Efficiency of Callogenesis and Amenability to *Agrobacterium* Mediated Transformation of Selected Traditional Sri Lankan Rice Varieties

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Abstract

Traditional rice (*Oryza sativa* L.) varieties possess many important traits such as medicinal and nutritional properties, drought and pest resistance, salt tolerance, *etc.* Efficient plant regeneration culture and transformation protocols are important in utilizing such genes. Therefore, the aim of this study was to optimize an efficient protocol for callus induction and transformation of selected Sri Lankan traditional rice varieties. *Dahanala, Kaluheenaty, Kalubalawee, Madael, Kahatawalu, Kaharamana, Gonabaru, Alwee, Wannidahanala, Kottiyaran, BG380* and *H4 varieties* were selected for the study. Twelve different concentrations of 2,4-D and kinetin combinations in MS basal medium were used to determine the effect of above hormone concentrations on callus induction. Calli were transformed using *Agrobacterium* mediated transformation. For calli transformation *Agrobacterium tumefaciens* GV3101 containing pBI121 vector harboring GUS gene was used. Efficiency of transformation through GUS expression was calculated for each variety. There was a significant difference in response with respect to 12 treatments used for callus induction of tested varieties except *Gonabaru*. Varieties *Kaluheenaty, Madael, Gonabaru, Kottiyaran* and *H4* have shown the best callus induction for the same combination of hormones (2.5mg/L 2,4-D and 0.5 mg/L kinetin). Other six varieties showed best callus induction for different combinations with majority of them being in the range of 2.5mg/L 2,4-D and 0.5 mg/L kinetin. In accordance with previous work on indica rice regeneration, our results also showed that callus induction was genotype dependant. Higher GUS expression efficiencies starting from 100% were reported for most varieties. Results of this study indicate the possibility of using optimized hormone concentrations and *Agrobacterium* mediated transformation for selected Sri Lankan traditional rice varieties in future genomic studies.

Key words: *Agrobacterium*, Callus induction, Rice, Transformation

Introduction

As a major food, rice (Family: Poaceae) has high demand with the increasing population which creates an enormous need to increase yield. Rice varieties with high yielding and improved nutritional value can be obtained through genetic modifications. There are records of more than thousand traditional rice varieties in Sri Lanka, some of these are Heenati, Dahanala, Hodarawalu, Kuruwee, Kuruluthuda, Mawee, Polael, Rathel, Suwadel. Most of the traditional varieties have been reported to have nutritional or medicinal characteristics and resistance to harsh environments. However, the knowledge about such genes is limited and requires functional genomic studies. Transformation can be applied as a technique to identify new genes and their functions could be introduced into new rice varieties. Method for production of transgenic varieties involves in-vitro plant regeneration and genetic transformation methods. Among transformation methods, simplest and easiest is the Agrobacterium mediated transformation.

Information on amenability for transformation could be a great help in studying expression of genes in these traditional varieties. Therefore, this research provides information for functional analysis of important genes of Sri Lankan traditional rice varieties which can improve the production of improved rice varieties.

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Materials and Methods

Traditional rice varieties Dahanala, Kaluheenaty, Kalubalawee, Madael, Gonabaru, Kahatawalu, Kaharamana, Alwee, Wannidahanal, Kottiyaran, BG 380 and H4 were obtained from Rice Research and Development of Institute, Bathalegoda, Sri Lanka.

Callus induction medium

MS basal medium (Murashig and Skoog, 1962) supplemented with different concentration combinations of 2,4-Dichlorophenoxyacetic acid (2,4-D) and kinetin was used for the callus induction (Table 1).

Table 1. Concentration combinations of 2,4-D and Kinetin used for callogenesis

Medium	Growth hormone	
	2,4-D (mg/L)	Kinetin (mg/L)
1	0	0
2	0	0.5
3	0	1
4	2	0
5	2	0.5
6	2	1
7	2.5	0
8	2.5	0.5
9	2.5	1
10	3	0
11	3	0.5
12	3	1

Vector and Agrobacterium strain

Agrobacterium tumefaciens strain GV3101 harboring pBI121 was used for transformation of rice calli.

Callus induction

Seeds were de-husked and surface sterilized using 0.1% (w/v) $HgCl_2$ and 20% NaOCl. Then seeds were transferred to MS medium. Ten replicates were prepared for each variety and it was repeated. Surface area of each calli was measured using an indirect method under sterilized condition.

Calli transformation

An hour prior to co-cultivation, calli were transferred into plasmolysis medium (½ MS + 4% sucrose). Overnight culture of Agrobacterium was pelleted by centrifugation (5000 g, 10 min) and suspended to an OD 600 of 0.2 in plant inoculation medium (½ MS + 3.0% sucrose + 200 µM acetosyringone). Plasmolyzed calli were transferred to inoculation medium. Calli were incubated for 10 min shaking and 20 min stationary. Calli were blotted on sterile filter papers and transferred to co-cultivation medium (½ MS + 3.0 % sucrose + 8.0 g/L agar + 200 µM acetosyringone). They were co-cultivated for 2 days under darkness. Then calli were transferred to MS medium complemented with 500 µg/mL cefotaxime.

Gus analysis

Calli were dipped in 100 µg/ml solution of 5-bromo-4-chloro-3-indolyl-β-D-glucuronide XglcA at 37 °C under darkness for 24 hours. Then number of blue calli with blue spots was recorded. Transient transformation efficiency was calculated according to following formula.

$$\text{Transformation efficiency} = \frac{\text{Number of callus with blue spots}}{\text{Number of total callus}} \times 100\%$$

Experimental design and Data Analysis

Randomized Complete Block Design (RCBD) was used for the callus induction. Data were analyzed using Kruskal-Walis test in SPSS package. Least Significant Difference (LSD) was obtained for the analysis of the transformation efficiencies using SAS software package 9.1.3.

Results and Discussion

Effect of growth regulators on callus induction

Eleven varieties out of twelve showed a significant

difference for different hormone combinations ($p<0.05$). *Gonabaru* did not show a difference in effect among different combinations of growth regulators ($p>0.05$). The reason for this result could be the best hormone combination for *Gonabaru* was not included in the tested combinations. Requirement of another factor also could be a reason for this result.

the only growth regulator in callus induction medium according to Afrasib and Jafar (2011). In this study *BG380, Alwee* and *Dahanala* varieties showed best callus induction with the combinations having only 2,4-D. *BG380* gave best callus initiation with 2.5 mg/ L 2,4-D and *Dhanala* gave best callus initiation with 3mg/ L 2,4-D. Additionally, several varieties have shown callus induction responses only with 2,4-D.

Table 2. Best hormone combination for callus induction and mean value of transient transformation efficiencies

Variety	Best hormone combination		Mean of transient transformation efficiency (%)
	2,4-D (mg/L)	Kinetin(mg/L)	
Dahanala	3.0	0	98.3
Kaluheenaty	2.5	0.5	91.6
Kalubalawee	3.0	1.0	96.5
Madael	2.5	0.5	89.9
Gonabaru	2.5	0.5	61.6
Kahatawalu	3.0	0.5	45.0
Kaharamana	2.0	0.5	76.6
Alwee	3.0	0	100
Wannidahanala	3.0	1.0	86.6
Kottiyaran	2.0	0.5	64.9
BG380	2.5	0	68.3
H4	2.5	0.5	45.0

LSD = 21.329

Kaluheenaty, Madael, Gonabaru, Kottiyaran and *H4* showed best callus induction for the combination of 2.5 mg/L 2,4-D and 1 mg/L kinetin. *Dahanala* and *Alwee* showed best callus induction with 3 mg/L 2,4-D. *Kalubalawee* and *Wannidahanala* showed best callus growth with the combination of 3 mg/L 2,4-D and 1 mg/L kinetin. Combination of 2 mg/L 2,4-D and 0.5mg/L kinetin was the best for callus induction of *Kaharamana.* But BG 380 variety exhibited the best callus induction in MS medium with 2.5 mg/L 2,4-D and kinetin 0 mg/L (Table 2).

Embryogenic callus induction is dependent on the interaction between the genotypes and growth hormones. Most reports show that 2,4-D is sufficient as

A requirement for a specific combination of auxin and cytokinin for callogenesis has also been reported in several studies. Afrasib and Jafar (2011) reported about the use of MS medium supplemented with 2, 4-D and Kinetin for best callus induction of rice.

Effect of genotype on callus induction

Best hormone combination for callus induction varied with the genotype of tested rice varieties. This result indicated that callus induction of different varieties have been differently affected by hormones. There are several reports which have indicated about the same effect for *indica* rice varieties (Agrawal *et al.*, 2006; Hussain *et al.*, 2010).

Efficiency of genetic transformation of callus

Transformed calli showed blue color which facilitates the clear identification of transformed calli from non transformed calli. Highest transformation efficiency was obtained for *Alwee* (Table 2) and it was 100%. Lowest was 45% for *H4* and *Kahatawalu*. Higher efficiencies were recorded for most varieties. In the formula which was used to calculate transient transformation efficiencies, calli having at least one blue spot were counted as transformed calli. Counting of callus with at least one blue spot and the detection of transient expression could be reasons for higher efficiencies. In transient expression, gene constructs which are not integrated to plant cell genome can give a positive result. Therefore, transient transformation also could be another reason for detecting higher transformation efficiencies.

For the accurate detection of transformation efficiencies, it should be calculated at the regeneration level. Sahoo *et al.* (2011) have obtained transformation efficiencies ranging between 40-46% which was lower than efficiencies at callus level.

Conclusions

Majority of the tested varieties (Kaluheenaty, Madael, Gonabaru, Kahatawlu, Kaharamana, Kottiyaran and H4) showed best callus induction in MS basal medium with 2.5 mg/L 2,4-D and 0.5 mg/L kinetin. *Agrobacterium* mediated transformation is efficient for transformation of the selected traditional rice varieties. Tested twelve varieties showed higher transformation efficiencies ranging from 45% - 100%.

References

Afrasiab, H., and Jafar, R., 2011. Effect of different media and solidifying agents on callogenesis and plant regeneration from different explants of rice varieties super basmati and IRRI-6. *Pak. J. Bot.* 43:487-501.

Agrawal, P.K., Gosal, S.S. and Sidhu, G.S. 2006. Sequential reduction of 2,4,D improves whole plant regeneration from long-term maintained calli in some *indica* cultivars of rice. *Oryza* 43:10-15.

Hussain, Z., Khan, M.H., Bano, R., Rashid, H. and Chaudhry, Z. 2010. Protocol optimization for efficient callus induction and regeneration in three pakistani rice cultivars. *Pak. J. Bot.* 42:879-887.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15:473-497.

Sahoo, K.K., Tripathi, A.K., Pareek, A., Sopory, S.K. and Singla-Pareek, S.L. 2011. An improved protocol for efficient transformation and regeneration of diverse indica rice cultivars. Plant Methods 7:49.