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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.

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4 **Short title:** Priming mitigates stress

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24

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 CO_2 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

43

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

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

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

The response to drought stress in crops will vary from partial stomatal closure under moderate stress to desiccation and plant death at the wilting point. In wheat, a gradual decline in stress sensitivity to drought is expected as grains develop (Saini and Westgate, 1999). 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 CO_2 assimilation under drought stress can be attributed to direct factors such as

76   a reduction in the CO_2 diffusion through the leaf mesophyll and consequently the CO_2 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 reprograming 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 CO_2,

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 $50.4 \pm 13.1\%$.

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 µmol m^{-2} 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 ±2.3˚C; RH%

177   59.0 ± 11.4 and PPFD of 350 µmol m^{-2} 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 CO_2 (A/Ci) responses were measured *in vivo* on

184   leaves using a portable gas exchange fluorescence system (Walz GFS-3000, Walz, Eiffeltrich

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 cm^2 leaf cuvette with PPFD set at 500 µmol m^{-2} s^{-1}, flow rate of 750 mL

190 min^{-1} and reference [CO_2] was set at 407 ppm (equal to the ambient CO_2 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 [CO_2] 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 $\Delta T = T_{leaf} - T_{cuvette}$, 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 [CO_2] were plotted against intracellular CO_2

208 concentration (C_i) to obtain a response curve. The biochemical parameters were normalized

209 to 25°C for comparation. Photosynthetic parameter limitations: maximum carboxylation

210 velocity of Rubisco ($V_{c,max25}$), electron transport demand for RuBP regeneration (J_{25}), day

211 respiration (R_{d25}), mesophyll conductance (g_{m25}) and the rate of use of triose phosphate

212 (TPU$_{25}$) 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 CO_2 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 μmol m^{-2} 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 $\alpha = 1 - \rho - \tau$

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)] × 100.*

245

246 *Statistical analyses*

247 All of the results are reported as mean ± 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 agricolea. 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 CO_2 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 CO_2

280   (Ci) 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 CO_2 (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 CO_2 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   paramenter 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   imposion (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

Discussion

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

The capability to sustain grain yield under abiotic stress is regarded as a characteristic of tolerance in crops. The photosynthetic performance determines the concurrent photosynthates available to the plant. Any constrain in photosynthesis can limit yield and biomass accumulation (Fischer et al., 1998). Our investigation showed that priming upregulates CO_2 assimilation (A_{max}) under drought stress, suggesting that the priming plants would had better grain-filling substrate supply to early developing grains, which was expected to be translated on to yield improvements. However, our yield results could not show yield improvments in either the studied cultivars due to priming. Drought reduction in yield during early grain initiation is reported to be due to a smaller sink size of initiated grains (Saini and Westgate, 1999) and the correlation between yield components such as the increase of TKW in spikes with reduced grain number is a key adaptive mechanism for restoring yield losses inflicted by stress in plants (Blum, 1996). 'Gladius' yield reduction under drought stress is attributed to a reduction in TKW, while 'Paragon' yield loss is shown to be explained by a reduction of grain number. Again, priming presented no significant effect on the ability to reallocate photo assimilates to grain filling (TKW) or prevent grain abortion on the studied cultivars, suggesting that the cultivars studied were not able to maintain carbohydrate accumulation in the reproductive organs throughout the drought stress treatment, even when assimilation was 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 CO_2 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 CO_2

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 CO_2

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 CO_2 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 [Ci] experienced during drought priming imposition, therefore balancing the Ci

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.

458

459 **Conflict of Interest**: The authors declare that they have no conflict of interest.

460

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607

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 ± 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 25ºC. . Data represents mean values +/- SEM, n = 3.*

633

634   *Figure 4: Chlorophyll fluorescence parameters measured at a PPFD of 900 µmol m^{-2} 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 ± 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