Agrobacterium-mediated high frequency transformation in dwarf recalcitrant rice cultivars

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Abstract

The *Agrobacterium*-mediated transformation was done in rice (*Oryza sativa* L. var. *indica*) cv. HKR126 and elite cross-bred cv. Pusa Basmati1 (PB1), using strain LBA4404 containing pCAMBIA1300 cloned with gene cassettes; potato proteinase inhibitor and *Bacillus thuringiensis* endotoxin (plasmid JDW53) or mannitol-1-phosphate dehydrogenase (plasmid RKJ108). Co-cultivation with scutellar-calli derived from mature seeds showed stable and highly efficient transformation. In cvs. HKR126 and PB1, 35 % and 41 % of hygromycin resistant calli were obtained. The transformation efficiency in PB1 (22.0 %) was much higher than in HKR126 (12.5 %). Similarly, PB1 had higher plant regeneration efficiency than HKR126. The shoots regenerated per callus were, 3 - 4 in HKR126 and 5 - 6 in PB1. The transformation efficiency with pRKJ108 (18.6 %) was higher than pJDW53 (15.9 %). Polymerase chain reaction (PCR) analysis showed the presence of transgenes in regenerated transgenic plants of both cultivars.

Additional key words: *Bacillus thuringiensis* endotoxin, hygromycin, kinetin, napthaleneacetic acid, PCR, potato proteinase inhibitor.

Introduction

Biotic and abiotic stresses are major constraints for rice production. It is estimated that biotic stress causes annual rice yield loss up to 40 % (Oerke and Dehne 2004). Current strategies aimed at reducing losses incurred by insect-pest rely primarily on chemical pesticides. Alternatively, the use of gene transfer technology to introduce insect resistance genes into crop plants provides an economical and environmentally sustainable substitute to pesticides (Giri and Laxmi 2000, Tu *et al*. 2000, Datta *et al*. 2002). Transgenic rice expressing individual *Bacillus thuringiensis* endotoxin genes (Tu *et al*. 2000, Khanna and Raina 2002, Wu *et al*. 2002) and genes encoding protease inhibitors (Duan *et al*. 1996) and lectins (Rao *et al*. 1998) have shown resistance against various insects. The problem that could arise from extensive use of insect resistance transgenic plants is the evolution of tolerance in different pest populations (Ballester *et al*. 1994, Tabashnik *et al*. 2000). However, agronomical practices for pest management and simultaneous introduction of more than one insect resistant gene that has different mode of action against the same pest could be applied to restrict the evolution of resistance in pests (Ruud *et al*. 1999).

Abiotic stress such as drought and salinity restricts rice production primarily in non-irrigated areas (Datta 2002). Various strategies to combat with abiotic stress include transfer of genes that synthesize osmoprotectants, stress protein (*e.g*. late embryogenesis abundant protein), ion transporters, signalling and control of transcription factors (Bajaj *et al*. 1999). Osmotic adjustment is an effective parameter for drought and salinity tolerance in crop plants, including rice (Datta 2002). Ectopic expression or overexpression of several genes for regulation of osmoprotectants such as proline (Zhu *et al*.

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Abbreviations: CH - casein hydrolysate; *cry1Ab* - *Bacillus thuringiensis* endotoxin; 2,4-D - 2,4-dichlorophenoxyacetic acid; *hpt* - hygromycin phosphotransferase; hyg^R - hygromycin resistant; KIN - kinetin; MS medium - Murashige and Skoog's medium; NAA - 1-napthaleneacetic acid; *pinII* - potato proteinase inhibitor; Tp-*mtlD* - transit peptide linked mannitol-1-phosphate dehydrogenase.
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1998), trehalose (Garg *et al*. 2002) and glycine-betaine (Mohanty *et al*. 2002) increased osmotolerance in rice.

Developing rice cultivars with traits governing resistance to insect-pest and/or enhanced abiotic stress tolerance would undoubtedly have an enormous impact on increasing global food production. *Indica* and *japonica* are the two major varieties of rice growing in different regions of the world. *Indica* rice alone accounts for approximately 80 % of the cultivated rice. However, major achievement in rice transformation has been obtained in *japonica rice*, largely due to better response to *in vitro* culture (Hiei *et al*. 1994, Visarada *et al*. 2002, Visarada and Sarma 2004, Lin and Zhang 2005). Transformation in *indica* rice cultivars remains difficult (Mohanty *et al*. 2002, Martinez-Trujillo *et al*. 2003, Lin and Zhang 2005). Recalcitrant nature of this sub-species has been a major limiting factor for successful transfer of available useful genes (Khanna and Raina 2002). Even in previous studies, in which transformation succeeded in *indica* rice, the results showed low transformation efficiency (Aldemita and Hodges 1996, Rashid *et al*. 1996).

In this study, we have targeted to increase the transformation efficiency in high yielding dwarf *indica* rice cultivars. Here we reports the efficient and successful *Agrobacterium*-mediated transformation in cv. HKR126 and cross-bred PB1 (*indica* × Basmati) with genes responsible for resistance against insect-pest; *Bacillus thuringiensis* endotoxin (*cry1Ab*) and potato proteinase inhibitor (*pinII*) and gene providing enhanced osmo-tolerance; mannitol-1-phosphate dehydrogenase (*mtlD*).

Materials and methods

Plant material and callus induction: Dehusked rice (*Oryza sativa* L.) seeds of 9 cultivars (var. *indica* pure lines IR72 and HKR126, Basmati pure lines Dehradun Basmati, Basmati370 and HBC19, cross-breds *indica* × basmati Pusa Basmati1, Super Basmati and HKR228 and var. *japonica* cv. Taipei309) were surface sterilized for 2 min with 70 % ethanol, 10 min in 0.1 % mercuric chloride having a drop of *Tween-20* and washed several times with sterile distilled water. Sterile seeds were cultured on callus induction medium and incubated in dark. Proliferated scutellar region was sub-cultured on to fresh callus induction medium for 3 weeks. Cultures were kept at 25 ± 2 °C under dark or 16-h photoperiod with irradiance of 140 μmol m^{-2} s^{-1}. The media used for tissue culture and transformation studies are given in Table 1.

***Agrobacterium* strains and transformation vectors:** *Agrobacterium* strain LBA4404 containing pCAMBIA1300 was used for transformation experiments. Plasmid CAMBIA1300 is a binary vector (obtained from the Center for the Application of Molecular Biology to International Agriculture, Canberra, Australia) and was cloned with *pinII* and *cry1Ab* (plasmid JDW53) or Tp-*mtlD* (plasmid RKJ108) gene cassettes in T-DNA region. Plasmids were generously provided by Prof. Ray Wu, Cornell University, Ithaca, NY, USA. The *pinII* and *cry1Ab* genes were driven by potato proteinase inhibitor II and maize ubiquitin promoters, respectively (Fig. 1*A*). In Tp-*mtlD,* Tp refers to rice rbc (small subunit gene of ribulose bisphosphate carboxylase/oxygenase) transit peptide ligated to *mtlD* gene for the targeting of gene product into chloroplast. Tp-*mtlD* was driven by an ABA (abcisic acid) inducible promoter comprised of rice actin1 and ABRC with an HVA22 intron (Fig. 1*B*). Tp-*mtlD* gene was flanked by MAR (matric attachment regions) sequences. Both the plasmids had *hpt* gene

Table 1. Media used in plant tissue culture and *Agrobacterium* transformation. All media were prepared in MS basal medium (Murashige and Skoog 1962). CH - casein hydrolysate, 2,4-D - 2,4-dichlorophenoxyacetic acid; KIN - kinetin; NAA - 1-naptha-leneacetic acid

Culture medium	Additives
Callusing medium	2.5 mg dm^{-3} 2,4-D, 560 mg dm^{-3} proline, 300 mg dm^{-3} casein hydrolysate (CH), 30 g dm^{-3} maltose, 8 g dm^{-3} phytagel, pH 5.8
Co-cultivation medium-I	100 μM acetosyringone, 560 mg dm^{-3} proline, 300 mg dm^{-3} CH, 68.5 g dm^{-3} maltose, 36 g dm^{-3} glucose, pH 5.2
Co-cultivation medium-II	100 μM acetosyringone, 560 mg dm^{-3} proline, 300 mg dm^{-3} CH, 30 g dm^{-3} maltose, and 10 g dm^{-3} glucose, 8 g dm^{-3} phytagel, pH 5.2
Selection medium	2.5 mg dm^{-3} 2,4-D, 560 mg dm^{-3} proline, 300 mg dm^{-3} CH, 30 g dm^{-3} maltose, 8 g dm^{-3} phytagel, pH 5.8
Regeneration medium-I (RM-I)	560 mg dm^{-3} proline, 300 mg dm^{-3} CH, 30 g dm^{-3} maltose, 10 g dm^{-3} agarose, 0.5 mg dm^{-3} NAA, 2.0 mg dm^{-3} KIN, pH 5.8
Regeneration medium-II (RM-II)	560 mg dm^{-3} proline, 300 mg dm^{-3} CH, 30 g dm^{-3} maltose, 5 g dm^{-3} agarose, 0.5 mg dm^{-3} NAA, pH 5.8

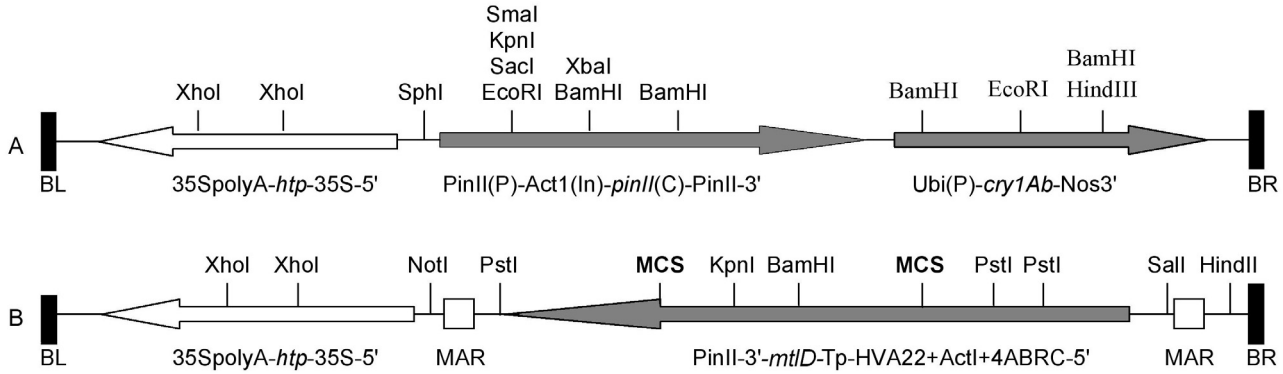

Fig. 1. Gene cassettes cloned in T-DNA region of binary vector pCAMBIA1300 with different restriction sites. *A* - JDW53 plasmid having *pinII* and *cry1Ab* gene. BL - left border, BR - right border, 35S-5' - CaMV35S promoter, *hpt* - hygromycin phosphotransferase, 35SpolyA - termination signal of CaMV, PinII(P) - potato proteinase inhibitor promoter, Act1 - rice actin1 intron, *pinII(C)* - coding region of *pinII*, PinII-3' - termination signal of Pin2, Ubi(P) - ubiquitin promoter, *cry1Ab* - synthetic insecticidal protein gene from *B. thuringiensis*, Nos3'- termination signal of nopaline synthase. *B* - RKJ108 plasmid having *mtlID* gene. MAR - matrix attachment regions, MCS - multiple cloning site, 4ABRC+Act1+HVA22 - abscisic acid inducible promoter comprised of rice actin1 and *HVA22* intron, *mtlD* - mannitol-1-phosphate dehydrogense derived from *E. coli*, Tp - rice rbc (small subunit of ribulose bisphosphate carboxylase/oxygenase) transit peptide.

within T-DNA region for plant selection and kanamycin resistance gene outside the T-DNA region for bacterial selection.

***Agrobacterium* culture and co-cultivation:** Single colony of *Agrobacterium tumefaciens* strains containing plasmids JDW53 or RKJ108 were inoculated in 5 cm^3 of liquid ABG medium (Chilton *et al*. 1974) containing kanamycin (50 mg dm^{-3}) and tetracyclin (5 mg dm^{-3}) and were grown overnight on a rotary shaker (10 g) at 28 °C. The culture (0.5 - 1.0 cm^3) was then inoculated in 50 cm^3 and 100 cm^3 of ABG medium containing same antibiotics on rotary shaker (10 g) for 2 d to raise actively proliferating secondary culture of *Agrobacterium*. The cultures were centrifuged at 3 000 *g* for 10 min at room temperature and resuspended in liquid co-cultivation medium-I (Table 1), adjusted to the absorbance of 1.0 (600 nm). The calli were inoculated by immersing in bacterial suspension for 10 min with gentle shaking. The *Agrobacterium* infected calli were dried on sterile filter paper and cultured on co-cultivation medium-II (Table 1). Co-cultivation was carried out in dark at 28 °C for 3 d.

Selection of transformants and regeneration: The *Agrobacterium* infected calli were thoroughly washed 5 - 6 times with sterile distilled water containing cefotaxime 250 mg dm^{-3} and blotted on filter paper. The calli were cultured on to selection medium containing hygromycin (50 mg dm^{-3}) and cefotaxime (250 mg dm^{-3}) for 4 - 6 weeks, maintained in dark for proliferation of transformed colonies. The calli were sub-cultured on fresh selection medium at 2 weeks interval. The hyg^R calli were transferred to regeneration medium (RM-I, Table 1) having hygromycin (50 mg dm^{-3}) and cefotaxime (250 mg dm^{-3}). These calli were incubated in dark for 1 week and then transferred to light for 3 weeks. Plantlets regenerated were sub-cultured into glass bottles containing regeneration medium-II (RM-II) for multiple shoots and root formation (Table 1). Remaining calli were kept on fresh RM-I for another 2 weeks to observe further regeneration. The transgenic plants were transferred to sterilized sand and peat mixture (50:50) in pots and grown in greenhouse, maintained at 35 ± 2 °C day and 25 ± 2 °C night temperatures.

DNA isolation and PCR analysis: The genomic DNA was isolated by using modified CTAB method according to Saghai-Maroof *et al*. (1994), from leaf tissues of putative transgenic plants. The genes *mtlD*, *pinII*, *cry1Ab* and *hpt* were amplified on genomic DNA using PTC-100^{TM} thermocycler (*MJ Research, Inc.*, Waltham, MA, USA). The plasmid DNA was isolated from *Agrobacterium* strains using a rapid miniprep method according to Li *et al*. (1995). The plasmid DNA amplified with respective primers was used as positive control. The PCR analysis was carried out in 50 µl reaction mixture with the following cycles: 30 s at 94 °C, 45 s at 62 °C and 45 s at 72 °C (40 cycles) with final extension of 5 min at 72 °C for amplification of *mtlD*, *pinII* and *rice actinI* genes. Whereas, for *cry1Ab* and *hpt* genes, the following cycles were used: 30 s at 94 °C, 45 s at 55 °C and 45 s at 72 °C (40 cycles) and final extension of 5 min 72 °C. The PCR products were separated on 1.5 % agarose gel stained with ethidium bromide.

The sequences of primers (5'-3') used were: *hpt* forward-CTACATAGCCATCGGTCCAGA, reverse-CGCAAGGAATCGGTCAATACA; *rice actin1* forward-AGATCAGGAAGAGGGGAAAAGGGCA, reverse-GCATTCGGTAAACGAAACCACCCTG; *mtl* forward-GTTTCCGGCGTCAATGCTGTCA, reverse-

CACCTGCGGATTTTCTCGTC; *pinII* forward-GATTCAGCGGGCCCGAGTTTAC, reverse-GATCAACATCCATCGCGCTTACAA; *cry1Ab* forward-GATTCAGCGGGCCCGAGTTTAC, reverse-ATGGGATTTGGGTGATTTGAGAGG.

Results

The callus induction and shoot regeneration was checked in 8 *indica* cultivars and a *japonica* cv. Taipei309. The callus induction was > 80 % in all the cultivars, with highest in Taipei309. The shoot regeneration frequency in cross-bred improved *indica* cultivars was significantly higher than either of pure lines of *indica* or basmati (Table 2). The Taipei309 is highly responsive to tissue culture (Visarada and Sarma 2002) and was taken as positive control and has shown best results for regeneration. For further transformation experiments we have selected two cultivars; HKR126 and PB1. The germination occurred at the same time in both the cultivars; 72 h after transferring on callusing medium (Fig. 2*A*,*B*). The HKR126 had delayed callus appearance, whereas, PB1 calli were fast proliferating (Fig. 2*C*,*D*).

The scutellar calli of cultivars HKR126 and PB1

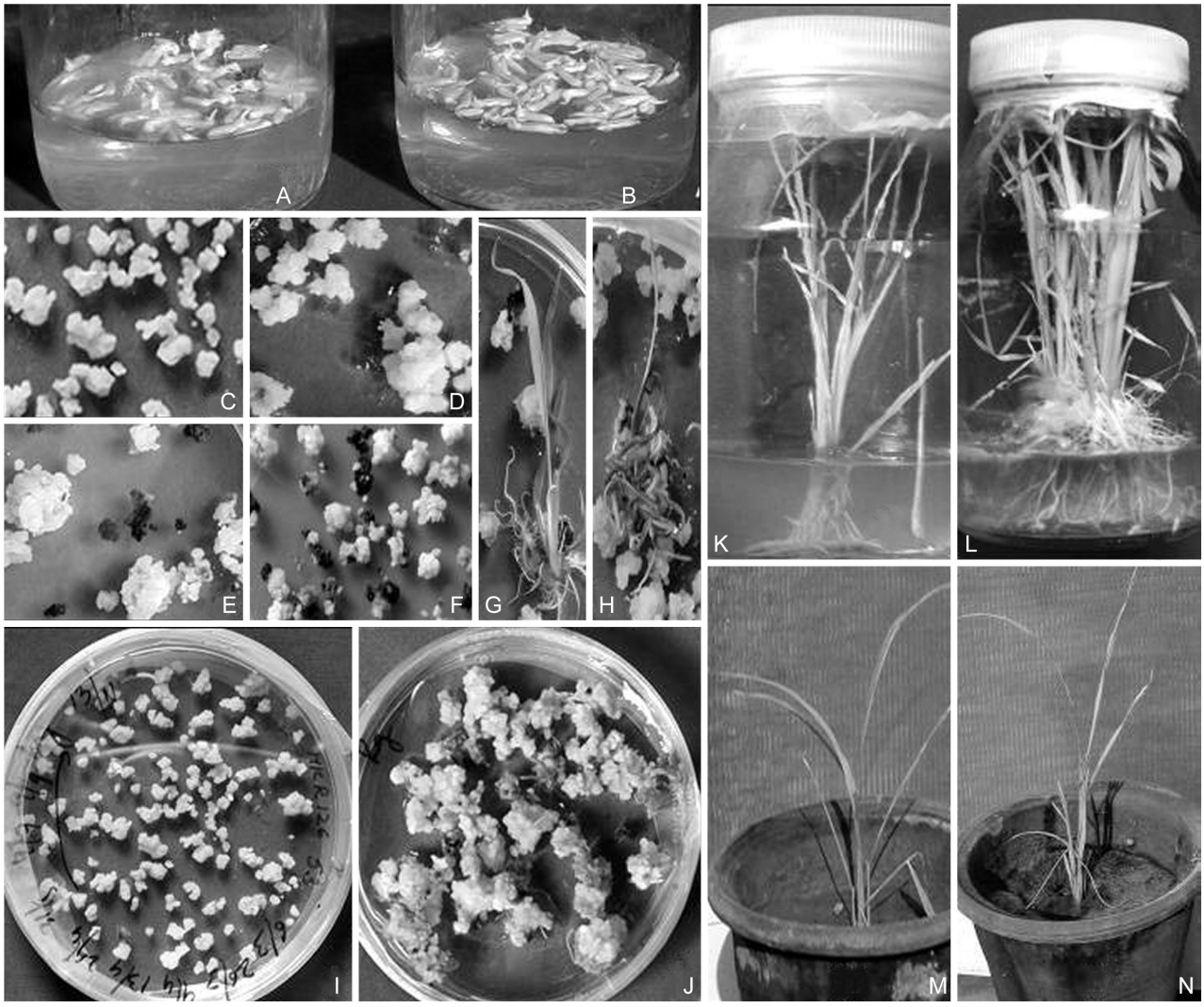

Fig. 2. *Agrobacterium*-mediated transformation stages in *indica* rice cv. HKR126 and cross-bred PB1. *A*,*B* - germinating seeds; *C*,*D* - calli proliferation; *E*,*F* - selection and proliferation of hyg^R calli, on selection medium; *G*,*H* - shoot regeneration on regeneration media-I (RM-I), regenerated shoots were transferred to RM-II; *I*,*J* -second round of regeneration on RM-I; *K*,*L* - shoot multiplication and rooting on RM-II; *M*,*N* - transgenic plants in greenhouse. On each transformation stage left photograph is showing HKR126 and right is PB1.

Table 2. Callus induction and shoot regeneration in different rice cultivars. Means of three replicates ± SE. Values in parentheses are number of shoots per regenerated callus.

Cultivar	Callus induction [%]	Shoot regeneration [%]
IR72	86.8 ± 3.8	55.8 ± 2.9 (4 - 5)
HKR126	80.2 ± 2.9	54.5 ± 2.4 (3 - 4)
Dehradun Basmati	83.7 ± 2.3	45.8 ± 2.2 (2 - 3)
Basmati370	80.8 ± 2.5	41.7 ± 3.2 (2 - 3)
HBC19	83.9 ± 2.8	39.7 ± 2.8 (2 - 3)
Pusa Basmati1	89.0 ± 3.5	71.7 ± 3.2 (5 - 6)
Super Basmati	90.5 ± 3.6	72.2 ± 2.4 (4 - 6)
HKR228	87.0 ± 2.5	68.3 ± 2.9 (4 - 5)
Taipei309	92.1 ± 3.3	81.1 ± 3.4 (5 - 6)

were co-cultivated with *Agrobacterium* containing plasmids pJDW53 and pRKJ108. The average hygromycin resistant (hyg^R) calli recovered on selection medium were 35 % and 41 % for HKR126 and PB1, respectively (Table 3). The hygromycin restricted the growth of non-transformed calli, which turned brown and the transformed calli showed active cell proliferation in both the cultivars (Fig. 2*E*,*F*). The hyg^R calli were transferred on to RM-I. Among 101 hyg^R calli (co-cultivated with pJDW53), only 34 calli (34 %) regenerated on RM-I in HKR126; whereas, the calli regeneration frequency was much higher (54 %) in PB1. Similarly, the calli regeneration frequency was 33 % and 54 % in HKR126 and PB1, respectively, when co-cultivated with pRKJ108 (Table 3). The regenerated calli on RM-I (kept for 3 - 4 weeks) showed green spots, regenerated into single shoots in HKR126; whereas, in PB1 calli produced more than one shoot (Fig. 2*G*,*H*). The shoot-regenerating calli were transferred to RM-II and the remaining calli were kept on fresh RM-I for another 2 weeks to check further regeneration potential. Most of these calli in HKR126 could not produce shoots and showed unembryogenic proliferation (Fig. 2*I*). However, in PB1, the calli had more potential showing more regenerating shoots (Fig. 2*J*).

The plantlets on RM-II medium have produced multiple shoots, 3 - 4 shoots in HKR126 and 5 - 6 shoots in PB1 (Fig. 2*K*,*L*) and initiated root formation. Total number of plants regenerated from hyg^R calli in HKR126 was 41 %, whereas, in PB1, plant regeneration was much higher (61 %, Table 3). The putative transgenic plants were shifted to greenhouse for acclimatization (Fig. 2*M*,*N*) and grown till maturity. The transformation efficiency (plants regenerated with transgenes/total calli co-cultivated) was also higher in PB1 (22.0 %) than in HKR126 (12.5 %). The transformation efficiency with pRKJ108 (18.6 %) was higher than pJDW53 (15.9 %, Table 3), by calculating mean efficiency of individual plasmid.

The presence of transgenes in putative transgenic plants was confirmed by PCR analysis. The PCR amplification of 4 genes was done separately (Fig. 3). The presence of genes *hpt*, *mtlD*, *pinII* and *cry1Ab* is shown by PCR amplified fragment of 555 bp (Fig. 3*A*), 600 bp (Fig. 3*B*), 490 bp (Fig. 3*C*) and 600 bp (Fig. 3*D*), respectively. *Rice actinI* gene was amplified as internal control in all the plants showing gene fragment of 304 bp (Fig. 3*B*,*C*).

Table 3. *Agrobacterium*-mediated transformation of 300 scutellar calli using LBA4404 with pCAMBIA1300 harboring *pinII*+*cry1Ab* (pJDW53) and *mtlD* (pRKJ108) gene cassettes showing hyg^R calli and plant regeneration in *indica* rice cultivars HKR126 and PB1. hyg^R - hygromycin resistant; SM - selection medium; RM - regeneration medium.

Cultivar	Plasmid	hyg^R calli on SM	Calli regenerated on RM-I	Regenerated plants	Plants with transgene	Transformation efficiency [%]
HKR126	JDW53	101	34	42	33	11.0
	RKJ108	114	38	47	42	14.0
PB1	JDW53	116	63	73	62	20.7
	RKJ108	130	70	77	70	23.3

Discussion

The aim of present study was to optimize *Agrobacterium*-mediated transformation efficiency in *indica* rice cvs. HKR126 and *indica* × Basmati cross-bred; PB1. For developing insect resistant transgenic plants *cry1Ab* and *pinII* and for osmotolerance Tp-*mtlD* genes were used. The expression of *cry1Ab* and *pinII*, derived from *Bt* and potato, respectively, has previously shown resistance against stem borers and sap-sucking insects in rice (Ramesh *et al*. 2004, Duan *et al*. 1996). Transgenic wheat plants with *mtlD* gene has been reported to accumulate mannitol for osmotic and salinity stress tolerance (Abebe *et al*. 2003).

The regeneration experiment for various cultivars indicated that elite cross-bred had higher regeneration potential than pure *indica* or basmati lines (Table 2). The shoot regeneration frequency with ordinary *in vitro*

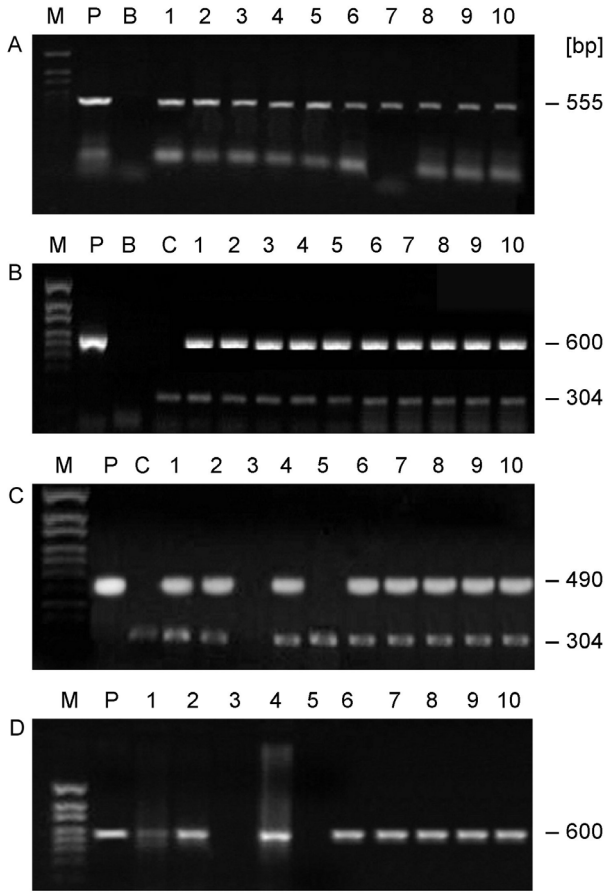

Fig. 3. Genomic DNA amplification by PCR of transformed plants of rice cultivars HKR126 and PB1. *A* - *hpt* gene showing fragment of 555 bp; *B* - *mtlD* gene (600 bp) and *rice rctin1* gene (304 bp); *C* - *pinII* (490 bp) and *rice actin1* gene (304 bp); *D* - *cry1Ab* gene (600 bp). *Lane M* - 100 bp DNA ladder; *lane P* - plasmid DNA as a positive control; *lane B* - water as a negative control; *lane C* - genomic DNA from non-transformed plants; *lanes 1* to *5* - amplified genomic DNA of transformed HKR126 plants; *lanes 6* to *10* - amplified genomic DNA of transformed PB1 plants.

culture (Table 2) was higher than tissue culture for transformation (Table 3), because the co-cultivated calli in transformation has to undergo stringent selection on antibiotic media (Lin and Zhang 2005). The transformation efficiency directly depends on the explant used and regeneration ability after the *Agrobacterium* infection. The genotypic differences play an important role in determining the regeneration efficiency (Abe and Futsuhara 1986, Lin and Zhang 2005). In *japonica* rice, widely accepted protocols for *in vitro* culture and regeneration have been developed (Hiei *et al*. 1994), whereas, in *indica* rice standardized protocol are still lacking and are largely genotype-dependent (Lin and Zhang 2005). To achieve high efficiency transformation, a highly competent and robust tissue culture technique is required. The protocol used in the present study and mentioned elsewhere in our previous work (Verma *et al*. 2002) has given higher number of plant regeneration.

Maltose was used in place of sucrose as carbon source in callus induction and regeneration media (Table 1). The results presented in this experiment indicate that maltose was preferred carbon source for tissue culture of *indica* rice (Verma *et al*. 2002, Lin and Zhang 2005), may be due to maltose supports the growth of embryogenic callus and high-frequency shoot formation (Jain *et al*. 1997). In our protocol we have used two kinds of regeneration media (RM-1 and -II), having agarose in each (Table 1). Use of agarose in media preparation is also mentioned in some previous reports (Jain *et al*. 1997). However, important feature in our media was that, higher concentration of agarose (1.0 %) was used in RM-I, which facilitated better organogenesis in calli, and resulting in more regeneration per calli. The regenerating shoots were transferred to RM-II, which had lower agarose content (0.5 %) that has enhanced the multiple shoots and rooting (Fig. 2*E-J*).

The *Agrobacterium*-mediated transformation efficiency in PB1 (22.0 %) was much higher than in HKR126 (12.5 %). The difference in transformation efficiency for two cultivars could be due to differences in the embryogenic potential and morphogenic competence in rice cultivars (Ayers and Parks 1994, Potrykus *et al*. 1995) and differential sensitivity of cultivars to *Agrobacterium* infection (Lin and Zhang 2005). The cross-bred cultivars exhibits stronger heterosis than parental pure line for several agronomical traits (Bharaj *et al*. 1994, Zeng *et al*. 1997).

Strikingly, the transformation efficiency in our experiments specifically with PB1 was higher than efficiencies reported previously in various rice cultivars; Zhang *et al*. 1997 (9 - 13 %), Khanna and Raina 1999 (4.4 %), 2002 (9 %). Even the efficiency observed here with HKR126 (12.5 %) was fairly high. Previous reports have ascribed low efficiency of *indica* rice transformation to possible toxicity of antibiotics to callus growth (Rashid *et al*. 1996, Khanna and Raina 1999) and have suggested withholding use of antibiotics on regeneration media. However, in the present study pressure of hygromycin was maintained up to regeneration medium, resulted in the selective proliferation of resistant calli with transgenes. The high efficiency transformation observed here correlates that regeneration of non-transformants was restricted thereby less competition for regeneration of positive transformants. Further it is interesting that transformation efficiency with pRKJ108 (18.6 %) was higher than pJDW53 (15.9 %, Table 3). The higher efficiency of pRKJ108 to transfer the transgene into plants could be that pRKJ108 had MAR (matrix attachment regions) sequence, which decrease gene silencing and position effect and increase the level of gene expression and also increase the frequency of low copy number transformants (Holmes-Davis and Comai 1998, Allen *et al*. 2000).

Taken together our results clearly explain that, although the transformation efficiency in cross-bred PB1 was higher than pure line *indica* cv. HKR126, still HKR126 has shown considerably efficient transformation. The protocol used in this study for transformation and regeneration experiments could be successfully used in developing large population of transgenic plants in recalcitrant *indica* rice cultivars.

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Huang, B. (ed.): **Plant-Environment Interactions.** 3rd Edition. - CRC Press, Taylor and Francis Group, Boca Raton - London - New York 2006. 388 pp. ISBN 978-0-8493-3727-7.

Climate continues to change and natural resources are more and more limited due to increasing demand. Therefore, elucidation of interactions of plants with their environment is of vitally importance. This book examines some of the most important abiotic stresses limiting plant growth and productivity in their natural and managed environments. Recent progress in molecular biology and biotechnology is reflected in changes made in this third, thoroughly revised edition.

The book is introduced by the chapter focused on cellular membranes as the sites of sensing of stress and sites of regulation of plant responses. The second chapter concerning heat stress emphasizes identification of optimum temperature for each plant species before examination of heat injury and establishment of heat tolerance. Mechanisms of plant adaptation to chilling or freezing (namely the role of cold-responsive genes, COR proteins, osmolytes, and phospholipase D) are considered in the third chapter. The fourth chapter deals with short-term and long-term responses of plants to changes in irradiance with the emphasis of photoprotective mechanisms. Involvement of hormones, especially abscisic acid (ABA), in detection and signaling of water stress, and in development of drought tolerance are the main topics of chapter five. It is also shown how different ABA-deficient and ABA-insensitive mutants have recently contributed to improvement of our knowledge in this field. In close relation is the sixth chapter devoted to salinity as salt and dehydration stresses show a high degree of similarity with respect to physiological, biochemical, molecular, and genetic effects. However, in addition of osmotic effects, specific ion toxicity is evaluated. The topic of the chapter seven is waterlogging and this chapter is focused mostly on adverse effects of lowered oxygen supply. As concern possible stresses caused by insufficient or excessive mineral nutrition, only one chapter (chapter eight) deals with low phosphorus availability as a primary constraint to plant productivity. The chapter nine is an overview of the physiological effects of copper, iron, manganese, molybdenum, zinc, cobalt, nickel, vanadium, cadmium, chromium, lead, mercury and other heavy metals. The chapter ten partially repeats the previous chapters and summarizes function of roots under drought, salinity, waterlogging and soil compaction. The chapter eleven points to the different methods for determination of stress injury and stress tolerance and the last, chapter twelve, is the survey of genomic approaches to improving abiotic stress tolerance.

The readable text of each chapter is accompanied by illustrative figures and tables, and comprehensive list of references. The information presented in this book can help readers in better understanding mechanisms of abiotic stress tolerance on whole-plant, cellular, and molecular levels.

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