

Maintenance of callus growth during subculturing is a genotype-dependent response in rice: Mature seed-derived callus from IR 54 rice cultivar lacks culturability

Following the finding that modified *Agrobacterium tumefaciens*-mediated transformation system works at high-efficiency for stable genetic transformation of rice¹, there is a great deal of activity as well as success in production of transgenic rice plants for varied applications². A common protocol to achieve rice transformation

through this approach involves co-cultivation of competent *A. tumefaciens* cells (harbouring the gene of interest) with calli pieces derived from the scutellar portion of mature seeds^{1,3}. This method warrants that mature seed-derived calli pieces must be able to survive a series of subcultures, in order

to (i) produce and maintain sufficient amounts of calli; (ii) subject calli to different concentrations of selection agent; (iii) check over-growth of *A. tumefaciens* cells on the culture plates following co-cultivation for a limited period and then washing off of the excess bacterial cells; (iv) subject calli to various

pre-treatments (such as with abscisic acid) for inducing high-level regeneration response; (v) transfer calli to regeneration medium, etc³. For the large-scale exploitation of the transgenic rice technology, it is therefore imperative that diverse rice cultivars must withstand and respond favourably to different treatments during the tissue culture experiments. It has been shown that anther culturability of rice is significantly variable among rice species, subspecies or varieties⁴. The regeneration response in rice is also found to be variable amongst different genotypes⁵⁻⁷. In the course of optimizing for a genetic transformation of diffe-

rent rice cultivars in our laboratory, we encountered the problem that mature seed-derived calli from IR 54 rice cultivar fail to withstand the subculturing treatment. On the other hand, there was no such problem with culturability of seed-derived calli from Pusa Basmati 1, Basmati 370 and Taipei 309 rice cultivars.

Seeds of different rice cultivars were obtained from diverse sources [Taipei 309 and IR 54, International Rice Research Institute (IRRI), Philippines; Basmati 370 and Pusa Basmati 1, Indian Agricultural Research Institute (IARI), New Delhi] and were multiplied at IARI.

Mature seeds of the above cultivars were dehusked, surface-sterilized with 70% ethanol for 30–45 s and 0.1% HgCl₂ for 20 min and rinsed five times with sterile distilled water (SDW). Seeds of Taipei 309 and Pusa Basmati 1 cultivars were soaked overnight in dark and were cultured on NB medium⁸ containing 2 mg l⁻¹ 2,4-D (NB callus induction medium, Figure 1 a) at 26 ± 1°C, in the dark³. After 5–8 days, the scutellar portions excised from these seeds were inoculated onto fresh NB callus induction medium (Figure 1 a). The proliferating daughter calli were selected and subcultured every fourteen days on the same medium at

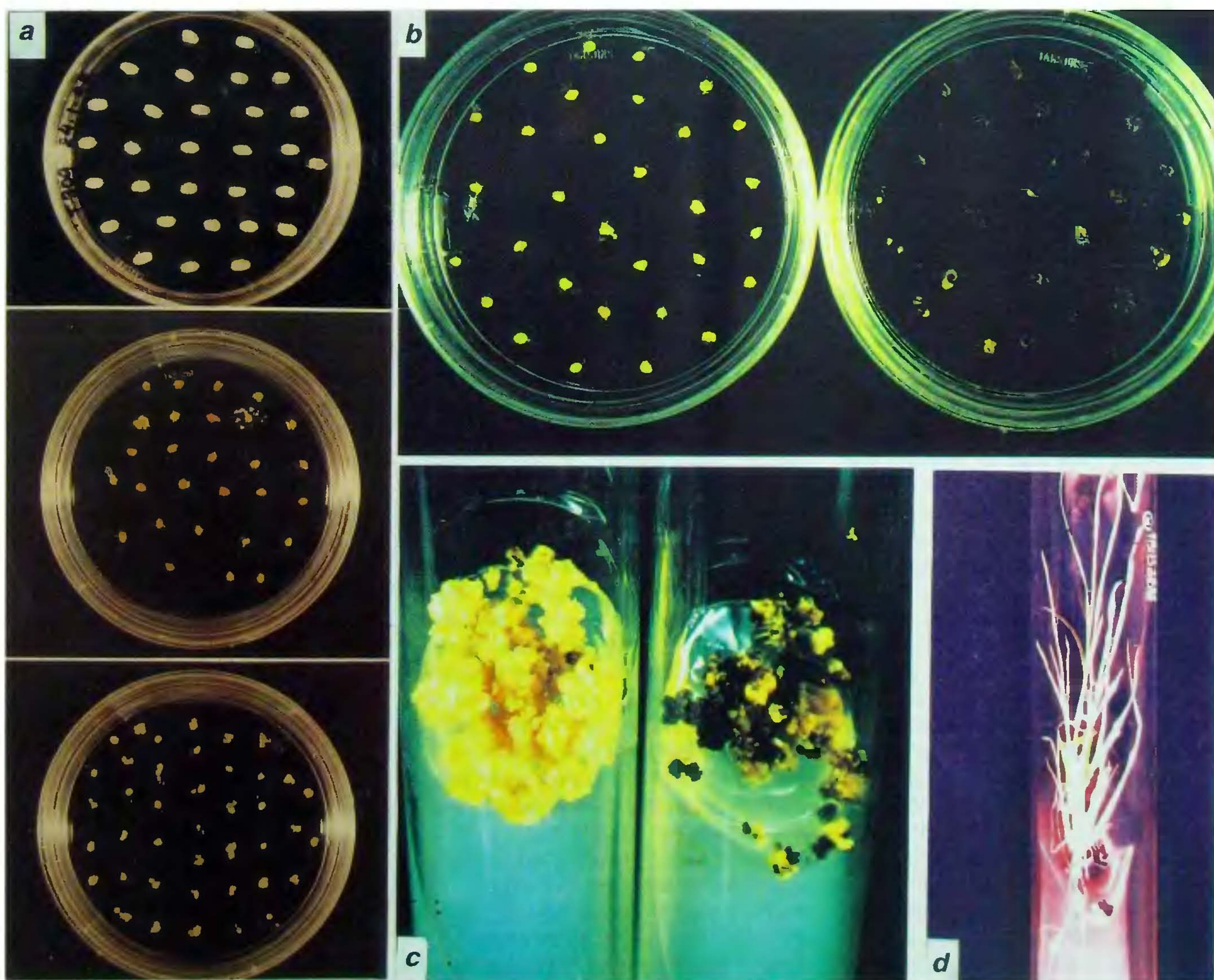


Figure 1. Tissue culture response of different rice cultivars. *a*, Stages in procuring scutellar calli for use in *A. tumefaciens*-mediated transformation of rice. Seeds of cultivar Taipei 309 (*top panel*), Excised scutella showing initiation of callus formation (*middle panel*), 1–2 mm-sized calli pieces employed as explants for co-cultivation with *A. tumefaciens* cells (*bottom panel*). Seeds, scutella and calli were inoculated on NB callus induction medium; *b*, Scutella of Taipei 309 (*left*) and IR 54 (*right*) after 7 days of inoculation on NB callus induction medium. Note that the scutellar portions of IR 54 have become necrotic upon subculturing whereas no such browning response is seen in Taipei 309 scutella; *c*, Response of Pusa Basmati 1 (*left*) and IR54 (*right*) calli after 15 days in secondary culture. Three-week-old primary calli (initiated from the mature seeds) were used for secondary culturing. MS callus induction medium was used; *d*, Regenerants of IR 54 from one-month-old primary callus.