



Effects of Different Gradients of Citric Acid Application on the Biomass and Photosynthetic Characteristics of Black Barley Flag Leaves

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Abstract. It is of great research significance to study the effects of different gradients of citric acid on the biomass and photosynthetic characteristics of flag leaf of black barley. Using black barley in Longzi as experimental material, a one-way randomized block design was adopted, and five citric acid gradient treatments, CK (0 kg/hm²), T1 (15 kg/hm²), T2 (30 kg/hm²), T3 (45 kg/hm²), and T4 (60 kg/hm²), were set to compare the effects of different gradients of citric acid on net photosynthetic rate, stomatal conductance, transpiration rate, and biomass of the flag leaf of black barley, stomatal conductance, intercellular carbon dioxide concentration, transpiration rate and biomass of black barley flag leaves under different gradients of citric acid treatment. The results showed that each gradient of citric acid treatment could improve the photosynthetic capacity of black barley flag leaves after flowering, and the best effect was achieved when the citric acid gradient was T2, and the photosynthetic indexes such as SPAD, net photosynthesis rate, stomatal conductance, inter-cellular CO₂ concentration and transpiration rate of the flag leaves after flowering were all higher than that of the blank photo-comparison CK ($P < 0.05$); the aboveground biomass, root biomass and total biomass increased significantly and the root-crown ratio increased significantly with the different gradients of citric acid treatment, while the root-crown ratio increased significantly. biomass, root biomass and total biomass of aboveground part increased significantly, and root-crown ratio increased. In conclusion, the application of different gradients of citric acid can promote the production and accumulation of photosynthetic products of black barley, and then increase the biomass of black barley.

Keywords: Black barley, citric acid, Biomass, photosynthetic characteristics

1 Foreword

Barley (*Hordeum vulgare* L. var. *coeleste* Linnaeus), also known as naked barley, belongs to the barley varieties, the separation of the palea shell and the glumes at maturity to the bare seed, [1], due to the deposition of pigments in the pericarp and

paste powder layer, a variety of colored barley is formed, [2], according to the distinction of the colour of the glumes, which can be divided into white barley, black barley, blue barley, yellow barley and purple barley, [3-4]. Research surface, black barley not only has a unique flavour and taste, but also rich in nutrients. Among them, the protein content is high, and contains a variety of amino acids needed by the human body; dietary fibre content is also high, help promote intestinal peristalsis, improve the function of the digestive system; mineral content is rich, such as calcium, iron, zinc, etc., has an important role in human health. Black barley is also rich in a variety of vitamins, such as vitamin B, E, etc., with antioxidant, anti-aging and other effects, [5-7]. Therefore, the planting and development of black barley in the plateau region has important economic significance.

Photosynthesis is the material basis of plant energy metabolism and the most important factor in the composition of its productivity, [8-9]. Photosynthesis is affected by internal and external environmental factors such as atmospheric temperature, photosynthetically active radiation, net photosynthetic rate, stomatal conductance, transpiration rate, and intercellular CO₂ concentration, [10]. Wang Jianlin et al, [11]. experimental study found that under the improved distribution of fertilizers, the photosynthetic characteristics of black barley were improved, and the yield was also improved, but the excessive infertility of the soil will still affect the growth of black barley. Among them, citric acid, as an efficient fertilizer additive, plays an important role in improving fertilizer utilization and promoting crop growth. Citric acid is an important organic acid, involved in many physiological metabolic processes in plant tissues, and is also an effective abiotic antagonist, [12]. Citric acid can alleviate the toxicity of heavy metals in plants, increase photosynthesis and promote their growth and development, [13-14]. It is important to study the effect of citric acid on photosynthesis in plants to regulate their growth and development. In a previous study, exogenous citric acid treatment significantly increased net photosynthetic rate, stomatal conductance, transpiration rate, intercellular CO₂ concentration, water use efficiency, and water vapor pressure deficit in pomegranate leaves, [15], which was consistent with the findings on oilseed rape, [14]. A large number of results have been obtained on the application of citric acid to crops, but no studies have been seen on citric acid on photosynthetic performance and biomass accumulation in flag leaf of black barley after flowering. In this study, we designed four research protocols on the effects of applying different gradients on the physiological characteristics of black barley, to investigate the effects of applying different gradients on the biomass and photosynthetic characteristics of black barley, and to provide a reasonable fertilization program for barley cultivation.

2 Materials and Methods

2.1 Generalization of the Material

The experiment was conducted from March to September 2024 in Mangcuo Village, Longzi County, Shannan City, Xizang (28°07'28.52"N, 91°53'93.06"E), at an altitude of 3,878 m. Longzi County belongs to the plateau temperate arid monsoon climate

zone, which is a plateau temperate semi-arid monsoon climate. The average annual sunshine hours are 3007.9 h, the annual precipitation is 295.41 mm, the average annual temperature is 5.5°C, the annual frost-free period is 228.3d, the solar radiation is strong, the sunshine time is long, the temperature is low, the temperature difference between day and night is big, the dryness and wetness are clear, more night rain, winter and spring are dry, and there are many windy days. Basic physical and chemical properties of soil: organic matter content 14.59 g/kg, pH value 8.69, total nitrogen content 0.85 g/kg, effective phosphorus content 6.21 mg/kg, quick-acting potassium content 115.37 mg/kg. overall soil nutrients are not high, and the fertility level is low.

2.2 Test Materials

The black barley variety for test was the hexagonal Longzi black barley local variety (provided by the Agriculture and Rural Affairs Bureau of Longzi County, Shannan City, Xizang Autonomous Region). Citric acid, produced by Shandong Yimei Chemical Co., Ltd. and calcium superphosphate (effective phosphorus P_2O_5 content of 12.0%, water-soluble phosphorus P_2O_5 content of 7.0%, and S content of 8.0%) were produced by Gansu Baiyin Hubao Chemical Co. The remaining conventional fertilizers were urea (containing N46.0%) and potassium sulfate (containing K_2O 50.0%, S 18.0%).

2.3 Experimental Design

A large field randomized block design was used with five treatments (Table 1), namely: (1) no citric acid 0 kg/hm² (CK); (2) citric acid 15 kg/hm² (T1); (3) citric acid 30 kg/hm² (T2); (4) citric acid 45 kg/hm² (T3); and (5) citric acid 60 kg/hm² (T4), which were uniformly applied using a Seeder sowing, plot area 16.8 m², row spacing 30 cm, 3 biological replications. Before sowing, the standard fertilizer amount (N amount 9 kg/mu, 9 kg/mu P_2O_5 , 9 kg/mu K_2O) was used as the standard base fertilizer, and the application of N, P and K fertilizers were all 135 kg/hm², with conventional irrigation, and the field management was consistent with that of the local area.

Table 1. Fertilizer rates for each treatment in the experiment

Experimental plot	Processing	Proportion of application	CA/kg/hm ²
18	CK	Conventional fertilization	0
34	T1	Conventional fertilization combined with 100g of citric acid (standard: 1kg/acre)	15
35	T2	Conventional fertilization combined with 200g of citric acid (standard: 2kg/acre)	30
36	T3	Conventional fertilization combined with 300g of citric acid (standard: 3kg/acre)	45
37	T4	Conventional fertilization combined with 400g of citric acid (standard: 4kg/acre)	60

2.4 Measurement Items and Methods

2.4.1 Biomass Measurement

Referring to the method of Dongqiang, [16], single-plant samples were taken from black barley at the maturity stage, and the aboveground biomass of stalks, leaves, kernels, rachis and roots were determined respectively. The above parts were uniformly put into the oven, first killed at 105°C for 20 min, and then baked at 65°C until constant weight, and the dry weight was weighed by scales.

2.4.2 Measurement of Photosynthetic Characteristic Indicators

The net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (Tr) and inter-cellular CO₂ concentration (Ci) of black barley flag leaves were measured by using Li-6400XT. 10 black barley plants with basically the same growth condition were selected in sunny and cloudless weather at 9:00-11:00 in the 7, 14, 21, 28, and 35d after the flowering of black barley.

2.4.3 Determination of Chlorophyll Content in Flag Leaves

At 9:00-11:00 a.m. in sunny and windless weather, after the flag leaves were fully unfolded, the flag leaves of 10 black barley plants with basically the same growth in each plot were labeled, and the relative chlorophyll content of the flag leaves was measured using the SPAD-502 chlorophyll meter (Konica, Japan) at 7, 14, 21, 28, and 35d after anthesis, avoiding the veins and taking 3 points on each side of the leaf veins for measurement in 3 repetitions. avoiding the leaf veins, taking three points on each side of the leaf veins for measurement, repeating three times, and calculating the mean value.

2.5 Data Analysis

Excel 2019 was used for data statistics, DPS 7.05 data processing system for ANOVA and significance of difference test, and Origin2022 for graphing.

3 Results and Analysis

3.1 The Effect of Applying Different Gradients of Citric Acid on the SPAD Value of Flag Leaves after Flowering in Black Barley

SPAD value represents the relative value of chlorophyll content of plant leaves, which reflects the size of plant photosynthetic rate to a certain extent, and is an indicator of plant growth and development. As can be seen from Figure 1, the relative chlorophyll content of flag leaves of black barley in different treatments showed a trend of increasing and then decreasing with the developmental process, and reached the maximum value at 14d after anthesis because it was at the peak of the grain filling rate at that stage; the relative chlorophyll content decreased rapidly at 28d~35d after anthesis. At each measurement period, the SPAD values of different treatments showed

that $T2 > T3 > T1 > T4 > CK$, and all treatments were significantly different from each other ($P < 0.05$). All citric acid treatments significantly increased the photosynthetic capacity and the best result was observed in T2 (30 kg/hm^2) treatment.

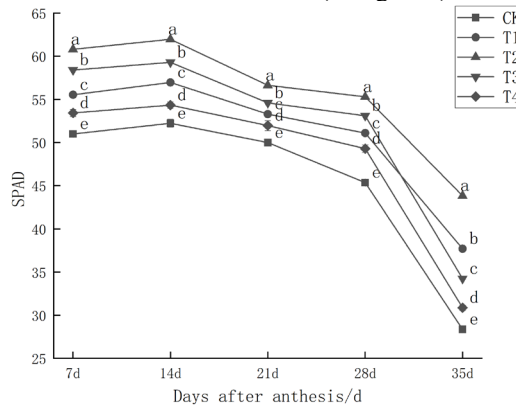


Fig. 1. The effect of different gradients of citric acid on SPAD in flag leaves of black barley after flowering.

Note: During the same period, different letters in the column indicate significant differences between treatments ($P < 0.05$), the same applies below.

3.2 The Effect of Applying Different Gradients of Citric Acid on the Net Photosynthetic Rate of Flag Leaves in Black Barley after Flowering

Net photosynthetic rate is the most favorable photosynthetic index to illustrate the production status of assimilates, and a higher net photosynthetic rate facilitates the entry of air into the interior of the leaf, thus weakening the resistance between the outside air and the carboxylated parts of the leaf, thus facilitating the plant itself to carry out photosynthesis. As can be seen from Figure 2, the net photosynthetic rate of flag leaves of black barley in different treatments showed a decreasing trend with the post-flowering fertility process. In 7d after flowering, the net photosynthetic rate of black barley flag leaves of CK, T1, T2, T3 and T4 treatments were the highest, which were 21.4, 27.07, 27.84, 24.79 and 23.07, respectively, and the higher net photosynthetic rate could help the black barley to accumulate the substances required for growth faster during the flowering period and filling period, which is important for the increase of black barley yield. All citric acid treatments significantly increased the net photosynthetic rate, and the T2 (30 kg/hm^2) treatment increased the net photosynthetic rate of black barley flag leaves most significantly in all periods, which was favorable to the accumulation of nutrients required for the growth of black barley.

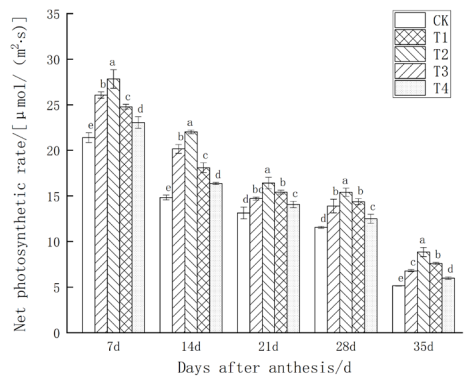


Fig. 2. The effect of different gradients of citric acid on the net photosynthetic rate of flag leaves in black barley after flowering

3.3 The Effect of Applying Different Gradients of Citric Acid on Stomatal Conductance in Flag Leaves of Black Barley after Flowering

As can be seen from Figure 3, stomatal conductance of flag leaves of black barley in different treatments showed a trend of increasing and then decreasing with the post-flowering fertility process, and the stomatal conductance reached a peak at 14d post-flowering, followed by a gradual decline. At 7, 14, 21, 28 and 35d after anthesis, the stomatal conductance of T2 treatment increased by 34.8%, 36%, 18.75%, 33.3% and 97.6%, respectively, compared with that of CK. Stomatal conductance of black barley flag leaves increased at 14d after anthesis, with significant differences between the control CK treatment and the other treatments ($P<0.05$), and significant differences among treatments ($P<0.05$); after ANOVA, all citric acid treatments significantly increased stomatal conductance at all periods after anthesis, and the T2 (30 kg/hm²) treatment increased stomatal conductance of black barley flag leaves at all periods most significantly, which in turn affected the growth and development of black barley.

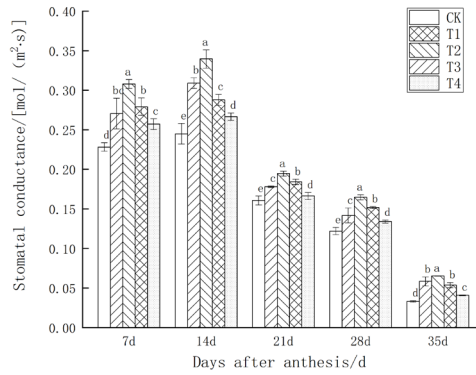


Fig. 3. The effect of different gradients of citric acid on stomatal conductance in flag leaves of black barley after flowering

3.4 The Effect of Applying Different Gradients of Citric Acid on the Intercellular Carbon Dioxide Concentration in the Flag Leaves of Black Barley after Flowering

As can be seen from Figure 4, intercellular CO_2 concentration in the flag leaves of black barley under different treatments showed an increasing trend with the post-flowering fertility process, and the intercellular CO_2 concentration peaked at 35d after flowering. At 7d after anthesis, the concentration of intercellular CO_2 in black barley flag leaves was $\text{T2} > \text{T1} > \text{T3} > \text{T4} > \text{CK}$, of which the concentration of intercellular CO_2 in flag leaves under the treatment of T2 was $246 \mu\text{mol/mol}$, which was higher than that of the control by $53.33 \mu\text{mol/mol}$ at 35d after anthesis. $260.00 \sim 339.33 \mu\text{mol/mol}$ floated, and the differences between CK and the treatments were significant. After analysis of variance, all citric acid treatments significantly increased intercellular CO_2 concentration in black barley flag leaves at all periods after flowering, and the T2 (30 kg/hm^2) treatment most significantly increased intercellular CO_2 concentration in black barley flag leaves at all periods, which in turn affected the growth and development of black barley.

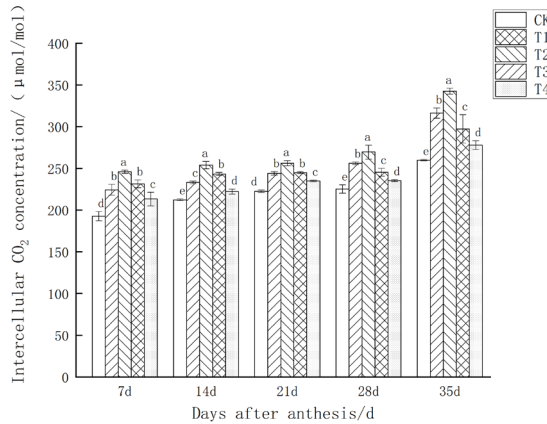


Fig. 4. The effect of different gradients of citric acid on intercellular carbon dioxide concentration in flag leaves of black barley after flowering

3.5 The Effect of Applying Different Gradients of Citric Acid on Transpiration Rate of Flag Leaves after Flowering in Black Barley

As can be seen from Figure 5, the transpiration rate of black barley flag leaves under different treatments reached the peak at 14d after flowering, and then gradually decreased. At 14d after flowering, the transpiration rate of black barley flag leaf was $\text{T2} > \text{T1} > \text{T3} > \text{T4} > \text{CK}$, in which the transpiration rate of flag leaf under T2 treatment was $9.70 \text{ mmol}/(\text{m}^2 \cdot \text{s})$, which was higher than that of CK by $2.45 \text{ mmol}/(\text{m}^2 \cdot \text{s})$; at all times after flowering, the transpiration rate of black barley flag leaf under all treatments was higher than that of CK and the differences between the treatments were significant ($P < 0.05$). In T2 (30 kg/hm^2) treatment in all periods of black barley flag

leaf transpiration rate increased most significantly, the effect of each treatment on black barley flag leaf transpiration rate and the effect of stomatal conductance of the same trend. This indicates that the application of citric acid affects the transpiration rate of black barley flag leaves by increasing the stomatal conductance, and the increase of transpiration rate will affect the fertility process of black barley.

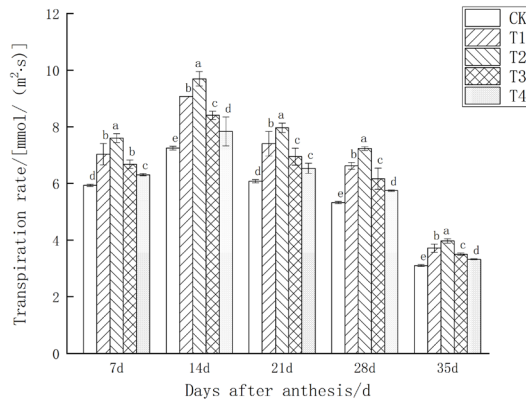


Fig. 5. The effect of different gradients of citric acid on transpiration rate of flag leaves of black barley after flowering

3.6 The Effect of Applying Different Gradients of Citric Acid on Biomass And Inter Organ Distribution of Black Barley

As can be seen from Table 2, the aboveground biomass, root biomass and total biomass of black barley with the increase in citric acid concentration showed a trend of increase and then decrease, were manifested as $T2>T1>T3>T4>CK$, the aboveground biomass, root biomass and total biomass of the T2 treatment was significantly higher than the rest of the treatments; citric acid application of the treatments of the root-crown ratio was significantly higher than that of the CK, among which, the root-crown ratio of the highest in the T2 root-crown ratio of 0.083, but the difference between T1, T3 and T4 treatments was not significant. 0.083, but the differences among T1, T3 and T4 treatments were not significant. Overall, compared with CK, total biomass was increased by 58.42% and root-crown ratio by 7.79% in T2; in T1, T3 and T4, total biomass was increased by 35.73%, 21.30% and 7.94%, respectively. Application of citric acid significantly increased the root-crown ratio of black barley and the best result was observed in T2 (30 kg/hm²) treatment. It indicated that the application of citric acid promoted the root growth and development of black barley.

Table 2. Effects of applying different gradients of citric acid on biomass and inter-organ allocation of black barley

Processing	Above-ground biomass	Root system/g	Total biomass/g	Root-crown ratio
CK	30.23e	2.34e	32.57e	0.077b
T1	41.03b	3.36b	44.39b	0.082a

T2	47.89a	3.96a	51.76a	0.083a
T3	36.67c	2.99c	39.66c	0.082a
T4	32.63d	2.66d	35.30d	0.082a

Note: Different lowercase letters after the data indicate significant differences between treatments ($P < 0.05$).

4 Discussion

The root system is the main organ of crop nutrient absorption, the root-crown ratio can directly reflect the plant's absorption capacity, and its size is closely related to crop yield and photosynthetic characteristics. Crop root-crown ratio reflects the distribution relationship between above-ground and below-ground parts, and the larger the root-crown ratio is, the higher the functional activity of the root system, and the lower the opposite, [17]. In this study, it was found that the application of citric acid could promote the growth of black barley biomass and increase the root-crown ratio of black barley to different degrees. Gong Fangfang et al. showed that the application of exogenous citric acid would change the root morphology of prickly pear seedlings on calcareous yellow soil, and could improve the root vigor and enzyme activity of prickly pear seedlings, and this change showed differences according to the amount of citric acid applied, [18]. The results of this study showed that the application of appropriate amount of citric acid could increase the aboveground biomass, root dry mass, and root-crown ratio, which was beneficial to the morphology of black barley plants. This is basically consistent with the results of previous studies.

Photosynthesis is the material basis for the formation of crop yields, 90% to 95% of the dry mass of the plant comes from photosynthesized organic matter, and higher photosynthetic performance can promote the accumulation of crop biomass, [19]. Guo Jiang et al. found that crop growth depends on photosynthesis to synthesize organic matter, and the strength of photosynthetic capacity determines crop yield, [20]. Potassium and phosphorus are involved in sugar metabolism and affect the synthesis and transport of photosynthetic products, and when deficient, the accumulation of photosynthetic products is impeded, thus affecting the rate of photosynthesis, [21]. Citric acid, by providing a carbon source to soil microorganisms, can change the population structure of soil microorganisms, increase the number of functional bacteria such as phosphorus- and potassium-solubilizing bacteria, and then promote the solubilization of mineral phosphorus and mineral potassium in the soil to increase the content of effective phosphorus and effective potassium, [22-23], thus improving the photosynthetic rate of the crop. In this study, citric acid treatment significantly increased net photosynthetic rate, stomatal conductance, transpiration rate, and intercellular CO_2 concentration of black barley leaves, which is consistent with the findings on mustard, [24]. The net photosynthetic rate of plants is also affected by, [25-26], factors, and the effects of citric acid on the photosynthetic phosphorylation of flag leaves of black barley, photosynthetic enzymes and their gene expression will be investigated in depth at a later stage. To explore the mechanism of citric acid regulation of black barley photosynthesis. At present, there is basically no research on citric acid on the

photosynthetic properties of black barley, so the results of this study for the study of black barley photosynthesis, production and other aspects of a certain reference.

5 Conclusion

Application of different gradients of citric acid can increase the aboveground biomass and total biomass of black barley, and increase the root-crown ratio. The application of citric acid has a positive effect on the growth and development of black barley, which can enable black barley to rapidly complete the accumulation of biomass, and has a positive effect on the increase of black barley yield. With the advancement of post-flowering fertility process, different citric acid dosage can enhance the chlorophyll content of black barley leaves, as well as increase the net photosynthetic rate and transpiration rate of black barley flag leaves. In conclusion, citric acid application improved the photosynthetic efficiency and biomass production of black barley, with the T2 gradient (30 kg/hm²) producing the most significant promotion, which could provide new ideas and methods for black barley cultivation.

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