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Differences in Growth, Photosynthesis, and Stress Resistance of Mulberry and Paper Mulberry under Bicarbonate Treatment (Postprint)

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Abstract

To investigate the effects of HCO3- on plant growth and physiological characteristics under bicarbonate stress in karst habitats, seedlings of Broussonetia papyrifera and Morus alba were used to examine plant growth, photosynthetic capacity, antioxidant enzyme activities, osmotic adjustment substance contents, and cell membrane system damage under different concentrations of NaHCO3 (0, 15, 30 mmol · L-1) treatment. The results showed that: (1) Under HCO3- treatment, the growth and photosynthetic capacity of both B. papyrifera and M. alba were inhibited, and significant antioxidant and anti-osmotic stress physiological responses occurred in leaf cells. (2) The inhibitory effect of HCO3- on the growth of B. papyrifera and M. alba was concentration-dependent and showed significant differences ($P < 0.05$). (3) The inhibitory effects of 30 mmol · L-1 HCO3- treatment on plant growth, photosynthesis, antioxidant enzyme system, and osmotic adjustment system, as well as damage to plant cells, were significantly stronger than those of 15 mmol · L-1 HCO3- treatment. (4) Under the same concentration of HCO3- treatment, the growth, photosynthetic capacity, antioxidant enzyme activities, and osmotic adjustment substance contents of B. papyrifera were significantly higher than those of M. alba, while its leaf cell damage was significantly lower than that of M. alba. Collectively, these results indicate that B. papyrifera possesses superior tolerance to bicarbonate stress compared to M. alba. This study provides scientific support for elucidating the adaptation mechanisms of Moraceae plants to karst environments.

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Full Text

Differences in Growth, Photosynthesis, and Stress Resistance of Morus alba and Broussonetia papyrifera Under Bicarbonate Treatments

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Abstract

This study investigated the effects of bicarbonate (HCO) stress on plant growth and physiological characteristics in karst habitats. Using seedlings of Broussonetia papyrifera and Morus alba as experimental materials, we examined growth performance, photosynthetic capacity, antioxidant enzyme activities, osmotic regulation substance content, and cell membrane system damage under three NaHCO concentrations (0, 15, and 30 mmol · L ^{1}). The results demonstrated: (1) Under HCO stress, both species exhibited inhibited growth and photosynthetic capacity, with significant physiological responses to antioxidant and osmotic stress in leaf cells. (2) The inhibitory effects of HCO on growth were concentration-dependent and showed significant differences among treatments ($P < 0.05$). (3) The 30 mmol · L ^{1} HCO treatment produced significantly stronger inhibition of growth, photosynthesis, antioxidant enzyme systems, and osmotic regulation systems, along with greater plant cell damage, compared to the 15 mmol · L ^{1} treatment. (4) Under identical HCO concentrations, Broussonetia papyrifera showed significantly higher growth rates, photosynthetic capacity, antioxidant enzyme activity, and osmotic regulation substance content than Morus alba, while its leaf cell damage was significantly lower. These findings indicate that Broussonetia papyrifera possesses superior tolerance to bicarbonate stress compared to Morus alba. This research provides scientific support for elucidating the adaptation mechanisms of Moraceae plants to karst environments.

Keywords: Morus alba; Broussonetia papyrifera; bicarbonate; antioxidant enzymes; osmotic regulation; cell damage

Introduction

In karst biogeosystems, the dissolution of limestone and dolomite by water creates stable HCO pools in soil and water, with concentrations significantly higher than in non-karst regions (Stokes & Griffiths, 2019). Previous research indicates that HCO concentrations in karst rivers and lakes typically range from 1–5 mmol · L ^{1} (McCray & Matocha, 1992), while concentrations in calcareous soils are approximately 4.5 mmol · L ^{1} (Zhang et al., 2012). Furthermore,

when environmental pH exceeds 7, HCO concentrations become several times higher than CO concentrations (Hussner et al., 2016). Given these elevated HCO levels in karst soils and water, investigating its effects on plants in karst habitats represents an important research direction.

Under saline-alkaline stress conditions such as NaCl, NaHCO , and Na CO , plant physiological activities are readily disrupted, causing osmotic and ionic stress that affects normal growth and development (Zhu et al., 2009; Ahmad & Sharma, 2010). Neutral salt stress is typically referred to as salt stress, while alkaline salt stress is termed alkali stress, with alkali stress causing significantly greater damage to plants than salt stress (Shi & Yin, 1993; Lu et al., 2009). Bicarbonate, as an alkaline salt, releases substantial HCO in soil, elevating pH and subjecting plants to both alkali stress and high pH stress (Hartung et al., 2002; Li et al., 2005; Chen et al., 2012). Under HCO stress, activities of antioxidant enzymes such as SOD, POD, and CAT increase in plant cells, demonstrating clear stress physiological responses (Dou et al., 2008). Additionally, HCO significantly affects plant acquisition of multiple mineral elements, increasing Na concentration while decreasing contents of Fe^{2} , Zn^{2} , Ca^{2} , Mg^{2} , Mn^{2} , Cu^{2} , and K (Michael et al., 2012). Importantly, HCO serves as a carbon source for plant carbon assimilation (Salbitani et al., 2020), especially under stress conditions when stomata close, with rhizosphere HCO accounting for a large proportion of carbon absorbed by plants (Keeley et al., 1984). Root-derived dissolved inorganic carbon (DIC) for photosynthesis constitutes approximately 20% of total carbon acquired by plants under drought stress (Rao & Wu, 2017). Beyond its role as a photosynthetic substrate, HCO directly affects the structural and functional integrity of the photosynthetic system. During photosynthesis, HCO binds tightly within the PSII reaction center, playing an important role in electron transfer and enhancing photosynthetic rates (Tikhonov et al., 2018). Higher HCO concentrations can also reduce chloroplast content by affecting the quantity of reduced iron ions in plants (Shahsavandia et al., 2020). These multifaceted roles of HCO in plant growth, antioxidant enzyme activity, and photosynthesis underscore the importance of investigating its effects on plant physiological characteristics for understanding plant adaptation mechanisms in karst environments.

Moraceae plants are typical pioneer species commonly found in karst regions (Wu et al., 2009). Among them, Morus alba L., a deciduous tree in the Morus genus, possesses rich germplasm resources, can adapt to strong stress conditions, and provides ecological functions such as soil and water conservation and environmental beautification, attracting widespread attention (Ren et al., 2017). Broussonetia papyrifera L., another Moraceae species, is a widely distributed deciduous tree with rapid growth, strong adaptability, and tolerance to drought, salt, and barren conditions, commonly used for soil and vegetation restoration (Gao, 2020; Tian et al., 2020). However, no studies have investigated the differential responses of these two species to varying HCO concentrations. Therefore, this research employed two Moraceae species (Broussonetia papyrifera and Morus alba) as study subjects, conducting seedling cultivation in an artificial

climate greenhouse and using bicarbonate to simulate stress conditions. By analyzing changes in growth characteristics and physiological-biochemical indices under different bicarbonate treatments, we aimed to address three questions: (1) What are the physiological response mechanisms of Broussonetia papyrifera and Morus alba under HCO stress? (2) What differences exist between the two species in their responses to HCO stress? (3) How do their tolerances to HCO stress compare? This study provides a scientific basis for elucidating the adaptation mechanisms of Moraceae plants to karst environments.

1. Materials and Methods

1.1 Experimental Material Cultivation

This experiment employed a cultivation method starting from seed germination. Broussonetia papyrifera seeds were collected from the old campus of the Institute of Geochemistry, Chinese Academy of Sciences in Guiyang, Guizhou Province, while Morus alba seeds were collected from the Guizhou Academy of Agricultural Sciences in Guiyang. Plump, full seeds were selected and placed in seedling boxes containing a certain volume of perlite, covered with a thin layer of perlite. The water reservoir of the seedling tray was filled with distilled water to a level that would not submerge the seeds. The cultivation room was maintained at 25 °C with 50–60% humidity and a 12-hour photoperiod.

Approximately 12 days later, seeds began to germinate. When seedlings developed four leaves, uniform and robust seedlings were selected and transplanted into 12-cell seedling boxes. Each box contained two seedlings spaced appropriately to ensure mutual interference would not occur during growth to experimental size. After transplanting, the boxes were placed in an artificial climate chamber with a 12-hour photoperiod, photosynthetic photon flux density (PPFD) of 300 $\text{mol} \cdot \text{m}^{2} \cdot \text{s}^{1}$, daytime temperature of 25 °C, nighttime temperature of 20 °C, and relative humidity of 55–65%. Both Morus alba and Broussonetia papyrifera seedlings were cultivated hydroponically using half-strength Hoagland nutrient solution to provide nutrients and water.

1.2 Bicarbonate Stress Treatment

When plant height reached 16–18 cm, different concentrations of NaHCO were added to the nutrient solution to prepare treatment solutions adjusted to pH 7.8 for the bicarbonate treatment experiment. Three concentration gradients were established (0, 15, and 30 $\text{mmol} \cdot \text{L}^{1}$). Previous research indicates that Na at these concentrations does not significantly inhibit or affect plant growth and photosynthetic capacity, with HCO being the primary influencing factor in this experiment. The NaHCO solutions were replaced daily at a fixed time. Plant growth and photosynthetic indices were measured every other day, and two leaves approximately 80 mm wide were collected and stored at -80 °C for subsequent measurement of physiological-biochemical indices under bicarbonate stress. All measurements were replicated three times.

1.3 Plant Growth Parameter Measurement

To measure the dynamic changes in growth indices of Broussonetia papyrifera and Morus alba under bicarbonate treatment, this study assessed plant growth by measuring above-ground parameters, minimizing interference with normal plant growth during the process. Growth indices were measured on days 0, 2, 4, 6, 8, 10, and 12 after bicarbonate treatment. Plant height (H), basal stem diameter (Db), leaf number (N), and number of leaves with width 80 mm (N) were selected as indicators for evaluating plant growth conditions.

1.4 Plant Leaf Photosynthetic Index Measurement

Following bicarbonate stress treatment, photosynthetic indices were measured on days 0, 2, 4, 6, and 8, with measurement times fixed between 14:00–16:00 to avoid potential midday depression of photosynthesis. A LI-6400XT portable photosynthesis system (LI-COR, Lincoln, NE, USA) was used to determine net photosynthetic rate (Pn), stomatal conductance (gs), intercellular CO concentration (Ci), and transpiration rate (E). Stomatal limitation value (Ls) was calculated using the formula Ls = 1 - Ci/Ca, where Ca represents ambient CO concentration (Farquhar & Sharkey, 1982; Han et al., 2007).

1.5 Plant Leaf Antioxidant Enzyme Activity Measurement

Enzyme extraction followed the method of Peng et al. (2007). Superoxide dismutase (SOD) activity was determined according to Zhang et al. (2000), peroxidase (POD) activity according to Zhang et al. (2000), and catalase (CAT) activity according to Aebi (1984).

1.6 Plant Leaf Osmotic Regulation Substance Content Measurement

Proline content was determined following Lei et al. (2007), and soluble sugar content according to Zou (2003).

1.7 Thiobarbituric Acid Reactive Substances (TBARS) Measurement

The method of Heath and Packer (1968) was employed.

1.8 Data Statistical Analysis

Data were organized using Microsoft Excel 2019. Two-way ANOVA was performed using IBM SPSS Statistics 20.0 to test the significance of effects of different tree species and NaHCO concentrations on plant growth, photosynthetic capacity, antioxidant enzyme activity, osmotic regulation substance content, and cell membrane system damage. Independent samples t-tests were used to examine significant differences between tree species for these parameters. Significance levels among different treatments were determined at the 0.05 level. Figures were prepared using Origin 2019b 32Bit.

2. Results

2.1 Effects of Bicarbonate Treatment on Growth Indices of Broussonetia papyrifera and Morus alba

Table 1 shows that for the same tree species under different HCO concentrations, all growth indices were more strongly inhibited under 30 mmol · L ^{1} HCO treatment than under 15 mmol · L ^{1} HCO treatment. With increasing concentration, all growth indices of both species differed significantly from the control group ($P < 0.05$). Between different tree species at the same HCO concentration, Broussonetia papyrifera showed significantly higher growth indices than Morus alba under 15 mmol · L ^{1} HCO treatment ($P < 0.05$), indicating superior HCO stress resistance and stronger adaptability in Broussonetia papyrifera.

Table 1 Growth indices of Morus alba and Broussonetia papyrifera after 8 days of HCO treatments

Species	Treatment	ΔH (cm)	ΔDb (mm)	ΔN (n)	ΔN (n)
Morus alba	Control	6.80±0.54Aa	0.59±0.03Ba	2.67±0.15Aa	2.33±0.58Aa
Morus alba	15 mmol · L ^{1}	1.81±0.17Bb	0.21±0.05Bb	1.33±0.58Ba	0.67±0.58Bb
Morus alba	30 mmol · L ^{1}	1.73±0.13Bb	0.22±0.02Ab	0.33±0.58Bb	0.00±0.00Bc
Broussonetia papyrifera	Control	5.87±0.42Ba	0.74±0.02Aa	2.67±0.58Aa	2.00±0.58Ba
Broussonetia papyrifera	15 mmol · L ^{1}	3.43±0.13Ab	0.57±0.04Ab	2.33±0.58Aa	1.33±3.58Aa
Broussonetia papyrifera	30 mmol · L ^{1}	1.84±0.26Ac	0.21±0.03Ac	1.33±3.58Ab	0.33±3.58Ab

Note: Data are mean ± standard deviation. Different lowercase letters indicate significant differences among different HCO concentrations for the same species ($P < 0.05$). Different capital letters indicate significant differences between species at the same HCO concentration ($P < 0.05$). The same below.

2.2 Effects of Bicarbonate Treatment on Photosynthetic Characteristics of Broussonetia papyrifera and Morus alba

As shown in Table 2, for the same tree species under different HCO concentrations, all photosynthetic indices of both species were more strongly inhibited under 30 mmol · L ^{1} HCO treatment than under 15 mmol · L ^{1} HCO treatment. With increasing concentration, Pn, gs, and E of both species decreased significantly. Ci decreased and Ls increased in Morus alba, while Ci first decreased then increased and Ls first increased then decreased in Broussonetia papyrifera.

All photosynthetic indices under 30 mmol · L ¹ HCO treatment differed significantly from the control group ($P < 0.05$). Between different tree species at the same HCO concentration, Broussonetia papyrifera showed significantly higher photosynthetic indices than Morus alba under 15 mmol · L ¹ HCO treatment ($P < 0.05$), with less photosynthetic inhibition in Broussonetia papyrifera.

Table 2 Photosynthetic parameters of Morus alba and Broussonetia papyrifera after 8 days of HCO treatments

Species	Treatment	Pn (mol · m ² · s ¹)	gs (mol · m ² · s ¹)	E (mol · m ² · s ¹)	Ci (mol · mol ¹)	Ls (mol · mol ¹)
Morus alba	Control	7.06±0.54Aa	0.16±0.01Aa	3.25±0.35Aa	472.73±19.58Aa	8.21±0.02Aa
Morus alba	15 mmol· L ¹	2.26±0.15Bb	0.06±0.01Ab	0.99±0.10Ab	372.18±13.79Bab	1.38±0.02Bab
Morus alba	30 mmol· L ¹	1.26±0.10Ac	0.02±0.00Ab	0.72±0.05Ab	296.25±18.65Bb	1.19±0.02Bb
Broussonetia papyrifera	Control	8.08±0.25Aa	0.23±0.03Aa	3.46±0.28Aa	549.38±31.01Aa	1.09±0.01Aa
Broussonetia papyrifera	15 mmol· L ¹	4.67±0.15Ab	0.12±0.01Ab	1.78±0.10Ab	431.58±15.76Ab	6.28±0.03Ab
Broussonetia papyrifera	30 mmol· L ¹	1.73±0.12Ac	0.02±0.00Ac	0.58±0.02Ac	469.69±19.23Aa	3.21±0.02Bb

2.3 Antioxidant Enzyme Activities of Broussonetia papyrifera and Morus alba Under Bicarbonate Treatment

As shown in Figure 1, for the same tree species under different HCO concentrations, SOD activity in both species first increased then decreased with increasing treatment concentration and duration. The inhibitory effect of 30 mmol · L ¹ HCO treatment on SOD activity was significantly stronger than that of 15 mmol · L ¹ HCO treatment ($P < 0.05$). Under 15 mmol · L ¹ HCO treatment, SOD activity in Broussonetia papyrifera leaves gradually increased, reaching maximum on day 8, while SOD activity in Morus alba leaves peaked on day 6 then declined. Under 30 mmol · L ¹ HCO treatment, SOD activity in Broussonetia papyrifera leaves was highest on day 2, while Morus alba leaves showed peak SOD activity on day 4, after which both species declined. SOD activity in Broussonetia papyrifera leaves was significantly higher than in Morus alba ($P < 0.05$).

Figure 1 Activities of SOD in Broussonetia papyrifera and Morus alba leaves

under 0 mmol · L ¹ HCO , 15 mmol · L ¹ HCO , and 30 mmol · L ¹ HCO treatments.

As shown in Figure 2, for the same tree species under different HCO concentrations, POD activity in both species first increased then decreased with increasing treatment concentration and duration. The inhibitory effect of 30 mmol · L ¹ HCO treatment on POD activity in Morus alba leaves was significantly stronger than that of 15 mmol · L ¹ HCO treatment ($P < 0.05$). Under 15 mmol · L ¹ HCO treatment, POD activity in Broussonetia papyrifera leaves increased continuously from day 2 to day 8, while Morus alba leaf POD activity first increased, then decreased, then increased again. Under 30 mmol · L ¹ HCO treatment, POD activity in Broussonetia papyrifera leaves increased continuously from day 2 to day 6, reaching maximum values, then declined. In contrast, Morus alba leaf POD activity peaked on day 4, then decreased significantly. POD activities between the two species showed significant differences ($P < 0.05$).

Figure 2 Activities of POD in Broussonetia papyrifera and Morus alba leaves under 0 mmol · L ¹ HCO , 15 mmol · L ¹ HCO , and 30 mmol · L ¹ HCO treatments.

Figure 3 indicates that for the same tree species under different HCO concentrations, CAT activity in both species first increased significantly then decreased with increasing treatment concentration and duration. The inhibitory effect of 30 mmol · L ¹ HCO treatment on CAT activity in Morus alba leaves was significantly stronger than that of 15 mmol · L ¹ HCO treatment ($P < 0.05$). Under 15 mmol · L ¹ HCO treatment, CAT activity in both species peaked on day 6. Under 30 mmol · L ¹ HCO treatment, CAT activity in Broussonetia papyrifera leaves increased continuously from day 2 to day 6, reaching maximum values, while Morus alba leaf CAT activity peaked on day 4 then decreased significantly. CAT activities between the two species showed significant differences ($P < 0.05$).

Figure 3 Activities of CAT in Broussonetia papyrifera and Morus alba leaves under 0 mmol · L ¹ HCO , 15 mmol · L ¹ HCO , and 30 mmol · L ¹ HCO treatments.

2.4 Osmotic Regulation Substance Content of Broussonetia papyrifera and Morus alba Under Bicarbonate Treatment

As shown in Figure 4, for the same tree species under different HCO concentrations, proline content in leaves of both species first increased significantly then decreased with increasing treatment concentration and duration. The inhibitory effect of 30 mmol · L ¹ HCO treatment on proline content was significantly stronger than that of 15 mmol · L ¹ HCO treatment ($P < 0.05$). Under 15 mmol · L ¹ HCO treatment, proline content in Broussonetia papyrifera leaves increased continuously from day 2 to day 6 then remained stable, while proline content in Morus alba leaves peaked on day 4 then decreased significantly. Under 30 mmol · L ¹ HCO treatment, proline content in Broussonetia papyrifera

leaves increased continuously from day 2 to day 6, reaching maximum values, while Morus alba leaf proline content decreased continuously from day 2 to day 8. Proline content between the two species showed significant differences ($P < 0.05$).

Figure 4 Proline content in Broussonetia papyrifera and Morus alba leaves under 0 mmol · L ^{1} HCO , 15 mmol · L ^{1} HCO , and 30 mmol · L ^{1} HCO treatments.

As shown in Figure 5, for the same tree species under different HCO concentrations, soluble sugar content in leaves of both species increased slightly then decreased significantly with increasing treatment concentration and duration. Both HCO treatments showed significantly stronger inhibitory effects on soluble sugar content than the control ($P < 0.05$). Under 15 mmol · L ^{1} HCO treatment, soluble sugar content in Broussonetia papyrifera leaves increased to approximately 120 mg · g ^{1} on day 4 then remained relatively stable, while Morus alba leaf soluble sugar content began to decrease after day 4. Under 30 mmol · L ^{1} HCO treatment, soluble sugar content in Broussonetia papyrifera leaves reached maximum on day 4 then decreased slowly, while Morus alba leaf soluble sugar content decreased continuously from day 2 to day 8.

Figure 5 Soluble sugar content in Broussonetia papyrifera and Morus alba leaves under 0 mmol · L ^{1} HCO , 15 mmol · L ^{1} HCO , and 30 mmol · L ^{1} HCO treatments.

2.5 Cell Damage in Broussonetia papyrifera and Morus alba Under Bicarbonate Treatment

As shown in Figure 6, for the same tree species under different HCO concentrations, the degree of leaf cell damage in both species was significantly higher under 30 mmol · L ^{1} HCO treatment than under 15 mmol · L ^{1} HCO treatment. With increasing treatment concentration and duration, TBARS content in leaves of both species was highest under 30 mmol · L ^{1} HCO treatment ($P < 0.05$). Between different tree species at the same HCO concentration, TBARS content in Morus alba leaves increased significantly under 15 mmol · L ^{1} HCO treatment, stabilizing at approximately 110 nmol · g ^{1} on day 4, which differed significantly from the control ($P < 0.05$). In contrast, TBARS content in Broussonetia papyrifera leaves showed no significant difference from the control ($P > 0.05$). Under 30 mmol · L ^{1} HCO treatment, TBARS content in both species increased continuously from day 2 to day 8, reaching maximum values. Morus alba consistently showed higher TBARS content than Broussonetia papyrifera, indicating greater leaf cell damage in Morus alba.

Figure 6 TBARS content in Broussonetia papyrifera and Morus alba leaves under 0 mmol · L ^{1} HCO , 15 mmol · L ^{1} HCO , and 30 mmol · L ^{1} HCO treatments.

3. Discussion

3.1 Changes in Seedling Growth of Broussonetia papyrifera and Morus alba Under Bicarbonate Treatment

Bicarbonate treatment exerts multiple effects on plants. HCO not only increases environmental pH but also affects mineral element absorption (Maria et al., 2014) and impedes water uptake through osmotic effects. Conversely, HCO is an indispensable component of the plant photosynthetic system and can serve as a photosynthetic substrate through root absorption (Terentyev & Zharmukhamedov, 2020). The effects of different bicarbonate concentrations on the same plant, or the same concentration on different plants, can be entirely distinct (Hajiboland et al., 2003).

External morphological characteristics can intuitively reflect plant tolerance to saline-alkaline stress (Lu et al., 2015). This experiment evaluated the alkali stress resistance of both species by assessing their growth indices. Results showed that 30 mmol · L ^{1} and 15 mmol · L ^{1} NaHCO treatments inhibited growth to varying degrees in both species, with a tendency toward growth cessation. However, some scholars have reported that Na concentrations at or below 30 mmol · L ^{1} promote plant growth (Anas & Vivekanandan, 2000; Liu et al., 2017), with significant inhibition occurring only when Na concentration exceeds 100 mmol · L ^{1} (Zhu, 2001; Li et al., 2009; Yan et al., 2020). The growth inhibition observed in both species following 15 mmol · L ^{1} and 30 mmol · L ^{1} NaHCO treatments in this study suggests that HCO stress, rather than Na , was responsible.

In this experiment, the 30 mmol · L ^{1} HCO treatment showed stronger inhibition of growth indices than other concentrations, likely because increasing HCO concentration raises pH, weakening root water and mineral absorption (Guo et al., 2016) and thereby affecting normal growth and development. Furthermore, under 15 mmol · L ^{1} HCO treatment, Broussonetia papyrifera demonstrated significantly better growth than Morus alba, indicating superior HCO stress resistance in Broussonetia papyrifera.

3.2 Changes in Photosynthetic Characteristics of Broussonetia papyrifera and Morus alba Under Bicarbonate Treatment

Photosynthesis provides plants with necessary materials and energy for growth, and photosynthetic rate reflects plant growth status (Greenway & Munns, 1980; Bai et al., 2008). Saline-alkaline stress affects photosynthesis and inhibits plant growth (Guo & Zhao, 2001). In this study, photosynthetic indices of both species showed that photosynthesis was inhibited to varying degrees under 30 mmol · L ^{1} and 15 mmol · L ^{1} HCO treatments, with stronger inhibition under 30 mmol · L ^{1} HCO .

Under NaHCO stress, photosynthesis inhibition may result from stomatal limitation caused by high pH-induced stomatal closure that hinders CO entry into

leaves, and non-stomatal limitation caused by direct toxic damage to photosynthetic organs from alkali stress (Lauteri, 1991). When Pn decreases with reduced Ci and increased Ls, stomatal limitation is considered the cause; when Ci increases with decreased Ls, non-stomatal limitation is indicated (Farquhar & Sharkey, 1982; Mediavilla et al., 2002). Under HCO stress in this study, Morus alba showed simultaneous decreases in Pn and Ci with increased Ls, indicating stomatal limitation as the primary factor reducing photosynthetic rate. In contrast, Broussonetia papyrifera showed decreased Pn with Ci first decreasing then increasing and Ls first increasing then decreasing, suggesting both stomatal and non-stomatal limitations affected its photosynthetic rate reduction. This demonstrates that stomatal and non-stomatal limitations on photosynthesis under HCO stress are not independent (Liu et al., 2012), and their relative contributions change with increasing HCO concentration and duration.

In this study, photosynthesis in both species decreased significantly under both HCO concentrations. Although Broussonetia papyrifera experienced both stomatal and non-stomatal limitations, its photosynthetic capacity was less inhibited than that of Morus alba. According to Wu et al. (2011), this is because Broussonetia papyrifera, as a karst-adapted species, has higher utilization capacity for rhizosphere HCO . However, when high-concentration HCO is added to the rhizosphere, Morus alba experiences severe alkali stress and hyperosmotic stress that causes further stomatal closure and reduces HCO absorption capacity by roots, leading to more severe damage to photosynthetic and membrane systems (Cirillo et al., 2019) and dramatically decreased photosynthetic assimilation capacity. Therefore, Broussonetia papyrifera' s superior HCO utilization capacity can provide more photosynthetic substrates, helping maintain its photosynthetic capacity under HCO stress.

3.3 Changes in Antioxidant Enzyme Activity, Osmotic Regulation Substance Content, and Cell Damage in Broussonetia papyrifera and Morus alba Under Bicarbonate Treatment

Under saline-alkaline stress, plant antioxidant enzyme protection and osmotic regulation coexist and cooperate (Yuan et al., 2020). Alkali stress exacerbates high pH effects, inhibiting ion absorption and disrupting ion balance (Guo et al., 2010; Javid et al., 2012; Lin et al., 2012), generating reactive oxygen species (ROS) that cause oxidative damage (Liu et al., 2008). Antioxidant enzymes such as SOD, CAT, and POD serve as biochemical selection indicators for NaHCO tolerance (Ahmad et al., 2014), effectively scavenging ROS and protecting membrane systems from oxidative damage (Chen et al., 2017; Chen et al., 2019). Soluble sugars and proline, as indicators of salt-alkaline resistance, can accumulate under stress to maintain intracellular osmotic balance and enhance cell structural stability (Smirnoff & Cumbes, 1989; Bohnert & Jensen, 1996; Zhang, 2010).

In this study, both antioxidant enzyme and osmotic regulation systems were affected by NaHCO treatment in both species. The antioxidant enzyme systems

of both species were activated during the initial stage of bicarbonate stress treatment to scavenge increased ROS in leaf cells. Under long-term 15 mmol · L ^{1} HCO treatment, SOD, POD, and CAT activities in both species could be maintained at relatively high levels. However, under 30 mmol · L ^{1} HCO treatment, antioxidant enzyme activities in both species increased then decreased, indicating that HCO stress intensity strengthened with increasing concentration, and persistent stress made it difficult for plants to maintain high antioxidant enzyme activity levels. Antioxidant enzyme activity indices showed that under 30 mmol · L ^{1} HCO treatment, POD and CAT activities in Broussonetia papyrifera leaves increased continuously from day 2 to day 6, remaining higher than the 0 mmol · L ^{1} HCO treatment. In contrast, under 30 mmol · L ^{1} HCO treatment, SOD, POD, and CAT activities in Morus alba leaves peaked on day 4, then decreased significantly from day 6, remaining lower than the 15 mmol · L ^{1} HCO treatment. Broussonetia papyrifera could maintain normal metabolic levels for longer periods under high-concentration HCO stress, indicating stronger alkali stress tolerance (Liu et al., 2006; Gao et al., 2018).

Proline and soluble sugar contents in both species under 15 mmol · L ^{1} HCO treatment showed that Broussonetia papyrifera' s proline and soluble sugar contents increased gradually and remained stable, maintaining the osmotic regulation system at relatively high levels, while Morus alba contents began to decrease from day 4, failing to maintain leaf osmotic regulation substance levels. Under 30 mmol · L ^{1} HCO treatment, osmotic regulation substance contents in both species increased then decreased. Specifically, Broussonetia papyrifera proline content under 30 mmol · L ^{1} HCO treatment increased continuously from day 2 to day 6, reaching maximum values higher than other treatments. In contrast, Morus alba proline and soluble sugar contents under 30 mmol · L ^{1} HCO treatment decreased continuously from day 2 to day 8, remaining lower than the 15 mmol · L ^{1} HCO treatment, demonstrating weaker osmotic regulation capacity in Morus alba. These results also indicate that Broussonetia papyrifera' s antioxidant enzyme and osmotic regulation systems have significantly stronger HCO stress tolerance than Morus alba.

TBARS can reflect not only cell membrane system damage but also react with intracellular proteins and nucleic acids, causing their denaturation (Smirnoff, 1993). In this study, under 30 mmol · L ^{1} HCO treatment, cell membrane systems in both species suffered severe damage with continuous deterioration. However, under 15 mmol · L ^{1} HCO treatment, both species could maintain cell damage at certain levels through antioxidant enzyme and osmotic regulation systems, with less damage in Broussonetia papyrifera leaves than in Morus alba.

In summary, HCO stress significantly inhibited photosynthetic capacity, antioxidant enzyme activity, and osmotic regulation substance content while causing leaf cell damage. The results demonstrate that Broussonetia papyrifera has superior HCO stress resistance compared to Morus alba. This study provides a scientific basis for understanding growth, photosynthetic, and stress resistance differences between Morus alba and Broussonetia papyrifera under bicarbonate

treatment, offering guidance for protecting Moraceae plants in karst environments.

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