Electronic Journal of Plant Breeding, 1(4): 1249-1256 (July 2010)

Research Article

Regeneration study of some *indica* rice cultivars followed by *Agrobacterium* – mediated transformation of highly regenerable cultivar, Pusa Basmati 1.

N.Aananthi , C.R.Anandakumar , R.Ushakumari and P.Shanthi

Abstract
Five *indica* rice (*Oryza sativa* L.var.) cultivars viz., ASD 16, White Ponni, Pusa Basmati 1, Pusa Sugandh 4 and Pusa Sugandh 5 were subjected to tissue culture to study their regenerability in terms of regeneration percent and total number of regenerated plantlets obtained for a fixed sample size per variety. Regeneration potential was found to be highest (56.03%) for Pusa Basmati 1 and the lowest (30.37%) for White Ponni. The highly regenerating *indica* rice cultivar Pusa Basmati 1 was subjected to genetic transformation mediated by *Agrobacterium tumifaciens* EHA 105 harbouring the virulent Plasmid Pcambia 1305.1. Sucessful transformation events in the infected calli with this strain were assayed by transient GUS assay using 5-bromo 4-chloro 3-indolyl-D glucuronide (X-gluc) as a substrate. The frequency of transformation in terms of transient GUS assay was found to be 44.0 ± 2 S.E

Key words: regeneration**,** *indica* rice, *Agrobacterium* –mediated transformation

Introduction

Rice, one of the most important staple food crops in the world, is consumed by more than one third of the world population. Researches on rice biotechnology have been actively pursued to produce transgenic rice plants with improved yield and quality, increased resistance to biotic and abiotic stresses, and value added grains such as golden rice(Lee *et al*., 2002).Tissue culture via somatic embryogenesis is a key step in gene transfer and plant regeneration in rice biotechnology. The genetic transformation of rice can be accomplished through *Agrobacterium* cocultivation of embryogenic calli (Hiei *et al*., 1994). In general, embryogenic calli, rather than direct tissues such as shoot spices, immature inflorescences, roots and leaves are used for genetic transformation and regeneration of rice plants because the callus culture, compared with organogenesis, is more suitable for gene delivery and regeneration of transgenic rice plants. Therefore, proliferation of the embryogenic calli with the high regeneration capacity is a prerequisite for the successful production of transgenic rice plants.

Callus induction and regeneration potential are affected by the genotypes, explant, carbohydrate sources, plant growth regulators, culture medium and culture conditions, among others. In particular genotype, and explants are important factors for the successful embryogenic callus induction and regeneration of rice plants (Rueb *et a*l. 1994). In this study, optimization of the genetic transformation technique was attempted for highly regenerable *indica* rice Pusa Basmati 1

Materials and Methods

Plant material

The rice genotypes chosen for the study included ASD 16, White Ponni, Pusa Basmati 1, Pusa Sugandh 4 and Pusa Sugandh 5 which were obtained from Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore and Agricultural College and Research Institute, Madurai.

Assistant Professor, Rice Research Station, Ambasamudram-627401

Callus induction

Mature dehusked seeds were sterilized sequentially with sterile distilled water to remove any impurities and then with 70 per cent ethanols for 120 sec followed by 0.1 per cent mercuric chloride solution for 6 – 8 min. with shaking and then were rinsed several times thoroughly with sterilized water. Two basal media MS (Murashige and Skoog, 1962), and N6 (Chu, 1978) were tested for the induction of embryogenic and non embryogenic calli. The MS media were supplemented with 30g l^{-1} maltose , 2,4-D (1.5 mgl^{-1} - ASD 16; 2.0 mg l^{-1} - White Ponni and IAA 2.5 mg l^{-1} - Pusa Basmati 1, Pusa Sugandh 4, Pusa Sugandh 5) with 0.5 mg l^{-1} kinetin and 1.0 g l^{-1} C.H , Coconut Milk 50 mll^{-1} , 8 g l^{-1} Agar, pH 5.8 and N6 media is supplemented with N6 basal + 2 mg l^{-1} 2, 4- D + kin 0.5 mg l^{-1} + 30 g l^{-1} sucrose, 8 g l^{-1} Agar, pH 5.8 .The cultures were maintained at a temperature of 25 ± 2^0 C under dark conditions for callus initiation. Number of explants giving callus response was recorded in each treatment and callus induction per cent was worked out as follows

$$\text{Callus induction per cent} = \frac{\text{Number of explants giving callus induction}}{\text{Total number of explants inoculated}} \times 100$$

The calli were subcultured and embryogenic calli transferred to fresh medium four days prior to cocultivation.

Plant regeneration

The embryogenic calli were transferred to MS regeneration media after 4 weeks of culture on callus induction medium. In order to get more organogenic response, 2,4 - D was avoided and different growth hormones *viz*., auxin (NAA) and cytokinins (BAP and Kinetin) were added to induce shooting and rooting simultaneously. BAP was tried at 0.5, 1.0, 1.5, 2.0 mg l^{-1}; NAA at 0.5, 1.0 mg l^{-1} keeping 1.0 mg l^{-1} of kinetin as constant. The percentage of plant regeneration was worked out as follows

$$\text{Percentage of Regeneration} = \frac{\text{Number of plants regenerated}}{\text{Total number of explants inoculated}} \times 100$$

Young plantlets with good shoot and root growth were taken out of the tubes for hardening. The plantlets were washed repeatedly to remove adhering agar and kept in water for three days under culture conditions. Plantlets were then transferred into small plastic cups containing autoclaved soil and covered with poly bags to check evapotranspiration loss (Biswas and Mandal, 1999). For one week plantlets were allowed to be under culture conditions and later for another one week at room temperature. Poly bags were removed and plantlets were transferred to mud pots. The experiments revealed that the regeneration performance of Pusa Basmati 1 is 84.67% from mature embryo derived callus.

Plant material and callus induction for *Agrobacterium*-mediated transformation

Six weeks old matured embryo derived high regenerable Pusa Basmati 1 seed calli were used as explants for genetic transformation.

Agrobacterium growth and infection

Agrobacterium strain EHA 105 harbouring pCAMBIA 1305.1 with GUS and HPT gene in the transfer region was used for transformation. One loopful of bacterial culture was streaked on AB minimal medium (Chilton *et al*.1974) supplemented with 0.5% glucose, rifampicin (10 mg l^{-1}) and kanamycin (50 mg l^{-1}) and grown at 28^0C in the dark and were cultured for 2 days. From this culture, 2-4 single colonies of the bacterium were transferred to 30ml of AB liquid medium containing rifampicin (10 mg l^{-1}) and kanamycin (50 mg l^{-1}). The culture was grown overnight and bacteria were collected by certrifugation at 3000 rpm for 10 min. The supernatant was removed and the pellet was re suspended in 30 ml of MS broth or AAM medium supplemented with 100 µM AS. The bacterial cell suspension adjusted to 1.0 O.D (A_{600}) with sterile sucrose was directly used for infection and 100 µM AS was added as optimum concentration to the co cultivation medium. The calli were then submerged in the prepared bacterial suspension for 10 minutes, blotted dry on a sterile filter paper and plated in cocultivation medium containing MS +30g l^{-1} maltose , 10 mg l^{-1}glucose, IAA 2.5 mg l^{-1} with 0.5 mg l^{-1} kinetin and 1.0 g l^{-1} C.H , Coconut Milk 50 mll^{-1} , 8 g l^{-1} Agar, pH 5.8, with or without 100 µM AS and pH adjusted to 5.8 for 72 hours in dark at 27^0C by placing the plates upside down. This was followed by thorough washing in sterile water containing cefotaxime 100 mg and carbenicillin 250 mg. The washed calluses were blotted on sterile filter paper to remove excess moisture and transferred to selection medium MS +30g l^{-1} maltose , IAA 2.5 mg l^{-1} with 0.5 mg l^{-1} kinetin and 1.0 g l^{-1} C.H , Coconut Milk 50 mll^{-1} , 8g l^{-1} Agar, 100 mg l^{1} cefotaxime + 250 mg l^{-1} carbenicillion + 30 mg l^{-1} hygromycin B (Boehringer Mannheim, GmbH, Germany), pH 5.8 at

28^0C in dark for 15 days. Hygromycin resistant calluses were transferred to fresh selection medium for every 15 days. White proliferating calluses were transferred to regeneration medium MS basal + 1.0 g l^{-1}tryptophan, 30 mg l^{-1}maltose, 2.0 mg l^{-1}BAP, 0.5 mg l^{-1} NAA and 1.0 mg l^{-1}kinetin, 1.0 g l^{-1} C.H , Coconut Milk 50 mll^{-1} , 8 g l^{-1} Agar, pH 5.8 under 16 hour photoperiod. Following regeneration, the shooted plants were transferred to rooting medium containing half strength MS, 30gl-1 maltose, pH5.8 and no hormone for rooting.

Transient GUS assay

Transient expression of GUS activity in transiently transformed calli was assayed histochemically as described by Jefferson (1987). Infected calli were assayed for transient GUS expression using X-gluc as sustrate for 16 hours at 37^oC.

Results

Optimization for callusing and regeneration

To determine the optimum conditions for callusing and regeneration, both MS and N6 medium were tried. Results presented in Table 1 indicate that the MS was a better medium than N6 and the best calluses were obtained in the presence of 2,4-D ,1.5 mgl^{-1} for ASD 16; 2.0 mg l^{-1} for White Ponni; IAA 2.5 mg l^{-1} for Pusa Basmati 1, Pusa Sugandh 4, Pusa Sugandh 5 with 0.5 mg l^{-1} kinetin,30 mg l^{-1} maltose, 1.0 g l^{-1} C.H , Coconut Milk 50 mll^{-1} .In order to study the time requirement for obtaining embryogenic calluses, seeds were incubated in the medium for 21, 42, 63 and 84 days on the same medium before testing for their regeneration potential, our results revealed that the second treatment (42 days) was found to be better than the others. Frequency of callusing was found to be higher in MS medium compared to N6 medium. On testing the regeneration with BAP at 0.5, 1.0, 1.5, 2.0 mg l^{-1}; NAA at 0.5, 1.0 mg l^{-1} keeping 1.0 mg l^{-1} of kinetin as constant, it was observed that the 42 days old calluses uniformly showed higher regeneration as against the 21 days old calluses. The best regeneration frequency was observed at 2.0 mg l^{-1} BAP+0.5 mg l^{-1}NAA +1.0 mg l^{-1} kinetin. The experiments revealed that the regeneration performance of Pusa Basmati 1 is 84.67% from mature embryo derived callus. Greening was observed within week following subculture such calluses were used for the genetic transformation studies (Table 2) .

Optimization of cocultivation conditions

To find out the optimum conditions for cocultivation, different concentrations of acetosyringone and the duration of cocultivation were tested. For optimizing the concentration of acetosyringone, the percentage of calluses showing GUS expression was worked out as follows

$$\text{Frequency of transient GUS expression} = \frac{\text{Number of explants with blue foci}}{\text{Total number of explants inoculated}} \times 100$$

It was found that the 100 µM acetosyringone was ideal for transformation. In our study it was observed that a maximum of 53 percent of calluses showing GUS expression for three days of cocultivation compared to others. Cocultivation for a period more than three days resulted in the calluses becoming prone to repeated *Agrobacterium* infection, which ultimately resulted in the loss of regeneration potential. Thus cocultivation for a period of three days was found to be the most suitable for the optimum transformation. Table 3.

Regeneration and recovery of transgenic plants

To study the optimum dose of antibiotics during rice regeneration six levels *viz*., 0, 50, 100, 150, 200, 250 mg l^{-1} of cefotaxime and carbenicillin were used in the rice regeneration medium. Antibiotics strongly reduced regeneration capacities of rice calli. In the presence of 50 and 100 mg l^{-1} cefotaxime and carbenicillin, a slightly inhibitory effect was seen. The highest dose of 100 mg l^{-1} cefotaxime and carbenicillin (250 mg l^{-1}) inhibited the regeneration dramatically Table 4.

Discussion

Studies have indicated that in rice, efficient transformation and subsequent regeneration using *Agrobacterium* –mediated methods are dependent on several factors. These include choice of the explant, hormonal composition of the medium used, nutritional supplements, temperature and duration of cocultivation, virulence of the *Agrobacterium* strain ,concentration and composition of the bacteriostatic agent used, concentration of antibiotic selection marker (Saharan *et al.*2004; Katiyar *et al* .1999 and Tyagi *et al.*2007).Among the several type of explants (Park *et al*. 1996 and Vijayachandra *et al*. 1995) matured seed derived calluses has been found to be most amenable to transformation (Hiei *et al.*1994) .The potential for callus formation ,regeneration and successful transformation in rice is a varietal

characteristics (Maggioni *et al.*1989 and Tyagi *et al*.2007) and *indica* rice reported to be inferior to *japonica* in this respect.(Tyagi *et al*. 2007).We tested two media, MS and N6 with different hormones namely 2,4 D and IAA for Pusa Basmati1 for callusing response ,involving different incubation periods. Our results indicated that MS media containing 2.5 mg l^{-1} IAA, 0.5 mg l^{-1} kinetin added with 1.0 mg l^{-1} CH, 50 ml l^{-1} Coconut milk gave the better results. But earlier reports stated that the MS media containing 2.0 mg l^{-1} 2,4 D added with CH was suitable for the most of the *indica* varieties. (Visarada *et al*. 2002; Lin and Jhang, 2005; Khanna and Raina, 1999; Wang *et al*. 2002; Zhang *et al*.1997; Mohanty *et al.*1999; Aldemita and Hodges, 1996 and Sridevi *et al*.2005 and Tyagi *et al*.2007).

On anlaysing the parameters like callusing, cocultivation and regeneration, it was seen that best callusing response was observed on MS medium with 2.5 mg l^{-1} IAA, 0.5 mg l^{-1} kinetin added with 1.0 mg l^{-1} CH, 50 ml l^{-1} Coconut milk, following subculturing for a total of 42 days. In this study, the maximum callus induction per cent was noticed on MS media and it was lower on N_6 media .Similar results were already reported by Sudha (2000) and Khaleda and Al-Forkan (2006). In contrast Lee et al. (2002) reported N_6 media showed higher callus induction and embryogenic callus formation. Studies on the effect of callus age on transient GUS expression have shown that the 42 days old calluses performed better and exhibited maximum number of blue foci in the variety Pusa Basmati 1(Table 5).

Optimization of cocultivation period showed that three days of cocultivation was the best and resulted in maximum Gus expression .It has been reported that the addition of high concentration of bacteriostatic agents like cefotaxime and carbenicillin may reduce the regenerability of the calluses. In this study it was seen that reduction of the time period for which the calluses were subjected to the bacteriostatic agent led to a substantial increase in the regeneration potential of the transformed calluses. Regeneration was best achieved in the presence of 2.0 mg l -1 BAP, 0.5 mg l -1 NAA, and 1.0 mg l-1 kinetin which supports with earlier reports that describe the use of 0.5 -2.0 mg l-1 BAP for different varieties of rice(Visarada *et al*. 2002; Lin and Jhang, 2005; Khanna and Raina, 1999; Wang *et al*. 2002; Zhang *et al*.1997; Mohanty *et al.*1999; Aldemita and Hodges, 1996 and Sridevi *et al*.2005 and Tyagi *et al*.2007).

References

Aldemita, R. R., and T. K. Hodges. 1996. *Agrobacterium tumefaciens*- mediated transformation of *japonica* and *indica* rice varieties. Plants, 199: 612-617.

Biswas, B. and A. B. Mandal. 1999. Varietal specificity in callus induction and plant regeneration in rice (*Oryza sativa* L.). Crop Improv., 26 135-140.

Chilton .M.D., T.C. Currier, S.K. Farrand, N. Baisakh, N. Oliva and S.K. Datta. 1974. Agrobacterium tumefaciens DNA and PS8 bacteriophage not detected in crown gall tumors.Proc.Natl Acad Sci USA 71: 3672-3676.

Chu, C. C. 1978. The N_6 medium and its applications to anther culture of cereal crops. P. 43-50. In Proc. Symp. Plant Tissue Culture, 25-30.

Hi ei, Y., S. Ohta, T. Komari and T.Kumashiro. 1994. Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA. Plant J., 6: 271-282.

Jefferson, R. A. 1987. Assaying chimeric genes in plants: The GUS gene fusion system. Plant Mol. Biol. Rep., 5 : 387-405.

Katiyar, S. K., G. Chandel, Singh and R. Pratibha. 1999. Genetic variation and effect of 2,4-D on *in vitro* plant regeneration in *indica* rice cultivars**.** Oryza, 36 (3): 254-256.

Khaleda, L. and M.Al. Forkan. 2006. Genotypic variability in callus induction and plant regeneration through somatic embryogenesis of five deepwater rice (*Oryza sativa* L.) cultivars of Bangladesh. African J. Biotech., (5): 435-1440.

Khanna, H. K. and S. K. Raina. 1999. *Agrobacterium* – mediated transformation of *indica* rice cultivars using binary and superbinary vector. Aust. J. Plant Physiol., 26: 311-324.

Lee, K., H. Jeon and M. Kim. 2002. Optimization of a mature embryo based *in vitro* culture system for high frequency somatic embryogenic callus induction and plant regeneration from *japonica* rice cultivars. Plant Cell Tiss. Ogan. Cult., 71: 237-244.

Lin, Y. J. and Q. F. Jhang. 2005. Optimizing the tissue culture conditions for high efficiency transformation of *indica* rice. Plant Cell Rep., 23: 540-547.

Maggioni, L., M. C. Lusardi and E. Lupotto. 1989. Effects of cultural conditions on callus induction and plant regeneration from mature and immature embryos of rice varieties (*Oxyza sativa* L.). J. Genet. Breed., 43: 99-106.

Mohanty, A., H. Kathuria, A. Ferjani, A. Sakamoto, P.Mohanty, N. Murata and A. K. Tyagi. 2002. Transgenics of an elite *indica* rice variety Pusa Basmati 1 harbouring the *coda* gene are highly tolerant to salt stress. Theor Appl. Genet., 106: 51-57.

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

Park, S. H., S. R. M. Pinson and R. H. Smith. 1996. T-DNA integration into genomic DNA of rice following *Agrobacterium* inoculation of isolated rice shoot apices. Plant Mol. Biol., 32: 1135-1148

Rueb, S., M. Leneman, R. A. Schilperoot and Hensgens Lan. 1994. Efficient plant regeneration through somatic embryogenesis from callus induced on mature rice embryo. Plant cell, Tissue and Organ. Cult., 36: 259-264.

Saharan, V., R. C. Yadav, N. R. Yadav and K. Ram. 2004. Studies on improved *Agrobacterium* – mediated transformation in two *indica* rice (*Oryza sativa* L.). African J. Biotech., 3(11): 572-575.

Sridevi, G., M. Dhandapani and K. Veluthambi. 2005. *Agrobacterium*- mediated transformation of white ponni, a non-basmati variety of *indica* rice (*Oryza sativa* L.). Curr. Sci., 88 : 128-132.

Sudha, C. 2000. Identification of best *indica* type for calls induction and regeneration capacity including hybrid rice parents (*Oryza sativa* L.) M.Sc., (Ag.) Thesis, TNAU, Coimbatore.

Tyagi, H., S. Rajasubramanian and I. Dasgupta. 2007. Regeneration and *Agrobacterium* – mediated transformation of a popular *indica* rice variety, ADT 39. Curr. Sci. 99 (5): 678-683.

Vijaya Chandra, K., K. Palani Chelvam and K.Veluthambi. 1995. Rice scutellum induces *Agrobacterium tumefaciens vir* genes and T-strand generation. Plant Mol. Biol., 29: 125-133.

Visarada, K. B. R. S., M. Sailaja and N. P. Sarma. 2002. Effect of callus induction media on morphology of embryogenic calli in rice genotypes. Biol. Plant., 45: 495-502.

Wang, L. T., X. T. Ming, C. C. An, H. Y. Yuan and Z. L. Chen. 2002. Callus induction and regeneration from mature seeds of *indica* rice Minghui 63 and anti-fungal assay of transgenic rice plants. Sheng Wu Gong Cheng Xue Bao., 18: 323-326.

Zhang, J., R.J. Xu, M.C. Elliott and D.F. Chen. 1997. *Agrobacterium* – mediated transformation of elite *indica* and *japonica* rice cultivars. Mol. Biotechnol ., 8 : 223-231.

Table 1. Effect of media on callus induction per cent in seed culture (expressed in per cent)

Media / Genotypes	MS + 2 mg l^{-1} 2, 4-D + 0.5 mg l^{-1} K + 30 g l^{-1} sucrose**MS**				N6 + 2 mg l^{-1} 2, 4- D + Kin 0.5 mg l^{-1} + 30 g l^{-1} Sucrose **N6**			
	A	**B**	**C**	**D**	**A**	**B**	**C**	**D**
ASD 16	85.13	71.14	16.50	40.08	77.00	64.89	19.53	27.88
White Ponni	**88.75**	**75.22**	13.29	**47.27**	**80.07**	**67.68**	18.01	**30.98**
Pusa Basmati 1	84.97	70.18	14.78	39.33	76.89	63.81	20.78	26.24
Pusa Sugandh 4	78.47	66.44	18.21	35.31	67.12	62.70	21.65	19.71
Pusa Sugandh 5	65.71	61.55	**23.43**	19.41	51.74	42.27	**37.23**	15.15

		A	**B**	**C**	**D**
Media	**SEd**	0.13	0.13	0.12	0.12
	CD (0.01)	0.36	0.33	0.33	0.33
Genotype	**SEd**	0.40	0.32	0.32	0.32
	CD (0.01)	0.91	0.87	0.85	0.85
M x G interaction	**SEd**	0.48	0.46	0.45	0.45
	CD (0.01)	1.29	1.23	1.20	1.20

Callus induction (A), embryogenic callus formation (B), rhizogenic callus formation (C) and regeneration (D)

Table 2. Regeneration per cent of plant lets from callus of seed at various levels of hormones

Treatments / Genotypes	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	Mean
G_1	11.01 (19.38)	10.08 (18.51)	11.32 (19.66)	18.90 (25.77)	37.37 (37.69)	54.23 (47.43)	75.30d (60.20)	54.73 (47.72)	34.12d (34.54)
G_2	11.00 (19.37)	10.19 (18.62)	10.25 (18.67)	11.60 (19.91)	20.01 (26.57)	55.34 (48.07)	72.51e (58.38)	52.03 (46.16)	30.37e (31.97)
G_3	37.22 (37.60)	43.33 (41.17)	55.30 (48.04)	53.11 (46.78)	54.23 (47.23)	59.19 (50.30)	**84.67a** (66.94)	61.23 (51.49)	56.03a (48.72)
G_4	31.12 (33.91)	42.12 (40.47)	50.12 (45.07)	45.65 (42.51)	53.34 (46.92)	54.21 (47.42)	82.11b (64.98)	58.32 (49.79)	52.12b (46.38)
G_5	27.19 (31.43)	38.15 (38.15)	40.39 (39.46)	41.19 (39.93)	50.96 (45.55)	51.62 (45.55)	80.54c (63.83)	55.76 (48.31)	48.22c (44.07)
Mean	2.51h (28.34)	28.77g (31.38)	33.48f (34.18)	34.09e (34.09)	43.18d (40.83)	54.92c (47.83)	79.02a (62.87)	56.41b (48.69)	44.17 (41.14)

	SEd	**CD (0.01)**
Genotypes	0.003 (0.002)	0.008 (0.005)
Treatments	0.004 (0.002)	1.00 (0.007)
G x T interaction	0.008 (0.006)	0.022 (0.015)

R1=0.5 mg l^{-1} BAP+0.5 mg l^{-1} NAA +1.0 mg l^{-1} kin

R2=0.5 mg l^{-1} BAP+1.0 mg l^{-1} NAA +1.0 mg l^{-1} kin

R3=1.0 mg l^{-1} BAP+0.5 mg l^{-1} NAA +1.0 mg l^{-1} kin

R4=1.0 mg l^{-1} BAP+1.0 mg l^{-1} NAA +1.0 mg l^{-1} kin

R5=1.5 mg l^{-1} BAP+0.5 mg l^{-1} NAA +1.0 mg l^{-1} kin

R6=1.5 mg l^{-1} BAP+1.0 mg l^{-1} NAA +1.0 mg l^{-1} kin

R7=2.0 mg l^{-1} BAP+0.5 mg l^{-1}NAA +1.0 mg l^{-1} kin

R8=2.0 mg l^{-1} BAP+1.0 mg l^{-1} NAA +1.0 mg l^{-1} kin

Table 4. Effect of different concentrations of cefotaxime and carbenicillin on plant regeneration from the calli of *indica* rices* (Mean ± SE)

Concentration of cefotaxime / carbenicillin (mg^{-1})	Cefotaxime concentration (mgl^{-1})		Carbenicillin concentration (mgl^{-1})	
	No.of Shoots / Callus	Precent regeneration	No.of shoots / callus	Percent regeneration
0	6	60 ± 2.0	6	62 ± 3.0
50	3.9	52 ± 2.0	4.2	50 ± 2.0
100	3.5	50 ± 1.0	3.9	48 ± 2.0
150	3.0	15 ± 1.0	3.5	31 ± 1.0
200	1.0	9 ± 1.0	3.0	30 ± 1.0
250	0.0	0.0 ± 0.0	2.2	25 ± 1.0

* The values are average of five replicates.

Table 3. Effect of cocultivation time on GUS activity in rice genotype Pusa Basmati 1 transformed with p CAMBIA 1305.1. in the presence of 100 µM AS

Duration of Co cultivation (days)	Percentage of Calluses GUS ± *		
	Experiment I	Experiment II	Mean ± SE
1	0.0	0.0	0.0
2	18.0	14.0	16.0 ± 2.0
3	61.0	45.0	53.0 ± 4.0
4	44.0	36.0	40.0 ± 4.0
5	29.0	35.0	32.0 ± 2.0

- GUS assay was performed on 50 calluses per treatment.

Table 5. Effect of callus age on GUS activity of rice cultivar Pusa Basmati 1 after transformation with p CAMBIA 1305.1 using 100 µM AS in the co cultivation medium

S.No	Age of Callus (days)	Percentage of Calluses GUS ± *		
		Experiment I	Experiment II	Mean ± SE
1.	21	29.0	35.0	32.0 ± 4.0
2.	42	44.0	36.0	40.0 ± 4.0
3.	63	23.0	17.0	20.0 ± 2.0
4.	84	14.0	8.0	11.0 ± 2.0

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