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*Drought priming effects on alleviating the photosynthetic limitations of wheat cultivars (*Triticum aestivum* L.) with contrasting tolerance to abiotic stresses*

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1 **Title:** Drought priming effects on alleviating the photosynthetic limitations of wheat cultivars
2 (*Triticum aestivum* L.) with contrasting tolerance to abiotic stresses.

3

4 **Short title:** Priming mitigates stress

5

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24

25 **Abstract**

26 Abiotic stress tolerance in plants is said to be induced by pre-stress events (priming) during
27 the vegetative phase. We aimed to test if drought priming could improve the heat and drought
28 tolerance in wheat cultivars. Two wheat cultivars ‘Gladius’ and ‘Paragon’ were grown in a
29 fully controlled gravimetric platform and subjected to either no stress or two drought cycles
30 during the tillering stage. At anthesis, both batches were subjected to either high temperature
31 stress, drought stress, or kept as control. No alleviation of grain yield reduction due to
32 priming was observed. Higher CO₂ assimilation rates were achieved due to priming under
33 drought stress. Yield results showed that priming was not damage cumulative to wheat.
34 Priming was responsible to alleviated biochemical photosynthetic limitations under drought
35 stress and sustained photochemical utilization under heat stress in ‘Paragon’. Priming as a
36 strategy in abiotic stress alleviation was better evidenced in the stress susceptible cultivar
37 ‘Paragon’ than tolerant cultivar ‘Gladius’, therefore the type of response to priming appears
38 to be cultivar dependable, thus phenotypical variation should be expected when studying the
39 effects of abiotic priming.

40

41 **Keywords:** heat stress; chlorophyll fluorescence; gas exchange; grain yield; photosynthesis;
42 stomatal conductance.

43

44 **Introduction**

45 Predictions about future climate scenarios, such as intensification in the frequency and
46 severity of extreme climate events, are reported to negatively affect crop yield and global

47 food production (Deryng et al., 2014). Crop yield is of key importance when discussing the
48 improvement of crop production under water-limitation (Farooq et al., 2009) and elevated
49 temperature scenarios (Wahid et al., 2007). The extent and nature of the damage, as well as
50 the plants' capacity of recovery from abiotic stress are, among other factors, depended on the
51 developmental stage at which the crop encounters the stress (Saini and Westgate, 1999). The
52 most critical costs on crop yield have been reported to occur when stress coincides with the
53 onset of meiosis, anthesis and early grain initiation (Garg et al., 1984, Saini and Westgate,
54 1999). Although the vulnerability of cereals to abiotic stresses are well studied, the progress
55 to overcome this problem has been very slow. Thus, improving crop's tolerance to abiotic
56 stress happening during their reproductive stages is of high importance to future crop
57 production.

58 Elevated temperatures during anthesis can affect the yield components by accelerated rate of
59 development, accelerated leaf senescence, inhibition of photosynthesis and carbohydrate
60 synthesis, increase in respiration as well as flower abortion, pollen sterility/viability, pollen
61 germination and floral asynchrony (Dupuis and Dumas, 1990). While accounts of grain
62 weight losses are often recurrent over stress occurring after anthesis during the grain filling
63 phase (Abid et al., 2017), the reduction in seed set is mainly during the two main phases of
64 ontogeny: the meiosis and anthesis (Wahid et al., 2007). The main factor influencing the final
65 grain yield in crops is often shown to be the grain number, while the grain weight is of lesser
66 importance.

67 The response to drought stress in crops will vary from partial stomatal closure under
68 moderate stress to desiccation and plant death at the wilting point. In wheat, a gradual decline
69 in stress sensitivity to drought is expected as grains develop (Saini and Westgate, 1999).
70 Although early drought can reduce tillering and the number of ears per square meter, plants at

71 the tillering stage are regarded as more plastic in their stress responses due to their smaller
72 leaf area, moderated rates of physiological activity, lower water demand as well as their
73 flexibility to compensate stress damages in favourable soil moisture conditions after drought
74 episodes (Garg et al., 1984)

75 The decline in CO₂ assimilation under drought stress can be attributed to direct factors such as
76 a reduction in the CO₂ diffusion through the leaf mesophyll and consequently the CO₂ supply
77 to Rubisco (Chaves et al., 2009) or by indirect factors such as metabolic constraints caused
78 by a decrease in ATP synthesis and a limited RuBP (Ribulose 1,5-bisphosphate) synthesis
79 (Lawlor and Cornic, 2002). Reproductive processes in crops are very vulnerable to changes
80 in the water status. The reduction of photosynthetic flux can lead to a reduction on starch
81 accumulation and invertase activity what can ultimately lead to pollen sterility and ovary
82 abortion (Cattivelli et al., 2008, Farooq et al., 2014). At anthesis, mild drought stress can lead
83 to a reduction of yield (Gupta et al., 2001), with a minimal effect on grain number but a direct
84 effect on grain size (Ji et al., 2010). Positive correlations among plant height, leaf area and
85 grain yield (Gupta et al., 2001) and among relative water content, stomatal conductance and
86 grain yield (Akram, 2011) were reported for wheat plants under drought stress imposed at
87 anthesis. Thus, the need to advance strategies to improve the tolerance of the crop to drought
88 stress occurring during reproductive phases are acute.

89 The occurrence of high temperature or soil water depletion can result in a range of
90 morphological, anatomical, physiological and biochemical changes in plants. It can directly
91 induce alterations in existing physiological processes, or indirectly promote alterations in the
92 pattern of the plant's development (Chaves et al., 2009). A transient stress can prime a plant
93 against a subsequent stress, the retention of a stress memory is evident from acclimation
94 responses (Chinnusamy and Zhu, 2009). The priming state can be triggered by biotic and

95 abiotic stress events as well as chemical elements and interspecies communication (Bruce et
96 al., 2007, Conrath, 2006). It is defined as a state which by a plant responds more rapidly and
97 or more efficiently to a stress (Balmer et al., 2015). The length of the priming state can
98 include the post-challenge primed state in the same generation and a) be transient (if
99 dependable of the half-life of stress induced proteins, RNAs and metabolites) or b) be
100 maintained throughout plant's live (if morphology/phenology reprogramming occur), it can
101 also be passed on to the next generation, a term defined as transgenerational tolerance (when
102 stable or heritable DNA methylation and histone modification occurs) (Chinnusamy and Zhu,
103 2009).

104 Improvements in the antioxidant defence system, changes in hormones, metabolites and sugar
105 signals are reported to be induced by priming, which are believed to increase plant tolerance
106 under abiotic stress (Wang et al., 2017). Previous results have described a positive effect of
107 drought priming by alleviating both drought and heat stresses during the grain filling stage in
108 wheat (Abid et al., 2017, Wang et al., 2014, Wang et al., 2015). Improvements in grain yield,
109 photosynthetic capacity, oxidative stress mitigation (Wang et al., 2014), alleviation of
110 photoinhibition (Wang et al., 2015) and improvements in regulation of growth hormones
111 (Abid et al., 2017) at grain-filling stage are also attributed to drought priming. Taking in
112 account the current climate crises (IPCC, 2019), both post-challenge primed state in the same
113 generation and transgenerational priming can act as an approach for abiotic stress mitigation.

114 Advancing the understanding of the effects of the physiological basis of abiotic stress
115 induced priming to maintain or improve the yield is needed. To our knowledge, no advances
116 regarding the effects of drought priming on heat and drought events during critical ontogeny
117 phase anthesis has been made. A improvement in photosynthetic performance due to abiotic
118 priming has been suggest in previous literature (Wang et al., 2017). Therefore we aimed to

119 investigated if the effect of mild drought priming on the photosynthetic apparatus could be
120 responsible for improving yield under drought and heat stress conditions during anthesis in
121 two commercial wheat cultivars from different origins.

122 **Materials and Methods**

123 Plant Material

124 The experiment was conducted at the National Phenomics Centre at the Institute of
125 Biological, Environmental & Rural Science (IBERS) at Aberystwyth University, Wales, UK
126 (52.43N, 4.01W) during spring of 2017. Four seeds from two wheat (*Triticum aestivum* L.)
127 cultivars ‘Paragon’ and ‘Gladius’ were sown in 3.5 L plastic pots filled with potting grown
128 mix ICL Levinton F2 (added nutrients: 144 N, 73 P, 239 K) in a greenhouse at ambient CO₂,
129 light intensity of 350 μmol m⁻² s⁻¹ photosynthetic photon flux density (PPFD), with day length
130 following the photoperiod regime. The average temperature of the duration of the experiment
131 was 19.8 ± 3.2 °C and relative air humidity of 50.4 ± 13.1%.

132 The cultivars studied in this experiment are commercial cultivars from two different origins.
133 ‘Gladius’ is an Australian cultivar adapted to more heat and drought prone climates (Fleury et
134 al., 2010), while ‘Paragon’ is an UK cultivar bred to a cool and temperate climate (Mendanha
135 et al., 2018, Sikder et al., 2015).

136 Treatments

137 At three fully developed leaves stage, four replicate pots per treatment were allocated to a
138 fully controlled gravimetric platform and randomly assigned across scales. Following the set-
139 up described by Wang et al. (2015), half of the pots were subjected to a drought priming (P)
140 by reducing the soil relative water content (SRWC) to 35% (withholding watering for five
141 days), followed by a recovery period (SRWC of 80%) until the full extension of the fifth leaf

142 when the drought priming process was repeated by withholding watering for five days. The
143 other half of the pots were kept well-watered at SRWC of 80% as non-primed control plants
144 (C).

145 At the developmental stage when 50% of the primary head was visible (Zadoks 55), plants
146 were subjected to either a high temperature stress (H, 32/28°C), drought stress (D; SRWC of
147 25%) or kept as non-stressed control plants (C) for seven days. The treatments subjected to
148 high temperature took place in a controlled climate chambers (Sanyo Fitotron, Weiss
149 Technik, Leicestershire) set at 14 hours of light in a diurnal cycle, at a photosynthetic photon
150 flux density of 350 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (PPFD) and relative humidity (RH%) of $82 \pm 4.0\%$. In total,
151 six treatments were established (Figure 1): no priming + no stress (CC); no priming + drought
152 stress (CD); no priming + heat stress (CH); priming + no stress (PC); priming + drought
153 stress (PD); priming + heat stress (PH).

154

155 Destructive harvest

156 *Growth and morphological development*

157 During the experiment, growth and morphological development data were collected from the
158 three leaves stage (Zadoks 13) until end of anthesis (Zadoks 69). Once a week, plant height,
159 the number of leaves in main shoot, developmental stage (Zadok scale), and number of tillers
160 were noted. Chlorophyll index (SPAD-502, Konica Minolta, Osaka, Japan) was determined
161 on the mid portion of the youngest fully developed leaf in three non-overlapping
162 measurements, once a week in all plants per pot.

163

164 *Biomass accumulation*

165 One plant in each pot was sampled for the destructive harvest two weeks after the end of the
166 stress event and both cultivars were harvested at the milk developmental stage (Zadoks 75-
167 77). Fresh weight (FW) was determined separately for plant fractions in order to establish
168 biomass allocation: main shoot (leaves, stem and head) and tillers (leaves, stems and heads)
169 were weighed individually. Dry weight (DW) was determined after 48h at 80°C in constant
170 flux oven; leaf area (LA) was determined for green leaves (primary tiller was measured
171 separately) using WinDias (Delta-T Devices Ltd., Cambridge, The UK). Green leaves were
172 counted separately from dead leaves in order to establish green leaves retention and to adjust
173 the final total leaf area after the stress event.

174

175 *Grain yield components*

176 Three plants per pot were kept in the greenhouse (average temperature of $22.0 \pm 2.3^\circ\text{C}$; RH%
177 59.0 ± 11.4 and PPFD of $350 \mu\text{mol m}^{-2} \text{s}^{-1}$) until they reached full grain maturity. Primary
178 spikes were harvested individually from the rest. Spikes were manually threshed and yield
179 recorded. Numbers of spikes, primary spike dimensions, kernel number per spike and
180 thousand-kernel weight (TKW) were measured.

181 *Photosynthesis measurements*

182 *Gas exchange*

183 Photosynthetic light (A/Q) and intracellular CO₂ (A/Ci) responses were measured *in vivo* on
184 leaves using a portable gas exchange fluorescence system (Walz GFS-3000, Walz, Eifeltrich
185 Germany) with an integrated red-blue LED light source with a chlorophyll fluorescence
186 module (Walz 3055-FL). Measurements were done on one randomly selected plant per pot.

187 During priming, the youngest fully developed leaf was chosen for measurements and during
188 anthesis the primary flag leaf was used at the end of the stress period. The leaf mid-portion
189 was placed in a 4.0 cm² leaf cuvette with PPFD set at 500 μmol m⁻² s⁻¹, flow rate of 750 mL
190 min⁻¹ and reference [CO₂] was set at 407 ppm (equal to the ambient CO₂ concentration). The
191 cuvette temperature was set as growth temperature so the control and drought stressed plants
192 were measured at 20° C and heat stressed plants at 32° C. The vapour pressure deficit (VPD)
193 was kept below 10.0 (control and drought stress treatments) and 20.0 Pa/kPa (heat stress
194 treatment). Before measurements were initiated, leaves were acclimatised to the highest light
195 level by increasing PPFD stepwise to 2000 μmol m⁻² s⁻¹ until steady-state carbon assimilation
196 and stomatal conductance (g_s) rates were obtained. Curves were performed by decreasing
197 light from 2000 μmol m⁻² s⁻¹ to the following light levels: 1500, 1200, 1000, 700, 500, 350,
198 250, 150, 150 and 75 μmol m⁻² s⁻¹. Following the end of the A/Q curve, the same leaf portion
199 was used to attain the A/C_i curve. The PPFD was set to 1500 μmol m⁻² s⁻¹ and [CO₂] was
200 varied according to the sequence: 407, 300, 250, 150, 100, 50, 407, 600, 800, 1100 and 1500
201 ppm. Light response curves were fitted to a non-rectangular hyperbola (Ögren, 1993), to
202 estimate dark respiration (R_{dark}), maximal quantum efficiency of photosynthesis (α), light
203 compensation point (LCP), maximum net assimilation (A_{max}) and curve convexity (θ). The
204 leaf cooling during the A/Q measurements (ΔT) was calculated as ΔT = T_{leaf} - T_{cuvette}, in which
205 the negative numbers indicate cooling. Water use efficiency at the leaf level (WUE_{leaf}) is
206 defined as the ratio of A_{max} to transpiration rate (E), under saturating light intensity.
207 Assimilation rate obtained at varying [CO₂] were plotted against intracellular CO₂
208 concentration (C_i) to obtain a response curve. The biochemical parameters were normalized
209 to 25° C for comparison. Photosynthetic parameter limitations: maximum carboxylation
210 velocity of Rubisco (V_{c,max25}), electron transport demand for RuBP regeneration (J₂₅), day
211 respiration (R_{d25}), mesophyll conductance (g_{m25}) and the rate of use of triose phosphate

212 (TPU₂₅) were fitted according the equations presented by Sharkey et al. (2007), using an
213 estimator utility (available at: <http://www.blackwellpublishing.com/plantsci/pcecalculation/>).
214 Saturated assimilation rate (A_{sat}) was determined from assimilation values obtained at 1500
215 ppm CO₂ concentration.

216

217 *Chlorophyll fluorescence*

218 All treatments were subjected to chlorophyll *a* fluorescence analysis. Plants were moved to a
219 dark room and a primary flag leaf was dark-adapted for 30 minutes at room temperature. A
220 pulse amplitude modulation (PAM) fluorimeter (PAM-2500, Walz Eiffeltrich, Germany) was
221 used to calculate PSII operating efficiency (F_q'/F_m'), non-photochemical quenching (NPQ),
222 maximum quantum efficiency of PSII photochemistry (F_v/F_m) and electron transport rate
223 (ETR) on the adaxial surface of the leaves. All quenching parameters were measured at 900
224 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

225

226 *Leaf absorbance measurements*

227 Leaf light absorptance (Abs_{Leaf}) was measured using an integrated sphere (Spectroclip-JAZ,
228 Ocean Optics, Duiven, The Netherlands) in three non-overlapping areas in the mid-portion of
229 the primary flag leaf. Measurements were performed after gas exchange measurements and
230 absorbance was calculated as:

$$231 \quad \alpha = 1 - \rho - \tau$$

232 where α is the absorptance, ρ is the reflectance and τ is the transmittance *i.e.* the absorbed,
233 reflected and transmitted fractions of light, respectively. The mean absorptance in the

234 wavelength range 400-700 nm was used to calculate the absorbed PPFD when calculating
235 ETR.

236

237 *Leaf relative water content (LRWC)*

238 After seven days of stress treatment (heat or drought), a 5-cm long mid-portion segment of
239 the flag leaf of the main tiller (primary leaf) was harvested and immediately weighed to
240 record fresh weight (FW). The leaf piece was subsequently transferred to a petri dish and
241 immersed in distilled water for four hours at room temperature. Turgid weight (TW) was
242 determined and leaf samples were dried at 80°C for 48 hours in a constant flux oven to obtain
243 dry weight (DW). The LRWC of each leaf was calculated as:

244 $LRWC (\%) = [(FW-DW)/(TW-DW)] \times 100.$

245

246 *Statistical analyses*

247 All of the results are reported as mean \pm standard error of the mean of at least three replicates,
248 the number of replicates (n) is indicated in every figure or table in the results section. Data
249 was checked for variance homogeneity and normal distribution before statistical analysis. The
250 data was analysed for each cultivar separately. Following the imposition of stress (S), a two-
251 way analysis was performed to indicate the effect of priming (P), stress (S) and their
252 interaction (P x S), and the level of significance of each factor is indicated as *P<0.05,
253 **P<0.01, ***P<0.0001. When a interaction between factors was detected, one-way ANOVA
254 was used to determine significant difference between treatments and is indicated by small
255 letters in the figures and tables. The means were compared using Duncan's multiple

256 comparison tests with R package agricolae. Statistical analyses were performed using R
257 (Team, 2017) open source statistical computing software (Version 3.4.3, The R Foundation,
258 Vienna, Austria),. The figures presented were generated using Sigmaplot program (version
259 11.0, Systat Software Inc).

260

261 **Results**

262 Biomass accumulation

263 In ‘Gladius’, specific leaf area (SLA) and plant height were not affected by priming or stress
264 imposition (Supporting information). In ‘Paragon’, plant height and SLA decreased with
265 priming but not by the stress imposition during anthesis (Supporting information). Biomass
266 accumulation and SPAD index did not differ between treatments in any of the studied
267 cultivars.

268 Gas exchange

269 Priming (P1 and P2) imposition during the vegetative stage did not affect most of the light or
270 intercellular CO₂ response parameters (Supporting information). Only g_s was reduced in
271 ‘Gladius’ during P1 (Supporting information).

272 Priming did not improve LRWC (Table 1 and 2) under stress imposition. In Paragon, heat
273 and drought reduced LRWC, but no difference was observed in ‘Gladius’. In light response
274 parameters, the interaction between priming and drought stress (PD) upregulated the
275 maximum assimilation (A_{max}) in ‘Paragon, and the same trend was observed in ‘Gladius’
276 (P<0.08). However, no difference under non-stress conditions or heat stress was observed
277 between primed (PC; PH) and non-primed (CC, CH) treatments (Figure 2).

278 Under drought stress, the stomatal conductance (g_s) of ‘Gladius’ was upregulated by priming
279 (PD), but the same was not observed in ‘Paragon’. The transpiration rate (E), internal CO₂
280 (C_i) and water use efficiency (WUE_{leaf}) were affected by stress imposition in both cultivars,
281 but no effect due to priming was noticed (Table 1 and 2). In both cultivars, a decrease in
282 intracellular CO₂ (C_i) was observed under drought stress, while no change was detected in
283 heat stress treatments. As for WUE_{leaf} , values increased with drought and decreased with heat
284 stress in both cultivars (Table 1).

285 As for intercellular CO₂ response parameters, A_{sat} values were upregulated by priming in
286 ‘Gladius’. In ‘Paragon’ only stress imposition affected this parameter, as a decreased in A_{sat}
287 due to drought and an increase due heat stress was observed (Figure 3a and b, Table 2). The
288 estimated values of $V_{c,max25}$ and J_{25} were not affect by priming or by the interaction between
289 priming and stress in ‘Gladius. In this cultivar, $V_{c,max25}$ increased in stressed plants (both under
290 heat and drought stress), while J_{25} decreased under heat but was unchanged under drought
291 (Figure 3c and e). In ‘Paragon’, the same parameters ($V_{c,max25}$ and J_{25}) were upregulated in
292 primed plants under drought stress (PD), but no difference in heat stressed plants due to
293 priming was noticed (PH) (Figure 4d and f).

294 Chlorophyll fluorescence

295 In ‘Paragon’, priming decreased values of F_v/F_m , the same was not observed in ‘Gladius’
296 Stress imposition affected the parameter in both cultivars, with a significant reduction of the
297 parameter in heat stressed treatments (Figure 4a and b). An interaction between priming and
298 stress was detected for values of the photochemical efficiency (F_q'/F_m') and ETR in both
299 cultivars, while under heat stress those values were upregulated by priming, under non-stress
300 condition the parameter decreased (Figure 4c, d, g and h).

301 Values of non-photochemical quenching (NPQ) in ‘Gladius’ were not affected by any of the
302 two factors studied (Figure 4e). In ‘Paragon’ stress affected NPQ as plants under heat stress
303 presented significantly lower values compared to drought and non-stressed (PC) plants
304 (Figure 4f).

305 Grain Yield

306 Stress imposition decreased grain number and grain yield in both cultivars. No effect of
307 priming was observed for those parameters (Figure 5a, b, c and d). The decrease in the
308 number of grains and grain yield was more accentuated by heat stress than by drought stress.
309 Yield of the primary ear showed a positive interaction between priming and heat and drought
310 stress in ‘Gladius’. The same parameter in ‘Paragon’ decreased by priming and stress
311 imposition (Figure 5e and f).

312 While heat stress increased values of TKW for both cultivar (Figure 5g and h, Table 3), no
313 difference was observed by either drought treatments or by priming in ‘Gladius’ (Figure 5g,
314 Table 3). The interaction between priming and drought stress decreased TKW in ‘Paragon’
315 (PD) (Figure 5h).

316

317 **Discussion**

318 Abiotic stress may result in substantive losses in crop production. Following the exposure to
319 a stress, plants may become more tolerant to future stress through the acquisition of a “stress
320 memory”, here defined as priming (Martinez-Medina et al., 2016). We tested if drought
321 priming during the tillering stage could alleviate photosynthetic limitations and yield
322 reduction of abiotic stresses imposed during anthesis in two wheat cultivars.

323 The capability to sustain grain yield under abiotic stress is regarded as a characteristic of
324 tolerance in crops. The photosynthetic performance determines the concurrent photosynthates
325 available to the plant. Any constrain in photosynthesis can limit yield and biomass
326 accumulation (Fischer et al., 1998). Our investigation showed that priming upregulates CO₂
327 assimilation (A_{max}) under drought stress, suggesting that the priming plants would had better
328 grain-filling substrate supply to early developing grains, which was expected to be translated
329 on to yield improvements. However, our yield results could not show yield improvements in
330 either the studied cultivars due to priming. Drought reduction in yield during early grain
331 initiation is reported to be due to a smaller sink size of initiated grains (Saini and Westgate,
332 1999) and the correlation between yield components such as the increase of TKW in spikes
333 with reduced grain number is a key adaptive mechanism for restoring yield losses inflicted by
334 stress in plants (Blum, 1996). ‘Gladius’ yield reduction under drought stress is attributed to a
335 reduction in TKW, while ‘Paragon’ yield loss is shown to be explained by a reduction of
336 grain number. Again, priming presented no significant effect on the ability to reallocate photo
337 assimilates to grain filling (TKW) or prevent grain abortion on the studied cultivars,
338 suggesting that the cultivars studied were not able to maintain carbohydrate accumulation in
339 the reproductive organs throughout the drought stress treatment, even when assimilation was
340 upregulated. Ji et al. (2010) showed that the drought stress applied during anthesis had a

341 larger cost effect on grain weight than on grain number in wheat, however the difference in
342 the germplasm studied indicated that the genetic control for grain number and size is different
343 under drought stress, as they observed that the germplasm that was able to maintain grain
344 number was not better in maintaining grain weight. The same authors showed that grain
345 weight of drought-tolerant varieties is usually strongly reduced when drought stress occurs
346 during anthesis. The difference in origins of the two cultivars may as well explain the reasons
347 their yield response differed to drought stress.

348 Although no reduction in CO₂ assimilation was observed, heat stress decreased grain number
349 and grain yield in both cultivars and no alleviation was detected in primed plants regarding
350 their yield components. The reduction in yield of the heat stressed plants (CH, PH) was
351 attributed to a reduction in grain number rather than a drop in TKW. Under heat stress
352 conditions, plants are reported to allocate resources in order to cope with the stress, reverting
353 less photo assimilates for reproductive development (Wahid et al., 2007) .When CO₂
354 assimilation is taken in consideration, no changes were observed in the maximum
355 carboxylation efficiency of Rubisco ($V_{c,max25}$), but a reduction in the regeneration of the
356 substrate RuBP driven by photosynthetic electron transport (J_{25}) was observed under all heat
357 stress treatments included here. Ratios of $J/V_{c,max}$, linked to differences in nitrogen
358 partitioning within the photosynthetic apparatus, were lower in all heat stress treatments,
359 indicating that heat-stressed plants spent less nitrogen in RuBP regeneration processes (e.g.
360 electron transport) than Rubisco synthesis (Yamori et al., 2010). The reduction observed on
361 the $J/V_{c,max}$ ratio at anthesis indicate a potential unbalance in the photosynthetic CO₂
362 assimilation during the re-allocation of leaf assimilates to the reproductive organs. Yet, this
363 change was not affected by priming nor did decreased overall CO₂ assimilation at anthesis.

364 While discussing the fitness costs of priming, Martinez-Medina et al. (2016) indicated that
365 priming fitness is only a clear advantage in times of stress, as primed plants are expected to
366 outperform non-primed plants, otherwise allocation costs are usually expected under non-
367 stress condition. Non-stressed drought primed wheat plants have both been reported to reduce
368 grain yield (Liu et al., 2017, Wang et al., 2015) as well as not (Abid et al., 2016). In our
369 study, the priming imposition (SRWC was lowered to 35%) was fairly mild as no effect was
370 seen on the photosynthesis parameters, as a result, primed non-stressed plants (PC) were able
371 to maintain a high yield when not stressed during anthesis. The lack of allocation costs may
372 be explained by the fact that our priming consisted of two brief stress periods at tillering,
373 followed by a recovery phase. As post-stress resembled pre-stress conditions, plants did not
374 progress into a new developmental stage during priming and therefore morphological costs
375 were avoided.

376 The moderate temperature used (32/28°C) during heat stress may be the reason for the lack of
377 clearer differentiation between primed and non-primed heat treatments (PH and CH). Heat
378 induced effects on photosynthesis are usually only permanent in temperatures above 36°C
379 (Sharma et al., 2015), however in our previous study 'Paragon' plants did not withstand
380 day/night temperatures of 36/32°C during heat stress (Mendanha et al., 2018), therefore lower
381 temperatures were used to impose heat stress in the current study.

382 Although a decrease in g_s was observed during the first priming (P1) in 'Gladius', the
383 reduction did not affect A_{max} , transpiration rate or the ability to restore assimilation under
384 elevated $[CO_2]$ (A_{sat}). Under drought stress, primed plants of 'Gladius' presented g_s similar to
385 control. The stomatal closure is said to be the first response to mild drought and the main
386 limitation of photosynthesis (Flexas and Medrano, 2002). Stomatal closure will lead to a
387 reduction in CO_2 assimilation, transpiration cooling and nutrient uptake, and it is a trait of

388 tolerance when water loss preservation compensates the negative costs (Farooq et al., 2009).
389 Results of g_s in 'Gladius' shows that priming did not improved stomatal control under
390 drought stress for this cultivar.

391 Primed plants of both cultivars were able to maintain carbon assimilation under drought
392 stress (PD) to values similar to control conditions. The A/C_i response curves enable us to
393 quantitatively estimate the internal limitations of carbon assimilation in plants. Under
394 saturating light and high $[CO_2]$, values of J_{25} and $V_{c,max25}$ are positively correlated with A_{sat} in
395 wheat (Law and Crafts-Brandner, 1999). Drought stress in 'Gladius' (CD and PD) increased
396 values of A_{sat} , $V_{c,max25}$ and J_{25} when compared to the control treatment (CC). Drought stressed
397 plants of 'Paragon' (CD and PD) were not able to restore assimilation under elevated
398 concentrations of CO_2 (A_{sat}) and values of $V_{c,max25}$ and J_{25} contrasted between primed and non-
399 primed plants (CD and PD). Primed and heat stressed plants (PD) of 'Paragon' were able to
400 sustain values of $V_{c,max25}$ and J_{25} similar to those of non-stressed plants (CC and PC). As g_s
401 decreases under moderated drought stress, internal CO_2 (C_i) is expected to decrease compared
402 to well-watered plants, leading to a reduction in carbon assimilation. Hence, drought stress is
403 believed to decrease Rubisco activity due to the restriction of CO_2 availability for
404 carboxylation (lower level or function of Rubisco activase) (Galmés et al., 2007), leading to a
405 reduction in RuBP content as well (Tezara et al., 1999). The $V_{c,max}$ correlates with the
406 apparent activity of Rubisco *in vivo*, which will vary both with the amount of Rubisco and its
407 activation state (Long and Bernacchi, 2003). In plants acclimated to drought stress, both
408 $V_{c,max}$ (Flexas et al., 2009) and Rubisco activase (Cramer et al., 2007) have been upregulated
409 under moderate drought stress. Cramer et al. (2007) proposed that a possible explanation for
410 either an unchanged or increased value of $V_{c,max}$ seen in 'Gladius' and primed 'Paragon' under
411 moderated drought stress could be an upregulation of Rubisco activase in response to the
412 lower $[C_i]$ experienced during drought priming imposition, therefore balancing the C_i

413 reduction in the chloroplast (C_c) and compensating net carbon assimilation. Therefore, we
414 believe that the unchanged values of $V_{c,max25}$ observed in our experiment were associated with
415 increases in Rubisco activase due the tolerance trait of ‘Gladius’ and to priming in ‘Paragon’.
416 Although Rubisco content or activity was not measured in our study, the results by Abid et al.
417 (2016) and Wang et al. (2014) are in agreement with our data studies. They showed that
418 wheat plants subjected to drought priming either at tillering or jointing stages had a)
419 significant higher Rubisco content (Abid et al., 2016), and b) were able to upregulate Rubisco
420 small subunit and Rubisco activase (Wang et al., 2014) when compared to non-primed plants
421 under drought stress. The decrease in $V_{c,max25}$ observed in non-primed plants can also be
422 attribute to oxidative stress affecting Rubisco, as drought priming improved the tolerance to
423 oxidative stress by induction of antioxidant defence in wheat (Selote and Khanna-Chopra,
424 2006, Wang et al., 2014).

425 Chlorophyll fluorescence provides information about the extent to which PSII is utilising or
426 being damaged by excess light. Among the fluorescence parameters, F_v/F_m has been used
427 widely to quantify the damages in PSII during heat stress (Poudyal et al., 2018, Sharma et al.,
428 2015). The lower values of F_v/F_m found for heat stressed plants (CH and PH) are in
429 accordance with literature (Sharma et al., 2015), although it is worth to mention that the
430 lowest value (0.71) cannot be considered as extremely stressed. Non-primed ‘Paragon’ plants
431 showed a decrease in the F_q'/F_m' and ETR under heat stress (CH) and a greater heat
432 dissipation via NPQ when compared to primed plants (PH). Primed ‘Paragon’ plants (PH)
433 were able to maintain higher rates of F_q'/F_m' with lower values of NPQ. Our results indicate
434 that the primed plants (PH) of the heat susceptible ‘Paragon’ had a higher photochemical
435 efficiency than non-primed (CH) and could thus better mitigate the damages of heat stress.
436 However, no decrease in carbon assimilation due to heat stress was observed, suggesting that
437 plants in our experiment were able to regulate to the prevailing temperature (Law and Crafts-

438 Brandner, 1999). While neither carboxylation nor RuBP regeneration were altered due to
439 priming during heat stress, the upregulation of ETR in ‘Paragon’ can be linked to its dynamic
440 behaviour to NPQ decrease. Other than improvements in F_q'/F_m' due to priming, none of the
441 other results in our study suggested that drought priming enhanced heat tolerance. A previous
442 report by Wang et al. (2015) showed that drought priming could improve cross-tolerance to
443 heat stress and reduce grain loss, by sustaining higher photosynthetic rates and dissipating a
444 lower energy rates when compared to non-primed plants.

445 We found that priming alleviated photosynthetic limitations in carbon assimilation under
446 drought stress and enhanced photochemical utilization under heat stress, within the life span
447 of the susceptible cultivar ‘Paragon’. Short periods of drought stress were not cumulatively
448 damaging to the wheat cultivars studied. The type of response to priming appears to be
449 cultivar dependent, thus phenotypical variation also should be explored when studying the
450 effects of abiotic priming.

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458

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460

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607

608 **Figures legends**

609 *Figure 1: Design scheme of the experiment. Illustration of the six treatments showing 1st*
610 *priming (P1), 2nd priming (P2) and heat and drought stress at anthesis: no priming + no*
611 *stress (CC); no priming + drought stress (CD); no priming + heat stress (CH); priming + no*
612 *stress (PC); priming + drought stress (PD); priming + heat stress (PH).*

613 *Figure 2: Measured light response curves of 'Gladius' (a, b and c) and 'Paragon' (d, e and f)*
614 *in the end of the stress treatments at anthesis where the panels are non-stressed control (a*
615 *and d), drought stressed (b and e) and heat stressed (c and f) plants. The effects of the two*
616 *factors studied: priming (P) and stress (S) and their interaction are indicated in the figure.*
617 *Treatments presented are: no priming + no stress (CC); no priming + drought stress (CD);*
618 *no priming + heat stress (CH); priming + no stress (PC); priming + drought stress (PD);*
619 *priming + heat stress (PH). Different lower letters indicate significant difference at $P < 0.05$*
620 *within each cultivar for fitted values of A_{max} while ns indicate no significant difference. Data*
621 *represents mean values \pm SEM, $n = 3$.*

622

623 *Figure 3. Parameters derived from A/C_i curves for 'Gladius' (a, c, e) and 'Paragon' (b, d, f):*
624 *saturated net photosynthetic rate (A_{sat}) (a and b); maximum carboxylation of Rubisco*
625 *($V_{c,max25}$) (c and d) and electron transport demand for RuBP regeneration (J_{25}) (e and f). Heat*
626 *stressed treatments were measured at 32°C after seven days of stress at heat treatment; all*
627 *other treatments were measured at the normal growth temperature 20°C. The effects of the*
628 *two factors studied: priming (P) and stress (S) and their interaction are indicated in the*
629 *figure. Treatments presented are: no priming + no stress (CC); no priming + drought stress*
630 *(CD); no priming + heat stress (CH); priming + no stress (PC); priming + drought stress*

631 (PD); priming + heat stress (PH). A_{sat} is given at actual leaf temperature while $V_{c,max25}$, J_{25}
632 and TPU_{25} are given at standardized 25°C. . Data represents mean values +/- SEM, n = 3.

633

634 Figure 4: Chlorophyll fluorescence parameters measured at a PPFD of 900 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of
635 'Gladius' (a, c, e, g) and 'Paragon' (b, d, f, h). F_v/F_m in dark adapted leaves (a and b),
636 quantum efficiency of PSII (F_q'/F_m') (c and d), non-photochemical quenching (NPQ) (e and f)
637 and electron transport rate (ETR) based on absorbed light (g and h). The effects of the two
638 factors studied: priming (P) and stress (S) and their interaction are indicated in the figure.
639 Treatments presented are: no priming + no stress (CC); no priming + drought stress (CD);
640 no priming + heat stress (CH); priming + no stress (PC); priming + drought stress (PD);
641 priming + heat stress (PH). Different lower letters indicate significant difference at $P < 0.05$,
642 ns indicate no significance between treatments within each cultivar. Data represents mean
643 values \pm SEM, n = 3.

644 Figure 5. Effects of drought priming during vegetative growth stages on grain yield
645 parameters of 'Gladius' (a, c, e, g) and 'Paragon' (b, d, f, h): total grain number (a and b),
646 total grain yield (c and d), yield of primary ear (e and f) and thousand-kernel weight (TKW)
647 (g and h) of wheat plants exposed to heat or drought stress during anthesis. The effects of the
648 two factors studied: priming (P) and stress (S) and their interaction are indicated in the
649 figure. Treatments presented are: no priming + no stress (CC); no priming + drought stress
650 (CD); no priming + heat stress (CH); priming + no stress (PC); priming + drought stress
651 (PD); priming + heat stress (PH). Different lower letters indicate significant difference at
652 $P < 0.05$ between treatments for each cultivar individually. Data represents mean values +/-
653 SEM, n = 4.

654