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GAMMA IRRADIATION EFFECTS ON OIL PALM (ELAIES GUINENSIS JACQ.) POLLEN VIABILITY, FRUITS AND BUNCH FORMATIONS

***Aida Nazlyn Nazari*[1], *Azhar Mohamad*[2] *and Shuhaimi Shamsudin*[2]**

[1]Applied Agricultural Resources Sdn. Bhd., Kota Damansara,
Selangor, Malaysia
[2]Agrotechnology and Biosciences Division, Malaysian Nuclear Agency, Bangi,
Selangor, Malaysia
Correspondence author: azhar_m@nm.gov.my

ABSTRACT

Assessing performance and genetic diversity of the wild material of oil palm is important for under- standing genetic structure of natural oil palm populations towards improvement of the crops. This in-formation is important for oil palm breeding programs, and also for continued ex-situ conservation of the germplasm and breeding program in Malaysia. Mutation induction is one of the approaches in creating variants for selection in the breeding program. In this study, the effect of irradiated pollen towards pollen viability, bunches formation and number of parthenocarpic fruits were evaluated. Elaies guineensis Jacq. *pollens were exposed to series of acute gamma radiation at dose 0, 10, 20, 40, 50, 100, 200, 300, 500, 100 and 2000 Gy . Pollen viability and pollen tube formation were disrupted in which unable the pollen to reach the ovule. At this stage, embryo was aborted towards formation of parthenocarpic fruits and rotten bunches. The study suggested that at low levels of irradiation i.e. < 200 Gy, generative nucleus partially damage and it is still maintaining capacity of fertilizing the egg cells for hybridization. It is important for breeders in understanding this finding towards novel variants of oil palm via mutation induction.*

Keywords: Acute gamma radiation, doses, *Elaies guineensis*, parthenocarpic, pollen viability

INTRODUCTION

African oil palm (*Elaies guineensis* Jacq.) is the most productive oil crop in the world as it produces eight to ten times more oil per hectare than rapeseed and soybean (Nelson et al., 2009). Significantly, the demand for oil palm increased as it meets the future vegetable oil needs (for both foods and industry) against the background of a rising world population (Arias et al., 2012). Consequently, intensive breeding have become a sudden surge within the oil palm industry to further develop better elite planting materials.

The oil palm commercial planting material is the "Tenera" which is the thin-shell fruit type and more productive in oil. Tenera is a hybrid between the thick-shell "Dura" with shell-less "Pisifera". Reciprocal recurrent selection (RRS) breeding scheme was used to make crossings and progeny testing where selection of the breeding populations and the parents for creating seeds for commercial hybrid production take place. Selection was done based on their performance within their family, block-trials and even against the performance of their relatives (Soh et al., 2003).

Due to the perennial nature of the crop and the long generation cycles needed which approximately 19 years per cycle, the progress of oil palm is breeding have been quite slow (Wong and Bernardo, 2008). Besides, the narrow genetic base of the African oil palm origin in Malaysia also contributed as one of the factors that slow down the rate of oil palm breeding progress. History showed that the Deli Dura populations in Malaysia derived from only four palms introduced in the Bogor Botanical Garden back in the 1948 (Ajambang et al., 2012; Arias et al., 2012; Rohani et al., 2012). In 1973, Malaysia Palm Oil Board (MPOB) had initiated worldwide germplasm collection program with the aim to broaden the genetic base of the oil palm planting materials. Besides, other efforts to broad- en the genetic basis of oil palm include genetic engineering technique and mutation technique (Arias et al., 2012; Rohani et al., 2012)

Mutation through the gamma irradiation have long been known as one of the applicable method to increase genetic variability within a crop and hence expedite the development of better crop cultivars worldwide (Khan et al., 2005; Rohani et al., 2012; Sutarto et al., 2009). Examples of crops developed through mutation technique are soybean, citrus, chicpea, rice and ornamental plants (Mudibu et al., 2012; Rohani et al., 2012).

Among all available mutagens, gamma rays are widely used due to the fact that they have shorter wavelength and delivered more energy per photon as compared to x-ray. Besides, gamma rays have the capability to penetrate deep into the plant tissues and were noted as one of the mutagens that gave rise to viable mutants (El-Degwy, 2013). Based on literature review, a few studies that involved mutation technique were conducted on oil palm *in vitro* cultures with the aim to produce mutants with slow height increments traits (Che Radziah et al., 2009; Rohani et al., 2012). However, to date no success results were reported

In this study, oil palm pollen was irradiated with various doses of gamma rays instead of the oil palm in-vitro cultures. The pollen is more sensitive to radiation as compare to in vitro plantlets. Besides, low growth performance of in vitro plantlets making the pollen more reliable in mutation induction study.

Our aim is to investigate the effect of pollen irradiation towards the pollen viability, bunches formation and no. of parthenocarpic fruits. The findings from this study could be used as the basis for designing future mutation breeding experiments for oil palm.

MATERIALS AND METHODS

Plant Material

Field experiment was conducted in 2013 at Applied Agricultural Resources Sdn. Bhd. field station. Twenty seven Deli Dura palms were chosen as the female parent and only a single Pisifera palm was chosen as the male parent.

Pollen Irradiation

Pollen from the Pisifera was subjected to gamma irradiation using a gamma cell BioBeam 8000 which used Cesium-137 as the radiation source with a dose rate of 17.2 Gy/min. Eight different doses were used which were 10, 50, 100, 200, 300, 500, 1000 and 2000 Gy.

Pollen Viability Test

The viability of the pollen was tested for 12 consecutive days upon exposure to the radiation. Sucrose solution which was made of 50 ml distilled water 5 g of sucrose and a drop of boric acid was use as the pollen setting solution. Wet filter paper was placed on a petri dish and a glass slide was placed on top it. Two drops of sucrose solution were place on top of the glass petri dish. A small portion of the irradiated pollen was placed in the sucrose solution. It was mixed well and dried for 3 hours before it could be viewed under the light microscope. After 3 hours, a cover slip was placed on top of the slide and the remaining excessive sucrose solution was dried out by using a tissue paper. The slide was then ready to be viewed under the light microscope. The pollen viability was based on the percentage of counts of 300 pollen grains (3 replicates of 100 pollen grains each) and can then be determined by the formula as stated bellows:

$$\text{Pollen viability} = \frac{\text{Total number of viable pollen}}{\text{Total number of pollen}} \times 100$$

Pollination and Bunch Harvesting

The female flowers from all the Dura palms were bag by using tyrelene bag one week before anthe- sis. Control pollination was carried out during the anthesis period where the irradiated pollen was puffed into the female flowers between 7 am and 11 am. After 6 months, the ripened bunches were harvested. These bunches were sent to the laboratory for morphology analysis.

Ripe Bunch Morphology Analysis

The fruit sets of each of bunches were recorded before the fruitlets were stripped down from each of bunches. The fruitlets were then counted and differentiated between the fertile and parthenocarpic fruitlets.

RESULTS AND DISCUSSION

Plant Materials

In order to determine the optimal radiation dosage for oil palm pollen, a single pollen source with twenty seven sib-matting female palms was used to minimise the contribution of the genotype factors towards the different radiation doses. Dolcet-Sanjuan et al. (2005) reported that extensive number of genotypes affected the rate of parthenogenic embryos produced by the cucumber where the female plants were determined as the main contributor. Other studies on four different watermelon cultivars also indicated that there was a significant difference in the number of seeds produced when irradiated pollen was used during the pollination process (Sari and Abak, 1994).

Effect of Irradiation on the Pollen Structure

The pollen structures were captured at 400 magnification during the pollen viability test which were carried out for 12 consecutive days upon exposed to radiation. Based on all the captured images (Figure 1), there was no difference observed between the different radiation doses ranged between 10 Gy and 2000 Gy. The viable pollen was identified based on a small sperm shape with a tiny white colour tail while the dead pollen identified as a small sperm shape without any tail and totally dark in color (Figure 2).

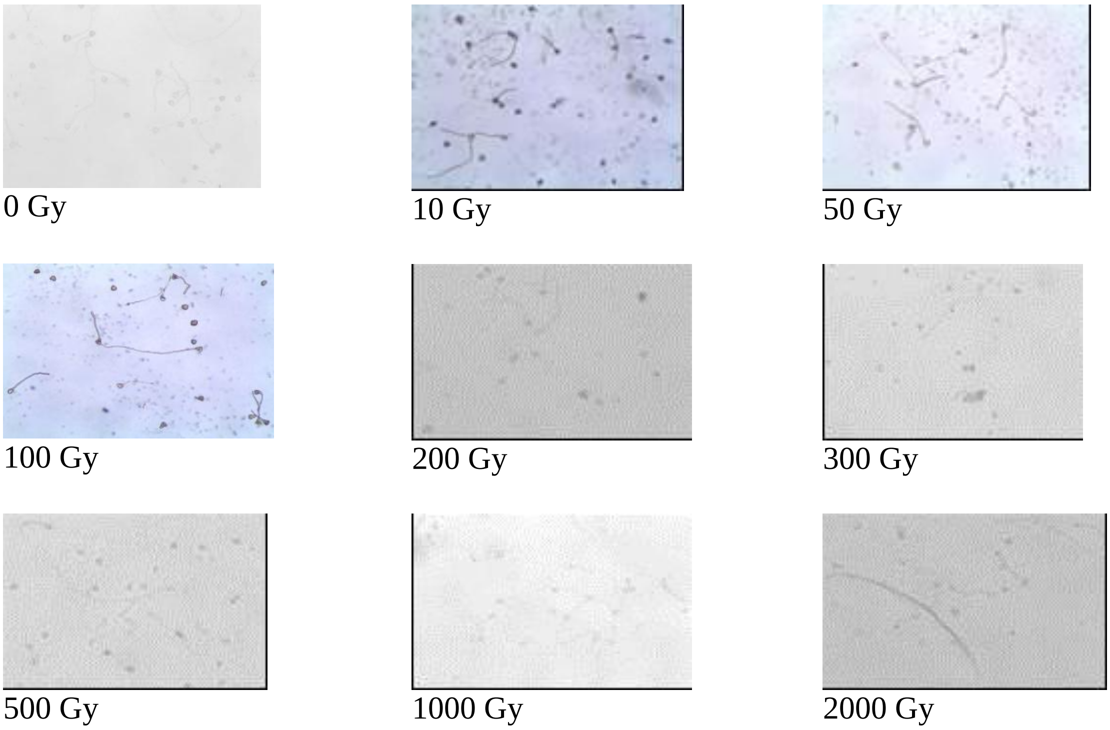

Figure1: Pollen structure images captured at 400 magnifications for radiation dose range between 10 Gy and 2000 Gy

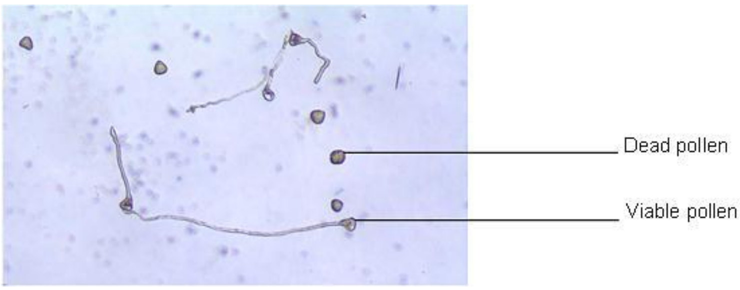

Figure 2: Differences of viable pollen structures and dead pollen structures at 400 magnifications

Effect of Irradiation on *In Vitro* Pollen Germination

Wide ranges of irradiation doses were tested in this study which was between 10 Gy to 2000 Gy. Pollen viability results were used as one of the means to determine the optimal dose for pollens of oil palm. Pollen viability is crucial in determining the successive rate of pollination through the information on the ability of the pollen grain to form pollen tube when they reach the stigma (Musial and Przywara, 1998). In Applied Agricultural Resources Sdn. Bhd, pollen viability bellows 60% will not be used for either plant breeding program or commercial oil palm production. This is because, poor fruits are expected if the pollen viability is bellow than 60%. In general more than 90% is required and considered as good pollen viability.

In this study, pollens that were exposed to radiation above 200 Gy had caused the pollen to become inviable with the pollen viability percentage ranged between 35.36% and 46.74% (Table 1, Figure 3). In contrast, the pollens that were exposed to doses bellow 200 Gy were still viable with pollen viability ranged between 69.08% and 78.44% (Table 1, Figure 3). These results suggested that the pollen tubes were disrupted if they were exposed to the irradiation doses above 200 Gy. Similar results were also reported by Peixe et al. (2000) where irradiated pollen significantly affected the pollen viability mainly for doses higher than 200 Gy in plum.

Table 1: Pollen viability and ripe bunch morphology recorded for different radiation doses

Radiation Dose (Gy)	Pollen Viability Before Radiation (%)	Pollen Viability After Radiation	Pollen Viability Percentage of Different	Total Fertile Fruitlets (%)	Total Parthenocarpic Fruitless (%)
0	67.26	69.08	-1.82	74.84	25.16
10*	69.70	78.44	-8.74	100.00	0
50	70.10	70.65	-0.55	100.00	0
100	72.97	73.93	-0.96	100.00	0
200*	64.55	45.77	18.78	20.56	79.44
300*	66.88	46.74	20.14	51.02	48.98
500*	65.78	45.38	20.41	0	0
1000*	67.98	37.97	30.01	51.02	48.98
2000*	74.38	35.36	39.02	17.44	82.56

* indicates significant different between the pollen viability test before and after radiation based on t-test

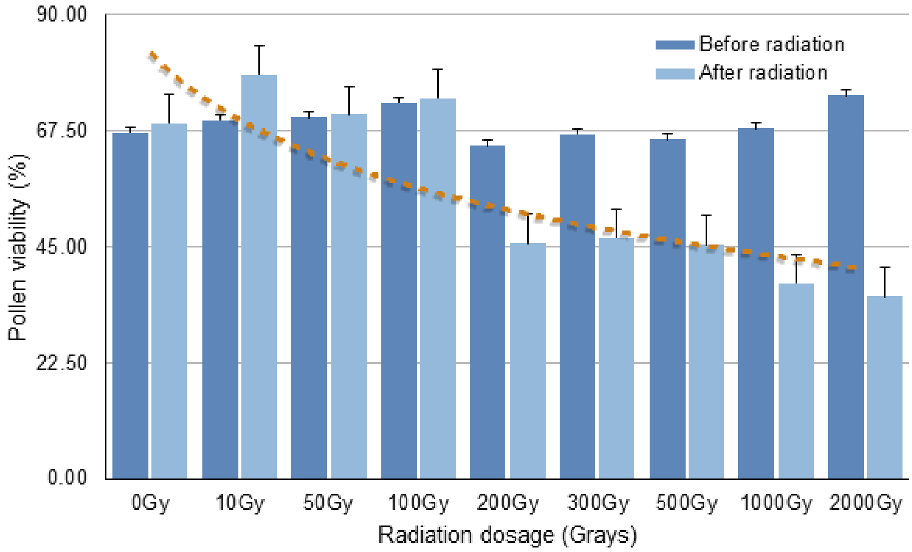

Figure 3: Pollen viability test for different doses of radiation

Effect of Irradiation on Fruit and Bunch Formation

Since irradiating pollens causes a decrease in the pollen viability, the bunches fruit sets percentage and the number of fertile fruits produced per bunch were also affected as well (Table 2). Irradiated pollen induced the formation of parthenocarpic fruits in comparison to the control. The number of parthenocarpic fruits was recorded the highest for pollen irradiated at 2000 Gy.

Naturally parthenocarpic fruits does occur in oil palm, but at a very low percentage where it indicates pollination problem that arise due to the low pollinator population, high rainfall, high sex-ratio among the palms and the female flower morphology with uneven stealth opening. However, consistent increasing in the percentage of parthenocarpic fruit formation (> 40%) with the increased doses of irradiation showed that the pollination process was disrupted by the irradiated pollen source.

Table 2: Bunch formation status for the different irradiation doses

Dose (Gy)	Bunch Status	Fruit Set (%)
0	Normal	78.13
10	Normal	80.00
50	Normal	79.07
100	Normal - Partially rotten	79.53
200	Partially rotten - rotten	60.00
300	Partially rotten	69.77
500	Rotten & Aborted	Nil
1000	Partially rotten	69.77
2000	Partially rotten - rotten	60.00

Numerous literatures indicated that irradiated pollen have the ability to germinate on the stigma, growth within the style and reach the embryo sac. Depending on the dose of radiation, some of the irradiated pollens were able to fertilise the egg cell and the polar nuclei like normal plants while some of the irradiated pollens were unable to fertilise the egg cell and the polar nuclei, which therefore induce the formation of parthenocarpic fruits (Musial and Przywara, 1998; Sugiyama and Morishita, 2002).

Results in Table 2 shows strong correlation between the pollen viability with the fruits and bunches formation where pollen with viability below 60% resulted bunches with poor fruit sets, partially rotten or aborted bunches and high number of parthenocarpic fruits formation (Figure 4).

Normal bunch | Partially rotten bunches | Rotten bunch

Figure 4: Different types of bunches formation

CONCLUSIONS

Radiation doses greater than 200 Gy caused the pollen tube formation to be disrupted and consequently resulted in parthenocarpic fruits formation. Since the pollens were unable to reach the ovule, the embryo was aborted and consequently contributed to the rotten and aborted bunches. Low level of irradiation (< 200 Gy) may damage only part of the generative nucleus while maintaining its capacity to fertilize the egg cells and lead to hybridization. This finding in study can be used as a guide in designing mutation breeding experiments for oil palm.

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REFERENCES

Ajambang, W., Sudarsono, Asmono, D. and Toruan, N. (2012). Microsatellite markers reveal Cameroon’s wild oil palm population as a possible solution to broaden the genetic base in the Indonesia-Malaysia oil palm breeding programs, *Afr. J. Biotechnol.* 11(69): 13244-13249.

Arias, D., Montoya, C., Rey, L. and Romero, H. (2012). Genetic similarity among commercial oil palm materials based on microsatellite markers, *Agronomia Colombiana* 30(2): 188-195.

Che Radziah, C.M.Z., Salwa, A.S. and Mohamad, O. (2009). Determination of gamma irradiation dose in oil palm for tilling study. In: *Proceedings of the 8th Malaysia Congress on Genetics*, 4-6 August 2009, Genting Highlands, Malaysia.

Dolcet-Sanjuan, R., Claveria, E. and Garcia-Mas, J. (2005). Optimization of cucumber doubled haploid line production using in vitro rescue of in vivo induced parthenogenic embryos, *J. American Soc. Hort. Sci.* 130(4): 555-560.

El-Degwy, I.S. (2013). Mutation induced genetic variability in rice (*Oryza sativa* L.), *Int. J. Agri. Crop. Sci.* 5 (23): 2789-2794.

Khan, M.R., Qureshi, A.S., Hussain, S.A. and Ibrahim, M. (2005). Genetic variability induced by gamma irradiation and its modulation with gibberellic acid in M2 generation of chickpea (*Cicer arietinum* L.), *Pak. J. Bot.* 37 (2): 285-292.

Mudibu, J., Nkongolo, K.K.C., Kalonji-Mbunyi, A. and Kizungu, R.V. (2012). Effect of Gamma Irradia- tion on Morpho-Agronomic Characteristics of Soybeans (*Glycine max* L.), *American J. Plant Sci.* 3: 331-337.

Musial, K. and Przywara, L. (1998). Influence of irradiated pollen on embryo and endosperm develop- ment in kiwifruit, *Annals of Botany* 82: 747-756.

Nelson, S.P.C., Wilkinson, M.J., Dunwell, J., Forster, B.P., Wenig, S., Sitorus, A., Croxford, A., Ford, C. and Caligari, P.D.S. (2009). Breeding for high productivity lines via haploid technology. In: *Proceedings International Palm Oil Congress*, 9-12 November 2009, Kuala Lumpur, Malaysia. 203-225.

Peixe, A., Campos, M.D., Cavaleiro, C., Barrosa, J. and Pais, M.S. (2000). Gamma-irradiated pollen induces the formation of 2n endosperm and abnormal embryo development in European plum (*Prunus domestica* L., cv. "Rainha Claudia Verde"), Sci. *Hort*. 86: 267-278.

Rohani, O., Samsul Kamal, R., Rajindeer, S. and Mohd Nazir, B. (2012). Mutation induction using gamma irradiation on oil palm (*Elaeis guineensis* Jacq.) cultures, *J. Oil Palm Res.* 24 (2): 1448-458.

Sari, N. and Abak, K. (1994). Induction of Parthenogenetic Haploid Embryos after Pollination by Irradi- ated Pollen in Watermelon, *Hort. Sci.* 29(10): 1189-1190.

Soh, A.C., Gan, H.H., Wong, T.Y. and Tan, C.C. (2003). Estimates of within family genetic variability for clonal selection in oil palm, *Euphytica* 133: 147-163.

Sugiyama, K. and Morishita, M. (2002). New method of producing diploid seedless watermelon fruit, *JARQ* 36(3): 177-182.

Sutarto, I, Agisimanto, D. and Supriyanto, A. (2009). Development of Promising Seedless *Citrus* Mutants through Gamma Irradiation. Induced Plant Mutations in the Genomics Era. Food and Agriculture Organization of the United Nations, Rome, 306-308.

Wong, C.K. and Bernardo, R. (2008). Genomewide selection in oil palm: increasing selection gain per unit time and cost with small populations, *Theor*. *Appl. Genet*. 116: 815-824.