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In vitro Clonal Propagation of *Cassia tora* L. (Coffee Pod): A Medicinal Plant

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Abstract: *Cassia tora* L. is a leguminous annual herb of tropical part of India. It is an important medicinal plant with commercially valuable seeds. Micropropagation of this medicinal legume via bud culture method has been first time reported here. Nodal explants from field grown plants were used to initiate *in vitro* culture. Heavy black leaching was observed in initiation medium from cut ends of the explants and all the explants were eventually died. Leaching was effectively checked by incorporation of an absorbent and an antioxidant -25 μM polyvinyl pyrrolidone -40 and 476 μM citric acid in the initiation medium, respectively. Best 90% shoot bud initiation with 1.5±0.1 shoots per explant was recorded on MS medium supplemented with 2.2 μM 6-benzyl adenine. Elongated shoots from original explants were divided into nodes and best shoot production was observed on MS basal medium, which also induced roots from the propagule. These shoots on the basal medium were longest (3.3 cm±0.38) also with highest number of nodes. There was no leaching observed at this stage therefore the mixture of polyvinyl pyrrolidone -40 and citric acid was not incorporated into the medium. For better quality roots and 100% rooting in shoots, elongated shoots were transferred into ½ strength MS medium supplemented with 2.5 or 4.9 μM indole-3-butyric acid. Regenerated plantlets were successfully hardened off in greenhouse with 70% survival.

Key words: Micropropagation, medicinal plant, leaching, rooting, acclimatization

INTRODUCTION

India is one of the world's top 12 mega-diversity countries (Singh and Chowdhery, 2002) also major exporters of crude drugs and *Cassia tora* seeds are among the maximum demanded (Planning Commission, Government of India, 2000). India has more than one fourth of the world's medicinal plant species (8,000/30,000). Medicinal plants related trade in India is more than US$ 200 billion annually and approx 7800 manufacturing units are in India. Most of the herbs are collected from forest, consequently the increasing demand for medicinal plants leading to continuous depletion of some of the species and forest land is continuously losing its natural flora at alarming pace. Thus propagation and conservation of the fast depleting genetic diversity of medicinal plants is one of the major concerns and immediate efforts are required in this regard.

Plant tissue culture technique has the capacity to produce large numbers of plants within limited space and time irrespective of the season, under controlled conditions of temperature, light intensity and photo period. Thus *in vitro* technique offers a sustainable and viable tool for rapid propagation, uniform progenies and disease free plants of desired genotypes (Bhojwani and Razdan, 1996). There is a widespread interest in the application of plant tissue culture methods and biotechnological approaches to the production of good variety of medicinal plants and isolation of medicinal secondary products. When compared to traditional agricultural growth techniques, plant tissue culture production of medicinal plants offers a number of unique advantages like possibility of year-round continuous production of plant medicinal compounds under highly controlled conditions (Kayser and Quax, 2007).

Cassia tora is an important medicinal plant, belongs to the family of Fabaceae. It is an annual herb grown in tropical parts of India; pinnate leaves and yellow flowers blooming in the month of August to September. Leaves used in ringworm and other skin troubles; seeds used as substitute for coffee and as a mordant in dying (Ramachandran *et al*., 1992). *Cassia tora* acts as a liver

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stimulant, mild laxative and heart tonic. The consumption of cooked leaves or use of extract of leaves of this plant helps the body in maintaining the normal level of cholesterol. The alcoholic or vinegar maceration of pounded fresh leaves is used externally to treat eczema and dermatomycosis. The extract of leaves of *Cassia tora* acts as a nerve tonic. Its powder proves useful in combating indigestion, toning up heart muscles and purifying blood. It is also used as an antidote in case of various poisonings. The plant extract has been reported to possess hyperglyceimic actions (Mukherjee, 2003). *In vitro* technique has proven as a potential technology for clonal propagation and conservation of medicinal plant species; micropropagation of different species of *Cassia* has been reported: *C. sophera* (Parveen and Shahzad, 2010), *C. siamea* (Parveen *et al*., 2010), *C. angustifolia* (Siddique and Anis, 2007). There is no report so far on *in vitro* clonal propagation on this valuable medicinal plant. The present study was undertaken to develop the *in vitro* technique for clonal propagation of *C. tora*.

MATERIALS AND METHODS

Fresh twigs with nodal segments were collected in September, from healthy natural growing *C. tora* plants, at Pt. Ravishankar Shukla University, Raipur campus; to initiate shoot culture. They were thoroughly washed in running tap water and then treated with 2-3 drops of Tween 80 aqueous solution for 10 min. Now the leaves were trimmed from twigs and divided into nodal segments with single axillary bud. These nodal segments were surface disinfested under laminar air flow cabinet, by quick dip in 70% ethanol followed by 0.2% mercuric chloride treatment for 15 min. Surface disinfectant was decanted and nodal segments were rinsed 3 to 4 times in sterilized distill water. Surface disinfested nodal explants were again cut aseptically in to final size <1 cm, using sterilized scalpel; to remove the cutting ends damaged during the surface disinfection. Each nodal explant was transferred individually onto shoot bud initiation medium. Murashige and Skoog (1962) (MS) medium as used to culture *C. tora*. For culture initiation, MS medium was used with different concentrations of 6-Benzyl Adenine (BA); the initiation medium also contained 25 μM polyvinyl pyrrolidone-40 (PVP-40) and 476 μM citric acid. Sucrose (3%) was used as carbon source and media were solidified with 0.8% agar. The media were adjusted to pH 5.7 with 1 N NaOH and sterilized by autoclaving for 20 min at 1.05 kg cm^{-2} pressure at 121°C. The cultures were incubated in 16 h light and 8 h dark regime, at 25°C±2 with 40 μmol m^{-2} sec^{-1} light intensity provided by cool white fluorescent tubes. After 4 weeks of incubation, observations have been recorded for bud break, shoot number, shoot length, number of nodes and root induction in nodal explants.

For shoot multiplication, shoots which were elongated on original nodal explants were excised, there leaved were trimmed and divided into nodal segments. Then the nodal segments were transferred aseptically onto multiplication medium for 4 weeks of incubation. MS medium with different levels of BA was tested for optimum shoot production; however PVP and citric acid were not incorporated into the multiplication medium.

Elongated shoots were harvested individually from multiplication medium and transferred vertically into rooting medium. Rooting medium is ½ strength MS medium with or without Indole-3-Butyric Acid (IBA). For acclimatization, plantlets were taken out from vessels and washed with tap water to remove culture medium. Washed plantlets were treated with fungicide solution (1% Bavistin) for 20 min and then transplanted on pots containing coco-pit and irrigated with the same fungicide solution; kept in green house with >80% RH and 30±2°C for 6 weeks. Followed by their transfer into poly-bags filled with soil mixture-sand: Soil: FYM :: 1:1:1. In all the above experiments, at least 10 replicates were taken and repeated thrice. The data were analyzed by Analysis of Variance (ANOVA).

RESULTS AND DISCUSSION

All the nodal explants of *C. tora* inoculated on initiation medium were died, due to excessive black leaching in the medium from the cut end of the explants. Leaching is generally associated with the woody plants; however severe leaching was observed with this annual herb. On the basis of our previous experience with *Azadirachta indica* (Quraishi *et al*., 2004); a mixture of absorbent and antioxidant: 25 μM PVP-40 and 476 μM citric acid had been incorporated, respectively into the initiation medium to overcome this problem (Fig. 1). This treatment successfully prevented the leaching and nodal explants survived and exhibited bud break response (Fig. 2). Also in medicinal plant *Glycyrrhiza glabra*, PVP effectively controlled the leaching during *in vitro* culture (Mousa *et al*., 2007). Similarly citric acid was one of the content of the mixture in initiation medium, used to check leaching from explants of medicinal plant *Leptadenia reticulata* in micropropagation (Arya *et al*., 2003). Nodal explants on MS basal medium showed rooting; however incorporation of BA at any concentration in the initiation

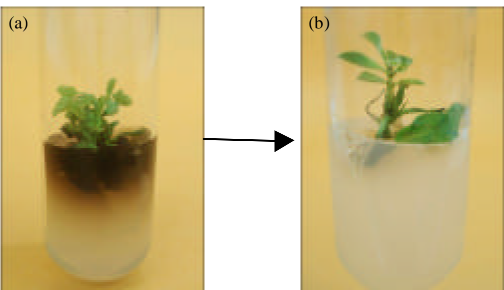

Fig. 1(a-b): *In vitro* leaching control in *Cassia tora* L. nodal explants, on MS medium containing 25 µM PVP-40 and 476 µM citric acid. Plantlet showing leaching plantel without leaching

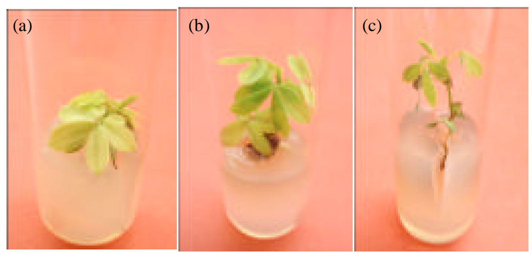

Fig. 2(a-c): *In vitro* shoot bud initiation from *Cassia tora* L. nodal explants, on MS medium containing 25 µM PVP-40 and 476 µM citric acid supplemented with different concentrations of BA, for 4 weeks

Table 1: *In vitro* shoot bud initiation from *Cassia tora* L. nodal explants, on MS medium containing 25 µM PVP-40 and 476 µM citric acid supplemented with different concentrations of BA, for 4 weeks

BA (µM L^{-1})	Bud break (%)	Shoot No.	Shoot length (cm)	Node No.	Rooting (%)
0	90	1.2 ± 0.1^{abc}	2.5 ± 0.4^{a}	2.7 ± 0.3^{ab}	90
0.44	90	1.3 ± 0.1^{abc}	1.3 ± 0.1^{bc}	2.2 ± 0.1^{b}	0
1.11	90	1.3 ± 0.1^{ab}	1.4 ± 0.1^{bc}	2.6 ± 0.2^{ab}	0
2.22	90	1.5 ± 0.1^{a}	1.9 ± 0.2^{b}	3.1 ± 0.3^{a}	0
4.44	80	1.2 ± 0.1^{bc}	1.8 ± 0.2^{b}	2.5 ± 0.2^{ab}	0
8.88	80	1.4 ± 0.1^{ab}	1.1 ± 0.1^{c}	2.8 ± 0.2^{ab}	0
17.78	50	1.0 ± 0^{c}	1.4 ± 0.1^{bc}	1.2 ± 0.1^{c}	0

Values with the same letters in a column are not significantly different

medium checked the rooting response in the nodal explants (Table 1). Maximum shoots induced from nodal explants in presence of 2.2 µM BA containing MS medium.

Elongated shoots were excised and their nodes were used as propagule for further shoot production. There was no leaching observed from these regenerated nodes; therefore PVP-40 and citric acid were not incorporated in the multiplication medium. Best shoot production response was recorded on MS basal medium (Table 2).

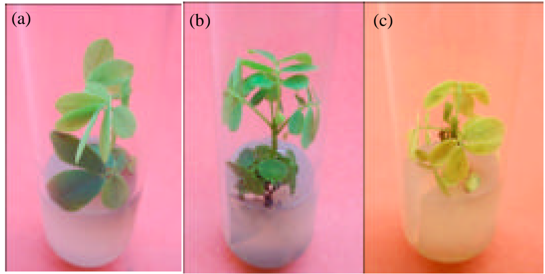

Fig. 3(a-c): *In vitro* shoot multiplication of *Cassia tora* L., on MS medium containing different concentrations of BA, for 4 weeks

Table 2: *In vitro* shoot multiplication of *Cassia tora* L., on MS medium containing different concentrations of BA, for 4 weeks

BA (µM L^{-1})	Bud break (%)	Shoot No.	Shoot length (cm)	Node No.	Rooting (%)
0	90	1.1 ± 0.05^{ab}	3.3 ± 0.38^{a}	3.9 ± 0.27^{a}	90
2.22	90	1.2 ± 0.09^{a}	1.7 ± 0.02^{b}	2.9 ± 0.18^{b}	0
4.44	80	1.0 ± 0^{ab}	1.4 ± 0.13^{bc}	3.0 ± 0.16^{b}	0
8.88	80	1.0 ± 0.03^{ab}	0.9 ± 0.05^{c}	2.2 ± 0.13^{c}	0

Values with the same letters in a column are not significantly different

Table 3: *In vitro* rooting of regenerated shoots of *Cassia tora* L., on ½ MS medium with different concentration of IBA

IBA (µM L^{-1})	Root No.	Root length (cm)	Rooting (%)
0	3.9±.40	4.3±0.21	90
2.5	2.9±0.24	5.5±0.25	100
4.9	3.4±0.34	4.4±0.23	100

Here also 90% rooting was recorded from propagule on basal medium; combined with the longest shoots and hence maximum number of nodes per culture was obtained (Fig. 3). Number of nodes produced in culture is important because they are the source of propagule for further shoot production. The shoots elongated on basal medium were not only much longer but also thick and healthy than those elongated on BA containing MS medium. Better quality shoot production on basal medium was observed may be due to the presence of root; which might improve the absorption of nutrients from the medium. Also probably due to the availability of endogenous hormones and other chemical factors produced by the roots (Bonga, 1987).

Elongated shoots were harvested and transferred on rooting medium. Although roots are present in 90% shoots during multiplication. But elongated shoots were subjected to rooting for the production of better quality roots with secondary branches and also to achieve root induction in all the 100% shoots. On ½ strength MS basal medium, 90% rooting was observed in the shoots but the roots were thin and without secondary branching (Fig. 4). Healthy elongated roots with secondary branches were recorded in 100% shoots in the presence of IBA

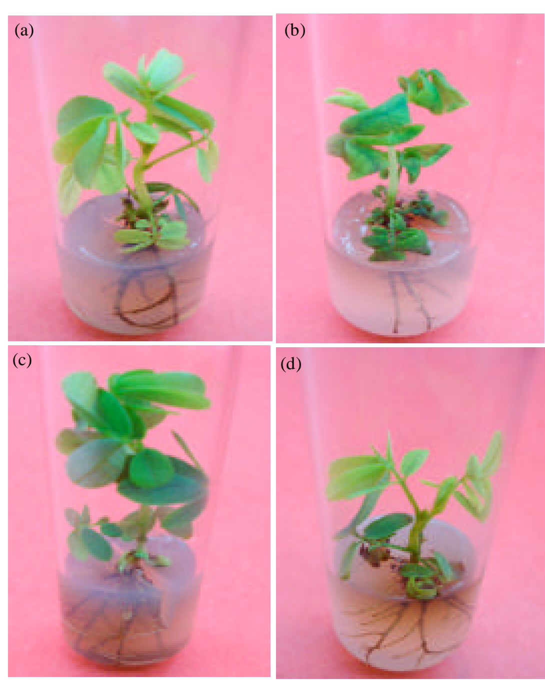

Fig. 4(a-d): *In vitro* rooting in shoots of *Cassia tora* L., on ½ strength MS medium containing 4.9 µM IBA

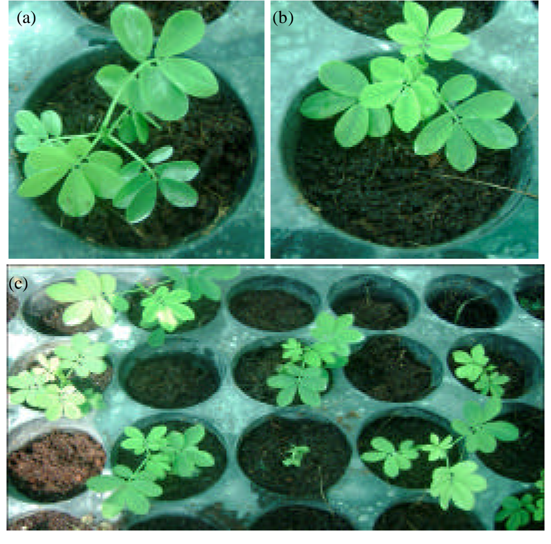

Fig. 5(a-c): Hardened *Cassia tora* plantlets in greenhouse

(2.5 or 4.9 µM) (Table 3). Also in *Cassia siamea*, best rooting (84%) was reported in the same medium: ½ strength MS medium with 2.5 µM IBA (Parveen *et al*., 2010). *C. sophera* shoots showed best rooting (93.7%) in ½ strength MS medium with 1.0 µM IBA (Parveen and Shahzad, 2010). *C. angustifolia* shoots were difficult to root; best 52% rooting achieved in MS medium by pulse treatment with 60 µM IBA and 1% activated charcoal for 1 week (Siddique and Anis, 2007).

Plantlets with the well developed root system were removed from culture medium and hardened in greenhouse as described in materials and methods. These *C. tora* plantlets were successfully acclimatized in with 70% survival rate and all the plantlets exhibited normal morphology compared with naturally grown plants (Fig. 5).

CONCLUSION

Cassia tora L. being a very important medicinal plant has the quality of attracting attention with its medicinal values and properties. Conservation and propagation of all medicinal plant is a very important task to be performed in today's world for the conservation of our floral heritage and get useful products from them. Micropropagation of *Cassia tora* L. plant was performed and an effective protocol for the micropropagation of *C. tora* via nodal explants has been standardized. Micropropagation of *C. tora* was performed including all the four stages till acclimatization, plant gave best response of growth and propagation with lower hormonal concentrations and roots were observed to be well developed with secondary branching. The result of 62.5% plant survival was obtained.

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