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Short Communication

Local Adaptation of Invasive Plant, *Synedrella nodiflora*, in Urban Tropical Lowland Landscape, Universitas Indonesia

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ABSTRACT
Synedrella nodiflora is an invasive species that originated from tropical America and now has spread throughout Indonesia. We analysed the ability of *Synedrella nodiflora* from the level of HSP70 gene expression at different heat stress in urban tropical lowland landscape Universitas Indonesia. We used the qPCR to quantify the level of HSP70 gene expression and analysed using the Pfaffl model. We found the level of HSP70 gene expression got higher related to elevated temperature from 29°C to 39°C with a range of fold from 123.1 to 1676.9. This ability reflects the adaptive plasticity of *Synedrella nodiflora* in the course of the invasion process.

Keywords: *Synedrella nodiflora*, adaptation, HSP70, qPCR, Pfaffl model

Phenotypic plasticity is defined as the capability of a certain genotype to express a phenotype under different environmental conditions. The variation of genotype support on the different development stage of plants. Therefore, it is important for the adaptation of plants (Bradshaw 2006). In the case of plant invasion, the variation of genotype should not be measured as a critical factor for invasion. The plasticity should be measured at the phenotypic and molecular level because it is likely to persist in changing environments (Gratani 2014). As an example, *Synedrella nodiflora*, which has a wide distribution in Java, showed low genetic differences (Susanto et al. 2018). The fixation of genetic variation caused by the evolution of plasticity mechanism as a result of local adaptation. These mechanisms allowing to produce different phenotypes from a single genotype (Schlichting & Smith 2002).

The remaining tropical lowland urban forest in Indonesia is limited. One of the representatives is Universitas Indonesia Campus Depok, located in two cities, Jakarta and Depok. The university landscape has six reservoirs and an urban forest representing a green and sustainable campus (Sheherazade et al. 2017; Anis et al. 2018). The campus also has well-documented data about plant communities, included family Asteraceae. Many studies have been conducted about Asteraceae, such as species diversity (Oktarina & Salamah 2017), chromosome variations (Salamah et al. 2018), and pollen morphology (Salamah et al. 2019).

Synedrella nodiflora is a member of the family Asteraceae that originated from tropical America. Found firstly in Java in 1888 and now has spread throughout Indonesia (Kostermans et al. 1986). *Synedrella nodiflora* is known as the dominant invasive weed in the urban ecosystem. The allelopathic activity from this species reduced the chance of other species in the surrounding to survive (Ghayal et al. 2010). Humans' continuous disturbance also promotes many plants weeds' response to a new environment and becomes invasive (Mooney & Cleland 2001). Plant invasion depends on the response with the flexibility to changing environment. Phenotypic plasticity can be advantageous for plants because it may increase adaptive response (Liu et al. 2016).

Climate changes influence the distribution of plants with thermotolerance because it allows for photosynthesis during periods of high temperatures (Godoy et al. 2011). Plants have evolved by regulating thermotolerance protein that responds to an elevated temperature that minimizes damage and ensures protection of cellular homeostatic (Kotak et al. 2007). Heat shock proteins (HSPs) level is different in accumulation in organisms adapted to heat and can reflect the local adaptation or acclimatization (Moseley 1997). The increased level of heat shock protein 70 (HSP70) showed better survival in invasive species (Hammann et al. 2016).

The present study was undertaken to address how the local adaptation of *Synedrella odiflora* in urban tropical lowland landscape Universitas Indonesia regarding different temperature stress by measuring the level of Hsp70 gene expression.

Young leaves from *Synedrella nodiflora* with ± 2 cm in length were collected from several locations at UI Depok Campus from September to November 2018. The samples were collected from 11:00 to 13:00 pm. The temperature was recorded to find the minimum, maximum, and average temperatures. We also planted the *Synedrella nodiflora* at room temperature as a control. The leaf samples were placed in a 50 ml centrifuge tube with 15 ml of NAP buffer (Camacho-Sanchez et al. 2013) before RNAs extraction.

Total RNA isolation was carried out using the modified cetyltrimethylammonium bromide (CTAB) method based on Zeng & Yang (2002). The concentration and purity of total RNA obtained were measured with a BioDrop spectrophotometer, and the total RNA was visualized with 1% agarose gel electrophoresis to check the RNA integrity. Total RNA was treated with RNase-Free DNase to remove DNA contaminants. The RNA obtained was then stored in -86°C or can be used directly in the next step.

Total RNA converted to cDNA using the RevertAid First Strand cDNA Synthesis Kit [Thermo Scientific™]. Furthermore, cDNA was amplified using QuantiNova SYBR® Green PCR Kit [Qiagen] and Hsp70 *Arabidopsis thaliana* primer based on Sung et al. (2001) with forward sequence 5'-TCAAGCGGATAAGAGTCACT-'3 (CG258F) and reverse sequence 5'-CTCGTCCGGGTTAATGCT-3' (CG259R) with targeted Hsp70 at cytosol. We performed qPCR in a total volume of 20ul comprising 1-5ul cDNA template, 10ul 2x QuantiNova SYBR Green PCR Master Mix, 1.4ul each primer (0.7uM), and RNase-Free water up to 20ul. This reaction mixture was subjected to a qPCR condition as follows: pre-denaturation at 95° C for 2 mins, 40 reaction cycles consisting of denaturation at 95o C for 5 secs, primer annealing at 55° C for 10 secs. We also put GAPDH as an internal reference gene for relative quantification with forward sequence 5'-TGAGAAGGCAGCCACCTATG -3' and reverse sequence 5'-TGCTGTCACCCTGGAAGTCA -3' (Yang et al. 2015). All the qPCR reactions ran at eco 48 qPCR machine [PCR Max].

Relative quantification of HSP70 gene expression was calculated with Pfaffl (2001) model using the equation:

$$\text{ratio} = \frac{(E_{\text{target}})^{\Delta CP_{\text{target}}}(\text{control} - \text{sample})}{(E_{\text{ref}})^{\Delta CP_{\text{ref}}}(\text{control} - \text{sample})}$$

The average temperature during observation from September to November 2018 at 11.00-13.00 was 34°C with a minimum of 29°C and a maximum of 39°C. We collected the young leaf of *Synedrella nodiflora* at three different heat stress that we recorded during the observation. Total we collected 134 samples of young leave and successfully extracted the RNA and got the cDNA template for qPCR analysis (Figure 1).

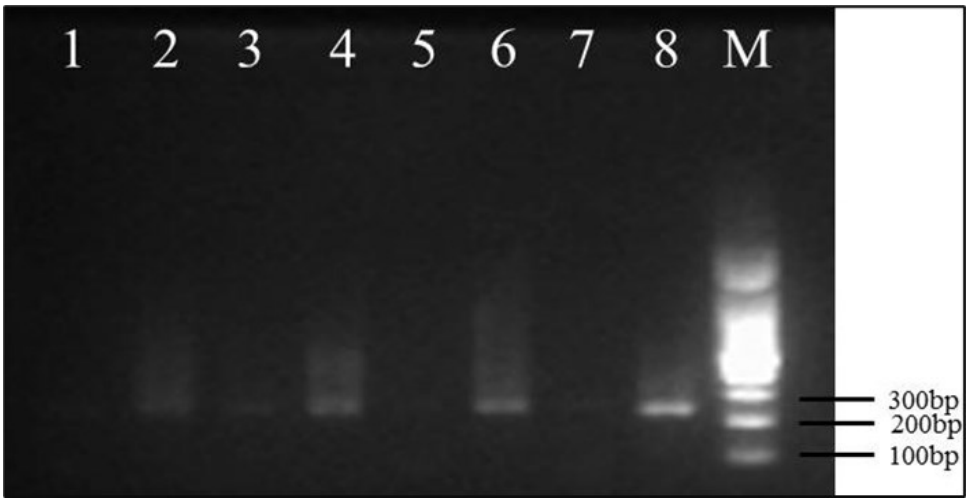

Figure 1. The electrophoresis visualization of Hsp70 amplification on cDNA template of *Synedrella nodiflora*. Line M is a 100bp marker of DNA, lines 1-8 are the representative samples.

Expression of HSP70 in *Synedrella nodiflora* has different levels related to heat stress. We found that the relative expression of HSP70 to GPADH as an internal reference gene got higher along with the temperature. At 29°C, the expression of HSP70 was 123,1 fold to the control and up to the highest at 39°C with 1676.9 fold (Figure 2).

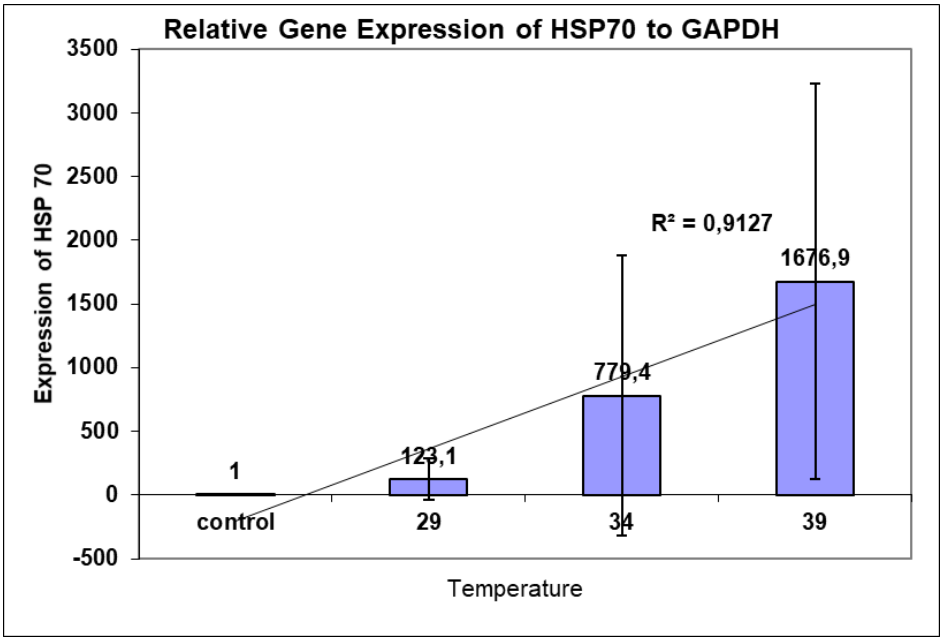

Figure 2. Expression of HSP70 in *Synedrella nodiflora* with different heat stress in urban tropical lowland landscape Universitas Indonesia.

On the basis of our result, the population of *Synedrella nodiflora* had developed local adaptation or acclimatization in urban tropical lowland landscape Universitas Indonesia. The resistance to heat shock could have evolved in the course of the invasion process—either during the transport or in the new area (Hamman et al. 2016). As we know, *Synedrella nodiflora* is a native of tropical America and now has spread throughout Indonesia (Kostermans et al. 1986). From the molecular level, heat shock proteins are the molecular chaperon that aids the refolding of macromolecules due to heat and other stressors. Induction of HSP is to be greater for invasive species relative to native (Kelley 2014).

Response to and survival of heat stress is a complex phenomenon in plants (Kotak et al. 2007). Only aware of few examples of elevated chaperon expression in non-native species or populations of terrestrial non-native organisms, based on the light heat-shock protein expression may be a more relevant determinant of invasion success in aquatic organisms than in terrestrial organisms (Hammann et al. 2016).

From our result, we provided a clear observation about the local adaptation of terrestrial plant, *Synedrella nodiflora*, in urban tropical lowland landscape with the elevated of HSP70 gene expression due to the different heat stress.

AUTHORS CONTRIBUTION

A.E.M and A.S. designed the research, analysed data, wrote the manuscript, and supervised all the process, C.K.A. and M.S. collected and running the samples.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this research.

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