Adequate potassium application enhances salt tolerance of moderate-halophyte *Sophora alopecuroides*

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ABSTRACT

A sand-culture experiment was carried out to investigate the effects of exogenous K_2CO_3 on salt resistance of moderate-halophyte *Sophora alopecuroides*. Thirty-day-old seedlings of *S. alopecuroides* were treated by two levels of salinity (0 and 200 mmol/L NaCl) in combination with four levels of K_2CO_3 (0, 5, 10 and 15 mmol/L). Gas exchange, chlorophyll content, K^+/Na^+ ratio, relative electrical conductivity (REC) and antioxidant enzymes activities were monitored after 15-day treatments. The results showed that adequate K^+ application effectively counteracted the adverse effects of salinity, in which gas exchange, K^+/Na^+ ratio and chlorophyll content significantly increased, while REC and antioxidant enzymes activities considerably decreased. The results suggest that adequate potassium application may effectively enhance plant photosynthetic capacity and reduce the oxidative stress in the salinity-stressed *S. alopecuroides*.

Keywords: leaf senescence; ascorbate-glutathione cycle; superoxide dismutase; defense against lipid peroxidation

Sophora alopecuroides L. is a perennial herb and a wild moderate halophyte which is widely distributed in the Desert Steppe, Northwest China (Wang et al. 2012). Over the past several decades, little precipitation combined with high evaporation has induced increasing soil salinization (Cao et al. 2010). Soil salinization has further caused serious limitations in the vegetation formation and the ecological conservation (Yang 2008, Cao et al. 2010). As a result, some salt-intolerant species are disappearing, while some salt-tolerant species are widely expanding in those areas (Wang et al. 2012). *S. alopecuroides* shows moderate salt resistance due to its developed roots, and plays the key role for the ecological conservation of ecosystem. More importantly, it contains many biologically active constituents such as antioxidative metabolites (flavonoids, stilbenes), toxic alkaloids which have promising prospects for making pharmaceuticals and biological pesticides (Lin et al. 2013). In addition, *S. alopecuroides* is a leguminous plant which has the function of green fertilizer due to its characteristics of nitrogen fixation. However, *S. alopecuroides* is just a moderate-halophyte, and its growth has been limited by increasingly serious soil salinization in recent years.

One of the primary plant responses to salinity is net influx of Na^+ and outflux of K^+, resulting in excessive Na^+ toxicity and K^+ starvation (Tomemori et al. 2002, Cakmak 2005). Generally, K^+ is utilized

by plant cells as the major cation, with its chief functions of maintaining electroneutrality and osmotic equilibrium (Zheng et al. 2008). In addition, K^+ is also involved in protein activities and depends on K^+-protein interactions, and those interactions cannot be mimicked by Na^+ or any other cation. Therefore, salinity-induced reduction of K^+/Na^+ ratio in saline soil may decrease the K^+ absorption probability and cause K^+ starvation, resulting in limitation of plant growth (Cakmak 2005). Our previous studies showed that adequate exogenous K^+ could effectively alleviate the salinity injuries in winter wheat (glycophyte). Nevertheless, little is known about the effects of external K^+ application on the salt adaptation ability of moderate-halophyte *S. alopecuroides*.

The objective of this study is to find out whether external K^+ application can slow salinity-induced leaf senescence and reduce oxidative stress in *S. alopecuroides*. The results may further demonstrate the feasibility of potassium application enhancing plant growth of *S. alopecuroides* under saline conditions.

MATERIAL AND METHODS

Plant culture. Thirty-day-old pot-culture seedlings were exposed to four modified Hoagland solutions containing increased levels of K_2CO_3 (0, 5, 10, and 15 mmol/L) or 200 mmol/L NaCl in combination with the four K_2CO_3 treatments. Water lost by evapotranspiration was replenished each day. The average day/night temperature was kept at 16–26°C and 10–16°C, respectively, with a mean photoperiod being 14 h and a photosynthetic photo flux density (PPFD) of 300 µmol/m^2/s. Leaves from the third branches were collected after 15 day treatments for measuring chlorophyll, Na^+, K^+ contents, relative electrical conductivity (REC) and antioxidant enzymes activities.

Gas exchange. Gas exchange was measured on the most recently fully-expanded leaves using an open system (CIRAS-2, PP Systems, Norfolk, UK) after 15 day treatments. Net photosynthetic rate (P_n), stomatal conductance (g_s), intercellular carbon dioxide concentration (c_i), transpiration rate (E) were recorded at a photosynthetic photon flux density of 300 µmol/m^2/s from an internal light source in the leaf chamber. Relative humidity was maintained at 70%, leaf temperature was set at 25°C, the flow rate was set at 200 µmol/s, and CO_2

concentration was maintained at 380 µmol/mol in the leaf chamber.

K^+/Na^+ ratio. Oven-dried leaf samples were finely ground to pass through a 2-mm sieve. About 0.5 g samples were soaked for 12 h in digesting tubes with 10 mL concentrated nitric acid and 3 mL perchlorate acid, then digested at 300°C for 6 h. The extractions were completed to 50 mL with deionized water. The amounts of K^+ and Na^+ contents were measured using an atomic absorption spectrophotometer (SP9-400, PYE, Cambridge, UK), and then the K^+/Na^+ ratio was calculated.

Chlorophyll content. Leaf samples were cleaned with deionized water and dried with filter papers. Samples were incubated in centrifuge tubes containing 10 mL 80% acetone for 48 h under dark conditions, and then were centrifuged at 5000 × g for 5 min. The absorbance of the extracts was measured at 663 nm and 646 nm with a UV/visible spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan). Chlorophyll contents were calculated using the following formula:

$$Chl = Chla\ (12.21A_{663} - 2.81A_{646}) + Chlb\ (20.13\ A_{646} - 5.03\ A_{663})$$

Where: A_{663}, A_{646} – absorbance at 663 and 646 nm.

Membrane permeability. Membrane permeability was performed by measuring REC following the method described by Yu et al. (2006). Ten pieces of 1 cm middle leaf section were placed in a tube containing 5 mL deionized water, and then were incubated in a water bath containing 0.01% Tween-20 at 25°C for 2 h. The initial electrical conductivity of the medium (EC_1) was analyzed using an electrical conductivity analyzer (KL-220, Jiangsu, China). The samples were autoclaved at 121°C for 20 min to release all electrolytes and cooled to 25°C, then the final electrical conductivity (EC_2) was measured. The REC was calculated using the formula:

$$REC\ (\%) = EC_1/EC_2 \times 100.$$

Antioxidant enzymes activities. Exactly 0.5 g fresh leaves were ground to a fine powder in 5 mL cooled phosphate buffer (pH 7.8) containing 0.1 mmol/L EDTA-Na_2, 1%(w/v) PVP. The homogenate was then centrifuged (12 000 × g at 4°C for 20 min). Ascorbate peroxidase (APX), catalase (CAT) and peroxidase (POD) activities were determined. Superoxide dismutase (SOD) activity was determined by measuring the amount of crude enzyme

extract required for inhibiting the reduction rate of nitro-blue tetrazolium (NBT) by 50% (Zheng et al. 2008). All measurements were performed at 4°C using a UV-visible spectrophotometer (UV-2550, Shimadzu, Japan).

Statistical analysis. One-way analysis of variance (ANOVA) was performed to assess the effects of K_2CO_3 application on alleviating NaCl stress. Significant effects were determined at $P < 0.05$.

RESULTS AND DISCUSSION

Gas exchange. Salinity (200 mmol/L NaCl) significantly decreased net photosynthetic rate (Figure 1a), stomatal conductance (Figure 1b), intercellular CO_2 concentration (Figure 1c) and transpiration rate (Figure 1d) of *S. alopecuroides*. Adequate external K_2CO_3 application effectively improved salt adaptation ability. Plants respond to environmental conditions by characteristic changes in gas exchange parameters (Pavlík et al. 2012,

Pavlíková et al. 2014), which are closely linked to oxidative stress, induction of senescence and stress metabolism. Stomatal limitation is often considered as an early physiological response to stress condition, which results in decreasing P_n through limiting CO_2 availability in the mesophyll (Cornic 2000). Therefore, the decreases of gas exchange might be due to both salinity-induced stomatal closure and induction of senescence and stress metabolism. Accordingly, external K^+ application effectively reduced the decrease of g_s under salinity condition, resulting in improving the capacity of CO_2 assimilation (Zheng et al. 2008). However, excessive K^+ application also performed harmful effects on plant gas exchange of *S. alopecuroides*.

K^+/Na^+ ratio. Salinity (200 mmol/L NaCl) significantly increased Na^+ but decreased K^+ concentration and K^+/Na^+ ratio in moderate-halophyte *S. alopecuroides* (Table 1). Under 200 mmol/L NaCl condition, 5–15 mmol/L K_2CO_3 application significantly enhanced K^+ content and K^+/Na^+ ratio in both shoot and root, with the optimal K^+/Na^+
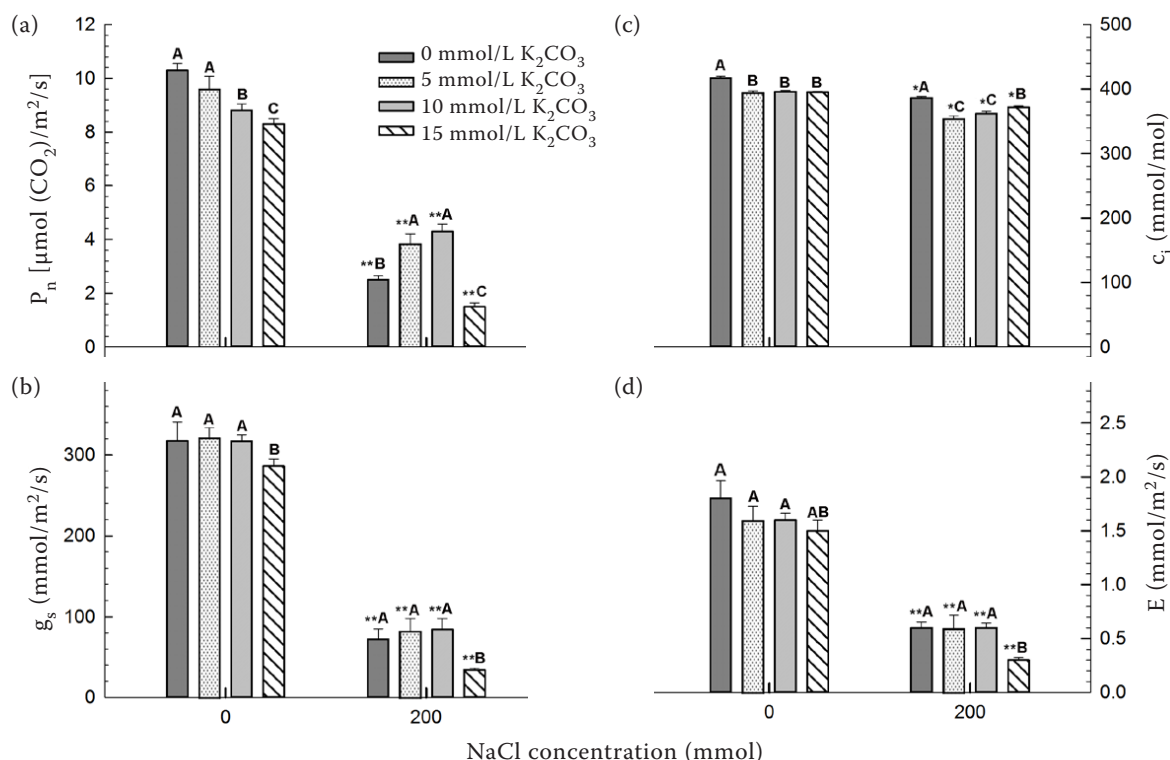

Figure 1. Effects of K_2CO_3 application on net photosynthetic rate (P_n); stomatal conductance (g_s), intercellular CO_2 content (c_i) and transpiration rate (E) of *Sophora alopecuroides* in 0 and 200 mmol/L NaCl treatments, respectively. Vertical bars are means ± standard error (*n* = 6). Within each treatment, significant differences between two salt concentrations are marked by *, ** at 0.05, 0.01 level respectively while different K_2CO_3 treatments are marked by letters at $P \leq 0.05$

Table 1. K^+, Na^+ contents (mg/g) and K^+/Na^+ ratio in shoots and roots of *Sophora alopecuroides* exposed to salinity and/or K_2CO_3 (mmol/L) addition for 15 days

Treatment		Shoots			Roots		
NaCl	K_2CO_3	K^+	Na^+	K^+/Na^+	K^+	Na^+	K^+/Na^+
0	0	31.20 ± 0.27[d]	1.37 ± 0.35[a]	23.80 ± 6.08[b]	27.41 ± 0.52[d]	3.67 ± 0.27[a]	7.47 ± 0.41[c]
	5	41.36 ± 0.63[c]	1.08 ± 0.17[ab]	38.88 ± 5.61[ab]	29.15 ± 0.33[c]	3.20 ± 0.45[ab]	9.10 ± 1.20[bc]
	10	47.39 ± 0.26[b]	0.91 ± 0.28[ab]	55.64 ± 17.6[a]	32.52 ± 0.15[b]	2.85 ± 0.41[ab]	11.42 ± 1.63[ab]
	15	50.97 ± 0.33[a]	0.85 ± 0.22[b]	62.91 ± 17.2[a]	35.22 ± 0.74[a]	2.47 ± 0.59[b]	14.24 ± 3.31[a]
200	0	29.53 ± 0.72[c]	3.79 ± 0.37[a]	7.83 ± 0.58[b]	23.38 ± 0.25[c]	14.28 ± 0.57[a]	1.64 ± 0.05[c]
	5	36.21 ± 0.30[b]	3.52 ± 0.19[a]	10.30 ± 0.47[b]	26.81 ± 0.16[b]	11.46 ± 0.56[b]	2.34 ± 0.10[b]
	10	45.69 ± 0.16[a]	2.16 ± 0.31[b]	21.44 ± 3.03[a]	27.30 ± 0.67[b]	10.94 ± 0.84[b]	2.50 ± 0.13[b]
	15	20.91 ± 0.17[d]	2.05 ± 0.44[b]	10.55 ± 2.38[b]	29.53 ± 0.82[a]	8.32 ± 0.22[c]	3.55 ± 0.01[a]

Data are means ± standard error (n = 6). Different letters within a column indicate significant differences at $P \leq 0.05$

ratio being noted in 10 mmol/L K_2CO_3 treatment in shoot while in 15 mmol/L K_2CO_3 treatment in the root. Salinity-induced ion imbalance and single salt toxicity are owing to redundant substitution of K^+ with Na^+, leading to excessive Na^+ toxicity and K^+ starvation in plant tissues (Zhu 2001, Parida and Das 2005). Suitable K^+/Na^+ ratio has a key role for maintaining plant growth under saline condition (Grewal 2010). Here adequate K_2CO_3 addition significantly alleviated the salinity-induced K^+ deficiency and effectively reduced the reduction of K^+/Na^+ ratio in both shoot and root. Compared with winter wheat (Zheng et al. 2008), *S. alopecuroides* performed much higher K^+/Na^+ ratio in both saline and non-saline conditions. This might be considered as one of the key roles for halophyte keeping higher salt tolerance than glycophyte.

Chlorophyll content. Salinity (200 mmol/L NaCl) induced significant decreases in leaf chlorophyll (Chl*a*, Chl*b* and total Chl) contents of *S. alopecuroides* (Table 2). However, adequate K_2CO_3 application effectively alleviated the salinity-induced injuries. Under saline condition, the contents of Chls were enhanced by 5 and 10 mmol/L K_2CO_3 application, while those values decreased sharply at 15 mmol/L K_2CO_3 treatment. The values of Chl*a*, Chl*b* and Chl contents all peaked in 10 mmol/L K_2CO_3 treatment.

Salinity admittedly hinders numerous morpho-physiological attributes like plant photosynthetic

Table 2. Chls contents (mg/g dry matter) of *Sophora alopecuroides* exposed to salinity and/or K_2CO_3 (mmol/L) addition for 15 days

Treatment		Chl *a*	Chl *b*	Chl
NaCl	K_2CO_3			
0	0	3.673 ± 0.023[b]	0.972 ± 0.024[b]	4.646 ± 0.042[b]
	5	3.847 ± 0.018[a]	1.128 ± 0.030[a]	4.976 ± 0.051[a]
	10	3.556 ± 0.025[c]	0.982 ± 0.016[b]	4.538 ± 0.068[c]
	15	3.415 ± 0.055[d]	0.874 ± 0.036[c]	4.289 ± 0.012[d]
200	0	2.537 ± 0.013[c]	0.625 ± 0.038[c]	3.161 ± 0.032[c]
	5	2.676 ± 0.028[b]	0.699 ± 0.025[b]	3.375 ± 0.061[b]
	10	2.943 ± 0.053[a]	0.788 ± 0.026[a]	3.731 ± 0.063[a]
	15	1.591 ± 0.026[d]	0.335 ± 0.014[d]	2.926 ± 0.042[d]

Data are means ± standard error (n = 6). Different letters within a column indicate significant differences at $P \leq 0.05$
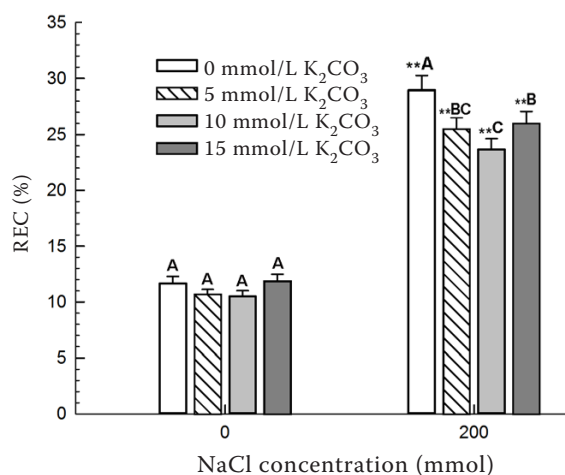
Figure 2. Effects of K_2CO_3 application on relative electric conductivity (REC) of *Sophora alopecuroides* in 0 and 200 mmol/L NaCl treatments, respectively. Vertical bars are means ± standard error (n = 6). Within each treatment, significant differences between two salt concentrations are marked by ** at 0.01 level while different K_2CO_3 treatments are marked by letters at $P \leq 0.05$

capacity by reducing Chls contents (Abbasi et al. 2012). Chlorophyll is the basis of photosynthesis and its content is a key indicator for plant growth under saline conditions (Mousavi et al. 2008). Here adequate K_2CO_3 application significantly increased Chls contents under saline condition. This is consistent with the results of Abbasi et al. (2014) who reported that potassium application may effectively alleviate the salinity-induced decreases of Chls contents of maize hybrids. More importantly, high Chl content is the prerequisite of plant with high photosynthetic capacity.

Membrane permeability. Non-significant differences were noted in the values of REC among K_2CO_3 treatments (0, 5, 10 and 15 mmol/L) under non-salinity condition (Figure 2). However, under salinity condition, 5–10 mmol/L K_2CO_3 application significantly decreased REC, with the optimal effect being noted in the 10 mmol/L K_2CO_3 treatment.

REC is a physiological and biochemical index which indicates plant cellular membrane perme-
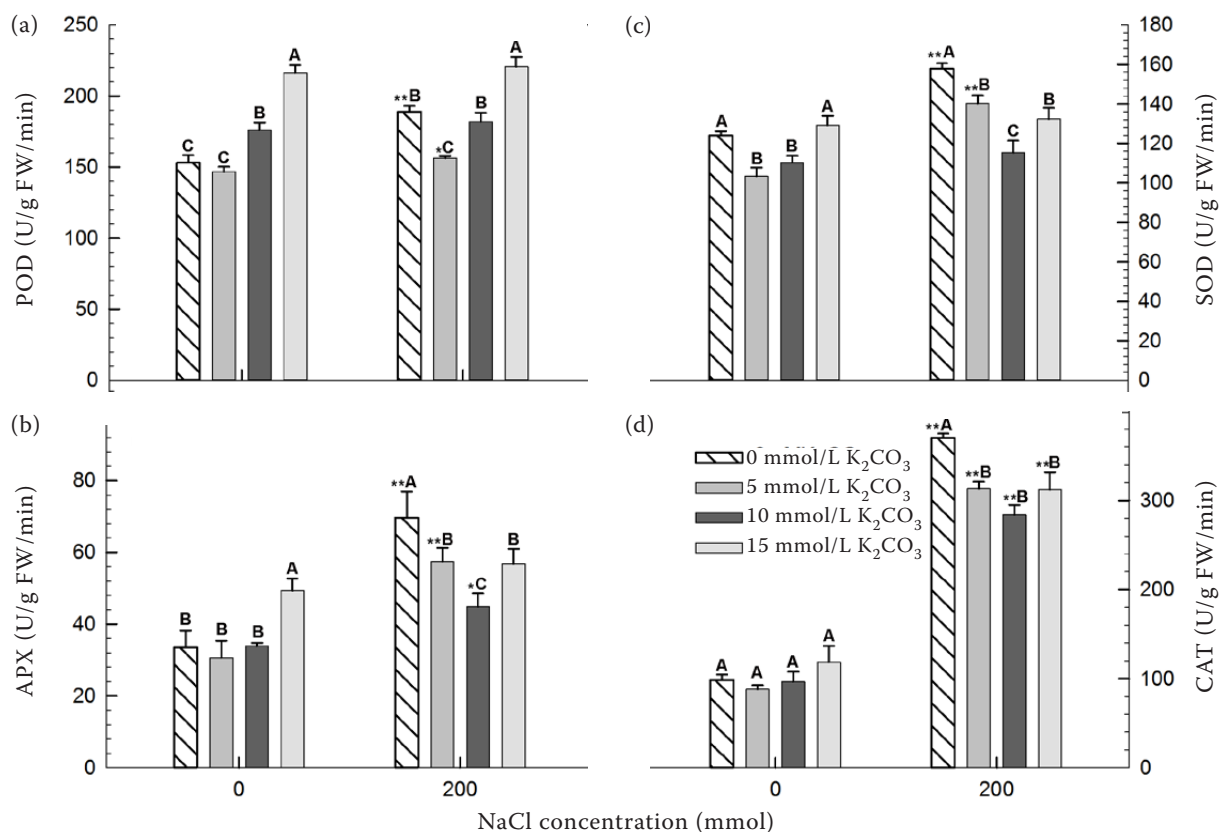

Figure 3. Effects of K_2CO_3 application on antioxidant enzymes (POD – peroxidase; SOD – superoxide dismutase; APX – ascorbate peroxidase; CAT – catalase) activities of *Sophora alopecuroides* in 0 and 200 mmol/L NaCl treatments, respectively. Vertical bars are means ± standard error (n = 6). Within each treatment, significant differences between two salt concentrations are marked by *, ** at 0.05, 0.01 level respectively while different K_2CO_3 treatments are marked by letters at $P \leq 0.05$; FW – fresh weight

ability (Feng et al. 2003). Here REC was remarkably increased by salinity, but it was effectively alleviated by adequate K$_2$CO$_3$ application. Salinity-induced oxidative stress causes peroxidation of unsaturated fatty acids and activates antioxidant system (Wu et al. 2012). The results demonstrate that moderate exogenous K$^+$ application may effectively diminish the salinity-induced damage to cellular membrane. Adequate K$^+$ supply may reduce the peroxidation of unsaturated fatty acids, and increased content of unsaturated fatty acid helps to improve the membrane fluidity (Zemanová et al. 2015). However, excessive K$^+$ addition is also harmful to the plant growth of *S. alopecuroides*.

Antioxidant enzymes activities. Sodium chloride considerably enhanced the activities of POD (Figure 3a), SOD (Figure 3b), APX (Figure 3c) and CAT (Figure 3d). However, adequate K$_2$CO$_3$ application effectively alleviated the adverse effects of salt stress. Under salinity condition, the above parameters significantly reduced by K$_2$CO$_3$ addition, with the optimal effects occurring in 5–10 mmol/L K$_2$CO$_3$ treatments.

Salinity-induced oxidative stress usually aggravates membrane lipid peroxidation and limits plant growth (Gill and Tuteja 2010). Antioxidant enzymes activities are key parameters for indicating the capability of plant to scavenge ROS (Gama et al. 2009). The enhancements of antioxidant enzymes activities might contribute to alleviating ROS-induced oxidative stress. On the contrary, the decreases of antioxidant enzymes activities in adequate K$_2$CO$_3$ treatments might indicate an alleviation of oxidative stress.

In conclusion, adequate K$^+$ supply may effectively enhance salt tolerance of moderate-halophyte *S. alopecuroides*. Therefore, adequate potassium fertilizer application might be a reliable strategy for promoting vegetation coverage in the salinized areas all over the world.

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