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Evaluation of the Expression Patterns of Mesocarp-specific Promoter MSP-C6 in Transiently Transformed Oil Palm Tissues

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ABSTRACT

Genetic manipulation of oil synthesis in the mesocarp tissue of oil palm may ensure sustainability of palm oil production and fulfil the high demand for palm oil. The suitability of newly isolated promoters in driving a gene must be tested before they can be used for gene manipulation, hence an easy, rapid and manageable method for large sample size is needed. This study aimed to examine the mesocarp-specific expression of the MSP-C6 promoter in oil palm. The MSP-C6 promoter fragments of varying lengths were tested in regulating the *red fluorescent protein* (*DsRED*) gene in oil palm tissues through biolistic- and polyethylene glycol (PEG)-mediated transient transformation. Our results showed that the MSP-C6 promoter fragments were not able to express the *DsRED* gene in the bombarded mesocarp tissue. However, the DsRED signals were observed in the bombarded polyembryoid tissue driven by the MSP-C6-F1 (2014 bp) promoter. Meanwhile, the *DsRED* expression was also not observed in all the protoplasts isolated from mesocarp tissue transformed by PEG-mediated method. Based on these results, the transient assays of MSP-C6 promoter fragments in oil palm tissues may need further optimisation. This is important to confirm the mesocarp-specific expression of the MSP-C6 promoter as in previous stable transformation of tomato plants. Confirmation of the tissue specificity of the MSP-C6 promoter is crucial before this promoter can be used for the modification of lipid composition in mesocarp tissue oil palm fruit.

Keywords: Biolistic-mediated transient transformation, mesocarp-specific promoter, oil palm, polyethylene glycol (PEG)-mediated transient transformation.

INTRODUCTION

The *MSP-C6* gene was identified as a stearoyl-acyl carrier protein desaturase (SAD), which catalyses the initial double bond insertion into stearic acid to produce monounsaturated oleic acid. MSP-C6 plays a key role in controlling unsaturated fatty acid levels in palm oil. According to a differential expression analysis by RNA-Seq, the *MSP-C6* transcript was found to be highly expressed during oil synthesis, from the maturation phase at 18 weeks after anthesis (WAA) until the end of the ripening phase (24 WAA) of mesocarp development (Badai et al., 2023). In addition, the *MSP-C6* gene was shown to be preferentially expressed in oil palm mesocarp tissues by reverse transcription quantitative real-time PCR (RT-qPCR) (Badai et al., 2023). Both analyses showed that the promoter region of MSP-C6 could be a potential tool for manipulating oil biosynthesis and lipid composition in the mesocarp tissue of oil palm.

The activity and specificity of the promoter in regulating transgene expression can be examined using stable and transient transformation methods. Stable transformation involves the integration of foreign DNA permanently into the host genome and maintenance of the inherited gene during mitotic and meiotic cell divisions. Meanwhile, transient transformation involves the introduction of foreign DNA into the cell for temporary gene expression (Altpeter et al., 2016). Functional characterisation of the MSP-C6 promoter using *Agrobacterium*-mediated stable transformation of tomato showed that the MSP-C6-F1 (2014 bp) promoter was able to drive mesocarp-preferential expression in both unripe and ripe transgenic tomato fruits (Badai et al., 2023).

The transient gene expression in plants has considerable advantages over stable expression because it does not require regeneration of the transformed cells, has no effect on the stability of the host genome, is independent of the position effects of the T-DNA integration sites, and can provide rapid screening of functional promoters for a large number of candidate genes (Tyurin et al., 2020; Xian et al., 2023). These advantages have made transient expression systems the preferred choice for short-term study of promoter activity in oil palm because of the long regeneration time around 20 - 25 years, inconsistent callus performance during tissue culture processes, and time-consuming and laborious screening of oil palm transgenics (Jamaludin et al., 2023). In this study, we conducted a functional analysis of a series of MSP-C6 promoter fragments through biolistic- and polyethylene glycol (PEG)-mediated transient expression in oil palm tissues, to examine the feasibility of replacing the laborious and time-consuming stable plant transformation assay with a faster transient assay of promoters in the laboratory. A fast and easy method is necessary for screening and characterising new promoters; however, the method must also reflect the actual expression regulated by the promoters.

MATERIALS AND METHODS

Amplification of Promoter Sequences

Promoter and 5'UTR sequences that are ~ 2 kb upstream from the translation start site of the *MSP-C6* gene were obtained from the Malaysian Oil Palm Genome Programme (MyOPGP) (http://genomsawit.mpob.gov.my/). The MSP-C6 promoter sequence can be found in the GenBank database under the accession number OR459827. Nested polymerase chain reaction (PCR) was performed to amplify the promoter region using promoter-specific primers (Table 1). The first set of primers amplified the upstream sequences (~ 2 kb) relative to the translational start site and a part of the coding region (~ 6 bp). The PCR product amplified with the first set of primers was used as template for second round PCR using the second pair of primers. The PCR mix contained 125 ng of DNA, 1X high fidelity buffer, 6 mM of $MgSO_4$, 0.4 mM dNTPs, 0.4 µM forward and reverse primer and 1 unit of high-fidelity platinum *Taq* DNA polymerase (Thermo Fisher Scientific) in a 25 µL total volume. The PCR was conducted using an Eppendorf™ Mastercycler™ Nexus Thermal Cycler (Thermo Fisher Scientific) with a thermal profile consisting of 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 sec, 52 - 55 °C for 30 s (depending on the annealing temperature) and 72 °C for 2 min 20 s, with a final extension at 72 °C for 7 min. A-overhang for PCR product was generated by adding GoTaq® Green Master Mix (Promega) and further incubated at 72 °C for 10 min. PCR products were checked by electrophoresis in 1% (w/v) agarose gel, at 120 V for 60 min.

Cloning of MSP-C6 Promoter Fragments into paMDsRED Vector

A series of MSP-C6 promoter fragments with different lengths were obtained by PCR using two reverse primers and five forward primers located at different sites of the regulatory region of the promoter. The primers were designed with restriction sites at the 5'-end (*Pst*I in the forward primers and *Xho*I in the reverse primers) (Table 1). The PCR-amplified promoter was then ligated into pGEM®-T Easy vector and transformed into *Escherichia coli* DH5α competent cells. Plasmid that contains the candidate promoter was

isolated, digested with *Pst*I and *Xho*I restriction enzymes and confirmed using DNA sequencing. A paMDsRED vector carrying *red fluorescent protein* (*DsRED*) gene derived from *Discosoma* sp. (Fizree et al., 2019) was used as a reporter gene for monitoring the activity of promoter fragments in biolistic- and PEG-mediated transient expression assays. The fragment harbouring the MSP-C6 promoter was then inserted into the *Pst*I/*Xho*I sites of paMDsRED.

Biolistic-Mediated Transient Expression Assay

Various oil palm tissues in this study were obtained from commercial *tenera* planting materials (*dura* × *pisifera*). Mesocarp tissues at 9 WAA, 12 WAA, 15 WAA and 17 WAA were sterilised in 20% (v/v) commercial bleach (Clorox), which contained 5.25% of sodium hypochlorite for 20 min followed by rinsing with sterile distilled water seven times for 2 min each. Meanwhile, spear leaves were rinsed with sterile distilled water and sterilised with 70% (v/v) ethanol. Stem, lateral roots and polyembryoid tissues were obtained from tissue culture materials. All tissues were excised into 1 × 1 cm sections except for the polyembryoid. All explants were placed at the centre of a petri dish (in an area of approximately 3 cm in diameter) containing solidified Y3A-4 medium [Y3A media supplemented with 1 µM 1-naphthaleneacetic acid (NAA) (Sigma-Aldrich) and 0.1 µM 6-benzyleadenine (BA) (Sigma-Aldrich) (Masani et al., 2013) and incubated at 28 °C overnight before bombardment.

Particle bombardment was conducted using PDS-1000/He^{TM} biolistic particle delivery system (Bio-Rad). The plasmid DNA with the promoter sequence (20 µg) was mixed with 20 µg of plasmid 35ShrGFP harbouring the *GFP* gene driven by CaMV35S promoter. Plasmid 35ShrGFP was used as a quantitative reference for the comparison of the candidate promoter of interest with CaMV35S promoter. The pre-mixed plasmid DNA mentioned above (40 µg), 100 µL of 2.5 M $CaCl_2$, and 40 µL of 0.1 M spermidine were added sequentially to 100 µL 1 µm of gold particles (Bio-Rad), with continuous mixing for 3 min, followed by centrifugation at 9503 x*g* for 30 sec. The supernatant was removed and the particles were washed twice with 500 µL of 100% (v/v) ethanol, followed by centrifugation at 9503 x*g* for 30 sec. Finally, DNA-coated gold particles were resuspended in 120 µL of absolute ethanol.

For each co-bombardment, 10 µL of DNA-coated gold particles was dispensed onto the center of a macrocarrier and air dried under sterile conditions. The sample was placed in the bombardment chamber in vacuum condition at 27 mmHg. Transient transformation of oil palm tissues was performed based on the parameters optimised by Parveez et al. (1997) using rupture disks at 1,550 psi for mesocarp, male inflorescence, spear leaves, stem and lateral roots. Rupture disks at 1,100 psi were used for polyembryoid tissues (Parveez, 2000). Each tissue type was bombarded with three technical replicates. The bombarded tissues were incubated at 28 °C in the dark for 2 days. Visualisation of transiently red, fluorescent signals and green fluorescent signals on the bombarded samples was carried out using the Nikon AZ100 stereo-microscope equipped with ET-mCherry, Texas Red and ET-EGFP (FITC/Cy2) filter set (Chroma). The images were captured using QICAM-12bit (QImaging) and analysed using the NIS-BR software (Nikon).

Transformation of Oil Palm Protoplasts Mediated by PEG

Transformation of protoplasts isolated from mesocarp at 15 WAA was performed according to the method described by Fizree et al. (2021) and Masani et al. (2022).

Table 1. List of promoter-specific primers for amplification of MSP-C6 promoter in different lengths.

Gene name	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	Annealing temperature (°C)	Position relative to translational start site
	(First round PCR)			
MSP-C6	MSP-C6-F-NPCR AATGATATAGCTCCAAATTACACTCGAAGC	MSP-C6-R-NPCR CGCCATTGCCTCTTCTCCTTTTC	52	-2040 to +6
	(Second round PCR)			
MSP-C6 (F1)	MSP-C6-F1 GGCTGCAGGATATAGCTCCAAATTACAC *Pst*I	MSP-C6-R1 CCCTCGAGCCTTCTTCTTCCTTCTTTTC *Xho*I	54	-2037 to -24
MSP-C6 (F2)	MSP-C6-F1 GGCTGCAGGATATAGCTCCAAATTACAC *Pst*I	MSP-C6-R2 CCCTCGAGAGTGTGTAAATTAGATGCAC *Xho*I	54	-2037 to -320
MSP-C6 (F3)	MSP-C6-F2 GGCTGCAGGCACCGTCTAAGTTGAG *Pst*I	MSP-C6-R1 CCCTCGAGCCTTCTTCTTCCTTCTTTTC *Xho*I	55	-1618 to -24
MSP-C6 (F4)	MSP-C6-F3 GGCTGCAGTTTCATATCCCTCTCGTCAC *Pst*I	MSP-C6-R1 CCCTCGAGCCTTCTTCTTCCTTCTTTTC *Xho*I	55	-1133 to -24
MSP-C6 (F5)	MSP-C6-F4 GGCTGCAGAAGTCCAGAAGCACATAG *Pst*I	MSP-C6-R1 CCCTCGAGCCTTCTTCTTCCTTCTTTTC *Xho*I	55	-782 to -24
MSP-C6 (F6)	MSP-C6-F5 GGCTGCAGAGATAGTAAATTTCATTCAATGG *Pst*I	MSP-C6-R1 CCCTCGAGCCTTCTTCTTCCTTCTTTTC *Xho*I	53	-594 to -24

RESULTS AND DISCUSSION

Isolation of the MSP-C6 Promoter and Transient Expression Analysis in Oil Palm Tissues

The *MSP-C6* gene consists of three exons flanked by two introns (Figure 1A). The 5'-upstream sequence of MSP-C6 amplified by nested PCR (Figure 1B) consists of 283 bp of 5'UTR and a 1731 bp-promoter sequence (Figure 1C). The results of DNA sequencing showed that the amplified products of MSP-C6 matched 100% with the sequence obtained from the MyOPGP. The upstream sequence of MSP-C6 (2014 bp) was cloned into the paMDsRED vector and fused to the DsRED from *Discosoma* sp. as a reporter protein for gene expression, i.e., pMSP-C6-F1-DsRED (Figure 2).

Our previous RT-qPCR study showed that the expression level of the *MSP-C6* gene was more than 10-fold in all mesocarp tissues and below 3-fold in polyembryoid tissue (Badai et al., 2023). In addition, another gene expression study of the *SAD1* gene, which encodes a different isoform of MSP-C6, also produced similar results (Hanifiah et al., 2018) whereby the *SAD1* gene was highly expressed in the mesocarp tissues from the cell division phase (7 WAA) to the maturation phase (19 WAA). The stable expression of the MSP-C6-F1 promoter in tomato was also found to express the *GUS* gene preferentially in the mesocarp tissue of both unripe and ripe transgenic tomato fruits (Badai et al., 2023). The upstream region of MSP-C6 was postulated to regulate the expression of the reporter gene in the mesocarp tissues by binding to specific transcription factors and their associated cofactors at a specific time in development enabling the oil palm to tune its gene expression in a tissue-specific manner (Swift and Coruzzi, 2017).

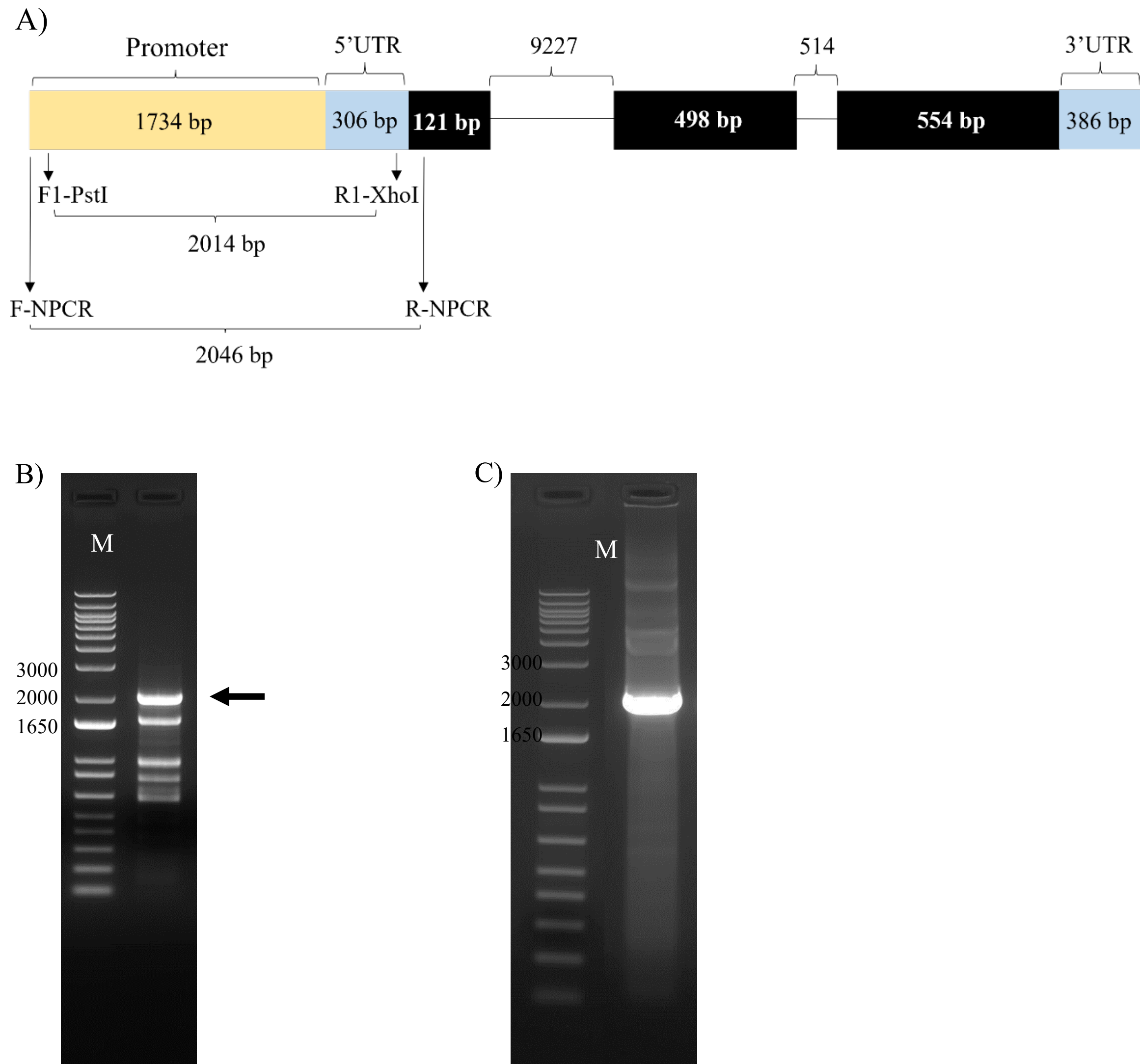

Figure 1. Cloning of the MSP-C6 promoter. A) Schematic representation of *MSP-C6* gene structure. Based on the MyOPGP, the upstream region of MSP-C6 consists of 306 bp of 5'UTR and 1734 bp of promoter sequence. B) The nested PCR using the first set of primers (F-NPCR and R-NPCR). A few PCR products including the expected PCR product (2046 bp) were observed on the gel. C) The nested PCR using the second set of primers (F1-*Pst*I and R1-*Xho*I). A single specific PCR product of the expected size (2014 bp; consisting of 283 bp of 5'UTR and 1731 bp promoter sequence) was obtained. M, marker 1 kb plus DNA ladder.

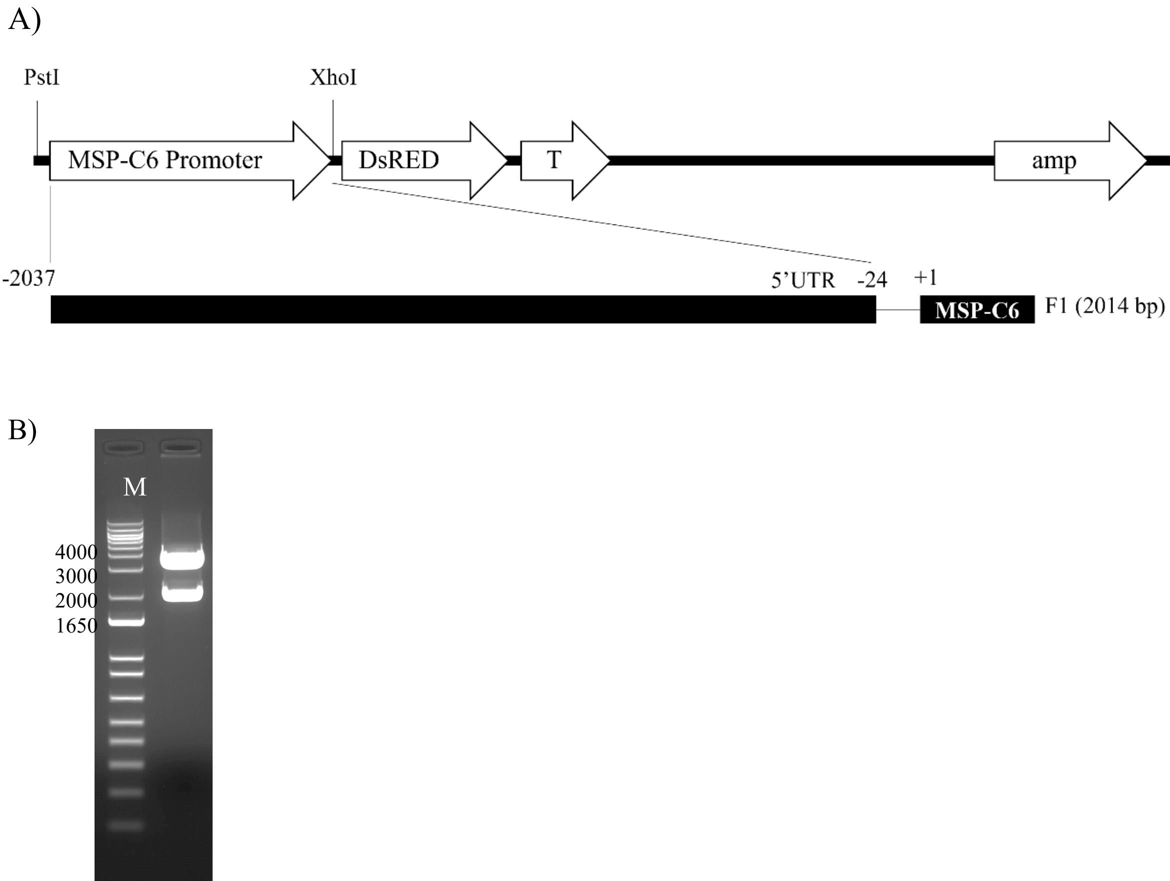

Figure 2. Cloning of MSP-C6 promoter into pamDsRED expression vector. A) A schematic diagram of the pamDsRED vector with a reporter gene encoding DsRED, a red fluorescent protein from *Discosoma* sp. driven by the MSP-C6 Fragment 1 (2014 bp). The translated start site of *MSP-C6* gene is defined as +1; T, translation terminator; amp, ampicillin. B) The digestion products of recombinant plasmid pMSP-C6-F1-DsRED with the pamDsRED vector (3616 bp) backbone, and a MSP-C6-F1 (2014 bp) promoter fragment flanked by *Pst*I and *Xho*I restriction sites. M, marker 1 kb plus DNA ladder.

Hence, the transient expression of MSP-C6 promoter was analysed in this study to examine whether the findings of transient assays correlate with the endogenous *MSP-C6* gene or the stable expression in transgenic tomato. The tissue-specificity of the upstream sequence of MSP-C6 was investigated through biolistic delivery of pMSP-C6-F1-DsRED into the mesocarp tissues at 9 WAA, 12 WAA, 15 WAA and 17 WAA, spear leaves, stem, lateral roots and polyembryoid tissues (Figure 3). The green fluorescent signals (from the 35ShrGFP co-bombarded with the construct) were detected in all tested tissues, indicating successful DNA delivery into target tissues (Figure 3) but the red fluorescent signals were not observed in the mesocarp tissues bombarded with the pMSP-C6-F1-DsRED plasmid (Figure 3A-G) except the polyembryoid tissue (Figure 3H).

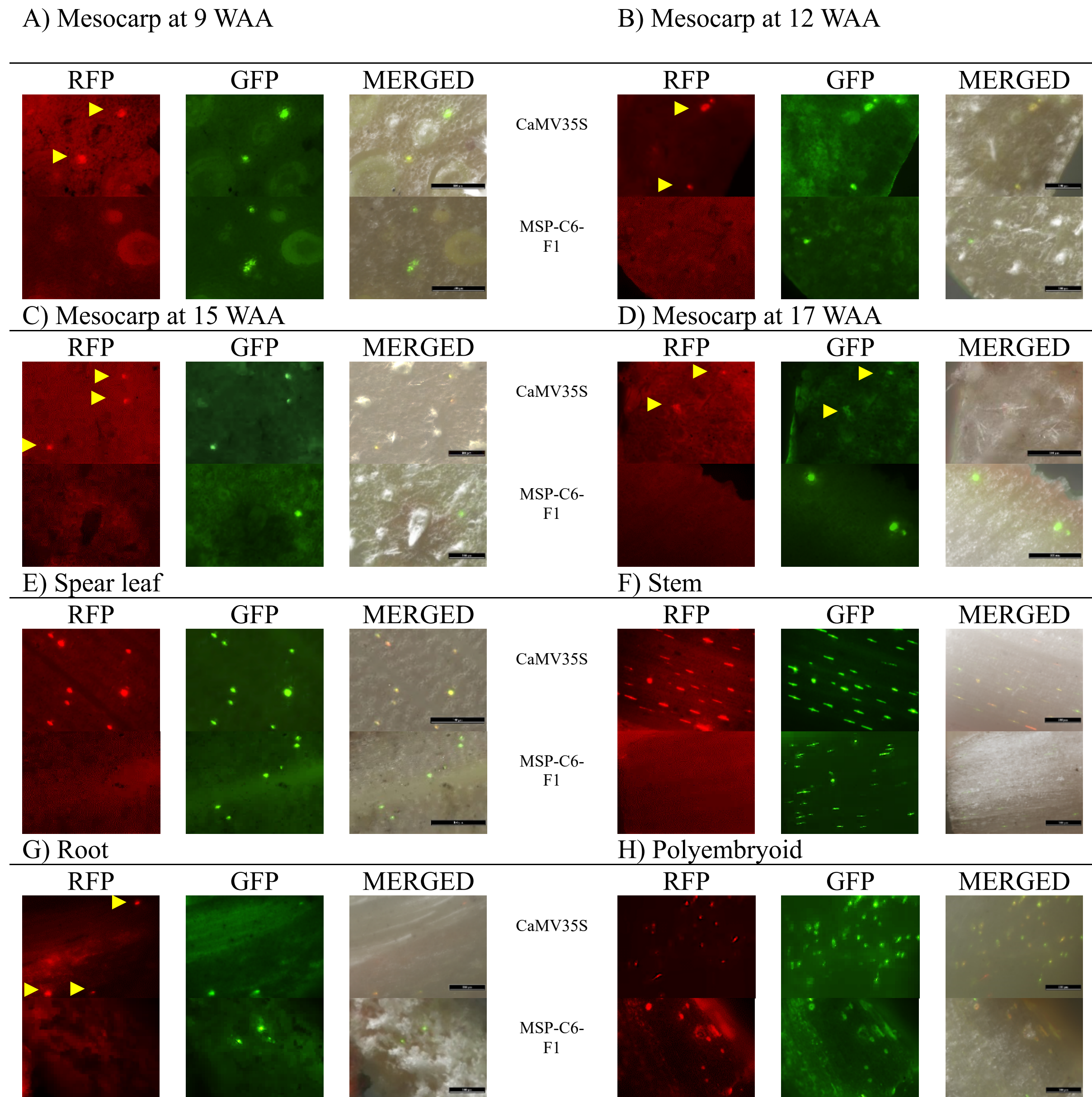

Figure 3. Biolistic-mediated transient expression analysis of CaMV35S and MSP-C6 promoters. The upstream sequence of MSP-C6 was fused to the *red fluorescent protein* (*DsRED*) gene in the pamDsRED vector. The tissue-specificity and activity of MSP-C6 promoter was examined through biolistic delivery of pMSP-C6-F1-DsRED into eight different tissues of oil palm (A) mesocarp at 9 WAA; (B) mesocarp at 12 WAA; (C) mesocarp at 15 WAA; (D) mesocarp at 17 WAA; (E) spear leaf; (F) stem; (G) root; and (H) polyembryoid. The *DsRED* driven by the CaMV35S promoter (pCaMV35S-DsRED) was used as a positive control. The plasmid 35ShrGFP carrying a *GFP* gene driven by the CaMV35S promoter was used as an indicator or reference for transformation efficiency. Biolistic transformation was carried out by co-bombardment of plasmid DNA from 35ShrGFP and pCaMV35S-DsRED or pMSP-C6-F1-DsRED; with three technical replicates of each tissue type. Scale bar, 100 µm.

Biolistic-Mediated Transient Expression Analysis of the MSP-C6 Promoter Fragments in Various Oil Palm Tissues

The pMSP-C6-F1-DsRED was used as a template to generate five fragments of different lengths through PCR. The fragments (F2-F6) from MSP-C6 promoter were denoted according to the size given in the brackets: MSP-C6-F2 (1718 bp), MSP-C6-F3 (1595 bp), MSP-C6-F4 (1110 bp), MSP-C6-F5 (759 bp), MSP-C6-F6 (571 bp). All fragments of MSP-C6 promoter were fused with the *DsRED* reporter gene in the paMDsRED vector (Figure 4).

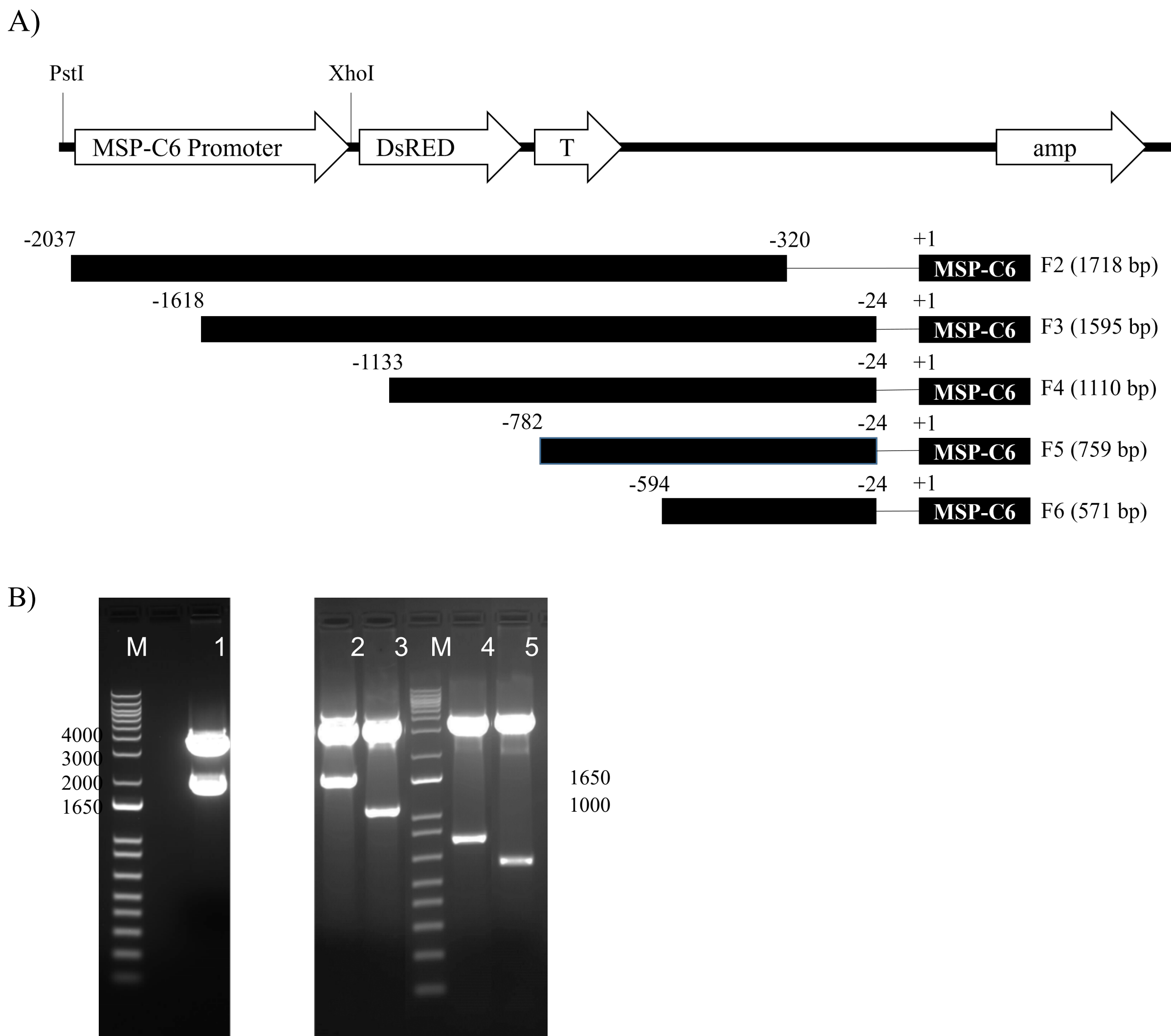

Figure 4. Cloning of promoter fragments from positions -2037 to -594 of MSP-C6 promoter into the pamDsRED expression vector. A) The *DsRED* reporter gene driven by different lengths of MSP-C6 promoter (F2-F6). The translated start site of *MSP-C6* gene is defined as +1. Salient features of pamDsRED construct are as follows: DsRED, *red fluorescent protein* (*DsRED*) gene from *Discosoma* sp.; T, terminator; amp, ampicillin. B) The digestion products of recombinant plasmids, the pamDsRED vector (3616 bp) with MSP-C6 promoter fragments (F2-F6) flanked by *Pst*I and *Xho*I. The digested products were observed on each lane. Lane 1, MSP-C6-F2 (1718 bp); Lane 2, MSP-C6-F3 (1595 bp); Lane 3, MSP-C6-F4 (1110 bp); Lane 4, MSP-C6-F5 (759 bp); Lane 5, MSP-C6-F6 (571 bp); M, marker 1 kb plus DNA ladder.

The promoter region of MSP-C6 may comprise CREs associated with variable functions, enhancers, insulators and silencers. Complex interplay among these elements can affect the quantitative variation in temporal and spatial gene expression of this gene. Dissection of the upstream region of the MSP-C6 promoter is important to identify the CREs that tightly control the temporal and spatial gene expression.

The MSP-C6-F2 (1718 bp), MSP-C6-F3 (1595 bp), MSP-C6-F4 (1110 bp), MSP-C6-F5 (759 bp) and MSP-C6-F6 (571 bp) were not able to express *DsRED* gene in all the bombarded tissues including the polyembryoid tissue (Figure 5) although the MSP-C6-F1 (2014 bp) was able to drive the expression of *DsRED* gene in the polyembryoid tissue (Figure 3H). The promoter region from position -2037 to -1618 may be required for the regulation of the transcription of the reporter gene in the polyembryoid tissue. Deletion of 419 bp promoter region from position -2037 to -1618 might suppress the transcriptional regulation of the MSP-C6-F3, F4, F5 and F6 in the polyembryoid tissues. Unknown sequence motifs may exist in the promoter region between -2037 to -1618, and play a role in gene regulation in the polyembryoid tissue. Although the MSP-C6-F3, F4, F5 and F6 promoters have several seed-, mesophyll- and root-specific elements (Badai et al., 2023), the *DsRED* was not expressed by MSP-C6-F3, F4, F5 and F6 promoter fragments in the mesocarp, spear leaf, stem, root and polyembryoid tissues (Figure 5).

The MSP-C6-F2 (1718 bp) lacking the 5'UTR region (Figure 4) was not able to express the DsRED signal in the bombarded polyembryoid tissue (Figure 5H) suggesting that the 5'UTR region may play crucial roles in the post-transcriptional regulation of gene expression driven by MSP-C6 promoter. Untranslated regions of messenger RNAs (mRNAs) can affect gene expression by controlling their translation efficiency, subcellular localisation, mRNAs stability and ribosome recruitment to the mRNAs (Peer et al., 2019). Without the 5'UTR region, which contains the necessary component for gene expression, the MSP-C6-F2 (1718 bp) promoter failed to express *DsRED* gene in all the bombarded tissues (Figure 5).

PEG-Mediated Transient Expression Analysis of the MSP-C6 Promoter Fragments in Oil Palm Protoplasts

PEG-mediated transient transformation of oil palm protoplasts isolated from the mesocarp at 15 WAA was conducted to verify the results of transient assays of MSP-C6 promoter fragments by biolistic bombardment. The green fluorescent signals (from 35ShrGFP) were detected in all transformed protoplast samples indicating successful DNA transfection after the cellular uptake of plasmid DNA (Figure 6). The protoplasts transformed with the positive control with CaMV35S promoter showed strong DsRED expression (Figure 6A). However, none of the fragments of the MSP-C6 promoter was able to express the *DsRED* gene in the transformed protoplasts isolated from mesocarp tissue (Figure 6B-G). These results showed good agreement with the experimental data in biolistic-mediated transient expression analysis.

Factors Influencing the Transient Expression in Oil Palm Tissues

The discrepancy between transient and stable expression of MSP-C6 in transformed tissues may be caused by the mRNA instability in the tissue samples that were transiently transformed. Instability of mRNA in the transiently transformed mesocarp tissues at 9, 12, 15 and 17 WAA may be due to many factors, such as endogenous and exogenous stimuli, handling and processing of the oil palm fruit during the sampling and limitation of mesocarp tissue at different developmental stages. Zhao et al. (2019) reported that the application of transient transformation for gene functional studies in strawberry can be affected by the variation of transient expression among individual fruits and the limitation of developmental stage. In addition, the efficiency of transient assays can be influenced by experimental variables and the physiological condition of plant tissues (Wroblewski et al., 2005).

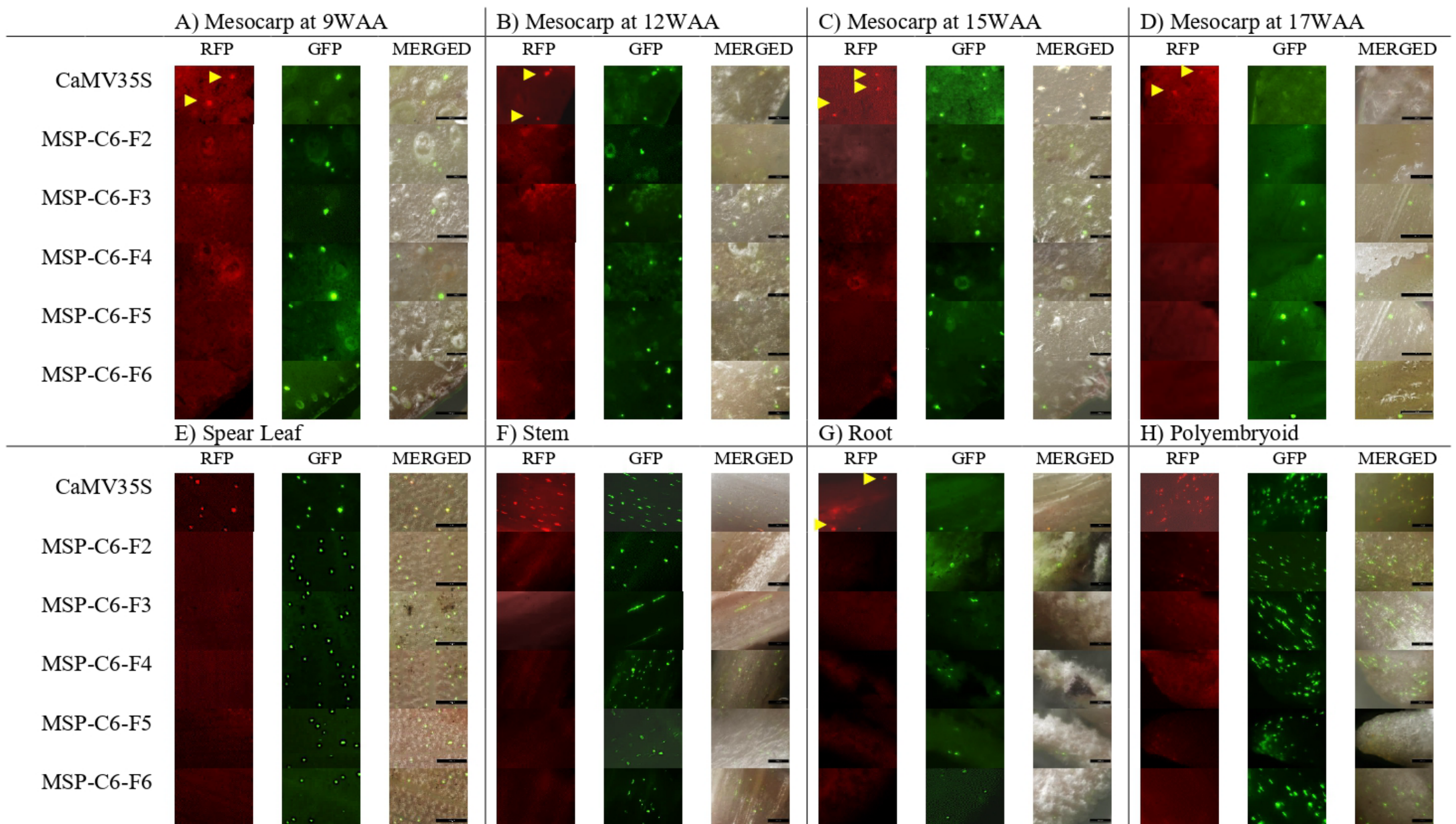

Figure 5. Biolistic-mediated transient expression analysis of the MSP-C6 promoter fragments (MSP-C6-F2 to F6) in mesocarp tissues at A) 9 WAA, B) 12 WAA, C) 15 WAA and D) 17 WAA, E) spear leaves, F) stem, G) lateral roots and H) polyembryoid tissues. The *DsRED* gene driven by the CaMV35S promoter (pCaMV35S-DsRED) was used as a positive control. The plasmid 35ShrGFP carrying a *GFP* gene driven by CaMV35S promoter was used as an indicator or reference for transformation efficiency. The expression of *DsRED* driven by the MSP-C6 promoter fragments (MSP-C6-F2 to F6) showed that none of the fragments was able to express the *DsRED* gene in all bombarded oil palm tissues. Scale bar, 100 µm.

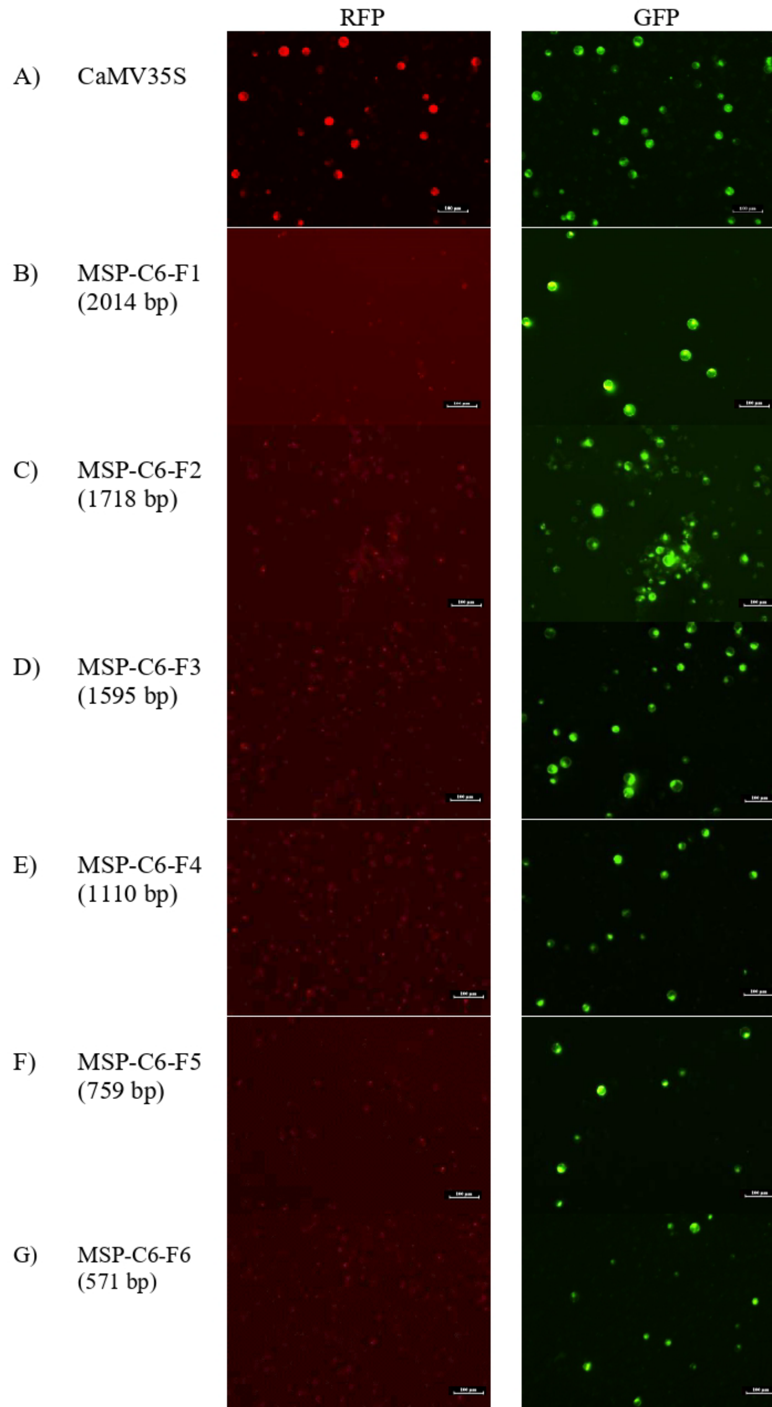

Figure 6. PEG-mediated transient transformation of different fragment lengths of the MSP-C6 promoter. Protoplasts that were isolated from mesocarp tissue at 15 WAA were transformed with A) CaMV35S; B) MSP-C6-F1 (2014 bp); C) MSP-C6-F2 (1718 bp); D) MSP-C6-F3 (1595 bp); E) MSP-C6-F4 (1110 bp); F) MSP-C6-F5 (759 bp); G) MSP-C6-F6 (571 bp) mediated by PEG. Green fluorescent signals were detected in all transformed samples, indicating successful DNA delivery into target tissues. None of the red fluorescent signal was detected in the protoplasts transformed with different lengths of MSP-C6 promoter fragments. RFP, red fluorescent protein; GFP, green fluorescent protein. Scale bar, 100 µm.

The biolistic is a useful device for rapid gene transfer and is not restricted to specific cell types. It is necessary to tailor particle bombardment protocols to each type of target tissue, which involves adjusting numerous crucial factors such as type and size of the particles, pre-culture conditions, distance from the target material and helium pressure (Lacroix and Citovsky, 2020). For the transformation of oil palm protoplasts mediated by PEG, a strict control of the quality and quantity of protoplasts and plasmid DNA is necessary to increase transfection efficiency (Wang et al., 2015). Biolistic- and PEG- mediated transformation have the possibility to cause damage to targeted cells. The impact of microparticles and tissue disrupted in the protoplast isolation may trigger a stress response that alters the transgene expression and potentially affects the outcome of functional analysis (Grafi et al., 2011; Lacroix and Citovsky, 2020). Future studies should focus on the optimization of a highly efficient transient expression system, which is critical for the study of promoter function, particularly in oil palm.

CONCLUSION

The MSP-C6 promoter fragments transiently transformed by biolistic- and PEG-mediated methods were not able to express DsRED in the mesocarp oil palm tissue as in stable transformation of tomato plants. The transient assays of MSP-C6 promoter fragments in oil palm tissues through biolistic- and PEG-mediated transient transformation may require further optimisation before these methods can be used routinely in the laboratory to ease the analysis of new oil palm promoters or to replace stable transformation.

AUTHORS CONTRIBUTION

SSB: conducted the experiments, writing – original draft, the manuscript and revised the manuscript, assisted with the experiments. OAR: supervision, supervised the experiments, assisted with the experiments. MYAM: supervision, supervised the experiments, assisted with the experiments. NAS: supervision, supervised the experiments. MPA: supervision, supervised the experiments. GKAP: supervision, supervised the experiments. CLH: writing – original draft, the manuscript and revised the manuscript, supervision, supervised the experiments.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest with regard to the publication of this paper.

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