



**Full Length Article**

# Water Deficit and Recovery-Induced Changes in Growth, Photosynthetic Characteristics, Antioxidant Enzymes and Yield of Two Tartary Buckwheat Genotypes

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## Abstract

Tartary buckwheat (*Fagopyrum tataricum*) is an important food crop that is widely adaptable to hostile environments. In this study the responses of two Tartary buckwheat genotypes: drought-susceptible Chuanqiao No. 1 (CQ) and drought-tolerant Jingqiao No. 2 (JQ) in terms of morphology, photosynthesis, physiology and yield to a progressive water deficit and recovery treatment (WD-R) were evaluated. Plants in the well-watered (WW) treatment were watered throughout the experiment. Compared to the WW treatment, water deficit in the WD-R treatment caused decreases in plant height, stem diameter, branch number, stem node number, biomass, seed number, soil water content (SWC), leaf relative water content (RWC), net photosynthesis rate (Pn), intercellular CO<sub>2</sub> concentration, stomatal conductance (Gs), transpiration rate (Tr) and Fv/Fm in both CQ and JQ plants. Leaf wilting, malondialdehyde content, superoxide dismutase activity, peroxidase activity, initial fluorescence (F<sub>0</sub>) and root-to-shoot ratio were significantly increased under water stress in the WD-R treatment. Under the WD-R treatment, compared to CQ, JQ maintained higher RWC, SWC, Pn, Gs, WUE, Fv/Fm, plant height, branch number, stem node number, root biomass, stem biomass, leaf biomass, total biomass, root-to-shoot ratio, seed number per plant, and yield, but a lower Tr and F<sub>0</sub>. By correlation analysis, Gs was positively correlated with leaf RWC and SWC. These differential growth indexes, biochemical traits and physiological responses might be useful for understanding drought-tolerance genotypes that can grow under water-deficit conditions with minimum yield loss. © 2021 Friends Science Publishers

**Keywords:** Water deficit and recovery; Photosynthetic parameter; WUE; Antioxidant enzyme; Yield loss

## Introduction

Water deficit is a primary constraint for terrestrial plants (Chimungu *et al.* 2014) and drought stress greatly limits crop production and food stability in arid and semiarid regions (Delpérée *et al.* 2003; Farooq *et al.* 2009). Plants have evolved various strategies to deal with drought stress, which can be divided into drought avoidance, and the development of adaptation mechanisms that contribute to drought tolerance (Claeys and Inze 2013; Hussain *et al.* 2018). Adaptive strategies that are induced during the drought process help plants survive (Nguyen *et al.* 2016). Plants such as maize (*Zea mays* L.) reduce tissue dehydration, either via tolerance to lower tissue water potential or by maintaining the water potential (Wu and Cosgrove 2000; Farooq *et al.* 2017, 2018). To successfully minimize water loss, plants limit transpiration by decreasing leaf area, closing their stomata and accelerating leaf

senescence, resulting in improved water-use efficiency (WUE) (Franks *et al.* 2015; Farooq *et al.* 2019) or reduced photosynthesis (Shi *et al.* 2016). To protect against drought-induced oxidative damage to the cells, osmotically active metabolites rapidly increase (Vasquez-Robinet *et al.* 2008; Claeys and Inze 2013). Plants also respond to water deficit by inducing sets of both regulatory and functional genes (Yoshida *et al.* 2014; Simmons and Bergmann 2016). Recent studies in *Arabidopsis* (*Arabidopsis thaliana*) have shown that phytohormones such as cytokinin or abscisic acid induce signaling pathways that activate or negatively regulate the genes necessary for drought-acclimation responses (Yoshida *et al.* 2014; Nguyen *et al.* 2016). Transcriptional regulation is a vital regulatory mechanism mediating drought tolerance (Yoo *et al.* 2010; Simmons and Bergmann 2016).

Tartary buckwheat (*Fagopyrum tataricum*) is an important traditional edible and medicinal crop with

excellent nutritional and pharmacological properties (Zhu 2016). Tartary buckwheat is usually cultivated in the mountainous areas of western China, northern India, Bhutan, and Nepal at high altitude. Therefore, it is extremely well-adapted to harsh climatic conditions (Zhou *et al.* 2015; Zhang *et al.* 2017) and it regularly suffers from drought stress in its main growing regions (Xiang *et al.* 2020). Thus, Tartary buckwheat is an ideal plant to study adaptation mechanisms under drought conditions. Therefore, to better understand the specific drought-adaptive responses with respect to morphological plasticity, biochemical indexes, photosynthetic and chlorophyll fluorescence parameters, WUE and seed yield, two contrasting Tartary buckwheat genotypes [drought-susceptible Chuanqiao No. 1 (CQ) and drought-tolerant Jinqiao No. 2 (JQ)] were sown under progressive drought and recovery at flowering stage.

## Materials and Methods

### Growth conditions and materials

The experiment was conducted in a greenhouse at Chengdu University (30°39'N, 104°11'E, altitude 490 m asl). Two Tartary buckwheat genotypes, previously evaluated for their yield responses to water stress, were used in this experiment: the drought-tolerant cv. Jinqiao No. 2 (JQ) and drought-susceptible cv. Chuanqiao No. 1 (CQ), selected for by the Shanxi Academy of Agricultural Sciences and the Agricultural Science Institute of Zhaojue County, Liangshan Yi Autonomous Prefecture, respectively. The experiment was initiated on 4 Mar 2018. Seeds were sterilized with 0.1% potassium permanganate solution for 30 min and then rinsed three to four times with distilled water. They were then sown in plastic pots (20 cm high, 25 cm diameter) in a waterproof shelter. One plant was allowed to grow per plastic pot to the three-and-a-half leaf stage. The plastic pots were allowed to drain freely from the bottom and contained 15 kg soil (sandy loam, with 1.87 g kg<sup>-1</sup> total nitrogen, 17.3 g kg<sup>-1</sup> organic matter, 1.49 g kg<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, and 15.8 g kg<sup>-1</sup> total potassium). At the experimental site of Chengdu University, the annual mean air temperature was 16°C, total precipitation was 900–1300 mm, and average sunshine duration was 1042–1412 h overall year.

### Drought treatment and recovery

The pots were weighed daily. A fully watered pot was set at 100% water-holding capacity. For the well-watered (WW) treatment, plants were watered daily to maintain 90% water-holding capacity. For the water deficit and recovery (WD-R) treatment, plants from bud-appearing stage were exposed to gradual soil water depletion (50% water-holding capacity) for 14 days, then all plants were re-watered daily to the initial pot water-holding capacity for 9 days of recovery. Pots were weighed daily during both

water-deficit and recovery periods. There were 60 pots for each treatment. Each treatment had four independent replicates, arranged in a completely randomized designed. The imposition of drought simulated possible conditions in the field.

### Microenvironment for plants

During the experiment, soil water content (SWC) and soil electrical conductivity (SEC) were measured in the top 20 cm of the soil every 2 days using a WET-2-K1 time-domain reflectometry probe (Delta-T Devices Ltd., Cambridge, U.K.).

### Evaluation of leaf wilting (LW) and leaf relative water content (RWC)

To analyze LW, 10 plants from 10 pots of each treatment were monitored daily at 08:00 h. LW was graded on a five-point scale: level 1, normal; level 2, curling slightly; level 3, curling slightly with middle and lower leaves drooping; level 4, curling heavily with all leaves drooping; level 5, rolling into a cylindrical shape with all leaves drooping and the tip growth point wilting.

To measure leaf RWC, leaf samples were weighed for fresh weight (FW) and drenched with deionized water at 4°C for saturated weight (SW), then oven-dried at 80°C for 72 h for dry weight (DW). Leaf RWC (%) was calculated as:

$$\left[ \frac{FW-DW}{SW-DW} \right] \times 100 \quad (\text{Barrs and Weatherley 1962})$$

### Gas exchange traits

The net photosynthesis rate (Pn), intercellular CO<sub>2</sub> concentration (Ci), stomatal conductance (Gs) and transpiration rate (Tr) of the last fully developed and expanded leaf was examined on days 5, 9, 14, 19 and 23 of the experimental period with a portable photosynthesis system (LI-6400, LI-COR Biosciences, Lincoln, NE, USA). These parameters were automatically recorded by the machine at 10:00–12:00 h under atmospheric CO<sub>2</sub> and full sunlight. WUE was calculated as:

$$Pn/Tr$$

### Chlorophyll (Chl) *a* fluorescence

Chl *a* fluorescence of the same leaves used for the gas-exchange measurements was determined in leaf discs by pulse-amplitude modulated fluorescence spectrometry (Mini-PAM, Heinz Walz, Pfullingen, Germany). The initial Chl *a* fluorescence (F<sub>0</sub>) was recorded after 30 min dark adaptation using a beam of 0.2 μmol m<sup>-1</sup> s<sup>-1</sup>. The maximum Chl *a* fluorescence (F<sub>m</sub>) was recorded at 8,000 μmol m<sup>-1</sup> s<sup>-1</sup> with a 0.8-s saturating pulse. The Overall Chl *a* fluorescence

(Fv) was calculated as  $F_m - F_0$ . Fv/Fm, the maximum efficiency of photosystem II (PSII) photochemistry in the dark, was calculated as:

$$\frac{(F_m - F_0)}{F_m} \quad (\text{Dias and Bruggemann 2010})$$

#### Malondialdehyde (MDA) and soluble protein (SP) contents, and activities of superoxide dismutase (SOD) and peroxidase (POD)

Fresh leaf samples (last fully developed leaves) were taken on the same days as the photosynthesis measurements to determine MDA and SP contents, and the activities of SOD and POD. Leaf samples were immediately frozen in liquid nitrogen after collection and then stored at  $-80^\circ\text{C}$  for further use. Contents of MDA and SP were measured with suitable modifications of Dhindsa's method (Dhindsa *et al.* 1981) and Bradford's method (Bradford 1976), respectively. The activities of SOD and POD were determined by Giannopolitis and Ries' method (Giannopolitis and Ries 1977) and Nakano and Asada's method (Nakano and Asada 1980), respectively.

#### Morphological measurements and grain yield

In each treatment, 20 plants from 20 pots of each treatment were harvested at physiological maturity to determine plant height, branch number, stem diameter, and number of nodes on the main stem. Seed numbers for each plant, 1000-seed weight and grain yield were recorded.

#### Statistical analysis

Statistical analyses were carried out using Excel 2010, S.P.S.S. 13.0 (Chicago, I.L., U.S.A.) and SigmaPlot 10.0 (Aspire Software International, Ashburn, V.A., U.S.A.). Two-way ANOVA was used to determine the significance level, and means were compared by Duncan's multiple range tests at a significance level of  $P < 0.05$ .

## Results

#### Leaf RWC and LW

During the experiment, no significant fluctuations in leaf RWC (Fig. 1A) or LW (Fig. 1B) were observed in either CQ or JQ plants under the WW treatment. A gradual decrease in RWC for both Tartary buckwheat cultivars were observed during the drought part of the WD-R treatment, but the JQ plants maintained a higher RWC level than the CQ plants. On day 14 of the WD-R treatment, the RWC of CQ leaves was 48.2% and that of JQ was 72.5%. Upon re-watering, RWC increased in both cultivars. When subjected to water deficit, LW markedly increased for the two genotypes, but was significantly lower for JQ plants vs. CQ plants. On day 14 of the WD-R treatment, CQ leaves

showed a high level of drooping and tip growth point wilting, whereas JQ leaves were slightly curled with middle and lower leaf drooping. After re-watering, the LW in both genotypes gradually returned to the WW levels.

#### Soil water content (SWC) and soil electrical conductivity (SEC)

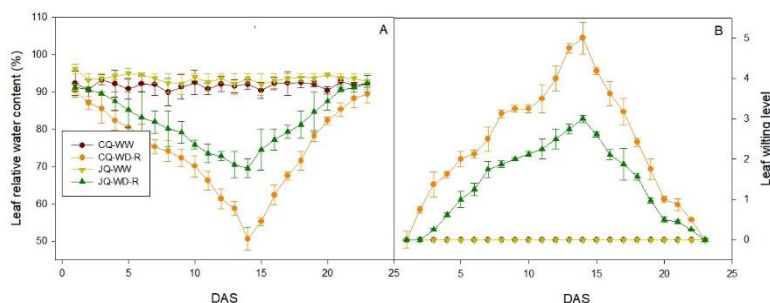
As shown in Fig. 2A and B, neither SEC nor SWC changed significantly in the CQ or JQ pots under the WW treatment. SEC in the WD-R pots of both genotypes increased significantly during the drought part of the treatment compared to the WW treatment. On day 14 of the WD-R treatment, SEC in the CQ pots was significantly higher than that in the JQ pots. After re-watering, SEC in the WD-R pots of both genotypes gradually decreased to normal WW values. Opposite trends were observed for SWC: SWC in CQ and JQ pots decreased by 44.6–51.1% during the dehydration part of the WD-R treatment compared to the WW treatment. On day 14 of the WD-R treatment, SWC in CQ pots had decreased more than in the JQ pots. During the process of recovery in the WD-R treatment, the SWC in CQ and JQ pots increased back to WW values.

#### Temporal dynamics of photosynthetic characteristics and WUE

All measured photosynthetic parameters and WUE of CQ and JQ plants in the WW treatment remained stable during the experiment (Fig. 3A–D). Pn, Ci, Gs and Tr of both genotypes declined consistently with decreasing soil water availability during drought phase of the WD-R treatment. Pn, Gs, Ci and Tr progressively increased with water application during the recovery phase of the WD-R treatment. There were significant differences in Pn and Gs between the two genotypes: JQ plants showed higher Pn than CQ plants during the experiment. Pn, Gs, and Tr of the CQ and JQ plants differed significantly ( $P < 0.05$ ) between WW and WD-R treatments on days 5, 9, 14, 19 and 23, while Ci differed on days 5, 9, 14 and 19. On day 14, Pn and Ci in CQ plants under the WD-R treatment were lowest among all treatments and genotypes, reduced by 81.08 and 56.44%, respectively, compared to JQ plants in the WW treatment ( $P < 0.01$ ). Gs and Tr in JQ plants under WD-R treatment were significantly lower than in the other treatments. There was no difference in WUE between the two genotypes in the WW treatment (Fig. 4). WUE in the JQ plants under the WD-R treatment was significantly highest for all treatments and genotypes on days 5, 9, 14 and 19. Especially on day 14, WUE in JQ plants under WD-R treatment was at its highest point in the whole experimental phase.

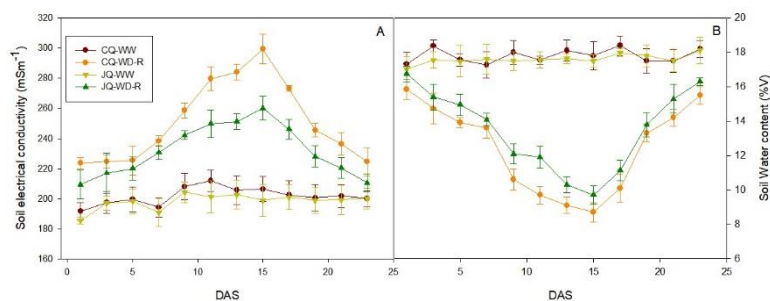
#### Dynamic changes in Chl a fluorescence characteristic

$F_0$  and Fv/Fm of CQ and JQ plants in the WW treatment were relatively constant throughout the experiment (Fig. 5A and B). However,  $F_0$  was significantly increased by water



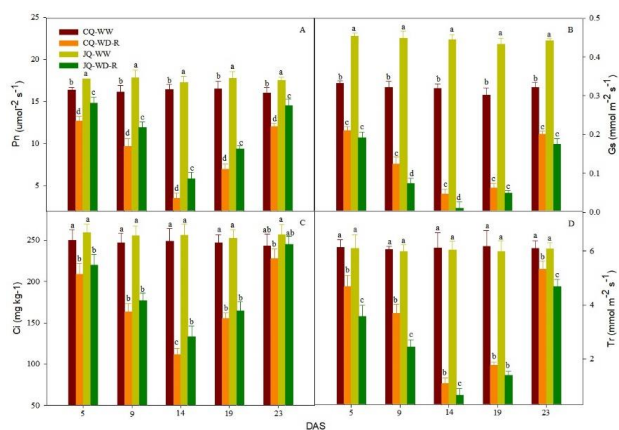
**Fig. 1:** Effect of drought stress and recovery phase on leaf relative water content (A) and leaf wilting rate (B) of two Tartary buckwheat genotypes

WW= Well-watered; WD-R= Water deficit to recovery; CQ= Chanqiao No.1; JQ= JingQiao No.2; DAS= Days after imposition of drought stress



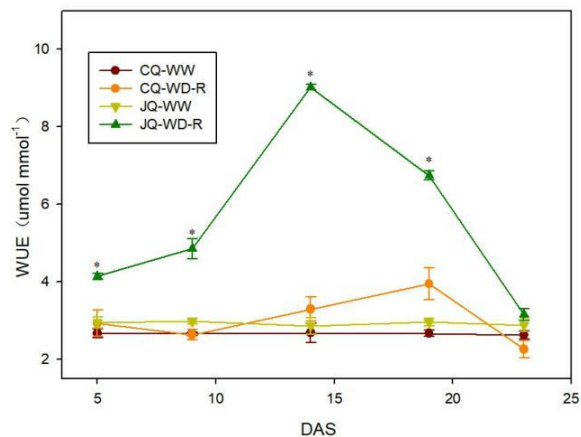
**Fig. 2:** Effect of drought stress and recovery phase on soil electrical conductivity (A) and soil water content (B) of two Tartary buckwheat genotypes

WW= Well-watered; WD-R= Water deficit to recovery; CQ= Chanqiao No.1; JQ= JingQiao No.2; DAS= Days after imposition of drought stress



**Fig. 3:** Effect of drought stress and recovery phase on Pn (A), Gs (B), Ci (C) and Tr (D) of two Tartary buckwheat genotypes. Different lowercase letters in each figure indicate significant differences ( $P < 0.05$ ) between treatments on each sampling days WW= Well-watered; WD-R= Water deficit to recovery; CQ= Chanqiao No.1; JQ= JingQiao No.2; DAS= Days after imposition of drought stress; Pn=net photosynthetic rate; Gs= stomatal conductance; Ci= intercellular CO<sub>2</sub> concentration; Tr= transpiration rate

deficit in the WD-R treatment in both CQ and JQ plants on days 9 and 14, while Fv/Fm was significantly decreased on day 14. Re-watering induced a decline in F<sub>0</sub> and an increase in Fv/Fm. On days 9 and 14, F<sub>0</sub> of CQ under the WDR treatment was highest among all treatments and genotypes, while Fv/Fm of CQ under WD-R treatment was lowest on day 14.



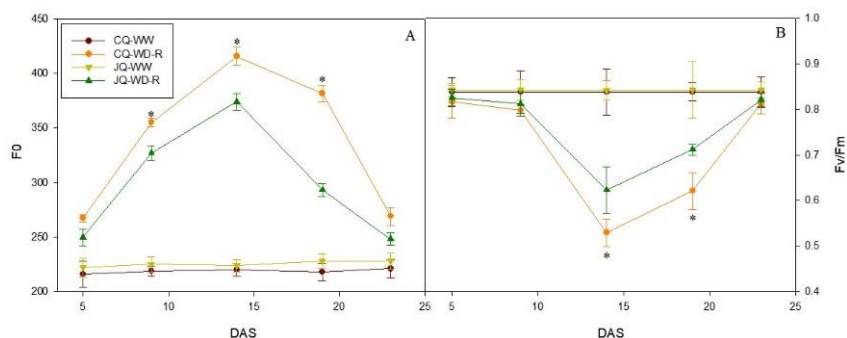
**Fig. 4:** Effect of drought stress and recovery phase on WUE of two Tartary buckwheat genotypes

WW= Well-watered; WD-R= Water deficit to recovery; CQ= Chanqiao No.1; JQ= JingQiao No.2; DAS= Days after imposition of drought stress; WUE=water-use efficiency; Asterisks \* represent significant differences between treatments on corresponding days ( $P < 0.05$ )

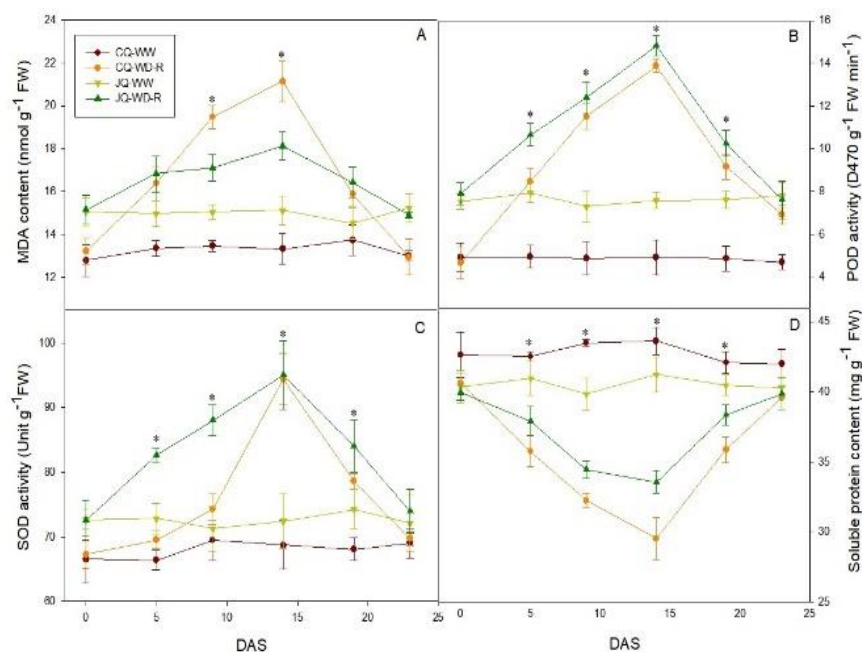
### Enzymatic antioxidants

Across the experiment, MDA and SP contents, and SOD and POD activities in both genotypes under WW treatment were relatively constant (Fig. 6A–D). During the drought phase of the WD-R treatment, MDA content, and SOD and POD activities were significantly enhanced in both





**Fig. 5:** Effect of drought stress and recovery phase on F0 (A) and Fv/Fm ratio (B) of two Tartary buckwheat genotypes WW= Well-watered; WD-R= Water deficit to recovery; CQ= Chanqiao No.1; JQ= JingQiao No.2; DAS= Days after imposition of drought stress; F0= initial fluorescence; Fv/Fm ratio= the maximum efficiency of photosystem II (PSII) photochemistry; Asterisks \* represent significant differences between treatments on corresponding days ( $P < 0.05$ )



**Fig. 6:** Effect of drought stress and recovery phase on MDA content (A), POD activity (B), SOD activity (C) and SP content (D) of two Tartary buckwheat genotypes WW= Well-watered; WD-R= Water deficit to recovery; CQ= Chanqiao No.1; JQ= JingQiao No.2; DAS= Days after imposition of drought stress; MDA= Malondialdehyde; POD= peroxidase; SOD= superoxide dismutase; SP= soluble protein; Asterisks \* represent significant differences between treatments on corresponding days ( $P < 0.05$ )

genotypes, and then dropped back to normal levels during the recovery phase. SP showed the opposite trend. MDA content of CQ plants under WD-R treatment was highest out of all treatments on days 9 and 14, leading to a 58.6% increment compared to CQ plants under the WW treatment on day 14 of the experiment. POD activity in JQ plants under WD-R treatment was highest among all treatments on days 5, 9, 14 and 19. During the drought phase of the WD-R treatment, SOD activity in JQ plants increased steadily, whereas that in CQ plants increased sharply from day 9 to day 14, then decreased back to normal WW levels on day 23. SOD activity in JQ plants under WD-R treatment was highest—significantly higher than that in JQ plants and CQ plants under the WW treatment—on days 5, 9, 14 and 19. The SP content of CQ and JQ plants differed significantly ( $P$

$< 0.05$ ) between WW and WD-R treatments on days 5, 9, 14 and 19. Among all treatments, SP content was significantly lowest in CQ plants under the WD-R treatment.

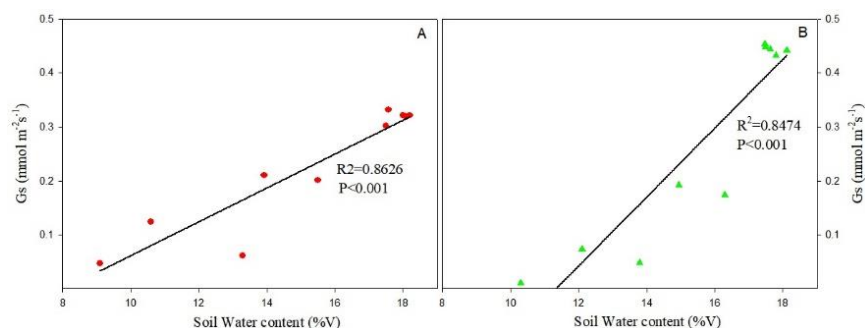
### Growth, biomass and root-to-shoot ratio

Plant height, branch number, stem diameter and stem node number for JQ were higher than for CQ under both WW and WD-R conditions (Table 1). The WD-R treatment caused a significant reduction in plant height, branch number, stem diameter and stem node number compared to the WW treatment due to soil dehydration. Among all treatments and genotypes, plant height, stem diameter, branch number and stem node number were significantly lowest for CQ under the WD-R treatment. The same trend

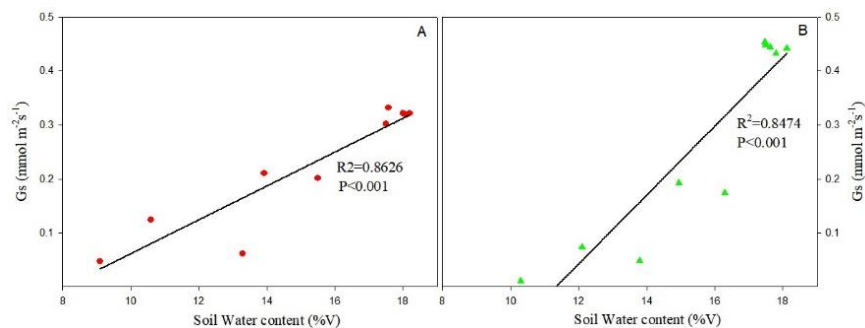
**Table 1:** Effect of drought stress and recovery phase on growth, biomass, root to shoot ratio, yield component and yield of two Tartary buckwheat genotypes

Varieties	Treatments	Plant height (cm)	Stem diameter (mm)	Branch Number	Stem node number	Biomass (g/plant)				Root to shoot ratio	Seeds number per plant	1000-grain weight (g)	Yield per plant (g)
						Root	Stem	Leaf	Total				
CQ	WW	63.8b	3.71b	9.9b	10.0bc	0.64c	1.83b	2.67b	5.14b	0.12c	158.2b	22.97a	3.63a
	WD-R	51.3c	3.37c	7.0d	9.7c	0.56d	1.23c	2.25c	4.04c	0.14b	112.7c	22.13a	2.49b
JQ	WW	76.4a	4.28a	10.7a	11.1a	0.72b	2.04a	3.12a	5.88a	0.12c	179.5a	22.85a	4.10a
	WD-R	65.1b	3.90b	9.1c	10.3b	0.92a	1.75b	2.74b	5.41b	0.17a	166.5ab	22.62a	3.76a

Within each column, different small letters denote significant differences among treatments at  $P < 0.05$   
 WW= Well-watered; WD-R= Water deficit to recovery; CQ= Chanqiao No.1; JQ= JingQiao No.2



**Fig. 7:** Relationship between leaf RWC and Gs in CQ (A) and JQ (B) plants during the water stress and recovery phase  
 CQ= Chanqiao No.1; JQ= JingQiao No.2; Gs= stomatal conductance; RWC=relative water content



**Fig. 8:** Relationship between Gs and SWC in CQ (A) and JQ (B) plants during the water stress and recovery phase  
 CQ= Chanqiao No.1; JQ= JingQiao No.2; Gs= stomatal conductance; SWC= soil water content

was observed for biomass. There were significant differences in biomass (root, stem, leaf and total) between the two genotypes. Root biomass, stem biomass, leaf biomass and total biomass were significantly lower under the WD-R treatment than under the WW treatment. Biomass (root, stem, leaf and total) of CQ in the WD-R treatment was the lowest for all water conditions and genotypes. JQ plants had a higher root-to-shoot ratio than CQ plants. Root-to-shoot ratios of CQ and JQ were significantly increased, by 16.7 and 41.7%, respectively, under the WD-R treatment compared to the corresponding WW treatment. Among all treatments, JQ plants under the WD-R treatment had the significantly highest root-to-shoot ratio.

### Yield components and total yield

Under WW conditions, there were no significant differences in seed yield between the two genotypes. However, the seed

yield of CQ decreased more than that of JQ under the WD-R treatment (Table 1). The yield of CQ reached under the WD-R treatment was 31.4% of that under the WW treatment, but there was no significant yield loss for JQ grown under the WW vs. WD-R treatments. Seed number per JQ plant was significantly higher than that per CQ plant under both WW and WD-R conditions. Seed number per CQ and JQ plant decreased under the drought part of the WD-R treatment. The number of seeds per CQ and JQ plant decreased significantly, by 28.8 and 12.8%, respectively, under the WD-R treatment compared to the corresponding WW treatment. There were no significant differences in grain weight among genotypes or treatments.

### Correlation analysis

Relationships between Gs, leaf RWC and SWC were studied to determine those that might indicate responses to

drought stress and recovery in CQ and JQ plants. The Gs was positively correlated with leaf RWC in CQ and JQ plants ( $P < 0.01$ ; Fig. 7). Positive correlations were also found in CQ ( $R^2 = 0.8626$ ,  $P < 0.001$ ; Fig. 8A) and JQ ( $R^2 = 0.8474$ ,  $P < 0.001$ ; Fig. 8B) plants between SWC and Gs.

## Discussion

Water loss, photosynthetic inhibition, growth restriction, and cell membrane damage are the most common symptoms in plants under drought stress (Farooq *et al.* 2009; Bodner *et al.* 2015; Hussain *et al.* 2017). Some of these effects can be restrictive or even devastating. In this study, the CQ and JQ plants survived 14 days of progressive water deficit. A significant difference in LW was found between WW and WD-R treatments. Compared to JQ plants, CQ plants were more sensitive to dehydration, as wilting appeared earlier and were more severe. The leaf is an important organ for photosynthesis. Under normal conditions, with sufficient water for photosynthesis, plants utilize a large fragment of light constant. When subjected to drought, the equilibrium between light capture and water utilization can be disrupted (Chaves *et al.* 2003). At first, CQ and JQ plants showed higher WUE to cope with decreasing water availability. To minimize water loss and maintain adequate leaf water status during dehydration in the WD-R treatment, CQ and JQ plants initially reduced Gs to adapt to the drought, along with a significant reduction in Tr (Chaves *et al.* 2002; Pompelli *et al.* 2010; Tardieu 2012). Furthermore, the dramatic reduction in Pn, Gs and Ci suggested that photosynthesis is mainly restricted by stomatal limitations in the two Tartary buckwheat genotypes (Flexas and Medrano 2002; Hu *et al.* 2018). A decline in leaf RWC is another way for plants to increase water availability, contributing to increased carbon delivery to the roots (Rosales-Serna *et al.* 2004). As expected, a large decrease in leaf RWC was found for both genotypes under the WD-R treatment. In addition, correlation analysis suggested that the two plants lost part of their leaf RWC at low stomatal aperture. At the same time, they reduced Gs to cope with the decreasing SWC. A similar finding was reported in *Jatropha curcas* (*Jatropha curcas* L.) (Sapeta *et al.* 2013). Compared to the CQ plants, JQ plants exhibited higher WUE by lowering transpiration through a decrease in Gs, supporting JQ as a drought-tolerant genotype (Blum 2009; Rahbarian *et al.* 2011). Upon re-watering, the stomata reopened and leaf RWC recovered rapidly, with increasing Pn. Comparatively higher Pn of WD-R-treated JQ plants was also detected after re-watering, implying better photosynthetic recovery from water deficit in the JQ plants.

On the other hand, drought usually causes photoinhibition when light exceeds the capacity for photosynthesis (Shi *et al.* 2016). The two Tartary buckwheat plants had to subtract excess light by preventing (Havaux and Tardy 1999) or dissipating (Chaves *et al.* 2003) absorbance. For the WD-R-treated plants, increased LW on days 5, 9 and 14 was an effective way to minimize

light absorption. At the same time, a decrease in Fv/Fm was observed in the two genotypes during the drought phase of the WD-R treatment. Fv/Fm is an important indicator of PSII photochemical efficiency. Thus, plants exhibited a reduction in PSII photochemical efficiency (Rahbarian *et al.* 2011), together with yield losses, under drought conditions (Pathan *et al.* 2014). In the current study, decreased Fv/Fm occurred after net assimilation decreased, reinforcing the notion that stomatal limitations primarily limit Pn, rather than photochemistry (Sapeta *et al.* 2013). PSII efficiency was limited due to reduced CO<sub>2</sub> supply, which has a negative effect on the Calvin cycle (Sapeta *et al.* 2013). JQ plants maintained a higher Fv/Fm ratio compared to CQ plants during the drought, suggesting rapid adjustments in the former to avoid the decline in PSII photochemical efficiency. Upon re-watering, Fv/Fm quickly recovered to pre-drought levels, suggesting that the damage had been successfully repaired.

Reactive oxygen species (ROS) are frequently generated under drought stress, causing serious oxidative damage to plants (Oliver *et al.* 2010). Antioxidant enzymes function in quenching the ROS (Luna *et al.* 2005). Of these, SOD, the first key enzyme in the active oxygen-scavenging system, plays a crucial role in catalyzing superoxide free radical dismutation into H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub> (Blokina *et al.* 2003). SOD activity increased in the leaves of the two Tartary buckwheat genotypes under water stress. The same trend was found for leaf POD activity at the end of the drought stress. Increased activities of SOD, POD, and catalase in response to water deficit have been reported in potato (*Solanum tuberosum* L.) (Boguszewska *et al.* 2010). Drought-tolerant white clover (*Trifolium repens* L.) retained significantly higher POD and SOD activity in roots and leaves during drought treatments (Li *et al.* 2012). We also found that JQ leaves maintained higher SOD and POD activity on days 5 and 9 of the drought phase compared to CQ leaves. The steadily decreasing trend of SOD and POD activity during the recovery phase revealed the successful reinstatement of osmosis and antioxidation. Typically, ROS-induced oxidative damage under water stress can be adjusted by osmoregulatory molecules, such as proline, soluble sugars, SP, flavonoids, and late-embryogenesis abundant proteins (Reddy *et al.* 2004; Claeys and Inze 2013). These substances help the membranes defend against drought stress and stimulate plants to absorb more water to retain normal metabolic functions (Claeys and Inze 2013). The MDA, a marker of oxidative stress stemming from lipid peroxidation, is generally employed to assay oxidative damage (Sarker and Oba 2018). In the present study, MDA content in the leaves of both Tartary buckwheat genotypes increased during the drought phase, but a slower increase in MDA was found in the JQ vs. CQ plants, which could be attributed to the former's better tolerance to drought stress.

Along with the physiological responses to water stress, morphological adaptations were observed. The most prominent of these in the two Tartary buckwheat genotypes

were the reductions in plant height, stem node number and branch number in the water-stressed plants. Genotypes with maximum root length and minimum shoot length under water deficit are regarded as drought-tolerant (Chaves *et al.* 2003; Oliver *et al.* 2010). A significant increase in root-to-shoot ratio was found in WD-R plants compared to WW plants, greater in JQ vs. CQ plants. Such responses might be attributed to root system development: root growth in JQ was relatively more enhanced than in CQ during the drought phase.

Drought-induced yield loss is a major problem in many plants (Bodner *et al.* 2015). The primary yield-determining components—grain number and grain weight—are influenced by water stress, depending on the duration and magnitude of the drought, and the phenological stage at which it occurs (Farooq *et al.* 2014; Bodner *et al.* 2015). In the present study, water deficit at the flowering stage markedly reduced seed number in CQ plants. Yield loss due to lack of water in CQ plants reached 31.4%, mainly due to the reduced number of seeds, whereas neither yield nor seed number were significantly affected by the water deficit in JQ plants. Thus, the higher yield and higher number of seeds in the JQ plants might have been a result of the higher photosynthetic activity with more functional leaves and lower transpiration, due to increased WUE, in those plants under water stress.

## Conclusion

Findings of this study imply that physiological acclimation of Tartary buckwheat variety JQ to progressive drought includes elevated water-use efficiency through stomatal closure, and decreased photosynthesis rate, transpiration rate and PSII photochemical efficiency. At the same time, JQ has a more active ROS-scavenging system with higher activities of superoxide dismutase and peroxidase, which may be one of the crucial routes to avoiding oxidative membrane damage and lipid peroxidation. Furthermore, accumulation of malondialdehyde helped maintain cell turgor and metabolic functions. Upon re-watering, these parameters eventually returned to, or close to, normal levels. These combined strategies enable JQ plants to survive without significant yield loss under drought conditions with unreliable precipitation.

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## Author Contributions

Y.W and D.X analyzed the data, wrote the manuscript and revised the manuscript; J.O, X.G and L.L conducted the experiment and collected data; X.W, Q.W, L.Z and G.Z reviewed and supervised the manuscript; all authors mentioned approved the final manuscript.

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