

Multiple shoot induction and regeneration of pigeon pea (*Cajanus cajan* (L.) Millsp) cv. Vamban 1 from apical and axillary meristem

Pigeon pea is an important world crop species widely cultivated in the tropics and subtropics as a good source of dietary protein, especially in developing countries. Genetic improvement of this crop through biotechnological methods has not yet been achieved mainly due to its recalcitrance in culture. In *Cajanus cajan*, the available reports draw attention to haploid embryogenesis¹, callus regeneration of irradiated seeds² and leaf discs³, multiple shoot induction from cotyledonary node⁴ and cotyledon⁵, and hybridization⁶ of *Cajanus cajan* with *Alyosia platycarpa* with an emphasis on incorporation of pod borer resistance into *Cajanus cajan* and, finally, organogenesis⁷. Though multiple shoots were induced successfully from cotyledonary nodes⁴, they failed with shoot tip. Hence the present investigation focuses on the shoot multiplication from cotyledonary node as well as shoot tip.

Seeds of *Cajanus cajan* var. Vamban 1 obtained from National Pulses Research Centre, Vamban, Pudukkottai, were sterilized with 70% ethanol for 30 sec followed by 3 min of soaking in 0.1% HgCl₂. The sterilized seeds were washed thoroughly 5 times with sterilized double-distilled water and were implanted in culture tubes containing 15 ml of medium (MS0-MS basal medium without hormone, MS3-MS basal medium + 13.31 µM BAP). MSR medium (1/2 strength MS inorganic + full strength organic addenda + 0.49-24.6 µM IBA) was used for rooting of regenerated node and shoot tip. The effect of the presence of seed coat on multiple shoot formation was studied by culturing the seed explants with or without seed coat separately on MS3 directly. The morphogenetic responses of cotyledonary region and shoot tip were also studied. In another experiment, 8-day-old seedlings from MS0 medium were placed horizontally on the MS3 medium so as to allow cotyledonary node and shoot tip to touch the medium. The mean number of shoots produced from different explants was calculated after 30 days. Explants with mass of shoot initials were transferred to MS0 or subcultured in MS3 after 30 days of inoculation for the elongation of shoots. The well-developed shoots were transferred to MSR

with varying concentrations of IBA (0.49 to 24.6 µM). Rooted plantlets were transferred to vermiculite in paper cups and supplied with Hoagland medium⁸. Acclimatized plants were then transferred to soil in the glass house.

Table 1 gives the results of the morphogenetic responses of different explants. Seeds germinated on MS3 callused with bulged primary roots. Cotyledonary nodes from the germinating seeds produced multiple shoot initials (Figure 1 a); an average

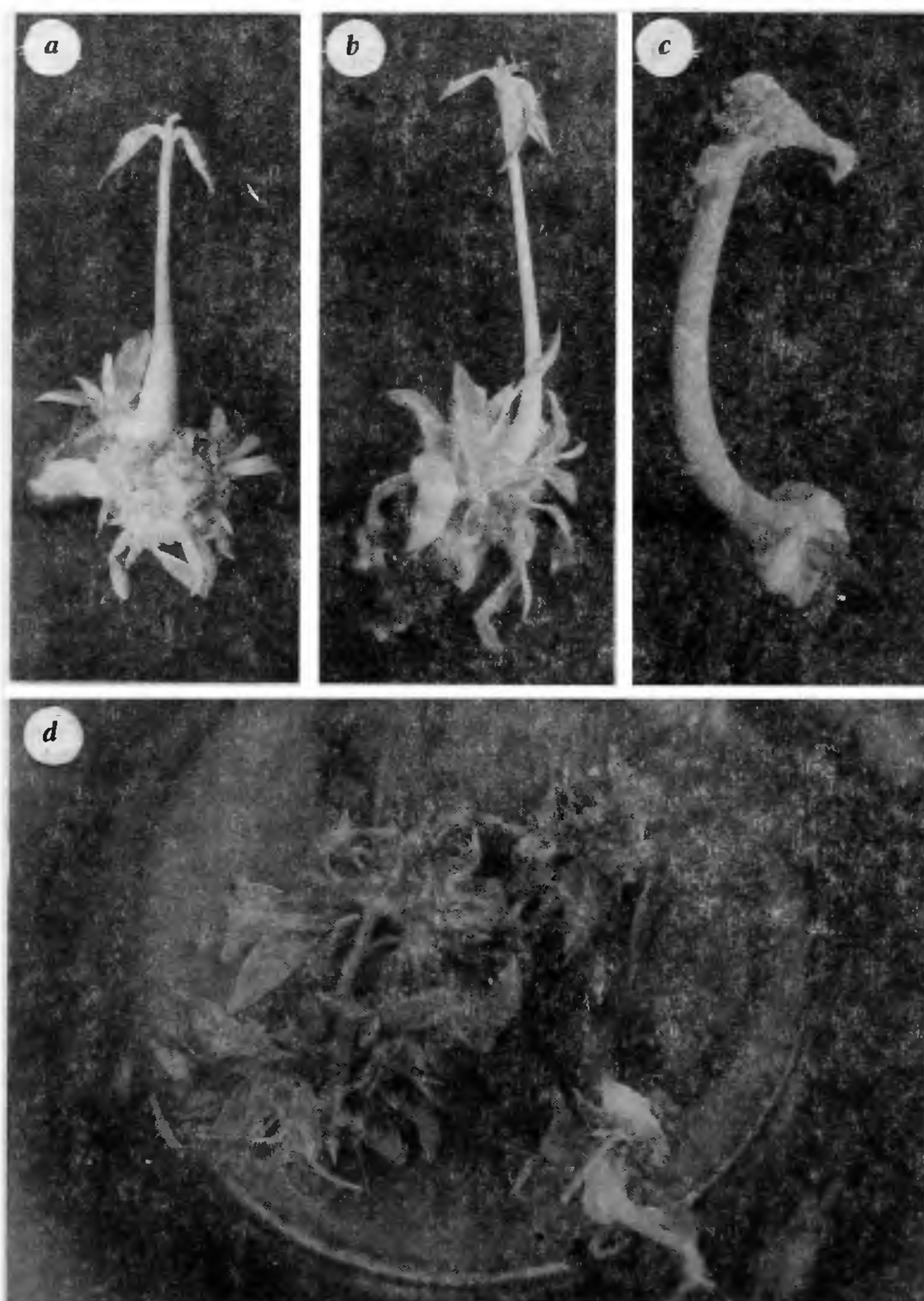


Figure 1. Multiple shooting of *Cajanus cajan*. a, Seed explants with multiple shoots at cotyledonary nodes in MS3 after 30 days of culture. b, Seed explant without seed coat showing less number of shoot regeneration from cotyledonary nodes. c, Seedling explant showing shoot initials at both cotyledonary node and shoot tip on MS3 after 30 days of culture. d, Elongation of shoots from cotyledonary nodes of the seed explants on MS3.

Table 1. Morphogenetic responses of different explants on MS3 medium

Explants	Average* number of shoots		
	Cotyledonary node	Shoot tip	Total
Seedling (cotyledonary node and shoot tip in contact with medium)	26	9	35
Seedling (only shoot tip in contact with medium)	16	12	28
Excised cotyledonary node	5	—	5
Excised shoot tip	—	—	—
Seed with seed coat	49	—	49
Seed without seed coat	7	—	7

*Average of 10 replicates.

of 49 shoots per explant were recorded in seeds with seed coat; the decoated seeds produced only an average of 7 shoots per explant (Figure 1 b). All the shoot initials from the cotyledonary node could elongate in the same medium (Figure 1 d) but the elongation rate speeded up when transferred to MS0. When cotyledonary nodes with multiple shoot initials alone were cultured by removing epicotyl, root and cotyledons, the rate of elongation was slow. Cotyledon might be an important source of some growth factors for the development of axillary meristem⁴. Seedling explants showed different types of morphogenetic responses. Multiple shoots were induced at both cotyledonary node and shoot tip when seedling explant with cotyledonary node and shoot tip was placed horizontally on the medium and also when the shoot tip of the seedling explant alone touched the medium. This result is in sharp contrast to an earlier report⁴ where shoots were not successfully regenerated from shoot tip region. Shoots which developed from shoot tips could not grow well in the same medium (MS3) and hence they were transferred to basal medium (MS0).

When the seedling explant was placed with the shoot tip touching the medium, it produced lesser number of shoots at the shoot tip region (Figure 1 c). When excised cotyledonary node and shoot tip were used

as explants, cotyledonary node produced an average of 5 shoots and the shoot tip did not produce any shoot (Table 1).

Elongated shoots (3–5 cm) were rooted on MSR with different concentrations of IBA. IBA at 2.46 μ M produced an optimum number of roots (8 roots/plantlet) within 13 days. Rooted plantlets were successfully transferred to vermiculite and then to soil in the glasshouse where the hardened plants produced normal viable seeds.

Since callus regeneration in pigeon pea is difficult, methods which rely on the proliferation of shoots from pre-existing meristem may provide practically ideal system for the transformation through particle-bombardment-mediated^{9,10} gene transfer. This protocol may be further extended to tissue-culture-based selection for stress tolerance. Since shoots are produced from the same explant in large number, nodal culture will provide true-to-type progenies through rapid micro-propagation. Proliferation of shoot tips to regenerate shoots will provide ideal protocol for viral elimination in pigeon pea. The method of regeneration of pigeon pea from cotyledonary node explants and shoot tips via multiple shoot initiation described in this investigation is similar to that for *Phaseolus*¹¹.

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