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Full Length Article

Nitrogen Alleviates Seedling Stage Drought Stress Response on Growth and Yield of Tartary Buckwheat

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Abstract

Drought is a major hindrance which faces crop growth and yield. Appropriate nitrogen (N) nutrition can ameliorate the effects of drought on crops. However, it is unclear whether the growth and yield of Tartary buckwheat under drought stress can be improved by N application. This study explored the effects of different N application rates on growth of drought-stressed Tartary buckwheat seedlings during 2017 and 2018. Tartary buckwheat seedlings were exposed to three drought levels (well-watered, moderate and severe drought) at low N (0.05 g N kg^{-1}) or high (0.2 g N kg^{-1}) rates, and their physiological activities, growth, and grain yields were determined. Severe drought significantly decreased photosynthesis, chlorophyll (*Chl*) content, and soluble protein (SP) content of leaves, and decreased the relative growth rate (RGR) and dry matter (DM) production, leading to 31.5–34.2% (drought at three-leaf stage) and 15.2–23.9% (drought at five-leaf stage) reductions in grain yield compared with the control. Under severe drought, plants with low N had lower *Chl* and SP contents, lower photosynthesis, and greater reductions in grain yield. Plants grown with high N tolerated drought by maintaining higher relative water content (RWC), water potential (Ψ_w), *Chl* and SP contents, photosynthetic rate (P_n), and superoxide dismutase (SOD) activity. Compared with plants at low N, those in high N showed significantly higher RGR, DM production, and grain number per plant, 1000-grain weight, and yield. Appropriate N application mitigated the adverse effects of drought on Tartary buckwheat by promoting osmoregulation, alleviating lipid peroxidation, and improving plant physiological traits. © 2020 Friends Science Publishers

Keywords: Chlorophyll content; Dry matter; *Fagopyrum tataricum*; Photosynthesis; Yield

Introduction

Drought stress has a negative influence on to the growth and development, resulting in crop yield reduction (Farooq *et al.* 2009). Crops show various morphological and physiological responses to drought stress (Qi *et al.* 2010) causing water deficits, leaf gas exchange decrease, and metabolic changes in plants (Anjum *et al.* 2011), which limits crop productivity (Farooq *et al.* 2009, 2014), reducing average yields by 50% or more (Wang *et al.* 2003; Farooq *et al.* 2014). Photosynthesis is an important biosynthetic reaction and the foundation of crop yield. The contribution of gas exchange, and especially the rate of photosynthesis, to crop productivity under sub-optimum conditions has received much attention worldwide (Samarah *et al.* 2010). Photosynthetic rate and chlorophyll (*Chl*) content are important indicators to evaluate plant health and environmental situation (Amane 2011). Senthil-Kumar *et al.* (2007) found that drought stress affected leaf gas exchange and enzymatic antioxidants activity, resulting in imbalance of the production of enzymatic system and the electron-transfer chain. In this case, the excess electrons can cause the production of reactive oxygen species (ROS) (Abid*et al.* 2016b). In this way, drought stress harms structure and function of cell, which leads to cell death (Sergi and Josep 2003). Consequently, plants have evolved an antioxidant system to protect them from ROS (Blokhina *et al.* 2003; Carvalho 2008). Unluckily, drought stress can affect the function of the antioxidant enzymes, thus induce lipid peroxidation damage of membrane to plant (Dat *et al.* 1998).

Plants' responses to drought are extremely complicated and vary among different crops and growth stages (Aslam *et al.* 2015). The growth and development of crops are significantly affected by water limitation. Sarker *et al.* (1999) found that drought stress decreases the water potential (Ψ_w) and relative water content (RWC), which lead to the changes of water status in wheat. Some crops have adaptive strategies to withstand adverse conditions. Morphological plasticity, improved water use efficiency, and gene regulation are possible mechanisms of plants which respond to drought during the vegetative stage (Lotscher and Hay 1997). Optimal cultivation conditions are critical for plants to withstand subsequent drought, such as optimal rates of fertilizer application, water, and light.

Nitrogen (N) is required by crops for the synthesis of chlorophyll, proteins, and enzymes. Certainly, N is very important to increase stromal and thylakoid proteins to affect photosynthetic capacity (Ahmad *et al.* 2014). In agricultural production, it is one of important strategy for crop productivity increase by applying N (Ataulkarim *et al.* 2016). Brennan (1992) found the N availability has a great impact to the functional activity of photosynthetic apparatus. A previous study reported that plant growth and development are limited by water restrictions, especially under the condition of low N availability. Water deficit and limited N have been shown to affect plant-water relations and photosynthetic ability, which lead to premature senility and low productivity of crops (Madani *et al.* 2010). Furthermore, appropriate N application has been shown to alleviate drought stress damage by allowing plants to maintain metabolic activity (Wu *et al.* 2018).

Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) is an excellent plant resource grown worldwide and processed into foods and drinks (Bonafaccia *et al.* 2003; Fabjan*et al.* 2003; Xiang *et al.* 2016). It has the concomitant function of both medicine and foodstuff because of various pharmaceutical ingredients, such as rutin, quercetin and isoquercetin in the different organs of plant (Zhao *et al.* 2012). Due to its abundant nutrition ingredients and health value, Tartary buckwheat is becoming highly attractive (Fabjan *et al.* 2003; Kreft 2016). However, Tartary buckwheat is mainly cultivated in marginal land of Southwest China. Owing to infrequent rain in these areas, drought stress has become a major hindrance for production of Tartary buckwheat (Ohnishi and Tomiyoshi 2005; Xiang *et al.* 2013). A previous study found that Tartary buckwheat could not tolerate drought stress during its initial growth stages (Zhao and Shang 2009). Therefore, it is important to find ways to ameliorate the adverse effects of drought on Tartary buckwheat at the seedling stage to improve its growth and yield. Previous studies found that N application can reduce the negative influence of drought on yield in other crops (Saneoka *et al.* 2004; Dinh *et al.* 2017). However, the reported results differ among studies because of differences in crops or species, environmental conditions, N application rates, drought stress levels, and growth stages of crops (Ping *et al.* 2011; Shi *et al.* 2014; Wu *et al.* 2018). A comprehensive understanding of compensation effect of N nutrition under water stress is scarce in Tartary buckwheat.

The specific objective of this study was to assess the effects of N application under different water regimes on the growth and development, physiological activities, and yield of Tartary buckwheat. The results will provide information about the physiological mechanism by which N promotes the growth of Tartary buckwheat, and highlight the potential of N to improve yield in arid or semiarid regions.

Materials and Methods

Experimental materials and site description

A trial was conducted during two years (2017 and 2018) at experimental farm of Chengdu University (30°39′ N, 104°11′ E, 490 m altitude), Sichuan Province, China. In each growing season, the Tartary buckwheat cultivar (XiQiao-1), was obtained from Chengdu University and is the most widely grown cultivar in southwest China. Before sowing, the 10% (v/v) hydrogen peroxide was used for seed sterilization, rinsed four times with deionized H_2O, then collected and stored for further use. Ten seeds were sown in each plastic pot (30 cm height × 25 diameter). The pots were filled with 9 kg air-dried soil with 13% soil moisture. The soil was alkaline (pH 7.6) containing 48.2 mg kg^{-1} available N, 20.3 mg kg^{-1} Olsen-P, 52.5 mg kg^{-1} available K, 1.8 g kg^{-1} organic matter, 0.62 g kg^{-1} total N, 0.41 g kg^{-1} total P, and 14.6 g kg^{-1} total K, 0.43 dS m^{-1} electrical conductivity (EC), 1.31 g cm^{-3} bulk density, and 37.9% field capacity (FC) by volume, respectively. When soil was added to the pots, the base fertilizer (0.6 g P_2O_5 and 1.2 g K_2O) was added to each pot. The seedlings were thinned to three plants per pot. Thirty pots were used for each treatment and five plants were maintained after thinning at 7 days after germination. Each pot was maintained with soil moisture at 80% of FC until drought stress was imposed. The pots were placed randomly and moved to a different place every week to ensure that all plants had equal growth conditions.

Experimental design and management

The experiment was arranged in completely randomized design. There were three soil water levels (well-watered (WW); moderate drought stress (MD); severe drought stress (SD)) and two N rates (0.05 and 0.2 g N kg^{-1} soil, represented as low N (N1) and high N (N2), respectively). The N fertilizer was applied at sowing (50%) and the five-leaf stage (50%), respectively. Drought was imposed on Tartary buckwheat seedlings at the three-leaf and five-leaf stages. At each stage, three drought levels (35–40%, 55–60% and 80% FC, respectively) were maintained by water application to the desired FC (Zlobin *et al.* 2018). At each stage, drought stress was maintained for 10 days. After the drought stress, the soil of each pot was re-watered to 80% of FC and the plants were grown until maturity. The experimental design and management were consistent during two growing seasons.

Data collection

Fully expanded leaves were randomly selected at 1 day before drought stress (0 D), days 5 and 10 of drought stress

(5 D, 10 D), and 1 and 3 days after irrigating (1 DR, 3 DR). These samples were used to analyze the RWC, Ψ_w, *Chl* and soluble protein (SP) content, and gas exchange parameters.

Leaf water status

To determine the RWC, leaf fresh weight (FW) was measured (Sartorius CPA225D balance, Sartorius Co., Beijing, China) immediately after leaves were cut from the Tartary buckwheat plants. Later, the leaves were floated on deionized water for 18 h and weighed to determine turgid weight (TW). These leaves were dried in a drying oven for 72 h at 75°C to measure dry weight (DW). The RWC was calculated as: RWC (%) = [(FW−DW)/(TW − DW)] × 100. The Ψ_w was measured as described by Canny (1997) using portable pressure chamber 3115 (Soil moisture Equipment Cor., California, U.S.A.).

Chlorophyll and soluble protein content

The *Chl* content was measured following the method of Xiong (2009). Leaf samples was ground and placed in centrifuge tube with 80% acetone and then covered with black cloth and kept at darkroom until the leaf changed to white. The *Chl* content was measured using a spectrophotometer at the wavelength of 645 and 663 nm. A leaf sample of 0.5 g was used to determine SP content using the method (Coomassie brilliant blue G-250 staining) described by Xiong (2009).

Leaf gas exchange

The gas exchange of fully expanded leaves was measured using portable photosynthesis system (GFS-3000, WALZ Inc., Effeltrich, Germany) between 09:00 and 11:30 h. During the measurement, a photosynthetic active radiation of 1200 µmol m^{-2} s^{-1} was provided by an automatic light source. The net photosynthetic rate ($P_{n)}$ and stomatal conductance (g_s) of Tartary buckwheat leaf were recorded by this photosynthesis system.

Malondialdehyde (MDA) content and superoxide dismutase (SOD) activity

The SOD activity and MDA content were measured following the method of Beauchamp and Fridovich (1971) and Wang *et al.* (2018), respectively. 0.5 g of frozen leaf sample was homogenized in a mortar and pestle, and the homogenate was centrifuged (4°C) at 10,000 × *g* for 30 min. Later, the supernatant was used to analyze the SOD activity and MDA content.

Dry matter, relative growth rate, drought index, yield, and yield components

Whole plants were cut at the three-leaf, five-leaf, anthesis, and maturity stages to determine FW. These samples were dried in a drying oven for 72 h at 75°C to measure DM. The RGR, RGR = (1/DM) × (△DM/△d), DI, DI = YD/YW were calculated according to Zhang *et al.* (2007). △DM and △d were assessed by the change in DM and days between two adjacent samplings stages, respectively. The YD and YW represented the yield of Tartary buckwheat under the conditions of drought stress and WW, respectively. Certainly, the 1000-grain weight and grain number per plant of each treatment were also measured at the maturity stage.

Statistical analysis

Two yeas data were analyzed by SPSS Statistics 17.0 (IBM, Chicago, I.L., U.S.A.). There were consistent physiological characteristics of Tartary buckwheat in 2017 and 2018, and no significant differences were found in across years and in interaction effects (Year × Nitrogen; Year × Drought and Year × Nitrogen × Drought). Hence, data was analyzed from the mean of two years, and the differences among treatments were assessed by Duncan's multiple range test ($P < 0.05$).

Results

Leaf RWC and Ψ_w

The leaf RWC and Ψ_w of Tartary buckwheat decreased under drought stress (Fig. 1). The difference in RWC and Ψ_w between the treatments of two N rates under drought stress conditions were significantly, but not under WW conditions. The RWC and Ψ_w were lower at low N than at high N treatments, and the decreases in RWC and Ψ_w under drought stress were greater at the three-leaf stage than at the five-leaf stage. After re-watering, the RWC and Ψ_w recovered to different extents among the different treatments. The RWC and Ψ_w of drought-stressed plants showed greater recovery at high N than at low N.

Leaf *Chl* and SP contents

Drought and N application significantly affected the *Chl* and soluble protein (SP) contents of Tatary buckwheat leaves ($P < 0.05$) (Fig. 2). Under drought stress, the *Chl* and soluble protein contents decreased significantly under both N application rates and decreased to lower levels at low N treatments than at high N treatments under drought stress and WW conditions. After re-watering, the *Chl* and SP contents recovered slowly in plants subjected to drought at the three-leaf and five-leaf stages, but the recovery was stronger in the high N treatments than in the low N treatments.

Leaf gas exchange

The leaf P_n was significantly influenced by N application, which was lower in the low N than in the high N treatments.

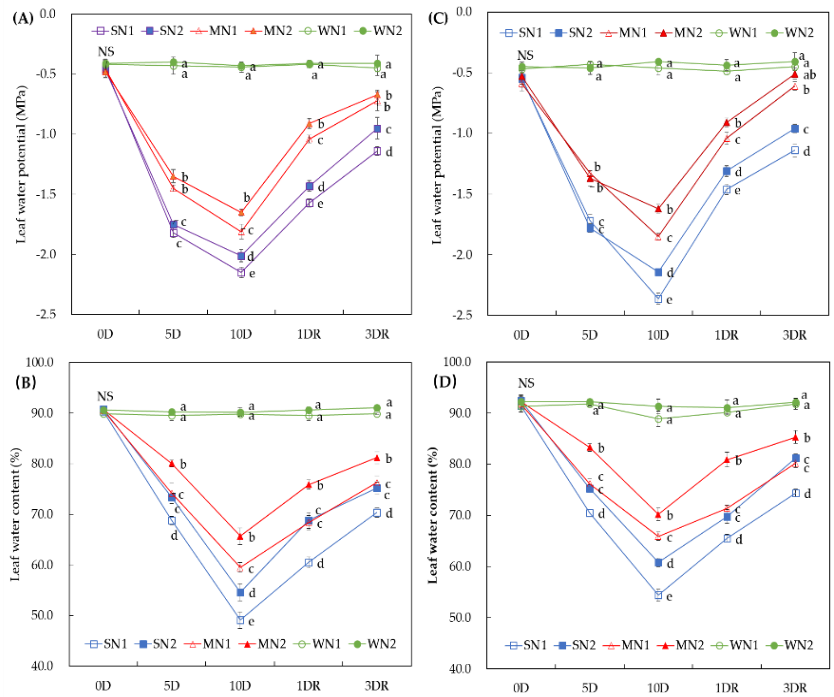

Fig. 1: Effect of drought stress on leaf relative water content (RWC) and water potential (Ψ_w) of Tartary buckwheat under two nitrogen (N) application rates. Panels **A–B** and **C–D** show results obtained when drought was applied at three-leaf and five-leaf stage, respectively. S, M, severe and moderate drought stress, respectively; W, well-watered conditions. 0 D, 1 day before drought stress; 5 D, 10 D, day 5 and 10 of drought stress, respectively; 1 DR, 3 DR, 1 and 3 days after re-watering, respectively. N1 and N2 represent the low and high N levels, respectively. Data are means ± SD of two years (2017 and 2018). Different letters denote significant differences between treatments at the same time. NS: non-significant difference

However, N application had no remarkable effects on g_s (Fig. 3). The P_n and g_s of Tartary buckwheat leaf responded to the different drought levels significantly. The P_n and g_s declined with increasing intensity of drought, and the lowest values of P_n and g_s were in the low N treatments. Seedlings subjected to drought at the three-leaf and five-leaf stage showed the same trends, but the decreases in P_n and g_s were greater when drought was applied at the three-leaf stage. After re-watering, the P_n and g_s of Tartary buckwheat showed better recovery in the high N treatments than in the low N treatments.

SOD activity and MDA content

The SOD activity and MDA content were higher in drought-stressed plants than in plants in the WW treatment (Fig. 4). There were no remarkable differences in MDA contents and SOD activity between the two N levels under WW conditions. Under drought stress, the MDA content tended to decline and SOD activity tended to increase with increasing N application rate, and the same trend was observed in drought-stressed plants at the three-leaf stage and at the five-leaf stage. After re-watering, SOD activity and MDA contents decreased in the drought-stressed plants, but decreased more in the treatments of high N than low N.

Dry matter and relative growth rate

In both growing seasons, drought stress at the three-leaf and five-leaf stages significantly reduced the DM accumulation and RGR (except FLA, five-leaf to anthesis stage) of Tartary buckwheat plants ($P < 0.01$; Table 1). The reduction in RGR was lower from the three-leaf to five-leaf stage (TFL) and from anthesis to the maturity stage (AM), and higher from the five-leaf to anthesis stage (FLA) under drought stress than in WW conditions. The DM accumulation showed similar decreasing trends in the two drought treatments, and decreased more in the severe drought treatments (by 13.1% in ST and 4.9% in SF) than in the moderate drought treatments. Consistently, drought stress had less impact on RGR and DM accumulation in the high N treatments than in the low N treatments at the four growth stages, and the patterns were similar in 2017 and 2018. Additionally, no significantly difference were observed in across years and in interaction effects ($Y \times N$; $Y \times D$ and $Y \times N \times D$).

DI, yield, and yield components

The drought and N application significantly affected the grain number per plant, 1000-grain weight, grain yield, and

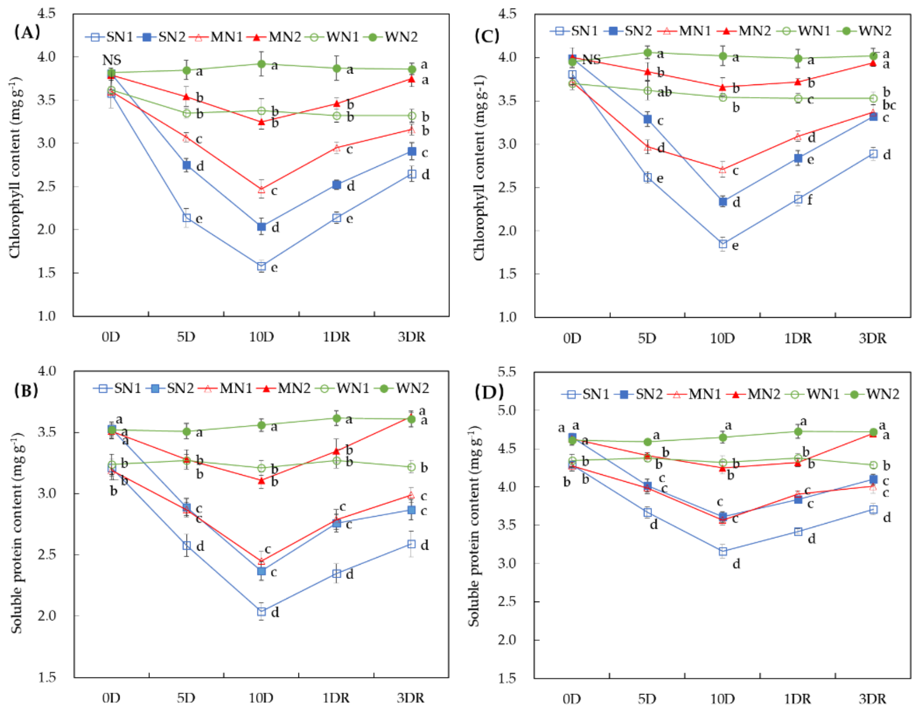

Fig. 2: Effect of drought stress on the chlorophyll (Chl) and soluble protein (SP) content of Tartary buckwheat under two nitrogen (N) application rates. Panels **A–B** and **C–D** show results obtained when drought was applied at three-leaf and five-leaf stage, respectively. S, M, severe and moderate drought stress, respectively; W, well-watered conditions. 0 D, 1 day before drought stress; 5 D, 10 D, day 5 and 10 of drought stress, respectively; 1 DR, 3 DR, 1 and 3 days after re-watering, respectively. N1 and N2 represent the low and high N levels, respectively. Data are means ± SD of two years (2017 and 2018). Different letters denote significant differences between treatments at the same time. NS: non-significant difference

DI, and the magnitude of the effects depended on the severity of drought, growth stage, and N levels, and the patterns were similar during two years (Table 2). The loss of yield was greater when drought was applied at the three-leaf stage than at the five-leaf stage. The grain yield decreased with increasing drought stress intensity, but increased with increasing N level. The yield in ST, MT, SF, and MF was reduced by 34.2, 21.9, 23.9 and 10.3%, respectively, in low N, and by 31.5, 18.2, 15.2 and 4.2%, respectively, in high N, compared with grain yields in WW conditions. The DI of moderate drought stress was greater than severe drought stress, and this trend was observed in both the low N and high N treatments of two years. However, the DI was higher in high N treatments than in low N treatments under the same degree of drought stress. The DI in ST, MT, SF, and MF was 4.3, 4.9, 10.6 and 6.3% lower, respectively, in low N than in high N.

The number of grains per plant significantly increased with increasing N application rate in all treatments. Compared with the WW treatments, the ST, MT, SF, and MF treatments showed 32.9, 25.4, 19.8 and 12.3% decreases, respectively, in grain number per plant in low N, and 34.6, 22.5, 13.9 and 5.1% decreases, respectively, in grain number per plant in high N. The 1000-grain weight was impacted by drought and N application. Compared with WW treatment, all stress treatments (except MF in N2) indicted significantly reduced 1000-grain weight. The weight of 1000-grain in ST, MT, SF and MF was 5.9, 2.4, 7.3 and 2.3% higher, respectively, in high N than in low N.

Discussion

The present study assessed the influences of N nutrition on the growth and development, physiological performance and yield of Tartary buckwheat under drought conditions. The Tartary buckwheat plants at the three-leaf and five-leaf stages showed different responses to severity of and N levels. The responses to drought included a decrease in Ψ_w, which resulted in stomatal closure and reduced photosynthesis (Fig. 1 and 3). Flexas and Medrano (2002) suggested that Ψ_w is very important for normal crop growth, and a decrease in Ψ_w adversely affects CO_2 assimilation and water use efficiency (WUE) due to metabolic impairment of photosynthesis. Thus, under a higher N application rate, the maintenance of Ψ_w allowed Tartary buckwheat plants to sustain leaf processes under conditions of drought stress, and then to recover faster after re-watering than under a lower N application level. In this case, the stomatal activity was higher under a higher N rate than under a lower N rate. These results may be attributed to the decreased Ψ_w under lower N application, which could lead to the limited growth and development of cell. The decline in Ψ_w may decrease

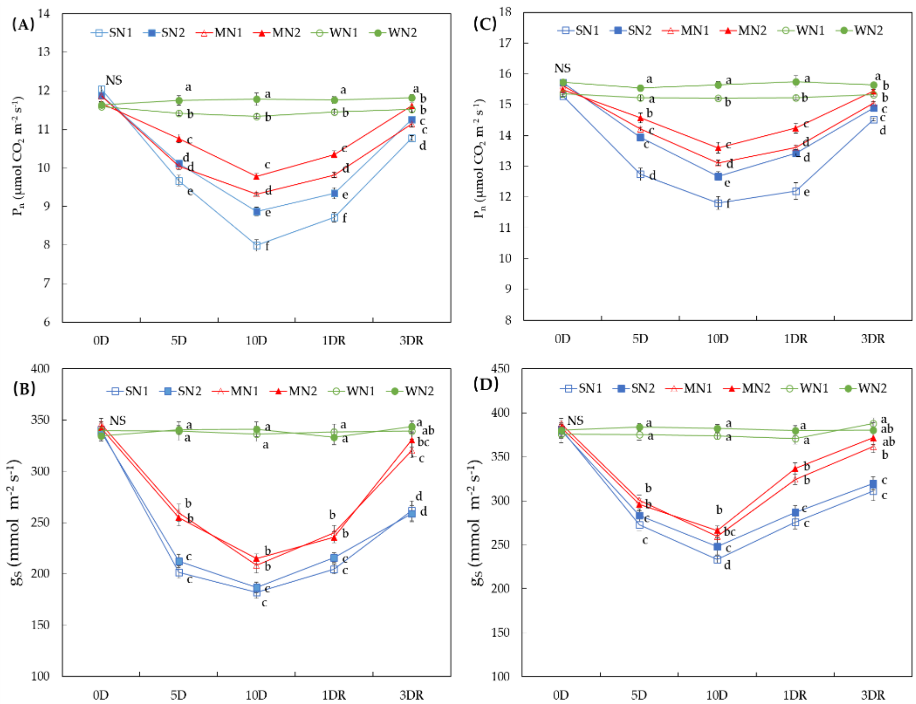

Fig. 3: Effect of drought stress on the net photosynthetic rate (P_n) and stomatal conductance (g_s) of Tartary buckwheat under two nitrogen (N) application rates. Panels **A–B** and **C–D** show results obtained when drought was applied at three-leaf and five-leaf stage, respectively. S, M, severe and moderate drought stress, respectively; W, well-watered conditions. 0 D, 1 day before drought stress; 5 D, 10 D, day 5 and 10 of drought stress, respectively; 1 DR, 3 DR, 1 and 3 days after re-watering, respectively. N1 and N2 represent the low and high N levels, respectively. Data are means ± SD of two years (2017 and 2018). Different letters denote significant differences between treatments at the same time. NS: non-significant difference

mesophyll conductance (Warren *et al.* 2004). Lower WUE under low Ψ_w has been shown to decrease DM accumulation and yield (Grassi and Magnani 2005).

Chlorophyll plays a key role in determining the intensity of photosynthesis, which is strongly affected by adverse conditions. Mafakheri *et al.* (2010) found that drought stress significantly decreased the *Chl* content also evident from present study (Fig. 2). The decrease in *Chl* content under drought stress would reduce the photochemical activity of chloroplasts, leading to decreased photosynthesis. In this study, the *Chl* content in Tartary buckwheat leaves increased by higher N application rates under drought and well-watered conditions (Fig. 2). Because N is an important component of *Chl* and proteins, it strongly affects plant metabolism during drought stress (Amane 2011). Sufficient N can enhance the recovery of photosynthesis, and so N-deficient crops show limited recovery after severe drought stress conditions (Grassi and Magnani 2005). Therefore, enough N may increase the photosynthetic capacity and stomatal control under drought conditions, owing to more than half of the N nutrient in plants' green tissues take part in collecting solar energy to drive photosynthesis (Sinclair and Jamieson 2006). Certainly, photosynthesis can be improved by increasing the total *Chl* content *via* appropriate N fertilization. Further research is required to explore the detailed mechanism by which N enhances photosynthesis in Tartary buckwheat under drought stress.

Drought-stressed plants produce excess H_2O_2, which can cause oxidative damage through the formation of ROS which damage proteins (Mohammadi *et al.* 2018). Sofo *et al.* (2010) suggested that the MDA content was a vital indicator of oxidative damage in plants, and closely related to the serious degree of drought and N available. In this study, Tartary buckwheat plants under the condition of severe drought with Low-N application caused excess MDA accumulation. Hence, a higher content of MDA under conditions of Low-N application during drought stress may reduce the ability of antioxidation in cell, resulting in greater ROS accumulation (Jiang *et al.* 2007). Lipid peroxidation can lead to further damage such as the loss of *Chl*, improved the permeability of cell membrane, breakdown of macromolecules, reduction of nutrient availability, and early senescence, which eventually lessen the growth period of grain (Calatayud *et al.* 2001). It was observed that lower MDA contents and higher SOD activity in the high N treatments than in the low N treatments, indicating that greater N availability increased the ROS scavenging capability of drought-stressed Tartary buckwheat. Cheng (2013) also suggested that greater SOD activity and lower MDA content in plants in a High-N treatment was indicative

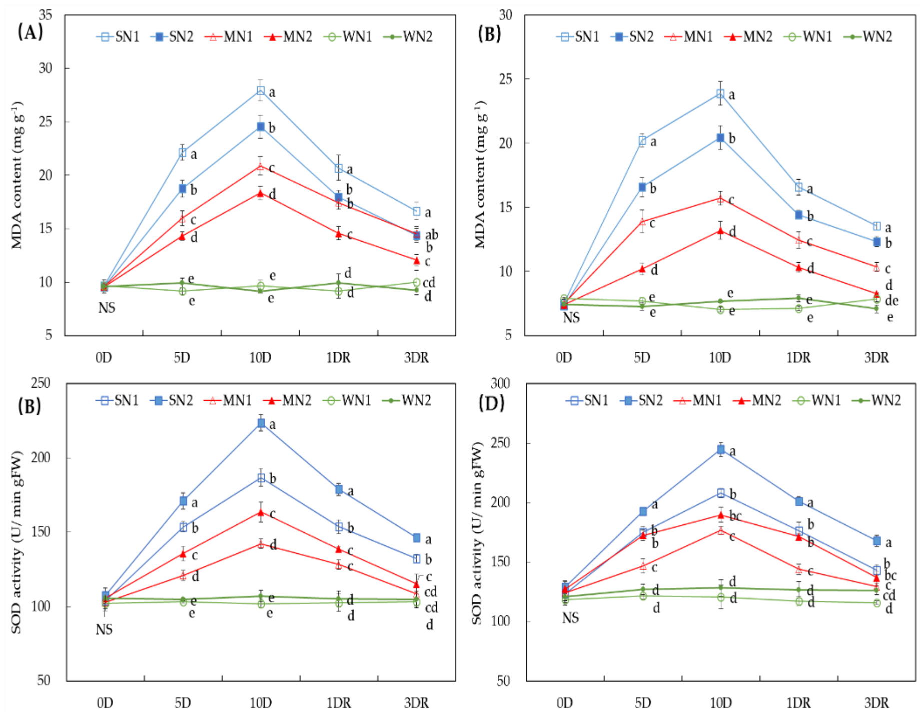

Fig. 4: Effect of drought stress on the MDA content and SOD activity of Tartary buckwheat under two nitrogen (N) application rates. Panels **A–B** and **C–D** show results obtained when drought was applied at three-leaf and five-leaf stage, respectively. S, M, severe and moderate drought stress, respectively; W, well-watered conditions. 0 D, 1 day before drought stress; 5 D, 10 D, day 5 and 10 of drought stress, respectively; 1 DR, 3 DR, 1 and 3 days after re-watering, respectively. Data are means ± SD of two years (2017 and 2018). N1 and N2 represent the low and high N levels, respectively. Different letters denote significant differences between treatments at the same time. NS: non-significant difference

of improved redox defenses to scavenge ROS. In this study, the drought-stressed Tartary buckwheat plant under higher N rates had the stronger activity of the ROS-detoxifying antioxidant system, thus may have protect the photosynthetic process, consistent with Zandalinas *et al.* (2017). Hence, present study results indicate that appropriate N fertilization can improve the production and drought tolerance of Tartary buckwheat by enhancing antioxidant enzyme activities and reducing lipid peroxidation.

It was also found that severe drought stress during seedling stage affected normal physiological processes (Fig. 3 and 4), leading to the inhibition of growth, development (Table 1) and yield formation in Tartary buckwheat (Table 2). The decrease in the RGR of Tartary buckwheat under drought stress was greater under a lower N application rate than under a higher N, and the recovery of photosynthesis after re-watering was also slower at the lower N rate. Abid *et al.* (2016a) suggested that the decline in P_n under stress conditions leads to the imbalance of photosynthesis and respiration, resulting in a decreased crop growth rate. In the present study, the RGR recovered after re-watering (Table 1), which was indicative of the reversibility of some physiological damage caused by drought. However, the DM accumulation in Tartary buckwheat at maturity was lower in the drought treatments than in the WW treatments under both N levels (Table 1). In this sense, drought stress had some irreversible effects on the growth and development of Tartary buckwheat, consistent with the report of Xu *et al.* (2010). Certainly, other factors may have affected the plants pre-drought, but further studies are required to explore this.

Drought stress has been shown to limit the growth, development and yield formation of crops under Low-N supply (Tuong *et al.* 2002; Bernier *et al.* 2007), and its effects were studied in detail by analyzing individual yield components (Hattori *et al.* 2010). In this study, Tartary buckwheat under higher N application rates had higher grain number per plant, higher 1000-grain weight, and produced higher yield than under lower N application rates (Table 2). The drought-stressed plants under lower N level showed significantly weaker performance in terms of yield and yield components. The results showed that there were significant differences in the growth, yield and its component of Tartary buckwheat plants among different drought and N conditions (Table 1 and 2).

Interestingly, the magnitude of yield loss differed depending on the timing of the drought treatment, with greater reductions when drought was applied at the three-leaf stage than at the five-leaf stage. These results indicate that Tartary buckwheat plants are more sensitive to drought stress at an early stage than at a later stage during vegetative growth. Zhao and Shang (2009), also found that Tartary buckwheat could not tolerate drought stress at the early

Table 1: Effect of drought stress and nitrogen application rate on dry matter accumulation and relative growth rate of Tartary buckwheat during several growth stages

Year	N Levels	Drought Treatment	Relative growth rate (mg/g/d)			Dry matter accumulation (g)			
			TFL	FLA	AM	Three-leaf	Five-leaf	Anthesis	Maturity
2017	Low N	ST	73.1	164.1	29.4	1.40	2.74	9.47	18.66
		MT	76.8	146.9	36.0	1.58	3.16	10.11	22.11
		SF	78.4	127.4	32.1	1.77	3.58	10.42	21.44
		MF	81.2	126.0	34.1	1.84	3.78	10.92	23.21
		WW	81.9	136.1	38.5	1.85	3.82	11.62	26.35
	High N	ST	89.3	190.0	31.1	1.61	3.48	13.4	27.13
		MT	79.8	182.0	38.2	1.93	3.93	14.66	33.12
		SF	93.3	128.6	36.2	2.21	4.89	14.32	31.42
		MF	92.0	138.1	39.1	2.26	4.95	15.21	34.81
		WW	95.3	148.7	40.6	2.26	5.06	16.35	38.25
2018	Low N	ST	71.0	159.9	30.8	1.41	2.71	9.21	18.56
		MT	73.4	159.6	33.7	1.59	3.11	10.56	22.30
		SF	82.9	121.5	33.5	1.75	3.64	10.26	21.61
		MF	83.9	123.4	34.1	1.85	3.86	10.99	23.36
		WW	81.7	137.1	37.1	1.89	3.89	11.89	26.44
	High N	ST	88.9	184.2	32.0	1.63	3.52	13.24	27.22
		MT	80.7	179.6	38.2	1.95	3.99	14.73	33.30
		SF	91.0	128.8	36.5	2.24	4.88	14.32	31.57
		MF	91.7	142.6	38.5	2.24	4.91	15.41	35.00
		WW	94.0	148.8	39.9	2.29	5.09	16.46	38.11
Mean (2017 and 2018)	Low N	ST	75.1c	166.0c	30.6f	1.47g	2.84h	9.63i	19.06j
		MT	79.8bc	153.6d	35.5d	1.65f	3.30g	10.62h	22.72h
		SF	83.9b	126.4f	33.7e	1.83e	3.75e	10.63h	22.11i
		MF	85.0b	127.3f	34.8d	1.91d	3.94d	11.24g	23.83g
		WW	84.6b	138.7e	38.7b	1.93d	3.99d	12.04f	27.02f
	High N	ST	91.7a	190.2a	32.0e	1.69f	3.62f	13.60e	27.64e
		MT	83.1b	182.6b	38.8b	2.00c	4.10c	14.98c	33.79c
		SF	95.9a	128.8f	36.7c	2.29b	5.06b	14.61d	31.96d
		MF	94.5a	141.5e	39.3a	2.31ab	5.08b	15.60b	35.43b
		WW	96.6a	150.7d	40.6a	2.34a	5.20a	16.69a	38.66a
ANOVA									
F-value		Year (*Y*)	0.036^{NS}	0.036^{NS}	0.035^{NS}	1.33^{NS}	2.18^{NS}	0.56^{NS}	1.77^{NS}
		Nitrogen (*N*)	120.85^{**}	156.97^{**}	50.02^{**}	1185.46^{**}	5334.9^{**}	2883.57^{**}	2028.01^{**}
		Drought (*D*)	16.53^{**}	196.07^{**}	38.47^{**}	392.89^{**}	1374.48^{**}	131.2^{**}	1868.1^{**}
		$Y \times N$	0.23^{NS}	0.10^{NS}	0.030^{NS}	0.16^{NS}	0.12^{NS}	0.029^{NS}	0.0037^{NS}
		$Y \times D$	0.31^{NS}	1.67^{NS}	1.17^{NS}	0.41^{NS}	0.36^{NS}	1.22^{NS}	0.41^{NS}
		$N \times D$	3.76^{*}	9.98^{**}	3.50^{*}	15.49^{**}	51.69^{**}	2.64*	70.24^{**}
		$Y \times N \times D$	0.89^{NS}	2.17^{NS}	0.47^{NS}	0.32^{NS}	2.10^{NS}	0.42^{NS}	0.22^{NS}

TFL: from three-leaf to five-leaf stage. FLA: from five-leaf to anthesis stage. AM: from anthesis to maturity stage. ST and SF represent severe drought stress at three-leaf and five-leaf stage, respectively. MT and MF represent moderate drought stress at three-leaf and five-leaf stage, respectively. Within each column, different small letters denote significant differences among treatments ($P < 0.05$). For ANOVA, $Y \times N$ represents interaction between year and N. $Y \times D$ represents interaction between year and drought. $N \times D$ represents interaction between N and drought. $Y \times N \times D$ represents interaction among year, N and drought. ^{NS}, not significant. *, significant ($P < 0.05$). **, significant ($P < 0.01$)

stage of vegetative growth. Davatgar *et al.* (2009) also suggested that plants' responses to water deficit rely on the stress condition and plant status, such as stress time, severity, duration and growth stage. For Tartary buckwheat, the root system is smaller in seedlings than in older plants, so seedlings' ability to take up water from the soil is weaker than older plants. Eneji *et al.* (2008) reported that the N uptake and utilization under drought stress is crucial for improving growth and productivity of crops. The response of plants to N is strongly related with the ability of roots to absorb nutrients and water (Ding *et al.* 2015). This explains why adverse environmental conditions during this earlier growth stage resulted in greater decreases in physiological activities (Fig. 2, 3 and 4) and grain yields (Table 2).

Drought-stressed Tartary buckwheat had a significantly higher grain yield under high N treatment than in the low N treatment. Similar promoting effects of N were observed in the WW treatments, but the effect of N to increase yield was stronger in the drought treatments than in the WW treatments. In general, a higher N application rate resulted in stronger growth, higher physiological activity, and improved yield performance of drought-stressed Tartary buckwheat. These findings show that appropriate N application can decrease drought damage during the seedling stage by enhancing the growth potential of Tartary buckwheat plants. Optimal nitrogen nutrition is fundamental to improve the growth and yield of Tartary buckwheat in in arid and semi-arid zones.

Conclusion

The results demonstrated that the combination of drought stress and N level during the seedling stage severely affected the growth potential and physiological performance

Table 2: Effect of nitrogen application rate and drought on yield and its components in Tartary buckwheat

Year	N Levels	Drought Treatment	Grains Number per Plant	1000-Grain Weight (g)	Yield per Pot (g)	DI
2017	Low N	ST	109.9	18.5	10.2	0.66
		MT	117.9	20.6	12.0	0.78
		SF	123.5	19.2	11.8	0.76
		MF	130.2	21.4	13.8	0.90
		WW	142.5	21.5	15.4	0.00
	High N	ST	115.1	19.5	11.2	0.68
		MT	127.0	21.5	13.5	0.81
		SF	136.8	20.5	14.1	0.85
		MF	145.8	22.1	15.6	0.94
		WW	150.3	21.9	16.6	0.00
2018	Low N	ST	110.8	18.7	10.1	0.65
		MT	117.9	21.0	12.2	0.78
		SF	123.5	19.4	11.8	0.76
		MF	131.7	21.1	14.0	0.90
		WW	144.0	21.8	15.6	0
	High N	ST	116.3	20.0	11.4	0.70
		MT	128.5	21.1	13.6	0.83
		SF	135.9	20.8	13.9	0.85
		MF	144.5	21.6	15.9	0.98
		WW	150.3	22.3	16.3	0
Mean	Low N	ST	110.4j	18.6g	10.2i	0.66h
		MT	117.9h	20.8d	12.1f	0.78e
		SF	123.5g	19.3f	11.8g	0.76f
		MF	131.0e	21.3c	13.9d	0.90b
		WW	143.3c	21.7b	15.5c	0
	High N	ST	115.7i	19.7e	11.3h	0.69g
		MT	127.8f	21.3c	13.5e	0.82d
		SF	136.4d	20.7d	14.0d	0.85c
		MF	145.2b	21.8ab	15.8b	0.96a
		WW	150.3a	22.1a	16.5a	0
ANOVA						
F-value		Year (*Y*)	3.00^{NS}	2.86^{NS}	2.31^{NS}	1.87^{NS}
		Nitrogen (*N*)	1456.54^{**}	133.46^{**}	725.16^{**}	188.56^{**}
		Drought (*D*)	2061.37^{**}	213.8^{**}	977.18^{**}	669.72^{**}
		$Y \times N$	1.60^{NS}	0.39^{NS}	0.38^{NS}	1.61^{NS}
		$Y \times D$	1.13^{NS}	4.39^{NS}	0.96^{NS}	0.90^{NS}
		$N \times D$	42.55^{**}	7.28^{**}	15.46^{**}	9.98^{**}
		$Y \times N \times D$	2.05^{NS}	1.59^{NS}	1.23^{NS}	0.57^{NS}

TFL: from three-leaf to five-leaf stage. FLA: from five-leaf to anthesis stage. AM: from anthesis to maturity stage. ST and SF represent severe drought stress at three-leaf and five-leaf stage, respectively. MT and MF represent moderate drought stress at three-leaf and five-leaf stage, respectively. Within each column, different small letters denote significant differences among treatments ($P < 0.05$). For ANOVA, $Y \times N$ represents interaction between year and N. $Y \times D$ represents interaction between year and drought. $N \times D$ represents interaction between N and drought. $Y \times N \times D$ represents interaction among year, N and drought. ^{NS}, non-significant. **, significant ($P < 0.01$)

of Tartary buckwheat plants while N application effectively ameliorated the adverse effects of drought stress. An adequate N fertilizer application under drought stress could promote increased antioxidant activity, Ψ_w, RWC, *Chl*, and SP content as well as photosynthesis ability, which ultimately results in high-yield of Tartary buckwheat. Appropriate culture techniques may optimize these traits to enhance drought tolerance and achieve higher yields of Tartary buckwheat in field production. These results provide insights into the role of N nutrition to improve the performance of drought-stressed Tartary buckwheat, which also provide a theoretical and practical guide for cultivation of Tartary buckwheat crops under drought stress.

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Author Contributions

DBX, WW and LXP designed the experiments and wrote the manuscript, WW, JYOY and LQL performed the experiments, GZ, LXP and YW statistically analyzed the data, reviewed the manuscript and made illustrations.

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