

Article

Appropriate Sodium Bicarbonate Concentration Enhances the Intracellular Water Metabolism, Nutrient Transport and Photosynthesis Capacities of *Coix lacryma-jobi* L.

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Abstract: Karst ecological stresses are harmful to plant growth, especially high bicarbonate concentrations, drought, high pH, etc. In this study, the effects of 0, 2.0, 7.0 and 12.0 mmol L⁻¹ sodium bicarbonate concentrations on the biomass, electrophysiological properties, intracellular water metabolism, nutrient transport, photosynthesis and chlorophyll fluorescence of *Coix lacryma-jobi* L. were investigated. The results show that 2.0 mmol L⁻¹ sodium bicarbonate effectively improved the biomass formation of *Coix lacryma-jobi* L., notably increased its intrinsic capacitance (IC) and decreased its intrinsic resistance (IR), intrinsic impedance (IZ), intrinsic capacitive reactance (IXc) and intrinsic inductive reactance (IXL) as well as reliably enhanced its intracellular water metabolism, nutrient transport and photosynthetic capacities. However, 7.0 and 12.0 mmol L⁻¹ sodium bicarbonate concentrations exhibited marked inhibitory effects on the plants' photosynthetic rate, stomatal conductance, transpiration rate and dry weight, whereas they did not significantly change the intracellular water metabolism or the nutrient transport capacity of *Coix lacryma-jobi* L. This study highlights that appropriate bicarbonate levels could enhance the intracellular water metabolism, nutrient transport, photosynthesis and growth of *Coix lacryma-jobi* L., which can be rapidly monitored by the plant's electrophysiological properties. Importantly, plant electrophysiological measurement is significantly superior to photosynthesis measurement. In the future, plant electrophysiological measurement can be used as a means to quickly and effectively evaluate the physiological response of plants to the external environment.

Keywords: dissolved inorganic carbon; karst; *Coix* seed rice; electrical signal; substance transport



Citation: Li, H.; Lv, J.; Su, Y.; Wu, Y. Appropriate Sodium Bicarbonate Concentration Enhances the Intracellular Water Metabolism, Nutrient Transport and Photosynthesis Capacities of *Coix lacryma-jobi* L.. *Agronomy* **2023**, *13*, 1790. <https://doi.org/10.3390/agronomy13071790>

Academic Editor: Guanfu Fu

Received: 5 June 2023

Revised: 27 June 2023

Accepted: 30 June 2023

Published: 3 July 2023



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1. Introduction

Strong karstification leads to high bicarbonate concentrations, drought, high pH, high calcium and magnesium levels and low nutrition in karst soil environments [1]. In karst ecological systems, stable bicarbonate (HCO₃⁻) reservoirs in soil and water can be formed by the dissolution of limestone and dolomite by water, as their HCO₃⁻ concentrations are notably higher than those of non-karst areas [2]. For instance, in the present literature, it was reported that the HCO₃⁻ concentration in calcareous soils is usually 1~5 mmol L⁻¹ [3,4]; Zhang et al., showed that the HCO₃⁻ concentration in karst rivers and lakes is usually about 4.5 mmol L⁻¹ [5], and Hussner et al., reported that the HCO₃⁻ concentration was usually several times higher than that of carbon dioxide if the ambient pH value was above 7 [6]. Plants grown in karst areas often suffer from various degrees of karst ecological stresses, such as high bicarbonate levels, drought, high pH, etc. [7]. Consequently, plants' adaptive mechanisms to karst environments is a permanent research hot spot.

Previous studies have reported that karstification has many negative effects on plant growth and metabolism. It affects the growth and photosynthesis of plants by inducing HCO_3^- stress in *Broussonetia papyrifera* L. [8] and tomatoes [9] as well as through decreasing the activity of key enzymes in wheat under drought conditions [10]. Alkali stress affects the growth and physiological characteristics of melon seedlings [11]. High concentrations of HCO_3^- can reduce the uptake of Fe in plants by limiting the expression of Fe acquisition genes, leading to Fe deficiency [12,13]. Fe deficiency strongly limits the biosynthesis of chlorophyll [14], which will result in a significant decrease in photosynthesis. In fact, plants are very tenacious, and they have developed a variety of unique adaptive mechanisms to adversity [15]. Some reports show that HCO_3^- not only provides short-term carbon and water sources for plants under drought stress, but also promotes stomatal opening and restores photosynthesis [16–20]. Meanwhile, HCO_3^- can promote carbon and nitrogen metabolism by regulating the activities of key enzymes involved in carbon and nitrogen metabolism and by participating in the regulation of complex physiological processes, including carbon assimilation and nitrogen reduction in plants [15,21]. Zhou et al., reported that 3~4 mmol L⁻¹ sodium bicarbonate concentration added to cultures was conducive to growth and the improvement of carbonic anhydrase activity in macroalgae [22]. Additionally, HCO_3^- can also change the activity of glycolysis and pentose phosphate pathways via the distribution of their substrates to enhance the stress resistance of plants and obtain survival opportunities [8]. Wu et al., systematically summarized the karst-adaptive mechanisms of plants in terms of morphology, ecology, photosynthetic capacity, utilization of inorganic nutrients, carbonic anhydrase activity, biodiversity, calcium regulation root organic acid exudation, etc. [1].

Coix lacryma-jobi L., a typical karst and oldest grass crop, is rich in protein, oil and medicinal components, such as fatty acids, amino acids, triterpenes, vitamins and various minerals [23,24]. Due to its high nutritional and medical value, it is widely used in improving immunity, inhibiting tumor angiogenesis, invigorating stomach and spleen tissue and regulating blood sugar; in addition, it possesses anti-tumor, anti-convulsant and apocenosic effects [25,26]. As a promising economic crop and natural medicine, *Coix lacryma-jobi* L. is widely planted in Southwestern China, especially in Guizhou Province. Currently, the planting area and yield of *Coix lacryma-jobi* L. in China is estimated to be around 73,000 ha and 0.22 million tons, respectively [24,27]. Recently, Guizhou's *Coix lacryma-jobi* L. industry has developed rapidly, becoming the largest producer and distribution center for *Coix lacryma-jobi* L. in Southeast Asia [24]. Moreover, it has made great contributions in alleviating poverty, revitalizing rural areas and controlling rocky desertification, and its planting area reached over 48,600 ha by 2021 [27,28]. In Guizhou Province, however, *Coix lacryma-jobi* L. is mostly planted in karst soil environments, and its growth and development are influenced by many adversities such as high bicarbonate content, drought and high pH levels. Therefore, it is worth exploring whether high levels of bicarbonate can promote the growth of *Coix lacryma-jobi* L. Moreover, to date, there are no data available about the adaptive mechanisms of *Coix lacryma-jobi* L. to high bicarbonate levels.

The electrophysiological activities of plants, including charge separation, electron movement, proton and dielectric transport, etc., control almost all of their biological processes [29–33]. Alterations in plants' electrical parameters are considered to be their fastest response to environmental stresses [34–37]. Numerous findings have shown that the passive electric properties of plants, such as their capacitance (C), impedance (Z), resistance (R), capacitive reactance (Xc) and inductive reactance (XL), can be used to evaluate their physiological status [33–45]. For instance, Zhang et al. found that the theoretical relationships among the clamping force (F) and leaf Z, R, Xc or XL were revealed to be $Z, R, Xc \text{ or } XL = y + ke^{-bF}$ based on bioenergetics. Then, the intrinsic R (IR), intrinsic Z (IZ), intrinsic Xc (IXc) and intrinsic XL (IXL) of plant leaves were successfully described as $IR, IZ, IXc \text{ or } IXL = y + k$ when the clamping force is 0 ($F = 0$) [44,45]. Meanwhile, they also developed intracellular water metabolism and nutrient transport parameters based on the IR, IZ, IXc, IXL and intrinsic capacitance (IC) of plants, which accurately

revealed the intracellular water metabolism and nutrient transport strategies of plants and allowed them to be evaluated [33,43–45]. Thus, whether plants' electrophysiological properties can be faster and more effective to determine the appropriate bicarbonate level for *Coix lacryma-jobi* L. growth compared to common photosynthesis indicators is worth further attention.

In this study, the effects of sodium bicarbonate on the growth of *Coix lacryma-jobi* L. were evaluated. Moreover, the effects of sodium bicarbonate on the electrophysiological properties, intracellular water metabolism, nutrient transport, photosynthesis and chlorophyll fluorescence of *Coix lacryma-jobi* L. were also investigated. This work provides a scientific basis for rapidly screening the appropriate bicarbonate level for *Coix lacryma-jobi* L. grown in karst environments.

2. Materials and Methods

2.1. Materials

Sodium bicarbonate (AR, $\geq 99.8\%$) was provided by Rhawn Reagent Chemical Technology Co., Ltd. (Shanghai, China). All reagents were analytical grade. Three-leaf seedlings of *Coix lacryma-jobi* L. 'Yizhu 1' with similar growth were used as experimental materials. The seedlings were grown in hydroponic conditions for 14 days. The weights of the aboveground parts were as follows: 0.38 ± 0.01 (fresh); 0.04 ± 0.01 (dry). The weights of the root were as follows: 0.17 ± 0.03 (fresh); 0.02 ± 0.01 (dry). Hoagland nutrient solution contained the following (in mmol L^{-1}): 3.0 mmol KNO_3 , 0.125 mmol $\text{NH}_4\text{H}_2\text{PO}_4$, 0.1875 mmol $(\text{NH}_4)_2\text{SO}_4$, 1.0 mmol MgSO_4 , 2.0 mmol $\text{Ca}(\text{NO}_3)_2$, 2.0 μmol KCl , 25 μmol H_3BO_3 , 2 μmol MnSO_4 , 2 μmol ZnSO_4 , 0.1 μmol CuSO_4 , 0.1 μmol Na_2MoO_4 , 0.043 μmol CoCl_2 and 50 μmol Fe-EDTA .

2.2. Sodium Bicarbonate Hydroponic Experiment Using *Coix lacryma-jobi* L.

The hydroponic method was used for studying the growth effects of sodium bicarbonate on *Coix lacryma-jobi* L. The culture solution was 1/2 Hogland nutrient solution containing different sodium bicarbonate concentrations. Based on the preliminary experimental results and the sodium bicarbonate concentration ($1.2\sim 3.3$ mmol L^{-1}) in field planting soils of *Coix lacryma-jobi* L., four treatments were designed with 0 (CK), 2.0, 7.0 and 12.0 mmol L^{-1} sodium bicarbonate. Each treatment had three replicates, and each replicate contained nine plants as biological replicates. Three plants were randomly selected for each replicate. The original culture solution was replaced with fresh culture solution with a pH of 8.00 ± 0.10 each day, and the whole treatment cycle was 14 days. The culture temperature, illumination time, illumination intensity and relative humidity of *Coix lacryma-jobi* plants were $25.0 \pm 2.0/19.0 \pm 2.0$ °C (light/dark), 12 h/12 h (light/dark), 500.0 ± 25.0 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 55.4~60.6%, respectively.

2.3. Determination of Biomass Parameters, Electrophysiological and Photosynthetic Characteristics and Chlorophyll Fluorescence

The height and weight of *Coix lacryma-jobi* L. plants were measured. The plant height of *Coix lacryma-jobi* L. was measured using a ruler. The fresh weights of the aboveground parts and root were measured by weighing them, and their dry weights were determined after drying them to a constant weight. The fresh weight of a whole *Coix lacryma-jobi* L. plant was the sum of the fresh weights of its aboveground parts and roots, and its whole dry weight was the sum of the dry weights of the aboveground parts and roots.

The intracellular water metabolism and nutrient transport parameters of *Coix lacryma-jobi* L. were determined as described by Zhang et al. [33,44,45]. The fresh second fully expanded leaves of *Coix lacryma-jobi* L. were sampled after measuring their photosynthetic parameters and chlorophyll fluorescence. Leaf samples were immediately soaked in distilled water for 30 min, and then the water on the leaf surface was removed. The electrophysiological parameters of the plant leaves were determined using a LCR-6300 tester (Gwinstek, Taiwan, China) in parallel connection mode with a tested

voltage and frequency of 1.5 V and 3 kHz, respectively. The leaf was first put between the two electrodes of a self-made parallel-plate capacitor, and then the leaf's passive electric properties, including C, R and Z under different clamping forces (1.139, 2.149, 3.178, 4.212 and 5.245 N), were continuously recorded, yielding 11~13 data points for each clamping force. Different clamping forces were obtained by adding iron blocks. Subsequently, leaf Xc and XL were calculated based on Equations (1) and (2), respectively.

$$X_c = \frac{1}{2\pi f C} \quad (1)$$

$$\frac{1}{-X_L} = \frac{1}{Z} - \frac{1}{R} - \frac{1}{X_c} \quad (2)$$

where π is 3.1416 and f is frequency.

The fitting equations for the clamping force and Z, R, Xc, XL and C of the leaves were constructed as Equations (3)–(7):

$$Z = y_1 + k_1 e^{-b_1 F} \quad (3)$$

$$R = y_2 + k_2 e^{-b_2 F} \quad (4)$$

$$X_c = y_3 + k_3 e^{-b_3 F} \quad (5)$$

$$X_L = y_4 + k_4 e^{-b_4 F} \quad (6)$$

$$C = y_0 + hF \quad (7)$$

Furthermore, the real-time intrinsic Z, R, Xc, XL, C and specific effective thickness (d) of the leaves ($F = 0$ N) were calculated using the corresponding parameters from Equations (3)–(7):

$$IZ = y_1 + k_1 \quad (8)$$

$$IR = y_2 + k_2 \quad (9)$$

$$IX_c = y_3 + k_3 \quad (10)$$

$$IX_L = y_4 + k_4 \quad (11)$$

$$IC = \frac{1}{2\pi f IXC} \quad (12)$$

$$d = \frac{U^2 h}{2} \quad (13)$$

where π is 3.1416, f is frequency and U is the tested voltage.

Subsequently, the intracellular water metabolism parameters of *Coix lacryma-jobi* L. including intracellular water-holding capacity (IWHC), intracellular water use efficiency (IWUE), intracellular water-holding time (IWHT) and water transfer rate (WTR) were calculated according to Equations (14)–(17):

$$IWHC = \sqrt{(IC)^3} \quad (14)$$

$$IWUE = \frac{d}{IWHC} \quad (15)$$

$$IWHT = IC \times IZ \quad (16)$$

$$WTR = \frac{IWHC}{IWHT} \quad (17)$$

Additionally, the intracellular water metabolism parameters of *Coix lacryma-jobi* L., such as nutrient flux per unit area (UNF), active transport flow of nutrient (NAF), nutrient

transfer rate (NTR), nutrient transport capacity (NTC) and nutrient active transport capacity (NAC), were calculated according to Equations (18)–(22):

$$\text{UNF} = \frac{\text{IR}}{\text{IXc}} + \frac{\text{IR}}{\text{IXL}} \quad (18)$$

$$\text{NAF} = \frac{\text{IXc}}{\text{IXL}} \quad (19)$$

$$\text{NTR} = \text{WTR} \quad (20)$$

$$\text{NTC} = \text{UNF} \times \text{NTR} \quad (21)$$

$$\text{NAC} = \text{UAF} \times \text{NTR} \quad (22)$$

The chlorophyll content of the second fully expanded leaf of each *Coix lacryma-jobi* L. specimen was measured using a SPAD-502Plus chlorophyll meter (Konica Minolta Inc., Tokyo, Japan). The net photosynthetic rate (Pn), stomatal conductance (Gs) and transpiration rate (Tr) of the second fully expanded leaf of each *Coix lacryma-jobi* L. specimen was determined using a portable Li-6800 photosynthesis measurement system (LI-COR Inc., Lincoln, NE, USA) between 8:00 and 10:00 a.m. The measurement conditions were a flow rate of gas of 500 mmol s⁻¹, photosynthetic active radiation of 500 μmol m⁻² s⁻¹, leaf temperature of 27 °C and CO₂ concentration of 400 μmol mol⁻¹. The chlorophyll fluorescence parameters of *Coix lacryma-jobi* L. leaves were also measured using a portable Li-6800 photosynthesis measurement system after dark adaptation for 1 h. First, the initial fluorescence (Fo) and maximum fluorescence (Fm) of *Coix lacryma-jobi* L. leaves were determined under dark adaptation conditions, and the maximum photochemical efficiency (Fv/Fm) of PSII was calculated according to Equation (23). Moreover, the maximum fluorescence (Fm') and stable fluorescence (Fs) of *Coix lacryma-jobi* L. leaves were determined under light adaptation conditions, and the actual photosynthetic efficiency (Φp) of PSII was calculated according to Equation (24).

$$\frac{F_v}{F_m} = \frac{F_m - F_0}{F_m} \quad (23)$$

$$\Phi_p = \frac{F_{m'} - F_s}{F_{m'}} \quad (24)$$

2.4. Statistical Analyses

Data are displayed as means ± standard deviation (SD) of three replicates, and all analyses were carried out using SPSS 18.0 (SPSS Inc., Chicago, IL, USA). The data were subjected to analysis of variance (ANOVA) tests. The means of the different groups were compared via Tukey's test ($p < 0.05$). Small letters indicate significant differences between treatments ($n = 3$; $p < 0.05$).

3. Results

3.1. Responses of the Growth in *Coix lacryma-jobi* L. to Sodium Bicarbonate

The effects of sodium bicarbonate on the growth of *Coix lacryma-jobi* L. are shown in Figure 1. A 2.0 mmol L⁻¹ concentration of sodium bicarbonate significantly ($p < 0.05$) promoted plant growth, shoot fresh weight and plant fresh weight compared to CK. A 7.0 mmol L⁻¹ concentration of sodium bicarbonate decreased the plant height, above-ground part fresh weight and root fresh weight of *Coix lacryma-jobi* L., whereas the difference between 7.0 mmol L⁻¹ sodium bicarbonate and CK did not reach a significant level. A 12.0 mmol L⁻¹ concentration of sodium bicarbonate further reduced *Coix lacryma-jobi* L. growth compared with 7.0 mmol L⁻¹ sodium bicarbonate.

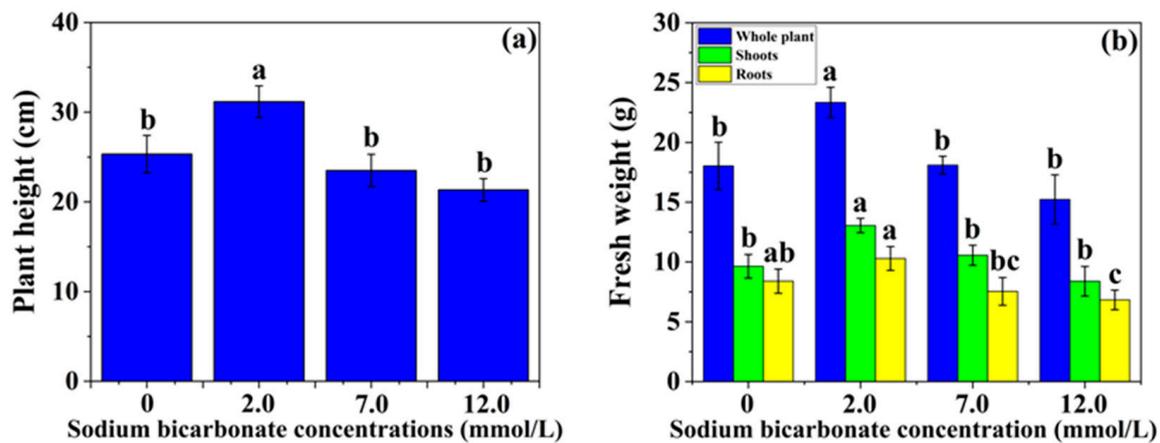


Figure 1. Effects of sodium bicarbonate on the growth of *Coix lacryma-jobi* L. Small letters indicate significant differences between treatments ($n = 3$; $p < 0.05$ according to Tukey's HSD). (a) plant height; (b) fresh weight.

3.2. Responses of Electrophysiological, Intracellular Water Metabolism and Nutrient Transport Parameters of *Coix lacryma-jobi* L. to Sodium Bicarbonate

The effects of sodium bicarbonate on the electrophysiological parameters of *Coix lacryma-jobi* L. are shown in Table 1. The plant treated with 2.0 mmol L⁻¹ sodium bicarbonate became vigorous, its IC value was higher and its IR, IZ, IXc and IXL values were lower. Sodium bicarbonate effectively increased the IC of *Coix lacryma-jobi* L. and decreased its IR, IZ, IXc and IXL. A 2.0 mmol L⁻¹ concentration of sodium bicarbonate significantly ($p < 0.05$) enhanced the IC of *Coix lacryma-jobi* L. and decreased its IR, IZ, IXc and IXL compared to CK. Although 7.0 and 12.0 mmol L⁻¹ sodium bicarbonate also decreased the IC of *Coix lacryma-jobi* L. and increased its IR, IZ, IXc and IXL, these electrophysiological parameters were not significantly ($p < 0.05$) different compared to the 0 mmol L⁻¹ sodium bicarbonate treatments. These findings emphasize that 2.0 mmol L⁻¹ sodium bicarbonate can effectively enhance the IC of *Coix lacryma-jobi* L., decrease its IR, IZ, IXc and IXL and promote its healthy growth.

Table 1. Effects of sodium bicarbonate on the electrophysiological parameters of *Coix lacryma-jobi* L.

[HCO ₃ ⁻] (mmol L ⁻¹)	IC (pF)	IR (MΩ)	IZ (MΩ)	IXc (MΩ)	IXL (MΩ)
0	80.35 ± 22.18 ^b	0.79 ± 0.39 ^a	0.52 ± 0.20 ^a	0.70 ± 0.20 ^a	1.26 ± 0.46 ^a
2.0	359.70 ± 44.70 ^a	0.07 ± 0.02 ^b	0.06 ± 0.01 ^b	0.15 ± 0.02 ^b	0.19 ± 0.03 ^b
7.0	108.20 ± 13.75 ^b	0.41 ± 0.04 ^{ab}	0.31 ± 0.02 ^{ab}	0.50 ± 0.06 ^{ab}	0.77 ± 0.04 ^{ab}
12.0	97.49 ± 36.47 ^b	0.37 ± 0.22 ^{ab}	0.32 ± 0.17 ^{ab}	0.61 ± 0.27 ^a	0.83 ± 0.41 ^{ab}

[HCO₃⁻]: NaHCO₃ concentration added to the culture medium. The data are shown as the mean ± SD. The data were subjected to analysis of variance (ANOVA) tests. The means of the different groups were compared using Tukey's test. Small letters indicate significant differences between treatments ($n = 3$; $p < 0.05$).

The effects of sodium bicarbonate on the intracellular water metabolism characteristics of *Coix lacryma-jobi* L. are shown in Table 2. A 2.0 mmol L⁻¹ concentration of sodium bicarbonate significantly ($p < 0.05$) increased the IWHC and WTR of *Coix lacryma-jobi* L. compared to the 0, 7.0 and 12.0 mmol L⁻¹ sodium bicarbonate treatments. Moreover, 2.0 mmol L⁻¹ sodium bicarbonate significantly ($p < 0.05$) decreased the IWHT of *Coix lacryma-jobi* L. compared to CK. At the same time, the d, IWUE, IWHT and WTR of *Coix lacryma-jobi* L. did not show significant ($p < 0.05$) differences between the 0, 7.0 and 12.0 mmol L⁻¹ sodium bicarbonate treatments. This indicates that there was little difference in the intracellular water-use efficiency of leaves in the 0, 7.0 and 12.0 mmol L⁻¹ sodium bicarbonate treatments. The present findings show that 2.0 mmol L⁻¹ sodium bicarbonate

could effectively improve the IWHC and WTR of *Coix lacryma-jobi* L. and promote its intracellular water metabolism.

Table 2. Effects of sodium bicarbonate on the intracellular water metabolism characteristics of *Coix lacryma-jobi* L.

[HCO ₃ ⁻] (mmol L ⁻¹)	d	IWHC	IWUE	IWHT	WTR
0	153.57 ± 99.08 ^a	734.05 ± 298.17 ^b	0.27 ± 0.28 ^a	38.52 ± 7.86 ^a	20.51 ± 12.48 ^b
2.0	407.40 ± 63.75 ^a	6848.49 ± 1263.66 ^a	0.06 ± 0.01 ^a	21.87 ± 1.96 ^b	316.87 ± 80.77 ^a
7.0	238.29 ± 176.15 ^a	1130.00 ± 217.07 ^b	0.20 ± 0.11 ^a	33.86 ± 3.24 ^{ab}	33.22 ± 3.83 ^b
12.0	181.56 ± 40.55 ^a	997.09 ± 524.67 ^b	0.22 ± 0.11 ^a	27.80 ± 4.85 ^{ab}	36.93 ± 19.39 ^b

[HCO₃⁻]: NaHCO₃ concentration added to the culture medium. The data are shown as the mean ± SD. The data were subjected to analysis of variance (ANOVA) tests. The means of the different groups were compared using Tukey's test. Small letters indicate significant differences between treatments ($n = 3$; $p < 0.05$).

The effects of sodium bicarbonate on the nutrient transport characteristics of *Coix lacryma-jobi* L. are shown in Table 3. Compared to CK, 2.0 mmol L⁻¹ sodium bicarbonate significantly ($p < 0.05$) increased the NAC of *Coix lacryma-jobi* L., significantly ($p < 0.05$) enhanced its NAF and NTC and significantly ($p < 0.05$) decreased its UNF. Compared to the 7.0 and 12.0 mmol L⁻¹ sodium bicarbonate treatments, 2.0 mmol L⁻¹ sodium bicarbonate also significantly ($p < 0.05$) increased the NAC of *Coix lacryma-jobi* L. Moreover, the UNF, NAF, NAC and NTC of *Coix lacryma-jobi* L. did not show significant ($p < 0.05$) differences between the 0, 7.0 and 12.0 mmol L⁻¹ sodium bicarbonate treatments. These findings here emphasize that 2.0 mmol L⁻¹ sodium bicarbonate could effectively improve the NAF, NAC and NTC of *Coix lacryma-jobi* L. and promote its nutrient transport processes.

Table 3. Effects of sodium bicarbonate on the nutrient transport characteristics of *Coix lacryma-jobi* L.

[HCO ₃ ⁻] (mmol L ⁻¹)	UNF	NAF	NAC	NTC
0	1.70 ± 0.55 ^a	0.58 ± 0.12 ^b	12.67 ± 10.15 ^b	36.39 ± 4.65 ^b
2.0	0.84 ± 0.08 ^b	0.77 ± 0.02 ^a	245.58 ± 70.30 ^a	43.53 ± 0.73 ^a
7.0	1.36 ± 0.20 ^{ab}	0.64 ± 0.06 ^{ab}	21.30 ± 1.70 ^b	39.13 ± 2.03 ^{ab}
12.0	1.03 ± 0.19 ^{ab}	0.75 ± 0.05 ^{ab}	28.07 ± 15.51 ^b	42.71 ± 1.64 ^{ab}

[HCO₃⁻]: NaHCO₃ concentration added to the culture medium. The data are shown as the mean ± SD. The data were subjected to analysis of variance (ANOVA) tests. The means of the different groups were compared using Tukey's test. Small letters indicate significant differences between treatments ($n = 3$; $p < 0.05$).

3.3. Responses of the Photosynthetic Characteristics of *Coix lacryma-jobi* L. to Sodium Bicarbonate

The effects of sodium bicarbonate on the photosynthetic characteristics of *Coix lacryma-jobi* L. are shown in Figure 2. A 2.0 mmol L⁻¹ concentration of sodium bicarbonate effectively increased the chlorophyll content of *Coix lacryma-jobi* L. compared to CK. Plants treated with 2.0 mmol L⁻¹ sodium bicarbonate possessed a superior photosynthetic rate of leaves of 20.86 $\mu\text{mol}\cdot\text{CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, which was significantly ($p < 0.05$) higher than that of the plants treated with 0, 7.0 and 12.0 mmol L⁻¹ sodium bicarbonate (1.15-, 1.19- and 1.8-fold higher, respectively). Compared to CK, 7.0 mmol L⁻¹ sodium bicarbonate significantly ($p < 0.05$) increased the chlorophyll content of *Coix lacryma-jobi* L. while significantly ($p < 0.05$) decreasing its stomatal conductance and transpiration rate; 12.0 mmol L⁻¹ sodium bicarbonate significantly ($p < 0.05$) decreased its photosynthetic rate, stomatal conductance and transpiration rate. These results indicate that 2.0 mmol L⁻¹ sodium bicarbonate can enhance the photosynthesis of *Coix lacryma-jobi* L. plants and promote their growth, and that 7.0–12.0 mmol L⁻¹ sodium bicarbonate inhibits the growth of *Coix lacryma-jobi* L.

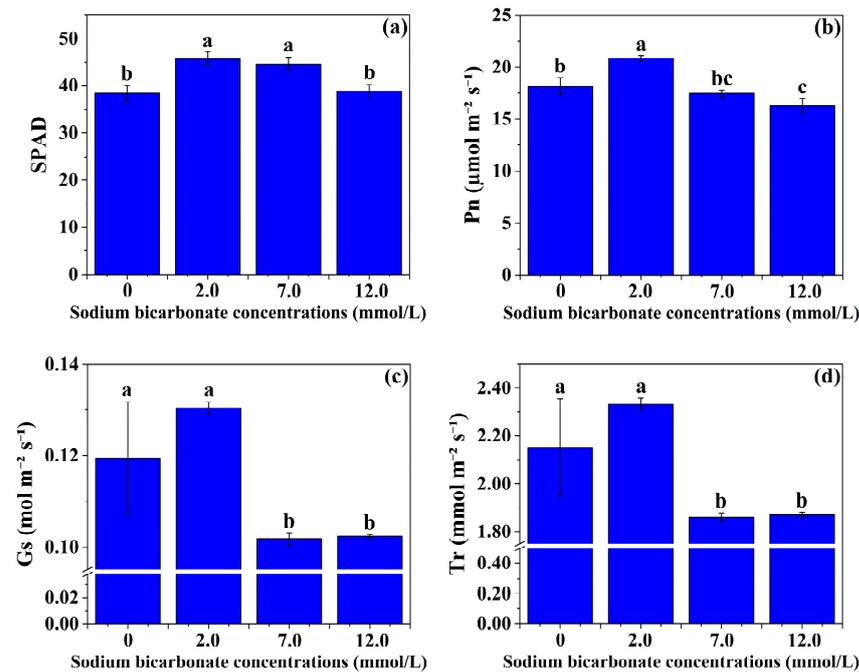


Figure 2. Effects of sodium bicarbonate on the chlorophyll content (SPAD), net photosynthetic rate (Pn), stomatal conductance (Gs) and transpiration rate (Tr) of *Coix lacryma-jobi* L. Small letters indicate significant differences between treatments ($n = 3$; $p < 0.05$ according to Tukey's HSD). (a) the chlorophyll content (SPAD); (b) net photosynthetic rate (Pn); (c) stomatal conductance (Sc); (d) transpiration rate (Tr).

3.4. Responses of the Chlorophyll Fluorescence of *Coix lacryma-jobi* L. to Sodium Bicarbonate

The effects of sodium bicarbonate on the chlorophyll fluorescence characteristics of *Coix lacryma-jobi* L. are shown in Figure 3. There were no significant ($p < 0.05$) differences in the Fv/Fm of *Coix lacryma-jobi* L. between the 0, 2.0, 7.0 and 12.0 mmol L^{-1} sodium bicarbonate treatments. A 2.0 mmol L^{-1} concentration of sodium bicarbonate effectively increased the Φ_p of *Coix lacryma-jobi* L. compared to CK, although the difference was not significant. Concentrations of 7.0 and 12.0 mmol L^{-1} decreased the Φ_p of *Coix lacryma-jobi* L. compared with 0 mmol L^{-1} sodium bicarbonate, and there was a significant ($p < 0.05$) difference between the 0 and 12.0 mmol L^{-1} sodium bicarbonate treatments. The present findings show that 2.0 mmol L^{-1} sodium bicarbonate can increase the Φ_p of *Coix lacryma-jobi* L., thereby enhancing its photosynthetic capacity.

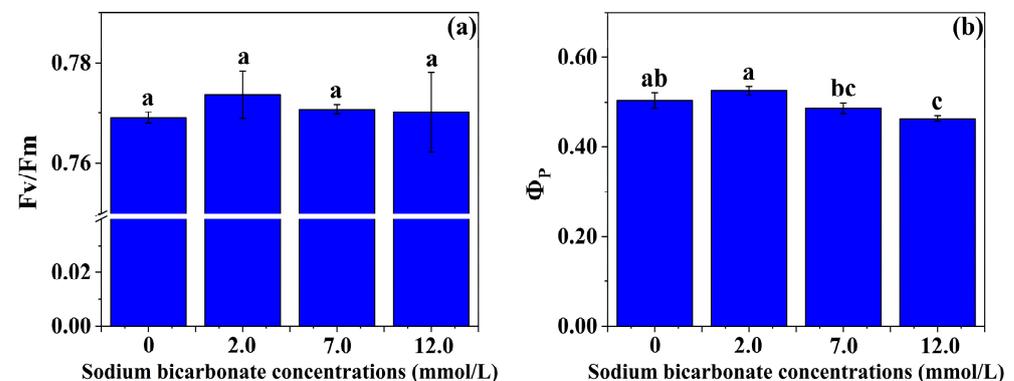


Figure 3. Effects of sodium bicarbonate on the maximum photochemical efficiency (Fv/Fm) and actual photosynthetic efficiency (Φ_p) of PSII in *Coix lacryma-jobi* L. Small letters indicate significant differences between treatments ($n = 3$; $p < 0.05$ according to Tukey's HSD). (a) the maximum photochemical efficiency (Fv/Fm); (b) actual photosynthetic efficiency (Φ_p) of PSII.

4. Discussion

Sodium bicarbonate can not only increase the pH of the environment, but it also affects plants' absorption of water and mineral elements [46]. At the appropriate concentrations of sodium bicarbonate, the positive effect is greater than the negative effect, such as being conducive to carbon assimilation, stimulating stomatal opening and improving drought resistance, etc. In contrast, at excessively high levels of sodium bicarbonate, it can bring anionic toxicity, causing effects such as physiological drought, stomata closure, reducing metabolite synthesis and transport rates, etc. It has been reported that high concentrations of HCO_3^- can reduce the uptake of Fe in plants by limiting the expression of Fe acquisition genes, leading to Fe deficiency [12]. Generally, salt stress occurs when the concentration of sodium is in the range of 25–200 mM [47,48]. Hence, the *Coix lacryma-jobi* L. seedlings did not suffer from salt stress because the concentration of sodium bicarbonate was far below 25 mM in this study. The results here demonstrate that 2.0 mmol L⁻¹ sodium bicarbonate can effectively enhance the growth of *Coix lacryma-jobi* L., which is probably derived from the long-term domestication of *Coix lacryma-jobi* L. with the appropriate sodium bicarbonate concentration. With increasing HCO_3^- concentrations, the pH of the culture solution increased gradually, which led to a reduction of the absorption of water and inorganic salts by plant roots, thereby affecting the plants' normal growth and development [12,49]. Rao and Wu found similar results in other plant species (*Camptotheca acuminata* seedlings) [15]. The water potential of plant leaves decreased gradually with the increase in sodium bicarbonate concentration, and the transpiration rate of plants under bicarbonate stress decreased significantly. These effects could explain how the 12.0 mmol L⁻¹ sodium bicarbonate treatment on *Coix lacryma-jobi* L. affected its growth. We also found that the sodium bicarbonate concentration in the soils of Guizhou's *Coix lacryma-jobi* L. planting areas was about 1.2~3.3 mmol L⁻¹.

Plants' physiological activities are vigorous in the healthy state, and its cells store more charge (ions, ion groups and electric dipoles), which can be considered as a generalized charging phenomenon [33]. Thus, the stronger the growth of plants, the higher its charge and C value and the lower its R, Z, Xc and XL values. The findings here emphasize that 2.0 mmol L⁻¹ sodium bicarbonate can effectively enhance the IC of *Coix lacryma-jobi* L., decrease its IR, IZ, IXc and IXL and promote its healthy growth, whereas these electrophysiological information parameters were not significantly ($p < 0.05$) different between the 0, 7.0 and 12.0 mmol L⁻¹ sodium bicarbonate treatments. These results demonstrate that the electrical parameters of *Coix lacryma-jobi* L. rapidly responded to bicarbonate stress, and the appropriate bicarbonate concentration can enhance the growth of *Coix lacryma-jobi* L.

The intracellular water metabolism and nutrient transport statuses of plants strongly reflects their growth and development. Zhang et al. reported that *Broussonetia papyrifera* plants grown in agricultural soils had higher IC, d, IWHC, WTR (or NTR), NTC and water content values and lower IZ and IXc values than those grown in moderately rocky, desertified soils [44,45]. The present findings show that 2.0 mmol L⁻¹ sodium bicarbonate can effectively improve the IWHC, WTR (or NTR), NAF, NAC and NTC values of *Coix lacryma-jobi* L. and promote its intracellular water metabolism and nutrient transport. Although the d, IWUE, IWHT, WTR (or NTR), IWUE UNF, NAF, NAC and NTC values of *Coix lacryma-jobi* L. were not significantly ($p < 0.05$) different between the 0, 7.0 and 12.0 mmol L⁻¹ sodium bicarbonate treatments, the 7.0 and 12.0 mmol L⁻¹ sodium bicarbonate treatments can improve the IWHC, WTR (or NTR), NAF, NAC and NTC of *Coix lacryma-jobi* L. Zhang et al. also reported four nutrient transport strategies in the tested plants: (1) low UNF, high NTR and high NTC (e.g., *Broussonetia papyrifera* grown in agricultural soils, *Ipomoea batatas* L., *Solanum tuberosum* L.); (2) high UNF, low NTR and low NTC (e.g., *Broussonetia papyrifera* grown in moderately rocky desertified soils, *Senecio scandens* Buch.-Ham. ex D., *Capsicum annuum* L.); (3) high UNF, low NTR and high NTC (e.g., *Rhus chinensis* Mill.); (4) low UNF, high NTR and low NTC (e.g., *Toona sinensis*) [45]. In this study, three nutrient transport strategies were found: (1) high UNF, low NTR and low NTC (0 mmol L⁻¹); (2) low UNF, high NTR and high NTC (2.0 mmol L⁻¹); (3) high UNF, low NTR and high

NTC (7.0 and 12.0 mmol L⁻¹). From this study, we can conclude that plant leaves with high intracellular water-holding capacity (IWHC) have a faster water transfer rate (WTR). These results also show that the appropriate bicarbonate concentration can improve the intracellular water metabolism and nutrient transport of *Coix lacryma-jobi* L., and that *Coix lacryma-jobi* L. can adapt to bicarbonate environments by improving its intracellular water metabolism and nutrient transport capacities. In addition, the inorganic carbon metabolism of *Coix lacryma-jobi* L., which is dependent on both atmospheric and soil inorganic carbon, can be captured by measuring the plant's electrophysiological parameters.

Shahsavandia et al., found that high HCO₃⁻ concentrations can reduce the chloroplast content of plants by affecting their reduced iron ion amounts [49]. Li et al., reported that high HCO₃⁻ concentration is a type of abiotic stress, which could affect the water use of plants and thereby inhibit their photosynthetic capacity [50]. Rao and Wu found that the water potential of plant leaves decreased gradually with the increase in sodium bicarbonate concentration, and the transpiration rate of plants under sodium bicarbonate stress decreased significantly [15]. In this work, 7.0 and 12.0 mmol L⁻¹ sodium bicarbonate had inhibitory effects on the chlorophyll content, photosynthetic rate, stomatal conductance and transpiration rate of *Coix lacryma-jobi* L. The intensification of osmotic stress and the decrease in transpiration rate caused by high HCO₃⁻ concentrations might be the main reasons for the decrease in photosynthetic capacity of *Coix lacryma-jobi* L. Nevertheless, plants treated with 2.0 mmol L⁻¹ sodium bicarbonate possessed a superior photosynthetic rate of 20.86 μmol·CO₂·m⁻²·s⁻¹, which was significantly ($p < 0.05$) higher than those of plants that received the 0, 7.0 and 12.0 mmol L⁻¹ sodium bicarbonate treatments. These positive effects were probably related to the utilization of HCO₃⁻ from the soils by *Coix lacryma-jobi* L.; this HCO₃⁻ is kept in the reaction center of PSII, and it plays an important role in the transfer of electrons in photosynthesis [1,8,15,51–53]. Our previous work has shown that in karst environments, plants can alternatively use bicarbonate from soil and atmospheric carbon dioxide, and when plants encounter some adversity, their stomatal conductance decreases, carbonic anhydrase activity increases, and their use of bicarbonate also increases, thereby improving their intercellular water status, which in turn increases the use of atmospheric carbon dioxide by these plants [1,15,16,20]. Bicarbonates can stimulate plants to release oxygen, which is called the bicarbonate effect [54]. Recently, Wu proposed that sodium bicarbonate may directly participate in photosynthetic O₂ evolution, resulting in the chemical equilibria, HCO₃⁻ + H⁺ → 1/2O₂ + 2e⁻ + 2H⁺ + CO₂, which provides electrons and concentrating CO₂ in the Calvin cycle, which is involved in photosynthetic carbon assimilation [51,53].

There are many studies on the impact of plants' photosynthetic characteristics [55–57]. Giovanna et al. reported that added HCO₃⁻ is beneficial for plant growth and improving photosynthetic efficiency [58]. The present findings show that there was no significant ($p < 0.05$) difference in the Fv/Fm of *Coix lacryma-jobi* L. in the 0, 2.0, 7.0 and 12.0 mmol L⁻¹ sodium bicarbonate treatments, which indicates that sodium bicarbonate treatment does not damage the reaction center of PSII. Moreover, 2.0 mmol L⁻¹ sodium bicarbonate increased the Φ_p of PSII in *Coix lacryma-jobi* L. and thereby enhanced photosynthesis, which further demonstrates that the appropriate bicarbonate concentration can enhance the photosynthesis and growth of *Coix lacryma-jobi* L.

Plants grown in karst areas often suffer from various degrees of karst ecological stresses including high bicarbonate concentrations, drought and high pH levels [1,7]. In this work, the results show that 2.0 mmol L⁻¹ sodium bicarbonate effectively enhanced the photosynthetic rate and Φ_p of *Coix lacryma-jobi* L., notably increased its IWHC, WTR (or NTR), NAF, NAC and NTC and improved its intracellular water metabolism and nutrient transport, thereby markedly enhancing its growth and biomass formation. Meanwhile, 12.0 mmol L⁻¹ sodium bicarbonate exhibited inhibitory effects on the chlorophyll content, photosynthetic rate, stomatal conductance, transpiration rate and biomass of *Coix lacryma-jobi* L. Generally, the utilization of bicarbonate from soil by plants is difficult to measure. The results indicate that the inorganic carbon used by *Coix lacryma-jobi* L.,

which comes from both atmosphere and soil, can be studied using measurements of plants' electrophysiological parameters. This work highlights that the appropriate bicarbonate concentration can improve the intracellular water metabolism, nutrient transport, photosynthetic capacity and growth of *Coix lacryma-jobi* L., and plants' electrophysiological parameters can be used for determining the appropriate bicarbonate level.

5. Conclusions

In conclusion, the present study indicates that 2.0 mmol L⁻¹ sodium bicarbonate effectively improves the biomass formation of *Coix lacryma-jobi* L. and notably increases its IC and decrease its IR, IZ, IXc and IXL as well as reliably enhances its intracellular water metabolism, nutrient transport and photosynthesis capacity. Moreover, 7.0 and 12.0 mmol L⁻¹ sodium bicarbonate exhibited inhibitory effects on the chlorophyll content, photosynthetic rate, stomatal conductance, transpiration rate and biomass of *Coix lacryma-jobi* L. This study highlights that the appropriate level of bicarbonate can enhance the intracellular water metabolism, nutrient transport, photosynthetic capacity and growth of *Coix lacryma-jobi* L., and its appropriate bicarbonate level can be rapidly obtained using plants' electrophysiological parameters.

Author Contributions: Y.S. constructed the project; Y.W. and H.L. designed the experiments; J.L. and H.L. performed the experiments; H.L. and J.L. analyzed the data; H.L., Y.W. and Y.S. wrote the paper. All authors have read and agreed to the published version of the manuscript.

Funding: This work was jointly supported by the Science and technology innovation talent project of Guizhou Province [(2016)5672], the Support Plan Projects of Science and Technology of Guizhou Province [(2021)YB453], and the Key project of Guizhou Vocational College of Agriculture [YJKT2020-9].

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: We declare that we do not have any commercial or associative interest that represent conflicts of interest in connection with the work submitted.

Abbreviations

HCO ₃ ⁻	sodium bicarbonate
C	capacitance
Z	impedance
R	resistance
F	force
d	specific effective thickness
Xc	capacitive reactance
XL	inductive reactance
IC	intrinsic capacitance
IR	intrinsic resistance
IZ	intrinsic impedance
IXc	intrinsic capacitive reactance
IXL	intrinsic inductive reactance
IWHC	intracellular water-holding capacity
IWUE	intracellular water use efficiency
IWHT	intracellular water-holding time
WTR	water transfer rate
UNF	nutrient flux per unit area
NAF	active transport flow of nutrient
NTR	nutrient transfer rate
NTC	nutrient transport capacity
NAC	nutrient active transport capacity

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