TRANSFORMATION OF OIL PALM USING *Agrobacterium tumefaciens*

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ABSTRACT

*Transgenic oil palm (*Elaeis guineensis *Jacq.) plantlets were regenerated after using* Agrobacterium tumefaciens*-mediated transformation of embryogenic calli derived from young leaves of oil palm. The calli were transformed with a* Agrobacterium *strain, LBA4404, harbouring the plasmid pUBA, which carries a selectable marker gene (*bar*) for resistance to the glufosinate-ammonium and is driven by a maize ubiquitin promoter. Modifications of the transformation method, treatment of the target tissues using acetosyringone, exposure to a plasmolysis medium and physical injury via biolistics were applied. The main reasons for such modifications were to activate the bacterial virulence system and to subsequently increase the transformation efficiency. Transgenic oil palm was selected and regenerated on a medium containing glufosinate-ammonium. Molecular analyses revealed the presence and integration of the introduced* bar *gene into the genome of the transformants. This is the first report of a successful transformation of oil palm using* A. tumefaciens *for selection against glufosinate-ammonium.*

Keywords: transgenic oil palm, *Agrobacterium*-mediated, *bar* gene, glufosinate-ammonium.

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INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is the most important economic crop for Malaysia. The Malaysian oil palm industry has become one of the most highly organized sectors of any national agricultural system in the world. It is able to compete with other vegetable oil products from developed and developing countries. The total exports of oil palm products, constituting palm oil, palm kernel oil, palm kernel cake, oleochemicals, biodiesel and finished products increased by 11.1% or 2.18 million tonnes to 21.75 million tonnes in 2008 from 19.57 million tonnes recorded in 2007 (MPOB, 2009). In order for oil palm to maintain its premier position and to remain competitive, increasing yield per unit area as well as producing novel high value products using approaches such as genetic engineering is essential (Parveez, 1998). To genetically engineer oil palm for modifying fatty acid composition or producing novel products, establishment of a reliable transformation method is essential.

Agrobacterium tumefaciens is one of nature's most successful plant genetic engineers, and is routinely used to transfer desirable genes into dicotyledonous plants. *Agrobacterium*-mediated transformation is relatively efficient and leads to a low copy number of intact and non-rearranged transgenes, frequently integrated into the plant genome (Stanton, 1998). The concept of using *Agrobacterium* as a tool to create transgenic plants is promising for monocotyledonous plants even though it was at one time thought to be an obstacle because monocots are not the natural host for *Agrobacterium* (Ignacimuthu *et al*., 2000; Wei *et al*., 2000). The first transgenic monocot plant mediated by *Agrobacterium* was *Asparagus officinalis* (Bytebier *et al*., 1987). This was achieved by inoculating asparagus calli with *Agrobacterium* containing a Ti plasmid carrying *neomycin phosphotransferase* (*npt* II) in the T-DNA region as a selectable marker. As for commercial crops, rice (Raineri *et al*., 1990) and maize (Gould *et al*., 1991) were the first monocotyledonous plants transformed with *Agrobacterium*. Following these achievements, transgenic plants obtained via *Agrobacterium*-mediated transformation have been regenerated in more than a dozen monocotyledonous

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species, ranging from the most important cereal crops to ornamental plant species. Efficient transformation protocols for agronomically important cereal crops such as rice, wheat, maize, barley and sorghum have been developed, and transformation for some of these species has become routine (Cheng *et al*., 2004).

Several approaches to improve gene delivery by *Agrobacterium* into monocots have been reported. The use of phenolic compounds like acetosyringone (Stanton, 1998), a plasmolysis medium (Uzé *et al*., 1997), physical injury of the target tissue (Zuker *et al*., 1999), and also construction of a vector containing extra *virulence* (*vir)* genes (Komari 1990; Rossi *et al*., 1996; Tzfira *et al*., 2000; Scott *et al*., 2001) are among the most common approaches.

Acetosyring one is one compound used successfully to enhance *A. tumefaciens*-mediated transformation in various plant species. Acetosyringone increased significantly the stable transformation efficiency in cotton (Sunilkumar and Rathore, 2001). For *vir* induction, acetosyringone is recommended in most of the monocot transformation protocols (Tingay *et al*., 1997; Zhao *et al*., 2000). When acetosyringone was omitted, the level of transient GUS expression was reduced, and stable transformed plants could not be regenerated for rice or onion (Rashid *et al*., 1996; Hiei *et al*., 1997; Zheng *et al*., 2001). Even though a different approach for wounding was used, the addition of acetosyringone has proven to enhance transformation efficiency. Kumar *et al*. (2006) reported that wounding through sonication followed by acetosyringone treatment enhanced transformation frequency substantially.

Microprojectile bombardment and sonication (Flores Solis *et al*., 2003; Kumar *et al*., 2006) are effective approaches for wounding or causing physical injury to promote transformation. Wounding by bombardment yields highly efficient transformation in carnation (Zuker *et al*., 1999). Compared to bombardment, sonication is a very easy and low cost method to substantially enhance the efficiency of the transformation of low or non-susceptible plant species (Singh and Chowla, 1999). The cavitation caused by sonication results in thousands of microwounds on and below the surface of the plant tissue. This wounding pattern permits *Agrobacterium* to travel deeper and more completely throughout the tissue compared to conventional microscopic wounding, thus increasing the probability of infecting the plant cells. The earlier inefficient transformation of monocotyledonous species was thought to be caused by a lack of production of virulence-inducing substances (Chilton *et al*., 1986). Phenolic plant metabolites released from damaged cells are required for the activation of virulence functions within *Agrobacterium* (Stachel *et al*., 1985). Cells at the wound site initiate the synthesis of phenolic compounds, which are thought to be produced as anti-bacterial agents, are recognized by invading *A. tumefaciens* and serve to initiate the DNA transfer.

Apart from the above, pre-treatment by a plasmolyzing medium produced a rapid and efficient transformation of *Panax ginseng* (Choi *et al*., 2001), and in rice, 10% sucrose pre-treatment of immature embryos enhanced the frequency of *Agrobacterium*-mediated transformation (Uzè *et al*., 1997). An efficient transformation system was established for rice when the pre-cultured immature embryos were plasmolyzed (Uze *et al*., 1997). A plasmolyzing pre-treatment of ginseng cotyledon explants was found to greatly increase the production of single cells derived from direct somatic embryogenesis (Choi and Soh, 1997; Choi *et al.,* 1999). It is not known why the plasmolyzing pre-treatment enhances the frequency of transformation. The plasma membrane is the main obstacle to getting DNA into the cells (Fennell and Hauptmann, 1992; Wu and Cahoon, 1995). The enhanced *Agrobacterium*-mediated gene transformation following sucrose pre-treatment is most likely to be related to damage to the cell membrane during the process of plasmolysis (Choi *et al*., 2001).

The above successes make it possible to apply the *Agrobacterium*-mediated method to transform monocot plants such as oil palm. Studies are on-going to modify this monocotyledonous plant through genetic engineering to improve the quality of the palm oil (Parveez *et al*., 1999; Parveez, 2003). Transgenic oil palm has been produced previously using the biolistic method (Parveez, 1998). However, this transformation method tends to introduce multiple copies of the transgenes which could lead to gene silencing. The *A. tumefaciens* method, on the other hand, has been proven to introduce single or low copies of transgenes (Cheng *et al*., 1997).

The objectives of this study are to optimize the parameters affecting *A. tumefaciens*-mediated transformation of oil palm embryogenic calli, to select for transformed cells on a medium containing glufosinate-ammonium, to regenerate transgenic oil palm, and finally to confirm the integration and expression of transgenes in the regenerated transgenic oil palm.

MATERIALS AND METHODS

Bacterial Strain and Plasmid

Agrobacterium tumefaciens, strain LBA4404, harbouring a T-DNA, a pUBA plasmid containing a *npt* II gene for bacterial selection and a *bar* gene (coding for phosphinothricin acetyltransferase) as the plant selectable marker driven by a maize ubiquitin constitutive promoter, was used.

Transformation of Oil Palm Embryogenic Calli

The composition of the medium used for tissue culture is given in *Table 1*. Embryogenic suspension calli were pre-treated onto a plasmolysis medium (PM) containing acetosyringone for an hour prior to bombardment with gold particles for the purpose of wounding, using the method described by Parveez (2000). The bombarded calli were later transferred into flasks containing an *Agrobacterium* suspension and the flasks were slowly shaken on a rotary shaker for 2 hr. The calli were then blotted dry on sterile filter paper before being transferred onto the PM medium for one day. Calli were transferred onto a co-cultivation medium (CM) and kept for three days at 27ºC in the dark. After three days, the calli were washed several times using a liquid EC medium with addition of timentin and a plant preservative mixture (PPM) to kill the bacteria. The calli were blotted dry and were cultured onto the EC medium containing timentin (100 mg litre^{-1}) and PPM (0.2% v/v). After one month, the calli were transferred onto a selection medium contained glufosinate-ammonium (commercial name: herbicide BASTA®, Bayer CropScience). After seven months of subculture, putative embryoids were transferred onto the EC medium. Continuous subculturing was carried out until shoots appeared before the plantlets were transferred into test tubes containing rooting medium.

Transgenic Plants Analysis

Plant DNA extraction. Extraction of genomic DNA was carried out according to Doyle and Doyle (1987) with some modifications. Total DNA was isolated from 4 g of leaf tissues and the tissues were ground to fine powder with a mortar and pestle in liquid nitrogen.

Polymerase chain reaction (PCR) analysis. The presence of the *bar* gene in embryoids and shoots was confirmed by PCR using two sets of specific primers for the gene (primer BAR-F1: 5′ GGTCTACACCATCGT CAACCC 3′; primer BAR-R2: 5′ GTCATGCCAGTTCCCGTCCT 3′), and (primer BARM-F: 5′ TGCACCATCGTCAACCACT 3′; primer BARM-R: 5′ ACAGCGACCACG CTGTTGAA 3′). Internal control primers specific to oil palm were also used (primer POR-12: 5′ CCCTCATCCATAGCACA 3′; primer POR-38: 5′ CAGGGAGCAAAGAAGCA 3′). Reaction conditions were as follows: the DNA was denatured at 95ºC for 5 min followed by 35 cycles of 95ºC for 1 min, 65ºC for 1 min, 72ºC for 1 min, and a final extension at 72ºC for 5 min. The PCR product was separated by electrophoresis in 1% (w/v) agarose gels.

Southern Blot analysis. Total genomic DNA was extracted from freshly collected leaves of PCR-positive transgenics and from untransformed leaves using a modified protocol described by Doyle and Doyle (1987). Southern Blot analysis was performed using a non-radioactive DIG High Prime Kit (Roche) according to the manufacturer's instructions. The undigested genomic DNA samples (100 μg) were run overnight on a 0.8% (w/v) agarose gel at 25 V. DNA was transferred onto a Hybond-N$^+$ membrane (GE Healthcare) by capillary blotting (Sambrook *et al*., 1989).

Dot Blot analysis. Nylon membranes were soaked in 2X SSC buffer for 5 min and were placed onto the dot blot apparatus. The apparatus was assembled as directed in the manufacturer's manual. Samples of 60 μg genomic DNA in 100 μl of dH_2O were denatured, and an equal volume of 1 M NaOH was added. Samples were incubated at room temperature for 20 min. DNA samples were loaded into each well and a vacuum was used to transfer the DNA onto the nylon membrane. After UV crosslink, the membrane was ready to use for hybridization, or kept at 4ºC. Hybridization was performed in a way similar to the Southern Blot analysis, using the DIG High Prime Kit.

TABLE 1 . MEDIUM COMPOSITION

Medium	Composition
EC	MS salts (Murashige and Skoog)+ Y_3 vitamins + 0.0375 g litre^{-1} NaF eEDTA+ 0.1 g litre^{-1} myo-Inositol + 0.1 g litre^{-1} L-glutamine + 0.1 g litre^{-1} L-asparagine + L-arginine + 3% sucrose + 5 μM α-Naphtaleneacetic acid (NAA) + 0.8% agar.
Liquid EC	EC medium without agar.
PM	EC medium with addition 200 μM acetosyringone, while sucrose was increased to 6%.
CM	EC medium with addition of 200 μM acetosyringone.
RM	MS salts + Y_3 vitamins + 0.0375 g litre^{-1} NaF eEDTA+ 0.1 g litre^{-1} myo-Inositol + 0.3 g litre^{-1} L-glutamine + 6% sucrose + 9 μM α-Naphtaleneacetic acid (NAA) + 0.15% activated charcoal.

Note: All media were adjusted to pH 5.7 prior to autoclaving.

RESULTS AND DISCUSSION

Transformation of Oil Palm Embryogenic Calli

Embryogenic suspension calli *(Figure 1a*) were chosen as the starting material for transformation because they can be easily regenerated (Siti Habsah, 2004). The use of highly regenerative tissue as transformation target tissue often results in the regeneration of a large number of independently transformed lines as observed in several monocot crop species such as wheat and rice (Kim *et al*., 1999; Yara *et al*., 2001). Prior to transformation, the suspension calli were cultured onto plasmolysis medium (*Figure 1b*). Pre-treatment of the embryogenic calli onto plasmolysis medium which contained acetosyringone will help to induce the *vir* gene transfer (Hie *et al*., 1994). Biolistic approach to create wounding was carried out to promote transformation process. Biolistic bombardment has been shown to be an efficient tool for wounding plant tissues prior to *Agrobacterium* transformation (Zuker *et al*., 1999). Kumar *et al*. (2006) reported that wounding followed by the addition of acetosyringone gave excellent results in terms of transformation frequency in each explant. Pre-treatment using plasmolysis medium, acetosyringone and biolistic were applied to improve the standard method of the transformation.

Selection and Regeneration of Transformed Embryogenic Calli

Oil palm embryogenic calli transformed with *Agrobacterium* were selected on medium containing glufosinate-ammonium at a concentration of 50 μg ml^{-1}. The transformed embryogenic calli were cultured on a medium without the selective agent for around one to three weeks. Upon transferring to a medium containing glufosinate-ammonium, untransformed cells began to die (*Figure 1c*), allowing only resistant cells to proliferate. The transformed embryogenic calli were subcultured onto a fresh selection medium every month (30 days). Normally, glufosinate-ammonium-resistant embryogenic calli (a new colony) appeared after five to six months in the selection medium (*Figure 1d*).

The glufosinate-ammonium-resistant embryogenic calli were continuously subcultured onto fresh selection medium for proliferation and regeneration. The size of the colonies became bigger and turned into embryoids. The transgenic embryoids began to regenerate into whitish embryoids followed by greenish polyembryogenic calli after three to five months of culture on the EC medium (*Figures 1e* and *1f*). After two to three months, shoots appeared from the polyembryogenic calli. The shoots were individually isolated and cultured in flasks containing the EC medium for shoot elongation. After two to three months, the elongated shoots were transferred into test tubes containing the RM medium for root initiation and further development. Plantlets with strong and healthy roots were obtained after about two months (*Figure 1g*). It was observed that the selection and regeneration process for the production of transgenic oil palm using *Agrobacterium*-mediated transformation is similar to the production of transgenic oil palm using the microprojectile bombardment method (Parveez, 1998). Fourteen lines of glufosinate-ammonium-resistant oil palm embryogenic calli were produced from the *Agrobacterium*-mediated transformation, and a total of 67 plantlets were regenerated.

PCR Analysis

Genomic DNA was extracted from putative embryoids and leaves that survived on the selection medium containing glufosinate-ammonium. The extracted DNA was subjected to PCR analysis to detect the presence of the *bar* gene in the genome of the transformants. It was discovered that 23 DNA samples extracted from embryoids showed positive results using two different sets of *bar* gene primers, and amplified PCR products of expected sizes of 311 bp (*Figure 2a*) and 461 bp (*Figure 2b*). Similarly, another 14 DNA samples extracted from leaves were also positive for the PCR analysis using the *bar* gene primers (*Figure 3*). No amplified product was observed for the untransformed plants. The results provide early indication of the transgene integration into the oil palm genome.

Dot Blot Analysis

After successfully confirming the presence of the transgene using PCR analysis, further Dot Blot analysis using DNA from 13 transgenic leaves was carried out. Integration of the *bar* gene into the oil palm genome was detected using the DIG High Prime Kit. It was observed that four out of 13 samples tested showed a positive signal (*Figure 4*: Lanes 2, 5, 9 and 13). No signal was detected for the untransformed samples. To further confirm the integration of the transgene into the oil palm genome, Southern Blot analysis was also carried out.

Southern Blot Analysis

PCR analysis of the *bar* gene indicated the presence of the gene in the DNA samples of the transgenic oil palm tested. However, the PCR results are not definitive evidence of stable integration of transgenes into the host genome. Potrykus (1990) proposed that a number of factors need to be determined before a stable integration of a transgene can be confirmed. Among the main requirements are: effective controls for the treatment and analysis, a correlation between treatment and predicted results,

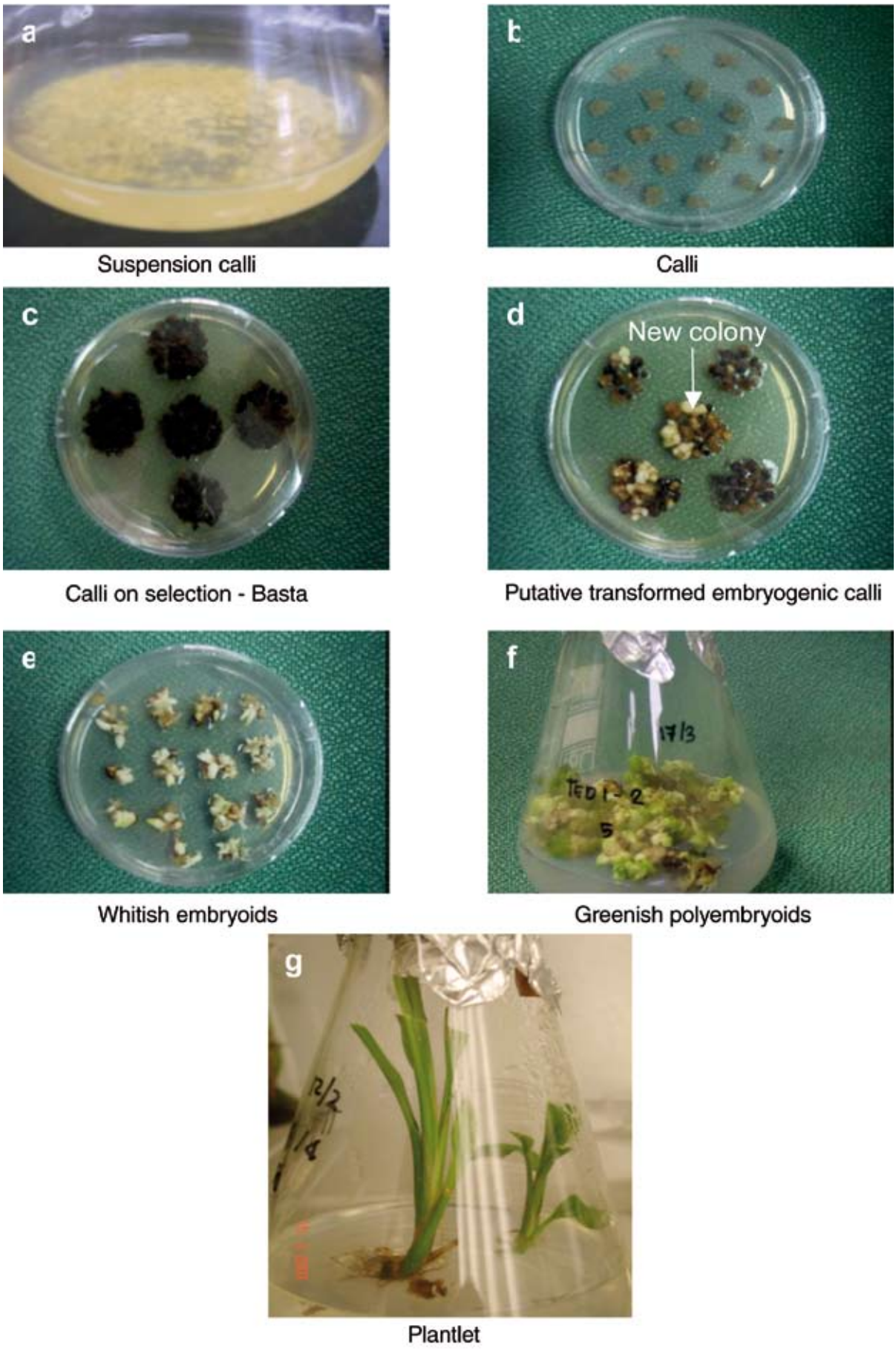

Figure 1. Development of transgenic oil palm.

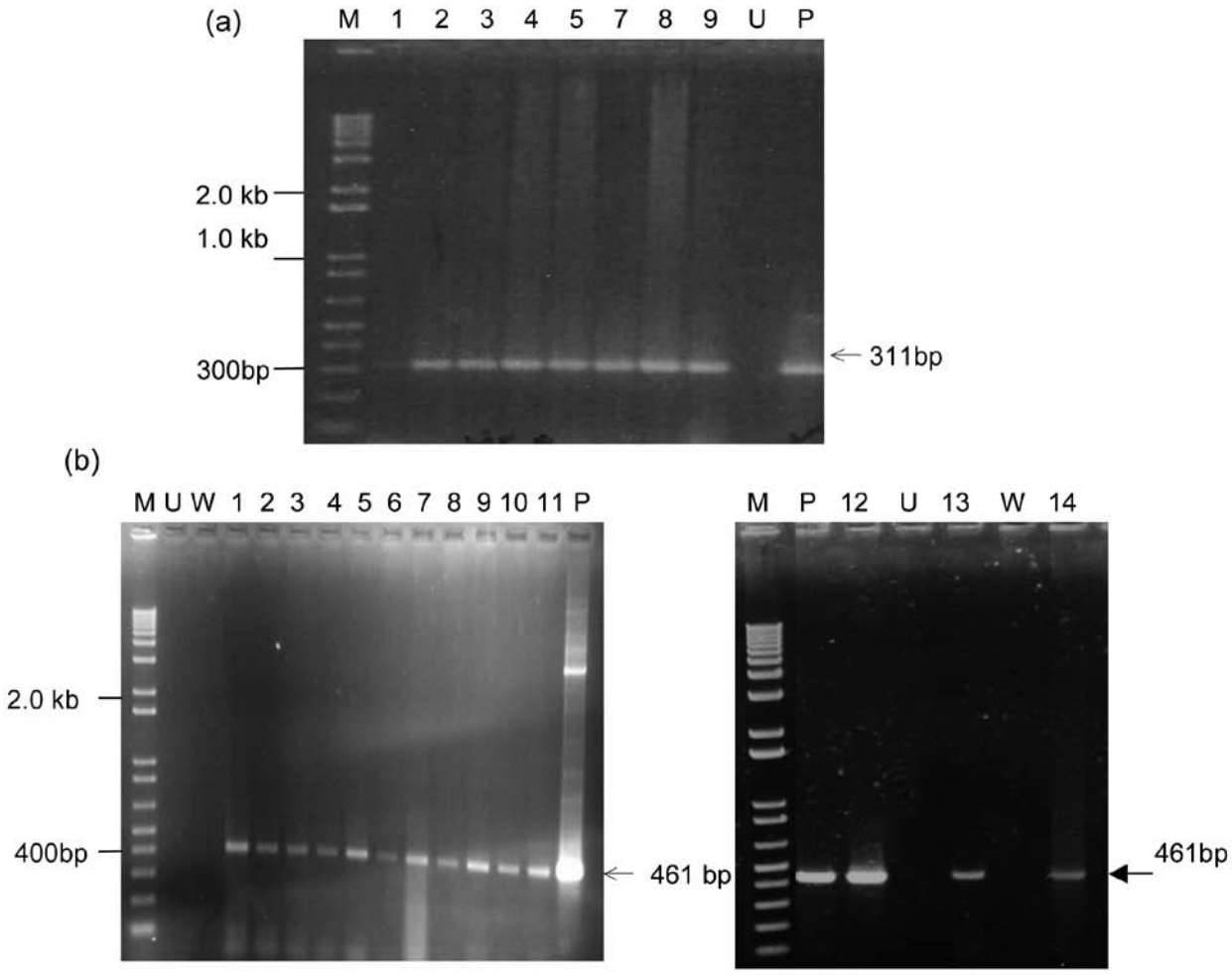

Figure 2. (a) Polymerase chain reaction (PCR) analysis on genomic DNA of oil palm embryoids with bar gene-specific primers (BARM-F and BARM-R). Lane M: 1 kb Plus DNA ladder; lane P: plasmid pUBA; lanes 1-9: DNA samples; and lane U: untransformed plant. Arrow indicates PCR products of 311 bp. (b) PCR analysis on genomic DNA of oil palm embryoids with bar gene-specific primers (BAR-F1 and BAR-R2). Lane M: 1 kb plus DNA ladder; lane P: plasmid pUBA; lanes 1-14: DNA samples; lane W: water; and lane U: untransformed plant. Arrows indicate PCR products of 461 bp.

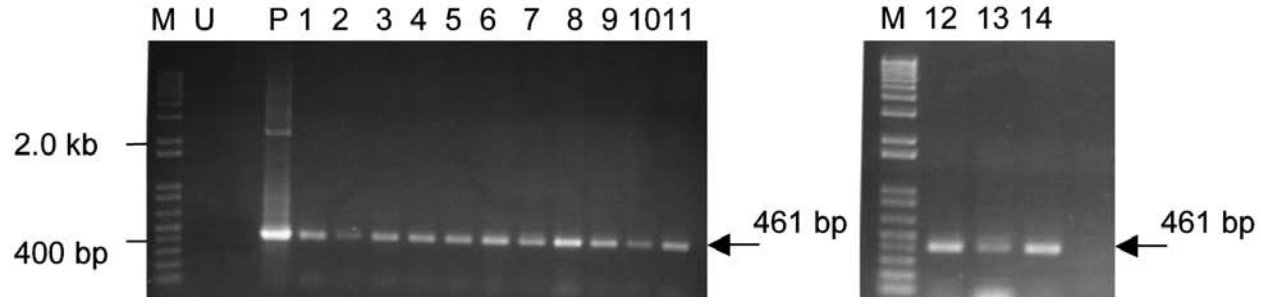

Figure 3. Polymerase chain reaction (PCR) analysis on genomic DNA of oil palm leaves with bar gene-specific primers (BAR-F1 and BAR-R2). Lane M: 1 kb Plus DNA ladder; lane P: plasmid pUBA; lanes 1-14: DNA samples; and lane U: untransformed plant. Arrows indicate PCR products of 461 bp.

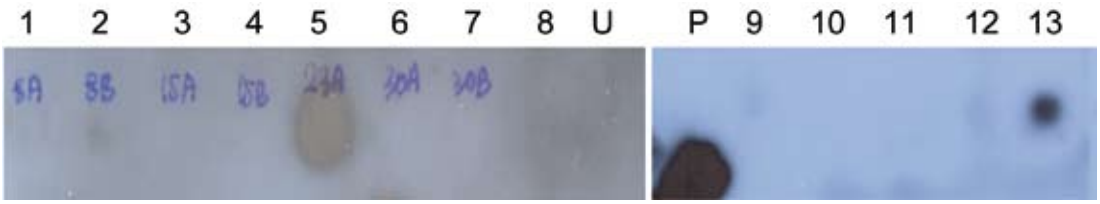

Figure 4. Dot Blot hybridization of 60 μg genomic DNA from leaf samples (lanes 1-13), untransformed oil palm (lane U) and plasmid pUBA (lane P). DNA blot was hybridized with a DIG-labelled PCR product of bar gene (~0.4 kb) at 65°C.

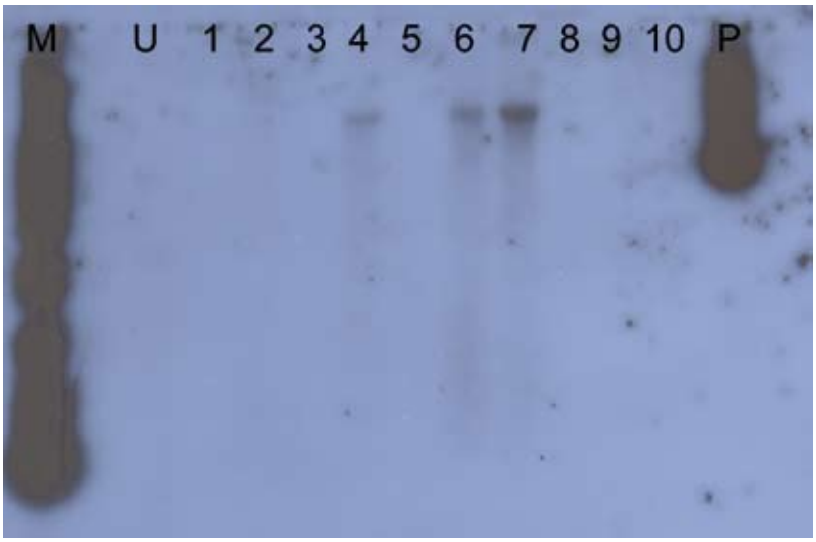

Figure 5. Southern Blot analysis of undigested genomic DNA of transgenic oil palm plants. DNA blot was hybridized with a DIG-labelled PCR product of bar gene at 45°C. Lane M: 1 kb Plus DNA ladder; lane U: untransformed plant, lane P: plasmid pUBA; lanes 1-10: samples of transgenic oil palm.

and complete Southern Blot analysis including positive signals on high molecular weight DNA for undigested genomic DNA. Southern Blot analysis using a non-radioactive DIG High Prime Kit was carried out to ultimately confirm the integration of the *bar* gene. The 100 μg genomic DNA from the transgenic oil palm samples were transferred onto a nylon membrane before being subjected to Southern hybridization. Three out of 10 undigested DNA samples (randomly chosen) showed positive signals (*Figure 5*: lanes 4, 6 and 7). A positive control plasmid pUBA generated a very strong positive hybridization signal. No signal was observed in the untransformed plant. This provides good evidence of successful integration of the transgene into the oil palm genome mediated by *Agrobacterium*.

CONCLUSION AND FUTURE DIRECTIONS

Based on the molecular analyses that were carried out, it was confirmed that the T-DNA was successfully integrated into the oil palm genome. The transformation rate was 0.7% which is slightly lower than the rate from microprojectile bombardment which is 1%-1.5% (Parveez, 2000). However, the *Agrobacterium*-mediated method is preferred compared to other transformation methods due to several advantages, such as low copy number, less co-suppression problems and stable integration (Gheysen *et al*., 1998; Shibata and Liu, 2000). In this study, glufosinate-ammonium-resistant transgenic oil palm was successfully produced after improvement of the *Agrobacterium*-mediated method for oil palm transformation. Confirmation of the transgenic status was carried out using PCR, Dot Blot and Southern Blot analyses. A higher transformation rate can still be achieved by optimizing several other factors affecting the transformation of monocots, such as bacterial density and type of strain, pH, temperature, antibiotics, physical injuries, and the selectable marker. Therefore, further studies should continue to be carried out to improve and increase the efficiency of *Agrobacterium*-mediated oil palm transformation. Using this improved method, it is now possible to introduce useful genes into oil palm to produce transgenic oil palm with value-added traits.

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REFERENCES

BYTEBIER, B F; DEBOECK, F and GREVE, H D (1987). T-DNA organization in tumor cultures and transgenic plants of the monocotyledon *Asparagus officinalis*. *Proc. Natl. Acad. Sci. USA, 84*: 5345.

CHENG, M; FRY, J E; PANG, S; ZHOU, H; HIRONAKA, C M; DUNCAN, D R; CONNER, T W and WAN, Y (1997). Genetic transformation of wheat mediated by *Agrobacterium tumefaciens*. *Plant Physiol., 115*: 971-980.

CHENG, M; LOWE, A B; SPENCER, M T; YE, X and ARMSTRONG, C L (2004). Factors influencing *Agrobacterium*-mediated transformation of monocotyledonous species. In vitro *Cell. Dev. Biol. Plant, 40*: 31-45.

CHILTON, M D; HOOD, E E; HALMER, G L and FRALEY, R T (1986). The hypervirulence of *Agrobacterium tumefacience* A281 is encoded in a region pTIB0542 outside of T-DNA. *J Bacteriol., 168*: 1291-1301.

CHOI, Y E and SOH, W Y (1997). Enhanced somatic single embryoformation by plasmolyzing pretreatment from cultured ginseng cotyledons. *Plant Sci., 130*: 197-206.

CHOI, Y E; YANG, D C; YOON, E S and CHOI, K T (1999). High efficiency plant production via direct somatic single embryogenesis from pre-plasmolysed cotyledons of Panax ginseng and possible dormancy of somatic embryos. *Plant Cell Rep., 18*: 493-499.

CHOI, Y E; YANG, D C; KUSANO, T and SANO, H (2001). Rapid and efficient *Agrobacterium*-mediated transformation of Panax ginseng by plasmolyzing pre-treatment of cotyledons. *Plant Cell Rep., 20*: 616-621.

DOYLE, J J and DOYLE, J L (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissues. *Phytochemical Bulletin, 19*: 11-15.

FENNELL, A and HAUPTMANN, R M (1992). Electroporation and PEG delivery of DNA in maize microspores. *Plant Cell Rep., 11*: 567-570.

FLORES SOLIS, J I; MLEJNEK, P; STUDENÁ, K and PROCHÁZKA, S (2003). Application of sonication-assisted *Agrobacterium*-mediated transformation in *Chenopodium rubrum* L. *Plant Soil Environ., 49(6)*: 255-260.

GHEYSEN, G; ANGENON, G and MONTAGUE, M V (1998). *Agrobacterium*-mediated plant transformation: a scientifically intriguing story with significant application. *Transgenic plant Research* (Lindsey, K ed.). Harwood Academic Press, Netherlands. p. 1-33.

GOULD, J; DEVERY, M; HASEGAWA, O; ULIAN, E C; PETERSON, G and SMITH, R H (1991). Transformation of *Zea mays* L. using *Agrobacterium tumefaciens* and shoot apex. *Plant Physiol., 95*: 426-434.

HIEI, Y; OHTA, S; KOMARI, T and KUMASHIRO, T (1994). Efficient transformation of rice (*Oriza sativa*) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA. *The Plant Journal, 6*: 271-282.

HIEI, Y; KOMARI, T and KUBO, T (1997). Transformation of rice mediated by *Agrobacterium tumefaciens*. *Plant Mol. Biol., 35*: 205-218.

IGNACIMUTHU, S; AROCKIASAMY, S and TERADA, R (2000). Genetic transformation of rice: Current status and future prospects. *Current Science, 79(2):* 186-195.

KIM, H K; LEMAUX, P G; BUCHANAN, B B and CHO, M J (1999). Reduction of genotype limitation in wheat (*Triticum aestivum* L.) transformation. P-1021. *Congress* In vitro *Bioll*. New Orleans, LA. 5-9 June 1999.

KOMARI, T (1990). Transformation of callus cells of *Chenopodium quinoa* by binary vectors that carry a fragment of DNA from the virulence region of pTiBo542. *Plant Cell Rep., 9*: 303-306.

KUMAR, V; SHARMA, A; PRASAD, B C N; GURURAJ, H B and RAVISHANKAR, G A (2006). *Agrobacterium rhizogenes* mediated genetic transformation resulting in hairy root formation is enhanced by ultrasonication and acetosyringone treatment. *Electronic Journal of Biotechnology, 9(4)*: 349-357.

MPOB (2009). Overview of the Malaysian oil palm industries. MPOB website: http://www.mpob.gov.my/

PARVEEZ, G K A (1998). *Optimization of Parameters Involved in the Transformation of Oil Palm Using the Biolistics Method*. Ph.D thesis, Universiti Putra Malaysia.

PARVEEZ, G K A (2000). Production of transgenic oil palm (*Elaeis guineensis* Jacq.) using biolistic techniques. *Molecular Biology of Woody Plants* (Jain, S M and Minocha, S C, eds.). Kluwer Academic Publishers, Dordrecht, The Netherlands. p. 327-350.

PARVEEZ, G K A (2003). Novel products from transgenic oil palm. *AgBiotechNet.5* (*ABN113*): 1-8.

PARVEEZ, G K A; RAVIGADEVI, S; ABDULLAH, S N A; OTHMAN, A; RAMLI, U S; RASID, O; MASRI, M M and CHEAH, S C (1999). Production of transgenic oil palm - current success and future considerations. *Proc. of the 1999 PORIM International Palm Oil Congress*. 1-6 February 1999, Kuala Lumpur. p. 283-297.

POTRYKUS, I (1990). Gene transfer to cereals: an assessment. *Bio/Technol*., *8*: 535-542.

RAINERI, D M; BOTTINO, P; GORDON, M P and NESTER, E W (1990). *Agrobacterium tumefaciens*-mediated transformation of rice (*Oryza sativa* L.). *Bio/Technol*., *8*: 33-38.

RASHID, H; YOKOI, S; TORIYAMA, K and HINATA, K (1996). Transgenic plant production mediated by *Agrobacterium* in *Indica* rice. *Plant Cell Rep*., *15*: 727-730.

ROSSI, L; HOHN, B and TINLAND, B (1996). Integration of complete transferred DNA units is dependent on the activity of virulence E2 protein of *Agrobacterium tumefaciens*. *Proc. Natl. Acad. Sci. USA, 93*: 126-130.

SAMBROOK, J; FRITSCH, E F and MANIATIS, T (1989). *Molecular Cloning - A Laboratory Manual.* Cold Spring Harbor, New York.

SCOTT, M; LOHRKE, M S; YANG, H and JIN, S (2001). Reconstitution of acetosyringone-mediated *Agrobacterium tumefaciens* virulence gene expression in the heterologous host *Escherichia coli. J. Bacteriology*, *183*: 3704-3711.

SHIBATA, D and LIU, Y G (2000). *Agrobacterium*-mediated plant transformation with large DNA fragments. *Trends Plant Sci*., *5*: 354-357.

SINGH, N and CHOWLA, H S (1999). Use of silicon carbide fibers for *Agrobacterium*-mediated transformation in wheat. *Genetics and Plant Breeding*. G B Pant University of Agriculture and Technology, Pantnagar, India. p. 145.

SITI HABSAH, R (2004). Agrobacterium-*mediated Transformation of Oil Palm* (Elaeis guineensis J*acq*.) *Suspension Culture*. Master thesis, Universiti Putra Malaysia.

STACHEL, S E; MESSENS, E; VAN MONTAGU, M and ZAMBRYSKI, P (1985). Identification of the signal molecules produced by wounded cells that activate T-DNA transfer in *Agrobacterium tumefaciens*. *Nature, 318*: 624-629.

STANTON, G B (1998). The introduction and expression of transgenes in plants. *Current Opinion in Biotechnol*., *9*: 227-232.

SUNILKUMAR, G and RATHORE, K S (2001). Transgenic cotton: factors influencing *Agrobacterium*-mediated transformation and regeneration. *Molecular Breeding, 8*: 37-52.

TINGAY, S; McELROY, D; KALLA, R; FIEG, S; WANG, M; THORNTON, S and BRETTELL, R (1997). *Agrobacterium*-mediated barley transformation. *Plant J*., *11*: 1369-1376.

TZFIRA, T; RHEE, Y; CHEN, M H and CITOVSKY, V (2000). Nucleic acid transport in plant-microbe interactions: the molecules that walk through the walls. *Annu. Rev. Microbiol*., *54*: 187-219.

UZÈ, M; WÜNN, J; KAERLAS, J P; POTRYKUS, I and SAUTTER, C (1997). Plasmolysis of precultured immature embryos improves *Agrobacterium*-mediated gene transfer to rice (*Oryza sativa* L.). *Plant Sci*., *130*: 87-95.

WEI, L; GUANGQIN, G and GUOCHANG, Z (2000). *Agrobacteriu*m-mediated transformation: state of the art and future prospect. *Chinese Science Bulletin, 45 (17)*: 1537-1546.

WU, F S and CAHOON, F (1995). Plasmolysis facilitates the accumulation of protein and DNA into extra-plasmalemma spaces of intact plant cells. *Plant Sci., 104*: 210-214.

YARA, A; OTANI, M; KUSUMI, K; MATSUDA, O; SHIMADA, T and IBA, K (2001). Production of transgenic japonica rice (*Oryza sativa*) cultivar, Taichung 65, by *Agrobacterium*-mediated method. *Plant Biotechnology, 18(4)*: 305-310.

ZHAO, Z Y; CAI, T; TANGLIANI, L; MILLER, M; WANG, N; PHANG, H; RUDERT, M; SCHROEDER,

S; HONDRED, D; SELTZER, J and PIERCE, D (2000). *Agrobacterium*-mediated sorghum transformation. *Plant Mol. Biol*., *44*: 789-798.

ZHENG, S J; KHRUSTALEVA, L; HENKEN, B; JACOBSON, E; KIK, C and KRENS, F A (2001). *Agrobacterium*-mediated transformation of *Allium cepa* L.: the production of transgenic onions and shallots. *Mol. Breed*., *7*: 101-115.

ZUKER, A; AHRONI, A; TZFIRA, T; BEN-MEIR, H and VAINSTEIN, A (1999). Wounding by bombardment yields highly efficient *Agrobacterium*-mediated transformation of carnation (*Dianthus carophyllus* L.). *Mol. Breeding*, *5*: 367-375.