CHARACTERIZING *OsHSBP2* GENE SEQUENCE AND ITS EXPRESSION IN RESPONSE TO DROUGHT AND HEAT STRESS IN THE BC15 RICE VARIETY (*Oryza sativa* L.)

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ABSTRACT

Rice production is significantly affected by challenging environmental factors, particularly drought and heat. Heat shock proteins (HSPs) are essential for enhancing stress tolerance related to temperature and oxidative stress, protecting cells and macromolecules from harmful effects. In certain plant species, the regulation of HSP activity is linked to Heat Shock Factor Binding Protein (HSBP). This study investigates the functional roles and regulatory mechanisms of *OsHSBP2* in rice, focusing on its expression patterns under drought and heat stress in the prominent BC15 rice variety. The coding sequences of *OsHSBP2* was isolated and fully sequenced to identify a 300-base pair segment that encodes a polypeptide of 99 amino acids, featuring an α helix and demonstrating 73.68% to 100% similarity to previously studied homologous HSBP proteins. These results lay the groundwork for further exploration of *OsHSBP2*'s functional role, aiming to enhance drought and heat stress tolerance in major rice varieties like BC15 through genetic engineering.

Keywords: *BC15, drought stress, heat stress, OsHSBP2.*

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1. INTRODUCTION

Rice production is significantly influenced by abiotic factors, particularly drought and heat. When rice plants encounter these stress conditions, they undergo a series of physiological and biochemical changes. These responses include alterations in photosynthesis, respiration, transpiration, and metabolism, which help stabilize cell membranes and regulate osmotic pressure for self-protection. The molecular mechanisms underlying abiotic stress responses in plants are complex and regulated at multiple levels, including transcription, post-transcription, translation, and post-translation. These processes involve various components and signaling pathways that ultimately activate functional proteins essential for protection and repair, enabling cells to endure periods of stress [1].

HSPs play a crucial role in helping plants respond to adverse abiotic environmental factors related to temperature. These proteins are activated when cells experience various types of stress, including oxidative stress, heat and cold [2]. HSPs function as molecular chaperones, safeguarding proteins and cellular structures from damage, thereby enhancing cell survival under abiotic stress conditions. Additionally, they facilitate recovery following periods of stress [3, 4].

HSF proteins (Heat Shock Factors) are pivotal in the regulatory pathways of HSP expression. They represent the final components of the signaling cascade activated by temperature and oxidative stress, mediating the expression of genes encoding stress-responsive proteins, including HSPs. The activity of HSFs is negatively regulated by HSBPs, which are small proteins (< 10 kDa) characterized by two conserved domains (> 60% similarity) containing seven hydrophobic repeat motifs [5]. In Arabidopsis, following a stress

period, HSBPs are synthesized and bind to HSFs, forming a complex that leads to the degradation of the active homotrimeric structure of HSF [5, 6]. Research indicates that HSBPs play a significant role in mediating responses to temperature-related stress factors across various plant species, including maize and *Arabidopsis* [5]. In rice, two HSBP copies, *OsHSBP1* and *OsHSBP2*, are continuously expressed in all tissues under non-stress conditions [5]. However, the specific functional roles of *OsHSBP* in mediating abiotic stress responses in rice have not been thoroughly investigated.

BC15 is the primary rice variety widely cultivated in Northern Vietnam. Despite its high productivity, the cultivation of this variety is significantly impacted by adverse environmental conditions such as drought and heat. While traditional rice breeding methods have certain limitations, the application of advanced breeding technologies, such as gene editing, has demonstrated considerable potential for enhancing agronomic traits, particularly those related to drought and heat tolerance [7]. To effectively apply gene editing technology for improving the characteristics of rice varieties, a comprehensive understanding of the structural features and functional activities of target genes is essential in any breeding program. Therefore, this study aims to elucidate the relationship between *OsHSBP2* and drought and heat stress responses in the BC15 rice variety. Expression pattern analyses under controlled artificial stress conditions and performed a detailed analysis of the coding region sequence of *OsHSBP2* were conducted.

The findings from this research will provide critical insights into the role of *OsHSBP2* in stress response mechanisms, potentially guiding future studies focused on the functional characterization of this gene. By understanding how *OsHSBP2* interacts with other signaling pathways and stress-responsive genes, its potential in breeding programs aimed at developing rice varieties with enhanced resilience to drought and heat stress can better harnessed. This approach not only contributes to the sustainability of rice production in challenging environments but also supports food security in regions heavily reliant on rice cultivation.

2. RESEARCH METHODS

2.1. Materials

The rice variety BC15 was obtained from the Plant Resources Center. The oligonucleotides utilized in this study were designed based on sequences available in GenBank and were ordered from Phusa Biochem (Vietnam) (Table 1).

2.2. Methods

2.2.1. Plant treatment

Table 1. Oligonucleotides used in the study

Name	Sequence	Targeted gene/vector (Code)	Experiment	Size (bp)
qPCR-HSBP2-F	AAGGCTGATCAGGACTCGGATG	*OsHSBP2* (*XM_015786025.3*)	Gene expression	114
qPCR-HSBP2-R	GATGTTCTCTGACATAGAT			
Actin-F	TGATGGTGTCAGCCACACT	*OsActin* (KX302608.1)	Gene expression	249
Actin-R	TGGTCTTGGCAGTCTCCATT			
HSBP2-F	CGCAGGCCCACTTTCGAAAACC	*OsHSBP2* (*XM_015786025.3*)	Gene isolation	626
HSBP2-R	GCGCAGTACAGTACAATATCCA			

The stress treatment for young rice plants was conducted following the methodology established by Qin *et al.* (2007) [8]. Initially, rice seeds were incubated in water at 37°C for 2 days to promote germination. Once germinated, the seeds were transferred to a Murashige and Skoog (MS) solution and grown at 28°C for 2 weeks. To simulate artificial drought stress, the roots of young rice plants were immersed in an MS solution containing 20% polyethylene glycol (PEG), which effectively induces osmotic stress, at 28°C. For heat stress treatment, the young rice plants were exposed to a temperature of 42°C. At designated time points (0, 0.5, 1, 2, 3, 6, 12 and 24 hours), rice samples were collected. Leaf and root samples were taken for the drought treatment experiments, while whole plants were harvested for the heat treatment experiments. All samples were immediately stored in liquid nitrogen to preserve RNA integrity for subsequent extraction and analysis. The artificial stress treatment was conducted in a growth chamber (RUMED) under full lighting conditions and 80% humidity. For the control experiments, rice plants were cultivated in the same growth chamber in the MS solution, maintaining full lighting, 80% humidity and a temperature of 28°C and collected at the same time points.

2.2.2. Evaluation of OsHSBP2 expression

Total RNA was extracted from root and stem tissues (100 mg) using Trizol reagent, following the manufacturer's instructions from Invitrogen (USA). The extracted RNA was then utilized for qPCR experiments. cDNA synthesis was conducted using the Maxima H Minus First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA), adhering to the manufacturer's guidelines and employing an oligo dT primer. The reaction mixture (20 µl) consisted of: 1.0 µg of purified RNA sample, 1.0 µL of 100 mM dNTPs, 1.0 µL of oligo dT (0.5 µg/µl), 4.0 µL of 5 X buffer, 1.0 µL of 5 U/µl Reverse Transcriptase, and the appropriate volume of H_2O. The reaction tube was incubated at 42°C for 60 minutes to facilitate cDNA synthesis.

For the qPCR experiments, the primer pair qPCR-HSBP2-F/qPCR-HSBP2-R was used. The components of the qPCR reaction included: 5.0 µL of 2 X SYBR Green Master Mix, 0.25 µL of each primer (10 pmol/µl), 1.0 µL of cDNA template, 0.15 µL of ROX fluorescent signal internal standard, and 3.35 µL of H_2O. The thermal cycling conditions were set as follows: 94°C for 30 seconds, 60°C for 20 seconds and 72°C for 30 seconds, repeated for 35 cycles using the Real-Time PCR System 7500 from Life Technologies. *OsActin* was employed as the internal standard gene for normalization. Each experiment was conducted in triplicate.

The relative expression levels of the target genes in stress-treated rice samples were compared to those in non-stress-treated samples using the ΔΔCt method as described by Jain *et al.* (2006)[9]. Each stress treatment experiment involved three rice plants per stress condition and time point, and the experiment was repeated three times.

2.2.3. Isolation and sequencing of OsHSBP2

The open reading frame (ORF) of *OsHSBP2* from the BC15 rice was amplified from cDNA of samples subjected to heat stress treatment at different time points during 24 hours using the primer pair HSBP2-F/HSBP2-R. The PCR mixture consisted of: 1.0 µL of template DNA, 1.2 µL of each primer (10 pmol/µL), 3.0 µL of 2 mM dNTPs, 3.0 µL of 10 X *Taq* DNA polymerase buffer, 1.0 µL of *Taq* DNA polymerase (5 U/µL) and 19.6 µL of deionized distilled water. The PCR amplification was conducted over 35 cycles with the following thermal profile: 95°C for 30 seconds, 55°C for 30 seconds and 72°C for 1 minute and 30 seconds.

After amplification, the PCR product was purified and to VNDAT Company (Vietnam) for sequencing. The nucleotide sequence of the isolated gene was processed using BioEdit 4.0 software and then was compared against the GenBank database using the BLAST tool. Subsequently, the protein structures encoded by the *OsHSBP2* gene were analyzed using the SWISS-MODEL tool [10].

3. RESULTS AND DISCUSSION

3.1. Evaluation of *OsHSBP2* expression under drought stress condition

Quantitative PCR analysis of *OsHSBP2* mRNA levels in BC15 rice (Figure 1) revealed significant alterations in both shoot and root tissues under simulated drought stress conditions. In shoot tissue, the expression of the target gene exhibited a rapid increase, peaking at a 5.5-fold enhancement after 30 minutes of stress exposure. The highest expression levels were recorded in shoot tissue, with 9.9-fold and 8.3-fold increased observed at 1 hour and 6 hours post-treatment, respectively. Conversely, the expression of *OsHSBP2* in root tissue demonstrated a more gradual response, reaching a maximum of 6.0-fold increase after 2 hours, followed by a gradual decline to baseline levels. Interestingly, a modest resurgence in expression was noted in root tissue at 24 hours post-drought treatment, achieved a 4.1-fold increase. These findings suggest that *OsHSBP2* expression is significantly responsive to drought stress in BC15 rice, suggesting its potential involvement in the plant's molecular response to water deficit conditions.

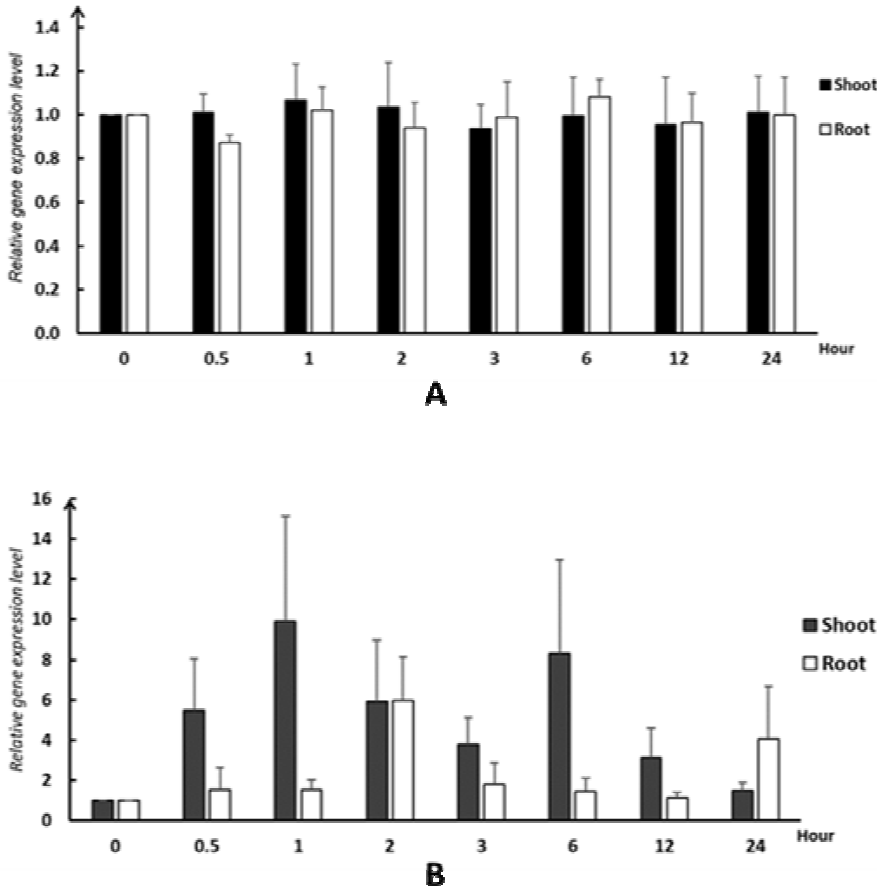

Figure 1. Expression levels of *OsHSBP2* under drought condition

Note: Graph presents the expression levels of OsHSBP2 in the BC15 rice variety under normal condition (A) and artificial drought condition (PEG 20%) (B) at various time intervals: 0 hour, 0.5 hour, 1 hour, 2 hour, 3 hour, 6 hour, 12 hour and 24 hour. OsActin served as the internal control gene for normalization. The expression level of rice samples at baseline (0 hour) is set to a value of 1.0. The values depicted in the graph represent the average results from three independent RT-qPCR experiments, with error bars indicating the standard deviation.

Numerous studies have established that *HSBP* is expressed across various tissues and organs in plants [5, 11, 12]. To date, there is a paucity of literature addressing HSBP expression patterns specifically under drought stress conditions. However, research conducted on *Brassica rapa* has indicated that *BrHSBP* expression is modulated in response to treatments with reactive oxygen species, such as H_2O_2, as well as phytohormones including abscisic acid, jasmonic acid and salicylic acid [12]. Additionally, several studies have demonstrated that HSBP plays a role in the regulation of raffinose biosynthesis [12, 13]. Raffinose is a compound that accumulates significantly in response to abiotic stressors, including drought, in plants [14]. The expression analysis of *OsHSBP2* conducted in this study suggests that this gene may be implicated in the drought tolerance mechanisms of BC15 rice. Further functional studies would be necessary to elucidate the specific role of *OsHSBP2* in drought stress tolerance mechanisms.

3.2. Evaluation of *OsHSBP2* gene expression under heat stress conditions

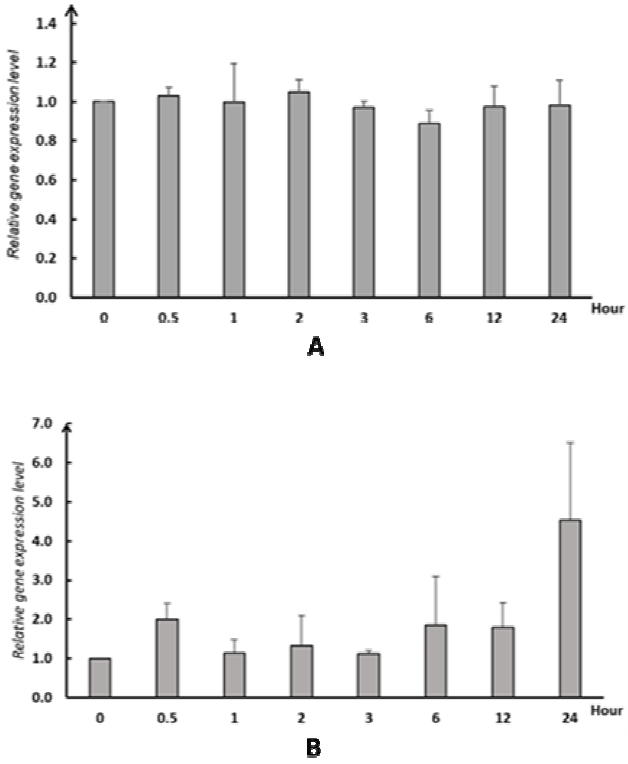

Figure 2. *OsHSBP2* expression level under hot conditions

Note: Graph comparing OsHSBP2 expression levels of rice varieties BC15 under normal condition (A) and artificial heat condition (42°C) at various time intervals 0 h, 0.5 hour, 1 hour, 2 hour, 3 hour, 6 hour, 12 hour and 24 hour. OsActin was used as the internal standard gene. The gene expression level of rice samples without stress treatment (0 h) has a value of 1.0. The value shown on the graph is the average RT-qPCR result of 3 experiments. The bar represents the standard deviation value of the experiments.

The expression level of *OsHSBP* in the BC15 rice variety exhibited minimal variation during the majority of the observation time points. Following exposure to heat stress, *OsHSBP* mRNA content increased rapidly, achieving a maximum of 2.0-fold enhancement. Subsequently, the transcription level of *OsHSBP* returned to near baseline levels, remaining stable until 12 hours post-treatment.

Notably, a significant increase in *OsHSBP* expression was observed, peaking at 24 hours after heat stress treatment, with a 4.5-fold increase compared to the initial levels. These results suggest that *OsHSBP* may play a more significant role in the late-phase responses of BC15 rice plants to high-temperature stress.

The increased expression of *OsHSBP2* in BC15 rice observed in this study indicates a significant relationship between *OsHSBP2* and the heat stress response mechanism. In *Arabidopsis*, the expression of *AtHSBP* is elevated during the recovery phase following heat shock treatment. Similarly, *ZmHSBP* in maize exhibits increased expression in response to heat stress factors. In contrast, *BrHSBP* demonstrates a decrease in expression levels under heat stress conditions, while its expression is upregulated when exposed to low-temperature conditions [12]. These contrasting patterns highlight the diverse roles of HSBP proteins in modulating heat stress tolerance responses across different plant species.

3.3. Analysis of BC15 *OsHSBP2* sequence characteristics

To facilitate functional studies of *OsHSBP2*, the coding sequence of this gene was isolated from total RNA samples of two rice varieties subjected to heat stress. The PCR analysis yielded a single DNA band approximately 0.63 kb in size (Figure 3), which aligns with the theoretically calculated size of the cloned *OsHSBP2* gene segment (624 bp).

Nucleotide sequence analysis revealed that the isolated DNA segment is 624 bp long and exhibits 100% similarity to the homologous sequence available in GenBank (accession code XM_015786025.3), confirming the successful isolation of the *OsHSBP2* gene segment. This segment contains an open reading frame of 300 bp, which encodes a protein comprising 99 amino acids, with a molecular mass of 10.48 kDa and an isoelectric point (pI) of 3.98. Structural analysis indicates the presence of an α-helical structure spanning positions 19 to 75. The degenerate amino acid sequence of the isolated gene shows a similarity of 67.47% to 96.97% with various homologous HSBP proteins previously studied (Figure 4), with the α-helix structural region exhibiting 73.68% to 100% similarity. The high sequence similarity, particularly in the α-helix region, suggests that *OsHSBP2* in BC15 rice may have a similar structure to HSBP proteins in other plant species. [5, 11, 15, 16]. Further functional studies would be needed to confirm its specific role in regulating HSF activity in BC15 rice.

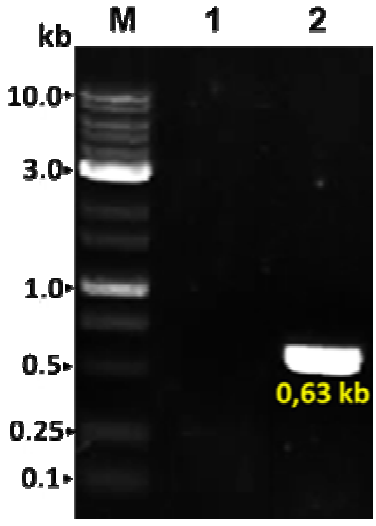

Figure 3. Isolation of the *OsHSBP2* gene segment

Note: Isolation of OsHSBP2 by PCR; (M): 1.0 kb DNA ladder (Cleaver Scientific); (1): blank sample (no template DNA); (2): template is cDNA of hot-treated rice samples BC15.

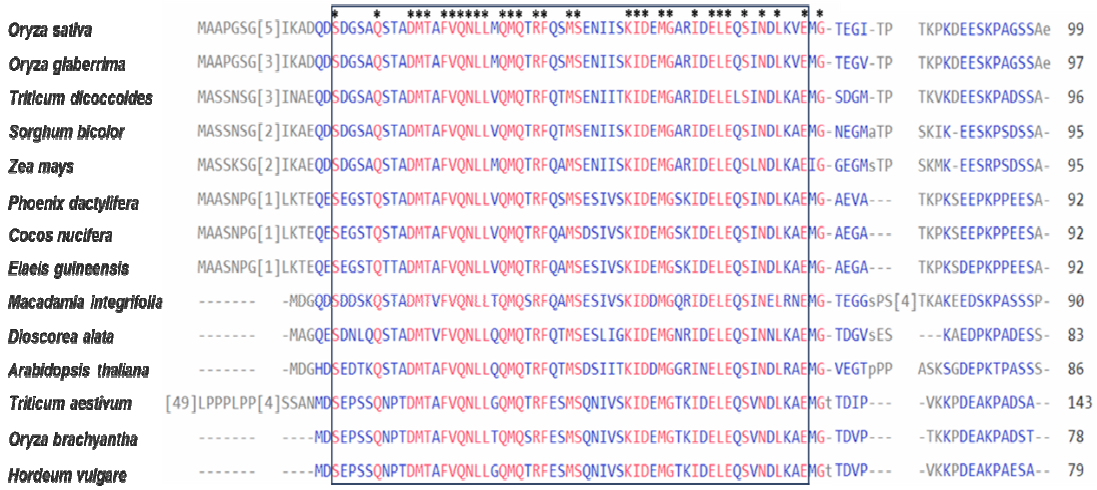

Figure 4. Amino acid sequence comparison of *OsHSBP2*.

Note: The degenerate amino acid sequence of isolated OsHSBP2 (O. sativas) was compared to the HSBP protein sequence of wild rice O. brachyantha (XP_015696858.1), wheat T. aestivum (XP_044381204.1), barley H. vulgare (XP_044946504.1), sorghum S. bicolor (XP_002436852.1), date palm P. dactylifera (XP_008782055.1), wheat T. dicoccoides (XP_037466802.1), African rice O. glaberrima (XP_052158029.1), E. guineensis oil palm (XP_010913595.1), Z. mays corn (NP_001150088.1), C. nucifera coconut (KAG1326961.1), D. alata yam (KAH7678721.1), M macadamia integrifolia (XP_042518414.1) and A. thaliana (NP_849392.4). An asterisk (*) represents a conservative amino acid residue position. The position of the sequence that creates the α-helix structure is boxed.

4. CONCLUSION

This study reveals significant alterations in *OsHSBP2* expression in BC15 rice under drought and heat stress, indicating its crucial role in stress response mechanisms. The results show successfully isolate and sequence the *OsHSBP2* gene segment from BC15 rice, confirming its identity and predicted structural similarity to HSBP proteins in other plant species. These findings provide a foundation for future functional studies aimed at enhancing drought and heat stress tolerance in rice varieties.

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