1	The impacts of flowering time and tillering on grain yield of sorghum hybrids across
2	diverse environments
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18	
19	Abstract

20	Sorghum in Australia is grown in water-limited environments of varying extent, generating
21	substantial genotype \times environment interaction (GEI). Much of the yield variation and GEI
22	results from variations in flowering time and tillering through their effects on canopy
23	development. The confounding effects of flowering and tillering complicate the
24	interpretation of breeding trials. In this study, we evaluated the impacts of both flowering
25	time (DTF) and tillering capacity (FTN) on yield of 1741 unique test hybrids derived from
26	three common female testers in 21 yield testing trials (48 tester/trial combinations) across
27	the major sorghum production regions in Australia in three seasons. Contributions of DTF
28	and FTN to genetic variation in grain yield were significant in 14 and 12 tester/trial
29	combinations, respectively. The proportion of genetic variance in grain yield explained by
30	DTF and FTN ranged from 0.2% to 61.0% and from 1.4% to 56.9%, respectively, depending
31	on trials and genetic background of female testers. The relationship of DTF or FTN with grain
32	yield of hybrids was frequently positive, but varied across the genetic background of testers.
33	Accounting for the effects of DTF and FTN using linear models did not substantially increase
34	the between trial genetic correlations for grain yield. The results suggested that other
35	factors affecting canopy development dynamics and grain yield might contribute GEI and/or
36	the linear approach to account for DTF and FTN on grain yield did not capture the complex
37	non-linear interactions.

38

39 Keywords

Flowering time; Genotype × environment interaction; Grain yield; Sorghum; Tillering
capacity; Water stress.

42

43 **1. Introduction**

44	In rain-fed environments, crops rely on within-season rainfall and the stored water
45	accumulated during a previous fallow [1,2]. Varying in depth and water holding capacity,
46	soils can generally accumulate a small proportion (e.g. 25-30% in Australia) [2] of the
47	precipitation received during the fallow period and rarely store adequate water for a crop to
48	produce grain without some rainfall during the growing season [1]. This often results in
49	cereal crops grown in various water-limited conditions [3–7], especially in terminal water
50	stress conditions when soil moisture is depleted during the grain-filling period due to limited
51	in-season precipitation.
52	Plant researchers have successfully classified crop growing environments into different
53	environment types (ETs) based on the temporal dynamics of a crop water stress index [3,6-
54	9], which is the ratio of potential soil water uptake to crop water demand. These ETs, which
55	vary in frequency across regions and seasons, generate differing scenarios of water
56	availability for crop breeding programs. The range of water availability in different ETs and
57	the associated range in timing and intensity of water shortage generate substantial
58	genotype × environment interactions (GEIs) across locations and seasons [10]. Cereal crop
59	improvement in dryland conditions is substantially impeded by the complexity of GEIs that
60	re-rank genotypes across environments [7,10,11]. Plant breeders can use one of two
61	strategies, either ignore GEIs and select for broad adaptation or exploit the interactions by
62	selecting for specific adaptation to types of environments. An understanding of the causes
63	of the GEIs can be useful for designing breeding strategies and agronomic approaches for
64	either scenario.

65	The impact of water stress on grain yield varies depending on the physiological stage of the
66	crop at which it occurs and the intensity of the water stress [8]. For example in sorghum,
67	grain yield under drought conditions is highly influenced by the temporal water use patterns
68	between pre- and post-anthesis stages [12–14]. In water limited environments, when a total
69	of only 150 mm water is available during the whole crop life cycle, a sorghum crop will
70	produce only about 1.6 t ha ⁻¹ of grain if all water is used by anthesis and no water remains
71	for use after anthesis. However, if 60mm of water is shifted from pre-anthesis to post-
72	anthesis by changes in management or genetics, grain yield can be more than doubled,
73	potentially achieving 3.5 t ha ⁻¹ [14].
74	While flowering and tillering are relatively simple traits with high heritability, they may
75	interact in a complex way with the growing environment to affect the timing and intensity
76	of water stress during the crop life cycle via their effects on canopy development dynamics,
77	and hence generate GEIs for grain yield. Flowering time is related to two important canopy
78	development attributes, total plant leaf area and canopy leaf area expansion rate, through
79	the effects of leaf number and leaf appearance rate [15,16]. Tillering, on the other hand, can
80	change canopy size by introducing more culms per plant [17–19]. Non-productive tillers
81	normally cease leaf area expansion early in the crop life cycle and die consecutively
82	between full expansion of the final leaf on the main culm and plant maturity, therefore
83	having minor effects on canopy size [20,21]. In contrast, tillers that continue to grow and
84	become fertile may account for up to 63% of leaf area index [17].
85	Thus, phenotypic variations in flowering time [22] and tillering [23] can complicate the
86	interpretation of breeding trials and the selection of superior parents for hybrid production.
87	To improve the accuracy of selection in sorghum breeding programs, it will be beneficial to

88	minimise these confounding effects of flowering time and tillering on grain yield. Although
89	the impact of flowering time on sorghum grain yield has been reported in some early
90	research [24,25], those studies were conducted only on dozens of hybrids and in a limited
91	number of environments. Similarly, although the contribution of fertile tiller number per
92	plant (FTN) to grain yield has been investigated by growing a single hybrid at various
93	densities [21], FTN is not normally incorporated into the analysis of yield of a crop breeding
94	program due to the intensive labour requirement for data collection.
95	Conceptually, flowering and tillering contribute to GEI in sorghum trials [8] but their
96	contribution has not been determined directly. This study aims to examine the impacts of
97	flowering time (DTF) and FTN on grain yield using data from large-scale yield testing trials.
98	These trials involved a large number of elite male parents and F_1 hybrids grown across the
99	major sorghum production regions in Australia during the 2015-17 summer growing seasons
100	The proportions of genetic variation in grain yield explained by linear mixed models
101	including DTF or FTN as a fixed effect were determined.

102 **2. Materials and Methods**

103 2.1. Breeding Trials

104 A total number of 1741 unique hybrids were grown in 21 trials at 15 locations across the major sorghum growing regions of central Queensland (CQ), southern Queensland (SQ), and 105 106 northern New South Wales (NNSW) in the three consecutive summer growing seasons of 107 2015-17. Hybrids were derived from crosses between 1078 elite male parents and three female testers at both the preliminary (PYTMales) and advanced yield testing (AYTMales) 108 109 stages of the Australian sorghum pre-breeding program [1]. Trials were arranged in partially replicated designs [26] with around 23-35% of hybrids replicated at least twice 110 (Supplementary Table S1). A different design was used in each individual trial to reduce the 111 possibility of error effects due to spatial variations specific to each trial [1]. The number of 112 hybrids grown per trial ranged from 445 to 925 depending on the season and location, with 113 entries in the trials including both test hybrids and a range of commercial hybrids. 17-25% of 114 115 test hybrids were replicated twice per trial, while the remaining test hybrids were not 116 replicated and commercial hybrids were replicated from once to twelve times depending on the trial. All plots consisted of two rows of 5-metre length. The trials used a solid row 117 configuration. Row spacing was 0.76 metre for 2015 trials at Warwick and Gatton, and 2015-118 119 16 trials at Blackville, Warwick and Pirrinuan, whereas it was 1 metre for the other trials. Trials were managed according to local management practices. Trials are indicated by a 120 combination of trial type ("AYTM" for AYTMales and "PYTM" for PYTMales), season (such as 121 "16" for 2016), and location (such as "WAR" for Warwick). The details of the trials are 122 presented in Supplementary Table S1. 123

- 124 Table 1 Quantitative comparisons of stay-green rating, flowering time (DTF), fertile tiller
- number per plant (FTN), and yield potential for the three female testers. Stay-green was
- visually rated from 1 to 9, with 1 indicating less than 10% green leaves and 9 indicating over
- 127 90% green leaves.

Female parent	Stay-green rating			DTF (Days after sowing)			FTN (tillers plant ⁻¹)	Yield potential (t ha ⁻¹)				
	2015	2016	2017	2	015	2016	2017	Overall	2	015	2016	2017
B010054	1.9	_a	-	,	55.5	-	-	0.4	4	.34	-	-
B963676	3.5	5.7	3.8 ^b	Ę	55.5	64.3	71.3	0.5	5	.50	5.03	4.21
B986604	3.6	5.3	3.7 ^b	ŗ	55.1	63.6	70.4	0.9	5	.47	5.02	4.14

^aB010054 hybrids were not grown in the 2016 and 2017 seasons; ^bstay-green ratings of
B963676 and B986604 in 2017 were estimated from one single trial in the 2017 season as
the phenotype was only expressed in this trial.

131

The three female testers used in this study, B010054, B963676, and B986604, were 132 133 originally selected to provide contrasting levels of stay-green. While they also vary in flowering, tillering and yield potential (Table 1), they share varying degrees of ancestry. The 134 PYTMales trial at Warwick in 2015 (PYTM15WAR) was in the first-year yield testing scheme 135 of the pre-breeding program. The male lines evaluated in the AYTMales trials were either 136 advanced from PYTMales trials with poor performing lines removed or retested from 137 AYTMales trials in the previous years. The male lines advanced from this early-stage 138 selection still possessed substantial genetic variance for grain yield, flowering and tillering 139 140 capacity. In each season, the plan was to produce a complete factorial combination of 141 hybrids by crossing all males to all three female testers in 2015 and two of them, B963676

142	and B986604, in 2016 and 2017. The actual number of hybrid combinations that were
143	evaluated in a specific season and location was constrained due to failures in seed
144	production. There was no planned selection for or against certain combinations of females
145	and males. Different sets of males were assessed in different seasons but with a number of
146	males in common between years. Some males were tested in a single season and then
147	removed from further testing due to bad performance, whereas others were evaluated in all
148	three seasons. The male lines shared various levels of ancestry as they included many sets of
149	siblings from the same bi-parental crosses.
150	Flowering time (days to flowering after sowing, DTF), defined as the number of days from

151 sowing to the time when 50% plants in the plot had flowered half way down the panicle, was recorded in each plot. The number of plants and fertile tillers bearing grain were 152 manually counted after maturity on a 1-metre length of row to obtain the average number 153 of fertile tillers per plant (FTN) per plot. Establishment of each plot was scored on a 1-9 scale 154 155 after either emergence or maturity, with 1 indicating very good establishment and 9 156 indicating no established plants. Plots were harvested separately after maturity and the grain weight per plot was recorded automatically by the plot harvester and later converted 157 158 to tonnes per hectare.

2.2. Data analysis 159

DTF and FTN were collected in 17 and 11 of the 21 trials. Spatial variations in DTF, FTN, and 160 grain yield were accounted for in each trial and a variance structure was subsequently 161 162 generated to create correlations between trials in a factor analytic (FA) framework [27]. Establishment was fitted as a covariate into the analysis to adjust for its effects on FTN and 163 grain yield. Together with a genetic variance per trial derived from the analysis, FA loadings 164

165	were obtained and used to create a pair-wise correlation matrix across the trials. GEI for
166	each trait was quantified by considering the correlation matrix between trials [1]. Genetic
167	correlations of DTF across the 17 trials ranged from 0.29 to 1, with a mean value of 0.66;
168	whereas genetic correlation of FTN across the 11 trials ranged from 0.41 to 1, with a mean
169	value of 0.76 (supplementary Figure S1). These results indicated low G×E interactions for the
170	two individual traits, suggesting that an overall BLUP (best linear unbiased prediction) for
171	DTF and FTN from the analyses of multi-environment trials (MET) could be used to indicate
172	the flowering time and tillering capacity, respectively, for each hybrid across all trials.
173	Therefore, MET analysis was conducted for each trait and the overall BLUPs for DTF and FTN
174	were predicted for each genotype to determine the impact of DTF and FTN on yield. In
175	contrast, between-trial genetic correlations for yield adjusted for establishment varied from
176	-0.71 to 0.62 with an average of 0.08 (supplementary Figure S1; supplementary Table S2),
177	indicating substantial G×E interaction for grain yield, which suggested an overall BLUP for
178	grain yield could not be used to indicate hybrid yield potentials across trials. Thus, it was
179	appropriate to use individual trial estimates of hybrid grain yield to investigate its
180	relationships with DTF and FTN. Broad-sense heritability was estimated according to the
181	formula of [26] due to the implementation of spatial models in this study.
182	To quantify the contribution of DTF and FTN to genetic variance of grain yield (hereafter
183	referred to as "genetic yield variance"), male lines were modeled within female testers.
184	Firstly, initial genetic yield variances of males within each tester were estimated by setting
185	effects of males within testers (Male Tester) as a random term in Model 1 (initial model).
186	DTF and FTN were fitted individually into the models for yield analysis. While the three
187	female testers have different flowering and tillering potential, they were expected to display
188	different general combining abilities for the three traits, which could probably confound the

189	impacts of DTF and FTN on grain yield investigated in this study. Therefore, effects of DTF
190	(DTF Tester) and FTN (FTN Tester) were fitted as a fixed effect within female testers in
191	Model 2 and 3 respectively. After accounting for the spatial variation in yield, genetic yield
192	variance of males within each tester was quantified. The proportion of genetic yield
193	variance of males within each tester due to variations in DTF and FTN was subsequently
194	calculated according to equation (1) and (2) respectively.
195	Yield = TestGeno + establishment + Tester + Male Tester + spatial variation + residuals
196	(Model 1),
197	Yield = TestGeno + establishment + Tester + Male Tester + DTF Tester + spatial variation +
198	residuals (Model 2),
199	Yield = TestGeno + establishment + Tester + Male Tester + FTN Tester + spatial variation +
200	residuals (Model 3),
201	where TestGeno is a vector of logical variables with 'yes' representing the male parent of a
202	test hybrid and 'no' representing a commercial hybrid; establishment is a score of 1-9
203	indicating the establishment of a plot.
204 205	Proportion of genetic yield variance contributed by DTF = Genetic yield variance_Model 1-Genetic yield variance_Model 2 Genetic yield variance_Model 1 (1),

	Proportio	on of genetic yield variance contributed by FTN =
	Genetic yiel	d variance_Model 1-Genetic yield variance_Model 3 × 1000%
206		Genetic yield variance_Model 1
207	(2),	

208	where Genetic yield variance_Model 1, Genetic yield variance_Model 2, and Genetic yield
209	variance_Model 3 are the genetic yield variances estimated from Model 1, 2, and 3
210	respectively.
211	To assess the relationship of DTF or FTN to grain yield of sorghum hybrids in production
212	environments, hybrids were grouped within female testers to remove the main effects of
213	testers on DTF, FTN, and grain yield. The effects of female testers, DTF, and FTN were fitted
214	as fixed terms, whereas the effects of hybrids and spatial variation were considered as
215	random terms in Model 4 and 5.
216	Yield = establishment + Tester.present + Tester + $B_1 \times DTF$ Tester + hybrids + spatial
217	variation + residuals (Model 4),
218	Yield = establishment + Tester.present + Tester + $B_2 \times FTN Tester + hybrids + spatial$
219	variation + residuals (Model 5),
220	where Tester.present indicates the presence of female testers, with 'yes' for test hybrids
221	and 'no' for commercial hybrids; B_1 and B_2 are the regression coefficients of DTF and FTN on
222	grain yield respectively.
223	For each trial, a mixed model was implemented in the asreml package [28] in R software in
224	Rstudio [29,30] for the analysis.
225	As only three and two hybrids were involved from crosses with female B010054 and

- 226 B986604, respectively, for trial PYTM15WAR, the regression coefficients of DTF and FTN on
- 227 yield for hybrids of these two testers in that trial were not reported. Therefore, the
- regression coefficients (B₁ and B₂) from the remaining 48 tester/trial combinations were

- 229 presented and plotted against the mean yield of all hybrids within the corresponding
- 230 tester/trial combinations.

231 **3. Results**

232 3.1. Phenotypic evaluations

233 DTF data were collected for 17 of the 21 trials. A large range in DTF was observed for the 17 trials. Differences in DTF of the latest and earliest genotypes within individual trials varied 234 from 10 to 20 days, with average DTF varying from 48.4 to 81.7 days after sowing (Table 2). 235 236 Heritability for DTF was consistently high, varying from 60.5% to 87.8% across the trials with a mean of 72.2% (Figure 1A). As DTF of the three testers was very similar (Table 1), with less 237 than one-day difference, and the inheritance of DTF is predominantly additive, their 238 corresponding hybrids differed little in DTF across the trials (supplementary Figure S2). 239 240 FTN data were collected for 11 of the 21 trials. A large range in FTN was observed for the 11 trials. Differences in FTN of the highest and lowest tillering hybrids in individual trials varied 241 from 1.0 to 5.0 fertile tillers per plant (Table 2). Heritability for FTN was moderately high, 242 varying from 41.4% to 70.7% with an average of 60.7% (Figure 1A). High tillering tester 243 parents tend to produce hybrids with more tillers; FTN of B010054 hybrids was the lowest, 244 while that of B986604 hybrids was generally the highest (Table 1; supplementary Figure S3). 245 Mean yield of the 21 trials varied between 2.63 and 8.10 t ha⁻¹ with an average of 5.5 t ha⁻¹ 246 (Table 2), which was consistent with the range of yields commonly observed in sorghum 247 pre-breeding trials [1]. Heritability of yield adjusted for establishment ranged from 20.6% to 248 77.1% with an average of 55.6% (Figure 1A). Mean yield of hybrids derived from the tester 249 B010054 was consistently lower than that of hybrids from the other two testers in the 2015 250 251 trials, whereas the relative yield of hybrids within the other two female testers varied across years and locations (supplementary Figure S4). This was consistent with the yield potential 252

- of the three testers as B010054 had the lowest yield potential, while the yield of B963676
- and B986604 were similar (Table 1).
- Table 2 Summary of yield, flowering time (DTF) and fertile tiller number per plant (FTN) for
- the 21 trials. The trials were sorted in ascending order for mean grain yield.

		Yield	D	TF	FTN		
Trial	(t ha⁻¹)	(days afte	er sowing)	(tillers plant ⁻¹)		
	Mean	Range	Mean	Range	Mean	Range	
aytm16EME	2.63	0.00-8.07	49.0	45-60	naª	na	
aytm16ORI	2.90	0.07-6.39	58.0	51-64	na	na	
aytm15CAP	3.01	1.08-4.57	51.9	46-58	na	na	
aytm15EME	3.13	1.00-6.10	48.4	44-55	0.5	0.0-1.0	
aytm17CAR	3.59	1.00-6.60	69.2	64-84	0.2	0.0-1.5	
aytm17MAC	3.97	1.60-6.10	76.4	68-79	na	na	
aytm15JIM	4.34	1.50-7.00	58.3	53-65	0.2	0.0-1.8	
aytm17PIN	5.18	1.00-8.70	71.0	68-84	0.3	0.0-2.5	
aytm16JAN	5.32	1.42-7.89	57.9	51-66	0.2	0.0-1.5	
aytm16CRO	5.76	2.66-8.33	71.6	66-81	0.4	0.0-2.3	
aytm16SPR	6.24	0.00-21.30	na	na	na	na	
aytm17EME	6.07	0.00-8.46	58.8	54-66	na	na	
aytm15GAT	6.27	3.10-9.30	63.2	60-74	na	na	
aytm16PIR	6.44	3.11-9.01	na	na	0.8	0.0-3.3	
aytm16DAL	6.59	1.28-9.22	67.5	62-78	1	0.0-3.0	
aytm15SPR	6.84	2.20-10.10	na	na	na	na	
aytm15WAR	6.97	3.10-10.10	74.3	68-78	0.4	0.0-2.7	
aytm16WAR	7.25	2.64-12.66	65.7	62-75	1.2	0.0-5.0	
aytm15DAL	7.30	1.40-10.80	na	na	na	na	
pytm15WAR	7.58	3.01-11.08	73.0	67-77	0.6	0.0-3.0	
aytm16BLA	8.10	2.57-13.21	81.7	70-90	na	na	

^aData not available.



258

259 Figure 1 Heritability of flowering time (DTF), fertile tiller number per plant (FTN) and grain

260 yield. The values above, in, and below each box indicate the maximum, mean and minimum

261 heritability respectively.

262

263	Correlations between overall BLUPs for DTF and FTN for hybrids across the three testers
264	were not significant, ranging from -0.049 to -0.030 (Table 3). Similarly , non-significant
265	correlations between the two traits were observed in almost all tester/trial combinations
266	except in 4 B963676/trial combinations. However, for those 4 B963676/trial combinations,
267	coefficients of determination (r^2) were less than 1.21% (supplementary Table S3). Therefore,
268	DTF and FTN were independent traits.

Table 3 Correlation between overall BLUPs for flowering time (DTF) and fertile tiller number per plant (FTN) for hybrids in combination with the three female testers. BLUPs for DTF and

271 FTN were predicted from the MET analysis of the 17 and 11 trials that had DTF and FTN data.

Female	Correlation coefficient	p value
B010054	-0.030	0.65
B963676	-0.047	0.10
B986604	-0.049	0.19

272 **3.2.** Impact of DTF on grain yield

Genetic yield contribution by DTF of male lines was significant in 15 of the 48 tester/trial 273 combinations (Table 4). Therefore, the proportion of genetic yield variance explained by 274 models including DTF as a fixed effect can only be accurately estimated in these 15 275 tester/trial combinations. The proportion of genetic yield contribution by DTF differed 276 277 across trials and females, ranging from 0.2% to 61% with an average of 13.6%. 278 For the relationship of DTF to grain yield of hybrids, statistically significant associations were observed in 22 tester/trial combinations, of which 14 were positive (Table 4). This general 279 trend towards a positive association between flowering time and grain yield was observed 280

281	in the data set as a whole, with coefficients being positive on 30 (i.e., 62.5%) of the 48
282	occasions. However, the directions of the association between DTF and yield were different
283	among hybrids derived from different female parents. For hybrids derived from B010054
284	and B963676, there were more significantly positive associations; all four significant
285	associations for the B010054 hybrids and seven of the eleven significant associations for the
286	B963676 hybrids were positive. A similar trend was observed in the whole data set, positive
287	associations were observed in 6 (i.e. 86%) of the 7 B010054 trials and 15 (i.e. 71%) of the 21
288	B963676 trials. In contrast, negative associations were present in four of the seven
289	significant cases for B986604 hybrids. Similarly, in the whole data set, there were 10
290	negative and 9 positive associations between flowering time and grain yield for the B986604

291 hybrids.

2.22 Table + reportion of genetic yield variance explained by nowering time (Dir) and parameters of wald test of Dir enect of grain yield of the 40 testery that

293 combinations for the three female testers in the 21 trials.

		Female B010	054			Female B963	676	Female B986604				
	Mean yield				Mean yield				Mean yield			
Trial	of female	Genetic yield			of female	Genetic yield			of female	Genetic yield		
Inal	specific	contribution	Regression	se	specific	contribution	Regression	se	specific	contribution	Regression	se
	hybrids t	by DTF (%) ^a	coefficient		hybrids t	by DTF (%) ^a	coefficient		hybrids t	by DTF (%) ^a	coefficient	
	ha ⁻¹				ha ⁻¹				ha⁻¹			
PYTM15WAR	_b	-	-	-	7.54	ns	-0.02	0.02	-	-	-	-
AYTM15CAP	3.00	ns ^c	0.02	0.03	3.00	ns	0.04	0.03	3.01	ns	-0.01	0.06
AYTM15DAL	6.93	5.4	<u>0.19</u> ^e	0.06	7.64	12.1	<u>0.17</u>	0.06	6.88	ns	0.16	0.13
AYTM15EME	2.80	ns	0.02	0.05	3.23	ns	0.05	0.04	3.62	ns	0.08	0.09
AYTM15GAT	5.73	11.1	<u>0.17</u>	0.07	6.67	11.2	<u>0.19</u>	0.06	6.48	ns	<u>0.47</u>	0.15
AYTM15WAR	5.99	ns	<u>0.13</u>	0.06	7.55	ns	0.08	0.05	7.74	ns	0.04	0.12
AYTM15JIM	4.01	4.4	<u>0.15</u>	0.05	4.45	ns	0.08	0.04	4.92	ns	-0.02	0.10
AYTM15SPR	6.79	ns	-0.01	0.06	6.81	ns	-0.10	0.05	7.06	ns	0.11	0.13
AYTM16BLA	-	ns	-	-	7.73	ns	<u>0.11</u>	0.05	8.06	ns	-0.02	0.06
AYTM16CRO	-	ns	-	-	5.66	ns	0.05	0.03	5.76	ns	0.04	0.04
AYTM16DAL	-	ns	-	-	6.70	-61.0 ^d	<u>-0.27</u>	0.03	6.41	-44.5	<u>-0.34</u>	0.04
AYTM16EME	-	ns	-	-	2.41	-2.1	<u>-0.10</u>	0.04	2.87	ns	<u>-0.11</u>	0.04
AYTM16WAR	-	ns	-	-	7.55	9.5	<u>0.18</u>	0.05	6.92	-0.2	<u>-0.13</u>	0.05
AYTM16JAN	-	ns	-	-	5.34	ns	0.07	0.04	5.33	3.9	<u>0.15</u>	0.05
AYTM16ORI	-	ns	-	-	2.66	13.6	0.08	0.02	3.14	ns	0.00	0.03

Preprints (www.preprints.org)		OT PEER-REVIEWED	Posted: 18	Septembe	er 2019	<u>doi:10.20944/</u>	preprints2019	<u>09.0198.v1</u>				
			Peer-reviewe	ed version a	available at Ag	ronomy 2020 ; <u>doi:1</u>	0.3390/agrono	my1001013	<u>.5</u>			
		115			0.11	5.5	<u></u>	0.05	0.50	115	0.00	0.00
AYTM16SPR	-	ns	-	-	6.21	ns	<u>0.08</u>	0.04	5.84	ns	-0.01	0.04
AYTM17EME	-	ns	-	-	6.18	ns	0.10	0.06	6.09	ns	0.06	0.06
AYTM17PIN	-	ns	-	-	4.88	-6.7	<u>-0.11</u>	0.03	5.30	ns	<u>-0.10</u>	0.04
AYTM17CAR	-	ns	-	-	3.60	-12.2	<u>-0.16</u>	0.03	3.59	ns	-0.08	0.04
AYTM17MAC	-	ns	-	-	4.00	ns	0.02	0.03	3.77	ns	<u>0.09</u>	0.04

^avalues estimated by modelling male parents within female parents; ^bResults were not presented due to very limited sample size for these two combinations

with PYTM15WAR; ^cns indicates the DTF effect on yield of male parents was not significant, therefore the genetic yield contribution cannot be estimated and

was not presented; ^dnegative values indicate negative effect of DTF on yield of male lines; ^evalues in bold and underlined text indicates significant DTF on

297 yield of hybrids.

298	The effect of DTF on grain yield was largely determined by the growing conditions, but also
299	modified by the genetic background of female testers. Directions of the association between
300	DTF and yield for hybrids across female testers were the same in 12 (positive in 8 and
301	negative in 4) of the 20 AYTMales trials. In the remaining eight AYTMales trials, hybrids from
302	different female parents displayed contrast directions of associations between flowering
303	and yield in the same individual trials.

After accounting for the effects of female tester and DTF, between trial genetic correlations for yield ranged from -0.82 to 1 with a mean of 0.08 (supplementary Table S4). Compared to the unadjusted between trial genetic correlation of grain yield, adjusting for DTF did not improve the between trial genetic correlation of yield.

308 3.3. Impact of FTN on grain yield

309 Genetic yield contribution by FTN of male lines was significant in 12 of the 48 tester/trial

310 combinations, with six significant each for male lines crossed with B93676 and B986604

311 (Table 5). Similar to that of DTF, the proportion of genetic yield variation explained by

312 models including FTN as a fixed effect varied across trials and female parents. Genetic yield

contribution by FTN ranged between 1.4% and 56.9% with an average of 18.3%.

For the relationship of FTN to grain yield of hybrids, statistically significant associations were

observed in 12 tester/trial combinations, of which nine were positive (Table 5). The general

316 trend towards a positive association between FTN and yield remained somewhat evident in

the data set as a whole, with 26 associations being positive and 21 negative.

Table 5 Proportion of genetic yield variance explained by fertile tiller number per plant (FTN) and parameters of Wald test of FTN effect on

319 grain yield of the 48 tester/trial combinations for the three female testers in the 21 trials.

		Female B01	0054		Female B963676					Female B986604			
Trial	Mean yield of female specific hybrids t ha ⁻¹	Genetic yield contribution by FTN (%) ^a	Regression coefficient	se	Mean yield of female specific hybrids t ha ⁻¹	Genetic yield contribution by FTN (%) ^a	Regression coefficient	se	Mean yield of female specific hybrids t ha ⁻¹	Genetic yield contribution by FTN (%) ^a	Regression coefficient	se	
PYTM15WAR	_b	-	-	-	7.54	-6.1 ^d	<u>-0.16</u> ^e	0.07	-	-	-		
AYTM15CAP	3.00	ns ^c	-0.38	0.21	3.00	ns	<u>0.33</u>	0.17	3.01	ns	-0.17	0.39	
AYTM15DAL	6.93	ns	-0.43	0.46	7.64	ns	0.09	0.39	6.88	ns	0.53	0.97	
AYTM15EME	2.80	ns	0.07	0.33	3.23	ns	-0.13	0.29	3.62	ns	0.00	0.63	
AYTM15GAT	5.73	ns	0.02	0.50	6.67	ns	0.69	0.42	6.48	ns	0.57	1.32	
AYTM15WAR	5.99	ns	-0.26	0.43	7.55	ns	-0.16	0.35	7.74	ns	-0.67	0.83	
AYTM15JIM	4.01	ns	-0.28	0.35	4.45	ns	-0.45	0.30	4.92	ns	-0.87	0.73	
AYTM15SPR	6.79	ns	-0.79	0.41	6.81	ns	0.04	0.36	7.06	ns	0.86	0.89	
AYTM16BLA	-	ns	-	-	7.73	16.9	<u>1.19</u>	0.33	8.06	22.3	<u>0.93</u>	0.39	
AYTM16CRO	-	ns	-	-	5.66	56.9	<u>1.16</u>	0.23	5.76	31.0	<u>0.73</u>	0.27	
AYTM16DAL	-	ns	-	-	6.70	ns	-0.47	0.26	6.41	ns	-0.10	0.27	

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				Peer-reviewed ve	ersion ava	ailable at Ag	gronomy 2020 ; <u>doi</u>	<u>:10.3390/agro</u>	<u>nomy1001</u>	<u>0135</u>				
AYTM	116EME	-	ns	-	-	2.41	ns	-0.26	0.30	2.87	ns	0.10	0.30	
AYTM	116WAR	-	ns	-	-	7.55	-2.5	-0.70	0.36	6.92	-7.6	<u>-0.77</u>	0.37	
AYTM	116JAN	-	ns	-	-	5.34	ns	0.34	0.27	5.33	-4.0	<u>-1.13</u>	0.32	
AYTM	1160RI	-	ns	-	-	2.66	ns	0.10	0.16	3.14	ns	0.21	0.19	
AYTM	116PIR	-	ns	-	-	6.41	ns	-0.28	0.22	6.38	ns	-0.01	0.22	
AYTM	116SPR	-	ns	-	-	6.21	32.0	<u>1.04</u>	0.26	5.84	1.4	<u>0.58</u>	0.27	
AYTM	117EME	-	ns	-	-	6.18	ns	0.12	0.44	6.09	ns	0.26	0.46	
AYTM	117PIN	-	ns	-	-	4.88	ns	0.54	0.28	5.30	ns	0.28	0.30	
AYTM	117CAR	-	ns	-	-	3.60	ns	0.30	0.27	3.59	ns	-0.23	0.30	
AYTM	117MAC	-	ns	-	-	4.00	23.5	<u>0.91</u>	0.28	3.77	15.2	<u>0.79</u>	0.30	

³²⁰ ^avalues estimated by modelling male parents within female parents; ^bResults were not presented due to very limited sample size for these two

321 combinations with PYTM15WAR; ^cns indicates the FTN effect on yield of male parents was not significant, therefore the genetic yield

322 contribution cannot be estimated and was not presented; ^dnegative values indicate negative effect of FTN on yield of male lines; ^evalues in

bold and underlined text indicates significant FTN on yield of hybrids.

324	Similar to the finding for DTF and grain yield, the number of positive and negative
325	associations between FTN and yield varied across hybrids derived from different female
326	testers. For hybrids in combination with B963676 and B986604, there was a general trend of
327	positive relationship, with five and four of the six significant cases being positive,
328	respectively. The trend towards a positive association between FTN and grain yield for the
329	B963676 and B986604 hybrids was confirmed in the whole data sets of the corresponding
330	tester groups, with 13 and 11 associations being positive and 8 each associations being
331	negative. In contrast, for B010054 hybrids, negative association was observed in five
332	occasions and positive in the other two, though none was significant.
333	Although the directions of the associations of FTN and yield varied across tester/trial
334	combinations, the effect of FTN on yield was largely determined by the environmental
335	conditions, and to a lesser extent, modified by the genetic background of female testers.
336	Directions of the association between FTN and yield for hybrids across testers showed the
337	same direction of associations in 13 (all positive in 8 and negative in 5) of the 20 AYTMales
338	trials. For the remaining seven AYTMales trials, the directions of the associations for hybrids
338 339	trials. For the remaining seven AYTMales trials, the directions of the associations for hybrids across female parents differed.

341 yield ranged from -0.79 to 1 with a mean of 0.06 (supplementary Table S5). Compared to

the unadjusted between trial genetic correlation for yield, adjusting for FTN did not improve

343 the all between trial correlations.

344 **3.4.** Relationships of DTF and FTN effects to mean grain yield

The linear regression coefficient quantifying the relationship between DTF and yield for
hybrids in combination with any of B010054, B963676, and B986604 was not related to the

347	mean yield of the tester/trial combination (Figure 2; Table 4). However, for the B010054
348	hybrids most associations (6 out of 7 trials) were positive. Similarly, there were generally
349	more positive associations (15 of 21 trials) for B963676 hybrids. However, this was not
350	obvious for B986604 hybrids, where the number of positive associations (9) were similarly
351	to the number of negative associations (11).
352	The linear regression coefficient quantifying the relationship between FTN and yield was
353	also not related to the mean grain yield of the tester/trial combinations (Figure 2; Table 5).
354	For B963676 and B986604 hybrids, the effect of FTN on yield was more likely to be positive,
355	but the opposite trend was found for the B010054 hybrids, with the FTN effect on yield
356	more likely to be negative.





Figure 2 Mean grain yield of the hybrids plotted against the coefficients of the regression of flowering time (DTF) or fertile tiller number per plant (FTN) on grain yield in individual trials by female tester. NS indicates non-significant DTF or FTN effect, * significant at the level of p < 0.05, ** significant at the level of p < 0.01, *** significant at the level of p < 0.001; vertical dashed lines represent regression coefficient of 0

363 **4. Discussion**

Sorghum production environments in dryland conditions such as Australia are highly 364 365 variable, particularly with regard to water availability during the growing season. While DTF and FTN are relatively simple traits, they can contribute to variations in grain yield by 366 367 altering canopy development dynamics, radiation interception and the temporal pattern of 368 water use. Depending on the environment, both traits may have a positive or negative effect on sorghum grain yield and hence contribute to GEI for grain yield, which complicates 369 370 selection and restricts genetic yield gain. In this study, we quantified the impacts of genetic variations in DTF and FTN on grain yield using data from 21 pre-breeding trials grown across 371 the major sorghum production regions in Australia in three growing seasons. The dataset 372 included 1741 unique test cross hybrids derived from 1078 elite male lines. The lines were 373 374 representative of the Australian sorghum pre-breeding program. For the purposes of the study, sets of test cross hybrids grown in a single environment were considered as an 375 376 experimental unit to explore the relationships between grain yield and the other two traits. The results provided insight that can be used to design breeding programs and cultivars 377 targeting this variable environment. 378

4.1. The impacts of DTF and FTN on grain yield varied across environments

The genetic variation in yield varied between sites [11,31] with the model including the across site estimates of DTF and FTN being significant in only 15 and 12 of the 48 tester/trial combinations, respectively (Table 4, 5). The average percentage of genetic yield variance explained by the across site estimate of FTN was slightly greater at 18.3% than the 13.6% that was explained by DTF (Table 4, 5). This may be because in addition to its indirect effect on grain yield by influencing the canopy size [17–19], tillering can affect yield through its

direct effect on the number of grain-bearing panicles [21], which can be exacerbated insituations of poor emergence.

388 Previous research has shown that grain yield is positively correlated with late flowering in favourable environments [8,24] but often negatively associated with yield in terminal 389 drought situations [8]. On the other hand, fertile tillers can produce 5-78% of grain yield 390 391 depending on the population density [21]. Under well-watered conditions, biomass accumulation is radiation limited, such that prolonged phenology and increased tillering can 392 393 increase canopy size (at least early in the season at low leaf area index), which increases light interception and hence biomass production and grain yield. However, in water-limited 394 environments, plant size is not only limited but leaves may also senescence earlier and 395 396 faster, leading to reduced grain yield. Hammer (2006) demonstrated the extreme sensitivity 397 of sorghum yield to water stress during the grain filling period. Our results are somewhat consistent with these findings, with both positive (30 and 26) and negative associations (18 398 399 and 22) between flowering and tillering, tillering and yield, respectively, being detected. 400 The more frequent detection of positive associations suggested that the mean DTF for the set of genotypes was potentially earlier than optimal and the mean FTN was potentially 401 402 lower than optimal for the environments that were sampled in the MET series. This may be related to sorghum breeders needing to select for broad adaption therefore taking a 403 404 conservative approach to selection for maturity and tillering. Sorghum is highly sensitive to 405 post flowering drought and the approach of selecting for early maturing and/or low tillering 406 genotypes reduces the risk of yield penalty under post-anthesis drought. Alternatively, it 407 may be that the environments sampled by these trials are not a good representative sample 408 of the target population of environments. Given that the average yield of grain sorghum was

2.90 t ha⁻¹ in Australia during the 2015-17 seasons [32,33] and the mean yield of the trials in
this study was 5.50 t ha⁻¹, the latter may have been the case.

411 Despite this, trial mean yield was not a good predictor of associations between DTF, FTN and yield. Results in this study suggested that the associations between DTF and yield, FTN 412 and yield were not related to the mean yield of the combinations. Approximately equal 413 414 amount of positive and negative associations were detected in either high or low yielding environments for hybrids derived from all testers except B010054. Previously, Jordan et al. 415 416 [1] investigated the role of stay-green on grain yield using hybrids from the same sorghum pre-breeding program. They found that the majority of associations between stay-green and 417 grain yield were positive for trials with a mean yield of less than 6 t ha⁻¹ and there were 418 similar amounts of positive and negative associations for trials with a mean yield of between 419 6 and 9 t ha⁻¹. As reduced tillering could result in the expression of stay-green via potential 420 restriction of pre-anthesis water use [16], this might indicate that reduced tiller number 421 422 would positively correlate with increased yield for trials with mean yield under 6 t ha⁻¹. 423 However, this was not observed in this study (Figure 2). This contrast may be because the stay-green trait can improve yield under drought conditions by reducing plant size at 424 anthesis through a number of mechanisms other than reducing tillering, including reducing 425 leaf number of the main stem, increasing sizes of upper leaves of the main stem [12,13], or 426 accelerating age-related senescence of lower leaves [34]. 427

428

429

background of female testers

Although the impact of environment conditions had the largest effect on the association
between DTF, FTN and yield, the frequency of significant associations varied across the

4.2. The impacts of DTF and FTN on grain yield were also affected by the genetic

432	genetic background of female testers. The directions of the associations between DTF and
433	yield, FTN and yield for hybrids derived from different female testers of the same trial were
434	the same in 12 and 13, respectively, of the 20 AYTMales trials (Table 4, 5). Contrasting
435	directions of associations between DTF and yield, FTN and yield for hybrids derived from
436	different female testers were observed in the other eight and seven AYTMales trials
437	respectively. The three testers have similar flowering date but possess various degrees of
438	stay-green and differ in tillering capacity (Table 1). Hence, it is very likely that they have
439	different patterns of canopy development and water uptake. These differences
440	consequently complicate the relationships of DTF and FTN with grain yield.

441 **4.3.** Implications for breeding programs

The relationships of DTF and FTN with grain yield were modified somewhat by the genetic
background of female parents. Hybrids with B963676 as a female parent had positive
associations between DTF and yield, FTN and yield more often than hybrids from the other
two parents. This result reinforces the importance of the selection of specific female parents
in hybrid breeding.

As independent traits, DTF and FTN were expected to show similar directions of associations 447 with grain yield as they both influence water use similarly. This was supported by the 448 449 observation that DTF and FTN displayed the same directions of association with yield in 28 450 of the 48 tester/trial combinations (Table 4, 5). However, static values of DTF and FTN, while indicative, do not provide robust estimates of the dynamics of canopy size and duration 451 452 throughout the whole crop life cycle. The use of recent developments in high throughput phenotyping platforms to capture canopy development dynamics during the crop life cycle 453 is likely to enhance the understanding and analysis of GEI on yield [35]. 454

In this study, the rationale for attempting to account for the confounding effects of DTF and 455 FTN on grain yield was to partition the GEI caused by canopy size and duration into more 456 heritable components that interact with the environment in complex ways. However, within 457 the materials and environments studied, the impacts of DTF and FTN on grain yield were 458 459 low. Following adjustment for variation in emergence, substantial GEI remained after further incorporating the effects of DTF and FTN in the linear mixed model analysis. This was 460 indicated by the low genetic correlation for yield between trials following the incorporation 461 462 of DTF and FTN (Supplementary Table S4 and S5). It is possible that this inability to further partition GEI was associated with the use of linear mixed models for the analysis of genetic 463 effects on yield. Linear mixed models assume linear relationships of yield with DTF and FTN, 464 whereas it is known that these effects are non-linear in their generation of GEI. To 465 overcome this defect, crop simulation models such as the sorghum module [36] 466 467 implemented in the APSIM platform [37] might be implemented to better account for the 468 impacts of DTF and FTN on yield and to dissect the GEI generated by canopy development dynamics due to differences in DTF and FTN. However, it is also likely that other genetic 469 factors, such as those affecting radiation use efficiency, transpiration efficiency, root angle, 470 height, seed number, and seed size play important roles in determining grain yield and 471 472 generating GEIs for grain yield. This was supported by the observation of the contrasting directions of associations of DTF and FTN with yield observed in 20 of the 48 tester/trial 473 combinations. Since sorghum breeders are dealing with a range of diverse environments 474 and materials with considerable genetic variations in a range of traits, these results 475 indicated no optimal DTF and FTN could be selected for broad adaptation across various 476 477 environments. While general trends were observed, it is clear that the complex milieu of 478 traits and environments and their dynamic interactions to generate GEIs could not be simply

deconstructed via the key major factors (DTF and FTN) known to affect canopy development
and duration. However, advances in the more integrated use of crop growth models in
conjunction with genomic selection [38] suggest the possibility for enhanced leveraging of
biological insight across multiple adaptive traits in the pursuit of more rapid genetic gain in
situation with confounding GEI.

484 **5.** Conclusions

DTF and FTN can influence the temporal water use patterns between pre- and post-anthesis 485 486 stages through their impacts on canopy development dynamics, and thus complicate yield 487 analysis and the selection of superior parents for hybrid production. Linear mixed models 488 were used to remove the confounding effects of the two traits on grain yield. The impacts of DTF and FTN were affected by both environmental conditions and genetic background of 489 female testers. Although both positive and negative relationships of DTF and FTN with grain 490 yield were observed, there was a general trend towards positive DTF and FTN effects on 491 492 grain yield.

In contrast to our expections, the results indicated that little additional GEI was associated with variations in DTF and FTN. It is possible that linear mixed models did not capture the non-linear effects of DTF and FTN on yield. In addition, it is likely that other genetic factors were also influencing the variation in grain yield across the diverse range of genotypes and environments studied.

498 Author contributions

- 499 X.W., D.J., and G.H. conceived the project. X.W. collected data of fertile tiller number per
- 500 plant and conducted all the analyses except predicting overall BLUPs for DTF and FTN from
- 501 MET analyses. C.H. conducted MET analyses to predict overall BLUPs for DTF and FTN. A.C.
- recorded flowering time. X.W. wrote the manuscript. The other authors read the manuscript.

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- 510 breeding project.

511 Conflict of interest

512 The authors declare that they have no conflict of interest.

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635 Supplementary Tables and Figures

- Table S1 Details of the 21 trials and the number of hybrid combinations for each female
- tester in the 2015-17 summer growing seasons.
- Table S2 Between trial genetic correlations for grain yield adjusted for establishment.
- Table S3 Correlation of overall BLUPs for flowering time (DTF) and fertile tiller number per
- 640 plant (FTN) for hybrids in combination with the three female testers. Overall BLUPs for DTF
- and FTN were predicted from the MET analyses of the 17 and 11 trials that had DTF and FTN
- 642 collected respectively.
- Table S4 Between trial genetic correlations for grain yield after accounting for the effect offlowering time (DTF).
- Table S5 Between trial genetic correlations for grain yield after accounting for the effect offertile tiller number per plant (FTN).
- 647 Figure S1 Between trial genetic correlations of flowering time (DTF), fertile tiller number per
- 648 plant (FTN), and grain yield. Yield indicates grain yield adjusted for establishment; Yield.DTF
- 649 indicates grain yield adjusted for establishment and DTF; Yield.FTN indicates grain yield
- adjusted for establishment and FTN. The values above, in, and below each boxplot indicate
- the maximum, mean, and minimum correlation coefficients respectively.
- Figure S2 Flowering time of hybrids by female testers for the 17 trials.
- Figure S3 Number of fertile tillers per plant of hybrids by female testers for the 11 trials.
- Figure S4 Grain yield of hybrids by female testers for the 21 trials.