Kandelia candel Thioredoxin f

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Water deficit caused by osmotic stress and drought limits crop yield and tree growth worldwide. Screening and identifying candidate genes from stress-resistant species are a genetic engineering strategy to increase drought resistance. In this study, an increased concentration of mannitol resulted in elevated expression of thioredoxin f (*KcTrxf*) in the nonsecretor mangrove species *Kandelia candel*. By means of amino acid sequence and phylogenetic analysis, the mangrove Trx was classified as an f-type thioredoxin. Subcellular localization showed that KcTrxf localizes to chloroplasts. Enzymatic activity characterization revealed that KcTrxf recombinant protein possesses the disulfide reductase function. *KcTrxf* overexpression contributes to osmotic and drought tolerance in tobacco in terms of fresh weight, root length, malondialdehyde (MDA) content, and hydrogen peroxide (H_2O_2) production. KcTrxf was shown to reduce the stomatal aperture by enhancing K^+ efflux in guard cells, which increased the water-retaining capacity in leaves under drought conditions. Notably, the abscisic acid (ABA) sensitivity was increased in *KcTrxf*-transgenic tobacco, which benefits plants exposed to drought by reducing water loss by promoting stomatal closure. *KcTrxf*-transgenic plants limited drought-induced H_2O_2 in leaves, which could reduce lipid peroxidation and retain the membrane integrity. Additionally, glutathione (GSH) contributing to reactive oxygen species (ROS) scavenging and transgenic plants are more efficient at regenerating GSH from oxidized glutathione (GSSG) under conditions of drought stress. Notably, *KcTrxf*-transgenic plants had increased glucose and fructose contents under drought stress conditions, presumably resulting from KcTrxf-promoted starch degradation under water stress. We conclude that KcTrxf contributes to drought tolerance by increasing the water status, by enhancing osmotic adjustment, and by maintaining ROS homeostasis in transgene plants.

Keywords: thioredoxin ; Kandelia candel ; mannitol ; drought ; water retaining capacity ; soluble sugar ; H2O2 ; stomatal aperture ; abscisic acid ; K+ flux ; guard cells ; noninvasive micro-test technique

1. Introduction and History

Water deficits caused by osmotic stress, drought, and salt result in the accumulation of reactive oxygen species (ROS), which impairs the function of biochemical processes, damages organelles, and ultimately results in cell death in stressed plants [1]. We have previously shown that seedlings of a non-secretor mangrove, *K. candel*, possess an efficient oxygen scavenging system against ROS under NaCl stress [2][3][4]. *K. candel* increased transcription of *CSD* gene encoding a Cu/Zn superoxide dismutase to reduce ROS in the chloroplast in a long-term and high saline environment [2]. Moreover, salt treatment increased transcription of a f-type thioredoxin (Trx) in *K. candel* [3]. Being as small and ubiquitous proteins (12-14 kD) with the conserved redox-active site (WCXPC), Trxs serve as a crucial important redox regulators in higher plants [5][6]. These proteins are able to catalyze the reduction of disulfide bonds in many target proteins to regulate their structure and function [5][6]. We have shown that *KcTrxf*-transgenic plants could scavenge the salt-elicited ROS in leaf cells through the up-regulation of catalase and ascorbate peroxidase and increased the activities of MDAR (monodehydroascorbate reductase) and GR (glutathione reductase) in chloroplast AsA-GSH cycle, leading to an increase in the ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) and non-protein thiols (NPTs) in the leaves [3]. Antioxidative systems also play an important role in the defense against negative consequences of drought stress [7]. However, the regulatory roles of *K. candel* Trx family genes in osmotic and drought tolerance are not yet fully understood.

2. Development

We attempted to explore the role of *KcTrxf* in plant adapting to water limited environments. Increasing concentration of mannitol resulted in elevated expression of thioredoxin f (*KcTrxf*) in *Kandelia candel*. This suggests that KcTrxf may contribute to osmotic tolerance. By means of amino acid sequence and phylogenetic analysis, the mangrove Trx was classified as an f-type thioredoxin. Subcellular localization showed that KcTrxf localized to chloroplast. Trx activity was analyzed using purified recombinant KcTrxf protein. Enzymatic activity characterization revealed that KcTrxf possessed the disulfide reductase function. *KcTrxf* overexpression contributed to osmotic and drought tolerance in tobacco in terms of fresh weight, root length, malondialdehyde content, and H_2O_2 production. KcTrxf was shown to reduce stomatal aperture by enhancing K^+ efflux in guard cells, which increased water retaining capacity in leaves under drought. Noteworthy, the abscisic acid (ABA) sensitivity was increased in *KcTrxf*-transgenic tobacco, which benefits the droughted plants to reduce water loss by promoting stomatal closure. *KcTrxf*-transgenic plants limited the drought-induced H_2O_2 in leaves, which could reduce lipid peroxidation and retain the membrane integrity. Noteworthy, *KcTrxf*-transgenic plants increased glucose and fructose under drought stress, presumably resulting from the KcTrxf-promoted starch degradation under water stress. We conclude that *KcTrxf* contributed to drought tolerance by increasing water status, enhancing osmotic adjustment, and maintaining reactive oxygen species homeostasis in transgene plants.

Our findings demonstrated the role of *K. candel Trxf* in the osmotic and drought tolerance. In particular, the following findings add new to our knowledge of mechanisms contributing to drought tolerance in higher plants, in brief,

1. KcTrxf contains a redox-active dithiol in the active site and serves as a redox regulator [5][6]. Therefore, KcTrxf might directly participate in the control of ROS under drought conditions.
2. KcTrxf contributes to regulating stomatal aperture by mediating the K^+ flow through plasma membrane in guard cell. This enabled the water-stressed plants to maintain water status, thus limiting the ROS production under drought stress [1].
3. KcTrxf could modify the osmolytes for osmotic adjustment to deal with drought stress [8][9]. KcTrxf increased glucose and fructose by enhancing starch degradation. Similarly, Trx f1-regulated b-amylase triggers diurnal starch degradation in *Arabidopsis* guard cells, and in mesophyll cells under osmotic stress [10]

References

1. Mingyi Jiang; Jian-Hua Zhang; Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and up-regulates the activities of antioxidant enzymes in maize leaves.. *Journal of Experimental Botany* **2002**, *53*, 2401-2410, 10.1093/jxb/erf090.
2. Xiaoshu Jing; Peichen Hou; Yanjun Lu; Shurong Deng; Niya Li; Rui Zhao; Jian Sun; Yang Wang; Yansha Han; Tao Lang; et al. Overexpression of copper/zinc superoxide dismutase from mangrove Kandelia candel in tobacco enhances salinity tolerance by the reduction of reactive oxygen species in chloroplast. *Frontiers in Plant Science* **2015**, *6*, , 10.3389/fpls.2015.00023.
3. Jing, X.; Sun, Y.; Xiang, M.; Qian, Z.; Lang T.; Zhao, R.; Shen, X.; Chen, S. Overexpression of KcTrxf in tobacco enhances salt tolerance through the regulation of ROS homeostasis under NaCl stress. J. Beijing For. Univ. 2015, 37, 17–26.
4. Niya Li; Xiaoyang Zhou; Ruigang Wang; Jinke Li; Cunfu Lu; Shaoliang Chen; Salt Compartmentation and Antioxidant Defense in Roots and Leaves of Two Non-Salt Secretor Mangroves under Salt Stress. *Mangrove Ecosystem Ecology and Function* **2018**, , , 10.5772/intechopen.75583.
5. Thioredoxin; thioredoxin reductases. *Science-Business eXchange* **2012**, *5*, 11-11, 10.1038/scibx.2012.11.
6. Peter Geigenberger; Ina Thormählen; Danilo M. Daloso; Alisdair R. Fernie; Press Enter Key For Correspondence Information; The Unprecedented Versatility of the Plant Thioredoxin System. *Trends in Plant Science* **2017**, *22*, 249-262, 10.1016/j.tplants.2016.12.008.
7. Mosfeq-Ul Hasan; Fanglu Ma; Zakaria Prodhan; Feng Li; Hao Shen; Yadong Chen; Xuede Wang; Molecular and Physio-Biochemical Characterization of Cotton Species for Assessing Drought Stress Tolerance. *International Journal of Molecular Sciences* **2018**, *19*, 2636, 10.3390/ijms19092636.
8. Andrea Polle; Shao Liang Chen; Christian Eckert; Antoine Harfouche; Engineering Drought Resistance in Forest Trees. *Frontiers in Plant Science* **2019**, *9*, , 10.3389/fpls.2018.01875.
9. A. Harfouche; Richard Meilan; Arie Altman; Molecular and physiological responses to abiotic stress in forest trees and their relevance to tree improvement. *Tree Physiology* **2014**, *34*, 1181-1198, 10.1093/treephys/tpu012.
10. Concetta Valerio; Alex Costa; Lucia Marri; Emmanuelle Issakidis-Bourguet; Paolo Pupillo; Paolo Trost; Francesca Sparla; Thioredoxin-regulated beta-amylase (BAM1) triggers diurnal starch degradation in guard cells, and in mesophyll cells under osmotic stress.. *Journal of Experimental Botany* **2010**, *62*, 545-55, 10.1093/jxb/erq288.