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Original Research Paper

Exogenous Hydrogen Sulphide Protection of *Cucurbita ficifolia* Seedlings against $NaHCO_3$ Stress

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ABSTRACT

Salinity is one of the main environmental stresses that affect plant growth and development. H_2S plays an important role in a variety of responses against abiotic stresses. This study was conducted to determine the effects of exogenous NaHS, a hydrogen sulphide (H_2S) donor on physiological characteristics of *Cucurbita ficifolia* seedlings under $NaHCO_3$ stress. The accumulation of chlorophyll content, malondialdehyde (MDA), soluble protein and soluble sugar along with the activities of superoxide dismutase (SOD) and peroxidase (POD) in leaves were estimated. $NaHCO_3$ stress decreased the chlorophyll content, increased the accumulation of MDA, soluble protein and soluble sugar, and improved SOD and POD activities in leaves, compared to the control. However, exogenous NaHS treatment (1.0 mmol/L) significantly increased the chlorophyll content, soluble protein and soluble sugar contents, and decreased the MDA content, SOD and POD activities of $NaHCO_3$-stressed seedlings. It is strongly suggested that $NaHCO_3$ stress inhibits the growth of *Cucurbita ficifolia* seedlings, while exogenous H_2S can effectively protect *Cucurbita ficifolia* seedlings against $NaHCO_3$ stress.

INTRODUCTION

Salinity is one of the major abiotic stresses that adversely affect crop productivity and quality, and puts various problems to the plants either at the population, organism or even at the molecular level (Mane et al. 2010). Nearly 20% of the world's cultivated area and nearly half of the world's irrigated lands are affected by salinity (Zhu et al. 1998). In China, about 2.6 million hectares land experience salinity problems. Further, the existing salinity problems are likely to worsen due to the rapidly growing human population in many countries and increasing concerns over limited water resources. Soil salinity diminishes the economic yield and quality of production in most of the agricultural crops by decreasing the osmotic potential of the soil solution and interfering with normal nutrient uptake, including ionic toxicity, and associating nutrient imbalances. Salinities of soil can be further classified as neutral salinities (NaCl and Na_2SO_4) and alkaline salinities ($NaHCO_3$ and Na_2CO_3), in terms of the characteristics of the salt ions. Some reports have clearly demonstrated that alkaline salinities are more destructive to plants than neutral salts (Shi & Sheng 2005, Shi & Wang 2005, Yang et al. 2008). However, up to date, a few studies have been performed on the alkaline salinities resistance aspect of plants (Shi & Sheng 2005, Shi & Wang 2005, Wang et al. 2011, Yang 2012).

H_2S together with nitric oxide (NO) and carbon monoxide (CO) have recently been identified as endogenous gaseous transmitters in animals, wherein they play various physiological roles (Yang et al. 2008). In plants, it has been confirmed that H_2S also acts as an antioxidative signal molecule to play a role in a variety of responses against abiotic stresses. Previous works have indicated that H_2S is associated with the tolerance of wheat seeds to Cu (Zhang et al. 2008) and Al (Zhang et al. 2010), sweet potato seedlings to osmotic stress (Zhang et al. 2009), wheat seedlings to water stress (Shan et al. 2011), and cucumber seeds to Ge (Yu et al. 2011). However, no study has been encountered concerning effects of H_2S on oxidative damage in plant seedlings under $NaHCO_3$ salinity condition until now.

Cucurbita ficifolia is used as graft material for cucurbit culture under protected cultivation conditions. The seed germination and seedling growth of *Cucurbita ficifolia* are significantly affected by increases in soil salinity (Sun & Luo 2013). Little is known about the effects of exogenous H_2S treatment on oxidative damage and antioxidant enzyme in *Cucurbita ficifolia* seedlings under $NaHCO_3$ stress, and its possible mechanism in protecting *Cucurbita ficifolia* seedlings against soil salinity stress is yet to be confirmed. In the present study, the effect of exogenous H_2S treatment on chlorophyll content, soluble protein, soluble sugar, antioxidant enzyme activity and lipid peroxidation in

Cucurbita ficifolia seedlings under $NaHCO_3$ stress were investigated. We conclude that exogenous H_2S acting as an antioxidative signal molecule, effectively protected *Cucurbita ficifolia* seedlings against soil salinity stress.

MATERIALS AND METHODS

Plant materials and treatments: In order to study the effects of exogenous NaHS, a hydrogen sulphide (H_2S) donor, on physiological characteristics of *Cucurbita ficifolia* seedlings under $NaHCO_3$ stress, an experiment was conducted in the lab of the School of Horticulture and Landscape Architecture, Henan Institute of Science and Technology, Xinxiang, China, during 2012. *Cucurbita ficifolia* was used to study the physiological characteristics. NaHS was purchased from Sigma and used as an exogenous H_2S donor. *Cucurbita ficifolia* seeds and seedlings were grown in a greenhouse under natural light conditions at 28-30°C and 60-70% relative humidity. *Cucurbita ficifolia* 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. Ten days later, *Cucurbita ficifolia* seedlings, at the fully expanded cotyledon stage, were transferred to plastic pots, which had been filled with coarse beach sand washed with freshwater before use. *Cucurbita ficifolia* seedlings were irrigated with half-strength Hoagland nutrient solution every 2 days. When the second true leaf of the seedlings has developed completely, uniformly growing seedlings were chosen to study the effects of H_2S on oxidative damage in *Cucurbita ficifolia* seedlings under $NaHCO_3$ stress. The seedlings were divided into two groups for further treatment. In the first group, seedlings were grown in distilled water for 96 h, as the control. In the second group, seedlings were cultivated in various concentrations of NaHS solutions (0, 0.5, 1.0, 2.0 and 3.0 mmol/L) for 48 h, which was used as exogenous H_2S donor, then were treated by 120 mmol/L $NaHCO_3$ solution for 48 h. Upon completion of the treatments, the second true leaf of seedlings was collected, frozen in liquid nitrogen, and then kept at -80°C until used for analyses.

Table 1: Effects of NaHS on chlorophyll contents of *Cucurbita ficifolia* seedlings under $NaHCO_3$ stress unit: mg/g

Treatment	Chlorophyll *a* (mg/g)	Chlorophyll *b* (mg/g)	Total chlorophyll (mg/g)
T1	0.77 ab	1.80 a	2.57 ab
T2	0.71 ab	1.56 ab	2.27 bc
T3	0.83 a	1.84 a	2.67 ab
T4	0.86 a	1.90 a	2.76 a
T5	0.85 a	1.89 a	2.74 a
T6	0.62 b	1.35 b	1.97 c

T1: seedlings were grown in distilled water for 96 h; T2-T6: seedlings were cultivated in various concentrations of NaHS solutions (0, 0.5, 1.0, 2.0 and 3.0 mmol/L) for 48 h, respectively, then were treated by 120 mmol/L $NaHCO_3$ solution for 48 h. The same explanation was used throughout this study. Note: Lower case letters indicate significant differences at $p<0.05$.

Determination of chlorophyll content and malondialdehyde (MDA) content: Chlorophyll content was determined in 80% acetone extract. After centrifugation (20,000 rpm, 20 min), the contents of chlorophyll *a*, chlorophyll *b* and total chlorophyll were analysed spectrophotometrically using a TU1810 spectrophotometer (Puxi, Beijing, China), according to the method of Zhang & Qu (2003).

MDA content was determined by the procedure described by Sudhakar et al. (2001). Leaves were ground in 10 mL of 0.1% trichloroacetic acid (TCA) solution. The homogenate was centrifuged at 4,000 rpm for 10 min and 2 mL of the supernatant fraction was mixed with 2 mL of 0.67% thiobarbituric acid (TBA). The mixture was heated at 90°C for 15 min, cooled and then centrifuged at 10,000 rpm for 5 min, and the spectrophotometric analysis was conducted on spectrophotometer.

Measurements of soluble protein and soluble sugar: For measurement of soluble protein, 0.2 g leaves were ground with 10 mL of distilled water and the homogenate was centrifuged at 6,000 rpm for 10 min. The supernatant was used for measurement of soluble protein content according to the method of Shi & Sun (2011).

For measurement of soluble sugar, 0.2 g leaves were mixed with 10 mL of distilled water and heated at 90°C for 30 min, cooled and then centrifuged at 4,000 rpm for 10 min. The supernatant was used for measurement of soluble sugar content according to the method of Fales (1951).

Assays of superoxide dismutase (SOD) and peroxidase (POD) activities: 0.5 g frozen leaves were homogenized in 3 mL of cold solution containing 50×10^{-3} mol/L Na phosphate buffer (pH 7.8), 1×10^{-3} mol/L EDTA and 2% (w/v) PVP. The homogenate was centrifuged at 10,000 rpm for 10 min at 4°C. SOD and POD activities were measured according to the method of Xu et al. (2011).

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 AND DISCUSSION

Effects of NaHS on chlorophyll content under $NaHCO_3$ stress: Among the cellular organs of plants, the chloroplast

is relatively sensitive to salinity stress. Srivastava et al. (1988) reported chlorophyll content as one of the parameters of salinity tolerance in crop plants. Hernandez et al. (1995) observed more chlorophyll degradation in a salinity-sensitive pea cultivar as compared to a tolerant cultivar. In the present study, we found that $NaHCO_3$ stress significantly affected the chlorophyll content of seedlings (Table 1). The contents of chlorophyll *a*, chlorophyll *b* and total chlorophyll were all decreased under $NaHCO_3$ stress, compared to the control (treated with distilled water). $NaHCO_3$-stressed seedlings treated with NaHS (from 0.5 to 2.0 mmol/L) maintained higher level of chlorophyll. For example, chlorophyll *a*, chlorophyll *b* and total chlorophyll levels all reached a minimum value after exposure to 1.0 mmol/L NaHS, which were 21.59%, 21.13% and 21.79%, respectively, compared to the $NaHCO_3$ stress.

Effects of NaHS on MDA content under $NaHCO_3$ stress: Plants suffering from salinity stress often exhibit symptoms associated with oxidative stress and membrane lipid peroxidation, which can result in accumulation of ROS and MDA. MDA is the product of cell membrane lipid peroxidation, its content in plants reflects the extent of oxidative stress and cell membrane homeostasis (Meloni et al. 2003). MDA content of $NaHCO_3$-stressed seedlings was significantly higher than that of the control. In comparison to the control, MDA content of $NaHCO_3$-stressed seedlings increased by 104.11%. However, NaHS treatment significantly reversed the accumulation of MDA in seedlings, and resulted in an MDA content that was intermediate between that of untreated $NaHCO_3$-stressed seedlings and the control (Fig. 1). Our results indicated that NaHS protected seedlings against oxidative stress due to salinity stress and that a treatment concentration of 1.0 mmol/L was optimal.

Effects of NaHS on the soluble protein and soluble sugar contents under $NaHCO_3$ stress: The accumulation of some organic solutes under salinity stress conditions has been considered as an adaptation of plants to osmotic stress. Soluble protein and soluble sugar are the primary substances

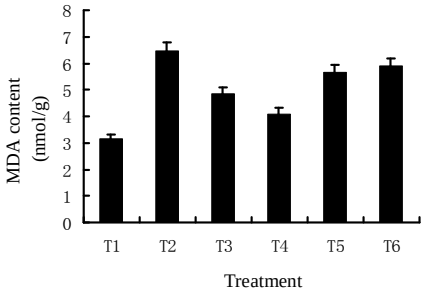

Fig. 1: Effects of NaHS on the MDA content of *Cucurbita ficifolia* seedlings under $NaHCO_3$ stress unit: nmol/g.

used for osmotic adjustment in plant cells. In this study, NaHS treatment resulted in obvious changes in the contents of soluble protein and soluble sugar (Fig. 2). Soluble protein content in seedlings with NaHS treatments all maintained a higher level than the control, and reached maximum values at 1.0 mmol/L. Soluble sugar contents of $NaHCO_3$-stressed seedlings and seedlings treated with different concentrations of NaHS were all higher than that of the control. Soluble sugar content of seedlings treated with 1.0 mmol/L NaHS was the highest of those tested. This revealed that NaHS treatment significantly increased the accumulation of soluble protein and soluble sugar in seedlings under $NaHCO_3$ stress, thereby improving their salinity tolerance , and that 1.0 mmol/L NaHS might be the optimal treatment concentration.

Effects of NaHS on the SOD and POD activities under $NaHCO_3$ stress: ROS over-production in plants happens when these are subjected to various abiotic stresses, and this makes them potentially harmful to cellular components. Fortunately, plants have the capacity to cope with these ROS by eliminating them with an efficient ROS-scavenging system (Gomez et al. 2004). In the present study, SOD and POD activities for all treatments are shown in Fig. 3. Following $NaHCO_3$ stress exposure, the SOD and POD activities increased by 15.09% and 95.06%, respectively, compared to the control. However, their activities were reduced when $NaHCO_3$-stressed seedlings were treated with NaHS

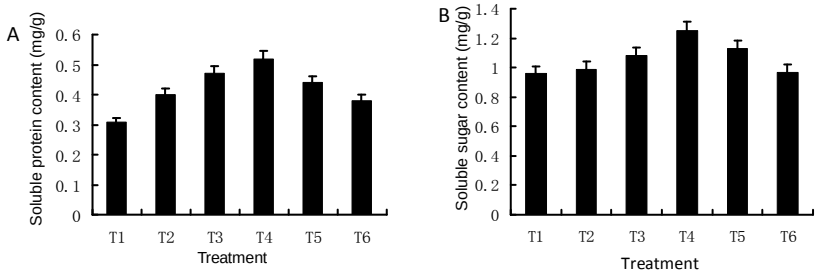

Fig. 2: Effects of NaHS on the soluble protein and soluble sugar contents of *Cucurbita ficifolia* seedlings under $NaHCO_3$ stress unit: mg/g.
A: soluble protein; B: soluble sugar

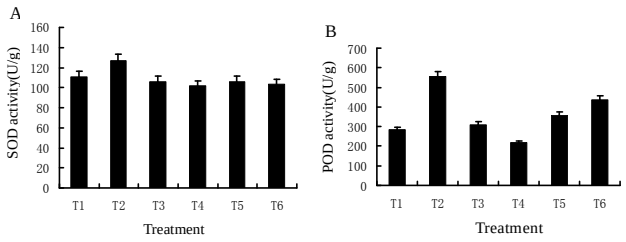

Fig. 3: Effects of NaHS on the SOD and POD activities of *Cucurbita ficifolia* seedlings under $NaHCO_3$ stress unit: U/g. A: SOD; B; POD

solutions. In comparison to the activity in untreated $NaHCO_3$-stressed seedlings, the SOD activities decreased by 16.63%, 20.14%, 16.57% and 18.68%, respectively, and POD activities decreased by 44.42%, 60.86%, 35.52% and 21.32%, respectively. This indicated that NaHS increased the $NaHCO_3$ tolerance of *Cucurbita ficifolia* seedlings and promoted their growth.

CONCLUSION

Soil salinity is a major limiting factor to agricultural land productivity, especially in arid and semi-arid regions of the world. $NaHCO_3$ stress inhibited the growth of *Cucurbita ficifolia* seedlings, while exogenous H_2S acting as an antioxidative signal molecule, effectively protected *Cucurbita ficifolia* seedlings against $NaHCO_3$ stress by increasing the accumulation of soluble protein and soluble sugar, and decreasing the MDA content. It is strongly suggested that H_2S plays an important role in protecting plants seedlings against soil salinity stress, and can be a promising source for the development of salinity soil-based agriculture.

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