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Genetic transformation of maize (*Zea mays* L.) using scutellar tissue of immature embryo mediated by *Agrobacterium tumefaciens*

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Abstract

Agrobacterium-mediated transformation emerges as a more promising avenue than the biolistic approach for genetically transforming maize plants. Within our transformation endeavours, we harnessed *Agrobacterium tumefaciens* strain EHA 105, equipped with the p CAMBIA 1305.1 plant transformation vector. This dynamic combination served as the driving force behind the alteration of both mature seed-derived calli and immature embryo explants. When considering the performance of these explants, immature embryos exhibited superior characteristics and were therefore favoured as the primary target material for transformation. This preference was underscored by their ability to yield plantlets with a remarkable transformation frequency of 24-30 % within the COH(M) 5 maize variety. Critical parameters were uncovered to heighten the success of the transformation process. Notably, collecting immature embryos at the 10-12 days after pollination (DAP) stage, boasting a size of 0.8 - 1.5 mm and subsequently inoculating them after 2 days of *Agrobacterium tumefaciens* cultivation (with an optical density of 1.0 at 600 nm) emerged as pivotal factors that significantly elevated the transient *GUS* expression rate. The art of cocultivation was found to be optimally executed through the immersion of explants within the bacterial suspension, while the subsequent washing step utilizing sterile distilled water infused with 150 mg L^{-1} of cefotaxime and 250 mg L^{-1} of carbenicillin yielded the most favourable outcomes in terms of transient GUS expression for the COH(M) 5 maize variety, surpassing alternative methods. Moreover, the application of cefotaxime up to 150 mg L^{-1} and carbenicillin up to 250 mg L^{-1} proved instrumental in securing the highest frequency of regeneration, underscoring their optimal range. Similarly, when considering the selection agent hygromycin, concentrations of up to 30 mg L^{-1} surfaced as the ideal range, fostering not only maximum regeneration but also a higher count of shoot formations.

Keywords

Agrobacterium; Immature embryo; GUS expression; co cultivation

Introduction

Maize (*Zea mays* L.) holds the distinction of being the world's third most crucial food and forage crop, trailing only wheat and rice in significance. Arguably, it claims the title of the most economically vital cereal crop on a global scale. Despite its paramount importance, the realization of maize's full potential in terms of yield and production frequently falls short due to a

plethora of challenges. These include abiotic stressors like drought, aluminium toxicity and nutrient scarcity, alongside biotic stressors such as pests, weeds and diseases (1). Addressing the escalating demand for maize necessitates the increasing application of biotechnological advancements for genetic enhancement. Manipulating the plant genome by introducing foreign genes from sources as diverse as bacteria, fungi, exotic plants, animals and even humans offer the promise of generating plants endowed not only with resistance against viral, bacterial and fungal ailments but also the capacity to withstand adverse environmental conditions (2). Currently, 2 prominent transformation protocols stand out for maize. Maize transformation can be achieved by either biolistic or *Agrobacterium* mediated methods (3). Among these, *Agrobacterium tumefaciens* has risen to prominence as the preferred vehicle for transgene delivery in maize transformation. This preference is largely attributed to the advantages inherent in T-DNA transfer compared to alternative gene delivery systems. Notably, the prevalence of straightforward insertion events accompanied by intact and enduring transgene expression and inheritance contributes to *Agrobacterium*-mediated transformation's allure (4). Early investigations into *Agrobacterium* infection in maize were documented by (5, 6). The feasibility of *Agrobacterium*-mediated transformation in cereal species, however, was initially hinted (7) who accomplished the transformation of immature embryos in rice using *Agrobacterium tumefaciens*. More recently, this technique has been effectively harnessed for maize, yielding transgenic maize plants at a remarkable frequency (8). Noteworthy advancements include the establishment of a high-throughput transformation system in maize employing *Agrobacterium*-mediated T-DNA delivery (9) as well as the successful transformation of maize using *Agrobacterium*'s standard vector system, attested (10) who reported an average transformation efficiency of 5.5 %. In this present study, the objective is to standardize the *Agrobacterium*-mediated transformation process for various maize genotypes, utilizing well-established marker genes such as *gus* and *hpt*. This endeavour aims to contribute to the refinement and optimization of the technique for enhancing maize genetic potential.

Materials and Methods

Plant material

Four maize genotypes (UMI 757, UMI 112, COH(M) 5 and UMI 285) were grown in Agricultural College and Research Institute, TNAU, Madurai. Immature ears free from pests and disease were harvested at 12-14 Days After Pollination (DAP). Immature kernels were sterilized with 70 % ethanol for 30 sec followed by 3 sterile distilled water washing, then sterilized with 0.1 % $HgCl_2$ for 30 sec and rinsed 3 times with sterile distilled water. Immature embryos 0.8 - 1.5 mm in length were aseptically excised from the kernels of the ears under the microscope.

Pre culture of explant

Explant selection and pre-culture

Excised immature embryos with the rounded scutellar side exposed and the flat plumule - radicle axis side in contact with the 2,4 D medium (Murashige and Skoog (MS) medium having $CaCl_2$, vitamins and sucrose from HI-MEDIA®). Around 50 embryos were placed in each plate and sealed with phytawrap™ (Himedia). Six weeks at 25 ± 2 ºC in both the light and dark with a continuous subculture in 15 days intervals to get embryogenic callus. The callus was selected and resized into 2 - 4 mm diameter and cultured in petri plates (90 x 15 mm) having pre-culture medium (PCM).

Agrobacterium growth and infection

Utilizing *Agrobacterium* strain EHA 105 containing the pCAMBIA 1305.1 plasmid (Fig. 1), received from Centre for Plant Molecular Biology, Tamil Nadu Agricultural University, Coimbatore and we initiated the transformation process. The p CAMBIA 1305.1 plasmid contains the *hpt* selectable marker gene (hygromycin) as well as the *gus* reporter gene, pivotal for bacterial selection. The *Agrobacterium* strain was streaked onto an AB plate supplemented with rifampicin (10 mg L^{-1}) and kanamycin (50 mg L^{-1}). These plated cultures were cultivated at a temperature of 28 ºC in complete darkness. Different cultivation periods spanning 1, 2, 3 and 4 days were explored to optimize the ideal duration for *Agrobacterium* cocultivation. It's worth highlighting that the presence of AC (an unspecified factor or compound) emerged as an indispensable component for ensuring the triumph of the transformation process, contributing significantly to achieving a robust and high-frequency transformation rate.

The *Agrobacterium* suspension, augmented by the vir gene inducer AC (Acetosyringone) at a concentration of 100 µM, was meticulously prepared in an Eppendorf tube. Subsequently, the plant explants were submerged in this concoction for varying intervals, namely 10, 20, 30 and 60 minutes. Following the inoculation process, the explants

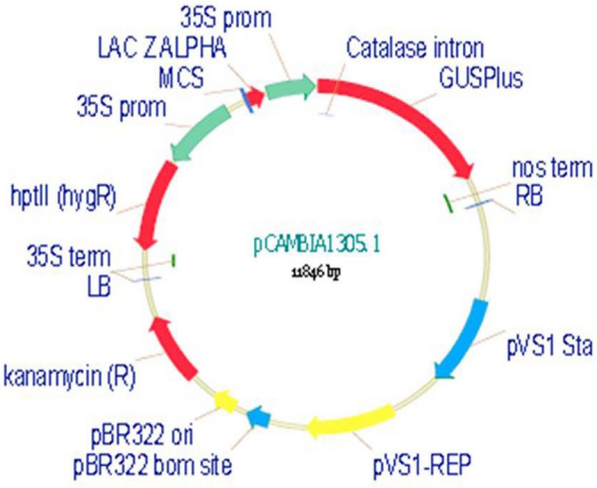

Fig. 1. Physical map of p CAMBIA 1305.1 harbouring *gus* and *hpt* gene.

were meticulously drained and gently blotted dry, using a sterile filter paper as the substrate. These prepared explants were then carefully transferred onto a cocultivation medium. To facilitate the cocultivation phase, the infected embryos were once again gently blotted dry on filter paper and then positioned on the cocultivation medium, ensuring the scutellum side was oriented upwards. The cultures were nurtured within a controlled environment maintained at a temperature of 22 ± 1 ºC, with darkness prevailing. As the cocultivation period concluded, the explants underwent a comprehensive washing process to effectively eliminate any surplus *Agrobacterium* growth that might have transpired during the incubation period.

For the purpose of determining the optimal dosage of antibiotics during the regeneration phase, a meticulous investigation involved the inclusion of cefotaxime and carbenicillin into the regeneration medium. These antibiotics were introduced at varying concentrations spanning 0, 50, 100, 150 and 200 mg/L. Within this context, it's worth noting that the regeneration medium was formulated with precision, comprising MS medium supplemented with cefotaxime at 100 mg/L, carbenicillin at 250 mg/L, hygromycin at 30 mg/L, maltose at 30 mg/L, casein hydrolysate at 1.0 g/L and agar at 8.0 g/L. The outcome of this intricate formulation was the promotion of the proliferation of antibiotic-resistant calli and immature embryos, specifically on the selection medium. It is imperative to highlight that this medium served as a discriminator, allowing only transformed explants to persevere, while non-transformed counterparts were effectively inhibited. This outcome was achieved by causing non-transformed explants to perish when subjected to the selection medium. Following a 14 days interval, during which proliferating calli underwent subculture onto fresh selection medium, these hardy calli - which had successfully weathered 3 rounds of selection, each spaced 15 days apart - were subsequently transferred to a regeneration medium. This regeneration medium was tailored, featuring MS medium supplemented with tryptophan at 1.0 g/L, maltose at 30 mg/L, BAP (Benzyl amino purine) at 2.5 mg/L, NAA (α-Naphthalene acetic acid) at 0.5 mg/L and kinetin at 1.0 mg/L. The incubation conditions for this stage encompassed a temperature of 25 ºC and a photoperiod of 16 h. As the final stage unfolded, the emerging shoot buds, emblematic of successful transformation, were meticulously transplanted onto a medium boasting half-strength MS formulation to facilitate the process of root development and growth.

Transient stable *GUS* assays

The assessment of transient *GUS* activity within the calli and immature embryos undergoing transient transformation was executed through a histochemical method (11). This involved subjecting leaf and root fragments extracted from potentially transformed plants to an incubation process within an X-Gluc staining solution, maintained at a temperature of 37 °C over the course of an overnight period.

Results and Discussion

Maize is one of the most important crops throughout the world and the second largest food crop in the United States. Hence, there is a great need to produce maize cultivars that are not only adaptable to different climates. Consequently, the ability to manipulate maize in tissue culture systems is not only to elucidate the genetic control of plant development but also to exploit its commercial application (12).

Efficient *in vitro* regeneration of normal and fertile plants from single cells, tissues and organs is a basic prerequisite for the production of genetically transformed plants (13). Plant regeneration through tissue culture of maize was first reported (14). Despite the extensive progress made in tissue culture studies of maize, genotype dependence still plays a role when culturing immature embryos (15). Immature embryo has proven to be the best source for the establishment of embryogenic callus and plant regeneration in maize.

Several factors have been reported to be related to the formation of callus as well as plantlet development from immature embryos in maize. Successful regeneration of plants depends on the genotypes, choice of tissue, development stage of the plant, culture media and the different stages of the tissue culture process (16). The age of embryos, placement of embryos on medium and composition of the culture medium are some of the major factors (17).

Agrobacterium mediated genetic transformation is an important and powerful tool. It is an efficient and reproducible transformation protocol is required for successful genetic transformation. *Agrobacterium*-mediated transformation of maize needs efficient regeneration systems, including highly responsive genotypes as well as culture conditions as critical factors to be identified and standardized.

Therefore, the present investigation was performed to standardize the tissue culture technique with respect to media composition, genotype, explant, hormonal combination, regeneration and hardening, also standardize the transformation technique for parameters such as genotype, explant, hormonal combination of medium, cocultivation method, cocultivation days, *Agrobacterium* density and concentration of antibiotics.

In the present study, an attempt was made to standardize the *Agrobacterium* - mediated transformation of maize inbreds using standard marker genes such as *gus* (β Glucouronidase) and *hpt* (Hygromycin phospho transferase).

Effect of genotype on transient *GUS* expression

A major advantage of *Agrobacterium* mediated transformation is that a small number of copies of relatively large segments of T- DNA with defined ends are integrated in to the plant genome with minimal rearrangement, resulting in transgenic plant of high quality. Transformation of maize remains difficult for a number of reasons. One pre requisite for high efficiency transformation is an exceedingly effective and robust tissue culture system. Response of

maize to callus induction and regeneration is genotype specific (18). Therefore, it is necessary to optimize these factors for each genotype that is to be transformed.

Frequency of transient *GUS* expression in four genotypes such as UMI 757, UMI 112, COH(M) 5 and UMI 285 were tested by cocultivation with *Agrobacterium.* Among the genotypes COH(M) 5- and UMI 285 showed superior *GUS* expression compared to UMI 757 and UMI 112. Transformation efficiency was higher in COH(M) 5 (24 %) followed by 8 % in UMI 285 but the genotype UMI 112 produced only 6 % of transgenic plant (Table 1).

Effect of explants on transient *GUS* expression

Attachment of the bacterium to the host plant cell is an initial step in the process of infection. Generally, the embryogenic calli of maize varieties have been poor starting material for *Agrobacterium* mediated transformation probably because callus tend to grow slowly. In contrast, freshly isolated immature embryo consists of actively dividing cells.

An immature embryo is the best explant source for transformation. Immature embryo collected from 10, 12, 14 and 16 DAP (Days After Pollination) were used to optimize the transformation of maize genotypes. Among the different immature embryo age, 12 DAP was showed the highest (69.0 %) and 16 DAP showed the lowest (45.8 %) GUS activity, after 3 days of cocultivation (Table 2). This is in agreement with the findings (19) who also isolated immature embryo from kernels 9-13 DAP and used for *Agrobacterium* infection. It was reported that use of immature embryos at the right development stage is a critical factor (20). The callus age was found to have a significant impact on transformation efficiency. Three and 12 weeks old calli showed the highest (42.0) and the lowest (10.0) *GUS* activity respectively, after 3 days of cocultivation. The results indicated that three weeks old callus and 12 DAP immature embryo were the best tissue for maize transformation. The results showed that three weeks old callus and 12 DAP immature embryo were the best tissue for maize transformation.

Effect of Acetosyringone (AC) on transient *GUS* expression

The addition of acetosyringone is essential for successful and higher frequency transformation but the concentration of acetosyringone in cocultivation medium varied between the varieties and explants. Acetosyringone is proved essential for any transformation and 100 mM gave the best results. The addition of acetosyringone to the inoculation and cocultivation media increased the efficiency of T- DNA delivery. In the present study, 100 µM was found to be the optimum concentration giving maximum *GUS* activity in the four genotypes assessed (Supplementary Table 3 and 3.1). For optimising the concentration of acetosyringone, the mean per cent of callus showing *GUS* expression obtained following each variation in cocultivation was taken as the transformation frequency. Although two levels of acetosyringone concentrations (100 and 200 mM) were tested, 100 mM, was found to be the optimum concentration giving maximum *GUS* activity in the four genotypes assessed. COH(M) 5 exhibited better performance followed by UMI 285, whereas UMI 112 had the least score.

Similar reports observed in the transformation of maize, (21-23) reported that 100 µM acetosyringone in the

Table 2. Effect of immature embryo age on *GUS* activity of maize genotypes at different DAP (Days After Pollination).

Genotypes	Immature embryo (DAP) and *GUS* expression			
	10 DAP	12 DAP	14 DAP	16 DAP
UMI 757	45.0	58.0	46.0	38.0
UMI 112	28.0	47.0	35.0	24.0
COH (M) 5	75.0	**89.0**	**85.0**	63.0
UMI 285	72.0	82.0	79.0	58.0
	55.0	69.0	61.3	45.8

Data were taken 3 days after *Agrobacterium* inoculation and means are from 50 embryo per treatment.

Table 3. Effect of acetosyringone concentration of *GUS* activity in calluses in maize genotypes.

Genotypes	Acetosyringone concentration (mM)	% of calluses showing *GUS* expression	
		Small zone	Large zone
UMI 757	0	0.0	0.0
	100	**12.0 ± 2.0**	**16.0 ± 3.0**
	200	6.0 ± 1.0	13.0 ± 2.0
UMI 112	0	0.0	0.0
	100	**8.0 ± 1.0**	**13.0 ± 2.0**
	200	3.0 ± 0.0	12.0 ± 2.0
COH (M) 5	0	0.0	0.0
	100	**21.0 ± 3.0**	**44.0 ± 3.0**
	200	10.0 ± 2.0	21.0 ± 3.0
UMI 285	0	0.0	0.0
	100	**18.0 ± 2.0**	**21.0 ± 3.0**
	200	5.0 ± 1.0	5.0 ± 1.0

Table 1. Transformation of maize genotypes by *Agrobacterium* strain EHA 105 p CAMBIA 1305.1 using immature embryo explant.

Genotype	Experiment number	No. of immature embryo inoculated	No. of explants producing hygromycin resistant plants	No. of explants producing hygromycin R and GUS plants	Transformation efficiency %
UMI 757	1	50	3	3	6.0
	2	50	4	4	8.0
UMI 112	1	50	3	3	6.0
	2	50	2	2	4.0
COH (M) 5	1	50	12	12	24.0
	2	50	11	1	22.0
UMI 285	1	50	5	5	10.0
	2	50	4	4	8.0

Table 3.1. Effect of AS and sucrose on maize transformation in COH(M) 5 variety using immature embryo explant.

Cocultivation	Number of embryo inoculated (x)	No. of *hyg*^{R} plant	No. of *hyg*^{R} and GUS f	No. of *hyg*^{E} + *GUS* + Plt regenerated (y)	Transformation frequency (%) (y/x)
With AS	50	23	12	12	24.0
Without AS	50	11	5	5	10.0

cocultivation medium gave maximum *GUS* activity. In contrast, used 200 µM acetosyringone in the cocultivation medium to get maximum *GUS* activity (24).

Effect of cocultivation method on recovery of transgenic plants

The periods of cocultivation differed according to plant species. Longer periods of cocultivation seem effective for efficient transfer of the Ti plasmid to plant cells. However, it was more difficult to eliminate, *Agrobacterium* after longer period of cocultivation. Cocultivation for 2-7 days is generally considered to be suitable for *Agrobacterium* mediated transformation reported for many plant species. Similar line of work was reported (23, 24) that cocultivation for 2 days was found to be suitable for maize transformation. Therefore, a 3 days cocultivation was better because the explant mortality was low and per cent explants showing higher *GUS* expression.

After co cultivation, the explants washing with sterile distilled water containing 150 mg L^{-1} cefotaxime and 250 mg L^{-1} carbencillin gave the best response of transient *GUS* expression. After washing the embryo transfer to regeneration medium containing antibiotics such as cefotaxime and carbenicillin have been used regularly in *Agrobacterium* mediated plant transformation to eliminate *Agrobacterium* after inoculation (25). The capable of carbenicillin and cefotaxime in controlling the growth of *Agrobacterium* on the regeneration of the calli showed that there was strong inhibition of the regeneration potential. Same results reported (26) on rice regeneration. Antibiotics strongly reduced regeneration capacities of maize transformation. The dose of 150 mg L^{-1} cefotaxime and 250 mg L^{-1} carbenicillin exhibited highest regeneration capacity (Table 4).

Effect of hygromycin on maize regeneration

After three cycles of selection *GUS* activities and hygromycin resistant calli were detected. The presence of hygromycin decreased the number of shoots and per cent regeneration. Antibiotic resistant plant proliferated on MS selection medium supplemented with 30 mg L^{-1} hygromycin (Table 5). Histochemical analysis revealed significant *GUS* activity in leaves and roots derived from immature embryo of selected hygromycin resistant plants. The intensity of blue staining varied between different tissues of the same plant.

Selection, multiplication and regeneration of transformed cells are the most important steps of transformation. *GUS* expression of the putative transformants was tested at 15 days intervals. Transfer of transformed calli to fresh selection medium every 15 days interval improved the selection efficiency (Table 6). All the genotypes exhibited significantly higher number of blue foci on the first day of selection. To eliminate non transformants, the transformed calli were subjected to three cycles of selection. Stable transformation of the *GUS* gene

Table 5. Effect of different concentrations of hygromycin on plant regeneration from the calli of maize genotypes.

Concentration of hygromycin (mg/L)	No. of shoots / callus	Per cent regeneration
0	7.0	62 ± 3.0
10	6.0	54 ± 3.0
20	4.5	42 ± 2.0
30	3.8	30 ± 2.0
40	2.1	18 ± 1.0
50	-	-

Table 6. Transgene expression in immature embryo derived to plants of maize genotypes transformed with *Agrobacterium* strain EHA 105 pCAMBIA 1305.1.

Genotypes	Histochemical *GUS* assay		
	Root	Shoot	Leaf
UMI 757 1	+++	++	++
2	++	+++	+
UMI 112 1	++	++	++
2	+	+	+
COH (M) 5 1	++++	+++	+++
2	+++	++	++
UMI 285 1	+++	++	+++
2	++	+	++

+ = low intensity ++ = medium intensity +++ = high intensity ++++ = very high intensity

Table 4. Effect of different concentrations of cefotaxime and carbenicillin on plant regeneration from calli of maize genotypes.

Concentration of cefotaxime / carbennicillin (mg^{-1})	Carbenicillin (mg^{-1})		Cefotaxime (mg^{-1})	
	No. of shoots / callus	Percent regeneration	No. of shoots / callus	Per cent regeneration
0	3.0	32 ± 2.0	2.2	24 ± 1.0
50	3.5	18 ± 1.0	3.0	32 ± 2.0
100	7.0	52 ± 2.0	3.6	30 ± 2.0
150	6.0	60 ± 3.0	3.8	46 ± 2.0
200	2.0	9 ± 1.0	4.3	50 ± 2.0
250	3.0	4.0	6.0	63 ± 3.0

The values are average of five replicates.

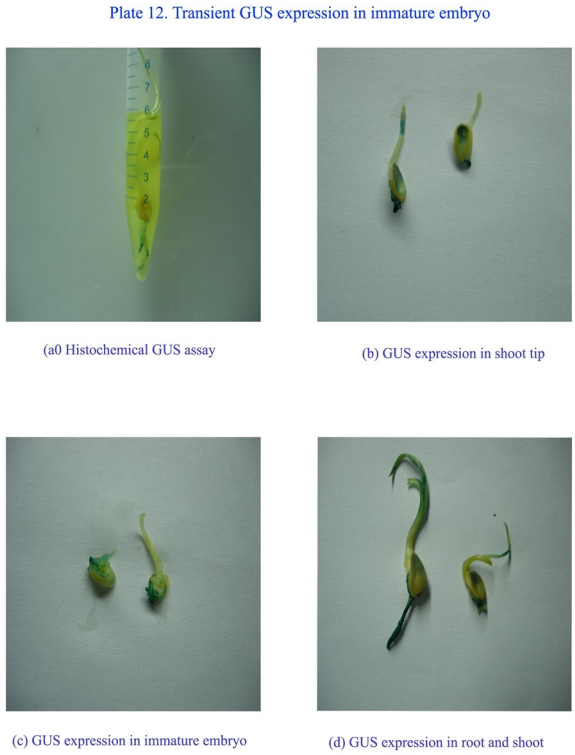

Fig. 2. Transient *gus* expressions in immature embryo

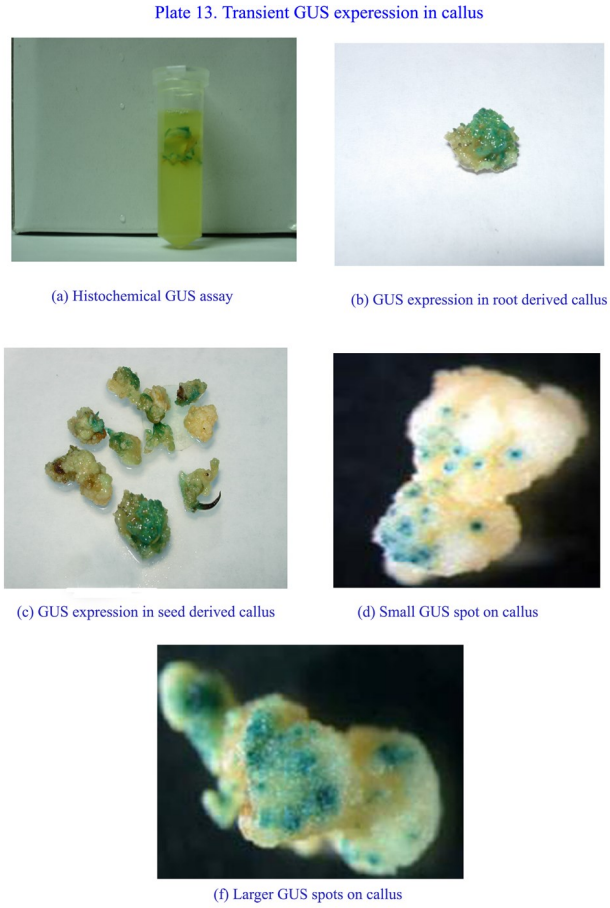

Fig. 3. Transient *gus* expressions in callus

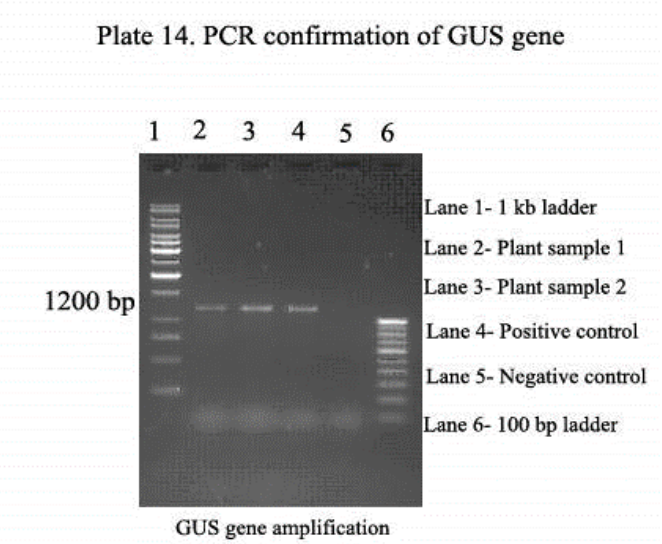

Fig. 4. PCR confirmation of *gus* gene.

was confirmed by histochemical assay after three cycles of selection in the mature embryo derived calli and immature embryo derived plantlets. (Fig 2) Resistant calli upon transfer to regeneration medium turned brown, some of the calli showed green regions after 15-20 days and others after 30 days, within the same genotype, putative resistant calli were regenerated on medium with (or) without hygromycin (Fig 3). All the plants exhibited sensitiveness to selection when they were screened in medium containing hygromycin. In COH(M) 5 three to four microscopic spots were visible in roots and leaves and so selection pressure was continued during regeneration.

A suitable genotype (S61), embryo size (1.5-2 mm), *A. tumefaciens* strain (LBA 4404), pretreatment culture and appropriate antibiotic (Timentin) for *Agrobacterium* mediated transformation of maize (26).

For further confirmation, *gus* positive plants were subjected to PCR analysis for stable integration of *gus* gene in genomic DNA. DNA isolated from plants was subjected to amplification of *gus* gene sequences using respective primers. This resulted in amplification of a 1200 bp fragment *gus* sequence (Fig 4). This conferred the stable integration of transgenes in the maize plant.

Conclusion

In conclusion, in tissue culture techniques immature embryo was best suitable explant because juvenile tissue response better than mature tissue for maize. Regarding transformation studies, immature embryo were preferred target material for transformation and 2 days of *Agrobacterium* culturing and three days of cocultivation period yielded effective transient *GUS* expression and stable integration of marker gene to this plant. The present studies pave the way for stable integration of novel genes such as disease and pest resistance in maize crop in future.

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Authors' contributions

NM carried out the transformation studies, drafted the manuscript. SH carried out the revision of the manuscript.

NM participated in the design of the study and performed the statistical analysis as well as validated and supervised the work. CRA validated and supervised the work. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: The Agrobacterium strain was used from our Tamil Nadu Agricultural University, Centre for Plant biotechnology and Molecular Biology, Coimbatore and it got permission to used.

References

1. Kanampiu F, Ranson J, Gressel J, Jewell D, Friesen D. Appropriateness of biotechnology to African agriculture: striga and maize as paradigms. Plant Cell Tissue Org Culture. 2002;69:105-10. https://doi.org/10.1023/A:1015241509808 .
2. Danilova SA. The technologies for genetic transformation of cereals. Russ J of PlantPhysiol. 2007;54:569-81. https://doi.org/10.1134/S1021443707050019 https://www.cabidigitallibrary.org/doi/full/10.5555/20073270769.
3. Kausch AP, Wang K, Kaeppler HF, Gordon-Kamm W. Maize transformation: history, progress and perspectives. Mol Breed. 2021;41:38. https://doi.org/10.1007/s11032-021-01225-0. https://pubmed.ncbi.nlm.nih.gov/37309443.
4. Shou H, Frame BA, Whitham SA, Wang K. Assessment of transgenic maize events produced by particle bombardment of *Agrobacterium* - mediated transformation. Mol Breed. 2004;13:201-08.https://doi.org/10.1023/B:MOLB.0000018767.64586.53 https://www.researchgate.net/publication/226695064.
5. Grimsley N, Hohn T, Davis JW, Hohn B. *Agrobacterium* - mediated delivery of infectious maize streak virus into maize plants. Nature. 1987;325:177-79. https://doi.org/10.1038/325177a0. https://www.nature.com/articles/325177a0.
6. Gould J, Devey M, Hasegawa O, Ulian EC, et al. Transformation of *Zea mays* L. using *Agrobacterium tumefaciens* and the shoot apex. Plant Physiol. 1991;95:426-34. https://doi.org/10.1104/pp.95.2.426. https://pubmed.ncbi.nlm.nih.gov/16668001.
7. Chan MT, Chang HH, Ho SL, et al. *Agrobacterium* mediated production of transgenic rice plants expressing a chimeric a-amylase promoter / b-glucouronidase gene. Plant Mol Biol. 1993;22:491-506.https://doi.org/10.1007/BF00015978. https://link.springer.com/article/10.1007/BF00015978.
8. Ishida Y, Saito SH, Ohta S, Hiei Y, et al. High efficiency transformation of maize (*Zea mays* L.) mediated by *Agrobacterium tumefaciens*. Nature Biotech. 1996;14:745-50. https://doi.org/10.1038/nbt0696-745. https://pubmed.ncbi.nlm.nih.gov/9630983.
9. Zhao ZY, Gu W, Cai T, Tagliani L, Hondred D, et al. High throughput genetic transformation mediated by *Agrobacterium tumefaciens* in maize. Mol Breed. 2001;8:323-33. https://doi.org/10.1023/A:1015243600325. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6358778.
10. Frame BR, Shai H, Chikwamba RK, Zhang Z, Xiang C, et al. *Agrobacterium tumefaciens* - mediated transformation of maize embryos using a standard binary vector system. Plant Physiol.2002;129:13-22.https://doi.org/10.1104/pp.000653. https://pubmed.ncbi.nlm.nih.gov/12011333.
11. Jefferson RA. Assaying chimeric genes in plants: The GUS gene fusion system. Plant Mol BiolRep.1987;5:387-405.https://doi.org/10.1007/BF02667740 https://link.springer.com/article/10.1007/BF02667740.
12. Al-Abed D, Rudrabhatla S, Talla R, Goldman S. Split-seed: a new tool for maize researchers. Planta. 2006;223:1355-60. https://doi.org/10.1007/s00425-006-0237-9 https://www.researchgate.net/publication/7288650.
13. Oduor RO, Njagi ENM, Ndung'u S, Machuka JS. *In vitro* regeneration of dryland Kenyan maize genotypes through somatic embryogenesis. International Journal of Botany. 2006;2:146-51.https://doi.org/10.3923/ijb.2006.146.151 https://www.researchgate.net/publication/26442648.
14. Green CE, Philips RC. Plant regeneration from tissue cultures of maize. Crop Sci. 1975;15:417-21.https://doi.org/10.2135/cropsci1975.0011183X001500030040x https://mnl.maizegdb.org/mnl/51/56.
15. Frame BR, Zhang H, Cocciolone SM, Sidorenko LV, et al. Production of transgenic maize from bombarded type II callus: effect of gold particle size and callus morphology of transformation efficiency. *In Vitro* Cell Dev Biol Plant. 2000;36:21-29. https://doi.org/10.1007/s11627-000-0007-5 https://www.researchgate.net/publication/225269006.
16. Armstrong CL. Regeneration of plants from somatic cell cultures: applications for *in vitro* genetic manipulation. In: M. Freeling and V. Walbot, Eds. The maize Hand Book. New York, NY, USA, Springer - Verlage; 1994.663-71. https://doi.org/10.1007/978-1-4612-2694-9_120.
17. Finar JJ. Direct somatic embryogenis. In: Gamborg UC and Phillips GC (Eds.) Plant Cell Tiss Org Cult. Narosa Publishing House, New Delhi - 110040, India; 1998.91-102. https://www.researchgate.net/publication/283756980_Somatic_Embryogenesis.
18. Armstrong CL, Green CE, Phillips RL. Development and availability of germplasm with high type II culture formation response. Maize Gen Coop Newsl. 1991;65:92-93. https://www.researchgate.net/publication/290825550.
19. Vega JM, Yu W, Kennon AR, Chen X, Zhang ZJ. Improvement of *Agrobacterium* - mediated transformation in Hi-II maize (*Zea mays*) using standard binary vectors. Plant Cell Rep.2008;27:297-305.https://doi.org/10.1007/s00299-007-0463-z https://www.researchgate.net/publication/273285856.
20. Ishida Y, Hiei Y, Komari T. *Agrobacterium* - mediated transformation of maize. Nature Protocols.2007;2:1614-21.https://doi.org/10.1038/nprot.2007.241 https://www.researchgate.net/publication/6252661.
21. Yang A, He C, Zhang K, Zhang J. Improvement of *Agrobacterium* – mediated transformation of embryogenic calluses from maize elite inbred lines. *In Vitro* Cell Dev Biol.2006;42:215-19.https://doi.org/10.1079/IVP2006768 https://link.springer.com/article/10.1079/IVP2006768.
22. Frame BR, McMurray JM, Fonger TM, Main ML, Taylor KW, et al. Improved *Agrobacterium* - mediated transformation of three maize inbred lines using MS salts. Plant Cell Rep. 2006;25:1024-34. https://doi.org/10.1007/s00299-006-0145-2 . https://www.researchgate.net/publication/228481966.
23. Huang X, Wei Z. Successful *Agrobacterium* - mediated genetic transformation of maize elite inbred lines. Plant Cell Tiss Org Cult. 2006;83:187-200. https://doi.org/10.1007/s11240-005-5772-8. https://www.researchgate.net/publication/226642605.
24. Opabode JT. *Agrobacterium*- mediated transformation of plants: emerging factors that influence efficiency. Biotech and Mol Bio Rev. 2006;11:12-20. https://www.researchgate.net/publication/253813518.
25. Pipatpanukul T, Bunnag S, Theera kulpisut P, Kostittrakul M. Transformation of *indica* rice (*Oryza sativa* L.) *cv*. RD 6 mediated by *Agrobacterium tumefaciens*. Songklanakarin J Sci Technol. 2004;26:1-12. https://www.academia.edu/60476267.
26. Takavar S, Rahnama H, Rahimian H, Kazemitabar K. *Agrobacterium* mediated transformation of maize (*Zea mays* L.). Journal of Sciences, Islamic Republic of Iran. 2010;21(1):21-29. https://www.researchgate.net/publication/228481966.