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OPTIMISATION OF BOMBARDMENT PARAMETERS IN OIL PALM EMBRYOGENIC CALLI BASED ON TRANSIENT EXPRESSION OF RFP GENE

A RAHMAN NURFAHISZA[1]*; MAT YUNUS ABDUL MASANI[1]*; SUBHI SITI MASURA[1]; MD PIJI MOHD AL AKMARUL FIZREE[1]; OMAR ABD RASID[1] and GHULAM KADIR AHMAD PARVEEZ[1]

ABSTRACT

Particle bombardment is a transformation method that uses a mechanical injury mechanism to deliver deoxyribonucleic acid (DNA) into plant cells. In early studies, a few aspects influencing the efficiency of DNA delivery through biolistic have been determined. However, the developed protocol yields low transformation efficiency, which requires further improvement. Re-evaluation of bombardment parameters by monitoring the expression of reporter genes for a longer duration post-bombardment will provide more conclusive results on stable expression than the previous two-day evaluation. Several bombardment parameters were evaluated; helium pressure, distance between the stopping screen and tissues, size of gold, DNA quantity and the bombardment number. Each parameter was assessed by monitoring the expression of red fluorescent protein (RFP) transiently in oil palm embryogenic calli (EC). The optimal parameters were determined based on the highest RFP signals retained in the EC after three months of bombardment. The optimal parameters were obtained when EC were bombarded three times at 2,000 psi pressure using 1.0 µm of gold particles coated with 1.0 µg of DNA and the distance between the stopping screen and tissues was fixed at 6 cm. The optimal parameters could improve DNA delivery through bombardment and lead to the production of edited oil palm at high efficiency.

Keywords: oil palm, optimal parameters, particle bombardment, red fluorescent protein, transient expression.

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INTRODUCTION

Malaysia is the world's major producer and exporter of palm oil. It is one of the sectors contributing to Malaysia's economic growth (Parveez *et al*., 2022). Furthermore, palm oil also contributes to the world's oil and fat supply and demand has increased over the years. The challenges such as climate change, disease and the limitations of arable farmland have affected palm oil production. Research on genetic enhancements is likely to be the potential solution for achieving maximum yields (Masani *et al*., 2018; Parveez *et al*., 2015). Genetic enhancements through genome editing, such as CRISPR/Cas9 technology, could provide opportunities for plant improvement by precisely targeting gene modification at a specific site. Recently, genome editing studies have been initiated to edit the oil palm genome precisely (Bahariah *et al*., 2023; Jamaludin *et al*., 2023; Yeap *et al*., 2021) which will lead to the production of improved planting materials and subsequently fulfil palm oil supply in the future.

One factor affecting genome editing efficiency is the success of deoxyribonucleic acid (DNA) delivery into oil palm tissues. A few DNA delivery systems have been developed for oil palm, such as particle bombardment or biolistic (Parveez, 2000; Parveez *et al*., 2015), *Agrobacterium*-mediated transformation (Izawati *et al*., 2009; 2015), polyethylene glycol-mediated transfection and DNA microinjection

[1] Malaysian Palm Oil Board,
6, Persiaran Institusi, Bandar Baru Bangi,
43000, Kajang, Selangor, Malaysia.

* *Corresponding authors e-mail: fahisza@mpob.gov.my; masani@mpob.gov.my*

(Masani *et al*., 2014; 2022). The transformation efficiency of oil palm is relatively low (0.7%-1.5%), with the highest at 1.5% obtained from DNA delivery using the bombardment method (Parveez *et al*., 2015). In contrast, other crops, such as wheat, have reported a transformation efficiency of up to 12.9% (Miroshnichenko *et al*. 2020). Thus, the transformation efficiency in oil palm must be improved by optimising bombardment parameters.

Particle bombardment is a mechanical injury technique that delivers the foreign gene into the cells. It delivers rapid DNA and can transfer multiple genes in one shot (Ramkumar *et al*., 2020). Since this system has no biological or host limitations, it can target various plant species and tissues (Lacroix & Citovsky, 2020). Particle bombardment as an efficient DNA delivery method has been applied in many plant species, such as soybean (Khalafalla *et al*., 2005), rice (Zhao *et al*., 2011) and wheat (Melchiorre *et al*., 2002). For oil palm, the bombardment protocol was established by Parveez (2000). The protocol has been applied to transform and regenerate transgenic oil palms. However, molecular analyses revealed that most regenerated oil palms were escapes and chimeric (Nurfahisza *et al*., 2014). To overcome these problems, the protocol must be re-established to increase DNA delivery efficiency and produce more transformants. In addition, Parveez (2000) might have used different oil palm clones, influencing the established bombardment protocol. Therefore, using the same clone to optimise bombardment parameters is also important.

Since oil palm requires a long regeneration time, stable transformation results cannot be obtained quickly (Masani *et al*., 2018). Therefore, optimal bombardment parameters were determined through transient expression studies. This approach allows an easy and rapid assay and serves as a reliable indicator for stable transformation. In plant studies, the most commonly utilised reporter gene expression systems include β-glucuronidase (GUS) (Xiong *et al*., 2011), green fluorescent protein (GFP) (Parveez & Majid, 2018; Stewart, 2001), red fluorescent protein (RFP) (Okwuonu *et al*., 2015; Sun *et al*., 2018) and luciferase (LUC) (Hastings & Morin, 2006). The bombardment protocol reported by Parveez (2000) was based on the optimal parameters obtained from the GUS transient assay. Although GUS can be easily observed by staining with a substrate, its assay is laborious, disruptive, and very toxic to the cells; subsequently, monitoring of expression along with cell development is almost impossible.

In contrast, RFP, GFP and LUC expression can be visualised and monitored in real-time in living tissues. More importantly, gene expression can be observed along with plant development (Hastings & Morin, 2006; Stewart, 2001). RFP, such as the Discosoma Red Fluorescent Protein (*DsRED*) gene, derived from coral (*Discosoma* sp.), was chosen as a visual reporter gene in our study due to the advantage of RFP expression that could be monitored in all stages of plant development. Moreover, Fizree *et al*. (2019) reported that *DsRED* produced distinct bright RFP signals compared to the pale background in the non-transformed samples and showed improved signal retention in oil palm embryogenic calli (EC). *DsRED* has a much higher extinction coefficient and fluorescence quantum yield as compared to GFP (Baird *et al*., 2000), which showed no abnormalities in the regenerated transgenic plants (Jach *et al*., 2001) and is much easier to detect *DsRED* expression in green tissues compared to GFP (Jach *et al*., 2001). Due to this characteristic, *DsRED* has been used widely in plant transformation, such as in soybeans (Cho *et al*., 2022), wheat (Tanaka *et al*., 2022) and walnuts (Zhang *et al*., 2015).

In this study, we used a visual reporter gene (*DsRED*) and performed assessment times longer than those previously developed by Parveez (2000). Transient expression occurs almost immediately after gene transfer, where non-integrated DNA is eventually degraded by nucleases. Thus, the highest signal retained over a longer duration may have a higher possibility of integration into the plant genome, leading to a stable transformation. This study presents the current optimal bombardment protocol utilising *DsRED* as a reporter gene, which is potentially reliable for producing stable transgenic palms with higher efficiency.

MATERIALS AND METHODS

Plant Materials

Oil palm friable calluses derived from cabbage of P164 clones were obtained from the Clonal Propagation Group of the Malaysian Palm Oil Board (MPOB). The friable calluses formed after being cultured for one to three months were then induced to suspension calli using liquid induction media [MS salts (Murashige & Skoog, 1962), Y3 vitamins (Eeuwans, 1976), 0.1 g/L myo-inositol and L-glutamine, 3% sucrose, 5 μM 2,4-dichlorophenoxyacetic acid (2, 4-D)] and incubated in the dark at 28°C for six months. The EC formed were collected and cultured on Y3A-4 solid media (Masani *et al*., 2013; 2022) and incubated at 28°C for a week before bombardment.

Particle Bombardment Transformation

This study used a construct, namely pAMDsRED (Fizree *et al*., 2019), carrying the *DsRED* gene driven by the CaMV35S promoter as a plasmid

DNA for precipitation with gold particles. High concentration (>2.0 µg/µL) and good quality plasmid DNA (an A260/280 ratio between 1.8 and 2.0) was isolated from bacterial culture using NucleoBond PC 500, Maxi kit (Macherey Nagel) following the manufacturer's instructions. The precipitation procedure to bind gold particles with DNA was performed according to Nurfahisza *et al*. (2020).

The bombardment was performed according to the method described by Parveez (2000). Five parameters (*Table 1*) were examined independently during the bombardment. Standard parameters that were optimised by Parveez (2000) (marked with * in *Table 1*) were used as a control for comparison. Four replicates (each containing about 0.5 g of EC per plate) were carried out for each tested parameter. The bombarded calli were then incubated at 28°C in dark conditions and subcultured on fresh Y3A-4 solid media (Masani *et al*., 2013; 2022) without selection for every four weeks.

TABLE 1. FIVE BOMBARDMENT PARAMETERS WERE EXAMINED INDEPENDENTLY

Parameters	Treatments
Helium pressure (psi)	650, 900, 1,100*, 1,350, 1,550, 1,800, 2,000
Distance from stopping screen to tissue (cm)	6.0, 7.5*, 9.0, 10.5, 12.0
Number of bombardments	1*, 2, 3
Size of gold (µm)	0.6, 1.0*, 1.6
DNA quantity (µg)	1.0, 1.5*, 2.0

Note: The asterisk mark (*) represents the standard conditions (Parveez, 2000) used as control during the bombardment.

RFP Monitoring and Transient Assessment

RFP signals were observed through a Nikon AZ100 fluorescence microscope paired with an ET-mcCherry, Texas Red® filter set (Chroma, USA). An imaging system, QICAM-12bit (QImaging, Canada), was also attached to a fluorescence microscope to capture the image in real time. The RFP signals from four replicates of each treatment were observed and scored at a week, a month, two months and three months after the bombardment. Then, the average number of RFP signals and the standard error were calculated based on three months of observation data from four replicate plates of each treatment. The significant differences among the treatments were analysed using Duncan's multiple range test (DMRT) with $p \leq 0.05$. In addition, a graph was plotted to identify the optimal parameter based on the highest average number of RFP signals retained after three months of bombardment.

RESULTS AND DISCUSSION

To efficiently transfer gene constructs through bombardment, it is necessary to determine specific parameters that affect the efficiency of DNA delivery. A brief overview of the optimised parameters is illustrated in *Figure 1*. Five parameters that affect DNA delivery were systematically evaluated. Since the efficiency of particle bombardment in most plants is species- and genotype-dependent, all the samples used as explants in this study were from a similar genetic background. This study was carried out using EC from the P164 clonal palm. P164 is a high-yielding hybrid resulting from crosses between the *Dura* and *Pisifera* varieties (Amanina *et al*., 2022). Using biological samples from a similar P164 genetic background could reduce experimental variation and improve data precision. The efficiency of bombardment parameters was evaluated by monitoring and counting the red fluorescing spots or RFP signals optically for a week, a month and up to three months after bombardment. Usually, none or, most likely, very few RFP signals will be observed after three months of bombardment. This occurred possibly due to the inevitable loss of vector DNA after several cell replication cycles. After three months of bombardment, the RFP signals may have a higher potential for stable DNA integration into the plant genome. Thus, the optimal parameter was identified based on the highest average number of RFP signals retained three months after bombardment, since the scores imply the number of successful DNA that might be integrated into the targeted tissues.

Effect of Helium Pressure

The pressurised helium gas accelerated the DNA-gold particles to penetrate through different cell layers of tissues. The strength of acceleration may influence the depth of penetration and distribution of gold-DNA particles into the target tissues (Mahdavi *et al*., 2014). In the first week after bombardment, the highest average number of RFP signals was observed when EC was bombarded at 650 psi (367.75 spots). This was followed by 1,100 psi (289.50 spots), 2,000 psi (252.25 spots), 1,800 psi (198.00 spots), 1,350 psi (181.25 spots) and the least using 900 psi (121.25 spots) (*Figure 2*). Meanwhile, bombardment using 1,550 psi only showed 29.50 spots of RFP signals. After a month and two months of bombardment, a pressure of 650 psi maintains the highest average number of RFP signals among the tested pressures. However, after three months of bombardment, the average number of RFP signals dropped about five-folds for 650 psi (from 33.25-6.50 spots) compared to two-folds for 2,000 psi (from 14.00-7.75 spots). Since the pressure at 2,000 psi could

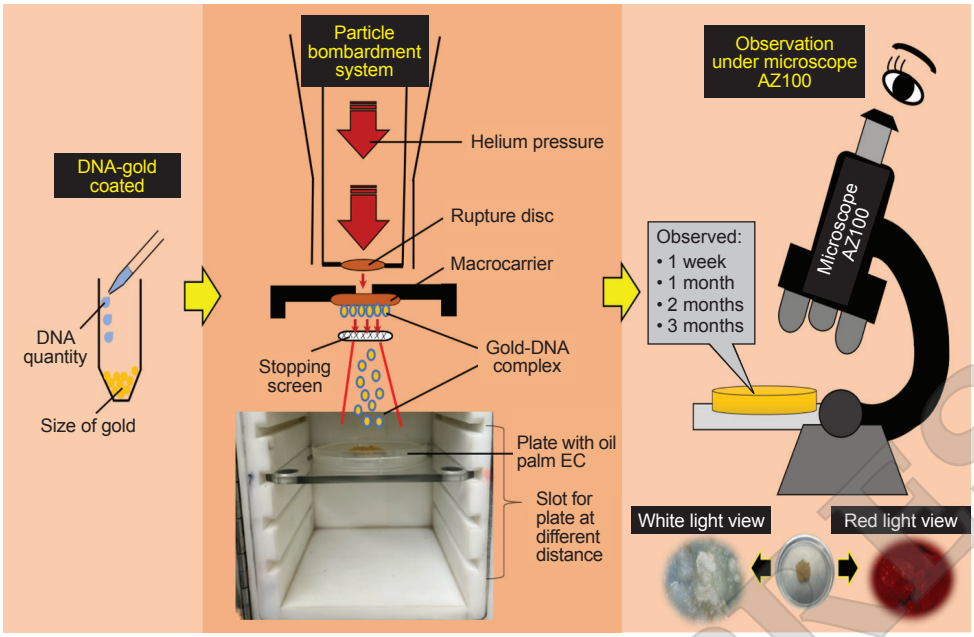


Figure 1. A brief overview for optimisation of bombardment parameters affecting the transient expression of DsRED *gene in oil palm EC.*

retain the highest average number of RFP signals after three months, this parameter was chosen as the optimal helium pressure for oil palm bombardment.

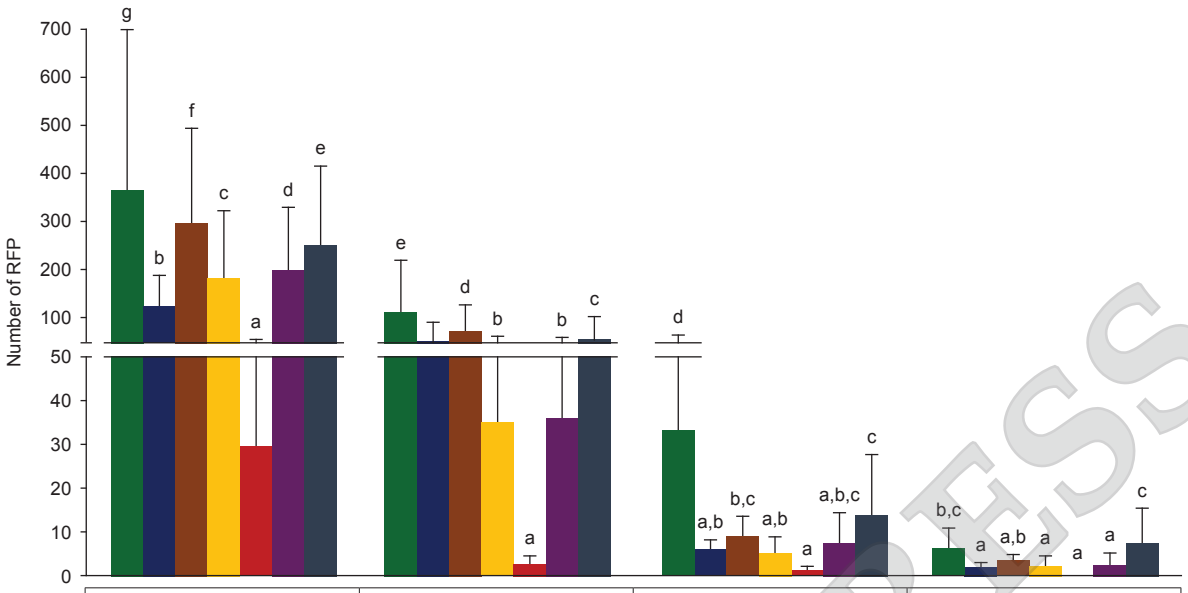
The result contrasted with the studies reported by Liu *et al*. (2024) in Chinese cabbage microspore, Mahdavi *et al*. (2014) in male banana flowers, and Mousavi *et al*. (2009) in date palm EC, which showed that helium pressure at 1,100 psi is the most optimal pressure to penetrate the target tissues. Previous studies in oil palm EC also showed 1,100 psi as the optimal pressure (Nurfahisza *et al*., 2020; Parveez, 2000). The contrasted results may be attributed to different biological materials for bombardment and various durations for evaluating transgene expression. This present study used EC derived from the cabbage of the P164 clone, while Parveez (2000) used EC derived from various clones and tissues, such as immature embryos, leaflets and roots. Meanwhile, the optimal pressure at 1,100 psi evaluated by Nurfahisza *et al*. (2020) was based on the number of putative transgenic shoots generated. A study on tobacco showed the best pressure at 1,350 psi (Davlekamova *et al*., 2023). Moreover, a study by Tee and Maziah (2005) showed the different optimal pressures obtained from the same species, *Dendrobium* Sonia 17, which were 1,100 and 650 psi for type-A and type-B calli, respectively. Our result showed that 2,000 psi was the best pressure to penetrate through oil palm P164 clonal EC. Using low-pressure bombardment may cause low penetration of gold-DNA into target tissues (Mousavi *et al*., 2009; Parveez, 2000).

Effect of Distance from Stopping Screen to Tissue

The distance between the stopping screen and the tissue can be adjusted according to the slot provided in the biolistic device, which is a distance of 6.0, 9.0 and 12.0 cm. The distance at 7.5 and 10.5 cm can be adjusted when an extra plate is put on stages at 9.0 and 12.0 cm slots, respectively. It is necessary to determine the optimal distance to distribute the DNA-gold particles evenly to the target tissue without causing severe tissue damage (Chernobrovkina *et al*., 2007).

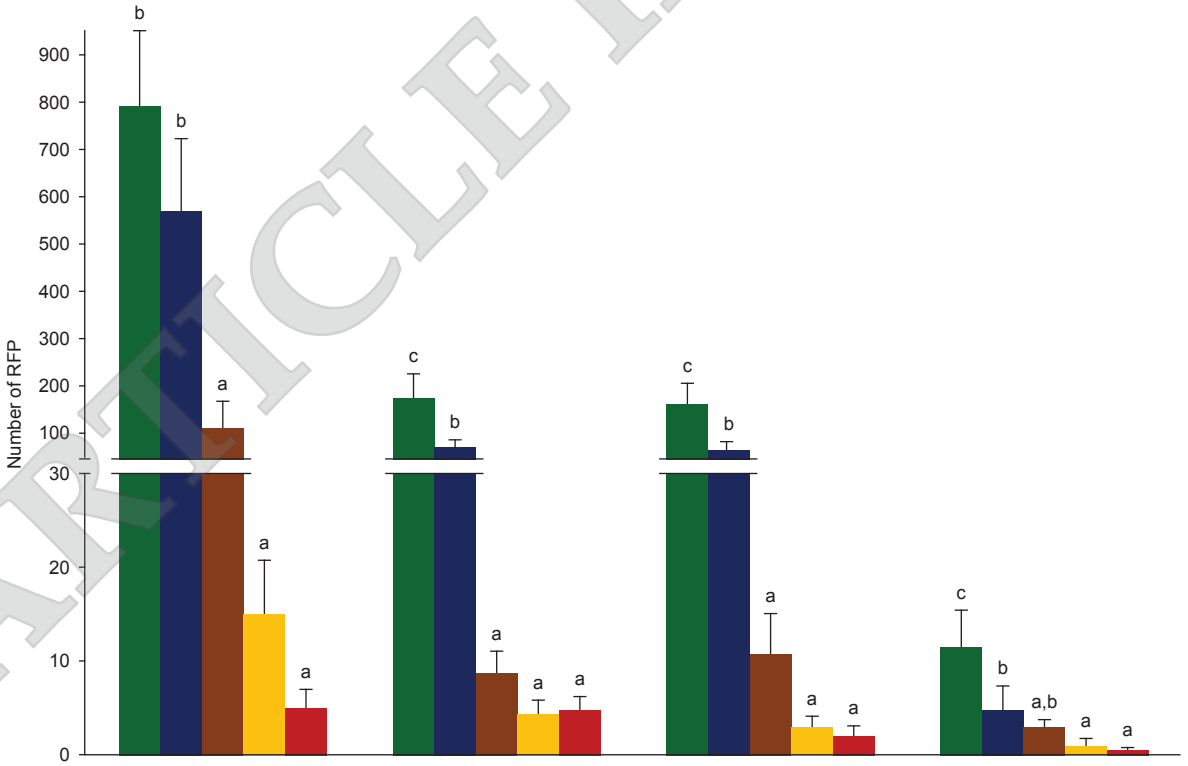
At a week of bombardment, the highest average number of RFP signals (789.75 spots) with significant differences ($p<0.05$) were obtained when using 6 cm. The average number of RFP signals observed in EC that were bombarded using a distance of 6 cm was continually maintained as the highest number (compared with other tested distances) for a month (141.25 spots), two months (132.25 spots) and three months (11.50 spots) after bombardment, even though the signal count has reduced throughout observation period (*Figure 3*). The second and third highest average number of RFP signals were recorded at a distance of 7.5 and 9.0 cm, respectively. Meanwhile, 10.5 cm and 12.0 cm showed very low signal counts from the first to the last observation.

Significant differences in RFP signal numbers were observed between the shortest (6 cm) and longest (12 cm) distances. The distance between the stopping screen and the target tissues possibly influences the number of RFP signals obtained. If the distance is too far, the DNA may not reach and



	1 week	1 month	2 months	3 months
650 psi	367.75	112.50	33.25	6.50
900 psi	121.25	51.00	6.25	2.25
1,100 psi	298.50	72.00	9.25	3.75
1,350 psi	181.25	35.00	5.50	2.50
1,550 psi	29.50	3.00	1.50	0.00
1,800 psi	198.00	36.00	7.75	2.75
2,000 psi	252.25	56.00	14.00	7.75

Figure 2. Effects of helium pressure on DsRED *expression in oil palm EC.*



	1 week	1 month	2 months	3 months
6.0 cm	789.75	141.25	132.25	11.50
7.5 cm	569.25	41.25	41.00	4.75
9.0 cm	107.25	8.75	10.75	3.00
10.5 cm	15.00	4.25	3.00	1.00
12.0 cm	5.00	4.75	2.00	0.50

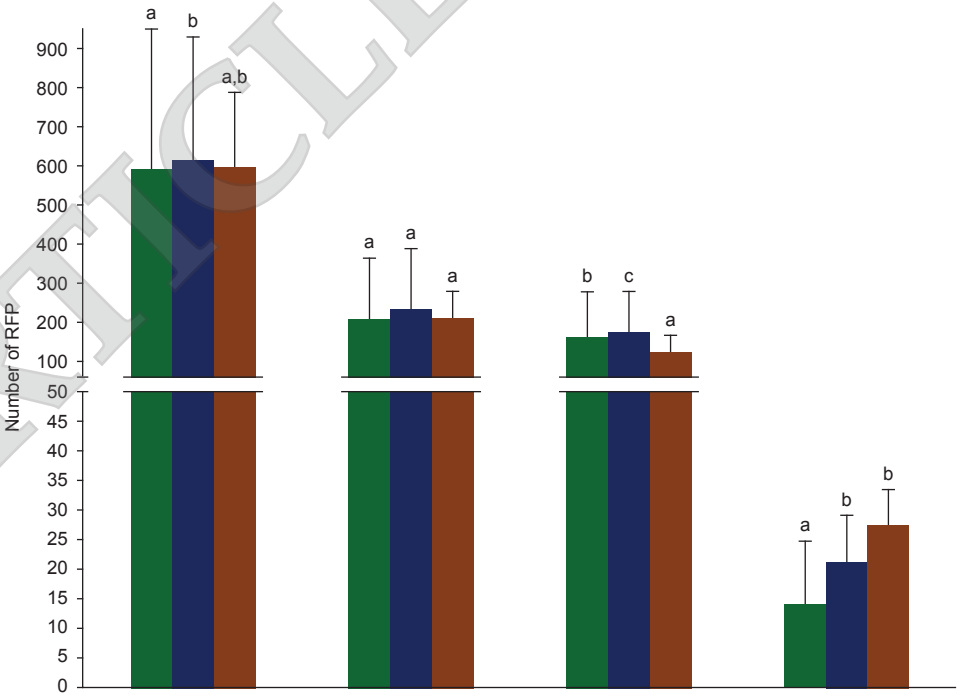
Figure 3. Effects of distance from stopping screen to tissue on DsRED *expression in oil palm EC.*

penetrate the target tissue efficiently (Nurfahisza *et al*., 2020). Moreover, the gold-DNA acceleration through the tissue may reduce across the distance (Parveez *et al*., 1997). This study showed that 6 cm was the optimal distance, whereby the RFP signals were consistently the highest during the period of observation. The result was in agreement with other studies conducted on soybeans (Khalafalla *et al*., 2005), bananas (Mahdavi *et al*., 2014) and Chinese cabbage (Liu *et al*., 2024), which showed 6 cm from the stopping screen to the tissue as the optimal distance for bombardment.

Effect of Number of Bombardments

Generally, three-times bombardments may deliver a high amount of DNA into tissue as more DNA is being penetrated into tissue. However, multiple bombardments tended to create severe injury and cause the cell not to be able to survive for tissue development. The injured cells may cause the delivered gene not to be expressed (Dhir *et al*., 2010; Mahdavi *et al*., 2014). Furthermore, the capacity of cell damage may also depend on the target tissue type. During the first week of observation, there was no significant difference in the number of bombardments tested on oil palm EC (*Figure 4*). The average numbers of RFP signals for one, two and three-times bombardment were 587.5, 611.0 and 594.5 spots, respectively. After three months of observation, the RFP signals were reduced for each treatment, but the lowest average of RFP signals was observed when EC was treated with a one-time bombardment (14.0 spots). Meanwhile, the highest average of RFP signals was observed when EC was bombarded three times (27.5 spots).

Multiple bombardments might cause the target tissue to undergo severe injury and subsequently reduce its ability to generate shoots. Studies in oil palm (Nurfahisza *et al*., 2020; Parveez *et al*., 1997), medical plant (*Erigeron breviscapus*) (Zhao *et al*., 2023) and banana (Sreeramanan *et al*., 2005) showed one-time bombardment was enough for high transgene expression. Contrary, studies in soybean (Khalafalla *et al*., 2005), Indian mulberry (Bhatnagar *et al*., 2002), *Centella asiatica* (Lai *et al*., 2011) and orchid (Da Silva & Tanaka, 2009) revealed that the highest transgene expressions were obtained when the samples were bombarded two-times compared to one or three-times of bombardment. These studies agree that multiple bombardments increase the transformation efficiency but may cause low tissue survivability when bombarded three times. However, our results showed that three-time bombardments gave much better RFP signals than one-time bombardments. Bombardment for more than one time might produce higher RFP signals as the coverage area of bombardment increases. Besides that, multiple bombardments may improve penetration through compensation from misfires caused by faulty or improper rupture disk



	1 week	1 month	2 months	3 months
1x	587.50	207.25	156.50	14.00
2x	611.00	232.50	172.75	21.25
3x	594.50	209.25	120.00	27.50

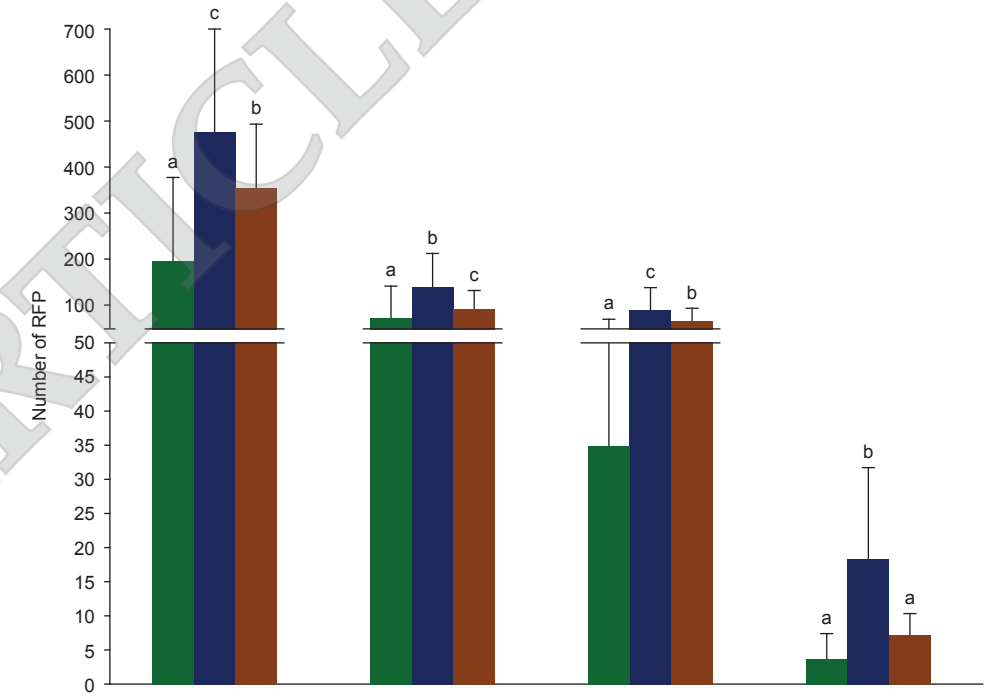
Figure 4. Effects of bombardment number on DsRED *expression in oil palm EC.*

set-up. Fitch *et al.* (1990), Kuehnle and Sugii (1992), and Zhao *et al*. (2018), also showed three-times bombardments as efficient transformations for liana, orchid and papaya, respectively. These types of plants belong to woody plants, which are related to the degree of lignification of the tissues that cause less injury (Zhao *et al*., 2018). Similarly, three-times bombardments were seen as the optimal number of bombardments to improve penetration and increase the transformation efficiency of oil palm. Higher signal expression at three times bombardment demonstrated the ability of oil palm EC to survive and subsequently regenerate into plants.

Effect of Size of Gold Particles

Gold particles act as a microcarrier to assist the DNA in penetrating target tissues. Even though gold particles are expensive, they are uniform in size and biologically inert (Bhatnagar *et al*., 2002; Dhir *et al*., 2010). The different sizes of gold particles (0.6, 1.0 and 1.6 µm) may affect the number of DNA carried into the target tissue and it also affects the capacity for tissue damage. The significantly highest average number of RFP signals was obtained at 1.0 µm followed by 1.6 and 0.6 µm at all observation periods (*Figure* 5). The 1.0 µm is the medium size of gold particles which showed better penetration into oil palm tissues compared to the smallest (0.6 µm) and largest (1.6 µm) sizes tested. Since a significant difference was observed among the treatments, the results conclusively indicated that the treatment with 1.0 µm size scored the highest number of RFP signals retained until three months (*Figure 6e-h*) compared to treatments with 0.6 µm (*Figure 6a-d*) and 1.6 µm of gold particles (*Figure 6i-l*).

Several studies showed that the smallest size of gold particles (0.6 µm) gave efficient DNA penetration into explants, such as in soybean (Khalafalla *et al.*, 2005), sorghum (Wang *et al*., 2021) and maize (Sreenu *et al*., 2016). At the same time, studies in date palm (Mousavi *et al*., 2009) and sunflower (Mohamed *et al*., 2006) were in contrast to our findings as the highest gene expressions were obtained when the DNA was precipitated using 1.6 µm of gold particles. However, most studies in plants such as rice (Mukhtar & Hasnain, 2018), banana (Mahdavi *et al*., 2014) and giant reed (Dhir *et al*., 2010) have documented that 1.0 µm is the suitable size of gold particles for bombardment. Theoretically, the large size of gold particles may carry a higher amount of DNA, which may increase the expression of the transgene. However, factors such as tissue types could have different impacts. A study by Ma *et al*. (2013) showed that hard tissues like stems needed 1.6 µm gold particles compared to calli, which only required 1.0 µm gold particles for better penetration.



	1 week	1 month	2 months	3 months
0.6 µm	196.50	72.75	35.00	3.75
1.0 µm	480.00	141.50	89.75	18.50
1.6 µm	356.75	92.00	66.25	7.25

Figure 5. Effects of gold particle sizes on DsRED *expression in oil palm EC.*

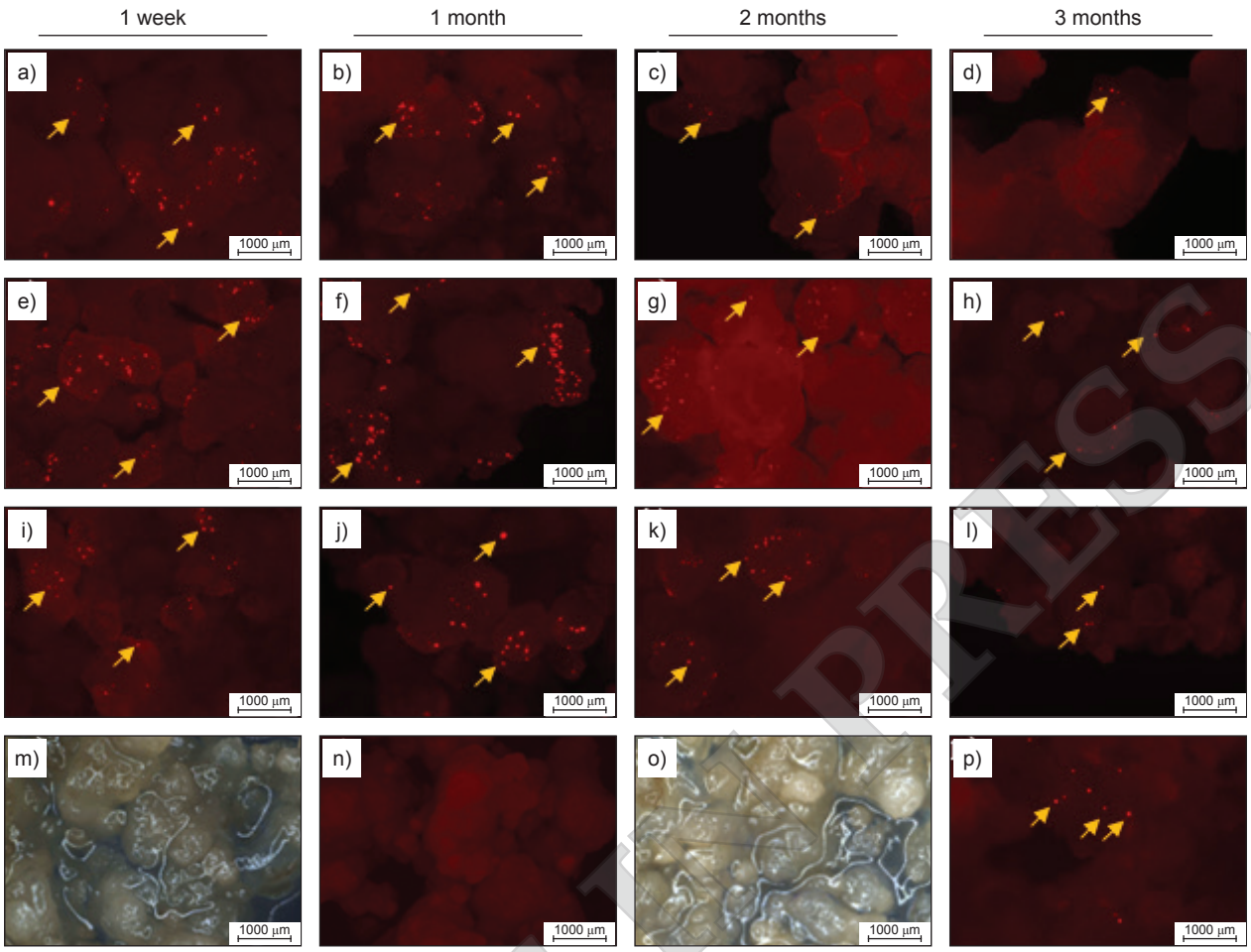


Figure 6. RFP spots observed in oil palm EC bombarded with gold particles, at size (a-d) 0.6, (e-h) 1.0 and (i-l) 1.6 µm. No RFP spots were observed for non-bombardment control, (m) under white light and (n) red light. No RFP spots were observed for bombarded calli, (o) under white light but the spots were observed under (p) red light. The yellow arrow indicated RFP spots in EC.

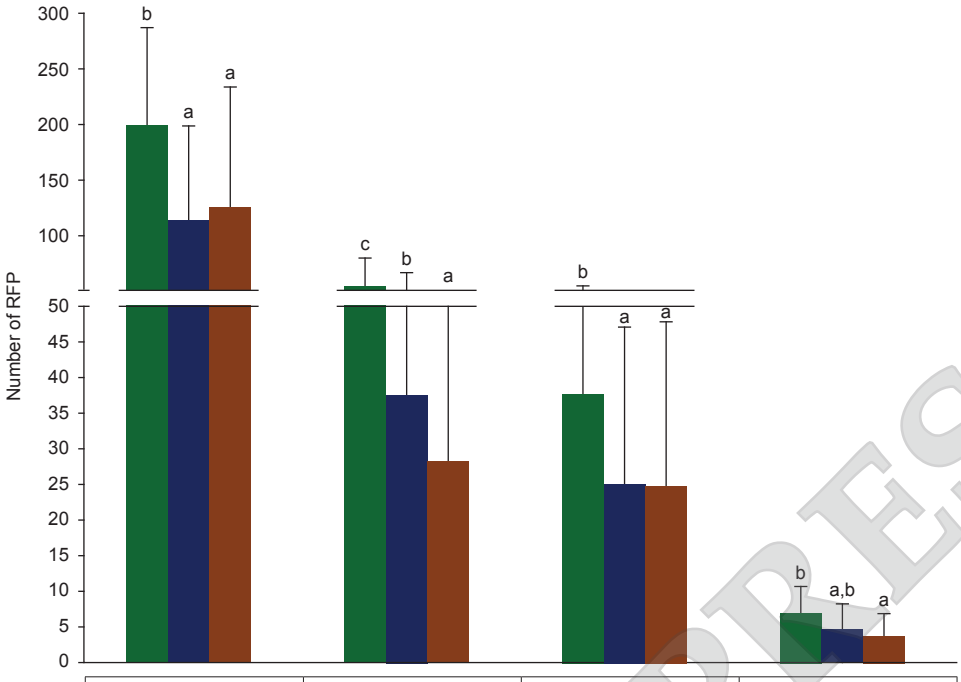
Effect of DNA Quantity

Gold particles could coat the appropriate amount of DNA for efficient delivery of DNA into tissue (Mookkan, 2018). The concern is choosing the right amount of DNA to avoid using a high concentration of DNA that may cause large aggregation. The large aggregation may cause inefficient DNA delivery. Thus, three different DNA quantities were evaluated: 1.0, 1.5 and 2.0 µg. In this study, RFP signals observed in the first week up to three months showed bombardment with 1 µg of DNA, resulting in the highest average number of RFP signals compared to 1.5 and 2.0 µg of DNA (*Figure 7*). The results contrasted with our expectations, as the lowest DNA quantity consistently showed the highest RFP signals throughout the observation.

Generally, the transgene expression may reduce when less DNA is transferred into the targeted tissues. The results may be due to several factors, such as the ratio of gold-DNA precipitation to form aggregates, which might affect the DNA transfer. Our results demonstrated that 1.0 µg of DNA was the appropriate ratio of DNA to bind with gold particles. It was noticeable that the RFP signals reduced when the DNA quantity increased, indicating an amount greater than 1.0 µg may cause improper DNA coating. This may contribute to improper particle aggregation, resulting in inefficient DNA delivery. A study by Mahdavi *et al*. (2014) on bananas showed that 1.5 µg of DNA per bombardment was the optimal DNA quantity. They also concluded that transient gene expression was reduced at lower (less than 1.5 µg) and higher (more than 1.5 µg) DNA quantities due to insufficient DNA-microcarrier binding and poor cell penetration efficiency, respectively.

Optimal Bombardment Parameters of Oil Palm EC

In this study, the efficient bombardment transformation system was determined by studying the effect of different bombardment parameters. Simultaneously, we are systematically evaluating the amenability of the P164 clone for oil palm transformation. The optimal parameters were identified based on the highest number of RFP



	1 week	1 month	2 months	3 months
1.0 µg	199.25	55.25	37.75	6.75
1.5 µg	114.25	37.50	25.00	4.50
2.0 µg	126.50	28.00	24.75	3.50

Figure 7. Effects of DNA quantity per bombardment on DsRED *expression in oil palm EC.*

signals retained after three months of bombardment. This study revealed that, except for gold particle sizes for bombardment, the optimal parameters differed from those identified by Parveez *et al*. (1997; 1998). The differences in penetration impact during bombardment may relate to genotype and target tissue types. Parveez *et al*. (1997; 1998) used EC from diverse genetic backgrounds, whereas this study focused solely on P164 clone calli, potentially explaining the optimal parameter discrepancy. Additionally, Parveez *et al*. (1998) showed varying GUS expression in EC derived from different tissue types, with calli from immature embryos exhibiting the highest GUS expression compared to those from leaflets or roots. However, most researchers will prefer using leaflets for the successful initiation of EC (Yarra *et al*., 2019). Therefore, using biological materials from the same genetic background in empirical studies is preferable for generating precise data with fewer variables.

On the other hand, in previous studies by Parveez *et al*. (1997; 1998), the transient GUS expressions were monitored after two days of bombardment, contrary to our data which monitored for a longer time, three months after the bombardment. The signals of expression remaining at a longer period showed the stability of transformation compared to the shortened time. Besides that, the highest signals observed at the early observation did not exactly remain high after a long time. For example, in helium pressure and the number of bombardment studies, the high signals observed in the first week were scored at 650 psi and two times bombardment, respectively. Later observation at three months after bombardment showed the highest signal for helium pressure, and the number of bombardments was at 2,000 psi and three times of bombardment, respectively. The reduced number of signals over the period may be because of the DNA not integrated into the plant genome or the foreign DNA being degraded in the cytoplasm. Thus, it is not too speculative to suggest that data obtained over a longer time of monitoring is much more convincing as it reflects the reliability of stable transformation.

CONCLUSION

Further improvement of DNA delivery into the oil palm through bombardment is necessary since the developed protocol produced a low transformation efficiency. Modification of transient expression involving a longer duration of assessment using *DsRED* as a reporter gene gave a more definitive conclusion on stable expression over the previous two-day evaluation using the GUS destructive assay. By monitoring the highest *DsRED* expression that was retained in plant cells for up to three months and using sample from the same genetic background, a new optimal protocol was determined. The 2,000 psi helium pressure,

the 6 cm distance of stopping screen to tissue, three-times bombardment, using 1µm of gold and 1.0 µg of DNA per bombardment were identified as optimal parameters for oil palm transformation. These optimal parameters could lead to further improvements in oil palm transformation, leading to the production of transgenic or edited palms at a higher efficiency in the future.

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