

Physiological Mechanism of Drought-Resistant Rice Coping with Drought Stress

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1 **Physiological mechanism of drought-resistant rice coping with**
2 **drought stress**

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29 **Abstract**

30 Drought stress is one of major threats to rice production. The weakening of leaf
31 photosynthesis due to drought is the main reason for the reduction of grain yield, but
32 its mechanism is still obscure. The objectives of this study were to reveal the
33 physiological mechanism of drought stress affecting photosynthetic capacity and grain
34 yield. Pot experiments were conducted with three rice cultivars, Hanyou113 (HY113),
35 Huanghuazhan (HHZ) and Zhonghan3 (ZH3) under four water management treatments
36 (traditional flooding (CK), mild drought stress (LD), moderate drought stress (MD) and
37 severe drought stress (HD)) in 2013 and 2014. Compared with CK, grain yield was
38 significantly reduced by 14.9%, 30.8% and 12.8% in HY113, HHZ and ZH3 under mild
39 drought stress, 32.9%, 33.7% and 22.9% in HY113, HHZ and ZH3 under moderate
40 drought stress and 53.6%, 45.6% and 30.7% in HY113, HHZ and ZH3 under severe
41 drought stress, respectively. The photosynthetic rate (P_n) decreased by 49.0% from 20.0
42 to 10.2 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in HY113, and 67.6% from 23.4 to 7.58 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in HHZ, and
43 39.3% from 23.4 to 14.2 in ZH3. The P_n of HHZ was similar to that of ZH3 under CK
44 conditions. The yield reduction of drought-resistant cultivars was smaller than that of
45 conventional cultivars. Maintaining leaf water potential (LWP), P_n , photosystem II (PSII)
46 original light energy conversion efficiency, non-photochemical quenching coefficient
47 (NPQ), and increasing in the ratio of photochemical reaction energy in fluorescence
48 and antioxidant enzyme activity, is the physiological basis to achieve a relatively high
49 photosynthesis. These traits could be the target for breeder to developing drought-
50 tolerant varieties.

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57 **Introduction**

58 Rice (*Oryza sativa* L.), a staple food crop over the world, more than 65% of the
59 population in China take it as their staple diet ¹. At present, 95% of rice is produced
60 under traditional puddled transplanted conditions, which leading to consumption of
61 large amounts of fresh water. Previous studies suggest that rice production consumes
62 about 65% of total agricultural water consumption ². However, with the global climate
63 change, the uneven spatial-temporal distribution of precipitation has caused a severe
64 shortage of freshwater resources, and seasonal drought stress has become a crucial
65 abiotic factor restricting the development of traditional puddled transplanted rice
66 production system in Central China ³⁻⁵. If per capita rice consumption is maintained at
67 the current level, with population increasing, rice production need to be increased by
68 about 20% by 2030 ⁶. Water deficit could occur at any time from booting to mature
69 stage, and the intensity of stress depends on the duration of water-deficit ⁷. However,
70 the sensitivity to water deficit shows significant difference at different growth period
71 of rice, many previous studies have shown that reproductive stage is the most
72 susceptible period for rice ⁸⁻⁹. The physiological activity and metabolism of rice flag
73 leaves were significantly reduced under drought stress at heading stage, which resulted
74 in a conspicuous decrease in dry matter accumulation. Although the proportion of dry
75 matter allocated to grains increased, it was not enough to prevent the yield declining ¹⁰⁻
76 ¹². Therefore, to better understand the physiological mechanism of different genotypes
77 cultivars coping with drought stress will bring us novel insight for future rice cultivation.
78 Leaf photosynthesis is the basis of crop yield formation, chloroplasts convert light
79 energy into chemical energy, which is used to form carbohydrates by capturing and
80 assimilating carbon dioxide (CO₂)¹³. Recent studies show that a clear correlation
81 relationship between leaf photosynthesis and grain yield has been observed. Therefore,
82 improvement of leaf photosynthesis was a great strategy to increase rice yield ¹⁴⁻¹⁵. The
83 depression of leaf photosynthesis under mild drought stress might due to CO₂ diffusion
84 path from atmosphere to site of carboxylation was blocked, however, the decrease in

85 leaf photosynthesis under moderate and severe drought stress is considered to be
86 dominant by significant reduction in the quantum efficiency, maximum photochemical
87 efficiency, and carboxylation efficiency¹⁶⁻¹⁹. Leaf as a primary photosynthetic organ in
88 rice, the level of the leaf water potential and the amount of chlorophyll content have a
89 significant effect on photosynthesis²⁰⁻²¹. Previous studies have shown that leaf water
90 potential participates in the regulation of cellular stomata movement²². Under drought
91 stress, leaf water potential will decrease, leading to stomatal closure and xylem
92 embolization, which ultimately leads to a significant increase in resistance of water to
93 the soil-plant-atmosphere transport process. If the leaf water potential is kept low level
94 for too long, it will cause plant carbon starvation and death²³⁻²⁴. Lack of CO₂ will
95 promote photo-oxidative stress in the chloroplasts, which can lead to the photochemical
96 activity of PSII decreased and the formation of reactive oxygen species (ROS). In
97 addition, the probability of chloroplast structure damage and chlorophyll
98 decomposition will be greatly increased under drought stress²⁵. However, some studies
99 have found that high chlorophyll content is not a necessary condition for high
100 photosynthetic rate of leaves, and the electron transport chain is not affected by the
101 reduction of chlorophyll content²⁶. Appropriately reducing the chlorophyll content of
102 the leaves can reduce the absorption of excessive light energy and light suppression,
103 which can improve the PSII efficiency. On the other hand, it can reduce the
104 consumption of nitrogen in the chlorophyll synthesis process of the leaves with rubisco
105 enzyme content increasing, ultimately increased P_n of the leaves²⁷⁻²⁸.

106 Chlorophyll fluorescence is generated during the photochemical reaction of chlorophyll
107 molecules in the ground state stimulated by light during the initial reaction of
108 photosynthesis. Chlorophyll fluorescence analysis is widely used to investigate the
109 mechanism of photosynthesis and to identify variation in photosynthetic physiological
110 circumstances²⁹⁻³¹. Measuring chlorophyll fluorescence kinetics parameters such as the
111 maximal quantum yield of PSII photochemistry (F_v/F_m), photochemical quenching (q^p),
112 effective quantum yield of PSII photochemistry (Φ_{PSII}) and NPQ can give a better

113 understanding the mechanism of the change in the activity of PSII under drought stress
114 in different genotypes³²⁻³⁴. Different genotypes are expected to respond differently to
115 drought stress in chlorophyll fluorescence parameters. The PSII photochemical
116 activities of drought-tolerant species could be maintained at a high level under drought
117 stress³⁵⁻³⁶. As a specific organelle for electron transfer, chloroplast is the main source
118 of ROS, and particularly susceptible to adversity stress. Under drought stress conditions,
119 an excess of light energy absorption lead to efficient photorespiration and excessive
120 energy in the excited state, which may increase the accumulation of ROS, and oxidative
121 damage³⁷⁻³⁹. Plants have evolved a balanced mechanism to maintain ROS generation
122 and clearance under normal conditions. Antioxidant enzymes system, such as
123 superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), can effectively
124 eliminate the ROS accumulation. However, under drought stress, the balance of this
125 antioxidant system is broken, and membrane lipid peroxidation occurs in the cell
126 membrane, producing a large amount of malondialdehyde (MDA). MDA not only
127 inhibits the activity of antioxidant enzymes, but also interacts with nucleic acids to
128 deactivate them⁴⁰⁻⁴⁴.

129 However, the coordination between q^p and NPQ in different cultivars and the
130 coordination between them and photosynthetic capacity in response to drought stress
131 still need further study. Furthermore, these studies attempted to assess the effects of
132 drought stress on photosynthetic physiology in upland rice, and comparison in
133 photosynthetic physiology among different rice ecotypes are still scarce⁴⁵⁻⁴⁶. To better
134 understand the physiological mechanism of different genotypes cultivars coping with
135 drought stress, we explored the changes of leaf water potential, chlorophyll content,
136 photosynthesis, chlorophyll fluorescence, ROS, yielding and their interrelationships
137 using three different genotypes cultivars at at heading stage to analyze their responses
138 to different level of drought stress.

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141 **Results**

142 **Chlorophyll content and ratio of Chlorophyll a (Chla) and Chlorophyll b (Chlb)**

143 Except for carotenoids, the content of Chla and Chlb of flag leaves were significantly
144 decreased under drought stress at heading stage and the chlorophyll content reduce
145 along with the increasing of drought stress level. However, changes in chlorophyll
146 content under drought conditions show differences among rice varieties, for HY113 and
147 HHZ, they were reduced by 16.1% and 17.8% respectively under severe drought stress,
148 while ZH3 was reduced by 9.85%, and the variation tendency is accordant in 2013 and
149 2014 (Table 1). As shown in Figure 1, the ratio of Chla to Chlb was increased
150 significantly with the increase of drought stress level at heading stage, the ratio was
151 increased by 13.8% in HY113, 10.7% in HHZ and 16.0% in ZH3 on average across two
152 seasons respectively.

153 **Response of LWP to drought stress**

154 The drought stress treatment to these tress cultivars (HY113, HHZ and ZH3) resulted
155 in water deficit. The response of flag leaves towards water deficit were compared by
156 analyzing LWP. During the drought periods from mild drought stress to severe drought
157 stress at heading stage, the LWP reduced 31.9% from -0.94 Mpa to -1.24 Mpa, and 54.8%
158 from -0.97 Mpa to -1.50 Mpa, and 15.7% from -0.89 Mpa to -1.03 Mpa in HY113, HHZ
159 and ZH3, respectively (Fig. 2). Maintain of leaf water relations parameters to that CK
160 was more stable in drought tolerant cultivar ZH3 than HY113 and HHZ, the differences
161 between these cultivars were more pronounced at severe drought conditions.

162 **Changes of photosynthetic parameters under drought stress**

163 Figure 3 A shows that the P_n of flag leaves decreased gradually as compared to CK
164 during the drought stress periods at heading stage and the magnitude of decline was
165 greater for all cultivars under severe drought stress. The P_n decreased 49.0% from 20.0
166 to 10.2 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in HY113, and 67.6% from 23.4 to 7.58 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in HHZ, and
167 39.3% from 23.4 to 14.2 in ZH3. The P_n of HHZ was similar to that of ZH3 under CK
168 conditions. However, it is found in Figure 3 B that the intercellular carbon dioxide

169 concentration (C_i) decreases at first and then increases as the degree of drought stress
170 increases. Figure 3 C and D shows that the stomatal conductance (G_s) and transpiration
171 rate (T_r) variable tendency of flag leaves is identical to the P_n . The G_s decreased 64.0%
172 from 0.50 to 0.18 mol m⁻² s⁻¹ in HY113, and 68.8% from 0.61 to 0.19 mol m⁻² s⁻¹ in
173 HHZ, and 54.3% from 0.35 to 0.16 mol m⁻² s⁻¹ in ZH3. The T_r decreased 71.7% from
174 12.7 to 3.60 mmol m⁻² s⁻¹ in HY113, and 73.6% from 13.8 to 3.65 mmol m⁻² s⁻¹ in HHZ,
175 and 71.2% from 12.5 to 3.60 mmol m⁻² s⁻¹ in ZH3.

176 **Effects of drought stress on the photosynthetic response curve parameters**

177 Photosynthesis-light response curve is useful to estimate the adaptation of plants to
178 adversity stress. The photosynthetic rate increases with the increase of
179 photosynthetically active radiation within a certain range, but the increase amplitude
180 was different. However, the photosynthetic curve was separated with different drought
181 stress degree, and the greater the stress degree was, the smaller the increase amplitude
182 of the light response curve was (Fig. 4). Drought stress at the heading stage resulted in
183 a significant decrease in maximum photosynthetic rate (P_{max}), apparent quantum yield
184 (ϕ) and light saturation point (LSP), and a significant increase in light compensation
185 point (LCP), but it had no significant effect on dark respiration (R_d) (Table 2). The
186 P_{max} decreased 53.8% from 26.4 to 12.2 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in HY113, and 58.2 from 26.1
187 to 10.9 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in HHZ, and 45.3% from 25.6 to 14.0 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in ZH3. The ϕ
188 decreased 50.8% from 0.063 to 0.031 mol μmol^{-1} in HY113, and 70.4% from 0.071 to
189 0.021 mol μmol^{-1} in HHZ, and 47.5% from 0.061 to 0.032 mol μmol^{-1} in ZH3. The LSP
190 decreased 33.6% from 1840 to 1221 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in HY113, and 33.0% from 1862 to
191 1247 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in HHZ, and 32.0% from 1874 to 1274 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in ZH3. While
192 the LCP increased 48.4% from 38.6 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in HY113, and 56.9% from 33.9 to
193 53.2 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in HHZ, and 25.8% from 32.2 to 40.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in ZH3.

194 **Effects of drought stress on chlorophyll fluorescence characteristics**

195 Chlorophyll fluorescence technology is an important means to reflect the interaction
196 between plant leaf photosynthesis and survival environment. The chlorophyll

197 fluorescence characteristics including F_v/F_m , Φ_{PSII} , q^p of flag leaves all significantly
198 decreased under moderate and severe drought stress at heading stage, except for NPQ .
199 and the magnitude of decline and increase was greater for all cultivars under severe
200 drought stress (Fig. 5). The F_v/F_m of flag leaves reduced by 15.2 %, 19.7% and 21.3%
201 in HY113, HHZ and ZH3, respectively (Fig. 5 A). The NPQ of HY113, HHZ and ZH3
202 flag leaves increased by 150%, 97.6% and 218%, respectively (Fig. 5 B). The Φ_{PSII} of
203 flag leaves reduced by 20.3%, 11.9% and 22.1% in HY113, HHZ and ZH3, respectively
204 (Fig. 5 C). The q^p of flag leaves reduced by 19.3%, 21.7% and 19.7% in HY113, HHZ
205 and ZH3, respectively (Fig. 5 D).

206 Monitoring the light energy conversion, electron transfer, distribution and heat
207 dissipation parameters absorbed by the leaf through chlorophyll fluorescence
208 technology can better reflect the true situation of photosynthesis. In the distribution and
209 transformation of light energy absorbed by flag leaves, the PSII photochemistry (P)
210 decreased significantly under drought stress, while excess energy (E) and antennas heat
211 dissipation (D) increased significantly (Table 3). The P reduced by 24.6% in HY113,
212 27.0% in HHZ, and 13.4% in ZH3, while the E and D was increased by 66.7%, 48.0%
213 in HY113, 57.1%, 34.8% in HHZ, and 25.0%, 47.8% in ZH3.

214 **Effects of drought stress on antioxidant enzyme activity and MDA**

215 The enzymatic activities of SOD, POD, CAT was gradually increased under mild and
216 moderate drought stress, but decreased significantly under severe drought stress at
217 heading stage (Table 4). The enzymatic activities of SOD, POD, CAT increased by
218 11.4%,18.0% and 21.8% in HY113, and 13.2%, 14.3% and 30.9% in HHZ, 13.4% 21.7%
219 and 17.6% in ZH3 under moderate on average across two seasons. Compared with
220 moderate drought stress, the activity of SOD, POD, CAT is significantly reduced by
221 16.1%, 24.1% and 14.7% in HY113, and 17.2% 20.1% and 7.6% in HHZ, and 9.8%,
222 26.5% and 7.7% in ZH3. The MDA content of HY113, HHZ and ZH3 had an increasing
223 trend under mild drought stress at the heading stage, but there was no statistical
224 difference. Moreover, the MDA content increased significantly by 14.7%, 15.3% and

225 35.3% under severe drought stress, respectively (Table 4). It shows that under severe
226 drought stress, the cell membrane is seriously damaged due to the accumulation of
227 peroxides.

228 **Effects of drought stress on grain yield and yield components**

229 Drought stress at heading stage led to decreases grain yield of all cultivars, and the
230 falling magnitude of yield was greater with the increase of drought stress. The grain
231 yield reduced by 14.9%, 30.8% and 12.8% in HY113, HHZ and ZH3 under mild
232 drought stress, respectively and 32.9%, 33.7% and 22.9% in HY113, HHZ and ZH3
233 under moderate drought stress, respectively and 53.6%, 45.6% and 30.7% in HY113,
234 HHZ and ZH3 under severe drought stress, respectively (Table 5). Yield components
235 of the setting rate was significantly reduced by 17.6%, 18.6% and 17.8%, respectively.
236 Which can account for the yield loss under the mild drought stress. With the increase
237 of drought stress, especially under the severe drought stress, the reduction of effective
238 panicles, number of spikelets per panicle, and 1000-grain weight contributed to the
239 reduction of grain yield. In addition, the average grain yield in 2014 was lower about
240 32.9%, 39.7% and 41.7% than in 2013, respectively.

241 **Path analysis of photosynthetic**

242 Path analysis of photosynthetic rate and pigment, chlorophyll fluorescence,
243 antioxidative enzymes in drought-resistant type (HY113, ZH3) and non-drought-
244 resistant type HHZ in Figure 6-A and B, which could be useful for understanding the
245 true relationship between photosynthesis and related parameters. The results showed
246 that for the drought-resistant varieties, the order of the magnitude of the impact on
247 photosynthesis is $G_s > F_0 > \Phi_{PSII} > SOD > NPQ > Caro$, when it comes to no-drought-
248 resistant varieties, the order turns to be $\Phi_{PSII} > C_i > CAT > F_v/F_m$.

249 **Discussion**

250 In this experiment, different levels of drought stress were treated at the heading stage
251 of rice through pot experiments. Our results showed that drought at the heading stage
252 significantly reduced the grain yield, and the yield was negatively correlated with the

253 degree of drought stress (Table 5), which was consistent with the previous studies ^{9,53}.
254 Compared with HY113 and HHZ, the yield of ZH3 decreases slightly under the same
255 drought stress conditions. Studies have shown that drought-resistant cultivars could be
256 more likely to develop ideal root characteristics when cultivated in arid and semi-arid
257 regions. In addition, their leaves cell soluble protein, chlorophyll content, cell osmotic
258 adjustment and antioxidant enzyme system activities are significantly higher than
259 conventional rice. However, drought-resistant cultivars demonstrating advantages
260 requires being in a certain arid environment ⁵⁴. Yield components are the basis for the
261 formation of rice output and the changes in yield components caused by drought stress
262 at different growth stages of rice was the key to determine the final grain yield. The
263 accumulation of photosynthetic products from heading stage to maturity stage is crucial
264 for yield formation. Drought stress at heading stage has an effect on number of spikelets
265 per panicle and 1000-grain weight, but the significant reduction in seed setting rate is
266 an important factor led to the reduction of rice yield (Table 5). This study shows that
267 the LWP reduced 31.9%, 54.8% and 15.7% in HY113, HHZ and ZH3, respectively (Fig.
268 2). Studies have shown that reduction in LWP led to panicle birth defect and a negative
269 correlation was found between LWP and spikelet sterility under drought stress at
270 flowering stage. The lower LWP with higher leaf temperature may be an important
271 cause of spikelets sterility in rice ⁵⁵.

272 The dry matter accumulated through photosynthesis is the basis of plant growth and
273 yield, and improving photosynthetic production efficiency is the goal of crops to cope
274 with drought stress. Photosynthetic rate decline is affected by stomatal limitation and
275 non-stomatal limitation. Studies have found that the main factor leading to the decrease
276 of P_n under mild and moderate drought stress is stomatal limitation, while under severe
277 drought stress, non-stomata limitation take the lead ⁵⁶. In this experiment, under mild
278 and moderate drought stress at heading stage, G_s and P_n of leaf decreased almost
279 simultaneously, indicating that the decrease in P_n was mainly due to stomatal limitation.
280 However, under severe drought stress, C_i increased significantly, and P_n was still falling,

281 indicating that stomatal limitation became the main reason for restricting P_n , which is
282 consistent with the results of previous studies. (Fig. 3A). In addition, the P_n of ZH3 and
283 HY113 under different drought stress levels are higher than that of HHZ, indicating that
284 drought-resistant cultivar could show better performance in P_n after suffer drought
285 stress. Analysis of the light response curve shows that HHZ will cause photoinhibition
286 when the light intensity reaches $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ under drought stress. For ZH3 and
287 HY113, the light intensity needs to reach $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ photoinhibition will occur,
288 and the maximum photosynthetic potential of ZH3 and HY113 is significantly higher
289 than HHZ.

290 The photosynthetic rate depends on the Rubisco activity and the electron transfer rate.
291 By monitoring the process of light energy conversion, electron transfer, distribution,
292 and heat dissipation absorbed by the leaves, our research found that the chlorophyll
293 fluorescence characteristics including F_v/F_m , Φ_{PSII} , q^p of flag leaves all significantly
294 decreased under moderate and severe drought stress at heading stage, except for *NPQ*.
295 and the magnitude of decline and increase was greater for all cultivars under severe
296 drought stress (Fig. 5). Which indicates that drought stress reduced the open ratio of
297 PSII reaction centers in the leaves and reduced the ability of PSII to transfer electrons,
298 resulting in a significant decrease in PSII's original light energy conversion efficiency,
299 and ultimately leading to insufficient ATP and NADPH energy required for CO_2
300 assimilation in the photosynthetic system. The decrease of PSII reaction center activity
301 under severe drought stress is the main reason for the decrease of photosynthetic rate⁵⁷.
302 The generation of residual light energy leads to an increase in active oxygen, which led
303 the enzymatic activities of SOD, POD, CAT was gradually increased under mild and
304 moderate drought stress, and decreased significantly under severe drought stress at
305 heading stage (Table 4). Plants activates the antioxidant enzyme system to remove the
306 superoxide anion O_2^- formed under strong light due to carbon assimilation hindered by
307 SOD, and the generated H_2O_2 can be further detoxified by POD and CAT to form H_2O
308 to reduce the damage of free radicals to plants. The reduced activity of PSII reaction

309 center leads to insufficient carbon assimilation capacity in leaves. The excess energy is
310 consumed in the form of heat dissipation NPQ , and the unconsumed part of the energy
311 causes damage to photosynthetic organelles such as cell chloroplasts, which will be
312 produced in plants. A large amount of reactive oxygen species breaks the balance of the
313 ROS scavenging system⁵⁸. At the same time, due to the accumulation of a large amount
314 of reactive oxygen species, the permeability of the cell membrane and the accumulation
315 of MDA content increase, all of which ultimately lead to a significant decline in the
316 photosynthetic capacity of the leaves.

317 **Conclusion**

318 The changes in rice yield are determined by the coordination of photosynthetic capacity
319 and photosynthetic efficiency to promote the formation and accumulation of
320 assimilation under drought stress. Photosynthetic capacity is regulated by chlorophyll
321 and LWP, photosynthetic efficiency is affected by the interaction between q^p and NPQ
322 and the activity of PSII, and the protective enzymes in the leaves can ensure the activity
323 of PSII, avoid chlorophyll degradation, and ensure photochemical energy conversion
324 efficiency. Drought-resistant cultivars can maintain higher LWP, P_n , PSII primary light
325 energy conversion efficiency, NPQ after being exposed to drought stress, and maintain
326 a higher photochemical reaction energy ratio and resistance in fluorescence. Indicators
327 such as oxidase activity jointly stabilize the function of photosynthetic machinery,
328 which is also the physiological basis for drought-resistant cultivars to maintain
329 relatively high photosynthesis under drought conditions. Therefore, Therefore,
330 increasing the energy distribution of the photoreaction center is of great significance
331 for stabilizing food production under adversity.

332

333 **Materials and methods**

334 **Site description**

335 Pot experiments were conducted in glass greenhouse at Huazhong Agricultural
336 University, Hubei Province, China (30°28'N, 114°21'E) during rice growing season of

337 2013 and 2014 (May to October). The soils were taken at 0–30 cm depth from a paddy
338 field, then air-dried and 2-mm sieved. The sieved soil was mixed with sand at a mass
339 ratio of 4:1, and then 18 kg soil filled in plastic pots (30 cm×30 cm×25 cm) to 1.50 g
340 cm⁻³ of bulk density. The pH, total nitrogen (N), available phosphorus, potassium and
341 organic matter of soil were 5.99, 0.21 g kg⁻¹, 22.3 mg kg⁻¹, 167 mg kg⁻¹ and 1.20 g kg⁻¹
342 ¹, respectively, in 2013 and 6.00, 0.18 g kg⁻¹, 19.2 mg kg⁻¹, 188 mg kg⁻¹ and 1.04 g kg⁻¹
343 ¹, respectively, in 2014. The glass greenhouse equipped with irrigation and cooling
344 system, which is convenient for precise quantitative irrigation of each pot, while
345 reducing the impact of high temperatures.

346 **Experiment design**

347 In this study, the experiments were laid out in a randomized complete block design
348 using 12 replicates. Four water management treatments (traditional flooding (CK), mild
349 drought stress (LD), moderate drought stress (MD) and severe drought stress (HD))
350 were compared at heading stage with three different genotype rice cultivars
351 (Hanyou113 (HY113), Huanghuazhan (HHZ), Zhonghan3 (ZH3)) in both 2013 and
352 2014. Among them, HHZ was a conventional rice, HY113 was a water-saving and
353 drought resistance rice, ZH3 was an aerobic rice. These three cultivars were mega
354 varieties widely planted in Central China; drought resistance level is expressed as
355 ZH3>HY113>HHZ. To better control soil water status, 3 cm water level were kept
356 during the whole growth season for CK, while soil drought stress was monitored by soil
357 moisture tensiometer (Procheck, Decagon Devices, Pullman, WA, USA) at 9:00, 14:00
358 and 17:00, maintain 85%, 70% and 55% of saturated soil moisture for LD, MD and HD,
359 respectively. From then on, 3 cm of water level was kept in the pots until 1 week before
360 harvest. Except for rainfall, other meteorological data (daily average temperature) were
361 collected through weather monitor (TPJ-20, Tuopu Instruments Ltd, Zhejiang, China),
362 installed close to the experimental site.

363 Twenty-day-old seedlings were manually transplanted into well prepared soil with two
364 seedlings per pot on 15 May in both 2013 and 2014. A commercial compound fertilizer

365 (N:P₂O₅:K₂O=15:15:15) dose of 12 g per pot was applied at soil preparation. In addition,
366 4.48 g N was applied at tillering and panicle initiation following the ratio of 5:5 in the
367 form of urea. Weeds, diseases, and insects were intensively controlled throughout the
368 whole growing season in both years to avoid yield loss.

369 The recorded parameters are described below.

370 **Chlorophyll content and LWP**

371 Chlorophyll was extracted from uppermost fully expanded leaves with 80% acetone,
372 and chl a, chl b, Car were determined throughout UV-1900 spectrophotometer
373 (Shimadzu, Japan) as described ^[47]. Leaf vein were removed, then soaking with
374 anhydrous ethanol, dark treatment at 4 °C for 48. Chlorophyll content were estimated
375 on the basis of the absorbance at 470 nm, 649 nm and 665 nm. Leaf water potential
376 (LWP) was determined according to the method used by Xu and Zhou, six fully
377 expanded leaves (those for which gas exchange measurements) were sampled and cut
378 into small pieces and mixed immediately (<20 s) at midday (12:00-14:00). A WP4 Dew-
379 point potential meter (WP4C, Decagon Devices Inc., USA) was used for LWP
380 measurement.

381 **Leaf gas exchange measurements**

382 P_n, stomatal conductance (G_s), transpiration rate (T_r) and inter-cellular CO₂
383 concentration (C_i) were measured with LI-6400XT portable photosynthesis
384 measurement system (Li-Cor, Lincoln, NE, USA). The same leaves as LWP were
385 selected, between 9:00 AM and 12:00 without cloud days when the photosynthetic
386 active radiation (PAR) was equal to 1200 μmol m⁻²s⁻¹. Flow rate through the chamber
387 was 400 μmol s⁻¹ with open path, artificial light source was 1500 μmol m⁻²s⁻¹, the vapor
388 pressure deficit was 1.2 kPa and leaf chamber temperature was 30 °C.

389 30 s measurements were conducted immediately after a stable decrease in chamber CO₂
390 concentration was achieved. Six fully expanded leaves were measured for each
391 treatment.

392

393 **Chlorophyll fluorescence measurements**

394 Chlorophyll fluorescence was determined using a portable FMS–2 Pulse Modulated
395 Fluorometer (Hansatech, England). The minimal fluorescence level (F_0), variable
396 fluorescence (F_v) and maximal fluorescence level (F_m) was measured after dark
397 adaption for 20 min. The minimal fluorescence parameters level in light adapted state
398 (F'_0) was observed by illumination the leaf with far-red light. The parameters were
399 calculated following the method of Gauthami ^[48]. The maximal (dark–adapted) quantum
400 yield of PSII photochemistry, photochemical quenching coefficient, non–
401 photochemical quenching coefficient and effective (light–adapted) photochemical
402 efficiency were calculated as $F_v/F_m=(F_m-F_0)/F_m$, $qp=(F'_m-F_s)/(F'_m-F_0)$, $NPQ=(F_m-$
403 $F'_m)/F'_m$, $\Phi_{psII}=(F'_m-F_s)/F'_m$, respectively.

404 **Superoxide dismutase (SOD), Peroxidase (POD), Catalase (CAT),**

405 The full expanded leaves without vein (0.5 g) were crushed into powder in a mortar
406 with pestle under liquid nitrogen. In addition, a small amount of quartz sand also needs
407 to be added during grinding. A reaction mixture was prepared by adding 50 mM
408 phosphate buffer (pH 7.0) and 1% (w/v) polyvinyl pyrrolidone at 0 °C for extracting
409 crude enzyme of the powder. The homogenate was centrifuged at 15000 g for 20 min,
410 and the supernatant was taken for enzyme activity measurement. The SOD activity was
411 measured by nitroblue tetrazole (NBT)-illumination method, 50% inhibition of
412 photochemical reduction as 1 unit of enzyme activity ^[49]. POD activity was assayed
413 according to the guaiacol method, 1% optical density under 470 nm per minute as 1
414 unit of enzyme activity ^[50]. CAT activity was assayed according to the ultraviolet
415 absorption method, 10% optical density under 240 nm per minute as 1 unit of enzyme
416 activity ^[51].

417 **Malondialdehyde (MDA)**

418 The MDA content was determined through thiobarbituric acid (TBA) method ^[52]. Leaf
419 sample (0.5 g) were crushed into powder in a mortar with pestle under liquid nitrogen,
420 then adding 10 ml trichloroacetic acid (TCA) and grind to homogenate. The

421 homogenate was centrifuged at 4000 g for 10 min, and 2 ml supernatant was taken for
422 enzyme activity measurement. A reaction mixture was prepared by adding 2 ml 0.6%
423 terminated through ice cold water (0 °C) followed by centrifugation at 4000 g for 15
424 min. The absorbance of the supernatant was measured at 532 nm, 600 nm and 450 nm
425 using UV-1900 spectrophotometer (BMS, Canada), respectively. The MDA content was
426 calculated as $6.45 \times (\text{OD}_{532} - \text{OD}_{600}) - 0.56\text{OD}_{450}$.

427 **Harvesting**

428 At maturity stage, 6 pots of unsampled plants were harvested for grain yield
429 determination and grain weight was adjusted to a standard moisture content of 0.14 g
430 H₂O g⁻¹. Panicle number was counted in each pot to determine the panicle number per
431 pot, and then all spikelets were parted from panicles by hand-threshing were taken to
432 count the spikelets number. Filled grains and shrunken grains (half-filled grains and
433 unfilled grains) were separated through water selection method, while half-filled grains
434 and unfilled grains could be future separated by a winnowing cleanliness appliance (FJ-
435 1, China Rice Research Institute, China). Grain-filling percentage was calculated as
436 $\text{filled spikelets number} / \text{total spikelets number} \times 100$.

437 **Statistical analysis**

438 Data were analyzed using SPSS15.0 (IBM, USA) statistical software and Canoco
439 (StatSoft Inc., Tulsa, Oklahoma, USA). Statistical differences of the parameter means
440 were determined by the Tukey's honestly significant difference (HSD) test. Path
441 analysis analysis was used to analysis correlation between the photosynthetic rate and
442 pigment, chlorophyll fluorescence, and antioxidative enzymes by CANOCO 5.0
443 (Microcomputer Power Ithaca, USA; Braak and Smilauer, 2012).

444

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628

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634 **Author contributions statement**

635 Cougui Cao and Ping Li conceived the experiments. Benfu Wang, Xiaolong Yang,
636 Liang Chen and Yuanyuan Jiang conducted the experiments and collected the data.
637 Benfu Wang analyzed the data and wrote the manuscript. All authors revised the
638 manuscript.

639

640 **Competing financial interests**

641 The authors declare that they have no competing interests.

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645 **Figure legend**

646 **Figure 1.** Different response of the ratio of Cha and Chb to severe drought stress at
647 heading stage in 2013 and 2014. CK indicates traditional flooding and HD is severe
648 drought stress at heading stage. Different letters indicate statistical significance in
649 variables mean among treatment according to LSD ($P \leq 0.05$), vertical bars represent
650 standard errors.

651

652 **Figure 2.** Effects on LWP under different drought stress levels at heading stage in 2013
653 and 2014. CK indicates traditional flooding, LD is mild drought stress, MD is moderate
654 drought stress and HD is severe drought stress at heading stage. Vertical bars represent
655 standard errors.

656

657 **Figure 3.** A P_n ; B C_i ; C G_s ; D T_r in photosynthetic traits changes under different drought
658 stress levels at heading stage in 2013 and 2014. CK indicates traditional flooding, LD
659 is mild drought stress, MD is moderate drought stress and HD is severe drought stress
660 at heading stage. Vertical bars represent standard errors

661

662 **Figure 4.** Effects on photosynthetic light-response curve of under different drought
663 stress levels at heading stage. CK indicates traditional flooding, LD is mild drought
664 stress, MD is moderate drought stress and HD is severe drought stress at heading stage.

665

666 **Figure 5.** A F_v/F_m ; B NPQ; C Φ_{PSII} ; D q_p changes of chlorophyll fluorescence
667 parameters under different drought stress levels at heading stage in 2013 and 2014. CK
668 indicates traditional flooding, LD is mild drought stress, MD is moderate drought stress
669 and HD is severe drought stress at heading stage.

670

671 **Figure 6.** Path analysis of photosynthetic rate and pigment, chlorophyll fluorescence,
672 antioxidative enzymes in non-resistant type HHZ (A) and resistant type HY113 and
673 ZH3 (B).

Table 1. Effects of drought stress on chlorophyll content at heading stage (2013,2014)

Year	Varieties	Treatments	Chla mg/g	Chlb mg/g	Caro mg/g
2013	HY113	CK	2.95 a	1.24 a	0.45 a
		LD	2.82 b	1.11 b	0.43 a
		MD	2.55 c	0.99 b	0.44 a
		HD	2.52 c	0.92 c	0.45 a
	HHZ	CK	3.01 a	1.25 a	0.34 a
		LD	2.85 b	1.12 b	0.35 a
		MD	2.65 c	1.03 c	0.35 a
		HD	2.50 d	0.94 d	0.34 a
	ZH3	CK	2.30 a	0.65 a	0.30 a
		LD	2.28 a	0.55 b	0.31 a
		MD	2.12 b	0.51 c	0.31 a
		HD	2.11 b	0.51 c	0.31 a
2014	HY113	CK	2.34 a	0.90 a	0.28 a
		LD	2.14 b	0.80 b	0.24 a
		MD	2.05 c	0.70 c	0.25 a
		HD	2.02 c	0.69 c	0.24 a
	HHZ	CK	2.79 a	0.98 a	0.30 a
		LD	2.76 ab	0.94 a	0.30 a
		MD	2.61 bc	0.85 b	0.28 a
		HD	2.49 c	0.79 c	0.28 a
	ZH3	CK	2.04 a	0.68 a	0.29 a
		LD	2.01 a	0.60 ab	0.30 a
		MD	1.85 b	0.56 c	0.29 a
		HD	1.83 b	0.55 c	0.29 a

675 CK: Traditional flooding; LD: Mild drought stress; MD, Moderate drought stress; HD severe
676 drought stress. Lowercase letters indicate significant differences ($P \leq 0.05$) among means across
677 water treatments within varieties for each year according to an ANOVA.

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680

681 **Table 2.** Effect of drought stress on parameters derived from photosynthetic light response curve at
 682 heading stage

Varieties	Treatments	Pmax $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$	ϕ $\text{mol}\cdot\mu\text{mol}^{-1}$	LCP $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$	LSP $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$	Rd $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$
HY113	CK	26.4 a	0.063 a	38.6 c	1840 a	2.30 a
	LD	22.7 b	0.064 a	39.0 c	1948 a	0.94 c
	MD	16.8 c	0.048 b	45.3 b	1878 a	1.45 b
	HD	12.2 d	0.031 c	57.3 a	1221 b	2.17 a
HHZ	CK	26.1 a	0.071 a	33.9 d	1862 a	2.31 a
	LD	14.0 b	0.062 b	36.2 c	1844 a	2.03 a
	MD	10.9 c	0.048 c	40.2 b	1536 b	2.16 a
	HD	10.9 c	0.021 d	53.2 a	1247 b	2.02 a
ZH3	CK	25.6 a	0.078 a	32.2 c	1874 a	1.99 a
	LD	18.9 b	0.037 b	32.9 c	1867 a	1.86 a
	MD	16.5 c	0.034 b	35.4 b	1784 a	1.82 a
	HD	14.0 d	0.066 a	40.5 a	1274 b	1.73 a

683 CK: Traditional flooding; LD: Mild drought stress; MD, Moderate drought stress; HD severe
 684 drought stress. Lowercase letters indicate significant differences ($P\leq 0.05$) among means across
 685 water treatments within varieties for each year according to an ANOVA.

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Table 3. Effects of drought stress on light energy distribution in rice leaves

Varieties	Treatments	P (%)	E (%)	D (%)
HY113	CK	0.69 a	0.06 c	0.25 d
	LD	0.65 ab	0.06 c	0.29 c
	MD	0.63 b	0.07 b	0.31 b
	HD	0.52 c	0.10 a	0.37 a
HHZ	CK	0.63 a	0.14 b	0.23 c
	LD	0.55 b	0.20 a	0.25 c
	MD	0.49 c	0.23 a	0.28 b
	HD	0.46 c	0.22 a	0.31 a
ZH3	CK	0.67 a	0.08 b	0.23 c
	LD	0.64 ab	0.08 b	0.28 b
	MD	0.62 b	0.08 b	0.29 b
	HD	0.58 c	0.10 a	0.34 a

699 CK: Traditional flooding; LD: Mild drought stress; MD, Moderate drought stress; HD severe
700 drought stress. Lowercase letters indicate significant differences ($P \leq 0.05$) among means across
701 water treatments within varieties for each year according to an ANOVA.

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716 **Table 4.** Effects of antioxidant enzyme activity and malondialdehyde content in leaves
 717 of different types of rice under drought stress.

Year	Varieties	Treatments	SOD U/g	POD U/g	CAT U/g	MDA nmol/g
2013	HY113	CK	340 b	19.4 b	29.4 b	15.2 b
		LD	367 a	21.3 a	33.2 a	15.5 b
		MD	383 a	23.0 a	34.2 a	16.9 ab
		HD	313 c	18.1 b	26.4 b	17.7 a
	HHZ	CK	457 c	32.7 b	17.1 c	14.7 b
		LD	473 b	33.0 ab	20.9 b	15.0 b
		MD	512 a	35.9 a	24.9 a	16.3 ab
		HD	448 c	27.3 c	22.5 ab	17.7 a
	ZH3	CK	265 c	20.9 b	23.9 c	25.0 c
		LD	273 b	25.4 a	25.6 b	27.7 bc
		MD	316 a	24.6 a	28.4 a	29.7 b
		HD	284 b	18.0 b	26.5 b	31.5 a
HY113	CK	433 bc	42.0 c	30.0 c	14.0 b	
	LD	459 b	45.5 b	32.3 b	15.1 a	
	MD	477 a	49.3 a	38.2 a	15.0 a	
	HD	411 c	40.7 c	36.1 ab	15.8 a	
2014	HHZ	CK	427 b	50.3 b	30.6 c	17.6 b
		LD	428 b	50.5 b	32.6 bc	17.6 b
		MD	488 a	59.8 a	38.7 a	18.3 ab
		HD	381 c	39.0 c	33.6 b	19.4 a
ZH3	CK	399 b	36.2 bc	25.4 d	23.8 c	
	LD	391 b	39.6 b	54.4 c	27.2 bc	
	MD	429 a	45.5 a	83.0 a	30.9 b	
	HD	388 c	33.6 c	75.7 b	34.4 a	

718 CK: Traditional flooding; LD: Mild drought stress; MD, Moderate drought stress; HD severe
 719 drought stress. Lowercase letters indicate significant differences ($P \leq 0.05$) among means across
 720 water treatments within varieties for each year according to an ANOVA.

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723 **Table 5.** Effects on yield and components under drought stress at heading stage in 2013
 724 and 2014

Varieties	Treatments	Panicles (no./pot)	Spikelets (no./panicles)	Setting rate (%)	Grain weight (g)	Grain yield (g/pot)
HY113	CK	13.8 ab	142 a	80.5 a	27.0 a	95.2 a
	LD	13.3 bc	141 a	67.4 b	25.7 b	81.1 b
	MD	14.2 a	128 c	56.7 c	25.4 b	66.1 c
	HD	12.6 c	136 b	50.0 d	24.8 c	51.4 d
HHZ	CK	14.9 a	170 a	73.4 a	18.5 a	89.4 a
	LD	14.6 ab	166 b	70.3 a	17.0 b	59.9 b
	MD	13.8 b	148 c	62.3 b	16.7 b	55.6 b
	HD	12.5 c	148 c	49.5 c	16.9 b	34.3 c
ZH3	CK	11.6 ab	161 b	80.8 a	25.6 a	91.2 a
	LD	12.3 a	173 a	64.1 b	25.1 a	87.4 b
	MD	10.5 c	154 c	62.9 b	23.9 b	72.9 c
	HD	10.9 c	156 c	59.0 c	23.6 b	61.4 d
HY113	CK	13.2 a	139 a	69.5 a	21.6 a	68.3 a
	LD	13.6 a	126 b	56.3 b	20.2 b	58.1 b
	MD	13.2 a	114 c	44.6 c	20.7 b	44.1 c
	HD	12.7 b	118 c	43.1 c	20.9 b	26.5 d
HHZ	CK	13.5 a	139 a	69.2 a	22.6 a	46.2 a
	LD	13.2 a	118 b	56.3 b	21.2 b	33.0 b
	MD	12.7 ab	114 c	44.6 c	21.7 b	32.5 b
	HD	12.1 b	114 c	43.1 c	21.9 ab	32.5 b
ZH3	CK	11.2 a	104 a	75.6 a	31.0 a	56.3 a
	LD	10.9 ab	96.0 c	64.3 b	31.9 a	44.2 b
	MD	11.3 a	101 b	61.8 b	31.9 a	41.7 c
	HD	10.6 b	97.0 c	51.6 c	32.0 a	40.1 c

725 CK: Traditional flooding; LD: Mild drought stress; MD, Moderate drought stress; HD severe
 726 drought stress. Lowercase letters indicate significant differences ($P \leq 0.05$) among means across
 727 water treatments within varieties for each year according to an ANOVA.

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Figures

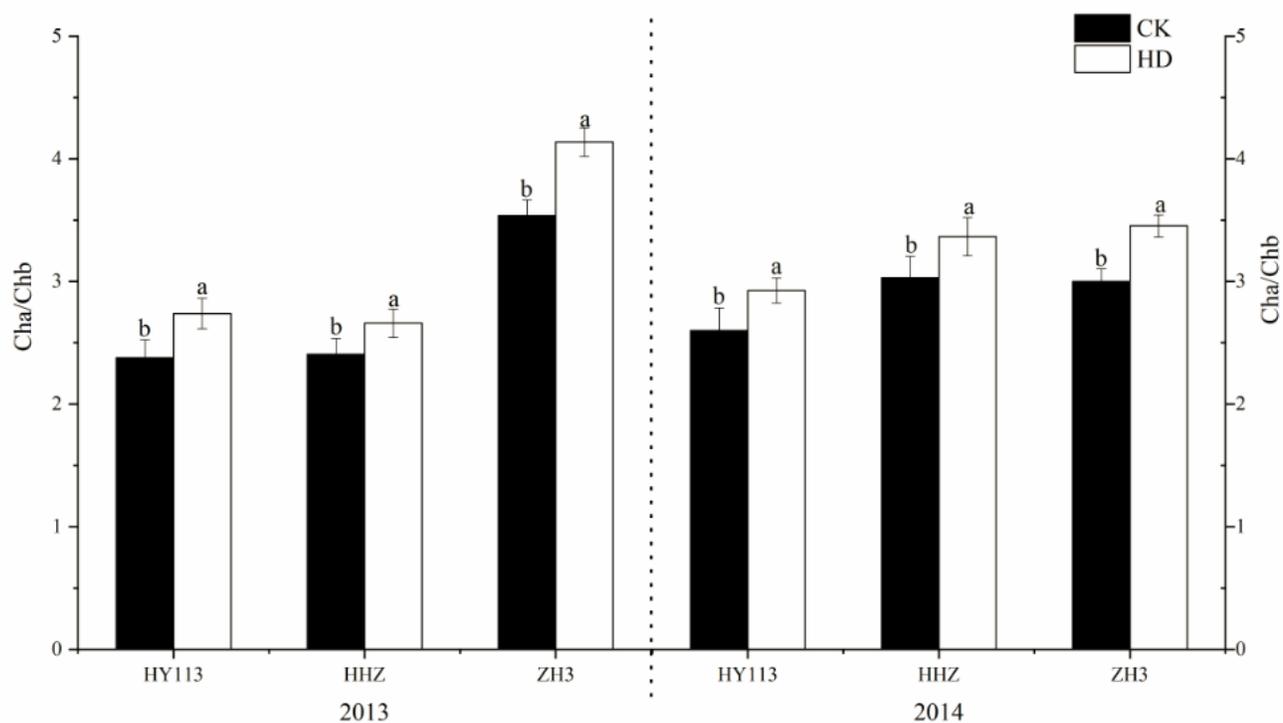


Figure 1

Different response of the ratio of Cha and Chb to severe drought stress at heading stage in 2013 and 2014. CK indicates traditional flooding and HD is severe drought stress at heading stage. Different letters indicate statistical significance in variables mean among treatment according to LSD ($P \leq 0.05$), vertical bars represent standard errors.

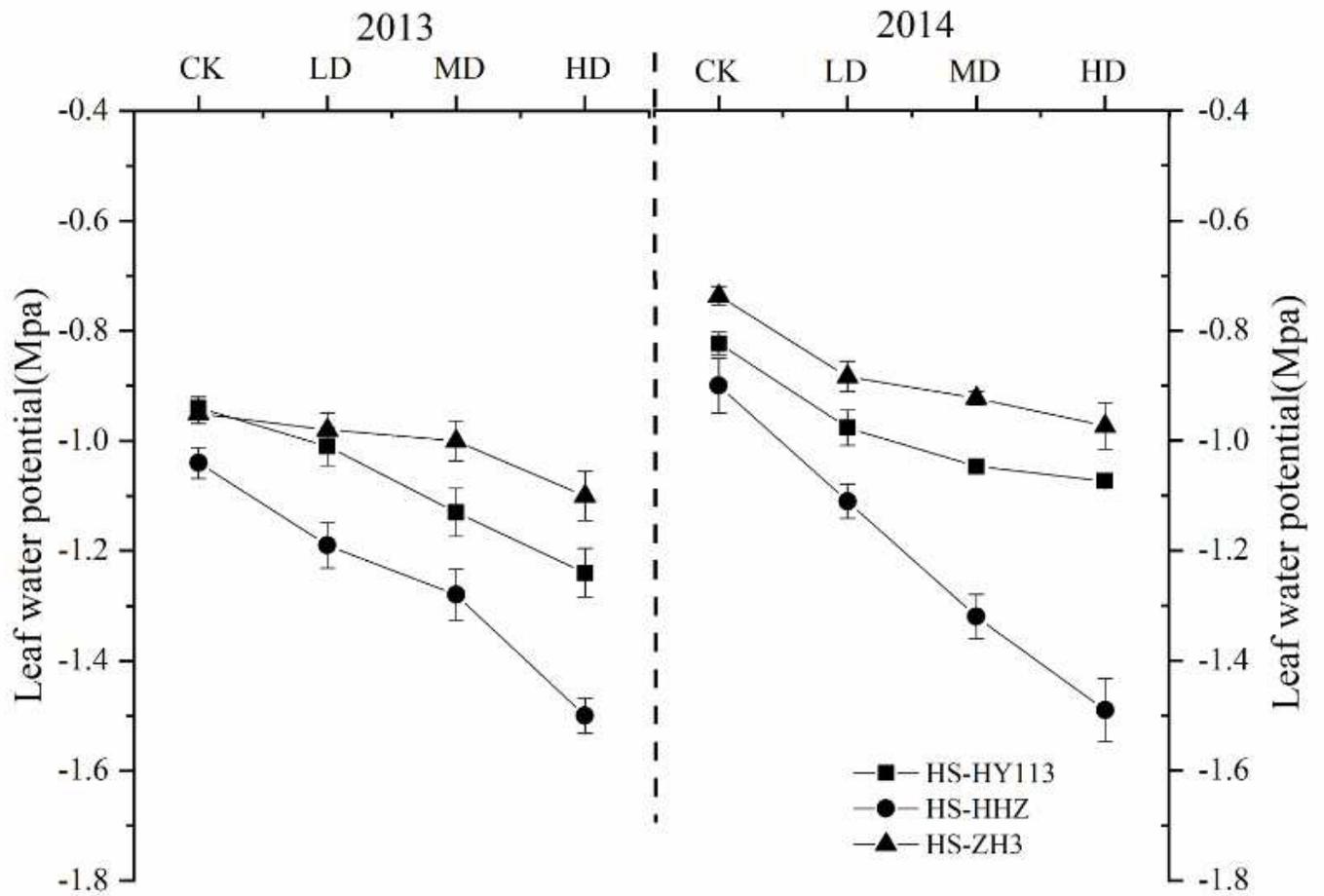


Figure 2

Effects on LWP under different drought stress levels at heading stage in 2013 and 2014. CK indicates traditional flooding, LD is mild drought stress, MD is moderate drought stress and HD is severe drought stress at heading stage. Vertical bars represent standard errors.

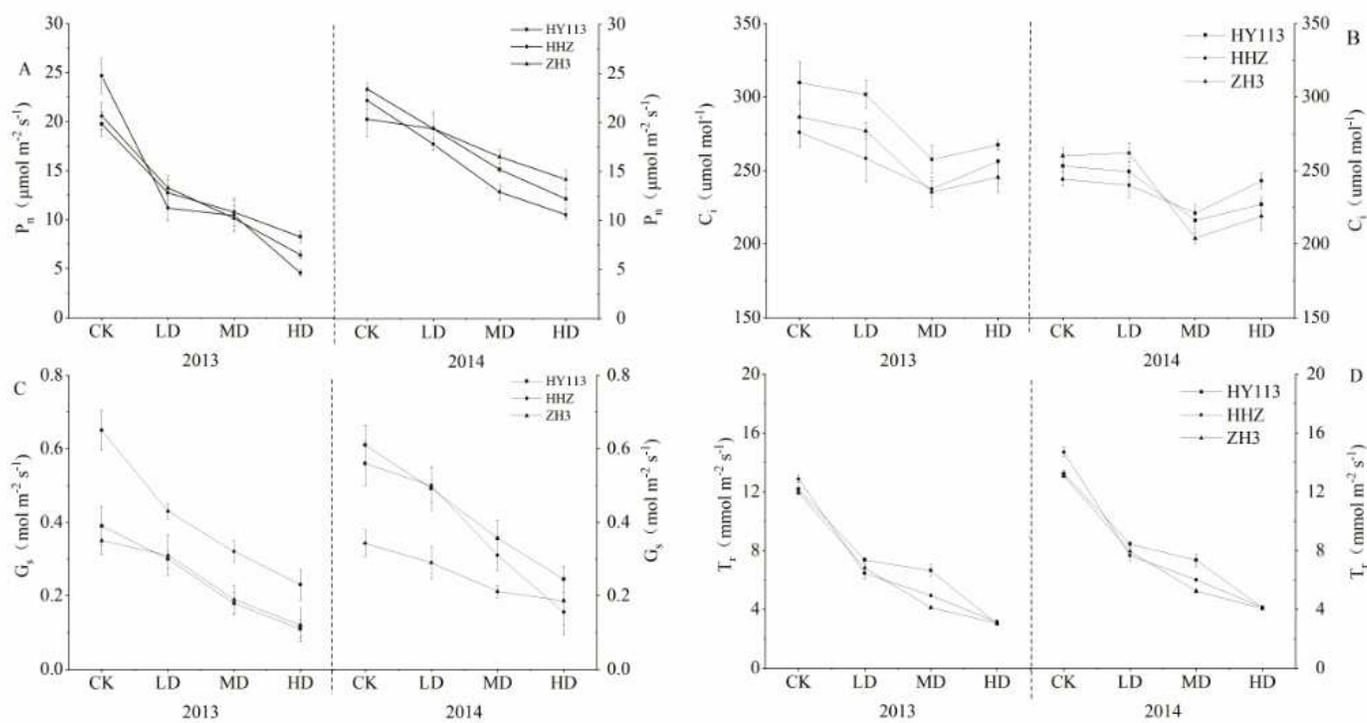


Figure 3

A P_n ; B C_i ; C G_s ; D T_r in photosynthetic traits changes under different drought stress levels at heading stage in 2013 and 2014. CK indicates traditional flooding, LD is mild drought stress, MD is moderate drought stress and HD is severe drought stress at heading stage. Vertical bars represent standard errors

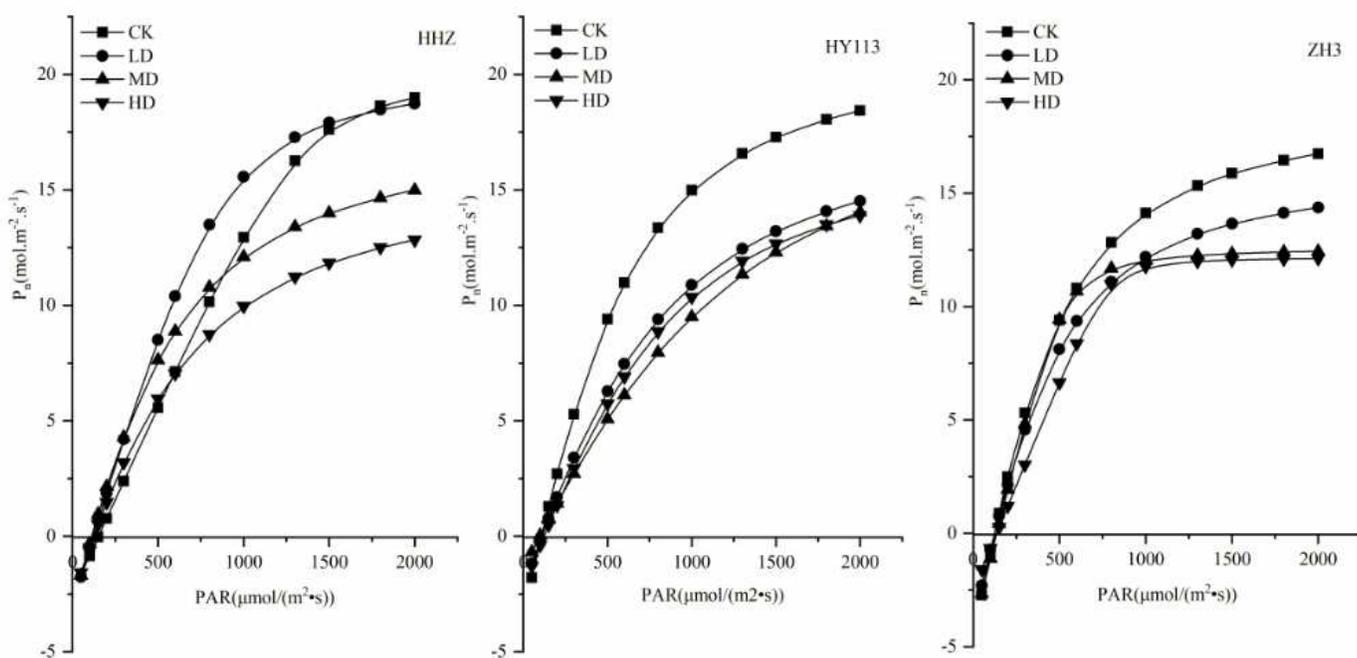


Figure 4

Effects on photosynthetic light-response curve of under different drought stress levels at heading stage. CK indicates traditional flooding, LD is mild drought stress, MD is moderate drought stress and HD is severe drought stress at heading stage.

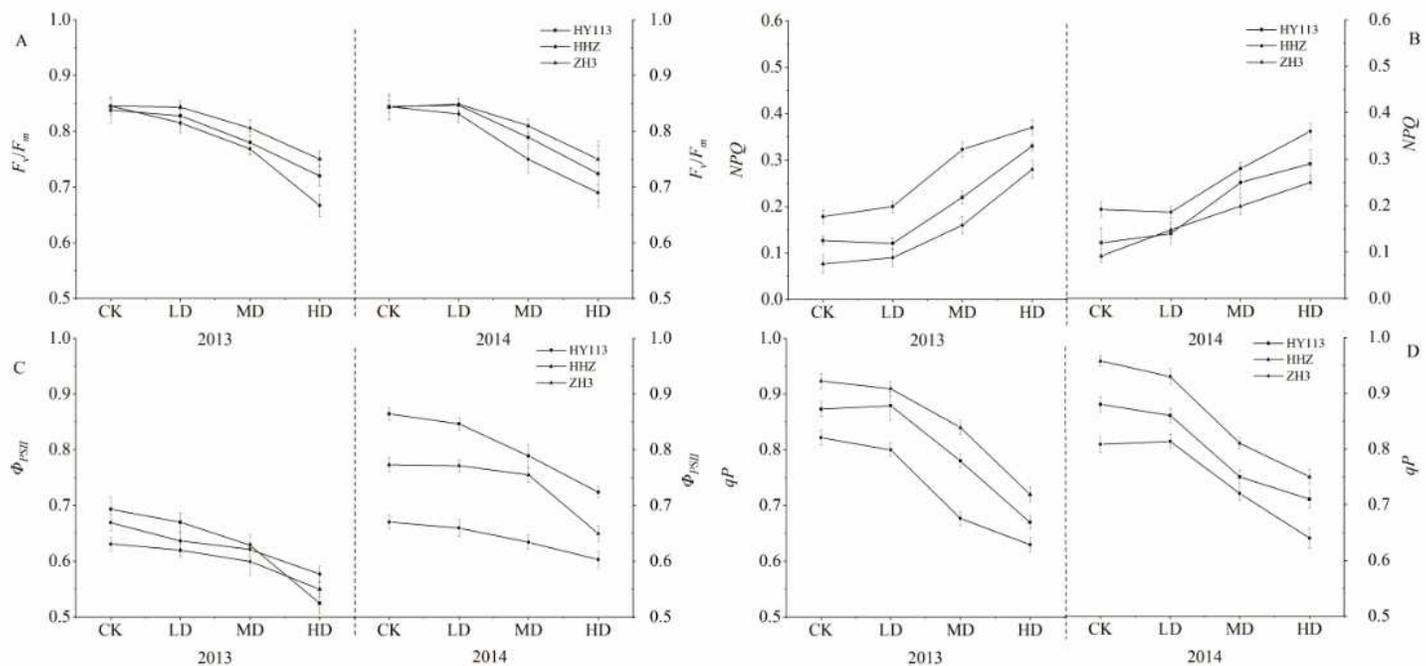


Figure 5

A F_v/F_m ; B NPQ; C Φ_{PSII} ; D q_p changes of chlorophyll fluorescence parameters under different drought stress levels at heading stage in 2013 and 2014. CK indicates traditional flooding, LD is mild drought stress, MD is moderate drought stress and HD is severe drought stress at heading stage.

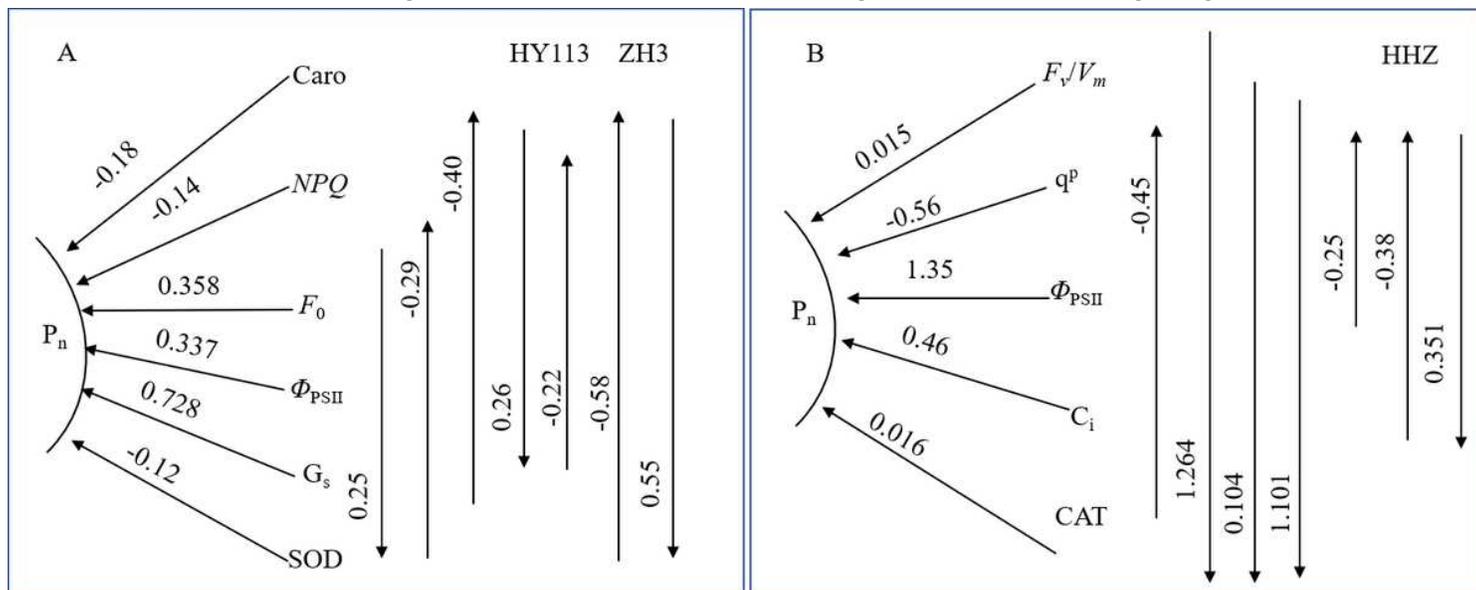


Figure 6

Path analysis of photosynthetic rate and pigment, chlorophyll fluorescence, antioxidative enzymes in non-resistant type HHZ (A) and resistant type HY113 and ZH3 (B).