

1 **Short title: Wheat heat stress water use and carbohydrates**

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7 **Tolerance of combined drought and heat stress is associated with transpiration**
8 **maintenance and water soluble carbohydrates in wheat grains**

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17 **One sentence summary:** Higher yield following drought and heat stress in wheats that
18 maintain transpiration and have higher water-soluble carbohydrates content in grains.

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21 A.E. performed experiments, analysed and interpreted data; N.J. analysed and interpreted
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23

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33 **Abstract**

34 Wheat (*Triticum aestivum* L.) production is increasingly challenged by simultaneous drought
35 and heatwaves. We assessed the effect of both stresses combined on whole plant water use
36 and carbohydrate partitioning in eight bread wheat genotypes that showed contrasting
37 tolerance. Plant water use was monitored throughout growth, and water-soluble
38 carbohydrates (WSC) and starch were measured following a three-day heat treatment during
39 drought. WSC were predominantly allocated to the spike in modern Australian varieties,
40 whereas the stem contained most WSC in older genotypes. Combined drought and heat stress
41 increased WSC partitioning to the spike in older genotypes but not in the modern varieties.
42 Glucose and fructose concentrations in grains measured 12 days after anthesis were
43 associated with final grain weight in the main spike. At the whole plant level, combined
44 drought and heat stress differentially altered daily water use and transpiration response to
45 vapour pressure deficit during grain filling, compared to drought only. Final grain yield was
46 increasingly associated with aboveground biomass and total water use with increasing stress
47 intensity. Ability to maintain transpiration, especially following combined drought and heat
48 stress, appears essential for maintaining wheat productivity.

49

50

51 **Introduction**

52 Recent decades have witnessed severe drought and heatwaves worldwide, including in major
53 wheat producing regions such as India, the U.S.A., Russia, Western Europe and Australia.
54 These climatic conditions have a significant impact on global wheat production, with
55 dramatic social and economic consequences (van Dijk et al., 2013). Current climate
56 projections predict drought and heatwaves will become more common and more intense in
57 the future (Rosenzweig et al., 2014). One way to limit the impact of weather variability on
58 productivity is to develop wheat varieties better adapted to the changing climate. This can be
59 assisted by understanding the mechanisms underlying plants' responses to complex stresses
60 so as to identify the traits that characterise stress tolerant varieties for breeding.

61 A small number of studies document the impact of combined drought and high temperature
62 on wheat productivity and biological processes, especially during the reproductive
63 developmental stage. The effect of combined stresses is more detrimental than the effect of
64 an individual stress (Mittler, 2006). Both drought and high temperatures reduce expansive
65 growth, accelerate flowering and shorten grain filling duration, resulting in a low grain set,
66 size and weight (Asana and Williams, 1965; Pradhan et al., 2012). In combination, drought
67 and high temperature impair the photosynthetic system, reduce stomatal conductance and gas
68 exchange, and disrupt plants' water relations (Machado and Paulsen, 2001; Shah and Paulsen,
69 2003). These additive alterations of morphological, physiological and cellular processes
70 result in severe reductions in final grain weight.

71 Although the major impact of combined drought and high temperature on wheat productivity
72 is well described, there is scarce information on the mechanisms that determine the ability to
73 maintain grain weight in these unfavourable environments (also called tolerance). Wheat
74 harvested grain mass consists of 85 % carbohydrates, of which ~80 % is starch (Stone and
75 Morell, 2009). During grain filling, water-soluble carbohydrates (WSC) are delivered to

76 grains either from current photosynthesis in photosynthesising organs or from remobilisation
77 of WSC stored during the vegetative stage (Borrell et al., 1989; Schnyder, 1993). Abiotic
78 stress after anthesis can limit gas exchange and damage the photosynthetic system, in which
79 case stored carbohydrates become a major source of carbon for grain filling (Blum, 1998). In
80 addition to the contribution from stem reserves, spike organs, especially awns, are thought to
81 contribute to the grain filling process due to their active photosynthesis, especially in dry
82 environments (Evans et al., 1972; Rebetzke et al., 2016).

83 Carbohydrate synthesis and transport are closely related to water movements in plants. Open
84 stomata are necessary for carbon capture and plants trade-off between maximising carbon
85 assimilation and limiting water loss through transpiration under adverse conditions such as
86 drought. Carbohydrate transport via the phloem and distribution throughout the plant rely on
87 water exchange with adjacent xylem (Hölttä et al., 2009), and the impact of water shortage on
88 xylem water transport also impairs phloem function (Sevanto, 2018). Soluble carbohydrates
89 also play an important role during drought by acting as compatible osmolytes to maintain cell
90 turgor and favourable plant water status, thereby sustaining biological processes and soil
91 water uptake (Blum, 2017). Maintaining plant hydration and enhancing carbohydrate
92 remobilisation to grains are considered key factors for crop productivity in limiting
93 environments (Blum, 2006), and the interplay between plant water relations and carbohydrate
94 metabolism and distribution highlights the importance of studying both mechanisms together.
95 In this work, we describe the impact of combined drought and heat stress on whole plant
96 water use and carbohydrate partitioning during grain filling in diverse wheat genotypes. We
97 hypothesised that the combination of both stresses would alter plant water use and
98 carbohydrate partitioning in the stem and spike, and that WSC availability would be a
99 limiting factor for optimal grain weight under combined drought and heat stress.

100

101

102 **Results**

103 **Combined drought and heat stress differentially reduced total grain weight at harvest**

104

105 The effect of drought and combined drought and heat stress (D&H) on total grain weight per
106 plant at harvest (per plant yield) depended on genotype (Table 1). Drought reduced per plant
107 yield in Currawa, Odessa, Frame, Young and Gladius (Fig. 1). Interaction of drought with
108 high temperature further reduced per plant yield in Odessa, Mendos and Young. In contrast,
109 heat stress did not exacerbate the effect of drought on yield in Currawa, Frame and Gladius.
110 Per plant yield in Koda was not sensitive to either drought or D&H stress. Overall, the
111 combination of drought and high temperature was more detrimental to per plant yield in some
112 genotypes, but not all (Fig. 1a).

113 In order to assess the impact of drought and combined D&H on grain filling, grain dry weight
114 in the main spike was measured at 12 days after anthesis (DAA) and at harvest. At 12 DAA,
115 total grain weight in the main spike was different among genotypes (Fig. 1b) but there was no
116 effect of the treatments or genotype x treatment interaction (Table 1). At harvest, total grain
117 weight in the main spike was reduced by drought in Gladius, and reduced by combined D&H
118 in Odessa, Koda, Mendos and Frame (Fig. 1c). There was no effect of drought or combined
119 D&H on the main spike total grain weight at harvest in Currawa, Synthetic W7984 and
120 Young.

121

122 **The correlation between aboveground biomass, water use and plant yield increased** 123 **with increasing stress intensity**

124 Plant yield was associated with aboveground vegetative biomass and total water use to
125 different degrees depending on treatments (Fig. 2). Total grain weight was linearly related to

126 aboveground biomass and total water used by the plant throughout the experiment; these
127 coefficients increased with stress intensity from $r^2 = 0.2$ and $r^2 = 0.31$ in well-watered
128 conditions (WW), to $r^2 = 0.35$ and $r^2 = 0.56$ under drought, and $r^2 = 0.46$ and $r^2 = 0.67$ under
129 combined D&H, respectively (Fig. 2a). This dependence of plant yield on total water use and
130 interaction with treatment was confirmed in a repeated experiment (Supp. Fig. S1). During
131 the 3d heat treatment, plants generally used similar amounts of water as compared to well-
132 watered conditions (Supp. Fig. S2), although soil water potential was halved.

133 When comparing modern Australian genotypes (Frame, Young and Gladius) to older
134 genotypes (Currawa, Odessa, Koda, Mendos and Synthetic W7984), the relationship between
135 per plant yield and aboveground biomass was similar in both groups ($r^2 = 0.48$ and $r^2 = 0.46$, $p <$
136 0.001 , respectively). Despite this, the slope of the regression for modern varieties was higher
137 compared to older genotypes ($a = 0.8$ and $a = 0.3$, respectively, Fig. 2). The higher slope for
138 modern genotypes was explained by the lower biomass required to produce similar grain
139 weight compared to older genotypes under well-watered conditions and reflects the high
140 harvest index of modern genotypes in favourable conditions. However, the dependence of
141 plant yield on total water used was higher in modern genotypes compared to older genotypes
142 ($r^2 = 0.80$ and $r^2 = 0.63$, $p < 0.001$, respectively).

143

144 **Combined drought and heat stress differentially reduced transpiration response to** 145 **vapour pressure deficit**

146 As total water use was strongly dependent on plant biomass (Supp. Fig. S3), water use was
147 normalised to the final aboveground biomass and expressed as unit of water per unit of
148 biomass to allow for comparison between plants. Water use differed between genotypes
149 following combined D&H when all treated plants (D and D&H replicates) were in the same
150 droughted conditions (Fig. 3a). Interaction of drought and 3d high temperature reduced daily

151 water use in Odessa, Koda, Mendos and Young for the subsequent 30d, whereas daily water
152 use following combined D&H was similar to D alone in Currawa, Frame, Synthetic W7984
153 and Gladius.

154 Plant water use is the summation of transpiration, which is driven by changes in vapour
155 pressure deficit (VPD). Transpiration response to VPD was differentially altered by the three-
156 day high temperature treatment depending on genotype over the same grain-filling period
157 when D and D&H plants were in the same droughted conditions (Fig. 3b). For both
158 transpiration rate (TR) and specific transpiration rate (STR), statistical significance was
159 confirmed ($p < 0.05$) for the genotype \times treatment interaction component of the VPD slope
160 parameter. That is, the VPD effect on transpiration exhibited genotype \times treatment interaction
161 both before and after normalisation to final aboveground biomass.

162 Heat stress reduced subsequent transpiration rate at VPD > 0.5 kPa in Odessa, at VPD > 0.7
163 kPa in Young and at VPD > 1.0 kPa in Koda and Mendos (Fig. 3b). In contrast, transpiration
164 response to VPD was not altered following combined D&H stress in Currawa, Frame,
165 Synthetic W7984 and Gladius. Transpiration response to VPD was affected in the same
166 genotypes where daily water use was reduced by combined D&H. During drought, Young
167 had the highest transpiration rate at VPD > 1.5 kPa whereas Currawa and Frame had the
168 lowest transpiration rates. Following combined D&H, Synthetic W7984 had the highest
169 transpiration rate at VPD > 1.5 kPa whereas Currawa and Odessa had the lowest transpiration
170 rates (Supp. Fig. S4b).

171

172 **Combined drought and heat stress increased WSC partitioning to the spike in old**
173 **genotypes, but not in modern varieties**

174 WSC were quantified in the main stem and the spike tissues following all treatments 12
175 DAA, i.e. immediately following the heat stress for the D&H replicates. There was a clear

176 contrast between older genotypes (Currawa, Odessa, Koda, Mendos, Synthetic W7984) and
177 more modern varieties (Frame, Young, Gladius) for WSC partitioning in stem parts compared
178 with the spike (Fig. 4). In well-watered conditions, stem parts contained 67-87 % of total
179 WSC in older wheat genotypes compared to 28-50 % in modern varieties. The spike tissues
180 (excluding grains) contained 49-71 % of total WSC in modern varieties, whereas WSC in the
181 spike were 12-33 % of total WSC in older genotypes.

182 Drought and combined D&H differentially affected WSC distribution in the main stem and
183 spike depending on genotype (Fig. 4). Drought significantly increased WSC partitioning into
184 the spike in Currawa and Odessa and reduced the WSC fraction in the stem in Frame (Supp.
185 Table 1). Combined D&H significantly increased WSC partitioning into the spike in older
186 genotypes, whereas there was no change in WSC partitioning in modern varieties in both
187 treatments. Changes in WSC allocation to the spike following combined drought and heat
188 stress did not affect WSC partitioning to grains, except in the Synthetic genotype where WSC
189 partitioning to grains was significantly increased by combined D&H compared to drought
190 only.

191

192 **The relationships between WSC concentrations and plant yield depended on plant**
193 **organ, individual carbohydrate and the date of release of the variety**

194 The relationship between WSC concentration at 12 DAA and plant yield depended on tissue
195 and date of variety release. Total WSC concentration in the stem at 12 DAA was positively
196 related with per plant yield in modern genotypes ($r^2 = 0.53$) whereas there was no significant
197 regression observed in older genotypes ($r^2 = 0.1$) (Table 2, Fig. 5a). In contrast, total WSC
198 concentration in awns at 12 DAA was positively related with plant yield in the two, awned
199 older genotypes ($r^2 = 0.85$) whereas there was no relationship in modern genotypes ($r^2 = 0.06$,
200 Fig. 5b). Similarly, in grains, total WSC concentration at 12 DAA was positively related with

201 plant yield in older genotypes ($r^2 = 0.41$) whereas there was no relationship in modern
202 genotypes ($r^2 = 0.13$) (Table 2, Fig. 5c).

203 To determine whether individual WSC varied similarly to total WSC, we quantified glucose,
204 fructose and sucrose concentrations in the stem, awns and grains. In the stem, a similar
205 contrast was observed for individual WSC between older and modern varieties as was
206 observed for total WSC (Table 2, Fig. 5a). Glucose and sucrose concentrations in the stem at
207 12 DAA were positively related with plant yield in modern varieties ($r^2 = 0.34$ and 0.56 ,
208 respectively). Stem fructose concentrations at 12 DAA were negatively related to plant yield
209 in older varieties ($r^2 = 0.31$). In awns, glucose, fructose and sucrose concentrations at 12 DAA
210 were each negatively related with plant yield in the two awned older genotypes ($r^2 = 0.84$,
211 0.81 and 0.67 respectively) but there were no significant relationships between these sugars
212 and plant yield in more modern types (Table 2, Fig. 5b). In grains, unlike other tissues,
213 sucrose concentrations at 12 DAA were low compared to glucose and fructose concentrations
214 (Fig. 5c). Glucose and fructose concentrations were positively related with total grain weight
215 at harvest ($r^2 = 0.43$ and 0.40 , respectively – Fig. 5c). Two (unknown) fructans also appeared
216 important for plant yield in modern varieties: fructan 1 in awns ($r^2 = 0.47$) and fructan 2 in the
217 stem ($r^2 = 0.73$). In contrast with other sugars in the awns, fructan 1 concentrations at 12 DAA
218 and yield per plant were positively related. There was no relationship between starch
219 concentrations at 12 DAA and plant yield (Fig. 5d).

220

221 **Drought and combined drought and heat stress altered WSC and starch balance in** 222 **grains**

223 In order to quantify WSC availability for starch synthesis, WSC and starch concentrations
224 were measured in grains 12 DAA, immediately following D&H treatments. There was a
225 significant interaction between genotype and treatment for WSC and starch concentrations in

226 grains (Table 1). Drought significantly reduced WSC concentration in grains in the Synthetic
227 type and heat stress did not exacerbate the effect of drought (Fig. 6). WSC concentration in
228 grains was reduced by combined D&H in Odessa and Koda compared to WW and was
229 specifically reduced by combined D&H in Currawa, Young and Gladius. Starch
230 concentrations offset the reduction in WSC concentration in Currawa, Odessa and Synthetic
231 W7984, resulting in a similar total non-structural carbohydrates (NSC) concentration in
232 grains in all conditions. The balance between WSC and starch concentrations was altered in
233 Koda, Young and Gladius (Fig. 6). Total NSC concentration was reduced under drought in
234 Koda and Young, and combined D&H reduced total NSC in Gladius. There was no
235 significant effect of drought and combined D&H on WSC and total NSC concentration in
236 grains in Currawa, Odessa, Mendos and Frame. Overall, there was a significant interaction
237 between genotypes and treatments for total carbohydrates concentration in grains (Table 1)
238 that was mainly driven by interaction between genotypes and treatments for WSC
239 concentration.

240

241 **Discussion**

242 **Higher water use and responsiveness to evaporative demand are indicators of higher** 243 **yield under combined drought and heat stress**

244 In this study, the impact of drought and combined D&H on total grain weight at harvest was
245 assessed in eight diverse wheat genotypes released between 1912 and 2007. The detrimental
246 effect of heat stress combined with drought depended on genotype, illustrating genetic
247 variation in grain weight response to combined D&H in wheat.

248 Per plant yield was increasingly dependent on both aboveground biomass and total water use
249 with increasing stress intensity (drought, then additional heat stress) (Fig. 2), highlighting the
250 important relationships between biomass, water use and grain weight under stress previously

251 reported (Reynolds et al., 2006; Blum, 2009; Reynolds and Langridge, 2016). Biomass and
252 water use are linearly related (de Wit, 1958) and mutually dependent during the plant's
253 lifecycle. During the vegetative stage, transpiration drives biomass accumulation, which in
254 turn results in high water use during grain filling when water is available. The maintenance of
255 water use ensures the favourable water status of plant tissues and assimilate transport to
256 grains.

257 We observed a high association between per plant yield and total water used in two
258 independent experiments (Fig. 2b, Supp. Fig. S1) regardless of transpiration sensitivity to
259 heat stress: the higher the water use, the higher the total grain weight at harvest. This
260 indicated that maintaining transpiration following heat stress was a desirable trait in our
261 conditions, confirming the strong relationship between plant transpiration and yield (de Wit,
262 1958; Fischer and Turner, 1978; Sinclair et al., 2005).

263 The amount of water used in transpiration is driven by the evaporative demand in the
264 atmosphere (Grantz, 1990). Our work illustrated the genetic variation in transpiration
265 response to VPD previously observed in diverse wheat genotypes grown under well-watered
266 and water-limited conditions (Schoppach and Sadok, 2012; Schoppach et al., 2016; Medina et
267 al., 2019). In addition, we identified genetic variation in transpiration response to combined
268 D&H (Fig. 3b). A three-day heat treatment altered transpiration response to VPD in the
269 subsequent drought-only treatment in some genotypes.

270 As transpiration largely depends on green leaf area, the dynamics of senescence in response
271 to heat stress could potentially have explained genotypic differences in transpiration response
272 to heat stress; water use would be quickly reduced in genotypes with faster heat-induced
273 senescence compared to genotypes with slower senescence rate following heat stress.
274 However, no significant differences or genetic variation for drought and heat stress-induced
275 chlorophyll content (greenness) in comparison with drought were found in these genotypes in

276 repeated experiments (Schmidt et al., 2020). The combination of high evaporative demand
277 and water scarcity can lead to the disruption of the water column in the xylem and cause
278 cavitation. Cavitation damage might explain the lack of recovery in water use following heat
279 stress observed in some genotypes in our experiments.

280 Here, with both drought and combined D&H stress, grain water use efficiency (WUE) was
281 more important than WUE *per se*. In our experiments, we measured the effects of stress
282 during grain filling when vegetative biomass was already accumulated, rather than during
283 vegetative biomass production. In the field, where increased rooting depth to access available
284 water and early vigour to enhance canopy coverage and reduce soil evapotranspiration are
285 important, this might not be the case. Nonetheless, the transpiration driven water use changes
286 observed here following heat stress during grain-filling, when plants were all subjected to
287 drought, influenced final grain weight. This trait – ability to maintain transpiration following
288 heat stress under drought - separated more from less tolerant types.

289 Although studied in a limited number of genotypes, we observed clear increased grain weight
290 per unit of biomass and per unit of water in more modern compared with older genotypes. In
291 addition to improved plant architecture for assimilation and partitioning (harvest index), we
292 also observed striking differences in the partitioning of assimilates between vegetative and
293 spike tissues before the imposition of stress.

294

295 **The spike is the main storage tissue for WSC in the more modern wheat varieties**

296 Excess assimilates that are not used for growth and defence may be stored for further use
297 during reproductive stages. The stem is considered an important source of stored WSC for
298 grain filling, and the ability to store and remobilise stem reserves is regarded as a beneficial
299 trait for wheat productivity under stress (Bidinger et al., 1977; Blum, 1998; Rebetzke et al.,
300 2008). At 12 DAA, WSC content in the stem is at its peak (Zhang et al., 2015;

301 Shirdelmoghanloo et al., 2016). Our results showed that the stem was the main storage organ
302 for WSC in tall genotypes in which the stem was the largest organ by weight, but not in more
303 modern varieties where stems are much shorter as a consequence of the introduction of semi-
304 dwarfing *Rht* genes (Borrell et al., 1993). More recent varieties partitioned more of the
305 biomass to spikes, and the reproductive organ was also the major store of WSC in modern
306 varieties (Fig. 4). Interestingly, in our experiment, a positive relationship between WSC
307 concentration in the stem and total grain weight in the main spike at harvest was only
308 observed in modern genotypes, which suggests that the important contribution of WSC
309 content stored in the stem to grain filling may be a consequence of the introduction of semi-
310 dwarfing genes (Richards, 1992; Miralles et al., 1998). Alternatively, it might suggest that
311 plant breeders have selected for varieties that partition more of their WSC to spike tissues in
312 the hot and dry conditions of South Eastern Australia, the origin of the more modern
313 varieties. Older genotypes had large reserves of WSC in the stem for a limited sink in the
314 spike (lower grain number), which could explain the absence of a relationship between both
315 traits as stored WSC in the stem may not have been used (Borrell et al., 1993). In contrast
316 with older types, more modern varieties had relatively low WSC concentration in the stem,
317 indicating an opportunity to increase stem capacity for WSC storage in modern varieties.

318

319 **WSC availability in grains rather than grain capacity limited grain weight under stress**

320 Starch is the main component of final grain mass. It is synthesised from stored WSC or
321 produced from current photosynthesis. Drought and heat stress alter WSC supply to grains,
322 either by limiting carbon assimilation through photosynthesis or by interrupting assimilate
323 remobilisation, thus WSC availability in grains might be a limiting factor for starch synthesis
324 and grain filling (Jurgens et al., 1978). In a field study on wheat genotypes grown in well-
325 watered conditions, Fahy et al. (2018) quantified WSC and starch content, and key starch

326 biosynthesis enzyme activity in grains at different grain developmental stages. They did not
327 find any correlation between carbohydrate content in grains and final grain yield, suggesting
328 that assimilate availability for starch synthesis is not a limiting factor for grain filling in
329 wheat in favourable growing conditions. These findings are in accordance with our results in
330 well-watered conditions where there was no relationship between WSC and starch content
331 and plant yield. However, WSC concentration was reduced with increased stress intensity,
332 and grains with relatively higher WSC concentrations at 12 DAA had higher yield (Fig. 5c).
333 Glucose and fructose are the first substrates in the starch biosynthesis pathway (Emes et al.,
334 2003). Genotypes with higher glucose and fructose concentrations in grains at 12 DAA had
335 higher yield, implying that shortage in glucose and fructose might have limited starch
336 biosynthesis later during grain filling and consequently final grain weight. Accelerated starch
337 biosynthesis under stress depleted glucose and fructose in grains without any increase in
338 sucrose content, indicating that insufficient sucrose supply to grains probably limited starch
339 biosynthesis under stress.

340 Many studies propose sink strength (grain capacity) is the limiting factor for starch
341 accumulation and grain filling in favourable environments (Borrás et al., 2004; Borrill et al.,
342 2015; Fahy et al., 2018). In our study, genotypes with higher grain capacity, represented by
343 grain dry weight at 12 DAA, had a higher yield in the well-watered treatment (Fig. 1a-b)
344 suggesting that grain sink strength was a major determinant of grain weight at harvest when
345 conditions were favourable. With D&H stress, however, genotypes with higher grain capacity
346 did not have higher yield, suggesting that high grain capacity was not sufficient to determine
347 grain weight at harvest under stress, as has also been observed in barley (Savin and Nicolas,
348 1996). In our experiments, drought and combined D&H did not immediately reduce grain
349 weight at 12 DAA. Reduction in grain filling occurred after 12 DAA, which corresponds to
350 the end of cell enlargement and beginning of carbohydrate accumulation (Emes et al., 2003).

351 Reduced grain weight under stress was due to altered grain filling, probably as a consequence
352 of limited WSC supply to grains.

353

354 **Conclusions**

355 Drought and heat stress have rarely been studied together, despite their co-occurrence being a
356 common scenario in wheat-growing regions. This work illustrated the effect of morphological
357 changes introduced in wheat over a century on plant water use and carbohydrates
358 partitioning. Results showed that heat stress occurring during grain filling, while plants were
359 suffering from water stress, changed subsequent water use immediately so that some
360 genotypes were unable to recover. Sensitivity to increased stress intensity was associated
361 with low transpiration response to high VPD following heat stress and to genetic variation in
362 transpiration. Reduced availability of WSC in grains following combined D&H was also
363 identified and important for final grain weight. This suggested that measurements of
364 transpiration and WSC content in grains following heat stress might be used to identify
365 genetic variation for tolerance of combined drought and heat stress.

366

367 **Material and methods**

368 **Experiment 1**

369 **Genetic material and growth conditions**

370 Eight bread wheat (*T. aestivum* L.) genotypes were selected from a diverse panel of 534
371 wheat accessions from 44 countries described in Garcia et al. (2019). The diversity panel was
372 previously subjected to post-anthesis drought and combined drought and heat stress in a pilot
373 experiment and evaluated for plant total grain weight (yield) at harvest (data not shown). The
374 selected genotypes contrasted for grain weight following drought or combined drought and
375 heat stress, and consisted of three Australian older varieties (Currawa, Koda, Mendos), three
376 Australian modern commercial varieties (Frame, Young, Gladius), one synthetic line from

377 CIMMYT (Synthetic W7984) and one landrace from Ethiopia (Odessa ES19565) (Supp.
378 Table S2). The selected genotypes were released between 1912 and 2007. In this study,
379 Frame, Gladius and Young were considered as modern genotypes; the remaining genotypes
380 were considered as older genotypes.

381 Single seeds were sown in 40 cm x 15 cm round pots containing 8.2 kg of a mixture of 1:1:1
382 (v/v/v) clay/loam:UC Davis mix:cocopeat mix. Seeds were sown on 11 August 2016, late
383 winter in the southern hemisphere. From 13 days after sowing (DAS) until the end of the
384 experiment, plants were grown in a glasshouse (34°58'17.8"S, 138°38'23.4"E) on a
385 gravimetric platform (Droughtspotter, Phenospex, Heerlen, The Netherlands) that
386 automatically irrigated to the pre-defined pot weight and recorded weights and water added
387 (details in *Water use and transpiration* below). The 168 pots were randomized to 168
388 Droughtspotter cells using a factorial, randomized complete block design, such that each
389 block comprised one replicate of each Genotype–Treatment combination, except in three
390 blocks that contained one empty pot each to estimate soil evaporation. The three treatment
391 groups comprised well-watered (WW), drought (D) and combined drought & heat stress
392 (D&H). In particular, all plants were well-watered (soil water potential = - 0.3 MPa,
393 gravimetric soil water content = 20 % (g/g)) and grown in temperate conditions (22 °C/ 15 °C
394 day/ night) until anthesis of the main spike. Anthesis date was the first day anthers were
395 observed on the main spike. One third of the plants (WW) were maintained in well-watered,
396 cool conditions until harvest. The remaining plants (D, D&H) were subject to a 6d drought
397 treatment (soil water potential = - 0.6 MPa, gravimetric soil water content = 12 % (g/g))
398 starting 3d after anthesis on the main spike of each individual; this was followed, in half of
399 these plants (D&H), by a 3d heat treatment at 37 °C/ 27 °C day/ night (n=7 for each accession
400 in each treatment). Heat treatment was imposed in an adjacent glasshouse where plants were
401 watered to weight manually. Drought was maintained until harvest in the D and D&H groups.

402 LED lights ($400 \mu\text{E}/\text{m}^2/\text{s}$) were installed above plants to minimize variations due to light
403 intensity. A graphical representation of the experimental design is shown in Supp. Fig. S5.
404 Environmental data are shown in Supp. Fig. S6.

405

406 **Water use and transpiration**

407 The gravimetric platform was configured to weigh each pot at regular time intervals. All
408 weight and water values were automatically logged and water usage estimated hourly for
409 each pot throughout the experiment. During the heat treatment in an adjacent glasshouse,
410 plants were watered manually at similar times as the drought treatment and weights
411 recorded. Pots were watered at least six times daily (6am, 10am, 12pm, 2pm, 4pm and
412 10pm). Pots containing soil only were weighed to estimate non-transpirational water loss
413 under WW, D and D&H treatments. The water usage is a combination of plant transpiration
414 and evaporation from the soil surface, which was negligible in all treatments as estimated
415 from pots containing soil only.

416

417 **Carbohydrates quantification**

418 The main stem and spike of three plants per genotype per treatment were sampled 12d after
419 anthesis (DAA), i.e. one day after heat treatment in drought and heat stressed plants, and
420 stored at -80°C for further analysis. Measurements were conducted separately on the stem,
421 flag leaf sheath, covered peduncle, exposed peduncle, rachis, grains, palea, lemma, awns and
422 glumes. Dry weight was obtained by weighing the samples after freeze-drying. Total WSC in
423 each tissue were determined using the anthrone method (Yemm and Willis, 1954) with some
424 modifications: soluble sugars were extracted with 80 % ethanol at 80°C for 1h, then
425 extracted with distilled water at 60°C for 1h. The extraction was repeated as many times as
426 needed until no coloration was observed. Supernatants were combined in the same tube for

427 colorimetric assay. Starch content in grains was measured using the Megazyme Total Starch
428 HK (K-TSHK 08/18, Megazyme, Bray, Ireland) according to the manufacturer's instructions.
429 Individual WSC measurements in grains were performed in four genotypes (Frame, Odessa,
430 Synthetic and Young). As plant morphology and grain number varied greatly between the
431 genotypes, WSC and starch contents were expressed as g/g DW to allow for comparison
432 between genotypes. Glucose, fructose and sucrose were analyzed in the same samples used
433 for total WSC analysis using high performance anion exchange chromatography with pulsed
434 amperometric detection HPAEC-PAD (Dionex ICS-5000; Thermo Fisher Scientific,
435 Sunnyvale, USA). Separations were performed at 30 °C and the flow rate was 0.5 mL/min. A
436 25 µL sample was injected on a Guard CarboPac PA20 (3 × 30mm) in series with an
437 analytical CarboPac PA20 (3 × 150mm). The elution program consisted of 0.1M NaOH from
438 0 to 2 min, followed by increasing 1M sodium acetate concentration up to 20 % from 2 min
439 to 35 min, followed by increasing 1M sodium acetate concentration up to 100 % from 35 min
440 to 36.5 min, a steady concentration from 36.5 min to 37.5 min, followed by a 0.1M NaOH
441 wash until return to equilibrium.

442 Glucose, fructose and sucrose were identified based on glucose, fructose and sucrose
443 standards. Fructans were identified by acid hydrolysis. Two WSC samples from the stem and
444 awns were incubated with 0.2M trifluoroacetic acid (TFA) at 80 °C for 30 min together with
445 untreated samples. Treated and untreated samples were analyzed using HPAEC-PAD as
446 described above. Glucose, fructose and sucrose were quantified using external standards and
447 peak areas determined using the instrument's Chromeleon software. Fructans were quantified
448 using peak areas.

449

450 **Harvest data at maturity**

451 Four plants per genotype per treatment were harvested at maturity to measure grain yield
452 components. Total grain weight was determined for the main spike and for the whole plant.
453 Seed number was counted using an automatic seed counter (Contador, Pfeuffer GmbH,
454 Germany). Biomass weight included tillers, leaves and spikes but excluded grains. Plant
455 height was measured from the base of the main stem to the top of the highest spike excluding
456 awns. Biomass water use efficiency (bWUE) was calculated as the ratio of total aboveground
457 biomass to total water use per plant. Grain water use efficiency (gWUE) was calculated as the
458 ratio of total grain weight to total water use per plant.

459

460 **Experiment 2**

461 In order to test the reproducibility of water use results, the 2016 experiment described above
462 was replicated in an independent experiment with four genotypes (Currawa, Synthetic
463 W7984, Mendos and Young) in 2017 with the same settings used for plant growth and
464 treatments, except that plants were sown one month earlier. Plant water use was recorded
465 using the gravimetric platform and three plants per genotype and per treatment were
466 harvested at maturity and measured as before.

467

468 **Statistical analyses of yield components**

469 The data were analysed by two-way ANOVA with genotype and treatment as fixed factors
470 for all measured yield component and biomass traits and for the analysis of carbohydrates
471 within each tissue. Treatment means within genotypes were compared using Tukey's HSD
472 (honestly significant difference) test at $p = <0.1$. Statistical analyses (ANOVA, Tukey's tests,
473 correlation analyses) and graphical representation were performed using R software (version
474 3.4.4, R Core Team, 2019) and ASReml-R (Butler et al., 2009).

475

476 **Statistical analyses of water use and transpiration**

477 The recorded water use data were used to identify genotype and treatment effects on hourly
478 transpiration rate (TR, mL/hr) and specific transpiration rate (STR, mL/hr/g biomass), with
479 the proviso that soil evaporation and plant transpiration water losses were indistinguishable.

480 VPD was computed hourly from vapour capacity (VC, kPa) using the following formula:

481
$$VC = 0.611 \exp(17.62 T / (T + 243))$$

482
$$VPD = (1 - RH/100) VC$$

483 where T is temperature and RH is relative humidity. It was further decided to model TR or
484 STR (henceforth denoted y) as a simple linear function of VPD with (a) genotype \times treatment
485 interaction incorporated into the VPD slope and intercept parameters, and (b) error variance
486 modelled as a function of treatment (but not genotype). The resulting model comprises 2×8
487 $\times 3 = 48$ fixed effects of interest (i.e. slope and intercept parameters) and 3 variance
488 estimates, represented symbolically as

$$y_{ij} = \text{Pot}_i + \text{spatial}_i + \text{Gen}_i \times \text{Tr}_i + \text{VPD}_j \times \text{Gen}_i \times \text{Tr}_i + \sigma_{\text{Tr}}^2$$

489 where (a) y_{ij} is the TR or STR value for Pot i on hour j , (b) Pot_i is a random-effects term for
490 variation between pots, and (c) *spatial* comprises fixed-effects terms for spatial variation
491 within the greenhouse. The model was fitted separately to each of TR and STR using the R
492 package `ASReml-R4` (Butler et al., 2017). Analysis of the effect of 3d heat stress on
493 transpiration was narrowed to 30d (0 to 30 days after treatment – DAT) after heat stress,
494 while all genotypes were still using water in the well-watered treatment, to limit the effects of
495 intrinsic differences of grain-filling duration on transpiration and distinguish the effects of
496 treatments.

497

498 **Supplemental data**

499 **Supplementary figure S1.** Relationship between total water used and final grain weight per
500 plant during Experiment 2 (2017).

501 **Supplementary figure S2.** Water use per plant during the three-day heat treatment,
502 normalised to aboveground biomass.

503 **Supplementary figure S3.** Relationship between aboveground biomass (excluding grains)
504 and total water used.

505 **Supplementary figure S4.** Genotypic differences in final grain weight (a), transpiration rate
506 at VPD = 2 kPa (b), plant height (c), harvest index (d), grain water use efficiency (e) and
507 WSC concentration in the stem (f).

508 **Supplementary table S1.** Statistical significance of differences in percentages of water-
509 soluble carbohydrates in different parts of wheat plants: stem, grains and spike, between WW
510 and D, D and D&H, or WW and D&H.

511 **Supplementary table S2.** Origins and pedigrees of the eight wheat genotypes used in the
512 study.

513 **Supplementary figure S5.** (a) Schematic of the treatment design. (b) Images of plants of
514 different genotypes following combined D&H stress at 12 DAA.

515 **Supplementary figure S6.** Daily maximum and minimum temperature (a), maximum daily
516 light intensity (b), and daily maximum and minimum VPD (c) in the glasshouse experiments
517 in 2016 and 2017.

518

519

520 **Acknowledgments**

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522 the Australian Plant Phenomics Facility, which is supported by the Australian Government's
523 National Collaborative Research Infrastructure Strategy (NCRIS). In particular, we thank
524 Chris Brien of the APPF for his constructive comments and advice on analysis of these data.

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526 assistance. We acknowledge and thank Vincent Bulone and Jelle Lahnstein for helpful
527 comments and assistance with the carbohydrates analysis.

528

529

530

531 **Table 1.** Analysis of variance (ANOVA) showing the statistical significance of the traits
532 measured for genotype, treatment and interaction between genotype and treatment.

Traits	Genotype	Treatment	Interaction
Total grain weight per plant	***	***	**
Total grain weight in the main spike at 12 DAA	***	ns	ns
Total grain weight in the main spike at harvest	***	***	ns
Aboveground biomass (excluding grains)	***	***	ns
Plant height	***	ns	ns
Total water use	***	***	ns
Water use after treatment	**	**	ns
biomass WUE	***	***	*
grain WUE	***	***	***
Harvest index	***	***	***
WSC concentration in grains	***	***	**
Starch concentration in grains	***	***	***
Total carbohydrates concentration in grains	***	**	*

* p < 0.1; ** p < 0.01; *** p < 0.001; ns not significant

533

534

535

536

537 **Table 2.** r^2 and p-values of linear regressions between individual carbohydrates'
 538 concentrations in different plant organs at 12 DAA and final grain weight per plant in modern
 539 vs. older genotypes.

540

Carbohydrate	Plant tissue	Modern		Old	
		r^2	p	r^2	p
WSC	Stem	0.53	0.03	0.1	0.26
	Awns	0.06	0.54	0.85	0.01*
	Grains	0.13	0.33	0.41	0.01
Glucose	Stem	0.34	0.1	0.01	0.73
	Awns	0.14	0.33	0.84	0.1
	Grains	0.47	0.13	0.00	0.9
Fructose	Stem	0.08	0.45	0.31	0.03
	Awns	0.11	0.39	0.81	0.01
	Grains	0.54	0.09	0.06	0.63
Sucrose	Stem	0.56	0.02	0.13	0.18
	Awns	0.07	0.49	0.67	0.05
	Grains	0.44	0.15	0.05	0.66
Fructan1	Stem	0.17	0.27	0.1	0.25
	Awns	0.47	0.04	0.35	0.22
	Grains	0.04	0.7	0.08	0.6
Fructan2	Stem	0.73	0.003	0.23	0.07
	Awns	0.05	0.56	0.44	0.15
	Grains	0.47	0.13	0.24	0.32
Starch	Grains	0.08	0.46	0.14	0.18

541 * Only two older genotypes were awned.

542

543

544

545 **Figure legends**

546

547 **Figure 1. Combined drought and heat stress differentially reduced final grain weight.**

548 (a) Mean total grain weight per plant at harvest (n=4). (b) Mean total grain weight in the main
549 spike at 12 days after anthesis (n=3). (c) Mean total grain weight in the main spike at harvest
550 (n=4). Error bars are standard error. Letters indicate the results of Tukey's test comparing
551 treatment effect within each genotype ($p < 0.1$). Plants were grown under well-watered
552 conditions (black), drought (dark grey) or combined drought and heat (light grey).

553

554 **Figure 2. Aboveground biomass and water use explained more of the variation in grain** 555 **weight under increasing stress intensity than in well-watered conditions.** Relationships

556 between final grain weight per plant and (a) aboveground biomass excluding grains, (b) total
557 water use. Each point represents one plant, grown under well-watered conditions (blue),
558 drought (orange) or combined drought and heat stress (red). Ellipses circle modern genotypes
559 (in green) and older genotypes (in grey). Table shows r^2 and p-value of linear regressions ('*' $p < 0.1$,
560 '**' $p < 0.01$, '***' $p < 0.001$) for each treatment (well-watered, drought, combined
561 drought and heat stress) and genotype group (modern, older).

562

563 **Figure 3. Interaction of high temperature and drought differentially reduced daily** 564 **water use and transpiration response to vapour pressure deficit.** (a) Daily water use per

565 plant estimated as total irrigation per day, normalised to aboveground biomass. Plants grown
566 in well-watered conditions (black), drought (dark grey) or in drought following three-day
567 heat stress (light grey). 0 DAT is the first day post heat treatment (12 days after anthesis).
568 Trend lines are loess regressions. Values are means of four replicates (n=4). The confidence
569 interval (0.95) is displayed around smoothed regressions in grey. (b) Hourly transpiration rate
570 response to VPD normalised to aboveground biomass. Plants grown in drought (orange) or in
571 drought following three-day heat stress (red). Graphs include data from 0 DAT to 30 DAT.
572 Trend lines are smooth regression lines.

573

574 **Figure 4. Combined drought and heat stress increased WSC partitioning to the spike in**
575 **old genotypes, not in modern varieties.** Genotypes are shown in order of date of release
576 (top – bottom, oldest = Currawa to newest = Gladius). Total water-soluble carbohydrates
577 (WSC) as in different organs of plants as a percentage of total WSC (n=3). Plant organs are
578 colour-coded as shown in the legend.

579

580 **Figure 5.** Relationship between total WSC, glucose, fructose, sucrose and starch
581 concentrations in (a) the stem, (b) awns, (c-d) grains at 12 DAA, and final grain weight per
582 plant. Each point represents an average of carbohydrate concentration (n=3) in the main tiller
583 for one genotype and one treatment. Ellipses circle old genotypes (grey) and modern
584 genotypes (green). Plants grown under well-watered conditions (blue), drought (orange) or
585 combined drought and heat stress (red). Regression lines include all datapoints. r^2 and p-value
586 of linear regressions ('ns' not significant, '*' $p < 0.05$) are indicated.

587

588 **Figure 6. Drought and combined drought and heat stress altered WSC and starch**
589 **balance in grains at 12 DAA.** Water-soluble carbohydrates (solid) and starch (transparent)
590 concentration in grains at 12 DAA from plants grown under well-watered conditions (black),
591 drought (dark grey) or combined drought and heat stress (light grey). The sum of WSC and
592 starch concentrations constitutes the non-structural carbohydrate (NSC). Values are means of
593 three replicates (+/- SE). Letters (top: NSC, bottom: WSC) indicate the results of Tukey's test
594 comparing treatment effects within each genotype ($p < 0.1$).

595

596 **Literature cited**

- 597 **Asana R, Williams R** (1965) The effect of temperature stress on grain development in
598 wheat. *Australian Journal of Agricultural Research* **16**: 1-13
- 599 **Bidinger F, Musgrave RB, Fischer RA** (1977) Contribution of stored pre-anthesis
600 assimilate to grain yield in wheat and barley. *Nature* **270**: 431-433
- 601 **Blum A** (1998) Improving wheat grain filling under stress by stem reserve mobilisation.
602 *Euphytica* **100**: 77-83
- 603 **Blum A** (2006) Drought adaptation in cereal crops: a prologue,
- 604 **Blum A** (2009) Effective use of water (EUW) and not water-use efficiency (WUE) is the
605 target of crop yield improvement under drought stress. *Field Crops Research* **112**:
606 119-123
- 607 **Blum A** (2017) Osmotic adjustment is a prime drought stress adaptive engine in support of
608 plant production. *Plant, Cell & Environment* **40**: 4-10
- 609 **Borrás L, Slafer GA, Otegui MaE** (2004) Seed dry weight response to source–sink
610 manipulations in wheat, maize and soybean: a quantitative reappraisal. *Field Crops*
611 *Research* **86**: 131-146
- 612 **Borrell AK, Incoll LD, Dalling MJ** (1993) The Influence of the Rht1 and Rht2 Alleles on
613 the Deposition and Use of Stem Reserves in Wheat. *Annals of Botany* **71**: 317-326
- 614 **Borrell AK, Incoll LD, Simpson RJ, Dalling MJ** (1989) Partitioning of Dry Matter and the
615 Deposition and Use of Stem Reserves in a Semi-dwarf Wheat Crop. *Annals of Botany*
616 **63**: 527-539
- 617 **Borrill P, Fahy B, Smith AM, Uauy C** (2015) Wheat Grain Filling Is Limited by Grain
618 Filling Capacity rather than the Duration of Flag Leaf Photosynthesis: A Case Study
619 Using NAM RNAi Plants. *PLOS One* **10**: e0134947
- 620 **Butler D, Cullis B, Gilmour A, Gogel B. 2009.** ASReml-R reference manual. Brisbane.

- 621 **Butler D, Cullis B, Gilmour A, Gogel BJ, Thompson R. 2017.** ASReml-R Reference
622 Manual Version 4. Hemel Hempstead.
- 623 **de Wit CT** (1958) Transpiration and crop yields. *In.* Agricultural Research Reports 64.6,
624 Pudoc, Wageningen, p 88
- 625 **Emes MJ, Bowsher CG, Hedley C, Burrell MM, Scrase-Field ES, Tetlow IJ** (2003)
626 Starch synthesis and carbon partitioning in developing endosperm. *J Exp Bot* **54**
- 627 **Evans LT, Bingham J, Jackson P, Sutherland J** (1972) Effect of awns and drought on the
628 supply of photosynthate and its distribution within wheat ears. *Annals of Applied*
629 *Biology* **70**: 67-76
- 630 **Fahy B, Siddiqui H, David LC, Powers SJ, Borrill P, Uauy C, Smith AM** (2018) Final
631 grain weight is not limited by the activity of key starch-synthesising enzymes during
632 grain filling in wheat. *Journal of experimental botany* **69**: 5461-5475n
- 633 **Fischer RA, Turner NC** (1978) Plant Productivity in the Arid and Semiarid Zones. *Annual*
634 *Review of Plant Physiology* **29**: 277-317
- 635 **Garcia M, Eckermann P, Haefele S, Satija S, Sznajder B, Timmins A, Baumann U,**
636 **Wolters P, Mather DE, Fleury D. 2019.** Genome-wide association mapping of grain
637 yield in a diverse collection of spring wheat (*Triticum aestivum* L.) evaluated in
638 southern Australia. *PLOS One* **14**(2): e0211730.
- 639 **Grantz DA** (1990) Plant response to atmospheric humidity. *Plant, Cell & Environment* **13**:
640 667-679
- 641 **Hölttä T, Mencuccini M, Nikinmaa E** (2009) Linking phloem function to structure:
642 Analysis with a coupled xylem–phloem transport model. *Journal of Theoretical*
643 *Biology* **259**: 325-337
- 644 **Jurgens SK, Johnson RR, Boyer JS** (1978) Dry Matter Production and Translocation in
645 Maize Subjected to Drought during Grain Fill1. *Agronomy Journal* **70**: 678-682

- 646 **Machado S, Paulsen GM** (2001) Combined effects of drought and high temperature on
647 water relations of wheat and sorghum. *Plant and Soil* **233**: 179-187
- 648 **Medina S, Vicente R, Nieto-Taladriz MT, Aparicio N, Chairi F, Vergara-Diaz O, Araus**
649 **JL** (2019) The Plant-Transpiration Response to Vapor Pressure Deficit (VPD) in
650 Durum Wheat Is Associated With Differential Yield Performance and Specific
651 Expression of Genes Involved in Primary Metabolism and Water Transport. *Frontiers*
652 *in Plant Science* **9**
- 653 **Miralles DJ, Katz SD, Colloca A, Slafer GA** (1998) Floret development in near isogenic
654 wheat lines differing in plant height. *Field Crops Research* **59**: 21-30
- 655 **Mittler R** (2006) Abiotic stress, the field environment and stress combination. *Trends in*
656 *Plant Science* **11**: 15-19
- 657 **Munns R, Weir R** (1981) Contribution of Sugars to Osmotic Adjustment in Elongating and
658 Expanded Zones of Wheat Leaves During Moderate Water Deficits at Two Light
659 Levels. *Functional Plant Biology* **8**: 93-105
- 660 **Pradhan GP, Prasad PVV, Fritz AK, Kirkham MB, Gill BS** (2012) Effects of drought and
661 high temperature stress on synthetic hexaploid wheat. *Functional Plant Biology* **39**:
662 190-198
- 663 **R Core Team 2019**. R: A language and environment for statistical computing. Vienna: R
664 Foundation for Statistical Computing.
- 665 **Rebetzke GJ, Bonnett DG, Reynolds MP** (2016) Awns reduce grain number to increase
666 grain size and harvestable yield in irrigated and rainfed spring wheat. *Journal of*
667 *Experimental Botany* **67**: 2573-2586
- 668 **Rebetzke GJ, van Herwaarden AF, Jenkins C, Weiss M, Lewis D, Ruuska S, Tabe L,**
669 **Fettell NA, Richards RA** (2008) Quantitative trait loci for water-soluble

- 670 carbohydrates and associations with agronomic traits in wheat. Australian Journal of
671 Agricultural Research **59**: 891-905
- 672 **Reynolds M, Dreccer F, Trethowan R** (2006) Drought-adaptive traits derived from wheat
673 wild relatives and landraces. Journal of Experimental Botany **58**: 177-186
- 674 **Reynolds M, Langridge P** (2016) Physiological breeding. Current Opinion in Plant Biology
675 **31**: 162-171
- 676 **Richards R** (1992) The effect of dwarfing genes in spring wheat in dry environments. I.
677 Agronomic characteristics. Australian Journal of Agricultural Research **43**: 517-527
- 678 **Rosenzweig C, Elliott J, Deryng D, Ruane AC, Müller C, Arneth A, Boote KJ, Folberth
679 C, Glotter M, Khabarov N, Neumann K, Piontek F, Pugh TAM, Schmid E,
680 Stehfest E, Yang H, Jones JW** (2014) Assessing agricultural risks of climate change
681 in the 21st century in a global gridded crop model intercomparison. Proceedings of
682 the National Academy of Sciences **111**: 3268-3273
- 683 **Savin R, Nicolas ME** (1996) Effects of short periods of drought and high temperature on
684 grain growth and starch accumulation of two malting barley cultivars. Functional
685 Plant Biology **23**: 201-210
- 686 **Schnyder H** (1993) The role of carbohydrate storage and redistribution in the source-sink
687 relations of wheat and barley during grain filling — a review. New Phytologist **123**:
688 233-245
- 689 **Schmidt J, Tricker PJ, Eckermann P, Kalambettu P, Garcia M, Fleury D** (2020) Novel
690 alleles for combined drought and heat stress tolerance in wheat. Frontiers in Plant
691 Science. **10**:1800
- 692 **Schoppach R, Sadok W** (2012) Differential sensitivities of transpiration to evaporative
693 demand and soil water deficit among wheat elite cultivars indicate different strategies
694 for drought tolerance. Environmental and Experimental Botany **84**: 1-10

- 695 **Schoppach R, Taylor JD, Majerus E, Claverie E, Baumann U, Suchecki R, Fleury D,**
696 **Sadok W** (2016) High resolution mapping of traits related to whole-plant
697 transpiration under increasing evaporative demand in wheat. *Journal of Experimental*
698 *Botany* **67**: 2847-2860
- 699 **Sevanto S** (2018) Drought impacts on phloem transport. *Current Opinion in Plant Biology*
700 **43**: 76-81
- 701 **Shah NH, Paulsen GM** (2003) Interaction of drought and high temperature on
702 photosynthesis and grain-filling of wheat. *Plant and Soil* **257**: 219-226
- 703 **Shirdelmoghanloo H, Cozzolino D, Lohraseb I, Collins NC** (2016) Truncation of grain
704 filling in wheat (*Triticum aestivum*) triggered by brief heat stress during early grain
705 filling: association with senescence responses and reductions in stem reserves.
706 *Functional Plant Biology* **43**: 919-930
- 707 **Sinclair TR, Hammer GL, van Oosterom EJ** (2005) Potential yield and water-use
708 efficiency benefits in sorghum from limited maximum transpiration rate. *Functional*
709 *Plant Biology* **32**: 945-952
- 710 **Stone B, Morell MK** (2009) Carbohydrates. *In* K Khan, PR Shewry, eds, *Wheat*, Ed 4.
711 AACC International Press, pp 299-362
- 712 **van Dijk AIJM, Beck HE, Crosbie RS, de Jeu RAM, Liu YY, Podger GM, Timbal B,**
713 **Viney NR** (2013) The Millennium Drought in southeast Australia (2001–2009):
714 Natural and human causes and implications for water resources, ecosystems,
715 economy, and society. *Water Resources Research* **49**: 1040-1057
- 716 **Yemm EW, Willis AJ** (1954) The estimation of carbohydrates in plant extracts by anthrone.
717 *Biochemical Journal* **57**: 508-514

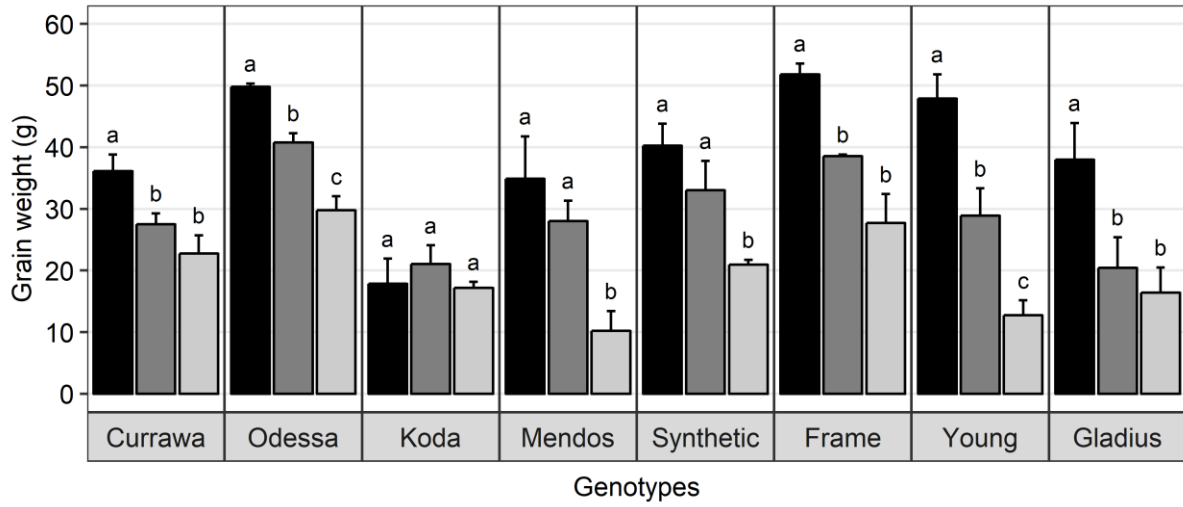
718 **Zhang J, Chen W, Dell B, Vergauwen R, Zhang X, Mayer JE, Van den Ende W (2015)**

719 Wheat genotypic variation in dynamic fluxes of WSC components in different stem

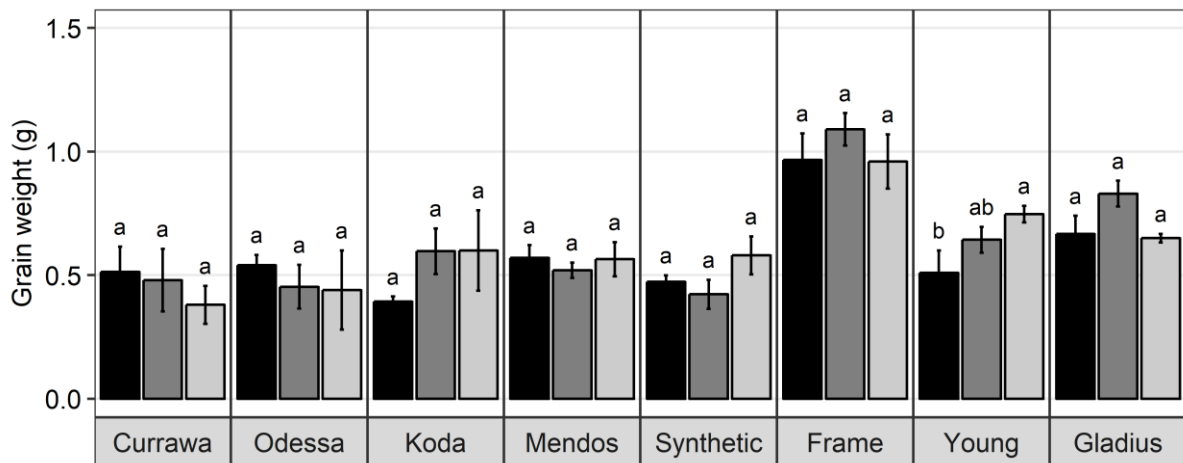
720 segments under drought during grain filling. *Frontiers in Plant Science* **6**: 624

721

(a) Whole plant at harvest



(b) Main spike at 12 DAA



(c) Main spike at harvest

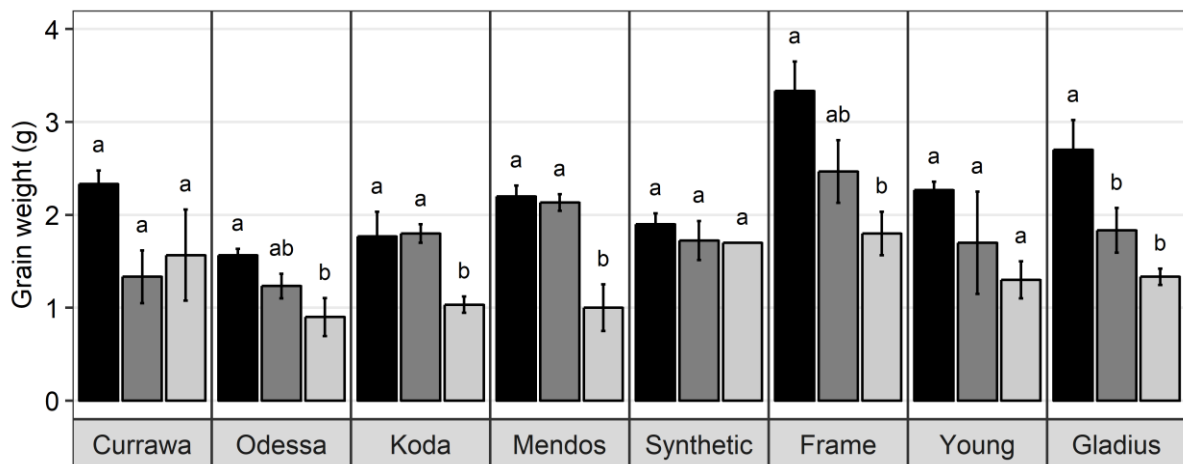
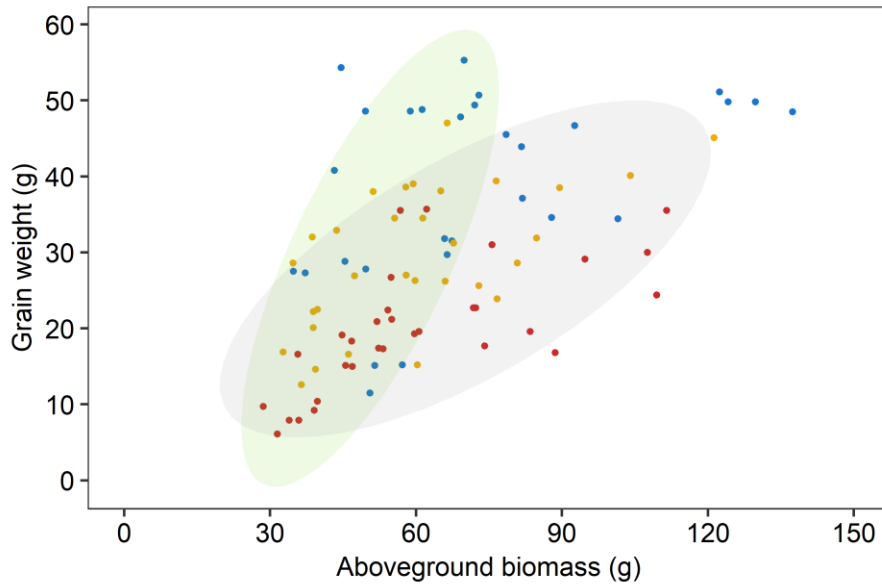


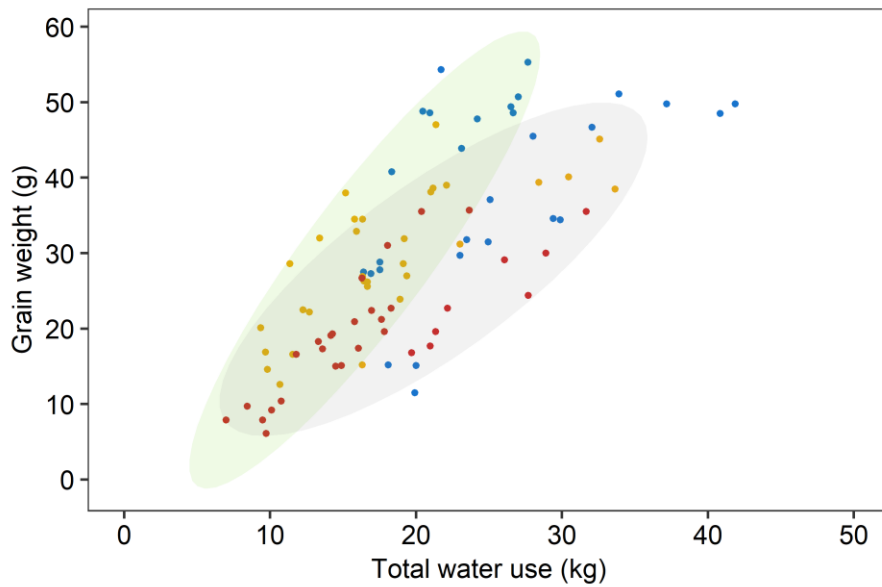
Figure 1. Combined drought and heat stress differentially reduced final grain weight.

(a) Mean total grain weight per plant at harvest (n=4). (b) Mean total grain weight in the main spike at 12 days after anthesis (n=3). (c) Mean total grain weight in the main spike at harvest (n=4). Error bars are standard error. Letters indicate the results of Tukey's test comparing treatment effect within each genotype ($p < 0.1$). Plants were grown under well-watered conditions (black), drought (dark grey) or combined drought and heat (light grey).

(a)



(b)

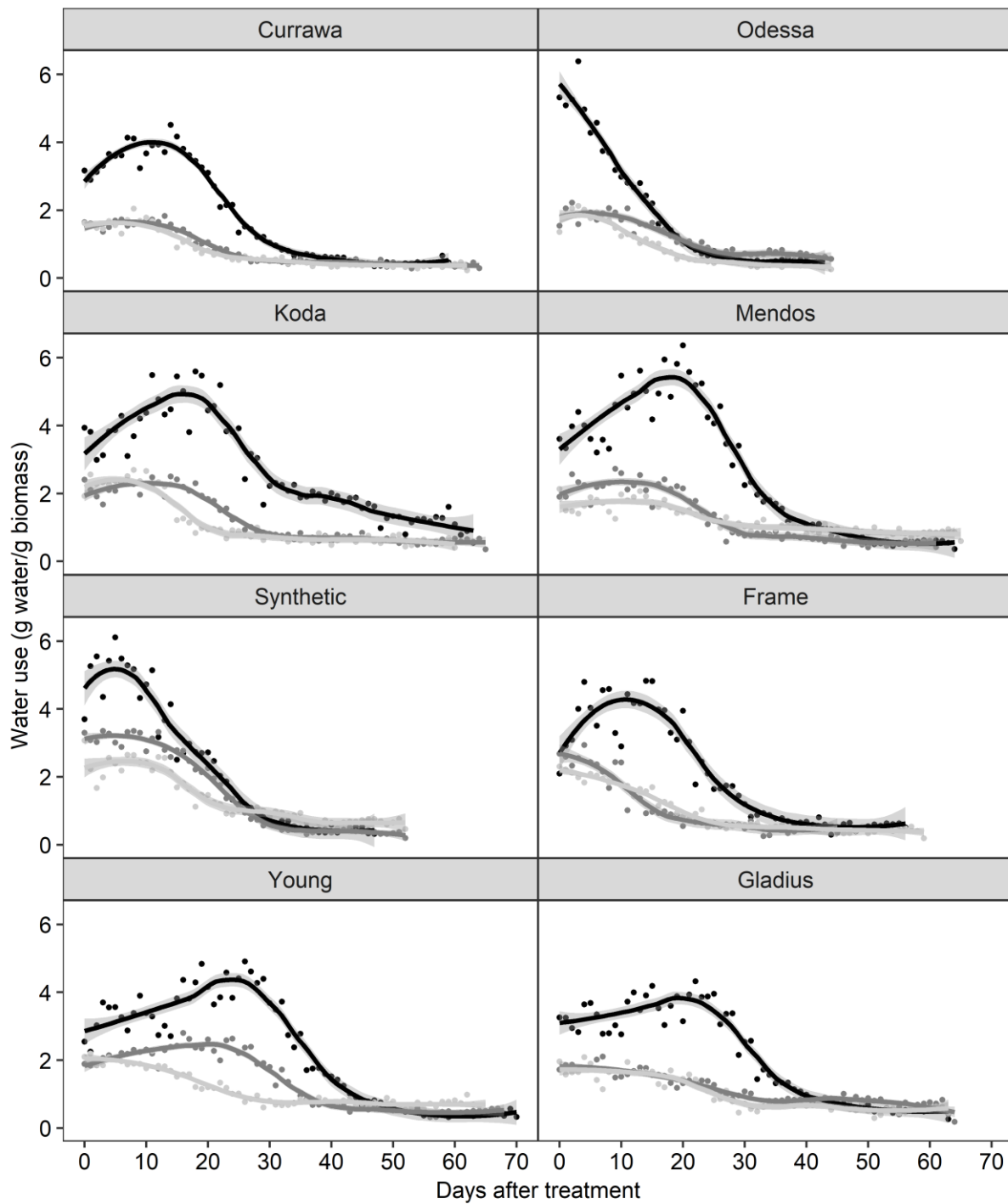


	Aboveground biomass		Total water use	
	r^2	p	r^2	p
Well-watered	0.2	*	0.31	**
Drought	0.35	*	0.56	***
Drought and heat	0.46	*	0.67	***
Modern genotypes	0.48	***	0.8	***
Older genotypes	0.46	***	0.63	***

* $p < 0.1$; ** $p < 0.01$; *** $p < 0.001$; ns not significant

Figure 2. Aboveground biomass and water use explained more of the variation in grain weight under increasing stress intensity than in well-watered conditions. Relationships between final grain weight per plant and (a) aboveground biomass excluding grains, (b) total water use. Each point represents one plant, grown under well-watered conditions (blue), drought (orange) or combined drought and heat stress (red). Ellipses circle modern genotypes (in green) and older genotypes (in grey). Table shows r^2 and p-value of linear regressions (*' $p < 0.1$, '**' $p < 0.01$, '***' $p < 0.001$) for each treatment (well-watered, drought, combined drought and heat stress) and genotype group (modern, older).

(a)



(b)

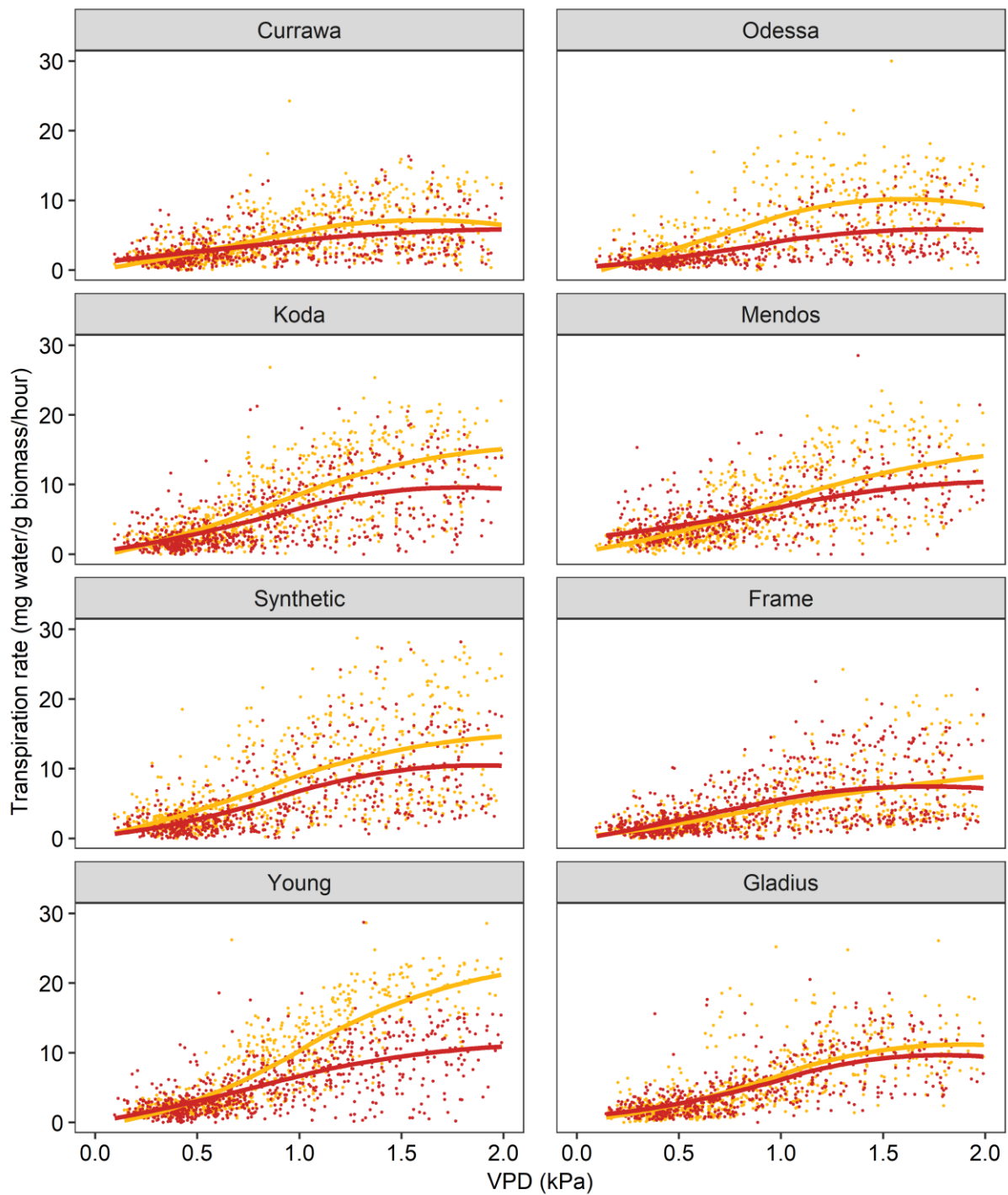
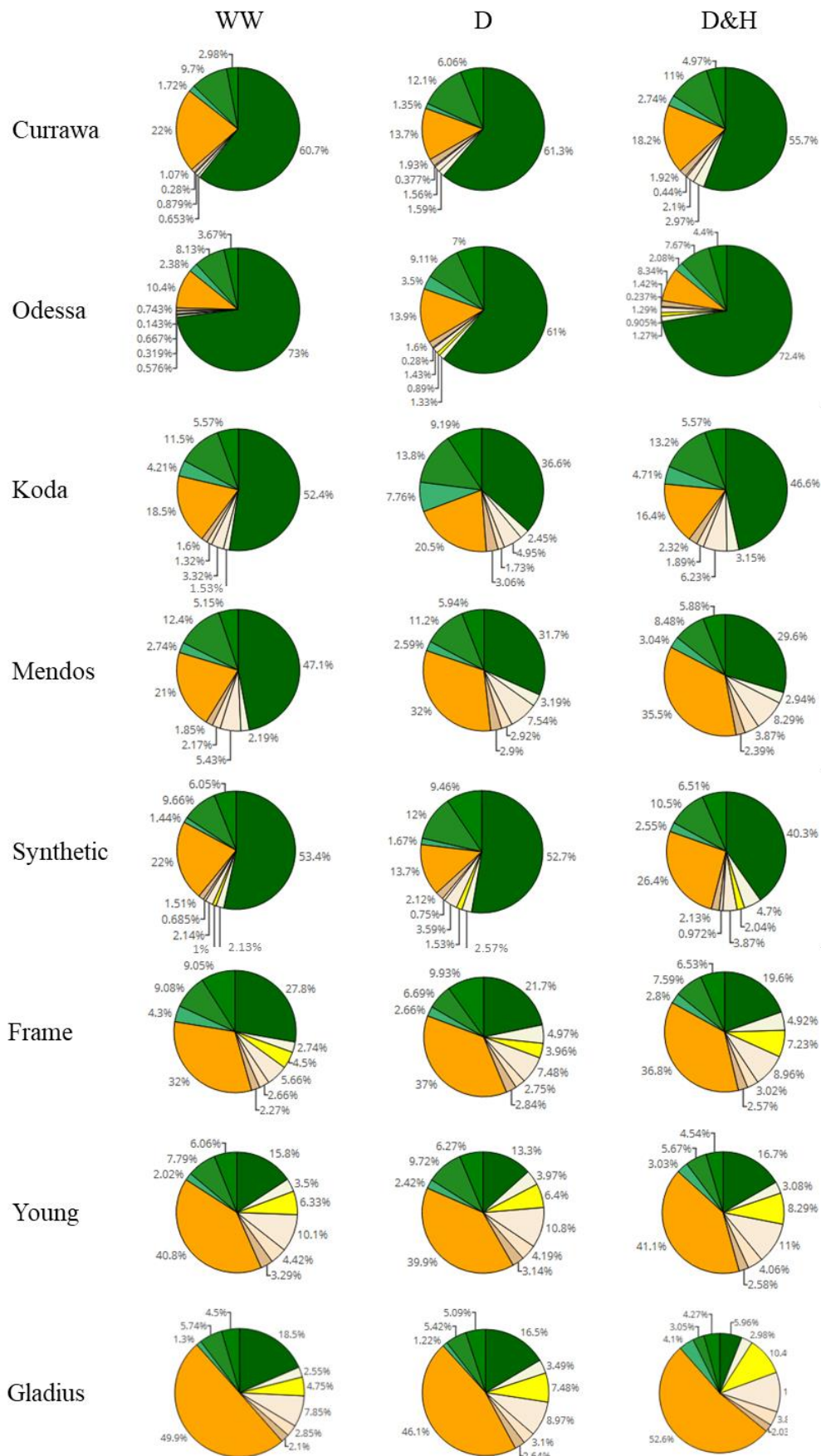


Figure 3. Interaction of high temperature and drought differentially reduced daily water use and transpiration response to vapour pressure deficit. (a) Daily water use per plant estimated as total irrigation per day, normalised to aboveground biomass. Plants grown in well-watered conditions (black), drought (dark grey) or in drought following three-day heat stress (light grey). 0 DAT is the first day post heat treatment (12 days after anthesis).

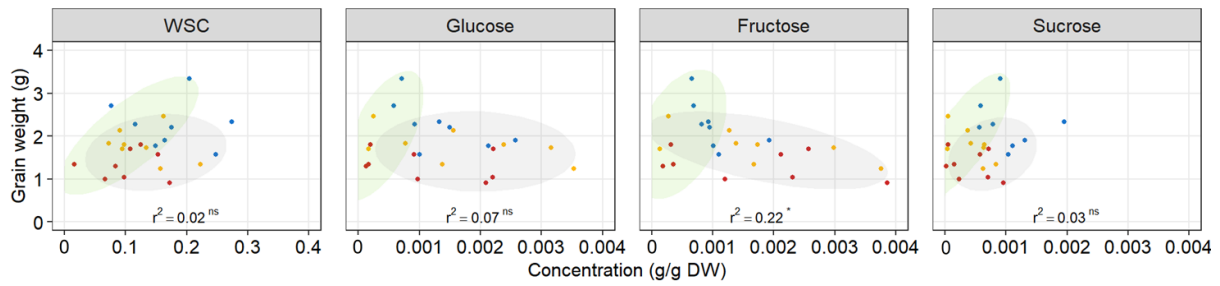
Trend lines are loess regressions. Values are means of four replicates (n=4). The confidence interval (0.95) is displayed around smoothed regressions in grey. (b) Hourly transpiration rate response to VPD normalised to aboveground biomass. Plants grown in drought (orange) or in drought following three-day heat stress (red). Graphs include data from 0 DAT to 30 DAT. Trend lines are smooth regression lines.



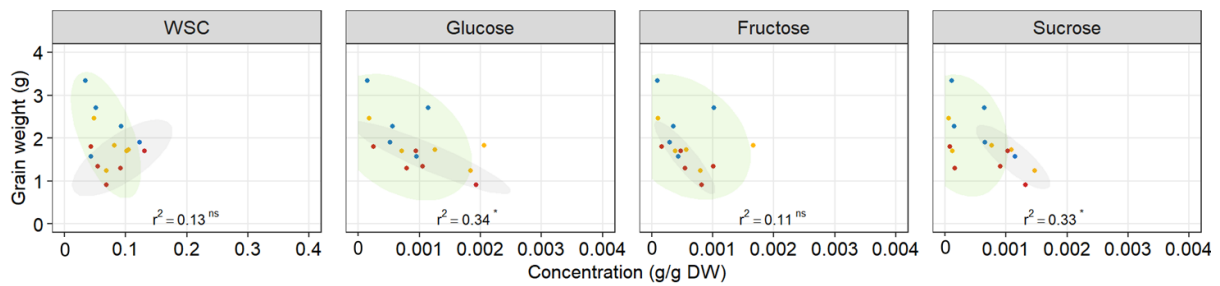
■ Stem ■ Leaf sheath ■ Peduncle (covered) ■ Peduncle (exposed)
■ Grains ■ Rachis ■ Palea ■ Lemma ■ Awns ■ Glumes

Figure 4. Combined drought and heat stress increased WSC partitioning to the spike in old genotypes, not in modern varieties. Genotypes are shown in order of date of release (top – bottom, oldest = Currawa to newest = Gladius). Total water-soluble carbohydrates (WSC) as in different organs of plants as a percentage of total WSC (n=3). Plant organs are colour-coded as shown in the legend.

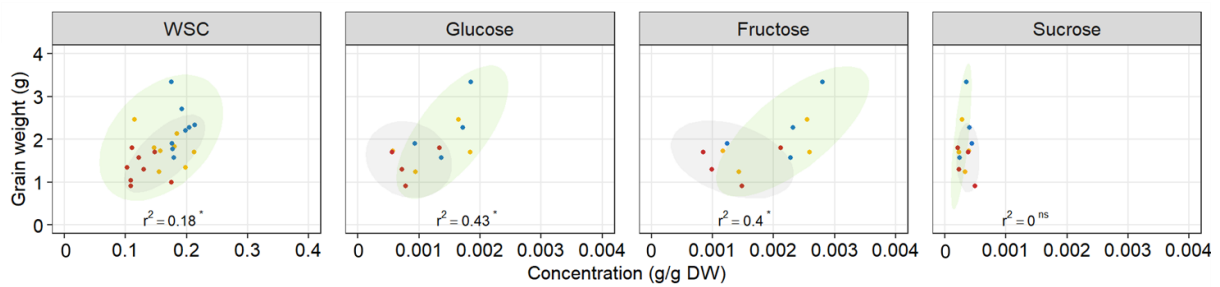
(a)



(b)



(c)



(d)

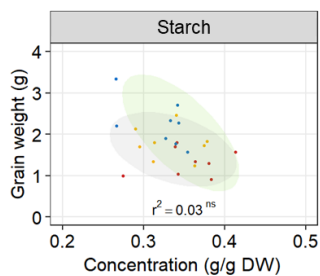


Figure 5. Relationship between total WSC, glucose, fructose, sucrose and starch concentrations in (a) the stem, (b) awns, (c-d) grains at 12 DAA, and final grain weight per plant. Each point represents an average of carbohydrate concentration (n=3) in the main tiller for one genotype and one treatment. Ellipses circle old genotypes (grey) and modern

genotypes (green). Plants grown under well-watered conditions (blue), drought (orange) or combined drought and heat stress (red). Regression lines include all datapoints. r^2 and p-value of linear regressions ('ns' not significant, '*' $p < 0.05$) are indicated.

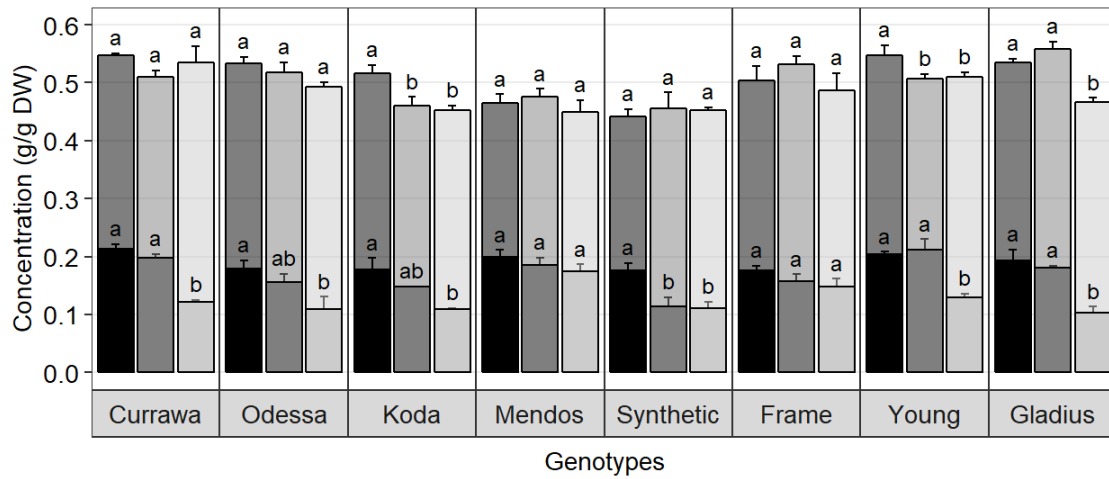


Figure 6. Drought and combined drought and heat stress altered WSC and starch balance in grains at 12 DAA. Water-soluble carbohydrates (solid) and starch (transparent) concentration in grains at 12 DAA from plants grown under well-watered conditions (black), drought (dark grey) or combined drought and heat stress (light grey). The sum of WSC and starch concentrations constitutes the non-structural carbohydrate (NSC). Values are means of three replicates (+/- SE). Letters (top: NSC, bottom: WSC) indicate the results of Tukey's test comparing treatment effects within each genotype ($p < 0.1$).

Parsed Citations

Asana R, Williams R (1965) The effect of temperature stress on grain development in wheat. *Australian Journal of Agricultural Research* 16: 1-13

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Bidinger F, Musgrave RB, Fischer RA (1977) Contribution of stored pre-anthesis assimilate to grain yield in wheat and barley. *Nature* 270: 431-433

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Blum A (1998) Improving wheat grain filling under stress by stem reserve mobilisation. *Euphytica* 100: 77-83

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Blum A (2006) Drought adaptation in cereal crops: a prologue,

Blum A (2009) Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. *Field Crops Research* 112: 119-123

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Blum A (2017) Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. *Plant, Cell & Environment* 40: 4-10

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Borrás L, Slafer GA, Otegui MaE (2004) Seed dry weight response to source-sink manipulations in wheat, maize and soybean: a quantitative reappraisal. *Field Crops Research* 86: 131-146

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Borrell AK, Incoll LD, Dalling MJ (1993) The Influence of the Rht1 and Rht2 Alleles on the Deposition and Use of Stem Reserves in Wheat. *Annals of Botany* 71: 317-326

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Borrell AK, Incoll LD, Simpson RJ, Dalling MJ (1989) Partitioning of Dry Matter and the Deposition and Use of Stem Reserves in a Semi-dwarf Wheat Crop. *Annals of Botany* 63: 527-539

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Borrill P, Fahy B, Smith AM, Uauy C (2015) Wheat Grain Filling Is Limited by Grain Filling Capacity rather than the Duration of Flag Leaf Photosynthesis: A Case Study Using NAM RNAi Plants. *PLOS One* 10: e0134947

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Butler D, Cullis B, Gilmour A, Gogel B. 2009. ASReml-R reference manual. Brisbane.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Butler D, Cullis B, Gilmour A, Gogel BJ, Thompson R. 2017. ASReml-R Reference Manual Version 4. Hemel Hempstead.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

de Wit CT (1958) Transpiration and crop yields. In. *Agricultural Research Reports* 64.6, Pudoc, Wageningen, p 88

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Ernes MJ, Bowsher CG, Hedley C, Burrell MM, Scrase-Field ES, Tetlow IJ (2003) Starch synthesis and carbon partitioning in developing endosperm. *J Exp Bot* 54

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Evans LT, Bingham J, Jackson P, Sutherland J (1972) Effect of awns and drought on the supply of photosynthate and its distribution within wheat ears. *Annals of Applied Biology* 70: 67-76

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Fahy B, Siddiqui H, David LC, Powers SJ, Borrill P, Uauy C, Smith AM (2018) Final grain weight is not limited by the activity of key starch-synthesising enzymes during grain filling in wheat. *Journal of experimental botany* 69: 5461-5475n

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Fischer RA, Turner NC (1978) Plant Productivity in the Arid and Semiarid Zones. Annual Review of Plant Physiology 29: 277-317

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Garcia M, Eckermann P, Haefele S, Satija S, Sznajder B, Timmins A, Baumann U, Wolters P, Mather DE, Fleury D. 2019. Genome-wide association mapping of grain yield in a diverse collection of spring wheat (*Triticum aestivum* L.) evaluated in southern Australia. PLOS One 14(2): e0211730.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Grantz DA (1990) Plant response to atmospheric humidity. Plant, Cell & Environment 13: 667-679

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Höittä T, Mencuccini M, Nikinmaa E (2009) Linking phloem function to structure: Analysis with a coupled xylem-phloem transport model. Journal of Theoretical Biology 259: 325-337

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Jurgens SK, Johnson RR, Boyer JS (1978) Dry Matter Production and Translocation in Maize Subjected to Drought during Grain Fill1. Agronomy Journal 70: 678-682

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Machado S, Paulsen GM (2001) Combined effects of drought and high temperature on water relations of wheat and sorghum. Plant and Soil 233: 179-187

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Medina S, Vicente R, Nieto-Taladriz MT, Aparicio N, Chairi F, Vergara-Diaz O, Araus JL (2019) The Plant-Transpiration Response to Vapor Pressure Deficit (VPD) in Durum Wheat Is Associated With Differential Yield Performance and Specific Expression of Genes Involved in Primary Metabolism and Water Transport. Frontiers in Plant Science 9

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Miralles DJ, Katz SD, Colloca A, Slafer GA (1998) Floret development in near isogenic wheat lines differing in plant height. Field Crops Research 59: 21-30

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Mittler R (2006) Abiotic stress, the field environment and stress combination. Trends in Plant Science 11: 15-19

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Munns R, Weir R (1981) Contribution of Sugars to Osmotic Adjustment in Elongating and Expanded Zones of Wheat Leaves During Moderate Water Deficits at Two Light Levels. Functional Plant Biology 8: 93-105

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Pradhan GP, Prasad PW, Fritz AK, Kirkham MB, Gill BS (2012) Effects of drought and high temperature stress on synthetic hexaploid wheat. Functional Plant Biology 39: 190-198

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

R Core Team 2019. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Rebetzke GJ, Bonnett DG, Reynolds MP (2016) Awns reduce grain number to increase grain size and harvestable yield in irrigated and rainfed spring wheat. Journal of Experimental Botany 67: 2573-2586

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Rebetzke GJ, van Herwaarden AF, Jenkins C, Weiss M, Lewis D, Ruuska S, Tabe L, Fettell NA, Richards RA (2008) Quantitative trait loci for water-soluble carbohydrates and associations with agronomic traits in wheat. Australian Journal of Agricultural Research 59: 891-905

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Reynolds M, Dreccer F, Trethowan R (2006) Drought-adaptive traits derived from wheat wild relatives and landraces. Journal of Experimental Botany 58: 177-186

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Reynolds M, Langridge P (2016) Physiological breeding. Current Opinion in Plant Biology 31: 162-171

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Richards R (1992) The effect of dwarfing genes in spring wheat in dry environments. I. Agronomic characteristics. Australian Journal of Agricultural Research 43: 517-527

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Rosenzweig C, Elliott J, Deryng D, Ruane AC, Müller C, Arneth A, Boote KJ, Folberth C, Glotter M, Khabarov N, Neumann K, Piontek F, Pugh TAM, Schmid E, Stehfest E, Yang H, Jones JW (2014) Assessing agricultural risks of climate change in the 21st century in a global gridded crop model intercomparison. Proceedings of the National Academy of Sciences 111: 3268-3273

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Savin R, Nicolas ME (1996) Effects of short periods of drought and high temperature on grain growth and starch accumulation of two malting barley cultivars. Functional Plant Biology 23: 201-210

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Schnyder H (1993) The role of carbohydrate storage and redistribution in the source-sink relations of wheat and barley during grain filling - a review. New Phytologist 123: 233-245

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Schmidt J, Tricker PJ, Eckermann P, Kalambettu P, Garcia M, Fleury D (2020) Novel alleles for combined drought and heat stress tolerance in wheat. Frontiers in Plant Science. 10:1800

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Schoppach R, Sadok W (2012) Differential sensitivities of transpiration to evaporative demand and soil water deficit among wheat elite cultivars indicate different strategies for drought tolerance. Environmental and Experimental Botany 84: 1-10

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Schoppach R, Taylor JD, Majerus E, Claverie E, Baumann U, Suchecki R, Fleury D, Sadok W (2016) High resolution mapping of traits related to whole-plant transpiration under increasing evaporative demand in wheat. Journal of Experimental Botany 67: 2847-2860

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Sevanto S (2018) Drought impacts on phloem transport. Current Opinion in Plant Biology 43: 76-81

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Shah NH, Paulsen GM (2003) Interaction of drought and high temperature on photosynthesis and grain-filling of wheat. Plant and Soil 257: 219-226

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Shirdelmoghanloo H, Cozzolino D, Lohraseb I, Collins NC (2016) Truncation of grain filling in wheat (*Triticum aestivum*) triggered by brief heat stress during early grain filling: association with senescence responses and reductions in stem reserves. Functional Plant Biology 43: 919-930

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Sinclair TR, Hammer GL, van Oosterom EJ (2005) Potential yield and water-use efficiency benefits in sorghum from limited maximum transpiration rate. Functional Plant Biology 32: 945-952

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Stone B, Morell MK (2009) Carbohydrates. In K Khan, PR Shewry, eds, Wheat, Ed 4. AACC International Press, pp 299-362

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

van Dijk AJM, Beck HE, Crosbie RS, de Jeu RAM, Liu YY, Podger GM, Timbal B, Viney NR (2013) The Millennium Drought in southeast Australia (2001–2009): Natural and human causes and implications for water resources, ecosystems, economy, and society. Water Resources Research 49: 1040-1057

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Yemm EW, Willis AJ (1954) The estimation of carbohydrates in plant extracts by anthrone. Biochemical Journal 57: 508-514

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Zhang J, Chen W, Dell B, Vergauwen R, Zhang X, Mayer JE, Van den Ende W (2015) Wheat genotypic variation in dynamic fluxes of WSC

components in different stem segments under drought during grain filling. *Frontiers in Plant Science* 6: 624

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)