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Ecophysiological Analysis of Mangrove Seedlings *Kandelia obovata* Exposed to Natural Low Temperature at Near 30°N

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Abstract: In this study, mangrove seedlings *Kandelia obovata* were firstly introduced to Zhoushan in Eastern China at 29° 93′ N from Xuwen in South China at 20° 34′ N in April 2016. In order to analyze ecophysiological differences of *K. obovata* seedlings domesticated in Zhoushan, the growth status and antioxidant system of *K. obovata* exposed to natural low temperature were studied through situ measurements. The results showed that *K. obovata* seedlings introduced artificially to Zhoushan grew slowly when subjected to natural cold stress. The chlorophyll contents exhibited a decreased tendency. In addition, 2-butanol and 2,3-butanediol were firstly found in *K. obovata* after being moved to Zhoushan, which are specific substances produced by *K. obovata* under low-temperature stress. Moreover, there was a synergistic competition mechanism in the antioxidant enzyme system in *K. obovata*, in which superoxide dismutase (SOD) would convert oxygen radicals to hydrogen peroxide, and then catalase (CAT) and peroxidase (POD) could work together to remove hydrogen peroxide. This study provides a foundation for better understanding of the response of mangroves to natural low temperature at high latitudes.

Keywords: *Kandelia obovata*; natural low temperature stress; high latitude area; volatile oil; antioxidant system

1. Introduction

Mangrove forest is one of the four most productive natural marine ecosystems with natural wetlands along tropical and subtropical shores, and possesses substantial ecological and commercial value [1], such as protecting shorelines from erosion, preventing dispersion of anthropogenic pollution into aquatic ecosystems [2], offering habitats for different species, etc. [3–5]. Temperature is considered as an important environmental factor limiting the growth and distribution of mangrove plants [6–8]. It is well known that the latitudinal distribution of mangroves is mainly limited by temperature. Low temperature can influence water and nutrient intake [9], stimulate reactive oxygen species production [10], and disturb normal photosynthesis [11] and metabolism [12]. The low-temperature

responses of higher plants have been widely studied and discussed in recent years [13–15]. However, there is little known about the mechanisms of low-temperature injury in mangrove plants at present.

K. obovata is one of the most widely distributed along the South China Coast, which has a higher cold resistance ability when exposed to low temperature [16]. In China, *K. obovata* is naturally distributed in the south of Fuding (27° 20′ N), Fujian Province, China, and the northernmost boundary of artificial domestication is in Yueqing (28° 25′ N), Zhejiang Province, China. As we know, mangroves are limited at their high-latitude areas by low temperature. However, climate change is facilitating range extensions of various mangrove species to higher latitude [17]. Thus, numerous attempts were made to transplant mangroves in higher latitude. Chen et al. investigated the adaptation of introduced mangrove species *Sonneratia apetala* from Bangladesh, and found that it had a competitive advantage over the native species [18]. Chimner et al. provided evidence that planting mangroves in non-native areas such as Hawaii could prove detrimental to existing vegetation as well as the natural functioning of the system [19].

Our research group successfully introduced *K. obovata* to Zhoushan (29° 93′ N), Zhejiang Province in 2016. To date, our understanding of species-specific mangrove responses to low temperature at high latitudes has been lacking, because low-temperature events are relatively uncommon and difficult to fully replicate via manipulative experiments [20–22]. Hence, there is a need for studies that use in situ measurements to improve our understanding of species-specific responses of mangroves to low temperature extremes at high latitudes.

The negative effects of natural low temperatures on the growth of mangrove plants are the inhibition of photosynthesis [8], the inhibition of the activities of superoxide dismutase (SOD) enzymes and other antioxidant enzymes, and increasing the content of MDA and electrolyte leakage [23]. It can be seen that the low temperature not only inhibits the growth of mangrove plants, but also has a great impact on the antioxidant system. Most studies of the antioxidant system of mangrove plants focused on the effects of salt, flooding, heavy metals, and other factors [24,25]. Furthermore, the above studies were conducted in tropical and subtropical regions, and there had been no relevant studies at higher latitudes.

This article investigated the effect of natural low-temperature stress on the growth status and antioxidant mechanism of *K. obovata*, and analyzed ecophysiological differences of *K. obovata* seedlings introduced into Zhoushan, compared with those in Zhanjiang, providing a scientific basis for the artificial introduction of *K. obovata* seedlings at high latitudes.

2. Materials and Methods

2.1. Plant Materials and Processing

In April 2016, 20,000 propagules of *K. obovata* collected from Xuwen(20° 34′ N), Zhanjiang, Guangdong province were introduced to Lujiazhi (29° 93′ N), Zhoushan, Zhejiang province, and 70%–75% seedlings survived after two winters. In January 2018, 100 g normal and 100 g half-wilted leaves of *K. obovata* seedlings were collected form Zhejiang, and 100 g normal leaves from Guangdong were collected for comparison at the same time, labelled as ZN (Zhoushan, Zhejiang), GN (Zhanjiang, Guangdong), and ZH (Zhoushan, Zhejiang), respectively. The leaves of each group were obtained from 80–100 mangrove seedlings, which were washed with distilled water, quickly dried at low temperature, and stored at −80 °C. When testing, each group was repeated three times.

2.2. Chlorophyll and Phenol Determination

The contents of chlorophyll a and chlorophyll b were calculated as described by Daid et al. [26]. For this, 0.1 g of leaves were taken into a mortar, 5 mL of 80% acetone was added, alongside some calcium carbonate and quartz sands, and the mixture was ground into a homogenate. After centrifugation (4000 r/min, 5 min), the absorbance values were measured using a spectrophotometer (UV2600, Shimadzu, Japan) at 663, 645, and 652 nm, respectively.

For the phenol determination, 0.1 g leaves were added into 2.5 mL 95% ethanol, and extracted by ultrasonic wave (300 W, 60 °C, 30 min). After centrifugation (12000 r/min, 10 min), the supernatant was collected; 50 μL supernatant and 250 μL 20 % Na_2CO_3 were set into a 1 mL volumetric flask with double-distilled water. The absorbances were measured with a spectrophotometer (UV2600, Shimadzu, Japan) at 760 nm [27].

2.3. Volatile Oils Detection

For the volatile oil detection, 50 g leaves were washed, dried, put in a steam distiller with 60 mL distilled water, and then extracted for 3 h. The obtained oil–water mixture was saturated with sodium chloride and extracted with diethyl ether, and a pale-yellow extract was presented. The upper layer of the extract was taken, anhydrous magnesium sulfate was added, and the mixture was allowed to remain overnight to remove water. Finally, magnesium sulfate was separated and removed, and the ether was evaporated to obtain a pale-yellow oily liquid. The volatile components of the extraction's oily liquid were measured by gas chromatograph–mass spectrometer (GC-MS, 2010, Shimadzu, Japan).

The GC-MS was equipped with a DB-5MS capillary column (30 m × 0.25 μm × 25 mm). The initial temperature was maintained at 50 °C for 1 min, and then raised to 250 °C at a rate of 10 °C/min. All samples were injected in split mode at 250 °C. The mass spectrometer was operated in EI mode (positive ion, 70 eV), and the quadrupole was 200 °C. Mass spectra were acquired in full scan mode with repetitive scanning from 20 *m/z* to 500 *m/z* for 1 s.

2.4. Enzyme Activity Detection

The enzymatic activities in the supernatant were determined using assay kits purchased from Nanjing Jiancheng Bioengineering Institute, China.

First, 0.1 g of leaves were taken into a mortar, and 1 mL of 50 mmol L^{-1} ice-cold sodium phosphate buffer solution (pH 7.0) mixed with 1.0 mmol/L ethylenediaminetetraacetic acid (EDTA) and 2% polyvinylpyrrolidone (PVP) was added in an ice bath. The homogenate was centrifuged at 10.0× *g*, 4 °C for 10 min, and the supernatant was collected for measurements of protein contents and antioxidant enzyme activities. Protein content was measured according to Bradford (1976) with bovine serum albumin as the standard [28].

SOD activity was determined by the method of the photoreduction of nitroblue tetrazolium (NBT) [29]. A 3-mL mixture containing 0.1 mmol/L EDTA, 2 mmol/L riboflavin, 50 mmol/L phosphate buffer (pH 7.8), 13 mmol/L methionine, 75 μmol/L nitroblue tetrazolium, and 0.5 mL of protein extract was reacted under a light intensity of approximately 120 μmol m^{-2} s^{-1} for 20 min. The absorbance was measured using a spectrophotometer (UV2600, Shimadzu, Japan) at 560 nm. One unit of SOD activity induced approximately 50% inhibition of the auto-oxidation of adrenaline.

Catalase (CAT) activity was measured according the method of Beer and Sizer (1952) [30], with minor modifications. The reaction mixture (1.5 mL) consisted of 100 mmol/L phosphate buffer (pH 7.0), 0.1 mmol/L EDTA, 20 mmol/L H_2O_2, and 50 μL enzyme extract. The reaction was started by addition of the extract. The decrease of H_2O_2 was monitored at 240 nm and quantified by its molar extinction coefficient (36 mol/L cm) and the result expressed as CAT units per min and mg of protein.

Peroxidase (POD) activity was measured according to Ryu and Dordick (1992) [31]. Protein extract (50 μL) was added to a mixture containing 50 mmol/L phosphate buffer (pH 7.0), 20 mmol/L guaiacol, and 10 mmol/L H_2O_2. The formation of tetraguaiacol was determined at 470 nm for 3 min. One unit of peroxidase activity was defined as the amount of the enzyme that causes an increase of 0.01 absorbance unit at 470 nm per min per mg protein.

2.5. MDA Detection

For the MDA detection, 0.5 g of leaves were placed in 3 mL 5% tri-chloroacetic acid, which was centrifuged at 1500× *g* for 10 min at 10 °C. The supernatant was mixed with 2 mL 0.67% thiobarbituric acid, which was heated at 100 °C for 30 min, then centrifuged at 1500× *g* for 10 min at 25 °C, and the

absorbances of the supernatant at 450, 532, and 600 nm were recorded [32]. The MDA content was calculated based on the following formula: C (μmol L^{-1}) = 6.452 × (A_{532} - A_{600}) - 0.559 × A_{450}.

2.6. Statistical Analysis

In all experiments, three replicates were performed for each sample, and each treatment was examined on two parallel samples. Data presented are the means ± SD. One-way analysis of variance (ANOVA) was followed by Student–Newman–Keuls. Each value represents the mean of six replicates ± SD. Means were compared using ANOVA. Data followed by different letters in a row are significantly different at $P < 0.05$.

3. Results

3.1. Chlorophyll Content

Chlorophyll is an essential component of photosynthesis for plants, and its content represents the strength of photosynthesis. As shown in Figure 1 Total chlorophyll, chlorophyll a, and chlorophyll b contents in the normal leaves in Zhanjiang, Guangdong (GN) were the highest, followed by the normal leaves from Zhoushan, Zhejiang (ZN), and the half-wilted leaves from Zhoushan, Zhejiang (ZH) had the lowest content. The chl. a/chl. b ratio in ZN was 5.76, the chl. a/chl. b in GN was 6.67, and the chl. a/chl. b in ZH was 4.32. In biology, the ratio of chlorophyll a to chlorophyll b reflects the utilization efficiency of the light energy of plants, and the higher the ratio, the higher the utilization efficiency. Hence, mangroves in GN had a higher utilization efficiency of light energy, and a higher rate of photosynthesis.

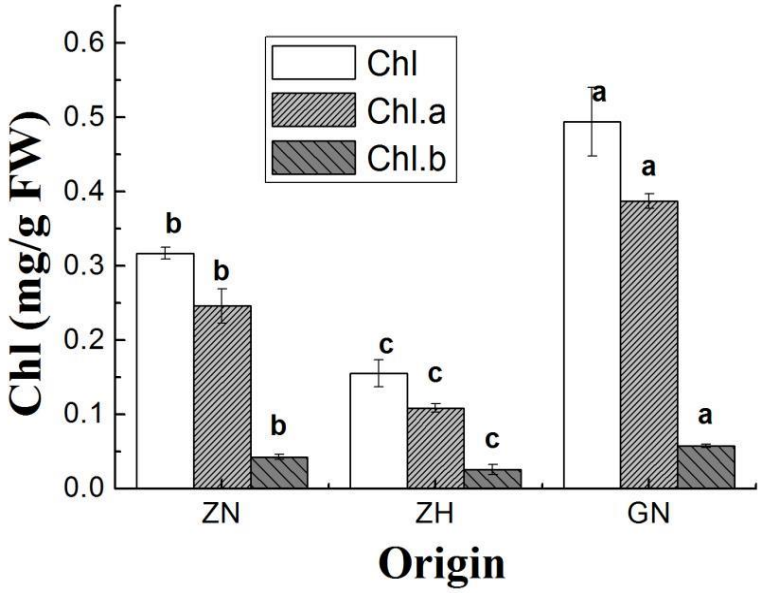

Figure 1. Total chlorophyll, chl. a, and chl. b contents of *Kandelia obovata* seedlings. FW stands for sample fresh weight. Different letters indicate significant difference among origin at the same sampling time according to one-way ANOVA at $P < 0.05$. GN: normal leaves from Zhanjiang, Guangdong; ZH: the half-wilted leaves from Zhoushan, Zhejiang; ZN: the normal leaves from Zhoushan, Zhejiang.

3.2. Total Phenol Content

Phenols are a group of substances formed by secondary metabolism in plants and belong to exogenous antioxidants. Most of them are plant growth hormones. As shown in Figure 2, the total phenol content of GN was the highest, that of ZN was not very different from that of ZH. The total phenol content of GN was three times that of ZN. It had been found that tree age and leaf fractions had

little effect on phenol content, while light time had a significant relationship with phenol content [33]. With the decrease of latitude, sunlight is more abundant and sunshine duration is longer; therefore, the total phenol content of GN was the highest.

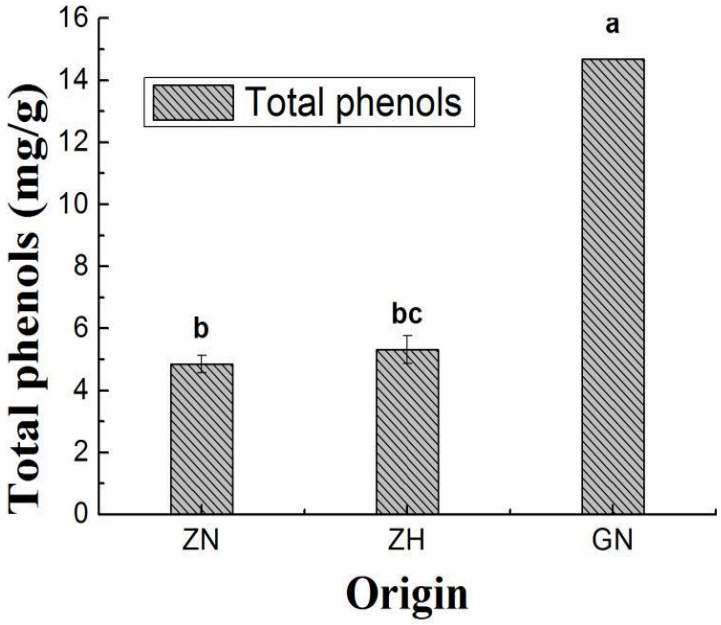

Figure 2. Total phenol contents of *K. obovata* seedlings. Different letters indicate significant difference among sources at the same sampling time according to one-way ANOVA at $P < 0.05$. GN: normal leaves from Zhanjiang, Guangdong; ZH: the half-wilted leaves from Zhoushan, Zhejiang; ZN: the normal leaves from Zhoushan, Zhejiang.

3.3. Volatile Oil Components

A total of 48 chemical constituents from the volatile oil of ZN leaves were identified, including alkane, alkene, acid, alcohol, ketone, benzene, etc. Among the identified compounds, 2-butanol, 2,3-butanediol, oxacycloheptadec-8-en-2-one, and tetradecanoic acid were predominant components, among which the 2,3-butanediol content was the highest in the volatile oil of ZN leaves, accounting for 3.42%, and 2-butanol and 2,3-butanediol were found for the first time in mangrove leaves.

A total of 44 chemical constituents from ZH leaves were identified, including ester, alcohol, phenol, ketone, acid, aldehyde, alkane, etc. Ethyl ester formic acid, tridecanoic acid, 2-butanol and 2,3-butanediol were predominant components, among which 2,3-butanediol content was the highest in the volatile oil of ZN leaves, accounting for 5.25%. It can be seen that 2-butanol and 2,3-butanediol were also present in ZH leaves.

A total of 36 chemical constituents from GN leaves were identified, including alkene, alkane, phenol, ketone, etc. Germacrene-D and 2,6-bis(1,1-dimethylethyl)-4-methyl phenol were predominant components, accounting for 14.87% and 35.2%, respectively.

3.4. Enzymatic Antioxidants

As shown in Figure 3, the SOD activity of ZH was significantly greater than that of the other two groups—up to 14 times greater than that of ZN and 8 times that of GN. The variation trend of CAT activity in the three samples was the same as SOD, and that of POD activity in the three samples was opposite to the trend of SOD. These results indicate that the specific activity of CAT and SOD enzymes is positively correlated. In addition, it is surprising that the POD activity was significantly different independent of the SOD activity or CAT activity. The POD activity of ZN was almost the same as that of GN, and it was nearly 10 times that of ZH.

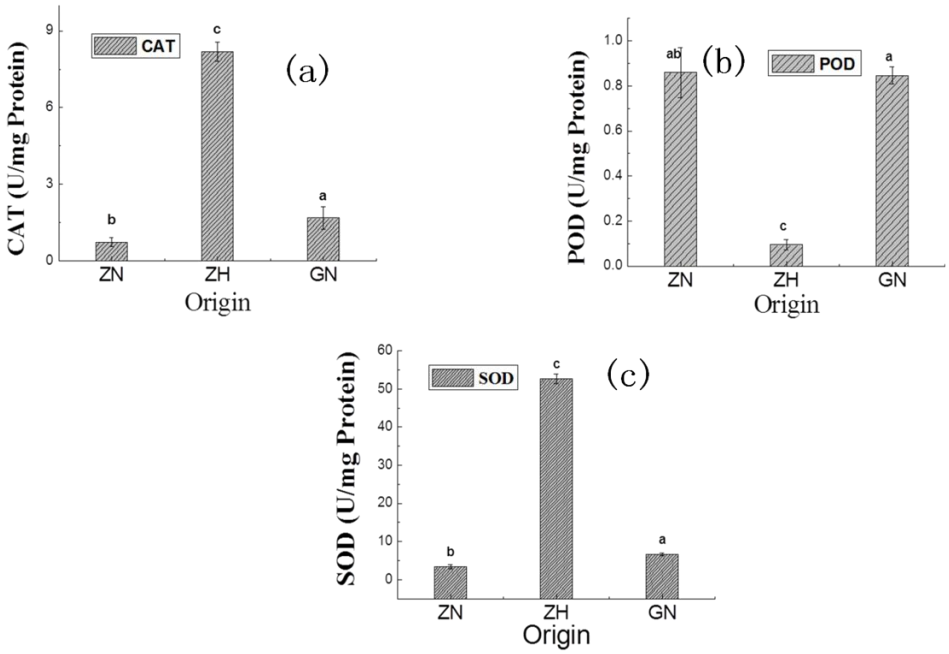

Figure 3. (**a**) Catalase (CAT), (**b**) peroxidase (POD), and (**c**) superoxide dismutase (SOD) enzyme activities of *K. obovata* seedlings. Different letters indicate significant difference among sources at the same sampling time according to one-way ANOVA at $P < 0.05$. GN: normal leaves from Zhanjiang, Guangdong; ZH: the half-wilted leaves from Zhoushan, Zhejiang; ZN: the normal leaves from Zhoushan, Zhejiang.

3.5. Non-Enzymatic Antioxidants

MDA is the final decomposition product of lipid peroxidation, and is used as an index for the status of lipid peroxidation due to increasing membrane damage. As shown in Figure 4, MDA contents in ZH were the lowest among the three samples, and the differences between ZN and GN were not obvious. When mangroves were introduced to Zhoushan, the MDA contents of leaves in ZN maintained a normal level, but those in ZH presented a remarkable decline.

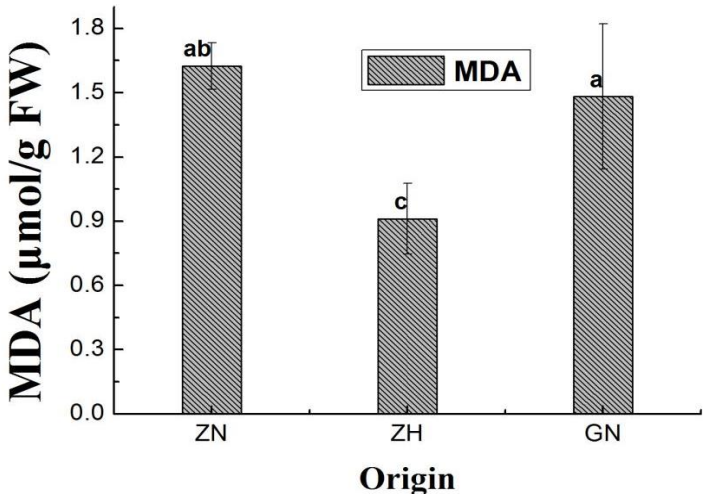

Figure 4. MDA contents of *K. obovata* seedlings. FW stands for sample fresh weight. Different letters indicate significant difference among sources at the same sampling time according to one-way ANOVA at $P < 0.05$. GN: normal leaves from Zhanjiang, Guangdong; ZH: the half-wilted leaves from Zhoushan, Zhejiang; ZN: the normal leaves from Zhoushan, Zhejiang.

4. Discussion

The growth rate of *K. obovata* seedlings in high latitudes is less than those in low latitudes under natural low-temperature stress. The natural distribution of *K. obovata* is that the higher the latitude, the lower the plant height after planting [34]. *K. obovata* from Jiulong River (24° 23′ N) could grow to 7.9 m, and those from Minami-Izu, Japan (34° 38′ N) could be up to 3–4 m [35]. One of the most important factors limiting mangrove growth is the low-temperature environment, which inhibits the synthesis of chlorophyll [36]. The synthesis of chl. a is sensitive to low temperatures, which could affect photosynthesis and may destroy chloroplast structure and inhibit enzyme activity [37]. Hence, the chlorophyll contents of *K. obovata* had a generally decreased tendency after being moved to Zhoushan.

Phenols are very important plant constituents which have radical scavenging ability due to their hydroxyl groups [38]. The phenolic content of *K. obovata* decreased significantly after being introduced into Zhoushan. The natural low temperature has no significant effect on the phenolic content, however, the length of light exposure has a great relationship with the phenol content [33]. Thus, the mangroves introduced to high-latitude areas lacked sufficient sunlight, and thus the phenol contents of ZN and ZH were inferior to that of GN.

Based on the GC-MS analysis, as can be seen from volatile oil components of three samples, *K. obovata* introduced to Zhoushan (ZN and ZH) contained more volatile oil substances than the GN sample. Besides, it is surprising that 2-butanol and 2,3-butanediol were only determined in mangroves from Zhoushan (ZN and ZH), and 2,6-bis(1,1-dimethylethyl)-4-methyl phenol (BHT) was only determined in mangroves from GN. Therefore, it is concluded that *K. obovata* generates different volatile oil components to adapt to low temperature. To our best knowledge, these results represent the first time that 2-butanol and 2,3-butanediol have been found in mangrove plants. Alcohols have been proved to lower freezing point [39], the possible mechanisms of which include: (i) a viscosity increase, retarding the diffusion of water during freezing and creating smaller crystals; (ii) "binding" of water, reducing the total amount of ice formed; and (iii) reduction in crystal growth rate, permitting the nucleation of additional crystals with an ultimate smaller size [40]. So, the results suggest that 2-butanol and 2,3-butanediol are specific substances produced by mangrove under low-temperature stress. The specific roles of alcohols in mangrove plants under cold stress still need to be studied.

Interestingly, 2,6-bis(1,1-dimethylethyl)-4-methyl phenol (BHT), also named butylated hydroxytoluene, a synthetic antioxidant, was detected in mangroves from GN, accounting for 35.2%, which is consistent with the previous studies [41–43] demonstrating that BHT is the highest volatile oil component of mangroves from South China. Thus, these mangroves from South China have the potential to be used as an alternative commercial source for BHT production. Besides, there has been a report of the occurrence of BHT in freshwater algae, which found that the quantity of BHT produced was related to the irradiation intensity of the growth conditions, exhibiting a positive correlation with the degree of antioxidative activity [44]. These results demonstrate that mangroves can produce BHT only in high-latitude areas, where there is abundant sunlight. However, when mangroves were introduced to lower-latitude areas, more alcohols emerged instead of BHT. Thus, it is suggested that BHT in mangroves is converted into 2-butanol and 2,3-butanediol in order to adapt to the low-latitude environment. Further studies focusing on these volatile oil components will enable better understanding of the cold-resistance mechanism in mangrove plants.

It is widely recognized that the low-temperature-induced damage of plant cells is mainly due to the excess ROS generated in plant cells, such as O_2, H_2O_2, and OH^-, which can cause lipid peroxidation, membrane damage, enzyme disorders, and even cell death [45]. The enzyme defense system is in a synergistic relationship in the antioxidant system. SODs constitute the first line of plant defense against ROS, which can dismutate O_2^- to H_2O_2 and O_2 [46]. The SOD activity of ZH increased, obviously due to excess low-temperature stress (Figure 3c), which indicates that ZH may have been challenged by higher oxidative stresses. However, when exposed to natural low temperature, the SOD activity of ZN was not changed obviously, which implies that ZN was not damaged by oxidative stress. SOD

activity increasing under low temperature stress could accelerate the removal of excess O_2^-, but it could also accelerate the production of H_2O_2. The accumulation of H_2O_2 would cause the damage of anti-oxidative enzymes, which in turn would aggravate the release of ROS and result in serious cell damage [47]. Thus, the scavengers of H_2O_2 play a critical role in plant chilling-resistant processes.

CAT has a high protein turnover rate in plant cells, and is found in peroxisomes, cytosol, and mitochondria [48]. As an enzymatic antioxidant, CAT can convert H_2O_2 into H_2O and O_2 [49]. In the present work, CAT activity increased significantly in ZH leaves (Figure 3a), which had the same trend as the SOD activity of ZH. POD is another important H_2O_2 scavenger. However, in the present work, ZH showed much lower POD activity than the other samples (Figure 3b). It is speculated that POD and CAT have a competitive relationship to scavenge H_2O_2.

As the peroxidation product of membrane lipids, MDA is a reliable indicator of membrane injury caused by ROS [50]. In the present work, a significant decrease in the content of MDA was observed in ZH, which indicated that ZH controlled the ROS generation when exposed to natural low temperature. The reduced MDA content in ZH may be due to an increased antioxidant defense system that scavenges ROS during or after low-temperature stress [51].

Our enzymatic antioxidant results show that excessive oxygen free radicals can be stimulated due to the effects of cold stress, which trigger the plant's initiation of the enzyme defense system. Firstly, the SOD acts to convert oxygen radicals to hydrogen peroxide, and then CAT and POD work together to remove hydrogen peroxide. Moreover, it is speculated that POD and CAT show a competitive relationship under the accumulation of oxygen free radicals that can be tolerated by seedlings under low-temperature stress, which explains that the specific activity of the POD enzyme was at a low level, while the specific activity of the other two enzymes was at a higher level.

5. Conclusions

This study demonstrated that mangrove (*K. obovata*) could survive at near 30° N. *K. obovata* seedlings grew slowly after being introduced into Zhoushan, and the contents of chlorophyll showed a remarkable decline. Surprisingly, for the first time, 2-butanol and 2, 3-butanediol were found in mangrove plants after being moved to Zhoushan, and BHT was only present in mangrove seedlings from Guangdong. Thus, it is speculated that BHT may be broken down to small molecular alcohol substances when introduced into high-latitude areas. In addition, there was a synergistic competition mechanism in the antioxidant enzyme system in *K. obovata*, which could help mangroves to adapt to natural low temperature; we propose that SOD acts to convert oxygen radicals to hydrogen peroxide, and then CAT and POD work together to remove hydrogen peroxide. Further research is needed to reveal the detailed regulatory mechanisms and functions of these specific volatile oil components. This will enable a better understanding of the cold-resistance mechanism of mangroves after being introduced into high-latitude areas.

Author Contributions: W.S., L.J., and C.Z. conceived and designed the experiments. Z.W. and D.Y. conducted the experiments. W.S., L.J., and Z.W. discussed the results. Z.W., L.C., Y.W., and J.G. wrote the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Ghasemia, S.; Moghaddamb, S.S.; Rahimib, A.; Damalas, C.A.; Naji, A. Ecological risk assessment of coastal ecosystems: The case of mangrove forests in Hormozgan Province, Iran. *Chemosphere* **2017**, *191*, 417–426. [CrossRef] [PubMed]
2. Qiu, Y.W.; Qiu, H.L.; Zhang, G.; Li, J. Bioaccumulation and cycling of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) in three mangrove reserves of south China. *Chemosphere* **2019**, *217*, 195–203. [CrossRef] [PubMed]
3. Tam, N.F.Y.; Wong, Y.S. Spatial variation of heavy metals in surface sediments of Hong Kong mangrove swamps. *Environ. Pollut.* **2000**, *110*, 195–205. [CrossRef]
4. Lewis, M.; Pryor, R.; Wilking, L. Fate and effects of anthropogenic chemicals in mangrove ecosystems: A review. *Environ. Pollut.* **2011**, *159*, 2328–2346. [CrossRef] [PubMed]
5. Chai, M.; Shen, X.; Li, R.; Qiu, G. The risk assessment of heavy metals in Futian mangrove forest sediment in Shenzhen Bay (South China) based on SEM–AVS analysis. *Mar. Pollut. Bull.* **2015**, *97*, 431–439. [CrossRef] [PubMed]
6. Berry, J.; Bjorkman, O. Photosynthetic response and adaptation to temperature in higher plants. *Annu. Rev. Plant Physiol.* **1980**, *31*, 491–543. [CrossRef]
7. Tomlinson, P.B. The Botany of Mangrove. *Q. Rev. Biol.* **1987**, *52*, 238.
8. Kao, W.Y.; Shih, C.N.; Tsai, T.T. Sensitivity to chilling temperatures and distribution differ in the mangrove species *Kandelia candel* and *Avicennia marina*. *Tree Physiol.* **2004**, *24*, 859–864. [CrossRef]
9. Chinnusamy, V.; Zhu, J.; Zhu, J.-K. Cold stress regulation of gene expression in plants. *Trends Plant Sci.* **2007**, *12*, 444–451. [CrossRef]
10. Fortunato, A.S.; Lidon, F.C.; Batista-Santos, P.; Leitao, A.E.; Pais, I.P.; Ribeiro, A.I.; Ramalho, J.C. Biochemical and molecular characterization of the antioxidative system of *Coffea* sp. under cold conditions in genotypes with contrasting tolerance. *J. Plant Physiol.* **2010**, *167*, 333–342. [CrossRef]
11. Posmyk, M.M.; Bailly, C.; Szafranska, K.; Janas, K.M.; Corbineau, F. Antioxidant enzymes and isoflavonoids in chilled soybean (*Glycine max* (L.) Merr.) seedlings. *J. Plant Physiol.* **2005**, *162*, 403–412. [CrossRef] [PubMed]
12. Ensminger, I.; Busch, F.; Huner, N.P.A. Photostasis and cold acclimation: Sensing low temperature through photosynthesis. *Physiol. Plant.* **2006**, *126*, 28–44. [CrossRef]
13. Huang, W.; Zhang, S.B.; Xu, J.C.; Liu, T. Plasticity in roles of cyclic electron flow around photosystem I at contrasting temperatures in the chilling-sensitive plant *Calotropis gigantea*. *Environ. Exp. Bot.* **2017**, *141*, 145–153. [CrossRef]
14. Liu, Y.-F.; Zhang, G.-X.; Qi, M.-F.; Li, T.-L. Effects of Calcium on Photosynthesis, Antioxidant System, and Chloroplast Ultrastructure in Tomato Leaves under Low Night Temperature Stress. *J. Plant Growth Regul.* **2015**, *34*, 263–273. [CrossRef]
15. Campos, M.D.; Nogales, A.; Cardoso, H.G.; Campos, C.; Grzebelus, D.; Velada, I.; Schmitt, B.A. Carrot plastid terminal oxidase gene (DcPToX) responds early to chilling and harbors intronic pre-mirnas related to plant disease defense. *Plant Gene* **2016**, *7*, 21–25. [CrossRef]
16. Lin, P. *Mangrove Ecosystem in China*; Science Press: Beijing, China, 1999.
17. Osland, M.J.; Enwright, N.; Day, R.H.; Doyle, T.W. Winter climate change and coastal wetland foundation species: Salt marshes vs. mangrove forests in the southeastern United States. *Glob. Chang. Biol.* **2013**, *19*, 1482–1494. [CrossRef] [PubMed]
18. Chen, L.; Nora, F.Y.T.; Huang, J.; Zeng, X.; Meng, X.; Zhong, C.; Wong, Y.; Lin, G. Comparison of ecophysiological characteristics between introduced and indigenous mangrove species in China. *Estuar. Coast. Shelf Sci.* **2008**, *79*, 644–652. [CrossRef]
19. Chimner, R.A.; Fry, B.; Kaneshiro, M.Y.; Cormier, N. Current extent and historical expansion of introduced mangroves on O'ahu, Hawai'i. *Pac. Sci.* **2006**, *60*, 377–383. [CrossRef]
20. Pickens, C.N.; Hester, M.W. Temperature tolerance of early life history stages of black mangrove *Avicennia germinans*: Implications for range expansion. *Estuar. Coast.* **2011**, *34*, 824–830. [CrossRef]
21. Patton, C.; Lehmann, S.C.M.; Parker, J.D. Convergence of three mangrove species towards freeze-tolerant phenotypes at an expanding range edge. *Funct. Ecol.* **2015**, *29*, 1332–1340. [CrossRef]
22. Coldren, G.A.; Proffitt, C.E. Mangrove seedling freeze tolerance depends on salt marsh presence, species, salinity, and age. *Hydrobiologia* **2017**, *803*, 159–171. [CrossRef]

23. Peng, Y.L.; Wang, Y.S.; Fei, J.; Sun, C.C.; Cheng, H. Ecophysiological differences between three mangrove seedlings (*Kandelia obovata*, *Aegiceras corniculatum*, and *Avicennia marina*) exposed to chilling stress. *Ecotoxicology* **2015**, *24*, 1722–1732. [CrossRef] [PubMed]
24. Li, R.Y.; Li, R.L.; Chai, M.; Shen, X.X.; Xu, H.L.; Qiu, G.Y. Heavy metal contamination and ecological risk in Futian mangrove forest sediment in Shenzhen Bay, South China. *Mar. Pollut. Bull.* **2015**, *101*, 448–456. [CrossRef] [PubMed]
25. Meeder, J.F.; Parkinson, R.W.; Ruiz, P.L.; Ross, M.S. Saltwater encroachment and prediction of future ecosystem response to the Anthropocene Marine Transgression, Southeast Saline Everglades, Florida. *Hydrobiologia* **2017**, *803*, 29–48. [CrossRef]
26. Jespersen, D.; Zhang, J.; Huang, B. Chlorophyll loss associated with heat-induced senescence in bentgrass. *Plant Sci.* **2016**, *249*, 1–12. [CrossRef] [PubMed]
27. Lamuela-Raventós, R.M.; Singleton, V.L.; Orthofer, R. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. *Methods. Mol. Biol.* **1999**, *299*, 152–178.
28. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. [CrossRef]
29. Beauchamp, C.; Fridovich, I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Anal. Biochem.* **1971**, *44*, 276–287. [CrossRef]
30. Beer, R.F., Jr.; Sizer, I.W. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J. Biol. Chem.* **1952**, *195*, 133–140.
31. Ryu, K.; Dordick, J.S. How do organic solvents affect peroxidase structure and function? *Biochemistry* **1992**, *31*, 2588–2598. [CrossRef]
32. Heath, R.L.; Packer, L. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* **1968**, *125*, 189–198. [CrossRef]
33. Bergqvist, J.; Dokoozlian, N.; Ebisuda, N. Sunlight exposure and temperature effects on berry growth and composition of Cabernet Sauvignon and Grenache in the central San Joaquin Valley of California. *Am. J. Enol. Viticult.* **2001**, *52*, 1–7.
34. Upadhyay, V.P.; Mishra, P.K. An Ecological Analysis of Mangroves Ecosystem of Odisha on the Eastern Coast of India. *Proc. Indian Natl. Sci. Acad.* **2014**, *80*, 647–661. [CrossRef]
35. Wakushima, S.; Kuraishi, S.; Sakurai, N. Soil salinity and pH in Japanese mangrove forests and growth of cultivated mangrove plants in different soil conditions. *J. Plant Res.* **1994**, *107*, 39–46. [CrossRef]
36. Zheng, C.F.; Ye, Y.; Liu, W.C.; Tang, J.W.; Zhang, C.N.; Qiu, Z.B.; Chen, J.N. Recovery of photosynthesis, sucrose metabolism, and proteolytic enzymes in *Kandelia obovate* from rare cold events in the northernmost mangrove, China. *Ecol. Process.* **2016**, *5*, 9. [CrossRef]
37. Taylor, A.O.; Rowley, J.A. Plants under Climatic Stress: I. Low Temperature, High Light Effects on Photosynthesis. *Plant Physiol.* **1971**, *47*, 713. [CrossRef]
38. Hatano, T.; Edamatsu, R.; Mori, A.; Fujita, Y.; Yasuhara, E. Eect of interaction of tannins with co-existing substances VI. Eect of tannins and related polyphenols on superoxide anion radical and on DPPH radical. *Chem. Pharm. Bull.* **1989**, *37*, 2016–2021. [CrossRef]
39. Pennycooke, J.C.; Towill, L.E. Cryopreservation of shoot tips from in vitro plants of sweet potato [*Ipomoea batatas (L.) Lam.*] by vitrification. *Plant Cell Rep.* **2000**, *19*, 733–737. [CrossRef]
40. Meryman, H.T. Mechanics of freezing in living cells and tissues. *Science* **1956**, *124*, 515–521. [CrossRef]
41. Hu, S.W.; Song, W.D.; Wang, H.; Yan, J.B.; Li, S.J. Study on the Volatile oil and Fatty Acids of the Leaves of the Mangrove Plants *Cerbera manghas*. *J. Fujian. Forest. Sci. Technol.* **2010**, *37*, 46–50.
42. Guo, X.X.; Tao, Z.; Song, W.D. Characteristics of chemical constituents of volatile oil from leaves of mangrove plant *Avicennia marina* by gas chromatography/mass spectrometry. *J. Trop. Oceanogr.* **2008**, *1*, 57–59.
43. Ji, L.L.; Song, W.D.; LIU, J.X. Determination of Volatile Oil and Fatty Acids in *Screw-pine*. *Mod. Food Sci. Technol.* **2008**, *06*, 588–592.
44. Babu, B.; Wu, J.T. Production of natural butylated hydroxytoluene as an antioxidant by freshwater phytoplankton 1. *J. Phycol.* **2008**, *44*, 1447–1454. [CrossRef] [PubMed]
45. Foyer, C.H.; Noctor, G. Redox homeostasis and antioxidant signaling: A metabolic interface between stress perception and physiological responses. *Plant Cell* **2005**, *17*, 1866–1875. [CrossRef] [PubMed]
46. Alscher, R.G.; Erturk, N.; Heath, L.S. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *J. Exp. Bot.* **2002**, *53*, 1331–1341. [CrossRef] [PubMed]

47. Jaleel, C.A.; Riadh, K.; Gopi, R.; Manivannan, P.; Inès, J.; Al-Juburi, H.J.; Zhao, C.-X.; Shao, H.-B.; Panneerselvam, R. Antioxidant defense responses: Physiological plasticity in higher plants under abiotic constraints. *Acta Physiol. Plant.* **2009**, *31*, 427–436. [CrossRef]
48. Feierabend, J. Catalases in plants: Molecular and functional properties and role in stress defence. In *Antioxidants and Reactive Oxygen Species in Plants*; Smirnoff, N., Ed.; Blackwell Publishing: Oxford, UK, 2007; pp. 101–140.
49. Apel, K.; Hirt, H. Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.* **2004**, *55*, 373–399. [CrossRef] [PubMed]
50. Bonnes-Taourel, D.; Guérin, M.C.; Torreilles, J. Is malonaldehyde a valuable indicator of lipid peroxidation? *Biochem. Pharmacol.* **1992**, *44*, 985–988. [CrossRef]
51. Kuk, Y.I.; Shin, J.S.; Burgos, N.R.; Hwang, T.E.; Han, O.; Cho, B.H.; Jung, S.; Guh, J.O. Antioxidative enzymes offer protection from chilling damage in rice plants. *Crop. Sci.* **2003**, *43*, 2109–2117. [CrossRef]