

**A peer-reviewed version of this preprint was published in PeerJ on 28 September 2018.**

[View the peer-reviewed version](https://doi.org/10.7717/peerj.5532) (peerj.com/articles/5532), which is the preferred citable publication unless you specifically need to cite this preprint.

Wang L, Sun J, Wang C, Shangguan Z. 2018. Leaf photosynthetic function duration during yield formation of large-spike wheat in rainfed cropping systems. PeerJ 6:e5532 <https://doi.org/10.7717/peerj.5532>



20 **ABSTRACT**

21 Improving photosynthetic capacity significantly affects the yield of wheat (*Triticum aestivum*  
22 L.) in rainfed regions. In this study, the physiological characteristics of eight large-spike wheat  
23 lines were compared with a multiple-spike cultivar as a control (CK) in a field over two  
24 consecutive seasons: 2010–2012. The tillering peak was 7–21 d after returning green for line 2040,  
25 the average rate of decline of relative water content was slower, and the average duration time of  
26 photosynthetic rate was longer than CK *in vitro*. There was a strong linear and positive correlation  
27 between photosynthetic rate and root activity at jointing, flowering, and grain-filling stages. In  
28 addition, average yields were higher in large-spike lines than CK (multiple-spike cultivar). The  
29 results suggest that large-spike lines might have greater water retaining capacity during yield  
30 formation under rainfed conditions.

31 **Key words:** Large-spike wheat, Drought resistance, Grain yield, Photosynthesis characteristics

32

33

34

35

36

37

38 **INTRODUCTION**

39 Wheat (*Triticum aestivum* L.) is the most important staple winter cereal crop in the North  
40 China Plain, water shortage is a serious issue threatening the sustainable development of  
41 agriculture in this area (Zheng *et al.*, 2014). Drought tolerance is considered a valid breeding  
42 target in the stabilization of crop performance by breeders (Riccardi *et al.*, 2016). With the world  
43 population continuously increasing, much of the future food will have to come from rainfed areas  
44 (Turrall, Svendsen & Faures, 2010). The imperative to develop drought-resistant crops is  
45 intensifying due to increasingly limited water supplies for crop production.

46 Leaf senescence comprises a series of biochemical and physiological events from the fully  
47 expanded state until death. The green leaf area duration is one of the important physiological traits  
48 with implication for yield potential related to increasing assimilate (i.e. source) availability. The  
49 leaf duration after full expansion depends strongly on the water conditions and crop species; some  
50 researchers have reported that the post-anthesis senescence in cereals affects the whole plant, with  
51 organs closest to the developing grains (i.e. flag leaves and glumes) generally senescing last  
52 (Distelfeld, Avni & Fischer, 2014). The leaf relative water content (RWC) was found to be  
53 correlated to the drought resistance and water saving of wheat cultivars (Dong *et al.*, 2008;  
54 Khakwani *et al.*, 2012). Using RWC and leaf senescence as indirect selection criteria for wheat  
55 grain yield is an interesting alternative approach because these traits are easily and rapidly  
56 screened, and relatively inexpensive, and we can gain a partial understanding of many  
57 physiological mechanisms that confer drought tolerance and lead to the development of wheat  
58 better adapted to environments.

59        Photosynthesis plays an important role in modern winter wheat cultivars, especially under  
60        rainfed conditions, and it is the most crucial source of biomass accumulation in plants; the  
61        chlorophyll content of leaves is one of the major indicators of photosynthetic capability of plant  
62        tissues (Pietrini *et al.*, 2017; Nageswara, Talwar & Wright, 2001). Large-spike wheat is  
63        characterized by large spikes, high numbers of grains per plant, and high yield potentials (Wang,  
64        Chen & Shangguan, 2016), and it also shows greater physiological advantages in root activity  
65        [measured by triphenyl tetrazolium chloride (TTC) method], photosynthetic properties, and the  
66        number of secondary roots compared with multiple-spike cultivars (Guo *et al.*, 2009; Wang *et al.*,  
67        2010). The photosynthetic function duration of leaves is closely correlated to grain yield of wheat,  
68        and previous studies have focused on the yield and photosynthetic traits at growth period (Raven  
69        & Griffiths, 2015; Gaju *et al.*, 2016; Merchuk-Ovnat *et al.*, 2016). Few integrated studies on the  
70        variation of photosynthetic function duration following the time *in vitro* and the formation of  
71        biomass and yield in rainfed environments are available, and poorly investigated in large-spike  
72        wheat.

73        The main objectives of this study were to (1) investigate the differences in the dynamic  
74        changes of chlorophyll relative value (SPAD), RWC, and photosynthetic rate ( $P_n$ ) following time  
75        *in vivo* between eight large-spike lines and one multiple-spike cultivar (CK), (2) analyze the  
76        relationship between TTC and  $P_n$  of large-spike lines and (3) evaluate the changes in  
77        photosynthetic pigments, dry weight of single spike and leaf + stem + sheath between eight  
78        large-spike lines and CK during the growth period. The findings should provide theoretical  
79        foundations for guiding breeders in selecting high drought-resistant wheat materials, and to  
80        improve production potential of large-spike wheat under rainfed conditions.

## 81 MATERIALS AND METHODS

### 82 Plant materials

83 In our study, eight new high-yield and large-spike wheat lines (2005, 2013, 2026, 2036, 2037,  
84 2038, 2039 and 2040) were used, which had been tested in the Shaanxi provincial wheat variety  
85 trial test in 2009 and the Shaanxi provincial wheat variety regional test in 2010, and these lines  
86 were bred through many generations over many years. The winter wheat cultivar of Xi'nong 979  
87 (*Triticum aestivum* cv. Xi'nong 979) has been planted across large areas of the Huang-Huai-Hai  
88 production region of winter wheat (*Wang & Shangguan 2015*), the detailed characteristics of these  
89 materials were provided in Table 1 and would be analyzed in the part of discussion. The seeds  
90 were also sown in Sufang Town, Wugong City, Shaanxi Province, northwestern China (*Wang,*  
91 *Chen & Shangguan 2016*).

### 92 Culture conditions and experiment design

93 The field experiment was conducted in the Institute of Soil and Water Conservation, Chinese  
94 Academy of Sciences and Ministry of Water Resources, Yangling, Shaanxi (34°16'N, 108°04'E)  
95 during the winter wheat growing seasons (October–June) in 2010–2011 and 2011–2012. The  
96 experiment was located in the sub-humid warm temperate continental monsoon climate zone and  
97 generally had flat topography. The soil of the experiment was a well aerated Eum-orthic Anthrosol  
98 soil characterized by favorable permeability, strong water and nutrient-retaining capacities, and  
99 wide crop adaptation. There were similar precipitation (231.1 and 229.8 mm) trends and other  
100 climate conditions during the two wheat growing seasons, so we chose to analyze the data from  
101 2011–2012.

102 The field experiment was a randomized block design involving nine treatments, and each plot  
103 size was 2 m × 2 m with 10 rows (20 cm spacing) of wheat sown at 110 seeds/row. Surrounding  
104 the experimental field, there were five guard rows of wheat. Plants were sampled from the central  
105 rows in each plot. Each year, winter wheat was planted on 10 October and the same quantity of  
106 chemical fertilizer (360 kg hm<sup>-2</sup> N and 70 kg hm<sup>-2</sup> P<sub>2</sub>O<sub>5</sub>) was applied to the top 20 cm of soil  
107 before planting. During the growing period, no irrigation and fertilizer were applied; the site was  
108 plowed to bury weeds and pests before sowing, and weeds were hand-hoed several times during  
109 the growing period.

#### 110 **Population tiller measurement**

111 In each plot, six rows of wheat were chosen and tagged at sowing stage, and the number of tillers  
112 were counted at seedling and tillering stages before winter. After wintering, wheat tiller number  
113 data were collected at intervals of 7 d from returning green stage to heading stage. The last survey  
114 date of the number of spikes was at mature stage.

#### 115 **Relative value of chlorophyll measurements for intact expanded and detached leaves**

116 A portable chlorophyll meter (SPAD 502, Minolta Camera Co., Osaka, Japan) was used to take  
117 SPAD values from the three uppermost fully expanded leaves of leaf 5, leaf 7, and the flag leaf on  
118 each plant at 10-d intervals (Fig. 1). A total of six plants were measured per plot, and three SPAD  
119 values per leaf, including one value around the midpoint of the leaf blade and two values at 3 cm  
120 away from the midpoint were averaged as the mean SPAD value of the leaf (*Peng et al., 1993*).

121 From a total of six repeat plants, leaf 5, leaf 7, and the flag leaf were cut when fully expanded  
122 and immediately placed in Petri dishes containing distilled water, and cultured in the plant

123 ZPW-280B (DongTuo, Inc., Heilongjiang, China) incubator *in vitro*. During growth, the  
124 photoperiod was 12 h (day, 100–120  $\mu\text{mol m}^{-2} \text{s}^{-1}$ )/12 h (night), temperature was  $25 \pm 2/18 \pm 2^\circ\text{C}$   
125 (day/night), and relative humidity was 60%–70% (day/night). Measurement methods and  
126 measured parts of detached leaves were the same as for intact expanded leaves.

#### 127 **Photosynthetic rate measurement**

128 For *in vitro*  $P_n$  measurement, the leaves were separated from plants and preserved in liquid  
129 nitrogen immediately, the interval times of measure were 30 min (jointing and flowering stages)  
130 and 10 min (grain-filling stage) during 09:00–11:00 AM on clear days with a wind speed below 1  
131  $\text{m s}^{-1}$ .

132 The  $P_n$  of fully expanded top-down penultimate and flag leaves for all lines and cultivars was  
133 measured with a LI-6400XT Portable Photosynthesis System (LI-6400, *Li-Cor*, Inc., Lincoln,  
134 Nebraska, USA) equipped with 2 cm  $\times$  3 cm leaf chamber and integrated light source. For the  
135 measurement, leaves were intact with no sign of insect or disease attack and the upper one-third of  
136 leaves was used. While being measured, the settings were leaf chamber temperature of  $25^\circ\text{C}$ ,  
137 mean  $\text{CO}_2$  concentration of  $382.6 \pm 2.5 \mu\text{L L}^{-1}$ , PAR generated by the LED (mixed red and blue)  
138 system of  $1300 \mu\text{mol m}^{-2} \text{s}^{-1}$ , humidity of 53%–56%, and the gas flow rate of  $5 \text{ mL min}^{-1}$ . The  $P_n$   
139 was measured for 3 min until  $P_n$  and transpiration rates stabilized; for each plot, three randomly  
140 selected and fully expanded leaves were measured.

#### 141 **Relative water content measurement**

142 The RWC was measured following the methodology of Barr and Weatherley (1962). First, six disc  
143 samples were cut from the youngest fully expanded leaf at jointing, flowering, and grain-filling

144 stages, and immediately weighed to obtain their fresh weight (FW). Second, the same discs were  
145 placed on distilled water for 24 h in Petri dishes in darkness and, after gentle blotting to remove  
146 excess water, they were weighed to obtain turgid weight (TW). Finally, discs were weighed after  
147 drying at 60°C for 48 h until constant dry weight (DW) was reached. Using these three parameters,  
148 RWC was calculated using the following equation (1):

$$149 \quad \text{RWC (\%)} = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW}) \times 100 \quad (1)$$

150 The RWC was measured at 30-min intervals at jointing and flowering stages, and 10-min  
151 intervals at grain-filling stage.

#### 152 **Root activity measurement**

153 In the field, the root system was excavated to a size of 20 cm (length) × 20 cm (width) × 30 cm  
154 (depth) at the sampling periods of jointing, flowering, and grain-filling stages. The wheat roots  
155 were washed with deionized water and excised at 5 cm from the root tips. Then, root activity was  
156 determined by the TTC method (*Lindström & Nyström, 1987*).

#### 157 **Photosynthetic pigment measurements**

158 Immediately after the leaf had fully expanded, six leaves were collected from each plot, and each  
159 leaf was soaked with 80% acetone and the concentrations of carotenoids (Car), chlorophyll (Chl) *a*,  
160 and Chl *b* were determined by measuring absorbance at 663, 646, and 470 nm, respectively, using  
161 an UV-2300 spectrophotometer (Techcomp, Inc., Shanghai, China). The Chl concentrations and  
162 contents were calculated using the following formulae (*Arnon, 1949*):

$$163 \quad C_a = (12.21 \times A_{663}) - (2.81 \times A_{646}) \quad (2)$$

164  $C_b = (20.13 \times A_{646}) - (5.03 \times A_{663})$  (3)

165  $C_{x\cdot c} = (1000 \times A_{470} - 3.27 \times C_a - 104 \times C_b)/229$  (4)

166  $\text{Chl contents (mg g}^{-1}\text{)} = (C \times V_T \times n)/FW \times 1000$  (5)

167 where  $C_a$ ,  $C_b$ , and  $C_{x\cdot c}$  are the concentrations of Chl *a*, Chl *b*, and Car, respectively;  $C$  is the Chl  
168 concentration ( $\text{mg L}^{-1}$ );  $V_T$  is the volume of extracting solution (mL);  $FW$  is the fresh weight of  
169 the leaves, and  $n$  is the dilution multiplier.

#### 170 **Dry weight measurement**

171 In each plot, 100 plants that had headed and flowered on the same days were chosen and tagged,  
172 and flowering date was defined as the time when 50% of the plants had flowered (*Wang &*  
173 *Shangguan, 2015*). Ten plants were collected as samples approximately every 5 d from the first  
174 day after the onset of flowering. Every plant was divided into single spike and leaf + stem +  
175 sheath parts. Then the two sample parts were used for DW measurement immediately after  
176 sampling by deactivating at 105°C and then drying at 80°C to a constant weight.

#### 177 **Yield trait measures**

178 At maturity, 20 plants were randomly selected in each plot for measurement of spike length,  
179 number of grains per spike, kernel weight per spike, number of spikes, and 1000-grain weight. At  
180 the same time, six rows (1 m length) were selected for estimation of grain yield, excluding borders  
181 in each plot.

#### 182 **Statistical analysis**

183 The data in the tables are the average value of three replicates in the form of means  $\pm$  SE (standard  
184 error). The significant differences ( $P < 0.05$ ) were tested by SPSS ver. 14.0 (SPSS Inc., Chicago,  
185 IL, USA), and the differences among the treatments were tested by Duncan's multiple range test.

## 186 **RESULTS**

### 187 **Dynamic changes in tiller death rate**

188 During the tillering period of seeding–heading and mature stage, there were fewer tillers for  
189 large-spike lines than for Xi'nong 979 (Fig. 2). There were different tillering peaks for different  
190 wheat materials, the peak for lines 2005, 2039, 2040 and CK was 2 weeks after returning green,  
191 and that for line 2013 was 3 weeks after returning green. Although there were two tillering peaks  
192 during the growth periods for lines 2036 and 2037, they were 7 d and 3 weeks after returning  
193 green. At mature stage, there was twice the number of tillers for Xi'nong 979 compared with  
194 large-spike lines, showing their obvious differences in tiller death rates.

### 195 **Time courses of declination of chlorophyll relative values**

196 The dynamic changes of SPAD were measured in intact expanded and detached leaves at different  
197 leaf positions for wheat lines. For the *in vivo* situation, the SPAD peaks were at different positions  
198 for wheat lines, the peaks for leaf 5 were around 0 and 6 d after expansion, and the highest values  
199 of leaf 7 and the flag leaf were around 24 d. In intact expanded leaves, the degenerative processes  
200 of SPAD values for leaf 7 and flag leaf included a relative steady phase and a rapid declining  
201 phase, and these were observed in the different wheat lines. The SPAD of leaf 5 showed a uniform  
202 decline for the different lines; the average SPAD of leaf 5, leaf 7, and the flag leaf of large-spike

203 lines was 4.29, 6.53, and 6.04 higher than CK, respectively (Fig. 3A–C).

204 In the situation *in vitro*, SPAD values showed continuous declines, and the average values of  
205 leaf 7 and flag leaf of large-spike lines were 5.64 and 4.62 higher than CK, respectively (Fig.  
206 3D–F).

#### 207 **Time courses of RWC and $P_n$ decline**

208 At the jointing stage, the leaf RWC of large-spike lines and CK showed declining trends; the rate  
209 of decline of leaf RWC for lines 2037, 2038 and 2040 was 1.74%, 2.32%, and 0.10% lower than  
210 for CK after 70 min *in vitro* (Fig. 4A). The  $P_n$  of lines 2036, 2037, and 2038 was higher than for  
211 CK, and values were 0.65, 0.55 and 1.18  $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$  after 130 min *in vitro*, respectively,  
212 compared to a negative  $P_n$  of CK for the same times. The average duration times of  $P_n$  of lines  
213 2037, 2038, and 2040 were longer than for the other lines and CK (Fig. 5A), indicating that the  
214 three lines had strong drought resistance.

215 At the flowering stage, the leaf RWC in all wheat lines slowly declined, and the values for  
216 lines 2013 and 2026 were higher than CK after 130 min *in vitro*; the decrease amplitudes of leaf  
217 RWC for lines 2005, 2013, 2026, and 2037 were smaller than for CK after 10–130 min *in vitro*,  
218 showing that they maintained a good water balance (Fig. 4B). The declining trend of  $P_n$  in eight  
219 lines and CK was stronger at flowering than at jointing stage; the  $P_n$  of lines 2036, 2038, 2039,  
220 and 2040 were higher than CK after 130 min *in vitro*; the decrease in amplitudes of  $P_n$  in lines  
221 2005, 2013, 2026, 2039, and 2040 were smaller than CK after 10–130 min *in vitro* (Fig. 5B),  
222 showing that they had a long photosynthetic duration.

223 At the grain-filling stage, the leaf RWC of all lines was within 78–90%; the values of lines  
224 2036, 2038, and 2040 were higher than CK after 100 min *in vitro*, and decreases in amplitudes of

225 the RWC of these lines were smaller than CK after 10–100 min *in vitro* (Fig. 4C). The variation  
226 tendencies of  $P_n$  for wheat materials differed, except for line 2036, the values of the large-spike  
227 lines were higher than for CK after 100 min *in vitro*, and the decrease amplitudes of  $P_n$  in all  
228 large-spike lines were lower than CK after 10–100 min *in vitro* (Fig. 5C), showing that large-spike  
229 lines had strong drought resistance and good water retaining capacity.

### 230 **Relationship between $P_n$ and TTC**

231 The pooled analysis showed a strong linear and positive correlation between  $P_n$  and TTC of  
232 large-spike lines at jointing, flowering, and grain-filling stages in this rainfed environment ( $P <$   
233 0.001; Fig. 6). The results indicate that TTC might be a good tool for indirect assessment of  $P_n$ .

### 234 **Photosynthetic pigment contents**

235 The differences in Chl *a* content between large-spike lines and CK were not significant (Table 2).  
236 At the jointing stage, the Chl *a* contents of lines 2036, 2037, 2039, and 2040 were higher than CK;  
237 the Chl *b* contents of large-spike lines were all lower than CK; and the Car contents of lines 2026,  
238 2036, 2037, 2039, and 2040 were higher than CK. At heading stage, the Chl *a* and *b* contents of  
239 lines 2026, 2036, 2037, 2039, and 2040 were higher than CK; and the Car contents of lines 2026,  
240 2036, 2037, and 2040 were higher than CK. At the flowering stage, the Chl *a*, Chl *b*, and Car  
241 contents of lines 2026 and 2040 were higher than CK. At the grain-filling stage, the Chl *a*, Chl *b*,  
242 and Car contents of line 2040 were higher than CK.

### 243 **DW of single spikes and other parts**

244 The DW of leaf + stem + sheath for large-spike lines and CK all increased after the flowering  
245 stage; with average maximum DM reached at 15 d after flowering being 3.57 and 2.21 g plant<sup>-1</sup>,

246 respectively, and then gradually declining. The DW of leaf + stem + sheath for large-spike lines  
247 and CK all reached their minimum values at 44 d after flowering: 2.11 and 1.21 g plant<sup>-1</sup>,  
248 respectively. The DW of leaf + stem + sheath of large-spike lines and CK began to decrease at 20  
249 d after flowering and the photosynthetic assimilation substances were gradually transported to  
250 grain, and were reduced to 59.17 and 54.78% of the maximum DW by 44 d after flowering,  
251 respectively (Fig. 7).

252 The DW of wheat grain showed an “S” growth pattern from flowering to maturity; the  
253 average DMW of single spikes for large-spike lines and CK all reached maximum values 44 d  
254 after flowering: 3.34 and 2.38 g plant<sup>-1</sup>, respectively. The average aboveground biomass of  
255 large-spike lines at 5 d after flowering and maturity stage were 4.13 and 5.45 g plant<sup>-1</sup>,  
256 respectively, which were 77.03 and 51.62% higher than for CK (Fig. 7).

### 257 **Yield and primary components**

258 The average spike length, number of grains per spike, kernel weight per spike, and 1000-grain  
259 weight of the eight large-spike lines were significantly higher than those of CK (Xi'nong 979) ( $P$   
260  $< 0.05$ ), and the number of spikes per hectare was significantly lower for large-spike lines than CK  
261 (Table 3). The yields of lines 2005, 2026, 2037, 2038, 2039, and 2040 were 3.00, 8.97, 8.33, 5.18,  
262 5.24, and 7.03% higher than those of CK, respectively. These results indicated future positive  
263 effects for improving grain yield of large-spike lines by coordinating the relationship among  
264 agronomic traits.

### 265 **DISCUSSION**

266 The carbon assimilation capacity and dry matter accumulation level of wheat plants at the

267 jointing stage have an important influence on the number of spikes, growth, and development at  
268 later periods. This study indicated different tillering peaks for different wheat lines, and the  
269 materials had one or two tillering peaks. At the mature stage, CK had twice the number of tillers  
270 compared with large-spike lines, showing obvious differences in tiller death rate between the  
271 large-spike lines and CK. The reason for this may be the large individual characteristics and  
272 storage capacity of large-spike wheat, in which most of the photosynthetic products made by the  
273 leaves might be used to supply the grain and self-growth – this would cause the low tillering rates  
274 due to nutritional deficiency, according to research on the accumulation and translocation of  
275 assimilation substance in large-spike wheat by *Wang, Chen and Shangguan (2016)*. Therefore, the  
276 tiller numbers of large-spike lines should be improved by regulating row spacing, density, sowing  
277 date (*Guo et al., 2009; Wang et al., 2010*). This will be the key to enhancing photosynthetic  
278 performance, increasing the tillering rate, and taking full advantage of spike grain weight for  
279 realizing high yield of large-spike wheat.

280       Loss of green (reduced chlorophyll content) in leaf was the first symptom of leaf senescence,  
281 and the duration of leaf life differed at different plant positions, as shown in SPAD differences.  
282 The leaf photosynthesis function duration at the late growth period was closely related to grain  
283 yield, and yield might be improved by the extension of leaf photosynthesis function duration (*Xie,*  
284 *Mayes & Sparkes, 2016*). This study showed two periods of SPAD changes in intact leaves –  
285 relatively steady and rapidly declining phases – at different positions of plants for different wheat  
286 materials. When leaves were detached from plants, the chlorophyll relative values continuously  
287 decreased; however, *Cao et al. (2001)* showed a similar change tendency for fully expanded and  
288 detached leaves in rice, and different crops may have different processes of decline. The “loss in

289 green” process was faster for detached than fully expanded leaves, because the chlorophyll content  
290 of detached leaves may be more easily degraded, but the fully expanded leaves might be regulated  
291 by the plant body and other organs. The SPAD for each leaf position, detached leaves, and fully  
292 expanded leaves differed among the different wheat lines.

293 The ability to maintain green leaf area duration during grain filling is one important  
294 physiological trait with implications for yield potential related to increasing assimilate (i.e. source)  
295 availability. For the nine wheat lines in our study, the duration of SPAD values for the flag leaf  
296 were much shorter than those for leaf 5 and leaf 7 after leaves were fully expanded (leaf 7 > leaf  
297 5 > flag leaf); however, when leaves were detached, the durations of SPAD values were in the  
298 order of leaf 5 > flag leaf > leaf 7. *Yang et al. (2014)* reported that the rate of decrease in SPAD  
299 readings increased with leaf age, as leaves were physiologically older in the lower than in the  
300 upper canopy, and the rate of decrease in SPAD readings was faster in lower leaves. Chl  
301 degradation is regulated by phytochrome, and continuously accelerating rate of leaves  
302 photosynthetic pigments at different position is the factor responsible for the difference in SPAD  
303 readings (*Mielke, Schaffer & Schilling, 2012*). This study also showed that the Chl *a*, Chl *b*, and  
304 carotenoid contents of large-spike lines were higher than CK at heading, flowering, and  
305 grain-filling stages. In addition to the difference in shading degree, the rates of decrease in SPAD  
306 readings for different leaf positions may be influenced by other environmental factors such as  
307 ambient temperature and humidity.

308 Water conditions influence photosynthetic activity of photosystem II by affecting the steady  
309 state contents of its primary functional protein complexes (*Slabbert & Kruger, 2011*). Varieties  
310 with high RWC under stress conditions show high drought tolerance and yield (*Belkheiri & Mulas,*

311 2013). Our study showed that the RWC and  $P_n$  for wheat materials showed a declining trend after  
312 being detached from the plant. At the jointing stage, lines 2037, 2038, and 2040 had a lower rate  
313 of decline of leaf RWC and a longer average duration time of  $P_n$  than CK, and so showed strong  
314 drought resistance. Lines of 2005, 2013, and 2026 maintained a good water balance and long  
315 photosynthetic duration at flowering stage, and the lines of 2036, 2038, and 2040 showed strong  
316 drought resistance and good water retaining capacity at grain-filling stage. Differences in RWC  
317 may be attributed to differences in their ability to absorb more water from the soil and or control  
318 water loss through the stomata during growth, it may also be a result of their varied genetic ability  
319 to absorb water in the existing rooting zone and or extending rooting depth to increase water  
320 reserve for crops (Siddique, Hamid & Islam, 2000; Blokina, Virolainen & Fagerstedt, 2003). The  
321 range of tolerance to dehydration would depend on the species and stage of development (Saint  
322 Pierre et al., 2012). For further progress in breeding for drought resistance, we will focus on  
323 subtle cultivar-level differences in expression of the gene networks involved in stress adaptation,  
324 and so improve final wheat yield.

325       Photosynthesis is among the plant physiological processes most sensitive to variations in soil  
326 moisture. Some researchers have shown that large-spike cultivars have more advantages in  
327 photosynthetic capacity and root activity than multiple-spike cultivars, and their carbohydrates  
328 storage capacity is large (Guo et al., 2009). The absorption of water and nutrients in maize roots  
329 was closely related to aboveground physiological processes, and strong root activity benefited the  
330 root system in absorbing water (Zhou et al., 2008). The degree of root activity indicated the ability  
331 of the root system to absorb water and transport water from the soil, and this influenced the leaf  
332 gas exchange parameters. Our pooled analysis showed a strong linear and positive correlation

333 between  $P_n$  and root activity of large-spike lines at jointing, flowering, and grain-filling stages in a  
334 rainfed environment, consistent with previous studies in rice and maize (*Yan et al., 2003; Zhou et*  
335 *al., 2008*). This indicated that  $P_n$  might be a good tool for indirect assessment of root activity.

336 Wheat yield mainly comes from photosynthetic carbon assimilation after heading. Our study  
337 showed that the DW of leaf + stem + sheath and single spikes was significantly higher than CK  
338 they increased after the flowering stage and then declined as the photosynthetic assimilation  
339 substances were gradually transported to grain; the DW of wheat grain experienced an “S” pattern  
340 of growth from flowering to maturity. The average aboveground biomass at 5 d after flowering  
341 and maturity stages of large-spike lines were 77.03 and 51.62% higher than that of CK,  
342 respectively, similar to results of some researchers (*Yang et al. 2002; Wang & Shangguan, 2015*.  
343 Improving crop biomass is the material base for very high yields, and the leaf assimilation product  
344 of large-spike lines may better satisfy the demand for grain filling, thus reducing the storage  
345 material in the leaf + stem + sheath prematurely transferred to grain and so increase grain yield.

346 In this study, based on the characteristics of wheat materials have been described in the first  
347 part of materials and methods, the average spike length, number of grains per spike, kernel weight  
348 per spike, and 1000-grain weight were significantly higher for the eight large-spike lines than CK,  
349 and the number of spikes per hectare of large-spike lines was significantly lower than for CK. The  
350 yields of lines 2005, 2026, 2037, 2038, 2039, and 2040 were higher than those of CK. Our results  
351 agreed with previous reports of the primary differences between large-spike and multiple-spike  
352 cultivars (*Jiang et al., 2000; Sui et al., 2010; Lu et al., 2015; Wang, Chen & Shangguan, 2016*).  
353 The large-spike lines maintained a higher production possibly by strengthening plant straw so that  
354 they appeared larger and had a smaller plant population than CK. These results indicated positive

355 effects for improving future grain yield of large-spike lines by coordinating the relationship among  
356 agronomic traits.

## 357 **CONCLUSIONS**

358 This study investigated the leaf photosynthetic function duration of different leaf positions  
359 among large-spike lines and a multiple-spike cultivar during wheat yield formation. It concluded  
360 that the average spike length, number of grains per spike, kernel weight per spike, and 1000-grain  
361 weight were significantly higher for the eight large-spike lines than CK. The large-spike lines had  
362 a slow rate of decline in RWC, long average duration time of  $P_n$ , and higher yield than Xi'nong  
363 979. There was a strong linear and positive correlation between photosynthetic rate and root  
364 activity at jointing, flowering, and grain-filling stages. The new large-spike lines might have  
365 advantages in individual plant development and growth, good drought-resistant capacity, and  
366 increasing grain yield in rainfed regions of China.

## 367 **ACKNOWLEDGEMENTS**

368 We thank Yaliang Zheng and Lei Deng for help and support with field management and technical  
369 assistance.

## 370 **Funding**

371 The study was sponsored by grants of the National Key Research and Development Program of  
372 China (2017YFD0300408, 2017YFD0301104), and the National Key Technology R&D Program  
373 of China (2015BAD22B01). The funders had no role in study design, data collection and analysis,  
374 decision to publish, or preparation of the manuscript. There was no additional external funding

375 received for this study.

## 376 REFERENCES

- 377 **Arnon D. 1949.** Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*  
378 **24**:1-15.
- 379 **Barr HD, Weatherley PE. 1962.** A re-examination of the relative turgidity technique for estimating water deficit  
380 in leaves. *Australian Journal of Biological Sciences* **15**:413-428.
- 381 **Belkheiri O, Mulas M. 2013.** Effect of water stress on growth, water use efficiency and gas exchange as related to  
382 osmotic adjustment of two halophytes *Atriplex* spp. *Functional Plant Biology* **40**:466-474.
- 383 **Blokhina O, Virolainen E, Fagerstedt KV. 2003.** Antioxidative damage and oxygen deprivation stress. *Annals*  
384 *Botany* **91**:179-194.
- 385 **Cao SQ, Lu W, Zhai HQ, Sheng SL, Gong HB, Yang TN, Zhang RX. 2001.** Research on the method to  
386 estimating flag leaf photosynthesis function duration at rice seeding stage by relative steady phase of  
387 chlorophyll content. *Chinese Journal of Rice Science* **15**:309-313. (in Chinese)
- 388 **Distelfeld A, Avni R, Fischer AM. 2014.** Senescence, nutrient remobilization, and yield in wheat and barley.  
389 *Journal of Experimental Botany* **65**:3783-3798.
- 390 **Dong BD, Liu MY, Shao HB, Li QQ, Shi L, Du F, Zhang ZB. 2008.** Investigation on the relationship between  
391 leaf water use efficiency and physio-biochemical traits of winter wheat under rained condition. *Colloids and*  
392 *Surfaces B: Biointerfaces*, **62**:280-287.
- 393 **Gaju O, DeSilva J, Carvalho P, Hawkesford MJ, Griffiths S, Greenland A, Foulkes MJ. 2016.** Leaf  
394 photosynthesis and associations with grain yield, biomass and nitrogen-use efficiency in landraces,  
395 synthetic-derived lines and cultivars in wheat. *Field Crops Research* **193**:1-15.
- 396 **Guo TC, Sheng K, Feng W, Xu LN, Wang CY. 2009.** Effects of plant density on physiological characteristics of  
397 different stems during tillering stage in two spike-types winter wheat cultivars. *Acta Botanica*  
398 *Boreali-Occidentalia Sinica* **29**:350-355. (in Chinese)
- 399 **Jiang GM, Hao NB, Bai KZ, Zhang QD, Sun JZ, Guo RJ, Ge QY, Kuang TY. 2000.** Chain correlation between  
400 variables of gas exchange and yield potential in different winter wheat cultivars. *Photosynthetica* **38**:227-232.
- 401 **Khakwani AA, Dennett MD, Munir M, Abid M. 2012.** Growth and yield response of wheat varieties to water  
402 stress at booting and anthesis stages of development. *Pakistan Journal of Botany* **44**:879-886.

- 403 **Lindström A, Nyström C. 1987.** Seasonal variation in root hardiness in container grown *Scots pine*, Norway  
404 *spruce*, and *Lodgepole pine* seedlings. *Canadian Journal of Forest Research* **17**:787-793.
- 405 **Lu DJ, Lu FF, Pan JX, Cui ZL, Zou CQ, Chen XP, He MR, Wang ZL. 2015.** The effects of cultivar and  
406 nitrogen management on wheat yield and nitrogen use efficiency in the North China Plain. *Field Crops*  
407 *Research* **171**:157-164.
- 408 **Merchuk-Ovnat L, Fahima T, Krugman T, Saranga Y. 2016.** Ancestral QTL alleles from wild emmer wheat  
409 improve grain yield, biomass and photosynthesis across environments in modern wheat. *Plant Science*  
410 **251**:23-34.
- 411 **Mielke MS, Schaffer B, Schilling AC. 2012.** Evaluation of reflectance spectroscopy indices for estimation of  
412 chlorophyll content in leaves of a tropical tree species. *Photosynthetica* **50**:343-352.
- 413 **Nageswara Rao RC, Talwar HS, Wright GC. 2001.** Rapid assessment of specific leaf area and leaf nitrogen in  
414 peanut (*Arachis hypogaea* L.) using chlorophyll meter. *Journal of Agronomy and Crop Science* **189**:175-182.
- 415 **Peng SB, García FV, Laza RC, Cassman KG. 1993.** Adjustment for specific leaf weight improves chlorophyll  
416 meter's estimate of rice leaf nitrogen content. *Agronomy Journal* **85**:987-990.
- 417 **Pietrini F, Di Baccio D, Iori V, Veliksar S, Lemanova N, Juskaite L, Maruska A, Zacchini M. 2017.**  
418 Investigation on metal tolerance and phytoremoval activity in the poplar hybrid clone "Monviso" under  
419 Cu-spiked water: Potential use for wastewater treatment. *Science of the Total Environment* **592**:412-418.
- 420 **Raven JA, Griffiths H. 2015.** Photosynthesis in reproductive structures: costs and benefits. *Journal of*  
421 *Experimental Botany* **66**:1699-1705.
- 422 **Riccardi M, Pulvento C, Patané C, Albrizio R, Barbieri G. 2016.** Drought stress response in long-storage  
423 tomatoes: physiological and biochemical traits. *Scientia Horticulturae* **200**:25-35.
- 424 **Saint Pierre C, Crossa JL, Bonnett D, Yamaguchi-Shinozaki K, Reynolds MP. 2012.** Phenotyping transgenic  
425 wheat for drought resistance. *Journal of Experimental Botany* **63**:1799-1808.
- 426 **Siddique MRB, Hamid A, Islam MS. 2000.** Drought stress effects on water relations of wheat. *Botanical Bulletin*  
427 *of Academia Sinica* **41**:35-39.
- 428 **Slabbert RM, Kruger GH. 2011.** Assessment of changes in photosystem II structure and function as affected by  
429 water deficit in *Amaranthus hypochondriacus* L. and *Amaranthus hybridus* L. *Plant Physiology and*  
430 *Biochemistry* **49**:978-84.
- 431 **Sui N, Li M, Meng QW, Tian JC, Zhao SJ. 2010.** Photosynthetic characteristics of a super high yield cultivar of

- 432 winter wheat during late growth period. *Agricultural Sciences in China* **9**:346-354.
- 433 **Turrall H, Svendsen M, Faures JM. 2010.** Investing in irrigation: Reviewing the past and looking to the future.  
434 *Agricultural Water Management* **97**:551-560.
- 435 **Wang LF, Chen J, Shangguan ZP. 2016.** Photosynthetic characteristics and nitrogen distribution of large-spike  
436 wheat in Northwest China. *Journal of Integrative Agriculture* **15**:545-552.
- 437 **Wang LF, Shangguan ZP. 2015.** Photosynthetic rates and kernel-filling processes of big-spike wheat (*Triticum*  
438 *aestivum* L.) during the growth period. *New Zealand Journal of Crop and Horticultural Science* **43**: 182-192.
- 439 **Wang XY, Feng W, Guo TC, Kang GZ, Wang CY. 2010.** Difference of carbon and nitrogen metabolism in  
440 leaves between main caulis and tillers during tiller senescence of two spike-type winter wheat. *Acta*  
441 *Agriculturae Boreali-occidentalis Sinica* **19**:38-42. (in Chinese)
- 442 **Xie Q, Mayes S, Sparkes DL. 2016.** Early anthesis and delayed but fast leaf senescence contribute to individual  
443 grain dry matter and water accumulation in wheat. *Field Crops Research* **187**:24-34.
- 444 **Yan JM, Zhai HQ, Wan JM, Jiao DM, Zhang RX. 2003.** Transportation Characteristics of Assimilate and  
445 Physiologic Mechanisms in Subspecific Heavy Ear Hybrid Rice (*Oryza sativa* L.). *Scientia Agricultura Sinica*,  
446 **36**:502-507. (in Chinese)
- 447 **Yang H, Li JW, Yang JP, Wang H, Zou JL, He JJ. 2014.** Effects of Nitrogen Application Rate and Leaf Age on  
448 the Distribution Pattern of Leaf SPAD Readings in the Rice Canopy. *Plos One* **9**:e88421.
- 449 **Yang JC, Peng SB, Zhang ZJ, Wang ZQ, Visperas RM, Zhu QS. 2002.** Grain and dry matter yields and  
450 partitioning of assimilates in Japonica/Indica hybrid rice. *Crop Science* **42**:766-772.
- 451 **Zheng CY, Yu ZW, Shi Y, Cui SM, Wang D, Zhang YL, Zhao JY. 2014.** Effects of tillage practices on water  
452 consumption water use efficiency and grain yield in wheat field. *Journal of Integrative Agriculture*  
453 **13**:2378-2388.
- 454 **Zhou XP, Zhang SQ, Yang XQ, Liu XF, Liu LS. 2008.** Heterosis of maize root activity and its relationship with  
455 photosynthetic characteristics. *Acta Agriculturae Boreali-Occidentalis Sinica* **17**:84-90. (in Chinese)
- 456
- 457
- 458

459

460 **Tables captions**

461 **Table 1 Main characteristics of wheat materials used in present study.**

462 **Table 2 The dynamic changes in chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and carotenoid (Car) contents**

463 **of wheat leaves during the developmental stages.** Values are means  $\pm$  SE ( $n = 3$ ); means followed by

464 different small letters in the same column are significantly different at  $P < 0.05$  according to LSD test.

465 **Table 3. Differences in spike length, number of grains per spike, kernel weight per spike, number of spikes,**

466 **1000-grain weight, and yield of eight large-spike genotypes and CK.** Values are means  $\pm$  SE ( $n = 3$ );

467 means followed by different small letters in the same column are significantly different at  $P < 0.05$

468 according to LSD test.

469

470

471

472

473

474

475

476

477

478

479

480

481 **Figure captions**

482 **Figure 1 The diagram of different leaf positions in this study.**

483 **Figure 2 The dynamic changes in tiller number in eight large-spike wheat lines and one multiple-spike**  
484 **cultivar (Xi'nong 979) from seeding to mature stage.** SS, seeding stage; TS, tillering stage before winter;  
485 GS, returning green stage; 7DAGS, 14DAGS, 21DAGS, 28DAGS, and 35DAGS indicate 7, 14, 21, 28, and  
486 35 d after returning green stage, respectively; HS, heading stage; MS, mature stage.

487 **Figure 3 Time courses of chlorophyll relative value (SPAD) decline in fully expanded (A–C) and detached**  
488 **(D–F) leaves at different positions (leaf 5, leaf 7, and flag leaf) for different wheat materials.**

489 **Figure 4 Time courses of relative water content (RWC) decline in detached leaves at jointing, flowering, and**  
490 **grain-filling stages for different wheat materials.**

491 **Figure 5 Time courses of photosynthetic rate ( $P_n$ ) decline in detached leaves at jointing, flowering, and**  
492 **grain-filling stages for different wheat materials.**

493 **Figure 6 The relationship between photosynthetic rate ( $P_n$ ) and root activity (TTC) of large-spike wheat**  
494 **lines during the growth period.**

495 **Figure 7 Dynamic changes in dry weight (DW) of single spike and leaf + stem + sheath in large-spike lines**  
496 **and CK (Xi'nong 979).** ■ and ● indicate DW for single spikes in large-spike lines and CK, respectively; □  
497 and ○ indicate DW for leaf + stem + sheath in large-spike lines and CK, respectively.

498

499

500