

## Effects of Drought Stress at Different Growth Stages on Growth and Physiological Characteristics of Diqing Tartary Buckwheat and Heifeng No. 1 Tartary Buckwheat (Postprint)

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### Abstract

To identify the water-sensitive periods of Diqing tartary buckwheat and Heifeng No. 1 tartary buckwheat, and to provide guidance for rational irrigation, stable-yield cultivation, and water-saving production, this study employed these two tartary buckwheat varieties as experimental materials in a pot experiment with artificial water control to investigate the effects of drought stress during three distinct growth stages—seedling, flowering, and maturity—on growth and physiological characteristics. The results demonstrated: (1) Drought stress significantly impacted the growth of both tartary buckwheat varieties, with plant height, stem diameter, leaf area, shoot dry weight, root volume, root surface area, root average diameter, root dry weight, root activity, and root soluble protein content all being significantly lower than those of the control; root SOD, POD, MDA, and free proline contents were significantly higher than the control, with Diqing tartary buckwheat exhibiting superior performance compared to Heifeng No. 1 under drought stress. (2) The magnitude of drought stress effects at different growth stages on the measured indicators of these two varieties followed the pattern: flowering stage > seedling stage > maturity stage. Drought stress during the flowering stage exerted the greatest influence on shoot dry weight; compared with the control, flowering-stage drought stress reduced shoot dry weight by 44.47% and 51.04% for Diqing tartary buckwheat and Heifeng No. 1, respectively. Drought affected the growth of both varieties, with flowering-stage drought stress having the most pronounced impact; Diqing tartary buckwheat demonstrated better growth under drought stress and was less severely affected. In production practice, timely water supply during the flowering stage of tartary buckwheat should be prioritized.

## Full Text

### Effects of Drought Stress on Growth and Physiological Characteristics of *Fagopyrum tataricum* (Diqing) and *F. tataricum* (Heifeng 1) at Different Growth Stages

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#### Abstract

To identify the water-sensitive periods of *Fagopyrum tataricum* (Diqing) and *F. tataricum* (Heifeng 1) and to inform rational irrigation strategies, stable cultivation practices, and water-saving production, this study investigated the effects of drought stress at three distinct growth stages—seedling, flowering, and maturity—on the growth and physiological characteristics of these two tartary buckwheat varieties using pot-based artificial water control experiments. The results demonstrated: (1) Drought stress significantly impacted the growth of both varieties, as evidenced by marked reductions in plant height, stem diameter, leaf area, shoot-leaf dry weight, root volume, root surface area, root average diameter, root dry weight, root activity, and root soluble protein content compared to control conditions. Conversely, root SOD activity, POD activity, MDA content, and free proline content were significantly higher under drought stress, with *F. tataricum* (Diqing) exhibiting superior performance compared to *F. tataricum* (Heifeng 1). (2) The magnitude of drought effects across different growth stages followed the pattern: flowering stage > seedling stage > maturity stage. Flowering-stage drought stress had the greatest impact on shoot-leaf dry weight, reducing it by 44.47% in Diqing and 51.04% in Heifeng 1 compared to controls. These findings indicate that drought affects the growth of both tartary buckwheat varieties, with flowering-stage drought causing the most severe impact. *F. tataricum* (Diqing) demonstrated better growth and was less affected under drought stress. In production practice, timely water supply during the flowering stage should be prioritized.

**Keywords:** *Fagopyrum tataricum* (Diqing), *Fagopyrum tataricum* (Heifeng 1), drought stress, different growth stages, growth and physiological characteristics

## Introduction

Tartary buckwheat (*Fagopyrum tataricum*), a dicotyledonous plant in the Polygonaceae family, is a unique dual-purpose crop in China valued for both medicinal and food applications. Growing consumer demand for health-promoting foods has stimulated expanded cultivation and development of this crop (Zhou et al., 2014). Tartary buckwheat exhibits physiological characteristics including cold tolerance, poor soil adaptation, short growth duration, and strong environmental adaptability, conferring distinct regional advantages for production in China's alpine and plateau areas (Zhang et al., 2013). However, drought tolerance does not equate to drought preference, and abiotic stress—primarily drought or seasonal drought—remains the main factor limiting yield improvement in the Loess Plateau region.

Drought represents one of the most significant abiotic stress factors affecting plant growth, development (Pereira et al., 2003), and distribution (Caruso et al., 2007; Xu et al., 2008). Previous research has demonstrated that drought impacts not only external morphological indicators such as plant height and leaf area (Nicotra et al., 2010) but also physiological characteristics including osmotic adjustment substances (soluble sugars, soluble proteins), SOD and POD activities (Shao and Shan, 2006), and ultimately yield. These indicators exhibit marked changes in response to drought stress (Zhao et al., 2016; Yang and Zhu, 2012). Crop drought resistance refers to the ability to maintain favorable growth status under water-limited conditions, with research focusing on external morphology, photosynthetic systems, antioxidant systems, osmotic adjustment systems, and stress-induced proteins. Some studies suggest crops cope with adversity through developed root systems or physiological adjustments, though consensus on specific diagnostic indicators for drought resistance remains elusive. While tartary buckwheat is considered drought-tolerant, it is not drought-loving, and water deficit severely affects its quality and yield under drought stress. Previous studies have shown that various indicators change and growth is inhibited in tartary buckwheat under drought conditions (Lu et al., 2018; Zhao et al., 2019). Drought stress first affects plant root systems, subsequently impacting whole-plant growth. Due to technical challenges in complete root extraction associated with tartary buckwheat's fibrous root structure, previous research has focused primarily on apparent morphology and physiological characteristics, with limited investigation into cellular and molecular mechanisms underlying drought tolerance and differential responses among varieties. To further elucidate potential mechanisms of drought tolerance in tolerant tartary buckwheat (Diqing), this study compared two varieties with contrasting drought resistance selected from preliminary screening, aiming to provide theoretical guidance for drought-resistant cultivation and variety breeding in arid regions.

For crops grown in arid and semi-arid regions, drought stress typically occurs as periodic events during the growth cycle, with different crops exhibiting varied responses to water deficit at different developmental stages and having distinct water requirements. Most crops possess a water-sensitive period during which

water deficiency severely impacts growth (Li et al., 2016). Therefore, identifying water-sensitive periods is crucial for water management and sowing date adjustment in crop production. Existing literature indicates that the flowering stage represents a water-sensitive period for tartary buckwheat, with water deficiency during this phase affecting grain filling and causing sterility (Hou and He, 2012). However, whether drought-resistant varieties differ in their responses and how morphological and physiological quantitative indicators vary under drought stress at different growth stages in varieties with contrasting drought resistance remain poorly documented. This study employed pot-based water control experiments to investigate quantitative changes in morphological and physiological indicators of two tartary buckwheat varieties with different drought tolerance under stage-specific drought stress, exploring the ecophysiological responses of these varieties to identify drought-sensitive periods and provide theoretical guidance for stable, drought-resistant cultivation.

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## Materials and Methods

### 1.1 Experimental Materials

The experimental tartary buckwheat varieties included the drought-tolerant ‘Diqing’ and drought-sensitive ‘Heifeng 1’, both previously screened (Lu et al., 2018) and provided by the Institute of Alpine Crops, Shanxi Academy of Agricultural Sciences. The test soil contained  $0.08 \text{ g} \cdot \text{kg}^{-1}$  total nitrogen,  $2.9 \text{ mg} \cdot \text{kg}^{-1}$  available phosphorus, and  $92.37 \text{ mg} \cdot \text{kg}^{-1}$  available potassium. Fertilizers used were analytical-grade reagents: urea (46% N), calcium superphosphate (15% P O), and potassium chloride (52% K O).

### 1.2 Experimental Design and Implementation

The experiment was conducted from May to September 2018 in a rainproof plastic shelter at Shanxi Normal University. A three-factor completely randomized design was employed. Factor A consisted of two tartary buckwheat varieties: drought-tolerant ‘Diqing’ (D) and drought-sensitive ‘Heifeng 1’ (H). Factor B comprised two water treatments: normal water supply (70–80% of field capacity, CK) and drought stress (30–40% of field capacity, S). Factor C included three drought stress periods: seedling stage, flowering stage, and maturity stage (each lasting 20 days). The experiment comprised 12 treatments with six replications each.

Implementation details: Pot experiments were conducted using 13 kg of air-dried, sieved, and homogenized soil-sand mixture as the growth medium per pot. Basal fertilizers were applied at rates of  $0.2 \text{ g} \cdot \text{kg}^{-1}$  urea,  $0.35 \text{ g} \cdot \text{kg}^{-1}$  calcium superphosphate, and  $0.21 \text{ g} \cdot \text{kg}^{-1}$  potassium chloride, mixed thoroughly with the soil-sand substrate. Uniform, plump, pest-free tartary buckwheat seeds were selected, sterilized with  $\text{H}_2\text{O}_2$ , rinsed repeatedly, and soaked in deionized

water for 12 hours before sowing on May 5 at 15 seeds per pot. All pots received equal normal watering after sowing to ensure successful germination. Seedlings were thinned to five plants per pot at the uniform seedling stage. Drought stress treatments were initiated at the three-leaf stage for the seedling treatment, at early flowering for the flowering treatment, and at late flowering for the maturity treatment. Except during the designated drought stress periods, all treatments maintained soil water content equivalent to the control. Water was supplemented daily at 18:00 using the weighing method to maintain target moisture ranges, with random repositioning of pots throughout the experiment.

### 1.3 Measurement Indicators

**1.3.1 Morphological Indicators** On the second day after drought stress completion at each growth stage (seedling, flowering, maturity), three plants per treatment were randomly selected. Plant height and maximum root length were measured with a ruler; stem diameter was measured with vernier calipers; leaf area was determined using a LI-3000C portable leaf area meter. Plants were separated into shoot and root portions, killed at 105 °C for 30 minutes, then dried at 75 °C to constant weight for dry matter determination. An additional three plants were analyzed using the Delta-T SCAN root analysis system (UK) to determine root surface area, root volume, and root average diameter.

**1.3.2 Physiological Indicators** On the second day after drought stress completion at each growth stage, three plants per treatment were randomly selected, washed clean, and dried with filter paper. Root activity in root tips was measured using the TTC method (Zhang et al., 2019). Main and lateral roots were analyzed for SOD activity, POD activity, MDA content, root soluble sugar content, root soluble protein content, and root free proline content using the nitroblue tetrazolium method, guaiacol colorimetric method, thiobarbituric acid method, anthrone colorimetry, Coomassie brilliant blue G-250 staining method, and acidic ninhydrin method, respectively (Zhang et al., 2019). Leaf chlorophyll content was determined using the acetone-ethanol direct extraction method (Zhang et al., 2019).

### 1.4 Data Processing

Statistical analysis was performed using SPSS 19.0 software, with Duncan's multiple range test for comparisons. Figures were generated using Microsoft Excel 2010.

## Results

### 2.1.1 Shoot Morphological Indicators

As shown in Table 1, variety differences and drought stress at different growth stages had highly significant effects ( $P < 0.01$ ) on shoot growth of tartary buckwheat. Comparisons between normal water supply and drought stress treatments consistently showed CK > S. Between the two varieties, Diqing consistently outperformed Heifeng 1. All shoot morphological indicators decreased to varying degrees under drought stress at different growth stages compared to stage-matched controls, with the magnitude of reduction following the pattern: flowering stage > seedling stage > maturity stage. Flowering-stage drought stress exerted the greatest impact. Under flowering-stage drought stress (S), Diqing and Heifeng 1 exhibited significant reductions ( $P < 0.05$ ) compared to controls (CK) in plant height (15.59% and 18.68%), stem diameter (20% and 24.28%), leaf area (23.16% and 35.87%), and shoot-leaf dry weight (44.47% and 51.04%). The reduction magnitude across indicators followed: shoot dry weight > leaf area > stem diameter > plant height, and Heifeng 1 > Diqing. Flowering-stage drought most severely affected shoot dry weight, with the drought-tolerant variety showing less growth reduction than the drought-sensitive variety.

**Table 1** Effects of drought stress on shoot growth of *Fagopyrum tataricum* at different growth stages

| Stress stage    | Plant Treatment | Plant height | Stem diameter | Leaf area (mm <sup>2</sup> ) | Shoot-leaf dry weight |
|-----------------|-----------------|--------------|---------------|------------------------------|-----------------------|
| Seedling stage  | CK              | 27.31±0.23g  | 3.20±0.11e    | 1328.74±98.99f               | 0.97±0.06gh           |
|                 | S               | 30.33±0.12g  | 3.71±0.11d    | 1547.62±11.19a               | 1.00±0.06gh           |
| Flowering stage | CK              | 21.44±0.32i  | 2.10±0.06g    | 749.57±14.23h                | 0.70±0.09h            |
|                 | S               | 24.61±0.28h  | 2.62±0.03f    | 949.14±13.34g                | 0.91±0.01bcd          |
| Mature stage    | CK              | 62.14±0.16e  | 5.00±0.01c    | 1878.46±28.85d               | 4.17±0.13e            |
|                 | S               | 73.62±1.63c  | 6.25±0.29b    | 2444.67±29.96a               | 7.51±0.11c            |
| A×B×C           |                 | 52.41±0.72f  | 3.99±0.09d    | 1306.55±10.96b               | 3.29±0.15f            |
|                 |                 | 64.45±2.47e  | 5.27±0.35c    | 2037.37±30.83b               | 6.72±0.25d            |
|                 |                 | 85.35±1.03b  | 6.31±0.17b    | 1801.04±46.51b               | 4.17±0.05b            |
|                 |                 | 92.87±1.45a  | 6.90±0.06a    | 2091.33±8.85b                | 9.22±0.06b            |
|                 |                 | 68.55±0.84d  | 5.37±0.04c    | 1575.17±28.71b               | 8.03±0.45c            |
|                 | 236.716**       | 103.238**    | 2045.067**    | 10.373**                     |                       |
|                 | 128.418**       | 74.132**     | 442.216**     | 2.143*                       |                       |
|                 | 437.363**       | 274.465**    | 487.314**     | 18.138**                     |                       |

Note: Data represent mean ± standard error. Different letters within columns indicate significant differences ( $P < 0.05$ ). Factor data (A, B, C) represent

ANOVA *F-values*. , \*\*, and ns denote significant ( $P < 0.05$ ), highly significant ( $P < 0.01$ ), and non-significant ( $P > 0.05$ ) differences, respectively. The same notation applies below.\*

### 2.1.2 Root Morphological Indicators

As shown in Table 2 , variety differences and drought stress at different growth stages significantly affected root growth ( $P < 0.05$ ). Comparisons between normal water supply and drought stress treatments showed CK > S for all parameters except main root length. Between varieties, Diqing consistently outperformed Heifeng 1. Root morphological indicators changed under drought stress at different growth stages, with the magnitude of change following: flowering stage > seedling stage > maturity stage. Flowering-stage drought stress exerted the greatest impact, with the drought-tolerant variety developing more extensive root systems than the drought-sensitive variety. Under flowering-stage drought stress (S), Diqing and Heifeng 1 showed significant reductions ( $P < 0.05$ ) compared to controls (CK) in root average diameter (20% and 21.95%), root surface area (9.71% and 10.87%), root volume (20.66% and 21.96%), and root dry weight (34.42% and 38.93%). Heifeng 1 showed a 27.66% reduction in main root length, whereas Diqing exhibited a 12.26% increase. The magnitude of change across indicators for Diqing followed: root dry weight > root volume > root average diameter > main root length > root surface area, while for Heifeng 1 it was: root dry weight > main root length > root volume > root average diameter > root surface area. Except for main root length, the reduction magnitude was greater in Heifeng 1 than in Diqing. These results indicate that flowering-stage drought most severely affected root dry weight, and that Diqing may enhance drought resistance by increasing root length to access deeper soil moisture, thereby mitigating drought effects more effectively than the sensitive variety.

**Table 2** Effects of drought stress on root system development of *Fagopyrum tataricum* at different growth durations

| Stress stage    | Treatment | Main root length | Root average diameter | Root superficial area (mm <sup>2</sup> ) | Root volume (cm <sup>3</sup> ) | Root dry wt |
|-----------------|-----------|------------------|-----------------------|--|--------------------------------|-------------|
| Seedling stage  | CK        | 21.94±0.52d      | 0.55±0.02gh           | 4466.83±176.72d                          | 15.03±0.03g                    | 0.29±0.01e  |
|                 | S         | 20.80±0.48d      | 0.655±0.01def         | 4662.50±152.01d                          | 17.56±0.25f                    | 0.33±0.02e  |
| Flowering stage | CK        | 15.03±0.03g      | 0.45±0.03i            | 3370.50±107.16e                          | 6.01±0.01g                     | 0.21±0.04e  |
|                 | S         | 17.56±0.25f      | 0.53±0.02h            | 3584.13±118.65e                          | 7.68±0.19c                     | 0.24±0.07e  |
| Mature stage    | CK        | 28.66±0.42a      | 0.76±0.03c            | 8733.63±219.26b                          | 7.45±0.18cd                    | 0.80±0.01cd |
|                 | S         | 25.53±0.60bc     | 0.95±0.06a            | 9673.33±239.49a                          | 15.48±0.44g                    | 1.22±0.03a  |
|                 | CK        | 21.40±1.60d      | 0.82±0.05b            | 8460.70±194.96b                          | 9.39±0.03a                     | 0.69±0.01d  |

| Stress stage | Treatment | Main root length | Root average diameter | Root superficial area (mm <sup>2</sup> ) | Root volume (cm <sup>3</sup> ) | Root dry wt  |
|--------------|-----------|------------------|-----------------------|--|--------------------------------|--------------|
| A×B×C        |           | 26.23±0.46ab     | 10.71±0.04cd          | 8673.33±239.49b                          | 21.15±0.98e                    | 1.01±0.16abc |
|              |           | 27.26±0.26ab     | 10.658±0.06de         | 8541.26±103.42b                          | 7.07±0.09de                    | 0.71±0.04cd  |
|              |           | 221.097**        | 111.638**             | 5.856*                                   | 71.967**                       | 11.333**     |
|              |           | 97.752**         | 172.708**             | 3.442*                                   | 134.007**                      | 18.991**     |
|              |           | 769.153**        | 1.672ns               | 274.097**                                | 121.866**                      | 311.329**    |

### 2.2.1 Root Activity, SOD Activity, POD Activity, and MDA Content

As shown in Table 3, variety differences and drought stress at different growth stages had highly significant effects ( $P < 0.01$ ) on root activity, SOD activity, POD activity, and MDA content. Comparisons between normal water supply and drought stress treatments showed  $S > CK$  for all parameters except root activity. Between varieties, Diqing outperformed Heifeng 1 for all parameters except MDA content. All indicators changed under drought stress at different growth stages, with the magnitude of change consistent with morphological indicators: flowering stage > seedling stage > maturity stage. Flowering-stage drought stress exerted the greatest impact. Under flowering-stage drought stress (S), Diqing and Heifeng 1 showed significant reductions in root activity (25.6% and 28%) and significant increases in SOD activity (41.64% and 33.29%), POD activity (43.78% and 37.5%), and MDA content (26.07% and 29.36%) compared to controls (CK) ( $P < 0.05$ ). The magnitude of change across indicators followed: POD activity > SOD activity > MDA content > root activity. Root activity and MDA content showed greater changes in Heifeng 1 than in Diqing, while enzyme activity changes were greater in Diqing than in Heifeng 1. These results suggest that under flowering-stage drought, the drought-tolerant variety experienced lower membrane lipid peroxidation, less impact on root activity, but greater effects on peroxidase activity compared to the drought-sensitive variety.

**Table 3** Effects of drought stress on root activity, SOD activity, POD activity, and MDA content in root of *Fagopyrum tataricum* at different growth stages

| Stress stage    | Treatment | Root activity ( $\mu\text{g} \cdot \text{g}^{-1}$ ) | SOD activity ( $\text{U} \cdot \text{g}^{-1}$ ) | POD activity ( $\text{U} \cdot \text{g} \cdot \text{min}^{-1}$ ) | MDA content ( $\text{nmol} \cdot \text{g}^{-1}$ ) |
|-----------------|-----------|---|---|--|---|
| Seedling stage  |           | 0.45±0.01ef   | 515.26±19.02b                                   | 46.36±1.75cde  | 4.81±0.23g  |
|                 |           | 0.55±0.03bc   | 378.22±6.48d                                    | 33.88±0.92f  | 4.04±0.15g  |
| Flowering stage |           | 0.32±0.02g  | 317.31±10.47e                                   | 33.91±1.85f  | 10.44±0.32de                                      |
|                 |           | 0.41±0.04f  | 254.95±19.05f                                   | 25.55±1.54g  | 8.53±0.53f  |
| Maturing stage  |           | 0.61±0.05b  | 690.25±26.72a                                   | 71.69±1.33a  | 9.96±0.48e  |

| Stress stage | Root activity (mg · g <sup>-1</sup> ) | SOD activity (U · g <sup>-1</sup> ) | POD activity (U · g · min <sup>-1</sup> ) | MDA content (nmol · g <sup>-1</sup> ) |
|--------------|---------------------------------------|-------------------------------------|---|---------------------------------------|
|              | 0.82±0.02a                            | 487.32±9.11b                        | 49.86±3.28bcd                             | 7.90±0.45f                            |
|              | 0.54±0.03bcd                          | 401.96±12.74cd                      | 44.77±1.85b                               | 16.52±0.61a                           |
|              | 0.57±0.04bc                           | 301.56±19.31e                       | 39.84±2.80ef                              | 12.77±0.27c                           |
|              | 0.60±0.01bc                           | 510.93±23.44b                       | 50.59±2.66bc                              | 11.11±0.32d                           |
|              | 0.45±0.03def                          | 288.26±6.64ef                       | 42.86±3.28de                              | 10.03±0.30e                           |
| A×B×C        | 37.963**                              | 44.7**                              | 72.381**                                  | 2.707*                                |
|              | 50.24**                               | 444.572**                           | 115.314**                                 | 6.295**                               |
|              | 73.566**                              | 83.737**                            | 73.671**                                  | 3.106*                                |

### 2.2.2 Root Osmotic Adjustment Substances

As shown in Table 4, variety differences and drought stress at different growth stages significantly affected root soluble sugar content, soluble protein content, and free proline content ( $P < 0.05$ ). Comparisons between normal water supply and drought stress treatments showed  $S > CK$  for all parameters except soluble protein content. Between varieties, Diqing outperformed Heifeng 1 for all parameters. All indicators changed under drought stress at different growth stages, with the magnitude of change following: flowering stage  $>$  seedling stage  $>$  maturity stage. Drought clearly affected osmotic adjustment substances, with flowering-stage drought stress exerting the greatest impact and the drought-tolerant variety showing superior performance. Under flowering-stage drought stress (S), Diqing and Heifeng 1 showed significant increases in soluble sugar content (36.78% and 29.04%) and free proline content (38.73% and 29.35%), and significant decreases in soluble protein content (22.31% and 24.68%) compared to controls (CK) ( $P < 0.05$ ). The magnitude of change across indicators followed: free proline content  $>$  soluble sugar content  $>$  soluble protein content. Increases in soluble sugar and free proline content were greater in Diqing than in Heifeng 1, while the opposite pattern was observed for soluble protein content. These results indicate that tartary buckwheat responds to drought stress by increasing free proline and soluble sugar osmotic adjustment substances to lower osmotic potential and resist water deficit.

**Table 4** Effects of drought stress on soluble sugar content, soluble protein content, and proline content in root of *Fagopyrum tataricum* at different growth stages

| Stress stage   | Soluble sugar content (mg · g <sup>-1</sup> ) | Soluble protein content (mg · g <sup>-1</sup> ) | Free proline content (g · g <sup>-1</sup> ) |
|----------------|---|---|---|
| Seedling stage | 2.31±0.12bcd                                  | 4.72±0.28de                                     | 37.21±2.22cde                               |
|                | 1.88±0.13de                                   | 5.34±0.12cd                                     | 28.84±3.73fg                                |

| Stress stage    | Treatment | Soluble sugar content (mg · g <sup>-1</sup> ) | Soluble protein content (mg · g <sup>-1</sup> ) | Free proline content (g · g <sup>-1</sup> ) |
|-----------------|-----------|---|---|---|
| Flowering stage |           | 1.92±0.24cde                                  | 3.48±0.16f                                      | 27.32±0.88fg                                |
|                 |           | 1.64±0.11e                                    | 4.14±0.22ef                                     | 21.47±1.83g                                 |
| Maturing stage  |           | 3.83±0.29a                                    | 5.50±0.15cd                                     | 62.50±2.55a                                 |
|                 |           | 2.8±0.08b                                     | 7.08±0.45a                                      | 45.05±2.26c                                 |
|                 |           | 3.51±0.17a                                    | 4.15±0.29ef                                     | 52.84±3.87b                                 |
|                 |           | 2.72±0.25b                                    | 5.51±0.31cd                                     | 40.85±3.61cd                                |
|                 |           | 2.84±0.21b                                    | 6.15±0.34bc                                     | 43.40±1.31c                                 |
|                 |           | 2.55±0.41bc                                   | 6.59±0.06ab                                     | 38.03±1.22cde                               |
|                 |           | 39.22**                                       | 6.191*  | 18.745**                                    |
| A×B×C           |           | 0.979ns                                       | 55.676**  | 23.521**                                    |
|                 |           | 23.72**                                       | 1.092ns   | 74.99**                                     |

### 2.2.3 Leaf Chlorophyll Content

As shown in Figure 1 [Figure 1: see original paper], variety differences and drought stress at different growth stages had highly significant effects ( $P < 0.01$ ) on leaf chlorophyll content. Comparisons between normal water supply and drought stress treatments showed CK > S. Between varieties, Diqing outperformed Heifeng 1. All indicators decreased under drought stress at different growth stages compared to stage-matched controls, with the magnitude of reduction following: flowering stage > seedling stage > maturity stage. Flowering-stage drought stress exerted the greatest impact, reducing leaf chlorophyll content by 25.51% in Diqing and 34.52% in Heifeng 1 ( $P < 0.05$ ).

**Table 5** Three-factor ANOVA F-values for effects on chlorophyll content in leaf of *Fagopyrum tataricum*

| Factor | F value   |
|--------|-----------|
| A×B×C  | 18.99**   |
|        | 23.83**   |
|        | 110.221** |
|        | 0.941ns   |

Different letters in the figure indicate significant differences among treatments ( $P < 0.05$ ).

**Figure 1** Effects of drought stress on chlorophyll content in leaf of *Fagopyrum tataricum* at different growth stages

## Discussion

Water is an indispensable element for plant growth, and plant responses to drought stress have long been a focal point of scientific research (Shen et al., 2017). Numerous studies have documented that drought stress induces visible morphological changes and even growth retardation (Liu, 2013; Feng et al., 2016), such as leaf wilting and significant alterations in plant height, stem diameter, and leaf area (Liu et al., 2016; Kong et al., 2010). In this study, both tartary buckwheat varieties exhibited consistent declining trends in plant height, stem diameter, leaf area, and shoot dry weight under drought stress at all growth stages, though the magnitude of decline differed between varieties. The drought-sensitive variety Heifeng 1 showed greater reductions across all indicators than the drought-tolerant variety Diqing. Comparing the impact of drought stress across growth stages revealed that, regardless of drought resistance level, flowering-stage drought caused the most severe effects on shoot morphological indicators with the greatest reduction magnitude, followed by seedling-stage drought, while maturity-stage drought had the least impact. Thus, the sensitivity of shoot morphological indicators to drought stress at different growth stages followed: flowering stage > seedling stage > maturity stage. However, across all indicators, the drought-tolerant variety demonstrated less growth reduction than the drought-sensitive variety under drought stress, exhibiting a relative growth advantage.

Drought stress affects root system architecture, water absorption and utilization, and internal water balance (Li et al., 2016), with root system growth being the first affected when crops experience water deficit (Zhang et al., 2018; Wang et al., 2018). This experiment yielded consistent results, with drought stress at different growth stages reducing root surface area, root volume, root dry weight, root average diameter, and main root length (in Heifeng 1) in both varieties, though the reduction magnitude differed. Heifeng 1 showed greater reductions than Diqing, indicating poorer drought tolerance. Notably, Diqing exhibited a significant increase in main root length, suggesting that drought stress stimulated primary root growth in this variety, enabling it to access deeper soil moisture to meet its needs and resist adverse conditions. This may represent one mechanism underlying its superior drought tolerance, consistent with findings by Wei et al. (2018) and Xie and Zhang (2018). Consistent with effects on shoot growth, flowering-stage drought stress had the greatest impact on root morphology with the largest change magnitude, followed by seedling-stage drought, while maturity-stage drought had the smallest effect. Among root parameters, root dry weight was most severely affected by flowering-stage drought. The drought-tolerant variety experienced less impact on root growth than the sensitive variety, with smaller changes across indicators and better adaptation to drought through root morphological adjustments.

Drought stress affects not only plant morphology but also physiological metabolism (Zhang et al., 2018). SOD and POD are crucial antioxidant enzymes that eliminate  $O_2$  and  $H_2O_2$  produced under stress, thereby mitigating

damage (Tatar and Gevrek, 2008). Soluble sugars, free proline, and soluble proteins are important osmotic adjustment substances. Under water stress, plants accumulate these substances to increase concentration, lower osmotic potential, and maintain cell turgor to retain required water (Zhang et al., 2015), thereby resisting adversity. Most studies report increased soluble sugar and free proline content under drought stress, while soluble protein results vary. MDA content reflects the degree of membrane lipid peroxidation, with drought inducing plasma membrane lipid peroxidation (Luo et al., 2014), making it an important drought resistance indicator. In this study, both varieties maintained high SOD and POD activities and increased soluble sugar and free proline content to adapt to and mitigate drought stress effects, consistent with previous research (Yang et al., 2018). Soluble protein content decreased, possibly due to amino acid binding for water loss defense or interactions increasing protein solubility and hydration while reducing precipitation. Root activity reflects root growth status and vitality level, with both varieties showing reduced root activity under drought stress. Across growth stages, flowering-stage drought stress had the greatest impact on root physiological indicators with the largest change magnitude, followed by seedling-stage drought, while maturity-stage drought had the smallest effect. Thus, the sensitivity of root physiological indicators to drought stress followed: flowering stage > seedling stage > maturity stage. Among these, POD content was most sensitive to drought stress during flowering. The drought-tolerant variety demonstrated stronger reactive oxygen species scavenging capacity, greater accumulation of osmotic adjustment substances, lower membrane lipid peroxidation, higher root activity, and less cellular damage under drought stress—physiological differences that likely contribute to its superior drought resistance. Drought stress damages chloroplast structure in leaves, reducing chlorophyll content and affecting photosynthesis. In this study, chlorophyll content decreased under drought stress, with the greatest reduction during flowering-stage drought. The drought-tolerant variety Diqing showed less impact on leaf chlorophyll content than the sensitive variety, with smaller reduction magnitude and consequently less impact on photosynthesis, consistent with its smaller reductions in root and shoot dry weight compared to Heifeng 1.

Drought stress at different growth stages affected shoot morphology, root morphology, enzyme activities, osmotic adjustment substances, root activity, and leaf chlorophyll content in both varieties, with impacts following: flowering stage > seedling stage > maturity stage. The flowering stage represents the most water-sensitive period for both varieties, emphasizing the need for timely irrigation during this phase in production practice. The drought-tolerant variety (Diqing) demonstrated stronger adaptability under drought stress, employing superior coping strategies through adjustments in shoot morphology, root morphology, and physiological systems, making it more suitable for cultivation in arid regions.

## Conclusion

This study investigated the effects of drought stress at different growth stages on the growth and development of Diqing and Heifeng 1 tartary buckwheat varieties. The results revealed consistent trends in indicator changes under drought stress for both varieties, though the magnitude of change differed. The drought-tolerant variety exhibited less impact on growth and physiological characteristics than the drought-sensitive variety, demonstrating stronger drought adaptability. Drought stress effects at different growth stages followed the pattern: flowering stage > seedling stage > maturity stage, indicating that flowering-stage drought stress most severely affected physiological growth and represents a water-sensitive period for tartary buckwheat. In production practice, careful variety selection is essential, and field management should prioritize timely water supply during the water-sensitive flowering stage. Under conditions where irrigation is unavailable, adjusting sowing dates to maximize overlap between the flowering period and local rainy seasons can minimize adverse drought effects.

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