

1 **Maize leaf functional responses to drought episode and rewatering**

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9

10 **Abstract**

11 Effects of crop growth and physiological activity to drought and irrigation regimes
12 have been extensively studied; however, the responses of plant growth, morphological
13 and photosynthetic behaviors to drought episodes and thereafter rewatering receive a
14 less attention. This field experiment was carried out directly *in situ* at an agricultural
15 ecosystem research station during 2015-2016, in a northeastern China, on the
16 renowned northeastern maize production belt, where is being threatened by severe
17 drought. A field automatic rain-shelter was used, and five irrigation regimes including
18 control, four drought episodes, and rewatering treatments were established. The
19 chlorophyll contents (SPAD values), light-saturated photosynthetic rate (A_{sat}), and
20 photosystem II actual quantum yield (Φ_{PSII}), maximum quantum yield (F_v'/F_m')
21 decreased at lower leaf positions and with plant development. Episodic drought
22 effects on plant growth, leaf morphological traits and photosynthetic processes at both
23 vegetative and reproductive stages were severely remarked, particularly at late
24 development stage and with longer drought duration. The recovery of leaf functional
25 traits of the plants experienced historical-drought following re-irrigating was not fully
26 restored to the level of the plants subjected to ample and normal water status; and the
27 strength of recovery was proportional to the persistence of pre-drought episodes. The
28 relationship of A_{sat} with SPAD depends on water status and plant development. A
29 principal component analysis can well denote the change patterns in responses to
30 water status treatments with plant development. The results may give an insight into

31 how to understand the maize traits' responses to drought episode and rewatering, and
32 this also might assist the drought-stricken crops to cope with future climatic change.

33 **Kew Words:** Climate Change; Drought Episode; Irrigation Regimes; Leaf Age;
34 Photosynthetic Potentials; *Zea Mays* L.

35

36 **1. Introduction**

37 Climate change results in abnormal changes in precipitation patterns in terms of both
38 its total amounts and episodic drought frequencies (Alley *et al.* 2003; Trenberth *et al.*
39 2014; IPCC 2014). Water shortage is a crucial constraint to crop growth, yield,
40 physiological processes in many areas around world, including rain-fed and
41 deficit-irrigation regions; meanwhile the abnormal occurrences of drought episodes
42 usually fluctuates at various spatial-temporal scales (Boyer 1982; Battisti & Naylor
43 2009; IPCC 2014; Rurinda *et al.* 2014; Myers *et al.* 2017). Intensifying drought also
44 may eliminate the expected benefits from some fewer favorite factors due to climate
45 change such as elevated CO₂ and enhanced anthropogenic nitrogen (N) deposit
46 (Iversen & Norby 2014; Gray *et al.* 2016; Xu *et al.* 2016), and climatic warming may
47 exacerbate drought disaster by further reducing soil moisture availability (Zeng *et al.*
48 2005; Lobell *et al.* 2011, 2014; Iversen & Norby 2014). As reported, due to
49 potentially adverse climate change, since 1950s to the present, agricultural
50 drought-inducible disaster area also had an increasing trend in China—the
51 drought-induced grain loss reached approximate 25-30 billion kg, accounting for 60%
52 of total loss of natural disaster (Jiao *et al.* 2014, Zhou 2015). It has been notable that
53 China's agricultural drought becomes more serious mainly due to the adverse climate
54 change and rapid social-economic development.

55 Maize is one of the most important three staple crops—maize, wheat, and rice, and
56 the main resources of the feed, industrial raw materials (Campos *et al.* 2004; Long *et al.*
57 2006; Ribaut *et al.* 2009), recent years it ranked first place among the three staple
58 crops (FAO 2017). In China, it also plays a critical role in food security and
59 husbandry industry development among agricultural and even entire economic sectors

60 at both regional and national levels (Meng *et al.* 2013; Ma & Ma 2017; PINC 2017).
61 Drought is one of major limitations to maize production (Boyer 1982; Sharp *et al.*
62 2004; Xu *et al.* 2008; Lobell *et al.* 2014; Avramova *et al.* 2015), resulting in a yield
63 reduction of 25-30% , even with no harvest in those years of extremely severe drought
64 (Campos *et al.* 2004; Zhang *et al.* 2011). In USA major maize production zone, the
65 drought sensitivity in maize production in recent two decades has been also reported
66 to increase, despite cultivar improvements and the agronomic practices with higher
67 sowing densities (Lobell *et al.* 2014). Climatic warming is projected to further
68 exaggerate drought's negative impact, leading to huge loss of maize production
69 (Ribaut *et al.* 2009; Lobell *et al.* 2014). Drought stress leads to reductions in maize
70 (*Zea mays* L.) and other crops' yields mainly by (i) reducing plant growth and
71 reproductive activities, (ii) reducing photosynthetic potentials and thereby radiation
72 use efficiency (RUE), and (iii) reducing harvest index (HI) (Saini & Westgate 1999;
73 Earl & Davis 2003; Barnabás *et al.* 2008; Xu *et al.* 2008). Contrastingly, if a maize
74 cultivar root system and its ear growth are not completely limited, and leaf survival is
75 enhanced despite water deficit, the cultivar may be recognized as high
76 drought-tolerant one (Ribaut *et al.* 2009). Nevertheless, the intermittent drought
77 imposition, and then following rewatering effects on crop plants grown in field still
78 receive a relatively scant attention.

79 Based on cyclic drought experiment using *Catalpa bungei* species, the
80 accumulative functional effects of progressive drought and subsequent re-watering on
81 plant growth, leaf and root parameters has been found as a useful adaptive mechanism
82 to drought successive drought and subsequent rewatering (Zheng *et al.* 2017). As
83 recently reported by Abid *et al.* (2016), the adaptability to drought, and recovery rate
84 and capacity was closely associated with wheat cultivars. The accumulation of
85 effective metabolites such as sugars, and some amino acids like proline and leucine
86 may exert an adaptive mechanism in response to the drought cyclic patterns (Meyer *et*
87 *al.* 2014; Foster *et al.* 2015; Sun *et al.* 2016; Zheng *et al.* 2017). In plants of *Lupinus*
88 *albus*, the new leaves can be produced more as quickly re-watered, although

89 restoration of other metabolites (e.g. sugar content) was lagged (Pinheiro *et al.* 2004).
90 Maize leaf length undergone one or several days of drought can restore completely
91 following rewatering, but its growth rate could not reach the control level, suggesting
92 that the growth resumption may be only a postponed event, no overcompensation
93 occurrence (Acevedo *et al.* 1971). It is implied that the magnitude and rate of
94 resumption might depend on pre-drought intensity and its duration (Hsiao 1973; Xu &
95 Zhou 2007; Xu *et al.* 2009, 2010). Thus, the extent of compensation for the limitation
96 of pre-drought by promoting plant growth as rewatering might determine the final
97 plant biomass or crop yield, which may link to drought severity and its duration.
98 Nevertheless, whether plant growth and physiological activities completely recovery
99 following rewatering, what are the rate and degree of recovery, and the ability of the
100 adaption to the drying-rewetting cycles might strongly depend on previous drought
101 strength and persistent duration, species and genetic types, and drying-rewetting cycle
102 patterns, which the underlying mechanism is elusive so far (Loewenstein & Pallardy
103 2002; Marron *et al.* 2003; Flexas *et al.* 2004; Yahdjian & Sala 2006; Xu *et al.* 2009,
104 2010; Sun *et al.* 2016; Zheng *et al.* 2017). Thus, responses of plant growth and leaf
105 functional processes to drought history and subsequent rewatering remain to be
106 clarified further, particularly *in situ* crop field.

107 As stated above, drought effects on plant/crop growth, photosynthesis, and other
108 crucial eco-physiological process have been investigated extensively (e.g., Ne Smith
109 & Ritchie 1992; Yordanov *et al.* 2002; Chaves *et al.* 2002; Harrison *et al.* 2014).
110 However, as just recently stated by Abid *et al.* (2016), “studying plants’ capability to
111 adapt and recover from drought stress is essential because of the ever-changing nature
112 of drought events”. Herein, the objectives of this present study were to: (1) examine
113 the effects of drought episode and rewatering on photosynthetic capacity and
114 chlorophyll fluorescence; (2) compare the leaf functional responses to drought
115 episode and rewatering at different leaf positions from bottom-most to upmost leaves,
116 at various plant growth developments; (3) determine changing patterns in responses to
117 drought episode and recovery after re-watering on photosynthetic capacity and
118 chlorophyll fluorescence with the leaf developments; and (4) elucidate the index for

119 drought adaptability and recovery ability following a pre-drought episode. Our
120 hypotheses are expected: i) drought-episode-induced negative responses may depend
121 on leaf ages/positions and leaf/plant development; ii) the amelioration of
122 drought-induced negative responses by rewatering in field grown maize plants may
123 mainly result from gas exchange behaviors relative to the chlorophyll fluorescence
124 performances—photosystem II (PSII) photochemical processes; iii) the morphological
125 and physiological functional traits may closely interacted, coordinately representing
126 the adaptive responses to episodic drought and following re-wetting.

127

128 **2. Materials and Methods**

129 **2.1. Site descriptions**

130 The present two-year field experiment was carried out directly *in situ* at an
131 agricultural ecosystem research station during 2015-2016 (41°49'N, 121°12'E, 27.4 m
132 a.s.l.), Jinzhou Ecology and Agricultural Meteorology Center, Jinzhou, Liaoning, a
133 northeastern Chinese province on the renowned northeastern maize production belt
134 (PINC 2017). This region is located in the northeast of the Eurasian areas, belongs to
135 the warm temperate semi-humid monsoon climate, and atmospheric circulation
136 mainly composed of westerlies and subtropical systems, with clear four seasons. The
137 mean annual temperature is 7.8-9.0 °C, with the extreme maximum temperature of
138 41.8 °C and the extreme minimum temperature of -31.3 °C; annual frost-free period is
139 144-180 days; average annual rainfall is 540-640 mm, with 60% - 70% of rainfall
140 concentrated in the summer. The soil is the typical brown soil, with a soil pH value of
141 6.3. The organic matter and total nitrogen content is 6.41-9.43 g kg⁻¹ and 0.69 g kg⁻¹,
142 respectively. The staple crop in the region is maize (Han *et al.* 2007).

143

144 **2.2. Experimental design**

145 This study, a maize water-controlled field experiment, was conducted using a huge
146 mobile rain-proof shelter during two growth seasons of 2015-2016. The two-year
147 experimental design and its results were similar; thus, here the 2016-year results were

148 mainly reported (for 2015-year experimental design and its results, see the Supporting
149 Information File: Table S1 & S2, and Figures S1-S3). In the 2016-year experiment,
150 the five irrigation treatments were designed: T₁, T₂, T₃, T₄, and T₅ treatments, which
151 denote Control, withholding water during jointing-tasseling, jointing-anthesis,
152 tasseling-milking, and silking-milking, with 260, 188, 138, 136, and 161 mm
153 irrigation amount in entire developmental stage, respectively (**Table 1**).

154 There were three replicates in each treatment and 15 plots in total. Each plot is 5 m
155 long and 3 m wide, surrounded by cement layer to avoid water permeation. The large
156 mobile water-proof shelter is 4 m high, which is used for simulated precipitation to
157 avoid the rainfall entrance. Maize cultivar used in this experiment was Danyu 405,
158 which has been planted widely in this region. Seeds were sowed on 23 May, 2016.
159 Controlled release fertilizer was used with 600 kg km⁻².

160

161 **2.3. Environmental variables and maize traits measurements**

162 **Soil relative water content (SRWC) measurements** We used weighing method to
163 measure the soil relative water content. Methods with soil auger were used to retrieve
164 soil samples (0-50 cm), then put the samples to the aluminum specimen box, and
165 weighed the samples to obtain the wet weight. Later, the samples were dried in an
166 oven at 105 °C until a constant weight, and then the dried soil sample was weighed.
167 There were three replicates in each treatment. The SRWC was calculated by the
168 equation below:

169

170 $SRWC = (\text{Wet soil weight} - \text{Dry soil weight}) / \text{Dry soil weight} / \text{F.C.} \times 100\%$

171 where F.C. is the soil water content measured 24 h after amply wetting the soil.

172

173 **Chlorophyll content measurements** The relative chlorophyll contents (i.e., SPAD
174 values) of maize leaves were measured by a SPAD-502 meter (Minolta Camera Co.
175 Ltd., Japan). We chose 3-5 plants grown healthily for each treatment, and each leaf
176 was measured three times on the leaf middle area to avoid the main vein, and then
177 averaged.

178 **Photosynthetic and chlorophyll fluorescence parameters** When measured the
179 photosynthetic parameters, three-five plants grown healthily were chosen for each
180 treatment, with a CIRAS-2 gas exchange system (PP Systems, Hertfordshire, UK). We
181 measured the upper (i.e., the youngest and expanded leaves, 1–2 leaves from the top
182 of the plants), middle (ear leaf or the leaves above or below ear leaf) and lower
183 positions (relative orderly leaves) of each plant, respectively. Instrumentation system
184 provided the red and blue built-in light source, and light intensity (Photosynthetically
185 active radiation, PAR) was set to $1500 \mu\text{mol m}^{-2}\text{s}^{-1}$. To ensure ample temperature and
186 humidity conditions, the photosynthetic parameters of the maize leaves were
187 measured at 9:00-11:00. The whole measuring process was used an open gas path, air
188 relative humidity was controlled in 50% - 70%, CO_2 concentration was controlled in
189 $380 - 390 \mu\text{mol mol}^{-1}$, and leaf temperature was set up at around $27 \text{ }^\circ\text{C}$. The
190 parameters included light-saturated net photosynthetic rate (A_{sat}), transpiration rate (E),
191 stomatal conductance (g_s); and the leaf water use efficiency (WUE) was calculated by
192 the formula: $\text{WUE} = A_{\text{sat}} / E$.

193 The chlorophyll fluorescence parameters were measured using a chlorophyll
194 fluorescence module (CFM) integrated with the CIRAS-2 gas exchange system at the
195 same part of the same leaves measured simultaneously for the gas exchange
196 parameters. First, the leaves were lighted at a light intensity of $1500 \mu\text{mol m}^{-2}\text{s}^{-1}$ after
197 15 min to measure the steady-state fluorescence (F_s), and then gave a strong flash
198 ($5100 \mu\text{mol m}^{-2}\text{s}^{-1}$, with pulse time of 0.3 s) to measure the maximum fluorescence
199 (F_m'); later put the leaves under dark adaptation for 3 s, opened the far red after 5 s to
200 measure the minimum light fluorescence (F_o'). According to the expressions, we
201 calculated chlorophyll fluorescence parameters: maximum quantum yield of PSII
202 photochemistry (F_v'/F_m'), quantum yield of PSII electron transport (Φ_{PSII}),
203 photochemical quenching (q_p), and non-photochemical quenching (NPQ) (Genty *et al.*
204 1989; van Kooten & Snel 1990; Maxwell & Johnson 2000; Kramer *et al.* 2004):

$$205 \quad F_v'/F_m' = (F_m' - F_o')/F_m'$$

$$206 \quad \Phi_{\text{PSII}} = (F_m' - F_s)/F_m'$$

207
$$q_p = (F_m' - F_s) / (F_m' - F_o')$$

208
$$NPQ = (F_m - F_m') / F_m'$$

209 **Plant height and leaf area measurements** Plant height and leaf area of maize were
210 measured at different stages, and the measured dates were approximately consistent
211 with those for the measurements of photosynthetic and fluorescence parameters.
212 Maximum length and width were measured for each leaf of a maize plant. A
213 conventional formula of the total leaf area per plant was used (Francis *et al.* 1969):

214 Total leaf area of an entire plant = $\sum_{i=1}^n (Li \times Wi \times 0.75)$

215 where i is the leaf order number of the measured plant, n is total number of the plant, Li is leaf
216 maximum, and Wi leaf maximum width.

217 **Leaf rolling index, leaf erection index, and leaf drooping angle determinations**

218 The upper leaf actual width (at natural state, L_n), maximum width (at unfolding state,
219 L_w), natural length (L_{nl}), maximum length (L_{sl}), basic angle of leaf (the angle
220 between leaf and stem), drooping angle (under the naturally bending down of the leaf,
221 the angle between the line from the pulvinus to the tip and the stem) were determined.
222 Then, the leaf rolling index (LRI, %), leaf erection index (LEI, %), leaf bend degree
223 (LBD, °) were calculated based on Xiang *et al.* (2012):

224
$$LRI = (L_w - L_n) / L_w \times 100$$

225
$$LEI = L_{nl} / L_{sl} \times 100$$

226
$$LBD = \text{Drooping angle} - \text{Basic angle.}$$

227 **The plant biomass and grain yield** At the end of grain-filling, the tagged plants for
228 measurements of these traits were retrieved, dried at 80°C to constant weight in a
229 drying oven, and weighed to obtain the plant root, stem, leaf, and grain biomass.

230

231 **2.4. Data statistics**

232 The statistical analysis was used with SPSS 20.0 software (SPSS Inc., Chicago, IL).
233 One-way ANOVA with Duncan's multiple comparison was used to test the differences
234 in the plant and leaf functional traits and morphological indicators between watering

235 treatments at a 0.05 significance level. The differences of functional parameters
236 among watering treatments, leaf positions and measurement dates were tested by the
237 three-way ANOVA at the 0.05 significance level. The correlations between leaf
238 functional and morphological traits were analyzed with Pearson method. The
239 comprehensive patterns of the responses of leaf functional and morphological traits to
240 episodic drought and rewatering were further analyzed with principal component
241 analysis (PCA, Jolliffe 2002).

242

243 **3. Results**

244 **3. 1. Photosynthetically physiological responses to drought episode and** 245 **rewatering**

246 ***3.1.1 Responses in upper leaves***

247 Photosynthetic physiological parameters were measured during the entire growing
248 season: on 1, July (V13, jointing); 14, July (V21); 25, July (VT, tasselling); 31, July
249 (R1, Silking); 7, August (R2, blistering); 14, August (R3, milking); 29, August (R4,
250 dough), respectively (Table 1). As shown in Figure 1, the upper leaf net light-saturated
251 photosynthetic rate (A_{sat}) of control treatment (T_1) increased with earlier plant
252 development, reaching the maximum of $45.4 \mu\text{mol m}^{-2}\text{s}^{-1}$ on 31 July (R1 stage),
253 thereafter sharply decreasing until to a lowest point of $14.1 \mu\text{mol m}^{-2}\text{s}^{-1}$ by 68.5% at
254 later grain-filling stage.

255 For the T_2 treatment (withholding water during jointing – tasseling, 27 days), the
256 episodic drought led to a significant decline of A_{sat} with a low level of $21.7 \mu\text{mol}$
257 $\text{m}^{-2}\text{s}^{-1}$ on 14, July (V21 stage), a 40.3% drop; and further down to a lower level of
258 $15.6 \mu\text{mol m}^{-2}\text{s}^{-1}$ on 25, July (VT stage), a 65.6% drop. Upon re-irrigating, it rose up
259 to $24.4 \mu\text{mol m}^{-2}\text{s}^{-1}$ by 56.4% relative to the previous value, but not to reach the level
260 of control treatment at the same stage—a maximum of $45.4 \mu\text{mol m}^{-2}\text{s}^{-1}$ for the control
261 plant. It indicated that a 27-day episodic drought from jointing to tasseling
262 significantly inhibited photosynthetic capacity, just a part of recovery when following
263 rewatering occurred, resulting in limitation to plant growth and development, and

264 final grain yield loss.

265 Leaf A_{sat} of the T₃ treatment (withholding water during jointing –anthesis, 41 days)
266 always markedly decreased, even it remained a low and stable level as rewatering and
267 during anthesis, thereafter declined rapidly after milking until to a low level of 6.6
268 $\mu\text{mol m}^{-2}\text{s}^{-1}$ at later grain-filling. The rewatering had a relatively-higher stimulation
269 relative to the control only at milking, indicating the mild stimulation appeared (17.0
270 vs: 15.4 $\mu\text{mol m}^{-2}\text{s}^{-1}$, Figure 1a).

271 While leaf A_{sat} of the T₄ treatment (withholding water during tasseling–milking, 41
272 days) had an increase at earlier withholding water (silking stage), then sharply
273 declined to a low level of 12.0 $\mu\text{mol m}^{-2}\text{s}^{-1}$ at blistering, thereafter remaining a lower
274 and stable level until the end of the grain-filling, although this time it seemed to
275 lightly rise relative to T₃ treatment, indicating that a stimulation occurred by the just
276 nearly rewatering. Leaf A_{sat} of the T₅ treatment (withholding water during
277 silking–milking, 34 days), similar to T₄ but less 7-day drought duration, declined even
278 more sharply. It may indicate that later drought at tasseling may result in more
279 sensitive effect on the gas exchange processes.

280 Other photosynthetically physiological parameters in the upper leaves showed
281 similar change patterns (Figure 1b-f): For T₂ and T₃ plants, SPAD, g_s , and E markedly
282 decreased 4 weeks after withholding water. However, Φ_{PSII} and F_v'/F_m' remained stable.
283 Rewatering for T₂ on 27, July, and for T₃ on 10, August, resulted in stimulations in
284 SPAD, g_s , E , Φ_{PSII} , and F_v'/F_m' ; However, they still could not fully recover to the
285 normal level. The drought stresses of T₄ and T₅ treatments also led to declines in the
286 gas exchange rates, while the chlorophyll fluorescence parameters remained relatively
287 stable except T₄ treatment decrease during drought episode and thereafter rewatering.

288 **3.1.2 Responses in middle leaves**

289 The changes in photosynthetic capacity of leaves at the plant middle position
290 exhibited a similar change pattern in the upper leaves (**Figure 2**): As compared to the
291 upper leaves, however, there were lower levels of A_{sat} , SPAD, g_s , and E during
292 drought episodes, while rewatering following the pre-drying also did not lead to a
293 complete restoration in the gas exchange parameters and SPAD values at later

294 grain-filling stage. The chlorophyll fluorescence parameters still maintained a high
295 level, implying that relatively mature middle leaves may have a drought-resistance in
296 terms of PSII photochemical activities as compared with the younger upper leaves.

297 **3.1.3 Responses in bottom leaves**

298 As measured in bottom leaves, there were greater levels in A_{sat} and SPAD under
299 various water treatments at jointing stage (1, July), declining with plant developing
300 (Figure 3). Plants at normal irrigation (control treatment) showed higher levels in the
301 gas exchange parameters (A_{sat} , g_s , and E) and SPAD values, while lower levels were
302 found during drought episodes, i.e., under T_3 , T_4 , and T_5 treatments. Only part
303 recovery was obtained upon rewatering at end of grain-filling stage in the plants
304 experienced pre-drying history (T_4 and T_5 treatments). The chlorophyll fluorescence
305 parameters such as Φ_{PSII} and F_v'/F_m' were maintained at a higher level as the plants
306 were subjected to withholding water treatments, particularly under T_3 treatment
307 (41-day withholding water during Jointing – anthesis). This again implicates an
308 adaptive response to drought episode in terms of chlorophyll PSII photochemical
309 activities.

310 The three-way ANOVA showed that watering treatment, leaf position, and
311 measured date produced significant effects on SPAD, A_{sat} , E and WUE, individually
312 ($P < 0.001$; Table 2). Only date alone and the interaction between leaf position and
313 date had significant effects on g_s ($P < 0.05$). The interactions between watering and
314 date, and between leaf position and date significantly affected SPAD, A_{sat} , and E ($P <$
315 0.001). Effect of leaf position and date alone, and their interaction on Φ_{PSII} were
316 significant; while the significant effect on F_v'/F_m' from date as single factor, and its
317 interaction with leaf position appeared. The interaction of the three factors was not
318 observed ($P > 0.05$).

319 The 2015-year experiment obtained the similar results on the responses of the
320 relative chlorophyll content (SPAD values) and photosynthetic potentials mainly
321 indicated by fluorescence parameters to the drought episode and the following
322 rewatering regimes at the same experimental site (see Figures S1-S3).

323

324 **3.2. Photosynthetically physiological responses in the leaves tagged at different**
325 **developments.**

326 We measured the same leaves tagged to examine the photosynthetically physiological
327 processes at different plant developments/leaf ages and the responses to episodic
328 drought and rewatering—just starting from emerging of the leaves to becoming fully
329 senesced. As showed in Figure 4, the leaf A_{sat} increased from the initial stage on first
330 July, reaching a maximum on 25 July; thereafter, linearly declining with plant
331 developing or leaf aging (Figure 4a). A gradual increase in SPAD values was observed
332 initially with a maximum occurrence on 31 July, and a marked decline at end of
333 grain-filling, i.e., 29 August (Figure 4b). Acute declines in E and g_s were obtained
334 after 31 July (Figure 4c,d); whereas the two chlorophyll fluorescence parameters
335 (Φ_{PSII} and F_v'/F_m') did not fluctuate greatly (Figure 4e,f).

336 As compared to the normal water supply, drought episodes of T_3 , T_4 , and T_5
337 treatments resulted in the declines in the tagged leaves' gas exchange
338 parameters— A_{sat} , g_s , and E , particularly with a longer drought persistence; while parts
339 of stimulations by rewatering occurred after an episodic drought (Figure 4a,c,d).
340 Decreases in SPAD values were often found as the drying episode occurred,
341 particularly at the end of grain-filling (Figure 4b). We found that the two chlorophyll
342 fluorescence parameters (Φ_{PSII} and F_v'/F_m') had a slight response to either drought
343 episodes or following rewatering, even with relative high levels upon the following
344 rewatering (Figure 4e,f). This indicated that, in terms of chlorophyll fluorescence
345 parameters, drought-tolerance may be enhanced with the leaf developing or its aging,
346 this issue may need to be investigated further for different crops and their cultivars.

347

348 **3.3. Responses of leaf morphological traits to drought and its relieving.**

349 Changes in maize plant canopy features (plant height, leaf area), leaf morphologic
350 traits (leaf size, leaf rolling index (LRI), leaf erection index (LEI), leaf bend degree
351 (LBD)) in responses to drought episodes and rewatering were shown in Table 3. The
352 results at first measurement (1 July 2016) showed that no significant changes in plant
353 height, plant individual leaf area, LRI, LEI, and LBD when the plants subjected to the

354 normally watering treatments. LRIs of T₂ and T₃ on 14 July increased significantly
355 due to their undergoing a 15-day drought episode duration. Plants of T₂ and T₃
356 reduced plant height and total leaf area, but increased LRI significantly on 25 July,
357 indicating that the two drought treatments significantly affected plant and leaf growth,
358 and morphological traits and canopy structure. LRI of T₃ was greatest under drought
359 on 31 July; and that of T₂ just following rewatering rapidly decreased relative to the
360 previous measurement; and the treatments of T₂ and T₃ had higher LBD. On 7 August,
361 LRIs under drought conditions, i.e., T₃, T₄ and T₅ treatments, were greater than that of
362 the control, while LEI and LBD were not affected significantly. At milking stage (R3,
363 On 14 August), LRI under T₄ treatment was greater significantly, and that of T₂
364 became lower close to control level due to rewatering. At the end of grain-filling stage
365 (measured on 29 August), T₂ plant leaf area was reduced significantly, whereas LRIs
366 were not significant between the watering treatments. LEIs of T₄ and T₅ treatments
367 were lower, indicating nearly rewatering did not trigger plant leaf erections with leaf
368 aging.

369

370 **4.4. Relationships between leaf morphological and functional traits in responses** 371 **to drought and rewatering.**

372 Relationships between the morphological and functional traits of upper leaves were
373 given in Table 4. The relationships among SPAD, A_{sat} , E , g_s , and WUE were
374 significant ($P < 0.05$) except the correlations of WUE with E and g_s . There were
375 significant relationships between the two chlorophyll fluorescence parameters— Φ_{PSII}
376 and F_v'/F_m' themselves; however, they did not correlate with SPAS values, and with
377 gas exchange parameters ($P > 0.05$). Among plant morphological traits, there were
378 significant and positive associations of plant height with total individual plant leaf
379 area, LBD; but negative with LEI. LEI was significantly and negatively correlated
380 with LBD. Between plant morphological and functional traits, there were significant
381 and negative correlations of plant height with A_{sat} , g_s , WUE, and also those of LRI
382 with SPAD, A_{sat} , E , g_s and WUE. Significant positive correlations of LEI with A_{sat} and
383 g_s were found, whereas significant negative correlations of LBD with SPAD, A_{sat} , g_s

384 and WUE appeared, implicating the two leaf morphological indicators (i.e., LEI and
385 LBD) may play an antagonistic role in the responses to drought episode and
386 rewatering.

387 To elucidate the critical linkage of the important functional traits such as
388 relationship between the chlorophyll content and photosynthetic capacity in responses
389 to watering treatment regimens at different growth stages, the correlations between
390 SPAD and A_{sat} were analysed. The results showed that no significant relationship was
391 observed from jointing to tasseling stages (Figure 5a), whereas significant-positive
392 relationships appeared from silking to blistering ($R^2 = 0.12$, $P < 0.001$), and from
393 milking to denting ($R^2 = 0.41$, $P < 0.001$; Figure 5b,c), indicating that their correlation
394 becomes stronger with plant developmental process, particularly at later grain-filling.
395 In addition, no significant relationship was found under normal irrigation condition
396 (Figure 5d), meanwhile the significant relationship appeared under drought episode or
397 rewatering treatments (Figure 5e,f), implying that their correlation can be enhanced
398 due to the watering treatments.

399 Based on the principal component analysis (PCA) on leaf functional and
400 morphological traits at silking stage, the first two principal components (PCs)
401 accounted for 60 % of the total variations of the maize traits (Figure 6). Gas exchange
402 parameters closely positively correlated with principle component one (PC1), while
403 the chlorophyll fluorescence parameters closely positively correlated with principle
404 component two (PC2). There were negative correlations of PC2 with SPAD, plant
405 height, and leaf area. The loadings were well typically distributed: the gas exchange
406 parameters were located on the right side, the two chlorophyll fluorescence
407 parameters upper part. SPAD was put alone on in quadrant III, while LRI was
408 separated separately in quadrant II. Finally, the two plant growth traits were located
409 on quadrant IV, contrasting to LRI. Plots of the two factor scores demonstrated that
410 the drought episode treatments (T_4 and T_5) were located on right-bottom parts, which
411 is far from the loadings of gas exchange, chlorophyll fluorescence quantum yields. It
412 implicates that the drought episode gave a severe impact on the photosynthetic
413 capacity, and PCA may well represent the change patterns in plant and leaf growth,

414 morphological and functional responses to the drought episode and rewatering
415 regimes.

416

417 **Discussion**

418 Maize, as one of most important staple crops, playing critical role in food security and
419 husbandry industry development (Campos *et al.* 2004; FAO 2017), is being hampered
420 severely by adverse climate change, such as abnormal precipitation alterations and
421 heat wave events. Drought that is been intensifying by global warming is one of major
422 limitations to maize growth and final grain yield (Boyer 1982; Sharp *et al.* 2004;
423 Lobell *et al.* 2014; Jiao *et al.* 2014; Avramova *et al.* 2015; Myers *et al.* 2017).
424 Although the effect on crop from drought as a single stress factor is studied
425 extensively, understanding the crop functional processes in responses to drought
426 episode and rewatering is still relatively scant, particularly across an entire plant
427 development. The present results indicated that drought during jointing period
428 produced severe effects on vegetative growth and photosynthetic capacity, while
429 continuous drought during tasseling greatly-negatively impacted the reproductive
430 growth and leaf photosynthetic capacity. Rewatering could alleviate adverse effects of
431 pre-drought, but not stimulate both vegetative and reproductive growth and
432 photosynthetic activities to recover fully to the levels of the control. Meanwhile, the
433 leaf chlorophyll fluorescence parameters showed a relatively-stable and adaptive
434 changes in response to episodic drying regimes. Moreover, the leaf functional traits
435 mostly significantly negatively correlated with morphological indicators, and leaf
436 rolling index (LRI) could be a sensitive indicator to assess the response of plants to
437 drought and rewatering. Moreover, the association of light-saturated net
438 photosynthetic rate (A_{sat}) with chlorophyll content (SPAD value) can be as an
439 effective proxy of leaf aging and the drought episodic durations. The present results
440 may provide a newly profound insight into understanding the crops' adaptive
441 mechanism to drought cycle and rewatering regimes, and might be useful for the
442 drought-resistant breeding practices and water-saving irrigation managements.

443 Nevertheless, the future models have well predicted that more extreme climate
444 events will happen by the end of this century, making drought severer and heat waves
445 more frequently (Luterbacher *et al.* 2004; Schär *et al.* 2004; IPCC 2014). The
446 consequences of water availability loss have a marked influence on crop growth and

447 productivity (Stuhlfauth *et al.*1990; Ciais *et al.* 2005; Chaves *et al.* 2009), finally
448 reducing the quantity and quality of grain yield (Marco & Tricoli.1993; Serraj &
449 Sinclair. 2002; Barnabás *et al.* 2008; Lobell *et al.* 2014), and threatening food security
450 regionally even globally (Ghannoum 2009; Myers *et al.* 2017). Among them,
451 photosynthesis—the fundamental process for determination of crop growth and its
452 development, even the final grain yield, is primarily affected by crucial climatic
453 change factors such as drought (e.g., Boyer 1982; Chaves *et al.* 1991; Jefferies *et al.*
454 1994; Angelopoulos *et al.*1996; Farooq *et al.* 2009; Shen *et al.* 2015). The current
455 results indicated that changes in the photosynthetically physiological parameters of
456 the upper leaves are most significantly different between the different irrigation
457 treatments ($P < 0.05$). Compared with control, under drought episode, the net
458 light-saturated photosynthetic rate (A_{sat}), chlorophyll content (SPAD value), stomatal
459 conductance (g_s), and transpiration rate (E), significantly decreased, while the actual
460 quantum efficiency of PSII (Φ_{PSII}), the effective quantum yield of PSII
461 photochemistry (F_v'/F_m') were no significantly declined. The previous experiments
462 showed that the net photosynthesis rate decreases with g_s dropping (Tenhunen 1987;
463 Jarvis & Davies 1998; Miyashita *et al.* 2005). A decrease in g_s will reduce water loss
464 under drought stress, which also generates a drop in CO_2 uptake (Frederick *et al.* 1989;
465 Miyashita *et al.* 2005). When the plants of T_2 treatment (pre-anthesis drought episode)
466 was re-watered on 27 July, the photosynthetic parameters of leaves such as A_{sat} , SPAD,
467 g_s , E , Φ_{PSII} and F_v'/F_m' mostly harmonically increased by the rewatering following the
468 pre-drought episode; the similar responses to rewatering also occurred under T_3 , T_4
469 and T_5 treatments (**Figures 1-3**). As reported, precipitation events could generate a
470 rapid response of the plant biological processes, which can trigger plants growth and
471 development (e.g., Acevedo *et al.* 1971; Reynolds *et al.* 2004), but the values of
472 photosynthetic parameters generally were lower than control level (Xu *et al.* 2009; Xu
473 *et al.* 2010; Suralta *et al.* 2017). The results illustrated the continuous drought
474 constrained photosynthetic capacity of the upper leaves, and the following rewatering
475 is unable to totally recover to normal level (**Figures 1 & 4**). As reported previously,
476 the degree and rate of recovery of rehydration might rely on the duration and severity

477 of pre-drought (Xu & Zhou 2007; Chen *et al.* 2009; Xu *et al.* 2009, 2010; Abid *et al.*
478 2016).

479 The change patterns of middle leaves' photosynthetic parameters were similar to
480 those of the upper leaves (**Figures 1-3**): Under the normal treatment, SPAD, A_{sat} , g_s , E ,
481 remained at a higher level compared with drought episodes (T_3 , T_4 , and T_5 treatments).
482 Rewatering did not promote the photosynthetic capacities of T_4 , T_5 at the grain-filling.
483 In relation to the chlorophyll fluorescence parameters, which has been ones of the
484 most practical and extensively used indicators to analysis plant eco-physiological
485 processes (e.g., Maxwell & Johnson. 2000). Of them, F_v'/F_m' , as most useful one,
486 often ranges between 0.80 and 0.83 without photoinhibition (Björkman & Demmig
487 1987). After suffering from severe water deficit stress, however, F_v'/F_m' would
488 decrease (Epron *et al.* 1992; Souza *et al.* 2004). However, our results showed both
489 F_v'/F_m' and Φ_{PSII} can maintain a high level even under severe drought. The results may
490 explain that the middle leaves' period of growth and development is longer, compared
491 with the upper leaves. It may demonstrate a higher drought adaptability in the
492 photochemical process and play a critical role in plant biomass accumulation and final
493 grain yield (also see Allison & Watson 1966; Xu *et al.* 2008, 2011; Chen *et al.* 2016).
494 For the bottom layer leaves, SPAD and A_{sat} were highest at the jointing stage (first
495 measured on 1 July). Since then, they decreased with plant growing. The influence of
496 drought on the bottom leaves was also consistent with the upper ones. SPAD and gas
497 exchange parameters were also reduced markedly by the drought episode. At the end
498 of grain-filling stage (24 August), the stimulating effect of rewatering was also lost
499 partly. The two chlorophyll fluorescence parameters, however, remained higher under
500 and after drought, especially at the end of grain-filling stage (e.g., withholding water
501 from jointing to anthesis stages, total 41-day drought episode). It may again reflect the
502 adaptive ability of maize elder leaves' photochemical activity in responses to drought
503 episodes and thereafter rewetting.

504 The results of the tagged leaves showed that along with the growth of the same leaf,
505 the changing trends of the photosynthetic physiological parameters under each
506 treatment were various (**Figure 4**): under control treatment, A_{sat} remained at a low

507 level measured on 1 July, reaching a maximum on 25 July, thereafter linearly
508 declining. SPAD reached a maximum on 31 July, thereafter significantly decreasing
509 until grain-filling stage (29 August). Stomatal conductance and E decreased sharply
510 after 31 July, whereas the changes in chlorophyll fluorescence parameters (Φ_{PSII} ,
511 F_v'/F_m') remained stable. The relative chlorophyll content and leaf gas exchange
512 parameters of leaves in normally irrigated plants were at a high level, while those of
513 the plants drought-stricken treatments (T_3 , T_4 and T_5 treatments) were at a low level,
514 especially at the later grain-filling stage. It may highlight the leaf senescence process
515 and its enhancement by drought stress (also see Xu *et al.* 2008; 2010). Rewatering can
516 trigger gas exchange process; the chlorophyll fluorescence parameters (Φ_{PSII} , F_v'/F_m'),
517 however, were less affected by drought stress and rehydration (Lu & Zhang 1999; but
518 Ghannoum *et al.* 2003; Gallé *et al.* 2007). Above all, the results again indicated that
519 the drought-tolerance of the chlorophyll fluorescence parameters may increase with
520 the plant/leaf growth and development when as measured in the same leaves (e.g., the
521 tagged leaves). This issue remains debated, and needs the further research.

522 With the progresses of the maize plant and leaf growth, the photosynthetic
523 performances of the upper, middle and lower leaves and the marked leaves of the
524 maize may gradually decrease under moderate and severe drought stresses, and then
525 recover partly after rewatering, which was consistent with previous research results
526 (e.g., Flexas *et al.* 2009; Vaz *et al.* 2010; Xu *et al.* 2011). The current results also
527 found that the photosynthetic performance of the middle leaves was higher than those
528 of the both upper and lower leaves. The reasons may be due to the middle leaves
529 being longer and greater than the upper one, and continuous drought may exacerbate
530 further premature senescence of the bottom leaves (Iacono & Sommer 2000; Lu *et al.*
531 2001; He *et al.* 2002; Xu *et al.* 2011). Thus, the middle leaf has stronger
532 photosynthetic performances than both the upper and the lower ones, and it may
533 contribute to most of carbon accumulation as plants exposed to drought episode and
534 the following rewetting.

535 The sensitivity of crop yield to water deficits often markedly differs at different
536 plant growth stages, which has been a classic study topic (Taylor *et al.* 1983; Fereres

537 & Soriano 2007)—Generally, staple crops including maize are more sensitive to water
538 deficit during seedling emergence, flowering, and early grain-filling than those during
539 early plant growth and late grain-filling periods (e.g., Doorenbos & Kassam 1979).
540 Under non-lethal water deficit during early vegetative growth, marked reductions in
541 maize plant height and biomass were often found, and its crop phenology could delay,
542 but this may not closely link to a lower yield potential (Dampney & Aspinall 1976;
543 Abrecht & Carberry 1993). However, maize plant at jointing stage has exuberantly
544 metabolic activities; under drought condition, the inhibition of plant height from
545 jointing stage to tasseling stage was significantly greater than those at other stages
546 (NeSmith & Ritchie 1992; Earl & Davis 2003). Therefore, the treatment with
547 withholding water during the jointing period would mainly affect plant vegetative
548 growth, leading to vegetative growth inhibition with short plant size, small leaves and
549 internodes, and delays of tassel emergence, silk emergence, and onset of grain filling,
550 finally a grain yield loss by 15-25% (Dampney & Aspinall 1976; NeSmith & Ritchie
551 1992). The current results indicated that the pre-anthesis drought significantly affected
552 plant height and leaf size, even the canopy structure (and also see Earl & Davis 2003;
553 Ne Smith & Ritchie 1992). Cakir (2004) found that the water content in the early
554 growth stage of maize has great influence on plant height, consequently resulting in
555 decline in canopy absorption of photosynthetically active radiation (PAR, Earl &
556 Davis 2003). Thus, an early pre-anthesis water deficit might also not be neglected to
557 obtain high yield even high in maize field, particularly with the severe and
558 consecutive drought events (also see Ne Smith & Ritchie 1992). Our results
559 manifested that the pre-anthesis drought markedly limited vegetative growth, lead to
560 the declines in plant height and leaf area; and although the rehydration could alleviate
561 the adverse effects from drought, it could not return fully to normal level at either
562 early or later plant development stages. It highlights the drought constraints to maize
563 plant growth during not only drought-persisting but drought-relieving periods.
564 Additionally, leaf rolling index (LRI, Xiang *et al.* 2012), as a key parameter of plant
565 morphological characteristics, obviously increased during drought episodes, but
566 immediately decreased by rewatering. Therefore, it is suggested that the easy changes

567 in LRI can be as a sensitive indicator for sensing drought and rewatering during
568 almost entire plant growing season.

569 As stated above, drought stress seriously affected the photosynthesis of maize,
570 inhibited the growth and development, and eventually leading to drops in plant
571 biomass and yield (Irigoyen *et al.* 1996; Bruce *et al.* 2002; Zhang *et al.* 2012). Maize
572 grain yield is highly sensitive to drought during tasseling-silking period, mainly due
573 to the marked decline in grain number (Otegui *et al.* 1995; Bolanos & Edmeades 1996;
574 Barnabás *et al.* 2008). Our results also found that although the plants of T₄ and T₅
575 treatments were normally irrigated at jointing stage, the episodic drought at later
576 tasseling stage, the ear growth was still significantly constrained relative to the control
577 group. The current results of T₄ and T₅ treatments (pro-anthesis drought) showed no
578 significant effect of drought at tasseling stage on the earlier plant growth. It again
579 highlights that the later drought may exert a greatly adversely influence on the
580 reproductive growth of maize plants.

581 The analysis on the correlation and principal component analysis reveal that there
582 were positive correlations between the functional traits, except those with Φ_{PSII} and
583 F_v'/F_m' ; although a positive correlation themselves between Φ_{PSII} and F_v'/F_m' was
584 found. A significant-positive correlation between plant height and total leaf area, and a
585 negative correlation between functional traits and morphological parameters occurred.
586 SPAD is recognized an ideal non-destructive method for testing chlorophyll status in
587 plant leaves, it has been widely used to investigate the environmental adaptability of
588 crops (Manetas *et al.* 1998; Uddling *et al.* 2007; Steele *et al.* 2008; Ciganda *et al.*
589 2009; Pour-Aboughadareh *et al.* 2017). Interestingly, with the development of maize
590 growth, the correlation of A_{sat} and SPAD was more positive and stronger, particularly
591 under drought and rehydration conditions (Figure 5). Some results showed that the
592 values of SPAD had a higher correlation with actual chlorophyll content under the
593 conditions of lower actual chlorophyll content levels (Steele *et al.* 2008; Ciganda *et al.*
594 2009), depending on species and the chlorophyll distribution on leaf (Uddling *et al.*
595 2007). Therefore, probably maize plants grew at later growth stage and moisture
596 condition became stressful, it may lead to reductions in both actual chlorophyll

597 content and photosynthetic performance. Thus, the relationship between A_{sat} and
598 SPAD might become more positive and closer (**Figure 5**). It is indicated that the
599 relationships among the functional traits of leaves would alter with plant development
600 and environmental changes, which may become closer and stronger with senescing at
601 the later growth stage and especially under adverse environmental conditions.

602 In conclusion, from the current field experiment, episodic drought may exert
603 markedly negative effects on photosynthetic potentials at either pre-anthesis or
604 post-anthesis stages, particularly during early grain-filling; and the rapid recovery of
605 leaf photosynthetic activities following re-irrigating occurred, but the recovery
606 magnitude and rate might depend on the severity and persistence of the previous
607 drought, and the leaf age and plant development. Generally, the leaf gas exchange,
608 and leaf rolling index may demonstrate higher sensitivity to drought episode and
609 thereafter rewatering than these chlorophyll content and its fluorescence parameters.
610 The results would provide a profound insight into how to understand the crop
611 functional traits' responses to various drought stresses and precipitation or irrigation
612 regimes. Finding the appropriate indicators for delayed leaf senescence while
613 increased/stabilized photosynthetic potentials under changing water status may assist
614 us in well dealing with climatic change to ensure crop production stability (Xu *et al.*
615 2010; Chen *et al.* 2013; Ben-Ari *et al.* 2016). Nevertheless, unambiguous
616 understanding the underlying mechanisms of molecular and eco-physiological
617 responses to various drought events, and helping the staple crops to cope with future
618 climatic change to guarantee food security regionally and globally still remain a huge
619 challenge, and that needs to be investigated further (Lobell *et al.* 2014; Gray *et al.*
620 2016; Myers *et al.* 2017).

621

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627

628 **Availability of data and materials**

629 The data sets supporting the results of this article are included within the article and
630 its supporting information file.

631

632 **Authors' contributions**

633 ZX and GZ conceived and designed the study; HS, YL, LZ, ZX conducted the
634 experiment and performed the data analysis; HS and ZX drafted the manuscript. All
635 authors approved the final manuscript.

636

637 **Competing interests**

638 The authors declare that they have no competing interests.

639

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901

902 **Table 1** Simulated rainfall regimes in the field experiment under a rain-shelter.

Growth stage	Irrigation dates(Month/day)	T ₁	T ₂	T ₃	T ₄	T ₅
		Irrigating at every 7-day	Drought episode (27 days)	Drought episode (41 days)	Drought episode (41 days)	Drought episode (34 days)
Irrigation amount (mm)						
V3~V13	5/24	8.7	8.7	8.7	8.7	8.7
	5/30	0.8	0.8	0.8	0.8	0.8
	6/8	10	10	10	10	10
	6/15	10	10	10	10	10
	6/22	10	10	10	10	10
	6/29	24	24	24	24	24
V13~VT	7/6	24	0	0	24	24
	7/13	24	0	0	24	24
	7/20	25	0	0		25
VT~R4	7/27	25	25	0	0	0
	8/3	25	25	0	0	0
	8/10	25	25	25	0	0
	8/17	25	25	25	0	0
	8/24	24	24	24	24	24
Entire period	Total precipitation	260.5	187.5	137.5	135.5	160.5

903 Notes: The dates measuring photosynthetic parameters are 1, July (V13, jointing); 14, July (V21);
 904 25, July (VT, tasseling); 31, July (R1, Silking); 7, August (R2, blistering); 14, August (R3,
 905 milking); 29, August (R4, dough), respectively. Red parts indicate the drought episodes. Relative
 906 Soil water moisture (0 – 50 cm depth) decreased to a severe water deficit stress level of 30-40% at
 907 the ends of the drought episodes, meanwhile it maintained a normal level of 70-80% in the control
 908 plots or after rewatering. The maize growth stage nomenclature refers to Abendroth *et al.* (2009),
 909 http://lib.dr.iastate.edu/extension_pubs/26. Verified 21 July 2017.

910

911 **Table 2** A significance list based on ANOVA for the effects and their interactions on the traits
 912 from watering, leaf position, and date

	SPAD	A_{sat}	E	g_s	WUE	q_P	NPQ	Φ_{PSII}	F_v'/F_m'
W	<0.001	<0.001	<0.001	0.988	0.007	0.114	<0.001	0.215	0.946
LP	<0.001	<0.001	<0.001	0.589	<0.001	<0.001	0.018	0.005	0.063
Date	<0.001	<0.001	<0.001	0.009	<0.001	<0.001	<0.001	<0.001	<0.001
W×LP	0.768	0.578	0.469	0.515	0.271	0.825	0.194	0.633	0.399
W×D	<0.001	0.006	<0.001	0.324	<0.001	0.692	<0.001	0.307	0.512
LP×D	<0.001	0.002	<0.001	0.014	0.454	<0.001	0.178	<0.001	<0.001
W×LP×D	0.156ns	0.963ns	0.939ns	0.946ns	0.452ns	0.692ns	0.990ns	0.850ns	0.635ns

913 Note: P values are given. ns, no significant difference; W, LP, and D denote watering treatment, leaf
 914 position, and measurement data, respectively. ×, interaction sign; SPAD, relative chlorophyll content;
 915 A_{sat} , light-saturated photosynthetic rate; E , transpiration rate; g_s , stomatal conductance; WUE, water use
 916 efficiency; Φ_{PSII} , PSII actual quantum yield; F_v'/F_m' , maximum quantum yield; q_P , photochemical
 917 quenching; NPQ, non-photochemical quenching.

Table 3 Effects of drought and rewatering on the plant growth and morphological indicators of maize (2016).

Traits	Treatments	1 Jul	14 Jul	25 Jul	31 Jul	7 Aug	14 Aug	29 Aug
Plant height (m)	T ₁	1.434±0.066a	2.014±0.097a	2.667±0.084a	2.717±0.119a	2.734±0.134a	2.729±0.144a	2.717±0.134a
	T ₂	1.336±0.057a	1.897±0.051a	2.233±0.093b	2.240±0.084a	2.232±0.092b	2.428±0.224a	2.214±0.108a
	T ₃	1.476±0.07a	1.897±0.051a	2.233±0.093b	2.327±0.132a	2.436±0.148ab	2.407±0.157a	2.333±0.199a
	T ₄	1.477±0.043a	2.014±0.097a	2.670±0.045a	2.710±0.147a	2.745±0.104a	2.751±0.110a	2.730±0.099a
	T ₅	1.355±0.009a	2.014±0.097a	2.667±0.084a	2.463±0.224a	2.556±0.212ab	2.527±0.230a	2.520±0.272a
Total leaf area (m ² plant ⁻¹)	T ₁	0.389±0.025a	0.823±0.035a	0.998±0.018a	1.049±0.021a	1.037±0.044a	0.974±0.017a	0.848±0.009a
	T ₂	0.400±0.039a	0.769±0.064a	0.871±0.034b	0.890±0.065a	0.930±0.047a	0.809±0.038b	0.643±0.08b
	T ₃	0.437±0.01a	0.769±0.064a	0.871±0.034b	0.953±0.097a	0.871±0.054a	0.686±0.042b	0.692±0.082ab
	T ₄	0.423±0.038a	0.823±0.035a	0.988±0.023a	1.025±0.002a	0.974±0.016a	0.768±0.058b	0.781±0.012ab
	T ₅	0.363±0.006a	0.823±0.035a	0.998±0.018a	0.877±0.078a	0.879±0.067a	0.837±0.07ab	0.738±0.043ab
Leaf rolling index (LRI, %)	T ₁	9.801±0.814a	13.031±1.87b	7.993±2.824b	5.381±0.285c	9.712±1.127d	9.795±1.410c	12.907±0.682a
	T ₂	10.143±0.795a	19.274±0.614a	17.626±1.356a	11.702±2.561b	12.126±2.358cd	10.453±0.910c	18.344±4.505a
	T ₃	11.116±0.288a	19.274±0.614a	17.626±1.356a	23.194±1.653a	26.575±0.513a	17.24±1.212ab	18.692±1.192a
	T ₄	9.691±1.278a	13.031±1.87b	9.594±1.546b	10.163±0.466b	16.867±0.658b	21.926±2.889a	18.93±3.082a
	T ₅	11.322±1.539a	13.031±1.87b	7.993±2.824b	7.336±0.257bc	15.059±0.874bc	14.641±0.925bc	12.608±2.55a
Leaf erection index (LEI, %)	T ₁	71.734±2.621a	81.218±5.921a	87.103±2.10a	71.813±7.855a	70.960±2.811a	59.500±4.481a	79.330±7.217a
	T ₂	73.885±0.901a	81.710±6.650a	92.412±1.10a	59.130±6.364a	65.779±1.005a	54.834±5.317a	65.922±1.196a
	T ₃	70.300±1.434a	81.710±6.650a	92.412±1.10a	56.537±3.256a	61.011±4.763a	50.771±5.036a	68.788±3.498a
	T ₄	73.822±2.055a	81.218±5.921a	79.918±10.07a	65.282±7.415a	64.864±5.571a	50.230±1.623a	52.139±3.120b
	T ₅	71.160±6.133a	81.218±5.921a	87.103±2.10a	59.149±8.36a	56.684±7.818a	46.903±3.308a	49.341±4.209b
Leaf bend degree (LBD, °)	T ₁	37.417±1.856a	16.750±4.116a	9.333±1.764a	36.222±4.283c	53.567±6.19a	81.417±9.419a	59.983±20.037a
	T ₂	33.000±1.953a	16.000±6.948a	11.783±3.537a	50.694±5.022ab	41.833±2.485a	69.167±5.988a	44.233±13.638a
	T ₃	39.667±5.516a	16.000±6.948a	11.783±3.537a	55.833±4.757a	37.083±7.045a	71.117±7.148a	49.583±18.112a
	T ₄	33.917±1.609a	16.750±4.116a	13.833±2.167a	31.722±3.692c	45.167±6.749a	65.278±8.084a	83.694±11.916a
	T ₅	35.750±3.527a	16.750±4.116a	9.333±1.764a	37.944±1.811bc	50.733±2.709a	60.833±6.194a	55.361±4.835a

Note: T₁, T₂, T₃, T₄, and T₅ denote Control, withholding water during jointing-tasseling, jointing-anthesis, tasseling-milking, and silking-milking, with 260, 188, 138, 136, and 161 mm irrigation amount in entire development, respectively. Different little-letters with mean data indicate significant differences between the five watering treatments (bold parts, n = 3-5).

Table 4 Correlation coefficients between the functional and morphological traits of upper leaves

	SPAD	A_{sat}	E	g_s	WUE	Φ_{PSII}	F_v/F_m'	Plant height	Plant leaf area	LRI	LEI
A_{sat}	0.546**										
E	0.498**	0.813**									
g_s	0.427**	0.845**	0.816**								
WUE	0.304*	0.479**	0.042	0.186							
Φ_{PSII}	-0.152	-0.184	-0.039	-0.143	-0.206						
F_v/F_m'	-0.111	-0.045	0.045	-0.049	-0.119	0.911**					
Plant height	-0.232	-0.391**	-0.168	-0.316**	-0.344**	-0.036	-0.02				
Plant leaf area	-0.021	-0.211	-0.035	-0.222	-0.238	-0.015	0.021	0.854**			
LRI	-0.436**	-0.506**	-0.449**	-0.422**	-0.204	0.014	-0.039	0.18	0.094		
LEI	0.145	0.328**	0.217	0.264*	0.227	-0.166	-0.025	-0.298*	-0.185	-0.211	
LBD	-0.264**	-0.383**	-0.232	-0.310*	-0.279*	0.119	0.007	0.389**	0.139	0.151	-0.460**

* $P < 0.05$, ** $P < 0.01$ represent significant differences. LRI, leaf rolling index; LEI, leaf erection index; LBD, leaf bend degree. Others see table 2.

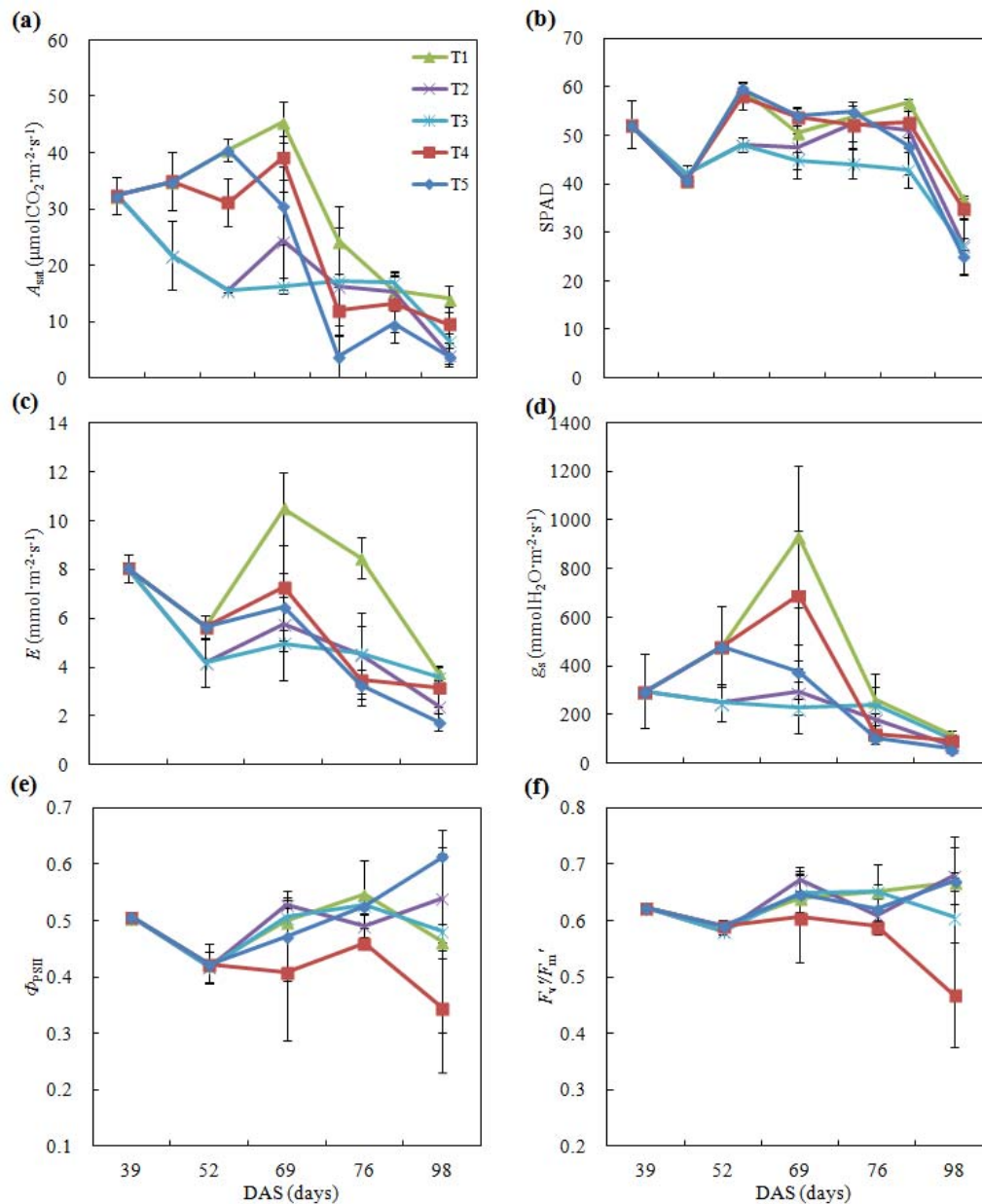


Figure 1. Effects of drought episode and rewating on photosynthetic physiological processes in upper leaves.

Notes: DAS, days after sowing; A_{sat} , light-saturated photosynthetic rate; SPAD, relative chlorophyll contents; g_s , stomatal conductance; E , transpiration rate; Φ_{PSII} , PSII actual quantum yield; F_v/F_m' , maximum quantum yield. T1, T2, T3, T4, and T5 denote Control, withholding water during jointing-tasseling, jointing-anthesis, tasseling-milking, and silking-milking, with 260, 188, 138, 136, and 161 mm irrigation amount in entire plant development, respectively.

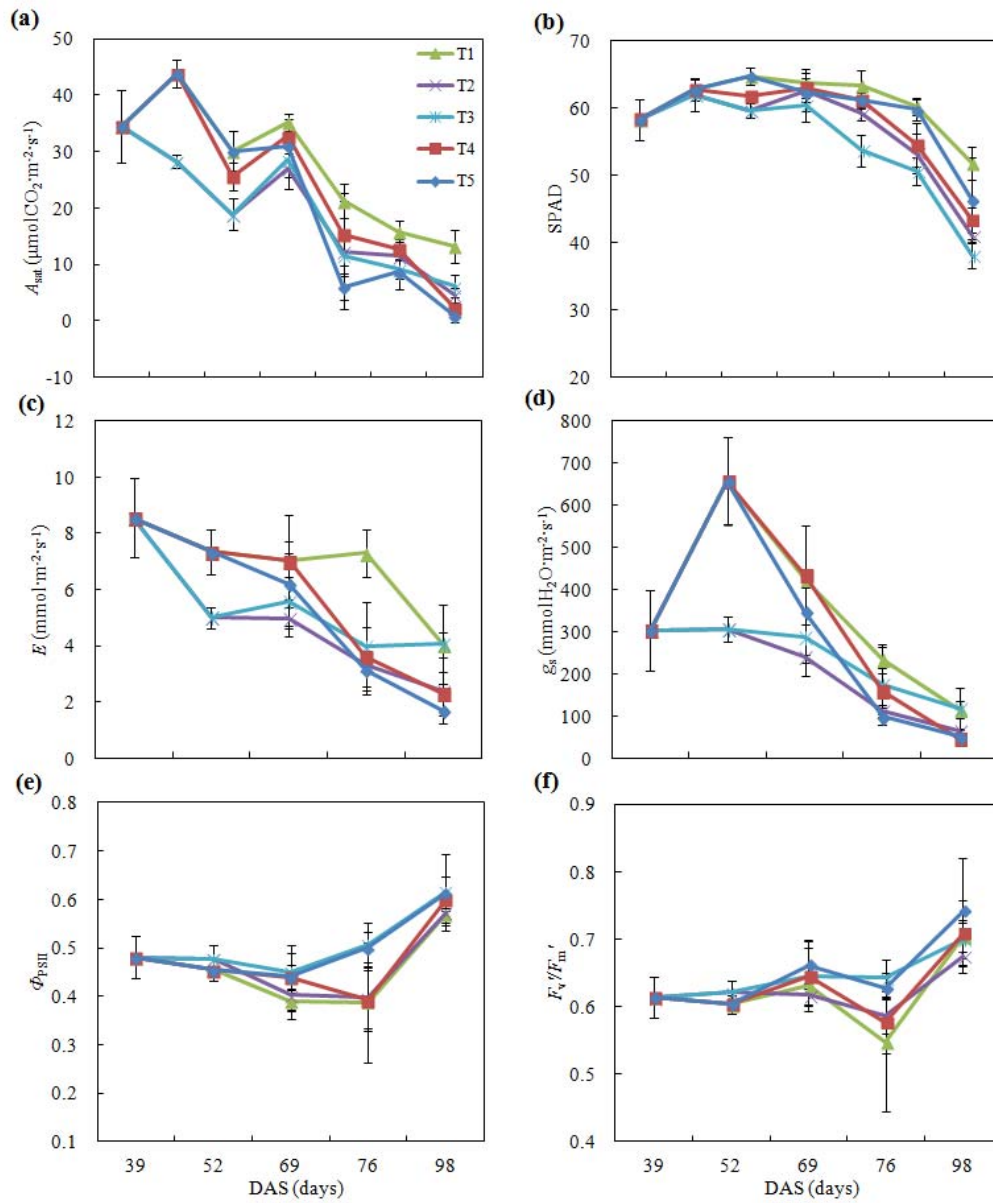


Figure 2. Effects of drought episode and rewatering on photosynthetic physiological processes in middle leaves.

Notes: DAS, days after sowing; A_{sat} , light-saturated photosynthetic rate; SPAD, relative chlorophyll contents; g_s , stomatal conductance; E , transpiration rate; Φ_{PSII} , PSII actual quantum yield; F_v/F_m' , maximum quantum yield. T1, T2, T3, T4, and T5 denote Control, withholding water during jointing-tasseling, jointing-anthesis, tasseling-milking, and silking-milking, with 260, 188, 138, 136, and 161 mm irrigation amount in entire plant development, respectively

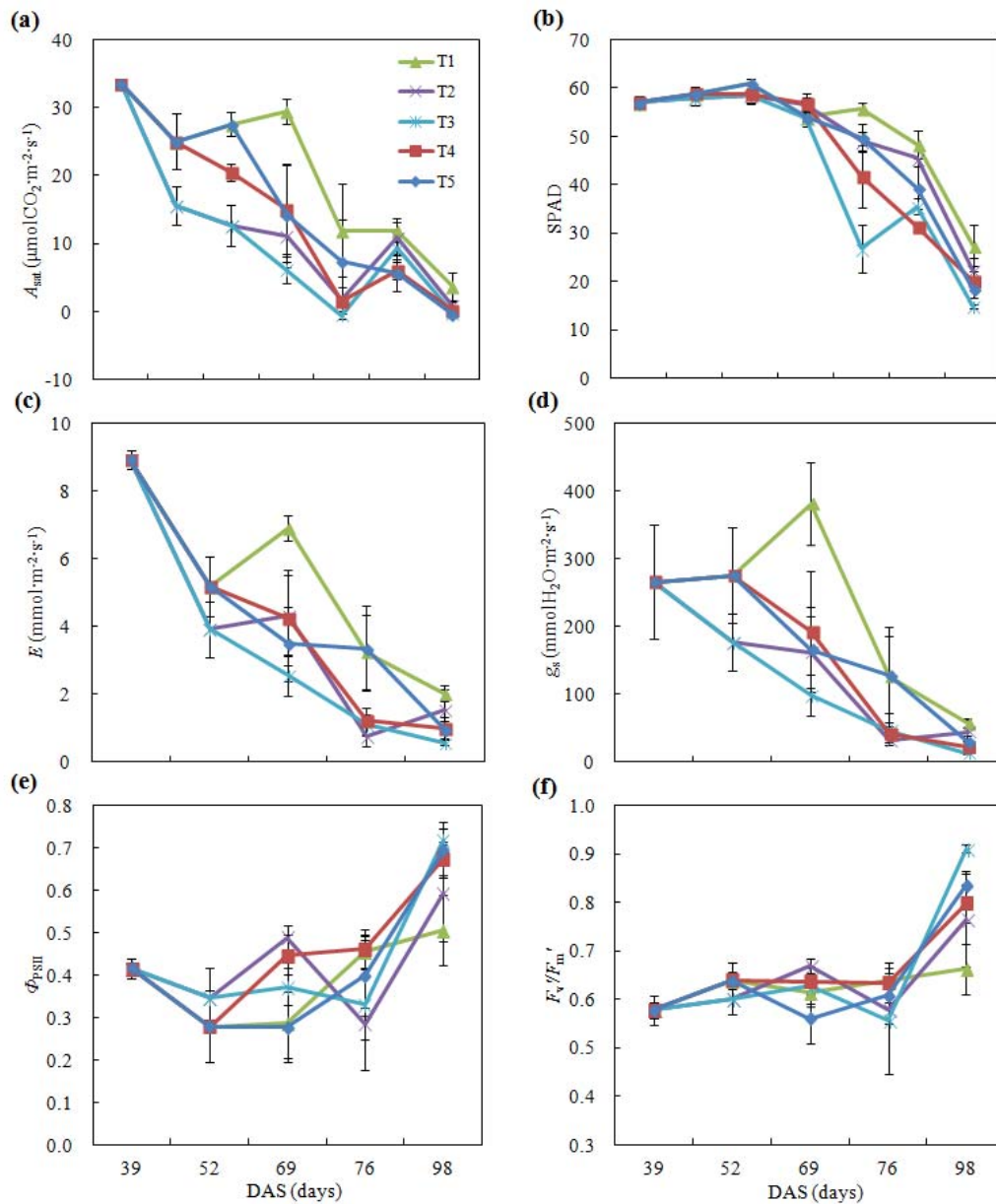


Figure 3. Effects of drought episode and rewatering on photosynthetic physiological processes in bottom leaves.

Notes: DAS, days after sowing; A_{sat} , light-saturated photosynthetic rate; SPAD, relative chlorophyll contents; g_s , stomatal conductance; E , transpiration rate; Φ_{PSII} , PSII actual quantum yield; F_v/F_m' , maximum quantum yield. T1, T2, T3, T4, and T5 denote Control, withholding water during jointing-tasseling, jointing-anthesis, tasseling-milking, and silking-milking, with 260, 188, 138, 136, and 161 mm irrigation amount in entire plant development, respectively.

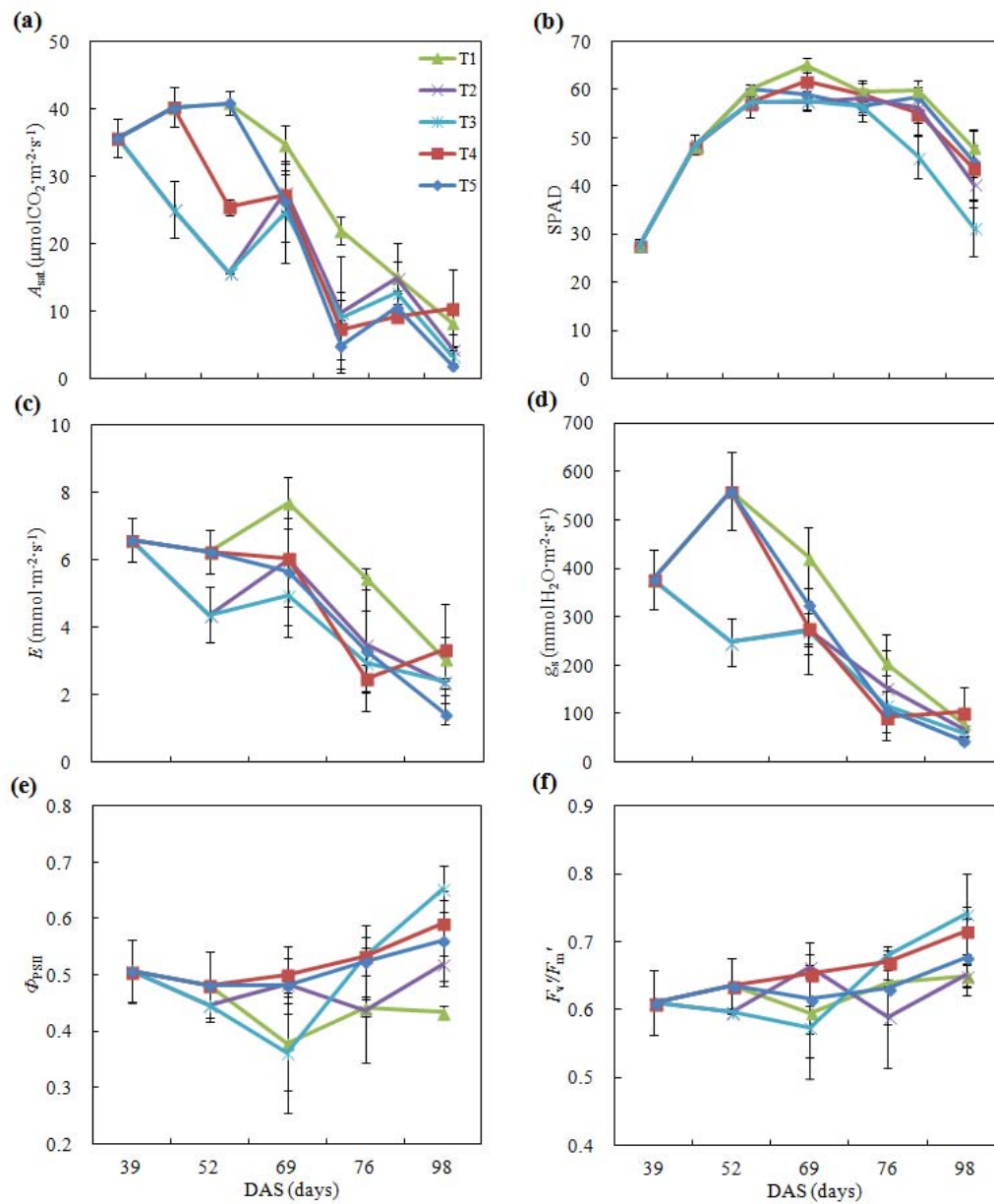


Figure 4. Photosynthetic physiological changes and the responses to drought episode and rewatering in the tagged leaves.

Notes: DAS, days after sowing; A_{sat} , light-saturated photosynthetic rate; SPAD, relative chlorophyll contents; g_s , stomatal conductance; E , transpiration rate; Φ_{PSII} , PSII actual quantum yield; F_v/F_m' , maximum quantum yield. T₁, T₂, T₃, T₄, and T₅ denote Control, withholding water during jointing-tasseling, jointing-anthesis, tasseling-milking, and silking-milking, with 260, 188, 138, 136, and 161 mm irrigation amount in entire plant development, respectively.

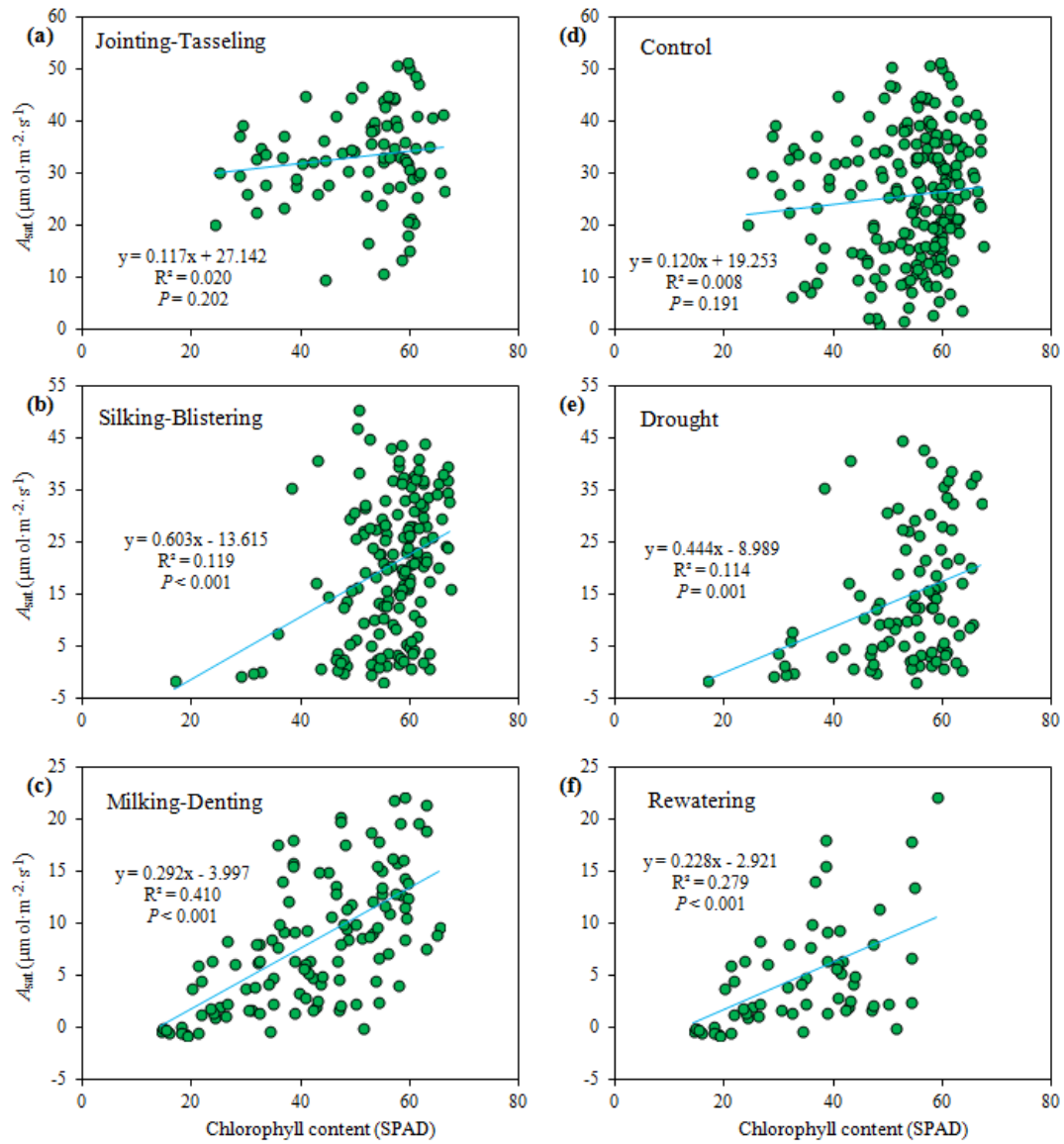


Figure 5 Effects of drought, rewatering, and plant developmental stages on relationships between light-saturated photosynthetic rate (A_{sat}) and relative chlorophyll content (SPAD readings).

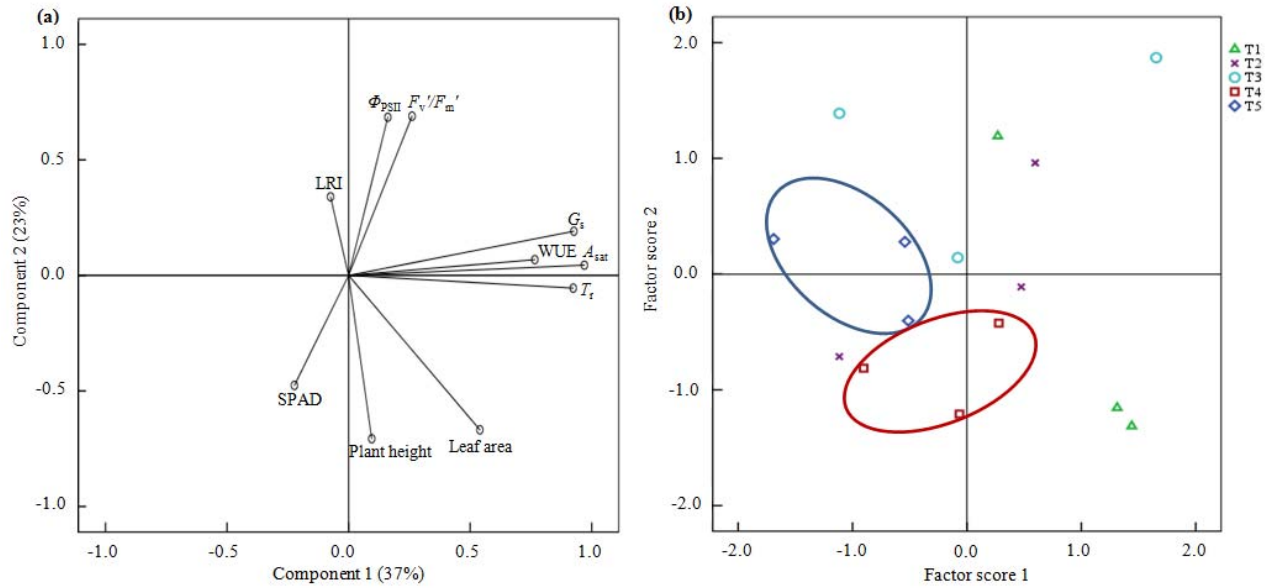


Figure 6 Principal component analysis on leaf functional and morphological traits and the effects of watering treatments at silking stage.

Notes: LRI, leaf-rolling index; WUE, water use efficiency; red ellipse is for T₄ treatment, while blue one is for T₅ treatment. For others see Fig. 1 and table 3.