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INCREASED FREQUENCY OF NORMAL PLANT REGENERATION FROM CROWN GALL CALLUS OF *SESBANIA ROSTRATA*

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REGENERATION of plants from callus cultures of leguminous species has been found to be difficult. A few exceptions are the reports on pea[1], soybean[2] *Vigna aconitifolia*[3] and clovers[4]. Currently there is wide interest in *Sesbania rostrata* an annual legume which in addition to the root nodules, produces nitrogen fixing nodules on the stem and the branches[5]. Tissue culture and plant regeneration experiments with *S. rostrata* have been reported recently[6]. Though callus induction from a variety of explants was easy, plant regeneration from callus was observed at a low frequency. We also observed easy induction of callus in leaf and stem explants of this species. In the course of these experiments, regeneration of normal, non-transformed, nopaline negative shoots from axenic *Agrobacterium tumefaciens* induced tumour tissue was observed in over 50% of the cultures. These results are reported in this paper.

Seeds of *S. rostrata* were treated with concentrated H_2SO_4 for 30 min and then surface-sterilized using 0.1% $HgCl_2$. After repeated washing with sterile water these were cultured on Murashige and Skoog[7] medium. One-month-old seedlings were the source of leaf and stem explants. MS basal medium with 6-benzyl-adenine (BA) and α-naphthaleneacetic acid (NAA) were used in various experiments. Medium was adjusted to pH 5.8 before solidifying with 0.8% Difco agar.

A. tumefaciens strain A208 containing pTiT37 was used for inducing crown galls on the plants grown in the green house. The in planta tumours were cultured *in vitro* on MS medium supplemented with 200 mg/l Claforan. The regenerated shoots were induced to root on MS + 0.1 mg/l NAA. All cultures were kept at a light intensity of 8.2 watts/m^2 at $25 \pm 2°C$.

The rooted plants were transferred to autoclaved soil and later to pots in the field net house. Tumour tissue and the shoots arising from axenic cultures were checked for the presence of nopaline as described earlier[8].

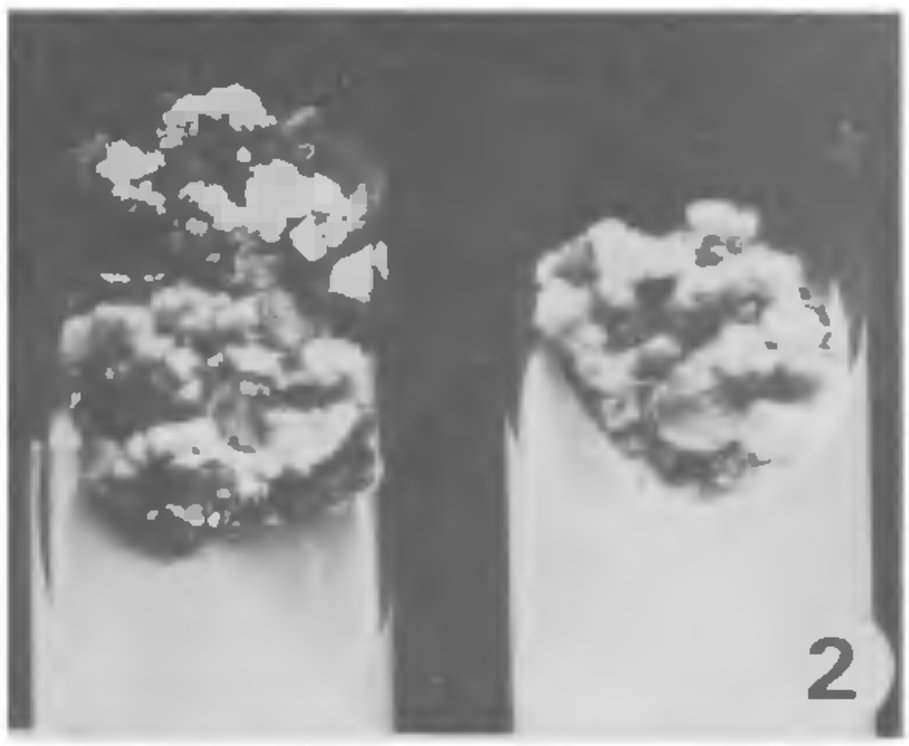
The *Agrobacterium* inoculated sites on stem produced tumours within ten days which gradually enlarged (figure 1). The tumour tissue could be cultured on MS medium alone without the addition of auxin or cytokinin and showed profuse growth (figure 2).

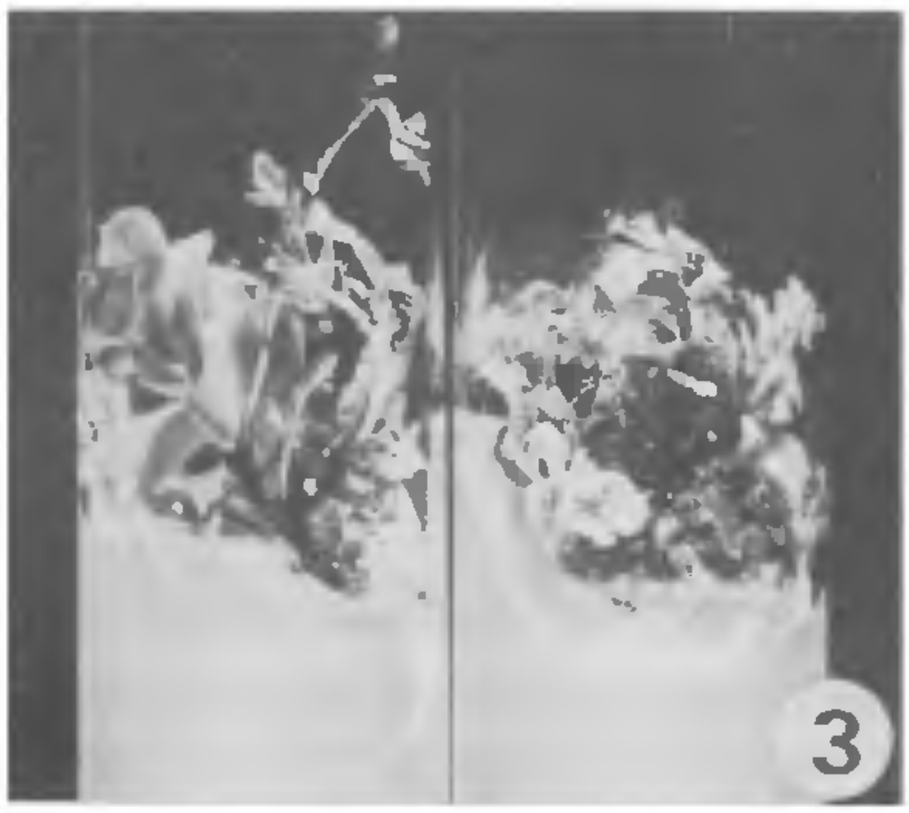
The response of leaf, stem and tumour explants cultured on MS and MS supplemented with BA and NAA is shown in table 1. Leaflets and stem segments showed scanty callus growth on MS medium. Profuse callus growth in these explants was observed in MS + BA + NAA medium.

Table 1 *Response of leaf, stem and tumour explants of Sesbania rostrata*

Medium/explant	Stem	Leaf	Tumour
MS	C^+	C^+	C^{++}
MS + 1.5 mg/l BA + 0.1 mg/l NAA	C^{++} ShR 1/48	C^{++} ShR 0/48	C^{++} ShR 26/48

Note: Number of cultures 48; Culture period 40 days; C^+ scanty callus; C^{++} profuse callus; ShR number of cultures showing shoot regeneration.





Figures 1–5. 1. In planta tumours on *Sesbania rostrata*; **2.** Tumour callus on MS medium; **3.** Shoot differentiation from tumour callus on MS + 1.5 mg/l BA + 0.1 mg/ml NAA; **4.** Rooting of tumour derived shoots on MS + 0.1 mg/l NAA, and **5.** Tumour derived normal plants in pots.

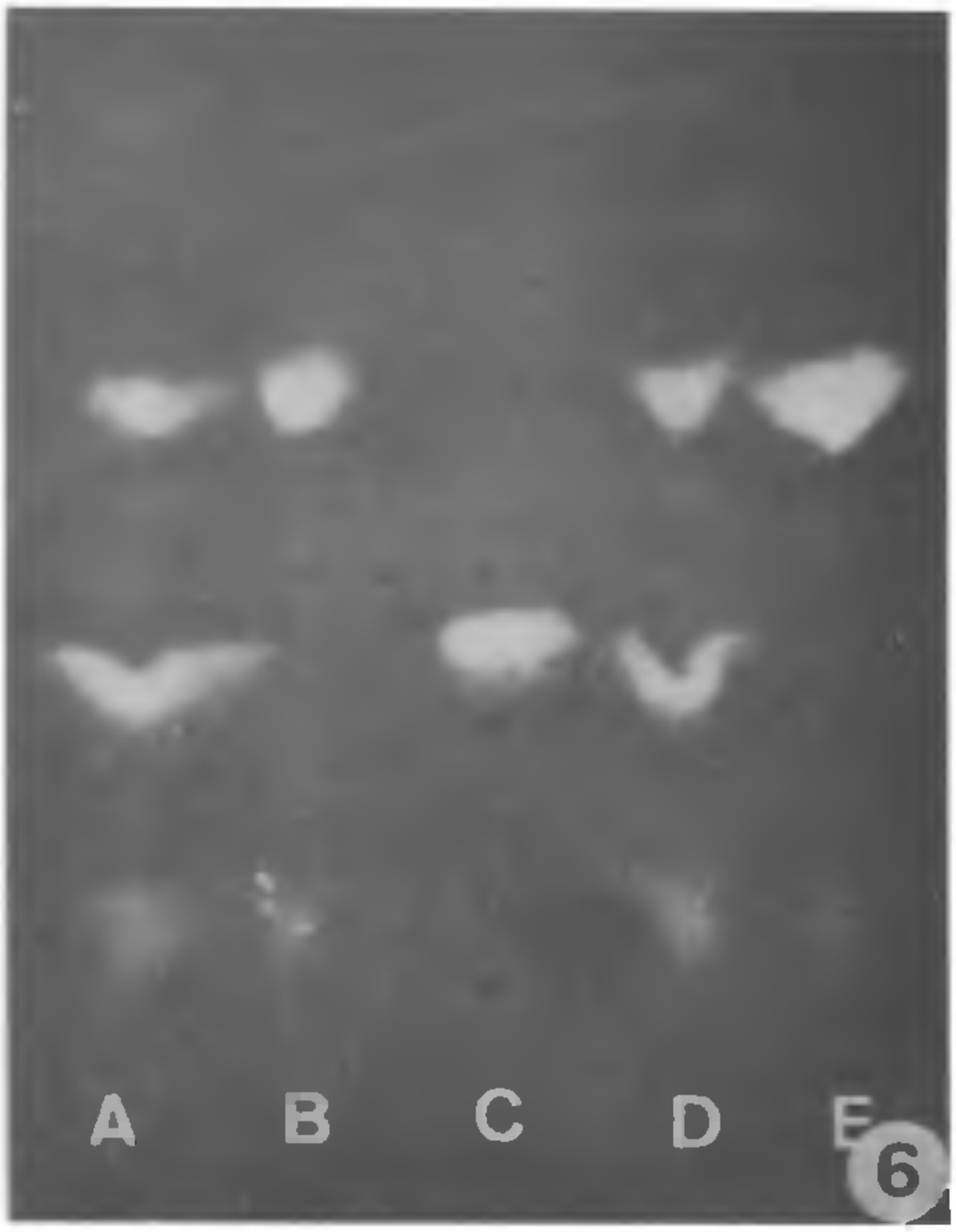


Figure 6. Nopaline assay of tumour callus and differentiated shoots; A and D tumour callus; B control, C nopaline standard, and E tumour shoot.

Shoot regeneration

Leaf callus did not show any shoot regeneration while less than 1% of the stem callus showed shoot regeneration. In contrast, more than 50% of the tumour callus gave shoot differentiation (figure 3). The tumour callus was nopaline positive but the shoots were negative (figure 6).

Root induction in tumour shoots

The excised shoots when transferred to MS + 0.1 mg/l NAA medium gave rooting in 46/48 explants (figure 4). The rooted plants were transferred to autoclaved soil in paper cups and later to pots (figure 5). Tumour shoots in tobacco[9], *Arabidopsis*[10] and *Brassica juncea*[8] were nopaline positive and did not root. The present observation that over 50% of the tumour callus cultures regenerated shoots compared to less than 1% in stem callus is interesting. Parallel observations have been reported previously in *Physalis* and *Nicotiana paniculata* where normal callus did not undergo differentiation but the *Agrobacterium* transformed callus differentiated[11]. It is likely that the tumour tissue of *Sesbania* was chimeric consisting of both transformed and non-transformed cells. The high phytohormone level of the transformed tumour cells along with the exogenous supply of growth regulators promotes shoot differentiation from the non-transformed cells. Deletion of T-DNA is yet another possibility.

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