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Full Length Research Paper

Factors affecting delivery and transient expression of *gusA* gene in Malaysian indica rice MR 219 callus via biolistic gun system

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The effect of the biolistic device parameters and callus factors affecting delivery and expression of *gusA* gene in indic*a* rice MR219 was optimised. Four weeks old embryogenic callus were bombarded with gold microparticles coated with pCAMBIA1305.2 plasmid harbouring the *gus*A gene which encodes-glucuronidase. The physical and biological factors investigated were the helium pressure, distance from stopping screen to target tissues, vacuum pressure, gold microparticles size, spermidine and calcium on DNA precipitation, the number of bombardments, age of callus, osmotic pre-culture length and osmotic pretreatment medium. The embryogenic calli were subjected to histochemical GUS assay after three days bombardment. The GUS activity (recorded as blue spots) detected histochemically and fluorometrically were further analysed by microscopy method. Mostly, the variables used in this experiment showed significant difference on the delivery of DNA and transient expression of the *gus*A gene in embryogenic rice calli. Combinations of selected optimized parameters and an effective selection were developed which allowed high-efficiency of DNA delivery combined with minimum damage to target indica rice MR219 embryogenic callus tissue.

Key words: Transient expression, *gusA* gene, biolistic gun, indica rice MR219.

INTRODUCTION

Rice (*Oryza sativa* L.) is an important primary cereal crop in the world. It is the staple food for more than two-third of the world's population. An increase in rice resistance to abiotic (low and high temperatures, drought and salinization) and biotic (phytopathogenic microorganisms and insect pests) factors is of great commercial value.

Genetic engineering is an essential method for improving agronomic characteristics of plants and supplementing the traditional breeding approaches (Morrish et al., 1993; Folling and Olsen, 2002; Sreeramanan et al., 2005; Fadeev et al., 2006). Although a number of laboratories in the world are involved in producing and improving transgenic rice, the transformation efficiencies achieved in Malaysia is mainly low. The transformation efficiency may be elevated via optimization of the transformation parameters and various methods for selection of transgenic line and regenerants (Fadeev, 2006; Andres et al., 2009). For these practical purposes, it is necessary to produce a large number of independent transgenic events. However, many significant factors

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Abbreviations: NAA, α-Naphthalene acetic acid; **2,4-D,** 2,4-dichlorophenoxyacetic acid; **EDTA,** ethylene diamine tetraacetic acid.

influences the production of transgenic rice lines through the biolistic gun system (Sivamani et al., 1996, Saker et al., 2006).

Optimization of the various physical and biological parameters affecting particle bombardment transformation frequency would be more easily and effectively performed if the transformed sectors could be detected and recovered for the regeneration purpose as possible after the experiment (Jain et al., 1996; Tadesse et al., 2003; Chong and Mahmood, 2005; Sreeramanan et al., 2005; Andres et al., 2009; Mousavi et al., 2009). Chong and Mahmood (2005), Sreeramanan et al. (2005) and Majid and Parveez (2007) optimized various physical and biological parameters for transient expression of *β*-glucuronidase (GUS) and GFP reporter genes in orchid, banana and oil palm. Reporter gene *gus*A encoding for enzyme GUS is used to provide a clear indication of the expression, transient or stable, of transferred genes in plant transgenic cells (Jefferson et al., 1987; Sreeramanan et al., 2005; Saker et al., 2006). Similarly, Mousavi et al. (2009) reported on the optimization of physical and biological parameters for transient expression of *uidA* gene (GUS gene) in embryogenic callus of date palm (*Phoenix dactylifera* L.) via particle bombardment.

Up till now, no efficient protocol has been described in the literature for the optimization of physical and biological parameters using biolistic gun system in embryogenic callus culture of indica rice MR219. Therefore, the objective of this research work was to optimize the transformation condition by microparticle bombardment in indica rice MR219 on the basis of transient *gusA* gene expression parameters.

MATERIALS AND METHODS

Plasmid

The plasmid pCAMBIA1305.2. (http://www.cambia.org.au/) (*uidA* version GUSPlus™ and the *hpt*gene) was used for the biolistic bombardment experiment. The reporter gene is an intron-containing *gusA* gene under the control of 35S promoter of Cauliflower Mosaic Virus (CaMV35S).

Callus preparation for transformation

Embryogenic calli used in this study were derived from mature seed. The calli were maintained on agar solidified embryogenic media containing MS including B5 vitamin (Murashige and Skoog, 1962) and supplemented with 1 mg/l 2,4-D and 10 mg/l NAA, 30 g/l sucrose and 3.5 mg/l agar (Zuraida et al., 2007) The medium was adjusted to pH 5.7 with KOH prior to autoclaving. Embryogenic calli were placed on basal medium with 10 mg/l maltose (MBO) at the centre of a petri dish (90 mm in diameter) and incubated overnight prior to bombardment then placed at 28°C in dark. For each parameter tested, six independent bombardments (replicates) were carried out. The controls include non-bombarded tissues and tissues bombarded with gold microcarriers only (no DNA). All bombarded and non-bombarded tissues were subjected to transient GUS histochemical assay 3 days after bombardment.

Biolistic transformation

The Biolistic ^{TM} PDS/1000 helium system (BioRad, USA) was used in this study. The biolistic device parameters analyzed were as follows: rupture disk pressure (helium pressure of 450, 650, 900, 1100, 1350 and 1550 psi); macrocarrier to stop screen distance (6, 11 and 16 mm), stopping plate to target tissue distance (60, 90 and 120 mm); vacuum pressure [26, 27 and 28 inches of mercury (inHg)]. Other parameters analyzed include gold microparticles size (0.6, 1.0 and 1.6 mm), presence of spermidine and calcium in DNA precipitation step, and number of bombardment (s) (1X, 2X and 3X) per target tissue plate.

GUS histochemical assay

GUS assay was performed according to Jefferson (1987). After bombardment of the cell, the tissues were incubated in the dark at 24 ± 1°C for at least two days to allow cell repair and DNA intergration (Jefferson, 1987). In this experiment, bombarded embryogenic calli were incubated for a week in order to differentiate the viable embryos from those damage tissues. The viable calli were transferred into histochemical reagent containing 0.1 M phosphate buffer, 0.5 mM ferricyanide, 0.5 mM ferrocyanide, 0.1% triton X-100, 10.0 mM EDTA, 20% methanol and 1.0 mM 5-bromo-3-indolyl-glucuronide (X-gluc) (Clontech). The samples were incubated for 18 to 24 h at 37°C, at which time the tissues were evaluated for their level of GUS expression. After staining, the samples were cleared with 70% ethanol. Transient GUS activity was recorded as blue spots (irrespective of size) using Olympus stereomicroscope and then photographed using Nikon camera.

Statistical analysis

The analyses of variances were done and means were compared by the Duncan's multiple range test (DMRT) using SPSS program 9.0 (SPSS Inc. USA).

RESULTS AND DISCUSSION

Effect of helium pressure

The preliminary bombardment data shows that the Biolistic PDS-lOOO/He apparatus was capable of delivering DNA into indica rice embryogenic calli. Transient expression of the *uidA* gene (defined by number of blue spots) was used during this study as an indicator to monitor the effects of various physical and biological conditions on the efficiency of this system (Figure 1A). Bombardment of the microcarries without DNA and unbombarded tissue did not show transient GUS gene expression (Figure 1B).

The ability of the microparticles to penetrate the different cell layers or tissue types is greatly dependent on the propelling force of the helium gas (Kirkkert, 1993). Results showed that changes in helium pressures were found to affect transient GUS expression significantly. It was observed that 1100 psi helium pressure gave highest (196) transient GUS gene expression compared to 1300 psi (130), 1550 psi (119), 900 psi (97) and 450 psi (65) (Figure 2), which was consistent with observations in rice

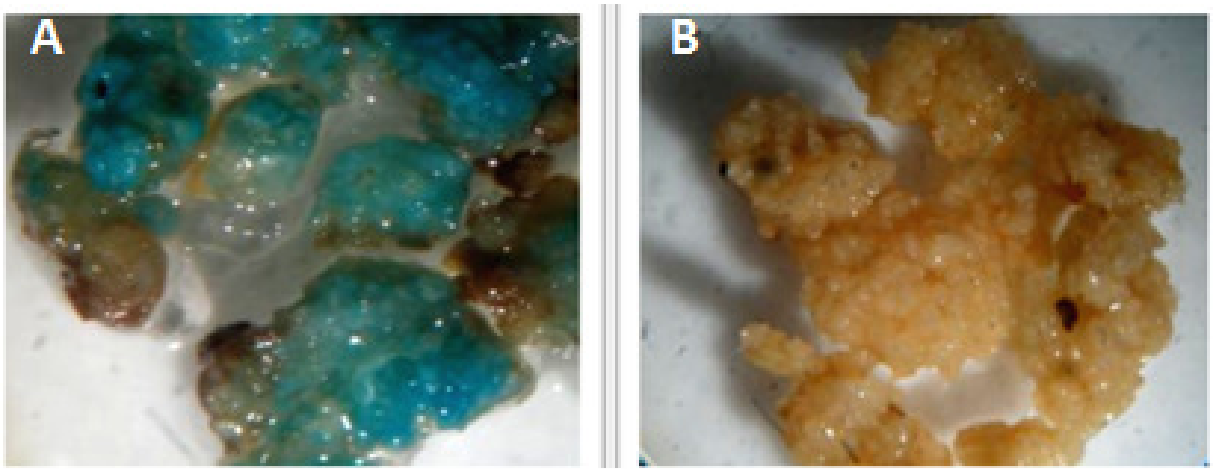

Figure 1. GUS expression in embryogenic calli. (A)GUS-positive (blue colour) and (B) control.

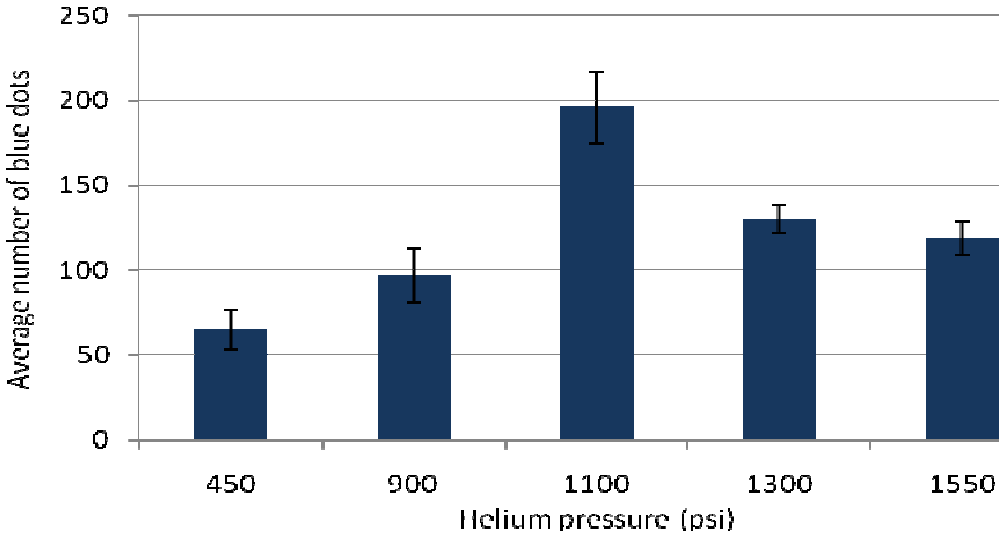

Figure 2. Effect of helium pressure on transient GUS expression in rice embryogenic calli.

(Zhang et al., 1996; Ramesh and Gupta, 2005) and cassava (Schopke et al., 1997). Bombarding using lower or higher pressures did not result in any significant increase in transient GUS expression. Lower expression at lower pressure could be attributed to the poor penetration capability of the microparticles as they moved towards the tissues. While at higher pressures, the high penetrating force of the microparticles might be injurious to the tissues.

Effect of distance from stopping screen to target tissue

Figure 3 compares the effect of distances (stopping plate to target tissue) on transient GUS expression at three different helium pressures. Changes in both helium pressure and distance were found to affect the level of transient GUS expression. For 1100 psi, the highest expression was observed at 9 cm.

Schopke et al. (1997) reported similar result of using 1100 psi with 9 cm combination that gave higher expression in their cassava cultures. For the same psi at 6 cm, a lower expression level was observed, which could be due to tissue damage as tissue dislocation was observed at this closed-up range. While at 12 cm, the transient gusA expression level was lowest, this could be due to decreased velocity of the microparticles with the long flight distance giving reduced penetration force and thereby fewer cells receiving the oncoming DNA. Increasing flight distance resulted in reduced transient expression which was also reported by Oard et al. (1990) and Parveez et al. (1997). For 1300 psi, the highest expression was observed at 12

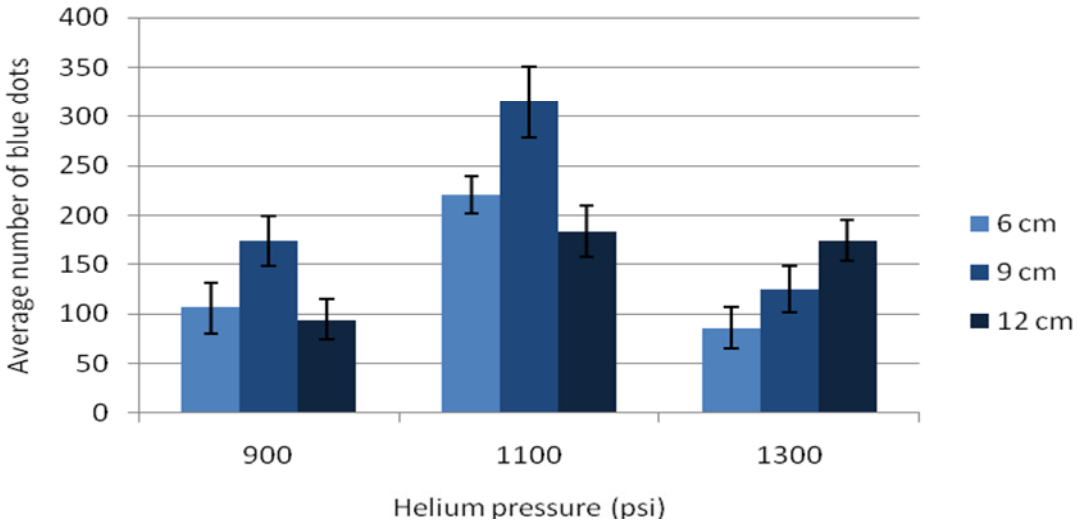

Figure 3. Effect of distance from stopping plate to target tissue and helium pressure on transient GUS expression in rice embryogenic calli.

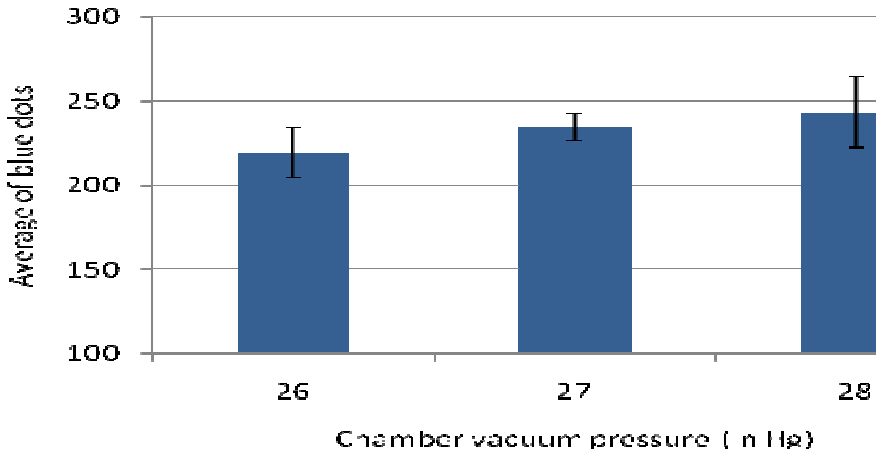

Figure 4. Effect of chamber vacuum pressure (in Hg) on transient GUS gene expression in indica rice embryogenic calli.

cm, while slightly lower expression levels were observed at 9 and 6 cm. At 900 psi, distance of 9 cm gave the highest expression level compared to 6 and 12 cm.

Effect of vacuum pressure

The vacuum in the bombardment chamber plays an important role in determining the drag forces that act on the micro particles during flight, thereby affecting the rate at which the micro particles lose velocity with distance. In short, the higher the vacuum, the better the microcarriers can maintain velocity (Kikkert, 1993).

However, there is a drawback of maintaining a high vacuum pressure in the chamber because too high a vacuum (<200 millibars) will cause the tissue to lose moisture rapidly and thereby reduce cell viability (McCabe and Christou, 1993). The best vacuum pressure that gave the highest transient GUS expression in the callus was accorded 28 in Hg (243), however there is no significant result when compared to other pressure (Figure 4). The lowest transient expression was observed at 26 in Hg (219). This is not surprising because as the vacuum pressure decreases, the velocity of the micro particles is slowed down; thereby the chances of getting a hit or a good penetration into the target tissues are greatly reduced.

Gold microcarrier size

Different gold micro particles sizes (0.6, 1.0 and 1.6 µm)

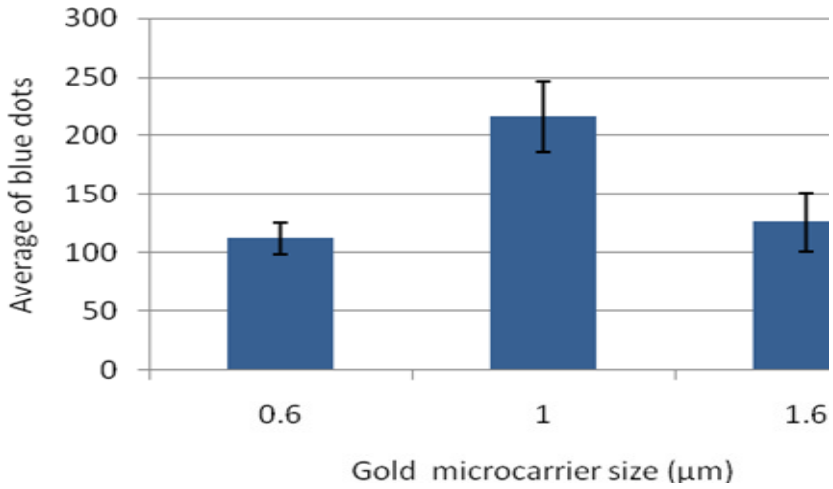

Figure 5. Effect of different gold microcarriers size on transient GUS expression in indica rice callus.

were compared for their efficiencies in delivering DNA into the target tissues. The combination size of 0.6 and 1.0 µm gave the highest (169) expression level when compared to other sizes used (Figure 5). High expression was also achieved when using 1.0 µm (119) and 1.6 µm (126) separately, when compared to 0.6 µm (55). It was suggested that 1 µm gold particle could be used as the superiority gold particle as reported in maize (Klein et al., 1988), wheat (Wang et al., 1988), tobacco (Russell et al., 1992), peanut (Clemente et al., 1992) and Norway spruce (Martinussen et al., 1994). The exceptions were white spruce embryogenic suspension cultures and einkorn wheat cells where 1.6 pm gold was better than that of 1.0 pm (Li et al., 1994; Takumi et al., 1994).

According to Parveez et al. (1997), a gold microcarrier of size 1.0 pm produced significantly higher transient GUS expression than the other microcarriers. Use of smaller size particles reduced the transient expression. However, increased size (above 1.3 µm) also resulted in significantly much lower transient expression. Folling and Olsen (2002) reported higher damaging effect with larger micro particles size in their wheat transformation. Choosing the right size of particle is important because different target cell may need a different size of microcarrier. A very small microcarrier will have a lower penetration force and a larger one will increase tissue damages (Klein et al., 1988). In future experiments for stable expression of indica rice callus, the combination of size of 0.6 and 1.0 µm gold particle would be used as the superior.

Number of bombardments

Increasing the number of bombardments significantly increased transient GUS expression in the rice callus (Figure 6). In this experiment, there was no significant different between double and triple bombardments, although triple bombardment gave higher transient GUS value. The same observation was reported; double and triple bombardments have been shown to increase transient expression proportionally in rice and wheat suspension cultures (Wang et al., 1988), banana (Sreeramanan et al., 2005) and triple bombardment in maize (Klein et al., 1988) were found to be better than single bombardment treatment.

On the other hand, double and triple bombardments in barley (Kartha et al., 1989), maize (Reggiardo et al., 1991) and peanut (Clemente et al., 1992) were shown to reduce the transient expression. Parveez et al. (1997), observed no significant differences between single and double bombardments in their oil palm although double bombardment gave higher expression.

$CaCl_2$ and spermidine in DNA-microcarrier precipitation

Before DNA can be transferred into plant cells, the DNA of interest must bind to the microcarrier. The result obtained from this experiment demonstrated the importance of $CaCl_2$ and spermidine for indica rice callus transformation. Spermidine was found to be more important for DNA to bind onto gold microcarriers than $CaCl_2$ (Figure 7). Generally, for both cases, the $CaCl_2$ and spermidine have a positive effect on the binding of DNA onto microcarriers.

Similar result was reported in cereal crop (Morrish et al., 1993), with the suggestion that both, $CaCl_2$ and spermidine are essential for good DNA precipitation onto micro carriers. Perl et al. (1992) suggested that spermidine was the major cause of particle aggregation

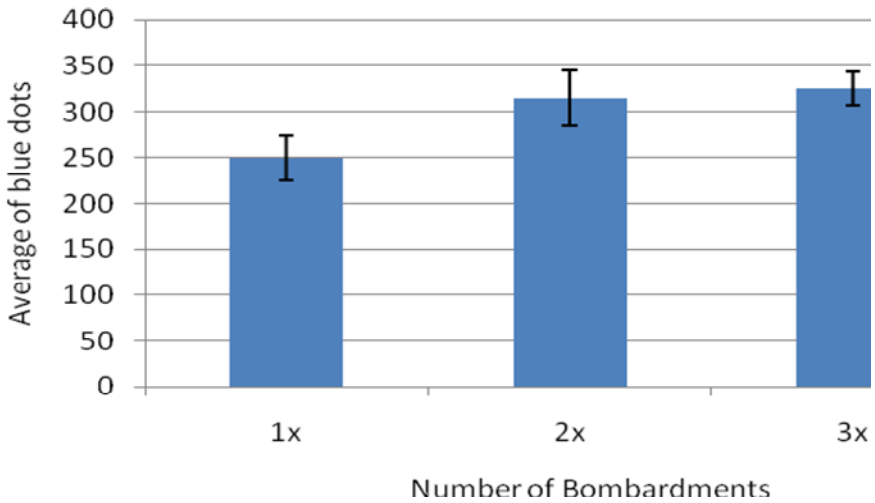

Figure 6. Effect of number of bombardments on transient GUS expression in indica rice callus.

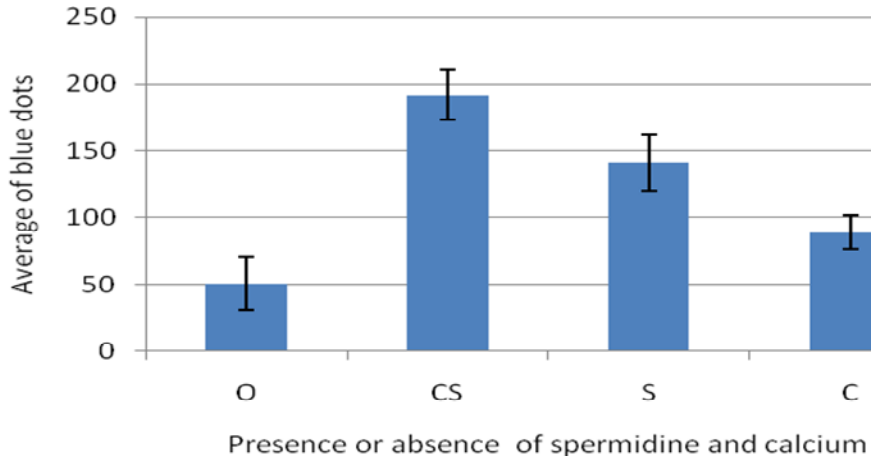

Figure 7. Effect of $CaCl_2$ and spermidine in the DNA-microcarrier cocktail mixture on transient GUS expression in indica rice callus**.** O = no spermidine or calcium; CS = both calcium and spermidine were added; S = spermidine only; C = calcium only.

which resulted in massive tissue wounding. Drastic reduction in transient GUS expression occurred in wheat immature embryo when spermidine was excluded from DNA precipitation procedure prior to bombardment treatment (Vasil et al., 1993). Calcium ions have also been reported to play a major role in binding of DNA onto metal in which the presence of calcium showed significant increase in transient GUS expression in wheat transformation (Perl et al., 1992).

Age of callus

Effect of age of callus tissue on transient expression of *gusA* gene is presented in Figure 8. Callus obtained from 3, 6, 9 and 12 week-old cultures, were subjected to bombardment, and then transient *GUS* expression. *GUS* expression was higher in 9 weeks old callus followed by 12, 6 and 3 weeks. Neither 3 nor 6-week-old cultures exhibited good *gusA* expression when compared to the 9 and 12-week-old callus. Observations have been previously reported for rice callus tissue, calli tested for *gusA* expression was highest in 44 days old callus tissues. The expression results scored for calli bombarded after 56 and 68 days old gradually decreased (Ramesh and Aditya, 2005).

The age of tissue is an important factor that needs to be considered for transformation work. Since particle bombardment involves the penetration of heavy metal particles into intact cells or tissues, microparticles hits

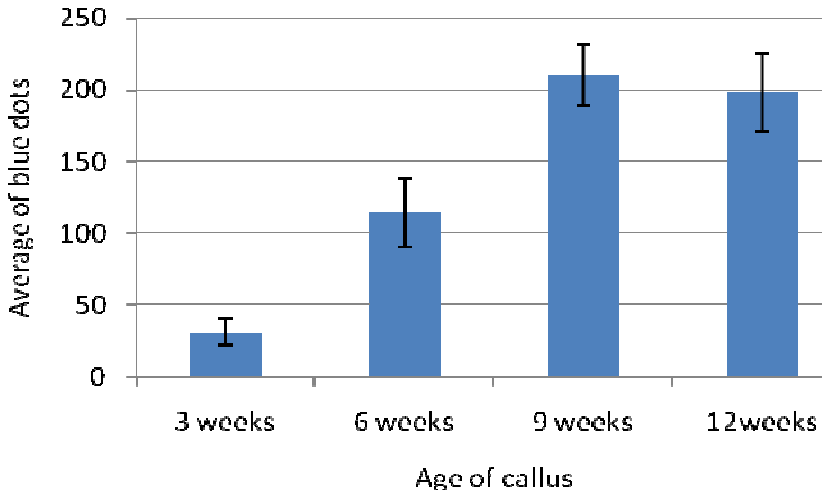

Figure 8. Effect of callus age on transient GUS expression in indica rice callus

Table 1. Effect of osmotic pre-culture length and osmotic pretreatment on transient GUS expression in indica rice embryogenic callus.

Length of osmotic pre-culture	Osmotic pre-treatment	Average of blue dots
	No osmoticum (Control)	56 ± 6
12 h	0.5 M Sorbitol	119 ± 14
	0.5M Mannitol	78 ± 7
	0.5 M Maltose	91 ± 21
24 h	No osmoticum (Control)	31 ± 5
	0.5 M Sorbitol	121 ± 15
	0.5M Mannitol	56 ± 11
	0.5 M Maltose	78 ± 7
48 h	No osmoticum (Control)	23 ± 4
	0.5 M Sorbitol	78 ± 13
	0.5M Mannitol	43 ± 5
	0.5 M Maltose	56 ± 4
72 h	No osmoticum (Control)	19 ± 7
	0.5 M Sorbitol	34 ± 9
	0.5M Mannitol	21 ± 11
	0.5 M Maltose	9 ± 3

may provoke various levels of tissue wounding and damage tissue (Tadesse et al., 2003). Increase in tissue damage in young explants indicates that this caused the softening of the tissue (Puddephat et al., 1999). Considering that majority of calli at ages ranging from 9 to 12 weeks are chosen, they are the most suitable culture age for bombardment of indica rice.

Osmotic impact on the callus

The length of osmotic pre-culture and osmotic pretreatment culture medium had a significant influence on the average of blue dots (Table 1). The highest number of blue spots was obtained when calli were cultured on medium supplemented with 0.5 M sorbitol for 24 h prior to particle bombardment. It was shown that shorter period, 12 to 24 h of preculture on osmotic agents resulted in a higher average of blue dots with transient *uidA* expression of Malaysian indica rice MR219. Rosillo et al. (2003) demonstrated that culture of *Coffea arabica* cv. Colombia suspension cultures for 4 h prior to bombardment with a 0.5 M mannitol and sorbitol mixture resulted in a higher number of blue spots.

Moreover, the use of high concentrations of mannitol, sorbitol or sucrose has improved transient *uidA* expression

in maize (Vain et al., 1993), rice (Jain et al., 1996), wheat (Ingram et al., 1999) and marigold (Vanegas et al., 2006). Mousavi et al. (2009) reported that mannitol with 179 ± 21 blue spots was found to be more effective than sorbitol (52 ± 9), glucose (2 ± 0.5) and sucrose (1 ± 0) with respect to transient *gusA* gene expression in embryogenic callus of date palm (*Phoenix dactylifera* L.). It is known that the type and concentration of osmotic agent may increase transient gene expression by reducing turgor pressure in cells. Therefore, the chance of cell survival increases by avoiding leakage following the shock wave created during bombardment (Rosillo et al., 2003). Moreover, a high concentration of osmotic agents may also induce changes in cell membranes, leading to increased cell tolerance to biolistic delivery impact (Ingram et al., 1999).

From this study, it is clear that optimization could improve the delivery and expression of foreign genes in rice callus. We anticipate that this improvement could lead to corresponding improvement in stable rice transformation.

REFERENCES

Andrés MG, Griselda A, Ana ME (2009). Optimization of coffee (*Coffea arabica*) transformation parameters using *uidA* and *hpt* genes: effect of osmotic pre-treatment, helium pressure and target distance. Rev. Biol. Trop. 57(1): 151-160.

Chong Siang Tee, Mahmood Maziah (2005). Optimization of biolistic bombardment parameters for *Dendrobium* Sonia 17 calluses using GFP and GUS as the reporter system. Plant Cell, Tissue Org. Cult. 80: 77-89.

Clemente TE, Robertson D, Isleib TG, Beute MK, Weissinger AK (1992). Evaluation of peanut *(Arachis hypoguea* L.) leaflets from mature zygotic embryos as recipient tissue for biolistic gene transfer. Transgenic Res. 1: 275-5284.

Fadeev VS, Blinkova OV, Gaponenko AK (2006). Optimization of biological and physical parameters for biolistic genetic transformation of common wheat (*Triticum aestivum* L.) using a particle inflow gun. Russian J. Genet. 42(4): 402-411.

Folling L, Olesen A (2002). Transformation of wheat (*Triticum aestivum* L.) microspore-derived callus and microspores by particle bombardment. Plant Cell Rep. 20: 1098-1105.

Ingram HM, Power JB, Lowe KC, Davey MR (1999). Optimization of procedures for microprojectile bombardment of microspore-derived embryo in wheat. Plant Cell Tissue Org. Cult. 57: 207-210.

Jain RK, Jain S, Wang B, Wu R (1996). Optimization of biolistic method for transient gene expression and production of agronomically useful transgenic Basmati rice plants. Plant Cell Rep. 15: 963-968.

Jefferson R A (1987). Assaying chimeric gene inplant: The *uid*A gene fusion system. Plant Mol. Biol. Rep. 5: 387-405.

Kartha KK, Chibbar RN, Georges F, Leung N, Caswell K, Kendall E, Qureshi J (1989). Transient expression of chloramphenicol acetyltransferase (CAT) gene in barley cell cultures and immature embryos through microprojectile bombardment. Plant Cell Rep. 8: 4299432.

Kikkert JR (1993). The Biolistic PDS-1000/He device. Plant Cell, Tissue Org. Cult. 33: 221-226.

Klein TM, Gradziel T, Fromm ME, Sanford JC (1988). Factors influencing gene delivery into *Zea mays* cells by high velocity microprojectiles. Bio/Technol. 6: 559-563.

Li YH, Trembley FM, Seguin A (1994). Transient transformation of pollen and embryogenic tissues of white spruce *(Picea gluuca* Moench.) resulting from microprojectile bombardment. Plant Cell Rep. 13: 661-665.

Majid NA, Parveez GKA (2007). Evaluation of green fluorescence protein (GFP) as a selectable marker for oil palm transformation via transient expression. Asia Pac. J. Biol. Biotechnol. 15(1): 1-8.

Martinussen I, Junttila 0, Twell D (1994). Optimization of transient gene expression in pollen of Norway spruce *(Picea abies)* by particle acceleration. Physiol. Plant. 92: 412-416.

McCabe D, Christou P (1993). Direct DNA transfer using electric discharge particle acceleration (ACCELL TM technology). Plant Cell, Tissue Org. Cult. 33: 227-236.

Morrish F, Songstad DD, Armstrong CL, Fromm M (1993). Microprojectile bombardment: A method for the production of transgenic cereal crop plants and the functional analysis of genes. In: A. Hiatt (Ed.), Transgenic Plants. Marcel Dekker, New York. pp. 133-171.

Mousavi M, Mousavi A, Habashi AA, Arzani K (2009). Optimization of physical and biological parameters for transient expression of *uidA* gene in embryogenic callus of date palm (*Phoenix dactylifera* L.) via particle bombardment. Afr. J. Biotechnol. 8(16): 3721-3730.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassay with tobacco tissue culture. Plant Physiol.15: 473-497.

Oard JH, Paige DF, Simmonds JA, Gradziel TM (1990). Transient gene expression in maize, rice, and wheat cells using an air gun apparatus. Plant Physiol. 92: 334-339.

Parveez GKA, Chowdhury MKU, Saleh NM (1997). Physical parameters affecting transient GUS gene expression in oil palm (*Elaeis guineensis* Jacq.) using the biolistic device. Ind. Crops Prod. 6:41-50.

Perl A, Kless H, Blumenthal A, Galili G, Galun E (1992). Improvement of plant regeneration and GUS expression in scutellar wheat calli by optimization of culture conditions and DNA-microprojectile delivery procedures, Mol. Gen. Genet., 235: 279-284.

Puddephat IJ, Thompson N, Robinson HT, Sandhu P, Henderson J (1999). Ballistic transformation of broccoli (*Brassica oleracea* var italic) for transient expression of the β-glucuronidase gene. J. Hort. Sci. Biotechnol. 74: 714-720.

Ramesh M, Gupta AK (2005). Transient expression of glucuronidase gene in indica and japonica rice (*Oryza sativa* L.) callus cultures after different stages of co-bombardment. Afr. J. Biotechnol. 4(7): 596-600.

Reggiardo MI, Arana JL, Orsaria LM, Permingeat HR, Spitteler MA, Vallejos RH (1991) Transient transformation of maize tissues by microparticle bombardment. Plant Sci. 75: 2377243.

Rosillo G, Acuña J, Gaitan A, Peña De M (2003). Optimized DNA delivery into *Coffea arabica* suspension delivery suspension culture cells by particle bombardment. Plant Cell Tissue Org. Cult. 74: 45-49.

Russell JA, Roy MK, Sanford JC (1992). Physical trauma and tungsten toxicity reduce the efficiency of biolistic transformation. Plant Physiol. 98: 1050-1056.

Saker MM, Abdallah SS, Youssef SS, Moursy HA, El-Sharkawy AM (2006) Development of transformation system for some Egyptian rice genotypes using particle bombardment. Ameriacn-Eurasian J. Agri. Sci. 1(1): 7-13.

Schopke C, Taylor NJ, Carcamo R, Beachy RN (1997). Optimization of parameters for particle bombardment of embryogenic suspension cultures of cassava (*Manihot esculenta* Crantz) using computer image analysis. Plant Cell Rep. 16: 526-530.

Sivamani E, Shen P, Opaika N, Beachy, RN, Fauquet CM (1996). Selection of large quantities of embryogenic calli from indica rice seeds for production of fertile transgenic plants using the biolistic method. Plant Cell Rep. 15: 322-327.

Sreeramanan S, Maziah M, Abdullah MP, Sariah M, Xavier R, Nor'Aini MF (2005). Physical and biological parameters affecting transient GUS and GFP expression in banana via particle bombardment. Asia Pacific J. Biol. Biotechnol. 13(1): 35-57.

Tadesse Y, Swennen R, Jacobs M (2003). Optimisation of transformation conditions and production of transgenic sorghum (*Sorghum bicolor*) via microparticle bombardment. Plant Cell Tissue Org. Cult. 75(1): 1-18.

Takumi S, Otani M, Shimada T (1994). Effect of sis promoter-intron combination on transient expression in einkorn, emmer and common wheat cells by particle bombardment. Plant Sci. 103: 161-166.

Vain P, Mcmullen MD, Finer JJ (1993). Osmotic treatment enhances particle bombardment- mediated transient and stable transformation of maize. Plant Cell Rep. 12: 84-88.

Vanegas PE, valdez-Morales M, valverde ME, Cruz Hernández A, Paredes-López O (2006). Particle bombardment, a method for gene transfer in marigold. Plant Cell Tissue Org. Cult. 84: 359-363.

Vasil V, Brown SM, Re D, Fromm ME, Vasil IK (1991). Stably transformed callus lines from microprojectile bombardment of cell suspension cultures of wheat. Bio/ Technol. 9: 743-747.

Wang YC, Klein TM, Fromm M, Cao J, Sanford JC, Wu R (1988). Transient expression of foreign genes in rice, wheat and soybean cells using particle bombardment. Plant Mol. Biol. 11: 4333439.

Zhang W, Chen L, Qu R, Marmey P, Beachy R, Fauquet C (1996). Regeneration of fertile transgenic indica (group 1) rice plants following microprojectile transformation of embryogenic suspension culture cells. Plant Cell Rep. 15: 465-469.

Zuraida AR, Hamidah G, Habibuddin H, Shaiful AS, Marzukhi H, Naziah B, Zulkifli AS (2007). Optimization of callus induction for Malaysian indica rice variety MR219. Proceeding of USM-UPM- PETA Conference 2007, Evergreen Laurel Hotel, Penang Malaysia. 28-29th November.