Physiological Responses of Zoysia matrella to Salt Stress (Postprint)

Authors: Chen Yilan, Li Xin, Wenzhong Zhao, Li Xinjie, Liao Haimin, Liu Dongming

Date: 2021-04-29T12:31:38+00:00

Abstract

To investigate the adaptability of Thuarea involuta to adverse environments and elucidate its salt tolerance mechanisms, the authors collected T. involuta from Dong Island of the Xisha Islands and selected robust plants propagated through division as experimental materials. By simulating the habitat of tropical coral islands, different concentrations of NaCl treatment were applied to examine the effects of varying degrees of salt stress on leaf malondialdehyde (MDA) content, antioxidant enzymes, and osmotic adjustment substances in the plants. The results demonstrated: (1) Under short-term (28 d) salt stress, increasing NaCl concentration did not accelerate membrane lipid peroxidation in T. involuta leaf cells, with only a minor increase in MDA content; as salt stress duration prolonged and NaCl concentration increased, the degree of membrane lipid peroxidation damage in T. involuta leaf cells intensified, with MDA content gradually rising and reaching its maximum at 400 $\text{mmol} \cdot \text{L}^{-1}$. (2) Under short-term (28 d) salt stress, low concentration (200 $\text{mmol} \cdot \text{L}^{-1}$) NaCl significantly enhanced superoxide dismutase (SOD) activity, while high concentration (400 $\text{mmol} \cdot \text{L}^{-1}$) NaCl significantly enhanced catalase (CAT) activity; peroxidase (POD) activity gradually increased with prolonged salt stress duration and increased concentration. (3) Under salt stress conditions, T. involuta tissues continuously accumulated soluble protein (SP) and proline (PRO) to enhance osmotic adjustment capacity, balancing intracellular and extracellular osmotic potential, thereby alleviating salt damage. Throughout the salt stress process, T. involuta integrated the antioxidant enzyme defense system with osmotic adjustment mechanisms, mitigating membrane lipid peroxidation damage and physiological drought caused by cellular water loss, thereby demonstrating strong salt tolerance. These findings provide scientific support for the application of T. involuta in artificial community construction and vegetation restoration on islands in the South China Sea, as well as for creating favorable ecological environments, and hold significant importance for the sustainable

development of tropical coral islands in China.

Full Text

Preamble

Physiological Response of Thuarea involuta to Salt Stress

CHEN Yilan[12], **LI Xin**[3], **ZHAO Wenzhong**[4], **LI Xinjie**[4], **LIAO Haimin**[1], **LIU Dongming**[2*]

[1] College of Life Sciences/Institute of Agro-bioengineering, Key Laboratory of Plant Resource Conservation and Germplasm Innovation in Mountainous Region (Ministry of Education), Collaborative Innovation Center for Mountain Ecology & Agro-bioengineering (CICMEAB), Guizhou University, Guiyang 550025, China
[2] South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China
[3] South China Agricultural University, Guangzhou 510642, China
[4] Hebei Qugang Expressway Development Co. Ltd., Dingzhou 073000, Hebei, China

Abstract

To investigate the adaptability of *Thuarea involuta* to adverse environments and elucidate its salt tolerance mechanisms, we collected specimens from Dongdao Island in the Xisha Islands and selected robust plants through vegetative propagation for experimental material. Simulating the habitat of tropical coral islands, we established different NaCl concentration treatments to examine the effects of varying salt stress levels on malondialdehyde (MDA) content, antioxidant enzymes, and osmotic adjustment substances in leaves. The results demonstrated: (1) Under short-term salt stress (28 days), increasing NaCl concentration did not accelerate membrane lipid peroxidation in leaf cells, with only minor increases in MDA content. However, as salt stress duration and NaCl concentration increased, membrane lipid peroxidation damage intensified progressively, with MDA content rising gradually and peaking at 400 $\text{mmol} \cdot \text{L}^{-1}$. (2) During short-term salt stress (28 days), low NaCl concentration (200 $\text{mmol} \cdot \text{L}^{-1}$) significantly enhanced superoxide dismutase (SOD) activity, while high concentration (400 $\text{mmol} \cdot \text{L}^{-1}$) significantly increased catalase (CAT) activity; peroxidase (POD) activity increased gradually with extended stress duration and higher concentrations. (3) Under salt stress, *T. involuta* continuously accumulated soluble protein (SP) and proline (PRO) to enhance osmotic regulation capacity, balancing intracellular and extracellular osmotic potential to alleviate salt damage. Throughout the salt stress period, *T. involuta* integrated antioxidant enzyme defense systems with osmotic regulation mechanisms to mitigate membrane lipid peroxidation damage and physiological drought from cellular water loss, demonstrating strong salt tolerance. These findings provide scientific support for the application of *T. involuta* in artificial community construction and vegetation restoration on islands in the South China Sea, and hold significant importance for the sustainable development of China's tropical coral islands.

Keywords: *Thuarea involuta*, salt stress, MDA, antioxidant enzymes, osmotic regulation, physiological response

Introduction

Thuarea involuta is a perennial herbaceous plant in the Poaceae family, distributed across the Ryukyu Islands, Southeast Asia, Oceania, and Madagascar. In China, it occurs in Guangdong, Hainan, and Taiwan (Chen, 1990), with additional distribution on Weizhou Island in Guangxi (Zhang et al., 2018). This species thrives on coastal sandy beaches, with creeping stems that send up leaves and inflorescences. The erect portions reach modest heights, bearing lanceolate leaves approximately 3 cm long and 3–8 mm wide, covered with fine hairs on both surfaces and often featuring wavy, crinkled margins [Figure 1: see original paper]. *T. involuta* is commonly planted on islands in the South China Sea for windbreak, sand fixation, and environmental greening (Ren et al., 2017).

China's tropical coral islands exhibit typical tropical and equatorial marine monsoon climates characterized by long sunshine hours, intense radiation, abundant heat, persistently high temperatures, and prevailing monsoons (Huang and Zhang, 2008). The soil is rich in calcium and phosphorus with high salt content and strongly alkaline pH values, creating harsh environmental conditions. Plants inhabiting these regions require specialized adaptations including salt tolerance, heat resistance, drought tolerance, and calciphilous traits (Xing and Deng, 2018; Jian, 2020). However, research on *T. involuta* remains limited internationally and domestically, focusing primarily on floral structure and development (Kuoh et al., 1998), cultivation and conservation in coral reef sandy soils (Wang et al., 2018), and relationships between leaf water potential, osmotic solutes, and environmental conditions (Allaway, 1984). No studies have reported on the species' stress-resistance biological characteristics or physiological-biochemical responses to adversity, leaving its adaptability to tropical coral island habitats undocumented.

Conducting research on vegetation degradation and restoration in tropical coral islands provides scientific support for vegetation conservation and restoration in the South China Sea islands and holds great significance for sustainable development of these ecosystems (Jian, 2020). Therefore, to explore *T. involuta*'s adaptability to adverse environments and reveal its salt tolerance mechanisms, we collected specimens from Dongdao Island in the Xisha Islands, selected robust plants through vegetative propagation, and established different NaCl concentration treatments simulating tropical coral island habitats. We investigated the effects of varying salt stress levels on MDA content, antioxidant enzymes, and osmotic adjustment substances in leaves, aiming to provide references for the application of *T. involuta* in artificial community construction, vegetation restoration, and ecological improvement on South China Sea islands.

Materials and Methods

1.1 Materials and Sampling Site Description

T. involuta was collected from Dongdao Island (112°43 13 –112°44 22 E, 16°39 34 –16°40 31 N) in the Xisha Islands. The island covers a land area of 1.7 km^2 with an elliptical shape and ranks among the three largest coral islands in the Xisha archipelago exceeding 1.5 km^2 (Tong et al., 2013; Wang et al., 2019). The island experiences an average annual temperature of 26–27 °C and annual precipitation of 1,500 mm, with distinct wet and dry seasons concentrated between June and November, supporting rich and dense vegetation (Liu et al., 2017).

1.2 Experimental Design

In April 2018, fresh *T. involuta* plants were collected from Dongdao Island and transported to the experimental greenhouse at South China Botanical Garden, Chinese Academy of Sciences. Vegetative propagation was conducted in 30 × 25 cm pots using a substrate mixture of coral sand:red soil:coconut coir:peat soil:organic fertilizer at a ratio of 80:10:5:3:2. In July 2019, uniform robust plants were selected for salt stress experiments.

Based on seawater salinity of 3.5% and coral sand salt content of 0.08%–0.6%, with higher salt content in soils closer to the coast due to seawater splash and salt spray effects, we established different NaCl concentration gradients for this experiment. Before treatment, *T. involuta* plants were divided into six groups labeled CK, S1, S2, S3, S4, and S5, with three replicates per group. The CK group served as the control with 0 $\text{mmol}\cdot\text{L}^{-1}$ NaCl, while experimental groups S1–S5 received NaCl concentrations of 100 $\text{mmol}\cdot\text{L}^{-1}$ (S1), 200 $\text{mmol}\cdot\text{L}^{-1}$ (S2), 300 $\text{mmol}\cdot\text{L}^{-1}$ (S3), 400 $\text{mmol}\cdot\text{L}^{-1}$ (S4), and 500 $\text{mmol}\cdot\text{L}^{-1}$ (S5), respectively. During the experiment, all plants were watered daily to maintain normal moisture conditions (soil water content of 40%–50% as determined experimentally). Starting from day one, 500 mL of NaCl solution was applied daily to maintain target concentrations. Healthy mature leaves were randomly collected from each group on days 1, 28, and 42 for laboratory analysis of various indicators.

1.3 Measurement Indicators and Methods

Malondialdehyde (MDA) content was determined using the thiobarbituric acid (TBA) method (Wang and Huang, 2015). MDA condenses with TBA to form a red product with maximum absorption at 532 nm, allowing colorimetric estimation of lipid peroxidation products. Absorbance at 600 nm was also measured, and MDA content was calculated using the difference between absorbance values at 532 nm and 600 nm.

Superoxide dismutase (SOD) activity was measured using the nitroblue tetrazolium (NBT) photochemical reduction method (Chen and Wang, 2002). The xanthine-xanthine oxidase reaction system generates superoxide anions (O_2^-), which reduce NBT to form blue formazan. SOD scavenges O_2^-, thereby inhibiting formazan formation. One enzyme activity unit was defined as the amount causing 50% inhibition in the reaction system.

Peroxidase (POD) activity was determined using the guaiacol colorimetric method (Lu and Li, 2012). POD catalyzes H_2O_2 oxidation of specific substrates, producing characteristic light absorption at 470 nm. One enzyme activity unit was defined as a change in A_{470} of 0.5 per minute per mg of tissue protein in the reaction system.

Catalase (CAT) activity was measured using the ultraviolet absorption method (Li, 2000). H_2O_2 has characteristic absorption at 240 nm, and CAT decomposes H_2O_2, causing absorbance at 240 nm to decrease over time. CAT activity was calculated based on the rate of absorbance change, with one enzyme activity unit defined as the degradation of 1 nmol H_2O_2 per minute per mg of tissue protein.

Soluble protein (SP) content was determined using the bicinchoninic acid (BCA) method (Wu et al., 2017). Under alkaline conditions, cysteine, cystine, tryptophan, tyrosine, and peptide bonds in proteins reduce Cu^{2+} to Cu^+. Two BCA molecules bind with Cu^+ to form a purple complex with absorption peaks at 540–595 nm, strongest at 562 nm where absorbance was read.

Proline (PRO) content was measured using the acidic ninhydrin staining method (Li, 2000). Proline was extracted with sulfosalicylic acid (SA), and after heating, reacted with acidic ninhydrin solution to produce a red color. Following toluene extraction, absorbance was measured at 520 nm.

1.4 Data Processing

Data were processed and graphed using Excel. Statistical analysis was performed with SPSS Statistics 17 software. Duncan's multiple range test was applied at the $P < 0.05$ level for multiple comparisons, and one-way ANOVA was used to test for significant differences among treatment groups.

Results

2.1 Changes in Malondialdehyde (MDA) Content in *T. involuta* Leaves Under Different Salt Stress Levels

MDA is a product of membrane lipid peroxidation, and its content reflects the degree of membrane damage (Kong et al., 2019). As shown in [Figure 2: see original paper], on day 1 of salt stress, MDA content in all treatment groups except S1 was slightly lower than or not significantly different from the control (CK), indicating no membrane lipid peroxidation occurred in any group. On day 28, MDA content in treatments S1–S4 increased only slightly, showing no significant differences from CK, demonstrating that membrane lipid peroxidation did not intensify with increasing NaCl concentration. Only treatment S5 showed a significant 30.80% increase in MDA content compared to CK. As salt stress progressed, membrane lipid peroxidation intensified, with MDA content in all treatment groups significantly higher than CK. Treatment S4 exhibited the highest MDA content, indicating the most severe membrane lipid peroxidation damage.

2.2.1 Changes in Superoxide Dismutase (SOD) Activity in *T. involuta* Leaves Under Different Salt Stress Levels

As illustrated in [Figure 3: see original paper], during short-term salt stress, moderate salinity enhanced SOD activity, with all treatment groups showing significantly higher activity than CK. SOD activity exhibited a rising-falling trend with increasing NaCl concentration, peaking at S3 on day 1 (47.74% higher than CK) and at S2 on day 28 (56.68% higher than CK). By day 42, SOD activity showed little variation across different NaCl concentrations but remained higher than CK.

2.2.2 Changes in Peroxidase (POD) Activity in *T. involuta* Leaves Under Different Salt Stress Levels

At the initial stage of salt stress, POD showed no response, with no significant differences in activity between any treatment group and CK. As salt stress duration increased, POD synthesis exceeded degradation rates, leading to a gradual upward trend in activity with higher NaCl concentrations [Figure 4: see original paper]. On days 28 and 42, POD activities in treatments S1–S2 showed no significant differences from each other, while activities in treatments S3–S5 also showed no mutual significant differences. However, all treatment groups except S1 on day 42 were significantly different from CK.

2.2.3 Changes in Catalase (CAT) Activity in *T. involuta* Leaves Under Different Salt Stress Levels

The NaCl concentration that enhanced CAT activity varied depending on salt stress duration [Figure 5: see original paper]. On days 1 and 42, CAT activity peaked in treatment S2, showing significant increases of 161.07% and 121.98% compared to CK, respectively. On day 28, CAT activity reached its maximum in treatment S4, with a significant 151.81% increase over CK. On days 1 and 28, CAT synthesis was slower than degradation in treatment S5, resulting in activity decreases of 18.96% and 21.71% compared to CK, respectively. However, with prolonged stress, CAT was rapidly synthesized, and by day 42, all NaCl treatments showed significantly higher CAT activity than CK.

2.3.1 Changes in Soluble Protein (SP) Content in *T. involuta* Leaves Under Different Salt Stress Levels

As shown in [Figure 6: see original paper], SP content exhibited a rising-falling trend with increasing NaCl concentration on days 1 and 42, with treatment S2 promoting SP accumulation to maximum levels (60.79% and 78.63% higher than CK, respectively). Treatment S5 showed the lowest SP content, with non-significant decreases of 10.11% and 8.07% compared to CK. On day 28, SP content in treatment S1 was significantly lower than CK by 3.61%, while treatments S2–S5 showed no significant differences among themselves but were all significantly different from CK.

2.3.2 Changes in Proline (PRO) Content in *T. involuta* Leaves Under Different Salt Stress Levels

At the initial stage of salt stress, PRO content was minimal in both control and treatment groups. As treatment duration progressed, PRO content increased with NaCl concentration [Figure 7: see original paper]. On day 28, PRO content in treatments S1–S4 increased slowly with no significant differences among them, while treatment S5 reached the highest level, 61 times that of CK. On day 42, treatments S1 and S2 showed no significant difference from each other but were both significantly different from CK, while treatment S3 reached 44.87 times the CK level.

Discussion

Under normal conditions, the production and scavenging of reactive oxygen species (ROS) in plants maintain dynamic equilibrium, with weak membrane lipid peroxidation and stable MDA content. In saline environments, ROS accumulation increases with NaCl concentration, exacerbating membrane lipid peroxidation and raising MDA content (Huang et al., 2018). Our study found that short-term salt stress did not intensify membrane lipid peroxidation damage in *T. involuta* leaves, with only minor MDA increases as NaCl concentration rose. However, with prolonged treatment, MDA content increased significantly. While maximum MDA content in *Chrysanthemum morifolium* cv. Chuju and *Salicornia europaea* occurred at 300 $\text{mmol} \cdot \text{L}^{-1}$ NaCl (Wang et al., 2014; Jiang et al., 2012), *T. involuta* showed peak MDA content at 400 $\mathrm{mmol \cdot L^{-1}}$, indicating stronger salt tolerance. The species can apparently activate antioxidant enzyme defense systems to alleviate membrane lipid peroxidation damage and employ osmotic regulation mechanisms to adjust cellular osmotic potential and enhance adaptation to saline environments (Jiang et al., 2020).

SOD, POD, and CAT are three key antioxidant enzymes in plant defense systems that scavenge ROS. SOD catalyzes the dismutation of superoxide anions to H_2O_2, POD catalyzes H_2O_2 oxidation of hydroxyl radicals to H_2O, and CAT directly converts H_2O_2 to H_2O, with their synergistic action reducing membrane lipid peroxidation damage (Ingram and Bartels, 1996). Our study revealed that at the initial salt stress stage, *T. involuta* tissues primarily enhanced SOD and CAT activities to scavenge ROS, while POD showed no response. Low salt concentrations can increase antioxidant enzyme activities to promptly remove ROS and reduce membrane damage, whereas high salt concentrations decrease these activities, causing loss of protective enzyme system self-regulation and consequent cellular injury (Lu et al., 2015). During short-term salt stress, low NaCl concentration (200 $\mathrm{mmol \cdot L^{-1}}$) significantly increased SOD activity, while high concentration (400 $\mathrm{mmol \cdot L^{-1}}$) significantly enhanced CAT activity. Throughout the salt stress period, *T. involuta* leaf tissues rapidly synthesized antioxidant enzymes to scavenge ROS, thereby preventing salt-induced cellular damage (Zhang et al., 2017). SOD and CAT activities showed initial increases followed by decreases with rising NaCl concentration, similar to trends observed in *Lycium ruthenicum* (Yang et al., 2019). However, POD activity increased gradually with extended stress duration and higher salt concentrations, consistent with findings in *Vicia faba* seedlings (Wang et al., 2017).

Plants can mitigate salt stress damage not only by activating antioxidant enzyme defense systems to eliminate harmful ROS but also by accumulating osmotic adjustment substances to reduce cellular osmotic potential, maintain water balance, and preserve cell turgor (Long, 2017). Some studies identify PRO as the primary osmotic adjustment substance (Sun et al., 2017), while others indicate both SP and PRO serve this function, generally increasing with NaCl concentration (Zhang et al., 2020). Our study found that at the initial salt stress stage, *T. involuta* leaf cells remained undamaged with balanced water dynamics and normal turgor, maintaining regular growth and development. During salt stress progression, plants under 100–200 $\mathrm{mmol \cdot L^{-1}}$ NaCl primarily accumulated SP to maintain cellular osmotic potential, while those under 300–500 $\mathrm{mmol \cdot L^{-1}}$ NaCl mainly accumulated PRO. The continuous accumulation of SP and PRO enhanced the osmotic regulation capacity of *T. involuta* tissues, effectively alleviating salt damage.

In summary, *T. involuta* exhibits strong salt tolerance. During short-term salt stress (28 days), increasing NaCl concentration did not exacerbate membrane lipid peroxidation damage, with no significant differences in MDA content among 200–500 $\mathrm{mmol \cdot L^{-1}}$ (S2–S5) treatments. As treatment duration extended, membrane lipid peroxidation damage gradually intensified with increasing NaCl concentration, but plant tissues could activate defense systems to synthesize antioxidant enzymes for ROS scavenging and accumulate SP and PRO to enhance osmotic regulation, thereby mitigating cellular damage. As a common plant species on tropical coral islands, *T. involuta* is primarily used for windbreak, sand fixation, and environmental greening. Therefore, these research findings provide valuable references for the application of *T. involuta* in artificial community construction, vegetation restoration, and ecological improvement on South China Sea islands.

References

Allaway WG, Pitman MG, Storey R et al., 1984. Water relations of coral cay vegetation on the great barrier-reef-water potentials and osmotic content[J]. *Aust J Bot*, 32(4): 449-464.

Chen JX, Wang XF, 2002. *Experimental Guidance of Plant Physiology* [M]. Guangzhou: South China University of Technology Press. [Chen Jianxun, Wang Xiaofeng, 2002. *Experimental Guidance of Plant Physiology* [M]. Guangzhou: South China University of Technology Press.]

Chen SL, 1990. *Flora of China* [M]. Beijing: Science Press, 10(1): 387-389. [Chen Shouliang, 1990. *Flora of China* [M]. Beijing: Science Press, 10(1): 387-389.]

Huang XL, Lin FF, Zhang MY, et al., 2018. Effects of salt stress on the growth and osmoregulatory substances in *Terminalia neotaliala* Capuron seedlings[J]. *J S Agric*, 49(7): 1364-1369. [Huang Xiangling, Lin Feifei, Zhang Mingyue, et al., 2018. Effects of salt stress on the growth and osmoregulatory substances in *Terminalia neotaliala* Capuron seedlings[J]. *Journal of Southern Agriculture*, 49(7): 1364-1369.]

Huang ZG, Zhang WQ, 2008. A discussion on the quaternary climate records of tropical coral reefs in China[J]. *Trop Geogr*: 11-15. [Huang Zhenguo, Zhang Weiqiang, 2008. A discussion on the quaternary climate records of tropical coral reefs in China[J]. *Tropical Geography*: 11-15.]

Ingram J, Bartels D, 1996. The molecular basis of dehydration tolerance in plants[J]. *Ann Rev Plant Biol*, 47(1): 377-403.

Jian SG, 2020. Vegetation of Tropical Coral Islands in China[J]. *Guihaia*, 40(3):443. [Jian Shuguang, 2020. Vegetation of Tropical Coral Islands in China[J]. *Guihaia*, 40(3):443.]

Jiang YC, Liu B, Guo H, et al., 2012. Preliminary study on the physiologic response of salt-dilution halophyte *Salicornia europaea* L. to salt stress[J]. *Xinjiang Agric Sci*, 49: 694-700. [Jiang Yongchao, Liu Bin, Guo Huan, et al., 2012. Preliminary study on the physiologic response of salt-dilution halophyte *Salicornia europaea* L. to salt stress[J]. *Xinjiang Agricultural Sciences*, 49: 694-700.]

Jiang YT, Li YM, Chen CX, et al., 2020. Effects of salt stress on physiological characteristics of *Begonia semperflorens* link et Otto seedlings[J]. *N Hortic*, (16):62-69. [Jiang Yuntian, Li Yumei, Chen Chenxia, et al., 2020. Effects of salt stress on physiological characteristics of *Begonia semperflorens* link et Otto seedlings[J]. *Northern Horticulture*, (16):62-69.]

Kong Q, Ma XH, Gong LX, et al., 2019. Growth and physiological responses of *Taxodium mucronatum*×*Cryptomeria fortunei* under different salt stress conditions[J]. *J Sw For Univ (Nat Sci Ed)*, 39(2): 179-183. [Kong Qiang, Ma Xiaohua, Gong Lixia, et al., 2019. Growth and physiological responses of *Taxodium mucronatum*×*Cryptomeria fortunei* under different salt stress conditions[J]. *Journal of Southwest Forestry University (Natural Sciences)*, 39(2): 179-183.]

Li HS, 2000. *Experimental Principles and Techniques of Plant Physiology and Biochemistry*[M]. Beijing: Higher Education Press. [Li Hesheng, 2000. *Experimental Principles and Techniques of Plant Physiology and Biochemistry*[M]. Beijing: Higher Education Press.]

Liu XT, Ge CD, Zou XQ, et al., 2017. Carbon, nitrogen geochemical characteristics and their implications on environmental changes in the lagoon sediments of the Dongdao Island of Xisha Islands in South China Sea[J]. *Acta Oceanol Sin*, 39(6):43-54. [Liu Xiaotong, Ge Chendong, Zou Xinqing, et al., 2017. Carbon, nitrogen geochemical characteristics and their implications on environmental changes in the lagoon sediments of the Dongdao Island of Xisha Islands in South China Sea[J]. *Acta Oceanologica Sinica*, 39(6):43-54.]

Long ZH, 2017. Study on the physiological response of salt stress and drought stress of *Bambusa tuldoides* cv. Swolleninternode[D]. Fujian Agricultural and Forestry University. [Long Zhihui, 2017. Study on the physiological response of salt stress and drought stress of *Bambusa tuldoides* cv. Swolleninternode[D]. Fujian Agricultural and Forestry University.]

Lu B, Hou YM, Li XY, et al., 2015. Physiological response and salt-tolerance of *Gleditsia microphylla* under NaCl stress[J]. *J Appl Ecol*, 26: 3293-3299. [Lu Bin, Hou Yuemin, Li Xinyang, et al., 2015. Physiological response and salt-tolerance of *Gleditsia microphylla* under NaCl stress[J]. *Chinese Journal of Applied Ecology*, 26: 3293-3299.]

Lu WJ, Li YS, 2012. *Experimental Course of Plant Physiology*[M]. Beijing: China Forestry Press. [Lu Wenjing, Li Yisong, 2012. *Experimental Course of Plant Physiology*[M]. Beijing: China Forestry Press.]

Ren H, Jian SG, Zhang QM, et al., 2017. Plants and vegetation of South China Sea islands[J]. *Ecol Environ Sci*, 26: 1639-1648. [Ren Hai, Jian Shuguang, Zhang Qianmei, et al., 2017. Plants and vegetation of South China Sea islands[J]. *Ecology and Environmental Sciences*, 26: 1639-1648.]

Sun CC, Zhao HY, Zheng CX, et al., 2017. Effects of NaCl stress on osmolyte and proline metabolism in *Ginkgo biloba* seedling[J]. *J Plant Physiol*, 53:470-476. [Sun Congcong, Zhao Haiyan, Zheng Caixia, et al., 2017. Effects of NaCl stress on osmolyte and proline metabolism in *Ginkgo biloba* seedling[J]. *Journal of Plant Physiology*, 53:470-476.]

Tong Y, Jian SG, Chen Q et al., 2013. Vascular plant diversity of the Paracel Islands, China[J]. *Biodivers Sci*, 21(3):364-374. [Tong Yi, Jian Shuguang, Chen Quan, et al., 2013. Vascular plant diversity of the Paracel Islands, China[J]. *Biodiversity Science*, 21(3):364-374.]

Wang F, Jian S, Liu D, et al., 2018. Constructing coral reef shelter by leveling coral reef sand, planting trees comprising arbor, shrubs, grassvine and herbaceous plant on coral reef sands, and performing conservation process[P]. CN108605572-A; CN108605572-B.

Wang SH, Zhu Y, Wang YF, et al., 2019. Effect of vegetation types on soil physicochemical property in East Island and Yongxing Island of Xisha Islands[J]. *J Trop Subtrop Bot*, 27(4):383-390. [Wang Senhao, Zhu Yijing, Wang Yufang, et al., 2019. Effect of vegetation types on soil physicochemical property in East Island and Yongxing Island of Xisha Islands[J]. *Journal of Tropical and Subtropical Botany*, 27(4):383-390.]

Wang XJ, Zhou Y, Wu Y, et al., 2014. Influence of salt stress on physiological characteristics of *Chrysanthemum morifolium* cv. Chuju[J]. *Guihaia*, 34(6): 828-832. [Wang Xuejuan, Zhou Yi, Wu Yan, et al., 2014. Influence of salt stress on physiological characteristics of *Chrysanthemum morifolium* cv. Chuju[J]. *Guihaia*, 34(6): 828-832.]

Wang XK, Huang JL, 2015. *Principles and Techniques of Plant Physiological and Biochemical Experiments*[M]. 3rd Ed. Beijing: Higher Education Press, 131-133. [Wang Xuekui, Huang Jianliang, 2015. *Principles and Techniques of Plant Physiological and Biochemical Experiments*[M]. 3rd Ed. Beijing: Higher Education Press, 131-133.]

Wang Y, Jin XX, Zhao H, et al., 2017. Effects of salt stress on photosynthesis and antioxidant enzyme activities of *Vicia faba* L. seedling[J]. *N Hortic*, (20): 25-30. [Wang Yun, Jin Xiaoxiao, Zhao Hui, et al., 2017. Effects of salt stress on photosynthesis and antioxidant enzyme activities of *Vicia faba* L. seedling[J]. *Northern Horticulture*, (20): 25-30.]

Wu SH, Chen HW, Jian SG, et al., 2017. The biological characteristics of *Cordia subcordata* on tropical coral island in China[J]. *Ecol Sci*, 36(6): 57-63. [Wu Shuhua, Chen Haowen, Jian Shuguang, et al., 2017. The biological characteristics of *Cordia subcordata* on tropical coral island in China[J]. *Ecological Science*, 36(6): 57-63.]

Xing FW, Deng SW, 2018. *Flora of the South China Sea Islands*[M]. Beijing: China Forestry Press. [Xing Fuwu, Deng Shuangwen, 2018. *Flora of the South China Sea Islands*[M]. Beijing: China Forestry Press.]

Yang WP, Ma R, Yang YY, et al., 2019. Effects of NaCl treatment on the growth and physiological indexes of *Lycium ruthenicum*[J]. *Mol Plant Breed*, 17(13):4437-4447. [Yang Wanpeng, Ma Rui, Yang Yongyi, et al., 2019. Effects of NaCl treatment on the growth and physiological indexes of *Lycium ruthenicum*[J]. *Molecular Plant Breeding*, 17(13):4437-4447.]

Zhang K, Li MN, Cao SH, et al., 2017. Response of *Carex rigescens* to different NaCl concentrations and its salinity threshold calculation[J]. *Pratac Sci*, 34(3):479-487. [Zhang Kun, Li Mingna, Cao Shihao, et al., 2017. Response of *Carex rigescens* to different NaCl concentrations and its salinity threshold calculation[J]. *Pratacultural Science*, 34(3):479-487.]

Zhang LP, Liu Y, Wang Y, et al., 2020. The effects of NaCl stress on the growth and accumulation of penetration adjustment substances in *Glycyrrhiza*[J]. *J Inn Mongol Agic Univ (Nat Sci Ed)*, 41:10-15. [Zhang Linping, Liu Yan, Wang Yang, et al., 2020. The effects of NaCl stress on the growth and accumulation of penetration adjustment substances in *Glycyrrhiza*[J]. *Journal of Inner Mongolia Agricultural University (Natural Science Edition)*, 41: 10-15.]

Zhang RP, Xin WW, Zhang SH, et al., 2018. New data on plants in Guangxi[J]. *Guihaia*, 38(8):1102-1105. [Zhang Ruopeng, Xin Weiwei, Zhang Shuhuan, et al., 2018. New data on plants in Guangxi[J]. *Guihaia*, 38(8):1102-1105.]

Note: Figure translations are in progress. See original paper for figures.