Maize leaf functional responses to drought episode and rewatering

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Abstract

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

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 CO_2 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, since1950s 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*

57 *al*. 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

3

89   restoration of other metabolites (e.g. sugar content) was lagged (Pinheriro *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

2. Materials and Methods

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 (41º49´N, 121º12´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 ºC, with the extreme maximum temperature of

138 41.8 ºC and the extreme minimum temperature of -31.3 ºC; 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

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: T_1, T_2, T_3, T_4, and T_5 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 ℃ 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 = (Wet soil weight - Dry soil weight) / Dry soil weight / F.C. × 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.

6

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 μmol m^{-2}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 μmol mol^{-1}, and leaf temperature was set up at around 27 ℃. 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: $WUE = A_{sat} / E$.

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 μmol m^{-2} s^{-1} after

197   15 min to measure the steady-state fluorescence (F_s), and then gave a strong flash

198   (5100 μmol m^{-2} 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            $F_v'/F_m' = (F_m'-F_o')/F_m'$

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

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 ech 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, Ln), maximum width (at unfolding state,

219 Lw), natural length (Lnl), maximum length (Lsl), 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 $$LRI = (Lw - Ln) / Lw \times 100$$
225 $$LEI = Lnl / Lsl \times 100$$
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℃ 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

3. Results

3. 1. Photosynthetically physiological responses to drought episode and rewatering

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 μmol $m^{-2}s^{-1}$ on 31 July (R1 stage),

253   thereafter sharply decreasing until to a lowest point of 14.1 μmol $m^{-2}s^{-1}$ 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 μmol

257    $m^{-2}s^{-1}$ on 14, July (V21 stage), a 40.3% drop; and further down to a lower level of

258   15.6 μmol $m^{-2}s^{-1}$ on 25, July (VT stage), a 65.6% drop. Upon re-irrigating, it rose up

259   to 24.4 μmol $m^{-2}s^{-1}$ 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 μmol $m^{-2}s^{-1}$ 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 T_3 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   μmol m^{-2}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 μmol m^{-2}s^{-1}, Figure 1a).

271   While leaf A_{sat} of the T_4 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 μmol m^{-2}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 T_3 treatment, indicating that a stimulation occurred by the just

276   nearly rewatering. Leaf A_{sat} of the T_5 treatment (withholding water during

277   silking–milking, 34 days), similar to T_4 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 T_2 and T_3 plants, SPAD, g_s, and E markedly

282   deceased 4 weeks after withholding water. However, Φ_{PSII} and F_v'/F_m' remained stable.

283   Rewatering for T_2 on 27, July, and for T_3 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 T_4 and T_5 treatments also led to declines in the

286   gas exchange rates, while the chlorophyll fluorescence parameters remained relatively

287   stable except T_4 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_{PSI} 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

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

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

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

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

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

12

354   normally watering treatments. LRIs of T_2 and T_3 on 14 July increased significantly

355   due to their undergoing a 15-day drought episode duration. Plants of T_2 and T_3

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 T_3 was greatest under drought

359   on 31 July; and that of T_2 just following rewatering rapidly decreased relative to the

360   previous measurement; and the treatments of T_2 and T_3 had higher LBD. On 7 August,

361   LRIs under drought conditions, i.e., T_3, T_4 and T_5 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 T_4 treatment was greater significantly, and that of T_2

364   became lower close to control level due to rewatering. At the end of grain-filling stage

365   (measured on 29 August), T_2 plant leaf area was reduced significantly, whereas LRIs

366   were not significant between the watering treatments. LEIs of T_4 and T_5 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 ware 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 gave the change patterns in plant and leaf growth,

414 morphological and functional responses to the drought episode and rewatering

415 regimes.

416

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 a*l. 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

16

477   of pre-drought (Xu & Zhou 2007; Chen e*t 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 a*l. 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 leave 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 (Damptey & 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% (Damptey & 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 e*t al*. 1995; Bolanos & Edmeades 1996;

574   Barnabás *et al*. 2008). Our results also found that although the plants of T_4 and T_5

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 T_4 and T_5 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 e*t 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).

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Availability of data and materials

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

Authors' contributions

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

Competing interests

The authors declare that they have no competing interests.

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

Growth stage	Irrigation dates(Month/day)	T_1 Irrigating at every 7-day	T_2 Drought episode (27 days)	T_3 Drought episode (41 days)	T_4 Drought episode (41 days)	T_5 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	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.

31

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: T_1, T_2, T_3, T_4, and T_5 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****					
Pant 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.
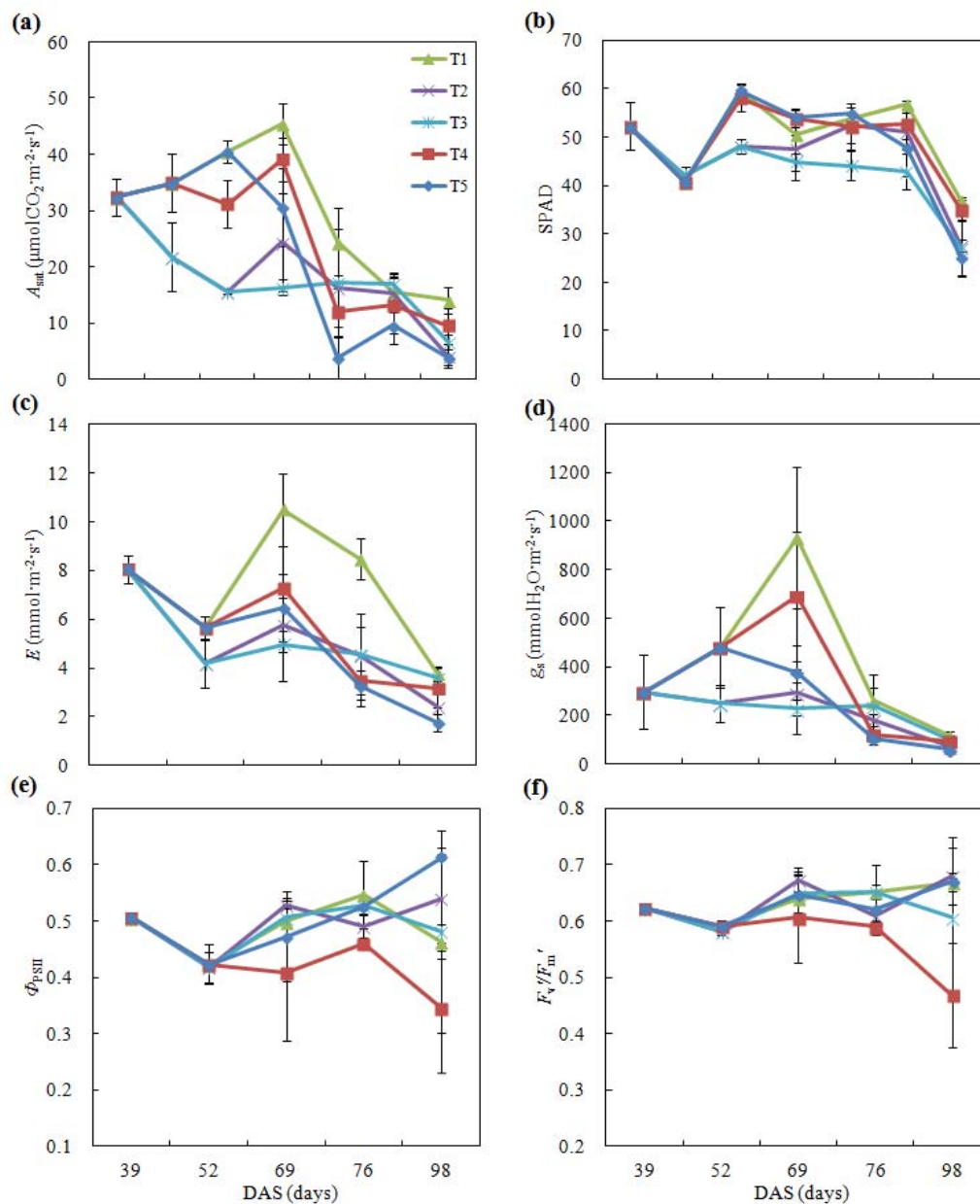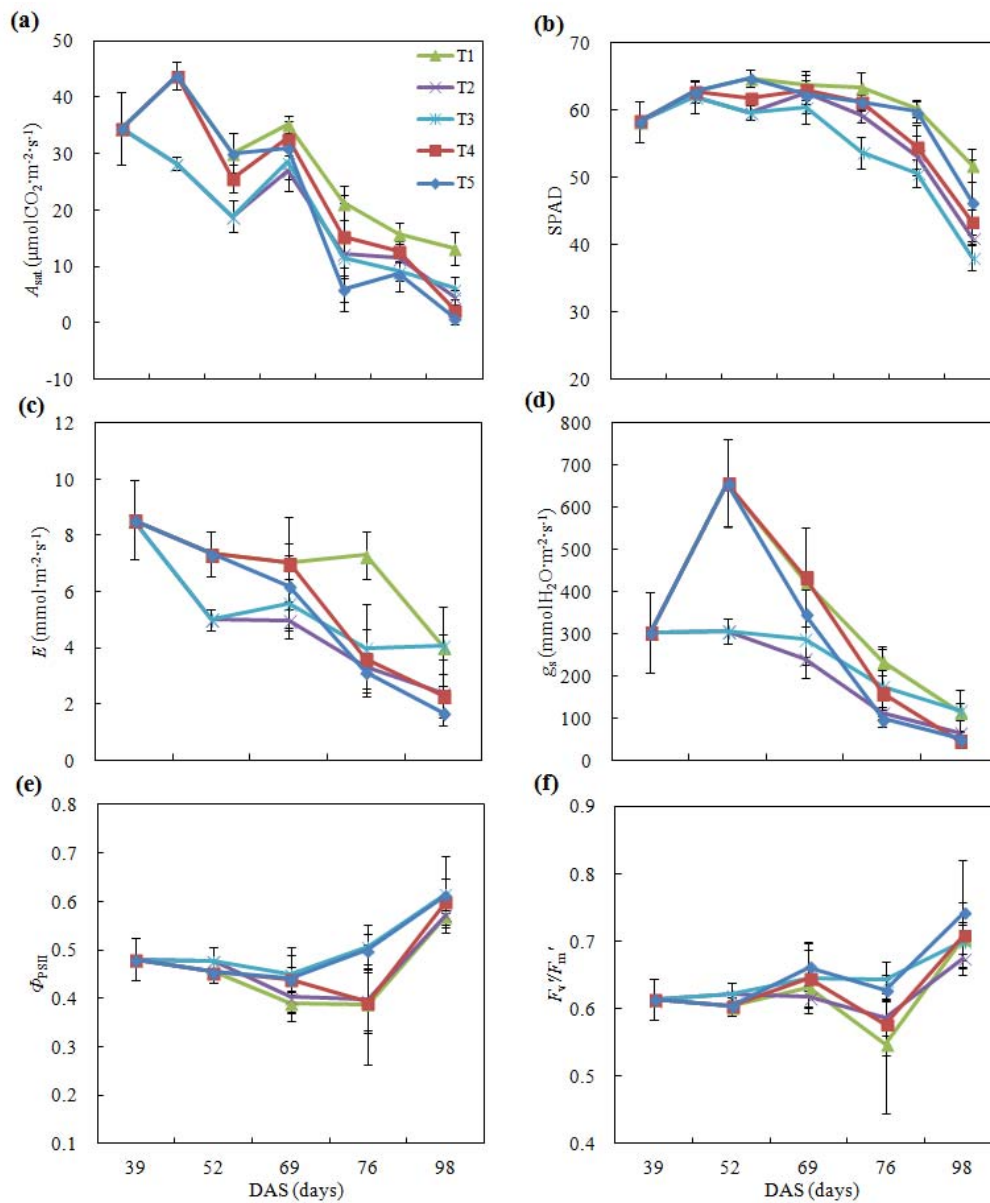
Figure 1. Effects of drought episode and rewatering on photosynthetic physiological processes in upper leaves.

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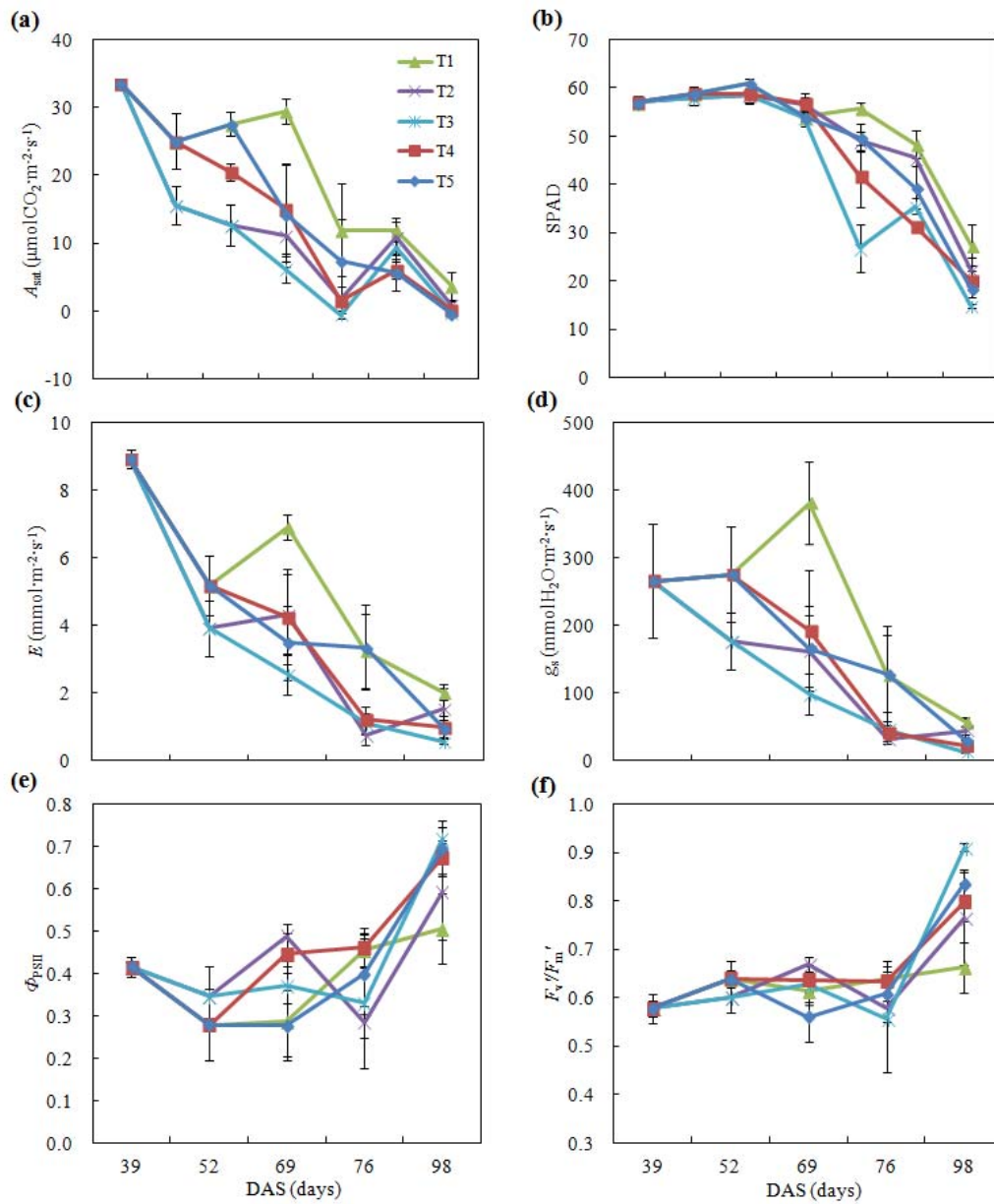
Figure 2. Effects of drought episode and rewatering on photosynthetic physiological processes in middle leaves.

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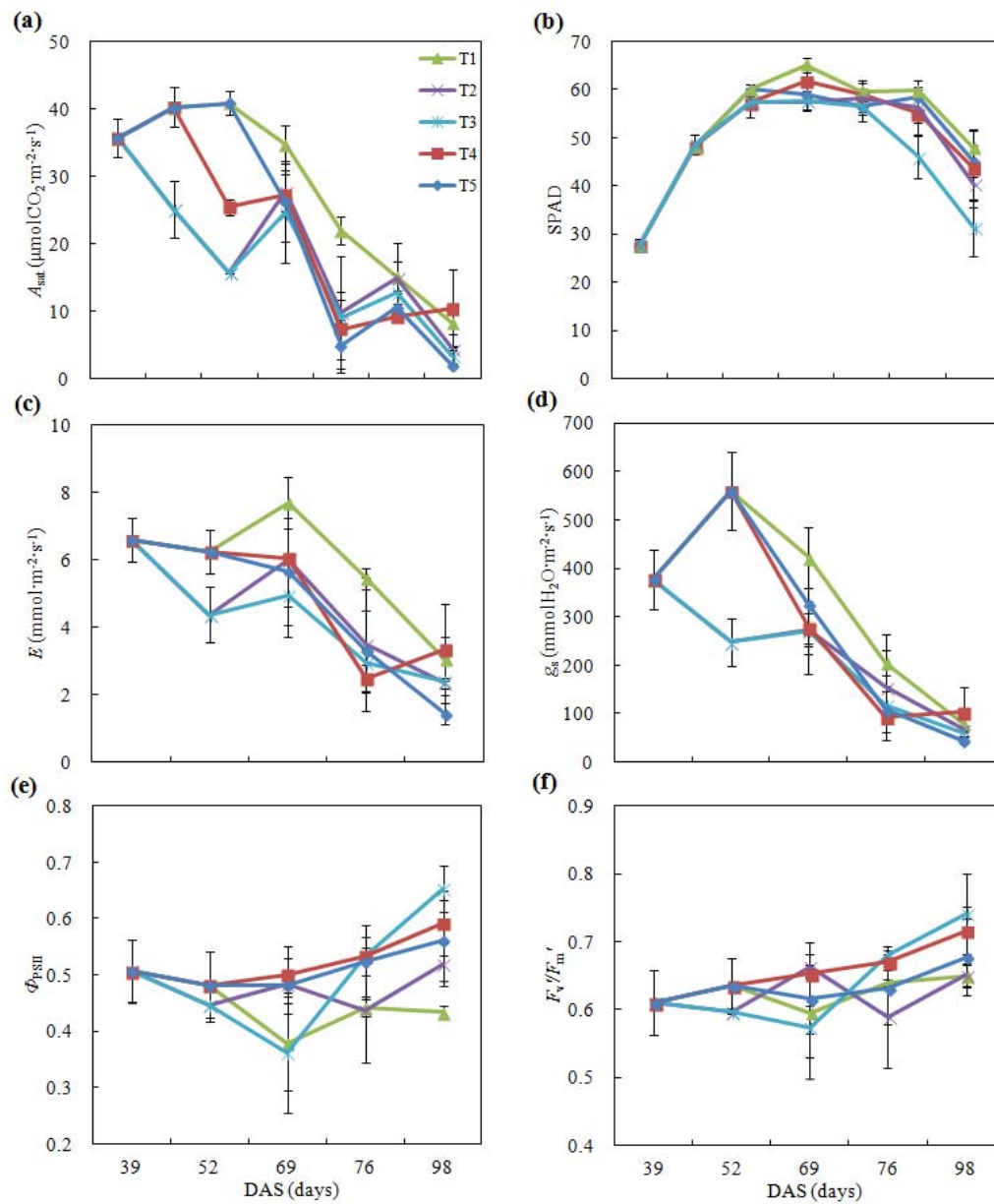
Figure 3. Effects of drought episode and rewatering on photosynthetic physiological processes in bottom leaves.

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. T_1, T_2, T_3, T_4, and T_5 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.
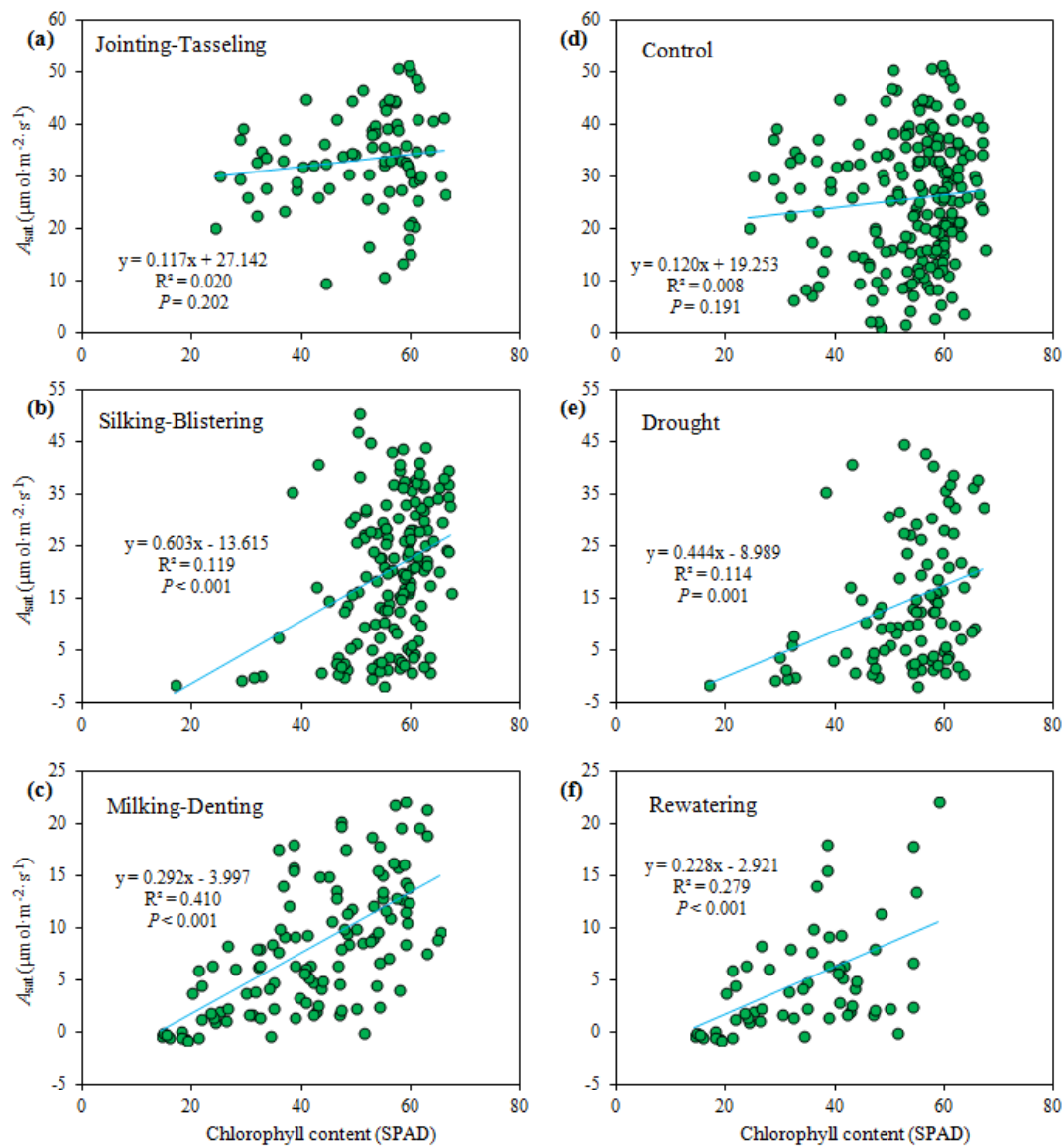
Figure 5 Effects of drought, rewatering, and plant developmental stages on relationships between light-saturated photosynthetic rate (A_{sat}) and relative chlorophyll content (SPAD readings).
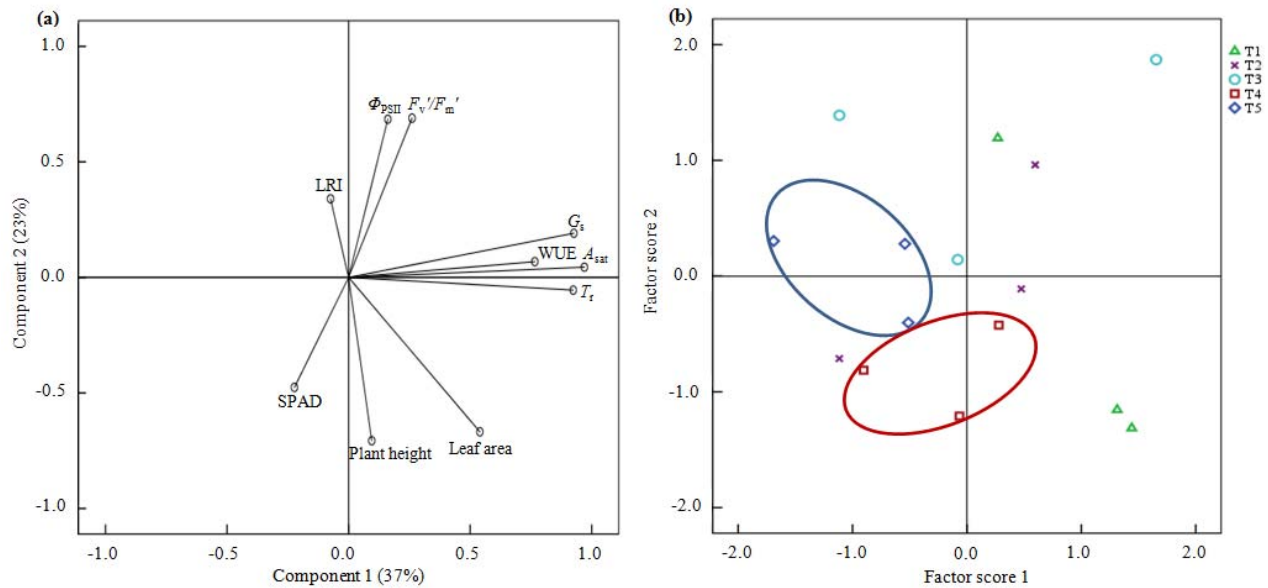
Figure 6 Principal component analysis on leaf functional and morphological traits and the effects of watering treatments at silking stage.

Notes: LRI, leaf-rolling index; WUE, water use efficiency; red ellipse is for T_4 treatment, while blue one is for T_5 treatment. For others see Fig. 1 and table 3.