Journal of Plant Physiology & Pathology

Mini Review A SCITECHNOL JOURNAL

A Rice bZIP Transcription Factor Regulates Duf630/632 Domain Protein in Response to Drought Stress

Jianfu Zhang[1*], Yunjie Xie, Xiangzhen Yu

Department of Agriculture, Fujian Academy of Agricultural Sciences, Fuzhou, China

***Corresponding Author:** Jianfu Zhang, Fujian Academy of Agricultural Sciences, Fuzhou, China; Email: jianfzhang@163.com

Received date: 03 August, 2024, Manuscript No. JPPP-24-144349;

Editor assigned date: 05 August, 2024, Pre QC No. JPPP-24-144349 (PQ);

Reviewed date: 20 August, 2024, QC No. JPPP-24-144349;

Revised date: 28 August, 2024, Manuscript No. JPPP-24-144349 (R);

Published date: 05 September, 2024, DOI: 10.4172/2329-955X.1000361

Abstract

Drought severely affects crop yields and poses a great threat to food security. It is an urgent task to mine drought tolerance genes and breed new varieties. In previous studies, transcription factors such as Basic Leucine Zipper (bZIP), Dehydration-Responsive Element Binding (DREB), DNA Binding with One Finger (DOF), Heat Shock Transcription Factor (HSF), v-Myb Myeloblastosis Viral Oncogene Homolog (MYB) etc., have been found to be key factors in response to drought stress, and the corresponding regulatory mechanisms have been gradually analyzed. Among them, the bZIP family has been shown to respond to drought stress through multiple pathways in plants. Recently, our team screened OsbBP1, the downstream target of drought tolerance regulator *OsbZIP72*, through Chip-seq technology. *OsBBP1*, a typical DUF630/632 domain protein, is localized in the nucleus and cytoplasm, and the DUF632 domain contributes to its localization in the cytoplasm. Through gene function identification, it is discovered that OsBBP1 was induced by Polyethylene Glycol (PEG) and Abscisic Acid (ABA) and positively regulated rice drought tolerance. Further, *OsBBP1* affects Reactive Oxygen Species (ROS) accumulation in plants by regulating ROS scavenging enzyme activity under drought conditions were found, thereby improving the survival rate of rice. The study revealed that the bZIP transcription factor family responds to drought stress by regulating DUF domain protein, and provided another regulatory pathway for plant resistance to abiotic stress.

Keywords: Transcription factor; bZIP family; DUF domain; ROS; Drought tolerance

Introduction

The increasing frequency and intensity of droughts due to the changing global climate pose a serious threat to the stability of agricultural production. Cultivation of drought-tolerant crops is an important strategy to improve the disaster resistance ability of agricultural production, ensure food supply, and realize resource conservation and environmental friendliness. In recent years, drought tolerance genes in plants have been discovered one after another and the molecular mechanisms of drought tolerance regulation have been gradually revealed. It mainly involves the activity of transcription factors, which act as switches responsible for "turning on" or "off" downstream target genes, and can also activate other transcription factors through cascades, forming complex signal networks, so that plants adapt to water shortage environment.

Plant response to drought is multi-pathway and the bZIP, Dehydration Responsive Element-Binding (DREB), DOF, Heat Shock Transcription Factors (HSF), Myeloblastosis (MYB), No Apical Meristem (NAC), Tricalcium Phosphate (TCP), WRKY and AP2/ERF family members act as key regulatory factors [1,2]. MYB and NAC transcription factors are widely involved in growth and development and stress response. For example, MYB46 has been found to regulate cell wall biosynthesis-related genes, and may be involved in drought tolerance with DREB [3,4]; *ANAC019* and *NtNAC053* regulate multiple target genes in drought response [5-7]. Some AP2/ERF transcription factors, such as P to ERF15, play a key role in response to ethylene and drought, affecting plant resistance to retroactivity. Members of the bZIP family plays an important role in abiotic stress ABA signaling pathway in plants, often binding the ABA-Responsive Elements (ABREs) containing an Adenine, Cytosine, Guanine and Thymine (ACGT) core motif, or the G-box motif [8-11]. In Arabidopsis, *AREB1*, *AREB2, ABF3* and *ABF1* belong to the group. A bZIP proteins, all of which play an integral role in ABA signaling response to drought stress [12-14]. In pepper plants, CaDILZ1, a member of subgroup D of the bZIP protein family, interacts with the RING finger protein CaDSR1, and involve in the ABA-mediated drought stress signaling pathway [15]. There are also multiple bZIP transcription factors that function together to cope with drought stress. For example, TaFDL2-1A and TabZIP8-7A, two bZIP transcription factors in wheat, can form transcriptional activation complex that synergistically promotes the expression of *ABA*-induced genes in response to drought stress [16]. In rice, *OsbZIP71* directly binds to the promoters of abiotic stress-related genes *OsNHX1* and *COR413-TM1*, and *OsbZIP72* directly binds to the promoters of the high-affinity potassium transporter gene *OsHKT1* and Sugars will eventually be exported transporter genes *OsSWEETs*, to regulate their expression separately, thereby maintaining the stability of plants under drought and salt stress [17,18]. Recently, our team discovered another target gene of the transcription factor *OsbZIP72, OsBBP1*, which positively regulates drought tolerance in rice [19].

OsbZIP72 binds to the promoter of *OsBBP1*

We screened the downstream target genes of *OsbZIP72* by Chip-seq technology, and *OsBBP1* was captured as a target candidate gene by enrichment analysis. Subsequently, it was verified that *OsbZIP72* could directly bind to the ACGT motif in the *OsBBP1* promoter region by yeast one-hybrid and EMSA assays. Meanwhile, *OsBBP1* expression was observed in overexpressing *OsbZIP72* rice plants and further verified by dual-luciferase reporter assay. It was found that *OsbZIP72* could positively regulate the expression of target gene *OsBBP1*. Although there may still be multiple targets that have not been verified in our Chip-seq data results, we can speculate that *OsbZIP72* is a transcription factor with multiple regulatory networks, including the existing reports on *OsbZIP72*.

OsBBP1 confers rice resistance to drought stress by regulating the accumulation of ROS

*Os*BBP1 is a typical Domain of Unknown Function (DUF) protein with a DUF630 domain at its N-terminal and a DUF632 domain at its C-terminal and *OsBBP1* is distributed in both the nucleus and cytoplasm, where the DUF632 domain contributes to its localization in the cytoplasm. We found that *OsBBP1* was induced by PEG and ABA, suggesting that this gene may be involved in abiotic stress. On the premise of verifying that the drought tolerance of rice was reduced after knocking out *OsbZIP72*, we identified the drought resistance of target *OsBBP1* in nippon bare variety. The *OsBBP1* transgenic plants were subjected to PEG treatment and drought treatment in soil, respectively. The results showed that *OsBBP1* could positively regulate rice drought tolerance. Under drought condition, *OsBBP1* can promote the expression of ROS scavenging-related gene *APX2*, *APX7*, *POD*, *CATB* and *CATC,* and improve the activities of Catalase (CAT) and Peroxidase (POD), suggesting that the antioxidant reaction was enhanced to reduce excessive ROS accumulation, thus improving the survival rate of plants. Not only that, but we also conduct field trials. Under normal condition, *OsBBP1* did not affect rice yield, but under field drought treatment, *OsBBP1* could enhance the ability of plants to resist water shortage and reduce the loss of rice yield.

Discussion

This study reveals another pathway by which *OsbZIP72* regulates rice drought tolerance, providing scientific basis for bZIP transcription factor family to regulate DUF domain protein, and also deepens the understanding of DUF domain protein responding to stress. *OsBBP1* has two DUF domains, and belongs to a typical DUF630/632 domain protein. Rice Stomata Developmental Defect (RSD1)/ Rolled and ERect LEaf (REL2), also belonging to the DUF630/632 domain protein, affects stomatal development and is closely related to water loss in plants under dehydration stress [20,21]. Although *OsBBP1* and RSD1/REL2 have similar protein domains, the molecular mechanisms for coping with drought are different. With the continuous excavation of DUF domain proteins, the discovery of these proteins in response to stress, especially drought, has been reported, such as *NtDUF868* genes in Nicotiana, *AtRDUF1* and *AtRDUF2* in Arabidopsis, *OsDUF668* genes and *OsDUF810.7* in rice [22-25]. Overall, the researchers have identified the importance of DUF domain proteins in plants, which may be potential targets in crop drought resistance breeding in the future.

Conclusion

However, the current research progress in this field is very limited and the interesting questions remain to be addressed, including but not limited to: (1) there are many types and quantities of DUF domain protein in plants, whether they are widely present in the downstream of abiotic stress regulators bZIP, DREB, DOF, HSF, MYB, etc., to regulate plant drought tolerance; (2) it is only revealed that DUF domain proteins are involved in regulating plant drought tolerance functionally, but the specific regulatory pathways are still unclear. For example, how do DUF domain proteins affect the accumulation of ROS in plants; and (3) at present, the differences between single DUF domain protein and double DUF domain protein are still vague.

In general, under drought condition, the transcription factor *OsbZIP72* can bind to the promoter of the target OsBBP1, to promote the expression of the latter, and finally improve the drought tolerance of rice by regulating the accumulation of ROS. This study provides direct evidence that the member of DUF family participates in bZIP-mediated drought resistance pathway.

Funding

The work was supported by the National Rice Industry Technology System of Modern Agriculture for China (grant no. CARS-01-20), the "5511" collaborative innovation project for high-quality development and surpasses of agriculture between the Government of Fujian and Chinese Academy of Agricultural Sciences (grant no. XTCXGC2021001), the special foundation of non profit research Institutes of Fujian Province (grant no. 2021R1023008) and free exploration of scientific and technological innovation project from Fujian Academy of Agricultural Sciences (grant no. ZYTS202403)

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