid (Fig. 6A, Table 3). A 20% extension of GFD resulted in simulated yield increases of between 10% and 13% (Fig. 6B, Table 3). Reducing the duration to flowering by 10 or 20% of the standard GFD to enable an extended grain filling duration by either 10 or 20% resulted in both positive and negative changes in yield relative to the standard genotype (Fig. 6 C-D).

Further evaluation of the simulated sorghum yields across the different environment types within each location revealed mean yields ranging from 2436 kg/ha in severely water limited environment type 4 (ET4) to 7154 kg/ha in environment type 1 (ET1) where water was mostly not limiting (Table 4). Extending GFD by 10% resulted in a yield increase of between 3.3% and 8.5% across the environment types, with the highest percentage increase observed in ET3. Changes of between 6.4% and 16.6% were observed across the environment types in scenarios where the GFD was extended by 20%. These yield increases were mainly significantly different from the standard in environments where water was not limited post anthesis (Table 4, Supplementary Table S3). In the revised scenarios where the shorter time to flowering was simulated, there was generally a non-significant change in yields across all the locations and environment types, with few exceptions at ET2 Dalby, ET1 and ET2 Emerald and ET4 Tamworth where significant positive and negative changes were observed (Supplementary Table S3). Reducing the flowering time to increase grain filling duration at Tamworth generally had negative impacts on the attainable yield across the different environment type simulations (Supplementary Table S3).

Increases in kernel weight were observed in all the environments and environment types with greater increases observed in ET1 and ET2, where water was mostly non-limiting post flowering. ET4 and ET5, which have significant post-flowering water limitation, had lower near zero increases in kernel weight (Supplementary Fig. S2).

4 Discussion

This study reports the extent of genetic variation in grain filling duration observed in a diverse panel of sorghum genotypes and highlights opportunities to use this trait in breeding for yield. A simulation study was conducted that suggested that increasing grain fill duration (GFD) has the potential to result in yield increases likely in Australian production environments.

4.1 Variation in GFD in sorghum is available beyond the commercial range but does not appear to have been utilized for improving yield

Appreciable genetic variation with moderate heritability was observed for GFD in the diversity panel with the phenotypic distribution indicating that the trait is controlled by multiple genes. We observed that 10% of genotypes evaluated have a longer GFD than the commercial check and did not seem to be negatively associated with important agronomic traits such as height and phenology. In combination, our results suggest that there is potential to increase yield by enhancing grain filling duration beyond current levels. However, previous reports on changes in GFD across sorghum hybrids in USA showed no changes in GFD over 60 years of breeding associated with a 0.5% per year increase in yield over the same period (Demarco *et al.*, 2023). The lack of association of GFD with yield in the Demarco *et al.* (2023) study could be explained in at least four ways; 1) that GFD was poorly estimated, 2) GFD is not associated with enhanced yield in sorghum, 3) GFD is correlated with other traits that have negative consequences on hybrid performance, and 4) that there is no genetic variation for GFD that can be exploited in elite US sorghum populations.

It is highly unlikely that GFD was poorly estimated by Demarco *et al.* (2023) given the narrow range of relative maturity in the tested hybrids and the limited difference in temperature across all the test locations. Regarding point 2, several studies in cereals have reported the contribution of GFD to yield and yield genetic gain. In maize, Fernández *et al.* (2022) reported that GFD contributed close to 50% of the genetic gain in kernel weight. Similar observations have been reported in an historical study of Chinese maize hybrids between 1964 and 2014 reporting that GFD contributed up to 54.46% of the observed variation in hundred kernel weight, with recent hybrids having longer filling duration and yield than their predecessors (Gao *et al.*, 2023). Reports of longer GFD association with yield have also been recorded in wheat (Chapman *et al.*, 2021) and rice (Jones *et al.*, 1979; Wang *et al.*, 2008; Yang *et al.*, 2008). These observations show that GFD is associated with yield in cereals and therefore likely to be similarly associated in sorghum, as observed by Gizzi and Gambin (2016). Regarding point 3, in the current study in sorghum, TKW has been shown to have a significant positive association with GFD. Additionally, we have no evidence to indicate that GFD is negatively associated with major agronomic traits such as height and flowering time, though the potential association between increased GFD, resulting in increased stem remobilization and increased lodging needs to be explored. Finally, regarding point 4, it is plausible that there is no substantial exploitable genetic variation for GFD in elite US sorghum germplasm potentially due to a small genetic pool that characterises many sorghum hybrid breeding programs. Most long GFD genotypes were from the guinea race (Fig. 4) which is seldom used in commercial hybrid programs. Sadras and Egli (2008), while reporting that grain filling rate dominated contribution to yield, appreciated that with wider diversity of genotypes, GFD's contribution to yield would be captured better. Understanding the GFD trait in sorghum therefore could provide opportunities for increasing genetic gains for grain size and therefore yield.

4.2 Simulation modelling indicates the potential of GFD to increase yield in sorghum

In this study grain fill duration was modelled by increasing the length of time between flowering and physiological maturity, assuming that rate of grain filling did not change, and that maximum grain size was constrained only by the assimilate partitioned to the grain during the period of grain filling. It was assumed that there was no association between GFD, and grain number and the study did not consider the potential increase in lodging that would likely be associated with an increase in demand for carbohydrates. With these assumptions the simulations suggest that extending the grain filling duration in sorghum could result in increases in grain yield in most Australian sorghum environments. The yield increases were greatest in environments where adequate water was available post flowering and had the smallest impacts in water limited environments (Table S3). Because of its perennial nature, if water is available during the grain filling period, sorghum will continue to produce additional carbohydrate which can be used to increase grain yield.

In Australia about 50% of the environments in the major sorghum growing regions have sufficient water available post flowering to enable extended GFD to result in increased yield. In environments where water is limited one might expect that the additional demand for assimilate created by longer GFD would result in remobilization stress which could increase the degree of lodging. It is likely that traits that achieve temporal water conservation such as stay green traits (Borrell *et al.*, 2001; Harris *et al.*, 2007; Jordan *et al.*, 2012), and canopy characteristics (Borrell *et al.*, 2014; Mantilla-Perez *et al.*, 2020) that favour increased storage of assimilates such as plant height (Fernandez *et al.*, 2009; George-Jaeggli *et al.*, 2011; George-Jaeggli *et al.*, 2021) could interact favourably with GFD to enhance its positive impacts of yield.

4.3 Different GxExM strategies for deploying GFD trait in sorghum breeding

All simulations conducted revealed that extending the grain filling duration in sorghum provided opportunities for increased or stable yield in the Australian production environments. The yield increases were consistent with the available water post anthesis with minimal and negative increases where sustained water limitation was more severe. The genetic variation in stay green traits (Borrell *et al.*, 2001; Harris *et al.*, 2007; Jordan *et al.*, 2012), plant height (Fernandez *et al.*, 2009; George-Jaeggli *et al.*, 2011; George-Jaeggli *et al.*, 2021), and canopy characteristics (Borrell *et al.*, 2014; Mantilla-Perez *et al.*, 2020) that can be employed to achieve temporal water conservation in sorghum could be leveraged along with extended GFD to improve sorghum yield. These strategies could be further tested by simulation modelling. Agronomic interventions, such as planting density (Wade and Douglas, 1990; Whish *et al.*, 2005) and time of planting, could also be important as a strategy to minimise risks of post anthesis water stress. They can also be assessed initially using simulation of GxMxE combinations as in the study of Hammer *et al.* (2014).

Reducing the time to flowering to achieve an extended grain filling duration by either 10 or 20 % showed increases in yield predominantly in environments where water was limiting (ET4 and 5), and opposite effects in environments where water was not severely limited post anthesis (ET1 and 2) (Fig. 6). These observations could primarily be due to (i) the early flowering scenarios provided an opportunity to conserve available moisture which was used during grain filling period increasing the potential yields attained and (ii) the early flowering in scenarios where water was not severely limited, resulted in less biomass accumulation pre flowering and led to a penalty in yield attainable potentially from a reduction in grain numbers (Van Oosterom and Hammer, 2008).

The Australian production environments simulated here are mostly constrained by timing and magnitude of water limitation, rather than by season length (Hammer and Muchow, 1994). In other production regions that might be constrained in length by low temperatures and frost, the extended GFD may have more downside consequences. Again, potential consequences could be explored by simulation in the first instance. In that situation, other strategies, such as maintaining overall crop duration but with extended GFD and slightly earlier flowering, might be advantageous in utilising the variation in GFD. A potential negative consequence of increasing GFD for yield advantage in sorghum could occur if the yield increases predispose the crop to lodging. Lodging could occur either because of the heavy panicles attained or due to weaker stems following excessive remobilisation of assimilates into the panicles late in the season (Rajewski and Francis, 1991). The potential impact of excess remobilisation has not been captured in the current simulation and would improve future studies of extending GFD. Finally, changes in the GFD may require changes in crop husbandry practices like fertiliser application, planning of rotations, and spray out activities before sorghum harvest. These should be considered with respect to resources needed and farm profitability.

4.4 Potential limitations of the study

Two methodological issues should be noted. First, the estimation of GFD in the current study was based on the observation of the abscission layer (black layer). This allowed at scale phenotyping to capture the genetic variation for this trait at the population level. In sorghum, (Eastin *et al.*, 1973; Vanderlip and Reeves, 1972), as well as in . In maize (Hunter *et al.*, 1991), the observation of the black layer is a confirmation that the plant reached physiological maturity, but the actual maximum dry weight may occur a few days earlier. Then our estimation of GFD based on black layer may be slightly overestimated. However, the second issue may be underestimating the duration of GFD. We based the estimation on grains from the tip of the panicle. Physiological maturity is observed typically at the base of the panicle at plant level in sorghum, but tracking GFD from the basal grains presents a complexity in terms of estimating flowering times at scale. While intra panicle patterns of growth and development differ among grain positions (Gambín and Borrás, 2005; Heiniger *et al.*, 1993a), sampling consistently from a specified potion of the panicle captures the estimates of GFD at scale appropriately as observed in variable pollination study (Heiniger *et al.*, 1993b). Consequently, estimates of GFD from the top of the panicle may not be necessarily representative of the base of the panicle due to flowering time and genotypic differences and warrants further investigation.

5 Conclusion

We have established that exploitable genetic variation is available for GFD in sorghum with some exotic lines having longer GFD than current commercial hybrids. Simulation studies revealed that extending the GFD increased yield in simulated scenarios in Australian production environments with non-limiting water post anthesis. While there are factors that require further study, we consider GFD is a potentially useful target trait for increasing sorghum yields and further work is needed to understand its genetic control and interactions with crop management.

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Acknowledgements

The authors acknowledge the contribution of the University of Queensland and Queensland Government's sorghum pre-breeding field team.

Author contributions

Conceptualization D.J., D.O and E.M.; Data curation D.O and C.H.; Formal analysis D.O. and C.H.; Funding acquisition D.J.; Investigation D.O.; Methodology D.O.; Project administration D.J and E.M.; Resources D.J., E.M. and A.C.; Software D.O., C.H., G.H. and GM.; Supervision A.K., E.M., A.C., C.H. and D.J.; Validation D.O. and C.H.; Visualization D.O.; Writing – original draft D.O.; Writing – review & editing D.O., D.J., E.M., A.K., Y.T., G.H., C.H. and G.M.

Conflict of interest

No conflict of interest declared.

Funding statement

The study was funded from investments by the Queensland Government, and The University of Queensland. D.O. is a beneficiary of the University of Queensland RTP Scholarship.

Data availability

All primary data to support the findings of this study are openly available in Dryad at https://doi.org/10.5061/dryad.hhmgqnkrn (Otwani *et al.*, 2025).

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Tables

Table 1: Number of genotypes in each experiment (diagonal) and genotype concurrence across experiments (off-diagonal elements)

Experiment	GAT	HRF1	HRF2
GAT	609		
HRF1	598	881	
HRF2	599	852	879

Table 2: Mean GFD, genetic variance, and estimate of broad sense heritability(H2) for each experiment, and genetic correlation of GFD across experiments for measures in days (above diagonal) or degree days (below diagonal in bold).

					Genetic correlations		
	Mean GFD (°Cd)	Genetic variance	Error Variance	H^2	GAT	HRF1	HRF2
GAT	500	757	1250	41	1	0.3	0.46
HRF1	506	1004	1027	61	**0.52**	1	0.92
HRF2	521	819	1584	56	**0.45**	**0.86**	1

Table 3: Simulated percentage change in yield relative to the standard hybrid for genotypes with 10 or 20% longer grain filling duration. Mean of all environments for each site has been used * Denotes significant difference at P<=0.05.

Site	Standard Mean yield Kg/ha	GFD +10% Change in yield (%)	GFD +20% Change in yield (%)
Tamworth	4423	5.7*	10.5*
Emerald	4231	7.5*	13.7*
Dalby	4438	5.6*	10.1*

Table 4: Simulated percentage change in yield relative to the standard hybrid for genotypes with 10 or 20% longer grain filling duration for each environment type (ET) at Dalby. * Denotes significant difference at P<=0.05, ns = not significant.

Dalby	Standard Mean yield Kg/ha	GFD +10% Change in yield (%)	GFD +20% Change in yield (%)
ET1	7154	7.7*	14.5*
ET2	6050	6.2ns	10*
ET3	3070	8.5ns	16.6ns
ET4	2436	4.4ns	9.2ns
ET5	3519	3.3ns	6.4ns

List of Figures

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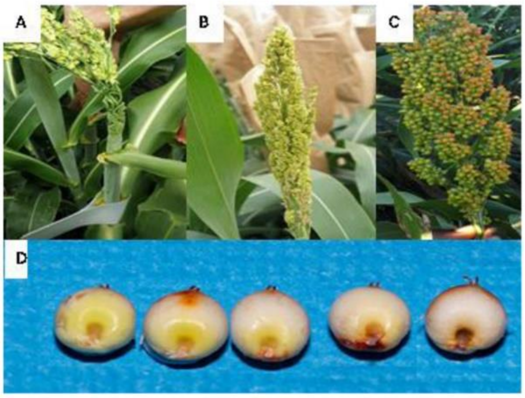

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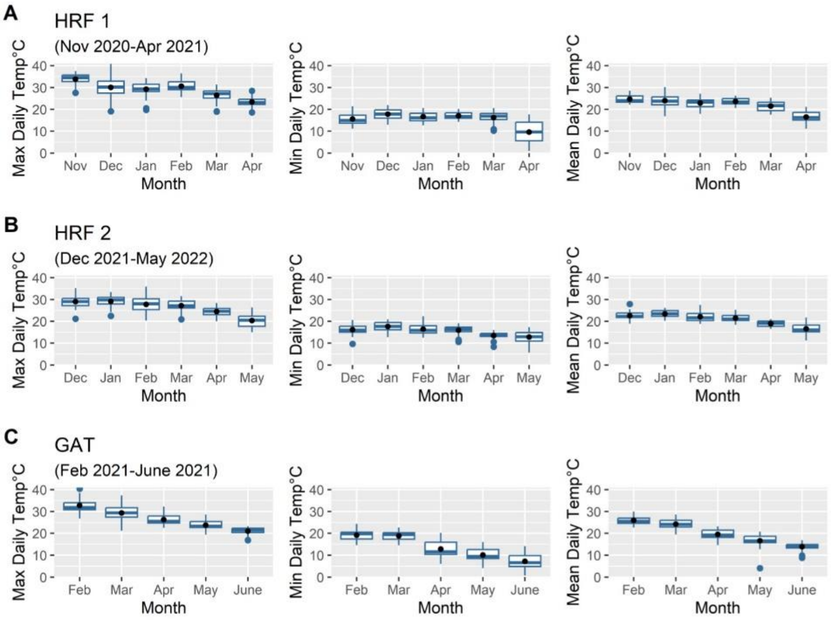

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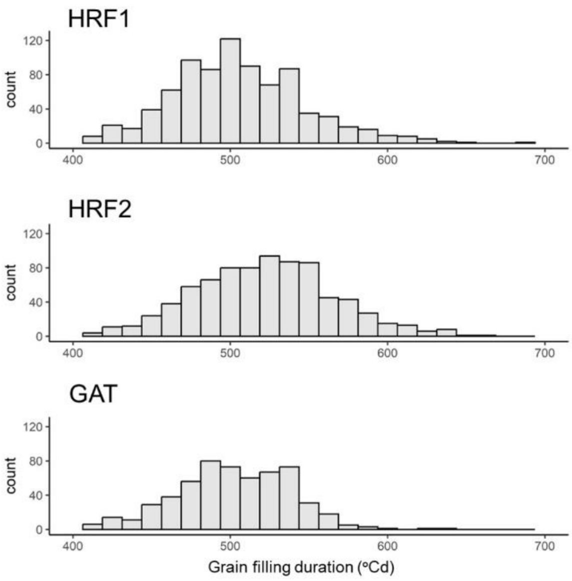

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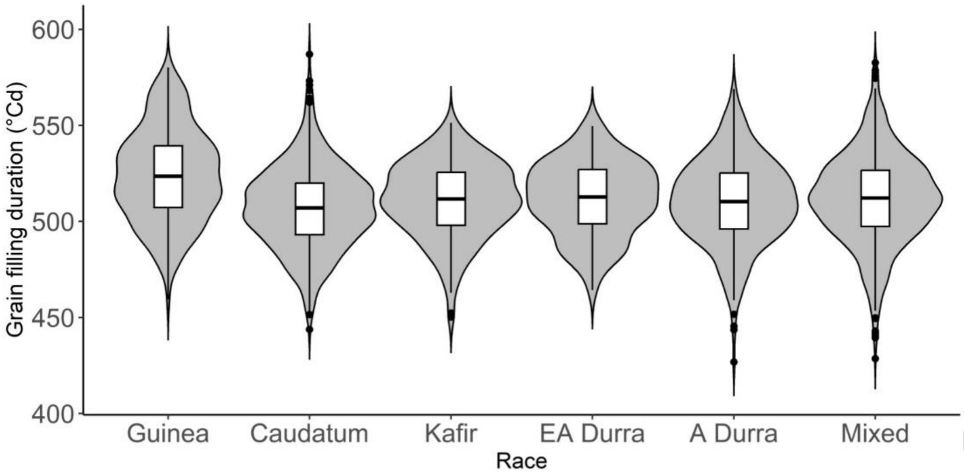

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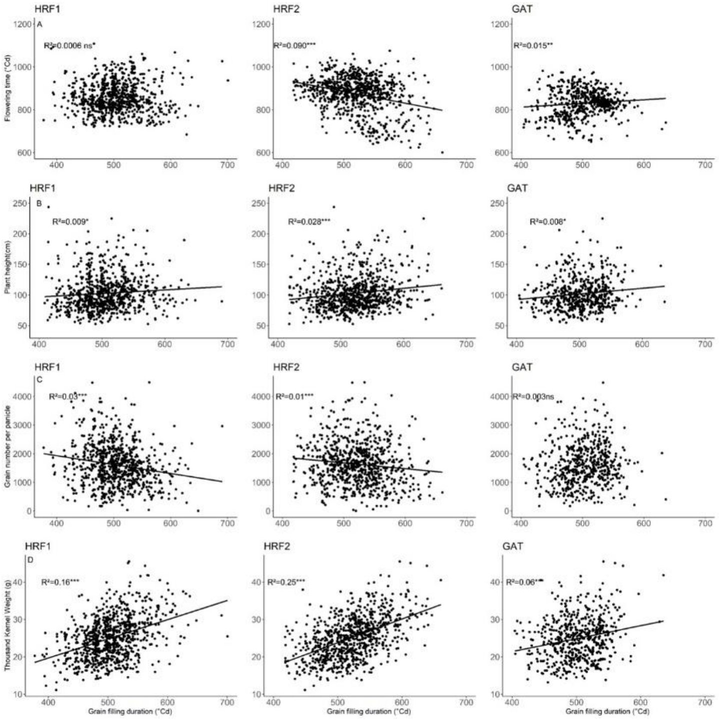

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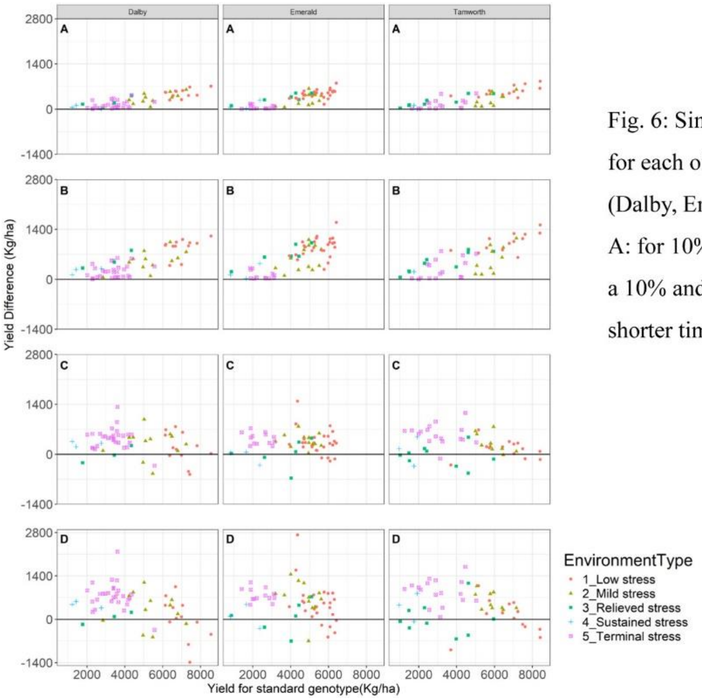

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