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PARTIAL SEQUENCES OF THE GENE THAT CODIFIES FOR THE TRANSCRIPTION FACTOR *VPHSFB1* IN *Vasconcellea pubescens*. FIRST REPORT

SECUENCIAS PARCIALES DEL GEN QUE CODIFICA PARA EL FACTOR DE TRANSCRIPCIÓN *VPHSFB1* EN *Vasconcellea pubescens*. PRIMER REPORTE

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ABSTRACT

Plant heat stress transcription factors (HSFs) are involved in the response to heat. In Arabidopsis thaliana the HSFs genes are completely identified, however there was no information available about these genes in Vasconcellea pubescens (Chamburo) until now. In this preliminary work we describe the VPHSFB1 gene of V. pubescens (gene expression evaluated by RT-PCR and the partial sequence) that was induced by the increment of temperature. From our results, VPHSFB1 could be used as a heat response marker gene in tropical species.

Key words: Caricaceae, gene expression, heat.

RESUMEN

Los factores de transcripción del estrés por calor en plantas (*HSFs*) están involucrados en la respuesta al calor. En *Arabidopsis thaliana* los genes *HSFs* están completamente identificados, sin embargo no había información disponible sobre estos genes en *Vasconcellea pubescens* (Chamburo) hasta ahora. En este trabajo preliminar describimos el gen *VPHSFB1* de *V. pubescens* (expresión génica evaluada por RT-PCR y la secuencia parcial) que fue inducido por el incremento de temperatura. A partir de nuestros resultados, se podría usar a *VPHSFB1* como un gen marcador de respuesta a calor en especies tropicales.

Palabras clave: Caricaceae, expresión génica, calor.

INTRODUCTION

Plant heat stress transcription factors (*HSFs*) are essential components of the signal transduction involved in the expression of genes responsive to this kind of abiotic stress (Nover *et al.*, 2001). In *A. thaliana* 21 members of *HSFs* belonging to three genes classes A, B and C, have been identified (Kotak *et al.*, 2004). Among these, *ATHSFB1* (Class B) is necessary for the expression of heat stress inducible genes (as heat shock protein genes) that are involved in thermotolerance (Ikeda et al., 2011).

Caricaceae is a family composed by six genera, two of them are *Vasconcellea* and *Carica*. The 21 species that belong to genus *Vasconcellea* (collectively known as highland papayas) are distributed in South America, endemically in some countries, as Ecuador (Scheldeman *et al.*, 2011). It has been estimated that *Vasconcellea* diverged from *Carica* 25 Ma ago (Carvahlo and Renner, 2012).

More specifically the exotic species *V. pubescens* has interesting properties and uses, ranging from high levels of antioxidants (Simirgiotis *et al.*, 2009), gastric ulcers treatments (Mello *et al.*, 2008), dermal antitumoral therapy (Dittz *et al.*, 2015) to biofilm production based on Papain against cavities (Torres and Obando, 2016).

In this preliminary work, we report the partial sequence of the *V. pubescens VPHSFB1* gene, a phylogenetic analysis with related sequences and the expression banding pattern of *VPHSFB1* after temperature increase.

MATERIALS AND METHODS

Oligonucleotides for RT-PCR amplification and further sequencing of the amplicons were designed from the *CPHSFB1* gene reported by Tarora *et al.* (2010). Germinated seedlings (75 days old) were subjected to increment of temperature (from 25° C to 33° C or 45° C) for a period of 4 hs; seedlings at 25° C were used as controls. After applying the temperature treatment, RNA was extracted from leaves (PureLink RNA MiniKit, Ambion), then RT-PCR was performed (Superscript III One Step RT-PCR, Invitrogen) and, finally, agarose gel electrophoresis (1% agarose, 45 min, 80 volts) was performed and documented. Amplicons were sequenced twice in UDLA research laboratory (ABI 3130 Genetic Analyzer). Phylogenetic analysis was made in comparison with *HSFs* selected sequences with MEGA7 (Kumar *et al.*, 2016).

RESULTS AND DISCUSSION

Phylogenetic analysis of partial sequences of the VPHSFB1gene

From a PCR product (plants at 25° C) we obtained two partial sequences of *V. pubescens* heat stress transcription factor (Figure 1), hereinafter referred to as *VPHSB1a* (340 bp) and *VPHSB1b* (330 bp).

Despite the fact that the sequences were only fragments of the *VPHSFB1* gene, the phylogenetic tree (Figure 2) exhibited one major clade comprising the *HSF* sequences of *V. pubescens*, *A. thaliana*, *C. papaya* and *Brassica rapa*. Within this clade, a subclade was formed with the Caricaceae members; this was the expected topology since *V. pubescens* and papaya are more related between them than with Arabidopsis. The other sequences in this analysis remained unsolved. Interestingly, the sequences in the Caricaceae subclade seemed to have accumulated changes earlier that the ancestral lineage split between Arabidopsis and *Brassica*. This may have been because *V. pubescens* and papaya are strictly tropical species, as Carvahlo and Renner (2012) have shown in their biogeographic study. Therefore, it is feasible that Caricaceae developed specialized *HSF* genes in order to cope with higher temperatures earlier than Arabidopsis or *Brassica*, which are less adapted to tropical climates.

From the alignment of all sequences (not shown), the highest identity percentages were obtained by comparing *VPHSFB1* with *CPHSFB1*, thus, we conclude that these sequences are orthologs among them.

Expression banding pattern of the VPHSFB1 gene

Although the expression of *VPHSFB1* is constitutive at the assayed temperatures, the intensity of bands obtained by gel electrophoresis (Figure 3) increased at higher temperatures. Previously Tarora *et al.* (2010) characterized the ortholog *CPHSFB1* gene in papaya. In a Northern blot analysis, it was observed that this gene accumulated transcripts differentially after temperature increase (from 24° C to 42° C) and, thus it is responsive to heat stress. This behavior is similar to the observed in our analysis, which revealed the involvement of *VPHSFB1* in the response to temperature increment and, probably, in heat stress.

We conclude that an ortholog *VPHSFB1* gene is present in the genome of *V. pubescens*, which is responsive to temperature increment, and that this gene could be used as a marker for heat stress assays in this tropical species.

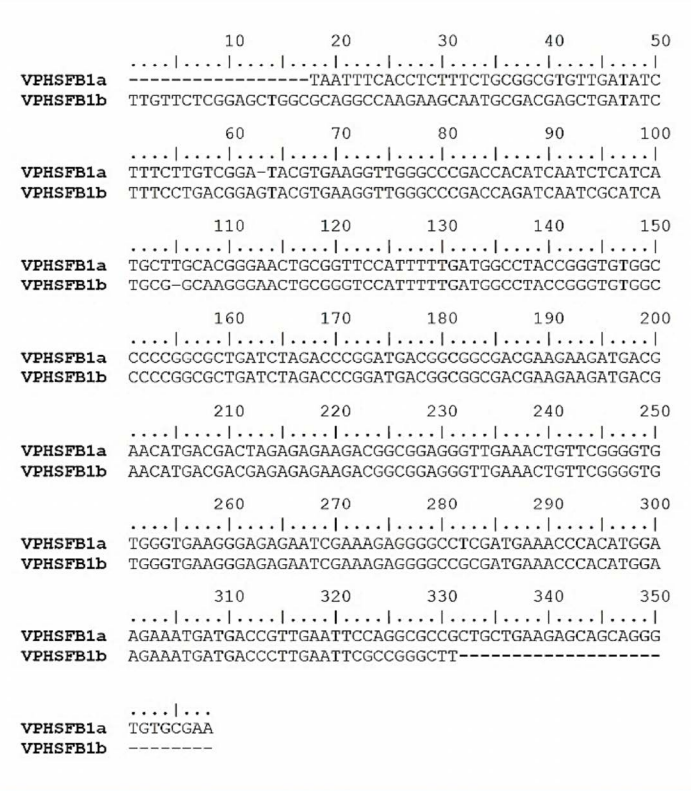
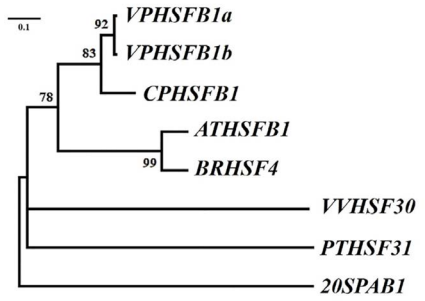


Figure 1. Clustal w alignment of partial sequences of the *VPHSFB1 gene.*



	VPHSFB1a	VPHSFB1b	ATHSFB1
VPHSFB1b	95,19		
ATHSFB1	66,31	66,84	
CPHSFB1	**86,10**	**88,24**	66,84

Figure 2. Maximum Likelihood phylogenetic tree based on a MUSCLE alignment of partial selected sequences *HSFs* genes (*C. papaya* CPHSFB1/AB506766.1, *A. thaliana ATHSFB1/ AT4G36990*, *Brassica rapa BRHSF*/EU186351.1, *Populus trichocarpa PTHSF31*/GI566202080, *Vitis vinifera VVHSF30*/NM001303086.1). The tree was rooted with 20SPAB1 *(AT1G16470.1)* that encodes for the 20S proteasome subunit PAB1 in *A. thaliana* (Iida *et al.*, 2009). The identity percentage of orthologs from *V. pubescens*, *A. thaliana* and *C. papaya* are shown below.

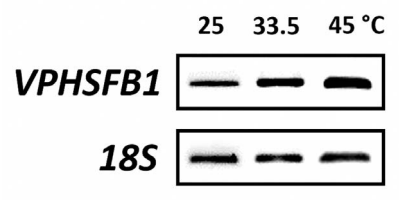


Figure 3. Banding pattern obtained from control plants (25° C) and plants under temperature increase (33.5° C and 45° C). 18S gene expression was used as a positive control. Controls with no template showed any band. The assay was made in triplicates with similar results.

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