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Expression profiling of BSD domain-containing genes in apricot during different developmental stages

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ABSTRACT

Plant transcription factors are evolutionarily conserved proteins that play an important role in the transcriptional regulation of gene expression by binding to their specific DNA sequences. BSD (mammalian BTF2-like transcription factors, synapse-associated proteins, and DOS2-like proteins) transcription factors are conserved in various species, from protozoa to humans, and are characterized by a typical BSD domain. However, little information is available about their possible roles in plant growth and development, and to date, members of this transcription factor family have not been systematically identified and analyzed in apricot. In this study, two BSD domain-encoding genes were identified in the apricot genome. Expression profile analysis by RT-qPCR revealed that both genes participate in different developmental stages of three different organs in apricot. *PaBSD1* was expressed higher than *PaBSD2* only in the stamen. Moreover, *PaBSD2* was higher expressed than *PaBSD1* in four different fruit stages, young leaf, leaf bud, sepal and petal. This study reveals the critical roles of BSD transcription factors in apricot development, with *PaBSD1* showing higher expression in stamen and *PaBSD2* in various fruit stages and leaf tissues. These findings provide a foundation for future functional studies and apricot breeding programs.

1. Introduction

Transcription factors (TFs) are genomic constituents that play important roles in controlling plant growth and development and response to stress factors by activating or repressing genes. The identification and characterization of these TFs, which play a critical role in the rearrangement of gene expression, are very important for elucidating extremely complex plant growth processes. *Arabidopsis thaliana* is an important model plant of plant molecular biology, and approximately 2000 TFs have been discovered in its genome. Nearly two decades ago, Doerks et al. (2002) discovered BSD (named after BTF2-like transcription factors, synapse-associated proteins, and DOS2-like proteins) TFs with a structurally conserved domain in various species from primitive protozoa to humans. The BSD domain is approximately 60 amino acids long, has two highly conserved adjacent tryptophan and phenylalanine residues at the C terminus, and three α-helix that are likely involved in DNA binding. Interestingly, the BSD domain also participates in the structures of different protein families. For example, the BSD domain is also found in members of the U-box family of proteins known to be involved in ubiquitination, indicating that BSD participates in different cellular regulations (Doerks et al. 2002).

Since it is a relatively newly discovered TF family, there are limited studies on BSD domain-encoding genes, which have a conserved domain in all living groups. For example, in yeast, BSD domain-containing DOS2 (DELOCALIZATION OF SWI6 2) has been shown to be involved in RNA interference and heterochromatic histone modification (Li et al. 2005). Reichmuth et al. (1995) showed that SAP47 (SYNAPSE-ASSOCIATED PROTEIN OF 47 kDa) is required for the short-term plasticity and association functions of synapses in *Drosophila melanogaster*. In mammals, BTF2 (BASIC TRANSCRIPTION FACTOR 2) is a component of the general transcription and DNA repair factor IIH core complex and is involved in the nucleotide excision repair of damaged DNA (Wang et al. 1995). To date, BSD domain-containing transcription factors have been identified in *Arabidopsis thaliana* (Park et al. 2009) and *Musa acuminata* (banana) (Ba et al. 2014). In *Arabidopsis*, ten genes encoding the BSD domain have been identified and of these, *AtBSD1* has been shown to be expressed in all tissues (Park et al. 2009). In banana, *MaBSD1*, a homolog of *AtBSD1*, has been shown to play a role in cell proliferation during somatic embryogenesis and its expression increases in parallel with ethylene accumulation and ripening (Ba et al. 2014; Shivani et al. 2017). Moreover, in tomato, *SlBSD1* positively regulates growth and fruit quality, but opposite pleiotropic effects on leaf senescence were detected in transgenic phenotypes obtained with knockdown or overexpression (Fan et al. 2020).

Apricot is a diploid species with eight pairs of chromosomes belonging to the genus Prunus, subgenus Prunophora Focke, and section Armeniaca (Lam.) Koch of the family Rosaceae (Olmsted 1941; Raji et al. 2014). Apricot (*Prunus armeniaca* L) cultivars are categorized into four eco-geographic groups: Central Asian, Iranian-Caucasian, European, and Dzhungar-Zailing. This fruit is commercially grown in 65 countries, highlighting its global agricultural importance (Kostina 1969). Although the roles of BSD TFs in plant growth and development have been described

in Arabidopsis, banana and tomato, no information is available about their possible functions in apricot. In this study, BSD TFs were identified for the first time in apricot and their expression patterns at different developmental stages were revealed by RT-qPCR. The results obtained from this study will not only contribute to the possible roles of BSD TFs in the plant kingdom but will also form the basis of functional characterization studies to be carried out in apricot in the coming years.

2. Materials and Methods

2.1. Plant materials and tissue sampling

Fifteen-year-old apricot trees from the Burdur Mehmet Akif Ersoy University Garden (37º, 01', 18" N; 30º, 17', 49" E) were selected for analysis. These trees represent a typical apricot growing environment in Burdur, Turkey. To analyze the expression pattern of *PaBSD* genes, 12 different tissues, including flower bud, leaf bud, young leaf (2 cm diameter), mature leaf (5 cm diameter), flower organs such as sepals, petals, stamens, carpels, young fruit (30 DAB), large green fruit (45 DAB), breaker fruit (60 DAB), and mature fruit (75 DAB), were sampled from the three different apricot trees. These samples were collected, separated and immediately frozen in liquid nitrogen and stored at −80°C.

2.2. Identification of BSD genes in apricot

To identify the BSD gene family in apricot, the BSD domain (IPR005607) was first obtained from InterPro (https://www.ebi.ac.uk/interpro/) and then used as a BLASTP query in The Genome Database for Rosaceae (GDR, https://www.rosaceae.org), *Prunus armeniaca* Genome v1.0 (apricot) with an e-value of 10^{-5} (Jung et al. 2019). The existence of BSD domain in candidate proteins obtained above were further confirmed by SMART (http://smart.embl-heidelberg.de/) (Letunic et al. 2021) and Conserved Domain Database (CDD) (https://www.ncbi.nlm.nih.gov/cdd/). Finally, sequences that did not contain BSD domains were eliminated, two genes encoding BSD domains were detected in apricot and named *PaBSD1* (PARG09221m01) and *PaBSD2* (PARG09221m02), respectively.

2.3. RNA extraction and RT-qPCR analysis of BSD genes in apricot

Total RNA extraction was conducted using the Plant/Fungi Total RNA Purification Kit (Norgen Biotek Corp., Thorold, ON, Canada) according to the manufacturers' instructions. Removal of DNA contamination was performed using the RNase-Free DNase I (Norgen Biotek Corp., Thorold, ON, Canada). The RNA concentration was measured with a microplate spectrophotometer (Epoch Microplate Spectrophotometer, Biotek Instruments, Inc.), and the quality of RNA was checked by agarose gel electrophoresis. The first strand of cDNA was synthesized using a VitaScript™ FirstStrand cDNA Synthesis Kit (Procomcure Biotech) according to the manufacturer's protocol. Gene-specific primers were designed Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). The apricot *TRANSLATION ELONGATION FACTOR 2* (*TEF2*) (Tong et al. 2009) and *ACTIN* (*ACT*) (Niu et al. 2014) genes served as the internal reference gene. The primer sequence details are given in Table 1.

The quantitative real-time PCR (qRT-PCR) was run using the iTaq Universal SYBR Green Super Mix (Bio-Rad Laboratories, Hercules, CA, USA). The reaction system contained 5 µl of iTaq Universal SYBR Green Super Mix, 1 µl of cDNA template, 0.5 µl of each forward and reverse primer, and 3.5 µl of Nuclease-free water. The reaction procedure's setting was as follows: 95°C for 30 s, followed by 40 cycles of denaturation at 95°C for 5 s, annealing and extension at 60°C for 40 s. Each sample had three biological and three technical replicates.

The relative expression levels of *PaBSD*s were calculated by applying the $2^{-\Delta Ct}$ method (Livak and Schmittgen 2001).

Table 1. Primer sequences specific to the *PaBSD* genes used in this study.

Primer name	Primer sequence (5'-3')
PaBSD1-F1	TGTCGTGTAGGCAAGTGGTGA
PaBSD1-R1	CGAACTTCGCAGCAGACGAG
PaBSD2-F2	TGTCGTGTAGGCAAGTGGTG
PaBSD2-R2	GAGGGGTCGTTTGGCCTGAA
TRANSLATION ELONGATION FACTOR 2-F	GGTGTGACGATGAAGAGTGATG
TRANSLATION ELONGATION FACTOR 2-R	TGAAGGAGAGGGAAGGTGAAAG
ACTIN-F	GTTATTCTTCATCGGCGTCTTCG
ACTIN-R	CTTCACCATTCCAGTTCCATTGTC

2.4. Statistical analyses

In this study, samples were collected from three different apricot trees in triplicate, and RT-qPCR data were statistically analyzed with SPSS software, version 17 (SPSS Inc., Chicago, IL, USA). The overall statistical significance of the data was revealed by Student's t-test at the $P<0.05$ level.

3. Results and Discussion

RT-qPCR is the most widely used analysis method to measure the expression of low-level expressed genes with high sensitivity and accuracy, combining traditional PCR and fluorescence techniques (Bustin 2000). In this study, to reveal the possible roles of *BSD* genes in apricot development, the expression patterns of their genes in 12 different stages of leaf, flower and fruit were analyzed by RT-qPCR (Figure 1). There is no statistical difference observed for the expression of both genes in the leaf bud and mature leaf stages. *PaBSD2* was expressed higher than *PaBSD1* in young leaf. *PaBSD2* was expressed higher than *PaBSD1* at the flower bud stage. In other flower organs, except carpel, *PaBSD2* was found to have higher expression, consistent with the flower bud. Apricot fruit development is a complex process in which many genes cooperate to alter numerous biochemical and physiological processes. According to the analysis results, expression of both *PaBSD* genes was detected, with *PaBSD2* being higher, at four different stages of apricot fruit. The differential expression of *PaBSD1* and *PaBSD2* across various tissues and developmental stages indicates their involvement in apricot leaf, flower, and fruit development. Specifically, the higher expression of *PaBSD2* in fruit stages suggests its pivotal role in fruit maturation and ripening, akin to its homologs in banana and tomato, which are known to influence the growth and ripening processes. These insights pave the way for future functional analyses and potential biotechnological applications in apricot cultivation.

Banana, which has a typical climacteric fruit, is an herbaceous perennial plant belonging to the Musa family (Liu et al. 2021). *MaBSD1* expression was investigated by RT-qPCR in

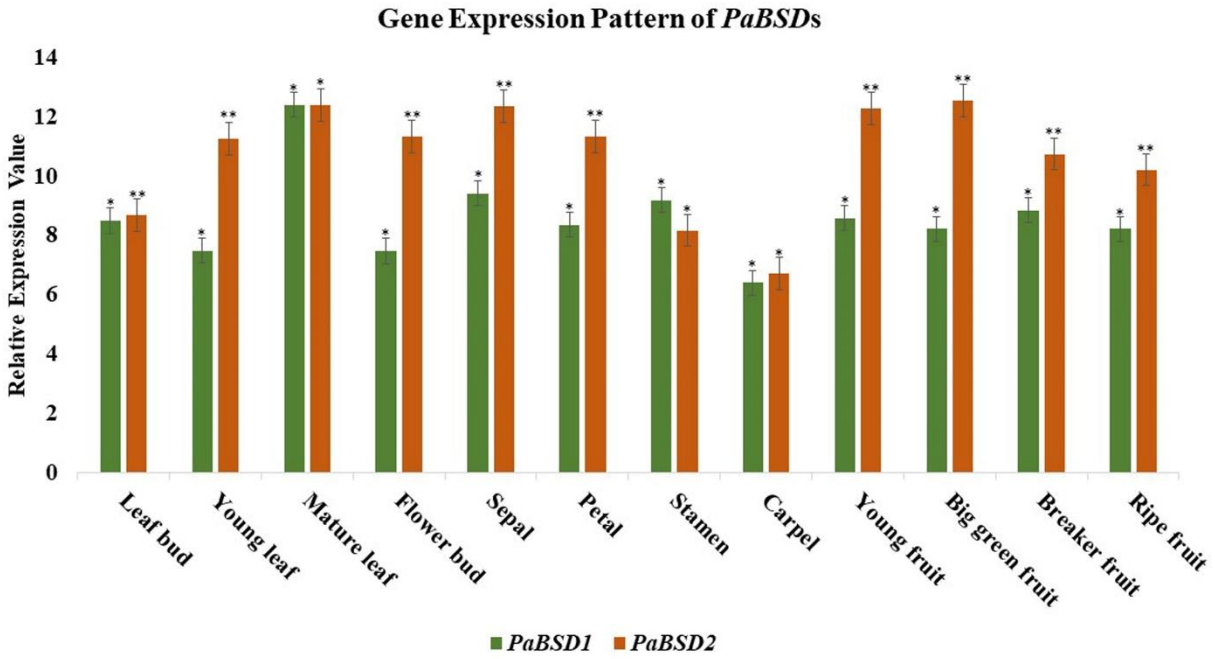

Figure 1. The expression profiles of *PaBSD* genes at the different developmental stages. Bars represent the mean of replicates ± standard deviation. * and ** indicate a significant difference at $P<0.05$ as determined by the Student's t-test.

banana under three different ripening conditions, including natural ripening, ethylene-induced and 1-MCP delayed ripening (Ba et al. 2014). *MaBSD1* expression was constant between days 0 and 7 of natural ripening, but started to increase on day 12 in accordance with the amount of ethylene, and on day 18, it was expressed 40 times more than on day 0. In contrast to natural ripening, there was a delay in *MaBSD1* expression in 1-MCP-treated fruits, and accumulation increased on days 30-36. Finally, *MaBSD1* expression increased rapidly 3-7 days after ethylene treatment. Fan et al. (2020) screened the expression of *SlBSD1*, the *AtBSD1* homolog in tomato, by RT-qPCR in six different tissues. The analysis showed that *SlBSD1* was expressed in all tissues examined, with the highest expression in the root and the lowest in the leaf.

A transcriptome is a snapshot of gene expression at a specific time and place in a tissue or cell, provided by capturing the total RNA within that tissue. This technique reveals, not only the expression of target genes, but also the combination of entire isoform sequences across cells and tissues. In this part of the study, to reveal the divergent roles of *BSD* genes in plant growth and development, digital expression profiles were examined based on transcriptome data from different model plants. In *Arabidopsis thaliana*, it was determined that both *BSD* genes expressed highest in the dry seed and lowest in the mature pollen stage among 48 developmental stages (Winter et al. 2007). In *Oryza sativa*, it was determined that the *AtBSD1* homolog was expressed highest in the shoot apical meristem and lowest in the seedling root at six different developmental stages (Jain et al. 2007). It has been shown that *SlBSD1* (Solyc04g077600), which is the *AtBSD1* homolog in *Solanum lycopersicum*, is expressed highest in the root and lowest in the leaf. In addition, *SlBSD1* showed the highest expression in the 3 cm fruit of *S. lycopersicum*, it also showed high expression in the immature green fruit stage in *Solanum pimpinellifolium*, suggesting that *BSD* genes have different functions in different species of the same genus (Sato and Orozco López 2012). *Prunus persica* is the taxonomically closest species to *Prunus armeniaca* and has two *BSD* genes. Based on the transcriptome data, it was determined that the first of these (Prupe.1G583500) was expressed highest in the shoot meristem and lowest in the bud, the second (Prupe.5G213500) was expressed highest in the fully opened flower and lowest in the bud and stem (Verde et al. 2013). Taken together with the RT-qPCR and transcriptome results obtained from these taxonomically distant species, it can be said that BSD domain-encoding homologues are firmly associated with plant growth and development.

4. Conclusion

In this study, two homologous genes encoding the BSD domain in apricot were identified for the first time, and their expression patterns in 12 different developmental stages of three different organs were revealed. The expression analysis results of *PaBSD* genes showed that these genes may play important roles in apricot growth and development, consistent with studies in other plants. As a result, this study will facilitate the understanding of the roles of *BSD* genes in growth and development in plants and will lay the foundation for future BSD-based molecular breeding studies in apricot.

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