Effects of Drought Stress on Phenotypic Plasticity in Prunus humilis Seedlings: Postprint

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Abstract

To investigate the effects of drought stress on phenotypic plasticity of Cerasus humilis, this experiment used Cerasus humilis as experimental material and studied its morphological characteristics, growth characteristics, and other aspects through drought stress treatments. The results showed that with increasing drought stress, root biomass, shoot biomass, total plant biomass accumulation, root-to-shoot ratio, and root-to-shoot ratio stress index of Cerasus humilis all exhibited a trend of first increasing and then decreasing, reaching maximum values under the T1 treatment and being significantly higher than other treatments (α=0.05). With decreasing soil water content, the root biomass allocation index of Cerasus humilis showed a trend of first increasing and then decreasing, while the shoot biomass allocation index showed the opposite pattern; under the T1 treatment, the root biomass allocation index was maximal and the shoot biomass allocation index was minimal (α=0.05). When water supply was 60%-80%, the plant height, crown width, basal diameter, number of secondary branches, main root length, main root diameter, and number of lateral roots of Cerasus humilis all reached maximum values (α=0.05), with no significant effect on the growth of primary branch number. With increasing water stress, leaf length began to decrease from the T2 treatment, while leaf width, single leaf area, and specific leaf area all showed a trend of first increasing and then decreasing (α=0.05). In conclusion, Cerasus humilis exhibited strong plasticity in response to different drought stress conditions by adjusting morphological characteristics, biomass accumulation and allocation of various organs.

Full Text

Preamble

Effects of Drought Stress on Phenotypic Plasticity of *Cerasus humilis* Seedlings

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Abstract

To investigate the effects of drought stress on phenotypic plasticity in *Cerasus humilis*, we conducted a controlled experiment examining morphological and growth characteristics under varying soil moisture conditions. Our results demonstrate that as drought stress intensified, root biomass, shoot biomass, total plant biomass accumulation, root-to-shoot ratio, and root-to-shoot stress index all exhibited an initial increase followed by a decrease, reaching maximum values under the T1 treatment (60–80% field capacity), which were significantly higher than other treatments ($\alpha = 0.05$). With decreasing soil water content, the biomass allocation index to roots showed a similar trend of initial increase then decrease, while leaf biomass allocation showed the opposite pattern. The root biomass allocation index peaked under T1, whereas leaf biomass allocation reached its minimum under the same conditions ($\alpha = 0.05$). When water supply ranged from 60% to 80% of field capacity, plant height, crown width, base diameter, number of secondary branches, main root length, main root diameter, and lateral root number all achieved maximum values ($\alpha = 0.05$), though primary branch number showed no significant response. As water stress intensified, leaf length began to decline from the T2 treatment onward, while leaf width, individual leaf area, and specific leaf area all displayed a pattern of initial increase followed by decrease ($\alpha = 0.05$). In summary, *C. humilis* exhibits strong phenotypic plasticity in response to different drought stress conditions through adjustments in morphological traits, organ biomass accumulation, and biomass allocation patterns.

Keywords: *Cerasus humilis*, drought stress, phenotypic plasticity

Introduction

Plants encounter diverse environmental conditions during growth and development, responding with corresponding phenotypic adjustments to adapt to heterogeneous habitats—a capability known as phenotypic plasticity (Pigliucci, 2005; Sultan, 2000). Phenotypic plasticity in plants comprises morphological, physiological, and ecological components (De Kroon et al., 2005). Typically, plants regulate their physiological, ecological, or morphological characteristics through phenotypic plasticity to optimize resource utilization and allocation, thereby adapting to local survival conditions (Zhu, 2007). Since the 1990s, Chinese scholars have systematically investigated plant phenotypic plasticity, focusing primarily on heterogeneous abiotic factors such as temperature, water, light, and nutrients (Schlichting, 1986; Wang et al., 2015; Le, 2015). Research has evolved from simple morphological descriptions to comprehensive studies of biomass accumulation and allocation among organs, as well as physiological and ecological responses (Xu et al., 2017; Pang et al., 2017; Wang et al., 2016). Water is an indispensable factor for plant growth and development, and both excess and deficit can affect normal plant growth. Consequently, the relationship between water availability and plant phenotypic plasticity has become a research hotspot in plant physiological ecology (Zhang et al., 2014).

Cerasus humilis, a small shrub in the Rosaceae family and genus *Prunus*, is a unique Chinese sand-dwelling medicinal plant. Despite its diminutive stature, it possesses a well-developed root system with strong sprouting capacity, and exhibits characteristics of cold resistance, drought tolerance, poor soil tolerance, and strong adaptability, making it a pioneer species for the Grain-for-Green Program in arid northwestern China. The fruit of *C. humilis* is considered a third-generation functional fruit with high nutritional and economic value, representing an exemplary integration of ecological and economic benefits. In recent years, research on *C. humilis* has attracted increasing attention from scholars, advancing steadily toward broader, deeper, and higher-level investigations. Significant achievements have been made in studies on drought resistance mechanisms (Zhu, 2006), breeding and propagation (Zhang and Liu, 1995), fruit nutrition, medicinal value, and molecular biology (Li et al., 2010; Zhang et al., 2011). However, research on phenotypic plasticity of *C. humilis* under drought stress remains unreported. Therefore, this study investigates the effects of drought stress on growth and morphological plasticity of *C. humilis* under controlled soil moisture conditions, providing a theoretical basis for future economic forest development of this species in arid and semi-arid regions and supporting its role in the sand industry development under the Belt and Road Initiative.

1.2 Experimental Design

The experiment was conducted in 2016 at the experimental field of the Desert Forestry Experiment Center, Chinese Academy of Forestry. Soil from farmland in the Ulan Buh Desert region was used as the substrate, sieved and equally filled into non-perforated plastic containers (40 cm top diameter × 40 cm height × 30 cm bottom diameter). PVC tubes were inserted for aeration. In late April, uniform *C. humilis* seedlings were selected from cold storage and transplanted into the containers under normal field management. The weighing method was employed to control soil water content, with field capacity maintained at 20.29%. Five treatments were established, each with 30 replicates. The control treatment (CK) maintained soil relative water content (SRWC) at 16–20%, representing 80–100% of maximum field capacity. Drought stress treatments corresponded to 60–80% (T1), 40–60% (T2), and 20–40% (T3) of field capacity. Water was replenished every other morning at 8:00 AM by weighing, and rain shelters were constructed to prevent natural precipitation from affecting the experiment.

Growth status was measured monthly from July to September.

1.3 Experimental Methods

Plant Growth Characteristics Measurement: Plant height, crown width, and base diameter were measured using vernier calipers.

Allocation Index: Plants were separated into main roots, lateral roots, and aboveground parts, then oven-dried to constant weight. The allocation index was calculated as:

Allocation Index = Organ Biomass / Total Biomass

Root-to-Shoot Ratio: Calculated as underground biomass divided by aboveground biomass.

Specific Leaf Area (SLA): Fifty healthy, mature leaves from the middle and upper portions of plants in each treatment were selected and brought to the laboratory. Leaf area, length, and width were obtained using a scanner. Leaves were then killed at 105°C for 30 minutes and oven-dried at 75°C to constant weight (W1). SLA was calculated as:

$$\text{SLA} = \frac{\text{LA}}{\text{W1}}$$

where LA is leaf area (cm^2) and W1 is leaf dry weight (g).

1.4 Data Processing

Data were organized and graphed using Excel 2003 software. Analysis of variance (ANOVA) was performed using SAS 9.0 software.

Results

2.1 Effects of Drought Stress on Biomass Accumulation in *Cerasus humilis*

As shown in , under different soil moisture conditions, root biomass, shoot biomass, and total plant biomass accumulation in *C. humilis* all exhibited a trend of initial increase followed by decrease, following the order T1 > T2 > CK > T3. The differences in biomass accumulation between T1 and CK were greater than those between T1 and T2 for all organs. For example, when water supply was reduced by 20% from normal (T1), root biomass accumulation increased by 6.17 g, a difference that was statistically significant ($\alpha = 0.05$). ANOVA revealed significant differences in shoot and total plant biomass accumulation among treatments ($\alpha = 0.05$). These results indicate that a water supply of 60–80% field capacity is optimal for *C. humilis* growth in the Ulan Buh Desert region. Water supply above 80% or below 40% of field capacity adversely affects growth and biomass accumulation in *C. humilis*.

2.2 Effects of Drought Stress on Biomass Allocation in *Cerasus humilis*

As shown in , with decreasing soil water content, the root biomass allocation index in *C. humilis* initially increased then decreased, reaching its maximum under T1 treatment. The ranking was T1 > CK > T3 > T2, with significant differences among treatments ($\alpha = 0.05$). Conversely, the shoot biomass allocation index showed an opposite trend, initially decreasing then increasing with declining soil moisture, following the order T2 > T3 > CK > T1, also with significant differences among treatments ($\alpha = 0.05$).

2.3 Effects of Drought Stress on Root-to-Shoot Ratio and Stress Index in *Cerasus humilis*

The root-to-shoot ratio reflects the allocation proportion between aboveground and belowground plant parts and serves as an important indicator of plant growth. As shown in , the root-to-shoot ratio of *C. humilis* was highest under mild water stress, significantly differing from other treatments ($\alpha = 0.05$). Lower root-to-shoot ratios under T2 and T3 treatments resulted from water deficiency limiting aboveground growth. The root-to-shoot stress index, defined as the ratio of stressed root-to-shoot ratio to that of the control group, also indicates plant growth status. The index was maximal under 60–80% water conditions, with the root-to-shoot ratio exceeding that under 80–100% conditions, demonstrating that T1 treatment is most favorable for *C. humilis* growth. Differences in root-to-shoot ratio across water treatments suggest that moderate irrigation maximizes this parameter, while either insufficient or excessive water supply is detrimental to growth and may affect fruit sugar accumulation.

2.4 Effects of Drought Stress on Morphological Characteristics of *Cerasus humilis*

Growth characteristics of *C. humilis* under different water treatments are presented in . Results show that normal water supply and various drought stress levels similarly affected plant height, base diameter, number of secondary branches, main root diameter, main root length, and lateral root number, all showing an initial increase then decrease with declining water content. The ranking for plant height, number of secondary branches, main root diameter, main root length, and lateral root number was T1 > CK > T3 > T2, while crown width followed T1 > T2 > CK > T3. At 60–80% water supply, plant height, crown width, base diameter, main root length, main root diameter, and lateral root number all reached maximum values with significant differences ($\alpha = 0.05$), indicating T1 treatment represents the optimal water requirement for *C. humilis* growth. From this optimum, all growth parameters decreased with further water reduction; for example, under T2 treatment, plant height, main root diameter, and main root length decreased by 15%, 16.5%, and 16.9%, respectively, demonstrating that reduced water content inhibits *C. humilis* growth. Primary branch number remained constant at 2 across all treatments, showing no significant effect of water stress. No significant difference in base diameter was observed between CK and T2 treatments, suggesting that base diameter growth increases significantly only under optimal water supply.

2.5 Effects of Drought Stress on Leaf Growth Characteristics of *Cerasus humilis*

Leaf length, width, area, and specific leaf area are all important indicators of plant growth. As shown in , with decreasing water gradients, leaf width, individual leaf area, and specific leaf area all exhibited an initial increase followed by a decrease. Average leaf length showed no obvious change under normal and mild stress conditions but gradually decreased when water supply fell below 60%, with reductions of 7.5% and 16.4% under T2 and T3 treatments, respectively ($\alpha = 0.05$). Leaf width and individual leaf area followed the same ranking: T1 > CK > T2 > T3. When water supply decreased by 20% from normal, leaf area increased by 3.2%, but decreased by 26.1% with a further 20% reduction, with significant differences among treatments ($\alpha = 0.05$). This indicates that plants grow most vigorously under optimal water supply, while water deficiency impairs normal growth. Specific leaf area followed the ranking T1 > CK > T3 > T2, with significant differences ($\alpha = 0.05$). Interestingly, when water decreased to T3 treatment, specific leaf area increased by 15.3% compared to T2, possibly due to reduced water loss capacity and accumulated dry matter under severe water deficit. However, the overall trend indicates that insufficient water supply limits normal growth and development of *C. humilis*.

Discussion

3.1 Phenotypic Plasticity of Growth Characteristics in *Cerasus humilis*

Environmental conditions influence plant growth, and numerous studies have demonstrated that drought stress affects plant growth and development to varying degrees (Xie et al., 2010; Yu et al., 2006; Zheng and Yan, 2006; Chen, 2013; Chen et al., 2004; Li et al., 2012; Li et al., 2010; Li et al., 2008). Growth characteristics represent morphological responses of green plants to different environmental conditions, and xerophytes maintain optimal growth states to effectively utilize environmental resources (Li et al., 2002). In arid and semi-arid regions, water is a critical factor affecting plant growth, with water supply directly influencing growth characteristics, biomass accumulation, and allocation patterns. Leaves are important water-consuming organs, and their responses under different water supply conditions reflect plant strategies for maintaining water balance, such as reducing light-exposed area or advancing leaf senescence to cope with arid and hot environments (Su and Yan, 2006). Our results show that compared with CK, all drought stress treatments caused root biomass, shoot biomass, total biomass accumulation, root-to-shoot ratio, and root-to-shoot stress index to initially increase then decrease, consistent with other studies (Guan et al., 2003).

This study demonstrates that drought stress significantly affects biomass accumulation and allocation in *C. humilis*. Under T1 treatment, biomass accumulation in all organs and total biomass reached maximum values, then decreased with further soil water reduction. Both excessively high and low soil water content adversely affected growth, reducing growth rate and biomass accumulation. Therefore, from a biomass accumulation perspective, T1 treatment is optimal for *C. humilis* growth. Previous research indicates that plants continuously adjust aboveground and belowground biomass proportions to adapt to environmental changes and mitigate damage. Our results show significant changes in biomass allocation indices across treatments, with maximum root biomass allocation index occurring under T1 treatment and the minimum shoot allocation index under the same conditions. This indicates that under drought stress, plants adjust their adaptive mechanisms to transfer more resources to roots for enhanced water and nutrient absorption, demonstrating drought resistance capacity.

Plants modify root distribution to absorb and transport more water, improving water retention to withstand drought stress. Thus, root-to-shoot ratio reflects competitive ability for environmental resources (Chen et al., 2004). Most studies show that drought stress increases root-to-shoot ratio, but our results show an increase-decrease-increase pattern with decreasing soil moisture, possibly related to cultivar-specific characteristics. The root-to-shoot stress index of *C. humilis* varied significantly across water treatments, following the same trend as the root-to-shoot ratio, representing another adaptive response to water variation.

3.2 Effects of Drought Stress on Morphological Plasticity in *Cerasus humilis*

Under drought stress, changes in plant morphological characteristics directly reflect damage status and serve as direct indicators for evaluating stress resistance (Zhou et al., 2010). Previous studies have shown that plant height growth rate slows with intensifying drought stress. Our results demonstrate that at 60–80% water supply, plant height, crown width, base diameter, number of secondary branches, main root length, main root diameter, and lateral root number all reached maximum values, indicating morphological plasticity differences across water treatments. Under this treatment, *C. humilis* exhibited stronger phenotypic traits than other treatments, suggesting that 60–80% water supply represents the optimal ecological niche for growth, consistent with previous findings (Xu et al., 2017). Primary branch number remained constant at 2 across all treatments, indicating no significant effect of water stress on this parameter.

Leaf structural traits characterize biochemical features and maintain relative stability in specific environments. However, leaf traits are not isolated and co-adapt with other characteristics. In arid and semi-arid regions, plants exhibit adaptive leaf traits to cope with dry and hot climates, with various interacting traits affecting growth and development. Leaf length, width, area, and specific leaf area are important growth indicators. Our results show that with decreasing water gradients, leaf length began to decline from T2 treatment, while leaf width and individual leaf area showed initial increase then decrease, reflecting adaptive characteristics to different drought stress levels. Decreases in leaf length, width, and area indicate strong drought adaptation capacity in *C. humilis*. Specific leaf area, representing leaf area per unit mass, is closely related to plant survival and growth. Studies show that plants with high specific leaf area exhibit high productivity and can adapt to resource-rich environments (Li et al., 2005). With intensifying drought stress, leaf area and specific leaf area gradually decrease (Wang, 2015). Our results show that specific leaf area peaked under T1 treatment, then gradually decreased. Although specific leaf area fluctuated under drought stress, these responses represent adaptations to different water conditions.

Conclusion

Drought stress significantly affected growth characteristics of *Cerasus humilis*. With intensifying drought stress, root biomass, shoot biomass, total plant biomass accumulation, root-to-shoot ratio, and root-to-shoot stress index all exhibited an initial increase followed by decrease, reaching maximum values under T1 treatment and significantly exceeding other treatments. With decreasing soil water content, root biomass allocation index initially increased then decreased, while leaf biomass allocation showed the opposite pattern, with maximum root allocation and minimum shoot allocation occurring under T1 treatment. Drought stress also significantly affected morphological characteristics. At 60–80% water supply, plant height, crown width, base diameter, number of secondary branches, main root length, main root diameter, and lateral root number all reached maximum values, while primary branch number showed no significant response. With intensifying water stress, leaf length decreased from T2 treatment onward, while leaf width, individual leaf area, and specific leaf area all displayed initial increase then decrease. In conclusion, *Cerasus humilis* exhibits strong phenotypic plasticity in response to different drought stress conditions through adjustments in morphological traits, organ biomass accumulation, and biomass allocation patterns.

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Note: Figure translations are in progress. See original paper for figures.