BIOLOGIA PLANTARUM 44 (4): 603-606, 2001

BRIEF COMMUNICATION

Micropropagation of *Cardiospermum halicacabum*

S. BABBER, K. MITTAL, R. AHLAWAT and T.M. VARGHESE

Department of Botany, CCS Haryana Agricultural University, Hisar-125004, Haryana, India

Abstract

The *in vitro* studies with *Cardiospermum halicacabum* indicated that the different explants, *i.e* cotyledon, hypocotyl, cotyledonary node, leaf , internode and node had the potential to produce calli on Murashige and Skoog (MS) medium supplemented with benzylaminopurine (BAP) and napthalene acetic acid (NAA). Calli of different explant origin showed variable growth responses on different BAP concentrations. The shoots were favourably formed from the calli of leaf and cotyledon explants. The maximum number of shoots were produced from calli subcultured on MS + BAP (17.8 μM). The roots were initiated on growth regulator free MS medium.

Additional key words: benzylaminopurine, *in vitro* culture, medicinal plants, plantlets multiplication.

Plants are not only the sources of food, fuel and fibre but also a rich source of medicines. Natural plant products are biologically more important and less toxic to human being as compared to synthetic ones. Collection of medicinal plants on a mass scale from natural habitats is leading to the depletion of plant resources. For the conservation of these valuable genotypes micropropagation is of special use.

Cardiospermum halicacabum L. (family *Sapindaceae*) leaves contain some pharmaceutically important compounds like saponin, quebrachitol, stigmasterol and apigenin. In the present investigation an attempt has been made to study micropropagation of this species. The purpose of this study was to initiate and maintain the calli produced from tissues of various plant parts (hypocotyl, cotyledon, cotyledonary node, leaf internode and node) and to define the nutritional conditions for regeneration.

Seeds of *Cardiospermum halicacabum* L. were surface sterilized for 1 min in 70 % (v/v) ethanol and for 20 min in 2.8 % (v/v) sodium hypochlorite solution, and washed 3 - 4 times continuously with sterilized double distilled water. The seeds were aseptically germinated on Murashige and Skoog (1962; MS) medium solidified with 8 g dm^{-3} *Bactoagar*. After 15 d various explants (hypocotyl, cotyledon and cotyledonary node) of seedlings were excised and used for inoculation. Similarly, various explants like leaf, internode and node collected from potted plants were subjected to surface sterilization with ethanol and sodium hypochlorite solution as mentioned earlier. The size of leaf and cotyledon explants varied from 0.5 to 1.0 cm^2, while hypocotyl, cotyledonary node, internode and nodal explants size was approximately 1.0 cm long. Three to five explants were placed in each flask.

MS medium was used without additives as well as supplemented with growth regulators like naphthalene acetic acid (NAA; 2.7 and 5.4 μM) and benzylaminopurine (BAP; 2.2 - 8.9 μM) in different combinations. Callus induction [%] was recorded 21 d after inoculation. For differentiation and regeneration 28-d-old calli from different explant sources were subcultured on MS medium solidified with 8 g dm^{-3} *Bactoagar* containing 30 g dm^{-3} sucrose and 8.9 - 26.7 μM BAP (15 flasks for each concentration). Cotyledonary nodes and nodal explants were placed directly on MS medium supplemented with different BAP concentrations. After 45 d the number of sprouted axillary buds was recorded.

The shoots formed from the calli as well as directly from the nodal explants were cut and subcultured to root-induction media (Table 3). After rooting the plantlets were transferred into small pots containing sterilized sand and soil (1:1) mixture. These pots were supplied with

Received 3 November 2000, *accepted* 8 March 2001.
Fax: (+91) 1662 34952, e-mail: babber.s@usa.net

half strength MS salt solution and covered with plastic bags to maintain high relative humidity. These were then placed in growth chamber at 25 ± 1 °C under irradiance of 25 μmol m^{-2} s^{-1} during 16-h photoperiod. After 30 d the plastic bags were removed and plantlets along with soil mixture transferred to the pots filled with garden soil and kept in the greenhouse.

On MS medium without growth regulators about 50 % callusing occurred after 15 - 20 d. However, the best callusing was observed at 5.4 μM NAA + 4.4 μM BAP for all the explants. The frequency of callus induction was 100 % for hypocotyl, leaf and cotyledonary explants while in cotyledonary node, node and internodal explants it varied from 82 - 95 %. The calli were induced between 9 - 12 d after inoculation in cotyledon, hypocotyl, leaf and internode segments on BAP (4.4 μM) + NAA (5.4 μM) combination, while they were delayed upto 18 d in cotyledonary node and nodal segments. From the response to various NAA + BAP combination it was noticed that a deviation in BAP concentration beyond 4.4 μM decreased callus induction. The role of auxin and cytokinin in callus induction was also proved by Rao and

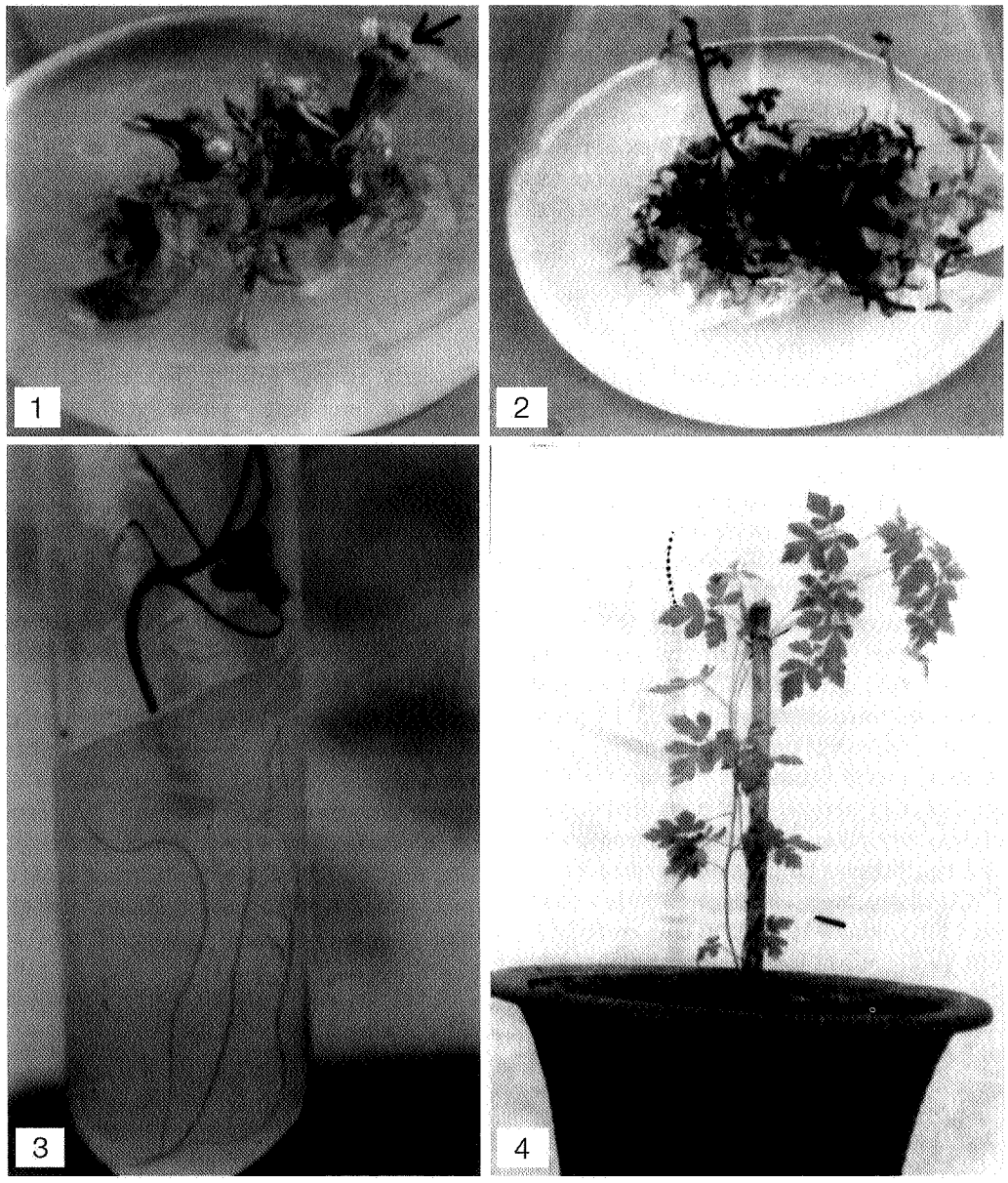

Fig. 1. The initiation of the callus from the cut ends of cotyledons.
Fig. 2. Formation of multiple shoots from the leaf callus.
Fig. 3. Formation of roots from cut shoot on hormone free MS medium.
Fig. 4. The acclimatized potted plant generated from leaf callus.

De (1987) in *Albizzia lebbek* and Kiran (1992) in *Cassia roxburghii*. The calli were maintained by subculturing on fresh medium after 30 d.

Calli from different explant sources (450 - 500 mg fresh mass) were transferred to MS medium with variable concentrations of BAP (8.9 - 26.7 μM). All the concentrations of BAP were effective for morphogenesis (Fig. 2), however, the efficiency varied with the concentrations of BAP as well as calli source (Table 1). There was linear correlation between the increasing concentration of BAP (upto 17.8 μM) and the frequency of multiple shoot formation. Such variability in the regenerative potential by the explants has also been reported in jute species (Singh and Chaturvedi 1997), peanut (Radhakrishnan *et al.* 2000, and *Tamarindus* (Jaiwal and Gulati 1991). In the present study, the leaf and cotyledonary calli were the most efficient to produce a considerable number of shoots.

Table 1. Number of shoots produced from callus from different explants on MS medium supplemented with BAP at different concentration after 45 d. Means ± SE, $n = 15$.

Explant	BAP [μM] 8.9	13.3	17.9	22.2	26.7
Cotyledon	6 ± 0.2	15 ± 1.5	25 ± 2.2	20 ± 1.5	21 ± 1.5
Leaf	4 ± 0.3	12 ± 1.2	20 ± 1.8	18 ± 1.7	19 ± 2.2
Hypocotyl	-	-	2 ± 0.2	1 ± 0.0	2 ± 0.3
Internode	-	-	2 ± 0.2	-	1 ± 0.0

Table 2. Number of shoots produced directly from cotyledonary nodes or nodes on MS medium supplemented with BAP at different concentrations after 45 d. Means ± SE, $n = 15$.

Explant	BAP [μM] 8.9	13.3	17.9	22.2	26.7
C. node	5 ± 0.7	10 ± 0.8	21 ± 1.5	19 ± 1.7	20 ± 1.5
Node	10 ± 1.2	8 ± 1.2	20 ± 1.2	15 ± 0.9	18 ± 1.3

The regeneration of shoots was also achieved directly from the cotyledonary node and nodal segment (Table 2) and it was also observed that the maximum number of shoot buds proliferated on 17.8 μM BAP. Lower concentration of BAP produced less number of shoots. The reason for the shoot proliferation directly from the explant like cotyledonary node and nodal segment could be attributed to the presence of pre-existing primordia which proliferated into shoots under adequate amount of cytokinin supplied in the nutrient medium which nullify the effect of apical dominance and thus enhanced the proliferation of lateral buds from the axis. This supports the studies of Vinod (1994) in chickpea, Rajkumar (1995) in *Camellia sinensis* and Harikrishnan and Hariharan (1996) in *Plumbago rosea*. The shoots when separated and implanted on root initiating media (Table 3) resulted in root initiation. Maximum number of roots were initiated on hormone free MS (full strength) medium (Fig. 3). This indicated that endogenous auxins are sufficient to induce rooting. Matsuoka and Hinata (1979) and Jyoti (1993) also reported initiation of roots on hormone free medium. After the root formation, the plantlets were transferred to pots containing sterilized soil and sand mixture and MS solution (Fig. 1). After one month, the pots were supplied with Knop's nutrient medium Later these plants were shifted to the garden soil. Under natural climatic condition these plants grew faster. Morphologically there was not much of the difference between the normal and *in vitro* raised plants except that the stem was comparatively weak (Fig. 4).

Table 3. Number of roots produced per shoot from different explants on MS medium supplemented with auxins in different concentrations [μM] and on full or half-strength hormone free MS medium. Means ± SE, $n = 8 - 10$.

Auxin	Cotyledon	Hypocotyl	Leaf	Node	C. node
NAA, 2.7	9 ± 1.6	2 ± 0.7	10 ± 0.7	10 ± 1.6	7 ± 1.3
NAA, 5.4	14 ± 0.9	2 ± 0.8	11 ± 1.5	12 ± 1.6	7 ± 2.0
IBA, 2,5	5 ± 1.2	-	6 ± 1.2	6 ± 1.2	1 ± 0.9
IBA, 5.0	2 ± 0.7	-	2 ± 0.7	3 ± 0.9	2 ± 0.9
1 MS	26 ± 1.6	4 ± 0.9	23 ± 1.6	19 ± 1.5	14 ± 0.7
1/2 MS	17 ± 1.3	2 ± 0.7	19 ± 1.6	17 ± 1.8	11 ± 1.5

References

Harikrishnan, K.N., Hariharan, M.: Direct shoot regeneration from nodal explants of *Plumbago rosea* Linn. a medicinal plant. - Phytomorphology **46**: 53-58, 1966.

Jaiwal, P.K., Gulati, A.: *In vitro* high frequency plant regeneration of a tree legume *Tamarindus indica* L. - Plant Cell Rep. **10**: 569-573, 1991.

Jyoti, M.: Selection and characterization of cadmium tolerant cells in *Solanum melongena* L. var. H-7. - Ph.D. Thesis. CCS Haryana Agricultural University, Hisar 1993.

Kiran, S.: Study on micropropagation of *Cassia roxburghii* DC through *in vitro* culture technique. - M.Sc. Thesis. CCS Haryana Agricultural University, Hisar 1992.

Matsuoka, H., Hinata, K.: NAA-induced organogenesis and embryogenesis in hypocotyl callus of *Solanum melongena* L. - J. exp. Bot. **30**: 363-370, 1979.

Murashige, T., Skoog, F.: A revised medium for rapid growth

and bioassay with tobacco tissue culture. - Physiol. Plant. **15**: 473-497, 1962.

Radhakrishnan, T., Murthy, T.G.K., Chandran, K., Bandyopadhyay, A.: Micropropagation in peanut (*Arachis hypogaea* L.). - Biol. Plant. **43**: 447-450, 2000.

Rajkumar, R.: Hormonal responses in shoot multiplication of *Camellia sinesis*. - Indian J. Plant Physiol. **38**: 340-341, 1995.

Rao, P.V.L., De, D.N.: Tissue culture propagation of tree legume *Albizzia lebbek* (L.) Benth. - Plant Sci. **51**: 263-267, 1987.

Singh, V.K., Chaturvedi, H.C.: Regenerating ability on *in vitro* grown jute species. - Indian J. Plant Physiol. **2**: 307-309, 1997.

Vinod, S.: *In vitro* studies on the effect of salinity on salt tolerant and sensitive genotypes of chickpea (*Cicer arietinum* L.). - Ph.D. Thesis. CCS Haryana Agricultural University, Hisar 1994.