Bicarbonate used as a buffer for controlling nutrient solution pH value during the growth of hydroponic lettuce

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Abstract: Unstable pH of nutrient solution in hydroponic systems is a major obstacle to production. To simplify the management of nutrient solution pH and attenuate negative effects of pH on plant growth, this study investigated the effect of different buffer salt additions on nutrient solution pH, ion uptake, growth and photosynthesis of Yamazaki lettuce, with the equivalent concentrations of macro-element unchanged. The results indicated that the pH of nutrient solution was buffered to varying degrees by the 2 mol/L HCO_3^-, 1 mol/L HCO_3^-, and 0.33 mol/L HPO_4^{2-} treatments. The 0.33 mol/L HPO_4^{2-} treatment fluctuated most with a pH of 1.74 units, while the addition of HCO_3^- buffered nutrient solution pH was better, with higher pH values (7.2-7.7). The CK-pH control solution, 1 mol/L HCO_3^- and 0.33 mol/L HPO_4^{2-} treatments all promoted the uptake of ion and then the growth of lettuce was better in comparison with the CK-pH no adjustment treatment, while the 2 mol/L HCO_3^- treatment inhibited K uptake and reduced the availability of Fe, the shoot and root fresh weight of lettuce decreased by 21.3% and 10.1%, respectively. In addition, although the chlorophyll content and net photosynthetic rate under 1 mol/L HCO_3^- and 0.33 mol/L HPO_4^{2-} treatments were significantly lower than the CK-pH control solution treatment, there were no significant differences in leaf length and leaf number of lettuce among them, and shoot fresh weight per plant was 83.09, 82.95, and 78.92 g, respectively. In conclusion, adding 1 mol/L HCO_3^- to the nutrient solution in a single dosage not only buffered the pH of nutrient solution better but also promoted ion uptake, it had no negative effects on the growth, photosynthesis and ion uptake of hydroponic lettuce in comparison with the CK-pH control solution treatment. Therefore, 1 mol/L HCO_3^- can be used as a buffer during the growth of hydroponic lettuce and reduce frequent addition of acids or alkalis, and then simplify the management of nutrient solution for hydroponic lettuce production.

Keywords: pH buffer, nutrient solution, hydroponic lettuce, growth, ion uptake
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1 Introduction

Hydroponics production is becoming a popular method of leafy vegetable cultivation in controlled environment agriculture due to its advantages of higher crop productivity, annual stability, shorter crop growth cycles compared with soil production[1-3]. In hydroponic systems, the nutrients required for crop growth are almost solely provided by the nutrient solution. When hydroponic nutrient solutions are poorly buffered[4], the ability of plants to absorb ions and the availability of nutrient elements are both affected by changes in nutrient solution pH, thereby influencing plant growth[5,6]. Therefore, fluctuations of nutrient solution pH are a major constraint on hydroponic crop production.

The main reason for pH changes in nutrient solution is the variety of the relative concentrations of H^+ and OH^- which is affected by the selective uptake characteristics of plants for nutrients. In order to absorb anionic and cationic nutrients, the plant root system releases OH^- and H^+ into solution for replacement, respectively, which leads to changes of the solution pH over time[4]. The regulation of nutrient solution pH can be divided into two types: 1) acid-base neutralization to adjust the pH of nutrient solution to a suitable range; 2) adjustment of nutrient solution formula to stabilize the acid-base changes in the nutrient solution within a certain range[7]. Current researches on pH regulation for nutrient solutions focus on the use of various acid-base solutions, but less are known about the pH buffering properties of nutrient solutions in hydroponics.

Various inorganic acids and alkalis are commonly used in production to adjust pH value of nutrient solution, such as HNO_3, H_2SO_4, H_3PO_4, KOH, and NaOH[8,9]. However, the large volume of nutrient solution requires proportionally large amount of acid and alkali added to adjust the pH to an appropriate range. High accumulation of acid anions in nutrient solution can adversely affect plant growth. For example, nitric acid can result in the accumulation of nitrate in plants[10]. When using sulfuric acid to adjust pH, the accumulation of sulfate ions can easily cause harm to plants[11]. Phosphoric acid leads to a large enrichment of phosphate in the nutrient solution, which cannot be fully absorbed and utilized by plants, and when it is discharged as waste liquid, it pollutes water systems[12]. The mixed 3:1:1 (v/v/v) HNO_3-H_3PO_4-H_2SO_4 was used to adjust nutrient solution pH of hydroponic water Spinach (*Ipomoea aquatica*), while failed to maintain the solution pH within the optimal range for 48 h[13]. Organic acids such as acetic acid and

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citric acid can also be used as pH modifiers for nutrient solution. Chen et al.[14] found that 0.25 mL/L wood vinegar instead of nitric acid can keep the pH of nutrient solution within the optimum range for lettuce growth. Singh et al.[15] adjusted the nutrition solution pH of the hydroponic leafy vegetable every other day with acetic acid, citric acid, and phosphoric acid, and the results showed that the pH in the phosphoric acid treatment was most stable and required the least amount of product. Current research on pH control methods makes it difficult to maintain the pH stability of nutrition solution for a long time, requiring a frequent addition of acids or alkalis. Therefore, substances that can reduce frequent addition of acids or alkalis are necessary to be discovered.

The buffering of nutrient solution pH in hydroponic systems received little attention. The main compositions of nutrient solution are inorganic salts, hence the buffer solutions in inorganic and analytical chemistry can be applied to the acid-base regulation of nutrient solution. The buffer solution contains a large amount of undissociated weak acid and its conjugate base, allowing it to resist small addition of acids, bases, or dilution while maintaining its own pH essentially unchanged[16]. The substances with buffering effect are known as buffers, which are generally weak acid or base and its salt[17]. Phosphate, citrate, borate and succinate are generally used as traditional buffers[18]. 2-[N-morpholino] ethanesulfonic acid (MES) is often known as a synthetic buffer, but it is expensive[4,19,20]. De Rijck et al.[16] investigated the effect of $H_2PO_4^-$,NH_4^+ and HCO_3^- on the pH of nutrient solution and proposed that all the three ions can buffer the pH of nutrient solution. Therefore, phosphate and bicarbonate as buffers are feasible to keep the pH of nutrient solution stable in hydroponic production systems.

Lettuce (*Lactuca sativa* L.) is often used as a model plant in plant factory with artificial lighting, due to its fast growth, short production period, low energy demand, and high nutritional values[21,22]. Therefore, the lettuce was used as the material in this study, the purpose is to find a compound with buffering effect that can reduce pH fluctuation and the growth of lettuce can be comparable with daily acid-base regulation treatment by monitoring the pH change of nutrient solution, ion uptake and lettuce growth during the growth of hydroponic lettuce. The results of this study contribute to simplify the management of nutrient solution pH for hydroponic lettuce production.

2 Materials and methods

2.1 Plant materials and growth conditions

Seeds of green leaf lettuce (*Lactuca staiva* L. cv. FrillIce) were sown in sponge cubes (length (L) 25 mm×width (W) 25 mm×height (H) 25 mm) filled with deionized water, and then were put in plastic containers (L520 mm×W360 mm×H90 mm) in plant factory (China Agricultural University, Beijing, China). Seeds were sprayed with water after sowing and covered them with plastic film with holes for moisturization. The seeds were left in the dark for two days until sprouting and then treated with light-emitting diode after germination. When the second true leaves were fully expanded, they were divided in 72-cell plastic cavity trays (L540 mm×W280 mm× H45 mm). Uniform lettuce seedlings were randomly selected on the 20th day after sowing and were transplanted to hydroponic beds (L1200 mm×W900 mm×H70 mm). Each bed held 54 plants.

A Photosynthetic Photon Flux Density (PPFD) of 200 μmol/(m^2·s) with a 16 h/d photoperiod was provided by LEDs (W-18W, Beijing Lighting Valley Technology Co., Beijing, China) with a red to blue ratio (R:B ratio) of 1.8. Air temperature and the relative humidity were maintained at (22±1)°C/(18±1)°C and 70%±5%/65%±10% at photoperiod/dark period, respectively. CO_2 concentration was maintained at (800±50) μmol/mol at photoperiod and without control at dark period. Yamasaki lettuce nutrient solution was used before transplanting. Tap water were applied to irrigate lettuce seedlings at cotyledon stage. At 1-2 true leaves stage, Yamasaki lettuce nutrient solution was used at 1/2 strength which is based on a full strength nutrient solution with the half mass concentration. A full strength of Yamasaki lettuce nutrient solution was applied after the second true leaf was fully expanded.

2.2 Treatment design

Phosphate and bicarbonate can be used as buffers to keep nutrient solution pH stable in hydroponic production systems. Therefore, they were added to the Yamazaki lettuce nutrient solution to achieve five nutrient solution treatments without changing macro-element equivalent concentrations (Table 1). The solution treatments were applied for 20 d after transplanting: 2 mol/L HCO_3^-, 1 mol/L HCO_3^-, 0.33 mol/L HPO_4^{2-}, CK-pH control solution and CK-pH no adjustment, respectively. Table 2 lists the ions composition and concentration of each treatment. The electrical conductivity was about 1.0-1.2 mS/cm. The initial pH of all nutrient solution treatments was adjusted to around 6.5, and the pH of CK-pH control solution treatment was adjusted every day at 6.0-6.5 with 10% H_2SO_4/NaOH, while the pH of other four treatments was not adjusted. The nutrient solution of all treatments was not renewed during the experiment.

Table 1 Molar concentration of macro elements in each treatment (mol/L)

Treatment	NH_4^+	NO_3^-	$H_2PO_4^-$	K^+	Ca^{2+}	Mg^{2+}	HCO_3^-	HPO_4^{2-}
2 mol/L HCO_3^-	0.5	5	0.5	5	1	0.5	2	0.00
1 mol/L HCO_3^-	0.5	6	0.5	4	1.5	0.5	1	0.00
0.33 mol/L HPO_4^{2-}	0.5	6	0.0	4	1	0.5	0	0.33
CK-pH control solution	0.5	6	0.5	4	1	0.5	0	0.00
CK-pH no adjustment	0.5	6	0.5	4	1	0.5	0	0.00

Note: the pH of CK-pH control solution treatment was adjusted daily, the pH of CK-pH no adjustment treatment was not adjusted except on the first day after transplantation.

Table 2 Compounds composition and mass concentration (mg/L) in each treatment

Compounds	2 mol/L HCO_3^-	1 mol/L HCO_3^-	0.33 mol/L HPO_4^{2-}	CK-pH control solution	CK-pH no adjustment
$Ca(NO_3)_2·4H_2O$	236	236	236	236	236
KNO_3	303	404	404	404	404
$MgSO_4·7H_2O$	123	123	123	123	123
$NH_4H_2PO_4$	57	57	-	57	57
$(NH_4)_2SO_4$	-	-	66	-	-
$KHCO_3$	200	-	-	-	-
$Ca(HCO_3)_2$	-	81	-	-	-
Na_2HPO_4	-	-	47	-	-
DTPA-Fe(7%)			30		
H_3BO_4			2.86		
$ZnSO_4·7H_2O$			0.22		
$CuSO_4·5H_2O$			0.08		
$MnSO_4·4H_2O$			2.13		
$(NH_4)_6Mo_7O_{24}·4H_2O$			0.02		

Note: the pH of CK-pH control solution treatment was adjusted daily, the pH of CK-pH no adjustment treatment was not adjusted except on the first day after transplantation.

2.3 Measurements

2.3.1 pH and EC of nutrient solution

A portable multi-parameter analyzer (HQ-40d, HACH Co., Ltd., USA) was used daily to measure the pH and EC values of hydroponic lettuce nutrition solution.

2.3.2 Growth characteristics of hydroponic lettuces

The lettuces were harvested on the 20th day after transplanting. All fully expanded true leaves were counted as the number of leaves, and the maximum leaf (2nd or 3rd fully expanded leaf from the apical shoot) was used to determine leaf length and width. The fresh weights of shoots and roots were measured by centesimal balance, and the shoots and roots were de-enzyme in an oven at 105°C for 3 h, then dried to a constant weight at 80°C. The dry weights of shoots and roots were measured by an electronic analytical balance (FA1204B, Bioon Group, Shanghai, China).

2.3.3 Logistic model simulation of lettuce growth

Six uniform lettuce plants were randomly selected from the five treatments for fresh weight weighing every other day after transplanting. Based on the shoot fresh weight of lettuce, the logistic model was fitted to analyze the growth of lettuce (Equation (1)), and the logistic model curve conformed to the S-shaped growth curve[23]. The absolute growth rate is the absolute amount of plant growth per unit of time. The absolute growth rate graph was created by determining a first-order derivative of the W number.

$$W = \frac{a}{1 + be^{-kt}} \tag{1}$$

where, W is the fresh weight, g; t is the number of growth days, d; a, b, and k are fitting parameters.

2.3.4 Chlorophyll contents of hydroponic lettuce leaves

The third completely expanded lettuce leaf from the top was picked and the leaf midvein was removed. 0.2 g of the sample was clipped and mixed, then put into 10 mL of 95% ethanol extract for 48 h in dark conditions. The absorbance of the extract at wavelength 665 nm and 649 nm were measured with an ultraviolet spectrophotometer (UV-1700, AUCY Scientific Inc., Shanghai, China). Chlorophyll a and chlorophyll b contents were calculated according to the method in reference and were used to calculate Chlorophyll a/b ratio and total chlorophyll content[24].

2.3.5 Photosynthetic characteristics of hydroponic lettuce leaves

A portable photosynthesis system (LI-6400XT, LI-COR Biosciences Corporation, Lincoln, USA) was used to measure the photosynthetic characteristics of lettuce leaves at the same leaf position before harvest. The standard red and blue light source leaf chamber was used, and the light intensity, leaf temperature, and CO_2 concentration in the leaf chamber were set to 250 μmol/(m²·s), 22°C, and 800 μmol/mol, respectively.

2.3.6 Nutrient element contents in nutrient solution

The nutrient solution was sampled daily from each treatment, stored at low temperature and shielded from light for the measurements of nutrient elements.

The ammonium nitrogen concentration was measured by ultraviolet spectrophotometer (UV-3150, Shimadzu Co., Ltd., Japan) at 630 nm based on indophenol colorimetric method[25]. Nitrate nitrogen concentration was measured by ultraviolet spectrophotometer at 210 nm[26]. Phosphate concentration was measured by ultraviolet spectrophotometer at 680 nm based on the molybdenum blue colorimetry[27].

Potassium was measured by atomic absorption spectrophotometer (AA-7002, Beijing East and West Analytical Instrument Co., Beijing, China) at 766.49 nm based on flame emission method[28]. Calcium, magnesium, and iron were measured by atomic absorption spectrophotometer at 22.67 nm, 285.21 nm and 248.33 nm based on flame emission method, respectively[29,30]. Sulfate concentration was measured by ultraviolet spectrophotometer based on barium sulfate precipitation method at 535 nm[31]. Bicarbonate was measured by bromophenol blue indicator-neutralization titration method[32].

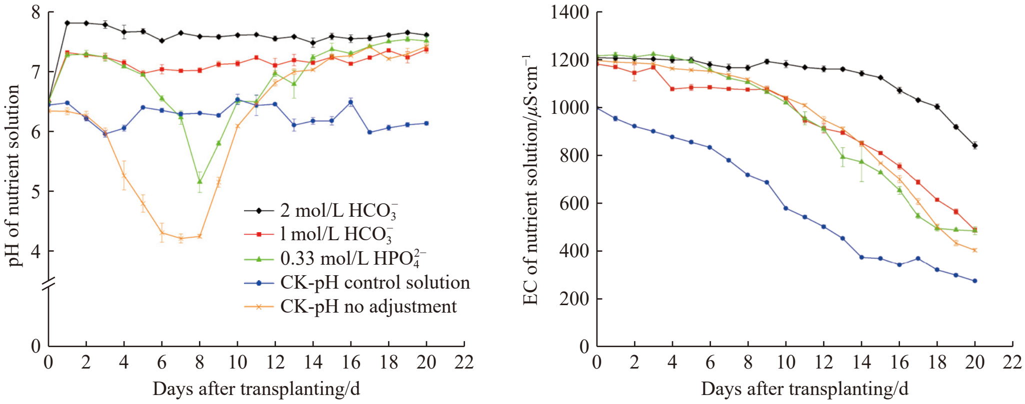
2.4 Statistical analysis

The data were processed by IBM SPSS Statistics 26 (IBM, Inc., Chicago, IL, USA). Before carrying out statistical tests, normality and homogeneity of the data was tested with Shapiro-Wilk test and Bartlett's test, respectively. Data satisfied the assumptions and further throughout the manuscript means are compared by one-way ANOVA followed by Duncan's multiple range test ($p \leq 0.05$). All replicates considered in this study were independent biological replicates originating from different plants.

3 Results

3.1 pH and EC of nutrient solution

The three treatments of 2 mol/L HCO_3^-, 1 mol/L HCO_3^-, and 0.33 mol/L HPO_4^{2-} all showed varying degrees of buffering impact on the pH of nutrient solution during the growth of hydroponic lettuce (Figure 1a). However, nutrient solution pH was more stable under 2 mol/L HCO_3^-, 1 mol/L HCO_3^- and CK-pH control solution treatment. The pH value was maintained at 6.0 to 6.5 under CK-pH control solution treatment by adjusting pH value on a regular basis. The pH value under 2 mol/L HCO_3^- treatment was maintained at 7.4 to 7.7, whereas the pH value under 1 mol/L HCO_3^- treatment was



a. pH b. EC

Note: Vertical bars represent standard deviation.

Figure 1 The variations of nutrient solution pH and EC values under five treatments during the growth of hydroponic lettuce maintained at 7.1 to 7.5. The pH values under 0.33 mol/L HPO_4^{2-} and CK-pH no adjustment treatment both decreased and then increased, reaching the lowest value on the 8th day after transplanting, 5.15 and 4.25, respectively. In contrast to the CK-pH no adjustment treatment, the 0.33 mol/L HPO_4^{2-} treatment showed a buffering effect on the pH of nutrient solution. The pH value under 0.33 mol/L HPO_4^{2-} treatment was maintained at 6.6 to 7.2 for the first 6 d, while the CK-pH no adjustment treatment showed a downward trend and decreased significantly quicker than the 0.33 mol/L HPO_4^{2-} treatment, reaching 4.30 on day 6. The pH changed the least under two HCO_3^- treatments among all treatments.

The initial EC values of the nutrient solution were maintained at 1.0 to 1.2 mS/cm for all treatments (Figure 1b), and showed a downward trend at different rates during lettuce growth. The EC value under 2 mol/L HCO_3^- treatment decreased at the slowest rate, realizing a value of 840 μS/cm at harvest, while the EC values of the other four treatments decreased similarly to reach 300-500 μS/cm on the day of harvest. The lowest EC value of 274 μS/cm was observed under the CK-pH control solution treatment.

3.2 Growth characteristics of hydroponic lettuces

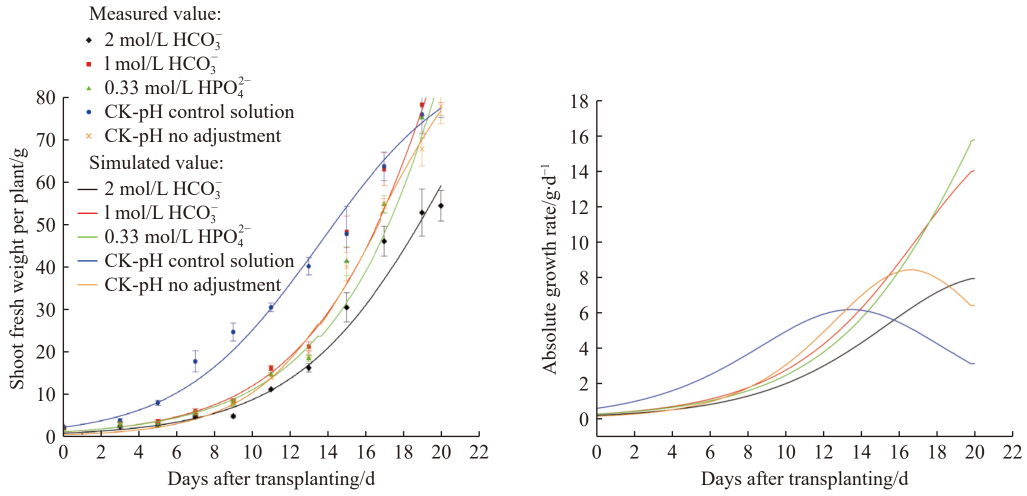
The addition of salts to the nutrient solution had a significant effect on leaf morphology and biomass accumulation of hydroponic lettuce (Table 3). The leaf length, leaf width and leaf number under 1 mol/L HCO_3^-, 0.33 mol/L HPO_4^{2-} and CK-pH control solution treatment were all higher than those under CK-pH no adjustment treatment, whereas 1 mol/L HCO_3^- treatment significantly inhibited the growth of lettuce, with leaf length and leaf width were only 10.8 cm and 9.5 cm, respectively. There was no significant difference in leaf length, leaf width, and leaf number among the 1 mol/L HCO_3^-, 0.33 mol/L HPO_4^{2-} and CK-pH control solution treatments. The biomass accumulation under five treatments showed a pattern similar to that of leaf morphology. The 1 mol/L HCO_3^-, 0.33 mol/L HPO_4^{2-} and CK-pH control solution treatment all promoted biomass accumulation in hydroponic lettuce plants, and the shoot fresh weight was significantly higher than CK-pH no adjustment treatment, whereas the shoot fresh weight per plant under 2 mol/L HCO_3^- treatment was only 56.31 g, and it was significantly lower than CK-pH no adjustment treatment. And the root fresh and dry weights under 2 mol/L HCO_3^- treatment also showed the lowest values among all treatments. It is noteworthy that the root fresh weight under 1 mol/L HCO_3^- treatment were significantly higher than the CK-pH control solution treatment.

Table 3 Effect of adding salts in nutrient solution on leaf morphology and biomass of hydroponic lettuce

Treatment	Leaf length/cm	Leaf width/cm	Leaf number	Fresh weight per plant/g		Dry weight per plant/g	
				Shoot	Root	Shoot	Root
2 mol/L HCO_3^-	10.8±1.0^b	9.5±0.7^c	20.3±1.0^c	56.31±5.50^c	8.33±1.23^b	1.91±0.16^c	0.24±0.03^b
1 mol/L HCO_3^-	12.7±0.3^a	11.5±0.3^{ab}	23.3±0.5^a	83.09±3.40^a	10.89±0.91^a	2.71±0.18^b	0.33±0.04^a
0.33 mol/L HPO_4^{2-}	12.9±0.5^a	10.9±0.8^b	22.4±1.1^{ab}	82.95±4.65^a	9.47±1.85^{ab}	2.47±0.45^b	0.26±0.04^b
CK-pH control solution	12.8±0.4^a	12.0±0.2^a	23.0±1.4^{ab}	78.92±1.61^a	8.20±0.26^b	3.13±0.13^a	0.30±0.04^{ab}
CK-pH no adjustment	11.7±0.7^{ab}	10.9±0.6^b	21.6±0.9^b	71.51±1.77^b	9.24±1.08^{ab}	2.38±0.03^b	0.27±0.02^b

Note: Data represent the mean±SE (n=6). Different letters in the same column indicate significant differences at the 5% level, according to Duncan's multiple range test at p<0.05.

The growth rate of lettuce was affected by the addition of salts in nutrient solution (Figure 2). The lettuce under CK-pH control solution treatment grew rapidly at the beginning of transplanting, while the other four treatments started to grow rapidly from day 10. The growth rate of lettuce under the 1 mol/L HCO_3^-, 0.33 mol/L HPO_4^{2-} and CK-pH no adjustment treatment was similar, while the growth of the lettuce under 2 mol/L HCO_3^- treatment was the slowest (Figure 2a). The absolute growth rate of lettuce under the CK-pH control solution and CK-pH no adjustment treatment reached the maximum on day 13 and day 17, respectively, subsequently declined (Figure 2b). The absolute growth rate of other three treatments kept increasing, but it was the lowest under 2 mol/L HCO_3^- treatment. The absolute growth rate of lettuce under 1 mol/L HCO_3^- and 0.33 mol/L HPO_4^{2-} treatment was still on the rise by day 20. According to the above results, the lettuce could be grown at a normal growth rate after a single addition of 1 mol/L



a. Logistic model simulation b. Absolute growth rate simulation

Note: Vertical bars represent standard deviation.

Figure 2 Logistic model simulation and absolute growth rate simulation of lettuce growth under five nutrient solution treatments

HCO_3^- or 0.33 mol/L HPO_4^{2-} to the nutrient solution, and its growth parameters and biomass were equivalent to the CK-pH control solution treatment.

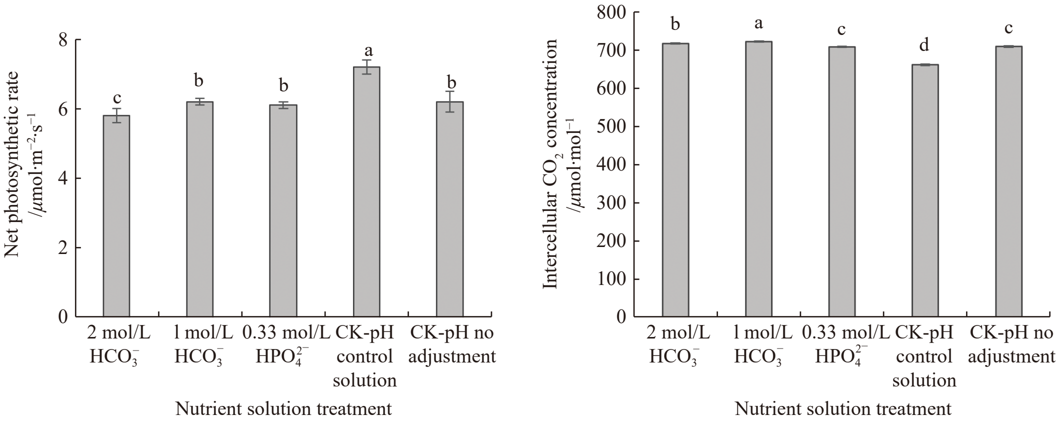
3.3 Chlorophyll content and photosynthesis of hydroponic lettuces

In comparison with CK-pH no adjustment treatment, CK-pH control solution treatment increased the photosynthesis of lettuce, while higher concentrations of HCO_3^- (2 mol/L) inhibited the photosynthesis of lettuce (Table 4, Figure 3). The highest contents of chlorophyll *a*, chlorophyll *b*, total chlorophyll, and chlorophyll *a*/*b* of hydroponic lettuce were all observed in CK-pH control solution treatment, whereas the chlorophyll *a* and total chlorophyll content under 2 mol/L HCO_3^- treatment were significantly lower than the other four treatments, and the chlorophyll *b* content was also the lowest. The chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents did not differ significantly among the 1 mol/L HCO_3^-, 0.33 mol/L HPO_4^{2-}, and CK-pH no adjustment treatment. The highest net photosynthetic rate of 7.2 μmol/(m²·s) and the lowest intercellular CO_2 concentration of 661 μmol/mol were observed under the CK-pH control solution treatment, while the net photosynthetic rate of lettuce under 2 mol/L HCO_3^- treatment was the lowest at 5.8 μmol/(m²·s), and there was no significant difference in the net photosynthetic rate of the other three treatments (Figures 3a and 3b). The intercellular CO_2 concentration, stomatal conductance, and transpiration rate of lettuce treated with HCO_3^- were significantly higher than the other three treatments, with 1 mol/L HCO_3^- treatment showed the highest values (Figures 3b-3d).

Table 4 Effect of adding salts in nutrient solution on photosynthetic pigment contents of hydroponic lettuce

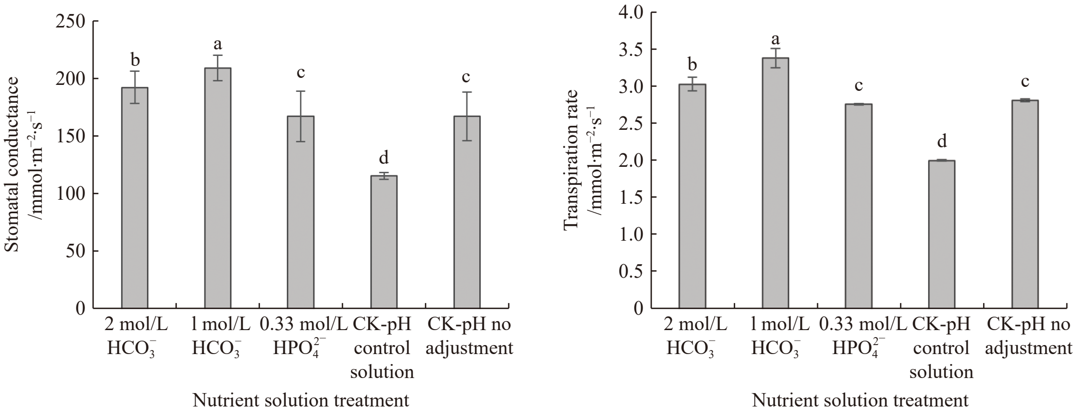
Treatment	Chlorophyll *a* content/ mg·g^{-1}	Chlorophyll *b* content/ mg·g^{-1}	Total chlorophyll content/mg·g^{-1}	Chlorophyll *a*/*b*
2 mol/L HCO_3^-	0.47±0.03^{d}	0.20±0.02^{b}	0.66±0.04^{d}	2.40±0.24^{ns}
1 mol/L HCO_3^-	0.54±0.03^{bc}	0.22±0.04^{ab}	0.76±0.05^{bc}	2.53±0.47^{ns}
0.33 mol/L HPO_4^{2-}	0.52±0.02^{c}	0.22±0.01^{ab}	0.73±0.02^{c}	2.47±0.06^{ns}
CK-pH control solution	0.63±0.04^{a}	0.24±0.01^{a}	0.87±0.05^{a}	2.62±0.16^{ns}
CK-pH no adjustment	0.57±0.02^{b}	0.24±0.01^{a}	0.80±0.02^{b}	2.43±0.04^{ns}

Note: Data represent the mean±SE (n=6). Different letters in the same column indicate significant differences at the 5% level, according to Duncan's multiple range test at p<0.05. ns represents no significant difference in the same column.



a. Net photosynthetic rate

b. Intercellular CO_2 concentration



c. Stomatal conductance

d. Transpiration rate

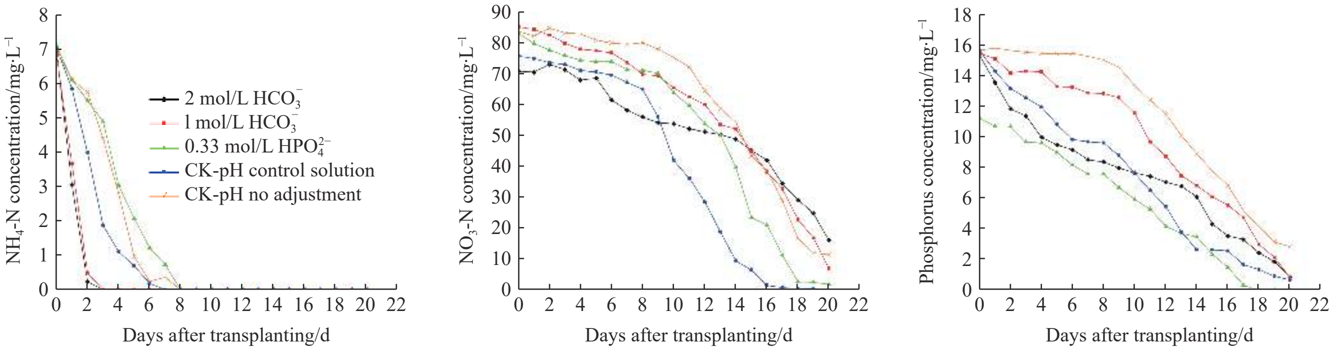
Note: Different letters in the same column indicate significant differences at 5% level, according to Duncan's multiple range test at p<0.05 (n=6). Vertical bars represent standard deviations.

Figure 3 Effect of adding salts in nutrient solution on photosynthetic characteristics of hydroponic lettuce

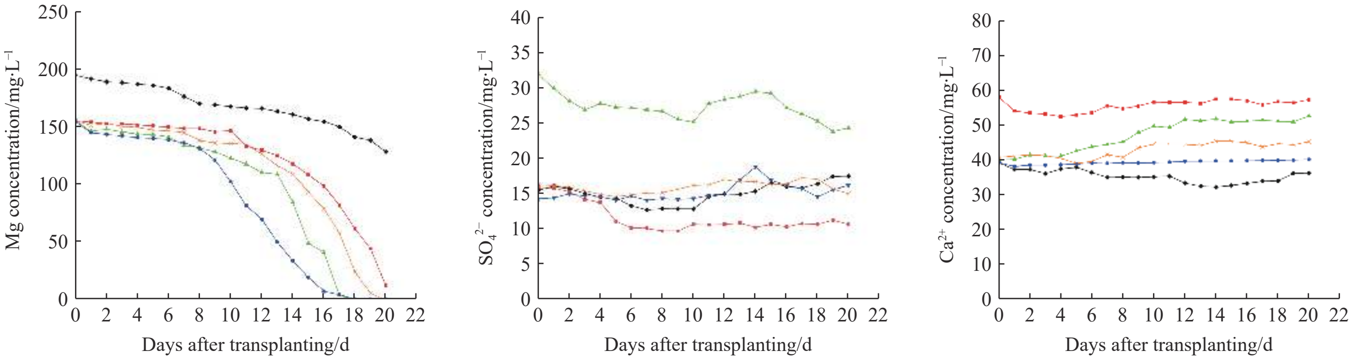
3.4 Variations of ion concentration in nutrient solution

The initial concentration of NH_4^+-N in the nutrient solution of all treatments was around 7 mg/L (Figure 4a). Compared with the CK-pH no adjustment treatment, 2 mol/L HCO_3^-, 1 mol/L HCO_3^- and CK-pH control solution treatment all promoted the uptake of NH_4^+-N. The rate of decline of NH_4^+-N with HCO_3^- treatments was the greatest. The NH_4^+-N concentration under 2 mol/L HCO_3^- and 1 mol/L HCO_3^- had dropped to 0 mg/L by day 3, while the NH_4^+-N concentration under the other three treatments dropped to 0 mg/L by day 8. And NH_4^+-N levels under the CK-pH control solution treatment decreased more quickly than 0.33 mol/L HPO_4^{2-} and CK-pH no adjustment treatment, decreasing rapidly in the first

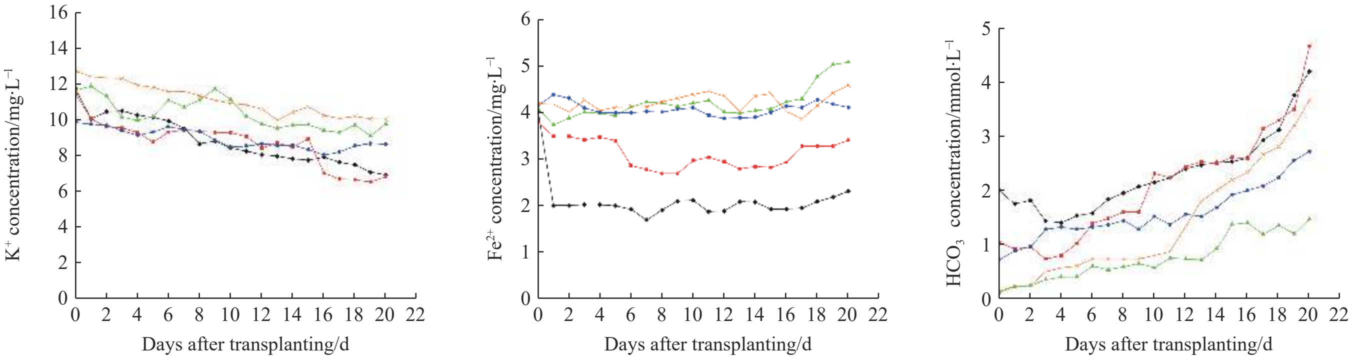
3 d after transplanting. It is worth mentioning that the uptake rate of NH_4^+-N by lettuce from 4th day after transplanting was slightly delayed under 0.33 mol/L HPO_4^{2-} treatment in comparison with the CK-pH no adjustment treatment.



a. NH_4^+-Nconcentration b. NO_3-Nconcentration c. phosphorus concentration



d. K^+ concentration e. SO_4^{2-} concentration f. Ca^{2+} concentration



g. Mg^{2+} concentration h. Fe^{2+} concentration i. HCO_3^- concentration

Figure 4 Variations of ion concentration in nutrient solution under five nutrient solution treatments during the growth of hydroponic lettuce

Compared with the CK-pH no adjustment treatment, 0.33 mol/L HPO_4^{2-} and CK-pH control solution treatment can promote the uptake of NO_3^--N, phosphorus and K^+. The addition of appropriate concentrations of HCO_3^- (1 mol/L) had no effect on the normal uptake of NO_3^--N, phosphorus and K^+ by lettuce, but the addition of excessive concentrations of HCO_3^- (2 mol/L) inhibited its uptake and utilization by lettuce (Figure 4b-4d). The NO_3^--N uptake pattern of lettuce under 1 mol/L HCO_3^-, 0.33 mol/L HPO_4^{2-} and CK-pH no adjustment treatment was similar, and the NO_3^--N concentration all started to drop rapidly from 11th day after transplanting, while the NO_3^--N concentration under the CK-pH control solution treatment decreased rapidly from 8th day after transplanting (Figure 4b). But the uptake rate of NO_3^--N by lettuce under 2 mol/L HCO_3^- treatment was the slowest from 13th day after transplanting. Compared with the CK-pH no adjustment treatment, the uptake rate of NO_3^--N were greater under CK-pH control solution and 0.33 mol/L HPO_4^{2-} treatment, the NO_3-N concentration was close to 0 on 16th and 18th day after transplanting, respectively. The NO_3-N concentration varied from 5 to 20 mg/L under 2 mol/L HCO_3^-, 1 mol/L HCO_3^- and CK-pH control solution treatment on day 20 after transplanting, with the highest NO_3^--N concentration of 16.3 mg/L under 2 mol/L HCO_3^- treatment.

The addition of salts to nutrient solution promoted the uptake of phosphorus compared with the CK-pH no adjustment treatment (Figure 4c). The phosphorus uptake pattern of lettuce under the 1 mol/L HCO_3^- and CK-pH no adjustment treatment was similar, with phosphorus concentration decreasing slowly in the early stage after transplanting. And it was rapidly decreased from 10th day after transplanting, while the uptake rate of phosphorus under 1 mol/L HCO_3^- treatment was greater. The lettuces under the other three treatments began to absorb phosphorus rapidly just after transplanting. The lettuces under 2 mol/L HCO_3^- treatment showed a lower absorption rate of phosphorus from day 10 onwards compared with the other four treatments. The phosphorus concentration of all treatments was closed to 0 except CK-pH no adjustment treatment at harvest.

The effect of different treatments on K^+ uptake and utilization by lettuce was consistent with the results for NO_3^--N (Figure 4d). The K^+ concentration decreased slowly in all treatments at the early stage after transplanting. Subsequently, compared with the CK-pH no adjustment treatment, the uptake rate of K^+ under the CK-pH control solution and 0.33 mol/L HPO_4^{2-} treatment was greater. The K^+ concentration under the CK-pH control solution treatment began to decrease rapidly from 8th day after transplanting and approached to 0 by day 18. The K^+ concentration under 1 mol/L HCO_3^-, 0.33 mol/L HPO_4^{2-} and CK-pH no adjustment treatment started to decrease rapidly from 12th day after transplanting, and the K^+ concentration under 0.33 mol/L HPO_4^{2-} treatment dropped to 0 mg/L by day 18 after transplanting. The addition of 2 mol/L HCO_3^- significantly inhibited the uptake of K^+ by lettuce, and its concentration only decreased by about 50 mg/L during the growth of hydroponic lettuce.

Different nutrient solution treatments affected the uptake of sulfur and calcium by lettuce (Figure 4e and 4f). The SO_4^{2-} concentration under 1 mol/L HCO_3^- and 0.33 mol/L HPO_4^{2-} treatment showed a decreasing trend, while the SO_4^{2-} concentration at harvest under the other three treatments was similar to the concentration before transplanting. The Ca^{2+} concentration did not change much under the 2 mol/L HCO_3^-, 1 mol/L HCO_3^- and CK-pH control solution treatment, while Ca^{2+} concentration increased slightly under the 0.33 mol/L HPO_4^{2-} and CK-pH no adjustment treatment. The variation trend of magnesium element under different nutrient solution treatments was consistent with Ca^{2+} during the growth of hydroponic lettuce, with a decreasing trend of Mg^{2+} concentration in all treatments (Figure 4g). And the addition of HCO_3^- promoted the uptake of Mg^{2+} compared with CK-pH no adjustment treatment.

The uptake of iron in hydroponic lettuce was affected by different nutrient solution treatments (Figure 4h). The Fe^{2+} concentration treated with HCO_3^- showed a decreasing trend. The Fe^{2+} concentration under 2 mol/L HCO_3^- treatment decreased rapidly to 2 mg/L on 1st day after transplanting and then subsequently remained around 2 mg/L. The Fe^{2+} concentration under 1 mol/L HCO_3^- treatment decreased more slowly compared with 2 mol/L HCO_3^- treatment, from initial 3.8 mg/L to 2.9 mg/L on day 6. The Fe^{2+} concentration under the other three treatments did not differ significantly, although those of the 0.33 mol/L HPO_4^{2-} and the CK-pH no adjustment treatment showed a small increasing trend from 17th day.

The HCO_3^- concentration in the nutrient solution under two HCO_3^- treatments decreased and subsequently increased rapidly, eventually reaching around 4.0 mmol/L by day 20 (Figure 4i). The other three treatments increased slowly in the first 11 days and then rapidly increased, while the HCO_3^- concentration under the 0.33 mol/L HPO_4^{2-} and CK-pH control solution treatment increased much slower than those in the CK-pH no adjustment treatment.

4 Discussion

4.1 pH buffering by salt addition in hydroponic lettuce systems

During the growth of hydroponic lettuces, the pH is changed because of the increased nutrient uptake and the changing ratio of NO_3^- to NH_4^+[4]. According to the charge balance requirements of plant, the uptake of NO_3^- means the precipitation of OH^- and leads to the increase of nutrient solution pH. The uptake of NH_4^+ results in H^+ addition and leads to decrease in nutrient solution pH. The lettuce preferentially absorbs NH_4^+-N and then NO_3^--N[33]. Therefore, the nutrient solution pH under CK-pH no adjustment treatment decreased firstly and reached 4.25 by day 8 (Figure 1a), the NH_4^+-N concentration decreased to 0 just now (Figure 4a), subsequently the lettuce began to absorb NO_3^--N and the pH started to increase (Figure 1a). The solution pH increased rapidly above 7.0 on the first day after transplanting (Figure 1a) due to the initial presence of greater quantities of HCO_3^- in the solution for both HCO_3^- treatments (Figure 4i), which formed a buffered system. Since the aqueous solution of HPO_4^{2-} is alkaline, the solution pH under 0.33 mol/L HPO_4^{2-} treatment increased slightly in the first 7 d (Figure 4a), the chemical equilibrium of phosphate species helps to buffer pH[4]. Nonetheless, the amount of phosphate that allows to be added to the nutrient solutions limits the buffering capacity before reaching nutrient imbalance caused by phosphorus[34]. Accordingly, the solution pH under 0.33 mol/L HPO_4^{2-} treatment showed a downward trend after the 5th day due to limited concentration of HPO_4^{2-} and the uptake of NH_4^+-N by lettuce (Figure 4a). When the NH_4^+-N concentration reached 0 mg/L under 0.33 mol/L HPO_4^{2-} treatment on day 8 after transplanting (Figure 4a), the pH reached its lowest value at the same time (Figure 1a). The pH started to rise under 0.33 mol/L HPO_4^{2-} treatment from day 8 due to the presence of HCO_3^- (Figure 4i). The pH of all treatments remained stable after day 11 (Figure 1a), when the solution pH was mainly influenced by the HCO_3^- concentration. The HCO_3^- concentration was increased under all treatments, but increasing the HCO_3^- concentration has only a slight effect on the pH[16]. Therefore, the addition of both HCO_3^- and HPO_4^{2-} can buffer pH, nutrient solution pH was more stable under both HCO_3^- treatments in comparison with 0.33 mol/L HPO_4^{2-} treatment due to finite HPO_4^{2-} in this experiment.

4.2 The uptake of nitrogen, phosphorous and potassium macro-elements

The uptake of mineral elements by plant is influenced by nutrient solution pH. There are two primary aspects: the availability of element and the uptake ability of nutrient element by plant affected by pH[6,35]. High pH reduces the availability of Fe, Mn, Zn, Cu, and P; low pH tends to reduce K, S, Ca, Mg, and P availability. The higher pH favored the uptake of NH_4^+-N, so the NH_4^+-N concentration under the HCO_3^- treatments decreased to 0 around the second day after transplanting (Figure 4a)[36]. As the lettuce growth became exponential (Figure 2a-2b), elements with active uptake such as N, P and K were rapidly absorbed and their concentrations were closed to 0 at harvest (Figure 4b-4d). The absolute grow rate under 2 mol/L HCO_3^- treatment was the lowest since the uptake of NO_3^--N, phosphorus and K^+ as macro-elements was inhibited, which accounts for its higher EC value compared with other treatments. Nitrogen element is a limiting factor for yield, the biomass of lettuce under 2 mol/L HCO_3^- treatment was significantly lower than the other treatments due to slower uptake rate of NO_3^--N (Figure 4b, Table 3)[37]. The uptake rate of NO_3^--N, P and K under 1 mol/L HCO_3^-, 0.33 mol/L HPO_4^{2-} and CK-pH control solution treatment was faster than CK-pH no adjustment treatment, thus the biomass of lettuce under CK-pH no adjustment was significantly lower than them (Figure 4b-4d, Table 3). There was no significant difference in lettuce biomass under 1 mol/L HCO_3^-, 0.33 mol/L HPO_4^{2-} and CK-pH control solution treatment, since the uptake rate of N, P, and K was similar among them. The net photosynthetic rate of leaves was greatest under CK-pH control solution treatment, which may be attributed to the faster uptake of N and K elements (Figure 4b and 4d), and the direct or indirect engagement of nutrients like N and K in the photosynthetic process[38]. The highest chlorophyll content was observed under the CK-pH control solution treatment, and the increase of chlorophyll content facilitated the increase of leaf photosynthesis, which resulted in higher yield. There was no significant difference in chlorophyll content or net photosynthetic rate among the 1 mol/L HCO_3^-, 0.33 mol/L HPO_4^{2-} treatment and CK-pH no adjustment treatment, perhaps due to small difference in uptake rates of N and K elements. Therefore, the single addition of 1 mol/L HCO_3^- and 0.33 mol/L HPO_4^{2-} to the Yamazaki lettuce nutrient solution not only buffered the pH of the nutrient solution, but also allowed lettuce to grow normally.

4.3 The uptake of calcium and magnesium middle elements

Mg is one of elements with intermediate uptake, so the Mg^{2+} concentration under different nutrient solution treatments showed a decreasing trend, but at lower rates than those with active uptake (Figure 4g). Ca belongs to elements with passive uptake, which is taken up at the same rate as water, and their concentrations remain close to initial level[39]. The uptake of Ca^{2+} is regulated by transpiration rate of plants[40], so that the Ca^{2+} concentration under both HCO_3^- treatments decreased slightly, while the Ca^{2+} concentration under other treatments was close to initial level (Figure 4f). Further Ca can regulate the uptake of Mg by plant, which may have resulted in higher uptake under both HCO_3^- treatments compared with the other three treatments (Figure 4g)[41,42]. Thus, the addition of 1 mol/L HCO_3^- promoted the uptake of Ca and Mg compared with other treatments.

4.4 The variation of HCO_3^- concentration

The decrease in HCO_3^- concentration under both HCO_3^- treatments during the first three days after transplanting was due to the neutralization of H^+ released from NH_4^+-N uptake during lettuce growth. Subsequently, the HCO_3^- concentration began to increase as the NH_4^+-N concentration decreased to 0 mg/L under both HCO_3^- treatments (Figure 4a and 4i). The other three treatments all had minor levels of HCO_3^- on the day of transplanting due to the exchange of anions and cations from atmospheric CO_2 into the nutrient solution[39]. The rapid increase of HCO_3^- concentration in nutrient solution from about day 10 after transplanting was caused by the rapid growth stage of lettuce and the vigorous respiration of root zone (Figure 2a-2b). The HCO_3^- concentration increased slowly as a result of daily acid-base adjustment under CK-pH control solution treatment. The addition of 0.33 mol/L HPO_4^{2-} to solution significantly inhibited the rise of HCO_3^-, but the exact reason remains to be further determined.

5 Conclusions

The results indicated that the addition of 2 mol/L HCO_3^-, 1 mol/L HCO_3^- and 0.33 mol/L HPO_4^{2-} to Yamazaki lettuce nutrient solution formulation all buffered pH. But the pH of nutrient solution treated with HCO_3^- was more stable. Compared with the CK-pH no adjustment treatment, the addition of 2 mol/L HCO_3^- significantly inhibited the uptake of ion, and then adversely affected the growth of lettuce, while the 1 mol/L HCO_3^-, 0.33 mol/L HPO_4^{2-} and CK-pH control solution treatment had a positive impact. Furthermore, 1 mol/L HCO_3^- treatment promoted the uptake of NH_4^+-N, the leaf morphology and biomass accumulation of lettuce showed equal levels in comparison with the CK-pH control solution treatment, and it had a higher absolute growth rate. Consequently, 1 mol/L HCO_3^- can be used as a buffer during the growth of hydroponic lettuces. The information from this study can help simplify the management of nutrient solution for hydroponic lettuces.

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