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Contrasting Effects of NaCl and NaHCO₃ Stresses on Seed Germination, Seedling Growth, Photosynthesis, and Osmoregulators of the Common Bean (*Phaseolus vulgaris* L.)

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Abstract: The common bean (*Phaseolus vulgaris* L.), the most important food legume for human nutrition globally, contributes greatly to the improvement of soil fertility in semi-dry lands where most of the soil is already salinized or alkalized, such as in the Songnen Plain of China. In this study, we investigated the effects of salt stress (neutral and alkaline) on the salt-tolerant common bean. Seed germination, seedling growth, photosynthesis, and osmotic adjustment were assessed. Neutral and alkaline salt growth environments were simulated using NaCl and NaHCO₃, respectively. The results indicated that at ≥ 60 mmol·L⁻¹, both NaCl and NaHCO₃ caused significant delays in seedling emergence and decreased seedling emergence rates. NaHCO₃ stress suppressed seedling survival regardless of concentration; however, only NaCl concentrations >60 mmol·L⁻¹ had the same effect. Alkaline salt stress remarkably suppressed photosynthesis and seedling establishment. The common bean compensated for the increase in inorganic anion concentration (influx of Na⁺) by synthesizing more organic acids and soluble sugars. This adaptive mechanism enabled the common bean to balance the large inflow of cations for maintaining a stable cell pH environment under alkaline salt stress.

Keywords: salt stress; alkali stress; seedling establishment; ionic balance; osmotic adjustment

1. Introduction

According to incomplete United Nations Educational, Scientific and Cultural Organization (UNESCO) and Food and Agriculture Organization of the United Nations (FAO) statistics, 950 million hectares of the global land area has saline-alkali soils [1]. Such land areas are mainly distributed in the arid and semi-arid climatic regions in the world [2]. Na⁺, Ca²⁺, Mg²⁺, and K⁺ are the main cations of dissoluble mineral salts, and Cl⁻, SO₄²⁻, HCO₃²⁻, CO₃²⁻, and NO₃⁻ are the corresponding main anions in saline and sodic soils. These ions are derived from neutral or alkaline salts, and thus the content and type of ions present in the soil differ according to the area and prevalent factors. The Songnen Plain is one of the largest saline-alkali land areas in the central part of northeastern China (43°30′–48°40′ N, 121°30′–127°00′ E), covering an area of 3.73 million ha saline-alkali land, and is one of the three major contiguous saline-alkali soil regions in the world [3]. Generally dominated by the presence of excess Na⁺ at the exchange sites and a high concentration of carbonate/bicarbonate anions, saline-alkali soils have a high pH (>8.5–10.8), high sodium absorption ratio (SAR), poor soil structure, and low nutrient level. Saline-alkali soils limit the productivity of agriculture and pasturage [4], experience rapidly increasing alkalization and desertification, and thus restrict the economic development of saline-alkali areas [5].

Salt stress has been defined as the stress of neutral salts, and alkali stress has been defined as the stress of alkaline salts [6]. To date, most studies have focused on salt stress in the soil, and its deleterious effect on various physiological responses (such as water deficiency, photosynthetic carbon metabolism, nutrient imbalance and membrane injury, etc.) have been studied in a range of plant species, and salinity-induced changes in these responses are well documented [7–10]. However, with the increasing recognition of the threat of alkalinity to agricultural production, some scientists have begun investigating the effects of alkali stress, which has the same stress factors as salt stress but with the added influence of high-pH stress on plant physiology at the mechanistic level. The high-pH environment surrounding the roots can directly cause Ca^{2+} , Mg^{2+} , and H_2PO_4^- precipitation [11], which may suppress ion uptake [12] and disrupt ion homeostasis of plant cells. Previous studies have clearly demonstrated the existence of alkali stress, and the increased severity of its effects compared to salt stress [13–16]. For instance, the most conspicuous symptom of alkaline salt stress in plants is the induction of leaf chlorosis and stunted growth, which is correlated with the precipitation of metal ions and phosphorus and the disruption of ionic balance and pH homeostasis in tissues [11,12]. Furthermore, these factors limit photosynthesis and increase lipid peroxidation. The activities of superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT), combined with the ascorbate–glutathione cycle play important roles in alleviating oxidative stress [17]. In addition, the accumulation of proline, organic acids, amino acids, soluble carbohydrates, and glycine betaine may be an adaptive response to the substantial influx of Na^+ induced by alkaline salt stress [18,19]. Meanwhile, the adaptability of plants to salt and alkali stresses not only depends on the species [20], but also varies among the different growth and developmental stages. The stage from seed germination to fully developed seedling is crucial and can define species distribution and population yield formation under saline-alkali conditions. Therefore, the study of plants under salt and alkali conditions during seed germination and at the seedling stage not only has theoretical significance in physiological ecology, but also has practical significance for crop production.

The common bean (*Phaseolus vulgaris* L.) is globally the most important food legume for human nutrition [21]. The common bean is grown in a wide range of environments and altitudes, from sea level to high elevations. Furthermore, the common bean and other leguminous species are regarded as appropriate crops for the enhancement of bioproductivity and reclamation of marginal lands because they not only yield nutritious fodder and protein rich seeds and fruits, but also are known to enrich soil nitrogen in symbiotic associations with *Rhizobium* [22]. Therefore, these plant species contribute greatly to the improvement of soil fertility in semi-dry lands where most of the soil is already salinized or alkalized, such as in the Songnen Plain in Northeast China [23,24]. Previous studies have mainly investigated the responses of the common bean to neutral salt (NaCl) stress conditions, i.e., growth and development, parameters for photosynthesis, and ionic balance [25,26], antioxidant system [27,28], molecular regulatory network [29–31], etc. However, the response characteristics and corresponding mechanisms of the common bean to alkaline salt stress have rarely been reported. In the present study, we used NaCl and NaHCO_3 solutions to simulate neutral salt and alkaline salt growth conditions, respectively, and investigated and compared seed germination, seedling growth, photosynthesis, and osmoregulatory response characteristics of the common bean to saline-alkali conditions from the germination to seedling stages. The findings of the present study improve our understanding of the mechanisms involved in alkali stress (high pH) damage in the common bean, provide a scientific basis for selective breeding and the promotion of saline-alkali-tolerant species, and may help guide common bean production in salt-alkaline soil areas.

2. Materials and Methods

2.1. Plant Material and Salt- and Alkaline Stress Treatment

A common bean (*P. vulgaris* L.) variety PV-HYD was selected as the experimental material as it is the main cultivated variety in the Songnen Plain of Northeast China and is tolerant to saline-alkali

conditions. All pots containing plants were placed in a glass greenhouse (daytime: 25 ± 1.5 °C, nighttime: 20 ± 1.5 °C). Plants were grown under uniform irradiance with a photoperiod of 14 h:10 h (light:dark), a photosynthetic photon flux density (PAR) of $400 \mu\text{mol quanta}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and 50–60% relative humidity.

Plastic pots (diameter: 25 cm, height: 25 cm, $n = 220$) were filled with washed sand (10 kg). The experimental design consisted of a control (C) treatment with complete Hoagland nutrient solution: $4 \text{ mmol}\cdot\text{L}^{-1} \text{Ca}(\text{NO}_3)_2$, $6 \text{ mmol}\cdot\text{L}^{-1} \text{KNO}_3$, $2 \text{ mmol}\cdot\text{L}^{-1} \text{MgSO}_4$, $1 \text{ mmol}\cdot\text{L}^{-1} \text{NH}_4\text{H}_2\text{PO}_4$, $80 \mu\text{mol}\cdot\text{L}^{-1} \text{Fe-EDTA}$, $46.3 \mu\text{mol}\cdot\text{L}^{-1} \text{H}_3\text{BO}_3$, $9.5 \mu\text{mol}\cdot\text{L}^{-1} \text{MnSO}_4$, $0.8 \mu\text{mol}\cdot\text{L}^{-1} \text{ZnSO}_4$, $0.3 \mu\text{mol}\cdot\text{L}^{-1} \text{CuSO}_4$, and $0.02 \mu\text{mol}\cdot\text{L}^{-1} (\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$, with neither NaCl and NaHCO_3 added, and 10 treatments of varying NaCl or NaHCO_3 levels. Based on the Hoagland nutrient solution, these consisted of 30, 60, 90, 120, and $150 \text{ mmol}\cdot\text{L}^{-1}$ NaCl treatments (neutral salt stress), and 30, 60, 90, 120, and $150 \text{ mmol}\cdot\text{L}^{-1} \text{NaHCO}_3$ treatments (alkaline salt stress). The pH of each neutral salt and alkaline salt stress treatment solution was measured using a digital pH meter (FE20K, Mettler-Toledo, Zurich, Switzerland), and ranged between 6.65 to 6.86 in the neutral salt stress groups and 8.17 to 8.27 in the alkaline salt stress groups. The experiment was arranged in a randomized, complete block design with 20 replicates, and each pot was considered as a single replicate. Seeds were sown equidistantly and singly in 15 holes per pot (the germination rate of seeds was 100% in the germination test under a filter paper bed). After planting, each pot was thoroughly watered with 1 L of nutrient solution corresponding to the different treatments. Thereafter, the pots were watered every 2 d at 17:00–18:00.

After 31 d from sowing, the leaf gas exchange parameters were measured before harvest; six plants from six pots under the same growth conditions were considered as one replicate, and experiments were conducted in triplicate. For each replicate, a part of the fresh sample was used to measure the leaf area, pigment content, pH of plant leaf tissue sap, and glycine betaine, proline, free amino acids content. The remaining fresh samples of each organ were placed in perforated paper bags, oven-dried at 105 °C for 30 h, then vacuum-dried at 80 °C for 48 h to a constant weight. The fresh weight (FW) and dry matter weight (DW) of the leaves, stems, and roots were recorded successively. Dry samples of leaves and roots were used to determine the inorganic ion, total soluble sugar, and organic acid content in the leaves and roots.

2.2. Seedling Emergence and Survival Investigations

After sowing, seedling emergence and time of emergence were recorded daily; i.e., when cotyledons were completely exposed above ground. The emergence rate was calculated from the number of seedlings and sown seeds. Seedling survival was confirmed when a seedling remained alive and was growing 15 days after emergence.

2.3. Measurement of Leaf Gas Exchange Parameters and Physiological Indices

Net photosynthetic rate (P_n), stomatal conductance (G_s), intercellular CO_2 concentration (C_i), and transpiration rate (E) of leaves of study plants under different treatments were determined between 9:00–11:00 using a portable open-flow, gas exchange system (LI-6400, LI-COR Biosciences, Lincoln, OR, USA). Briefly, fully expanded, first attached leaflets were clamped to the leaf chamber and the measurements were recorded when atmospheric relative humidity and CO_2 concentration reached a stable value. The photosynthetically active radiation, air temperature, relative humidity, and CO_2 concentration inside the sensor head were set at $1100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, 25 °C, 60%, and $360 \mu\text{mol}\cdot\text{mol}^{-1}$, respectively.

Fresh leaves (~0.2 g each) from each replicate were dipped in 5 mL of 80% acetone to determine chlorophyll *a* (Chl*a*), chlorophyll *b* (Chl*b*), and total carotenoid (Car) content. The concentrations of Chl*a*, Chl*b*, and Car ($\text{g}\cdot\text{kg}^{-1}$, FW) were determined by the absorption at 663.2, 646.8, and 470 nm, respectively, using a spectrophotometer (Specord210 PLUS, Analytikjena, Jena, German). The content of Chl*a*, Chl*b*, and Car was calculated using the formulae of Lichtenthaler [32]: $\text{Chl}a = 12.25A_{663.2} - 2.79A_{646.8}$; $\text{Chl}b = 21.50A_{646.8} - 5.10A_{663.2}$; $\text{Car} = (1000A_{470} - 1.82\text{Chl}a - 85.02\text{Chl}b)/198$.

Total plant water content (WC) was measured according to Lei et al. [33], which was calculated using the formula: $(FW - DW)/DW$, and expressed as percentage composition (%). The pH of leaf tissue sap was determined following the method of Yang et al. [12]; i.e., the leaves were crushed, the tissue sap was extruded, and the pH was measured using a digital pH meter (FE20K, Mettler-Toledo, Zurich, Switzerland).

2.4. Determination of Inorganic Ions and Organic Solutes

Inorganic ions were extracted and measured following the method described by Yang et al. [12]. Briefly, dry samples (~100 mg each) were treated with 20 mL of deionized water at 100 °C for 20 min, and the extract was used to determine the concentrations of free inorganic ions. The concentrations of NO_3^- , Cl^- , SO_4^{2-} , and H_2PO_4^- were determined by ion chromatography (ICS-600, Dionex, Waltham, MA, USA). A flame photometer (TAS-990, Persee, Beijing, China) was used to determine K^+ and Na^+ concentrations, and complexometric titration was employed to determine Ca^{2+} and Mg^{2+} concentrations.

The content of soluble sugars was determined using the phenol sulfuric acid method [34] with modifications [35]. Briefly, dry samples (~50 mg) were extracted with 80% ethanol (*v/v*) at 85 °C for 1 h. The solution was then centrifuged at 12,000 rpm for 10 min. The resulting supernatants were then combined, treated with activated charcoal, and evaporated to dryness in a vacuum evaporator. The residues were re-dissolved in distilled water to be subjected to soluble sugars analysis. Glucose was used as the standard. Soluble sugar content was determined as $\text{mmol}\cdot\text{kg}^{-1}$ DW, using a calibration curve.

Free proline was extracted and determined as described by Bates et al. [36]. Plant samples (~0.5 g) were homogenized in 5 mL of sulfosalicylic acid (3%); thereafter, 2 mL samples of the extracts were transferred to plastic tubes and 2 mL of glacial acetic acid and 2 mL of ninhydrin were added to each tube. The resulting mixtures were heated at 100 °C for 1 h in a water bath. The mixture was extracted with toluene, and the absorbance of the fraction aspirated from the upper liquid phase was read at 520 nm using a spectrophotometer (Specord 210 PLUS, Analytikjena, Jena, Germany). Proline content was determined using a calibration curve and expressed as $\text{mmol}\cdot\text{kg}^{-1}$ FW.

High performance liquid chromatography was used to determine and analyze the organic acids. According to Yang et al. [11], dry samples (~100 mg) were treated with 20 mL of $0.05\text{ mol}\cdot\text{L}^{-1}$ HCl at 100 °C for 1 h. All sample extract solutions were filtered through a Millipore system ($0.45\text{ }\mu\text{m}$) before use, and ultra-pure grade water was used throughout the study. A Hyersil C18 column ($4.6 \times 250\text{ mm}$, $5\text{ }\mu\text{m}$; Shimadzu, Kyoto, Japan) was used. The mobile phase was an aqueous solution containing 0.5% (in mass fraction) KH_2PO_4 and $0.5\text{ mmol}\cdot\text{L}^{-1}$ tetra-*n*-butylammonium hydrogen sulfate (pH 2.0), and was treated with an ultrasonic generator for 20 min for degassing. The flow rate was $0.5\text{ mL}\cdot\text{min}^{-1}$ and a Shimadzu RID-0A UV Detector (220 nm; Shimadzu, Kyoto, Japan) was used. The column temperature and pressure were maintained at 40 °C and 20 MPa, respectively, and the injection volume was 5 μL for all runs. Organic acid content was expressed as $\text{mmol}\cdot\text{kg}^{-1}$ DW.

Glycine betaine was estimated according to the method of Grieve and Grattan [37] and expressed as $\text{mmol}\cdot\text{kg}^{-1}$ FW. Briefly, plant sample extracts were prepared in 20 mL test tubes by chopping 0.5 g leaves or roots in 5 mL of toluene-water mixture (0.05% toluene). All tubes were shaken for 24 h at 25 °C. After filtration, 0.5 mL of the extracts were mixed with 1 mL of $2\text{ mol}\cdot\text{L}^{-1}$ HCl solution and 0.1 mL of potassium tri-iodide solution (i.e., 7.5 g iodine and 10 g potassium iodide in 100 mL of $1\text{ mol}\cdot\text{L}^{-1}$ HCl) and shaken in an ice cold water bath for 90 min. Thereafter, 2 mL of ice-cooled water was added and after gentle shaking, 10 mL of 1,2 dichloroethane (chilled at $-10\text{ }^\circ\text{C}$) was added. The upper aqueous layer was discarded and optical density of the organic layer was recorded at 365 nm, and the calculations were performed using the reference standard for glycine betaine ($50\text{--}200\text{ mg}\cdot\text{mL}^{-1}$).

The total amino acids in the common bean solutions were determined according to the methods of Yao et al. [38]. Briefly, hot distilled water (100 mL) was added to beakers that contained leaf or root fresh samples (~1 g), and each sample was allowed to infuse for 10 min. The infusions were filtered through Whatman filter paper No. 1 prior to analysis. The total free amino acids were determined by adding 0.5 mL of phosphate buffer solution (pH 8.0) and 0.5 mL of 2% ninhydrin solutions (containing $0.8\text{ mg}\cdot\text{mL}^{-1}$ of tin chloride) to 1 mL of the infusions in 25 mL volumetric flask. The mixtures were

heated for 15 min in a boiling water bath and cooled to 25 °C. The mixtures were then diluted to 25 mL with distilled water and allowed to rest for 10–15 min. The optical density of the solution was checked at 570 nm by spectrophotometer (Specord 210 PLUS, Analytikjena, Jena, German). Using leucine as a standard, a standard curve was prepared by plotting the absorbance of a series of working standards against their respective concentrations. The total amino acid content was represented as $\text{mmol}\cdot\text{kg}^{-1}$ FW.

2.5. Statistical Data Analysis

All experiments were based on three replicated measurements. Values represent mean $\pm$ standard deviation (SD) of the three replicates. Statistical analyses were performed using the analysis of variance (ANOVA) with SPSS 19.0 software (SPSS Inc, Chicago, IL, USA). Differences between treatments were determined by Duncan's new multiple range method (DMRT) and were considered statistically significant at $p < 0.05$.

3. Results

3.1. Effect of Neutral Salt and Alkaline Salt Stresses on Seedling Emergence and Survival

Under NaCl stress, the emergence time of common bean seedlings was delayed, but the emergence rate and seedling survival rate did not decrease significantly for 30 or 60 $\text{mmol}\cdot\text{L}^{-1}$ treatments. For the remaining three treatments (90–150 $\text{mmol}\cdot\text{L}^{-1}$), not only did the emergence time increase by 12–32%, but the emergence rate and seedling survival rate also obviously decreased by 10–25% and 12–34%, respectively. For the seedlings grown under NaHCO_3 stress, the change patterns of emergence time, emergence rate, and seedling survival were similar to those of the seedlings treated with NaCl, but they were changed sharply with increasing alkalinity (Figure 1A–C). Collectively, the NaCl/ NaHCO_3 stresses suppressed the growth of the common bean, and the effects of alkaline salt stress were more severe than neutral salt stress.

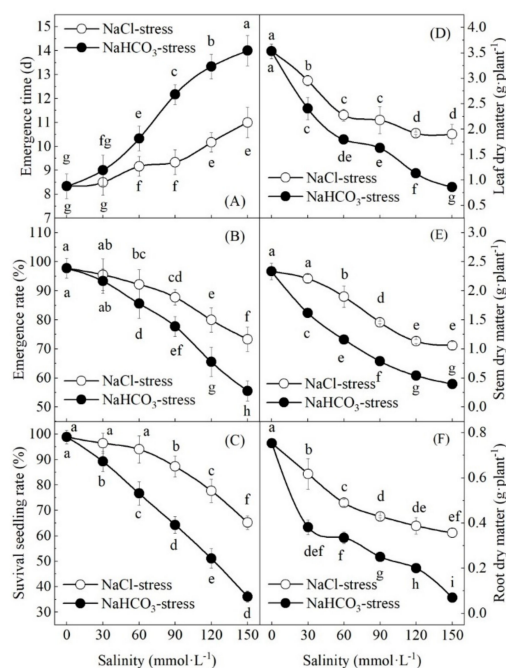


Figure 1. Effects of NaCl and NaHCO_3 stresses on seedling emergence time (A), emergence rate (B) and survival rate (C), dry mass of leaves (D), stems (E), and roots (F) of the common bean (*Phaseolus vulgaris*). Common beans were treated with NaCl (30–150 $\text{mmol}\cdot\text{L}^{-1}$, pH 6.65–6.86) and NaHCO_3 (30–150 $\text{mmol}\cdot\text{L}^{-1}$, pH 8.17–8.27) stresses. Data points and bars represent the mean $\pm$ SD of three replicates. Values followed by different letters in the same subgraph are significantly different at the 5% level among the various concentration treatments of neutral salt conditions or alkaline salt conditions, according to Duncan's new multiple range method.

3.2. Effect of Neutral Salt and Alkaline Salt Stresses on Growth

The biomass in leaves, stems, and roots of common bean plants significantly decreased with increasing concentrations of NaCl and NaHCO₃; the reductions under alkaline conditions were greater than those under saline conditions (Figure 1D–F). Leaf area, total plant WC, and tissue pH data are shown in Figure 2A–C, respectively. NaCl and NaHCO₃ stresses also had a significant effect on leaf area, with higher leaf areas observed when stress was minimal and lower leaf areas under more severe stress conditions (Figure 2A). NaCl stress only resulted in a slight decrease in WC, while alkaline salt-stressed plants had dramatically lower WCs, especially in plants treated with NaHCO₃ solutions ≥ 90 mmol·L⁻¹ (Figure 2B). Tissue pH of common bean leaves under neutral salt or alkaline salt stresses was almost consistent with that of the C treatment, except for 120–150 mmol·L⁻¹ NaHCO₃ treatments. Nevertheless, the mean tissue pH was 7.25 under neutral salt-stress, 7.17 under alkaline salt-stress, and 7.29 in the C treatment (Figure 2C).

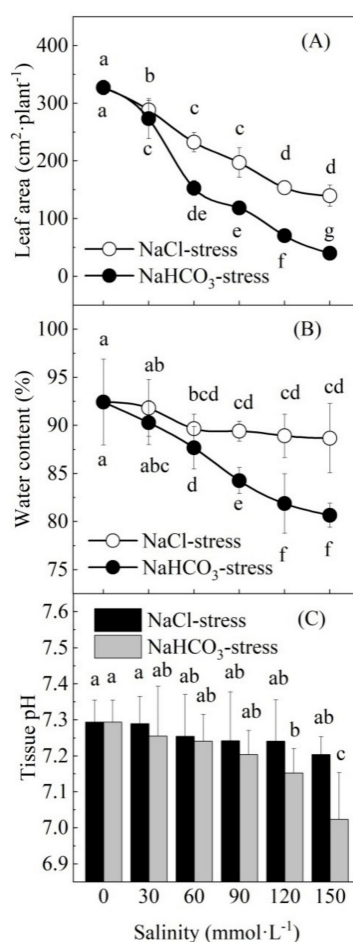


Figure 2. Effects of NaCl and NaHCO₃ stresses on leaf area (A), plant water content (B), fresh leaves tissue pH (C) of common bean (*Phaseolus vulgaris*) seedlings. Common beans were treated with NaCl (30–150 mmol·L⁻¹, pH 6.65–6.86) and NaHCO₃ (30–150 mmol·L⁻¹, pH 8.17–8.27) stresses. Data points and bars represent the mean $\pm$ SD of three replicates. Values followed by different letters in the same subgraph are significantly different at the 5% level among the various concentration treatments of neutral salt conditions or alkaline salt conditions, according to Duncan's new multiple range method.

3.3. Effects of Neutral Salt and Alkaline Salt Stresses on Gas Exchange and Pigments

The changes in P_n , E , and G_s of common bean were similar under the different stress conditions (Figure 3A–C). All values decreased with increasing salinity under both saline and alkaline conditions, with greater reductions under alkaline conditions. The effect of NaCl stress on C_i was not significant

except at the salinity of 120–150 mmol·L⁻¹ (Figure 3D). Compared with the C treatment, common bean leaves treated with NaHCO₃ stress at a low concentration (30 mmol·L⁻¹) showed a decreased *C_i*. However, at salinities ≥ 60 mmol·L⁻¹, *C_i* increased sharply with increasing salinity under alkali stress. As shown in Table 1, treatment with low concentrations of neutral salt stress had no significant effect on the photosynthetic pigment content of common bean leaves, and only treatments with high concentrations of neutral salt (120–150 mmol·L⁻¹) significantly suppressed the accumulation of photosynthetic pigments. Compared with neutral salt stress, Chl*a*, Chl*b*, and Car under alkaline salt stress decreased rapidly with the increase in stress intensity.

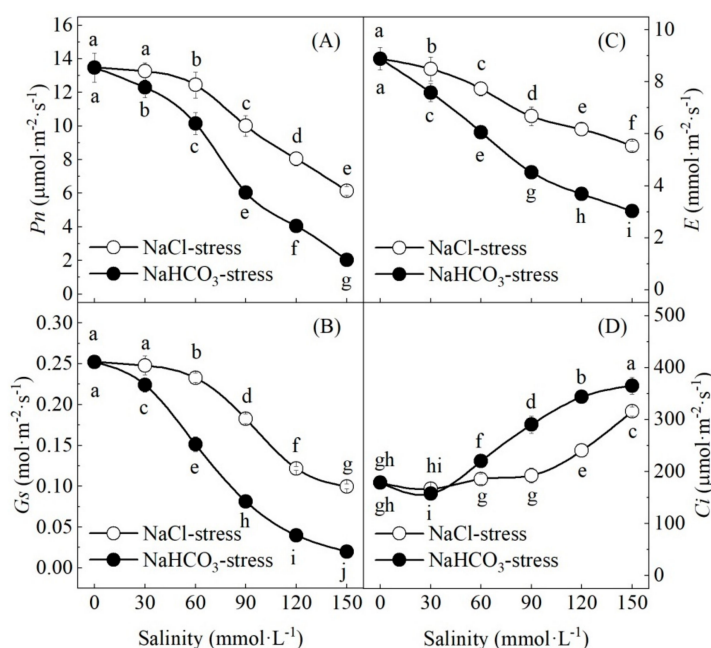


Figure 3. Effects of NaCl and NaHCO₃ stresses on the net photosynthetic rate (A), stomatal conductance (B), transpiration rate (C), and intercellular CO₂ concentration (D) of common bean (*Phaseolus vulgaris*) seedlings. Common beans were treated with NaCl (30–150 mmol·L⁻¹, pH 6.65–6.86) and NaHCO₃ (30–150 mmol·L⁻¹, pH 8.17–8.27) stresses. Data points and bars represent the mean ± SD of three replicates. Values followed by different letters in the same subgraph are significantly different at the 5% level among the various concentration treatments of neutral salt conditions or alkaline salt conditions, according to Duncan's new multiple range method.

Table 1. Effects of NaCl and NaHCO₃ stresses on the content of chlorophyll (Chl) *a*, *b*, and total carotenoids (Car) in common bean (*Phaseolus vulgaris*) seedlings.

Salinity (mmol·L ⁻¹)	NaCl Stress			Salinity (mmol·L ⁻¹)	NaHCO ₃ Stress		
	Chl <i>a</i> (g·kg ⁻¹)	Chl <i>b</i> (g·kg ⁻¹)	Car (g·kg ⁻¹)		Chl <i>a</i> (g·kg ⁻¹)	Chl <i>b</i> (g·kg ⁻¹)	Car (g·kg ⁻¹)
0	2.58 ± 0.16a	0.73 ± 0.06a	0.60 ± 0.05ab	0	2.58 ± 0.16ab	0.73 ± 0.06a	0.60 ± 0.05a
30	2.72 ± 0.13a	0.69 ± 0.02ab	0.66 ± 0.03a	30	2.66 ± 0.13a	0.66 ± 0.02b	0.58 ± 0.02ab
60	2.65 ± 0.06a	0.66 ± 0.03ab	0.62 ± 0.03a	60	2.34 ± 0.10b	0.61 ± 0.03b	0.52 ± 0.02bc
90	2.48 ± 0.10a	0.63 ± 0.04bc	0.55 ± 0.04b	90	2.01 ± 0.13c	0.48 ± 0.05c	0.47 ± 0.04c
120	2.21 ± 0.16b	0.57 ± 0.02cd	0.43 ± 0.03c	120	1.89 ± 0.09cd	0.40 ± 0.04d	0.39 ± 0.02d
150	2.20 ± 0.15b	0.55 ± 0.04d	0.40 ± 0.02c	150	1.74 ± 0.13d	0.39 ± 0.03d	0.34 ± 0.02d

Values represent the means of three replicates; means followed by different letters in the same column are significantly different at *p* < 0.05, according to the Duncan's new multiple range method.

3.4. Effect of Salt and Alkali Stresses on Inorganic Ion Content

As shown in Figure 4A,B, Na⁺ concentration in leaves and roots gradually increased with the increase in NaCl or NaHCO₃ concentrations. In particular, at concentrations ≥ 60 mmol·L⁻¹, the increase in Na⁺ concentration was significantly larger under NaHCO₃ stress than under NaCl stress. In contrast, K⁺ concentration showed changes following a single peak curve, i.e., it increased initially and then

decreased with the increase in concentrations of NaCl or NaHCO_3 . In particular, at 30–60 $\text{mmol}\cdot\text{L}^{-1}$, the K^+ concentration in leaves was significantly higher under NaHCO_3 stress than under NaCl stress at the same concentrations, but the difference in K^+ concentration in roots between NaCl and NaHCO_3 stress was not significant (Figure 4C,D). At $>90\text{ mmol}\cdot\text{L}^{-1}$, K^+ concentration in the leaves was relatively higher under NaCl stress. The difference in K^+ concentration in roots between NaCl and NaHCO_3 stress treatments was significant only when the stress concentration was $\geq 90\text{ mmol}\cdot\text{L}^{-1}$, with the K^+ concentration under NaCl stress treatment being higher. As shown in Figure 4I,J, the Na^+/K^+ ratio in the leaves and roots both increased with increasing salt concentrations. Although the Na^+/K^+ ratio changed relatively slowly under NaCl stress, the Na^+/K^+ ratio under NaHCO_3 stress (especially at $\geq 60\text{ mmol}\cdot\text{L}^{-1}$) increased rapidly and was significantly higher than that under NaCl stress.

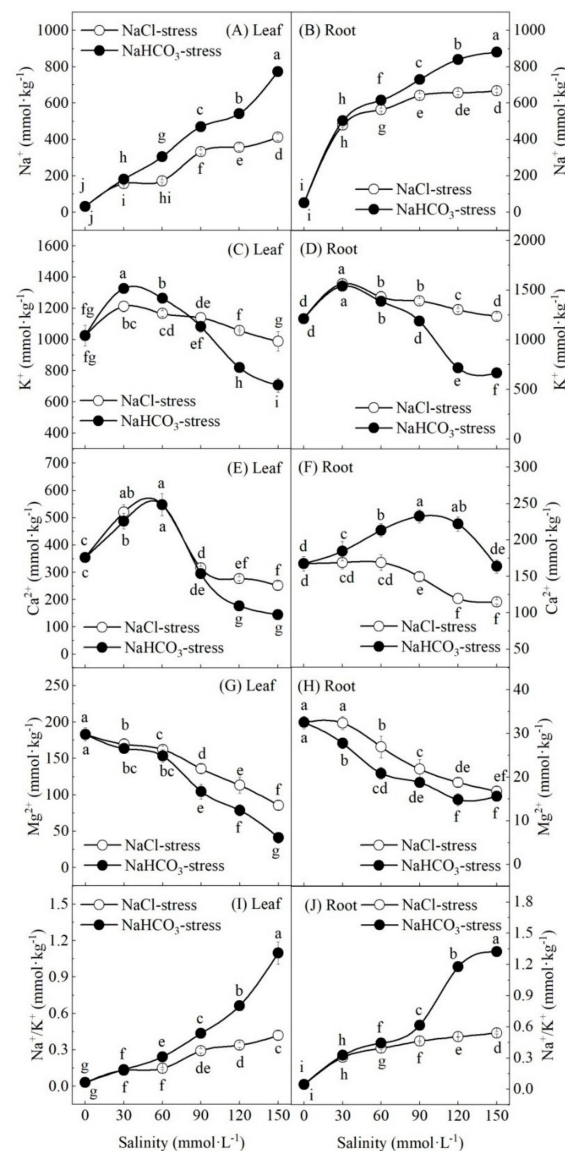


Figure 4. Effects of NaCl and NaHCO_3 stresses on the Na^+ (A,B), K^+ (C,D), Ca^{2+} (E,F), Mg^{2+} (G,H) concentrations, and Na^+/K^+ (I,J) in leaves and roots of the common bean (*Phaseolus vulgaris*). Common beans were treated with NaCl (30–150 $\text{mmol}\cdot\text{L}^{-1}$, pH 6.65–6.86) and NaHCO_3 (30–150 $\text{mmol}\cdot\text{L}^{-1}$, pH 8.17–8.27) stresses. Data points and bars represent the mean $\pm$ SD of three replicates. Values followed by different letters in the same subgraph are significantly different at the 5% level among the various concentration treatments of neutral salt conditions or alkaline salt conditions, according to Duncan's new multiple range method.

With the increase in NaCl and NaHCO₃ concentrations, Ca²⁺ concentration in leaves and roots increased initially and then decreased (Figure 4E,F). At concentrations of 0–90 mmol·L^{−1}, the Ca²⁺ concentration in leaves was not obviously different (at the same salt concentrations), regardless of being under NaCl or NaHCO₃ stress. However, at concentrations of ≥120 mmol·L^{−1}, Ca²⁺ concentration in leaves under NaHCO₃ stress decreased rapidly, and the difference under NaCl stress was significant. When the stress concentration was ≥60 mmol·L^{−1}, the Ca²⁺ concentration in roots was always greater under NaHCO₃ stress than under NaCl stress. In addition, under NaCl and NaHCO₃ stresses, Mg²⁺ concentration in leaves and roots showed a gradual decreasing trend with an increase in stress concentration (Figure 4G,H). Except in the roots under the 30 mmol·L^{−1} NaCl stress treatment, Mg²⁺ concentration in leaves and roots was significantly lower at each salt stress concentration than those in the C treatment, and the differences between NaCl and NaHCO₃ stress treatments were significant at concentrations ≥90 mmol·L^{−1} for leaves and 30–120 mmol·L^{−1} for roots. At a concentration of 150 mmol·L^{−1}, Mg²⁺ concentration in roots under NaCl and NaHCO₃ stress treatments reached similar levels.

Cl[−] concentration in leaves and roots increased significantly with increasing concentrations under NaCl stress; however, no significant changes were observed under NaHCO₃ stress (Figure 5A,B). In addition, H₂PO₄[−] concentration in both leaves and roots in the NaCl treatments increased with increasing concentrations. In contrast, H₂PO₄[−] concentration decreased with increasing NaHCO₃ stress concentration in the leaves, and decreased at first and then increased in the roots, reaching a minimum value under the 90 mmol·L^{−1} NaHCO₃ concentration (Figure 5C,D). With the increase in NaCl and NaHCO₃ concentrations, NO₃[−] concentration in leaves and roots gradually decreased, in particular, it decreased more rapidly under NaHCO₃ stress (Figure 5E,F). SO₄^{2−} concentration in both leaves and roots increased significantly with increasing NaHCO₃ stress concentration. In contrast, SO₄^{2−} concentration in the leaves and roots varied under NaCl stress. With the increase in NaCl concentration, no significant change in SO₄^{2−} concentration was observed in the leaves, but it increased significantly in the roots (Figure 5G,H).

3.5. Effect of Salt and Alkali Stresses on Organic Solute Content

The increase in NaCl and NaHCO₃ stress concentrations promoted the increase in soluble sugar content in leaves and roots (Figure 6A,B). The soluble sugar content in leaves and roots increased slowly with increasing NaCl concentrations, but increased initially (until reaching a peak) and then decreased with increasing NaHCO₃ concentrations. Both increases were significant and reached peaks at 120 mmol·L^{−1}. Regardless of NaCl or NaHCO₃ treatment, proline content in the leaves and roots increased initially and then decreased with increasing concentrations (Figure 6C,D). In particular, proline content in the leaves increased slowly at NaCl or NaHCO₃ concentrations of 30–60 mmol·L^{−1}, and then increased rapidly at a concentration ≥90 mmol·L^{−1}. Proline content in the roots began to increase significantly at NaCl concentrations ≥60 mmol·L^{−1} or at NaHCO₃ concentrations of ≥30 mmol·L^{−1}. The content of organic acids, betaine, and free amino acids in the leaves and roots increased with increasing NaCl and NaHCO₃ concentrations, and the magnitude of increase was more prominent in those under NaHCO₃ stress treatment (Figure 6E–J).

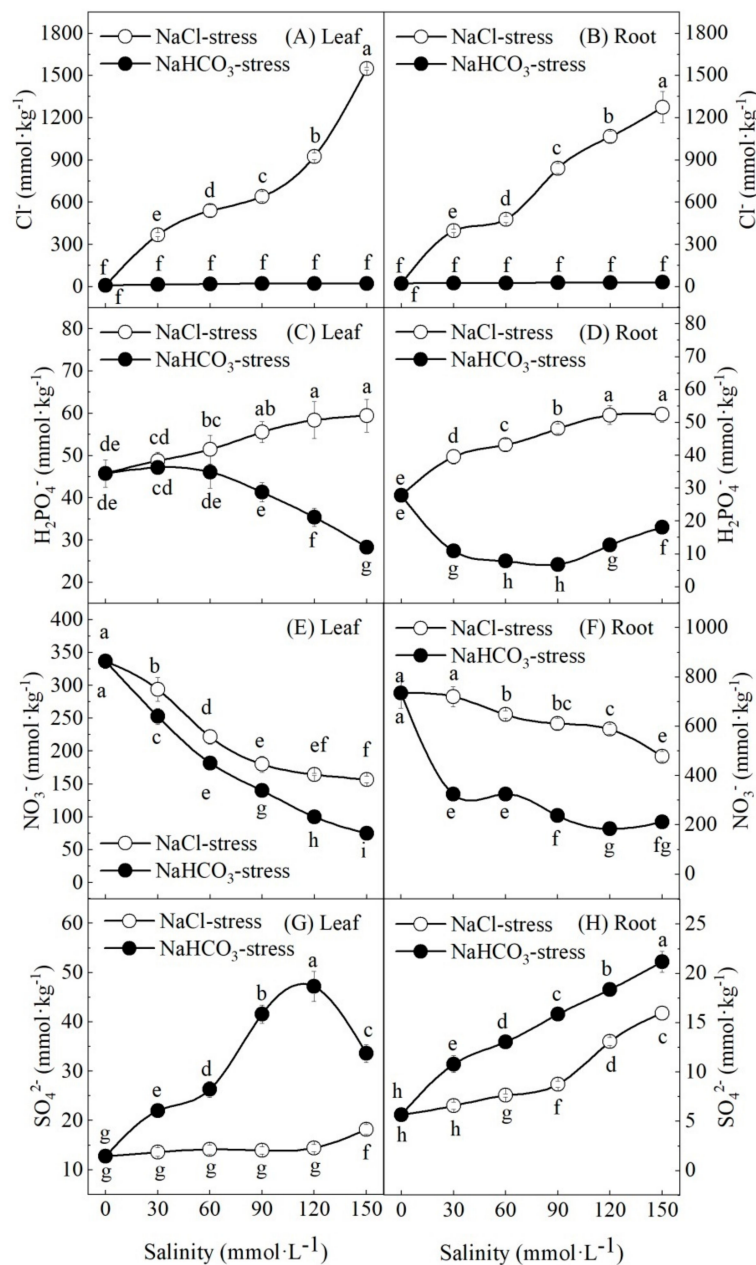


Figure 5. Effects of NaCl and NaHCO₃ stresses on Cl⁻ (A,B), H₂PO₄⁻ (C,D), NO₃⁻ (E,F), and SO₄²⁻ (G,H) concentrations in the leaves and roots of common bean (*Phaseolus vulgaris*). Common beans were treated with NaCl (30–150 mmol·L⁻¹, pH 6.65–6.86) and NaHCO₃ (30–150 mmol·L⁻¹, pH 8.17–8.27) stresses. Data points and bars represent the mean ± SD of three replicates. Values followed by different letters in the same subgraph are significantly different at the 5% level among the various concentration treatments of neutral salt conditions or alkaline salt conditions, according to Duncan's new multiple range method.

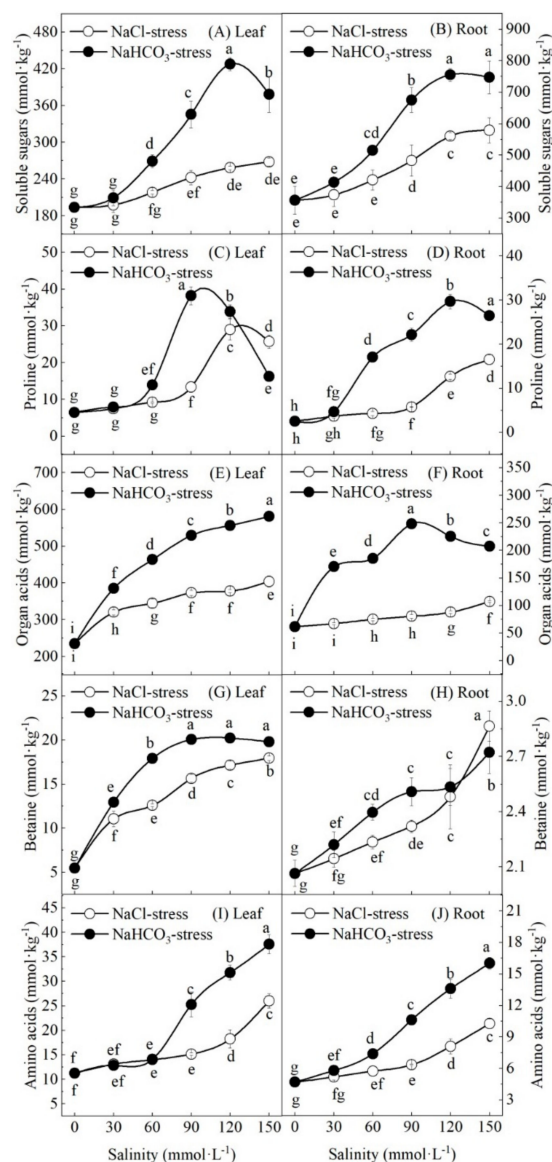


Figure 6. Effects of NaCl and NaHCO₃ stresses on soluble sugar (A,B), proline (C,D), organic acid (E,F), betaine (G,H), and amino acid (I,J) content in the leaves and roots of common bean (*Phaseolus vulgaris*). Common beans were treated with NaCl (30–150 mmol·L^{−1}, pH 6.65–6.86) and NaHCO₃ (30–150 mmol·L^{−1}, pH 8.17–8.27) stresses. Data points and bars represent the mean ± SD of three replicates. Values followed by different letters in the same subgraph are significantly different at the 5% level among the various concentration treatments of neutral salt conditions or alkaline salt conditions, according to Duncan's new multiple range method.

3.6. Effects of Neutral Salt and Alkaline Salt Stresses on Contribution Rates of Each Osmoregulators

Tables 2 and 3 demonstrate that the contribution rate of Cl[−] to the total negative charge in the leaves and roots was the highest under neutral salt stress, while the organic acids, soluble sugars, and NO₃[−] had relatively high contribution rates under either neutral or alkaline salt stress, with their contribution rates being higher under alkaline salt stress. In addition to the changes in the contribution rates of different osmoregulators with increasing salt- and alkali stress, the present study also demonstrated that neutral salt stress promoted a continuous increase in the contribution rates of Cl[−] and proline with an increase in stress intensity, while alkaline salt stress showed an enhancing effect on the content of SO₄^{2−}, soluble sugar, proline, organic acids, and free amino acids.

Table 2. Contribution of each solute molarity to the total determined osmoregulators molarity in common bean leaves under neutral salt and alkaline salt stresses. Percentages were calculated from the mean values of each solute.

Treatment		Na ⁺ (%)	K ⁺ (%)	Ca ²⁺ (%)	Mg ²⁺ (%)	Cl ⁻ (%)	H ₂ PO ₄ ⁻ (%)	NO ₃ ⁻ (%)	SO ₄ ²⁻ (%)	Soluble Sugars (%)	Proline (%)	Organ Acids (%)	Betaine (%)	Amino Acids (%)
NaCl Stress	Control	43.65	1.32	14.47	7.48	0.35	1.87	14.34	0.54	8.25	0.28	10.01	0.23	0.48
	30	37.43	4.88	15.61	5.08	11.41	1.46	9.08	0.42	6.11	0.23	9.90	0.34	0.41
	60	34.47	5.06	15.78	4.67	15.92	1.48	6.56	0.42	6.45	0.27	10.18	0.37	0.41
	90	30.52	8.91	9.09	3.91	17.16	1.60	4.82	0.37	6.50	0.36	10.00	0.42	0.41
	120	25.92	8.76	7.55	3.08	22.65	1.59	4.03	0.35	6.33	0.71	9.25	0.42	0.45
	150	20.08	8.38	5.91	2.01	31.46	1.40	3.19	0.37	5.44	0.52	8.21	0.36	0.53
NaHCO ₃ Stress	30	43.65	1.32	15.61	5.23	0.35	1.87	14.34	0.54	8.25	0.28	10.01	0.23	0.48
	60	43.32	5.92	16.50	4.62	0.46	1.51	8.25	0.72	6.83	0.26	12.58	0.42	0.42
	90	38.25	9.23	9.36	3.31	0.56	1.39	5.50	0.80	8.15	0.42	14.03	0.54	0.43
	120	30.10	13.08	6.09	2.72	0.66	1.31	3.89	1.16	9.61	1.06	14.72	0.56	0.70
	150	23.68	15.67	5.05	1.44	0.69	1.22	2.89	1.36	12.36	0.98	16.08	0.58	0.92

Table 3. Contribution of each solute molarity to the total determined osmoregulators molarity in common bean roots under neutral salt and alkaline salt stresses. Percentages were calculated from the mean values of each solute.

Treatment		Na ⁺ (%)	K ⁺ (%)	Ca ²⁺ (%)	Mg ²⁺ (%)	Cl ⁻ (%)	H ₂ PO ₄ ⁻ (%)	NO ₃ ⁻ (%)	SO ₄ ²⁻ (%)	Soluble Sugars (%)	Proline (%)	Organ Acids (%)	Betaine (%)	Amino Acids (%)
NaCl Stress	Control	45.83	1.99	6.23	1.21	0.87	1.05	27.74	0.21	13.48	0.10	2.33	0.08	0.18
	30	40.05	12.26	4.37	0.84	10.17	1.01	18.44	0.17	9.56	0.09	1.72	0.05	0.13
	60	36.33	14.30	4.36	0.69	12.14	1.10	16.42	0.19	10.69	0.11	1.90	0.06	0.15
	90	31.62	14.59	3.48	0.51	19.13	1.10	13.88	0.20	10.99	0.13	1.83	0.05	0.14
	120	28.01	14.10	2.66	0.42	22.92	1.12	12.65	0.28	12.07	0.27	1.90	0.05	0.17
	150	25.83	13.95	2.51	0.37	26.65	1.10	10.00	0.33	12.10	0.35	2.25	0.06	0.21
NaHCO ₃ Stress	30	47.58	15.51	5.72	0.86	0.82	0.34	10.00	0.33	12.80	0.14	5.27	0.07	0.18
	60	40.92	18.14	6.39	0.62	0.80	0.23	9.60	0.38	15.18	0.50	5.47	0.07	0.22
	90	34.21	21.03	6.80	0.55	0.80	0.20	6.85	0.46	19.47	0.64	7.16	0.07	0.31
	120	23.11	27.14	7.24	0.48	0.90	0.41	5.93	0.59	24.38	0.96	7.27	0.08	0.44
	150	21.54	28.49	5.45	0.52	1.02	0.58	6.84	0.68	24.18	0.86	6.72	0.09	0.52

4. Discussion

The emergence process reflects seed vigor and germination ability, and it is an optimum index used to measure degrees of stress and the responses of plants to various stresses at the seed germination stage. The speed of seed germination depends on the speed of water absorption. Liu et al. [39] reported that at solute concentrations of $<0.4 \text{ mol} \cdot \text{L}^{-1}$, seeds are only able to absorb water very slowly from soil solutions. In the present study, salt concentrations of $\geq 60 \text{ mmol} \cdot \text{L}^{-1}$ of both NaCl and NaHCO₃ resulted in significant delays in common bean seedling emergence, and the seedling emergence rate also dropped significantly (Figure 1A,B). Salinity may have reduced the rate of water absorption, impaired cell membrane reparations during imbibition, and even aggravated the damage to the membrane structure, leading to exudation of solutes from the seed [12] and even the entry of toxic ions (e.g., Na⁺). With increasing salinity and treatment duration, the damage intensified, and finally impacted seed germination and seedling survival rates. Compared with the C treatment, salt and alkaline stress concentration of $30 \text{ mmol} \cdot \text{L}^{-1}$ had no significant effect on the seedling emergence time or emergence rate of common bean, which could be attributed to the fact that relatively low NaCl or NaHCO₃ concentrations did not suppress water absorption in common bean seeds. Seedling survival was suppressed under all NaHCO₃ concentrations, but was only significantly suppressed at NaCl concentrations $>60 \text{ mmol} \cdot \text{L}^{-1}$, (Figure 1C). Therefore, in contrast to neutral salt stress, changes in seedling emergence time and rate, as well as survival rate, occurred more rapidly as the concentration of alkaline salt stress increased. This phenomenon indicates that high pH may be an important restrictive factor that affects the emergence process and survival of common bean seedlings. Although a high pH did not interfere with common bean seed water absorption at low alkaline salt concentrations, the radicles that broke through the seed coats were markedly injured by alkaline salts, and thus the

seeds were able to germinate but had difficulty forming normal seedlings that were able to survive in the long-term.

Leaf growth and biomass accumulation are ideal indicators for evaluating growth and development of common bean. In this study, despite the stress treatment (NaCl or NaHCO₃), dry matter accumulation in the leaves, stems, and roots, as well as leaf area and plant water content were all suppressed, and the degree of suppression was more severe under NaHCO₃ stress. In addition, the biomass and leaf area were all significantly low under all NaHCO₃ treatments than under all NaCl treatments, i.e., at all concentrations (Figure 1D–F; Figure 2A,B). At concentrations ≥ 90 mmol·L⁻¹, plant water content significantly differed between the NaHCO₃ and NaCl treatments, indicating that alkaline salt stress has a more obvious suppressive effect on common bean growth than neutral salt stress. Munns [7] concluded that neutral salt generally induces osmotic stress and ion-induced injury, while alkaline salt has an additional stress factor, i.e., high pH [16,40]. Numerous previous reports have shown that although it is a key limiting factor for plant growth and development, the effect of high pH on plant growth and development varies according to the different growth stages. In the present study, although NaHCO₃ stress at a low concentration (30 mmol·L⁻¹) did not significantly affect the emergence time and emergence rate of common bean seedlings, after emergence, the high pH environment around the radicle may have seriously reduced the supply of mineral nutrients and oxygen in the micro-domain of the root zone as the stress continued. Such stress may have also directly destroyed the cell structure and function in the roots, breaking the ionic balance [41], triggering disorders in nutrient absorption and metabolism [42,43], thereby resulting in more significant suppression of seedling growth and drastic reduction in the seedling survival rate.

The net photosynthetic rate of plant leaves usually decreases with increasing environmental stress intensity [44]. In the present study, the magnitude of decrease in P_n due to increasing NaHCO₃ stress was significantly higher than that due to NaCl stress (Figure 3A). These results not only verified that neutral salt environment and alkaline salt environment are two distinctive stress sources, but also indicated that common bean was more tolerant to neutral salt stress than to alkaline salt stress. Reduced plant P_n values under higher salt stress are generally considered to be the result of either reduced intracellular CO₂ partial pressure caused by stomatal closure or nonstomatal factors [45]. Koyro et al. [46] reported a significant correlation between the G_s and E of plant leaves and the water potential of the salt stress environment in which the plant exists. In the present study, WC, G_s , and E of the common bean all decreased gradually as NaCl and NaHCO₃ stress intensity increased, yet the magnitudes of decrease in G_s and E were greater under alkaline salt stress treatments (Figure 2B; Figure 3B,C). Neutral salt stress had relatively little effect on the C_i of common bean leaves, which was only significantly higher than that under C treatment at NaCl concentrations of 120–150 mmol·L⁻¹. However, at a low concentration (30 mmol·L⁻¹) of alkaline salt stress, the C_i decreased correspondingly. Nevertheless, at NaHCO₃ concentrations of ≥ 60 mmol·L⁻¹, the C_i of common bean leaves increased significantly (Figure 3D). This could be attributed to the high pH environment (as a result of the increase in alkaline salt stress intensity), which caused a gradual decrease in the amount of water absorbed by the roots, thereby leading to a physiological water shortage in the plant that indirectly affected stomatal opening and gaseous exchange. Nonstomatal factors mainly depend on the cumulative effects of leaf water potential and osmotic potential, biochemical constituents [47], photosynthetic pigment content [48], ion toxicity in the cytosol [49], etc. Under neutral salt stress conditions, photosynthetic pigment content (Chl a , Chl b , and Car) did not change significantly in common bean leaves under NaCl treatment, except at concentrations of 120–150 mmol·L⁻¹, which significantly suppressed the accumulation of Chl a , Chl b , and Car. In contrast, under NaHCO₃ stress conditions, the content of these three photosynthetic pigments decreased significantly with an increase in stress intensity. This indicated that compared to neutral salt stress, photosynthetic pigment content, net photosynthetic rate of common bean leaves, and leaf area per plant were more significantly suppressed as a result of resistance and adaptation to the alkaline salt stress environment. The photosynthetic capacity of

the seedlings was limited to a larger extent in the alkaline salt stress environment, which severely restricted quality, growth, and the development processes of common bean seedlings.

Under salt and alkali stresses, plants usually accumulate a large amount of Na^+ to reduce the cell water potential. As Na^+ is the main toxic ion in saline-alkali soil, low concentrations of Na^+ and high concentrations of K^+ are crucial in the maintenance of a series of enzymatic reactions in the plant cytoplasm [50]. Meanwhile, during plant growth and development, the intracellular ionic balance mechanism ensures that the pH of the plant body is stable, regardless of the pH of the living environment, to maintain the normal metabolism of the plant; provided that the plant has the ability to adapt to the environment [12]. The results of the present study revealed that NaHCO_3 stress (except at concentrations of 120–150 $\text{mmol}\cdot\text{L}^{-1}$) had no significant effect on the pH of common bean leaves, indicating that the common bean may have maintained a stable intracellular pH environment by utilizing an ionic balance mechanism in vivo, which was broken down by the high concentration of alkaline salt stress. K^+ concentration was generally higher than Na^+ concentration in the common bean leaves and roots (except for leaves under 150 $\text{mmol}\cdot\text{L}^{-1}$ NaHCO_3 stress and roots under 120–150 $\text{mmol}\cdot\text{L}^{-1}$ NaHCO_3 stress); this was consistent with the findings of previous studies [25,26]. NaCl or NaHCO_3 stresses at low concentrations had similar effects on Na^+ concentration in common bean leaves and roots, as well as on the Na^+/K^+ ratio. However, at concentrations $>60\text{ mmol}\cdot\text{L}^{-1}$, Na^+ concentration and Na^+/K^+ ratio under NaCl stress increased slowly as the stress concentration further increased, while both increased significantly under NaHCO_3 stress. This indicated that common bean root cells were able to relieve the adverse effect due to the invasion of a large amount of Na^+ into the intracellular environment under stresses from neutral salt at various concentrations (30–150 $\text{mmol}\cdot\text{L}^{-1}$) and alkaline salt at certain concentrations (30–90 $\text{mmol}\cdot\text{L}^{-1}$) via the adaptive accumulation of K^+ in vivo. In contrast, the alkaline salt environment with high pH values and high salt concentrations (120–150 $\text{mmol}\cdot\text{L}^{-1}$) may have restricted the extrusion of Na^+ from the solute of common bean root cells to the outer environment, resulting in excessive invasion of Na^+ into the leaves and roots, and thus a rapid increase in the Na^+/K^+ ratio.

Guo et al. [18] assume that Ca^{2+} is closely related to alkaline salt tolerance. In *Arabidopsis*, the Ca^{2+} -responsive AtSOS3-AtSOS2 (AtCIPK24-AtCBL4) protein kinase pathway mediates the regulation of expression and activities of Na^+ transporters, such as AtSOS1 and AtNHX, which is a Na^+/H^+ exchanger that mediates Na^+ compartmentalization into vacuoles with Ca^{2+} as the key signal component in the SOS system [51]. In the present study, stress due to the neutral and alkaline salts at 30–60 $\text{mmol}\cdot\text{L}^{-1}$ concentration promoted an increase in Ca^{2+} concentration in the leaves and roots, which is also consistent with the findings of Yang et al. [11]. However, at relatively high salt concentrations (90–150 $\text{mmol}\cdot\text{L}^{-1}$ NaCl or NaHCO_3 for the leaves and 90–150 $\text{mmol}\cdot\text{L}^{-1}$ NaCl or 120–150 $\text{mmol}\cdot\text{L}^{-1}$ NaHCO_3 for the roots), the Ca^{2+} concentration decreased. Based on the above data, we believe that the increase in Ca^{2+} concentration induced by low concentrations of neutral or alkaline salts can immediately trigger the Na^+ exclusion system, to reduce the damage caused by Na^+ to the common bean plant. With the increase of stress intensity, compared with that of the NaCl stress, NaHCO_3 stress strongly enhances Ca^{2+} accumulation in the roots to better adapt to adverse environment and leads to a decrease in Ca^{2+} concentration in the leaves. At the same time, the suppressive effects on Mg^{2+} accumulation in leaves and roots became more obvious with the increase in stress concentrations of both neutral and alkaline salts, and high rhizosphere pH of NaHCO_3 stress can inhibit absorption and accumulation of Mg^{2+} from the roots to leaves more seriously.

In addition, it has been suggested that under salt stress, plants can increase the accumulation of inorganic ions, such as Cl^- , NO_3^- , and SO_4^{2-} , as well as the synthesis of organic osmolytes, such as soluble sugars, prolines, betaines, and polyols, to reduce the cell water potential and osmotic potential, to regulate ionic balance in vivo, and enhance plant tolerance to salt stress [52–54]. The results of the present study indicated that under neutral salt and alkaline salt stresses, Cl^- , H_2PO_4^- , NO_3^- , SO_4^{2-} concentrations and soluble sugar, proline, organic acids, betaine, free amino acids content in leaves and roots showed different change patterns with an increase in stress concentration. Moreover,

differences in their contributions to the total molar mass of osmoregulators were also observed and were dependent on the type of salt- and alkali stress. Accumulation of proline and betaine under neutral salt and alkaline salt stresses has been reported to be similar to that of Na^+ [12,39]. In the present study, although the content of proline and betaine in common bean leaves and roots was significantly affected by salt- and alkali stress, its proportion among all osmoregulators was relatively small. In general, under neutral salt or alkaline salt stress, common bean accumulates a large number of cations *in vivo* by producing organic acids, soluble sugars, proline, betaine, and inorganic ions, such as Cl^- and SO_4^{2-} .

Under neutral salt stress, the main osmotic regulators are likely the inorganic ions, especially Cl^- , followed by the metabolic pathways of proline and betaine. However, under alkaline salt stress, common bean is able to compensate for the lack of negative charge due to the decrease in inorganic anion content by synthesizing more organic acids and soluble sugars. Carbohydrates are the primary source of reserve energy stored in the vegetative organs of plants and provide energy and metabolites for the biosynthetic processes and growth [55]. Higher carbohydrate concentrations before and during the stress period indicate better tolerance [56]. A more dramatic increase in the content of organic acids and soluble sugars also suggested that alkaline salt stress altered the profiles of organic acids and soluble sugars for growth-maintenance. Stoop and Pharr [57] reported that the utilization of carbohydrates could be a limiting factor of growth under stress. Therefore, the accumulation of soluble sugars with a concomitant sharp decrease in biomass and leaf area in common bean plants under alkali stress is probably due to reduced utilization of carbohydrates in actively growing tissues. However, sugars might also be effective candidates for the oxidative stress in plant tissues through sugar signaling, triggering the production of specific reactive oxygen species (ROS) scavengers under environmental stresses [58]. In addition, to counteract alkaline salt stresses, some plants induce intrinsic stress defense mechanisms, of which the exudation of organic acids is one of stress responses, for example, the exudation of citric acid has been reported to be closely related to alkaline-salt stress [59] and high salinity [60]; malate plays an important role in maintaining ion balance in cotton [61] and tomato [19] under alkaline-salt stress. Our results indicated that organic acids content in the leaves is higher than that in the roots, and that alkaline salt stress strongly stimulated organic acid accumulation, but neutral salt stress only produced a small effect on the accumulation of organic acids in common bean leaves and roots. However, the regulation of organic acid and soluble sugar metabolism is complex, and involves carbon assimilation [62], nitrogen metabolism [63], and other biochemical pathways, further studies are required to elucidate the regulatory mechanism of the common bean in accumulating organic acids and soluble sugars to adapt to neutral and alkaline salt environments.

5. Conclusions

In the process of resisting alkaline salt stress, the common bean not only needs to deal with a high concentration of toxic ions and an indirect barrier to water absorption, but also needs to regulate the relatively high pH environment in its root zone. Compared with neutral salt stress, alkaline salt stress was shown to more seriously suppress germination, growth, and photosynthesis in common bean, as well as curb its seedling establishment to a great extent. Under neutral salt or alkaline salt stress, common bean root cells were able to alleviate the adverse effects caused by a large influx of Na^+ into the intracellular environment through adaptive accumulation of K^+ *in vivo*, and thus an appropriate Na^+/K^+ ratio was maintained in the leaves and roots. However, this mechanism was more likely to be broken by alkaline salt stress. In addition, increased synthesis of organic acids, soluble sugars, and other compounds was found to be an important adaptive mechanism against alkaline salt stress, which helped to maintain a stable intracellular pH environment by balancing the large inflow of cations into common bean cells. Therefore, organic acids and soluble sugars are possible key indicators for the selective breeding of salt-alkali tolerant common bean varieties, and adjusted carbon partitioning and allocation *in vivo*, and exogenous organic acid application could have an important implication on common bean production in saline-alkali areas.

Author Contributions: S.Y., L.Y., and W.G. carried out all experiments concerning common bean seedlings. S.Y., Y.H., and Y.X. performed data acquisition and analysis. Y.Z. and L.Y. conceived and designed the study, wrote and revised the manuscript, and approved the final version for submission.

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