

Genetic transformation of *Terminalia chebula* Retz. and detection of tannin in transformed tissue

B. Shyamkumar, C. Anjaneyulu and C. C. Giri*

Centre for Plant Molecular Biology, Department of Genetics,
Osmania University, Hyderabad 500 007, India

Genetic transformation of *Terminalia chebula* Retz. was carried out using *Agrobacterium tumefaciens* strain C-58. Explants such as cotyledon, hypocotyl, excised mature zygotic embryo, cotyledonary node, *in vitro* leaf and shoot were used for genetic transformation. Different experimental methods were followed for infecting the explants. Cotyledon and hypocotyl explants showed swelling response on MS basal medium subsequent to genetic transformation. About $32.5 \pm 2.5\%$ cultures showed swelling response when 8–12 d *in vitro* pre-cultured cotyledon explants were used. Swelling response was not observed in non-transformed control cotyledon explants. Callus induction was observed in one of the swollen cotyledon explants infected with the bacterial suspension grown on media containing 0.1 mM acetosyringone. Callus initiation was not observed in cotyledon explants without co-cultivation, which were kept as control. The transformed callus was subjected to nopaline assay using paper electrophoresis. The analysis indicated the transformed nature of the callus with the presence of nopaline and its absence in non-transformed control callus. Transformed callus grown on fresh MS basal medium showed more than two-fold increase in the growth after four weeks of culture compared to normal control callus. Normally no growth was observed in untransformed control callus. The transformed callus was analysed for the presence of tannins using thin layer chromatography, which indicated the presence of tannic acid in the transformed callus. Genetic transformation of *T. chebula* and detection of tannin in transformed callus are reported here. This can be used to study the tannin biosynthetic pathway using biochemical and molecular approach.

Keywords: *Agrobacterium tumefaciens*, nopaline assay, tannic acid, *Terminalia chebula*, transformed tissue.

PLANTS produces a wealth of chemical compounds derived from both primary and secondary metabolic pathways. Secondary metabolites belong to a large molecule group and they broadly include phenolics, terpenes, steroids and alkaloids. It has been found¹ that more than 100,000 secondary metabolites are synthesized in plants, while the total number is estimated to exceed over 500,000. Tannins are among the array of chemical compounds widely dis-

tributed in the plant kingdom and belong to the phenolic class of secondary metabolites². Tannins are classified into two broad groups, namely hydrolysable and condensed². Amongst the few families of dicotyledons, particularly Combretaceae is a rich source of tannins. *Terminalia chebula* Retz. belongs to the family Combretaceae and is an important medicinal tree which contains hydrolysable type of tannins³. Maximum amount of tannin is present in the fruit pulp of *T. chebula*, popularly known as myrobalan^{4,5}. The dried pericarp of the seed contains tannin and 30–35% of astringent substances. Tannins such as chebulagic acid, chebulinic acid, tannic acid and gallic acid belong the hydrolysable group and are extensively used for medicinal purposes^{3,6}. *T. chebula* is called the 'King of medicines' and is always listed first in the ayurvedic materia medica⁷. The plant product of *T. chebula* is used against many diseases related to digestive disorders, urinary system, skin, parasitic infections, heart, ulcers, colic pain, hemorrhoids and also in wound healing^{5,8}. *T. chebula* has demonstrated anti-HIV and antibacterial activity^{9,10}. *T. chebula* has also shown cardioprotective effect against the potent chemical, isopreterenol¹¹. Recently, chebulagic acid from immature seeds of *T. chebula* was found to suppress the onset and progression of collagen-induced arthritis in mice¹². Besides its medicinal value, myrobalan is also used widely in the leather industry.

The natural regeneration of *T. chebula* from seeds is poor and it is a slow-growing tree, compared to other species of *Terminalia*^{13–15}. Recently, an *in vitro* propagation methodology has been developed by us^{16,17}. Plants are the potential source for discovery of new products and fine chemicals for drug development. Currently major pharmaceutical companies are showing renewed interest in the use of plants as a source of pharmacophore, and bioprospecting is becoming an increasingly important area of research for the search of new drug discovery. Further, compared to field production of plants, *in vitro* production of elite and valuable secondary metabolites by cultured plant cells is considered to be a suitable possible alternative¹⁸.

Recent advances in the genetic transformation of forest trees have made it possible to transfer genes of academic and agronomic importance^{19,20}. *Agrobacterium*-mediated genetic transformation methods are mostly used for developing transgenic tree species^{19,21,22}. Development of genetic transformation protocol will also be useful for the manipulation of genes involved in the secondary metabolic pathways^{23,24}. The present study was envisaged to evaluate the susceptibility of *T. chebula* tissue to *Agrobacterium* infection as well as the production of tannin from *in vitro* cultures of *T. chebula*. To the best of our knowledge, till date there is no report available on the genetic transformation of *T. chebula*. The present communication reports the successful genetic transformation of *T. chebula* and production of tannin in transformed callus using a specialized strain of *Agrobacterium tumefaciens*, i.e. C-58.

*For correspondence. (e-mail: giriccin@yahoo.co.in)

Seeds were collected from the identified elite plant of *T. chebula* in the Srisailem Tiger Forest Reserve (STFR), Srisailem, Andhra Pradesh, India. The pericarp of the seed was removed and kept in distilled water for 1–2 days at room temperature. The mature zygotic embryos were excised mechanically from seed testa using a mechanical device, e.g. bench vice. Excised embryos were surface-sterilized with 0.1% (w/v) bavistin for 10 min followed by 5–6 washes with sterile distilled water. Further sterilization was carried out with 0.1% (w/v) HgCl_2 for 4–5 min, followed by 8–10 washes with sterile distilled water. The excised embryos of 15–17 mm size were cultured in 2.5 cm diameter tube containing semisolid 0.9% (w/v) agar supplemented with MS^{25} nutrients, 3% (w/v) sucrose and different concentrations of GA_3 , i.e. 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/l. To circumvent the browning problem, the excised embryos were sub-cultured to fresh germination medium at regular intervals of two days. The pH of the media was adjusted to 5.8 ± 0.1 prior to autoclaving. All the cultures were incubated in dark in culture room conditions at $25 \pm 2^\circ\text{C}$ and 80% relative humidity. Subsequent to germination of excised embryos in darkness, the cultures were transferred to light. Each treatment was carried out with 50 seeds. All the experiments were repeated three times. The observations were taken after one month of culture. Latin square design was followed and mean and SE were calculated for all the parameters. Statistical analysis was done using Matlab version 5.3 and SPSS version 10.0 Math Works Inc, USA, statistical packages.

A. tumefaciens strain C-58 was tested in a genetic transformation study for induction of shooty teratomas from explants of *T. chebula*. The strain was obtained from M. A. Subroto (Department of Biotechnology, University of South Wales, Australia). This specialized strain of *A. tumefaciens* belongs to the nopaline type. *A. tumefaciens* C-58 strain was cultured and grown on LB medium. The cultures were maintained in the incubator at 29°C . Bacterial culture of the strain was initiated from fresh 1–2 d streaked plates. The suspensions were initiated by transfer of single bacterial colony grown on LB agar media to LB liquid medium. These bacterial cultures were incubated in a shaker at 29°C at 120 rpm. Acetosyringone was incorporated in the liquid bacterial culture medium at concentrations ranging from 0.05, 0.1, 0.6, 1, 10 to 25 mM after filter-sterilization. In another set of experiments, acetosyringone at concentrations 0.6, 1, 5, 10 and 25 mM was also used in the co-cultivation culture medium. The OD of the bacterial growth was taken after 12, 24, 48, 72 and 96 h using spectrophotometer at 600 nm.

Different types of explants of *T. chebula* were used for co-cultivation experiments. Explants such as cotyledons and hypocotyls of different age groups, i.e. pre-cultured *in vitro* for 8–12 d, 15–21 d in MS medium, excised mature zygotic embryos cultured in MS medium for 4–6 d, *in vitro*-grown first generation shoot, leaves and cotyledonary

node (CN), were used for co-cultivation experiments. Minimum of 25–200 explants were used for co-cultivation in different experiments. The bacterial suspension with active growth ($\text{OD} = 1.57$) after 48 h at the exponential phase was used for co-cultivation experiments. Different methods were followed for infecting explants in the co-cultivation experiments. Experimental methods such as injury/cut on explants with sterile scalpel blade dipped in bacterial suspension; incubation of explants in bacterial suspension for different time durations, i.e. 5, 10 and 15 min; pricking of explants with needle dipped in bacterial suspension; vortexing of explants in silicon carbide 5% (w/v) for 5 min and subsequent incubation in bacterial suspension for 20 min; incubation of explants in 12% (w/v) mannitol for 1 and 2 h, and subsequent incubation in bacterial solution for 5 min were evaluated. The explants were also injured/pricked with sterile blade without bacteria and placed on the MS basal medium as control. Cotyledon explants of size 0.5–0.8 cm from 8-day-old seedlings were cultured on MS + 2,4-D (1.0 mg/l) for callus initiation. These callus cultures were treated as normal/untransformed control callus for the present study. All cultures were incubated in dark at $27 \pm 2^\circ\text{C}$ for different co-cultivation periods, i.e. 24, 48 and 72 h. Finally, after 48 h of co-cultivation the explants were transferred to the MS basal medium supplemented with various concentrations of cefotaxime, i.e. 200, 250, 300 and 350 mg/l and incubated in culture room conditions in dark at $25 \pm 2^\circ\text{C}$. The concentration of cefotaxime showing best result in terms of complete control of bacterial growth was selected. Observations were recorded after 4–6 weeks of culture. Experimental data were recorded as the number of cultures showing swelling response and formation of callus per explant. Each method was treated with a minimum of 25 to 200 replicates. Latin square design was followed and mean and SE were calculated for all the parameters. Statistical analysis was done using Matlab version 5.3, and SPSS version 10.0 Math Works Inc., USA, statistical packages.

Bacteria-free callus obtained subsequent to genetic transformation with C-58 strain was analysed for nopaline assay using paper electrophoresis. The untransformed callus growing *in vitro* on MS + 2,4-D (1.0 mg/l) treated as normal/untransformed control callus was used for nopaline analysis. The nopaline analysis using paper electrophoresis was done according to Otten and Schilperoort²⁶ with minor modifications. Approximately 150 mg of callus from both transformed and untransformed control was homogenized with 1 ml of nopaline extraction buffer and centrifuged at 3500 rpm for 4 min. After centrifugation the supernatant was collected in a new eppendorf tube. Nopaline dehydrogenase activity was tested using a mixture containing arginine and α -ketoglutaric acid and was neutralized (pH 6.8–7.0) before adding to the mixture. One volume incubation mixture was added to one volume extract and incubated for 1 h at room temperature. From the above reaction mixture, 8–10 μl was taken and spotted on

a Whatmann 3 mm paper at the anodal end and dried at room temperature. The authentic nopaline (0.5 µg/ml) was prepared and 8 µl from the stock was used as a marker. Electrophoresis was performed in formic acid : acetic acid : water (5 : 15 : 80 v/v) for 1 h at 400 V. The paper was stained in 1 vol. 0.02% (w/v) phenantrenequinone/ethanol, 1 vol. 10% (w/v) NaOH/60% (v/v) ethanol according to Yamada and Itano²⁷. After drying, the spots were visualized under a long-wave ultraviolet lamp (366 nm). Photographs of the nopaline assay were taken using gel documentation system (Pharmacia, UK).

The callus induced subsequent to genetic transformation was excised and grown on MS basal media for further growth. These cultures were maintained in the same medium with a subculture passage of one month. Observations of callus growth were recorded after four weeks of culture. Growth kinetics study was undertaken and growth of the callus was estimated on fresh weight basis. Extraction of tannin from transformed, non-transformed control callus and pericarp of the seed was carried out. The dried powder of the callus and seed pericarp weighing approximately 400 mg was taken and extracted with 50 ml of methanol 3–4 times. Extraction was done by vigorous mixing of tissue powder to 50 ml of methanol, first for 6–8 h and the residue was then extracted for 16 h followed by two extractions for 4 h each. The extracted solution derived from individual samples was pooled and air-dried for 3 days at room temperature and the resultant liquid concentrate was used for thin layer chromatography (TLC). TLC was performed on 0.25 mm thick silica gel layer (silica gel 254F grade, Thomas Baker, Mumbai) using methanol : chloroform (2 : 1 v/v) solvent system, with minor modifications to the protocol by Pansera *et al.*²⁸. Equal volume (2 µl) of the extract from each of the samples was spotted on the TLC plate and compared with the standard authentic tannic acid. After air-drying for 20 min, the presence of tannic acid was viewed by spraying the plate with 0.1% (w/v) FeCl₃ reagent. A chromatogram was prepared for the calculation of R_f values.

Reduced frequency of germination of excised embryos (i.e. 11.1 ± 2.66) was observed after longer period of cul-

ture (9–12 days) on MS basal medium with 3% (w/v) sucrose (Table 1). On the contrary, 0.5 mg/l GA₃ stimulated the highest percentage of germination, i.e. 99.88 ± 0.16 within shortest time, i.e. 2–3 days (Table 1). There was a decline in the germination percentage on media containing beyond 1.0 mg/l GA₃. In the earlier studies germination of cultured, excised embryos was found to be enhanced by incorporation of GA₃ into the medium²⁹. Fully-grown healthy seedlings were obtained after 7 days of *in vitro* culture of excised embryos. As no literature was available on genetic transformation of *T. chebula*, standardization experiment to evaluate optimum cefotaxime concentration was undertaken. Cefotaxime concentration that inhibits complete bacterial growth around explants was considered optimum, when the explants were transferred to cefotaxime medium subsequent to co-cultivation for 48 h. Incorporation of higher concentration of cefotaxime, i.e. 300 or 350 mg/l showed complete inhibition of bacterial growth around the explant. Hence 300 mg/l cefotaxime was used for all the experiments. Different types of explants such as cotyledon, hypocotyls, CN, excised mature zygotic embryos, *in vitro* shoot and leaf were used for the present transformation experiments, which showed varied response in terms of degree of infection around the explants after 48 h of co-cultivation (Table 2). Cotyledon, hypocotyl and excised mature zygotic embryos showed moderate degree of infection after 48 h of co-cultivation (Table 2). Whereas CN, *in vitro* shoot and leaf showed high degree of infection after the co-cultivation period. Cotyledon and hypocotyl explants showed swelling response subsequent to genetic transformation with *A. tumefaciens* C-58. Swelling was not observed in non-transformed control cotyledonary explants. About 32.5 ± 2.5% cultures showed swelling response when 8–12 d *in vitro*, pretreated cotyledon explants were used, which exhibited subsequent callus induction within 20 days of transfer to co-cultivation medium following transformation. The use of cotyledon explants without prior *in vitro* culture did not show any swelling response. A swelling response of 15.5 ± 2.5 and 12.5 ± 2.5% was observed from 8 to 12 days and 15 to 21 days *in vitro* pre-cultured hypocotyl explants res-

Table 1. Effect of different concentrations of GA₃ supplementation on germination of excised embryos of *Terminalia chebula*

| Concentration of GA ₃ (mg/l) | No. of embryos inoculated | No. of days taken for germination | *Percentage of embryos germinated (mean ± SE) |
|---|---------------------------|-----------------------------------|---|
| 0.0 | 50 | 9–12 | 11.1 ± 2.66 |
| 0.1 | 50 | 7–9 | 38.3 ± 0.96 |
| 0.2 | 50 | 5–8 | 66.1 ± 0.55 |
| 0.5 | 50 | 2–3 | 99.8 ± 0.16 |
| 1.0 | 50 | 2–3 | 98.8 ± 0.46 |
| 2.0 | 50 | 4–5 | 91.6 ± 2.08 |
| 3.0 | 50 | 4–5 | 86.6 ± 1.52 |
| 4.0 | 50 | 5–6 | 83.8 ± 0.88 |
| 5.0 | 50 | 5–6 | 76.1 ± 3.64 |

*Each treatment tested with 50 excised embryos and calculated %mean ± SE of germination of embryos of three repeated experiments. Observations were taken after one month of inoculation of excised embryos to the germination medium.

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Table 2. Effect of different explants and co-cultivation techniques for genetic transformation of *T. chebula* using *Agrobacterium tumefaciens* strain C-58

| Type of explant | Co-cultivation technique | Time of treatment (min) | Co-cultivation response after 48 h | | **Percentage of explants showing culture response (mean \pm SE) |
|-----------------|--------------------------|-------------------------|--|-------------------------------------|---|
| | | | No. of explants used for cocultivation | *Degree of infection around explant | |
| Cot (0–12 d) | Inj. | – | 100 | + | 22.5 \pm 2.5, SwE |
| Hyp (8–12 d) | -do- | – | 100 | ++ | 13.4 \pm 0.3, SwE |
| Cot (8–12 d) | PN | – | 100 | + | 15.4 \pm 0.3, SwE |
| Hyp (8–12 d) | -do- | – | 100 | + | 13.5 \pm 2.5, SwE |
| Cot (8–12 d) | Inb. | 5 | 200 | ++ | 32.5 \pm 2.5, SwE |
| | | | | | (Callus induction 3.01%) |
| Cot (15–21 d) | -do- | -do- | 200 | ++ | 14.5 \pm 2.5, SwE |
| Hyp (8–12 d) | -do- | -do- | 200 | ++ | 12.5 \pm 2.5, SwE |
| CN | -do- | -do- | 25 | +++ | – NSI/BE |
| Ex. Em (4–6 d) | -do- | -do- | 100 | ++ | 21.5 \pm 2.5, Ger |
| IVL | -do- | -do- | 25 | ++ | BE |
| IVS | -do- | -do- | 50 | ++ | BE |
| Cot (8–12 d) | -do- | 10 | 200 | ++ | 12.4 \pm 0.3, SwE |
| Hyp (8–12 d) | -do- | -do- | 200 | ++ | 9.4 \pm 0.2, SwE |
| Cot (8–12 d) | -do- | 15 | 100 | +++ | BE |
| Hyp (8–12 d) | -do- | -do- | 100 | +++ | BE |
| Cot (8–12 d) | VSC (5%) | 5 min | 100 | +++ | BE |
| | +BS | | | | |
| | (Inb.) | | 20 min | | |
| Hyp (8–12 d) | -do- | -do- | 100 | +++ | BE |
| Cot (8–12 d) | Man. (1%) | 1 h | 100 | ++ | 28.6 \pm 1.20, SwE |
| | (Inb.) | | | | |
| Hyp (8–12 d) | -do- | -do- | 100 | ++ | 12.5 \pm 2.5, SwE |
| Cot (8–12 d) | -do- | 2 hr | 50 | ++ | 17.3 \pm 1.20, SwE |
| Hyp (8–12 d) | -do- | -do- | 50 | ++ | 11.5 \pm 2.5, SwE |

Hyp, Hypocotyl; Cot, Cotyledon; Inj, Injury; Inb, Incubation; Man, Mannitol; Ex. em, Excised embryo; CN, Cotyledonary node; BE, Browning of explant; IVL, *In vitro* leaf; IVS, *In vitro* shoot; NSI, No shoot initiation; VSC, Vortexing in silicon carbide (5%); PN, Pricking with needle. SwE, Swelling of explants; Ger, Germination. Each treatment of explants with given replicates and calculated %mean \pm SE. Observations were taken after one month of culture. *Bacterial infection around explant (+, less; ++, moderate; +++, high). Note that callus is generally not induced in MS basal medium in *T. chebula*. The transformed nature of the induced callus was confirmed by nopaline assay. **Before co-cultivation the bacterial suspension was treated with 0.1 mM concentration of acetosyringone. –, No treatment time.

pectively, but no callus induction was observed. No callus initiation was observed in untransformed cultures when transferred to MS basal medium.

There was no callus initiation observed from CN, *in vitro* shoot and leaf explants; rather they turned brown after 8–10 days of *in vitro* culture in co-cultivation medium. In overall evaluation, the cotyledons and hypocotyls showed best response as explants for genetic transformation. Therefore, further experiments on genetic transformation using different co-cultivation techniques were carried out using cotyledon and hypocotyl explants only. The number of cotyledon and hypocotyl explants showed swelling and the frequency was 15.4 ± 0.3 and $13.5 \pm 2.5\%$ respectively, when infected through pricking method (Table 2). High degree of infection, evident by increased area of bacterial growth around the explants, was observed in 8–12-day-old cotyledon and hypocotyl explants co-cultivated after vortexing in silicon carbide for 5 min. However, the explants co-cultivated through the method of vortexing in

silicon carbide turned completely brown within 2–3 days of transfer to co-cultivation medium.

Various concentrations of acetosyringone were used in bacterial suspension as well as in inoculation medium. Callus induction was observed in one of the swollen cotyledon explants infected with the bacterial suspension containing 0.1 mM acetosyringone (Table 2). But at higher concentrations of 10 and 25 mM, the explants turned brown and no swelling was observed. In contrast, no callus induction was observed in untransformed cultures. Normally no callus was induced by placing cotyledon explants of *T. chebula* on MS basal medium. Transformed callus of *T. chebula* induced subsequent to genetic transformation with C-58 was analysed for the presence of opine, i.e. nopaline. Isolates from control–untransformed callus, transformed callus, standard arginine, and standard authentic nopaline were used for the study. The presence of nopaline (Figure 1, lane 3) was confirmed and it is at a position similar to that of the standard nopaline (Figure 1,

lane 1). The presence of arginine in control–untransformed callus and transformed callus was confirmed by comparing with standard arginine (Figure 1, lane 4). Nopaline was not detected in the untransformed control callus (Figure 1, lane 2). This assay clearly indicated the transformed nature of the C58-induced callus with the presence of nopaline.

C58-transformed callus excised and grown on fresh MS basal medium showed profuse growth compared to control (Figure 2 *a, b*). From a study on the growth kinetics, it was found that there was more than two-fold increase in the growth of the callus after four weeks of culture compared to the normal callus (Figure 3). A slight decline in the growth of transformed callus was observed after four weeks compared to the third week of culture. After four weeks of culture, fresh weight of control callus was reduced when compared to initial weight of the callus at the time of inoculation. The control callus slightly turned brown compared to the transformed callus after four weeks of culture.

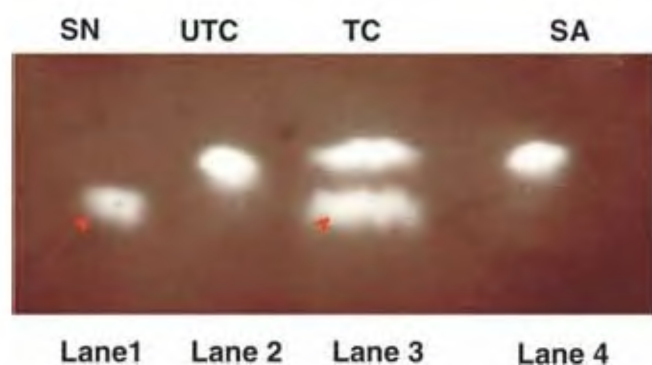


Figure 1. Nopaline assay by paper electrophoresis for confirmation of transformation in *Terminalia chebula* SN, Standard nopaline (lane 1, arrow); UTC, Untransformed callus (lane 2); TC, C58-induced transformed callus (lane 3, arrow); SA, Standard arginine (lane 4).

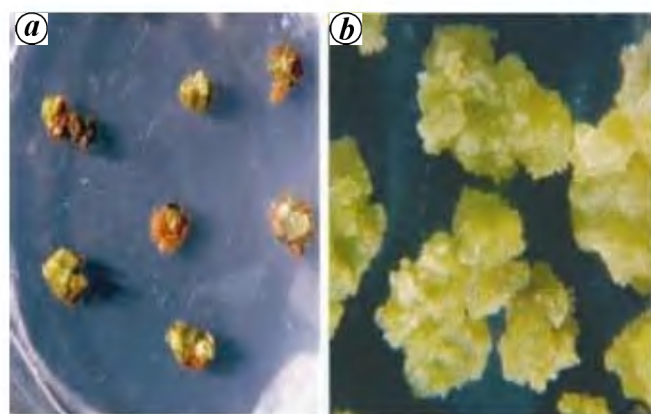


Figure 2. Genetic transformation of *T. chebula* and growth of untransformed and transformed callus using *Agrobacterium tumefaciens* strain C58. *a*, Reduced or meagre growth of untransformed control callus on MS basal medium after four weeks of culture. *b*, Growth of C58-transformed callus on MS basal medium after four weeks of culture.

The transformed callus was analysed for the presence of tannins using TLC. Isolates from the pulp of the seed, untransformed callus, transformed callus and standard authentic tannic acid were used for the study. The presence of tannin in the pulp of the seed (Figure 4, lane 2), untransformed callus (Figure 4, lane 3) and transformed callus (Figure 4, lane 4) was confirmed by comparing with authentic tannic acid (Sigma Co, USA). Rf of tannin in the

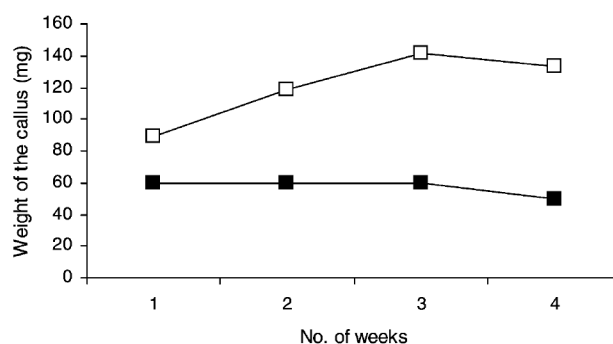


Figure 3. Growth curve of untransformed control callus and C58-induced transformed callus of *T. chebula*. Open square represents two-fold increase in the growth of C58-induced transformed callus after four weeks of culture. *b*, Closed square represents growth of untransformed callus after four weeks of culture.



Figure 4. Analysis of tannins in C58-induced transformed callus using thin layer chromatography in *T. chebula*. STA, Standard tannic acid (lane 1, arrow); PS, Pericarp of seed (lane 2, arrow); UTC, Untransformed callus (lane 3, arrow); TC, Transformed callus showing presence of tannic acid (lane 4, arrow).

transformed callus was 0.94 cm and it was similar to that of standard tannic acid, i.e. 0.94 cm (Figure 4). The Rf of tannin present in the pulp of the seed and untransformed callus was also found to be 0.94. The observation clearly indicated the presence of tannic acid in transformed callus.

In the present study, out of the overall swollen cotyledon explants (32.5 ± 2.5), only a single cotyledon showed callus formation when explants were co-cultivated in the bacterial suspension containing 0.1 mM acetosyringone. The other swollen cotyledons did not show any callus formation. This may possibly be attributed to the fact that though integration of the T-DNA occurred, due to the weak expression of bacterial genes in the plant genome, callus formation was not observed. Similarly, in an earlier report³⁰ the transformation response was not evident even though T-DNA integration was confirmed in *Betula pendula*.

The reduced frequency transformed callus formation response of *T. chebula* may be attributed to low susceptibility towards the C-58 strain. In an earlier study low susceptibility towards the strain C-58 was reported in *Acacia mangium*³¹. But, in this study the addition of acetosyringone did not show better response in callus formation on transformation with C58 in *A. mangium*³¹. Transformed cultures offer an additional advantage in the production of valuable secondary metabolites as evidenced in *Camptotheca acuminata*³², *Taxus baccata*³³ and many other species³⁴.

Genetic transformation study using *A. tumefaciens* revealed the susceptibility of *T. chebula* to *Agrobacterium* infection. Transformed callus was obtained from cotyledons subsequent to genetic transformation using wild strain of *A. tumefaciens*, C-58. Paper electrophoresis analysis revealed the presence of nopaline in transformed callus indicating its transformed nature compared to its absence in untransformed control. TLC analysis using authentic standard tannic acid showed the presence of tannin in the transformed callus, which is generally found in seed pulp. Susceptibility of *T. chebula* to *Agrobacterium* provides an opportunity to produce transgenic plants with desirable genes. Transformed callus obtained in the present investigation can be used to study the tannin biosynthetic pathway using the biochemical and molecular approach. The importance of tannin and its high expression has also been demonstrated in *Populus tremuloides*³⁵. This culture system may also be useful for metabolic profiling of *T. chebula* using recent metabolomics approaches.

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Luminescence studies on the sediments laid down by the December 2004 tsunami event: Prospects for the dating of palaeo tsunamis and for the estimation of sediment fluxes

M. K. Murari¹, H. Achyuthan² and A. K. Singhvi^{1,*}

¹Planetary and Geosciences Division, Physical Research Laboratory, Ahmedabad 380 009, India

²Department of Geology, Anna University, Chennai 600 020, India

The tsunami of 26 December 2004 was associated with an Mw 9.3 earthquake. This was the second largest earthquake ever recorded. Geodynamic processes leading to such earthquakes suggest that these are re-

petitive. It is therefore desirable to obtain their recurrence interval. Earlier attempts to date palaeo-tsunami sediments have assumed that the basic premises for the use of optically stimulated luminescence (OSL) are adequately met. The sediments transported by the 2004 tsunami provided a maiden opportunity to verify the basic premises of the zeroing of the luminescence at the time of deposition. Results on eight of the nine samples using the conventional single aliquot regeneration (SAR) method provided OSL palaeodoses in the range 0.5 to 0.7 Gy. The recently developed component-specific SAR (CS-SAR) consistently provided OSL palaeodoses in the range 0.5–0.7 Gy. Further, CS-SAR consistently provided evidence that in eight of the nine samples, a fraction of the sample did experience daylight bleaching during transport with the tsunami. This was suggested by the fast-component OSL palaeodose of ~0.2 Gy. This implies a maximum age offset of ≤ 50 yrs and suggests that despite its energy and wave amplitude, only the top ~20–50 cm layer of the sediment in the intertidal zone was mobilized. This inference can be used for sediment influx calculations and hence for regional disaster management.

Keywords: Coastal dynamics, luminescence dating, quartz, tsunami.

THE study area covered a 200 km track along the Pichavaram–Chennai transect (13°08'N, 80°19'E to 11°27'N, 79°47'E). Satellite remote sensing LISS III data were used to delineate the geomorphologic units. The physiography of the study area is a low, slightly undulating terrain with a general slope of 1° to 0.30' towards the east coast of Tamil Nadu. The beach is narrow, and within few tens of metres from the shoreline, the elevation reaches a local maximum of ~ +1 m asl. Moving landwards, the profile rises to +2 m over a distance of ~800 m from the shoreline. Along the transect, four major zones within the tidal flat were recognized, viz. an outer sand flat merging with the beach dunes complex and rock exposures, middle sand flat, sandy to silty inner flats (mixed flats of Reineck and Singh¹, and salt marsh). The tsunami samples were collected along the entire transect of East Coast Road (Figure 1). This area is covered in the Survey of India toposheets for the region bounded by 79°45'E; 11°45'N and 80°00'E; 12°15'N. The samples and their location are given in Table 1. These are tsunami-affected sites that suffered significant damage. The tsunami waters rose to nearly 25–30 m^{2,3}, inundated river mouths and tidal flat zones for nearly 3 h and deposited a sand sheet of thickness up to 100 cm. The tsunami-laid sands extended inland from 80 to 1500 m. The thickness of the sand decreased after 400 m inland. The samples were from areas away from human interference and were collected by inserting metal pipes into the sands about 10–15 cm below their surface, with due care to avoid any daylight exposure. These being recently laid tsunami deposits, the field identification was straightforward.

*For correspondence. (e-mail: singhvi@prl.res.in)