BIOLOGIA PLANTARUM 47 (1): 121-124, 2003/4

BRIEF COMMUNICATION

Micropropagation of *Sesbania sesban* from the mature tree-derived explants

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Abstract

The nodal and internodal explants excised from the orthotropic shoots of *Sesbania sesban* var. *bicolor* elicited the development of shoots directly from the explants as well as *via* an intervening callus phase on Nitsch (N) medium. On benzyladenine (BA) supplemented media, the adventitious shoot buds developed involving a callus phase. An average of 8.9 ± 4.1 shoots developed per nodal explant on N medium containing 0.5 mg dm^{-3} BA in 95 % cultures, whereas 65 % cultures of internodal explants developed shoots with an average of 5.9 ± 3.6 shoots per explant on N medium supplemented with 1.0 mg dm^{-3} BA. On kinetin (Kn) supplemented medium shoots developed directly from the surface of both the explants at all the concentrations tried. Nodal explants on N medium supplemented with 1.5 mg dm^{-3} Kn developed an average of 12.5 ± 7.9 shoots per explant in 100 % cultures, while internodal explants induced an average of 11.6 ± 7.4 shoots per explant in 75 % explants at 0.5 mg dm^{-3} Kn. The *in vitro* regenerated shoots developed roots when implanted on N medium supplemented with 2 mg dm^{-3} indole-3-butyric acid (IBA), after 30 d of inoculation. The *in vitro* developed plantlets were initially acclimatized under controlled conditions for four months, prior to their transfer to the field.

Additional key words: caulogenesis, Egyptian cattle pod, tree legume.

Micropropagation is an important technique of tissue culture, which ensures rapid and mass propagation of trees in limited time and space. For clonal propagation usually the explants derived from the mature trees are preferred as they are selected on the basis of past experience.

Sesbania sesban var. *bicolor*, commonly known as Egyptian cattle pod, is widely distributed from Africa to Asia. It is a fast growing avenue tree widely used as windbreak and shade tree for coffee, tea or turmeric plantations. It is also used for agroforestry and community forestry programs due to its high nitrogen fixing capacity and the ability to grow on the marginal, dry and degraded lands. The genus *Sesbania* comprises of about 50 species, but the *in vitro* morphogenic potential of this genus has not been thoroughly worked out. The present communication describes the regeneration protocol for *Sesbania sesban* var. *bicolor*, employing explants derived from the mature trees.

The young orthotropic shoots of *Sesbania sesban* var. *bicolor* (Wight & Arn.) were collected during January - March from the trees growing in the botanical garden of the University of Delhi. The offshoots after defoliation were washed with 1 % (v/v) polysan (*Polypharm Ltd.*, Mumbai, India) for 15 min and then left in running tap water for over an hour. The shoots were surface sterilized

Received 11 March 2002, *accepted* 22 November 2002.
Abbreviations: BA - benzyladenine; B5 - medium of Gamborg *et al.* (1968); IBA - indole-3-butyric acid; Kn - kinetin; MS - medium of Murashige and Skoog (1962); N - medium of Nitsch (1969); SH - medium of Schenk and Hildebrandt (1972).
Acknowledgements: The authors are thankful to the Council of Scientific and Industrial Research (CSIR), New Delhi for financial assistance in form of a research project to KN and SCG and a Senior Research Fellowship to AJ.
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by treating them with 0.1 % (m/v) mercuric chloride for 10 min and rinsed thoroughly with sterile distilled water four to five times. The explants were finally treated with 70 % (v/v) alcohol for 5 min, prior to inoculation. Under aseptic conditions, the explants were placed on MS medium (Murashige and Skoog 1962), B5 (Gamborg *et al.* 1968), White's medium (White 1943), SH medium (Schenk and Hildebrandt 1972) and N medium (Nitsch 1969) supplemented with 0.8 % agar (*Qualigens*, *Glaxo Fine Chemicals*, Mumbai, India) and 3 % sucrose (*Daurala Sugar Works*, Meerut, India). The medium was supplemented with different concentrations of benzyl-adenine (BA) or kinetin (Kn) alone. pH of media was adjusted to 5.8 with 1M HCl/1M NaOH prior to autoclaving at 1.05 kg cm^{-2} at 121 °C for 15 min. All the cultures were incubated at temperature of 25 ± 2 °C, relative humidity of 55 ± 5 % and maintained under 16-h photoperiod with irradiance of 40 μmol m^{-2} s^{-1} provided by cool daylight fluorescent tubes; 40 W (*Crompton*, Mumbai, India).

The *in vitro* developed shoots were rooted on N medium supplemented with 3 % sucrose, 0.8 % agar and different concentrations (1.0, 2.0 or 3.0 dm^{-3}) of indole-3-butyric acid (IBA). For hardening, 8 to 9-week-old rooted shoots were initially transferred to small disposable cups containing sterile mixture of *1*) sand:soil (1:1), *2*) sand:soil:vermiculite (1:1:1), and *3*) cowdung: sand:soil (1:1:1). These plantlets were initially irrigated with dilute inorganic salt solution of N medium for 10 - 15 d followed by tap water. After acclimatization for four months under culture room conditions (25 ± 2 °C, 55 ± 5 % RH and irradiance of 40 μmol m^{-2} s^{-1}), these were transferred to the field.

The response has been expressed in terms of percentage of responding explants, average number of shoots per explant and average length of shoots. A completely randomized block-design with two replications was used and the data were subjected to analysis of variance by Fischer's least significant difference (P = 0.05). Data expressed as percentage response were arcsine transformed before analysis. Each experiment was repeated at least once maintaining the same number of explants.

The offshoots collected during the months of January - March elicited caulogenic potential, while those collected during other months of the year failed to do so.

The nodal explants developed pale yellow-brown callus at the cut ends as well as on the surface of the explants within 7 d of inoculation on all the basal media (B5, MS, N, SH and White's). Shoot buds differentiated directly from the explants after 20 d of inoculation. The growth of the shoots increased significantly following subculturing, after 4 weeks. The shoots also differentiated from the calli. The percentage of explants developing shoots varied significantly (27 - 68 %) on all the five media tried. N medium proved to be the best, where 68 % explants developed shoots with an average of 2.1 shoots per explant having an average shoot length of

Table 1. Caulogenic response of nodal and internodal explants on different basal media, after 60 d of culture. Means ± SE, n = 48. Values followed by the same letter in each column are not significantly different (P = 0.05). Relative amount of calli ++ - moderate, +++ - profuse.

Basal medium	Responding explants [%]		Number of shoots [explant^{-1}]		Shoot length [cm]	
	node	internode	node	internode	node	internode
B5	64b++	6c+++	1.2 ± 0.6c	1.0 ± 0.0c	1.1 ± 0.6d	0.0 ± 0.0d
MS	85a+++	7c+++	1.7 ± 0.4b	1.0 ± 0.0c	0.9 ± 0.3d	0.5 ± 0.0c
N	68b+++	28a++	2.1 ± 2.1a	3.0 ± 1.6a	3.7 ± 2.5a	3.4 ± 1.5a
SH	27d++	0d+++	1.2 ± 0.4c	0.0 ± 0.0d	2.8 ± 2.3b	0.0 ± 0.0d
White	42c+++	20b++	1.5 ± 1.0b	2.1 ± 1.5b	1.9 ± 1.0c	1.4 ± 0.5b

Table 2. Caulogenic response of nodal and internodal explants on N medium supplemented with different concentrations of Kn, after 60 d of culture. Means ± SE, n = 48. Values followed by the same letter in each column are not significantly different (P = 0.05). Relative amount of calli ++ - moderate, +++ - profuse.

Kinetin [mg dm^{-3}]	Responding explants [%]		Number of shoots [explant^{-1}]		Shoot length [cm]	
	node	internode	node	internode	node	internode
0	42b++	10c++	2.0 ± 1.1d	1.5 ± 0.5d	2.2 ±1.6a	1.0 ± 0.0c
0.1	100a++	12c++	1.4 ± 0.9d	1.0 ± 0.0d	2.9 ± 1.7a	2.0 ± 0.0b
0.5	24c+++	75a+++	1.3 ± 0.4d	11.6 ± 7.4a	1.1 ± 0.9c	1.3 ± 0.2c
1.0	90a+++	18c+++	5.2 ± 4.8c	2.3 ± 0.5c	1.8 ± 0.7b	0.0 ± 0.0d
1.5	100a+++	70a+++	12.5 ± 7.9a	6.3 ± 4.6b	1.7 ± 0.6b	3.3 ± 0.8a
2.0	91a++	50b++	8.0 ± 6.5b	7.5 ± 4.4b	0.9 ± 0.6c	0.0 ± 0.0d

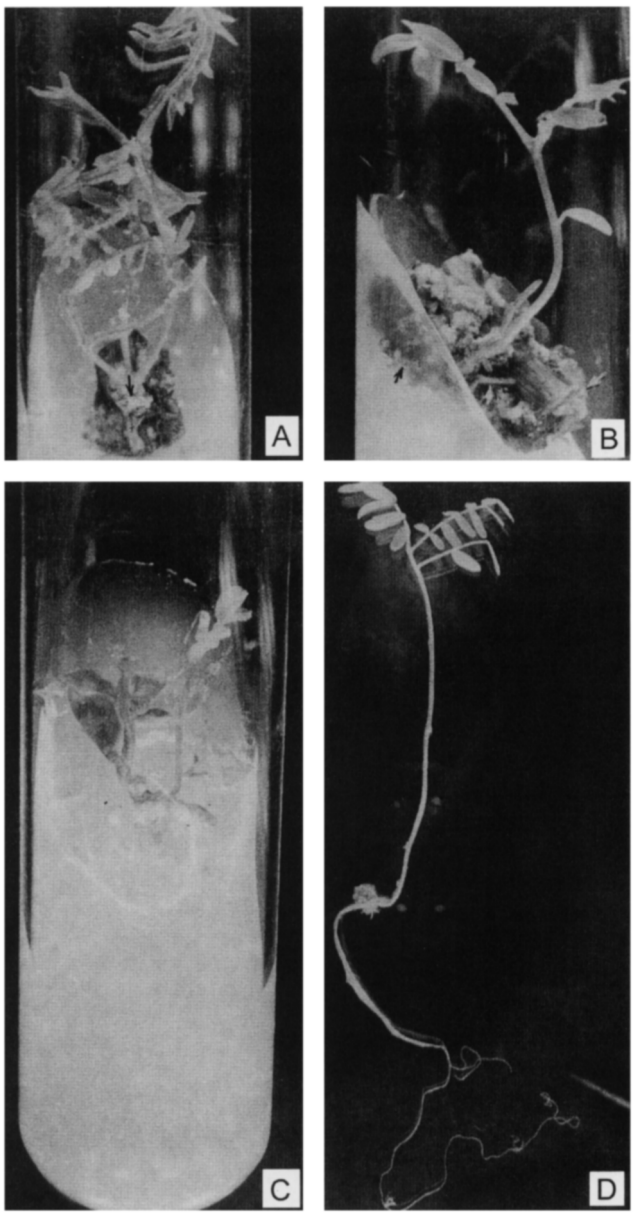

Fig. 1. *A* - Nodal explants of *Sesbania sesban* with profuse nodular compact calli with many adventitious shoots along with few elongated shoots developed on N medium + 0.5 mg dm^{-3} BA. after 57 d of inoculation (×2.1). *B* - Nodal explants of *Sesbania sesban* with multiple shoots developed on N medium + 1.5 mg dm^{-3} Kn, after 57 d of inoculation (×1.5). *C* - Internodal explants of *Sesbania sesban* with multiple shoots developed on N medium + 0.5 mg dm^{-3} Kn, after 57 d of inoculation (×0.8). *D* - *In vitro* regenerated plantlet of *Sesbania sesban* just prior to transfer to soil (×1.8).

3.7 ± 2.5 cm (Table 1). Likewise, for the internodal explants too, N medium proved to be the best. Though the percentage of explants developing shoots was more on White's medium, the average number of shoots per explant as well as the average shoot length was best on N medium. SH medium completely inhibited the caulogenic response. On B5 and MS medium, the internodal explants elicited a very low percentage of response, *i.e.* 6 % and 7 %, respectively (Table 1).

To accentuate the caulogenic response, N medium was supplemented with BA and Kn. Incorporation of BA enhanced the development of nodular, compact calli at all the concentrations. The shoot buds developed from the callus in next 10 - 15 d at all the concentrations. A maximum of 95 % explants developed adventitious shoots at concentration 0.5 mg dm^{-3} with an average of 8.9 ± 4.1 shoots per explant (Fig. 1*A*). The addition of Kn to the basal medium induced the development of multiple shoots from the surface of the explants without an intervening callus phase. The caulogenic response was promoted at the higher concentration, 1.5 mg dm^{-3} being the best (Fig. 1*B*). At this concentration, a maximum of 12.5 ± 7.9 shoots developed per explant with an average shoot length of 1.7 ± 0.6 cm. The elongation of shoots was better at the lowest concentration (0.1 mg dm^{-3}) of Kn (Table 2). The development of shoots on the Kn supplemented media was also accompanied by the development of nodular callus on the surface of the explants.

The internodal explants when cultured on BA supplemented N medium induced the development of nodular calli from the cut ends as well as on the surface of the explants. The adventitious shoot buds developed from the callus within 10 - 15 d of culture at all the concentrations of BA tried similar to the nodal explant. For internodal explants, 0.5 mg dm^{-3} BA proved to be the best, where an average of 9.3 ± 4.9 shoots developed per explant. The percentage of responding explants was more on 1 mg dm^{-3} BA. At all the concentrations of BA tried the elongation of shoots was drastically inhibited (data not shown). Pale yellow compact callus developed at the cut ends of the explants at all the concentrations of Kn within 15 d of culture. After 50 d the shoots developed directly from the surface of the explant as well as from the cut ends. The maximum response in terms of percentage response (75 %) as well as the average number of shoots per explant (11.6 ± 7.4) was recorded at 0.5 mg dm^{-3} Kn (Fig. 1*C*; Table 2). The shoot length was best at 1.5 mg dm^{-3} but at the higher concentrations of Kn, defoliation of leaves took place. The caulogenic response was least at the lowest concentration of Kn, *i.e.* 0.1 mg dm^{-3} (Table 2).

On the basal medium the development of roots took place after 2 weeks of inoculation however, the roots remained stunted. To further facilitate the root induction, 1 - 4 cm long *in vitro* regenerated shoots were implanted on N medium supplemented with different concentrations (0, 1.0, 2.0 or 3.0 mg dm^{-3}) of IBA. The incorporation of IBA enhanced the rooting of the shoots significantly. The induction of roots was accompanied with development of compact calli at all the concentration of IBA. A

maximum of 70 % shoot developed roots at 3 mg dm^{-3} IBA. Though the percentage shoots developing roots was less at 2 mg dm^{-3} IBA as compared to the 3 mg dm^{-3}, it was selected to be optimum, as the explants developed moderate calli and the elongation of roots was best at this concentration (Table 3).

Table 3. Effect of different concentrations of IBA supplemented to N medium on *in vitro* rhizogenesis, after 30 d of culture. Means ± SE, $n = 36$. Values followed by the same letter in each column are not significantly different ($P = 0.05$). Relative amount of calli ++ - moderate, +++ - profuse.

IBA [mg dm^{-3}]	Responding explants [%]	Number of roots [$shoot^{-1}$]	Root length [cm]
0	20c+++	1.9 ± 1.0d	2.5 ± 1.2c
1.0	29c+++	2.5 ± 1.5c	3.4 ± 1.3b
2.0	50b++	3.0 ± 1.9b	7.8 ± 1.5a
3.0	70a+++	4.3 ± 1.7a	1.6 ± 1.4d

In vitro raised 30-d-old plantlets (Fig. 1*D*) were transferred initially to small cups containing the combinations of autoclaved mixtures: *1*) sand:garden soil (1:1), *2*) garden soil:sand:vermiculite (1:1:1), and *3*) garden soil:sand:cow dung (1:1:1). The combination of garden soil and sand supported the growth of transferred plantlets best. The plantlets were initially irrigated with dilute inorganic salt solution of N medium for 10 - 15 d followed by the tap water. The plantlets were covered by perforated polythene bags to check the excess loss of water. They were maintained under controlled conditions for 3 - 4 months prior to transfer to earthen pots. The plants grew normally with no phenotypic aberrations.

To conclude, the present communication describes the regeneration protocol(s) for *S. sesban* var. *bicolor* employing nodal and internodal explants derived from the mature tree. Employing this protocol a large number of plants can be regenerated throughout the year from the elite tree therefore, eliminating the dependence on seeds for the large scale use of this taxon in agroforestry and social forestry programmes.

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