The estimated of 18.1 kDa class IV small heat shock protein (sHsp) from *Hevea brasiliensis* using of PHYRE² and SWISS-MODEL software

S A Pasaribu¹, M Basyuni²*, E Purba³ and Y Hasanah³

¹ Doctoral Program of Agricultural Sciences, Faculty of Agriculture, Universitas Sumatera Utara, Jl. Prof. A. Sofyan No. 3, Padang Bulan 20155, Indonesia
² Department of Forestry, Faculty of Forestry, Universitas Sumatera Utara, Jl. Tri Dharma Ujung No. 1 Medan, North Sumatera, 20155, Indonesia
³ Department of Agriculture, Faculty of Agriculture, Universitas Sumatera Utara, Jl. Prof. A. Sofyan No. 3, Padang Bulan 20155, Indonesia

*E-mail: m.basyuni@usu.ac.id

**Abstract.** The objective of research is *Hevea* and *Ricinus* which are two of the latex-producing plant species. The modelling of protein was obtained from *Hevea brasiliensis* and *Ricinus communis*. Identification of small heat shock heat protein 18.1kDa very different between *hevea* and *ricinus* to coverage 92% and 69% analyzed by SWISS-MODEL. In addition, the data from similarity, identity, range, and Q-mean were distinct. According to the PHYRE² analysis, we observed the residue presenting in *Hevea* and *Ricinus* not much different (*Hevea*: 12-158; *Ricinus*: 11-156). The 3D-ligand site prediction confirms is not found in *Hevea* as well as *Ricinus*. Appropriately, the small heat shock protein involved in different species will be helpful in the development of stress tolerance in plant crops, the different homology images and templates were identified as different binding site predictions. These models can be used for supporting the *Hevea* and *Ricinus* proteins modeling, mainly in the molecular study.

1. **Introduction**

Small heat shock proteins (sHsps) are proteins group with molecular masses of 15 to 42 kDa [1, 2] and usually named according to their molecular size. sHsp has been arranged in six classes based on DNA sequence similarity, immunological cross reactivity, and intracellular localization [3]. Three classes (CI, CII, and CIII classes) of sHsp are localized in the cytosol or in the nucleus [3,4] and three others in the plastid (P) [4], endoplasmic reticulum (ER) [5,6], and mitochondria (M). L) [7, 8]. HSPs are usually not detected in vegetative tissue under normal growth conditions, but can be caused by environmental stress drought [9], cold [10,11], salinity [12, 13]. The correlation between sHsp synthesis and stress response results in sHsp protecting cells from the adverse effects of stress. Response of sHsp in plant tolerance to drought stress made as an adaptive defense mechanism [14].

Water in enough quantities is needed by plants in cell expansion, growth and development of other parts plant. Various mechanisms at the morphological, physiological and molecular levels that contribute to drought tolerance in plants have been developed. Proteins that play a key role in stress signaling, transcription regulation, macromolecular protection, cellular detoxification, and various other cellular processes are the main factors associated with drought tolerance [15]. The HBsHSP23.8 expression was significantly regulated by drought and high light conditions in RRII 430 and RRIM
600 clones which were classified as drought tolerant clones [9]. The HSP chaperone and the resulting ROS scavenger enzyme were seen to be increased in Reyan '93-114 'cold tolerant rubber clones as indicated by physiological responses indicating that the expression of several enzymes encoding and non-enzymatic ROS scavenger encoding genes [10]. HSP genes expressed in leaves and stems, where the level of expression is relatively higher in cold-tolerant rubber tree clones of Reyan '93 -114' compared to cold-sensitive clones 'Reken501' [11].

Therefore, the present study describes about the source, model template and location of heat shock of a small protein with a molecular mass of 18.1 kDa on Hevea brasiliensis drought stress using Phyre² and Swiss Models software.

2. Materials and Method

2.1. Collection of protein
Database cDNA and amino acid of 18.1 kDa class I small heat shock protein (sHsp) from Hevea brasiliensis and Ricinus communis drought stressed obtained from National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/) from. Number accession of protein sequences followed the GenBank: XP_021660151.1 (Hevea brasiliensis) and XP_002533590.1 (Ricinus communis).

2.2. The sources of protein and modelling template
The selection template and builds of the 3-dimensional structure model of the protein was performed by PHYRE² online software (http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index) [16], compared with SWISS-MODEL (https://swissmodel.expasy.org/) [17]. The highest quality of the template was selected to structure protein model.

2.3. The location of sHps types
The location of sHsp types in Hevea and Ricinus and the DNA accession number were retrieved from National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/).

3. Results and Discussion

3.1. The PHYRE² and SWISS-MODEL protein
The small heat shock protein (sHsp) 18.1 kDa class I modelling using PHYRE² for hevea and ricinus can be seen in Table 1. The hevea and ricinus genes have same results in sequence aligned, confidence and coverage. Only the residue value have a different result, that is 147 (hevea) and 145 (ricinus).

| Gene               | Target ID | Seq.Aligned | Residue | Confidence | Coverage |
|--------------------|-----------|-------------|---------|------------|----------|
| Hevea brasiliensis | d1gmea    | 92.00       | 147     | 100.00     | 0.93     |
| Ricinus communis   | d1gmea    | 92.00       | 145     | 100.00     | 0.93     |

SWISS-MODEL protein results showed that a different result for all variable. The value of the variable on hevea seems to be higher than ricinus, except the variable of seq similarity (hevea: 0.49 and ricinus: 0.55). This shows that the structure and binding site of the sHsp analyzed has quite similar structure between hevea and ricinus which is one of latex producing plants.

| Gene               | Target ID  | Seq.Identity | Seq.Similarity | Range | Coverage | Q-mean |
|--------------------|------------|--------------|----------------|-------|----------|--------|
| Hevea brasiliensis | lgme.2.A   | 65.75        | 0.49           | 12-158| 0.92     | -1.95  |
| Ricinus communis   | lgme.2.A   | 52.63        | 0.55           | 11-156| 0.69     | -1.26  |
Based on gene expression analysis data, \textit{HbsHSP23.8} was identified to be strongly associated with drought responses in rubber clones RRIM 600 and RRII 430 (upregulation of 7-8 fold) whereas in RRII 105 and RRII 414 the upregulation was 4 fold and 3.3 fold, respectively under drought [9, 18]. In rice plants, sHsp 18.0 has also been shown to influence rice resistance to biotic and abiotic stresses [1], family in \textit{Salix suchowensis}, an important bioenergy woody plant showed potential mechanism under stresses [19].

3.2. \textit{The PHYRE}² and \textit{SWISS} image of protein models

Homology modelling showed that 147 residues (93% within the amino acid sequence) resulted 100.0% confidence of modelling from the single highest template scoring in \textit{Hevea brasiliensis} and \textit{Ricinus communis} using PHYRE² software (Figure 1A and 1B). The coverage was higher value compared the SWISS-MODEL predicted 92% and 69% respectively (Figure 2A and 2B).

![Figure 1. The PHYRE² protein modelling for hevea (A) and ricinus (B). Image coloured by rainbow N → C terminus. Model dimensions (Å): X: 76.228 Y: 42.742 Z: 44.638 (A); Model dimensions (Å): X: 76.228 Y: 44.727 Z: 45.594 (B)](image1)

![Figure 2. The SWISS-MODEL protein for hevea (A), and ricinus (B)](image2)

3.3. \textit{The location of sHsp types and DNA accession number in Hevea and Ricinus}

There are 13 types of sHsp class I and 2 types of sHps class IV in \textit{Hevea brasiliensis}. While as many as 8 types of class I sHsp and 2 types sHsp of class IV in \textit{Ricinus communis}. For more details, types of sHsp proteins and DNA accession numbers can be seen in Table 3.
The number of sHsp in *hevea* has been obtained compared to *ricinus*. This is evident from the number of sHsp that has been studied and obtained the genes associated with the sHsp due to *hevea* growth in sub-optimal environmental conditions, including drought, cold and salt environments. This also proves that the *hevea* is the largest source of natural rubber in the world which has been widely cultivated by the world’s rubber producing countries, including Indonesia. For the plant defense system, the role of HSPs showed a special focus on plant response to biotic and abiotic stresses, which will be helpful in the development of stress tolerance in plant crops [20]. Recently, triterpene proteins have also been reported using PHYRE2 and SWISS-MODEL for triterpene synthesis in mangrove species (KcMS, RsM1, RsM2, BgBAS, BgLUS) [21]. Moreover, two cycloartenol synthase protein (KcCAS and RsCAS) from *Kandelia candel* and *Rhizophora stylosa* have been reported in the lowest contact 3D-Ligand binding site [22].

| Table 3. Types of HSP and DNA access numbers of *hevea* and *ricinus* |
|-----------------|-----------------|-----------------|-----------------|
| **Species**     | **Protein**     | **DNA accesions number** | **Locations**   |
| **hevea brasilensis** |                 |                 |                 |
| Class I         | hsp 17.3 kDa    | 132aa     | XP_021660156.1  | C, N            |
|                 | hsp 17.3 kDa    | 157aa     | XP_021647702.1  | C, N            |
|                 | hsp 17.3 kDa    | 162aa     | XP_02638116.1   | C, N            |
|                 | hsp 17.3 kDa    | 156aa     | XP_021682446.1  | C, N            |
|                 | hsp 17.3 kDa    | 164aa     | XP_0217566.1    | C, N            |
|                 | hsp 17.4 kDa    | 160aa     | XP_021645554.1  | C, N            |
|                 | hsp 17.5 kDa    | 157aa     | XP_021664292.1  | C, N            |
|                 | hsp 17.5 kDa    | 157aa     | XP_021647702.1  | C, N            |
|                 | hsp 17.5 kDa    | 151aa     | XP_021682603.1  | C, N            |
|                 | hsp 17.5 kDa    | 149aa     | XP_02166152.1   | C, N            |
|                 | hsp 18.1 kDa    | 153aa     | XP_021666162.1  | C, N            |
|                 | hsp 18.1 kDa    | 155aa     | XP_021650950.1  | C, N            |
|                 | hsp 18.1 kDa    | 158aa     | XP_021660151.1  | C, N            |
| Class IV        | hsp 22.0 kDa    | 192aa     | XP_021684062.1  | P, ER, M        |
|                 | hsp 22.0 kDa    | 262aa     | XP_021636451.1  | P, ER, M        |
| **ricinus communis** |                 |                 |                 |
| Class I         | hsp 16.9 kDa    | 150aa     | XP_002527121.1  | C, N            |
|                 | hsp 17.3 kDa    | 153aa     | XP_002520482.1  | C, N            |
|                 | hsp 17.3 kDa    | 157aa     | XP_002520483.1  | C, N            |
|                 | hsp 17.3 kDa    | 158aa     | XP_002520481.1  | C, N            |
|                 | hsp 17.3 kDa    | 166aa     | XP_002530396.1  | C, N            |
|                 | hsp 18.1 kDa    | 156aa     | XP_015583500.1  | C, N            |
|                 | hsp 18.1 kDa    | 160aa     | XP_002520462.1  | C, N            |
|                 | hsp 18.1 kDa    | 166aa     | XP_002530399.1  | C, N            |
| Class IV        | hsp 22.0 kDa    | 190aa     | XP_002521274.1  | P, ER, M        |
|                 | hsp 22.0 kDa    | 192aa     | XP_015575066.1  | P, ER, M        |

Noted: C (cytosol), N (nucleus), P (plastid), ER (endoplasmic reticulum), M (mitochondria)

### 4. Conclusions

Protein modelling purposed for studying distinct small heat shock protein in *Hevea brasiliensis*. That protein contributes to the biotic and abiotic stress of the plant. The predict protein modelling image for *hevea* and *ricinus* has not similar results using PHYRE2 and SWISS-MODEL software. The *Hevea* showed a higher for sequence similarity, sequence identity, coverage, and Q-mean compared to date of *Ricinus*.
Acknowledgment
This study was supported by the Penelitian Disertasi Doktor project No. 11/AMD/E1/KP.PTNB/2020, from Directorate General of Research and Community Service, the Ministry of Research and Technology/National Agency for Research and Innovation of the Republic of Indonesia.

References
[1] Kuang Jie, Jianzhong Liu, Jun Mei, Changchun Wang, Haitao Hu, Yanjun Zhang, Meihao Sun, Xi Ning, Langtao Xiao and Ling Yang. 2017. A Class II small heat shock protein OsHsp18.0 plays positive roles in both biotic and abiotic defense responses in rice. Scientific Reports: 1-14; 11333, doi:10.1038/s41598-017-11882-x
[2] Guo, M.; Liu, J.H.; Ma, X.; Luo, D.X.; Gong, Z.H.; Lu, M.H. 2018. The plant heat stress transcription factors (HSFs): Structure, regulation, and function in response to abiotic stresses. Front. Plant Sci. 7, 114.
[3] Scharf, K.D.; Berberich, T.; Ebersberger, I.; Nover, L. 2012. The plant heat stress transcription factor (Hsf) family: Structure, function and evolution. BBA Gene Regul. Mech. 1819, 104–119
[4] Xue, G.P.; Sadat, S.; Drenth, J.; McIntyre, C.L. 2014. The heat shock factor family from Triticum aestivum in response to heat and other major abiotic stresses and their role in regulation of heat shock protein genes. J. Exp. Bot, 65, 539–557
[5] Huang, X.Y.; Tao, P.; Li, B.Y.; Wang, W.H.; Yue, Z.C.; Lei, J.L.; Zhong, X.M. 2015. Genome-wide identification, classification, and analysis of heat shock transcription factor family in Chinese cabbage (Brassica rapa pekinensis). Genet. Mol. Res., 14, 2189–2204.
[6] Hu, Y.; Han, Y.T.; Wei, W.; Li, Y.J.; Zhang, K.; Gao, Y.R.; Zhao, F.L.; Feng, J.Y. 2015. Identification, isolation, and expression analysis of heat shock transcription factors in the diploid woodland strawberry Fragariavesca. Front. Plant Sci. 6, 736.
[7] Wei, Y.; Hu,W.; Xia, F.; Zeng, H.; Li, X.; Yan, Y.; He, C.; Shi, H. 2016. Heat shock transcription factors in banana: Genome-wide characterization and expression profile analysis during development and stress response. Sci. Rep., 6, 36864.
[8] Wan, X.; Yang, J.; Guo, C.; Bao, M.; Zhang, J. Genome-wide identification and classification of the Hsf and sHsp gene families in Prunus mume, and transcriptional analysis under heat stress. Peer J., 7, e7312
[9] Pramod, S. S.K. Annamalainathan, Simitha. M.Xavier, K.V.Sumesh and A.Thulaseedharan. 2017. Expression analysis of genes encoding sHsp and Rab1 in Hevea brasiliensis. International Journal of Biotechnology and Biochemistry, 13 (4): 391-402
[10] Deng, X., Jianxiao Wang, Yan Li, Shaohua Wu, Shuguang Yang, Jinquan Chao, Yueyi Chen, Shixin Zhang, Mingji Shi & Weimin Tian. 2017. Comparative transcriptome analysis reveals phytohormone signalings, heat shock module and ROS scavenger mediate the cold-tolerance of rubber tree. Scientific Report, 8, 4931, 1-16; doi: 10.1038/s41598.23094.4
[11] Deng, D.; Wang, J.X.; Wang, J.; Tian, W.M. Two HbHsfA1 and HbHsfB1 genes from the tropical woody plant rubber tree confer cold stress tolerance in Saccharomycyes cerevisiae. Braz. J. Bot., 41, 711–724. doi.org/10.1007/s40415-018-0485-5
[12] Michael Derevyanchuk, M, Raisa Litvinovskaya, Vladimir Khripach, Volodymyr Kravets. 2015. Brassinosteroid-induced de novo protein synthesis in Zea mays under salinity and bioinformatic approach for identification of heat shock proteins. Plant Growth Regul: 1-9, doi: 10.1007/s10725-015-0093-3
[13] Turan, S. 2018. Single gene versus multigene transfer approaches for crop salt tolerance. Salinity Responses and Tolerance in Plant, Vol.1: 359-379
[14] Augustine, S.M. 2016. Function of heat shock protein in drought tolerance regulation in plants. Drought Stress Tolerance in Plant, Vol.1: 163-185
[15] Tester, M., Langridge, P., 2010, “Breeding technologies to increase crop production in a changing world,” Science, 327, pp. 818-822.
[16] Bilal, S., Shahzad, R., Khan, A. L., Al-Harrasi, A., Kim, C. K., and Lee, I. J. 2019. Phytohormones enabled endophytic *Penicillium funiculosum* LHL06 protects *Glycine max* L. from synergistic toxicity of heavy metals by hormonal and stress-responsive proteins modulation. *J. Hazard. Mater.*, 379, 120824.

[17] Venkanna, D., Südfeld, C., Bäier, T., Homburg, S. V., Patel, A. V., Wobbe, L., and Kruse, O. 2017. Knock-down of the IFR1 protein perturbs the homeostasis of reactive electrophile species and boosts photosynthetic hydrogen production in *Chlamydomonas reinhardtii*. *Front. Plant Sci.*, 8, 1347.

[18] Li, Yan, Wecan Yu, Yueyi Chen, Shuguang Yang, Shaohua Wu, Jinquan Chao, Xinlong Wang, and Wei-Min Tian. 2019. Genome-wide identification and characterization of heat shock transcription factors in rubber tree. *Forest*, 10, 1157: 1-13; doi: 10.3390/f10121157

[19] Jia, Zhiqiang Yue, Mengzhu Lu, Xuebing Xin and Jianjun Hu. 2018. Genome-wide characterization of the sHsp gene family in Salix suchowensis reveals its functions under abiotic stress. *International of Molecular Science*, 19, 1-16, 3246; doi:10.3390/ijms19103246

[20] Saeed ul Haq, Abid Khan, Muhammad Ali, Abdul Mateen Khattak, Wen-Xian Gai, Huai-Xia Zhang, Ai-Min Wei and Zhen-Hui Gong. 2019. Heat shock proteins: dynamic biomolecules to counter plant biotic and abiotic stresses. *International of Molecular Science*, 20, 5321, 1-31; doi:10.3390/ijms2015321.

[21] Basyuni M., Sulistiyono N., Wati R., Oku H., Baba S. and Sagami H. 2018. Predicted cycloartenol synthase protein from *Kandelia obovata* and *Rhizophora stylosa* using online software of Phyre2 and Swiss-model. *J. Phys. Conf. Ser.* 978 012077

[22] Basyuni M., Wati R., Sulistiyono N., Hayati R., Oku H., Baba S. and Sagami H. 2018. Protein modelling of triterpene synthase genes from mangrove plants using Phyre2 and Swiss-model. *J. Phys. Conf. Ser.* 978 012095.