International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018 :143-147
UGC Approved Journal No 48951

Original Article Open Access

In-vitro micropropagation of *Cassia absus* (l.) An Indian medicinal plant

Mungole Arvind*, Kharwade Priyanka and Wadekar Mohan

*Department of Botany, N. H. College, Bramhapuri, Dist. Chandrapur
Email: aru.mungole@gmail.com

Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Mungole Arvind, Kharwade Priyanka and Wadekar Mohan (2018) *In-vitro* micropropagation of *cassia absus* (l.) An Indian medicinal plant, *Int. J. of. Life Sciences*, Special Issue, A12: 143-147.

ABSTRACT

In the present study the protocol for callus induction and regeneration in *Cassia absus* was standardized. Young apical leaves, cotyledonary leaves, epicotyle and hypocotyle were used as explants for callus induction on Ms Medium containing IBA and kinetin in different concentrations also used BAP and kinetin in different concentrations. The maximum percentage of callusing was observed on the medium supplemented with 0.8mg/L IBA and 0.1mg/L Kinetin was found to be 100% for cotyledonary leaf & 90% for epicotyle explants. The calli in most of the cultures were brown and soft in nature. Initiation of shooting of *Cassia absus* established from cotyledonary leaf explants on MS medium supplemented with combination of hormones BAP 0.2 mg/L & Kinetin 0.6 mg/L. This study was aimed to develop standard protocol for callus induction, protocol for organogenesis & standardization of media and growth hormonal concentrations which may helps in conservation and cultivation of this species.

Key words: In-vitro Micropropagation, Regeneration, *Cassia absus*.

INTRODUCTION

In-vitro micropropagation is an important tool from rapid multiplication of medicinal plants (Atal Kapur 1982 a&b). *In-vitro* culture is one of the best and most successful examples of commercial application of plant tissue culture technology. The capability to regenerate and propagate plants from cultures cells and tissues is one of the most exciting and useful aspects of In-vitro cell and tissue culture. Increasing demand of those plants, which are specially use for the food and medicine, is one of the cause of their rapid depletion from the natural habitats. In-vitro micropropagation offer a great potential for conservation and large-scale multiplication of such useful species and subsequent exploitation as well as for the extraction of active ingredient. Thus, the exploration of tissue culture technique in medicinal plant is indeed desirable. Therefore, the whole world is diverting towards the multiplication of these plants.

Besides preventing from depletion of stocks of wild plants, the contamination of plant material may lead to inferior quality of product. Tissue culture is one way by which plant material can be supplied in a pure from and continuously throughout the year (Datta, 1993). *Cassia absus* is medicinally important plant, belongs to the family Fabaceae a small herbaceous plant grown as weed in the natural habitat it is widely distributed throughout the worlds tropics and subtropics. It has a long history of use by indigenous and tribal people in Ayurvedic natural herbal medicines. The leaves and fruits of *Cassia absus* is very useful source of drugs most commonly used for medicinal purposes, though the roots have also been studies. The leaves and fruit of *Cassia absus* are widely used to treat wound, ringworm, sores abscesses, ulcers and inflammation (Van der Maesen, 2008). In many parts of Africa and Asia, seeds are used to treat diabetes, conjunctivitis, ulcers and cataract (Ghani et al, 1997, Van der Maesen, 2008; Hussain et al., 2008). The seeds are also considered to be astringent and hypertensive (Aftab et al., 1996) and may help to reduce swelling and prevent hemorrhaging. The seeds and leaves of the plant are most commonly used for medicinal purposes though the roots have also been studied. The leaves are bitter, acrid and have been used traditionally for a cough, diseases of the nose and as an astringent to the bowel. It is regarded as useful enriching the blood as tonic, a bitter astringeunt for the bowels, applied locally to heal ulcers. (Ghani *et al.,*1997). Chloroform, petroleum ether and acetone extract of *Cassia absus* have anti-bacterial (Manjusha et al., 2009), hypertensive and anti-spasmodic effect (Aftab *et al*., 1996) seeds, leaves and roots contain two alkaloids, Chaksine and isochaksine (Aftab *et al.,* 1996).

The seeds also contain oils, fatty acids, sterols and flovonoids. The antroquinones, Chrysophenol and emoclin, isolated from Chaksu roots have laxative effect (Rao et al., 1979) & phytochemical studies involving the extracts of seed have shown antibacterial, antimalerial and blood pressure lowering effect (Aftab *et al*., 1996) A recent Study has also evaluated the antiinfommatory and the anti-histuninic activity of an eye drop formulation containing the seeds of the plant. A lot of work has been done antibacterial, antimalerial activity of this plant (Aftab *et al.,* 1996). A large number of publications on the Chemistry, Pharmacology and several other aspects have been made, but here have been a few reports on in-vitro regeneration of *Cassia absus*. Therefore it has attracted the attention of Botanists, Chemists, Pharmacologists because of its medicinal importance in Ayurvedic mixture. In nature, seed production in this plant is irregular, with a low germination percentage due to the impermeability of the integument. It is highly demanded by the different Pharmaceutical companies. Little work done on *in-vitro* regeneration of *Cassia absus*. Keeping entire importance of this taxa in mind decided to do in-vitro Micropropagation of it. The present study was undertaken to examine the potential of different explants with different concentrations of hormones in combination, to rapid initiation of callus and regeneration.

METHODOLOGY

Cassia absus plant used in the present study was collected from the wild population of campus of N. H. College, Bramhapuri, Dist. Chandrapur. Different explants were used for establishing callus including apical leaf, cotyledonary leaf, epicotyls and hypocotyls. They were washed thoroughly under running tap water for 10 min. subsequently sterilization was carried out in laminar air flow cabinet under asceptic conditions. Then explants were surface sterilized with 0.1% (W/v) mercuric chloride for 2-3 min. followed by 70% ethyl alcohol 2-3 min. then washed 2-3 time sterile double-distilled water and inoculated on agar solidified MS (Murashige & Skoog, 1962) medium Supplemented with different concentration of IBA, Kinetin & BAP in combination. All media contained 3% sucrose & 1% agar with pH 5.8 adjusts before sterilization. For shooting cultured on freshly prepared shooting medium containing MS medium with BAP 0.2 mg/L of kinetin - 0.6 mg/L hormone concentration cultures were maintained at 27 ^{0}C with 10 hr. photoperiod.

RESULT & DISCUSSION

The MS medium supplemented with various concentration of BAP and kinetin, IAA and IBA, IBA and Kinetin inducing callusing. The MS medium supplemented with all this combination showed brown and soft callus induction. The maximum percentage of callusing was observed at the medium supplemented with 0.8mg/L IBA and 0.1mg/L kinetin was found to be 100% for cotyledonary leaf & 90% for epicotyle explants. Callus induction were followed by the hormonal combination supplemented with 0.4mg/L BAP + 0.8mg/L Kinetin i.e. 90% for cotyledonary leaf & 60%

for epicotyle explants with 0.9mg/L IBA + 0.2mg/L Kinetin (Table 1). The MS medium supplemented with hormonal concentration 0.2mg/L BAP + 0.6mg/L Kinetin on which Hypocotyle explant was found to be more responsive and induced callus ie. 90% with brown and soft nature. It is followed by the combination 0.3mg/L IAA + 0.6mg/L IBA ie. 60% callus induction was reported (Table 1).

Table No.1: Induction of callus on MS media supplemented with different concentration of hormones.

Hormone concentrations	Explants used	Percentage of callus induction	Duration of induction of callus in days	Colour and nature of the callus
0.2mg/L BAP + 0.6mg/L Kinetin	Epicotyle	50	20	Brown and soft
	Hypocotyle	100	20	Brown and soft
0.4mg/L BAP + 0.8mg/L Kinetin	Cotyledonary leaf	90	15	Brown and soft
0.3mg/L IAA + 0.6mg/L IBA	Cotyledonary leaf	50	25	Brown and soft
	Hypocotyle	60	25	Brown and soft
0.7mg/L IBA + 0.1mg/L Kinetin	Hypocotyle	50	20	Brown and soft
0.8mg/L IBA + 0.1mg/L Kinetin	Cotyledonary leaf	100	20	Brown and soft
	Epicotyle	90	20	Brown and soft
0.9mg/L IBA + 0.2mg/L Kinetin	Epicotyle	60	25	Brown and soft
	Hypocotyle	50	20	Brown and soft
	Cotyledonary leaf	60	25	Brown and soft

Table 2: Effect of different concentration of hormones on shoot regeneration of *Cassia absus/*

Hormonal concentration	No of shoot per treatment	Shoot length in cm.	Shoot morphology
BAP 0.2mg /L Kinetin – 0.6 mg /L	3	2	Green & long
	2	1.5	Thin Short
	2	1.2	Thin Short
	1	1	Thin Short
	2	1	Thin Short

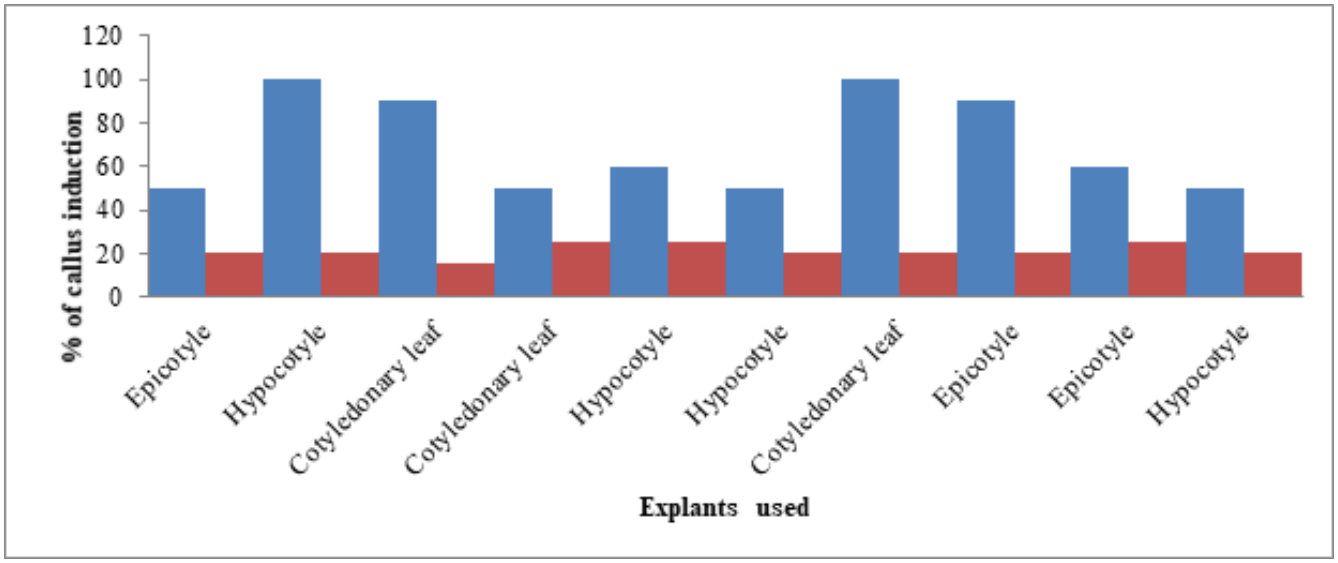
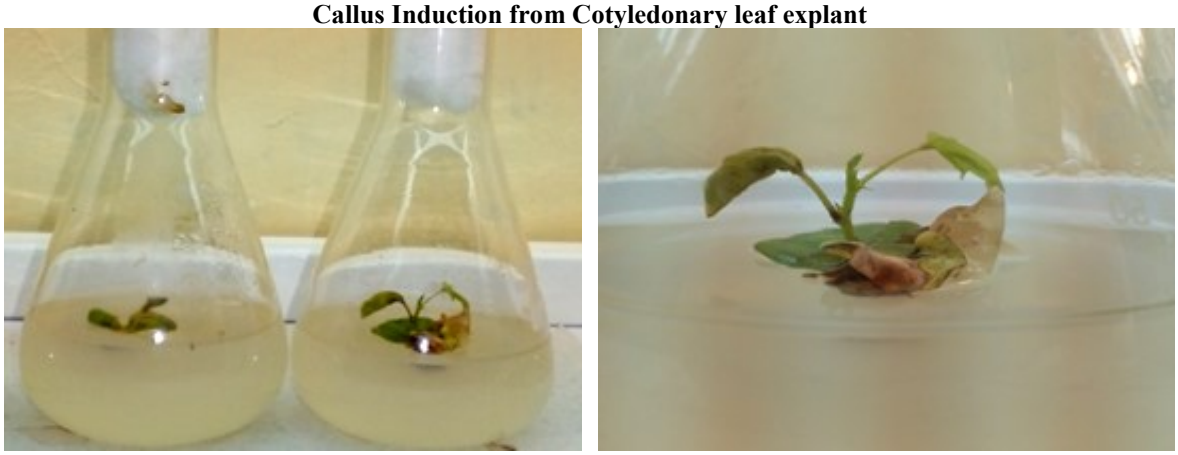


Fig. 1: Response of different explants to callus induction and duration of time

Habit and habitat of the *Cassia absus*

Callus Induction from Cotyledonary leaf explant



Direct regeneration and shooting of *Cassia absus*

Photo plate 1: Showing habit, habitat and different stages of in-vitro regeneration of *Cassia absus*

The cotylodanry leaf found to be more responsive explants on IBA and kinetin. Toker. *et. al.,* (2003) studied the formation of callus using different type of explants like stem, root, leaf and seed of *Ecbollium elaterium* where seed and root explants did not yield callus at all while, stem node and leaf explants formed the callus to a lesser extent. Thus the differential response of various explants can be attributed to differences in cultural requirements of explants and also the variation in endogenous hormone level (Ghosh and Sen, 1994). Further studies were carried out for shoot regeneration capacity by using cotylodanry leaf explants. Shoot were initiated from Cotyledonary leaf explants by showed indirect organogenesis. The best result of shooting (2 cm) was observed with MS medium supplemented with the combination of 0.2mg/L BAP and 0.6mg/L kinetin after. 10th day with good and long morphology in which 3 shoot per treatment wear

recorded. Followed by shoot length (2 cm) was recorded (Table 2). Tissue culture provides the best approach for preservation and multiplication of medicinal herbs. Bera and Roy (2000), proposed the plant tissue culture as a tool for rapid multiplication of plants. Advantages of *in vitro* culture method lie in its ability to produce huge number of true type individuals in a short time and limited space. Tissue cultural techniques a means for conserving and multiplying medicinal plants have been reported by Le (1994), Nin*, et. al.,* (1994) and Wawrosch *et. al.,* (2001).

CONCLUSION

Plant having medicinal importance where collected from wild condition. This lead to gradual depletion due to in discriminate collection. This wild population depletion can be prevented by cultivating such plant for commercial use through In-vitro micropropagation. From the all types of explants collected from *Cassia absus* that is apical leaf, cotyledonary leaf, epicotyls and hypocotyls, cotyledonary leaf was found to be better for callusing on IBA and Kinetin supplemented in MS medium. The best responsive combination of medium is one which supplemented with 0.8mg/L IBA and 0.1mg/L kinetin. Shoot were induced at concentration 0.2mg/L BAP and 0.6mg/L kinetin from cotyledonary leaf explants.

Acknowledgements:
Author is thankful to the Principal N. H. College, Bramhapuri for providing the Tissue culture Laboratory Facility & valuable guidance.

REFERENCES

Aftab K, Rahman Ahmed SI and Usmanghani K (1996). Traditional medicine *cassia absus* L. (Chakshu) pharmacological evalution. Phytomedicine, 2: 213-219.

Atal CK and Kapur BM (1982 a) Cultivation and Utilization of Aromatic plants. Regional research Laboratory CSIR, Jammu Tawi.

Atal CK and Kapur BM (1982 b) Cultivation and Utilization of Medicinal plants. Regional research Laboratory CSIR, Jammu Tawi.

Bera TK, Roy SC (2000) Plant tissue culture. A tool for rapid multiplication of medicinal plants. tylophora indica – the asthuma herb. *J. of the national botanical Society*. 50(1-2): 27-34.

Datta PC (1993) Biotechnology and Tissue culture of some medicinal plants. InGovil JN, SInghVK, Hashmi S (eds) Glimpses in plant research, Medicinal plant, New Vistas of research XI (II): 337-342.

Ghani UK, Saeed A and Alam MT (1997). Industyunic medicine. Department of pharmacognosy, University of Kerachi, Karachi, Pakistan. 310-311.

Ghosh B, Sen S (1994) Micropropagation of AAsparagus cooperi Baker as affected by growth regulators, Plant Sci 82:119-124.

Hussain K, Shahazad A and Hussain SZ (2008). An ethanobotanical survey of important wild medicinal plants of Hattar District, Haripur, Pakistan Ethnobotanical Leafletes, 12: 29-35.

Manjusha W, Aparna K and Sanyogita D (2009). Antibacterial activity and phytochemical analysis of *cassia absus* L. bionfolet – *A Quarterly Journal of Life Sciences*, 6: 326 - 330.

Nin S. Schif S, Bonnici A, Magherini R (1994) In vitro propagation of *Artemissia absinthium* L. Adv Hortic Sci 8: 145-147.

Rao KRV, Rao SJVLN and Vimaladevi M (1979). Phytochemical investigation of *cassia absus* (roots and leaves). *Journal of Natural Products*, 42: 299-300.

Murashige T and Skoog F (1962). *Physiol. Plant* 15:473-497.

Toker G, lu Memie M, Toker MC and Yeilada E (2003). Callus formation and Cucurbitacin B accumulation in Ecballium eleaterium callus cultures. Fitoterapia 74(7-8):618-623.

Van Der MAESEN LTG (2008). Chamaecrista absus (L.) H. S. Irwin & Barneby. In Plant Resources of Tropical Africa (11) – 1 Medicinal plants 1 (Schmelzer, G. H. and Gurib – Fakim, A., Eds). Fondation PRoTA, Wegeningen, The Netherlands, Bockhuys Publishers, Leiden, The Netherland. CTA, Wegeningen, The Netherlands, 181-183.