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Comparative transcriptomics of transgenic tobacco plants overexpressing *Miscanthus sinensis BTF3*

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Abstract

Background Plant basic transcription factor3 (*BTF3*) plays an important role in photosynthesis rate and plant growth and development. In addition, it is involved in resistance mechanisms related to abiotic and biotic stress and has a significant impact on plant growth phenotype. This study was conducted to expand our understanding on plant transcriptome changes as there are no reports on plant transcriptome changes in normal environments due to the overexpression of *Miscanthus sinensis BTF3* gene.

Results The amino acid sequence length of the *BTF3* gene isolated from *M. sinensis* was 158 aa, and it showed the highest homology (97.47 %) with that of *Paniocum virgatum* (*PvBTF3*). *Nicotiana benthamiana* (Nb) transgenic plant was produced by transforming a binary vector into which the *MsBTF3* gene was inserted into *Agrobacterium*. Up- and down-regulatory factors were classified through transcriptome analysis using transgenic plants overexpressing *MsBTF3*. Gene ontology (GO) analysis of up-regulated transcripts showed that the most expressed transcripts were involved in biological processes. Analysis of down-regulated differentially expressed genes (DEGs) showed that they were involved in metabolic processes that corresponded to biological processes. Blast analysis revealed that Nb03180T (chloroplast photosystem II 22 kDa component), Nb04871T (basic transcription factor 3-like), Nb13433T (expansin-B15- like), Nb15392T (receptor-like serine/threonine-protein kinase SD1-8 isoform X3), Nb17216T (3-bata-glucan endohydrolase), and Nb20214T (probable rhamnogalacturonate lyase B) were among the DEGs whose transcript expression was up-regulated more than 2-fold compared to the wild type. In particular, the reference gene, that is the *NbBTF3* gene, showed the highest expression owing to the overexpression of *MsBTF3*.

Conclusions Transcriptome changes in tobacco plants through *MsBTF3* overexpression revealed that *MsBTF3* caused broad-spectrum changes in the biological processes of plants. *MsBTF3* showed that various metabolic and defense responses could be activated by regulating the level of specific gene expression in plants. These data obtained through this process will greatly contribute to elucidating the mechanism by which *MsBTF3* helps strong physiological responses in plants.

Keywords Basic transcription factor3, *Miscanthus sinensis*, *Nicotiana benthamiana* transgenic plant, Transcriptome analysis, Gene ontology, Differentially expressed genes, *NbBTF3*

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Background

Transcription factors (TFs) are fundamental elements of gene expression associated with biological processes and play a central role in all cellular functions, including cell cycle progression, metabolism, growth, development, and reproduction [1, 2]. TFs become functional through the activation of cis and mixed proteins in the promoter region, which bind to DNA elements, and additional proteins at the corresponding sites [3]. In general, TFs perform similar functions in animal and plant biological processes, with many plant-specific TFs responding to photosynthesis, nitrogen fixation, and reproduction [4]. Plants can improve their genetic function through various types of DNA-protein and protein-protein interactions [5].

BTF3 was discovered in HeLa cells, where it is transcribed in an RNA polymerase II-dependent manner and exists in two isoforms: *BTF3a* and *BTF3b*. *BTF3a* encodes the *BTF3* protein and is responsible for stimulating transcription, whereas *BTF3b* encodes a shortened form of *BTF3a* that lacks the first 44 amino-terminal extensions [6]. BTF3b is a component of the β-subunit of the nascent polypeptide-associated complex (NAC), which participates in the regulation of protein positioning during translation [7]. *BTF3*, which has also been identified in plants, is associated with a large gene family. It plays an important role in various biotic and abiotic stress processes and is one of the TFs important for photosynthetic rate, growth, and development [8, 9]. In recent years, BTF3 has been studied in relation to stress-related genes, with reports of its role in plant growth and development [5, 10].

Silencing of *TaBTF3* in wheat (*Triticum aestivum* L.) results in the production of abnormal mesophyll cell structures and the enlargement of intercellular spaces due to disordered cell arrangement [11]. In addition, the silencing of *CaBtf3* in *Capsicum annuum* L. revealed that it regulates the transcription of pathogenicity-related genes [12]. In rice, overexpression of *OsBTF3* revealed that it functions as a key regulator of photosynthesis, growth, and development [13]. The importance of *OsBTF3* in seed germination and seedling growth in response to NaCl, abscisic acid (ABA), and gibberellic acid (GA3) stress was revealed through its regulation *Osj3 g1BTF3*, *Osj3 g2BTF3*, and *Osj10 gBTF3* expression in the Osj10 gBTF3 RNAi line [10]. *BTF3* was shown to have the characteristics of a drought-resistance gene in a cDNA library of water-stressed peanut (*Arachis hypogaea* L.) leaf tissues [14]. Moreover, the *AhBTF3* gene was found to improve cell resistance under osmotic stress in transgenic tobacco through heterologous overexpression [15].

In the present study, *BTF3* isolated from *Miscanthus sinensis* was cloned into a plant expression vector and overexpressed in transgenic tobacco plants. Total RNA from wild-type *BTF3* overexpressing plants was isolated and subjected to RNA sequencing for comparative transcriptomics. Using big data to compare the entire transcriptome, functions were grouped through GO analysis, and up- and down-regulated gene expression patterns analyzed. We identified a group of genes that changed through the overexpression of *BTF3* and tested the expression of reference genes to ensure the reliability of the transcriptomic analysis.

Methods

Sequence analysis and vector construction of *MsBTF3*

Using the amino acid sequence of the *BTF3* gene isolated from *Miscanthus sinensis* planted in the field of Kangwon National University, sequence homology analysis was performed by searching for genes with high identity to other plant species using BLAST on the NCBI website. *Agrobacterium tumefaciens* LBA4404 containing the *pMBP1* vector harboring the neomycin phosphotransferase gene (nptII) as a selectable marker was used. The *MsBTF3* gene was inserted into the SacI-XbaI site of the vector using restriction cloning. A single colony of *A. tumefaciens* LBA4404 was cultured on a shaker (200 rpm) for 24 hours at 28 °C in a yeast extract peptone (YEP) medium supplemented with 25 mg/L kanamycin and rifampicin.

Production of *Nicotiana benthamiana* transgenic plants

Nicotiana benthamiana seeds collected from greenhouse of Kangwon National University were placed in 70% EtOH, shaken for 1 min, placed in a 3% NaClO solution, and shaken again for 3 min. Afterwards, the cells were washed three times with sterilized water, placed on murashige and skoog (MS) medium, germinated, and grown for four weeks. Well-grown seedlings were transferred to sterile soil and grown for an additional three weeks. The leaves from these plants were used for transformation. The leaves were placed in a 0.1% NaClO solution, shaken for 10 min, washed three times with sterilized water, and cut into appropriate sizes using a mesh blade. The prepared leaves were inoculated with the *A. tumefaciens* broth cultured for 1 d, shaken for 10 min (200 rpm) at 24 °C, placed on co-culture medium supplemented with 200 μM acetosyringone, and cultured in the dark for 2 days. Afterward, the shoots were transferred to a shoot regeneration medium containing 50 mg/L kanamycin and 250 mg/L cefotaxime and cultured for 4 weeks. Next, they were transferred to a rooting medium containing 25 mg/L kanamycin. After rooting, the plants

were transferred to soil in a greenhouse, and the seeds were harvested at later time.

Acclimatization of the transgenic tobacco plants in the soil

To produce T1-generation transgenic plants, harvested T0 transgenic tobacco seeds were sterilized with NaOCl and placed on a germination medium containing kanamycin. After 4 weeks, the grown young plants were transferred to sterile soil and grown in a culture room for 4 weeks under 16 h light/8 h dark conditions, with the temperature maintained at 24 °C and humidity at 25–30%.

Isolation of genomic DNA and total RNA from transgenic tobacco plants

To confirm whether transformation took place, genomic DNA from control and T1 transgenic tobacco leaves grown in soil for 4 weeks was extracted using AccuPrep® Plant Genomic DNA Extraction Kit (BIONEER, Daejeon, Republic of Korea). Total RNA was isolated using the Hybrid-R kit (Gene All Biotechnology Co., Seoul, Korea), and quantified using the A2100 Bio System (Agilent Technologies Inc., Santa Clara, CA, USA). Samples with an RNA purity (RNA Integrity Number, RIN) of ≥ 8.0 were used for cDNA synthesis.

PCR analysis

Insertion of the *MsBTF3* gene into the genome was confirmed by PCR using gene-specific primers (Forward: 5'-GGGTCTAGAATGAACAAGGAGAGGCTCAT-3'; Reverse: 5'-GGGGAGCTCTCAGGCTCTTGCCTC CTGAG-3'). The conditions were initial denaturation at 94 °C for 5 minutes, 30 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min, and final extension at 72 °C for 5 minutes. To confirm the mRNA expression level of the inserted *MsBTF3* gene, cDNA synthesis was performed using the extracted total RNA. cDNA was synthesized using PrimeScript™ RT Master Mix (Perfect Real Time) (Takara Korea Biomedical Inc., Seoul, Korea) with PCR conditions the same as before. The expression of *NbActin* served as a control. Gene-specific primers were used for PCR analysis (*NbActin*_Forward: 5'-CAGCTCATC CGTGGAGAAGA-3'; *NbActin*_Reverse: 5'-AGGATA CGGGGAGCTAATGC-3'; *MsBTF3*_Forward: 5'-GGG TCTAGAATGAACAAGGAGAGGCTCAT-3'; *MsBTF3*_ Reverse: 5'-GGGGAGCTCTCAGGCTCTTGCCTC CTGAG-3'.

RNA sequencing and transcriptome analysis

Total RNA was extracted from fully expanded leaves of 4-week-old wild-type and MsBTF3-overexpressing *N. benthamiana* plants grown under normal conditions in a growth chamber. RNA quality and integrity were assessed using an Agilent 2100 Bioanalyzer, and samples with RNA Integrity Number (RIN) ≥ 8.0 were selected for sequencing. mRNA was enriched and fragmented from total RNA, and cDNA libraries were constructed using the Illumina TruSeq RNA Sample Preparation Kit v2 following the manufacturer's protocol. Paired-end sequencing (2 × 150 bp) was conducted on the Illumina NovaSeq 6000 platform (Macrogen Inc., Seoul, Republic of Korea). Raw reads were processed using Trimmomatic v0.39 to remove low-quality bases (Phred score < 20) and adapter sequences [16]. Clean reads of at least 50 bp were retained for downstream analysis. Reads were mapped to the *N. benthamiana* reference genome (NCBI BioProject accession number: PRJNA1136030) using HISAT2 v2.1.0. SAM files were converted and sorted with Samtools v1.13. Read counts were generated using HTSeq v0.11.2, and normalization and DEG (differentially expressed gene) analysis were performed using the DESeq v1.38.0 package in R. Genes with a log2 fold change > 2 and adjusted p-value < 0.05 were considered significantly differentially expressed. For functional analysis, DEGs were annotated using BLAST v2.12.0+ and InterProScan v5.56–89.0. GO (Gene Ontology) categorization was performed with BLAST2GO v6.0.3, classifying DEGs into biological process, cellular component, and molecular function categories. All raw sequencing data have been deposited at NCBI SRA under BioProject accession number PRJNA1136030.

Gene annotation and functional analysis

NCBI's BLAST v2.12.0+ (ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/LATEST/) and InterProScan v5.56–89.0 (https://www.ebi.ac.uk). After analyzing the function and structure of genes through (/interpro/search/sequence/), GO (Gene Ontology) analysis and annotation were performed using BLAST2GO v6.0.3 (https://www.blast2go.com/). GO analysis was conducted by dividing the data into three categories: molecular function, cellular components, and biological processes.

Real-time PCR analysis

In transgenic tobacco, 10 up- and 10 down-regulated genes were selected for real-time PCR analysis based on the log2 fold change values. cDNA for analysis was synthesized using PrimeScript™ RT Master Mix (Perfect Real Time) (Takara Korea Biomedical Inc., Seoul, Republic of Korea). The PCR mixture was prepared using TB Green® Premix Ex Taq™ (Tli RNaseH Plus) (Takara Korea Biomedical Inc., Seoul, Republic of Korea) and the analysis was conducted using the CronoSTAR™ 96 Real-Time PCR System (Takara Korea Biomedical Inc., Seoul, Republic of Korea). The analysis conditions were initial denaturation at 95 °C for 30 s, two-step amplification (denaturation at 95 °C for 5 s, annealing at 60 °C for 30 s)

for 40 cycles, followed by melting at 95 °C for 1 min, 60 °C for 15 s, and 98 °C for 5 s to check the melting curve. The primers used for here are listed in Table 1. All real-time PCR processes were performed in triplicate.

Results

Sequence analysis of the MsBTF3 protein

The entire nucleotide sequence corresponding to the full-length *MsBTF3* gene from *M. sinensis* was 474 base pairs (bps) long (Fig. 1A). The amino acid sequence size comprised 158 amino acids (aa). A BLAST analysis using aa sequences from NCBI revealed four BTF3 proteins with *Sorghum bicolor* (*SbBTF3*) and *Dichanthelium oligosanthes* (*DoBTF3*) showed similar homologies at 96.84%. The next highest was the *Zea mays BTF3* (*ZmBTF3*) protein, which had a 96.20% homology (Fig. 1B). Among these *BTF3* proteins, the aa sequence of the *PvBTF3* was found to be 203 aa long, 45 aa longer than that of the other *BTF3* proteins that were homologous (Fig. 1B).

Table 1 Primer sequences of up and down-regulated reference genes in MsBTF3-overexpressing *N. benthamiana* transgenic plant

Reference Gene	Primers
Nb00931T	F: GCAAGGGAGAAGCTGGACAT R: TTTCTGCCTCAACACCTGCT
Nb01570T	F: GAGCATCCTAACCCTAGAAGCT R: CACCTCATATGGCTCCGACC
Nb03180T	F: TTTGCGGCATCTTTGTTGGG R: CTCCAATGGCTCCAAGCAGA
Nb04871T	F: GGCAATCGAGGAGGTGAACA R: TTGTCAGGGCCAAGTTGGTT
Nb13433T	F: TGCTAGGGTTGCGTGTGATT R: GTAGTACTGCTGCTGCTGCT
NB14787T	F: ACAACGTCACCATCGAGACC R: GCTTCTTCAGCGAGATTGCG
Nb15392T	F: ACCGGTACCTGTTCAATTTGGA R: TGAGCTTGAGGTGAGCCAAG
Nb16959T	F: GCCCATGGAAGAGTTGTGGA R: CGGACTACGGGCTCATACAC
Nb17216T	F: GTTGCTCCTGCCATGCAAAA R: TGGGCGGGTAGGTATTCTCT
Nb20214T	F: GCAATTTTGGAGCGCTTGGA R: AGTTCTCGGCCCATTTCTCG
Nb01963T	F: GCAAAAGGTAAAGGCAGCCC R: TGACACAGGGACAAGCGTAC
Nb02842T	F: CCATTGCCACCCTGAAGTCT R: TCGGCTTCTGTGCTGATGTT
Nb03726T	F: TGGATCAACCGTGACACTGG R: GACCTTCGTGCTGGTCTTCA
Nb06225T	F: TGTTAACCGGTGGGACCCTA R: TCACGCCGACATCTGAACAA
Nb12091T	F: ATTGGCCAGATTTCGTCGGT R: AGGCATCCTCCTCTGACCAT
Nb13131T	F: TTGCGTGCAAAGAACGTCAG R: TGACTTGCGCCTTGGAATCT
Nb13558T	F: GATCCAAGGCCACACCATGA R: TGGGCTCTTGATCAGCAGTG
Nb14170T	F: TCCCTTCTGGTTTCCATCGC R: GGAGCCAGCAATGAGTCCTT
Nb18211T	F: AGTGTGCCGTTTGCTTAGGA R: AATCGGGCAATTTACGTGGC
Nb25885T	F: AGCAACGGCAGCAACATTTT R: TGCCAATCAAGCTGCCGATA

Characterization of transgenic tobacco plants overexpressing *MsBTF3*

For molecular biological testing, putative transgenic plants (Fig. 2A and B) selected on a medium containing kanamycin were acclimatized to soil and grown for 4 weeks for analysis. After collecting leaves from the control and transgenic plants overexpressing *MsBTF3*, the insertion of *MsBTF3* was confirmed via PCR (Fig. 2C). In addition, the expression of *MsBTF3* in the transgenic tobacco plants was confirmed using RT-PCR by assaying its expression against the tobacco *ACTIN* gene (Fig. 2D).

Transcriptome profiling in MsBTF3-overexpressing tobacco plants

The up-regulated GO analysis in transgenic tobacco plants overexpressing *MsBTF3* was divided into three categories: biological processes, molecular functions, and cellular components (Fig. 3). In the GO annotation of the up-regulated transcripts, the category with the largest number of transcripts was biological processes, which were divided into 19 sub-categories: organic substance metabolic processes, primary metabolic processes, cellular metabolic processes, nitrogen compound metabolic processes, biosynthetic processes, regulation of cellular processes, response to stress, regulation of metabolic processes, cellular response to stimulus, establishment of localization, and response to chemically up-regulated sub-categories (Fig. 3): The number of transcripts in these sub-categories ranged from 26 to 100. Less than 20 transcripts were up-regulated in cell communication, signal transduction, signaling, response to endogenous stimuli, small-molecule metabolic processes, and catabolic processes (Fig. 3). The GO annotations shown as molecular function subcategories were organic cyclic compound binding, ion binding, hydrolase activity, transferase activity, transferase activity, catalytic activity acting on a protein, small-molecule binding, transmembrane transporter activity, and oxidoreductase activity (Fig. 3). Among these, more than 50 transcripts were identified in the subcategories of organic cyclic compounds and ion binding (Fig. 3). GO annotation, classified as a cellular component, showed more than 50 transcripts in the subcategories of membrane, intracellular anatomical structure, and organelles (Fig. 3). Among the down-regulated

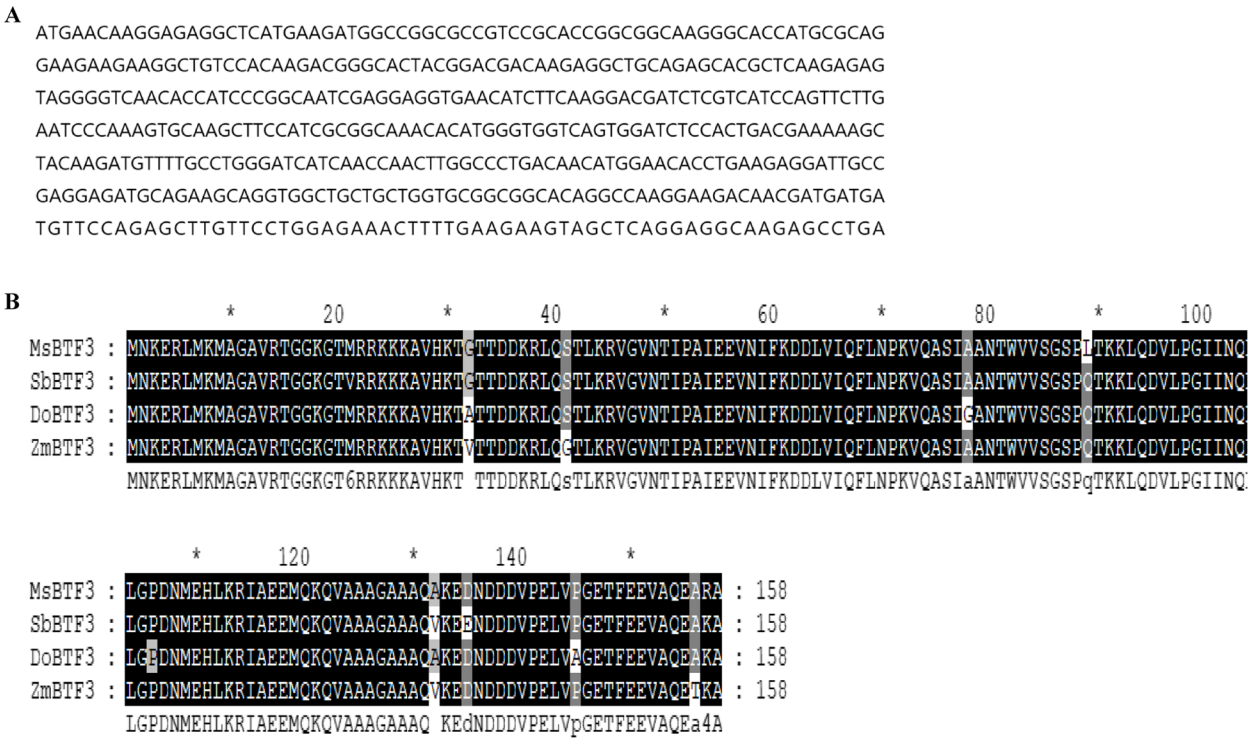

Fig. 1 Analysis of nucleotide sequence identity between *MsBTF3* and *BTF3* genes of other species. **A** Nucleotide and (**B**) amino acids

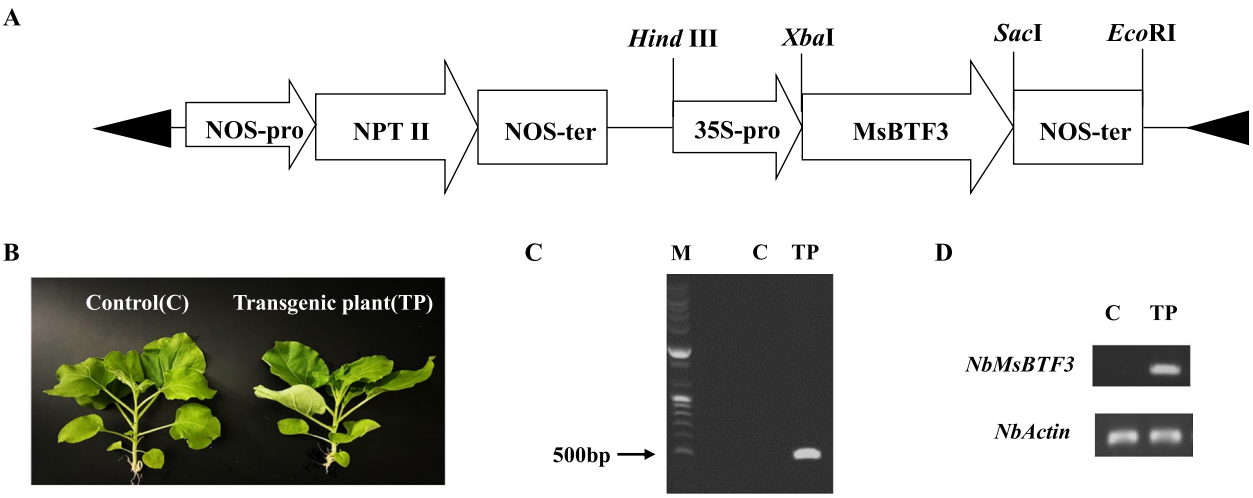

Fig. 2 Production and molecular analysis of *N. benthamiana* transgenic plant using the *MsBTF3* gene. **A** Vector construction, **B** Production of *N. benthamiana* transgenic plant, **C** PCR analysis using genomic DNA: M, DNA ladder marker, C, Control plant, TP, Transgenic plant and (**D**) mRNA transcriptional level of *N. benthamiana* transgenic plant: C, Control plant, TP, Transgenic plant

GO annotation categories, cellular components, molecular functions, and biological processes were found to be the most frequent (Fig. 4). Among the subcategories, those with an over-representation of more than 100 transcripts were organic substance metabolic processes and primary metabolic processes, cellular metabolic processes, and biosynthetic processes (Fig. 4). Among the down-regulated molecular function categories, carbohydrate derivative binding and protein binding were not in the up-regulated subcategories, proving to be the only down-regulated transcripts (Fig. 4). DEGs that were highly up-regulated and down-regulated in transgenic

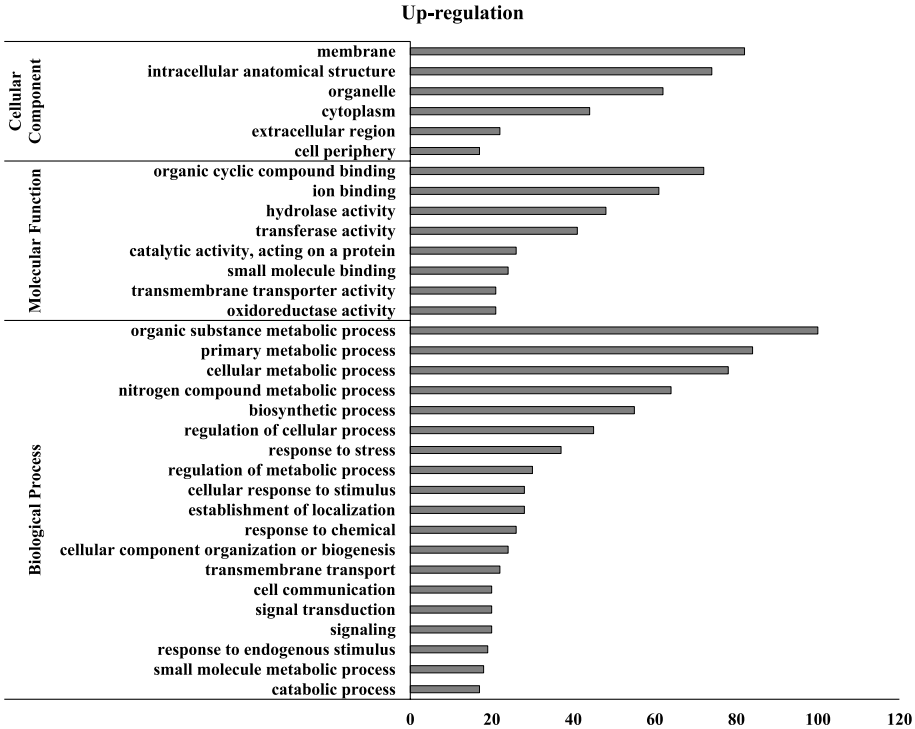

Fig. 3 GO classification of up-regulated genes in DEGs analysis by RNA sequencing in *MsBTF3*-overexpressing *N. benthamiana* transgenic plant

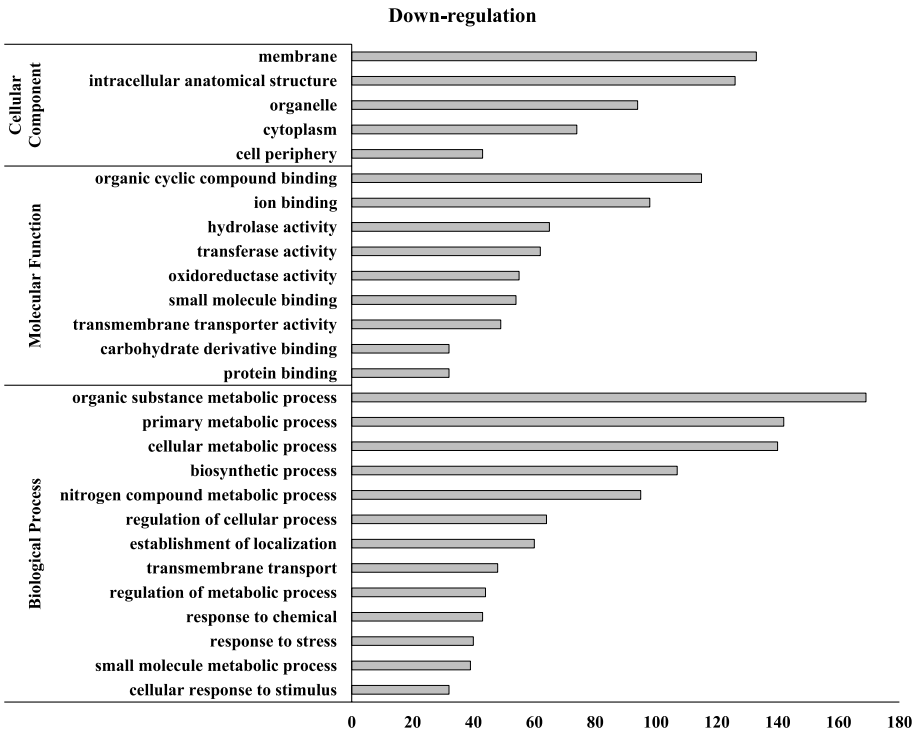

Fig. 4 GO classification of down-regulated genes in DEGs analysis by RNA sequencing in *MsBTF3*-overexpressing *N. benthamiana* transgenic plant

plants compared to those in WT plants were identified as organic substance metabolic processes in the biological process category. DEGs related to molecular function showed more subcategories in WT plants than in transgenic plants (Figs. 3 and 4).

Expression matching of reference genes using real-time PCR analysis

Among the up-regulated and down-regulated DEGs, 20 reference genes were selected for verification of the transcriptome analysis by real-time PCR using the same RNA samples (Fig. 5). Up-regulated reference genes included Nb03180T (chloroplast photosystem II 22 kDa component), Nb04871T (basic transcription factor 3-like), Nb13433T (expansin-B15-like), Nb15392T (receptor-like serine/threonine-protein kinase SD1-8 isoform X3), Nb17216T (3-bata-glucan endohydrolase), Nb20214 T (probable rhamnogalacturonate lyase B). Nb00931T, Nb01570T, Nb14787T, and Nb16959T were identified as uncharacterized proteins (Fig. 5). The down-regulated reference genes analyzed using real-time PCR included Nb01963T (glucan endo-1,3-beta-glucosidase 8-like), Nb02842T (anther-specific protein LAT52), Nb03726T (glucose-methanol-like), Nb (choline-gmc- oxidoreductase), Nb06225T (seine protease inhibitor 1-like), Nb12091T (ATPase 8), Nb13131T (PDR-type ACB transporter), Nb13558T (fructokinase-5), Nb18211T (ring-H2 finger protein ATL52-like), and Nb25885 T (WAT1-related protein At5 g64700-like) (Fig. 5). In the transgenic plants, the expression levels of the up-regulated reference genes were 2–40 times higher. In particular, gene expression level above 40 was interpreted as a meaningful result because it appeared to be the *BTF3* of tobacco, which is highly homologous to the gene used as the overexpressed gene in the transgenic plants.

Discussion

In plants, *BTF3* is a hydrophilic protein. Its DNA sequence contains five exons and four introns [8, 11]. The N-terminal region of BTF3 contains an NAC domain, which functions as a DNA-binding domain in a variety of species [9]. However, the function of NAC in plants has not been fully clarified, and it is known that several genes for α and β NAC exist in various plant genomes, similar to the human genome [5]. Phylogenetic analysis of BTF3 proteins revealed that plant BTF3 genes share a conserved evolutionary lineage, and their divergence patterns are consistent with the evolutionary relationships observed among various plant and animal species [17]. MsBTF3 showed a high identity of more than 95% in base sequence with *Penicum virgatum, D. oligosanthes*, *S. bicolor*, and *Z. mays*. The lengths of the aa sequences were similar, except for *Penicum virgatum*.

Recently, *BTF3* was shown to play an important role in plant growth and development [8]. The *JcBTF3* protein localizes to and functions in the nucleus and cytoplasm and its protein sequence is highly similar to *BTF3* of other plants; however, it has been reported to have no transcriptional activity [17]. Conversely, *ZmBTF3* is capable of transcriptional activation and shows high expression in immature embryos [18]. In this study, we produced transgenic tobacco plants overexpressing *MsBTF3* and compared their transcriptomes to that of wild-type plants under normal conditions. Transcriptome analysis showed that the overexpression of *MsBTF3* resulted in extensive transcriptome changes. A summary of the DEGs between

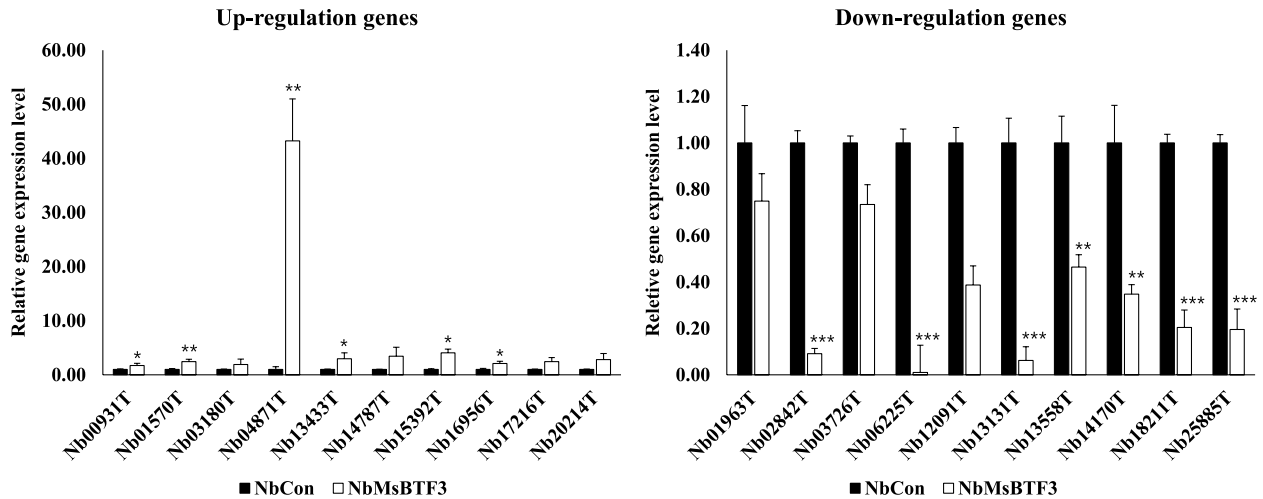

Fig. 5 Transcriptional level of reference genes selected for high or low expression in *N. benthamiana* transgenic plant overexpressing *MsBTF3*

the wild-type and transgenic plants is shown in Figs. 3 and 4. We found several DEGs related to organic substance metabolic processes and primary metabolic processes in the GO annotation classification. The function of *MsBTF3* under normal conditions is highly similar to that of *ZmBTF3* in terms of its aa sequence, indicating high transcriptional activation and widespread changes in the transcriptome.

MsBTF3 is classified as a TF. In the present study, 696 BLAST results were identified as TFs. Among these, 306 TFs were up-regulated and 390 down-regulated. Among the up-regulated TFs, *BTF3* plays an important role in plant growth and development [10]. It has been shown that when *BTF3* expression is reduced in rice, the production of the NAC complex is reduced, causing serious nutritional defects and preventing normal plant growth and development [8]. Lack of the *NAC* gene has been shown to cause morphological problems in tobacco [19]. In this study, 194 NAC transcripts expressed in the *MsBTF3*-overexpressing tobacco plants were analyzed. Among them, 89 transcripts were up-regulated and 105 were down-regulated. Among the up-regulated *NAC* transcripts, those with high expression levels were the NAC domain-containing proteins 90, 21, 22, and 29.

The basal transcription-like protein *BTF3* is known to exhibit a resistant phenotype to salt and high-temperature stress when overexpressed, as various stress response modules are activated [8, 15]. Transcriptome analysis of the *MsBTF3*-overexpressing tobacco plants revealed 90 stress-related response genes, of which 36 were down-regulated and 54 were up-regulated. Among the stress-related genes, oxidative stress 3 was the most highly up-regulated. This gene encodes a chromatin-related factor that has been reported to be related to heavy metal and oxidative stress resistance mechanisms [20]. This supports the need to study the phenotypes of transgenic tobacco plants expressing *MsBTF3* under stress.

When *MsBTF3* was overexpressed in tobacco, the gene with the highest expression level was *NbBTF3* (Nb04871T), indicating that *MsBTF3* expression induces *BTF3* expression in tobacco. Silencing of *NbBTF3* causes developmental defects by disrupting gene expression in the chloroplasts and mitochondria of higher plants [19]. In the case of BTF3, a significant TF movement was induced. Our results also showed that the movement of metastatic factors, such as MYB, WRKY, bZip, zinc finger, heat shock, disease resistance, stress, hormones, and NAC, actively occurs due to *MsBTF3* overexpression. Further research is needed to determine how *MsBTF3* affects these TFs.

Conclusion

Taken together, *MsBTF3* overexpression caused significant transcriptome changes in tobacco. Among the differentially expressed genes, NbBTF3 (Nb04871T) showed the greatest increase in expression, confirming that MsBTF3 overexpression strongly induces endogenous BTF3 expression in transgenic tobacco plants. Overexpression of *MsBTF3* resulted in the greatest changes in reference genes related to biological processes. This study provides important basic data for predicting the various functions and mechanisms of *BTF3*.

Authors' contributions

ESS contributed to study conceptualization. JWS and DYH conducted an experiment. JGL and HYK helped with data analysis. IYC and MJK led the formal analysis and figure development. ESS contributed to supervision. ESS contributed to write the original draft and manuscript editing. All authors read and approved the final manuscript.

Funding

Not applicable.

Data availability

Illumina reads have been deposited at the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1136030 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1136030). Additionally, the associated biosample is available at https://www.ncbi.nlm.nih.gov/biosample/42501032.

Declarations

Ethic approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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