Genetic Variation of *Aedes Aegypti* (Diptera : Culicidae) based on DNA Polymorphism

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Abstract- Dengue hemorrhagic fever (DHF) is a health problem in Indonesia, particularly in Jember. Dengue is a disease caused by Dengue virus (DENV). *Ae. aegypti* is the main vector for transmission of Dengue viruses into human. The spread of Dengue virus caused by the vector density. The objective of this research is to study the genetic variation of *Aedes aegypti* based on DNA polymorphism by Random Amplified Polymorphic (RAPD) PCR analysis. The mosquitoes were collected from Arjasa, Mumbulsari and Mayang in Jember district. The primer were used to RAPD analysis are OPE 16, OPE 17, OPE 19, OPF 2, OPF 4 and OPF 6. The result showed, the genetic variation of *Aedes aegypti* from Arjasa, Mumbulsari and Mayang based on DNA polymorphism were 22.58%, 23.53% and 21.21% respectively.

Keywords—genetic variation, *Aedes aegypti*, DNA polymorphism)

INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is an arthropod-borne diseases caused by a flavivirus and spread by the female *Aedes* mosquitoes. *Aedes aegypti* is the main vector for dengue virus. There are four serotypes of dengue viruses (DEN 1,2,3 and 4). Dengue viruses infection have been reported in over 100 countries and 2.5 billion people live in areas where dengue is endemic (3, 4, 13). Jember is endemic DBD, it cases was relatively high in some districts, such as at Arjasa, Mayang and Mumbulsari. Dengue vector control was conducted in two activities, that are the eradication of adult mosquitoes (fogging) and the eradication of mosquito larvae by controlling the larvae periodic. This program has not successfully to eradicate the vector and the use pesticides continuously to eradicate vector causing mosquitoes are more resistant. The resistant mosquitoes have an ability to transmit a dengue virus is higher.

One of the factors determining the spread of dengue virus are the vector density. There is a correlation between the genetic variability and the adaptation for survival. Organism has higher variability will survive and regenerate more easily than the lower variability (11). Vector insects have a higher chance to survive and would have high population. The vector which has dense population would be an effect of DHF increases.

The genetic variation can be revealed by conducting an analysis of the DNA polymorphism with RAPD (Random Amplified Polymorphic DNA). Since the discovery in 1990 of the RAPD technique, it has been extensively using for several purposes, for example, individual or strain identification, genetic variation of the population and the phylogenetic relationship (10). Random amplified polymorphic DNA (RAPD) and RFLP markers are most commonly used molecular markers to elucidate the genetic variations in *Ae. aegypti* (2). RAPD technique detects randomly amplified polymorphic DNA fragments in PCR with single arbitrary primer of 8-10 bp (12). The number of fragments amplified and the degree of polymorphism in eukaryotic species depend on the nucleotide sequence, the secondary structure and the number of primers used for each RAPD assay. These features of the RAPD assay make it possible to detect DNA polymorphism in the absence of specific nucleotide sequence information (6).

Based on the background above, the current study was designed to analyze DNA polymorphism of the *Ae. aegypti* populations to demonstrate the genetic variations. The result of this study to expect effective controlling a dengue vector and eliminate DHF cases particularly in Jember district.

MATERIALS AND METHODS

Mosquitoes larva and pupae were collected from both natural and artificial breeding places in endemic area from Jember district (Arjasa, Mumbulsari and Mayang). Mosquitoes were reared until adult in an insectary at 70-80% relative humidity, 28°C and with a 12-12h light–dark photoperiod. Larvae were fed on a finely-ground fish food. Male and female mosquitoes were kept together in cages with unlimited access to water and sugar until blood feeding.

Genomic DNA was extrated from individual female mosquitoes using CTAB method. Total genomic DNA was isolated from leg tissues. DNA was amplified in PCR reaction using single primer of arbitrary nucleotide sequence. Primer used in this analysis have the following sequences : OPE 16 : 5' GGTGACTGTG 3', OPE 17 : 5' CTACTGCCGT 3', OPE 19 : 5' ACGGCGTATG 3'. OPF 4 : 5' GGTGATCAGG 3' dan OPF 2 : 5' GAGGATCCCT 3' (10). The PCR products were analyzed by electrophoresis in 1.5% agarose. The sizes of amplified bands were compared to DNA marker (1 kb). All the DNA bands scored as present (1) or absent (0) for each sample. Ambiguous bands were not scored..

RESULT AND DISCUSSION

Ae. aegypti population were analyzed by RAPD-PCR using 10 oligonucleotides primer i.e OPE 16, OPE 17, OPE 19, OPF 2, 4 OPF, OPF 6. The number of fragment DNA from RAPD-PCR product by using primer OPE 16, OPE 17, OPE 19, OPF OPF 2 and 4, varied 4-10 bands and sizes ranging from 160-2349 bp. An example of RAPD-PCR product from sample Arjasa (sample 1 & 2), Mumbulsari (sample 3 & 4) and Mayang (sample 5 & 6) by using primer OPF-4 shown at figure 1. This primer can amplify DNA genome more numerous and varied or polymorphism.

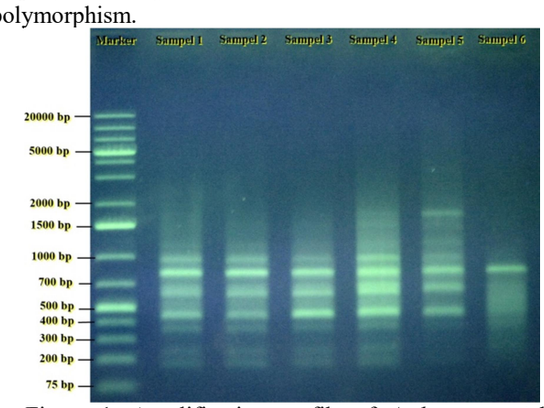

Figure 1. Amplification profile of *Aedes aegypti* by using primer OPF-4

Figure 2, show the amplification profile of *Aedes aegypti* from Arjasa (sample1 & 2), Mumbulsari (sample 3 & 4) and Mayang (sample 5 & 6) by using OPF- 6, the profile DNA of all samples are not varied or monomorphism.

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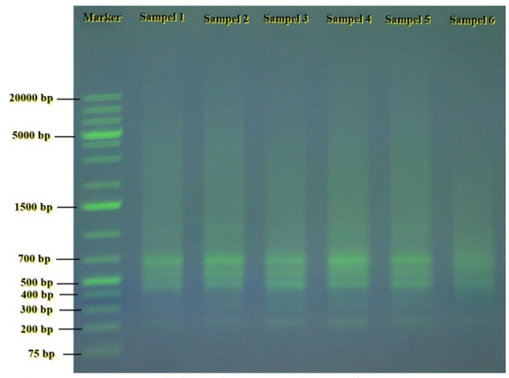

Figure 2. Amplification profile of *Aedes aegypti* by using primer OPF-6

Based on the differences and similarities of DNA band from PCR products by each primer, it can be determined the percentage of DNA band that is monomorphism or polymorphism. The percentage of polymorphism and monomorphism can be seen in the following table 1.

Table 1. Percentage DNA band monomorphism and polymorphism

Sample	Primer	Percentage monomorphism (%)	percentage polymorphism (%)
Arjasa	OPE 16 OPE 17 OPE 19 OPF 2 OPF 4 OPF 6	77.42	22.58
Mumbulsari	OPE 16 OPE 17 OPE 19 OPF 2 OPF 4 OPF 6	76.47	23.53
Mayang	OPE 16 OPE 17 OPE 19 OPF 2 OPF 4 OPF 6	78.79	21.21

Table 1 shows the percentage of DNA bands monomorphism and polymorphisms, the samples of *Aedes aegpyti* from Arjasa Mumbulsari and Mayang have polymorphism and percentage ranging 21.21%-23.53%. The level polymorphisms will indicate a level diversity. Genetic diversity of an organism would indicate tolerance range an organism for adapting to their habitats. An organism that has a high diversity will have a good survival compared to lower diverse. Vectors which have a low survival would have an impact on population numbers slightly, this would reduce dengue vectors that can infect humans. Insects have low genetic diversity are more susceptible to environmental disturbances (5). According to Passarge (8), homogeneous populations in genetic will have less adaptability to changes in the environment, while the populations with high genetic variation have a good adaptive.

This variability occurred by a migration of this vector and partly through human activity. The transportation considered as the main source of spread of mosquito-borne diseases, however, other factors like a use of insecticides and elimination of larval habitats in and around dwellings had a impact on gene flow and genetic structure of *Ae. aegypti* populations. According to Paupy genetic variation or genetic polymorphism in population is caused by diversity of genetic material (DNA) in a population or an individual. The two main sources of genetic variation are mutations and recombinations of genes as a result of sexual reproduction and gene flow (2,9). A mutation is a permanent change in the DNA within a gene. Some mutations, which affect all cells in an organism, are inherited from a parent. Other mutations develop during an organism's life and occur in only some cells. Genetic variation in populations of *Aedes aegypti* occurs in an isoenzyme and DNA polymorphism (7,14). The intensity of the use of insecticides is an important thing factor that led to the diversity or genetic polymorphisms in the population of *Ae aegypti*.

CONCLUSION

The genetic variation of *Aedes aegpyti* based on DNA polymorphism from all of the sampling collection site (Arjasa, Mumbulsari and Mayang) by using RAPD-PCR analysis, indicate the percentage polymorphism were 22.58%, 23.53% and 21.21% respectively.

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