Stomatal conductance bear no correlation with transpiration rate during their diurnal variation under high air humidity

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A Good understanding of the response of photosynthesis rate (P_N) and transpiration rate (Tr) to stomatal alteration during the diurnal variations is important to cumulative photosynthetic product and water loss of crops. Six wheat genotypes were studied for two years with pot cultivation in rain-shelter. Among different genotypes, stomatal conductance (g_s) was significantly correlated with both P_N and Tr. But for each genotype, though g_s was significantly correlated with P_N regardless of relative air humidity (RH) status and it was also significantly correlated with Tr under lower RH (LRH, 15.4%) and moderate RH (MRH, 28.3%), it was not correlated with Tr under higher RH (HRH, 36.7%) during the diurnal changes. The conditional correlation between g_s and Tr of wheat evoked new thinking on the relationships among g_s, P_N, and Tr. Path analysis was further carried out to clarify the correlations of g_s with the 4 atmospheric factors, that of Tr with g_s and the four factors, and the direct and indirect effects of the factors, during their diurnal dynamic variation. The effects of these factors on g_s or Tr were related to RH. All the 4 factors had a much higher correlation with g_s under HRH than that under LRH and MRH. Air temperature (T) had a rather higher direct effect than RH and photosynthetically active radiation (PAR). And the other factors had a much higher indirect effect on g_s through vapor pressure deficit (VPD) and T. Transpiration rate was highly correlated with g_s under LRH and MRH, with g_s having a higher direct effect on it. In comparison, Tr was not correlated with g_s under HRH but highly correlated with the atmospheric factors, with T, RH, and PAR having a higher indirect effect through VPD.

1 # Stomatal conductance bear no correlation with transpiration rate

2 # during their diurnal variation under high air humidity

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Abstract

A Good understanding of the response of photosynthesis rate (P_N) and transpiration rate (Tr) to stomatal alteration during the diurnal variations is important to cumulative photosynthetic product and water loss of crops. Six wheat genotypes were studied for two years with pot cultivation in rain-shelter. Among different genotypes, stomatal conductance (g_s) was significantly correlated with both P_N and Tr. But for each genotype, though g_s was significantly correlated with P_N regardless of relative air humidity (RH) status and it was also significantly correlated with Tr under lower RH (LRH, 15.4%) and moderate RH (MRH, 28.3%), it was not correlated with Tr under higher RH (HRH, 36.7%) during the diurnal changes. The conditional correlation between g_s and Tr of wheat evoked new thinking on the relationships among g_s, P_N, and Tr. Path analysis was further carried out to clarify the correlations of g_s with the 4 atmospheric factors, that of Tr with g_s and the four factors, and the direct and indirect effects of the factors, during their diurnal dynamic variation. The effects of these factors on g_s or Tr were related to RH. All the 4 factors had a much higher correlation with g_s under HRH than that under LRH and MRH. Air temperature (T) had a rather higher direct effect than RH and photosynthetically active radiation (PAR). And the other factors had a much higher indirect effect on g_s through vapor pressure deficit (VPD) and T. Transpiration rate was highly correlated with g_s under LRH and MRH, with g_s having a higher direct effect on it. In comparison, Tr was not correlated with g_s under HRH but highly correlated with the atmospheric factors, with T, RH, and PAR having a higher indirect effect through VPD.

Keywords: stomatal conductance; relative air humidity; net photosynthesis rate; transpiration rate; wheat genotypes

1. INTRODUCTION

The water shortage has been the most significant factor restricting plant growth and crop productivity with a deepening water-resource crisis worldwide. Wheat (*Triticum aestivum* L.) is one of the main crops consumed by humans and is cultivated in different environments. Only less than 30% of the rainfall occurs during the wheat growing season in the North China Plain (NCP), the main wheat production region of China, which meets only about 25-40% of the water requirements of wheat. As a result, more than 70% of the irrigation water is used for winter wheat (Mei et al. 2013). Irrigation usage for wheat threatens the sustainability of the groundwater resource (Zhang, Pei & Hu 2003). Therefore, how to conserve soil water to enable sustainable crop production and maximize harvest of fields are becoming the main goals for many efforts of

72   agriculture researchers.

73   Stomata, the gateway through which vapor and CO_2 pass, play an important role in
74   regulating both photosynthesis and transpiration. Low stomatal conductance (g_s) results in low
75   net photosynthesis rate (P_N) by restricting CO_2 uptake (Farquhar & Sharkey 1982), while high g_s
76   benefits higher P_N but at a greater expense of water loss via transpiration (Lawson & Blatt 2014).
77   The ability of stomata to exert rapid control of their aperture to minimize water loss while
78   maintaining CO_2 uptake is one of the primary evolutionary mechanisms that has allowed
79   terrestrial plants to survive and spread in an otherwise desiccating atmosphere (Hetherington &
80   Woodward 2003). A good understanding of the response and behavior of stomata and
81   transpiration in winter wheat is in urgent need of water saving through the pathway of cultivar
82   adoption and agricultural practices. Stomata adjusts aperture in response to diverse external
83   stimuli, such as vapor pressure deficit (VPD) (Devi, Sinclair & Vadez 2010; Leonardi, Guichard
84   & Bertin 2000), relative air humidity (RH) (Bakker 1991a; Bakker 1991b; Merilo et al. 2018;
85   Suzuki et al. 2015; Talbott, Rahveh & Zeiger 2003), soil moisture (Belko et al. 2012; Kholová et
86   al. 2010a; Kholová et al. 2010b), air temperature (T) (Haque et al. 2017; Hetherington &
87   Woodward 2003), photosynthetic photon flux density (PPFD) (McAusland et al. 2016), and CO_2
88   (Yoshimoto, Oue & Kobayashi 2005). These atmospheric factors continually vary at diurnal and
89   seasonal rhythms (Assmann & Wang 2001). Besides, the circadian clock was reported to control
90   g_s responses partly at least over the diurnal period (Dodd et al. 2005; Hassidim et al. 2017), with
91   phase of the circadian clock adjusting to environmental cues, such as T and PPFD, etc. (de Dios
92   et al. 2016; Yin & Johnson 2000). The majority of these studies were conducted with controlled
93   experiments. Few studies were performed utilizing different gradients of natural conditions,
94   which may be largely different from those carried out in climate chambers or green houses.
95   Besides, these researches seldom laid stress on how the influencing factors affect g_s during the
96   diurnal dynamic change.

97   Our previous study found that RH played an important role in determining the diurnal g_s
98   pattern of wheat. All tested genotypes under lower RH (LRH, 15.7%) and most genotypes under
99   higher RH (HRH, 40.7%) displayed a gradual decline pattern from morning through the
100   afternoon. All genotypes presented a single-peak curve pattern under moderate RH (MRH,
101   28.3%), but the peak time differed among genotypes (Zhang et al. 2019). Base on the previous
102   research, this study aims at 1) clarifying how the influencing factors contribute to the diurnal
103   variation of g_s along with transpiration rate (Tr) of wheat under different RH and soil moisture
104   regimes; 2) making clear how P_N and Tr of wheat are correlated with g_s during the diurnal
105   change under different regimes of soil moisture and RH. A deep understanding of the stomata
106   and transpiration traits of wheat, the influencing factors, and their effect on P_N and Tr under

107   different conditions have great implications for saving water through cultivars and agricultural
108   practices.

2. MATERIALS AND METHODS

2.1 Plant materials

111   Six winter wheat genotypes, Jinmai 47, Chang 6878, Jing 411, Zhoumai 18, Lankaoaizao 8,
112   and 12 Song 1 were used as materials, with the names and associated details being listed in Table
113   1. The 6 genotypes were selected from wheat germplasm nursery affiliated to Luoyang Academy
114   of Agriculture and Forestry, located in Henan Province, China. In April 2016, the jointing stage
115   of winter wheat, stomatal conductance was measured in three replications of different genotypes
116   in the nursery under rain-fed and well-watered conditions. According to the data collected from
117   9:00 to 11:00am, six genotypes, which were in a wide spectrum of g_s from very low to rather
118   high value in the two water conditions, were tentatively selected as materials.

2.2 Growth conditions

120   The experiments were carried out at Shunyi Scientific Experimental Station, Institute of
121   Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural
122   Sciences, Beijing, China (40°N and 116°E, altitude 34m) in 2017-2018 growing seasons of
123   winter wheat. The precipitation and air temperature of every month during the growing season
124   are presented in Figure 1. Pot cultivation was adopted in this experiment. Polyvinyl chloride
125   (PVC) pots were 30 cm depth and 35 cm in diameter, with a drainage hole on the bottom. The
126   pots were filled with 16 kg plow layer soil, which was sieved through a 5 mm sieve and then
127   fully mixed. The soil nutrients were determined as 0.109 g/kg of total nitrogen, 14.4 g/kg of
128   organic matter, 24.5 mg/kg of available phosphorus, 106 mg/kg of available potassium, and soil
129   pH was 7.7.

130   For each genotype, 6 pots were planted for two water treatments and three replications.
131   Twenty wheat seeds were sown in each pot on October 5, 2017. Thirteen uniform seedlings were
132   kept for overwintering, with the slender ones being removed on 30 d after emergence. After
133   recovering in the spring, 10 uniform seedlings were eventually selected and kept as materials,
134   with the unqualified ones being eliminated.

135   **2.3 Air humidity and soil water treatments**

136   The treatments were different regimes of air humidity and soil water. The environmental RH

137   gradient was taken as air humidity treatments. April 13, 28 and May 6, 2018, which were LRH

138   (15.4%), MRH (28.3%), and HRH (36.7%) respectively, but similar in T and PAR were

139   specifically selected. The specific diurnal meteorological conditions of the 3 days were shown in

140   Figure 2. Two water treatments were arranged: well-watered (WW) and drought stress (DS)

141   conditions, with soil water content being 75% and 50% of field water holding capacity (FC),

142   respectively. Water withholding for the two treatments was conducted by the weighing method.

143   The plants were watered daily to restore the initial soil water content, between 1 and 2 h after

144   sunset. Water withholding was kept for 30 d from April 6 through May 6. During water

145   treatment periods, rain-shelter was used to prevent the rain on rainy days and was kept rolled up

146   on sunny days to allow the crops to grow in the open-air conditions.

147   **2.4 Leaf gas exchange measurements**

148   The youngest fully expanded leaves of the main tiller were measured for measuring the

149   diurnal variation of gas exchange parameters under LRH, MRH and HRH from 8:00 to 18:00

150   with the 2 hours interval, utilizing a Licor-6400 portable infrared gas analyzer (LI-COR Inc.,

151   Lincoln, Nebraska USA). For each replication, the leaves of three plants were measured and

152   averaged as the value for each time span. With one measurement, 3 parameters concerned,

153   including g_s, P_N, Tr, were obtained in the meanwhile. To eliminate the disturbance to PAR from

154   cloud cover and shading of neighboring leaves, PAR was set as the average value of 5 days in

155   April or May for each time span. And chamber T was set as the same as the air temperature

156   outside the chamber. Relative air humidity could not be set for Li-cor-6400 gas analyzer, RH of

157   the air flowing into the chamber was as same as the air RH. At the same time with diurnal

158   measurement of gas exchange parameters, 4 atmospheric factors were also determined. Air

159   temperature and Relative air humidity were monitored once per minute by an automated

160   temperature and relative humidity system (TH12R, Miaoxin, CN). VPD was calculated by T and

161   RH as follows.

162      $$VPD = \left(1 - RH\right) \times 0.6108 \times e^{\frac{17.27 \times T}{T + 273.3}}$$

163   For investigating the relationships between g_s with P_N and Tr across genotypes under

164   identical soil water and meteorological conditions, leaf gas exchange parameters were measured

165   with 6 different genotypes under two water treatments at 9:30-11:00am on April 16, 29, and May

166   5, 2018, the LRH (16.4%) , MRH (25.9%), and HRH (37.8%) day respectively. The same gas

167   analyzer described above were used, with the leaf chamber conditions being set at the same

168   temperature of 25°C, PAR of 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

2.5 Statistical analysis

170   The collected data were statistically analyzed by SAS software (SAS 9.4, Cary, NC, USA).

171   A Pearson correlation analysis was used to assess correlations between parameters. Signifcance

172   was considered at P < 0.05 and 0.01. Data were presented as means $\pm$ standard errors (n = 3).

3. RESULTS

3.1 Main meteorological factors responsible for the diurnal variation of stomatal

conductance

176   The four meteorological factors, T, RH, VPD, and PAR interact and associate with each

177   other. Each factor might directly affect g_s, also indirectly affect g_s through other factors. Path

178   analysis was carried out to clarify the correlations of four terms with g_s and their direct and

179   indirect effects on g_s during diurnal dynamic variation. Table 2 showed how g_s related to

180   diurnally varying atmospheric factors depended on moisture regimes. All the 4 factors had a

181   much higher correlation with g_s under HRH than that under LRH and MRH. The correlation

182   between g_s and the four factors tended to be higher under WW than that under DS when RH was

183   the same level. Of the four factors, g_s was significantly correlated with T and PAR under all the

184   regimes except DS+MRH. All the 4 factors were not correlated with g_s under DS+MRH.

185   Temperature had a rather higher direct effect than RH and PAR on g_s. And other factors had a

186   much higher indirect effect on g_s through VPD and T on g_s.

3.2 Main factors responsible for the diurnal variation of transpiration rate

188   Stomatal conductance and the four main meteorological factors T, RH, VPD, and PAR were

189   closely related to each other. Path analysis was conducted to clarify the correlations between *Tr*

190   and the 5 factors, the direct effect and indirect effect through other factors on *Tr* during their

191   diurnal dynamic variation (Table 3).

192   Under LRH, *Tr* was highly correlated with g_s under both WW and DS, with higher direct

193   effect coefficient (DPC=0.7346) and a higher negative indirect effect through VPD and a

194 positive indirect effect through T under WW, with higher direct effect (DPC=0.8225) and a weak

195 indirect effect through other factors under DS. Transpiration rate was also significantly

196 correlated with T, RH, and VPD under WW. The temperature had a higher positive direct effect

197 and a higher negative indirect effect through VPD on Tr. Relative air humidity had a relatively

198 lower direct effect and a higher negative indirect effect through T and a higher positive indirect

199 effect through VPD on Tr. Vapor pressure deficit had a higher direct effect and a higher indirect

200 effect through T on Tr. All the 4 meteorological factors had a lower indirect effect through g_s on

201 Tr.

202   Under MRH, Tr had an extremely significant correlation with all the 5 factors, with the

203 correlation between Tr and g_s being much higher, that between Tr and RH being negative, and

204 that between Tr and T, VPD, and PAR being positive. Stomatal conductance had a rather higher

205 direct effect than the indirect effect through any other factors on Tr. Of the 4 meteorological

206 factors, VPD had the highest direct effect, and T, RH, and PAR had a higher indirect effect

207 through VPD on Tr. The indirect effect of 4 meteorological factors through g_s on Tr was rather

208 smaller.

209   Under HRH, Tr was not correlated with g_s but significantly correlated with the 4

210 meteorological factors except PAR under WW. Vapor pressure deficit had the largest direct

211 effect, while PAR had the smallest direct effect on Tr under DS. The indirect effect of T, RH,

212 and PAR on Tr through VPD was the largest, with that of RH being negative, and that of T and

213 PAR being positive. The indirect effect through g_s of the 4 meteorological factors on Tr was

214 rather smaller.

215 **3.3 Stomatal conductance bear no correlation with transpiration rate in the diurnal change**

216 **under HRH**

217   The meteorological factors, such as T, RH, and VPD, etc., vary with time diurnally.

218 Meteorological conditions were considered to be identical for different genotypes under the same

219 measurement time span (9:30-11:00am). Across different genotypes, g_s had extremely significant

220 correlations with P_N and Tr under identical meteorological conditions and soil water status

221 (Table 4). The correlations between g_s and Tr under WW were lower than those under DS in the

222 three different RH conditions. For the case of LRH, the correlations under WW were lower than

223 those under DS. While for MRH and HRH, the correlation between g_s and P_N was higher under

224 WW than that under DS. On the other hand, the correlations among g_s with P_N and Tr in the

225 diurnal dynamics were investigated with 6 genotypes individually under WW and DS conditions

226 (Table 5). There were the same results in WW and DS whether respectively or collectively.

227   Stomatal conductance was closely correlated with P_N and Tr under LRH and MRH. Interestingly,
228   though g_s was still closely correlated with P_N, it did not significantly correlate with Tr for each
229   genotype under HRH in the diurnal variation. This proved the high dependency between g_s and
230   P_N, also revealed the minor role of g_s on determining Tr diurnal variation under HRH. It might
231   be other factors, mainly the meteorological factors, that predominantly control Tr in the diurnal
232   variation in such case.

233   4. DISCUSSION

234   4.1 Factors influencing the diurnal variation of stomatal conductance and transpiration
235   rate

236   Stomata adjust aperture in response to environmental factors, such as soil water stress and
237   atmospheric factors, as reported by many studies (Xu et al. 2016; Hernandez-Santana et al. 2016).
238   The present study aimed at the relations between g_s of wheat with the influencing factors during
239   the diurnal dynamic variation, and found that the relations was associated closely with RH.
240   Stomatal conductance was not correlated with RH and VPD under LRH+DS and MRH+DS
241   regimes, but highly correlated with the two factors under HRH. Moreover, stomatal conductance
242   displayed a higher correlation with the influencing factors under WW than under DS. Thus, it
243   seems that only when g_s of wheat was relatively higher in the case of high air RH and soil
244   moisture, it was closely related to atmospheric factors during the diurnal variation. The reduced
245   stomatal aperture could not respond to diurnally varying factors sensitively under dry soil and air
246   moisture, which explain the rather lower correlation between them. Stomatal conductance of
247   wheat was significantly correlated with PAR in most of the regimes. Sabir & Yazar (2015) found
248   that g_s was better correlated with PAR for all the tested grapevines cultivars than the other
249   measured meteorological parameters, including RH and T, etc. This is consistent with the case
250   under DS+LRH and WW+MRH in our study, implying that sunlight had a significant effect on
251   stomatal diurnal response under the two regimes.

252   Transpiration rate is controlled by the plant itself through stomatal adjustment on one hand.
253   As a passive diffusion process of water, it was also regulated by surrounding factors, such as T,
254   RH, and solar radiation intensity (Yang et al. 2012). Which factors mainly restrict Tr during the
255   diurnal variation depended on RH, as the present study showed. Higher correlation between g_s
256   and Tr of wheat existed under LRH and MRH, but no correlation under HRH. This might imply
257   that increased g_s under HRH was not restricting factors for Tr, instead, atmospheric factors
258   restricted Tr in this case. While reduced g_s became the main limiting factor for Tr under lower

259   RH. Vapor pressure deficit had the highest direct effect of the four atmospheric factors, and other

260   factors had the highest indirect effect through VPD under almost all the regimes.

4.2 Correlations between stomatal conductance with transpiration rate and photosynthesis

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rate in the diurnal dynamics

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263   Across different genotypes under identical meteorological conditions and soil water status,

264   extremely significant correlations existed between g_s with P_N and Tr, as reported by some

265   previous studies (Wong, Cowan & Farquhar 1979; McAusland et al. 2016). This indicates the

266   important contribution of cultivars with different stomatal traits to photosynthesis and

267   transpiration, and in turn to yield formation and water consumption, in the case of identical

268   environmental conditions.

269   During the diurnal dynamics, g_s was significantly correlated with Tr in all the wheat

270   genotypes tested under LRH and MRH, but not under HRH (Table 5), indicating the correlation

271   between them depended on RH. As a physical process of water passive diffusion, transpiration is

272   not only controlled by the plant itself through stomatal adjustment but also driven by soil

273   moisture and atmospheric factors. In some cases, Tr is mainly regulated by RH or VPD, instead

274   of g_s. Devi et al. (2010) found that Tr increased over VPD rising, with a break point occurring in

275   some genotypes above which there was little or no further increase in Tr of peanut. Stomatal

276   conductance declined with VPD increasing (Leonardi, Guichard & Bertin 2000; Talbott, Rahveh

277   & Zeiger 2003), or RH declining (Fanourakis et al. 2016; Fanourakis et al. 2019), which implied

278   that Tr did not decline with g_s reducing, but increased with VPD rising. And the occurrence of Tr

279   breakpoint might be due to that g_s continuously declined and turned to be the restricting factor of

280   Tr. Also, Aliniaeifard et al. (2016) conducted an experiment with *Chrysanthemum morifolium*

281   plants in the growth chamber. Similarly, they found higher g_s but lower Tr under low VPD (high

282   RH) in comparison with growth under moderate VPD. Consistently, Giday et al. (2015) found

283   cultivar differences in plant transpiration rate at high relative air humidity were not related to

284   genotypic variation in stomatal responsiveness. The transpiration rate did not increase with g_s

285   rising but lowered down with increased RH. These indicated that it was VPD but not g_s that

286   controlled Tr in such cases. Whether g_s is closely related to Tr depends on if g_s is restricting Tr

287   under the specific circumstance. In the present study, the correlation coefficients between g_s and

288   Tr of wheat under LHR and HRH were contrasting (Table 5). This might be ascribed to the

289   different limiting effects of g_s on Tr. Stomatal conductance went down and turned to be the

290   limiting factor of Tr under LRH. While enhanced g_s is no more a limiting factor of Tr under

291   HRH, and weakly correlated with Tr. It might be either the regulating and limiting effect of g_s to

292   Tr or the synchronous response of g_s and Tr to the atmospheric factors that result in the high
293   correlation between g_s and Tr under MRH.

294   Stomatal conductance was highly correlated with P_N of wheat in the diurnal dynamics for
295   each genotype, also across different genotypes at identical environmental conditions,
296   highlighting the strong dependency between photosynthesis and stomatal regulation. However,
297   as reported, stomata did not respond to environmental cues with P_N synchronously, but an order
298   of magnitude slower than P_N (Lawson & Blatt 2014). The lag in stomatal behavior and the
299   temporal disconnect between P_N and g_s challenge the notion that stomata adjust the aperture to
300   regulate P_N. Moreover, Mott (1988) reported that it was CO_2 concentration inside the leaf (Ci)
301   rather than that outside the leaf influenced stomatal aperture. Afterwards, series of studies carried
302   out by Roelfsema et al. (2002; 2006) found that red light-induced stomatal opening is mediated
303   by the reduction of Ci which is in turn caused by the increased photosynthetic activity of
304   mesophyll cell. A recent study in maize (*Zea mays* L.) identified 2 *Ca* genes which encode
305   carbonic anhydrase mediated the response of plants to increased Ci (Kolbe et al. 2018). As an
306   organism of high auto-regulation, plants respond to environmental cues positively within its
307   adaptation limits. Under some specific circumstances, photosynthesis, the initiative anabolism
308   process, might be capable of regulating g_s according to its demand for CO_2 through affecting Ci,
309   though g_s has been proved to be a limiting factor of photosynthesis by most studies (Carmo-Silva
310   et al. 2012; Chastain et al. 2014).

5. CONCLUSIONS

312   Relative air humidity played an important role in affecting the correlation between g_s with
313   P_N and Tr of wheat during the diurnal variation. The transpiration rate was not significantly
314   correlated with g_s but mainly affected by the atmospheric factors under HRH. In particular, VPD
315   had a rather higher direct and indirect effect on Tr. The notion that stomata continuously adjust
316   aperture in response to environmental factors to optimize the trade-off between photosynthesis
317   and water loss was challenged. Thus the definite interrelationship among P_N, Tr, and g_s of wheat
318   need to be elucidated conditionally. In these cases that g_s is not the key influential factor for Tr,
319   any efforts to artificially reduce g_s of wheat may not contribute significantly to water saving, but
320   pay the price of photosynthetic reduction and yield loss. But when meteorological and soil water
321   conditions were identical, g_s was significantly correlated with Tr and P_N across different
322   genotypes. Thus, to select and adopt appropriate wheat cultivars with specific stomata traits is
323   undoubtedly a good strategy for realizing water saving. Taking yield issue into account,
324   genotype adoption has to compromise the requirement for yield and water saving, and needs to

325   match the water availability of areas with stomatal sensitivity of wheat genotypes to water stress.

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Genotypes' names and associated details

Genotype	Year of release	Breeding place	Pedigree
Jing 411	1993	Beijing	Fengkang 2/Changfeng 1
12 Song 1	line	Henan	LK 906/Yan 7961
Jinmai 47	1998	Shanxi	12057//Han 522/K37-20
Lankaoaizao 8	2003	Henan	Lankao 84(184)1/Lankao 90
Chang 6878	2003	Shanxi	Linhan 5175/Jinmai 63
Zhoumai 18	2005	Henan	Neixiang 185/Zhoumai 9

Path analysis of stomatal conductance with meteorological factors during diurnal dynamic variation

The measurement dates and diurnal meteorological conditions are shown in Figure 2 . T , air temperature ; RH , relative air humidity ; VPD , vapor pressure deficit ; PAR, photosynthetically active radiation; WW, soil well-watered; DS, soil drought stress; LRH, low er relative air humidity; MRH, moderate relative air humidity; HRH, high er relative air humidity. The daily average relative air humidity of LRH, MRH and HRH were 15.4%, 28.3% and 36.7% respectively. **, significant differen ce at p < 0.01; * , signi fi cant difference at p < 0.05.

Regimes	Independent variable	Direct path coefficient	Indirect path coefficient				Simple correlation coefficient
			T	RH	VPD	PAR	
WW LRH	T	1.8076		1.1045	-2.3004	-0.3287	0.2830**
	RH	-1.2985	-1.5376		2.1758	0.2603	-0.3999**
	VPD	-2.3397	1.7773	1.2075		-0.3159	0.3291**
	PAR	-0.3435	1.7297	0.9840	-2.1519		0.2183*
DS LRH	T	-7.4551**		-1.8020	8.8615	0.1602	-0.2355*
	RH	2.1159**	6.3492		-8.3855	-0.1270	-0.0474
	VPD	9.0120**	-7.3306	-1.9688		0.1540	-0.1335
	PAR	0.1675	-7.1306	-1.6041	8.2849		-0.2824**
WW MRH	T	0.0172		-0.3842	0.6178	0.0045	0.2553*
	RH	0.4345	-0.0152		-0.5996	-0.0044	-0.1847
	VPD	0.6316	0.0168	-0.4125		0.0047	0.2406*
	PAR	0.0051	0.0153	-0.3711	0.5763		0.2257*
DS MRH	T	-1.6275*		-0.4768	2.0112	0.1741	0.0810
	RH	0.5290	1.4669		-1.9455	-0.1709	-0.1205
	VPD	2.0436	-1.6017	-0.5036		0.1789	0.1172
	PAR	0.1994	-1.4211	-0.4535	1.8332		0.1580
WW HRH	T	-0.3377		0.1237	-0.4543	-0.0818	-0.7502**
	RH	-0.1346	0.3103		0.4425	0.0776	0.6959**
	VPD	-0.4605	-0.3332	0.1294		-0.0757	-0.7400**
	PAR	-0.1288	-0.2144	0.0812	-0.2705		-0.5325**

	T	2.0471		1.0820	-3.3811	-0.2891	-0.5411**
DS	RH	-1.2121	-1.8274		3.3177	0.2236	0.5018**
HRH	VPD	-3.4552	2.0031	1.1639		-0.2514	-0.5396**
	PAR	-0.4298*	1.3769	0.6306	-2.0211		-0.4433**

Path analysis of transpiration rate with stomatal conductance and meteorological factors during diurnal dynamic variation

The measurement dates and diurnal meteorological conditions are shown in Figure 2 . g_s, stomatal conductance; T , air temperature ; RH , relative air humidity ; VPD , vapor pressure deficit ; PAR, photosynthetically active radiation; WW, soil well-watered; DS, soil drought stress; LRH, low relative humidity; MRH, moderate relative humidity; HRH, high relative humidity. The daily average relative air humidity of LRH, MRH and HRH were 15.4%, 28.3% and 36.7% respectively. **, significant difference at $p < 0.01$; * , sign if icant difference at $p < 0.05$.

Regimes	Independent variable	Direct path coefficient	Indirect path coefficient					Simple correlation coefficient
			g_s	T	RH	VPD	PAR	
WW LRH	g_s	0.7346**		0.9623	0.3766	-1.1614	-0.1552	0.7569**
	T	3.4006**	0.2079		0.8009	-3.4695	-0.6801	0.2598*
	RH	-0.9416	-0.2938	-2.8927		3.2816	0.5386	-0.3079**
	VPD	-3.5288*	0.2418	3.3435	0.8756		-0.6536	0.2785**
	PAR	-0.7107**	0.1604	3.2540	0.7135	-3.2454		0.1718
DS LRH	g_s	0.8225**		0.0305	-0.0303	-0.1454	0.0678	0.7451**
	T	-0.1296	-0.1937		-0.5435	1.0710	-0.2296	-0.0253
	RH	0.6381	-0.0390	0.1103		-1.0134	0.1820	-0.1220
	VPD	1.0891	-0.1098	-0.1274	-0.5938		-0.2206	0.0376
	PAR	-0.2400	-0.2323	-0.1239	-0.4838	1.0013		-0.0787
WW MRH	g_s	0.7465**		0.0179	0.1135	-0.1575	0.0687	0.7890**
	T	0.0703	0.1906		0.5432	-0.6404	0.2707	0.4345**
	RH	-0.6143*	-0.1379	-0.0621		0.6215	-0.2601	-0.4529**
	VPD	-0.6547	0.1796	0.0687	0.5832		0.2779	0.4547**
	PAR	0.3045*	0.1684	0.0625	0.5247	-0.5974		0.4627**
DS MRH	g_s	0.7503**		0.0547	0.0912	-0.1643	0.0373	0.7692**
	T	0.6750	0.0608		0.6819	-1.3795	0.2062	0.2444*
	RH	-0.7566**	-0.0904	-0.6084		1.3344	-0.2024	-0.3234**

	VPD	-1.4017	0.0879	0.6643	0.7203		0.2118	0.2826**
	PAR	0.2361	0.1186	0.5894	0.6485	-1.2574		0.3353**
	g_s	0.5982**		0.2367	0.2545	-0.9967	-0.0338	0.0589
WW	T	-0.3155	-0.4488		-0.3360	1.3290	0.0403	0.2690*
HRH	RH	0.3658	0.4163	0.2899		-1.2945	-0.0383	-0.2609*
	VPD	1.3470	-0.4427	-0.3113	-0.3515		0.0373	0.2787**
	PAR	0.0635	-0.3186	-0.2003	-0.2205	0.7912		0.1153
	g_s	0.5807**		1.0599	0.5937	-1.8809	-0.2289	0.1245
DS	T	-1.9589	-0.3142		-1.0561	3.4108	0.3472	0.4289**
HRH	RH	1.1830	0.2914	1.7487		-3.3469	-0.2686	-0.3923**
	VPD	3.4856*	-0.3133	-1.9169	-1.1360		0.3020	0.4214**
	PAR	0.5162**	-0.2574	-1.3176	-0.6155	2.0388		0.3645**

Correlations of stomatal conductance (g_s) with net photosynthesis rate (P_N) and transpiration rate (Tr) among six genotypes

The measurements were conducted with 6 genotypes during 9:30-11:00 at each measurement day . WW , well-watered ; DS , drought stress ; LRH, low er relative air humidity; MRH, moderate relative air humidity; HRH, high er relative air humidity. The daily average relative air humidity of LRH, MRH and HRH were 16.4%, 25.9%, and 3 7 .8% respectively. **, significant difference at p < 0.01 ; *, significant difference at p < 0.0 5 .

Group	WW		DS	
	gs-P_{N}	gs-Tr	gs-P_{N}	gs-Tr
LRH	0.9460**	0.8968**	0.9663**	0.9820**
MRH	0.9100**	0.9376**	0.9096**	0.9837**
HRH	0.9547**	0.6741**	0.8222**	0.9491**

Correlations of stomatal conductance (g_s) with net photosynthesis rate (P_N) and transpiration rate (Tr) in diurnal dynamics of each genotype

The measurement dates and diurnal meteorological conditions are shown in Figure 2 . LRH, low er relative air humidity; MRH, moderate relative air humidity; HRH, high er relative air humidity. The daily average relative air humidity of LRH, MRH and HRH were 15.4%, 28.3% and 36.7% respectively. **, significant different at p < 0.01.

Genotype	LRH		MRH		HRH	
	gs-P_N	gs-Tr	gs-P_N	gs-Tr	gs-P_N	gs-Tr
12 Song	0.9726**	0.9441**	0.7655**	0.6879**	0.8910**	0.3538
Lankaoaizao 8	0.7316**	0.7537**	0.8859**	0.8071**	0.8437**	0.2141
Jinmai 47	0.8771**	0.9019**	0.8042**	0.8425**	0.7184**	0.2127
Chang 6878	0.8233**	0.7739**	0.8274**	0.8633**	0.7666**	0.3450
Jing 411	0.9240**	0.8909**	0.5985**	0.6794**	0.8501**	0.2251
Zhoumai 18	0.8453**	0.8514**	0.9162**	0.9560**	0.8163**	0.1919

Figure 1

The environmental condition during the 2017-2018 wheat growing season

The data are the total precipitation and the average values of air temperature in each month.
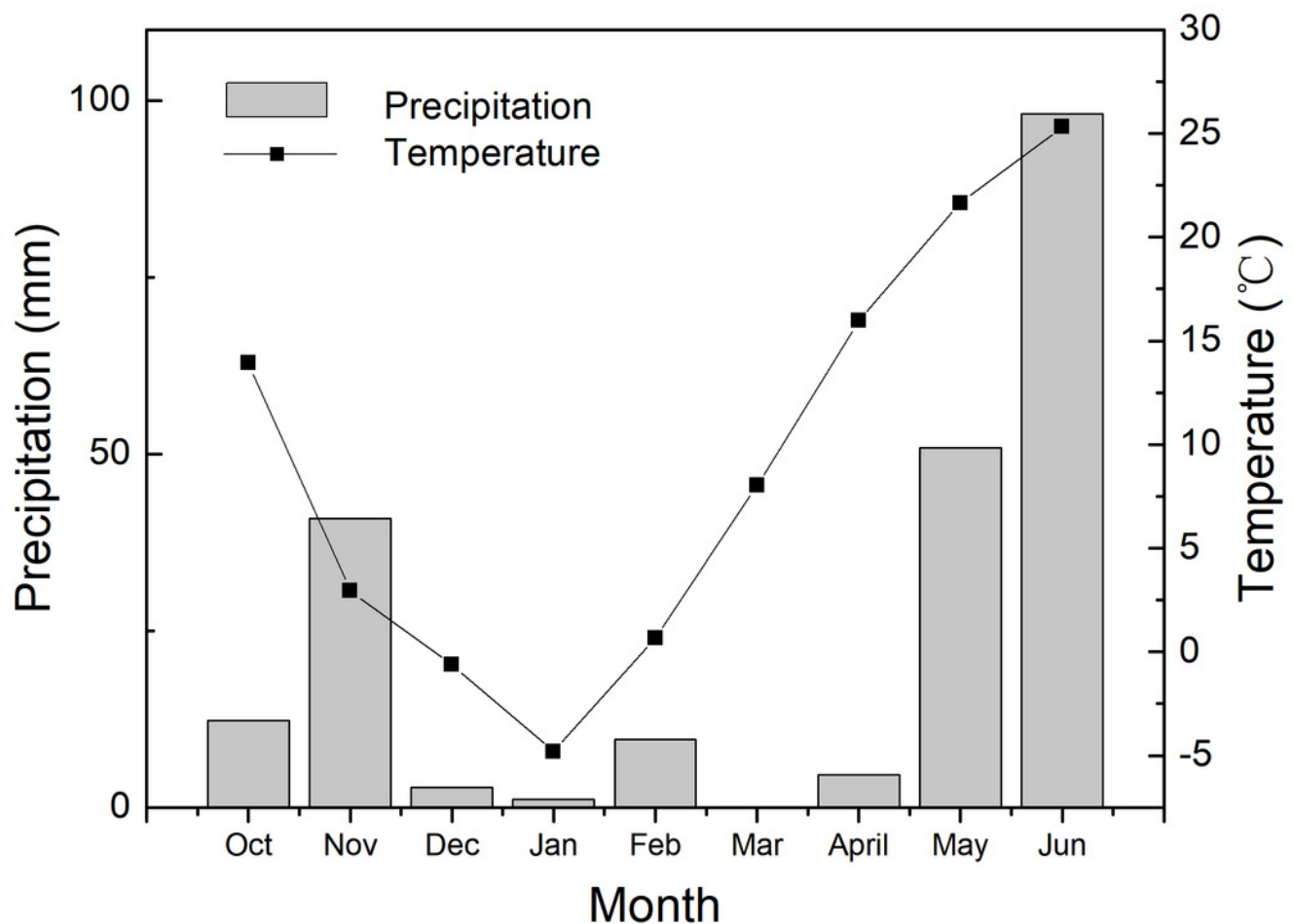
Figure 2

The diurnal meteorological conditions of measurement days

(A)Diurnal temperature (T) condition of each measurement day. (B) Diurnal relative air humidity (RH) condition of each measurement day. (C) Diurnal vapor pressure deficit (VPD) condition of each measurement day. (D) Diurnal photosynthetically active radiation (PAR) condition of each measurement day. Values represent means ± standard errors (n=3).