

Metabolic and film antitranspirants both reduce drought damage to wheat yield despite having contrasting effects on leaf ABA

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1 i. **Title:**

2 **Metabolic and film antitranspirants both reduce drought damage to wheat yield**
3 **despite having contrasting effects on leaf ABA**

4 ii. **Running title**

5 Drought mitigation with antitranspirants

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15 v. **Abstract and Keywords**

16 Abstract

17 Film antitranspirants (e.g. di-1-*p*-menthene) and metabolic antitranspirants (e.g.
18 exogenous ABA) can be used to protect grain crops from drought, particularly during
19 reproductive development. Here, we compared effects of di-1-*p*-menthene (1.0 L/ha)
20 and exogenous ABA (100 µM) on well-watered and droughted spring wheat in two
21 glasshouse experiments. Progressive drought was imposed in the first experiment,
22 controlled drought in the second, both from flag leaf emergence. Antitranspirants

23 were applied at flag leaf emergence, except in the controlled drought experiment
24 additional ABA applications were made at four further stages up to anthesis-
25 complete. Measurements were taken for endogenous ABA, stomatal conductance
26 and photosynthesis during reproductive development, and yield components at
27 maturity. Both progressive and controlled drought elevated leaf ABA, less so in di-1-
28 *p*-menthene-treated plants, whereas in exogenous ABA-treated plants leaf ABA was
29 elevated further. Overall, both antitranspirants improved yield under reproductive-
30 stage drought, more so for controlled drought. Grain yield was negatively associated
31 with reproductive-stage leaf ABA concentration except that raising leaf ABA
32 concentration with multiple exogenous ABA applications was associated with high
33 yield. In conclusion, both antitranspirants generally reduced effects of reproductive-
34 stage drought on yield despite having contrary effects on leaf ABA.

35 Keywords: abiotic stress, drought amelioration, drought mitigation, water deficit,
36 water stress.

37 vi. **Main Text**

38 1. Introduction

39

40 Abiotic stresses depress crop productivity to levels below their genetic potential (Fita,
41 Rodríguez-Burruezo, Boscaiu, Prohens, & Vicente, 2015). According to the
42 Intergovernmental Panel on Climate Change (IPCC, 2018), drought will increase due
43 to climate change. This poses a threat to world food security, especially in view of
44 the rapidly increasing world population, projected to reach 9.8 billion by 2050 (United
45 Nations, 2017). Wheat, one of the three most important cereals, accounts for about
46 44% of global trade (FAO, 2019) and 41% of calories from cereals (Khadka, Earl,

47 Raizada, & Navabi, 2020). Climate adaptation technologies are needed to improve
48 its production to help meet the global food security challenge. One such technique is
49 the use of antitranspirants (ATs) - products sprayed on plant leaves to reduce
50 transpiration (Iriti et al., 2009). Among the ATs are di-1-*p*-menthene (DpM), a film-
51 forming AT, and exogenous ABA (Exo-ABA), which is metabolic. Film-forming ATs,
52 reduce transpiration by covering stomata with a film of low permeability to water
53 vapour, while the metabolic type induce partial stomatal closure by acting on guard
54 cells (Mphande et al. 2020). Both ATs are being studied as possible tools for drought
55 amelioration. The use of ATs, i.e., reduction of transpiration, has the potential to
56 raise leaf temperature, which is one of the known non-target effects (Mphande et al.,
57 2020). This might aggravate drought impacts if it induces heat stress. A review by
58 Mphande et al. (2020) provides details on commercial ATs, how they reduce
59 transpiration and the potential effects on leaf temperature.

60 Drought, often accompanied with heat stress (Chaves et al., 2016; Lipiec, Doussan,
61 Nosalewicz, & Kondracka, 2013), limits crop productivity by supressing gas
62 exchange and damaging photosynthetic pigments and inactivating photosystem II
63 (Anjum et al., 2011; Batra et al., 2014). More than 90% of the water a plant absorbs
64 is lost through transpiration (Morison, Baker, Mullineaux, & Davies, 2008). As soil
65 water availability is limited during drought, reducing transpiration improves the plant
66 water status hence maintaining higher biological function within the cells (Farooq,
67 Wahid, Kobayashi, Fujita, & Basra, 2009). However, with the possibility of an
68 increase in temperature, improved biological activity is not to be expected where the
69 risk of heat stress is high. Drought elevates endogenous abscisic acid (endo-ABA)
70 concentration (Dayer et al., 2020; Norton, Xian, Kalic, Hughes, & Gubler, 2020) to
71 reduce transpiration via stomatal closure (Lee & Luan, 2012). Although endo-ABA

72 reduces stomatal transpiration, it may also lead to reproductive organ abortion (Pang
73 et al., 2017). ATs are an agronomic approach to reducing transpiration.

74 ATs also reduce photosynthesis, but benefits outweigh this detrimental effect by
75 improve yield under drought conditions if applied just before the most sensitive crop
76 growth stage (GS). Examples include Kettlewell and Holloway (2010) who found that
77 DpM applied at the boots swollen stage improved yield of droughted winter wheat by
78 as much as 42%; and Faralli et al. (2017) who found that DpM applied at the
79 initiation of flowering improved yield in droughted oil seed rape by 12%. Mphande, et
80 al. (2020) observed that DpM applied at the stem elongation stage in wheat resulted
81 in a 29% yield improvement, which was associated with reduced endo-ABA
82 concentration. Nevertheless, these yield improvements have not been seen in every
83 case and the mechanism of AT drought amelioration has not yet been established.
84 ATs may have positive effects on yield-influencing physiology due to reduced
85 transpiration. Understanding the mechanisms is likely to play a crucial role in
86 maximising AT potential and developing plant breeding traits for enhancing crop
87 tolerance to drought.

88 Therefore, glasshouse experiments were conducted to assess the impact of DpM on
89 gas exchange, endo-ABA concentration, yield and yield components in spring wheat
90 under both well-watered and drought conditions, and to compare these effects to
91 similar applications of Exo-ABA. Our hypothesis is that although both antitranspirants
92 are expected to reduce gas exchange, applying DpM maintains yield under drought,
93 in part by reducing leaf ABA; while applying Exo-ABA is less effective at maintaining
94 yield under drought as leaf ABA is elevated.

95 **2. Materials and Methods**

96

97 2.1 Experimental site description and design

98 Glasshouse experiments were conducted at Harper Adams University, UK (52°46'N,
99 2°25'W) in 2018 and 2019. The light in the glasshouse was set to 16 hrs per day.

100 Photosynthetically active radiation (PAR) was supplied using Osram sodium vapour
101 tubes of the model Vialox NAV-T 400 (with an output of 48, 800 lumens and colour
102 temperature of 2000k, the photosynthetic photon flux was 633 $\mu\text{mol/s}$ per tube
103 ([https://www.waveformlighting.com/horticulture/convert-lumens-to-ppf-online-](https://www.waveformlighting.com/horticulture/convert-lumens-to-ppf-online-calculator)

104 [calculator](https://www.waveformlighting.com/horticulture/convert-lumens-to-ppf-online-calculator)). The experiments were arranged in a randomised complete block design.

105 There were six treatments per block made up of two levels of irrigation, well-watered
106 (WW) and drought and three levels of spray type (unsprayed control, DpM and Exo-
107 ABA); making it a 2x3 factorial design.

108 2.2 Agronomy

109 Growth medium: In 2018, 5 litre pots were filled with John Innes No. 2 Compost
110 (William Sinclair Horticulture Ltd., UK). Its pot capacity (PC) and permanent wilting
111 point (PWP) expressed in volumetric water content (VWC) terms were determined by
112 Saeed (2008) as 50.33% and 7.00%, respectively. Therefore, its plant available
113 water (PAW) is $50.3 - 7.0 = 43.3\%$ VWC. In 2019, a mixture of air-dry sandy loam
114 soil and moist John Innes No. 2 Compost was used in a ratio of 9:1 by weight, to
115 allow for quicker soil drying. The PC and PWP of the mixture were determined using
116 the pressure plate method as 14.3% and 6.7%, respectively and hence the PAW
117 was 7.6%.

118 Fertilizer application: In 2018, we applied no additional fertilizer to John Innes no.2
119 Compost but in 2019, we added Chempak Formula 2 (NPK: 25:15:15; Chempak
120 Products, Hoddesdon, UK) by dissolving 30g of product in 10 litres of water and
121 applying 200ml of the nutrient solution per pot twice, at stem extension and anthesis
122 stages.

123 Planting: Thirteen seeds of the spring wheat variety, Chilham (KWS UK Ltd), were
124 planted in each pot in 2018. After establishment, thinning was done at GS24 to leave
125 11 plants per pot. In 2019, plants were thinned to seven and with tiller removal to
126 ensure that all measurements were based on main stems only, unlike in 2018.

127 Pest control: The common pests in the glasshouse were aphids and powdery
128 mildew. In 2018, aphids were controlled with Gazelle SG (a.s. 20% w/w acetamiprid)
129 at a rate of 250 g/ha while powdery mildew was managed with Eclipse at a
130 concentration of 250 g/litre (24.5% w/w) fenpropimorph and 84 g/litre (8.2% w/w)
131 epoxiconazole. In 2019, Rose Clear™ Ultra Gun 2, an insecticide-fungicide emulsion
132 mixer of 0.05 g/L cypermethrin and 0.075 g/L myclobutanil, was used for both pests.

133 Irrigation: Irrigation management was based on the mathematical relationship
134 described by Bellingham (2009): $L[U]SMT = VWC_{PC} - (VWC_{PC} - VWC_{PWP}) \times (MAD)$,
135 where L[U]SMT and MAD stand for lower (or upper) soil moisture target and
136 maximum allowable depletion. With the MAD of 30% for wheat (Hunsaker et al.,
137 2005, higher values may be used e.g. 50%, Bellingham, 2009), the LSMT for the
138 WW pots was 37.0% and 12.0% in 2018 and 2019, respectively. WW pots were
139 irrigated to PC twice a week, on Tuesdays and Fridays, to maintain the moisture
140 content above the LSMT. In the drought treatment, plants were droughted for part of
141 the growth cycle after which normal irrigation was restored. In 2018, drought was

142 progressive (i.e. progressive drought, PD) for 17 days, from GS37 to GS45. In 2019,
 143 it was controlled (i.e. controlled drought, CD), with partial irrigation used to sustain
 144 plants for a longer period of 50 days from GS37 to GS70. The upper and lower
 145 targets for the CD pots was 10.5% and approximately 9.0%, with the MADs of 50%
 146 and 70% (Hafez & Farig, 2019), respectively. Approximately 88 ml and 150 ml of
 147 water was applied on Tuesdays and Fridays, respectively, to maintain the soil
 148 moisture between 9% and 10.5% (VWC).

149 2.3 Spray application

150 Exogenous (+/-)- ABA (A1049; Sigmal Aldrich – UK) weighing 52.864 mg and
 151 dissolved in methanol (5 ml) and made up to 2.0 L with distilled water to obtain a
 152 concentration of 100 μ M (5.3 g/ha); and 10 ml DpM (96%, Miller Chemical and
 153 Fertilizer Corp., USA) shaken in 2.0 L tap water (1.0 L/ha) were applied using a
 154 hand-held boom sprayer (Lunch Box Sprayer, Trials Equipment (UK) Ltd). In each
 155 year, there was one DpM application made when the flag leaf was fully emerged
 156 (GS39, Zadoks, Chang, & Konzak, 1974). Under PD, a single Exo-ABA spray was
 157 applied at GS39. Under prolonged CD, there were multiple applications of Exo-ABA,
 158 once every week for five weeks between GS39 and GS70. The third set of plants not
 159 sprayed with any substance served as the unsprayed control.

160 The dates on which the spray products were applied are listed below (Table 1),
 161 alongside ABA sampling dates.

162 Table 1: Spray application and ABA sampling schedule.

Experiment	Spraying				Leaf sampling			Spike sampling	
	GS*	Spray types	DAP*	Date	GS	DAP	Date	DAP	Date
PD (2018)	GS39	DpM*	63	12 th Oct	GS42	68	17 th Oct		N/A

	GS39	Exo-ABA	63	12 th Oct	GS42	68	17 th Oct		N/A
	GS39	DpM	59	16 th Jan	GS62	80	6 th Feb	80	6 th Feb
	GS39	Exo-ABA	59	16 th Jan	GS62	80	6 th Feb	80	6 th Feb
CD (2019)	GS43	Exo-ABA	65	22 nd Jan					
	GS54	Exo-ABA	72	29 th Jan					
	GS62	Exo-ABA	80	6 th Feb					
	GS69	Exo-ABA	87	13 th Feb					

163 * DAP, DpM, Exo-ABA and GS stand for days after planting, di-1-*p*-menthene,
 164 exogenous ABA and growth stage, respectively.

165

166 Measurements

167 Temperature and relative humidity (RH) were monitored and regulated by the
 168 TomTech T200 logger (Tomtech (UK) Ltd, Spalding, UK).

169 Soil moisture: Measurement of soil moisture was done using the HD2 mobile reading
 170 device connected to the TRIME-PICO 64 two-rod soil moisture probe (IMKO
 171 Micromodultechnik GmbH, Ettlingen, Germany). Readings were recorded as %VWC.

172 Leaf and spike ABA: Five fully expanded flag leaves were collected from each pot
 173 into 5 ml plastic tubes and immediately flash frozen in liquid nitrogen and stored at -
 174 80°C for ABA assay. Sampling was done at five days after spraying (plants at GS42)
 175 under PD and at 21 days after spraying (plants at GS62) under CD. Under CD,
 176 spikes were also sampled. To perform an ABA assay, the frozen leaf and spike
 177 samples were transferred to an Edwards Modulyo 4K freeze drier (Akribis Scientific
 178 Ltd, UK) and dried for two days, after which they were ground and assayed
 179 according to the Cusabio ABA ELISA protocol, (Cusabio Biotechnology Co.,
 180 Ltd, Wuhan, China). The ABA standard used in the assay was a mixture of isomeric
 181 forms ((+/-) cis-Abscisic acid). Absorbance readings were taken at 450 nm using a

182 BioTek spectrophotometer (BioTek Instruments Ltd, UK). The leaf and spike ABA
183 concentrations in the sample extracts were calculated after a natural logarithmic
184 standard curve was fitted.

185 Infrared gas analysis: Stomatal conductance (g_s), net photosynthesis (A) and leaf
186 temperature were determined with a portable open-system infrared gas analyser
187 (IRGA) - LCpro-SD (ADC BioScientific Ltd, UK). Light (PAR) was set to 250 and
188 $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ in 2018 and 2019, respectively, and CO_2 to $400 \mu\text{mol mol}^{-1}$.
189 Measurements were taken between 10:00 hrs and 14:00 hrs and recorded when the
190 system was in a steady state (~ five minutes). Measurements were taken at five days
191 after spraying in 2018, and one day after spraying in 2019. Intrinsic water use
192 efficiency (WUE_i) was calculated as a ratio of net photosynthesis, P_N , to stomatal
193 conductance (Sikder et al., 2015).

194 Yield and yield components: Spikes from each pot were hand-harvested, threshed,
195 cleaned, weighed and oven dried at 105°C for 40 h. The number of grains per spike
196 was determined by counting the fertile spikes and grains per pot. A spike was
197 counted as fertile if it was bearing at least one grain. In 2018, 11 main stem and
198 between seven and 28 tiller spikes were harvested and mixed. In 2019 there were
199 only seven main spikes per pot, as tillers were trimmed. Thousand grain weight
200 (TGW) was determined by the method described by (Sylvester-Bradley et al., 1985).
201 Finally, grain yield per spike was obtained by weighing all the grain harvested from
202 each pot and dividing that weight by the number of fertile spikes. Both TGW and
203 grain yield were expressed at 15% moisture content. Shoot biomass was determined
204 at harvest by weighing above-ground plant parts, oven-dried at 105°C , and harvest
205 index (HI) calculated as a ratio of grain weight to shoot biomass (Huhn, 1991).

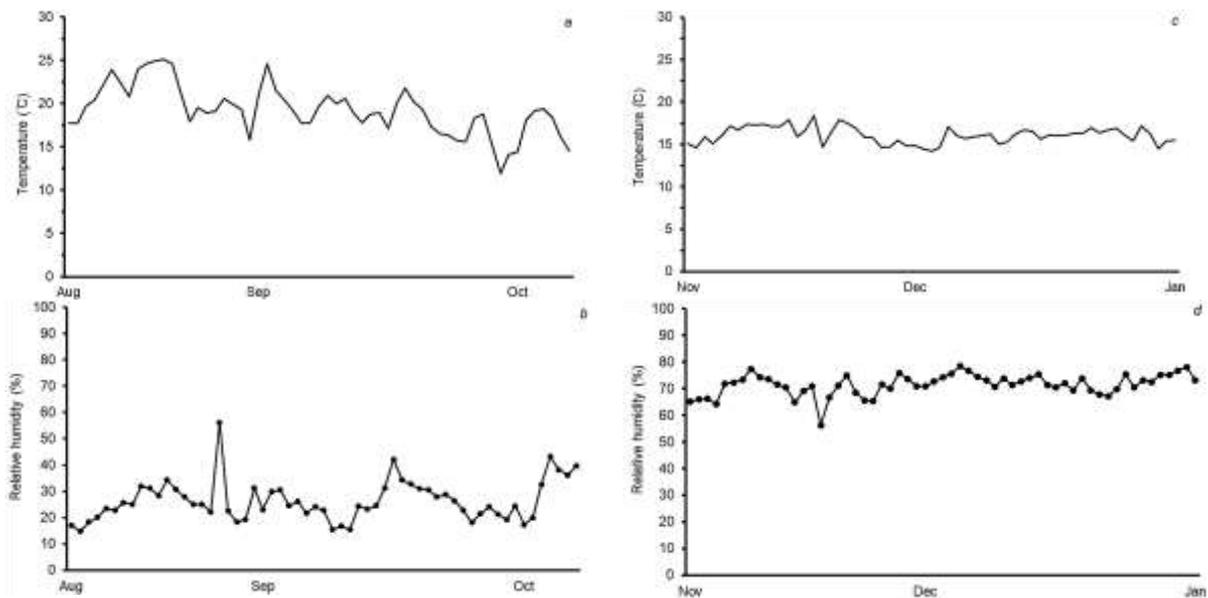
206 Statistical analysis: A two-factor analyses of variance (ANOVA) of gas exchange
207 variables, leaf and spike ABA concentration and yield and yield components were
208 performed using GenStat 20th Edition (Hemel Hempstead, UK). The Tukey test (at
209 5%) was used for *post hoc* analyses. Relationships between ABA and yield
210 components were analysed using simple linear regression. Curves were fitted and
211 modelling done using Microsoft Excel, but with parameters generated from GenStat.

212 3. Results

213 3.1 Temperature and relative humidity

214 The average, minimum and maximum daily temperature and RH inside the
215 glasshouse for 2018 and 2019 were 19.3 °C, 11.9 °C and 25.1 °C; and 26.4 %, 14.8
216 % and 56.1%; and 15.9 °C, 14.2 °C and 18.4 °C; and 71.5%, 56.3 %, 78.4%,
217 respectively. Thus, the average temperature for 2018 was significantly ($p < 0.001$)
218 higher than for 2019 while the opposite was true for RH and equally significant ($p <$
219 0.001) as the values above indicate (and Figure 1).

220



221

222 Figure 1: Average daily air temperature (a and c) and average daily relative
223 humidity (b and d) inside the glasshouse rain shelters recorded by the TomTech
224 T200 data logger (Tomtech (UK) Ltd, Spalding, UK).

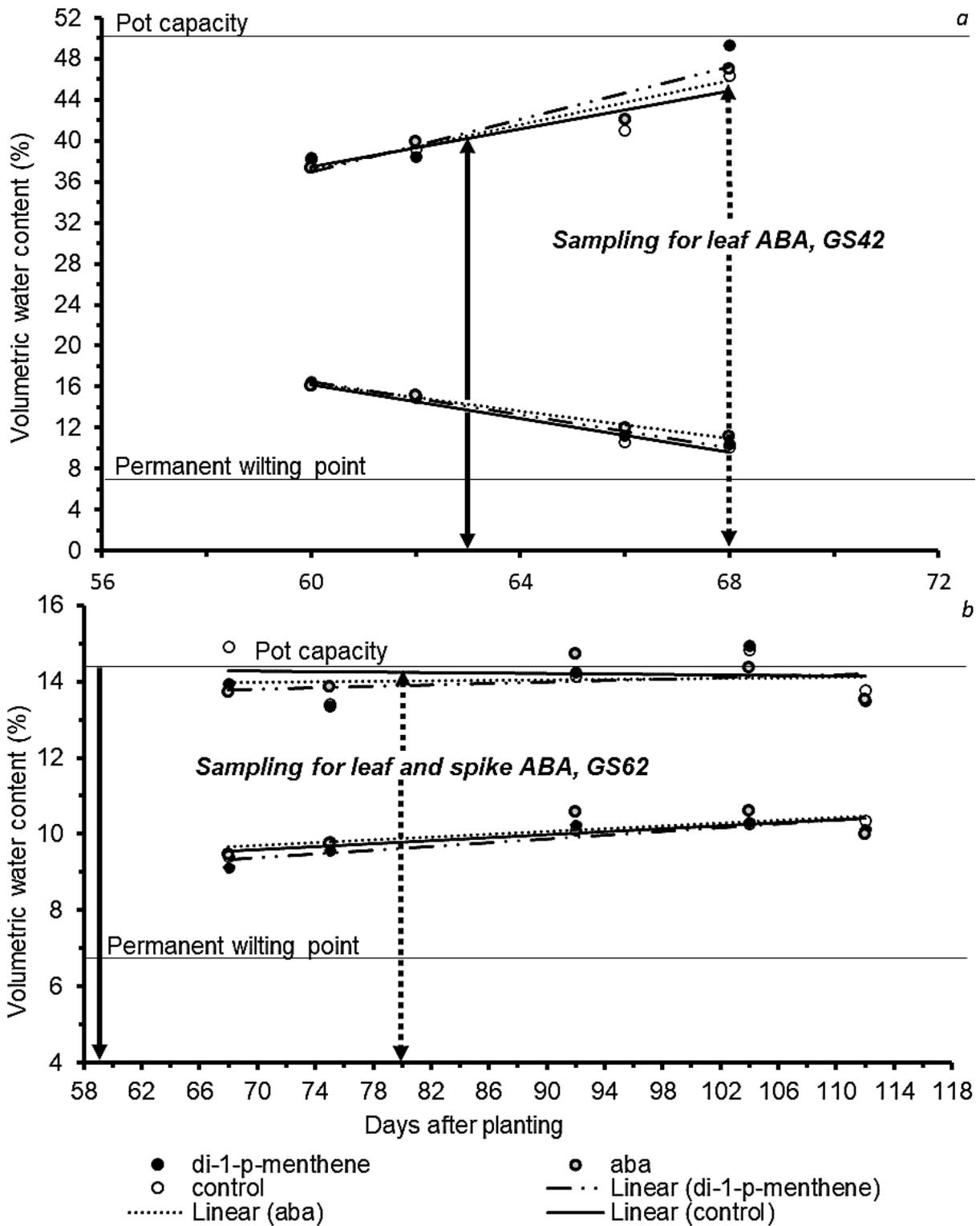
225

226 3.2 Soil moisture

227 Under PD (2018), the average fraction of PAW decreased from 22% at 60 DAP to
228 8% at 68 DAP (Figure 2), when watering was resumed. Under CD (2019), the
229 average proportion of remaining PAW fluctuated but measurements taken between
230 68 and 112 DAP ranged from 35% to 46%. WW plants were kept above their LSMT
231 of 37% and 12% (VWC) in 2018 and 2019, respectively.

232

233



234

235 Figure 2: Soil moisture readings from (a) progressive drought (PD) and (b)

236 controlled drought (CD) wheat plants between 60 and 68 days after planting (DAP)

237 and between 68 and 112 DAP, respectively. Note that the lower pot capacity for CD

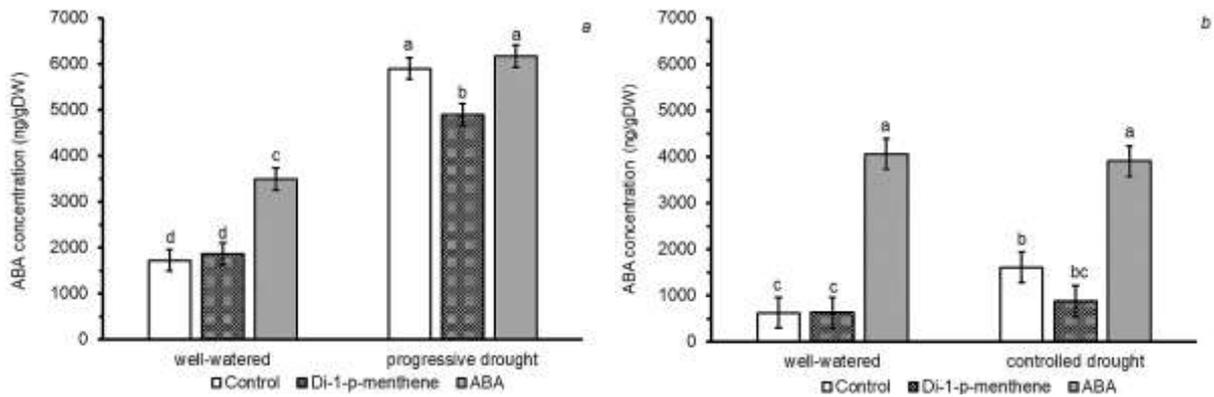
238 was due to a predominantly sandy growth medium instead of John Innes No. 2
239 Compost for PD. Undotted arrows indicate DAP on which the antitranspirants were
240 sprayed.

241

242 3.3 ABA concentration

243 The leaf and spike BA concentrations (ng/g DW) under PD were much higher than
244 under CD, reflective of the respective more severe and milder drought. Due to
245 drought, the ABA concentrations in flag leaves significantly increased above WW
246 plants by 243% and 159% under PD and CD, respectively, while in the spikes it rose
247 by 184% (determined under CD only). The effect of DpM on endo-ABA concentration
248 was consistent in the two experiments, but it varied for Exo-ABA. Application of DpM
249 reduced the endo-ABA concentration in droughted plants to the lowest values at five
250 days after spraying (Figure 3). Under drought conditions, Exo-ABA had no significant
251 effect on elevating endo-ABA at five days after spraying (PD). The only sampling for
252 which Exo-ABA significantly elevated leaf ABA in droughted plants was at one day
253 after the fourth spraying under CD (Figure 3 b). Under WW conditions, the effect of
254 DpM was negligible. In contrast, Exo-ABA significantly increased the leaf ABA
255 concentration in WW plants in both experiments, regardless of time lapse after
256 spraying. The effect was so large that the concentration rose to similar levels as
257 under droughted conditions at 80 DAP. However, at 68 DAP, it was significantly
258 lower than in the droughted plants but remained significantly higher than in the WW
259 unsprayed control and DpM-treated plants.

260



261

262 Figure 3: Flag leaf ABA levels under (a) progressive drought (PD) and (b) controlled
 263 drought (CD) wheat plants sampled at 68 days after planting (DAP i.e. five days
 264 after spraying, DAS, $p = 0.008$); and 80 DAP (i.e. 21 DAS DpM and one day after a
 265 fourth spray of Exo-ABA, $p = 0.015$), respectively. Different letters represent
 266 significant differences (Tukey test, $p < 0.05$). Error bars represent common standard
 267 errors of means from ANOVA table.

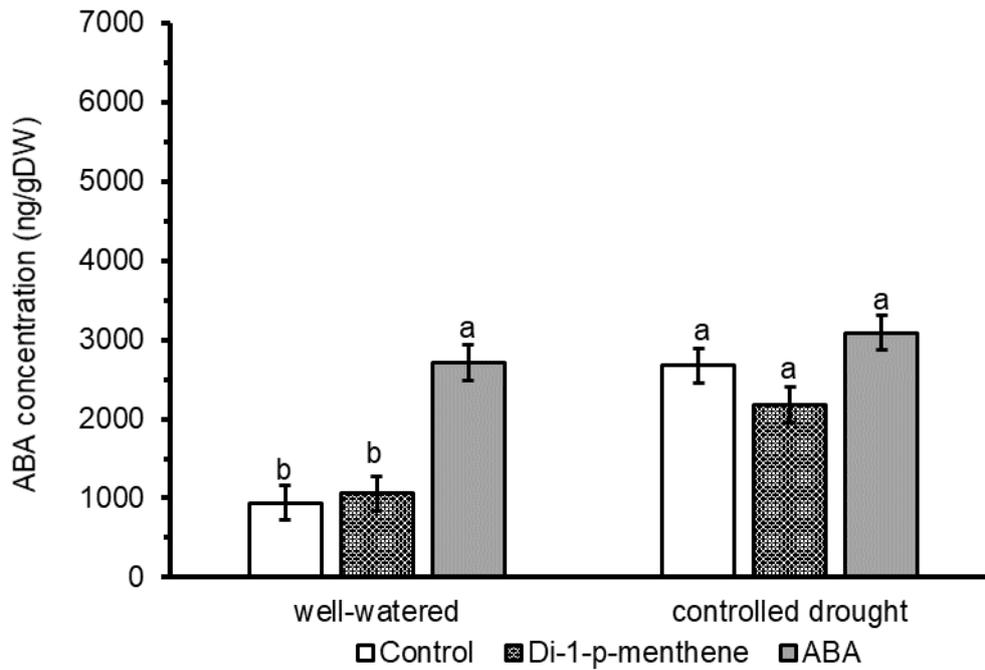
268

269

270 Spike ABA response to spray type treatments followed a similar pattern to foliar
 271 ABA, with DpM reducing the concentration to the least values and Exo-ABA
 272 elevating it (Figure 4). However, the effect of DpM was not significant at both levels
 273 of irrigation, while Exo-ABA significantly increased the leaf ABA only under WW
 274 conditions. Notice that the concentration of ABA in spikes was higher than in leaves
 275 of the unsprayed control and DpM-treated counterpart plants.

276

277



278

279 Figure 4: Spike ABA under controlled drought (CD) wheat plants ($p = 0.025$)
 280 sampled at 80 days after planting, i.e. 21 days after spraying di-1-*p*-menthene and
 281 one day after a fourth exogenous ABA spray under controlled drought. Different
 282 letters represent significant difference (Tukey test, $p < 0.05$). Errors bars represent
 283 common standard errors of means from ANOVA table.

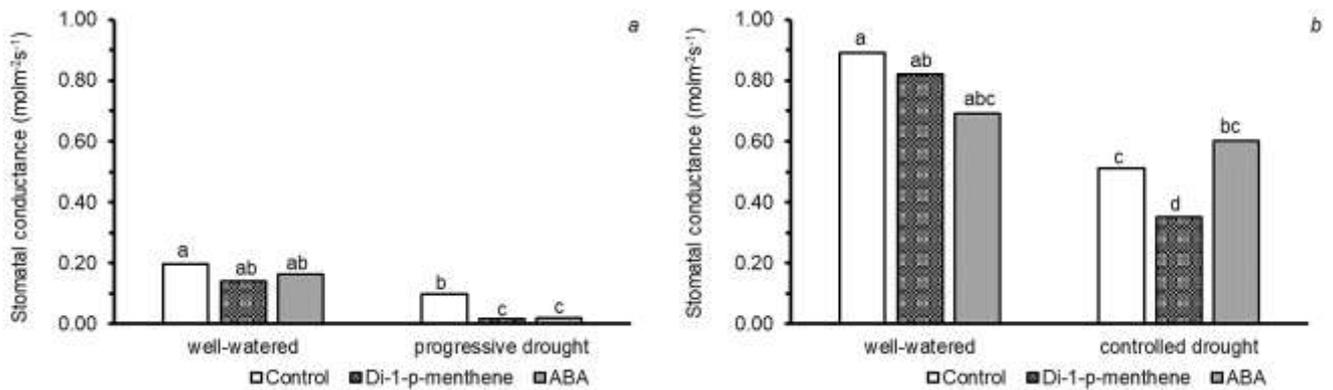
284

285 3.4 Gas exchange

286 Drought suppressed gas exchange both under PD and CD. Compared to the WW
 287 unsprayed control, stomatal conductance (Figure 5) under the PD treatment for
 288 unsprayed, DpM and Exo-ABA decreased significantly ($p = 0.025$) by 51%, 92% and
 289 90%, respectively. Under CD (2019), which was mild, the reductions ($p < 0.001$)
 290 corresponded to 43%, 61% and 33%. Compared to the unsprayed control, Exo-ABA
 291 significantly reduced stomatal conductance under PD but surprisingly induced a
 292 nonsignificant increase under mild CD. DpM consistently and significantly reduced

293 stomatal conductance. Under WW conditions, DpM and Exo-ABA negligibly reduced
 294 stomatal conductance in both years.

295



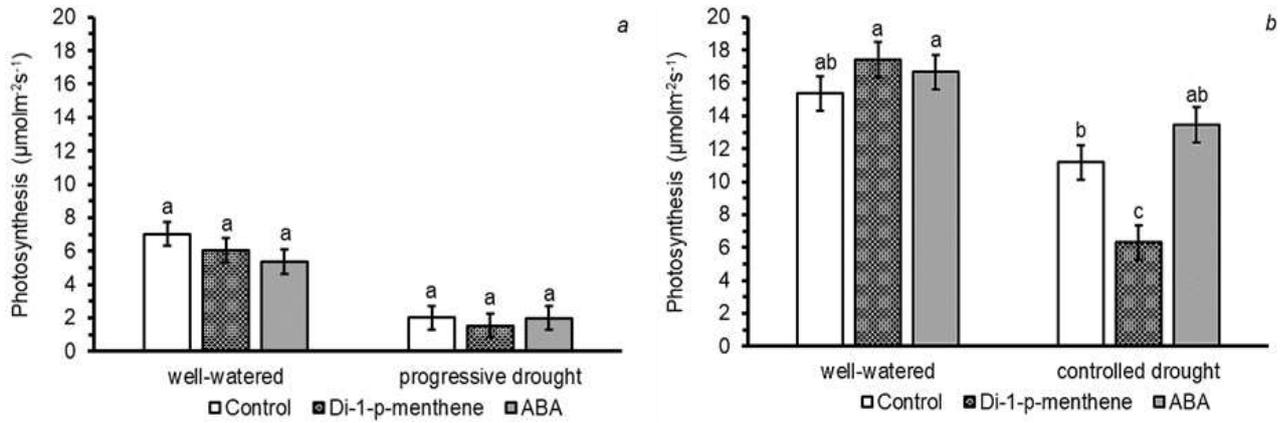
296 Figure 5: Stomatal conductance under (a) progressive drought (PD) and (b)
 297 controlled drought (CD) wheat plants measured five days after spraying (DAS, $p =$
 298 0.025) and one DAS ($p < 0.001$), respectively. The photosynthetically active radiation
 299 in 2018 was set at $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ in 2019. Different letters
 300 represent significant differences (Tukey test, $p < 0.05$). The graphs were constructed
 301 from back transformed values. The transformed means and their standard errors
 302 (SEM) in the order: well-watered and droughted (control, di-1-p-menthene and
 303 exogenous ABA, SEM); were: under PD (square root scale: 0.4434, 0.3759, 0.402 and
 304 0.3108, 0.1244, 0.1382, SEM: 0.02189) and CD (log 10 scale: -0.118, -0.195 and -0.367
 305 and -0.679, -1.043 and -0.514, SEM: 0.0739), respectively.

306

307 Photosynthesis values for PD (2018) were much lower than for CD (2019, where a
 308 higher light level was used during measurements (Figure 6). The effects of DpM and
 309 Exo-ABA on photosynthesis for PD were not significant at either water level. Under
 310 the mild CD and high level of light during measurements (PAR of $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$).

311 DpM significantly suppressed photosynthesis by 44% while Exo-ABA had no
 312 significant effect (Figure 6, b).

313



314

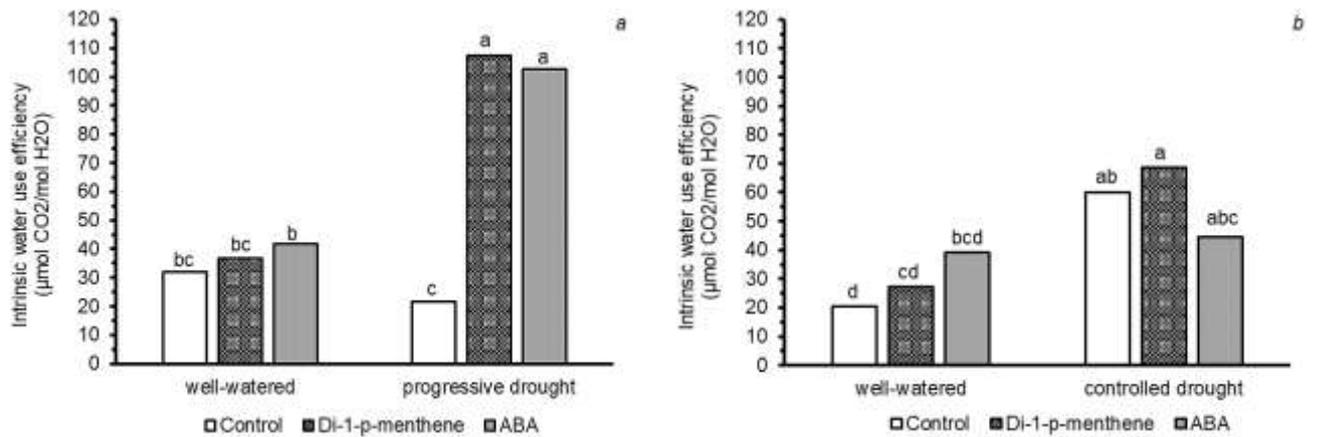
315 Figure 6: Net photosynthesis under (a) progressive drought (PD) and (b) controlled
 316 drought (CD) wheat plants measured five days after spraying (DAS) at
 317 photosynthetically active radiation (PAR) set at 250 µmol m⁻² s⁻¹ (p = 0.488) and one
 318 DAS at PAR of 1000 µmol m⁻² s⁻¹ (p = 0.004). Different letters represent significant
 319 differences (Tukey test, P < 0.05) while error bars are standard errors of means from
 320 ANOVA table.

321

322 Generally, both drought and ATs increased WUE_i (Figure 7). Under the severe PD,
 323 the ATs significantly increased the WUE_i to much higher levels than under CD.
 324 Under the milder CD, all plants had significantly higher WUE_i compared to
 325 unsprayed WW plants, although there were no significant differences for CD plants
 326 treated with ATs.

327

328

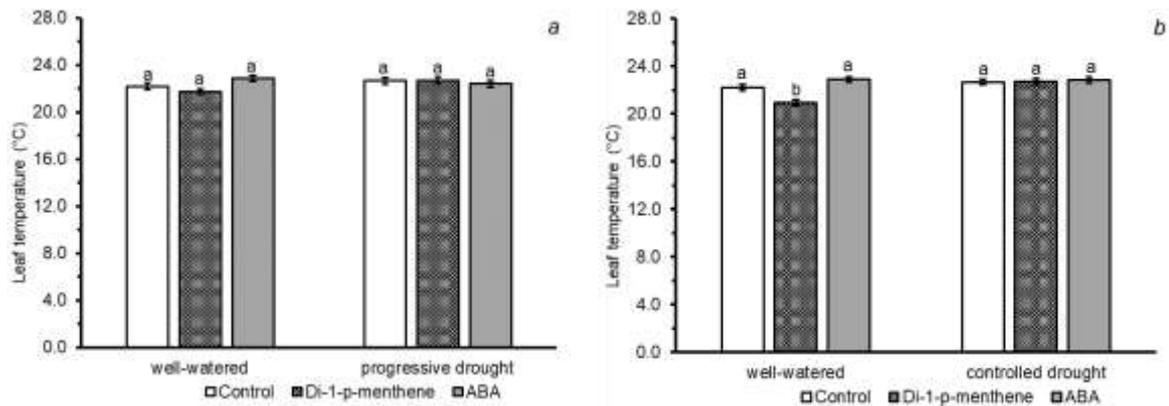


329 Figure 7: Intrinsic water use efficiency under (a) progressive drought (PD) and (b)
 330 controlled drought (CD) wheat plants determined on flag leaves ($p < 0.001$) and ($p =$
 331 0.007), respectively. Error bars represent standard error of means (SEM). Different
 332 letters represent significant difference (Tukey test, $p < 0.05$); graphs were constructed
 333 from back transformed values. The transformed means and their SEM in the order:
 334 well-watered and droughted (control, di-1-p-menthene and Exo-ABA, SEM); were:
 335 under PD (log base 10 scale: 1.50, 1.57, 1.62 and 1.34, 2.03, 2.01, SEM: 0.50) and under
 336 CD (square root scale: 4.51, 5.21, 6.25 and 7.75, 8.29, 6.68, SEM: 0.589), respectively.

337

338 3.5 Leaf temperature

339 Overall, PD had no effect on leaf temperature but CD significantly increased it by
 340 0.7°C , above well-watered plants. In 2018, the ATs had no significant effect on leaf
 341 temperature regardless of water treatment (Figure 8, a). However, in 2019, DpM
 342 significantly reduced leaf temperature compared to all other treatments (Figure 8, b).
 343 Although DpM did not significantly reduce leaf temperature under well-watered
 344 conditions in 2018, the tendency of the AT to suppress the variable is apparent in
 345 both years.



346

347 Figure 8: Water treatment and antitranspirant (AT) interaction effects on flag leaf

348 temperature, showing the tendency of di-1-p-menthene to reduce the variable under

349 well-watered conditions with non-significant effect (a, $p = 0.069$) and significant

350 effect (b, $p = 0.007$). The ATs had non-significant effects under both progressive

351 drought (PD) and controlled drought (CD). Error bars represent common standard

352 errors of means from ANOVA table. Different letters represent significant difference

353 (Tukey test, $p < 0.05$).

354

355 3.6 Yield and yield components

356 Drought generally reduced most yield components and significant differences were

357 observed but not in all cases (Figures 9 and 10). Under the more severe PD, with

358 tillers untrimmed, the number of fertile spikes per pot, grains per spike, grain weight,

359 shoot biomass per plant and HI were all reduced compared to WW plants, but there

360 were no significant differences (main stem and tiller spikes were mixed). Under the

361 CD treatment with the plants trimmed to seven main stems only, there were no

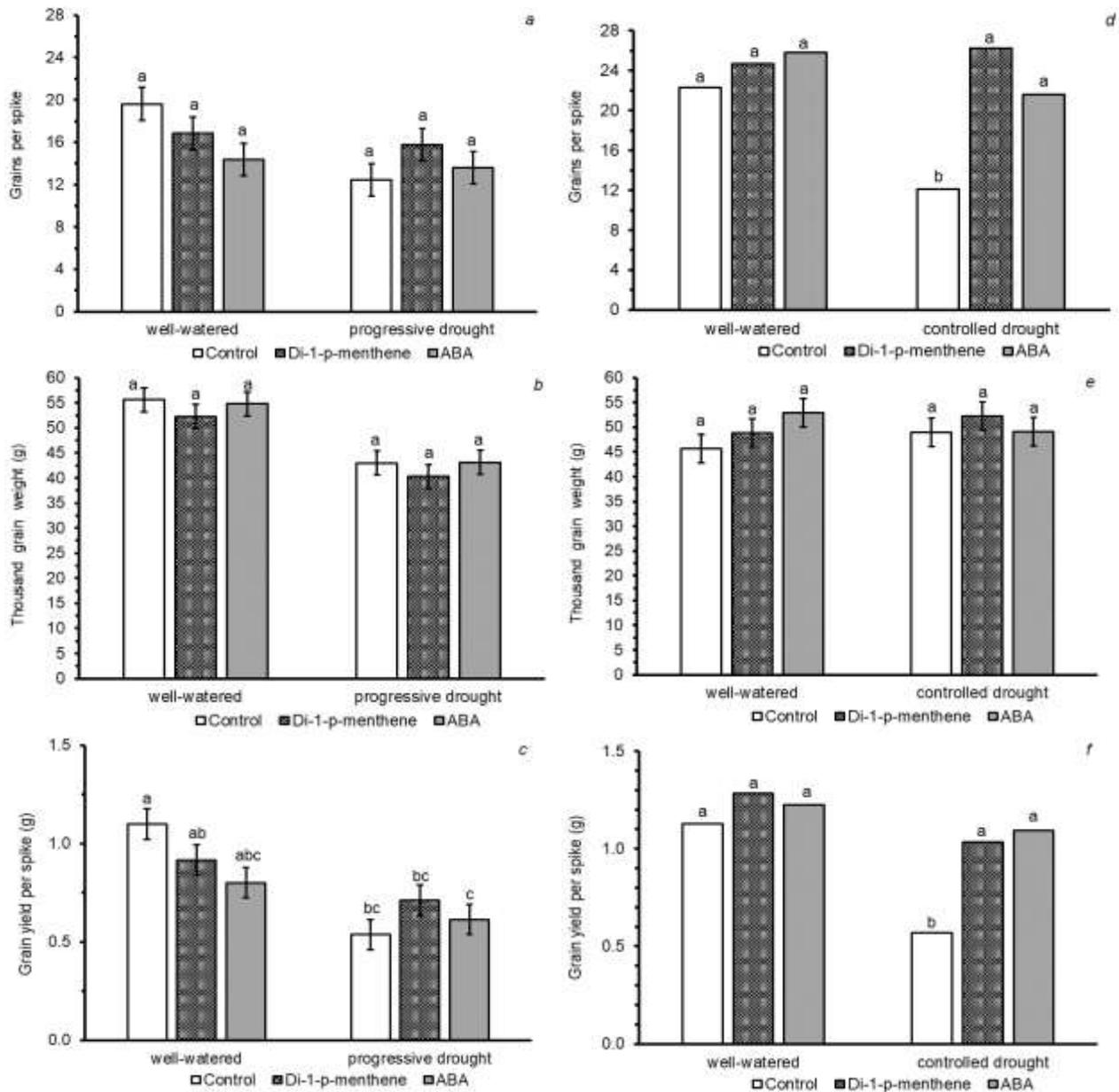
362 differences in the number of fertile spikes per pot (data not shown) or TGW. Grain

363 number per spike and grain yield per spike were significantly reduced by CD in

364 unsprayed plants, but application of DpM and Exo-ABA protected these yield

365 components. This was supported by greater biomass and the allocation of
 366 significantly higher proportions of shoot biomass to grains, as the improved HI show.
 367 Under CD, the average grain number per spike and grain yield per spike were
 368 significantly higher than under PD.

369



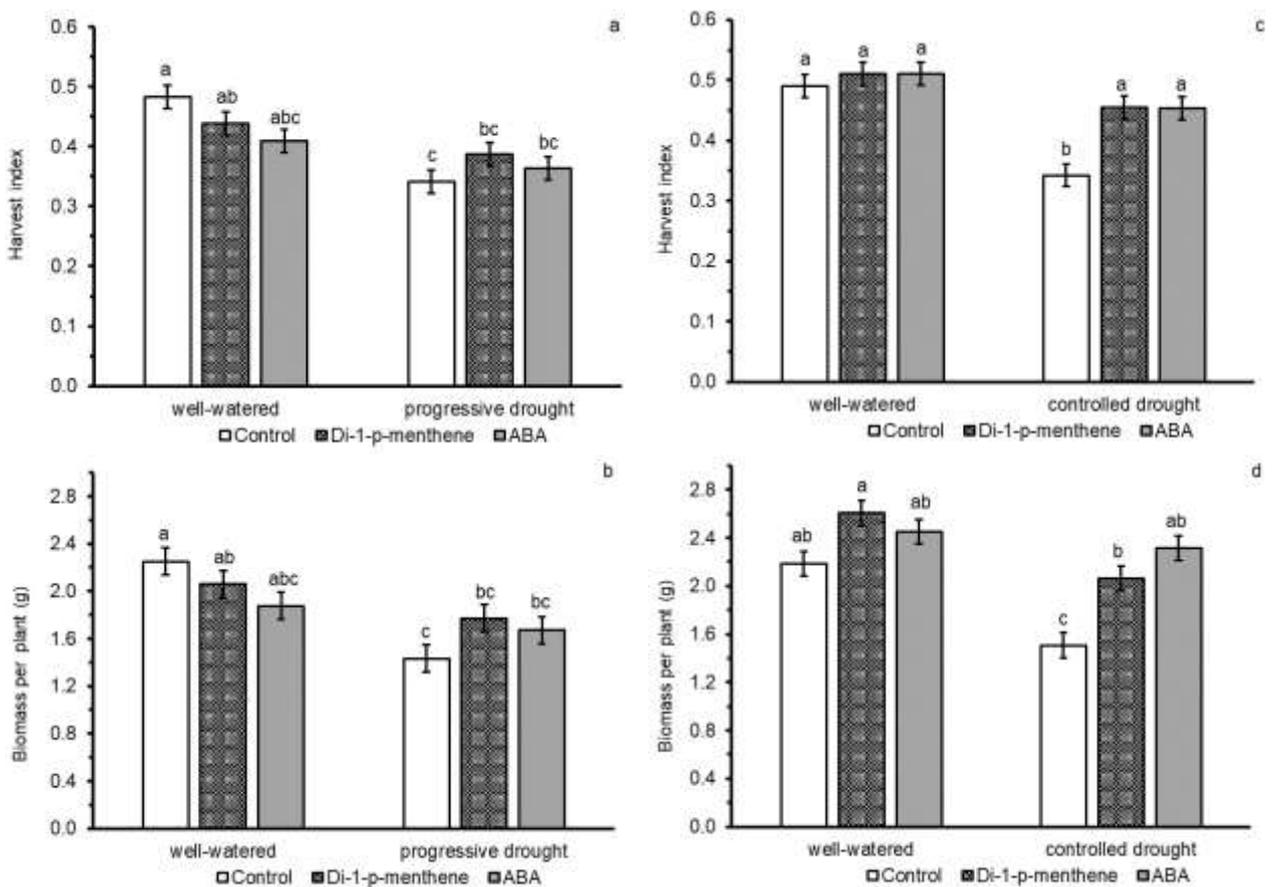
370

371 Figure 9: Number of grains per spike, thousand grain weight and grain yield per
 372 spike under (a, b and c) progressive drought (PD) and (d, e and f) controlled drought

373 (CD) wheat plants ($p = 0.083$, $p = 0.984$ and $p = 0.038$) and ($p = 0.043$, $p = 0.370$ and $p = 0.005$), respectively. Error bars represent common standard errors of means (SEM)
 374 = 0.005), respectively. Error bars represent common standard errors of means (SEM)
 375 from ANOVA. Different letters represent significant difference (Tukey test, $p < 0.05$).
 376 Figure 9 (d and f) were constructed from back transformed values; their means on
 377 the square root scale and SEM in the order: well-watered and droughted (control, di-
 378 1-*p*-menthene and exogenous ABA, SEM); were (4.72, 4.97, 5.08 and 3.48, 5.12, 4.65,
 379 SEM: 0.262) and on the logit scale (-4.474, -4.343, -4.390 and -5.162, -4.561, -4.504,
 380 SEM: 0.0750), respectively.

381

382



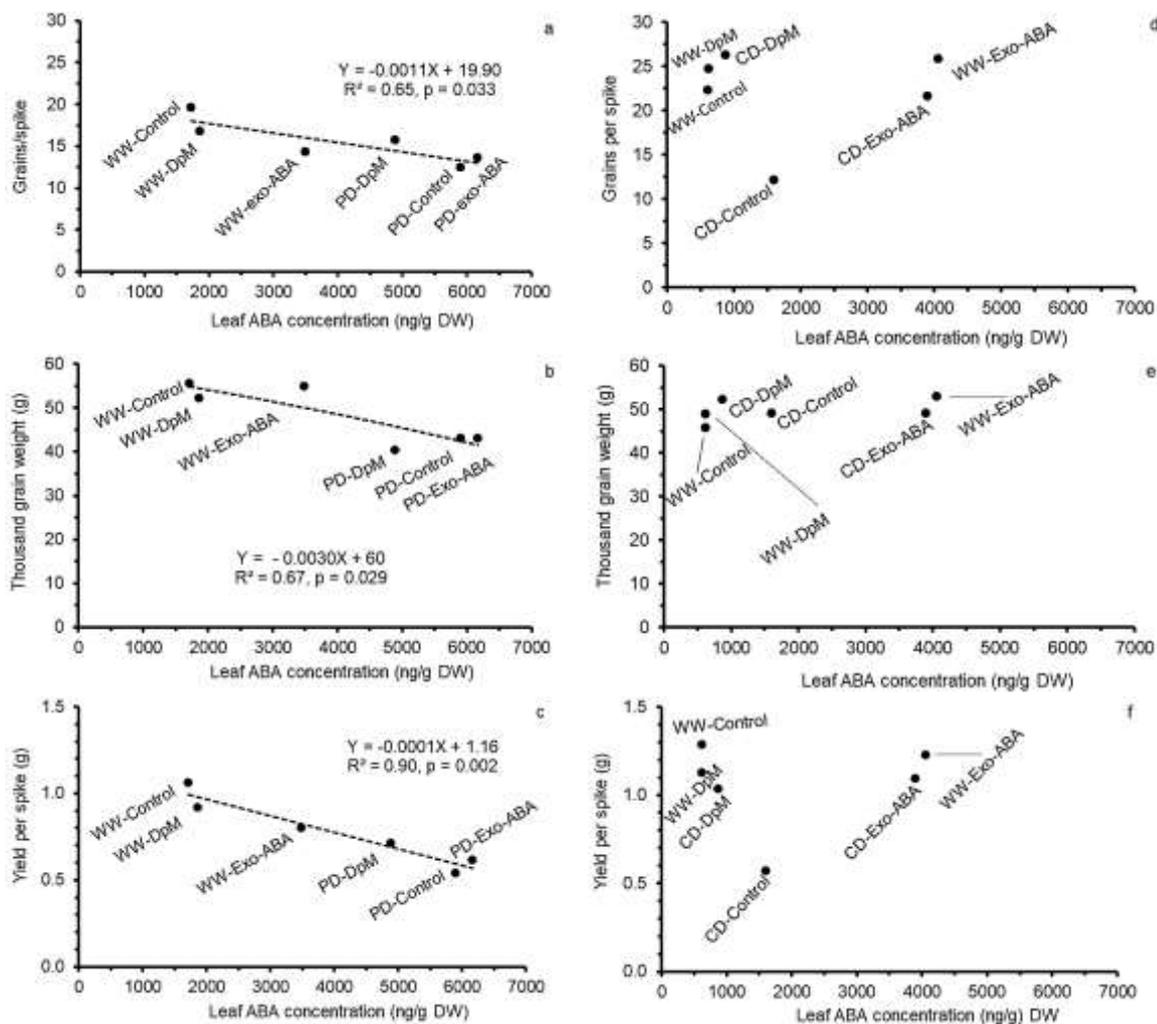
383

384 Figure 10: Harvest index and shoot biomass per plant under (a and b) progressive
 385 drought (PD) and (c and d) controlled drought (CD) wheat plants ($p = 0.036$ and $p =$

386 0.025) and ($p = 0.033$ and $p = 0.039$). Error bars represent common standard errors of
 387 means (SEM) from ANOVA. Different letters represent significant difference (Tukey
 388 test, $p < 0.05$).

389

390 Yield components were significantly correlated with leaf ABA sampled at the booting
 391 stage under PD (Figure 11). Leaf and spike ABA sampled at early anthesis under
 392 CD showed no significant associations (graphs on spikes not presented). The
 393 relationships between leaf ABA and the number of grains per spike, TGW and yield
 394 per spike under PD were all significant and linear, their values decreasing with
 395 increasing leaf ABA (Figure 11 a, b and c).



396

397 Figure 11: Linear regressions of flag leaf ABA concentration against fertile spikes per
398 pot, grain number per spike, thousand grain weight and grain yield per spike under
399 (a, b, c and d) progressive drought (PD) and (e, f g and h) controlled drought (CD)
400 wheat plants. Under controlled drought (CD) sampling for ABA assay was done at
401 booting (GS42), five days after spraying, and under CD at early anthesis (GS62), 21
402 days after spraying di-1-*p*-menthene and one day after a fourth exogenous ABA
403 spray under CD.

404

405 4. Discussion

406 Drought is an important yield-limiting abiotic stress because of its impact on key
407 physiological processes such as stomatal conductance, photosynthesis (Acevedo,
408 Silva, & Silva, 2002; Shakeel Ahmad Anjum et al., 2011) and on metabolic
409 processes that determine grain development (Mphande et al., 2021). ATs reduce the
410 negative impact of these processes on yield. In the two glasshouse experiments,
411 drought downregulated stomatal conductance and photosynthesis and upregulated
412 ABA concentration. The higher levels of stomatal conductance under CD reflect not
413 only the greater photosynthetic active radiation used but also the milder stress, as
414 water deficit was more severe under PD than under CD. The application of either
415 DpM or Exo-ABA just before booting (GS39) reduced the yield-limiting effects of
416 drought, despite distinct physiological effects of these products.

417 Drought induces an increase in the endo-ABA accumulation (Dayer et al., 2020;
418 Pang et al., 2017). Compared to the WW plants, drought upregulated the
419 accumulation of ABA in leaves by 3.4 and 2.6 times under PD and CD, respectively;
420 and in spikes by 2.8 times (determined under CD only). With PAW approaching

421 PWP, droughted plants under PD grew under more severe drought and warmer and
422 drier ambient conditions than under CD, as Figures 1 and 2 indicate. As expected,
423 application of DpM resulted in less accumulation of leaf ABA during droughted, while
424 Exo-ABA resulted in equal or greater leaf ABA during droughted (although the
425 differences were not always significant). In wheat, younger leaves accumulate higher
426 levels of ABA (Quarrie & Henson, 1981). This might explain the non-significant effect
427 of DpM on endo-ABA with sampling at 21 days after spraying (for DpM under CD),
428 being too late. In addition, the weekly irrigation intervals (on two different days)
429 meant that endogenous ABA levels were constantly fluctuating under CD. Under a
430 progressive drought scenario, accumulation of endogenous ABA is known to steadily
431 increase (Itam et al., 2020), but hydration promotes catabolism of ABA (Yang and
432 Zeevaart, 2006). Further, the sampling for endo-ABA at anthesis (under CD) was too
433 close to the time of determination of yield components. It was therefore too late for
434 the concentration of endo-ABA to influence grain number, and hence correlate with
435 this yield component and yield. The largest difference in the leaf ABA levels were
436 observed in Exo-ABA -treated plants under CD, which were sampled one day after a
437 fourth application. Although Exo-ABA consistently elevated leaf ABA when it was
438 low, under CD the increase was small and under PD there was no increase (as leaf
439 ABA was elevated due to drought). This suggests that endo-ABA reaches a ceiling
440 under severe drought, beyond which homeostatic mechanisms rapidly catabolise it,
441 probably in not more than two days. Endo-ABA concentration is controlled by a
442 balance between its biosynthesis and catabolism (Nambara & Marion-Poll, 2005).
443 There is evidence that once drought-induced endo-ABA accumulates to a certain
444 level, negative feedback mechanisms mediated by transcription factors, AhAREB1
445 and AhNAC2, limit its further accumulation (Liu et al., 2016). There are also

446 suggestions that ABA self-regulates (see references in Nambara & Marion-Poll,
447 2005); and that this is by modulating the negative feedback transcription factors
448 AhAREB1 and AhNAC2 (Liu et al., 2016).

449 Other than for the effect of Exo-ABA treatment at one day after the fourth spraying
450 (under CD), leaf ABA concentration was expected to be higher than in the spike
451 counterparts, as Mphande et al. (2021) found at ear emergence. It is known that
452 endo-ABA in spikes of wheat plants subjected to drought increases steadily reaching
453 maximum at the peak of grain filling, enhancing senescence and remobilisation of
454 fixed carbon from stems to spikes (Mphande et al., 2016; Yang et al., 2003).
455 Therefore, while the endo-ABA concentration in leaves declines as they age, it
456 increases in spikes as grain filling approaches (Kondhare et al., 2015; Yang, et al.,
457 2004); this might explain why at anthesis spikes accumulated higher levels of the
458 hormone than leaves at the same growth stage. It is known that drought (i.e. high
459 ABA) during grain filling is less damaging to yield than between stem elongation and
460 heading (Khadka et al., 2020). A high concentration of spike ABA pre-anthesis may
461 have damaging effects on some yield components. By physically blocking stomata,
462 DpM decreases the number of stomatal pores involved in gas exchange. In the
463 current study, DpM significantly reduced stomatal conductance to the lowest values
464 under both PD and CD conditions. Exo-ABA only reduced stomatal conductance
465 under PD, while it resulted in a negligible increase under CD. The non-significant
466 effect of spray type on photosynthesis under PD compared to CD may be attributed
467 to differences in the level of PAR at which measurements were taken. The level of
468 light used ($250 \mu\text{molm}^{-2}\text{s}^{-1}$) under PD was much lower than under ($1000 \mu\text{molm}^{-2}\text{s}^{-1}$)
469 and other studies (e.g. $1200 \mu\text{molm}^{-2}\text{s}^{-1}$, Faralli et al., 2017). Clearly, when the
470 photosynthetic machinery was operating below capacity, no significant differences

471 between treatments were detectable. Our results are consistent with a study on
472 beans by Iriti et al. (2009) who found DpM to be more effective in reducing stomatal
473 conductance than chitosan, another metabolic AT. Droughted plants had higher
474 WUE than well-watered plants, in agreement with previous research (e.g.
475 Mendanha, Rosenqvist, Hyldgaard, Doonan, & Ottosen, 2020). WUE_i can be
476 increased by reducing stomatal conductance or increasing photosynthesis or a
477 combination of both (from the formula: P_N/g_s , (Sikder et al., 2015). This explains why
478 DpM significantly increased the WUE_i under severe drought (PD) but not under mild
479 CD or WW conditions. Increasing the WUE is one of the drought tolerance traits as
480 water conservation outweighs the penalty on reduced carbon assimilation
481 (Mendanha et al., 2020). Under PD, both DpM and Exo-ABA improved WUE_i but did
482 not significantly increase the fertile spikes per pot (data not shown), grain number
483 per spike and grain yield per spike most likely due to the masking effect of mixing of
484 main stem and tiller spikes. Iriti et al. (2009) reported DpM to be most effective in
485 increasing WUE_i relative to chitosan and the control under drought.

486 There are two key concerns associated with the use of ATs and these are
487 suppression of photosynthesis and the potential to increase leaf temperature to
488 damaging levels due to reduction of transpiration (Mphande et al., 2020). In the
489 present study, ATs repressed photosynthesis, particularly under CD, nevertheless,
490 the benefit of improved yield outweighed the penalty on photosynthesis. However,
491 we did not find evidence suggesting that ATs elevate leaf temperature. This is
492 expected as the air temperature remained low throughout the experiments and light
493 levels were relatively low. It might be expected that AT induced heat stress would
494 impact yield under conditions of high temperature, high solar radiation and low wind
495 speed (Gale & Hagan, 1966; Fahey & Rogiers, 2018), but previous studies suggest

496 that this does not outweigh the potential for yield improvement under droughted
497 conditions (Kettlewell, Heath, & Haigh, 2010; Mphande et al. 2020). In fact, field
498 experiments done in summer on the same genotype of spring wheat also showed no
499 evidence of significant increase in leaf temperature due to AT application. Although
500 Mphande et al., (2021) did not report the DpM effect on leaf temperature, on average
501 values determined on 10th July, 2018, which was after the application of the AT, were
502 32.1 °C, 33.0 °C and 33.3 °C for the well-watered-unsprayed benchmark, droughted-
503 unsprayed and droughted-DpM-sprayed plants, respectively. Thus DpM-sprayed
504 leaves were 0.3 °C hotter than the unsprayed ones. Clearly, drought was a more
505 important factor driving temperature increase (by 0.9 °C in this case) than DpM.
506 Mphande et al., (2021) reported yield benefit in the same spring wheat genotype as
507 in the present study, with ambient temperature in rain-out shelters ranging from 37.1
508 °C to 40.4 °C on hot days – determined more than two weeks after DpM had been
509 sprayed. Nonetheless, further research is needed to determine the efficacy of DpM
510 under hot conditions, such as in the tropics where the interaction with heat stress
511 may be more critical.

512 Saini & Aspinall (1982) showed that high levels of ABA during booting completely
513 inhibited spike fertility in wheat, and Mphande et al., (2021) suggest that some of the
514 positive effects of AT under drought are due to increased spike fertility. The present
515 study has shown negative associations between leaf ABA at the booting stage and
516 yield/yield components (Figures 11 a, b, c and d). The number of grains per spike
517 was linearly related to leaf ABA in a negative way. This trait is fixed during spikelet
518 morphogenesis and development but is affected by environmental conditions
519 (Dolferus, Ji, & Richards, 2011; Lin et al., 2020). It is known that pre-anthesis
520 drought at this stage damages florets and entire spikelets, which are the

521 determinants of grain number per spike (Oosterhuis & Cartwright, 1983). In surviving
522 florets, grain number is further repressed by the sporadic effect of drought on pollen
523 meiosis, which has been identified as the most sensitive reproductive process to
524 abiotic stress (Saini & Aspinall, 1982). Reduction in grain number is linked to
525 elevated ABA concentration induced by drought (Morgan, 1980) or other abiotic
526 stresses. Saini & Aspinall (1982) demonstrated that elevating the endo-ABA
527 concentration just before or at the commencement of pollen meiosis significantly
528 reduced grain set and number of grains per spike, but these traits were unaffected if
529 increased concentrations were during pollen development and at the onset of
530 anthesis. This may explain why elevated leaf ABA concentration at booting in the
531 present study was correlated with number of grains per spike under PD but there
532 was no correlation at anthesis under CD. To the contrary, in a glasshouse study,
533 Weldearegay, Yan, Jiang, & Liu (2012) found that drought at anthesis elevated
534 spikelet ABA levels, reduced grain set and grain yield of spring wheat. The mixing of
535 main stem and tiller spikes under PD masked treatment effects on grain number per
536 spike and other agronomic traits. Unlike main stems, tillers were not at GS39 at the
537 time of spray application, hence drought was not expected to have also affected
538 pollen meiosis in tiller spikes. In addition, even by the time normal watering was
539 resumed (main spikes at GS45), most tillers were still at stem extension. Thus, the
540 treatment effect of stems that were at GS39 was likely overshadowed. The lower
541 number of grains per spike under PD can be attributed to source limitation resulting
542 in competition for growth resources, as there were 11 plants per pot and their tillers
543 against seven tiller-free main spikes under CD. The number of grains per spike
544 reduces with increasing plant density (Li et al., 2016), and water deficit (Lin et al.,
545 2020). Under severe stress, entire flowering heads are aborted, thereby reducing the

546 number of fertile spikes per unit area (Katerji et al., 2009). Thus, under high endo-
547 ABA concentration due to drought, reproductive failure increases. Figure 9 (a, b, and
548 c) demonstrates that accumulating high leaf ABA, of over 5000 ng/g DW in this case,
549 had damaging effects being associated with the lowest yield/yield component values.

550 The significant increase in grain number, yield per spike shoot biomass per plant and
551 HI under CD due to DpM and Exo-ABA application suggests that these compounds
552 had stimulatory or ameliorative effects on these agronomic traits. In droughted spring
553 wheat, Mphande et al. (2021) found an association between yield improvement and
554 reduced endo-ABA concentration attributed to DpM. DpM is also known to improve
555 plant water balance (Faralli et al., 2017) and this is likely the mechanism linked to
556 reduced endo-ABA. Increasing the plant water status of dehydrated plant cells
557 restores turgor and reduces elevated endo-ABA concentration (Liang and Zhang,
558 1999) through catabolic pathways of hydroxylation and conjugation (Nambara &
559 Marion-Poll, 2005). Maintenance of cellular turgor is crucial for sustaining biological
560 processes including photosynthesis, assimilate partitioning, growth and reproduction
561 (Farooq et al., 2009). Under water stress, high-yielding, drought-tolerant wheat
562 genotypes are associated with higher HI values (Kobata, Koc, Barutcular, Tanno, &
563 Inagaki, 2018). That DpM significantly improved the HI under CD despite repressing
564 photosynthesis suggest that there was an increased role of water-soluble
565 carbohydrates (remobilised from stems Mphande et al, 2016) in grain filling (Ehdaie,
566 Alloush, & Waines, 2008).

567 Application of Exo ABA provides signals to leaves to close the stomata and has been
568 shown to protect wheat from water loss during mild drought, however the amount
569 applied, the interaction with endogenously produced ABA and the timing of the
570 drought can all impact on crop response (Mphande et al., 2020). In wheat, Travaglia

571 et al. (2010) observed that under moderate drought, Exo-ABA sustained chlorophylls
572 *a* and *b*. This agrees with the present study as Exo-ABA increased net
573 photosynthesis with a PAR selection of $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ and not $250 \mu\text{mol m}^{-2} \text{s}^{-1}$,
574 thought the effect was not significant. Exo-ABA increasing of stomatal conductance
575 and photosynthesis has also been reported in soyabeans (Liu, Jensen, & Andersen,
576 2004). Further, Exo-ABA upregulates the expression of glutathione and ascorbate
577 antioxidant activities in droughted wheat (Wei et al., 2015). These mechanisms may
578 explain why Exo-ABA improved shoot biomass, HI, grain number and grain yield in
579 our study. Heightened expression of antioxidants is one of the traits conferring
580 tolerance to abiotic stresses like drought in crop genotypes (Vaidyanathan,
581 Sivakumar, Chakrabarty, & Thomas, 2003). However, application of very high levels
582 of Exo-ABA is counterproductive – (e.g. while 20 mg/l (~ 76 μM) during pollen
583 meiosis reduced grain set to less than 15% while 100 mg/l (~378 μM) completely
584 inhibited grain set in wheat, Saini & Aspinall, 1982). In the current study, 100 μM
585 increased grain set under CD by average of 116%. The inconsistent effects of Exo-
586 ABA concentrations (e.g. 76 μM vs 100 μM) on grain set might be attributed to
587 genotypic differences between the experiments. That the environment is also a
588 factor is clearly demonstrated by the effects of Exo-ABA and DpM on yield and yield
589 components in our study, being less effective under more severe PD than under mild
590 CD. Our results corroborate past findings. A study on oilseed rape by Faralli et al.
591 (2017) reported that the effect of DpM on pod number per plant significantly
592 decreased with increasing drought severity. In wheat, Travaglia et al. (2010) found a
593 significant increase in individual grain weight attributed to Exo-ABA under moderate
594 but not severe drought. Working with three Exo-ABA concentrations (30, 40 and 60
595 mg/L ~ 113, 151 and 227 μM) on spring wheat, Zhang et al. (2016) observed that

596 grain yield averaged over two years decreased with increasing concentration,
597 supportive of the damaging effects of increased endo-ABA. Other than for the more
598 severe PD, the effect of Exo-ABA on grain yield obtained in this study is consistent
599 with the finding of Zhang et al. (2016). They observed yield improvement in winter
600 wheat grown under both WW and droughted conditions. The effect of Exo-ABA
601 increased with drought from WW to moderate deficit and reached the highest under
602 severe drought. These studies suggest that the mitigative effects of ATs are
603 influenced not only by dose rate but also by genotype, timing and the magnitude of
604 drought. In contrast, Sivakumar, Virendranath, & Srivastava (2001) reported decline
605 in grain weight and grain number per spike due to Exo-ABA application of 28% and
606 22%, respectively, showing that the yield benefits are not in all cases. There is need
607 for establishing the interplay between Exo-ABA as an AT for drought mitigation and
608 its potential to cause damage to yield components by elevating ABA concentration.

609 **5. Conclusion**

610 In the present study, the effects of DpM and Exo-ABA on gas exchange and leaf and
611 spike ABA concentration were compared in WW and droughted spring wheat in two
612 glasshouse experiments. We found no evidence of Exo-ABA damaging yield or
613 number of grains per spike. DpM had no significant effect on endo-ABA in WW
614 plants but generally reduced it under drought. As such, the correlation between
615 reproductive-stage leaf ABA concentration and yield, seen here in one experiment
616 and in previous field experiments, might reflect a general association between endo-
617 ABA and drought stress rather than a direct effect of the hormone on grain
618 development, as previously proposed.

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851

852 ix. Tables

853 Table 1: Spray application and ABA sampling schedule.

Experiment	Spraying				Leaf sampling			Spike sampling	
	GS*	Spray types	DAP*	Date	GS	DAP	Date	DAP	Date
PD (2018)	GS39	DpM*	63	12 th Oct	GS42	68	17 th Oct		N/A
	GS39	Exo-ABA	63	12 th Oct	GS42	68	17 th Oct		N/A
	GS39	DpM	59	16 th Jan	GS62	80	6 th Feb	80	6 th Feb
CD (2019)	GS39	Exo-ABA	59	16 th Jan	GS62	80	6 th Feb	80	6 th Feb
	GS43	Exo-ABA	65	22 nd Jan					
	GS54	Exo-ABA	72	29 th Jan					
	GS62	Exo-ABA	80	6 th Feb					
	GS69	Exo-ABA	87	13 th Feb					

854 * DAP, DpM, Exo-ABA and GS stand for days after planting, di-1-*p*-menthene,
855 exogenous ABA and growth stage, respectively.

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857 x. **Figures Legends**

- 858 • Figure 1: Average daily air temperature (a and c) and average daily relative
859 humidity (b and d) inside the glasshouse rain shelters recorded by the
860 TomTech T200 data logger (Tomtech (UK) Ltd, Spalding, UK).

- 861 • Figure 2: Figure 2: Soil moisture readings from (a) progressive drought (PD)
862 and (b) controlled drought (CD) wheat plants between 60 and 68 days after
863 planting (DAP) and between 68 and 112 DAP, respectively. Note that the
864 lower pot capacity for CD was due to a predominantly sandy growth medium
865 instead of John Innes No. 2 Compost for PD. Undotted arrows indicate DAP
866 on which the antitranspirants were sprayed.
- 867 • Figure 3: Flag leaf ABA levels under (a) progressive drought (PD) and (b)
868 controlled drought (CD) wheat plants sampled at 68 days after planting (DAP
869 i.e. five days after spraying, DAS, $p = 0.008$); and 80 DAP (i.e. 21 DAS DpM
870 and one day after a fourth spray of Exo-ABA, $p = 0.015$), respectively.
871 Different letters represent significant differences (Tukey test, $p < 0.05$). Error
872 bars represent common standard errors of means from ANOVA table.
- 873 • Figure 4: Spike ABA under (a) progressive drought (PD) and (b) controlled
874 drought (CD) wheat plants ($p = 0.025$) sampled at 80 days after planting, i.e.
875 21 days after spraying di-1-*p*-menthene and one day after a fourth exogenous
876 ABA spray under controlled drought. Different letters represent significant
877 difference (Tukey test, $p < 0.05$). Errors bars represent common standard
878 errors of means from ANOVA table.
- 879 • Figure 5: Stomatal conductance under (a) progressive drought (PD) and (b)
880 controlled drought (CD) wheat plants measured five days after spraying (DAS,
881 $p = 0.025$) and one DAS ($p < 0.001$), respectively. The photosynthetically
882 active radiation in 2018 was set at $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ in
883 2019. Different letters represent significant differences (Tukey test, $p < 0.05$).
884 The graphs were constructed from back transformed values. The transformed

885 means and their standard errors (SEM) in the order: well-watered and
886 droughted (control, di-1-p-menthene and exogenous ABA, SEM); were: under
887 PD (square root scale: 0.4434, 0.3759, 0.402 and 0.3108, 0.1244, 0.1382,
888 SEM: 0.02189) and CD (log 10 scale: -0.118, -0.195 and -0.367 and -0.679, -
889 1.043 and -0.514, SEM: 0.0739), respectively.

890 • Figure 6: Net photosynthesis under (a) progressive drought (PD) and (b)
891 controlled drought (CD) wheat plants measured five days after spraying (DAS)
892 at photosynthetically active radiation (PAR) set at $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ ($p = 0.488$)
893 and one DAS at PAR of $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ ($p = 0.004$). Different letters
894 represent significant differences (Tukey test, $P < 0.05$) while error bars are
895 standard errors of means from ANOVA table.

896 • Figure 7: Intrinsic water use efficiency under (a) progressive drought (PD) and
897 (b) controlled drought (CD) wheat plants determined on flag leaves ($p <$
898 0.001) and ($p = 0.007$), respectively. Error bars represent standard error of
899 means (SEM). Different letters represent significant difference (Tukey test, $p <$
900 0.05); graphs were constructed from back transformed values. The
901 transformed means and their SEM in the order: well-watered and droughted
902 (control, di-1-p-menthene and Exo-ABA, SEM); were: under PD (log base 10
903 scale: 1.50, 1.57, 1.62 and 1.34, 2.03, 2.01, SEM: 0.50) and under CD
904 (square root scale: 4.51, 5.21, 6.25 and 7.75, 8.29, 6.68, SEM: 0.589),
905 respectively.

906 • Figure 8: Number of grains per spike, thousand grain weight and grain yield
907 per spike under (a, b and c) progressive drought (PD) and (d, e and f)
908 controlled drought (CD) wheat plants ($p = 0.083$, $p = 0.984$ and $p = 0.038$)

909 and ($p = 0.043$, $p = 0.370$ and $p = 0.005$), respectively. Error bars represent
910 common standard errors of means (SEM) from ANOVA. Different letters
911 represent significant difference (Tukey test, $p < 0.05$). Figure 8 (f and h) were
912 constructed from back transformed values; their means on the square root
913 scale and SEM in the order: well-watered and droughted (control, di-1-*p*-
914 menthene and exogenous ABA, SEM); were (4.72, 4.97, 5.08 and 3.48, 5.12,
915 4.65, SEM: 0.262) and on the logit scale (-4.474, -4.343, -4.390 and -5.162, -
916 4.561, -4.504, SEM: 0.0750), respectively.

917 • Figure 9: Harvest index and shoot biomass per plant under (a and b)
918 progressive drought (PD) and (c and d) controlled drought (CD) wheat plants
919 ($p = 0.036$ and $p = 0.025$) and ($p = 0.033$ and $p = 0.039$). Error bars represent
920 common standard errors of means (SEM) from ANOVA. Different letters
921 represent significant difference (Tukey test, $p < 0.05$).

922 • Figure 10: Linear regressions of flag leaf ABA concentration against fertile
923 spikes per pot, grain number per spike, thousand grain weight and grain yield
924 per spike under (a, b, c and d) progressive drought (PD) and (e, f g and h)
925 controlled drought (CD) wheat plants. Under controlled drought (CD) sampling
926 for ABA assay was done at booting (GS42), five days after spraying, and
927 under CD at early anthesis (GS62), 21 days after spraying di-1-*p*-menthene
928 and one day after a fourth exogenous ABA spray under CD.

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