

10. Yamaguchi, F., Ariga, T., Yoshimura, Y. and Nakazawa, H., Anti-oxidative and anti-glycation activity of garcinol from *Garcinia indica* fruit rind. *J. Agric. Food Chem.*, 2000, **48**, 180–185.
11. Selvi, A. T., Joseph, G. S. and Jayaprakasha, G. K., Inhibition of growth and aflatoxin production in *Aspergillus flavus* by *Garcinia indica* extract and its antioxidant activity. *Food Microbiol.*, 2003, **20**, 455–460.
12. Benzie, I. F. and Strain, J. J., The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': the FRAP assay. *Anal. Biochem.*, 1996, **239**, 70–76.
13. Rice-Evans, C. and Miller, N., Total antioxidant status in plasma and body fluids. *Methods Enzymol.*, 1994, **234**, 279–293.
14. Cao, G. and Prior, R. L., Measurement of oxygen radical absorbance capacity in biological samples. *Methods Enzymol.*, 2002, **299**, 50–62.
15. Lakshmi, B., Tilak, J. C., Adhikari, S., Devasagayam, T. P. A. and Janardhanan, K. K., Inhibition of lipid peroxidation induced by γ -radiation and AAPH in rat liver and brain mitochondria by selected mushrooms. *Curr. Sci.*, 2005, **88**, 484–488.
16. Lowry, O. H., Rosebrough, N. J., Farr, L. A. and Randal, R. J., Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 1951, **193**, 265–267.
17. Devasagayam, T. P. A. and Pushpendran, C. K., Changes in ascorbate-induced lipid peroxidation of hepatic rough and smooth microsomes during postnatal development and ageing of rats. *Mech. Ageing Dev.*, 1986, **34**, 13–22.
18. Cao, G., Sofic, E. and Prior, R. L., Antioxidant capacity of tea and common vegetables. *J. Agric. Food Chem.*, 1996, **44**, 3426–3431.
19. Lachnicht, D., Brevard, P. B., Wagner, T. L. and DeMars, C. E., Dietary oxygen radical absorbance capacity as a predictor of bone mineral density. *Nutr. Res.*, 2002, **22**, 1389–1399.
20. Tilak, J. C., Banerjee, M., Hari Mohan and Devasagayam, T. P. A., Antioxidant availability of turmeric in relation to its medicinal and culinary uses. *Phytother. Res.*, 2004, **18**, 798–804.
21. Devasagayam, T. P. A., Boloor, K. K. and Ramasarma, T., Methods for estimating lipid peroxidation: Analysis of merits and demerits. *Indian J. Biochem. Biophys.*, 2003, **40**, 300–308.
22. Hertog, M. G. L., Feskens, E. J. M., Hollman, P. C. H., Katan, M. B. and Kromhout, D., Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen elderly study. *Lancet*, 1993, **342**, 1007–1011.
23. Surh, Y.-J., Cancer chemoprevention with dietary phytochemicals. *Nature Rev. Cancer*, 2003, **3**, 768–780.
24. Krishnaswamy, K., Indian functional foods: Role in prevention of cancer. *Nutr. Rev.*, 1996, **54**, S127–S131.
25. Devasagayam, T. P. A., Tilak, J. C. and Singhal, R., Functional foods in India: history and scope. In *Angiogenesis, Functional and Medicinal Foods* (eds Losso, J. N. et al.), Marcel Dekker Inc, New York (in press).

ACKNOWLEDGEMENTS. We thank Dr T. K. Dongre for suggestions and Dr K. K. Boloor and Mrs Jayashree K. Boloor for help.

Received 16 June 2005; revised accepted 8 March 2006

Enhanced genetic transformation efficiency of mungbean by use of primary leaf explants

L. Sita Mahalakshmi, T. Leela, S. Manoj Kumar, B. Kiran Kumar, B. Naresh and Prathibha Devi*

Department of Botany, Osmania University, Hyderabad 500 007, India

Transgenic mungbean plants [*Vigna radiata* (L.) Wilczek] were developed via genetic transformation of primary leaf explants with disarmed *Agrobacterium tumefaciens* strain C-58 harbouring a binary plasmid, pCAMBIA-1301 [(containing genes for β -glucuronidase (*GUS*) and hygromycin phosphotransferase (*hpt*)]. A genotype independent, high frequency plant regeneration protocol was initially developed with a survival of about 90% in two cultivars of mungbean by use of primary leaf explants (cut at the node) from four-day-old and ten-day-old seedlings. The co-cultivated explants (taken from ten-day-old seedlings of the cultivar K-851) were cultured on hygromycin selection to recover putatively transformed plants, which were acclimatized and moved to the glasshouse. The time required for regeneration of transgenic plantlets from the transformed explants was nearly 12 weeks. The transformed plants were morphologically similar to the seed-germinated plants. Transformation was confirmed by histochemical assay for *GUS* activity. Stable integration of the marker gene in the T0 transgenics and its inheritance in the T1 transgenics has been confirmed through molecular analysis. The enhanced genetic transformation efficiency obtained presently is repeatable and can be used to mobilize genes of agronomic importance into elite cultivars of mungbean.

Keywords: *Agrobacterium*, enhanced genetic transformation, mungbean, primary leaf explants.

GENE transfer to plants has opened new ways for the use of recombinant DNA technology and is useful in complementing the conventional breeding programmes^{1,2}.

Mungbean [*Vigna radiata* (L.) Wilczek] ($2n = 22$) is an important foodgrain legume crop of Asia and a good source of dietary protein. It is cultivated in India, South America and Australia. However, its production is limited due to certain undesirable agronomic traits and its susceptibility to biotic stresses like diseases caused by fungi, bacteria, viruses and insect pests. Genetic improvement of mungbean is possible by transfer of agronomically important genes through genetic transformation.

Although efficient plant regeneration and genetic transformation protocols have been developed in some legumes^{1,3–18}, lack of an efficient regeneration system has limited the improvement of mungbean via genetic trans-

*For correspondence. (e-mail: prathibhadevi@osmania.ac.in)

formation. Direct shoot regeneration from explants taken from *in vitro* germinated seedlings of several cereals and pulses^{12,13,19–21} has been found suitable for genetic transformation, since uniform explant sources can be obtained all through the year. Although genetic transformation via microprojectile gun has been reported (mungbean³, pigeonpea¹²), there are quite a few reports on genetic transformation by *Agrobacterium*-mediated gene transfer using either cotyledonary nodes or axillary bud regions of nodes in several legumes, including mungbean (*Vigna radiata*¹⁰, *Trifolium repens*⁷, *Lotus japonica*⁸, *Glycine max*¹¹, *Cajanus cajan*^{13–17} and *Vigna mungo*¹⁸).

The present report describes the development of transgenic mungbean plants through an efficient *Agrobacterium*-mediated genetic transformation method using primary leaf explants that could be induced to directly regenerate shoots via a rapid, reliable and genotype independent protocol.

To standardize the protocol for high frequency plant regeneration as a pre-requisite for genetic transformation, seeds of two cultivars of mungbean (*Vigna radiata* (L.) Wilczek), viz. K-851 and LGG-407 were obtained from National Seed Corporation Hyderabad, India and LAM Agricultural Farm, Guntur, India respectively. The seeds were surface-sterilized with 0.1% mercuric chloride for 10 min, thoroughly rinsed 4–5 times in distilled water and seed-coats removed aseptically. The seeds were germinated on B5 basal medium²² supplemented with 3% (w/v) sucrose and 0.7% (w/v) phyta-agar (taken in culture tubes) and incubated in a plant growth room with 16 h photoperiod at 25 ± 1°C temperature, 75% humidity and 60 µE m⁻² s⁻¹ light intensity, to obtain sterile seedlings for harvest of primary leaf explants.

The primary leaf explants (cut at the node) were taken from four-day-old and ten-day-old seedlings. Based on certain preliminary experiments, only explants bearing the basal half of the lamina were used for the study, where the B5 culture medium with various concentrations of benzyladenine (BA), viz. 0.1, 0.5 and 1.0 mg/l was used to find the most suitable concentration for shoot induction (shoot induction medium, SIM). The regenerated shoots (2 cm long) could be efficiently rooted on a root-inducing medium (RIM) comprising B5 medium with 0.5 mg/l indolebutyric acid (IBA). The rooted plantlets were transferred (for hardening) to pots containing a 1:1 mixture of sand and soil and acclimatized for 1 week (by covering with a plastic bag and gradually exposing the plant to ambient environment) prior to transfer to the glasshouse. These experiments were carried out in three replicates (100 explants/replicate) and the results statistically analysed.

Agrobacterium-mediated genetic transformation of the mungbean cultivar K-851 was carried out using the primary leaf explants (from ten-day-old seedlings) for production of transgenics. A disarmed *Agrobacterium tumefaciens* strain C-58 harbouring a binary plasmid pCAMBIA-1301 was used (Figure 1). The plasmid contained the β-glu-

curonidase reporter gene (*GUS*)²³ from *Escherichia coli* with an intron, driven by the cauliflower mosaic virus (CaMV) 35 S promoter and *nos* poly-A terminator sequences and the selectable marker gene hygromycin phosphotransferase gene (*hpt*) under the control of CaMV 35 S promoter and CaMV 35 S poly-A terminator. Bacteria were maintained on LB agar plates (1% w/v tryptone, 0.5% w/v yeast extract and 1% w/v sodium chloride, pH 7.0) with 50 µg/l kanamycin sulphate.

For co-cultivation and transformation, a single bacterial colony was inoculated into 25 ml of liquid LB medium containing 50 mg/l kanamycin sulphate and incubated at 28°C on a shaker at 100 rpm for 16–18 h and used in the late log phase A₆₀₀ at 0.6. The bacterial culture was centrifuged at 5000 rpm and half-strength B5 liquid medium added to the bacterial pellet to make up a volume of 25 ml. Freshly cut explants were dipped in this suspension for 5 min, blotted on sterile filter paper and transferred to the previously standardized SIM. Twenty explants per petri plate were co-cultivated for 48 h and a total of 200 explants were used with three replicates. They were then transferred to SIM containing 200 mg/l cefotaxime (SIM-Cef) for a week to eliminate the bacteria and also to allow the explants to recover.

The explants were then transferred to hygromycin selection medium SIM-Sel-1 and later to SIM-Sel-2 (SIM with 2 and 5 mg/l hygromycin respectively) to apply selection pressure for recovery of regenerated transformed shoots by four weeks. The putatively transformed shoots were then transferred (for hardening) to RIM for rooting and subsequently transferred to pots and moved to the glasshouse after acclimatization (as explained in the plant regenera-

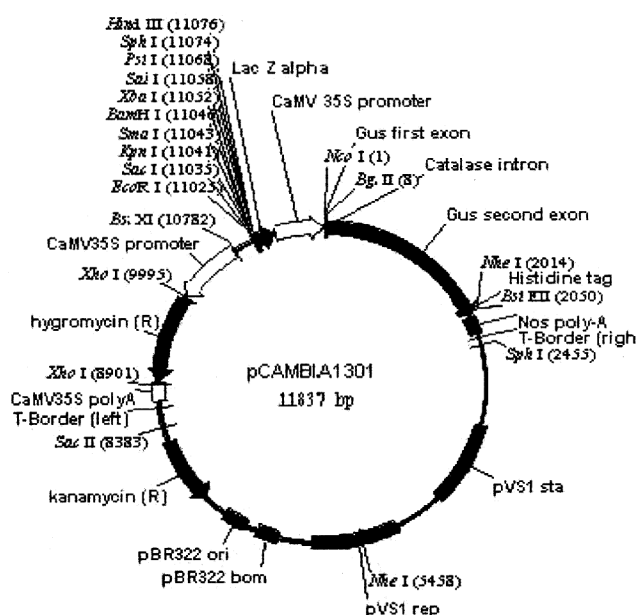


Figure 1. Restriction map of the plasmid pCAMBIA 1301.

tion protocol above). Explants were cultured without co-cultivation to regenerate untransformed control plants.

Histochemical *GUS* assay was carried out on two different occasions on the co-cultivated explants, viz. on the third day after co-cultivation to study the transient expression and three weeks after co-cultivation on explants bearing shoots and also on control explants, using a modified method of Jefferson *et al.*²³. The modified histochemical assay buffer consisted of 100 mM NaPO₄ buffer, 100 mM Na₂ EDTA and 50 mM K₄Fe (CN)₆·3H₂O and 0.1% Triton X-100 (pH 7.0). 5-Bromo-4-chloro-3-indolyl-β-D-glucuronic acid (X-gluc; Clontech Laboratories, Palo Alto, CA, USA) was dissolved in 50% (v/v) ethanol, stored at -20°C and added to the buffer to a final concentration of 0.5 mg/ml prior to the assay.

Apart from the preliminary *GUS* assay, which was carried out on the third day after co-cultivation to study the transient expression for initial confirmation, *GUS* analysis was carried out three weeks after co-cultivation. To rule out the possibility of *Agrobacterium* contamination, the co-cultivated explants (two weeks after co-cultivation) were cultured on antibiotic-free medium for one week before analysis for *GUS* activity to ascertain that the *Agrobacterium* did not appear on the culture medium. The putatively transformed material and controls were subjected to histochemical *GUS* assay by scoring one petri plate (containing 20 explants with shoots) from each replicate (out of a total of three replicates). For *GUS* assay, the material was immersed in *GUS* substrate mixture immediately followed by vacuum treatment for 10 min and incubated at 37°C. Histochemical localization of *GUS* activity was examined under a Zeiss SV8 stereomicroscope. Chlorophyll was extracted from the material by successive incubation in 70% (v/v) ethanol for 2 h and 100% ethanol overnight to facilitate better examination. The data from the experiments were evaluated as number of *GUS*-positive explants having at least one blue spot in 20 co-cultivated explants per replicate.

For molecular studies to confirm the integration of foreign genes in the putatively transgenic plants, genomic DNA

was isolated from the putatively transgenic plants and untransformed control plants by a modified method of Rogers and Bendich²⁴.

Polymerase chain reaction (PCR) analysis was carried out on the T0 putative transgenics and the T1 transgenics for amplification of the coding region of the *hpt* gene. One microgram of RNAase-treated DNA was used as template for PCR amplification. Each PCR reaction was performed in 25 µl (final volume) of reaction mixture consisting of 2.5 µl of 10X PCR amplification buffer, 2 µl of template DNA, 0.5 µl of 10 mM dNTPs, 0.75 µl of 50 mM MgCl₂, 100 ng (0.5 µl) of each primer, 10.5 µl of sterile distilled water, 7.5 µl of enhancer (Invitrogen) and 1 unit (0.25 µl) of Platinum Taq DNA polymerase (Invitrogen). The following primers were used to amplify the 819 bp fragment of the *hpt* gene – Forward primer: 5'-CGT TAT GTT TAT CGG CAC TTTG-3'; Reverse primer: 5'-GGG GCG TCG GTT TCC ACT ATCG-3'. The samples were heated to 94°C for 4 min and then subjected to 34 cycles of 1 min at 93°C, 1 min at 58.5°C and 90 s at 72°C followed by another 5 min final extension at 72°C. The amplified products were assayed by electrophoresis on 1.5% agarose gels, visualized and photographed with ethidium bromide under ultraviolet light.

Well-established T0 transformants were subjected to Southern blot hybridization analysis. Ten microgram of genomic DNA from the putatively transformed and untransformed control plants was digested with *Xba*I, which recognizes a unique site within the pCAMBIA 1301 plasmid DNA. The digested DNA was separated by electrophoresis through a 0.8% agarose gel and transferred onto Nylon N+ membrane (Amersham), according to the manufacturer's instructions. The blot was probed with a non-radioactively labelled (Alkphos Direct Labeling and Detection System of Amersham Biosciences, Uppsala, Sweden) 819-bp PCR-amplified *hpt* gene fragment. For positive control, the plasmid pCAMBIA-1301 was restricted with *Xho*I to release the *hpt* gene. The blot was exposed to X-Omat film (Kodak) for 15 min for autoradiography.

Table 1. Effect of different concentrations of BA on relative frequency of plantlet regeneration via multiple shoot differentiation in two cultivars of mungbean

Cultivar	Explant	Frequency of multiple shoot development on B5 medium containing different concentrations of BA (mg/l)*				Frequency of rooting on B5 + IBA 0.5 (mg/l)**	Percentage survival***
		0.0	0.2	0.5	1.0		
K-851	Primary leaf from four-day-old seedlings	Nil	63.6 ± 0.8	77.6 ± 1.4	87.0 ± 0.5	84.6 ± 0.5	87.0 ± 0.5
	Primary leaf from ten-day-old seedlings	Nil	67.0 ± 1.1	82.0 ± 1.1	95.3 ± 0.8	89.1 ± 0.6	92.3 ± 0.8
LGG-407	Primary leaf from four-day-old seedlings	Nil	62.6 ± 1.4	74.0 ± 1.1	84.0 ± 0.5	82.0 ± 1.1	84.3 ± 1.4
	Primary leaf from ten-day-old seedlings	Nil	66.0 ± 1.2	75.3 ± 1.2	95.0 ± 0.5	87.3 ± 0.5	89.6 ± 0.6

*Percentage of total cultured explants producing multiple shoots two weeks after inoculation presented as mean ± SE of three replicates.

**Percentage rooting and acclimatization of total cultured plantlets presented as mean ± SE of three replicates.

***Percentage survival of total transplanted plantlets presented as mean ± SE of three replicates.



Figure 2 a-h. *In vitro* plant regeneration and *Agrobacterium*-mediated genetic transformation of mungbean: **a** and **b**, Shoot regeneration from primary leaf explants; **c**, Explants co-cultivated with *Agrobacterium* strain; **d**, Regeneration of healthy shoots on selection medium containing hygromycin; **e**, Histochemical assay of co-cultivated explants with blue sectors; **f**, Regenerated shoot; **g**, Rooted shoot; **h**, Regenerated transformed plantlet.

Table 2. Histochemical *GUS* assay of putatively transformed mungbean explants (of cultivar K-851) three weeks after co-cultivation

Exp. no	No. of co-cultivated primary leaf explants	No. of primary leaf explants with blue spots	Transformation frequency (%) (<i>GUS</i>)
1	20	13	65
2	20	15	75
3	20	14	70

In the present study an efficient, rapid, reliable, reproducible, genotype-independent *in vitro* plant regeneration protocol was developed from primary leaf explants (cut at the node) of four-day-old and ten-day-old seedlings of two cultivars of mungbean (Table 1). In both cultivars, the primary leaf explants of ten-day-old seedlings showed higher response compared to those of four-day-old seedlings. However, the highest response in both the cultivars was recorded with 1 mg/l BA. About 6–8 multiple shoots developed in two weeks from each explant (Figure 2a and b). Efficient rooting of the regenerated shoots was achieved with about 90% survival (Table 1). The SIM containing 1.0 mg/l BA was the most suitable and was hence used for the culture of explants after transformation.

Healthy multiple shoots could be recovered from the co-cultivated primary leaf explants cultured on selection medium (containing hygromycin; Figure 2c and d).

Successful transformation was confirmed by *GUS* assay, which was found to be an easy and reliable way of establishing optimal conditions for transformation. The histo-

chemical evaluation revealed intense blue sectors in sub-epidermal tissue of the shoots regenerated after co-cultivation with *Agrobacterium* (Figure 2e). Strongest *GUS* expression was concentrated in one area with unidirectional distribution of the blue precipitate. Untransformed (control) shoots did not reveal any blue cells after histochemical staining (not shown here). Although regeneration in mungbean was previously reported from cotyledonary node and stem-tip explants⁴ through organogenesis and somatic embryogenesis¹⁷, the protocols were not favourable for genetic transformation, because of the low regeneration frequency and the long time taken for regeneration compared to the present report. The 70% transformation efficiency (average) obtained presently through *GUS* assay (Table 2) agrees with an earlier report¹⁰. This indicates that the transformed nodal meristem cells that produced β -glucuronidase kept their potential to undergo anticlinal divisions. Stringent hygromycin selection was applied presently to ensure the recovery of transformants with minimum escapes. While the delayed application of selective

Table 3. PCR analysis (and mean transformation frequency) for amplification of the 819 bp fragment of *hpt* gene and Southern blot hybridization in putatively transformed mungbean plants (of cultivar K-851)

Exp. no.	No. of co-cultivated primary leaf explants	No. of surviving shoots after four weeks on hygromycin selection	No. of rooted putative T0 transgenic plants	No. of putative T0 transgenic plants in glasshouse	No. of plants positive for PCR	No. of plants positive for PCR/ total number of co-cultivated explants (mean transformation frequency)	No. of plants positive for Southern hybridization/ total plants (PCR-positive plants) tested
1	200	130	96	10	5	5/200	6/12
2	200	110	84	8	4	4/200	—
3	200	98	86	6	3	3/200	—
Total	600	338	266	24	12	12/600	6/12
Mean transformation frequency = 2%							

Table 4. Inheritance of *hpt* gene in T1 generation of transgenic mungbean

Plant no.*	No. of T1 plants tested	PCR analysis of <i>hpt</i> gene		Segregation χ^2	Probability
		Positive plants	Negative plants		
P-2	5 (P-2-1, P-2-2, P-2-3, P-2-4 and P-2-5)	1 (P-2-2)	4 (P-2-1, P-2-3, P-2-4 and P-2-5)	8.06	0.0045
P-5	5 (P-5-1, P-5-2, P-5-3, P-5-4 and P-5-5)	3 (P-5-2, P-5-4 and P-5-5)	2 (P-5-1 and P-5-3)	0.60	0.43

*P-2 and P-5 are independent T0 plants and seeds of five replicates were taken from the above independent transgenic plants for inheritance studies in T1 generation. All calculated values are lesser than the tabulated value of $\chi^2 = 3.84$ at 1 degree of freedom and 0.05 probability.

agent leads to excessive escape¹³, an early selection pressure adversely affects shoot regeneration¹⁶. We however found a delay period of one week necessary for efficient recovery of transgenics, which became evident after molecular analysis.

The shoots regenerated on hygromycin selection were rooted on RIM to produce about 266 putatively transgenic plants (Figure 2*f-h*; Table 3). However, only 24 putative T0 transformants were successfully acclimatized and transplanted to pots and moved to the glasshouse. They produced normal flowers and pods with viable seeds. The seeds were collected to raise the T1 generation. Two of the T0 plants (selected after molecular analysis and explained below) were advanced to the T1 generation by sowing five seeds harvested from each plant.

Molecular analysis of putative T0 transformants was carried out by PCR (for amplification of the 819 bp fragment of *hpt* gene) and Southern blot hybridization (for the confirmation of *hpt* gene integration). Molecular analysis of the T1 plants was carried out by PCR to study the inheritance of the integrated *hpt* transgene.

Molecular analysis established the transgenic status of the T0 and T1 transformants. Out of the 24 T0 transformants (produced from 600 explants), only 12 plants (P-2, P-3, P-5, P-6, P-7, P-9, P-10, P-16, P-19, P-21, P-22 and P-24) were positive for PCR (Figure 3*a*) with a mean transformation frequency of 2% (Table 3). Further, transgene integration pattern in the nuclear genome of the putative transformed T0 plants was confirmed through Southern hybridization of the genomic DNA. Southern hybridization of

the twelve PCR-positive T0 transformants was carried out and the *hpt* gene hybridization signals were found in six plants, out of which a single copy of the gene was observed in four plants (P-5, P-7, P-22 and P-24) and two copies of the gene were observed in two plants (P-2 and P-10; Figure 3*b*). The 1.1 kb hybridization signal was observed in case of the positive control (the plasmid pCambia 1301 restricted with *Xho*I to release the *hpt* gene), but no hybridization signal was observed in case of the untransformed plant DNA (negative control).

Inheritance of the *hpt* gene in the ten T1 putative transformants (progeny of P-2 and P-5) was analysed by PCR. However, out of the ten plants, four (P-2-2, P-5-2, P-5-4 and P-5-5) tested positive for PCR (Figure 3*c*), indicating the presence of the *hpt* gene and thereby inheritance of the gene from T0 to T1 generation (Table 4).

The main focus of transgenic research is on finding cells (explant tissue) that are competent for both transformation and regeneration, choice of medium, selection criterion, *Agrobacterium* strain, co-cultivation and selection parameters^{9,11}.

Our results show that by fine-tuning the conditions of transformation, even a recalcitrant crop like mungbean can be transformed with an optimum frequency. An earlier study¹⁰ on *Agrobacterium*-mediated transformation of mungbean utilized cotyledonary node explant to achieve direct regeneration and primary leaf explant to achieve indirect regeneration through callus. It reports the development of only a few plantlets of T0 generation with the effective frequency of transformed shoots of less than

1%. The present communication is therefore an improvement with about 2% mean transformation efficiency with successful acclimatization and growth of plants to maturity. The reason for few positives of PCR may be attributed to

the stringent method of cDNA synthesis, which needs further investigation. The enhanced genetic transformation efficiency obtained presently is repeatable and can be used to mobilize genes of agronomic importance into elite cultivars of mungbean.

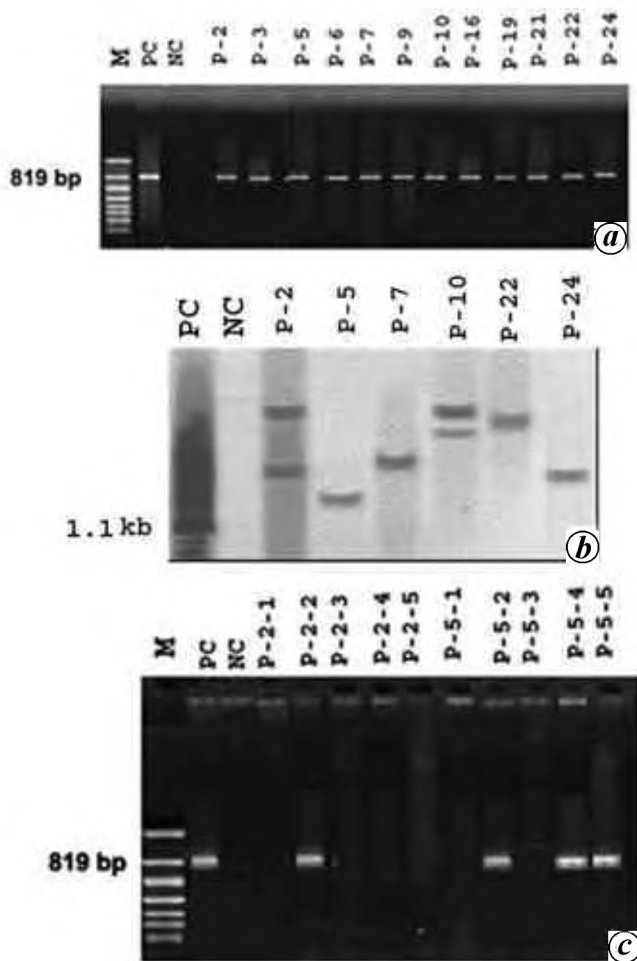


Figure 3. PCR and Southern blot hybridization of T0 transformants of mungbean obtained via *Agrobacterium*-mediated transformation of primary leaf explant using plasmid pCAMBIA-1301 and PCR of T1 transformants. **a**, PCR amplification of genomic DNA of T0 transformants showing amplification of the 819 bp fragment of *hpt* gene after *Agrobacterium*-mediated gene transfer using plasmid pCAMBIA-1301. Lanes 4–15, T0 transformants (P-2, P-3, P-5, P-6, P-7, P-9, P-10, P-16, P-19, P-21, P-22 and P-24); lane 3, Untransformed plant (negative control); lane 2, Plasmid pCAMBIA-1301 (positive control); lane 1, DNA size marker (50 bp marker). **b**, Southern blot hybridization of *hpt* gene in genomic DNA from putative T0 transformants of T0. The plant genomic DNA was digested with *Xba*I to provide a single cut within the plasmid. The blot was probed with non-radio Alkphos-labelled 819-bp PCR-amplified *hpt* gene fragment. Lanes 3–8 carry genomic DNA of six putative (T0) transgenic plants. Lane 2, Genomic DNA from untransformed plant (negative control; NC); lane 1, DNA of plasmid pCAMBIA-1301 restricted with *Xho*I to release the *hpt* gene (positive control; PC). **c**, PCR amplification of genomic DNA of T1 transformants (progeny of T0 transformants P-2 and P-5) showing amplification of the 819-bp fragment of *hpt* gene. Lanes 4–8, T1 transformants (P-2-1, P-2-2, P-2-3, P-2-4 and P-2-5); lanes 9–13, T1 transformants (P-5-1, P-5-2, P-5-3, P-5-4 and P-5-5); lane 3, Untransformed plant DNA (NC); lane 2, Plasmid pCAMBIA-1301 (PC); lane 1, DNA size marker (50 bp marker).

1. Puonti-Kaerlas, J., Stabel, P. and Eriksson, T., Transformation of pea [*Pisum sativum* (L.)] by *Agrobacterium tumefaciens*. *Plant Cell Rep.*, 1989, **8**, 321–324.
2. Hiei, Y., Komari, T. and Kubo, T., Transformation of rice mediated by *Agrobacterium tumefaciens*. *Plant Mol. Biol.*, 1997, **35**, 205–218.
3. Bhargava, S. C. and Smigocki, A. C., Transformation of tropical grain legumes using particle bombardment. *Curr. Sci.*, 1992, **66**, 439–442.
4. Gulati, A. and Jaiwal, P. K., *In vitro* induction of multiple shoots and plant regeneration from shoot tips of mungbean (*Vigna radiata* (L.) Wilczek). *Plant Cell Tissue Organ Cult.*, 1992, **29**, 199–205.
5. Van Wordragen, M. G. and Dons, H. J. M., *Agrobacterium tumefaciens*-mediated transformation of recalcitrant plants. *Plant Mol. Biol. Rep.*, 1992, **10**, 12–36.
6. Ramsay, G., Regeneration in grain legume tissue culture. *Grain Legumes*, 1993, **20**, 16–17.
7. Larkin, *et al.*, Transgenic white clover. Studies with the auxin-responsive promoter GH3, in root gravitropism and lateral root development. *Transgenic Res.*, 1996, **5**, 325–335.
8. Oger, P., Petit, A. and Dessaux, Y., A simple technique for direct transformation and regeneration of the diploid legume species *Lotus japonicus*. *Plant Sci.*, 1996, **116**, 159–168.
9. Nagl, W., Ignacimuthu, S. and Becker, J., Genetic engineering and regeneration of *Phaseolus* and *Vigna*. State of the art and new attempts. *Plant Physiol.*, 1997, **150**, 625–644.
10. Jaiwal, P. K., Kumari, R., Ignacimuthu, S., Potrykus, I. and Sautter, C., *Agrobacterium tumefaciens*-mediated genetic transformation of mungbean [*Vigna radiata* (L.) Wilczek] recalcitrant grain legume. *Plant Sci.*, 2001, **161**, 239–247.
11. Olhoft, P. M. and Somers, D. A., L-Cysteine increases *Agrobacterium*-mediated T-DNA delivery into soybean cotyledonary-node cells. *Plant Cell Rep.*, 2001, **20**, 706–711.
12. Dayal, S., Lavanya, M., Devi, P. and Sharma, K. K., An efficient protocol for shoot generation and genetic transformation of pigeonpea [*Cajanus cajan* (L.) Millsp.] using leaf explants. *Plant Cell Rep.*, 2003, **21**, 1072–1079.
13. Manoj Kumar, S., Syamala, D., Kiran Sharma, K. K. and Prathibha Devi, Protocol for efficient plant regeneration and *Agrobacterium tumefaciens* mediated genetic transformation pigeonpea [*Cajanus cajan* (L.) Millsp.]. *Indian J. Genet.*, 2003, **63**, 289–294.
14. Satyavathi, V. V., Prasad, V., Khandelwal, A., Saila, M. S. and Sita, G. L., Expression of haemoglobin protein of Rinderpest virus in transgenic pigeonpea [*Cajanus cajan* (L.) Millsp.] plant. *Plant Cell Rep.*, 2003, **21**, 651–658.
15. Manoj Kumar, S., Kiran Kumar, B., Sharma, K. K. and Devi, P., Genetic transformation of pigeonpea with rice chitinase gene. *Plant Breed.*, 2004, **123**, 485–489.
16. Manoj Kumar, S., Syamala, D., Kiran Sharma, K. and Prathibha Devi, *Agrobacterium tumefaciens* mediated genetic transformation of pigeonpea [*Cajanus cajan* (L.) Millsp.]. *J. Plant Biotechnol.*, 2004, **6**, 69–75.
17. Prathibha, Devi, Radha, P., Sita Mahalakshmi, L., Syamala, D. and Manoj Kumar, S., Plant regeneration via somatic embryogenesis in mungbean [*Vigna radiata* (L.) Wilczek]. *Sci. Hortic.*, 2004, **99**, 1–8.
18. Saini, R., Jaiwal, S. and Jaiwal, P. K., Stable genetic transformation of *Vigna mungo* L. Hepper via *Agrobacterium tumefaciens*. *Plant Cell Rep.*, 2004, **21**, 851–859.

19. Prathibha Devi, Zhong, H. and Sticklen, M. B., *In vitro* morphogenesis of pearl millet [*Pennisetum glaucum* (L.) R. Br.]. Efficient production of multiple shoots and inflorescence from shoot apices. *Plant Cell Rep.*, 2000, **19**, 546–550.
20. Syamala, D. and Prathibha Devi, Efficient regeneration of *Sorghum bicolor* (L.) Moench, from shoot-tip explants. *Indian J. Exp. Biol.*, 2003, **41**, 1482–1486.
21. Syamala, D. and Prathibha Devi, Development of plant regeneration and genetic transformation system from shoot apices of *Sorghum bicolor* (L.) Moench. *J. Plant Biotechnol.*, 2004, **2**, 77–85.
22. Gamborg, O. L., Miller, R. A. and Ojima, K., Nutrient requirements of suspension cultures of soybean root cells. *Exp. Cell Res.*, 1968, **50**, 151–158.
23. Jefferson, R. A., Assaying chimeric genes in plants the *GUS* gene fusion system. *Plant Mol. Biol. Rep.*, 1987, **5**, 387–405.
24. Rogers, S. O. and Bendich, A. J., Extraction of DNA from milligram amounts of fresh herbarium, and mummified plant tissues. In *Plant Molecular Biology Manual*, Kluwer, Dordrecht, 1988, vol. A6, pp. 1–10.

Received 10 March 2005; revised accepted 8 March 2006

Characterization of soils in the tsunami-affected coastal areas of Tamil Nadu for agronomic rehabilitation

D. R. Chaudhary*, Arup Ghosh and J. S. Patolia

Discipline of Phytosalinity, Central Salt and Marine Chemicals Research Institute, G.B. Marg, Bhavnagar 364 002, India

The coastal area of Tamil Nadu was stuck by tsunami in the morning of 26 December 2004. This caused huge loss of human and animal lives, damage to houses and agricultural lands. Based on the situation studied in the fields, various observations made at the sites and analysis of the soil and water samples collected from the affected areas, it was observed that soils of the area as well as water have turned saline due to inundation of sea water. The electrical conductivity of saturation extract of the surface soil varied from 3.1 to 62.0 dS m⁻¹, whereas that of surface crust showed very high values (110–117 dS m⁻¹), which indicated accumulation of salt on the surface. The chemical composition of soil saturation extract showed higher concentration of sodium, followed by calcium and magnesium as the major cations, whereas chlorides and sulphates were the major anions followed by bicarbonates, indicating that the soils turned saline, but not sodic. However, exchangeable sodium percentage (ESP) of soils varied from 10.2 to 27.4 for surface soil. These soils are low in available N and P (except few),

and medium to high in available K which needs regular monitoring of fertility status and soil test-based fertilizer application.

Keywords: ESP, reclamation, salt-affected soil, soil fertility, tsunami.

SOIL resource is of vital importance for survival and welfare of the people. Nevertheless, of late the pressure on this imperative resource has increased to such an extent that the relationship between the living beings and the soil has become critical. This has resulted in various kinds of land degradation, environmental pollution and decline in crop productivity and sustainability. Among the different types of land degradation, degradation due to salinization hazard affects crops of the agricultural land. Recently, the coastal districts of Tamil Nadu (TN) were struck with killer tidal waves ‘tsunamis’ in the morning of 26 December 2004, causing huge loss of human and animal life, damage to houses and agricultural lands. Inundation of sea water caused severe damage to agricultural lands in Nagapattinam and Cuddalore districts of TN (Figures 1a and b) and soils of the coastal belt turned saline along with contaminating groundwater (Figure 2). The affected agricultural lands need to be quickly rehabilitated to restore the production capacity and ensure food security in the rural areas. After the tsunami, in order to evaluate the soil salinity hazard and irrigation water quality caused by the massive inflow of sea water, impounded over the coastal districts of TN, a survey was conducted in some of the affected areas in February 2005.

Nagapattinam district lies in the centre of the coastal districts and is bound on the north by Cuddalore district, south by Palk Strait, east by the Bay of Bengal and west by Tiruvarur and Tanjavur districts. The mean annual rainfall of this district is 1337 mm. The north-east monsoon brings copious rainfall (75.7%), followed by the south-west monsoon (17.3%). Cuddalore district is bound on the north by Villupuram district, south by Nagapattinam, east by Bay of Bengal and west by the Perambalur district. The mean annual rainfall of this district is 1259 mm. The north-east monsoon brings 59% rainfall, followed by the south-west monsoon (29%).

For evaluation of quality of irrigation water, open-well water samples were collected from seven water sources [six contaminated with sea water (W-1 to 6) and one non-contaminated (NCW-1)] in Nagapattinam district and two samples (W-7 and 8) from Cuddalore district. Water samples were analysed for different chemical characteristics using standard laboratory methods¹. Samples were categorized on the basis of EC, sodium adsorption ratio [SAR = Na/(Ca + Mg/2)] and residual sodium carbonate [RSC = (CO₃ + HCO₃) – (Ca + Mg)].

Physiographically, the investigated area falls under marine landform with marine alluvium geological formation. The original soil series of the tsunami-affected areas of the

*For correspondence. (e-mail: dr_chaudhary@yahoo.com)