Vietnam Journal of Biotechnology **20**(1): 53-62, 2022

OPTIMIZATION OF *AGROBACTERIUM*-MEDIATED TRANSFORMATION PROCEDURE FOR AN *INDICA* RICE VARIETY-KHANG DAN 18

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Received: 16.11.2020
Accepted: 11.5.2021

SUMMARY

In addition to traditional methods, advanced biotechnologies, especially CRISPR/Cas9-mediated genome editing, have emerged as effective tools for improving important agronomic traits in rice. However, the critical step for utilizing these systems is to develope an effective system for rice transformation and regeneration. This study was performed to establish procedures for *Agrobacterium*-mediated transformation and regeneration of rice cultivar Khang Dan 18 (KD18) – a popular *indica* variety in the North of Vietnam. The tissue cultures procedure with optimized medium compositions showed high frequencies of callus induction and shoot regeneration, at 94.7% and 45.3% respectively. We found that 30 mg/L of hygromycin was an effective concentration for transgenic KD18 selection. Light-yellow friable calli were used as the starting material for transformation mediated by *A. tumefaciens* strain AGL1 harbouring pHUE411 vector containing *gus intron* and hygromycin resistance gene *(hptII)*. Important factors related to transformation procedure had been optimized in this study. The high transformation frequency (12.8%) was achieved by using the optimized procedure for KD18 cultivar. In which, bacterial density (OD_{600}), infection time and co-cultivation period were performed at 0.1, 20 minutes and 3 days, respectively. PCR analysis using specific primers and the histological GUS staining demonstrated the presence of *hptII* and *gus* genes in transgenic rice lines. This result provides a potential protocol to transfer genes of interest into KD18 as well as other *indica* rice cultivars.

Keywords: *A. tumefaciens*, *gus intron*, hygromycin, Khang Dan 18, transformation

INTRODUCTION

In Vietnam, rice is considered as the most important crop cultivated in over 7.8 million hectares with aproduction of 45.2 million tons. Vietnam's rice exports reached nearly 7 million tons and fetched 2852 million USD (General Statistics Office of Vietnam, 2015). However, rice production in Vietnam has been facing a lot of challenges such as the decrease of arable land, destructive insects, disease epidemics, water scarcity, drought and saline intrusion. Under the climate change scenario, sea-level rise is expected to go up to 13 cm in 2030, 25 cm in

2050 and 100 cm in 2100. If this happens, 16.8% of Red River Delta area and 38.9% of Mekong Delta area will experience flooding, resulting in a significant reduction in rice production and the salinization of a large land area (Ministry of Natural Resources & Environment, 2016). Therefore, it is necessary to generate rice varieties with high yield, good quality and strong tolerance to extreme environmental conditions, especially in the regions with unfavourable natural conditions.

In order to solve these problems, many traditional and modern approaches have been applied to improve quantity and quality of elite rice varieties including hybrid breeding (Miah *et al.,* 1996; Sathish *et al.,* 1997), mutation breeding (Oo, Lang, 2005), genetic transformation, molecular marker-assisted breeding (Vu *et al.,* 2012; Hoque *et al.,* 2015) and genome editing technology (Zhang *et al.,* 2014; Xu *et al.,* 2015). Of which, the CRISPR/Cas9-mediated genome modification system holds great promise in the improvement of stress tolerance in rice, and its efficiencies have been proven in recent studies (Arora, Narula, 2018). This technology allows for highly precise and purposeful genetic modifications and induces transgene-free mutants for a short time.

In Vietnam, *indica* varieties have been cultivated in all rice production areas. However, the lack of efficient generation and transformation protocols is a major bottleneck for *indica* rice genetic improvements. Most of *indica* rice genotypes showed their poor potential in regeneration (Sahoo *et al.,* 2011). Moreover, previous published protocols for rice transformation via *Agrobacterium tumefaciens (A. tumefaciens)* in Vietnam just mostly focused on *japonica* varieties such as Taichung 65 (Hoang *et al.*, 2015), J02 (Pham *et al.*, 2016). There is few research on *indica* varieties and the existing protocols are still lengthy and highly genotype specific (Cao *et al.*, 2019; Tran, Mishra, 2015). Therefore, establishing an appropriate, easy and rapidregeneration and transformationsystem is the first step in the genetic improvement of *indica* rice varieties, especially *indica* ones popularly grown in Vietnam, using biotechnologies. In this study, we selected KD18, a major rice variety in the North of Vietnam, as a research subject to enhance a regeneration and transformation protocol for *indica* varieties. Different factors influencing the efficiency of transformation method mediated by *A. tumefaciens* such as regeneration system, selected agentconcentration, bacterial density and infection time were optimized in order to performa rapid and effective transformation procedure for KD18 cultivar. This result paves the way for the application of biotechnologies, especially the CRISPR/Cas9 system, on KD18 as well as other *indica* rice cultivars.

MATERIALS AND METHODS

Materials

Mature seeds of *indica* rice variety KD18 provided by Thai Binh Seed Group Joint Stock Company.

A. tumefaciens strain AGL1 harbouring pHUE411 vector containing *gus intron* gene and hygromycin resistance gene *(hptII)* supplied by Plant Cell Biotechnology, Institute of Biotechnology.

The specific primers Gus-F/R (forward – 5'-ACCGTTTGTGTGAACAACGA-3' and reverse – 5'-GGCACAGCACATCAAAGAGA-3') and Hyg-F/R (forward – 5'-GCGAAGAATCTCGTGCTTTC-3' and reverse – 5'-GATGTTGGCGACCTCGTATT-3') were used to confirm the presence of transgenes in transgenic rice lines.

Methods

The culture media used in this study are modified and improved by the report of Nishimura *et al.* (2006) with the details below (Table 1). All experiments were conducted with 3 replicates.

Regeneration

Mature healthy rice seedsweredehusked

without damaging embryos and surface-sterilized in 70% ethanol for 1 minute, then in 60% Javel bleach solution supplemented with 1-2 drops of Tween 20 and intermittent shaking duration of 45 minutes, followed by five rinses in sterile water. The sterilized seeds were dried on sterile filter papers and cultured on callus induction medium (N6D) in dark at 26ºC. After 2 weeks, light-yellow friable calliderived from mature embryos were sub-cultured to fresh N6D mediumfor 5 days before incubating on differentiation medium (MSNK) for 9 days. The differentiated calli were subsequently transferred to light on regeneration medium (MSB). After 2 weeks post-transfer to MSB, buds induced from the callus were shifted to rooting medium (MS).

Identification of hygromycin concentrations for KD18 transgenic selection

To identify the optimal hygromycin concentrations for the transformation process, we added hygromycin at 25 mg/L, 30 mg/L, 40 mg/L and 50 mg/L to the callus multiplication and rooting media. The dead explants were recorded at 3 weeks on callus induction medium and at 2 weeks on rooting medium.

Table 1. Compositions of different media used in the study.

Medium	Label	Compositions
Callus induction	N6D	N6 (Chu *et al.*, 1975) + 2 mg/L 2,4-D + 100 mg/L myo-inositol + 300 mg/L casamino acid + 2.878 g/L L-proline + 30 g/L sucrose + 3 g/L gellan, pH 5.8
Co-cutivation	2N6-AS	N6 + 2 mg/L 2,4-D + 100 µM AS + 100 mg/L myo-inositol + 300 mg/L casamino acid + 10 g/L glucose + 30 g/L sucrose + 3 g/L gellan, pH 5.2
Callus differentiation	MSNK	MS (Murashige, Skoog, 1962) + 0.2 mg/L NAA + 2 mg/L kinetin + 100 mg/L myo-inositol + 2 g/L casamino acid + 30 g/L sucrose + 30 g/L sorbitol + 3 g/L gellan, pH 5.8
Regeneration	MSB	MS + 1 mg/L BAP + 30 g/L sucrose + 7.5 g/L agarose, pH 5.8
Rooting	MS	MS + 30 g/L sucrose + 7.5 g/L agarose, pH 5.8
Bacterial culture	YEP	10 g/L yeast extract + 10 g/L bacto peptone + 5 g/L NaCl + 15 g/Lbacto agar, pH 7.0
Suspension	N6 liquid	N6 salts + 68 g/L sucrose + 36 g/L glucose + 3 g/L KCl + 4 g/L $MgCl_2$ +100 µM AS, pH 5.2

Transformation method

A procedure for *Agrobacterium*-mediated transformation method of KD18 was improved with optimized conditions for regeneration and selection gained from the above result.

Rice callus preparation: Mature healthy rice seeds were dehusked, sterilized and then placed on N6D medium in the dark to induce callus. After 14 days, yellowish-white, friable and about 1–3 mm in size calli were inoculated on fresh N6D medium, incubated in darkness for 4–5 days. Mature seed-derived calliwere used as the target materials for transformation.

Agrobacterium suspension preparation: Single bacterial colonies were placed on YEP solid medium containing 50 mg/L kanamycin and 25 mg/L rifamycin and incubated at 28ºC in the dark for 2 days. The sterile spoons were used to collect *Agrobacterium* cells on medium plates and then suspended in N6 liquid medium to adjust to the optimal *Agrobacterium* cell density for transformation.

Infection: Four bacterial densities i.e. 0.05, 0.1, 0.3 and 0.6 (OD_{600nm}) were used for infection. Embryogenic calli were immersed in the *Agrobacterium* suspension with gentle shaking for 10, 20 and 30 minutes, then blotted on sterilefilter papers. The *Agro*-infected calli were transferred onto a sterilized filter paper on the

surface of 2N6-AS medium plates for co-cultivation at 26ºC in the dark.

Callus selection: After 2, 3 or 4 days co-cultivated on 2N6-AS, calli were blotted by sterilized filter papers and placed on N6D medium supplemented with 500 mg/L cefotaxime and 50 mg/L timentin. At 7 days later, calli were cultured in the first selection medium N6D containing 400 mg/L cefotaxime, 30 mg/L timentin and an appropriate hygromycin concentration for 10 days. Proliferated calli were transferred to the fresh selection medium and kept in dark for 7 days before transferring to MSNK medium (supplemented with 400 mg/L cefotaxime and the selected hygromycin concentration).

Plant regeneration: After 9 days post-transfer to MSNK, hygromycin resistant calli were transferred to regeneration medium MSB containing 400 mg/L cefotaxime and the selected hygromycin concentration under the light condition for 10–14 days. Then, regenerated shoots were transferred to rooting medium MS supplemented with 250 mg/L cefotaxime and the appropriate hygromycin concentration.

Transgene presence confirmation through histochemical GUS assay

Histochemical GUS assay was carried out after co-cultivated on 2N6-AS and at regeneration stages. Embryogenic calli and rooted shoots were incubated in X-Gluc solution (Tris/NaCl pH 7.2 + 0.1% X-Gluc + 10% Triton X-100) at 37ºC for 48 hours. Then the stained calli and shoots were rinsed and soaked in 70% ethanol to completely remove the chlorophyll. *Gus* expression was observed and photographed under a stereoscopic microscope. The frequency of transient *gus* expression was calculated based on GUS positive calli while that of stable *gus* expression were based on GUS positive shoots.

Transgene confirmation

Total genomic DNA was extracted from young leaves of the transgenic rice lines using the modified CTAB protocol originally published by Doyle, Doyle (1990). The presence of *gus intron* and *hptII* genes was confirmed by PCR analysis using specific primers. The samples were heated to 94°C for 4 minutes and then subjected to 30 cycles of 30 seconds melting at 94°C, 30 seconds annealing at 56°C and 45 seconds synthesis at 72°C and followed by another 7 minutes final extension at 72°C. PCR products were assayed by electrophoresis on 0.8% agarose gels, stained with ethidium bromide and then visualized and photographed under UV light.

RESULTS AND DISCUSSION

An optimized somatic regeneration procedure for KD18

A stable and highly efficient regeneration protocol is one of the prioritizing requirements when establishing a transformation system for a plant variety. In order to develop an optimized regeneration procedure for KD18, various media were subsequently compared for abilities to induce callus and regenerate whole plants. In previous studies of rice transformation, MS and N6 media were most frequently used for both *japonica* and *indica* rice varieties. In which, MS medium was more commonly utilized for callus induction of *indica* varieties (Cao *et al.,* 2019; Sahoo *et al.,* 2011). From preliminary tests of various regeneration media with different growth hormone concentrations and cultivation times, we conducted an efficient regeneration protocol for KD18 with selected medium components (Table 1). Regeneration results indicate that callus induction of KD18 was more potential on N6 basal medium (supplemented with 2 mg/L 2,4-D) with afrequency of 94.7%. Shoot regeneration frequency was 45.3%, in which the average number of regenerated shoots per callus cluster was 4.7 (Table 2). Allregenerated shoots from callus were rooted and well developed on MS basal medium. This stable and high regeneration frequency would be a prerequisite for successful gene transfer in KD18.

Determination of hygromycin concentrations for KD18 transgenic selection

The appropriate concentrations of antibiotics

were used for preliminary screening of non-transgenic calli throughout the transformation process. In this study, we used *A. tumefaciens* strain AGL1 harbouring pHUE411 vector containing *gus intron* and hygromycin resistance gene *(hptII)* for KD18 transformation. Hygromycin has been used as a selection agent for genetic transformation of different plant species. In previous reports, on the hygromycin-added media, non-transgenic calli turned brown and died, while cell proliferation continued in transgenic rice calli (Hoang *et al.*, 2015; Tran, Mishra, 2015). Moreover, the selected antibiotic concentrations were depended on rice varieties. The suitable hygromycin concentration for rice transgenic selection was previously reported between 25 and 50 mg/L depending on each rice varieties and regeneration stages (Hoang *et al.*, 2015; Nishimura *et al.*, 2006; Sahoo *et al.*, 2011). Therefore, we tested a range of hygromycin concentrations from 25 to 50 mg/L in order to determine the optimum concentration for callus induction and the rooting formation of KD18 cultivar.

Table 2. The result of somatic regeneration of KD18 cultivar on the optimized media.

Experiment	Total inoculated calli	Callus induction (%)	Shoot regeneration (%)	Number of shoots/callus cluster	Rooted shoots (%)
1	150	94.0	46.0	4.4	100
2	150	96.7	42.0	5.0	100
3	150	93.3	48.0	4.6	100
Mean		94.7 ± 1.76	45.3 ± 3.06	4.7 ± 0.32	100

Table 3. Effects of hygromycin on KD18 *in vitro* regeneration at different selection stages.

Hygromycin concentration (mg/L)	Callus multiplication stage		Rooting stage	
	Total inoculated calli	Callus browning/death (%)	Total inoculated shoots	Shoot death (%)
0	150	0	90	0
25	150	68.7 ± 1.15	90	82.2 ± 5.09
30	150	95.3 ± 3.06	90	100
40	150	100	90	100
50	150	100	90	100

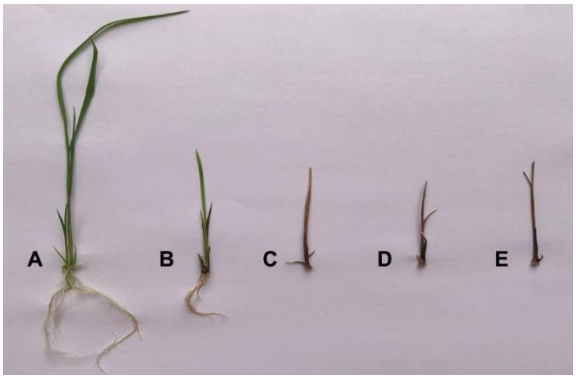

Figure 1. Effects of hygromycin on the rooting stage. A: Control, B: 25 mg/L hygromycin; C: 30 mg/L hygromycin; D: 40 mg/L hygromycin; E: 50 mg/L hygromycin.

After 2 weeks on callus induction medium N6D and 5 days of sub-culutre, rice calli derived from mature embryos were transferred to the hygromycin-supplemented medium. On medium added 25 mg/L hygromycin, the callus growth was inhibited and 68.6% of callus turned black and died at 3 weeks (Table 3). The KD18 calli were completely inhibited at 30 mg/L hygromycin or higher. The similar tendency was observed in the rooting stage. The rooting frequency was significantly reduced as hygromycin concentrations increased from 25 mg/L to 50 mg/L. On regeneration medium supplemented with 30 mg/L hygromycin or higher, rooting was completely inhibited and all plants died after 2 weeks (Table 3, Fig. 1). Taken together, hygromycin at 30 mg/L was found as the most effective in our transformation experiments of KD18. Sahoo *et al.* (2011) obtained similar results when screening transgenic *indica* rice varieties, while previous studies used a much higher concentration of hygromycin (50 mg/L) (Cao *et al.*, 2019; Hoang *et al.*, 2015; Pham *et al.*, 2016).

Optimization of *Agrobacterium*-mediated transformation of KD18

The efficiency of transformation depends on various factors including genotypes, bacterial strains, transformation processes etc… In this study, we focused on optimizing three important parameters influencing the T-DNA delivery efficiency such as bacterial density, inoculation time and co-cultivation period.

Effects of bacterial cell density on transformation efficiency of KD18

Bacterial density denotes the constant total number of bacteria cells per unit of volume. This factor was considered as an important parameter that directly affects the transformation efficiency. Low bacterial density might reduce the exposure of bacteria to infected cells, leading to low transformation efficiency; while excessive bacterial density was found to adversely affect the callus growth and cause risk of bacterial over-growth problems. To determine a suitable *Agrobacterium* cell density for KD18 transformation, calli were immersed in the bacterial suspension at densities (OD_{600nm}) of 0.05, 0.1, 0.3 and 0.6.

After co-cultivation, infected calli were collected for histological GUS staining to determine the transient expression of *gus* gene. In which, 87.3% of inoculated calli from the treatment using the bacterial density of 0.1 (OD_{600nm}) showed GUS stain indicating by large and small blue spots (Table 4). The highest percentages of calli producing shoots and stable *gus* expression (13.7% and 12.2%, respectively) were also obtained from this treatment. The suitable bacterial densities for infection are depended on rice varieties and transformation procedures. In the previous study on Bac Thom 7 transformation, the highest transformation efficiency was achieved at OD_{600nm} of 0.03. However, in the report by Sahoo *et al.* (2011), the density of *Agrobacterium* was used up to 0.3 for different *indian* rice varieties i.e. IR64, CSR10, PB1, and Swarna. In our research, the OD_{600nm} = 0.1 is the most appropriate bacterial density for KD18, and consistent to the results reported in *japonica* transformation by Nishimura *et al.* (2006) and Hoang *et al.* (2015).

Table 4. Effects of bacterial cell density on transformation efficiency.

Treatment	Total transformed calli	Transient *gus* expression (%)	Callus producing shoots on selection medium (%)	Stable *gus* expression (%)
0.05	270	82.5 ± 2.5	12.2 ± 1.1	9.3 ± 1.3
0.1	270	87.3 ± 2.5	13.7 ± 0.6	12.2 ± 1.1
0.3	270	56.7 ± 2.9	6.7 ± 1.1	6.7 ± 1.1
0.6	270	42.0 ± 2.7	4.8 ± 0.6	3.7 ± 0.6

Effects of inoculationtime on transformation efficiency

Infection duration is also an important parameter influencing genetic transformation efficiency. While a shorter time of infection decreased the ability of bacteria to penetrate plant cells, prolonged infection duration negatively affects the subsequent regeneration. As OD_{600nm} = 0.1 was selected as the most appropriate bacterial cell density during transformation, we optimized the duration of infection by immersing callus in *Agrobacterium* suspension for 10, 20, and 30 minutes. Twenty minutes infection period showed the highest frequency of GUS stained calli and regenerated shoots, at 81.9% and 12.6%, respectively. The highest rate of callus producing shoots on selection medium was also obtained from this inoculation treatment (12.6%). The shorter or longer infection time resulted in lower frequencies of shoot production and *gus* gene expression (Table 5).

Effects of co-cultivation timeon transformation efficiency

Co-cultivation time significantly impacts on infection and subsequent T-DNA integration into the host cell genome. In this experiment, we tested different co-cultivation durations ranging from 2 to 4 days. Our results suggested that the co-cultivation period of 3 days was the most suitable for genetic transformation of KD18 indicating by the highest frequency (93.1%) of transient *gus* expression observed after co-cultivation (Table 6). We also found the overgrowth of bacteria at subsequent steps of transformation when the co-cultivation time was prolonged. In addition, the highest frequencies of callus producing shoots (14.1%) and stable *gus* expression (13.3%) were also observed from the treatment of 3 days co-cultivation. This result was similar to previous rice transformation studies of the *japonica* variety J02 (Pham *et al.*, 2016), *japonica* variety Taichung 65 (Hoang *et al.*, 2015) and *indica* rice variety Bac Thom 7 (Cao *et al.*, 2019).

Table 5. Effects of infection time on transformation efficiency.

Treatment	Total transformed calli	Transient *gus* expression (%)	Callus producing shoots on selection medium (%)	Stable *gus* expression (%)
10 minutes	270	73.6 ± 2.4	7.8 ± 1.1	5.9 ± 0.6
20 minutes	270	81.9 ± 2.4	12.6 ± 0.6	12.6 ± 0.6
30 minutes	270	66.7 ± 4.2	7.8 ± 1.1	7.4 ± 0.6

Table 6. Effects of the co-cultivation time on transformation efficiency.

Treatment	Total transformed calli	Transient *gus* expression (%)	Callus producing shoots on selection medium (%)	Stable *gus* expression (%)
2 days	270	90.9 ± 4.5	5.9 ± 0.6	4.8 ± 0.6
3 days	270	93.1 ± 2.4	14.1 ± 0.6	13.3 ± 1.1
4 days	270	87.9 ± 5.2	6.7 ± 1.1	3.7 ± 0.6

Utilization of the optimized procedure for KD18 transformation at a pilot scale

Based on the above optimizations, we conducted an efficient *Agrobacterium*-mediated transformation for KD18 cultivar with optimal parameters including bacterial density (OD_{600nm} = 0.1), inoculation time (20 minutes), co-cultivation time (3 days) and selected agent concentration (30 mg/L hygromycin) (Fig. 2). This procedure was performed at a large scale for KD18 transformation with three replicates.

Table 7. The evaluation of the transformation protocol for KD18 with the established optimal conditions.

Experiment	Total transformed calli	Callus producing shoots on selection medium (%)	Stable *gus* expression (%)	PCR-positive samples (%)
1	120	12.5	12.5	12.5
2	120	15.0	13.3	13.3
3	120	14.2	12.5	12.5
Mean		13.9 ± 1.3	12.8 ± 0.5	12.8 ± 0.5

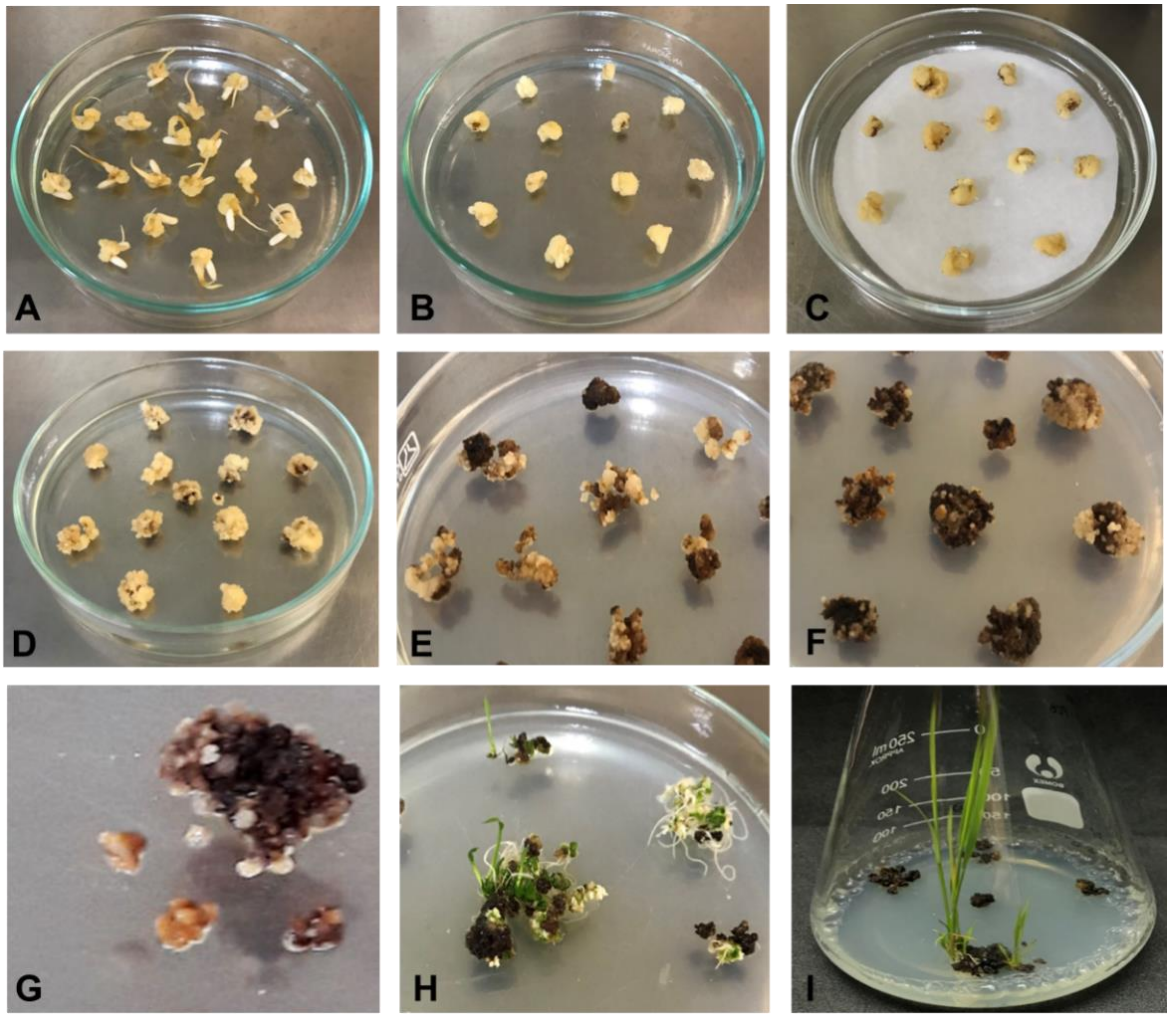

Figure 2. *A. tumefaciens*-mediated transformation procedure for KD18. A: Callus induction on N6D; B: Sub-culture callus on N6D; C: Co-cultivation of calli with *A. tumefaciens* on 2N6-AS; D: Development of callus on N6D after transformation; E: First selection cycle of transformed calli; F: Second selection cycle of transformed calli; G: Callus differentiation on MSNK; H: Shoot regeneration from callus on MSB containing hygromycin; I: Rooting and developing of transgenic shoots on MS.

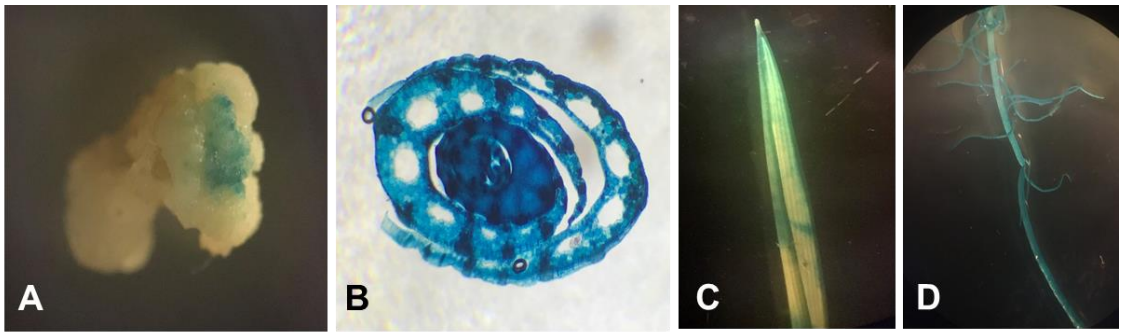

Figure 3. *Gus* expression in different organs of transgenic rice plants. A: Callus; B: Stem; C: Leaf; D: Root.

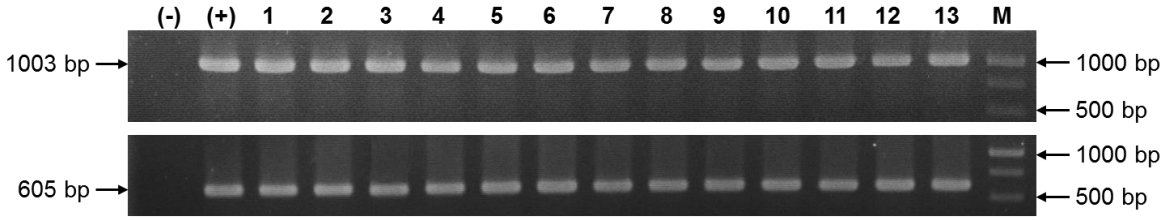

Figure 4. PCR confirmation of the presence of *gus* gene (above) and *hptII* gene (below) in transgenic rice lines. Lane (-): negative control (WT); Lane (+): positive control (plasmid); Lane 1-13: Individual transgenic rice lines; Lane M: Marker DNA 1kb.

At the large scale of transformation, the frequency of hygromycin-resistant regenerated shoots was achieved up to 13.9% (Table 7). Importantly, all regenerated shoots produced roots on MS medium added 30 mg/L hygromycin. The GUS assay showed expression of *gus* gene in the whole transgenic plant including leaves, stems and roots (Fig. 3). The frequency of stable *gus* expression reached up to 12.8%.

Total genomic DNA from leaf samples of transgenic rice lines was extracted for PCR reactions to confirm the presence of *gus* and *hptII* genes. The PCR and gel-electrophoresis showed expected products of transgenes, indicating by 1003 bp and 605 bp DNA bands of *gus* and *hptII* genes, respectively (Fig. 4). This result again demonstrated the potential procedure for KD18 transformation using *Agrobacterium*-mediated method.

Taken together, we successfully optimized an *Agrobacterium*-mediated transformation procedure for KD18 cultivar. This protocol has decreased total time from inoculation to generated transgenic shoots. In addition, performing bacterial suspension directly from a solid medium plate is another advantage compared to previous reports of rice transformation (Sahoo *et al.*, 2011; Tran, Mishra, 2015). Notably, this is the first study on *A. tumefaciens*-mediated transformation protocol for rice variety KD18 with an effective transformation frequency of 12.8%. On the other hand, previous studies indicated that the rice transformation efficiency was depended on varieties. Most of *japonica* rice varieties were reported to be more amenable to genetic modifications than *indica* rice varieties (Nishimura *et al.*, 2006). Previously, Cao *et al.* (2019) conducted the transformation efficiency of an *indica* rice variety (Bac Thom 7) by up to 24.32%. However, this procedure was not effective for KD18 indicated by the low transformation frequencies (<5%) in our preliminary tests. Therefore, we need to optimize

both regeneration and transformation as we start using genetic transformation with a new variety.

CONCLUSION

In this study, we have developed an efficient procedure for *Agrobacterium*-mediated method for KD18 cultivar by optimizing different transformation parameters. The optimal concentration of hygromycin for selection was 30 mg/L. The highest transformation frequency (12.8%) was obtained when embryonic callus was immersed in *Agrobacterium* suspension at OD_{600nm} = 0.1 for 20 minutes and then co-cultivated for 3 days. The procedure presented herein has the potential transfer genes of interest into KD18, as well as other *indica* rice cultivars.

Acknowledgements: *This study was funded by National Funded Independent Project coded ĐTĐL.CN.51/19 "Research and application of CRISPR/Cas9 technology to improve salinity tolerance in rice".*

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