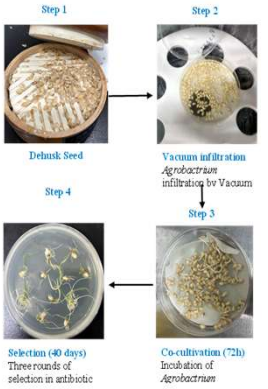
IOCAG 2025 Conference
The 3rd International Online Conference on Agriculture
22–24 October 2025 | Online
MDPI

Highly efficient direct seed transformation protocol for japonica rice (*Oryza sativa* L.) by *Agrobacterium* tumefaciens

Haque Md Maksudul[1,2], Shin-ichiro-Kidou[1,3]
[1]Graduate School of Science, Nagoya City University, Nagoya, Japan.
[2]Bangladesh Institute of Research and Training on Applied Nutrition (BIRTAN), Narayanganj, Bangladesh.
[3]Research Center for Biological Diversity, Nagoya City University, Nagoya, Japan.

Abstract

Graphical Abstract



Molecular breeding and gene function studies in plants require high transformation efficiency. Agrobacterium-mediated transformation has contributed significantly to molecular research in many plants, but is inefficient and inconsistent in rice that do not host Agrobacterium. Transformation efficiency in rice remains low. Therefore, this study aimed to establish a simple and efficient transformation method for rice using Agrobacterium. Two foreign genes (CISP1-GFP and CISP2-GFP) and a Agrobacterium strain (EHA105) were used in the experiments. Then, Agrobacterium infection of rice seeds that had absorbed water and germinated under reduced pressure infiltration conditions showed that an average of 14% of the seeds formed after growth (12% with CISP1-GFP and 16% with CISP2-GFP) carried the foreign gene, and it was also confirmed by PCR, Western blot, GFP fluorescence and TAIL-PCR. Since this method does not involve callus formation or re-differentiation of rice plants, no special equipment or complicated operations are required, and transformants can be obtained in only three months. Therefore, this method is expected to simplify rice genetic manipulation and promote molecular breeding of rice.

METHOD

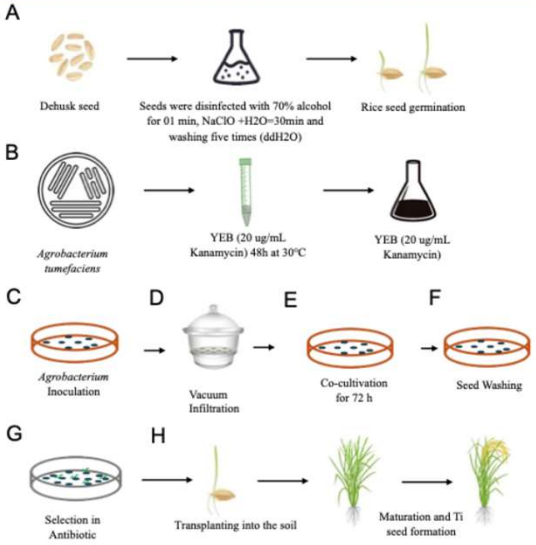


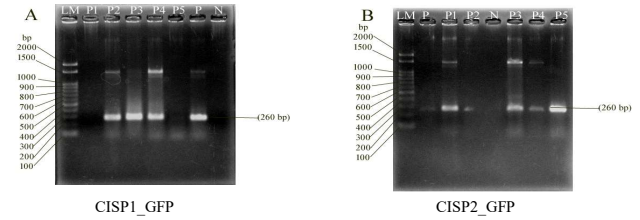
Figure.1 Agrobacterium-mediated rice seed transformation experiment design. The protocol's workflow involves a single-step ice seed infection and plant regeneration to create transgenic rice plants. The four main steps of the workflow are followed daily until the plant regeneration process, which can take up to eight weeks to complete before seedling acclimatization. The technique commences with the sterilization of Rice seeds and seed germination (A) concurrently with isolation and bacterial culture (B). The subsequent stage accomplishes the seed transformation by Agrobacterium-mediated transformation (AMT), culminating in tumefaciens infection (C), Vacuum Infiltration (D), Following seed infection, cocultivation transpires (E) promptly succeeded by Antibiotic Selection (G) and greenhouse acclimation and plant recovery. (H).

RESULTS

Table. 1 Summary Transformation efficiency in Rice cv. Kita Ake

Experiment Sl. No.	Plasmid	No of Infected seed	Selection by Hygromycin-B antibiotic	No of Plants positive for		Transformation efficiency (%)	Mean transformation efficiency (%)
				PCR	Western Blotting		
1	CISP1_GFP	25	05	3	3	12	14
2	CISP2_GFP	25	05	05	4	16	

RESULTS



CISP1_GFP CISP2_GFP

Figure . 2 PCR testing of DNA taken from the leaves of transformants. The binary vector plasmid-carrying Agrobacterium EHA105 transformed. Hygromycine-B resistance gene primers were used for PCR amplification agarose gel electrophoresis. Lane P: Plasmid DNA as a positive control; lanes P1–P5: genomic DNA from transgenic plant lines (P1, P2, P3, P4, and P5); lane N: genomic DNA from a non-transformed plant. CISP1_GFP and CISP2_GFP rice cultivar PCR findings. 'LM' marks the 100bp low marker in panels (A and B). The other lanes show 260 bp PCR products from converted Rice plants as 'GFP positive'.

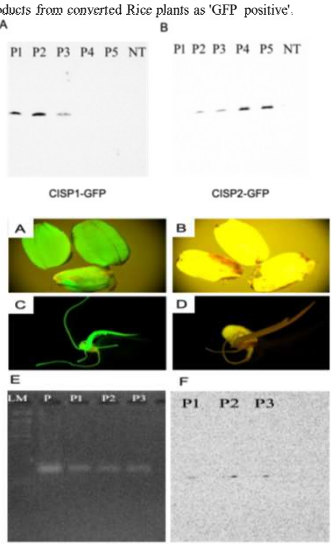


Fig. 4 Evaluation of expression of the GFP gene T_1 transgenic plants. (**A**) T_1 transgenic seeds; (**B**) T_1 non-transgenic seeds; (**C**) T_1 transgenic seedling; (**D**) T_1 non-transgenic seedling; (**E**) PCR of DNA from leaves of T_1 generations of CISP1-GFP transformants and (**F**) Western blot of the same T_1 generations.

Figure. 3 Western blot tests of transgenic and non-transformed rice cv. Kita Aka T_0 generations. Thirty micrograms of extracted protein was appliqued to each lane and subjected to SDS-PAGE. After swimming, the proteins were transferred from the gel to a PVDF membrane and subjected to Western blot analysis using an antibody against GFP. The protein expressed in the transformants (CISP1-GFP or CISP2-GFP) was then detected. Transgenic rice plants showed a 30 kDa band in western analysis. A. CISP1-GFP expresses P1, P2, and P3; B. CISP2-GFP expresses P2, P3, P4, and P5. Lane NT is a negative control from a non-transformed plant.

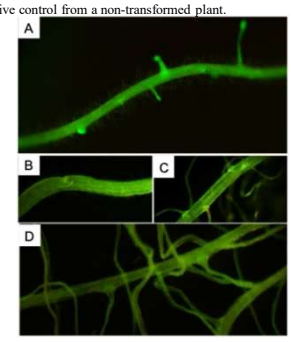


Fig. 5 Evaluation of CISP1-GFP protein expression in roots of T_1 transgenic plants. (A) GFP fluorescence image of 3-day-old T_1 transgenic rice seedling roots. (B-D) GFP fluorescence images of 58-day-old T_1 transgenic rice roots.

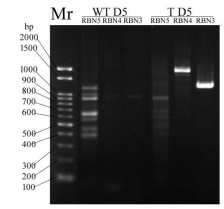


Fig. 6 TAIL-PCR products loaded on the agarose gel stained with ethidium bromide. Multiple bands may be caused through multiple T-DNA insertions or originated through nested AD primer binding. Mr: Marker (1-kb ladder), WT: Non transgenic Plant, T: Transgenic Plant. Single or multiple bands can be shown but size of the bands should be different in all lanes.

Fig. 7 Conformation of Transformed Rice T2 Geminated seed by GFP fluorescence

Brachypodium transformation was possible using this protocol.

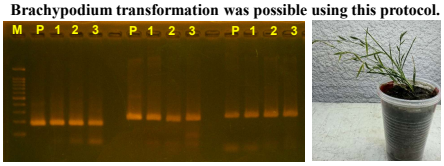


Fig. 8 Conformation of Transformed Brachypodium by PCR (CISP1_GFP) using three pair Primers.

CONCLUSION

Transgenic rice plants were successfully co-cultivated with *Agrobacterium* strain EHA101 and EHA105 with pRI 101-ON on medium. In conclusion, *Agrobacterium*-mediated transformation may produce transgenic rice plants, and transgene viability was proven for T_0 generations, thus it is important to verify it on subsequent generations. This approach skips tissue culture, making it beneficial. This approach is fast, inexpensive, and simple to use for plant genetic modification. This is the initial study of rice seed transformation utilizing mature seed explants, consequently it is important to preserve transgenic plants for large-scale in vitro multiplication to examine CISP gene overexpression. Finally, our technique produced stable transgenic rice plants in 3 months with 14% transformation efficiency. Brachypodium transformation was possible using this protocol. It was rapid, practical, highly reproductive, efficient, and dependable.