Effects of NaHCO3 Stress on Morphological Indices and Photosynthetic Parameters of Purple-Root Water Hyacinth Postprint

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Abstract

To investigate the effects of NaHCO stress on morphological indicators and photosynthetic parameters of purple-root water hyacinth, and to provide theoretical support for the application of purple-root water hyacinth in alkaline water bodies and the amelioration of water salinization, this study used purple-root water hyacinth as experimental material and treated mature plants with different concentrations of alkaline salt NaHCO solution. Morphological indicators including plant height, root length, root-to-shoot ratio, biomass, water content, and photosynthetic parameters (net photosynthetic rate (Pn), intercellular CO concentration (Ci), transpiration rate (Tr), stomatal conductance (Gs)) were measured under NaHCO stress. The results showed that the pH value of the aqueous solution was most stable at a NaHCO concentration of 20 mmol·L ^{1}; in low-concentration NaHCO solutions (40 mmol·L ^{1}), morphological indicators of purple-root water hyacinth exhibited increases or showed no significant effects compared to CK, whereas in high-concentration NaHCO solutions (60 mmol·L ^{1}), morphological indicators decreased significantly with increasing NaHCO concentration, showing a negative correlation with NaHCO concentration. NaHCO stress significantly affected photosynthetic parameters of purple-root water hyacinth: with increasing NaHCO concentration and prolonged experimental treatment, Pn showed a continuous decreasing trend, while Ci, Tr, and Gs showed overall increasing trends, indicating that photosynthesis was primarily limited by non-stomatal factors. Comprehensive analysis revealed that purple-root water hyacinth possesses certain NaHCO tolerance and can survive normally in water bodies with NaHCO concentrations not exceeding 40 mmol·L ^{1}, and can ameliorate water pH values at low NaHCO concentrations.

Full Text

Effects of NaHCO Stress on Morphological Indices and Photosynthetic Parameters of Purple Root Water Hyacinth

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Abstract

This study investigated the effects of NaHCO stress on morphological indices and photosynthetic parameters of purple root water hyacinth (*Eichhornia crassipes*) to provide theoretical support for its application in alkaline water bodies and remediation of water salinization. Adult purple root water hyacinth plants were treated with different concentrations of alkaline salt NaHCO solution, and morphological indicators (plant height, root length, root-shoot ratio, biomass, water content) and photosynthetic parameters (net photosynthetic rate (Pn), intercellular CO concentration (Ci), transpiration rate (Tr), stomatal conductance (Gs)) were measured under NaHCO stress. The results showed that the pH value of the solution was most stable at 20 mmol · L 1 NaHCO concentration. At low NaHCO concentrations (40 mmol · L 1), morphological indices exhibited growth or no significant difference compared to the control (CK), whereas at high concentrations (60 mmol · L 1), morphological indices decreased significantly with increasing NaHCO concentration, showing a negative correlation. NaHCO stress significantly affected photosynthetic parameters: Pn showed a continuous decreasing trend with increasing NaHCO concentration and treatment duration, while Ci, Tr, and Gs generally increased, indicating that photosynthesis was primarily limited by non-stomatal factors. Comprehensive analysis revealed that purple root water hyacinth possesses certain NaHCO tolerance and can survive normally in water bodies with NaHCO concentrations not exceeding 40 mmol · L 1, while also improving water pH under low NaHCO concentrations.

Keywords: NaHCO stress, purple root water hyacinth, morphological indices, photosynthetic parameter

Soil salinization has become a worldwide environmental problem. Different saline-alkali soils contain various salt types, mainly including NaSO , NaCl, Na CO , NaHCO , and calcium and magnesium salts, with soda saline-alkali soils predominantly composed of Na CO and NaHCO (Shi, 2011). The western Songliao Plain is the most concentrated distribution area of saline soil in China, where Na accounts for 71.18% of total cations and HCO accounts for 74.27% of total anions in the surface soil (Wu, 2012). Salinization is also a critical factor affecting and controlling aquatic environments, attracting significant global attention in arid and semi-arid regions (Sunil Mehta et al., 2000). According to the water-salt migration patterns of saline-alkali soils, although water mineralization varies, the main ions are Na and HCO (Xu, 2008). Research indicates that groundwater salinization depends not only on evaporation but primarily on water-soil interactions (Zhang et al., 2005). Introducing, screening, and cultivating plants tolerant to saline-alkali water bodies to restore aquatic vegetation and reduce water salinization can improve soil physicochemical properties through feedback mechanisms, achieving the dual benefits of environmental improvement and soil remediation. Therefore, selecting and cultivating plant varieties capable of purifying water bodies and tolerating alkaline salt NaHCO represents a promising biological approach to control water salinization.

Purple root water hyacinth, officially named "giant purple root with small petiole water hyacinth," is a new germplasm material developed by the Yunnan Ecological Agriculture Research Institute from common water hyacinth using Gene Phenotype Induction and Regulation Expression (GPIT) technology (Huang, 2015). It offers stronger water purification capabilities than common water hyacinth while overcoming the latter' s problems of excessive growth, easy decay, and high oxygen consumption (Huang, 2015). In recent years, purple root water hyacinth has been increasingly applied in aquatic ecological restoration, demonstrating advantages such as efficient and rapid water environment remediation without causing secondary pollution (Chen, 2018; Chen et al., 2016; Lu, 2016; Zhan et al., 2014).

Global saline-alkali land area is approximately 1 billion hectares, with China accounting for about 34.6 million hectares (Xie and Yang, 2003), mainly distributed in coastal regions of southeast China, the Songnen Plain in northeast China, and northwest China (Chai, 2013). Consequently, river water ecological environments in these saline soil distribution areas have suffered varying degrees of alkali damage through salinization. This study leverages the strong water purification capacity and adaptability of purple root water hyacinth to investigate its growth under NaHCO stress, providing a theoretical foundation for planting in alkaline water bodies, improving water salinization, and regulating water-soil balance.

Materials and Methods

In May 2018, purple root water hyacinth plants purchased from the Jinhua Aquatic Plant Base in Zhejiang were cleaned of surface soil and weeds. After removing severely damaged and decayed leaves, they were placed in 24 plastic aquatic planting boxes (66 cm × 45 cm × 47 cm) filled two-thirds with clean water. Following a 7-day acclimation period, healthy and uniform plants were selected for stress treatments. Six NaHCO concentration gradients were established: 0 $\text{mmol}\cdot\text{L}^{1}$ (control CK), 20, 40, 60, 80, and 100 $\text{mmol}\cdot\text{L}^{1}$, with three replicates per concentration and 30 plants per replicate. The experiment was conducted at the nursery base of Northeast Forestry University under consistent temperature, humidity, and light conditions, with plants placed in uniformly sized aquatic boxes. Solution pH was monitored daily at 17:00 Beijing time. Every three days, three plants from each NaHCO concentration were photographed individually to observe growth, and another three plants were selected for measurement of morphological indices and photosynthetic parameters. Normal pest and disease management was performed during the experimental period.

Plant Height and Root Length Measurement

On days 3, 6, 9, 12, 15, and 18 of the stress treatment, three plants from each NaHCO concentration were selected and transported to the laboratory in a foam box with ice packs. After washing the roots and rinsing with distilled water, surface moisture was absorbed with filter paper. Plant height and root length were measured with a steel ruler. The relative effect of NaHCO on plant height or root growth was calculated based on Sun et al. (2017) with modifications: Relative effect on plant height = (plant height under stress - plant height under non-stress) / plant height under non-stress × 100%; Relative effect on root length = (root length under stress - root length under non-stress) / root length under non-stress × 100%.

Root-Shoot Ratio and Water Content Measurement

After measuring plant height and root length, plants were separated by concentration and weighed for above-water and below-water fresh weight, then packaged and placed in an oven at 105°C for 30 minutes, followed by drying at 70°C to constant weight for dry weight measurement. Total biomass = above-water biomass + below-water biomass (Han et al., 2019); Root-shoot ratio = below-water biomass / above-water biomass (Han et al., 2019); Leaf water content = (above-water fresh weight - above-water dry weight) / above-water fresh weight × 100% (Wu et al., 2019); Root water content = (below-water fresh weight - below-water dry weight) / below-water fresh weight × 100%. The NaHCO tolerance coefficient ( ) was calculated based on Li and Wang (2010) with modifications: = average measured value under different concentrations / control measured value.

Leaf Photosynthetic Parameter Measurement

A LI-6400 portable photosynthesis system was used to measure the light response curve of CK on day 2 using a closed gas circuit with a CO cylinder to maintain gas balance. The LI-6400 red-blue light source simulated light intensities of 1,800, 1,600, 1,400, 1,200, 1,000, 800, 600, 400, 200, 100, 50, 25, and 0 mol · m ^{2} · s ^{1}, with the instrument automatically recording Pn, Ci, Tr, and Gs to determine the optimal light intensity for purple root water hyacinth. Subsequently, on days 3, 6, 9, 12, 15, and 18 of the experiment, measurements were taken between 09:00-11:30 Beijing time using the LI-6400 open gas circuit at atmospheric CO concentration of 400-420 mmol · mol ^{1} under the optimal light intensity. Three plants were randomly selected from each NaHCO treatment, and one functional leaf (the third leaf from the top) with similar growth and light exposure was chosen from each plant, with three measurements per leaf averaged.

Data Processing

SPSS statistical software was used for analysis of variance, with Duncan' s method for analyzing differences between data groups. Excel software was used for data statistics and chart preparation.

Results

Effects of NaHCO Stress on Plant Height and Root Growth

As shown in Table 1 , at 20 mmol·L^{1} NaHCO concentration, plant height increased by 13.36% and root length increased by 31.97% compared to CK. Root length also increased by 46.10% and 15.24% at 40 and 60 mmol·L^{1} concentrations, respectively, indicating that 20 mmol·L^{1} promoted plant height growth while 20, 40, and 60 mmol·L^{1} promoted root growth. At 40, 60, 80, and 100 mmol·L^{1} concentrations, the relative effect on plant height was negative and continuously decreasing, indicating inhibition of plant height growth by 7.66%, 42.04%, 52.87%, and 53.51% compared to CK, respectively. At 80 and 100 mmol·L^{1} concentrations, the relative effect on root growth was negative, indicating inhibition of root growth by 14.13% and 15.24% compared to CK. With increasing NaHCO stress duration, average plant height showed an initial increase followed by a decrease due to leaf wilting and shedding after prolonged stress. Average root length generally decreased over time because old roots initially shed, new roots grew, and then roots continued to shed.

Effects of NaHCO Stress on Root-Shoot Ratio and Water Content

As shown in Table 2 , at NaHCO concentrations of 20 and 40 mmol·L^{1}, the NaHCO tolerance coefficients ( ) for total biomass, root-shoot ratio, leaf water content, and root water content were all greater than or equal to CK, increasing by 0.99, 0.33, 0.01, 0.00 and 0.90, 0.13, 0.01, 0.00, respectively. At 60, 80, and 100 mmol·L^{1}, these coefficients were all lower than CK and continued to decrease, demonstrating a negative correlation between the NaHCO tolerance coefficients and NaHCO concentration. With increasing treatment duration, total biomass and root-shoot ratio followed the same trend as root growth, while leaf and root water content followed the same trend as plant height growth, consistent with the physiological characteristics of purple root water hyacinth.

Effects of NaHCO Stress on Photosynthetic Parameters

As shown in Figure 1 [Figure 1: see original paper], Pn, Ci, Tr, and Gs of purple root water hyacinth increased logarithmically with light intensity, with maximum Pn of 19.2 mol·m^{2}·s^{1} at 1,400 mol·m^{2}·s^{1} light intensity, which was selected as the optimal light intensity. Photosynthetic characteristics were then measured under this optimal light intensity at different NaHCO stress levels. SPSS analysis and Excel processing revealed significant effects of NaHCO stress on Pn, Ci, Tr, and Gs ($P<0.05$) (Figure 2 [Figure 2: see original paper]). Compared to CK, Pn under NaHCO stress was consistently lower. At 20 and 40 mmol · L^{1}, Pn initially decreased, then slowly increased, and finally dropped sharply, with maximum values on day 6 that were 23.64% and 29.70% lower than CK, respectively. At 60, 80, and 100 mmol · L^{1}, Pn continuously decreased until photosynthesis ceased, leaving only dark respiration. This indicates that purple root water hyacinth can adapt to low NaHCO concentrations, but suffers irreversible damage with prolonged stress or high concentrations.

Compared to CK, Ci, Tr, and Gs generally increased under NaHCO stress. Maximum values at 20, 40, 60, 80, and 100 mmol · L^{1} increased by 27.57%, 27.57%, 46.69%, 50.37%, 52.21% for Ci; 86.46%, 87.81%, 103.09%, 143.71%, 145.65% for Tr; and 17.83%, 57.36%, 133.73%, 173.49%, 181.84% for Gs compared to CK. The continuous increase in maximum values of Ci, Tr, and Gs with increasing NaHCO concentration indicates that photosynthesis in purple root water hyacinth was primarily limited by non-stomatal factors.

Changes in Water Solution pH Under NaHCO Stress

As shown in Figure 3 [Figure 3: see original paper], water solution pH values under different NaHCO concentrations increased substantially compared to CK and showed a roughly linear increasing trend over time. The pH increase was most gradual at 20 mmol · L^{1}, indicating significant NaHCO absorption by purple root water hyacinth and consequent improvement of water pH at this concentration.

Growth Changes of Purple Root Water Hyacinth Under NaHCO Stress

Single-plant photographs from days 9 and 18, which most intuitively demonstrate growth changes under NaHCO stress, are shown in Figures 4 [Figure 4: see original paper] and 5 [Figure 5: see original paper]. With increasing NaHCO concentration, purple root water hyacinth morphology changed significantly, with leaves gradually becoming sparse, dry, and shedding, and root quantity decreasing.

Discussion

Changes in Morphological Indices Under NaHCO Stress

Adversity stress inhibits chlorophyll synthesis, reduces photosynthetic rate, and decreases assimilate and energy supply, thereby affecting normal plant growth and development (Wang et al., 2014). Consequently, growth status and morphological phenotype are the most intuitive indicators in evaluating plant stress tolerance, with high-stress toxicity verified through reduced growth and survival rates (Wang L et al., 2013). This study found that at low NaHCO concentrations (40 mmol · L^{1}), plant height, root length, root-shoot ratio, and water content showed growth or no significant effect compared to CK, while at high concentrations (60 mmol·L ^{1}), these indices decreased significantly with increasing NaHCO concentration, showing a negative correlation. These findings are consistent with studies on NaHCO stress effects on kidney bean seedlings (Yu et al., 2018) and salt stress effects on *Astragalus mongholicus* seed germination and seedling growth (Ma et al., 2018). Additionally, with prolonged treatment duration, plant height, root length, root-shoot ratio, and water content all decreased substantially after day 12, indicating that cumulative low-concentration NaHCO stress can eventually cause damage equivalent to high-concentration stress.

Effects of NaHCO on Photosynthetic Parameters

As an emerging excellent plant for aquatic ecological restoration, purple root water hyacinth has attracted considerable attention, though few reports exist on applying its strong water purification capacity and adaptability to remediate saline-alkali water. This study investigated changes in photosynthetic parameters under NaHCO stress to explore its ecological adaptation. Alkali stress primarily causes osmotic stress, leading to photosynthesis inhibition (Zhou et al., 2014). This study found that Pn is an effective indicator of plant stress response and stress tolerance identification (Lu et al., 2017). With increasing NaHCO concentration and treatment duration, Pn continuously decreased while Ci, Tr, and Gs generally increased, consistent with findings that chlorophyll content and Pn in kidney bean leaves decreased while Ci increased with increasing saline-alkali stress (Yu et al., 2018). Xu (1997) emphasized that decreased Ci is an essential condition for identifying stomatal limitation of photosynthesis, while increased Ci is the most reliable criterion for non-stomatal limitation. This indicates that photosynthesis in purple root water hyacinth was primarily limited by non-stomatal factors.

Conclusion

This study demonstrates that purple root water hyacinth can improve water pH under low alkali concentrations, its photosynthesis is primarily limited by non-stomatal factors, and it can safely adapt to water solutions with alkali concentrations not exceeding 40 mmol·L ^{1}. However, the concentration gradient in this experiment was relatively large, particularly between 40-60 mmol·L ^{1} NaHCO , and the experimental period was relatively short. Future research could include more detailed investigations, including re-watering experiments at different concentration stages.

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