Study of Heat Shock Protein (Hsp) 70 gene expression of Emilia sonchifolia L. and Sphagneticola trilobata L. in Universitas Indonesia, Depok and Kebun Raya Cibodas

M Syadewi1, A Salamah1,2, A E Maryanto1,2 and N Andayani1

1Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Indonesia, Depok 16424, Indonesia
2Biodiversity and Environmental Genomics Research Cluster, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Indonesia, Depok 16424, Indonesia

Abstract. The temperature of earth surface is generally different according to altitude. Universitas Indonesia (UI) is located at 50–140 masl with an average temperature of 28.6 °C, while Kebun Raya Cibodas (KRC) located at 1.300–1.425 masl with an average temperature of 20.06 °C. Temperature differences are thought to affect plant responses, such as the expression of heat shock protein (Hsp) 70 genes. The research aims to find out the expression of Hsp70 genes on Emilia sonchifolia and Sphagneticola trilobata collected from UI and KRC. The study was conducted by isolating RNA from young leaves, which then converted into cDNA. The cDNA product was further amplified by polymerase chain reaction (PCR) using Hsp70 Arabidopsis thaliana primer. The amplification products were then sequenced and analyzed by in-silico. The results of amplification show that there is a band with approximately 250 bp in all samples which is thought to be a partial product of the Hsp70 gene. The sequencing results show that PCR amplification product is Hsp70 partial gene with a nucleotide variation in the 65th base which has no effect on amino acid changes. The results indicate that Hsp70 gene is expressed in Emilia sonchifolia and Sphagneticola trilobata grown in UI and KRC.

Keywords: Hsp, expression, Asteracea, UI, KRC

1. Introduction
Differences in earth surface temperature at a different geographical area, affecting plant responses, such as expression of heat shock protein (Hsp) gene [1, 2]. Heat shock protein (Hsp) acts as a molecular chaperone that helps to repair unfolded or aggregated proteins, which commonly formed in response to stress [3]. There are 12 subfamily Hsp70 genes in Arabidopsis thaliana, including Hsp70-1, Hsp70-2, Hsp70-3, Hsp70, Hsp70b, BiP-1, BiP-2, BiP-3, cpHsc70-1, cpHsc70-2, mtHsc70-1, mtHsc70-2 that play different roles in plants growth and development[4]. One of Hsp subfamily genes is Hsp70 that plays an important role in cell tolerance against heat stress [5].

There is limited information about temperature differences that affect the expression of Hsp70 genes in plant natural habitat. Such investigation can be done using Asteracea family species because they are widely distributed (composite), having a wide range of thermal sensitivities, and having the ability to dominate an area (invasive). Those characteristics are the result of their large seeds production, their
wide seed distribution ability, and their allelopathic compounds [6, 7]. Research of Hsp70 in Asteraceae had been done by Barua et al. [8] using Solidago altissima leaves. The result shows that Hsp70 was accumulated in response to heat and light exposure.

Based on Agassi [9], it is known that ten species of Asteraceae have the highest importance value in UI environment, among them are Symedrella nodiflora, Mikania micrantha, Tridax procumbens, Cyanthilium cinereum, Elephantopus scaber, Sonchus arvensis, Youngia japonica, Emilia sonchifolia, and Sphagneticola trilobata. There are only two species that can be found in both UI and KRC which are Emilia sonchifolia and Sphagneticola trilobata. Analysis Hsp70 gene expression in Emilia sonchifolia and Sphagneticola trilobata have never been done before. Therefore this study is conducted to asses Hsp70 gene expression in Emilia sonchifolia and Sphagneticola trilobata.

2. Method

2.1. Sample collection
Young leaves from Emilia sonchifolia and Sphagneticola trilobata with ± 2 cm in length were collected from UI and KRC. The samples collecting time was from 11:00 to 13:00 pm. Leaf samples collected from KRC were then put in a 50 mL centrifuge tube which already contains 15 mL of NAP buffer.

2.2. RNA isolation
Total RNA isolation was carried out using modified cetyltrimethylammonium bromide (CTAB) method based on Zeng et al. [10]. The concentration and purity of total RNA obtained was measured with BioDrop spectrophotometer, and the total RNA was visualized with 1 % agarose gel electrophoresis. Total RNA was then treated with RNase-Free DNase to take out the DNA contaminant. The RNA obtained was then stored in -80 °C or can be used directly in the next step.

2.3. Synthesis and cDNA amplification
Total RNA converted to cDNA using the GoScript™ Reverse Transcription System kit [Promega]. Furthermore, cDNA was amplified using GoTaq® Green Master Mix kit [Promega] and Hsp70 Arabidopsis thaliana primer based on Sung et al. [3] with forward sequence TCAAGCGGATAAGAGTCACT (CG258F) and reverse sequence CTCGTCCGGGTTAATGCT (CG259R). Primer from Arabidopsis thaliana was chosen because it’s usually used as a model organism for studying the responses to abiotic and biotic stresses. The result of PCR was visualized with 1 % agarose gel electrophoresis and stored in -20 °C.

2.4. In silico analysis
Nucleotide sequence and chromatogram from sequencing result was analyzed using Geneious version 11.1.4. to show the attach position from samples, and Bioedit version 7.2 .5., to compared nucleotide sequence with reference species (Arabidopsis thaliana and Saussurea medusa). Then, nucleotide sequence translated into amino acid sequences with the help of ORF finding website on NCBI.

3. Results and discussion
The average air temperature measurement when plant samples were taken showed that the average temperature of Emilia sonchifolia and Sphagneticola trilobata in Universitas Indonesia was higher than in Kebun Raya Cibodas (table 1).

Electrophoresis result of Hsp70 gene amplification shows that the gene can be amplified at 55 °C annealing temperature with band size 250 bp in all samples (figure 1).
Table 1. The average air temperature in Universitas Indonesia and Kebun Raya Cibodas

| Location                  | Species                  | Average air temperature (°C) |
|---------------------------|--------------------------|-----------------------------|
| Universitas Indonesia (UI)| *Emilia sonchifolia*     | 35.20                       |
|                           | *Sphagneticola trilobata*| 22.95                       |
| Kebun Raya Cibodas (KRC)  | *Emilia sonchifolia*     | 33.10                       |
|                           | *Sphagneticola trilobata*| 32.10                       |

Figure 1. Gel electrophoresis *Hsp70* gene amplification cDNA. Electrophoresis gel is performed using 1% agarose 100 V in 30 minutes with GelRed. (Lane 1) *E. sonchifolia* UI, (Lane 2) *Emilia sonchifolia* KRC, (Lane 3) *S. trilobata* UI, (Lane 4) *S. trilobata* KRC, (M) Marker.

Based on the BLAST result in NCBI site, it is known that the product length of *Hsp70* gene *Arabidopsis thaliana* is 862 bp, while product length obtained is 250 bp. The difference in product length is probably due to non-specific primer, so the primer is attached to the undesirable site, and the result is a partial product. Another possible reason is the difference in *Hsp70* gene sequence in different species leads to produce undesirable product size [11].

The alignment of all samples with *Arabidopsis thaliana* showed a corresponding *Hsp70* sequence in all four samples (figure 2). The black graph (marked with a black arrow in figure 2) is a part of samples sequence that homologous with the base sequence of the *Hsp70 Arabidopsis thaliana* gene. This supports the assumption that PCR amplification products are *Hsp70* genes.

Alignment of *Hsp70* gene of species reference (*Arabidopsis thaliana* and *Saussurea medusa*) with samples (figure 3) shows the difference of one nucleotide base at the 65th position (figure 3 (A)).

The nucleotide sequences are then translated into amino acid sequences with the help of ORF finding website. The results show all four samples have the same amino acid sequence, that is MFQGLGCSPIKAVRELGSERRETVRSISGVGVRALTRTR.
Figure 2. Alignment of all samples with Hsp70 gene of A. thaliana using Geneious version 11.1.4.

Figure 3. Alignment of Hsp70 gene of A. thaliana & S. medusa with all sample using Bioedit version 7.2.5. (A) difference of one nucleotide base at the 65th position.

Furthermore, the alignment of the four samples with the Hsp70 Arabidopsis thaliana and Saussurea medusa genes showed that in the 65th nucleotide base position, plants exposed to a normal range of temperature would encode thymine, while plants exposed at high temperature range would encode cytosine. The results obtained in accordance with Tonsor et al. [12] showing that there is a variation of Hsp101 genotype on Arabidopsis thaliana leaves that are exposed to different temperatures. The conversion of a nucleotide base sequence do not affect the structure and function of the protein, since they do not alter the amino acid sequence encoded by GGC under conditions of thermal stress and GGT encountered in the normal encoding environment amino acid glycine. However, based on literature nucleotide base changes can affect protein synthesis through the efficiency of mRNA splicing or control of the transcription process [13].

4. Conclusion
The results of amplification show that there is a partial product of the Hsp70 gene. The sequencing results show a nucleotide variation in the 65th base which has no effect on amino acid changes. The results indicate Hsp70 gene is expressed in Emilia sonchifolia and Sphagneticola trilobata grown in UI and KRC.
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