

MUTATION INDUCTION USING GAMMA IRRADIATION ON OIL PALM (*Elaeis guineensis* Jacq.) CULTURES

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ABSTRACT

This article reports on in vitro mutation induction in oil palm (*Elaeis guineensis* Jacq.) using gamma irradiation. The radiosensitivity tests were carried out to determine the level of gamma dose suitable for oil palm cultures with good regenerative capacity. Calli from Clone C3-5, Clone GP13-0, Clone GP13-1 and Clone C7-1 were exposed to various levels of gamma radiation (GR) from 0 to the highest 100 Gray (Gy). Of these, embryogenesis was observed in Clone C3-5 and Clone GP13-0 at 40 Gy and at lower doses of GR. The mean weight of callus growth was halved at about 40 Gy to 60 Gy of gamma rays. AFLP analysis was carried out on these callus cultures to monitor changes in the DNA profile. The DNA changes were observed even at a dosage as low as 10 Gy. The changes observed were basically random in nature. For a mutation induction study, leaf derived calli from a selected ortet was used. Friable callus from Clone CX49, Line A' and Line B' were exposed to both fractionated and acute irradiation. When fractionated irradiation was used, there were no significant differences in the establishment of polyembryoid cultures and shoot production. In this study, the highest gamma ray dose was 5 Gy repeatedly given for three and five times at 4-5 subculture intervals. Under acute irradiation with doses of 0, 20, 40, 60, 80 and 100 Gy, the embryoid growth decreased significantly at doses of 40 Gy and above, which also affected the shoot regeneration.

Keywords: mutation induction, callus cultures, gamma irradiation, radiosensitivity, fractionated and acute irradiation.

Date received: 13 July 2009; **Sent for revision:** 29 July 2009; **Received in final form:** 7 July 2012; **Accepted:** 10 July 2012.

INTRODUCTION

The genetic resource of oil palm (*Elaeis guineensis* Jacq.) in Malaysia is based on the Deli *dura* population derived from four palms introduced in Bogor in 1948 (Kushairi and Rajanaidu, 2000). Sizeable oil palm germplasm collections which have been made since 1973 (Rajanaidu and Rao, 1987), were evaluated and utilised for crop improvement. The breeding programme for oil palm carried out by conventional cross hybridisation is extremely

time-consuming. Oil palm has a long economic life cycle and selection for desirable traits takes more than 10 years. To fast track introgression of desirable genes into existing oil palm materials that currently have a relatively limited genetic base; several methods have been employed with the aim of broadening the variation for breeding. On-going genetic engineering research at MPOB (Parveez, 2003) is one of the attempts to meet the above objective. Another effort is to use a mutation breeding technique. Like other crops, mutation breeding has been employed with the aim of improving and increasing the availability of genetic resources.

Mutation induction by gamma ray irradiation has played an important role in the improvement of a number of crops such as rice (Domingo *et al.*, 2007), maize, banana (Novak *et al.*, 1990; Musoke *et al.*, 1999), sugar-cane (Nagatomi *et al.*, 1996), apple, pear (Masuda and Yoshioka, 1995a, b)

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and ornamental plants (Chitrapan and Siranut, 2002; Lamseejam *et al.*, 2002). *In vitro* mutant induction and selection can provide a rapid method for developing new products. Okamura (2006) indicated that chronic gamma irradiation gave a wider mutation spectrum than acute gamma irradiation. This was also shown by Nagatomi *et al.* (2000) in chrysanthemums. Ionising radiation especially with ion beams in combination with tissue culture would be useful for producing new commercial floriculture and ornamentals (Okamura, 2006). This is especially true when slight variations of outstanding cultivar are desired. This is the first attempt of mutation induction on oil palm using gamma irradiation.

A zygotic or somatic embryo is not an ideal material for mutation induction for oil palm. Being an organised structure, seed or somatic embryo exposed to mutagens can lead to chimeras (IAEA, 1986). The best option for oil palm is to use callus cultures derived from high yielding ortets that have high regenerative capacity. Induced mutants are known in some plant species to improve traits such as disease tolerance, short stature plants, early fruiting and high yielding. Some of the traits of interest such as plants with a shorter stem stature have been observed in banana using gamma irradiation (Lopez *et al.*, 2004). A majority of Malaysian oil palms are tall, with an annual height increment of 40-75 mm (Rajanaidu *et al.*, 2000), which makes them difficult to harvest after 20 years. Hence, the objective of this study was to produce mutants with slow height increment while maintaining the ortet's high yielding ability. A single genotype was used for the mutation induction studies.

MATERIALS AND METHODS

Radiosensitivity Test

Radiosensitivity test was carried out to determine the range of doses that were neither too low nor too high for the tissues under study. For oil palm, it is important that the treated calli are able to produce embryoids. This is because embryogenic cultures are a prerequisite to shoot production. To get an idea of irradiation dose for oil palm tissue, mature seed embryos were used since callus tissues were invaluable. The seed embryos were exposed to various levels of gamma rays (GR) from ^{60}Co source ranging from 0 to 160 Gray (Gy) at 40 Gy intervals. After irradiation, the seed embryos were cultured individually in 25 × 150 ml tubes containing 10 ml of basal nutrient medium as described by Rohani *et al.* (1997). The cultures were maintained in a light room with 12 hr photoperiod at $28^{\circ}\pm 1^{\circ}\text{C}$. After two months, the numbers of embryos exhibiting

shoot or both shoot and root development was determined indicating the germination success.

Based on the results from the seed embryo culture experiments, the first callus Clone C3-5 was exposed to 0 to 40 Gy of GR at 10 Gy intervals. Each coin-shaped callus culture, 10 mm in diameter, was weighed before exposure to different doses of GR. This was followed by transfer of the control and treated cultures to fresh basal nutrient medium (Rohani *et al.*, 2003) containing 7.5×10^{-5} M α -Naphthalene acetic acid (NAA), 1.5 g litre⁻¹ activated charcoal (AC) and gelled with 7 g litre⁻¹ agar. The calli were incubated in the dark at $28^{\circ}\pm 1^{\circ}\text{C}$. The fresh weight increase was calculated, as the difference between the weight of the callus at five weeks of culture and the initial weight. The number of callus cultures producing embryoids was also recorded and estimated as a percentage of the total number of cultures per treatment. Calli from three other clones, GP13-0, GP13-1 and C7-1 were also exposed to GR. Calli from Clone GP13-0 were exposed to 0 to 100 Gy of GR at 20 Gy intervals, while calli from Clones GP13-1 and C7-1 were exposed to 0 to 70 Gy and 0 to 60 Gy of GR at 10 Gy intervals, respectively. Calli from GP13-1 and GP13-0 are of the same clone but from different lines (callus initiation at different stages). For the above study, calli were obtained from the MPOB clonal palm production section.

Preparation of Genomic DNA for AFLP Analysis

Calli from Clones C3-5, GP13-1 and C7-1 were frozen in liquid nitrogen and stored in the -80°C freezer until required. DNA was prepared from the cultures using the method described by Doyle and Doyle (1990). AFLP analysis using the *EcoRI*/*MseI* enzyme pairs was carried out to examine for changes in DNA after irradiation. The *EcoRI*/*MseI* enzyme pairs were assayed by using the GIBCO BRL AFLP Analysis System 1, as described in the manufacturer's manual. The study involved: i) the use of different primer combinations and ii) the use of one common primer combination on three different callus clones. In this respect:

- i. Different primer combinations including ACT/MCTT, AAG/MCAC and ACT/MCAC were used on samples from Clone GP13-1.
- ii. Primer combinations of AAG/MCAC were used on calli from Clones C3-5, GP13-1 and C7-1 in an effort to see whether the changes occur on the same loci.

Mutation Induction

Explant culture and callus initiation. Immature leaf explants from a high yielding palm or ortet (Clone CX49) with fresh fruit bunch (FFB) of 175.78

kg and oil to bunch ratio (O/B) of 28.42% were excised into 2 mm strips, four to five strips per cluster. Four leaf explant clusters were placed in each 90 mm diameter Petri dish containing basal nutrient medium as described by Rohani *et al.* (2003) supplemented with various treatments: A. 10^{-3} M NAA + 2.5 g litre⁻¹ AC (Control); B. 7.5×10^{-4} M NAA + 2.0 g litre⁻¹ AC; C. 10^{-4} M NAA + 2.0 g litre⁻¹ AC; D. 7.5×10^{-5} M NAA + 1.5 g litre⁻¹ AC; E. 10^{-5} M NAA + 1.5 g litre⁻¹ AC and F. 10^{-5} M NAA. The media were gelled with 7 g litre⁻¹ of Agar. The cultures were incubated in dark room at $28^{\circ} \pm 1^{\circ}$ C for eight weeks for callus induction. Leaf explant clusters that produced callus were bulked on basal nutrient medium containing 7.5×10^{-4} M NAA + 2.5 g litre⁻¹ AC. The calli were further bulked onto a nutrient medium supplemented with 6×10^{-5} M NAA and gelled with 3 g litre⁻¹ Gelrite. At the third subculture, the calli were transferred to basal nutrient medium containing four different media for induction of embryogenic callus. The media were made up of basal nutrient medium supplemented with 5×10^{-5} M 2,4-D (2,4-dichlorophenoxyacetic acid) + 20 g litre⁻¹ sucrose, 5×10^{-5} M 2,4-D + 30 g litre⁻¹ sucrose, 6×10^{-5} M NAA + 20 g litre⁻¹ sucrose and 6×10^{-5} M NAA + 30 g litre⁻¹ sucrose. The media were gelled with 3 g litre⁻¹ Gelrite. Friable and granular calli were selected for the mutation induction experiments. One week prior to irradiation, each callus culture at 10 mm in diameter was spread thinly in the middle of a 90 mm diameter Petri dish containing the culture medium. After irradiation, the control and treated cultures were transferred immediately to sterile fresh bulking medium of the same nutrient, hormone and sucrose compositions. The cultures were then incubated in the dark room.

Isolation of somatic embryos and bulking. Primary embryoids which were whitish in colour, formed after two, four and seven weeks in culture, were isolated. This was to check whether their formation at different times had any effect on mutation induction during field observation. The embryoids were grouped in a cluster of 10 mm diameter each before transferring it to a 125-ml conical flask containing hormone-free solid nutrient medium as described by Rohani *et al.* (2003). The cultures were maintained for eight weeks and incubated in a 12-hr photoperiod room at $28^{\circ} \pm 1^{\circ}$ C. The embryoid clusters which developed into a polyembryoid (PE) mass were further subdivided into clumps of 15-20 mm in diameter and maintained at two monthly subculture cycles in the light room.

Shoot regeneration and rooting. As the PE cultures matured, shoots that regenerated were isolated for rooting. Shoots attaining a height of 30 mm were rooted individually in a 10 ml of liquid rooting medium (Rohani *et al.*, 2003), if the number of

shoots obtained were small. The double layer rooting method (Zamzuri, 1998) was used if larger number of shoots were obtained.

a. Fractionated irradiation

Nagatomi *et al.* (2006) indicated that chronic irradiation produced a wider variation than acute irradiation. It was advocated that doses used for targeting *in vitro* cultures should be as low as possible (IAEA, 1986). Since there was no gamma-field facility in Malaysia, a fractionated irradiation method was used. Line A' and Line B' (different sucrose levels) calli were exposed to low doses of GR given repeatedly at short intervals. Treatments A and B were Controls but calli in Treatment A did not undergo repeated subculture as in Treatment B in which the calli underwent five subcultures at four to five days subculture intervals. This treatment was to test the effect of repeated culturing at short intervals. Calli in Treatments C and D were repeatedly exposed to 5 Gy of GR, three and five times, respectively. All the treated cultures including Control A were transferred to fresh nutrient medium and were maintained in the dark for eight weeks as described in the Mutation Induction section. The number of cultures producing embryoids was recorded. Embryoid maintenance, shoot regeneration and rooting are as described above.

b. Acute irradiation

Line A' and Line B' calli from the same clone as above were exposed to 0, 20, 40, 60, 80 and 100 Gy of GR. A limited number of callus cultures were used for this experiment. After irradiation, the cultures were transferred to fresh nutrient medium and incubated in the dark for eight weeks as described above. The rate of embryogenesis, embryoid maintenance, shoot regeneration and rooting were as described above.

Statistical Analysis

Analysis of variance (ANOVA) was performed to test the significance effect of the treatments for PE establishment and shoot production at the third subculture stage. SAS 9.2 was used for data analysis.

RESULTS AND DISCUSSION

Radiosensitivity Test

The germination rates of seed embryos in the Control were 75% while seed embryos exposed to 40 Gy of GR were 4%. Seed embryos exposed to ≥ 40 Gy were mostly darkened (Figure 1) with no germination. This indicated that 40 Gy of GR would be adequate for oil palm tissues.

Since oil palm is a perennial crop, it takes longer to carry out tests based on its height and other field parameters after irradiation unlike other crops such as rice (Akbar and Babar, 2003) and other annuals. For oil palm, the radiosensitivity test was assessed not only on fresh weight increase of callus but also based on the number of callus cultures forming embryoids. As shown in *Table 1*, the first callus from Clone C3-5 that were exposed to 10 Gy-40 Gy of GR showed a decrease in the mean weight of callus growth and with embryogenesis (*Figure 2*) rates ranging from 50%-100%. This was followed by exposing calli from Clones GP13-0, GP13-1 and C7-1 to see the effects of higher doses of GR on callus growth and embryogenesis. Generally, the calli continued growing even at high GR doses. However, the mean callus fresh weight decreased with increased doses of GR except for Clone GP13-1 where the mean fresh weight of callus cultures treated with 20 Gy was slightly higher than the Control. The mean callus fresh weight of Clone C7-1 decreased when the calli were exposed to 10 Gy of GR after which there was no further decrease lower than 50 mg with higher GR doses. Embryogenesis was also observed in Clone GP13-0 at 0 and 20 Gy of GR but none in Clones

GP13-1 and C7-1. The line graphs (*Figure 3*) show that generally, the callus growth was halved at 40 Gy to 60 Gy (*Figures 3A to 3C*) except for clone C7-1 (*Figure 3D*), where the callus growth was severely restricted even at 10 Gy. Since the number of calli was limited, determination of the mean lethal and optimal irradiation for oil palm cultures was not carried out. However, it is pertinent to ensure that the treated cultures remain embryogenic and able to produce mutants.

AFLP Analysis

Although a dominant marker, AFLP analysis was preferred as it generates large number of polymorphism and has a high multiplex ratio (Rafalski *et al.*, 1996). Calli from Clone GP13-1 were analysed with different AFLP primer combinations namely ACT/MCTT, AAG/MCAC and ACT/MCAC as shown in *Figures 4A, 4B and 4C* respectively. Different primer combinations tend to amplify different segments of the genome. This allows an effective scan of the genome. The DNA changes were observed in almost all lanes as compared to the Control (Lane a). The changes however, appeared to be random.

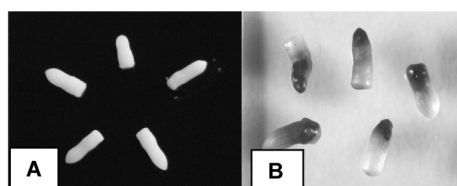


Figure 1. A. Fresh oil palm seed embryos, lengths ranging from 2.5 mm - 3.5 mm. B. Irradiated seed embryos.



Figure 2. Primary embryoids.

TABLE 1. EFFECTS OF GAMMA RADIATION (GR) ON CALLUS GROWTH AND EMBRYOGENESIS

Gamma dose (Gy)	Callus clones#							
	Mean wt. of callus growth (mg)				Embryogenesis rate (%)			
	C3-5	GP13-0	GP13-1	C7-1	C3-5	GP13-0	GP13-1	C7-1
0	320a	250a	48a	110a	83.3a	57.1	0	0
10	280a	-	40a	50b	50.0b	-	0	0
20	180b	190a	49a	60b	50.0b	71.4	0	0
30	190b	-	45a	50b	66.7ab	-	0	0
40	140b	100b	35b	50b	100a	0	0	0
50	-	-	36b	60b	-	-	0	0
60	-	100b	8c	60b	-	0	0	0
70	-	-	8c	-	-	-	0	-
80	-	70c	-	-	-	0	-	-
90	-	-	-	-	-	-	-	-
100	-	50c	-	-	-	0	-	-

Note: #Number of cultures per treatment: Clone C3-5, 6; Clone GP13-0, 7; Clone GP13-1, 8 and Clone C7-1, 7. Means with same letter are not significantly different.

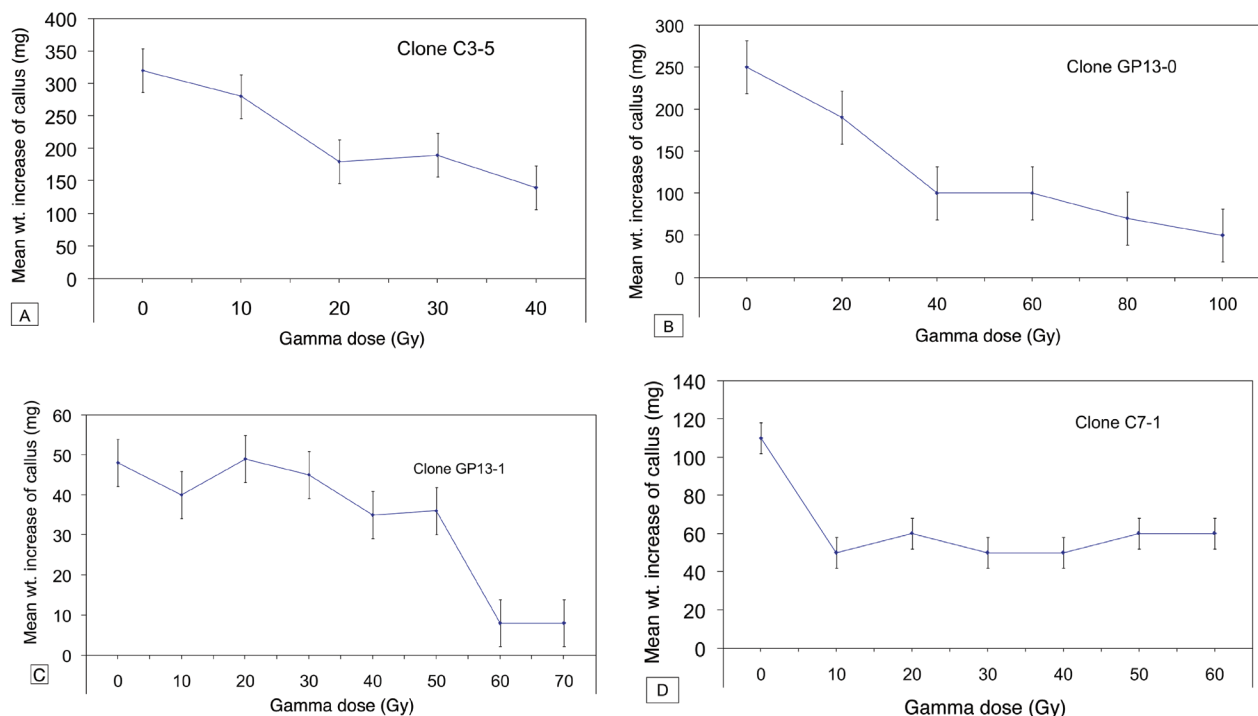


Figure 3. Line graphs of mean weight of callus cultures before and after gamma irradiation. A. Clone C3-5 calli exposed to 0 – 40 Gy; B. Clone GP13-0 calli exposed to 0 – 100 Gy; C. Clone GP13-1 calli exposed to 0 – 70 Gy and D. Clone C7-1 calli exposed to 0 – 60 Gy.

Figure 5 shows AFLP profiles of three batches of calli from Clones C3-5, GP13-1 and C7-1 using a common primer combination (AAG/MCAC). Except for Lane c1, there were not many changes seen in Figure 5A. Figures 5B and 5C show a number of additional bands (thin arrows) and missing bands (solid arrows). The changes were rather random.

Generally, AFLP analysis could not detect specific changes in the genome caused by GR either at increasing or decreasing doses. Changes occurred even at 10 Gy of GR (Figures 4 and 5). Radiation appeared to cause random changes in the genome and the changes did not appear to be restricted at specific sites, clones or lines. The appearance/disappearance of specific AFLP bands indicated that the GR had caused major genomic re-arrangements in the genome.

Mutation Induction

Callus induction. Callus was observed in leaf explants cultured on media A, B and F with callusing rates of 10.9%, 6.0% and 2.5% respectively (number of sterile leaf explant clusters ranged from 182-199 clusters). The Control medium was still the best for callus initiation. At the third subculture, calli were exposed to four media for induction of embryogenic callus as in Table 2. Calli cultured on media containing NAA and 20 g litre⁻¹ sucrose (Line A') or 30 g litre⁻¹ sucrose (Line B') produced embryoids while none were produced in media

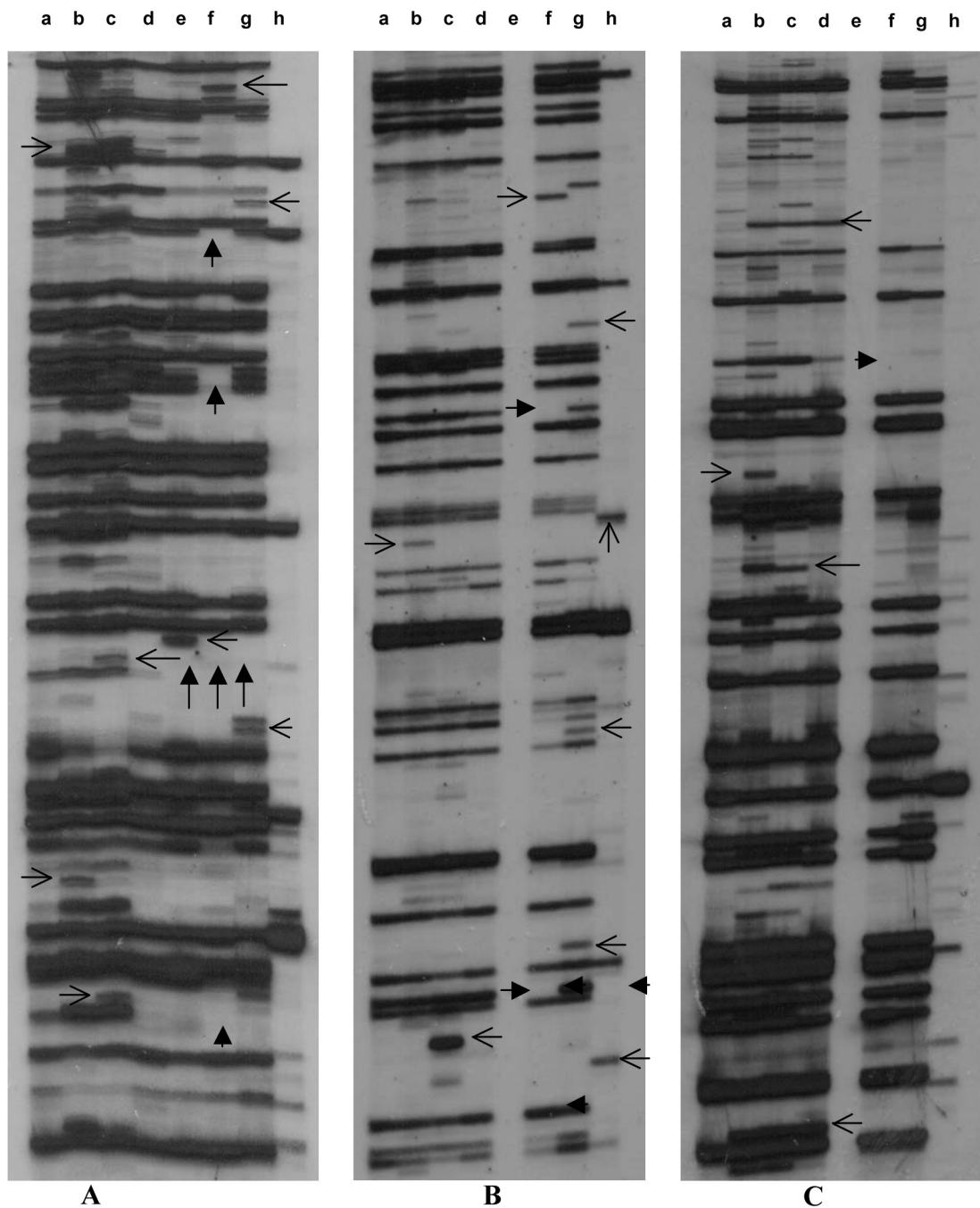
TABLE 2. EFFECTS OF AUXIN AND SUCROSE LEVELS ON EMBRYOGENESIS

Auxin (M)	Sucrose (g litre ⁻¹)	No. of sterile callus cultures	Embryogenesis rate (%)
5 x 10 ⁻⁵ 2,4-D	20	19	0
5 x 10 ⁻⁵ 2,4-D	30	20	0
6 x 10 ⁻⁵ NAA	20	30	33.0
6 x 10 ⁻⁵ NAA	30	29	34.5

Note: NAA – α-Naphthalene acetic acid.

containing 2,4-D. Calli from NAA were friable or granular in nature. In the current mutation induction study, only undifferentiated friable calli were used.

Embryogenesis. Embryogenesis occurred in all the treatments using fractionated or acute irradiation methods (Tables 3, 4, 5 and 6). The embryogenesis rates ranging from 40%-100% surpassed in general, the conventional average embryogenesis rate of oil palm cultures at 6% (Rohani *et al.*, 2000) indicating that the callus selected for this study has shown good regenerative potential. In the fractionated irradiation experiment, frequent transfer of callus cultures at shorter subculture intervals encouraged callus growth hence, providing more tissues for embryogenesis. This indicated that the number of embryoid clusters obtained (Tables 3 and 4, Treatments B, C and D) irrespective of cultures treated (Treatments C and D) or untreated with GR



A. Primers ACT/MCTT. A number of loci are missing in lanes e, f, g and h (thick arrow head). Additional loci are seen in lanes b, c, e, f and g (thin arrow head) compared to control.
 B. Primers AAG/MCAC. Some dense bands seen in control are missing in lanes f and h. Additional loci in majority of the lanes as compared with the control. No results in lane e (AFLP amplification failed).
 C. Primers ACT/MCAC. With the exception of lanes e and h, generally there are additional loci observed when compared to the control. No results in lane e and h, where amplification failed.

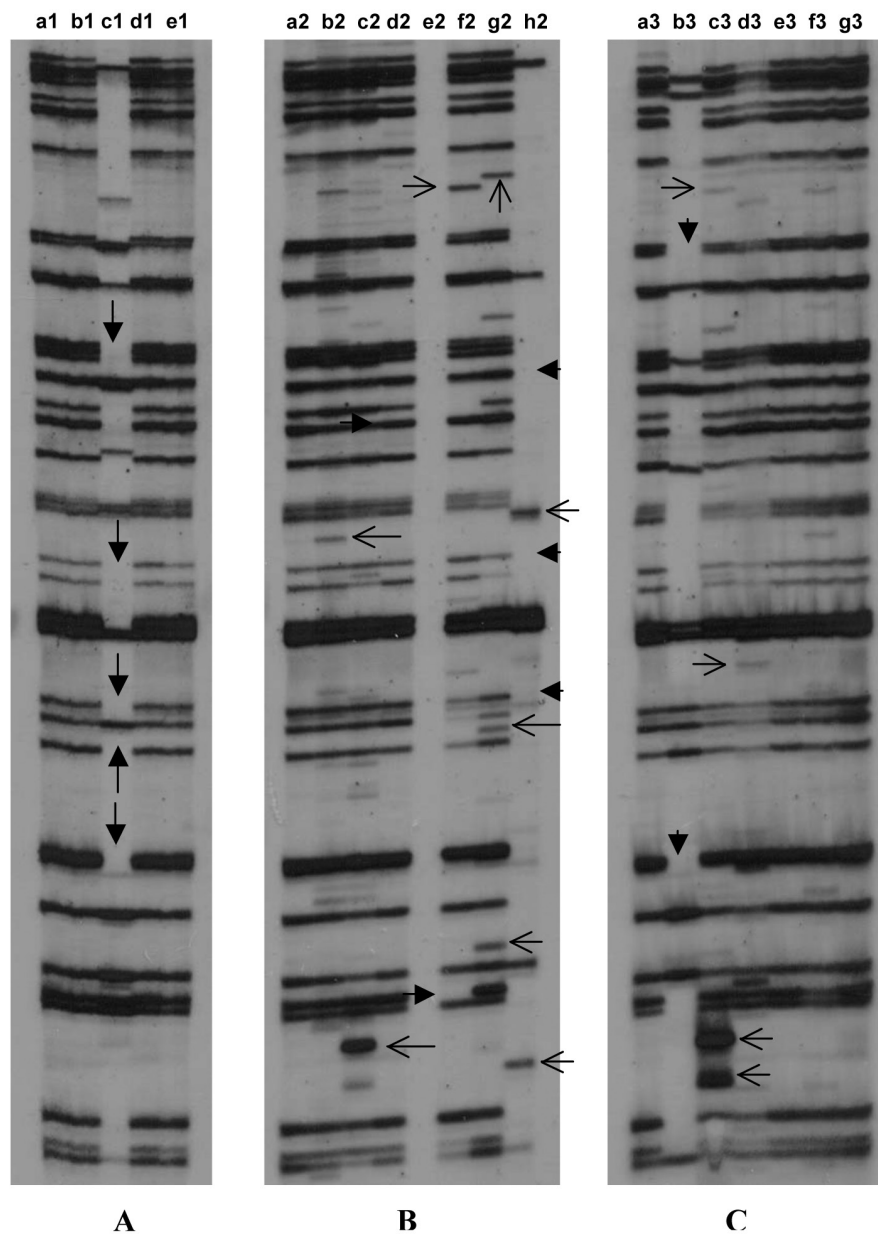
Figure 4. AFLP profiles of Clone GP13-1 calli exposed to 0, 10, 20, 30, 40, 50, 60 and 70 Gy (a-h) using different primer combinations.

(Control B) was higher than the Control A (Tables 3 and 4). The anther culture of wheat exposed to low doses of GR (1, 3 and 5 Gy) showed more embryoids and shoot regeneration than in the control (Ding *et al.*, 1991).

In the acute irradiation experiment, although embryogenesis occurred in all the treatments, the number of embryoid clusters generally decreased

with higher GR doses (Tables 5 and 6). This was related to the decrease in callus growth with higher GR doses as demonstrated in the radiosensitivity experiment (Table 1).

Establishment of PE cultures and shoot production. Primary embryoids formed at two, four and more than seven weeks were isolated and transferred



A. Gamma doses – a1 – e1 (0, 10, 20, 30 and 40 Gy) on Clone C3-5 calli. There are not many changes observed except for lane c1, there appears to be a lot of missing loci (thick arrow head).
 B. Gamma doses – a2 – h2 (0, 10, 20, 30, 40, 50, 60 and 70 Gy) on Clone GP13-1 calli. Additional loci seen in most lanes (thin arrow head) and missing loci are observed in lanes f2 and h2 (thick arrow head). No result in lane e2.
 C. Gamma doses – a3 – g3 (0, 10, 20, 30, 40, 50 and 60 Gy) on Clone C7-1 calli. Distinct additional bands are seen in lane c3 (thin arrow head). Few additional bands are seen in other lanes as compared to a3 (control). Lane b3 have many missing loci.

Figure 5. AFLP profiles of callus cultures exposed to gamma rays. The primer combinations used were AAG/MCAC.

TABLE 3. EFFECTS OF FRACTIONATED IRRADIATION ON EMBRYOGENESIS, EMBRYOID PROLIFERATION AND SHOOT PRODUCTION AT THIRD PE SUBCULTURE (Line A' callus)

Gamma dose (Gy)#	No. of sub-cultures	Embryogenesis rate (%)	No. of primary embryoid clusters	Mean No. of PE cultures established	Mean No. of shoots produced
A - 0	1	67	12	13.83a	57.60a
B - 0	5	100	24	16.33a	98.50a
C - 5	3	100	34	16.67a	55.83a
D - 5	5	100	31	6.83b	33.33a

Note: # Number of cultures per treatment: 6.
 Means with same letter are not significantly different.
 PE – polyembryoid.

TABLE 4. EFFECTS OF FRACTIONATED GR ON EMBRYOGENESIS, EMBRYOID PROLIFERATION AND SHOOT PRODUCTION AT THIRD PE SUBCULTURE (Line B' callus)

Gamma dose (Gy)#	No. of sub-cultures	Embryogenesis rate (%)	No. of primary embryoid clusters	Mean No. of PE cultures established	Mean No. of shoots produced
A - 0	1	100	4	11.43a	46.00a
B - 0	5	100	18	10.00a	84.14a
C - 5	3	100	12	11.14a	78.57a
D - 5	5	100	13	5.29b	55.86a

Note: # Number of cultures per treatment: 7.
Means with same letter are not significantly different.
PE – polyembryoid. GR – gamma radiation.

TABLE 5. EFFECTS OF ACUTE IRRADIATION ON EMBRYOGENESIS, EMBRYOID PROLIFERATION AND SHOOT PRODUCTION AT THIRD PE SUBCULTURE (Line A' callus)

Gamma dose (Gy)#	Embryogenesis rate (%)	No. of primary embryoid clusters	Mean No. of PE cultures established	Mean No. of shoots produced
A - 0	80	33	16.80a	57.80a
B - 20	100	35	12.20ab	71.00a
C - 40	60	14	8.40abc	9.40b
D - 60	60	11	6.00bc	5.80b
E - 80	60	3	1.80c	0.00
F - 100	40	2	1.20c	0.00

Note: # Number of cultures per treatment: 5.
Means with same letter are not significantly different.
PE – polyembryoid.

to a hormone-free nutrient medium for further bulking as PE cultures and for shoot regeneration. In oil palm tissue culture, embryoids obtained were often at different developmental stages, expressed in different forms such as fused torpedo-shaped (Figure 3), nodular, haustorium-like, wavy or globular (Rohani and Ong-Abdullah, 2003). Certain types of embryoids (nodular, torpedo-shaped) could proliferate well into PE cultures (Figure 6), while other types either produced limited growth or did not grow at all. The different embryoid morphologies not only affected the establishment of PE cultures (Rohani *et al.*, 2000; Rohani and Ong-Abdullah, 2003; Meilina *et al.*, 2005) but eventually shoot production. Hence, it is vital that the embryoids have the propensity to proliferate for shoot regeneration.

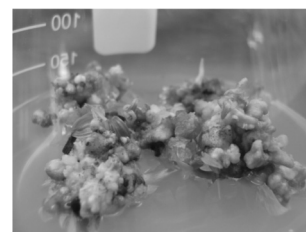


Figure 6. An established polyembryoid (PE) culture.

In the fractionated irradiation experiment, embryoids from both Line A' (Table 3) and Line B' (Table 4) calli proliferated into PE cultures. Subjected to ANOVA, the mean number of PE cultures established at the third subculture did not show significant differences between the

TABLE 6. EFFECTS OF ACUTE IRRADIATION ON EMBRYOGENESIS, EMBRYOID PROLIFERATION AND SHOOT PRODUCTION AT THIRD PE SUBCULTURE (Line B' callus)

Gamma dose (Gy)#	Embryogenesis rate (%)	No. of primary embryoid clusters	Mean No. of PE cultures established	Mean No. of shoots produced
A - 0	100	7	13.00a	143.00a
B - 20	100	13	16.00a	124.00a
C - 40	100	12	12.67a	102.00a
D - 60	100	6	4.33b	3.00b
E - 80	100	5	4.00b	1.00b
F - 100	100	3	1.33b	0.00

Note: # Number of cultures per treatment: 3.
Means with same letter are not significantly different.
PE – polyembryoid.

treatments, $p \geq 0.05$, for both lines (Tables 3 and 4). Here, a low dose of 5 Gy of GR was used, applied repeatedly three or five times at short intervals. The exposure of calli to low GR dose appears to have a detrimental effect on the embryoid quality as reflected by a production of a large number of shoots. The mean number of established PE cultures was lowest in Treatment D for both callus lines (Tables 3 and 4) as compared to the other treatments. However, the mean number of shoots produced was not significantly different among all the treatments, $p \geq 0.05$. It is hoped that a number of useful mutants can be obtained from fractionated GR.

In the acute irradiation experiment, the mean number of PE cultures established decreased significantly with higher GR doses of more than 20 Gy for Line A' (Table 5) and more than 40 Gy for Line B' (Table 6), $p \leq 0.01$. As reported earlier, the mean callus fresh weight decreased with higher GR doses (Table 1) and this would affect the number of embryogenic cultures obtained (Tables 5 and 6). The mean number of shoots produced from Line A' callus decreased significantly at more than 20 Gy of GR (Table 5), $p < 0.01$ and there was no significant difference in shoot production from Line B' callus (Table 6). The number of shoots produced depended on the established PE cultures. Not all embryogenic cultures would develop as prolific PE cultures as described earlier. As observed by Wooi (1995), 50% of the embryogenic cultures proliferated into PE cultures while 20% regenerated shoots with limited multiplication and 30% failed to produce plantlets.

Field planting. Rooted ramets from all the four experiments (Tables 3 to 6) were transferred directly to sand and soil mixture as described by Rohani *et al.* (2003). In vegetatively propagated plants like oil palm, it is not possible to eliminate undesirable mutations induced simultaneously with the desired ones at *in vitro* or *in vivo* (nursery) stages except those that showed discernable variations as described by Meilina *et al.* (2005). Three batches of a total of 793 ramets have been planted in the field in 2005 and 2006, and will be observed for any interesting traits to be reported later.

CONCLUSION

In an attempt to induce mutation in oil palm cultures, it is important to use callus that has previously shown to be embryogenic. However, it is not easy to acquire embryogenic cultures readily. We had to rely on the availability of granular or friable calli (normal selection requirement). Even after selection, these calli may turn out to be non-embryogenic.

From the radiosensitivity study, the calli continued their growth even at higher doses of GR (maximum dose: 100 Gy) but generally decreased with increased GR doses. However, embryogenesis was observed in the Controls and in the treated cultures exposed to 40 Gy of GR or less as shown in calli from Clones C3-5 and GP13-0.

The AFLP analysis has shown that the changes in DNA were random. The changes have been detected at the lowest dose of 10 Gy and did not occur at specific sites. Radiation caused major genomic rearrangements and it will be interesting to test if any of the changes are linked to agronomic trait improvement.

Embryogenic calli from a selected clone CX49 were used for mutation induction using both fractionated and acute irradiation methods. In the study using fractionated irradiation, the frequent transfer of cultures at short subculture intervals promoted callus growth and enhanced embryogenesis as compared to the Controls. Embryoid proliferation and shoot production did not differ significantly among treatments since only low GR dose of 5 Gy applied three or five times within four to five days of transfer cycles were used. It is essential that calli not only have the propensity to proliferate but at the same time are embryogenic. Readily established embryoids are the route to mutant shoot production. However, it is important to note that the response of cultures to gamma radiation is genotype dependent.

Calli exposed to acute irradiation produced embryoids in all the treatments (0, 20, 40, 60, 80 and 100 Gy) but the rate of embryogenesis and the number of embryogenic clusters decreased significantly with increased doses of GR. The results showed that the effective range of the doses for embryogenesis was at ≤ 40 Gy.

Both ramets from fractionated and acute irradiation methods were planted in the field in 2005 and 2006. It is hoped that a number of the mutants with desirable agronomic traits will be identified.

ACKNOWLEDGEMENT

The authors would like to thank the Director-General of MPOB for approving this article for publication. Thanks are also due to Mr Shuhaimi Shamsuddin from Malaysian Nuclear Agency for carrying out the irradiation work and Ms Noor Hariani Ismail of MPOB who initiated and maintained the cultures after irradiation treatment. Ms Jamaliah Ahmad, Ms Nor Adillah Dollah, Ms Masdiana Mastor and Ms Siti Maslia Adnan are also thanked for maintaining the PE and shoot cultures. The authors also appreciated the ideas mooted by

Dr N Rajanaidu on this mutation study. The help of Ms Rahimah Abdul Rahman who carried out the AFLP work, Mr Zamzuri Ishak from the Clonal Production Section for the supply of callus used in the radiosensitivity study, Ms Zaharah Rahmat for the statistical analyses is also acknowledged. We also extend our appreciation to Dr Ravigadevi Sambathamurthi, Dr Meilina Ong Abdullah and Dr Ooi Siew Eng for their critical comments on the manuscript. Lastly, our deep appreciation to Datuk Dr Mohd Basri Wahid for his encouragement on this project. The research work was funded by MPOB.

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