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A mutant variant of *Nix* gene on γ-irradiated *Aedes aegypti* as a male-determination factor

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Abstract. *Ernawan B, Surniyantoro HNE, Sugoro I, Tambunan USF. 2019. A mutant variant of Nix gene on γ-irradiated* Aedes aegypti *as a male-determination factor. Biodiversitas 20: 893-899.* This study was aimed to examine the effect of γ-irradiation on the *Nix* gene sequence as a male-determination factor on *Ae. aegypti*. The novelty of the present study is the investigation on the gene mutation level of the deoxyribonucleic acid as an impact of ionizing radiation exposure. Males *Ae. aegypti* at the pupal stage were sterilized by applying 70 Gy γ-rays in varies dose-rates, i.e., 300, 600, 900, 1200 and 1500 Gy/h utilizing panoramic irradiator and males group without γ-irradiation as controls. Adult males that emerged from the pupal stage were assessed for the *Nix* gene sequence using a Real-Time Polymerase Chain Reaction method and were sequenced using Clustal Omega and Bioedit software, respectively. The results indicated that mutation occurred in *Nix* gene sequences were categorized as a point mutation. The change of *Nix* gene sequences was occurred between the nitrogenous base of guanine to adenine (G>A), thymine to adenine (T>A) and cytosine to adenine (C>A) in certain sequence numbers. There were six and nineteen on the forward-strand and reverse-strand of the *Nix* gene, respectively, due to γ-irradiation treatment. We conclude that γ-irradiation exposure causes point mutation on the *Nix* gene of males *Ae. aegypti*.

Keywords: *Ae. aegypti*, *Nix* gene, point mutation, Real-Time Polymerase Chain Reaction, γ-irradiation

Abbreviations: DNA: deoxyribonucleic acid; WHO: World Health Organization; DDT: Dichloro-diphenyl-trichloroethane; SIT: Sterile insect technique; bp: base pairs; Co-60: Cobalt 60 radioisotope; RT-PCR: Real-time Polymerase chain reaction; IR: Ionizing radiation; Gy: Gray (Unit of ionizing radiation dose); FISH: Fluorescence in situ hybridization; γ: Gamma

INTRODUCTION

Aedes aegypti is a very popular mosquito species because of its role as a vector of several viral diseases in tropical countries, including dengue fever, chikungunya, yellow fever and Zika (Vorou 2016). Historically, *Ae. aegypti* originated from Africa, then spread to various continents, especially in tropical areas, including Central America, Central Asia to Southeast Asia and Northern Australia (Jansen and Beebe 2010). Distribution of *Ae. aegypti* to various areas is influenced by various factors, including the species ability to adapt in different conditions or climates, geographical conditions, mobility and population migration (Harrington et al. 2005; Chadee and Martinez 2016).

The incidence rate of dengue fever has grown rapidly around the world in recent decades. Over 2.5 billion people (40% of the world's population) are now at risk from dengue fever. The most significant dengue epidemics region in recent years have occurred in the Americas, Western Pacific, Africa, and Southeast Asia, with approximately 390 million dengue infections occur around the world per year. Of these, 500,000 cases develop into dengue hemorrhagic fever, a more severe form of the disease, which results in up to 25,000 deaths annually worldwide (WHO 2011). The Indonesian Ministry of Health noted that the number of dengue fever in Indonesia from January to February 2016 was 8,487 patients with 108 deaths. The highest number at the ages of 5-14 years reached 43.44% and 15-44 years reached 33.25% (Kemenkes RI 2016).

So far, the control of the *Ae. aegypti* mosquito has been focused on using pesticides such as DDT, temephos, malathion, and, more recently, pyrethroids (Maestre-Serrano et al. 2014). Unfortunately, most pesticides were failed and resulted in the development of mosquito resistance to insecticides. Metabolic resistance involved a large family of multi-gene enzymes: cytochrome P450s, glutathione S-transferases, and carboxylesterase. P450 cytochrome, in particular, has been involved in providing resistance to mosquitoes (Ngoagouni et al. 2016; Moyes et al. 2017). This encourages scientists to find new methods to control the spread of *Ae. aegypti*, one of which is by utilizing nuclear techniques for sterile insect technique (SIT). Until now, information related to the effect of gamma irradiation dose rates on the quality parameters of sterile *Ae. aegypti* mosquitoes in SIT is not sufficient. The information is needed to optimize sterilization process using gamma irradiation as one of the main components of SIT.

Utilization of nuclear techniques has been widely applied in various fields. Besides being used in sterile

insect techniques (Dyck et al. 2005; Ernawan et al. 2017; Ernawan et al. 2018), nuclear techniques can also be used in attenuating rodent malaria parasites of *Plasmodium berghei* in blood of mice (Syaifudin et al. 2013) and inhibition of *Plasmodium falciparum* multiplication supplemented with tritium-labeled hypoxanthine (Surniyantoro et al. 2016). *Ae. aegypti* has 1,376 Mbp of a genome and three chromosomes (Nene et al. 2007). The *Nix* gene is one of the target genes that control the masculinity of *Ae. aegypti*. This gene is located at the M locus on chromosome 1 (denoted 1.q.2.1), meaning it is located on chromosome 1, arm q, region 2 and band 1 as shown in Figure 1 (Hall et al. 2015; Fontaine et al. 2017). Gene mutation in the *Nix* gene can cause damage to the structure and function of the *Nix* protein. This study was aimed to examine the effect of gamma irradiation on the *Nix* gene sequence as a male-determination factor on *Ae. aegypti*.

MATERIALS AND METHODS

Procedures

Aedes aegypti *strain rearing*

Aedes aegypti used in this study was collected from South Tangerang, Banten Province, Indonesia in 2014 and then maintained in the Entomology Laboratory, Center for Isotopes and Radiation Application, National Nuclear Energy Agency of Indonesia, Jakarta. The mosquito colonies used in the study were the fourteenth generation from the field collection. The mosquito strain was maintained in a laboratory with controlled climate parameters, including temperatures of 26±1°C, relative humidity 70±10% and photoperiod (12: 12 hours). Adult mosquitoes were maintained in the Bugdorm-1® mosquito cage (30x30x30 cm) and supplied with 10% (w/v) sugar solution, while female mosquitoes are supplied blood feed every week for egg maturation.

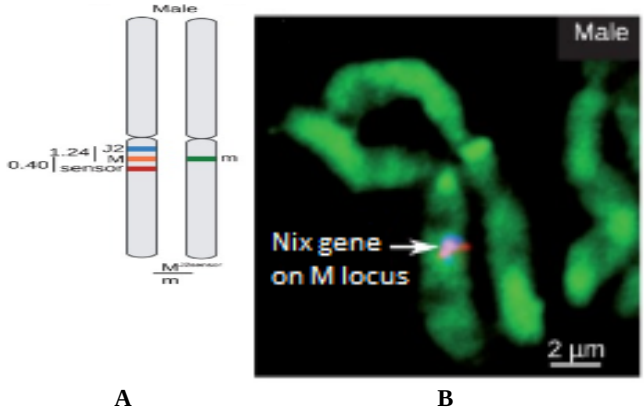

Figure 1. The position of Nix gene on M locus of *Aedes aegypti*'s chromosome 1; A. A representation of the relative position of the transgenes with respect to the M-locus and the m-locus; B. A karyotype of mitotic chromosomes from the khw strain of *Aedes aegypti* in males using Fluorescence in situ hybridization (FISH) technique (Hall et al. 2014)

Gamma irradiation procedure

Male pupa aged less than 24 hours were inserted into transparent plastic tubes and then were irradiated with a 70 Gy gamma irradiation in varies dose-rate, which are 300, 600, 900, 1200 and 1500 Gy/h (interval 300 Gy/h). The gamma irradiation process was carried out using a panoramic irradiator (Babha Atomic Research Center, India) at the Center for Isotopes and Radiation Application, National Nuclear Energy Agency of Indonesia, Jakarta. Radioisotope used as sources of gamma irradiation was Co-60. Control pupa was treated the same as experimental treatments, but without gamma irradiation. After the irradiation process, a plastic vial tube containing pupa is filled with water and placed into Bugdorm-1® until the pupa develops into an adult mosquito for further analysis.

Genomic deoxyribonucleic acid (DNA) isolation

The genomic DNA was isolated from 20 mg of males *Ae*. *aegypti* in each treatment using the GeneJET Genomic DNA Purification kit (ThermoFisher Scientific, Cat. No. 0721) and according to the manufacturer's instructions. The obtained DNA was stored at -20°C.

Amplification of Nix gene and sequence analysis

Amplification of *Nix* gene was performed using Real-Time Polymerase Chain Reaction (RT-PCR) technique, as described previously by Hall et al. (2015) with the forward primer was 5'- GATTTTTGTTTTTGTCGTGCAA -3' and the reverse primer was 5'- AATGCAAGTATCATAGGTCAGCA -3'. The PCR reactions were carried out with an enzyme activation of 95°C for 3 minutes, followed by denaturation of 95°C for 1-3 seconds and annealing of 60°C for 20 seconds. The amplified products were sequenced in the Integrated DNA Technologies Pte. Ltd., Singapore. The sequencing results were analyzed for *Nix* gene mutation using Clustal Omega and Bioedit software.

RESULTS AND DISCUSSION

In this study, the effect of gamma irradiation on the *Nix* gene sequence as a male-determination factor on *Ae. aegypti* was investigated. Gamma irradiation exposure in *Ae. aegypti* males pupa were carried out using a panoramic irradiator with Co-60 as a source of gamma irradiation. There was a 70 Gy exposure at various dose-rate treatments, 0 (control), 300, 600, 900, 1200 and 1500 Gy/h. The range and time of exposure-setting in each treatment are shown in Table 1, while the result of *Nix* gene amplification is shown in Figure 2.

Figure 3 demonstrates the sequencing results of *Nix* gene in each of treatment and control. They are divided into the forward sequence (3A) and reverse sequence (3B). The results indicated that mutation occurred in *Nix* gene sequences were categorized as a point mutation. The change of *Nix* gene sequences was occurred between the nitrogenous base of guanine to adenine (G>A), thymine to adenine (T>A) and cytosine to adenine (C>A) in certain sequence numbers. There were six points mutation on the

forward-strand (base number 7, 8, 12, 16, 311 and 312) and nineteen on the reverse-strand (base number 5-7, 9, 10, 13-16, 18, 19, 21-24, and 26-29) due to gamma irradiation treatment.

Table 1. The range and time of exposure-setting in each treatment

Radioisotope	Activity (Ci)	Dosage (Gy)	Dose-rate (Gy/h)	Time of exposure	Range between source and sample (cm)
Co-60	12,266.18	70	0 (controls)	-	-
			300	14 minutes	30.92
			600	7 minutes	21.86
			900	4 minutes 40 seconds	17.85
			1200	3 minutes 30 seconds	15.46
			1500	2 minutes 48 seconds	13.83

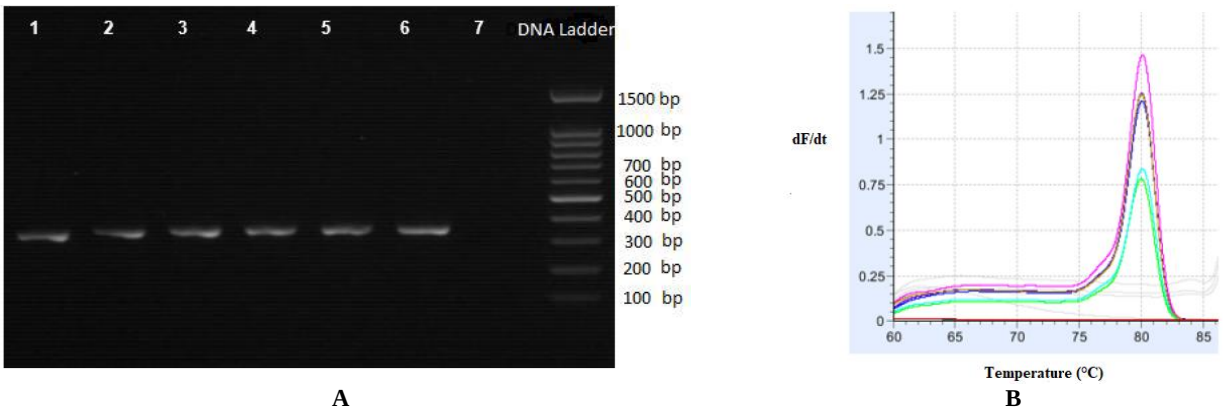

Figure 2. The result of *Nix* gene amplification on 0.8% electrophorese gel (A) and Real Time-PCR curve (B); number 1-6 were bands determining the *Nix* gene at 320 bp; number 7 was an un-template control and DNA ladder 100 bp; all of the gamma irradiation treatments were amplified at 80ºC. Controls are shown in a dark green graph. The gamma irradiation dose-rates in each treatment of 300, 600, 900, 1200 and 1500 Gy/h are shown in the light green, dark purple, light purple, blue and orange graph, respectively.

```
                    10         20         30         40         50         60         70         80         90         100
0 Gy/h      CCCACCGGAA ATAAAAGCTG AGATCATGCA GAATTTGATA TCATCAAAAA  ATATTGTATA TATATAGGAA ATATTCCATT CTTTGCATCA AAGAATGATG
300 Gy/h    -----AAAAA AAAAATGCTG AGATCATGCA GAATTTGATA TCATCAAAAA  ATATTGTATA TATATAGGAA ATATTCCATT CTTTGCATCA AAGAATGATG
600 Gy/h    -----AAAAA AAAAATGCTG AGATCATGCA GAATTTGATA TCATCAAAAA  ATATTGTATA TATATAGGAA ATATTCCATT CTTTGCATCA AAGAATGATG
900 Gy/h    ----AAAAAA AAAAATGCTG AGATCATGCA GAATTTGATA TCATCAAAAA  ATATTGTATA TATATAGGAA ATATTCCATT CTTTGCATCA AAGAATGATG
1200 Gy/h   ------AAAA AAAAATGCTG AGATCATGCA GAATTTGATA TCATCAAAAA  ATATTGTATA TATATAGGAA ATATTCCATT CTTTGCATCA AAGAATGATG
1500 Gy/h   ------AAAA AAAAATGCTG AGATCATGCA GAATTTGATA TCATCAAAAA  ATATTGTATA TATATAGGAA ATATTCCATT CTTTGCATCA AAGAATGATG
Clustal Co          ** * *** **** ********** ********** **********  ********** ********** ********** ********** **********

                    310        320
0 Gy/h      CTGACCTATG ATACTTGCAT TAA
300 Gy/h    CTGACCTATG TAACTTGCAT TAA
600 Gy/h    CTGACCTATG ATACTTGCAT TA-
900 Gy/h    CTGACCTATG ATACTTGCAT TAA
1200 Gy/h   CTGACCTATG ATACTTGCAT TAA
1500 Gy/h   CTGACCTATG ATACTTGCAT TA-
Clustal Co  ********** ********* **

                    10         20         30         40         50         60         70         80         90         100
0 Gy/h      -GGGATTTTG AAAGGAACTT AGTGGTAGAA CTATTAATAT CGTGTTGCCG  AATTTTGAAT TGTTTAAACA CAAAGACTTG TCTACACTTT TTCTCGAACG
300 Gy/h    GGGGAAATTG AAGGCAACTT AGTGGTAGAA CTATTAATAT CGTGTTGCCG  AATTTTGAAT TGTTTAAACA CAAAGACTTG TCTACACTTT TTCTCGAACG
600 Gy/h    --GGGAATTG AAAGCAACTT AGTGGTAGAA CTATTAATAT CGTGTTGCCG  AATTTTGAAT TGTTTAAACA CAAAGACTTG TCTACACTTT TTCTCGAACG
900 Gy/h    -GGGGATTTG AAG-GCAACT TAGTGGTAGA CTATTAATAT CGTGTTGCCG  AATTTTGAAT TGTTTAAACA CAAAGACTTG TCTACACTTT TTCTCGAACG
1200 Gy/h   --GGGAATGA AAG-GCAACT TAGTGGTAGA CTATTAATAT CGTGTTGCCG  AATTTTGAAT TGTTTAAACA CAAAGACTTG TCTACACTTT TTCTCGAACG
1500 Gy/h   --GGGAATGA AAA-GCAACT TAGTGGTAGA CTATTAATAT CGTGTTGCCG  AATTTTGAAT TGTTTAAACA CAAAGACTTG TCTACACTTT TTCTCGAACG
Clustal Co    **   *   **    *  *     *    * ********** **********  ********** ********** ********** ********** **********
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Figure 3. Sequences of Nix gene on forward (*above*) and reverse (*below*) strand of DNA using Clustal Omega and Bioedit software. Mutations are marked with red letters

Confirmation of the DNA amplification results by real-time PCR was carried out using 0.8% agarose gel electrophoresis. The results of the visualization of agarose gel are shown in Figure 2A. Number 1-6 is a sample of males *Ae. aegypti*'s *Nix* gene at a dose-rate of 0 Gy/h (control) up to 1500 Gy/h, respectively. Number 7 is an un-template control (without DNA template), while the last is a DNA ladder with an interval of 100 bp. Agarose gel electrophoresis results showed that the *Nix* gene DNA fragments in all gamma irradiation treatments were approximately 320 bp. It also indicated that a total length of the *Nix* gene in *Ae. aegypti* is 320 bp. Figure 2B demonstrates that the *Nix* gene in all of the gamma irradiation treatments amplified at 80ºC. Controls are shown in a dark green graph. The treatments of gamma irradiation dose-rates of 300, 600, 900, 1200 and 1500 Gy/h, respectively, are shown in the light green, dark purple, light purple, blue and orange.

The amplification results were sequenced to determine the sequence of nucleotide bases in the *Ae. aegypti*'s Nix gene. The DNA sequence in each gamma irradiation treatment is aligned to analyze the mutations that occur. The Nix gene DNA sequences are aligned using the Clustal Omega software from the European Molecular Biology Laboratory - The European Bioinformatics Institute (EMBL-EBI) and BioEdit software. Clustal Omega software has some advantages, including high accuracy, fast and can be used for large scale up to 190,000 sequences using one processor (Sievers et al. 2011).

Discussion

The strength and novelty of the present study is the investigation on the *Nix* gene mutation level of the deoxyribonucleic acid as an impact of ionizing radiation exposure. To our best knowledge, this research has not been done in Indonesia. This study can be used to develop the sterile insect technique (SIT) and to control the spread of *Ae. aegypti* by utilizing nuclear techniques. Until now, the development of SIT applications for controlling insect populations, especially mosquitoes, has undergone many developments. The method of mass mosquito breeding in the laboratory, the method of releasing mosquitoes to the target area and monitoring methods have been validated and standardized (Balestrino et al. 2014; Hapairai et al. 2014; Bourtzis et al. 2016; Zheng et al. 2015). However, research to evaluate the quality parameters of sterile mosquitoes and applications to target areas is still being carried out by researchers for the success of the program mosquito population control as a vector of disease. It can also be used as a pilot study to determine various kinds of genetic mutation information on *Ae. aegypti*'s genes. This study is the first experiment using Co-60 as a source of gamma irradiation exposure to make a genetic mutation in the *Nix* gene of *Ae. aegypti*'s DNA fragment. The selection gamma irradiation dose refers to the previous studies by Sasmita et al. (2014) that stated the optimal dose of gamma irradiation in *Ae. aegypti* was 70 Gy. This approach offers an opportunity to complete a preventing program of *Ae. aegypti* spread in the population and reducing the incidence of dengue fever in human.

As the disease vector, *Ae. aegypti*, viruses are also reported to spread in tropical countries. Dengue, a common viral disease transmitted by *Ae. aegypti* has infected 96 million people globally in 2010 (Bhatt et al. 2013). Asia contributes the highest number of infected (70%), followed by Africa (16%), America (14%) and Oceania (<0.2%). Chikungunya, first isolated in Tanzania, Africa in 1953. The virus then spread to Central and Southeast Asia (Weaver and Reisen 2010). In 2007, clinical cases of chikungunya were reported to be detected in southern Europe, namely in Italy (Rezza et al. 2007). It was also reported to be detected in the Caribbean Islands, South America in 2013-2014 (Van Bortel et al. 2014). Yellow fever, according to history as evidenced by phylogenetic studies originating in Africa and spread from the 16th century during the slave trade. Until the 20th century, yellow fever epidemics were reported in North and South America (Gubler 2004). The Zika virus was first isolated in 1947 in the Zika forest, Uganda, Africa (Li et al. 2012). Furthermore, the Zika virus spread to Asia, America, the Mediterranean, and Micronesia. In recent years, clinical cases of Zika virus have been reported in South America and are of concern to the world (Vorou 2016; Wiwanitkit 2016).

The results of our study showed that mutation in the *Nix* gene had occurred due to gamma irradiation treatment at some points. There were six point mutations on the forward-strand, base number 7 (G>A), 8 (G>A), 12 (T>A), 16 (A>T), 311 (A>T) and 312 (T>A) and nineteen on the reverse-strand, base number 5 (A>G), 6 (T>A), 7 (T>A), 9 (T>G), 10 (G>A), 13 (A>G), 14 (deletion), 15 (G>C), 16 (A>C), 18 (C>A), 19 (T>C), 21 (A>T), 22 (G>A), 23 (T>G), 24 (G>T), and 26 (T>G), 27 (A>T), 28 (G>A), 29 (A>G). Generally, the mechanisms of DNA mutation are substitution, deletion, and insertion (Sagermann et al. 2006). DNA mutation due to induction of gamma irradiation is random, cannot be targeted at DNA with a particular sequence number. The change in the DNA sequence also affects both secondary and tertiary structures. Changes in the structure of the DNA fragments can affect the genetic information encoded. The negative impact is chromosome aberration and instability of the genome (Koturbash et al. 2006). The point mutation that occurs in the *Nix* gene will be translated into different *Nix* protein than normal. There are three possibilities. Firstly, a silent mutation, which is a change in a base pair in the gene (at position 3 of the codon) which causes a change in one genetic code but does not result in changes of the encoded amino acid. Secondly, a missense mutation, which is a change in genetic code (generally at positions 1 and 2 at the codon). There is a change in the amino acids that are different from the natural amino acids making up the *Nix* protein. The last, nonsense mutation, which is a change in certain amino acid codons to be a stop codon. Almost all meaningless mutations lead to the inactivity of a protein resulting in a mutant phenotype. Most of the nonsense mutation will produce a protein that has an amino acid chain shorter than normal, so the protein produced will not working properly (Arodz et al. 2012). It should be done in further research to find out the changes in amino acids that occur due to a point mutation in the *Nix* gene.

The genetic factor plays an important role in any system of living organisms. Almost all structures and functions are controlled by genes, including diseases. In human, changes in genes both mutations and polymorphisms have an impact on their ability to survive. Most of the research showed the link between genetic mutations to risk factors of disease. For examples, the results of a epigenetic study demonstrated that Ala55Val and 45 bp I/D UCP2 polymorphisms play a role in increasing the risk of obesity in human (Surniyantoro et al. 2018), the other previous studies by Surniyantoro et al. (2018) stated that heterozygous allele 194Trp of XRCC1 exon 6 polymorphism showed a significantly higher micronuclei frequency in human exposure to ionizing radiation than controls. Micronuclei act as a biomarker of DNA damage. It is also demonstrated that ionizing radiation exposure, such as gamma radiation, can influence and damage the DNA fragments. Whereas, in *Ae. aegypti*, an F1552/C1552 point mutation was linked with permethrin resistance in the *Ae. aegypti* voltage-gated sodium channel. There was a single-base substitution (T>G) at the second position of codon 1552 resulting in the replacement of Phenylalanine by Cysteine in segment 6 domain III (Yanola et al. 2010). Saavedra-Rodriguez et al. (2007) also stated that a transition in codon 1011 encodes a Val replacement while a transition in codon 1016 encodes an Iso replacement. These mutations cause pyrethroid resistance in Latin American *Ae. aegypti*. All of the supporting studies above showed the importance of genetic factors in living organisms. Ionizing radiation (IR) exposure is a mutagenic agent, in addition to chemicals, which are widely studied, that produce various types of DNA damage, from single-strand breaks, modified nucleotides to double-strand breaks (Adewoye et al. 2015). Each of DNA damage has its own repair system, depending on the type of damage (Cooper 2000). The level of DNA damage is affected by the dose and dose-rate. A 70 Gy of the dose was the optimal dose for attenuating *Ae. aegypti* in the sterile insect technique (SIT) (Nurhayati et al. 2009). In human DNA, a previous study stated an equivalent dose has a significant correlation with micronuclei frequency among radiation-exposed workers, increasing the micronuclei frequency by 16.3 per 1 mGy of equivalent dose (Surniyantoro et al. 2018). This proves that the DNA damage tended to rise with equivalent dose, but various results in the different level of dose-rates.

Gamma irradiation at the pupa stage can also reduce sperm quantity. While in the mating competitiveness parameters, gamma irradiation at high doses can reduce the competitiveness of the mosquito species mating. Bellini et al. (2013) reported a semi-field test of the competitiveness of *Ae. albopictus* mosquito mating after gamma 30-60 Gray (Gy) dose irradiation. The results showed that competitiveness decreased with increasing gamma irradiation doses. Oliva et al. (2013) reported the effects of gamma irradiation on the age of *Ae. albopictus* mosquitoes in a semi-field trial on Reunion Island, one of the French regions in the Indian Ocean. The results showed that the age of mosquitoes in gamma irradiation was lower compared to field mosquitoes.

Some studies also report on the effects of gamma irradiation on *Ae. aegypti* mosquito species. Nurhayati et al. (2009) reported the effect of gamma irradiation on sterility. The results of the study stated that gamma irradiation had an effect on these two parameters. The optimum dose of gamma irradiation in *Ae. aegypti* mosquitoes ranges from 65-70 Gy. Sasmita et al. (2015) reported the effect of gamma irradiation on several quality parameters of *Ae. aegypti* mosquitoes, including the percentage of the appearance of adult mosquitoes, age of mosquitoes, sterility and mating competitiveness. The results of the study stated that gamma irradiation had no effect on the percentage of the appearance of adult mosquitoes. Gamma irradiation doses affect the age of mosquitoes, sterility and mating competitiveness. The results also provide information on the optimum dose of gamma irradiation in *Ae. aegypti* mosquitoes ranges from 60-80 Gy. Shetty et al. (2016) reported the effect of gamma irradiation on doses of 0-50 Gy on fertility, egg hatching, appearance and age of *Ae. aegypti* mosquitoes. The result of the study stated that gamma irradiation at low doses (< 2 Gy) had no effect on the parameters of the study.

In the context of SIT, gamma irradiation is used to induce sterility in the reproductive system of insects. gamma irradiation can damage chromosomes in gonadal cells, so chromosome fragmentation can occur and cause lethal dominant mutations, translocations and other chromosome aberrations (Robinson 2002). Chromosome damage results in unbalanced production of the gamete and subsequent zygote or embryo deaths occur (Klassen 2005; Robinson 2005). The impact of gamma irradiation on somatic cells is expressed in the development of abnormal organs, decreased ability to obtain nutrients, life skills, ability to fly, and ability to mate in these insects (Bakri et al. 2005).

In conclusion, our study reported that there are six and nineteen point mutations on the forward-strand and reverse-strand, respectively, in the *Nix* gene of males *Ae. aegypti* caused by gamma irradiation exposure. This result can be used as a pilot study to determine various kinds of genetic mutation information on *Ae. aegypti*'s genes. In the subsequent studies, it is necessary to examine the changes in amino acids that occur due to a point mutation in the *Nix* gene and the gene expression which can be used to develop the dengue vector *Ae. aegypti* decreasing program.

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