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

Arizala-Quinto E. D., Viteri G. 1, Idrovo-Espín F.M. 1,2

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 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.

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