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Effects of $NaHCO_3$ Stress on the Growth and Physiological Indexes of Pumpkin Seedlings

Yong-Dong Sun and Wei-Rong Luo
School of Horticulture and Landscape Architecture, Henan Institute of Science and Technology, Xinxiang, Henan, 453003, China

Abstract: Few studies have investigated the alkali-based salt resistance aspect of plants. Here, we used 2 pumpkin cultivars ('*Cucurbita ficifolia* Bouch.' and 'Chuandilong') to study the growth and physiological response mechanisms of pumpkin to different levels of $NaHCO_3$ stress. After second true leaf development, pumpkin seedlings were treated with varying levels of $NaHCO_3$ stress (0, 30, 60 and 90 mmol/L) during sand culture. The chlorophyll content, electrolyte leakage, malondialdehyde (MDA) contents, proline contents, soluble sugar contents and superoxide dismutase (SOD) and peroxidase (POD) activities of both cultivars were investigated. Finally, the growth and physiological indexes of pumpkin seedlings under $NaHCO_3$ stress were explored. The results showed that the chlorophyll a, chlorophyll b and total chlorophyll of both pumpkin seedlings increased with exposure to low $NaHCO_3$ concentrations (0-30 mmol/L) and then decreased with exposure to 30-90 mmol/L$NaHCO_3$ and all reached their minimum values with exposure to 90 mmol/L $NaHCO_3$. Under the latter stress, the rate of decrease in total chlorophyll content of '*C. ficifolia* Bouch.' was higher than that of 'Chuandilong'. However, electrolyte leakage, MDA content, proline content, soluble sugar content and SOD and POD activities increased with the escalation of $NaHCO_3$ concentration and peaked after exposure to 90 mmol/L$NaHCO_3$. The rates of increase in electrolyte leakage and MDA contents of '*C. ficifolia* Bouch.' were higher than those of 'Chuandilong' at this $NaHCO_3$ concentration. These results suggested that under greater $NaHCO_3$ stress, these pumpkin cultivars showed significant inhibition of growth, with '*C. ficifolia* Bouch.' being more sensitive than 'Chuandilong' to $NaHCO_3$ stress. Injury caused by $NaHCO_3$ stress was most likely mitigated by increasing the antioxidant activities of SOD and POD and accumulating proline and soluble sugar contents to facilitate osmotic adjustment.

Keywords: Growth, $NaHCO_3$ stress, physiological indexes, pumpkin seedlings

INTRODUCTION

The 2 major environmental factors currently responsible for reduction of plant productivity are drought and salinity. Salinity is one of the major obstacles to increasing production in crop growing areas throughout the world. The existing salinity problems are likely to worsen due to rapidly growing human populations in many countries and increasing concerns over limited water resources, which forces growers to use poor quality water for irrigation. Of the cultivated land, about 9.0×10^8 ha (60%) are saline and sodic, which influences plant growth and crop productivity (Läuchli and Lüttge, 2002). In China, about 2.6×10^7 ha lands experience salinity problems.

In saline and sodic soils, Na^+, Ca^{2+}, Mg^{2+} and K^+ are the main cations of dissoluble mineral salts and Cl^-, SO_4^{2-}, HCO_3^-, CO_3^{2-} and NO_3^- are the corresponding main anions (Läuchli and Lüttge, 2002). We can further classify neutral salts (NaCl and Na_2SO_4) and alkaline salts ($NaHCO_3$ and Na_2CO_3), in terms of the characteristics of the salt ions. Alkaline salts consequentially elevate the soil pH, which causes plants to experience the damaging effects of both salt and alkali stress. Thus, the problem of soil alkalization due to $NaHCO_3$ and Na_2CO_3 may be more severe than the problem of soil salinization caused by NaCl and Na_2SO_4. However, to date, an increasing number of reports on distribution, exploitation (Zhao *et al*., 2002) and physiological mechanisms (Peng *et al*., 2004) of NaCl-based salt resistance of halophytes have been published, while few studies have been performed on the alkaline salts resistance aspect of plants (Shi and Sheng, 2005; Shi and Wang, 2005; Wang *et al*., 2011; Yang, 2012).

Pumpkin (*Cucurbita moschata* Duch.) is a popular vegetable and is used as graft material for cucurbit culture under protected cultivation conditions. With aggravation of secondary salinization of soil, many factors that cause an increase in the salt tolerance of pumpkin have been identified by researching the physiological responses of pumpkin to salt stress. In the

Corresponding Author: Yong-Dong Sun, School of Horticulture and Landscape Architecture, Henan Institute of Science and Technology, Xinxiang, Henan, 453003, China

pumpkin, NaCl stress decreases the fresh weight of roots and shoots, the length of the main stem, the number of functional leaves and increases the MDA contents and activities of SOD, POD and CAT (Zhou *et al*., 2007). In addition, Sun *et al*. (2008) had found that Na_2CO_3 stress significantly inhibited the germination rate and increased the soluble sugar contents and SOD activities of pumpkin seeds. Zhao *et al*. (2006) investigated the difference between the effects of salt and alkali stress on pumpkin and indicated that alkali stress had more adverse effects than salt.

However, to date, little is known about the growth and physiological response mechanisms of pumpkin to different levels of $NaHCO_3$ stress. In this study, we describe the effects of $NaHCO_3$ stress on the growth, membrane permeability, MDA contents, proline contents, soluble sugar contents and antioxidant enzyme activities of 2 pumpkincultivars. Our data show that under higher $NaHCO_3$ stress, pumpkin cultivars demonstrated significant inhibition of growth and '*C. ficifolia* Bouch.' was more sensitive than 'Chuandilong' to $NaHCO_3$ stress and injury caused by $NaHCO_3$ stress was most likely mitigated by increasing the antioxidant activities of SOD and POD and increasing proline and soluble sugar contents.

MATERIALS AND METHODS

Plant materials and $NaHCO_3$ concentration treatments: Two pumpkin cultivars ('*C. ficifolia* Bouch.' and 'Chuandilong') were used. Pumpkin seeds and seedlings were grown in a greenhouse under natural light conditions at 28-30°C and 60-70% relative humidity. Pumpkin seeds were disinfected by soaking in 0.1% $HgCl_2$ for 10 min and washing 3 times with distilled water. Then, seeds were sown in 50 seedling plug trays filled with a 2:1 (v/v) mixture of peat and vermiculite. Seven days later, pumpkin seedlings, at the fully expanded cotyledon stage, were transferred to plastic pots, which had been filled with coarse beach sand washed with fresh water before use. Pumpkin seedlings were irrigated with half-strength Hoagland nutrient solution every 2 days.

When the second true leaf of the seedlings had developed completely, thirty-six pots of uniformly growing seedlings were chosen and randomly divided into 4 sets, 3 pots per set, each set contained 3 replicates. $NaHCO_3$ were dissolved into the nutrient solution directly and 4 levels of 0 (control), 30, 60 and 90 mmol/L $NaHCO_3$ concentrations were prepared. The 4 treatments levels were applied to the pumpkin seedlings of 4 sets, respectively.

Determination of physiological indexes: The true leaves of pumpkin seedlings grown for 7 days under different $NaHCO_3$ treatment conditions were excised and rapidly frozen in liquid nitrogen for the determination of physiological indexes. The contents of Chlorophyll a, Chlorophyll b, total chlorophyll were analyzed spectrophotometrically using a TU1810 spectrophotometer (Puxi, Beijing, China), according to the method of Zhang and Qu (2003). Membrane permeability of the excised leaves was measured as percentage electrical conductivity, as described by Alpaslan and Gunes (2001). MDA content was determined using the thiobarbituric acid reaction, as described by Sudhakar *et al*. (2001). Proline content of the leaves was determined following the method of Sairam *et al*. (2002). Soluble sugar content was quantified by the anthrone sulfuric acid method (Fales, 1951). SOD activity was determined according to the method of Meloni *et al*. (2003) and POD activity was determined using the guaiacol oxidation method of Zhou *et al*. (2003).

Statistical analysis: All data were subjected to Analysis of Variance (ANOVA) using the SPSS version 10.0 statistical package for Windows. Where the F-test showed significant differences among means, Duncan's multiple range tests were applied, at the 0.05 level of probability, to separate means.

RESULTS

Effects of $nahco_3$ stress on chlorophyll contents of pumpkin seedlings: $NaHCO_3$ stress significantly affected the chlorophyll contents of both pumpkin cultivars (Table 1). The chlorophyll a, chlorophyll b and total chlorophyll of both pumpkin cultivars increased at lower $NaHCO_3$ concentration (0-30 mmol/L) and then decreased with increasing levels of $NaHCO_3$ concentration. Chlorophyll a, chlorophyll b and total chlorophyll levels all reached a minimum value after exposure to 90 mmol/L $NaHCO_3$. The contents of chlorophyll a, chlorophyll b and total chlorophyll in '*C. ficifolia* Bouch.' increased by 102.11, 54.67 and 85.71%, respectively, over control values after exposure to 30 mmol/L $NaHCO_3$, whereas those in 'Chuandilong' increased by 81.10, 32.59 and 62.16%, respectively. Moreover the contents of chlorophyll a, chlorophyll b and total chlorophyll in '*C. ficifolia* Bouch.' decreased by 51.06, 54.67 and 109.66%, respectively, of the control values, after exposure to 90 mmol/L $NaHCO_3$, respectively, whereas those in 'Chuandilong' decreased by 59.58%, 37.27 and 103.56%, respectively. Under90 mmol/L $NaHCO_3$ stress, the rate of decrease in total chlorophyll content of '*C. ficifolia* Bouch.' was higher than that of 'Chuandilong'.

Effects of $NaHCO_3$ stress on electrolyte leakage and MDA contents of pumpkin seedlings: The electrolyte leakage and MDA contents of the two pumpkin cultivars increased with increasing $NaHCO_3$

Table 1: Effects of $NaHCO_3$ stress on chlorophyll contents of pumpkin seedlings

Materials	$NaHCO_3$ (mmol/L)	Chlorophyll a content (mg/g)	Chlorophyll b content (mg/g)	Total chlorophyll content (mg/g)
'*C. ficifolia* Bouch.'	0	5.68b	3.00b	8.68c
	30	11.48a	4.64a	16.12a
	60	6.49b	3.31b	9.80b
	90	2.78c	1.36c	4.14d
'Chuandilong'	0	7.67c	4.91b	12.58c
	30	13.89a	6.51a	20.40a
	60	11.10b	5.31b	16.41b
	90	3.10d	3.08c	6.18d

Lower case letters indicate significant differences at $p<0.05$

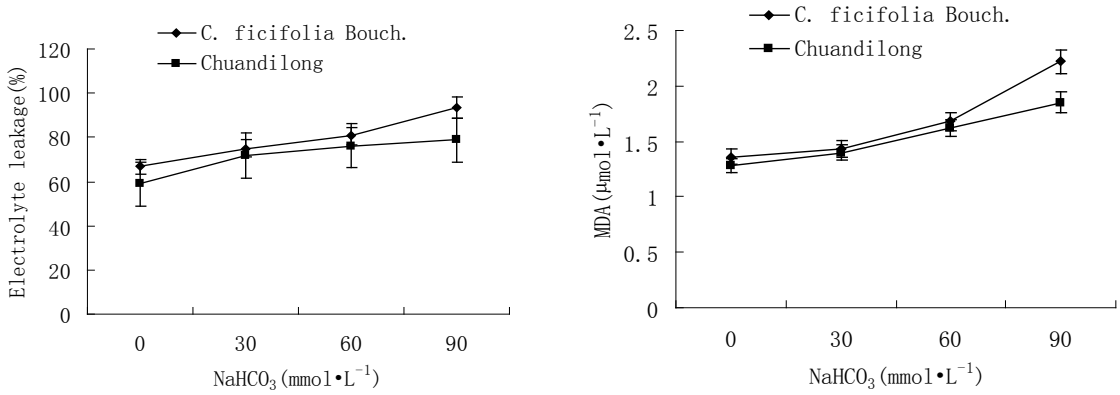

Fig. 1: Effects of $NaHCO_3$ stress on electrolyte leakage and MDA contents of pumpkin seedlings

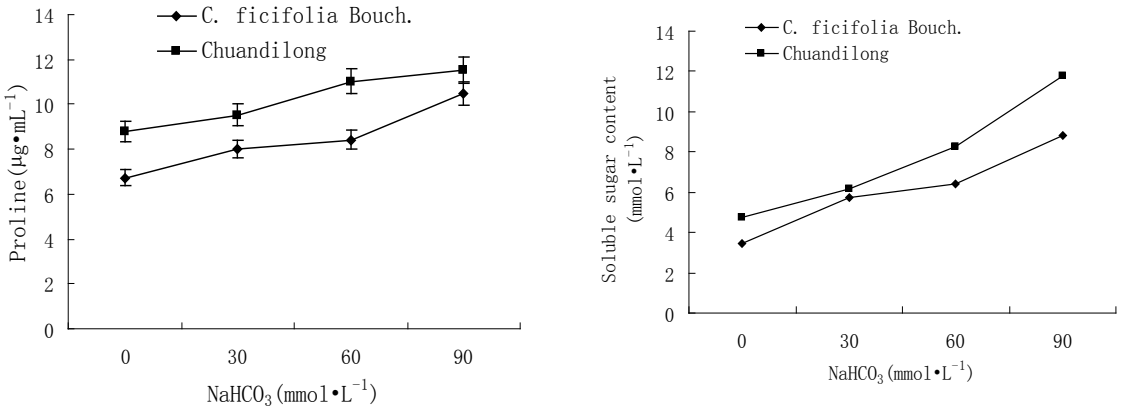

Fig. 2: Effects of $NaHCO_3$ stress on proline and soluble sugar contents of pumpkin seedlings

concentrations. The increased rate in electrolyte leakage of '*C. ficifolia* Bouch.' was higher than that of 'Chuandilong' with 90 mmol/L $NaHCO_3$ concentration. For instance, the electrolyte leakage of '*C. ficifolia* Bouch.' increased by 39.99%, while those of 'Chuandilong' increased by 34.09% over the control (Fig. 1). Under $NaHCO_3$ stress, MDA contents of '*C. ficifolia* Bouch.' were higher than those of 'Chuandilong'. In comparison to the control, the MDA contents of '*C. ficifolia* Bouch.' and 'Chuandilong' increased by 62.73 and 44.87% with 90 mmol/L $NaHCO_3$ concentration, respectively (Fig. 1).

Effects of $NaHCO_3$ stress on the proline and soluble sugar contents of pumpkin seedlings: Effects of $NaHCO_3$ stress on the proline and soluble sugar contents of pumpkin seedlings were shown in Fig. 2. $NaHCO_3$ stress progressively increased the proline and soluble sugar contents of both pumpkin cultivars. With the increase in $NaHCO_3$ concentration, more proline and soluble sugar contents accumulated in '*C. ficifolia* Bouch.' than in 'Chuandilong'. Specifically, the proline contents and soluble sugar contentswas 1.56- and 2.50-fold higher than those of the control for '*C. ficifolia* Bouch.' after exposure to 90 mmol/L $NaHCO_3$

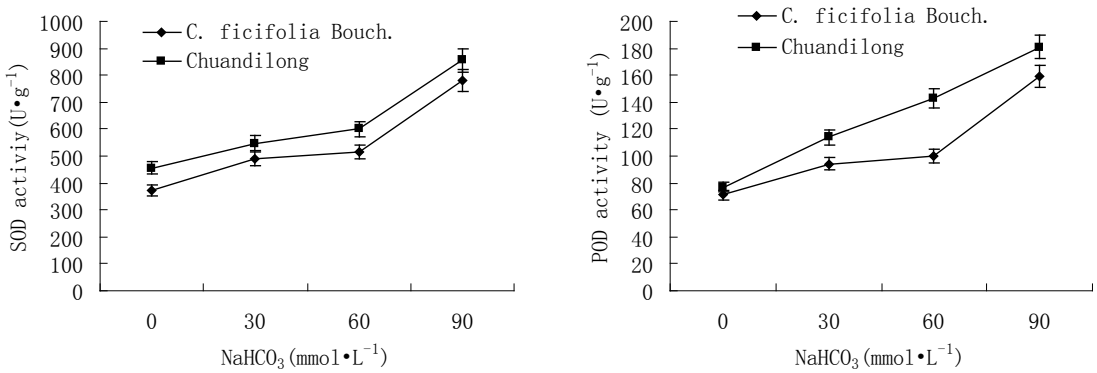

Fig. 3: Effects of $NaHCO_3$ stress on SOD and POD activities of pumpkin seedlings

concentration and 1.31-and 2.55-fold higher, respectively, for 'Chuandilong'.

Effects of $NaHCO_3$ stress on the activities of SOD and POD of pumpkin seedlings: Figure 3 shows the changes in SOD and POD; the activities of SOD and POD in the 2 pumpkin cultivars exhibited similar trends, viz., an increase with increasing $NaHCO_3$ stress. In comparison to the control, the activities of SOD and POD in '*C. ficifolia* Bouch.'increased by 109.12 and 123.94%, respectively, after exposure to 90 mmol/L $NaHCO_3$ concentration, while those in 'Chuandilong' increased by 87.72 and 135.06%, respectively. The SOD activities in '*C. ficifolia* Bouch.' were higher than those of 'Chuandilong' under $NaHCO_3$ stress, but POD activities were lower.

DISCUSSION

Soil salinity is a prevalent abiotic stress for plants. Among the cellular organs of plants, the chloroplast is relatively sensitive to salt stress. Srivastava *et al*. (1988) reported chlorophyll content as one of the parameters of salt tolerance in crop plants. Hernandez *et al*. (1995) observed more chlorophyll degradation in a NaCl-sensitive pea cultivar as compared to a tolerant cultivar. In the present study, the contents of chlorophyll a, chlorophyll b and total chlorophyll in both pumpkin cultivars all increased with lower $NaHCO_3$ concentrations and then decreased with exposure to higher concentrations of $NaHCO_3$. Under higher $NaHCO_3$ stress, the rate of decrease in total chlorophyll contents of '*C. ficifolia* Bouch.' was higher than that of 'Chuandilong', which suggested that '*C. ficifolia* Bouch.' was more sensitive than 'Chuandilong' to $NaHCO_3$ stress.

Membrane permeability is a sensitive test for determining salt stress and tolerance (Mansour and Salama, 2004). Higher membrane stability may be correlated with abiotic stress tolerance (Meloni *et al*., 2003). In the present study, electrolyte leakage and MDA contents increased significantly under higher $NaHCO_3$ stress and the increase was greater in '*C. ficifolia* Bouch.' than in 'Chuandilong', which indicated that membrane stability had been destroyed and lipid peroxidation had occurred and that '*C. ficifolia* Bouch'. was more sensitive than 'Chuandilong' to $NaHCO_3$ stress.

The accumulation of some organic solutes under saline conditions has been considered to represent an adaptation of plants against osmotic stress. Proline and soluble sugar are the primary substance for osmotic adjustment in plant cells (Gilbert *et al*., 1998). Our results revealed increased proline and soluble sugar contents under $NaHCO_3$ stress, which indicated that proline and soluble sugar can help lower the osmotic potential in the plant.

Reactive Oxygen Species (ROS) are easily produced in plant cells by osmotic stress and can injure the plant cells if they are not eliminated in time (Zhang *et al*., 2005). An efficient antioxidant system is important in combating salinity stress (Rout and Shaw, 2001). An increase in the activity of antioxidant enzymes under salt stress could be indicative of an increased production of ROS and suggests escalation of a protective mechanism to reduce oxidative damage triggered by stress. The key enzymes in eliminating ROS are SOD and POD (Gomez *et al*., 2004). In our study, the activities of SOD and POD in the 2 pumpkin cultivars increased significantly under $NaHCO_3$ stress, which suggested that the antioxidant defense system of the pumpkin seedlings was enhanced rapidly to scavenge all ROS generated by $NaHCO_3$ stress.

CONCLUSION

In conclusion, under higher $NaHCO_3$ stress, the 2 pumpkin cultivars studied here showed significant inhibition of the growth and '*C. ficifolia* Bouch.' was more sensitive than 'Chuandilong' to $NaHCO_3$ stress. Injury caused by $NaHCO_3$ stress was most likely mitigated by an increase in the antioxidant activities of SOD and POD and by accumulation of proline and soluble sugar contents, to facilitate osmotic adjustment.

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