TAP CHI SINH HOC 2016, 38(4): 505-514
DOI: 10.15625/0866-7160/v38n4.9170

AN EFFICIENT PROTOCOL FOR *Agrobacterium*-MEDIATED TRANSFORMATION OF *GUS/GUSPLUS* GENE INTO CASSAVA PLANTS (*Manihot esculenta* Crantz)

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ABSTRACT: In this study, to evaluate the ability to accept transgenes of the two cassava cultivars KM94 and KM140, which are grown widely in Vietnam, *A. tumefaciens* bacterial strains C58/pGV2260, EHA105 and LBA4404 containing vector pCB-gusplus or pPIPRA558 haboring selectable marker gene *gus/gusplus,* were co-inoculated with explants from four selected sources, including (1) immature leaves, (2) shoot apexes, (3) callus, and (4) somatic embryo cotyledons. Transgenic explants were selected using three antibiotics kanamycin, neomycin, and paromomycin, at concentrations of 25, 50, 75, and 100 mg/l, based on *nptII* gene. These experiments were conducted to optimize conditions for transferring *gus* gene to cassava plant. The transformation efficiency was evaluated based on the percentage of X-gluc positive stained explants 10 days after infection, somatic embryos, regenerated shoots and whole regenerated plants. The highest transformation efficiency was achieved when using *A. tumefaciens* C58/pGV2260, carrying expression vector pCB-gusplus, and cotyledons of cultivars KM94. In this protocol, cotyledons were cut into small pieces, then cultured on the callus induction medium for 2 days and submerged in bacterial suspension, supplemented with 100 µM AS, with shaking at 50 rpm for 15 minutes. Explants were then co-cultured on somatic embryo induction medium supplemented with 150 µM AS in the darkness for 2 days. Explants were then transferred to selective callus induction medium with 50 mg/l kanamycin for 3-4 weeks, followed by the culture on selective shoot induction medium with the same kanamycin concentration. Shoots, with 2-3 leaves, were transferred to a selective rooting medium to establish whole plants.

Keywords: Agrobacterium tumefaciens, *Manihot esculenta,* gene modification, *gus/gusplus*, cassava, *nptII.*

Citation: Do Hai Lan, Le Van Son, Le Tran Binh, 2016. An efficient protocol for *Agrobacterium*-mediated transformation of *gus/gusplus* gene into cassava plants (*Manihot esculenta* Crantz). Tap chi Sinh hoc, 38(4): 505-514. DOI: 10.15625/0866-7160/v38n4.9170.

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Received 7 December 2016, accepted 20 December 2016

INTRODUCTION

The genetic transformation efficiency is not only dependent on the regeneration system but also many factors, including plant genetic characteristics. In order to effectively transfer target gene into plant, it is necessary to perform the tranfromation of *gus* gene as a reference. Currently, there are number of transgenic vectors carrying the selectable marker gene *gus* such as pBI121, pCAM1301, pPTN289, of which pBI121 has been used widely in transgenic research in plants and successful procedures of *gus* gene transformation have been applied. Successful transformation of *gus* genes into cassava via *A. tumefaciens* bacteria are also reported (Sarria et al., 1993; Raemakers et al., 1993; Schöpke et al., 1993). In studies of the cassava crops, gusA (GUS) from *Escherichia coli*, encoding ß-glucuronidase, was used as a selectable marker gene (Jefferson et al., 1987). Glucuronidase enzyme activity is used to measure the number of embryos expressing selectable marker genes and gene transfer efficiencies through the blue staining of cells. It is also a measure of the ability of the

activity of the promoter and is useful in the study of gene cassettes and other gene structures such as promoter, enhancer and other regulation sequences. For the transfer of genes to cassava somatic embryos, the gene gun and some strains of *Agrobacterium* have been used. GUS homologous recombination was observed in only a few embryos after three months of infection. Only about 1% of GUS positive embryos detected in the first few weeks expressed enzyme activity after three months. However, their presence is a clear signal of stability of consolidation of *gus* gene in the cassava genome (Sarria et al., 1993).

MATERIALS AND METHODS

A. tumefaciens strains C58/pGV2260, EHA105 and LBA4404 containing vector expressing PCB-gusplus or pPIPRA558 were provided by Plant Cell Technology Division, Institute of Biotechnology.

Cassava variety KM94 and KM140 (Plant Resource Centre, Institute of Agricultural Science of Vietnam, An Khanh, Hoai Duc, Ha Noi) is used for the generation of transgenic cassava plants. KM94 cassava stems were cut into segments, 3-4 cm in length, and then rinsed under running water for 30 minutes and the cultured samples were surface sterilized by ethanol 70% for 1 minute and 0.1% (w/v) $HgCl_2$ for 7 minutes, followed by the removal of $HgCl_2$ and washing with distilled water 5 times. The samples, after sterilization, were cultured on Murashigh and Skoog (MS) medium with additional 3% sucrose with the density of 5 cut segments/conical V-250 ml flask containing 50 ml of medium solution. Four types of plant materials were used for infection including (1) immature leaves (2×2 mm) and (2) shoot apexes (1-1.5 cm in length) of the seedlings over 4 weeks old, (3) callus induced on MS medium supplemented with 12 mg/l picloram, and (4) cotyledons induced on MS medium supplemented with 0.3 mg/l BAP.

Optimizing conditions for transformation of *gus* gene into cassava plant:

Experiment 1. Selecting bacterial strains, vectors and materials for *A. tumefaciens* bacteria infection suitable for gene integration into cassava crops. Four materials were incubated with suspensions at OD_{600} of 0.8 of *A. tumefaciens* strains CV58/pGV2260, EHA105 and LBA4404 carrying the expression vector PCB-gusplus or pPIPRA558 and 100 μM AS for 10 minutes.

Experiment 2. Selecting the method of infecting cassava plants with *A. tumefaciens/pCB-gusplus*. Using the method of infection by cutting materials into pieces which were then shaken at 50 rpm with bacterial suspension OD_{600} of 0.8 for 10 minutes or using sonication for 1, 2, 3, 4, and 5 minutes.

Experiment 3. Selecting suitable period for infection and bacterial density of *A. tumefaciens carrying pCB-gusplus* suitable for gene transfer to the optimal material of optimal variety. Immediate infection after cutting or after the induction on somatic embryo induction medium CIM (MS + 12 mg/l picloram) for 2 days with the density of bacterial suspension at the OD_{600} of 0.2, 0.4 , 0.6, 0.8, and 1.0.

Experiment 4. Selecting the suitable concentration of Acetosyringon (AS) and the appropriate infection period of bacteria *A. tumefaciens/pCB-gusplus*. AS concentration used was 50, 100, 150, 200 μM with infectious period of 5, 10, 15, 20, 25, and 30 minutes with bacterial suspension having optimal OD on optimal materials.

Experiments from 1-4: co-culture was done for 2 days and antibiotic kanamycin (50 mg / l) was used for selection of transgenic explants. After 10 days of infection, X-gluc staining was carried out for evaluation.

Experiment 5. Choosing the appropriate co-culture period. Co-culture was done for 1, 2, 3, and 4 days; antibiotic kanamycin (50 mg/l) was used for selecting. After 10 days of infection, X-gluc staining was done for evaluation.

Experiment 6. Selecting suitable antibiotics and their suitable selective threshold. Kanamycin, neomycin, and paromomycin, at concentrations of 25, 50, 75, and 100 mg/l for each, were used to select cotyledon pieces infected by *A. tumefaciens/pCB-gusplus* in

optimal OD_{600} for an optimal period of infection time (shaking at 50 rpm), and optimal co-culture condition in the darkness for an optimum period with the addition of optimal AS concentration.

Transformation efficiency was evaluated based on the numbers of somatic embryogenesis tissues, regenerated shoots, and complete plants.

The presence of the *gus* gene in putative transgenic plants was tested by *ntpII* amplification with specific primers.

RESULTS AND DISCUSSION

Selection of *A. tumefaciens* strains, experession vectors and plant materials for transformation of cassava

In this study, to evaluate the ability of cassava cultivars KM94 and KM140 to accept selectable marker gene gus/gusplus, we used strains *A. tumefaciens* CV58/pGV2260, EHA105 and LBA4404 carrying vector expressing pCB-gusplus or pPIPRA558 and selected by the antibiotic kanamycin (50 mg/l) based on nptII gene expression.

KM94 and KM140 cassava explants were infected with *A. tumefaciens* suspension at OD_{600} 0.8. We used 4 types of plant materials, including immature leaves, shoot apexes, callus and cotyledons. Infection period was 10 minutes (Fauquet C. and Fargette D. (1990); and explants were co-cultured on MS medium supplemented with 12 mg/l picloram (Do Xuan Dong et al., 2012) and 100 µM AS for 2 days (Ihemere et al., 2006); bacterial removal from explants by washing was conducted using ½ liquid MS supplemented with cefotacim 500 mg/l, the excessive solution in the explants was removed and then explants were cultured on MS medium supplemented with 12 mg/l picloram, and selected by kanamycin (50 mg/l).

After 10 days, explants were subjected for X-Gluc staining to evaluate the transformation efficiency (table 1).

Table 1. Effects of *A. tumefaciens* strains, expression vectors and plant materials on the efficiency of *gus* gene transformation into cassava plants

Cassava cultivar	Plant materials	Percentage of gus gene expression in bacterial strains and vectors (%)						*Gus expression degree*
		CV58/pGV2260		EHA105		LBA4404		
		pCB-gusplus	*pPIPRA 558*	*pCB-gusplus*	*pPIPRA 558*	*pCB-gusplus*	*pPIPRA 558*	
Control	Young leaf	0	0	0	0	0	0	-
	Shoot apex	0	0	0	0	0	0	-
	Callus	0	0	0	0	0	0	-
	Cotyledon	0	0	0	0	0	0	-
KM94	Young leaf	75.33 ± 1.27	40.28 ± 0.35	51.47 ± 0.65	46.38 ± 0.58	53.59 ± 0.64	47.16 ± 0.43	+
	Shoot apex	84.19 ± 1.34	59.75 ± 0.58	69.58 ± 0.74	52.24 ± 0.57	59.68 ± 0.68	37.49 ± 0.40	++
	Callus	87.16 ± 1.14	63.29 ± 0.66	81.47 ± 1.63	68.23 ± 0.78	87.14 ± 1.48	78.47 ± 0.87	+
	Cotyledon	**95.36** ± 1.53	60.17 ± 0.73	87.55 ± 1.67	54.32 ± 0.56	84.11± 1.66	65.24 ± 0.85	++
KM140	Young leaf	70.27 ± 1.39	37.19 ± 0.47	53.46 ± 0.63	41.11 ± 0.43	49.10 ± 0.56	39.96 ± 0.54	+
	Shoot apex	79.25 ± 1.16	52.20 ± 0.42	59.89 ± 0.75	46.28 ± 0.57	48.79 ± 0.64	32.64 ± 0.47	++
	Callus	84.83 ± 1.22	58.36 ± 0.37	76.35 ± 0.68	60.21 ± 0.87	79.46 ± 0.87	69.33 ± 0.76	+
	Cotyledon	90.15 ± 1.20	71.17 ± 0.85	82.69 ± 1.06	48.57 ± 0.57	81.24 ± 1.45	59.69 ± 0.71	++

"-": non-transgenic; "+": expressed; "++": strongly-expressed.

After 10 days of transformation, in the control sample, tissue pieces infected with YEB medium without bacterium *A. tumefaciens* show negative results when stained with X-gluc solution (non-blue stained). Of all the experiments, materials of all types in both cassava cultivars showed X-gluc-positive; however, the percentage of expression was not the same. The highest percentage of *gus* expression belongs to strain CV58/pGV2260 containing vector pCB-gusplus; this result was the same in four types of materials of both cultivars; cotyledon pieces of cassava KM94 showed the highest percentage (95.36%) and the highest degree of expression (++). Despite the second highest percentage of gus expression (87.16%), the percentage was calculated by the number of calluses catching the blue of X-gluc/total number of calluses infected with *A. tumefaciens*, the percentage of blue spots/total tissue block was very low.

Based on above results, we selected KM94 cassava cotyledons as optimal material and *A. tumefaciens* containing PCB-gusplus vector for other experiments.

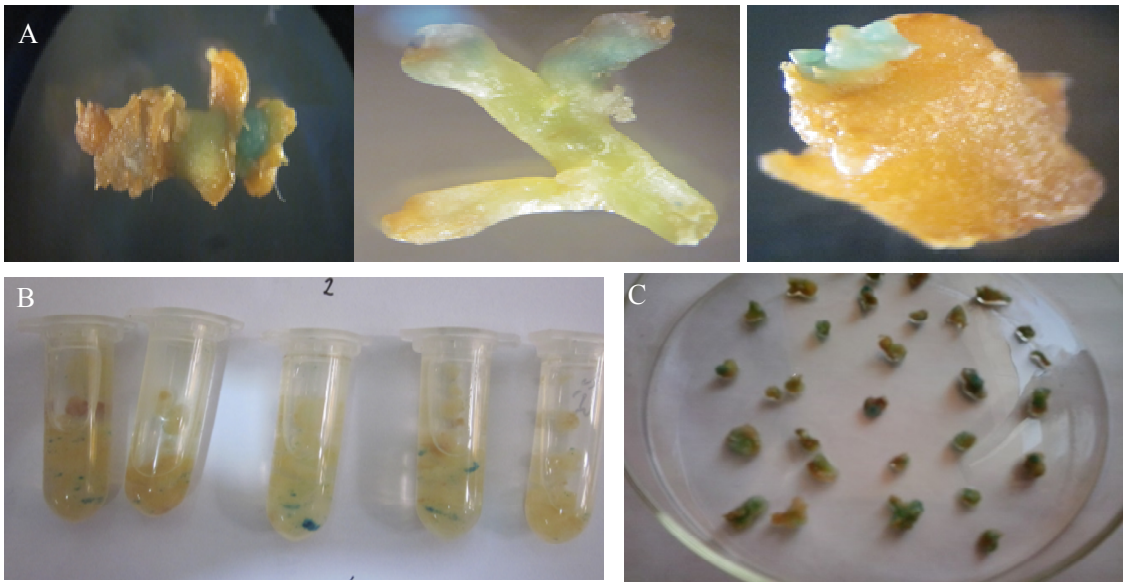

Figure 1. X-gluc staining of transgenic explants
A. shoot apex; B. somatic embryo; C. cotyledon piece.

Effect of *A. tumefaciens* infection method on transformation of cassava

To facilitate *A. tumefaciens* to infect explants, we created wounds for tissues by cutting tissues into small pieces; however, sonication can also be used to create small holes on membranes to facilitate the penetration of bacteria into cells. It was difficult to make wounds for calluses, among the materials used, so sonication method might be effective.

In this experiment, in three of four types of explants, including leaf, shoot apex and cotyledon, shaking (at 50 rpm) method showed higher effieciency compared to sonication method (table 2), whereas the sonication method showed higher efficiency when callus was used, indicating the effect of sonication on protoplast. In the treatment with callus, longer the duration of sonication, higher the percentage of callus dead. In addition, callus did not survived when transferring to shoot induction medium. Therefore, the method for infection using cotyledon and shaking at 50 rpm was the most suitable method.

Table 2. Effects of infection method and plant material on the efficiency of *gus* gene transfromation into cassava plants

Cassava variety	Plant material	Shaking at 50 rpm	Percentage of gus expression (%) Sonication 0 minute	1 minute	2 minutes	3 minutes	4 minutes	5 minutes
KM94	Young leaf	75.26	0	40.18	41.28	16.52	3.67	0
	Shoot apex	84.75	0	49.74	49.33	12.37	4.74	0.10
	Callus	87.64	0	93.49	91.49	68.74	35.39	1.36
	Cotyledon	96.85	0	60.36	57.48	24.28	8.84	0.31
KM140	Young leaf	70.27	0	47.15	43.46	21.17	9.12	0.56
	Shoot apex	79.25	0	42.23	49.89	16.36	8.74	0.43
	Callus	84.83	0	88.46	86.35	50.12	29.38	0.32
	Cotyledon	90.15	0	41.16	52.69	18.46	7.19	0.87

Effects of *A. tumefaciens* strains and infection time on transformation of KM94 cassava using cotyledon

Selection of infection time and *A. tumefaciens* bacterial density suitable for transformation of KM94 cassava using cotyledon.

Cotyledon pieces (2×2 mm) were directly infected with *A. tumefaciens* bacterial suspension at different densities or induced on MS added with 12 mg/l picloram for 2 days prior infection. After 10 days of infection, the examination of *gus* expressed explants was performed and the results were shown in table 3.

Table 3. Effect of bacterial density and infection time on to the transformation of KM94 cassava using cotyledons

		Bacterial density (OD_{600}) 0	0.2	0.4	0.6	0.8	1.0
Immediate infection	Number of initial explants	150	150	150	150	150	150
	Number of explants positively stained with X-gluc	0	8	16	56	69	12
	Percentage	0	5.33	10.67	37.33	46.00	8.00
Infection after 2 day induction	Number of initial explants	130	130	130	130	130	130
	Number of explants positively stained with X-gluc	0	10	15	52	67	12
	Percentage	0	7.69	11.54	40.00	51.53	9.23

Among the treatments, OD_{600} of 0.8 resulted in the highest percentage of X-gluc stained tissues and and the lowest value was obtained at OD_{600} of 0.2 (5.33 and 7.69%). It is clear that both low or high densities of bacteria reduced the infected rate. The tranformation rate using tissues immediately infected by bacteria was generally lower than those using 2 days induced tissue. Overall, cotyledon explants induced 2 days before infection with the bacterial suspension at OD_{600} of 0.8 showed the highest transformation rate (51.53%).

Effects of *A. tumefaciens* infection period and acetosyringone on transformation of cassava

Selection of acetosyringon (AS) concentration and the suitable time of infection with *A. tumefaciens.*

Based on this nature, we used AS at concentrations of 50, 100, 150, and 200 µM to enhance transformation efficiency. Time of infection by bacteria also directly affects the effectiveness of temporary gene expression and resilience of the sample after transformation. The too long infection time leads to bacterial overgrowth in the culture medium after infection, reducing the survival and regeneration of plant tissue after transformation. Both too long and too short infection time reduce the degree of gene expression. Therefore, we optimized the infection time using durations of 5, 10, 15, 20, 25, and 30 minutes. Samples were stained with X-gluc 10 days after infection (2 days co-culture).The results were shown in table 4.

Table 4. Effect of AS concentration and infecting time on efficiency of the transformation of cassava KM94

AS concentration (µM)		Infect time (in minute) of					
		5	10	15	20	25	30
50	Number of initial explants	120	120	120	120	120	120
	Number of explants positively with stained X-gluc	8	11	32	29	20	13
	Percentage	6.67	9.17	26.67	24.17	16.67	10.83
100	Number of initial explants	130	130	130	130	130	130
	Number of explants positively with stained X-gluc	12	20	47	37	25	17
	Percentage	9.23	15.38	**36.15**	28.46	19.23	13.08
150	Number of initial explants	130	130	130	130	130	130
	Number of explants positively with stained X-gluc	12	15	38	28	21	13
	Percentage	9.23	11.53	29.23	21.53	16.15	10.00
200	Number of initial explants	150	150	150	150	150	150
	Number of explants positively with stained X-gluc	10	13	37	26	20	14
	Percentage	6.67	8.67	2.47	17.33	13.33	9.33

The increase in AS concentration from 5 µM to100 µM resulted in the increase in the percentage of cotyledonary explants positively stained with X-gluc. However, when the increase in AS concentration was too high (150-220 µM) , the percentage of stained explants decreased. Similar changes could be found for infection time. The infection time increased from 5 to 15 minutes, resulting in the increase of the rate of blue-stained explants, but this rate decreased when the infection time was 20-30 minutes. The rate of explants blue-stained with X-gluc was the highest at AS concentrations of 100 µM and infection time of 15 minutes. The lowest rate was observed at AS concentrations of 50 and 100 µM and infect time of 5 minutes. The results are consistent with the studies of Ihemere et al. (2006).

Effect of co-culture period on transformation of cassava

Selection of suitable period of co-culture.

After infection with *A. tumefaciens* suspension at OD_{600} of 0.8 for 15 minutes, the cotyledonary explants were cultured for 1, 2, 3 and 4 days on MS callus inducion media supplemented with 12 mg/l picloram and AS at concentrations of 100, 150, 200, 250 µM.The explants were stained with X-gluc after 10 days of infection.

Among co-culture period treatments, 1 day resulted in the lowest percentage of blue-stained explants, while treatments with 2, 3 and 4 days of co-culture showed higher percentage and did not differ. However, after 3 days of co-culture, *A. tumefaciens* started to grow (0.48-1.02%),

and grew vigorously after 4 days of co-culture (11.58-13.27%). Hence, we chose 2 days of co-culture for later experiments.

Among AS concentrations, 150 µM generally resulted in higher percentage of blue-stained explants in all co-culture period treatments. For 2 days co-culture treatments, AS concentration of 150 µM gave the highest result (38%), hence was chosen.

Effects of antibiotics identity and concentration on transformation of cassava

Selection of antibiotics and suitable selective threshold.

Two genes encoding for Neomycin phosphotransferase used in selection of transgenic plants are neomycin phosphotransferase I (nptI) gene and neomycin phosphotransferase II (nptII) gene. NptII isolated from the transposon *Tn5* of *Escherichia coli* K12, is a selectable marker for transgenic plants. It can also be used in studies on gene expression and regulation of gene expression because N-terminal end is designed to preserve the activity of the enzyme. Therefore, to determine the antibiotics and their suitable concentration, we chose 3 antibiotics kanamycin, neomycin, and paromomycin at concentrations of 25, 50, 75, and 100 mg/L to select transgenic explants. The conditions for transformation were *A. tumefaciens* haboring *PCB-gusplus* with OD_{600} of 0.8, shaking at 50 rpm for 15 minutes, 2 days co-culture in the darkness, and 150 µM AS.

Table 5. Effect of co-culture period and AS concentration on transformation of KM94 cassava using cotyledons

Co-culture period (day)	Number of initial explants	AS concentration (µM)	Number of blue-stained explants	Percentage of blue-stained explants	Percentage of explants with *A. tumefaciens* growth on the surface (%)
1	150	100	4	2.66	0
2	150		28	18.67	0
3	150		28	18.67	0.82
4	150		28	18.67	13.27
1	150	150	11	7.33	0
2	150		57	**38.00**	0
3	150		57	38.00	0.85
4	150		58	38.6	12.75
1	150	200	7	4.66	0
2	150		35	23.33	0
3	150		36	17.33	1.02
4	150		36	17.33	11.58
1	150	250	7	4.66	0
2	150		33	22.00	0
3	150		33	22.00	0.48
4	150		33	22.00	12.47

In general, all explants were infected and fully covered by *A. tumefaciens* Although explants were repeatedly washed with ½ MS medium with supplement of cefotaxime, *A. tumefaciens* bacteria still grew. When using 50 mg/L antibiotics, the rate of explants forming somatic embryo increased, but differed when different antibiotics were used. The rate when using kanamycin, neomycin, and paromomycin was 11.67%, 5.00% and 4.17%, respectively. The rate decreased (even to zero) at selective concentrations of 75-100 mg /L.

Table 6. Effects of antibiotics and their selective threshold on transfomration of KM94 cassava using cotyledons

Antibiotic	Concentration (mg/l)	Number of initial explants	Number of explants forming somatic embryo	Percentage of explants forming somatic embryo (%)	Number of explants forming shoot
Kanamycin	25	120	0	0	0
	50	**120**	**14**	**11.67**	**3**
	75	120	8	6.67	1
	100	120	3	2.50	1
Neomycin	25	120	0	0	0
	50	120	6	5.00	1
	75	120	4	3.33	0
	100	120	0	0	0
Paromomycin	25	120	0	0	0
	50	120	5	4.17	1
	75	120	3	2.50	0
	100	120	0	0	0
Control	0	120	0	0	0

Antibiotic	Number of shoots	Percentage of shoots forming root	Number of complete plants *in vitro*	Number of transgenic plants confirmed by PCR
Kanamycin	0	0	0	0
	10	**100**	**10**	**8**
	2	100	2	1
	1	100	1	1
Neomycin	0	0	0	0
	2	100	2	1
	0	0	0	0
	0	0	0	0
Paromomycin	0	0	0	0
	2	100	2	1
	0	0	0	0
	0	0	0	0
Control	0	0	0	0

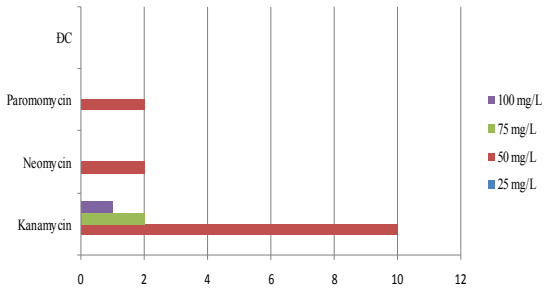

Figure 2. Number of transgenic *KM94* plants formed on selective media with antibiotics at different concentrations

After 3-4 weeks, when somatic embryos were completely formed, explants were transferred to CEM medium (MS supplemented with 0.3 mg/L BAP), maintaining selective antibiotic concentrations for plantlet formation. Only 7 of 43 formed somatic embryos (16.28%), of all treatments, were cultured for generating plantlets. On average, each explant formed 2-3 somatic embryos, and some formed 4-5. After the plantlet formation, 100% of the plantlets formed roots establishing 17 complete plants.

The use of kanamycin for selection of transgenic plants was more effective than neomycin and paromomycin, since the highest rate of transgenic plants was obtained when using kanamycin (50 mg/L). The use of *A. tumefaciens* strain CV58/pGV2260 haboring

pCB_gusplus was also effective for the transformation of cassava.

Molecular analysis of KM94 cassava transgenic plants

To confirm the incorporation of transgene into plant geneome, 17 putative transgenic complete plants were subjected for analysis. Leaves of transgenic KM94 lines and controls were used for isolation of total DNA. To detect the presence of selectable marker gene, we performed PCR using total isolated DNA as template and primers *nptII*_F/R to amplify a segment (963 bp) of *nptII* gene.

PCR products were checked on 0.8% agarose gel. Analysis result of 2 month-old plants grown in net house showed that 13/17 (76.47%) of transgenic cassava lines had bands located at the specific position of 1000 bp. No such band was observed for negative control (fig. 3).

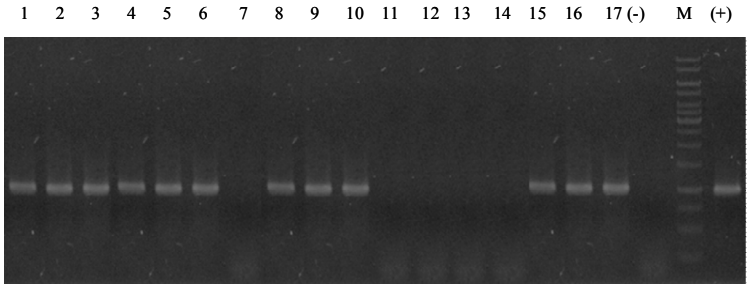

Figure 3. PCR analysis of putative transgenic cassava lines

M. 1 kb DNA marker; (+) positive control: PCR product from *nptII* carried by vector pCB-gusplus; (-) negative control: PCR products from *nptII* in wild type cassava KM94; 1-17 PCR from *nptII* in putative transgenic cassava KM94.

The transformation efficiency, in term of percentage of explants generating transgenic plants at 2 months old stage per total intitial explants, ranged from 0 to 0.9%. This result is consistent with the study of Wenham JE (1995) with the 1% percentage of forming cassava plants carrying *gus* gene.

CONCLUSIONS

We succeeded in transferring *gus* gene into cassava plants. The obtainment of cassava KM94 tissues, somatic embryos and plants carrying the selectable marker gene indicates that this protocol can be used for transformation of interest genes into cassava.

The transformation mediated by *A. tumefaciens* C58/pGV2260 carrying vector *pCB_gus* into cassava KM94 cotyledons was the most effective. In this protocol, cotyledons were cut into small pieces, induced for 2 days on callus induction medium and infected by shaking at 50 rpm in bacterial suspension for 15 minutes, at AS concentration of 100 µM. The explants were then co-cultured on somatic embryo induction medium supplemented with 150 µM AS in the darkness for 2 days, transferred to callus induction medium containing 50 mg/L kanamycin for 3-4 weeks, and transferred to selective regeneration medium containing kanamycin. After the emergence of 2-3 leaves, the plants were transferred to selective rooting medium.

REFERENCES

Claude F., 1990. African cassava mosaic virus: Etiology, epidemiology and control laboratoire de phytovirologie, ORSTOM, Abidjan, Ivory Coast. Denis Fargette, Laboratoire de Phytovirologie, ORSTOM, Abidjan, Ivory Coast. Plant Dis. 74:404-411. Copyright 1990 The American Phytopathological Society. DOI: 10.1094/PD-74-0404.

Do Xuan Dong, Do Hai Lan, Pham Bich Ngoc, Le Van Son, Le Tran Binh, Chu Hoang Ha, 2012. An efficient protocol for plant regeneration of cassava (*Manihot esculenta* Crantz) via somatic embryogenesis induction. Journal of Biotechnology, 10(3): 527-533.

Ihemere U., Diana A-G., Lawrence S., Sayre R., 2006. Genetic modification of cassava for enhanced starch production. Plant

Biotechnology Journal, 4: 453-465.

Jefferson R. A., Kavanagh T. A., Bevan M. W., 1987. GUS fusions: -glucuronidase as a sensitive and versatile gene fusion marker in higher plants. European Molecular Biology Organization Journal, 6: 3901-3907.

Raemakers C. J. J. M., Jacobsen E., Visser R. G. F., 1993. Cyclic somatic embryogenesis and plant transformation in cassava. In: W.M. Roca & A.M. Thro, eds. Proceedings of the First International Scientific Meeting of the Cassava Biotechnology Network, Cartagena, Colombia, August 1992. Working Document 123. Call, Colombia, CIAT.

Sarria R., Torres E., Angel F., Chavarriaga P., Roca W. M., 2000. Transgenic plants of cassava (*Manihot esculenta*) with resistance to Basta obtained by Agrobacterium-mediated transformation. Plant Cell Reports 19: 339-344. Q Springer-Verlag.

Sarria R., Gomez A., Cataño M., Herrera P. V., Calderon A., Mayer J. E., Roca W. M., 1993. Towards the development of Agrobacterium tumefaciens and particle bombardment-mediated cassava transformation. In: W. M. Roca & A.M. Thro, eds. Proceedings of the First International Scientific Meeting of the Cassava Biotechnology Network, Cartagena, Colombia, August 1992. Working Document 123. Cali, Colombia, CIAT.

Schopke C., Chavarriaga P., Fauquet C., Beachy R. N., 1993. Cassava tissue culture and transformation: improvement of culture media and the effect of different antibiotics on cassava. In: W.M. Roca & A.M. Thro, eds. Proceedings of the First International Scientific Meeting of the Cassava Biotechnology Network, Cartagena, Colombia, August 1992. Working Document 123. Cali, Colombia, CIAT.

XÂY DỰNG HỆ THỐNG CHUYỂN GEN CHỈ THỊ *GUS/GUSPLUS* VÀO CÂY SẮN (*Manihot esculenta* Crantz) THÔNG QUA VI KHUẨN *Agrobacterium tumefaciens*

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TÓM TẮT

Trong nghiên cứu này, để kiểm tra khả năng tiếp nhận gen của 2 giống sắn KM94 và KM140 được trồng phổ biến tại Việt Nam, các chủng vi khuẩn *A. tumefaciens* C58/pGV2260, EHA105 và LBA4404 chứa vector biểu hiện pCB-gusplus hoặc pPIPRA558 mang gen chỉ thị gus/gusplus được chuyển vào 4 loại vật liệu được lựa chọn là (1) lá chưa trưởng thành, (2) đỉnh chồi, (3) mô sẹo, (4) lá mầm phôi soma, và được sàng lọc bằng 3 loại kháng sinh kanamycin, neomycin, và paromomycin với các nồng độ cho mỗi loại là 25, 50, 75, 100 mg/l nhờ sự có mặt của gen nptII. Các thí nghiệm này được bố trí để tìm ra điều kiện tối ưu cho chuyển gen gus vào cây sắn. Đánh giá khả năng tiếp nhận gen gus qua số mô dương tính với X-gluc sau 10 ngày lây nhiễm, số mô tạo phôi soma, số mô tái sinh, số cây hoàn chỉnh. Kết quả cho thấy, chủng vi khuẩn *A. tumefaciens* C58/pGV2260 chứa vector biểu hiện pCB-gusplus hiệu quả nhất khi chuyển vào lá mầm của giống sắn KM94 bằng cách cắt lá mầm thành mảnh nhỏ, cảm ứng 2 ngày trên môi trường tạo mô sẹo rồi lây nhiễm bằng cách lắc 50 v/p với huyền phù vi khuẩn trong 15 phút với nồng độ AS 100 µM. Sau đó đồng nuôi cấy trên môi trường tạo phôi soma có bổ sung AS 150 µM trong tối 2 ngày, chuyển sang môi trường tạo mô sẹo có chọn lọc bằng kanamycin 50 mg/l, duy trì trong 3-4 tuần, chuyển sang môi trường tái sinh chọn lọc với nồng độ kanamycin duy trì, sau khi xuất hiện 2-3 lá thật thì chuyển sang môi trường ra rễ chọn lọc.

Từ khóa: Agrobacterium tumefaciens, cây sắn, chuyển gen, *gus/gusplus*, *nptII*.