

Identification of BES1 Transcription Factor Gene Family in Strawberry Species

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Abstract: The BES1 genes is a class of transcript factor in the brassinosteroid signal pathway associated with multiple biological processes of plants, such as stress response. To investigate the potential functions of BES1 transcription factors in strawberry. We identified the 42 *BES1* genes from three strawberry species by bioinformatics method. The BES1 protein can be further categorized into 3 distinct groups, based on the protein sequences encoded by *BES1* genes. An expression profile analysis based on RNA-seq data illustrated that the BES1 genes are involved in stress tolerance of cultivated strawberry. A qPCR analysis reveals the gene expression change under low temperature stress. This study provides an overview of BES1 gene families in strawberry which will facilitate the further understanding of BES1 function in strawberry plants.

Keywords: BES1 Gene Family; Strawberry; RNA-seq; Transcription Factor; Cold Stress.

1. Introduction

Brassinosteroids (BRs) are essential hormones for plant which regulate a wide range of growth and development processes including cell elongation, vascular differentiation, root growth, responses to light, resistance to stress and senescence [1,2]. Tremendous progress has been made in the model plant, *Arabidopsis*, contributing to identify a canonical BR signal transduction pathway which is comprised of multiple components from the cell surface receptors (brassinosteroid-insensitive 1, BRI1) to the core transcription factors and their target genes in the cell nucleus (Kim and Russinova, 2020). In the BRs signal transduction pathway, the transcription factors (TFs) play crucial role that directly regulate the expression of target genes for further response.

The BRASSINAZOLE RESISTANT1 (BZR1) and BRI1 EMS SUPPRESSOR1 (BES1) are the two key TFs in the BRs signal transduction pathway in *Arabidopsis* [3]. The BES1 shares high identity (88%) with BZR1 and thus was named BZR2 as well [4]. Both contain a N-terminal domain including a bipartite nuclear localization signal (NLS) and a DNA binding domain, a BIN2 phosphorylation domain, a PEST motif (polypeptide sequences enriched in proline(P), glutamate(E), serine(S), and threonine(T)), and a C-terminal transcriptional activation domain [5]. A basic helix-loop-helix (bHLH) like motif of BES1 functions as the DNA binding domain which can interact with the BR-response element (BRRE; CGTGC/TG) in the promoter regions of target genes (Nosaki et al.). By chromatin immunoprecipitation-microarray (ChIP-chip) studies in *Arabidopsis*, 1609 genes were identified as putative target genes controlled by AtBES2, 404 of which are either up- or down-regulated by BRs and BES1 involving in plant growth, BR biosynthesis and signalling. The central region of these proteins contains 22–24 putative phosphorylation sites for the GSK3 kinase family and several contain a putative PEST motif involved in protein degradation [6].

2. Method and Materials

2.1. Identification of BES1 Genes in Strawberry.

The genome data of cultivated strawberry (*Fragaria ananassa* cv ‘Camarosa’) and the its two progenitor wild strawberry, including *F. vesca* and *F. iinumae*, were download from GDR database (<https://www.rosaceae.org/>). The BES1 protein sequences from *Arabidopsis* were retrieved as BLASTP queries to performed a search against genome data using BLAST with an e-value cut off of 10^{-10} . Meanwhile, a HMM profile of BES1_N (PF05687) domain downloaded from PFAM protein family database (<http://pfam.xfam.org/>) was used as a query to perform a search against genome data base using HMMSearch of the HMMER software with the cutoff E-value setting. For the redundancy protein sequences coded by a same gene locus, the longest protein sequence among the different proteins of transcript iso-forms was retained.

2.2. Phylogenetic Analysis and Nomenclature of BES1s

The BES1 protein sequences were used for construction of phylogenetic tree. Mafft software (Version 5) (Katoh et al., 2005) was employed to align the sequences, then a unroot tree construction was performed by IQ-tree (Version 2.0). The parameter of bootstrap test and approximate likelihood ratio test in IQ-tree were set as 1000 times. The phylogenetic tree result was visualized by ggtree package (Version 3.0.4) and ggtreeExtra package (Version 1.2.3) in R platform (Version 4.1.1). The BES1s identified from strawberry species were named according to the phylogenetic tree structure. For the paralogs in cultivated strawberry in a same phylogenetic clade, a lower case letters were labeled in the end of gene names following the order of physical gene location annotation.

2.3. Gene Expression Analysis

RNA-seq data was used to analysis the gene expression

level of *FanBES1s*. The transcriptome raw data was download from NCBI-SRA database([https:// www.ncbi. nlm. nih. gov/sra](https://www.ncbi.nlm.nih.gov/sra)) according to PRJNA512251 accession number. The download data was processed as previous description [7].

The total RNA of the treated samples was extracted with a modified CTAB method. 1µg total RNA was used as template for the first-strand cDNA was synthesis in a reverse-transcription PCR reaction. In the qPCR analysis, FaActin2 was used as reference gene. The stress treatment were

conducted as described in previous research [7]. The primer targeting the FanBES1-2b is ACGGAGAGAGAGAGCGA GC and GGCGAATATCTT GGCCGCAA.

3. Results and Discussion

3.1. Identification of BES1 Genes in Diploid and Octoploid Strawberry

Table 1. Physical and chemical properties of BES1 proteins

Gene ID in GDR database	Polypeptides length	Molecular weight (Da)	Isoelectric point	Name	Species
maker-Fvb1-1-snap-gene-206.48-mRNA-1	322	34905.6	8.8	<i>FanBES1-1a</i>	<i>F.ananassa</i>
maker-Fvb1-2-augustus-gene-90.55-mRNA-1	342	36986.2	9.07	<i>FanBES1-1b</i>	<i>F.ananassa</i>
maker-Fvb1-3-augustus-gene-53.32-mRNA-1	421	46033.6	10.31	<i>FanBES1-1c</i>	<i>F.ananassa</i>
augustus_masked-Fvb1-4-processed-gene-68.9-mRNA-1	322	34857.6	9.25	<i>FanBES1-1d</i>	<i>F.ananassa</i>
maker-Fvb2-1-augustus-gene-258.60-mRNA-1	314	34304.9	10.01	<i>FanBES1-2a</i>	<i>F.ananassa</i>
maker-Fvb2-3-augustus-gene-29.62-mRNA-1	314	34321	10.15	<i>FanBES1-2b</i>	<i>F.ananassa</i>
maker-Fvb2-4-augustus-gene-261.74-mRNA-1	314	34344	9.87	<i>FanBES1-2c</i>	<i>F.ananassa</i>
maker-Fvb3-1-augustus-gene-282.46-mRNA-1	321	34264.9	8.47	<i>FanBES1-3a</i>	<i>F.ananassa</i>
maker-Fvb3-2-augustus-gene-38.65-mRNA-1	329	34957.6	8.55	<i>FanBES1-3b</i>	<i>F.ananassa</i>
maker-Fvb3-2-augustus-gene-38.72-mRNA-1	373	39810.4	9.95	<i>FanBES1-3c</i>	<i>F.ananassa</i>
maker-Fvb3-3-augustus-gene-21.51-mRNA-1	329	34957.6	8.55	<i>FanBES1-3d</i>	<i>F.ananassa</i>
maker-Fvb3-4-augustus-gene-256.36-mRNA-1	329	34959.6	8.55	<i>FanBES1-3e</i>	<i>F.ananassa</i>
augustus_masked-Fvb3-1-processed-gene-266.2-mRNA-1	159	16865.7	9.33	<i>FanBES1-4a</i>	<i>F.ananassa</i>
maker-Fvb3-2-augustus-gene-52.48-mRNA-1	161	17379.3	9.35	<i>FanBES1-4b</i>	<i>F.ananassa</i>
augustus_masked-Fvb3-3-processed-gene-37.9-mRNA-1	159	16911.7	9.33	<i>FanBES1-4c</i>	<i>F.ananassa</i>
augustus_masked-Fvb3-4-processed-gene-239.6-mRNA-1	159	16885.7	9.33	<i>FanBES1-4d</i>	<i>F.ananassa</i>
augustus_masked-Fvb3-1-processed-gene-217.1-mRNA-1	358	38937.2	8.02	<i>FanBES1-5a</i>	<i>F.ananassa</i>
augustus_masked-Fvb3-2-processed-gene-101.12-mRNA-1	358	38861.2	8.22	<i>FanBES1-5b</i>	<i>F.ananassa</i>
augustus_masked-Fvb3-3-processed-gene-85.11-mRNA-1	358	38943.1	7.83	<i>FanBES1-5c</i>	<i>F.ananassa</i>
augustus_masked-Fvb3-4-processed-gene-203.9-mRNA-1	358	38819	7.79	<i>FanBES1-5d</i>	<i>F.ananassa</i>
maker-Fvb5-1-snap-gene-165.45-mRNA-1	597	67965	6.73	<i>FanBES1-6a</i>	<i>F.ananassa</i>
maker-Fvb5-3-snap-gene-126.37-mRNA-1	546	62349.6	6.25	<i>FanBES1-6b</i>	<i>F.ananassa</i>
maker-Fvb5-4-snap-gene-136.46-mRNA-1	546	62306.5	6.31	<i>FanBES1-6c</i>	<i>F.ananassa</i>
augustus_masked-Fvb7-1-processed-gene-172.7-mRNA-1	699	77982.4	5.41	<i>FanBES1-7a</i>	<i>F.ananassa</i>
maker-Fvb7-2-augustus-gene-115.57-mRNA-1	695	77724.1	5.11	<i>FanBES1-7b</i>	<i>F.ananassa</i>
maker-Fvb7-2-augustus-gene-180.57-mRNA-1	695	77698.1	5.11	<i>FanBES1-7c</i>	<i>F.ananassa</i>
maker-Fvb7-3-augustus-gene-118.35-mRNA-1	719	80332.2	5.58	<i>FanBES1-7d</i>	<i>F.ananassa</i>
maker-Fvb7-4-augustus-gene-113.45-mRNA-1	697	77768.4	5.6	<i>FanBES1-7e</i>	<i>F.ananassa</i>
evm.model.scaf_79.1260	322	34803.5	9.03	<i>FiiBES1-1</i>	<i>F.iinumae</i>
evm.model.scaf_4.125	314	34335	9.95	<i>FiiBES1-2</i>	<i>F.iinumae</i>
evm.model.scaf_93.644	329	34957.6	8.55	<i>FiiBES1-3</i>	<i>F.iinumae</i>
evm.model.scaf_93.899	160	17272.1	9.33	<i>FiiBES1-4</i>	<i>F.iinumae</i>
evm.model.scaf_85.303	526	57322.3	7.68	<i>FiiBES1-5</i>	<i>F.iinumae</i>
evm.model.scaf_14.937	659	74594.4	6.05	<i>FiiBES1-6</i>	<i>F.iinumae</i>
evm.model.scaf_25.750	744	83473.8	5.98	<i>FiiBES1-7</i>	<i>F.iinumae</i>
FvH4_1g14040.1	322	34857.6	9.25	<i>FveBES1-1</i>	<i>F.vesca</i>
FvH4_2g40170.1	314	34401.1	10.03	<i>FveBES1-2</i>	<i>F.vesca</i>
FvH4_3g07150.1	329	34929.6	8.55	<i>FveBES1-3</i>	<i>F.vesca</i>
FvH4_3g10040.1	159	16860.7	9.33	<i>FveBES1-4</i>	<i>F.vesca</i>
FvH4_3g17990.1	335	35923.5	8.01	<i>FveBES1-5</i>	<i>F.vesca</i>
FvH4_5g24520.1	645	72815.3	5.83	<i>FveBES1-6</i>	<i>F.vesca</i>
FvH4_7g14210.1	695	77563.9	5.11	<i>FveBES1-7</i>	<i>F.vesca</i>

we identified 28 BES1 genes from *Fragaria ananassa* (FanBES1s), 7 BES1 genes from *Fragaria vesca* (FveBES1s)

and 7 BES genes from *Fragaria iinumae* (FiiBES1s) (Table 1). The predicted physical and chemical properties of BES1

proteins are diverse (Table 1). The polypeptides length of BES1s are predicted which are range between 159 and 744, with different molecular weights (Mw) falling into the intervals of 16865.7 ~ 83473.8 Da. The electrophoretic mobilities of BES1 proteins are diversified as the different theoretical isoelectric point (pI) of BES1 proteins ranging from 5.11 to 10.31.

3.2. Phylogenetic Analysis

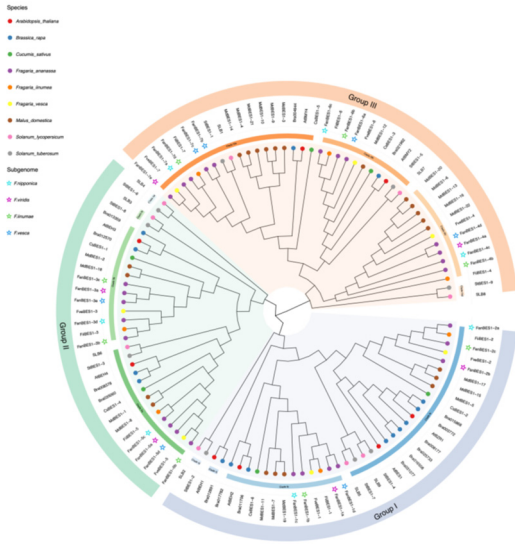


Figure 1. A phylogenetic tree with BESs protein sequences from three strawberry species

The evolutionary relationships of BES1 proteins was explored by constructing a maximum likelihood phylogenetic tree with BESs protein sequences from three strawberry species and 69 BES1 protein sequences identified from other six species by previous research [8-12]. Systematic nomenclature of BES1 genes were given according to the topology of the phylogenetic tree (Table 1). Consist with the previous report, the BES1 family from different species could be clearly classified into 3 groups [8,9,12]. This result indicated that the BES1 proteins in those species share common evolutionary ancestor proteins. However, the proportion of each group in different species are various. The

Table 2. The expression level of BES1 genes based on RNA-seq data

Gene	CK_TPM	LowTemperature_TPM	logFC	FDR
<i>FanBES1-1a</i>	4.28	1.08	-1.82	2.58E-08
<i>FanBES1-2b</i>	29.75	8.90	-1.58	5.63E-10
<i>FanBES1-2c</i>	19.92	6.21	-1.52	9.34E-09

To validate the expression pattern of these DEGs, we selected the *FanBES1-2* as target gene for further qPCR analysis. As shown in Figure3, the gene expression of *FanBES1-2* was down regulated during the treatment of cold stress. These data is consistent well with the RNA-seq which implies the role of *FanBES1-2* in response to cold stress.

4. Conclusion

The BES1 genes are crucial for signal transduction of brassinosteroid. In present work, we identified the 42 BES1 genes in three strawberry species. The BES1 genes could be

BES1 from four Rosacea species are enriched in the Goup III, while BES1 in two Brassicaceae are enriched in the Group I. This different enrichment are a result of evolutionary process in different species which may contribute some unique biological traits of these plants.

3.3. Gene Location of BES1s in Cultivated Strawberry

The BES1 genes from cultivated strawberry species are unevenly distributed on chromosomes (Figure 2). The *FanBES1* genes are located on four different sub-genomes including *Fni_like* subgenome, *Fii_like* subgenome, *Fvi_like* sub-genome and *Fve_like* subgenome. Some putative duplicated genes were found. These implied some putative gene loss and duplication events of BES1 genes along with the evolution process of cultivated strawberry.

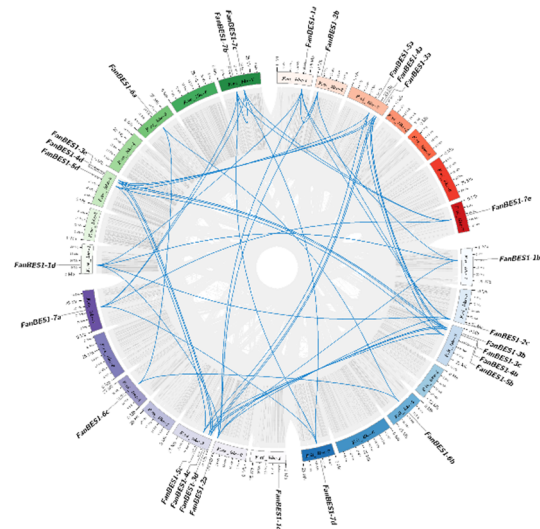


Figure 2. Gene Location of BES1s in cultivated Strawberry

3.4. Gene Expression under Cold Stress.

To reveal the potential gene function of *FanBES1* which might play roles in low temperature response. The gene expression was evaluated based on the RNA-seq data. As shown in 2, three BES1 genes were identified as DEGs. All the DEGs were downregulated under low temperature treatment.

classified into 3 groups. Based on the location of the BES1 genes showed that the BES1 were distributed in all chromosomes and may be derived from different accent donor species. Our further gene expression of *FanBES1* identified three BES1 gene from cultivated strawberry which may participate in the response to cold stress. Taken together, our data may shade light for further analysis of BES1 gene in strawberry.

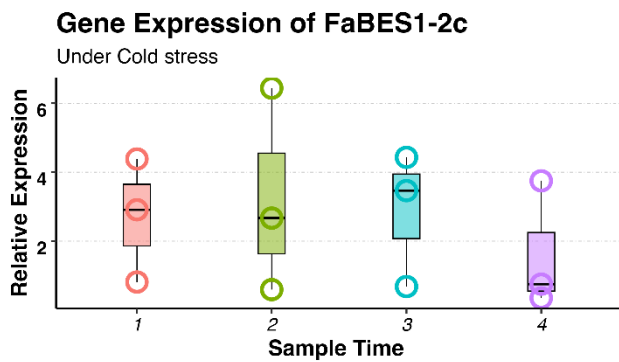


Figure 3. Gene Expression level of FanBES-2c under cold stress treatment

Acknowledgments

This work was financially supported by Sichuan Science and Technology Program (2023NSFSC1245).

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