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Proteomic analysis on the leaves of *TaBTF3* gene virus-induced silenced wheat plants may reveal its regulatory mechanism

*Guozhang Kang**, *Gezi Li*, *Hongzhen Ma*, *Chenyang Wang*, *Tiancai Guo*

The National Engineering Research Centre for Wheat, The Key Laboratory of Physiology, Ecology and Genetic Improvement of Food Crops in Henan Province, Henan Agricultural University, Zhengzhou, 450002, China

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ABSTRACT

Basic transcription factor 3 (BTF3) is involved in the transcriptional initiation of RNA polymerase II and is also associated with apoptosis. In this study, virus-induced gene silencing of TaBTF3 caused severe viral symptoms in wheat seedlings, which then displayed stunted growth, reduced height, and decreased total fresh and dry weights. A proteomic approach was further used to identify the protein species showing differential abundance between the *TaBTF3* virus-induced gene silenced wheat plants and the *barley stripe mosaic virus*-induced gene silencing green fluorescent protein transgenic wheat plants (control) with the objective of exploring its regulatory mechanism in higher plants. Using two-dimensional electrophoresis technologies, 59 protein spots showed significant changes, of which 54 were successfully identified by tandem mass spectrometry with matrix-assisted laser desorption/ionization-time of flight spectrometry. Analysis of protein abundance revealed that the differential protein species were associated with signal transduction, stress defense, photosynthesis, carbohydrate metabolism, and protein metabolism, and were mostly localized in both chloroplasts and mitochondria. Furthermore, the BTF3–responsive protein interaction network revealed 20 key protein species, most of which are regulated by abscisic acid, ethane, or oxidative stress. This suggested that changes of these protein species could be critical in the BTF3 pathway.

Biological significanceBasic transcription factor 3 (BTF3), the *β*-subunit of NAC, has originally been identified as a basic transcription factor that is both involved in the transcriptional initiation of RNA polymerase II and associated with diverse biological functions. Reports on BTF3 mainly focus in animals, however, there has been limited molecular information about BTF3 in higher plants so far.

In previous studies, we first isolated the *TaBTF3* gene from common wheat (*Triticum aestivum* L.) and obtained silenced transgenic wheat seedlings using the VIGS method. In TaBTF3-silenced transgenic wheat plants, the structure of the wheat mesophyll cell was seriously damaged and transcripts of the chloroplast- and mitochondrial-encoded genes were significantly reduced. These results suggested that the *TaBTF3* gene may be involved in regulating the growth and development of wheat seedlings. However, the induced or related genes by *TaBTF3* have not been identified.

The significance of this study is to first identify many protein species with the altered abundance between the *TaBTF3* virus-induced silencing wheat plants and the BSMV-VIGS GFP transgenic wheat plants (control) using the proteomic approach. In addition, 20 of these

* *Corresponding author at: The National Engineering Research Centre for Wheat, Henan Agricultural University, Zhengzhou, Henan Province, 450002, China.* Tel.: +86 371 6355 8205; fax: +86 371 6355 8200.
E-mail address: zkangg@163.com (G. Kang).

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identified protein species which might play critical roles in the BTF3 interaction network are identified using protein interaction network. These results help to further explore the molecular mechanism of BTF3 in higher plants.

1. Introduction

The nascent polypeptide-associated complex (NAC) directly interacts with the signal recognition particle (SRP) to ensure the proper folding and targeting of synthesized proteins, thereby inhibiting the targeting of nonsecretory proteins to the endoplasmic reticulum [1,2]. Basic transcription factor 3 (BTF3), the β-subunit of NAC, is involved in the transcriptional initiation of RNA polymerase II and is also associated with apoptosis in mammalian cells [3,4]. Previous reports regarding BTF3 have mainly focused on animals, with limited molecular information available for BTF3 in higher plants [5,6]. A *Capsicum annuum* BTF3 gene was found to be involved in hypersensitive response (HR) cell death and could function as a transcription factor in the nucleus through the transcriptional regulation of HR-related gene expression [7].

Wheat is the last cereal to be genetically transformed due to the inherent difficulties associated with gene delivery into regenerable explants and the recovery of plantlets with the introduced gene. Although particle bombardment and cocultivation with *Agrobacterium tumefaciens* have been attempted in wheat, the disadvantages of these two methods are always prominent and include multiple copy insertions, a low transformation efficiency, a high tendency for gene silencing, unstable transformation, time consumption, and a low transformation efficiency [8,9]. As an alternative, virus-induced gene silencing (VIGS) has emerged as an attractive option for the rapid generation of gene knockdown phenotypes. The technique is based on homology-dependent gene silencing achieved by mainly exploiting the *barley stripe mosaic virus* (BSMV)-mediated antiviral defense mechanism of plants to study the function of endogenous genes [10]. VIGS is of great importance in wheat as it can potentially speed up the characterization of candidate genes [11].

In previous studies, we first isolated the *TaBTF3* gene from common wheat (*Triticum aestivum* L.) and obtained silenced transgenic wheat seedlings using the VIGS method. In TaBTF3-silenced transgenic wheat plants, the structure of the wheat mesophyll cell was seriously damaged and transcripts of the chloroplast- and mitochondrial-encoded genes were significantly reduced. These results suggested that the *TaBTF3* gene may be involved in regulating the development of the chloroplast, mitochondria, and mesophyll cells in wheat [12]. However, the induced or related genes by *TaBTF3* have not been identified.

A survey of the current literature revealed that the analysis of BTF3-responsive physiological changes is not comprehensive, and analyses of transcriptomes are somewhat incomplete because many gene products cannot be detected due to post-translational modifications [13]. Thus, the small number of studies describing the physiological and transcriptional responses to BTF3 cannot reveal the possible mechanism of BTF3 action. Proteomics is an essential tool for gaining an improved understanding of the molecular mechanisms underlying the generation of mutant species [14]. As far as is known, no proteomic studies investigating BTF3 have been published.

In this study, we used two-dimensional electrophoresis (2-DE) and biological mass spectrometry (MS/MS) to compare the leaf proteins in BSMV-VIGS green fluorescent protein (GFP, control) and TaBTF3-silenced transgenic wheat plants. The protein species identified were then further analyzed using Pathway Studio software (Ariadne Genomics, Rockville, MD, USA) to identify specific key protein species that may be related to TaBTF3 and its function in the development of the wheat chloroplast, mitochondria, and mesophyll cells.

2. Materials and methods

2.1. Plant materials

The experimental setup of our analyses was schematically shown in Supplementary Fig. 1. Seeds of a common wheat (*T. aestivum* L.) cultivar "Yumai 34" were sterilized with 0.01% $HgCl_2$, germinated in distilled water for 3 days, and then transplanted into plastic pots (5 cm in height and 5 cm in diameter; one plant per pot) holding 100 g of potting mixture consisting of local soil:nutrient soil:vermiculite in a ratio of 4:1:1 by volume (20% water-holding capacity). The seedlings were maintained in an FPG-300C-30D illumination incubator (Ningbo Laifu Technology Co., Ltd., Beijing, China) under a 14-h photoperiod, 25/15 °C day/night temperatures, light intensity of 250 $\mu mol\ m^{-2}\ s^{-1}$, and relative humidities of 60%/75% (day/night). Two-week-old seedlings with two fully expanded leaves and almost identical heights (11.6 ± 0.2 cm) were inoculated with the control vector BSMV-GFP or target gene vector BSMV-TaBTF3, produced as described previously [12]. Samples whose uppermost leaves were fully expanded in every treatment 15 days after the inoculation of BSMV-GFP or BSMV-TaBTF3 vectors was collected, immediately frozen in liquid nitrogen, and stored at −80 °C for protein extraction.

2.2. Growth parameters

Growth parameters (plant height, fresh weight [FW], and dry weight [DW]) were recorded 15 days after inoculation of the BSMV-GFP and BSMV-TaBTF3 vectors. Three biological replicates with five technical replicates (5 plants) each were designed in growth experiment.

2.3. Protein extraction

For 2-DE, three biological replicates were performed with two technical replicates each (1.5 g each, coming from the upmost fully expanded leaves of 8 plants). Proteins from leaf samples

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