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ORIGINAL ARTICLE

Partial sequence analysis and relative expression of the *HSP70* gene of *Vasconcellea pubescens*

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Abstract

Environmental factors affect nearly all land areas on the planet. Global warming is one of the most destructive of these factors because it has adverse effects on crop production systems. Plants are sessile organisms that have evolved complex mechanisms to cope with stress factors. Heat shock proteins (HSPs) are one of those mechanisms. In this study, we analyzed a partial gene sequence that encodes for HSP70 protein in *Vasconcellea pubescens*. We also measured the relative expression of the gene in plantlets of *Vasconcellea pubescens* and performed biochemical assays under heat stress. The plantlets were exposed to three temperatures 25° C (control), 45 °C and 55 °C (stress temperatures) for 4 hours. The bioinformatic analysis led to the first description of a partial sequence of the HSP70 gene and its evolutionary history in *V. pubescens*. We found significant differences for relative expression of the *HSP70* gene, percentage of electrolyte leakage, and proline content between plants subjected to heat stress and those in the control group. Our results showed that *V. pubescens* displays thermotolerance even under extreme temperatures. *V. pubescens* is a poorly studied species that may contain genes of biotechnological interest (such as *HSP70*) that could be used for plant genetic modification.

Keywords: *VpHSP70* gene; *Vasconcellea pubescens;* heat stress; global warming; thermotolerance.

1. Introduction

Temperature increases appear to be one of the primary abiotic factors that impact crop yield. It is estimated that, in mild scenarios (2 °C increase), crop yield reduction ranges in the range of 8 % to 19 %, while under severe scenarios (4 °C increase), the reduction could range from 20 % to 48 % [1]. Every plant possesses a distinct range of minimum, maximum, and optimum temperatures for growth and development [2]. In their natural habitat, plants face heat stress during stretches of consecutive days with abnormally high temperatures, termed as heat waves [3]. Heat stress severity depends on heat wave frequency, intensity, and duration [4].

When high temperatures start to affect the biological processes of organisms, they activate a heat shock response (HSR) mechanism [5]. In plants that have developed thermotolerance, often through exposure to non-lethal temperatures, this response includes an increase in the expression of genes encoding Heat Shock Proteins (HSPs) [5, 6, 7]. These are a group of stress-related proteins that are conserved across evolution and are present in nearly all living cells. HSPs counteract the environmental stressors and genetic variations, thus mitigating the effects that these factors impose on the proteome. [8, 9]. These proteins are usually located in the cytosol, mitochondrion, endoplasmic reticulum, and nucleus [10].

Heat shock protein 70 (HSP70) is a class of molecular chaperon proteins ubiquitously expressed in both prokaryote and eukaryote organisms [11]. HSP70 is involved in protein folding, modulation between protein interactions, cell viability, and protein homeostasis processes such as degradation and translocation of damaged proteins [12, 13]. During stress periods, HSP70 prevents the formation of damaged protein aggregates, thus contributing to protein integrity preservation [14]. Structurally, HSP70s are formed by two functional domains, a nucleotide-binding domain (NBD) at the N-terminus and a substrate binding domain (SBD) at the C-terminus. These domains are connected by a short linker segment [15]. The chaperone activity of HSP70 begins with an allosteric conformational change, during which the SBD recognizes the substrate with high affinity. Then, HSP70 folds the substrate into its native state. Finally, the correctly folded substrate is release through ATP-hydrolysis. [11, 16].

Vasconcellea is a plant genus within the Caricaceae family, naturally occurring in the subtropical regions of South America, especially in the high regions of Colombia and Ecuador. Ecuador harbors at least 16 of the 21 *Vasconcellea* species previously described [17]. In 2020, Tineo et al. [18] identified 5 new *Vasconcella* species in Peru through the sequencing of six marker genes, expanding the total count of species within this genus to 26. As indicated by Scheldeman [19], *Vasconcellea* species (especially *Vasconcellea pubescens* and *Vasconcellea x heilbornii*) are primarily consumed in local contexts as fresh fruit, roasted, in juices, marmalades, preserves, sauces, pie fillings, and pickles. Despite efforts to market *V. x heilbornii* (babaco) in countries like New Zealand, there was a lack of consumer interest attributed to the organoleptic traits of this fruit [19]. Our research group focuses on native *Vasconcellea* species, driven by the evidence that wild papayas associated with this genus tend to exhibit increased resistance to abiotic stress [20]. We are particularly interested in *V. pubescens*, a high-altitude papaya crop, adapted to moderate temperatures (18 °C to 22 °C), and well-suited for cultivation around 800 m above sea level (masl) [19, 21]. We have previously studied how *V. pubescens* behaves under abiotic stress [22, 23]. This study aimed to show preliminary evidence of a homologous sequence to the *HSP70* gene in *V. pubescens* and evaluate this species response to heat stress through molecular and biochemical assays.

2. Materials and Methods

2.1. Plant material

Mature fruits of *V. pubescens* were collected in the surroundings of Sangolquí (altitude 2748 masl, average max temperature 19.93 °C, average min temperature 7.95 °C, [24]). Province of Pichincha in Ecuador. Seeds were extracted from the fruits and washed with 50 ml of 96 % ethanol for 5 min.

Five hundred seeds were sown in 100, 8-oz polyethylene plastic cups (upper diameter: 8.0 cm, lower diameter: 5.6 cm, height: 9.3 cm, approx. volume: 240 mL). The seeds were allowed to grow under a regime of 12 hours of light and 12 hours of shade and watered regularly by root drenching. After 17 weeks, 64 plants were randomly selected for trials.

2.2. Heat stress assay

The trials were divided into 2 parts. The first part comprised 32 plantlets, with 16 constituting the control group (kept at 25 °C), the remaining 16 plantlets were incubated in 45 °C ovens for 4 hours (mild heat stress). The second part comprised 32 plantlets, with 16 assigned to the control group and the remaining 16 assigned to the extreme heat stress group 55 °C for 4 hours.

2.3. Bioinformatics and molecular assays

We performed a local tBLASTx search to identify homologous genes of *HSP70* within various species: *Carica papaya* (Scontig1.398), *Carica papaya* var Maradol (KU065119.1), *Nicotiana tabacum* (AB689673.1), *Brassica napus* (NM_001315590.1), *Vitis vinifera* (XM_002263563.3), and *Populus trichocarpa* (XM_006378652.2). This was accomplished using the complete sequence of the *HSP70* gene from *Arabidopsis thaliana* (AT5G02500) as a query.

RNA extraction was performed according to the protocol described by Cevallos et al. [23]. The oligonucleotides (Forward: 5'ACTTGGTCTGGAAACCGCTGGC3', reverse: 5'ACCG TTGGCAATGTCGAAGCAG3') were designed from the *HSP70* sequence of *Carica Papaya* (Scontig1.398), which was retrieved from Phytozome database [25]. We amplified the *Vp18S* gene reported by Arizala et al. [22] with the following oligonucleotides (Forward: 5'ATGATAA CTCGACGGGGATCGC3', reverse: 5'CTTGGATGTGGTAGCCGTTTT3'), and used it as a gene expression control.

RT-PCR was performed using the SuperScript® III One-Step RT-PCR Platinum® Taq HiFi kit (InvitrogenTM), under the following conditions: cDNA synthesis at 55 °C for 20 min, one cycle at 94 °C for 2 min, 35 cycles (94 °C for 15 sec, 60 °C for 30 sec, 72 °C for 1 min), and one cycle at 72 °C for 5 min.

The PCR products were eluted in a 2 % agarose gel by electrophoresis (100 V for 30 min). The resulting agarose gel was analyzed with the ChemiDoc MP Image System (Bio-Rad). The Relative Expression (RE) of the HSP70 gene was was measured using Bio-Rad's Image LabTM 5.2.1 software through densitometric quantification, with the Vp18S band serving as a reference (assigned an RE value of 1).

The PCR product (referred as *VpHSP70*) was sequenced by UDLA Sequencing and Fragment Analysis Services (Quito-Ecuador) through second generation sequencing, Sanger method.

Multiple sequence alignments of the identified *HSP70* sequences and the sequenced *VpHSP70* fragment were performed using the MUSCLE algorithm [26]. To elucidate the phylogenetic relationships among the sequences, a phylogenetic tree was constructed using the Maximum Likelihood method with 1000 bootstrap replications. Both processes were made with MEGA XI [27]. The translated oligopeptides sequences were obtained from AGUSTUS [28]. Weblogo [28] was used to generate a graphical representation of the oligopeptides sequences.

2.4. Biochemical assays

The percentage of electrolyte leakage (EL) was determined following the protocol described by Camejo et al. [30]. Immediately after the heat treatment, four 5-mm diameter discs were punched from each leaf and washed with distilled water for 5 minutes. The discs were then placed in 25-ml beakers with 20 ml of distilled water. The conductivity (µS/cm) was measured for each temperature (C1) using a potentiometer (Fisher Scientific accument XL600).

The final measurement point (C2) was recorded 24 hours later, after autoclaving all plant disks. The EL was calculated using Equation 1.

$$\mathrm{EL} = \frac{C_1}{C_2} \times 100. \tag{1}$$

Catalase activity (CAT act) was measured according to the protocol described by Aebi [31] with modifications. We weighed 60 mg of leaf powder and added 100 µl of 1X PBS. The samples were then centrifuged at 4 °C for 30 min (14000 rpm). In a 0.7 ml microcentrifuge tube, 50 % of the supernatant was combined with an equal volume of the working solution containing H_2O_2 (30 %). Subsequently, 10 µl of this mixture was blended with 990 µl of the working solution and 500 µl of H_2O_2 (30 %). Absorbance was measured at 240 ηm in a 2 ml quartz cuvette. Measurements were taken 10 and 70 seconds after starting the reaction, using 1.5 ml of working solution as blank. Catalase (CAT) enzyme activity was calculated using Equation 2. The results were expressed as U/mg fresh weight.

$$\text{CAT act} = \frac{A_{10\,\mathrm{s}} - A_{70\,\mathrm{s}}}{0.01}. \tag{2}$$

The proline concentration assay followed a modified version of the protocol outlined by Bates et al. [32]. Leaves were collected and weighed (80 mg for each reaction), after which 5 µL of 3 % sulfosalicylic acid per milligram of fresh weight was added. The samples were centrifuged at room temperature for 15 min (14000 rpm). Then, 100 µL of supernatant was mixed with the following reaction solution: 3 % sulfosalicylic acid (100 µL), glacial acetic acid (200 µL), and acidic ninhydrin (200 µL). The mixture was incubated for 1 h at 96 °C. One milliliter of toluene was added to the reaction mixture and vortexed for 30 s. The new mixture was incubated for 5 min. The organic phase was removed and proline was measured by absorbance at 520 nm using toluene as reference. The concentration was calculated by extrapolation and expressed as mg/g of fresh weight. A curve of proline standard concentration/absorbance is required.

2.5. Statistical analysis

The data obtained from each of the biochemical tests and relative quantification of *VpHSP70* gene expression were analyzed through R commander [33]. Analysis of variance (ANOVA) was performed. Tukey's test ($P < 0.001$) was used to verify differences between treatment means.

3. Results and Discussion

3.1. Plantlets response to heat stress

When compared to the plantlets cultivated at 25 °C (Figure 1a), those exposed to low heat stress (45 °C for 4 hours) exhibited minimal deterioration in both petioles and leaves, without any noticeable wilting. The stems of the heat stress plantlets predominantly retained an upright posture (Figure 1b).

Plantlets exposed to high heat stress (55 °C for 4 hours) presented a high degree of deterioration. Leaves had markedly withered, rendering them not only limp but also fragile upon touch. The stems and petioles displayed a notable loss of turgidity, exhibiting a flaccid appearance. (**Figure 1d**). This is consistent with previous studies made by our research group [22], where subjecting the plantlets to a regimen of 45 °C for 4 hours did not induce significant harm. Consequently, we opted to incorporate a higher temperature to induce noticeable damage.

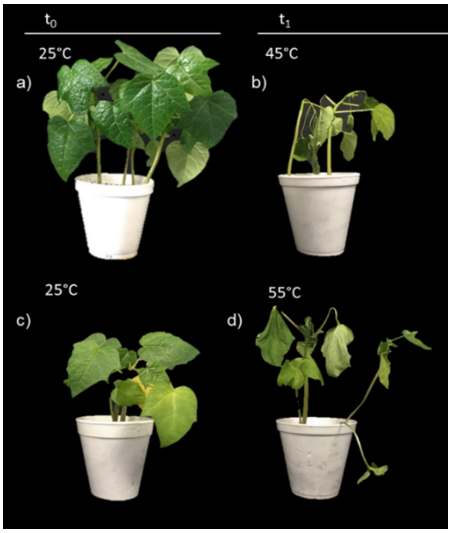

Figure 1. *V. pubescens* plantlets **a)** and **c)** Control 25 °C, **b)** low-stress temperature, 45 °C **d)** high-stress temperature 55 °C.

Most *Vasconcellea* species are confined to the northern Andean region and prefer cold climates with limited seasonality [34]. Potatoes (*Solanum tuberosum*), grow in similar geographical areas within the Andean region and show a similar temperature range preference [35]. Our results showed that, although *V. pubescens* thrives in cold climates, its seedlings can withstand temperatures as high as 55 °C. In contract, potatoes are notably sensitive to heat stress, with alterations in tuber yield observed at 38 °C [36]. This suggests that *V. pubescens* is thermotolerant.

3.2. Partial sequence analysis and relative expression of the *VpHSP70* gene

We identified a homologous sequence to the *HSP70* gene in Scontig 1.398 from papaya. Previously, Le et al. [37] identified the same *HSP70* sequence using bioinformatic methods. After conducting PCR experiments and sequencing, we obtained a 175-bp fragment of the gene of interest. When aligned with *HSP70* of other species, the DNA sequence showed high similarity (**Figure 2**). The highest percentage identity of *V. pubescens* was obtained with *C. papaya* (94.86 %) followed by *P. trichocarpa* (82.86 %). *C. papaya* cv. Maradol showed the lowest identity percentage (79.43 %). While it holds true that our comparison of *VpHSP70* encompassed tree, fruit, and shrub plant species, the resultant percentage identity values were notably elevated. These findings underscore the conservation of *HSP70* across the diverse plant species investigated in this study. Sequences that are conserved over millions of years of evolution are known to have similar functional roles [38].

C. papaya and *V. pubescens* are members of the same family, thus the higher percentage of identity found is consistent. The lower percentage of identity observed between *C. papaya* cv. Maradol and *V. pubescens* could be explained because *V. pubescens* has been subjected to different events throughout its natural history [39]. Domestication (in the middle of the last century for *C. papaya* cv. Maradol), selection, genetic drift, and inbreeding. It is likely that these events have had varying impacts on the genetic structures of both species. [39, 40]. It is interesting to note that the *Vasconcellea* genus shows greater diversity in South America, while the wild species of *Carica* are exclusively distributed in Central American. According to Hagen et al. [41] the movement of active tectonic plates favored the formation of mountains, such as the Andes in South America, as well as the appearance of archipelagos like those found in Southeast Asia. These processes

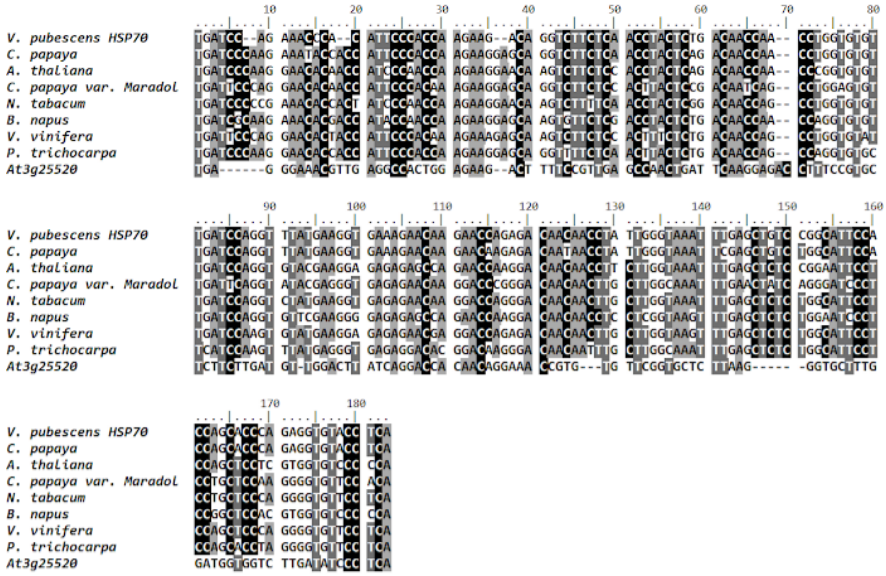

Figure 2. DNA Sequence alignment of selected *HSP70* genes and *VpHSP70*.

fostered the creation of diverse ecological niches, consequently leading to the emergence of a multitude of new species. Additionally, it is possible that the domestication of cultivars by Central American populations resulted in a reduction of *Carica* varieties[42].

The tree branches of the bootstrap consensus tree (**Figure 3.**) are color-coded according to the relationships between the analyzed species. We found two main clades and the outgroup. The first clade is further divided into two subclades. The first subclade (red) is formed by *V. pubescens, C. papaya, P. trichocarpa, C. papaya cv Maradol,* and *V. vinifera*. The second subclade (yellow) is formed by *B. napus* and *A. thaliana*. The second clade (green) is formed by just *N. tabacum*. As it was discussed before, *V. pubescens* is more closely related to *C. papaya var.* than to *C. papaya cv Maradol*. According to Ming et al. [43] *C. papaya* shares a greater number of genes with *P. trichocarpa*, these genes are related to cell expansion, plant height, and lignin biosynthesis. This might explain the formation of this subclade, which consists of *P. trichocarpa* and species that exhibit traits typical of woody plants or trees. The high similarity of gene coding sequences between *A. thaliana* and *B. napus* has been recorded previously [44]. Thus, explaining the formation of the yellow subclade.

A previous study by Zhang et al. [45] identified the conserved and regulatory motifs in the HSP70 protein sequences. We obtained a 53-amino acid fragment of the VpHSP70 protein by translating the partial *VpHSP70* sequence. The analysis with WebLogo showed a linear consensus of 4 highly conserved motifs (**Figure 4.**), which is consistent with Zhang et al [45].

Domains $\beta 3$ and $\beta 5$ encompass nine amino acid residues each, while domain $\beta 4$ comprises eight residues. We also found a small helix, $\eta 1$, of 3 residues and a small fragment (4 aa) that is part of the SBD [45, 46] and is implicated in binding misfolded proteins [47]. It has been established that the SBD undergoes conformational changes contingent on the presence or absence of the peptide substrate [48]. This domain commonly accommodates substrates such as antimicrobial peptides, transcription factors, stress related proteins (such as SOD1), and a wide variety of unfolded polypeptides [47]. The results described above suggest that the nucleotide sequence obtained represents a fragment of the *VpHSP70* gene.

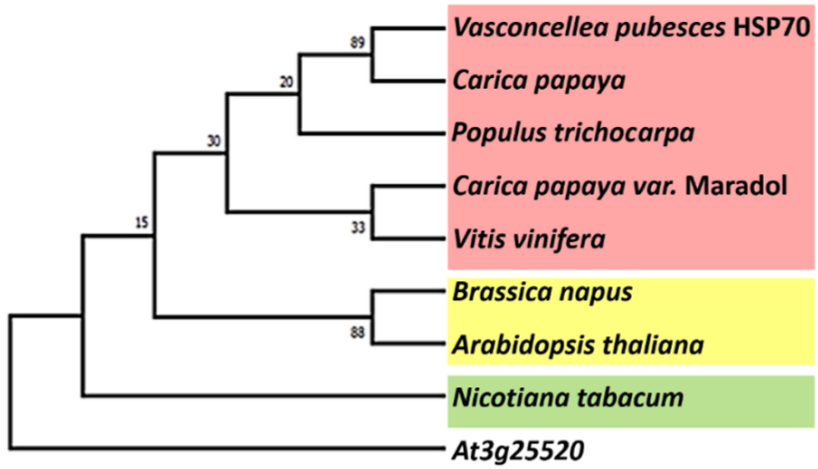

Figure 3. Bootstrap consensus tree from selected *HSP70* gene sequences and *VpHSP70*, two main clades are show.

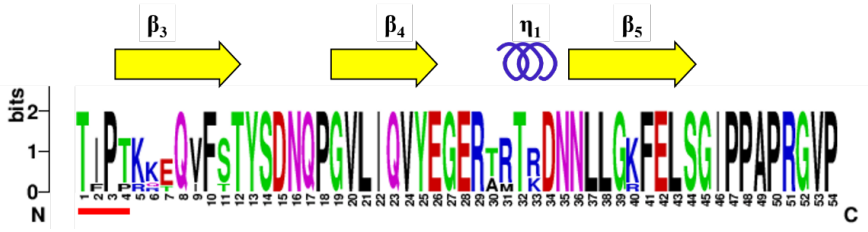

Figure 4. WebLogo sequence of the 53 aa showing sequence conservation and the conservation rate of each residue. We identified four domains: $\beta 3$, $\beta 3$, $\eta 1$, and $\beta 3$.

3.3. Relative expression profile of the *VpHSP70* gene

The RE of *VpHSP70* showed a notable increase with escalating temperatures (**Figure 5.**), rising from 1.278 (control temperature of 25 °C) to 3.67 (45 °C) and further to 4.776 (55 °C). Given the involvement of HSPs in maintaining protein integrity, the genes encoding them exhibit a basal expression level rather than being exclusively activated in response to temperature elevation [8]. Therefore, a certain level of *VpHSP70* expression was expected at 25 °C.

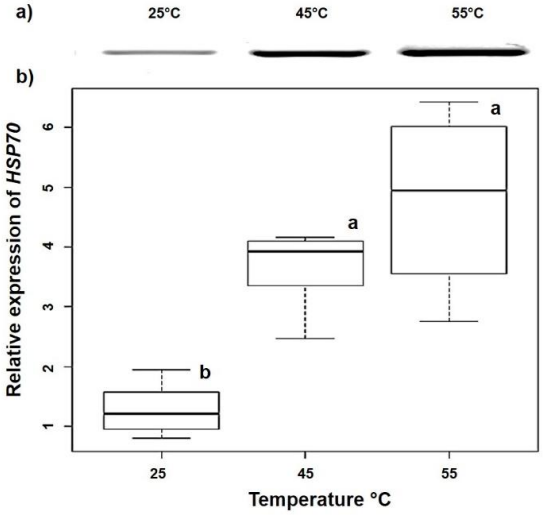

Figure 5. Relative expression (RE) of *VpHSP70* at 25, 45, and 55 °C. The expression of the *Vp18S* gene was used as a reference in all treatments (RE = 1).

Kumar et al. [49] evaluated *HSP70* expression at different developmental stages of thermotolerant and thermosensitive wheat plants (*Triticum aestivum*). They observed that thermotolerant cultivars expressed more *HSP70* than thermosensitive cultivars, both under normal growth and heat stress (30, 35, and 40 °C for 2 hours). In lettuce (*Lattuca sativa*), Liu et al. [50] observed that plants experiencing different levels of heat stress increased the RE of two *LsHSP70* genes. Our results are consistent with both previous studies.

3.4. Biochemical assays under heat stress

3.3. Percentage of electrolyte leakage

The stability and integrity of the membrane of leaf cells was evaluated through the percentage of electrolyte leakage (EL). Since the data we obtained did not exhibited a normal distribution, we carried out a 1/X transformation (**Figure 6.**). The electrolyte leakage (EL^{-1}) in *V. pubescens* decreased with rising temperatures, registering values of 0.038 (45 °C) and 0.028 (55 °C), in contrast to control value of 0.063 (25 °C).

When subjected to stress, plants experience alterations in the plasma membrane, ranging from denaturation to aggregation, depending on stress intensity. These changes increase membrane EL [51]. For example, the EL of *A. thaliana,* a cultivar highly susceptible to heat, surpasses 50 % within 15 min of exposure to 50 °C [52]. In contrast, *Brassica juncea,* which is heat tolerant, reaches an EL of 52.48 % after 4.5 h of heat exposure [53]. The highest electrolyte leakage (EL) recorded in our study was 35.33 % (equivalent to 0.028 EL^{-1}), we obtained this value after subjecting the plantlets to 55 °C for 4 hours. Therefore, *V. pubescens* shows a greater

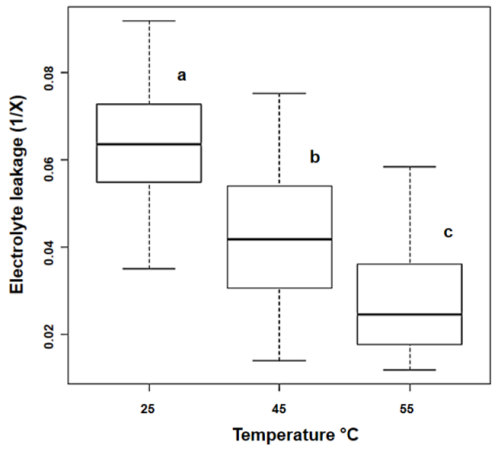

Figure 6. Electrolyte leakage changes in response to temperature fluctuation. Different letters indicate statistically significant differences ($P \leq 0.05$).

thermotolerance than genotypes previously described as tolerant to heat [53]. HSP70 is involved in protein movement across membranes [54]. Therefore, it is possible to establish a connection between the results of the EL assay and *VpHSP70* expression, which is required to ensure efficient protein translocation inside the cell.

3.4. Catalase activity assay

The CAT activity means across different treatments were as follows, 2.648 U/mg (at 25 °C), 2.705 U/mg (at 45 °C), and 1.766 U/mg (at 55 °C). The difference between these values was not statistically significant (Figure 7). This suggests that CAT activity was not affected by increasing temperatures.

The plant's antioxidant system effectively withstands both biotic and abiotic stress by generating redox chemicals, such as tocopherol, carotenes, ubiquinones, and plastoquinones, which localize to the chloroplasts, mitochondria, and peroxisomal membranes and prevent cell membrane oxidation [55]. CAT is an antioxidant enzyme, mainly located in peroxisomes, that breaks down H_2O_2 into water (H_2O) and oxygen (O_2) [56]. The accumulation of antioxidant proteins positively correlates with thermotolerance in different plant species [57]. However, CAT is not only involved in stress response but also in growth processes. Thus, the genes encoding this enzyme are constitutively active [58].

The constant levels of CAT activity observed in our study can be explained by the fact that plants have a wide array of antioxidant mechanisms and enzymes responsible for inactivating reactive oxygen species, as well as the fact that the plantlets in our assay were in early developmental stages. There is evidence that CAT activity (and CAT gene expression) may decrease with increasing temperature, while other enzymes (ascorbate peroxidase, glutathione reductase) increase their activity during heat stress. Genes that codify for antioxidant enzymes are highly regulated by each plant species [59].

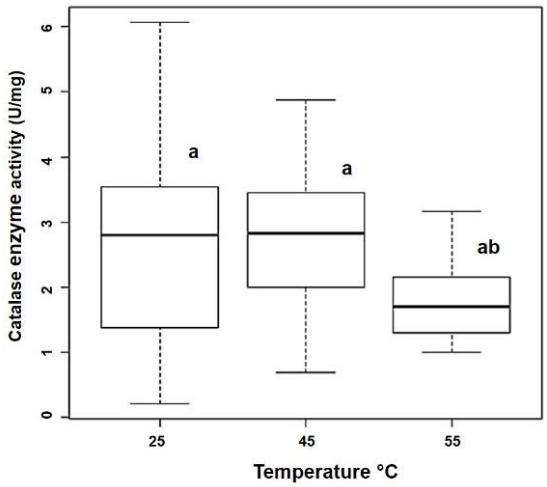

Figure 7. Catalase enzyme activity changes in response to temperature. Different letters indicate statistically significant differences ($P \leq 0.05$).

3.5. Proline content Analysis

Free proline content showed significant differences among the treatments (**Figure 8.**), with values of 8.4608 mg/g fresh weight in the control group (25 °C), 12.0983 mg/g fresh weight in the low-stress group (45 °C), and 28.5108 mg/g fresh weight (55 °C) in the high-stress group.

Proline accumulation is considered a strategy for plant protection and survival under abiotic stress conditions [60].

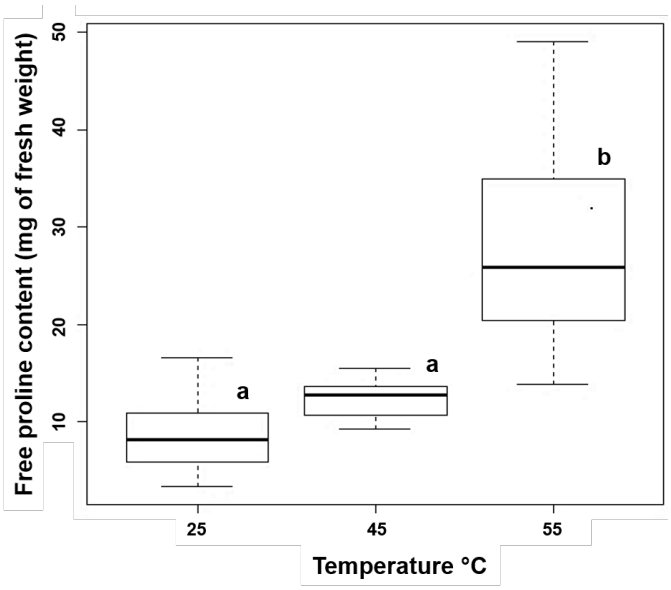

Figure 8. Changes in free proline content (mg/g of fresh weight) in response to temperature. Different letters indicate statistically significant differences ($P \leq 0.05$).

Proline serves as a compatible solute, acting as non-enzymatic antioxidant, low molecular weight chaperone, and regulatory osmolyte when plants are subjected to stress, thereby helping to mitigate water loss. [56, 61]. Zhu et al. [62] subjected papaya fruits to 7 °C and 35 °C and reported that proline content (and the expression of the *P5CS* gene involved in the biosynthesis of this amino acid) increased as a function of temperature. This finding aligns with our results and supports our assumption that proline accumulation is associated with thermotolerance in *V. pubescens*.

The results of the present work, suggest that wild type *V. pubescens* plantlets are adaptable plants that can tolerate high temperatures, even though they naturally grow in cold climates. Domesticated variants of a species undergo genotypic and phenotypic changes relative to their wild counterparts, designed to enhance factors like ease of harvest, yield, and crop phenology [63]. Therefore, *V. pubescens* retains the ability to tolerate high temperatures. In a recent paper Alvarez et al [20] found that wild varieties of papaya overexpressed stress response genes (CpWRKY and CpNAC/drought) and better tolerated this condition. The thermotolerance observed in this study could be explained by the accumulation of chaperone VpHSP70, but future studies need to confirm if this accumulation occurs. Plants are known to have a basal thermotolerance, which would coincide with the relative constitutive expression of *VpHSP70* at 25 °C. However, these observations can also be explained by a plasticity event (understood as the ability of a plant to adapt and cope with changes in its environment). Vanderauwera et al. [64] determined that *Arabidopsis* plants showed both types of thermotolerance (under light and heat stress regimes) as a measure to counteract oxidative stress. Hence, there is a need to assess how *V. pubescens* responds to various stress conditions and at different points in its phenological stages (as suggested by Scheepens et al. [65]). Moreover, contrasting these responses with those of other genus members can offer insights for discerning the mentioned conditions.

4. Conclusions

In the present study, we described a partial sequence of the *VpHSP70* gene from *V. pubescens* plantlets. Our bioinformatic analysis suggested that the nucleotide sequence and the corresponding translation to amino acids belongs to a member of the HSP70 protein family, which is highly conserved across species. The RE of *VpHSP70* increased with heat. Our biochemical assays suggest that there is a protein (possibly HSP70) acting as a chaperone in *V. pubescens*, a plant species that is typically found in cold climates but can tolerate extreme heat temperatures of up to 55 °C for 4 hours. *V. pubescens,* a species with widespread distribution in South America, is yet to be fully explored. Additional research could confirm the impact of heat stress on plantlet recovery, expanding to other developmental stages (flowering, fruiting) and plant tissues (flowers, fruits, roots, etc.). More sensitive methods such as real time RT-PCR (gene expression) or immunoprecipitation (protein isolation and analysis) could help to verify the presence of the chaperon. It's worth noting that poorly studied wild species could potentially have useful genes for plant genetic modification. So, future studies on other members of the *Vasconcellea* genus, endemic to Ecuador might be valuable. Our research could serve as an initial roadmap in this direction.

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6. Conflict of Interest

None declared

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Análisis de la secuencia parcial y expresión relativa del gen *HSP70* de *Vasconcellea pubescens*

Resumen: Los factores ambientales afectan prácticamente todas las áreas terrestres del planeta. El calentamiento global se encuentra entre los más destructivos de estos factores, ya que tiene efectos adversos en los sistemas de producción de cultivos. Las plantas son organismos sésiles que han desarrollado mecanismos complejos para enfrentar los factores de estrés. Las proteínas de choque térmico (HSPs) son uno de esos mecanismos. En este estudio, analizamos la secuencia parcial de un gen que sospechamos codifica la proteína *HSP70* en *Vasconcellea pubescens*. También medimos la expresión relativa del gen en plántulas y realizamos ensayos bioquímicos bajo estrés térmico. Las plántulas fueron expuestas a tres temperaturas: 25 °C (control), 45 °C y 55 °C (temperaturas de estrés) durante 4 horas. El análisis bioinformático llevó a la primera descripción de una secuencia parcial del gen *HSP70* y su historia evolutiva en *V. pubescens*. Encontramos diferencias significativas en la expresión relativa del gen *HSP70*, el porcentaje de fuga de electrolitos y el contenido de prolina entre las plantas sometidas a estrés térmico y las del grupo control. Nuestros resultados mostraron que V. pubescens presenta termotolerancia incluso bajo temperaturas extremas. V. pubescens es una especie poco estudiada que podría contener genes de interés biotecnológico (como *HSP70*) que podrían aprovecharse para cultivar variedades termotolerantes.

Palabras Clave: gen *VpHSP70*; *Vasconcellea pubescens*; estrés térmico; calentamiento global; termotolerancia.

Análise parcial da sequência e expressão relativa do gene *HSP70* de *Vasconcellea pubescens*

Resumo: Os fatores ambientais afetam praticamente todas as áreas terrestres do planeta. O aquecimento global está entre os mais destrutivos desses fatores ao ter efeitos adversos nos sistemas de produção de culturas. As plantas são organismos sésseis que evoluíram mecanismos complexos para lidar com fatores de estresse. As proteínas de choque térmico (HSPs) são um desses mecanismos. Neste estudo, analisamos a sequência parcial do gene suspeito de codificar a proteína *HSP70* em *Vasconcellea pubescens*. Também medimos a expressão relativa do gene em plântulas e realizamos ensaios bioquímicos sob estresse térmico. As plântulas foram expostas a três temperaturas: 25 °C (controle), 45 °C e 55 °C (temperaturas de estresse) por 4 horas. A análise bioinformática levou à primeira descrição de uma sequência parcial do gene HSP70 e sua história evolutiva em *V. pubescens*. Encontramos diferenças significativas na expressão relativa do gene *HSP70*, no percentual de vazamento de eletrólitos e no teor de prolina entre as plantas submetidas a estresse térmico e as do grupo de controle. Nossos resultados mostraram que V. pubescens apresenta termotolerância mesmo sob temperaturas extremas. V. pubescens é uma espécie pouco estudada que pode conter genes de interesse biotecnológico (como *HSP70*) que poderiam ser aproveitados para cultivar variedades termotolerantes.

Palavras-chave: gene *VpHSP70*; *Vasconcellea pubescens*; estresse térmico; aquecimento global; termotolerância.

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