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Advances in Micropropagation of a Highly Important *Cassia* species- A Review

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1. Introduction

The medicinal properties of plant species have made an outstanding contribution in the origin and evolution of many traditional herbal therapies. Over the past few years, however, the medicinal plants have regained a wide recognition due to an escalating faith in herbal medicine in view of its lesser side effects compared to allopathic medicine in addition the necessity of meeting the requirements of medicine for an increasing human population.

With an ever increasing global inclination towards herbal medicine for healthcare and their boom in recent years has imposed a great threat to the conservation of natural resources and endangered plant species. Currently 4,000-10,000 medicinal plants are on the endangered species list and this number is expected to increase (Canter et al., 2005). Most of the pharmaceutical industry is highly dependent on wild population for the supply of raw material for extraction of medicinally important compounds. The genetic diversity of medicinal plants in the world are getting endangered at an alarming rate because of ruinous harvesting practice and over-harvesting for production of medicines, with little or no regard to the future. Also, extensive destruction of the plant-rich habitat as a result of forest degradation, agriculture encroachments, urbanization, etc. is other factors.

In modern medicine, plants are used as sources of direct therapeutic agents, as model for new synthetic compounds and as a taxonomic marker for the elaboration of more complex semisynthetic chemical compounds (Akerele, 1992). Wide variations in medicinal quality and content in phytopharmaceutical preparations have been observed. These are influenced mainly by cultivation period, season of collection, plant- to- plant variability in the medicinal content, adulterants of medicinal preparations with misidentified plant species, a lack of adequate methods for the production and standardization of the plants, a lack of

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understanding of the unique plant physiology or efficacy with human consumption and consumer fraud. Generally, herbal preparations are produced from field-grown plants and are susceptible to infestation by bacteria, fungi and insects that can alter the medicinal content of the preparations (Murch et al., 2000). Also there is significant evidence to show that the supply of plants for traditional medicines is failing to satisfy the demand (Cunningham 1993). An efficient and most suited alternative solution to the problems by the phytopharmaceutical industry is development of *in vitro* systems for the production of medicinal plants and their extracts.

2. Role of biotechnology

Biotechnology involves modern tissue culture, cell biology and molecular biology offers the opportunity to develop new germplasms that are well adapted to changing demands. Biotechnological tools are also equally important for multiplication and genetic enhancement of the medicinal plants by adopting various techniques such as *in vitro* regeneration and genetic transformation. It can also be harnessed for the production of secondary metabolites using plant as bioreactors (Tripathi and Tripathi, 2003). In addition, modern biotechnology is being increasingly applied for plant diversity characterization and they have a major role in assisting plant conservation programmes.

3. Plant tissue culture

In recent years, tissue culture has emerged as a promising technique for culturing and studying the physiological behaviour of isolated plant organs, tissues, cells, protoplasts and even cell organelles under precisely controlled physical and chemical conditions. Tissue culture can be divided into three broad categories. The most common approach is to isolate organised meristems like shoot tips or axillary buds and induce them to grow into complete plants (Fig1A-F). This system of propagation is commonly referred to as micropropagation. In the second approach, adventitious shoots are initiated on leaf, root and stem segments or on callus derived from those organs. The third system of propagation involves induction of somatic embryogenesis in cell and callus cultures. The commercial technology is primarily based on micropropagation, in which rapid proliferation is achieved from tiny stem cutting, axillary buds and to a limited extent from somatic embryos, cell clumps in suspension cultures and bioreactors. This technique is being used for large scale propagation of a number of plant species viz. *Rauvolfia tetraphylla* (Faisal et al., 2005), *Tylophora indica* (Faisal and Anis, 2003), *Vitex negundo* (Ahmad and Anis, 2007), *Pterocarpus marsupium* (Husain et al., 2007), *Mucuna pruriens* (Faisal et al., 2006), *Balanites aegyptiaca* (Siddique and Anis, 2009). Although there are a number of reviews published on micropropagation of medicinal plants, they do not provide the comprehensive micropropagation reports on *Cassia* species. In this way, the present review highlighted *in vitro* regeneration of medicinally important *Cassia* species, their significance and the wide scope existing for investigations on mass cloning of these plants.

3.1 *Cassia angustifolia* Vahl. (Fabaceae)

Cassia angustifolia Vahl. is a small medicinal shrub commonly known as senna, a valuable drought resistant plant. It is mainly grown as a cash crop in various parts of the world (Anonymous, 1992). The leaves and pods of senna are chief source of anthraquinone, glycoside known as sennosides, which are extensively used as a laxative. It is also used as a

febrifuge in splenic enlargements, anaemia, typhoid, cholera, biliousness, jaundice, gout, rheumatism, tumours, foul breath and bronchitis and in leprosy (Pulliah, 2002). It is employed in the treatment of amoebic dysentery as an anthelmintic and as a mild liver stimulant. Poor seeds viability and low germination frequency restricts its propagation on a large scale. Therefore, micropropagation appears to be an alternative method in order to meet the demand for commercial production of this medicinal plant.

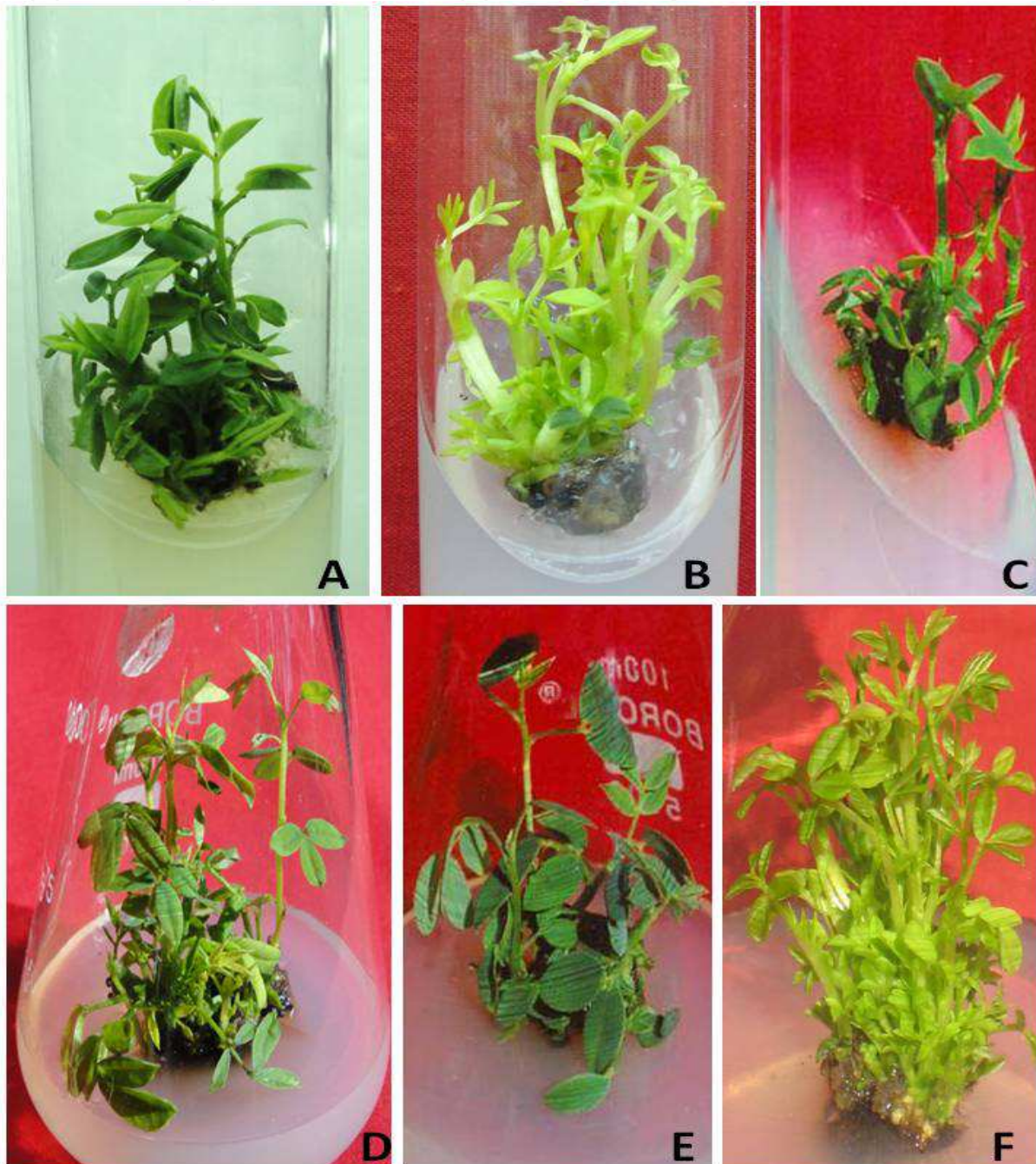


Fig. 1. (A-F). A. Shoots induction in *Cassia occidentalis* on MS medium containing BA. B. Shoot induction in *C. alata*. C. Multiple shoot induction from cotyledonary node explant of *C. angustifolia* D. Multiplication and elongation of shoots from cotyledonary node explant of *C. occidentalis* E& F. Shoot proliferation in *C. angustifolia* and *C. alata*.

3.1.1 Explant type

Multiple shoots were developed successfully from different explants (Cotyledonary node, nodal and shoot tips) excised from *in vitro* grown seedlings of *C. angustifolia* (Agrawal and Sardar 2003; Siddique and Anis 2007 a, b). Among the various explants, cotyledonary node gave the best response (Agrawal and Sardar, 2003), (Siddique and Anis, 2007a, b). In addition, plant regeneration has been successfully developed by using root explants (Parveen and Shahzad 2011). Root explants are advantageous over other explants in terms of their easy manipulation, higher regeneration potential and excellent susceptibility for *Agrobacterium* transformation (Knoll et al., 1997).

In the second approach a much faster rate of multiplication has been obtained through indirect organogenesis. Different explants (Cotyledons, leaflets and petiole) excised from axenic seedlings were used for inducing organogenic callus. Agrawal and Sardar (2006) used cotyledons and leaflets for *in vitro* propagation. Cotyledons were more responsive where 91% cultures produced about 12 shoots per explant. Siddique et al. (2010) used petiole for indirect shoot organogenesis in *C. angustifolia*.

3.1.2 Growth regulators

Plant growth regulators play an important role in growth and development (Little and Savidge, 1987). A range of auxins and cytokinins played a vital role in multiple shoot regeneration in many *Cassia* species. MS medium with optimal quantity of cytokinins (BA, Kn, 2-iP or TDZ) is required for shoot proliferation in many genotypes but inclusion of low concentration of auxins along with cytokinin triggers the rate of shoot multiplication (Tsay et al., 1989). BA individually within the range of 0.5-10.0 μM was common in most of the *in vitro* micropropagated plants. According to Agrawal and Sardar (2003) 1.0 μM 6-benzyladenine (BA) was found best to induce multiple shoots in CN. However, at higher concentration of BA (10.0 μM), a decreasing trend in response in terms of percentage responding explants, average shoot number per explants as well as average shoot length was seen. Also, CN gave the best response in MS medium fortified with 1.0 μM thidiazuron (TDZ). In case of nodal explants best result was obtained with 5.0 μM TDZ and 1.0 μM Indole-3-acetic acid (IAA). Further, transfer of shoot clusters in hormone free MS medium considered to increase the rate of multiplication (Siddique and Anis, 2007a, b). TDZ has also been successfully used to induce shoot bud in root explants. To avoid adverse effect of TDZ, culture were transferred to shoot regeneration medium, where 2.5 μM BA + 0.6 μM NAA gave the maximum response (Parveen and Shahzad, 2011).

In cotyledons and leaflets explants, multiple shoots were observed when green, morphogenic callus (1.0 μM 2,4-D + 1.0 μM BA) was transferred to BA + NAA (Agrawal and Sardar 2006). In case of petiole, highest number of shoots and shoot length was recorded on MS medium along with 5.0 μM TDZ and 1.5 μM IAA (Siddique et al., 2010).

3.1.3 Somatic embryogenesis

Somatic embryogenesis is the most striking confirmation of totipotency, it is a process where groups of somatic cells/tissues lead to the formation of somatic embryos which resemble the zygotic embryos of intact seeds and grow into seedlings on suitable medium. Types of

auxins and its interaction with cytokinins significantly influenced on somatic embryogenesis. Plant regeneration via somatic embryogenesis from single cells, that can be induced to produce an embryo and then complete plant, has been demonstrated in many plant species (Wann et al. 1987). In *Cassia angustifolia*, direct and indirect somatic embryos were observed on MS medium supplemented with auxin alone or in combination with cytokinins (Agrawal and Sardar, 2007). Efficient development and germination of somatic embryos are prerequisites for commercial plantlet production.

Besides this, antimutagenic and genotoxic potential of senna have been reported by Silva et al., (2007). Hence it is a commercially important medicinal plant which has diverse medicinal applications, there is pressing need to conserve the plant by *in situ* and *ex situ* multiplication in general and micropropagation.

3.2 *Cassia siamea* Lam

Cassia siamea Lam. (Caesalpinaceae), is an evergreen tree, commonly planted as an avenue and shade tree in tea estates and found useful for afforestation of degraded and wastelands where organic manure is deficient. It decreases soil erosion, while improving soil fertility in the plantation site, well adapted to a variety of climatic conditions within the tropics and highly resistant to drought. The anthraquinones and cassiamin B present in the plant is an antitumour promoting and chemopreventing agent (Sastry et al., 2003). The root and bark is used in folklore medicine to treat stomach complaints and as a mild purgative. Therefore, a systematic propagation of this valuable tree is important.

3.2.1 Explant type

Various explants, shoot tip, cotyledonary node and nodal segments excised from *in vitro* raised seedlings were used for multiplication. Maximum response was observed with nodal segments in MS macro salts + B₅ micro salts (Sreelatha et al., 2007). In contrast, according to Parveen et al., (2010), cotyledonary node gave the best response in MS medium supplemented with BA and NAA. Gharyal and Maheshwari (1990) used stem and petiole for *in vitro* propagation. Gharyal et al., (1983) used *C. siamea* for androgenesis also.

3.2.2 Growth regulators

Sreelatha et al., (2007) used the different medium MS, B₅ and MS macro salts + B₅ micro salts with various hormones alone or in combinations. 0.1mg/l Kn + 0.1 mg/l TDZ + 2.0 mg/l 2-iP gave the best response on MS macro salts + B₅ microsalts in nodal explants. Parveen et al., (2010) found that MS medium augmented with 1.0 µM BA + 0.5 µM NAA was the best medium for multiple shoot regeneration in CN explants. B₅ basal medium supplemented with 0.5 mg/l IAA + 1 mg/l BA gave the best response in stem segments (Gharyal and Maheshwari 1990).

3.3 *Cassia sophera* Linn

Cassia sophera Linn (Fabaceae) is an important medicinal plant. The whole plant extracts and leaves have expectorant properties, cures cough, asthma and acute bronchitis, anticancer and anti-inflammatory properties. They are specific to eliminate ring worms and also useful

in the treatment of gonorrhoea and syphilis. The bark is used in the treatment of diabetes; wounds and ascites (Anonymous, 1992). The root is administered internally with black pepper for snake bite. The seed extract exhibited important pharmacological effects like analgesic, hypnotic, sedative and antiepileptic effects (Bilal et al., 2005). Nature has provided us a rich store house of herbal remedies to cure most diseases. *In vitro* regeneration is the best alternative to overcome these hurdles. Conventionally *C. sophera* can be propagated through seeds. The seeds however remain viable for a short period and germinated poorly. Because of wide spectrum of its medicinal properties, Parveen and Shahzad (2010) has developed a protocol for rapid multiplication of this valuable plant through cotyledonary node, excised from *in vitro* raised seedlings.

3.3.1 Explant types and growth regulators

Cotyledonary node explants were cultured on Murashige and Skoog medium (MS) supplemented with thidiazuron (TDZ, 0.1 -10.0 μ M). 2.5 μ M TDZ proved to be optimal for the production of maximum number of shoots. For further multiplication and elongation, shoot clusters were transferred to various concentrations of BA. 1.0 μ M BA showed better response.

3.4 *Cassia fistula* Linn

Cassia fistula Linn. (Caesalpiniaceae) commonly known as 'Indian Laburnum' has been extensively used in Ayurvedic system of medicine for various ailments. The whole plant possesses medicinal properties useful in the treatment of skin diseases, rheumatism, anorexia, jaundice, antitumour, antiseptic and antimicrobial (Kirtikar and Basu 1991) and antifungal activity (Gupta, 2010). It possesses hepatoprotective, anti-inflammatory and antioxidant activities (Ilavarasan et al., 2005). Both the leaves and pods were widely used in traditional medicine as strong purgative and laxatives (Kirtikar and Basu 1975, Elujoba et al., 1999) due to presence of sennoside (Van, 1976). In Ayurvedic medicinal system, it was used against various disorders such as pruritus, leucoderma, diabetes and other ailments (Satyavati and Sharma 1989, Alam et al., 1990, Asolkar et al., 1992). Leaves were also found effective against cough and ring worm infections (Chopra et al., 1956, Biswas and Ghose 1973). It is also used in treating bone fracture (Ekanayak 1980). Kuo et al., (2002) have isolated and identified oxyanthraquinones, chrysophenol and chrysophanein from the seeds. Extensive studies have been carried out on its medicinal values and the synergistic actions. Patel et al., (1965) reported analgesic and antipyretic action. Mazumdar et al., (1998) reported sedative and analgesic action of *C. fistula* seeds. Gupta and Jain (2009) have reported hypolipidemic activity of this important legume. It has also been reported for anti-inflammatory (Suwal, 1993), hypoglycaemic activity (Alam et al., 1990; Esposito et al., 1991), antiperiodic (Kashiwada et al., 1990), anti rheumatic (Suwal, 1993), anti-tumor (Bodding, 1983; Gupta et al., 2000), hepato-protective (Bhakta et al., 1999), antioxidant (Luximon Ramma et al., 2002; Sidduraju et al., 2002), anti fungal and anti bacterial activities (Patel and Patel, 1956; Ramakrishna and Indragupta, 1997; Dhar and Qasba, 1984; Perumal et al., 1998).

3.4.1 Explant type and growth regulators

There are only few reports available for *in vitro* regeneration of *C. fistula*. Gharyal and Maheshwari (1990) used the stem and petiole for shoot regeneration. Stem and petiole were

cultured on B5 basal medium supplemented with 2 mg/l NAA + 0.5 mg/l BA (medium a) or 0.5 mg/l IAA + 1.0 mg/l BA (medium b). Medium b gave the best response where well differentiated shoots were developed.

3.5 *Cassia obtusifolia* L Syn *Cassia tora*

It is also an important medicinal plant. The seeds are effective for insomnia, headache, constipation, oliguria, cough, ophthalmia, dacryoliths, omblyopia and hypertension (Purohit and Vyas, 2005). The roots extract contain tannins, flavonoids, alkaloids (Olabiyi et al., 2008), betulinic acid, chrysophanol, physcion, stigmasterol and aloe-emodin (Yang et al., 2006). Doughari et al., (2008) reported that leaf extracts contain the activity against both gram positive and gram negative bacteria and fungi that can therefore be employed in the formulation of antimicrobial agents for the treatment of various bacterial and fungal infections including gonorrhea, pneumonia, eye infections and mycotic infections. Also Joshi (2000) reported that the plant extract is antiviral, spasmolytic and diuretic used against epilepsy, scabies and sores.

3.5.1 Explants and growth regulators

Hasan et al., (2008) used shoot tips for callus induction and shoot regeneration. Shoot tips were cultured in MS medium supplemented with different concentrations and combinations of 2,4-D and Kn. 2.0 mg/l⁻¹ 2,4-D + 0.2mg/l⁻¹ Kn were found best for shoot induction as well as elongation.

3.6 *Cassia occidentalis* Linn

Cassia occidentalis (Linn) (Caesalpinaceae) commonly known as Coffee Senna. It is an ayurvedic plant with huge medicinal importance. It is used for fever, menstrual problems, tuberculosis, diuretic, anemic, liver complaints (Kritikar and Basu 1999). This weed has been known to possess antifungal and anti-inflammatory activity. An infusion of the bark is given in diabetes (Anonymous 1998). Leaf extracts have antibacterial (Jain et al., 1998 and Saganuwan and Gulumbe 2006), antimalarial (Arya et al., 2010), antimutagenic (Tona et al., 1999 and Jafri et al., 1999), antiplasmodial (Sharma et al., 2000), anticarcinogenic (Tona et al., 2004) and hepatoprotective (Sharma et al., 2000) and analgesic and antipyretic (Sini et al., 2010) activity. A wide range of chemical compounds including achrosin, aloe-emodin, emodin, anthraquinones, anthrones, apigenin, aurantiobtusin, campesterol, cassiollin, chryso-obtusin, chrysophanic acid, chrysarobin, chrysophanol, chrysoeriol etc. have been isolated from this plant. Further, micropropagation of *C. occidentalis* Linn is being conducted in tissue culture laboratory of Botany department at AMU Aligarh.

3.7 *Cassia alata* L.

Cassia alata L. (Fabaceae) commonly known as Ringworm Bush is an erect medicinal shrub or small tree distributed mainly in the tropics and subtropics. The plant is a source of chrysoeriol, kaempferol, quercetin, 5,7,4'-trihydroflavanone, kaempferol-3-O-β-D-glucopyranoside, kaempferol-3-O-β-D-glucopyranosyl-(1->6)-β-D-glucopyranoside, 17-hydrotetratriacontane, n-dotriacontanol, n-triacontanol, palmitic acid ceryl ester, stearic

acid, palmitic acid (Liu et al., 2009). *C. alata* leaf is also credited for the treatment of haemorrhoids, constipation, inguinal hernia, intestinal parasitosis, blennorrhagia, syphilis and diabetes (Abo et al., 1998; Adjanahoun et al., 1991). The flowers and leaves are used for the treatment of ringworms and eczema. The other uses of *C. alata* are as an antihelminthic, antibacterial, laxative, diuretic, for treatment of snakebites and uterine disorders (Kirtikar and Basu, 1975). Besides the leaf extract of this species has shown several pharmacological properties such as antimicrobial, antifungal (Khan et al., 2001), antiseptic (Esimone et al., 2008), anti-inflammatory, analgesic (Palanichamy and Nagarajan, 1990) and anti-hyperglycemic activities (Palanichamy et al., 1988). It contains therapeutic (Damodaran and Venkataraman, 1994) and anti-ageing activities (Pauly et al., 2002) also.

3.7.1 Explant and growth regulators

FettNeto et al., (2000) used cotyledonary node along with one third of the hypocotyl and cotyledons. The best result was obtained on 0.5 micro MS salts + 0.38 mg/l⁻¹ of BA and 0.005 mg/l⁻¹ IBA.

3.8 Root development and acclimatization

The induction of roots *in vitro* is an important step in plant micropropagation and genetic transformation. *In vitro* root induction from growing shoots has been achieved in standard media containing auxin and in media in the absence of auxin depending on plant genotype (Rout et al., 1989) (Fig. 2 A-C). There is marked variation in the rooting potential of different plant species and systematic trials are often needed to define the conditions required for root induction. Agrawal and Sardar (2006) examined the effectiveness of various auxins on rooting of *C. angustifolia* microshoots and found that 10.0 µM IBA was superior to IAA or NAA. However 200 µM IBA was best for *ex vitro* rooting in *C. angustifolia* (Parveen and Shahzad 2011). According to Parveen et al., (2010) 2.5µM IBA gave the maximum roots in *C. siamea*. Sreelatha et al., (2007) reported that NAA (1.0 mg/l) + IBA (0.25 mg/l) produced long and well developed roots in *C. siamea*. 0.1 mg/l IAA exhibited the positive effect on root induction in *C. fistula* (Gharyal and Maheshwari 1990).

Prolific rooting on *in vitro* grown microshoots is critical for the successful establishment of these shoots in the greenhouse or field. Plantlets were developed within the culture vessels under low level of light, aseptic conditions, on a medium containing sugar and nutrients to allow for heterotrophic growth and in an atmosphere with high level of humidity. These contribute a culture- induced phenotype that cannot survive the environmental conditions when directly placed in a greenhouse or field. The physiological and anatomical characteristics of micropropagated plantlets necessitate that they should be gradually acclimatized to the environment of the greenhouse or field (Fig 2 D, E). In *C. siamea*, Sreelatha et al., (2007) reported that when micropropagated plantlets were transferred to pots containing (3:1) vermiculite: sand under greenhouse conditions, about 40 % of the plants survived. A high survival 85 % was recorded when plantlets of *C. siamea* were transplanted into 1:1 sterilized garden soil and garden manure (Parveen et. al., 2010). Siddique and Anis (2007) noted the highest survival of *C. angustifolia* when the plants were maintained inside the growth room in sterile soilrite for 4 weeks and eventually transferred to natural soil. Approximately 70 % of rooted plants of *C. obtusifolia* survived in pots containing a 1:1:1 mixture of sterile sand, soil and farmyard manure (Hasan et al., 2008).

Plant name	Explant	Media/ Adjuvant	PGRs used	Response	Optimal response	No of shoots	References
<i>Cassia angustifolia</i>	CN, N, St	MS	BA, Kn	Direct and Indirect	1.0µM BA	2	Agrawal and Sardar, (2003)
<i>C.angustifolia</i>	L, C	MS	2,4-D,BA, Kn,NAA	Indirect	5.0 µM BA+ 0.5µM NAA	12	Agrawal and Sardar, (2006)
<i>C. angustifolia</i>	N	MS	BA, TDZ, IAA, NAA	Direct	5.0 µM TDZ+ 1.0 µM IAA	12	Siddique and Anis, (2007a)
<i>C. angustifolia</i>	CN	MS	TDZ	Direct	1.0 µM TDZ	17	Siddique and Anis, (2007b)
<i>C. angustifolia</i>	C	MS	2,4-D, NAA ,BA,Kn, 2-iP Zeatin	Somatic embryo	10.0 µM 2,4-D+ 2.5 µM BA or 5.0 µM BA	19	Agrawal and Sardar (2007)
<i>C. angustiolia</i>	P	MS	2,4-D,TDZ, BA, Kn	Indirect	5.0 µM TDZ + 1.5 µM IAA	12	Siddique et al., (2010)
<i>C. angustifolia</i>	R	MS	BA, Kn, TDZ IAA, NAA	Indirect	2.5 µMBA+ 0.6 µM NAA	24	Parveen and Shahzad (2011)
<i>Cassia siamea</i>	A	B5/Cocon ut milk	2,4-D, Kn	Indirect	2mg/l 2,4-D, 0.5mg/l Kn, 15% coconut milk	Pollen Embryo-oids	Gharyal et al., (1983)
<i>C. siamea</i>	S, P	B5/PVP, PVPP	BA,IAA, NAA	Indirect	0.5 mg/l IAA + 1mg/l BA	Only green Meriste moid Observed	Gharyal and Maheshwari (1990)
<i>C. siamea</i>	St, CN, N	MS macro salt + B5 micro salt	BA,Kn,2-iP, TDZ, NAA, IBA, IAA	Direct	0.1 mg/l Kn+ 0.1mg/l TDZ+ 2mg/l 2-iP	20	Sreelatha et al., (2007)
<i>C. siamea</i>	CN	MS	BA,Kn, TDZ	Direct	1.0 µM BA +0.5 µM NAA	12	Parveen et al., (2010)
<i>Cassia sophera</i>	CN	MS	BA, TDZ	Direct	1.0 µM BA	14	Parveen and Shahzad, (2010)
<i>Cassia fistula</i>	S, P	B5/PVP,P VPP	BA, NAA, IAA	Indirect	0.5 mg/l IAA 1.0 mg/l BA	–	Gharyal and Maheshwari (1990)
<i>Cassia alata</i>	CN with H, C	MS macro + B5 micro salt	BA, NAA, IBA	Indirect	0.38mg/l BA, 0.05 mg/l IBA		Fett Neto et al., (2000)
<i>Cassia obtusifolia</i>	St	MS	Kn, 2,4-D	Indirect	2 mg/l 2,4-D+ 0.2 mg/l Kn	5	Hasan et al., (2008)

Abbreviations: CN- Cotyledonary node, N- Nodal, St- Shoot tip, L- Leaflet, C- Cotyledon, P- Petiole, R- Root, A- Anther, S- Stem, H- Hypocotyl

Table 1. *In vitro* multiplication of different *Cassia* species

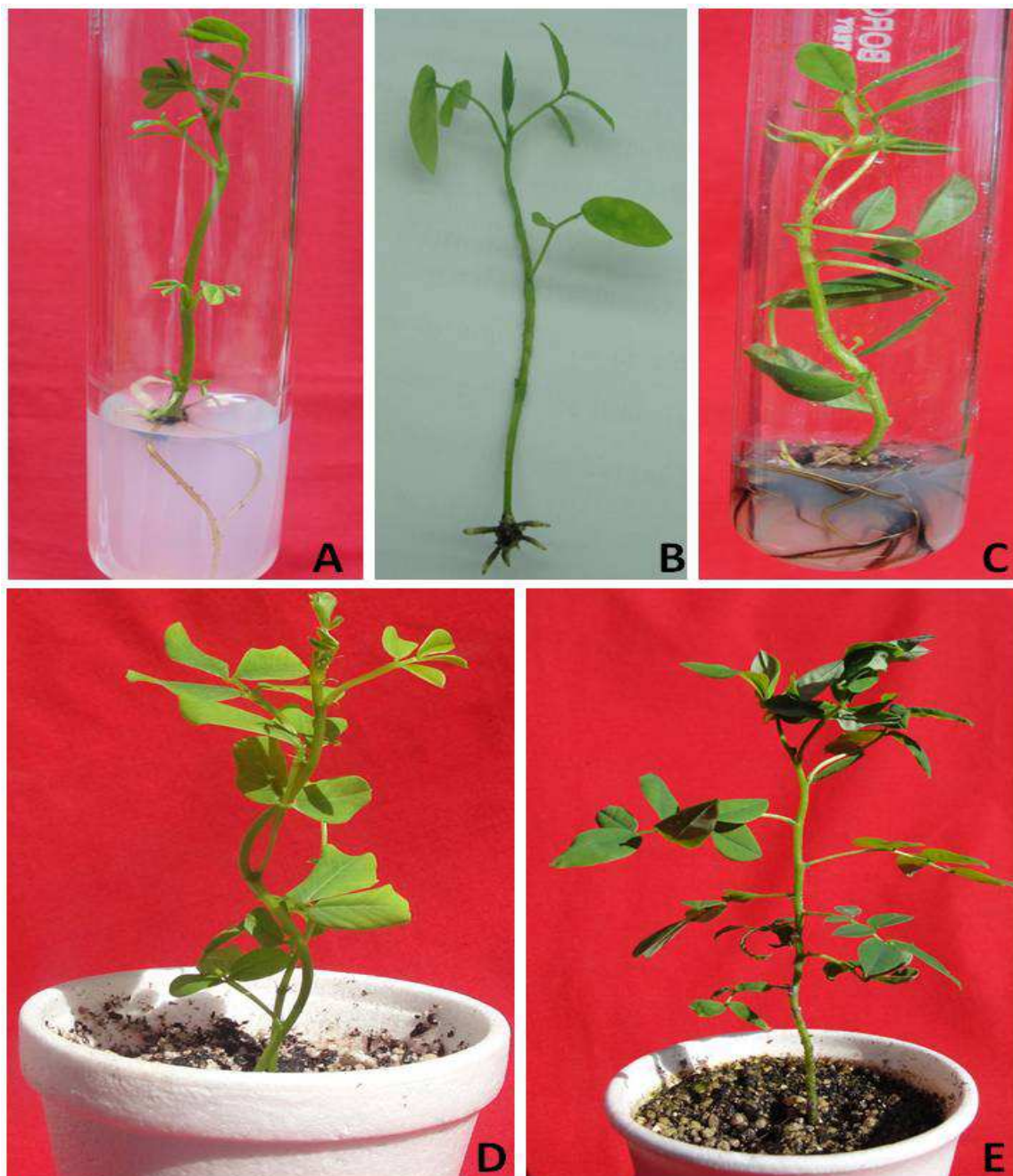


Fig. 2. (A-E) A *In vitro* rooting of *C. alata* B&C. *Ex vitro* and *In vitro* rooting in *C. occidentalis* D&E. Acclimatized plants of *C. alata* and *C. occidentalis*.

4. Conservation strategies used for the propagation

Due to growing demand, the availability of medicinal plants to the pharmaceutical companies is not enough to manufacture herbal medicines. There is need to conserve the economically important plants. Tissue culture techniques have been used as tools for germplasm conservation of rare or threatened as well as medicinal plants (Zornig, 1996).

The utilization of *in vitro* techniques for germplasm conservation is of great interest in plant species (Costa Nunes et al., 2003). The *in vitro* conservation can be for medium and long

periods. The conservation for a medium period is done by decreasing the growth of cultures. The long period conservation is done by cryopreservation techniques (Engelmann, 1998).

The establishment of *in vitro* germplasm banks in developing countries has great importance, but these techniques must be associated with other plant genetic resources conservation practices (Engelmann, 1997). The *in vitro* conservation techniques allow material exchanges among germplasm banks and the germplasm keeps its sanitary conditions and viability during the transport (Ashmore, 1998). The powerful techniques of plant cell and tissue culture, recombinant DNA and bioprocessing technologies have offered mankind a great opportunity to exploit the medicinal plants under *in vitro* conditions.

5. Conclusion and future prospects

The biotechnological strategies have opened up new vistas in all aspects of plant germplasm characterization, acquisition, conservation, exchange and genetic resource management. Future prospects are highly encouraging in terms of the development and application of new techniques and protocols within the context of germplasm conservation. It is useful for multiplying the species which are difficult to regenerate by conventional methods and save them from extinction. Further, the technology delivery with effective dissemination channels has to play a major role in the commercial production of micropropagated plants, it surely needs to be revived and utilized in a broader spectrum rather than confined to publications. For instance, adoption of tissue culture technology will have to facilitate the use of genetically engineered plants as soon as they become available in near future. Furthermore, technology has always to be understood in a dynamic way. Recent developments in transgenic plants can have multidirectional benefits. The benefits range from manipulating generation time, plant protection, wood quality, production of compounds of pharmaceutical value and improvements to polluted soil.

6. Acknowledgement

Authors are grateful to the University Grants Commission, Govt of India, New Delhi for providing research support under UGC- SAP (2009) program. The award of a Research Associateship to I. S. by the Council of Scientific and Industrial Research (CSIR), is gratefully acknowledged.

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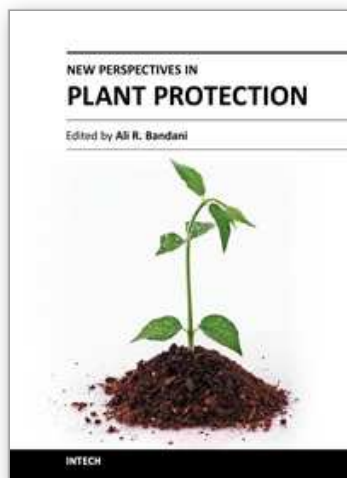
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New Perspectives in Plant Protection

Edited by Prof. Ali R. Bandani

ISBN 978-953-51-0490-2

Hard cover, 246 pages

Publisher InTech

Published online 11, April, 2012

Published in print edition April, 2012

Crop losses by pests (insects, diseases and weeds) are as old as plant themselves but as agriculture are intensified and cropping patterns including the cultivation of high yielding varieties and hybrids are changing over time the impact of the pests becoming increasingly important. Approximately less than 1000 insect species (roughly 600-800 species), 1500 -2000 plant species, numerous fungal, bacterial and nematode species as well as viruses are considered serious pests in agriculture. If these pests were not properly controlled, crop yields and their quality would drop, considerably. In addition production costs as well as food and fiber prices are increased. The current book is going to put Plant Protection approaches in perspective.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

M. Anis, Iram Siddique, Ruphi Naz, M. Rafique Ahmed and Ibrahim M. Aref (2012). Advances in Micropropagation of a Highly Important Cassia species- A Review, New Perspectives in Plant Protection, Prof. Ali R. Bandani (Ed.), ISBN: 978-953-51-0490-2, InTech, Available from:
<http://www.intechopen.com/books/new-perspectives-in-plant-protection/advances-in-micropropagation-of-a-highly-important-cassia-species-a-review>

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