Review of Literature

Plants have provided medicine and treatments for a variety of health problems (Bruneton, 1999). More than 50% of all modern clinical drugs have originated from natural products. Recent estimation reveals that more than two thirds of the global population relies on drugs of plant origin (Aufield, 1991). The ability of synthetic drugs to control major complicated diseases is limited. Natural drugs are preferred over synthetic drugs because of the lower incidence of adverse reactions to plants preparation and of their reduced cost.

The plants have given western pharmacopoeia about 7000 different pharmaceutically important compounds and a number of top selling drugs of modern time eg. Quinine, artemisinin, taxol, camptothecin, etc. (Tshibangu et al., 2002).

Endemic Flora of India

India is accredited with 17,000 species of higher plants of which 30% are endemic. In India, 26 plants species have reached the stage of extinction and are both threatened and likely to be threatened, due to habitat destruction. Ahmedullah and Nayar (1986) have reported 1932 endemic taxa. India harbors 5, 150 endemic species (Varaprasad et al., 2007). India recognizes over 3000 plants for their medicinal value. Over 6000 plants in India represent about 75% of the medicinal needs of the third world countries (Rajashekarhan, 2002). India harbors 140 genera but no endemic family is recorded (BSI, 1983).

Roughly 1/3 of the Indian medicinal flora is estimated to be endemic to the subcontinent. About 70% of the medicinal plants species is in the tropical and
subtropical forests and 30% in the temperate and high altitude forests. The Himalayan range, northeastern region, Western Ghats and central plains are the natural habitats of many of these medicinal plants. The Eastern Ghats region is very rich in natural wealth which is shown by its great biodiversity. Out of 2,000 species of flowering plants known so far to occur in Eastern Ghats, about 77 species (67 dicots, 9 monocots and 1 gymnosperm) are endemic (Pandravada et al., 2007).

Floristic Diversity of Western Ghats

Western Ghats, the 1600 km long hill chain than run parallel to the west coast of India between the river Tapti in Gujarat and Kanyakumari in Tamil Nadu covering approximately 1, 60,000 sq km. area is a treasure house of plants and animals next only to Himalayan tracts in terms of its diversity of unique species of the total recorded species 38% flowering plants are confined only in this Ghat region. It also harbors a large number of wild relatives of cultivated species including pepper, cardamom, mango, jack fruit and plantain along with highly valuable sandal wood, rose wood and teak.

Floristically, the Western Ghats is one of the richest regions of the country. It harbors more than 3,500 species of flowering plants; almost 27% is of the total of its kind in India. Forests habitats existing in the Western Ghats are in general tropical wet evergreen, semi – evergreen and moist deciduous, along with dry deciduous scrub jungles, sholas, savannahs, peat bogs and swamps. Ahmedullah and Nayar (1986) reported that in India, endemism is high in the Himalayan region and in the Western Ghats. The 10 dominant natural orders of plants in the Western Ghats are Poaceae, Leguminosae, Acanthaceae, Orchidaceae, Asteraceae, Euphorbiaceae, Rubiaceae,
Asclepiadaceae, Geraniaceae and Lamiaceae. The genera found in the region with more than 15 sps. each are *Crotalaria, Impatiens, Diospyros, Ipomoea, Eugenia, Strobilanthus, Ficus, Desmodium, Habernaria and Osbechia*.

Nearly 63% of the tree species of the low and medium elevation evergreen forests of Western Ghats are endemic. Hot spot status is conferred to the Western Ghats due to the high level of diversity and endemism (Nayar, 1996).

**Western Ghats – Threatened Biodiversity Hot Spot**

The Western Ghats in India is one of the most threatened biodiversity hot spots of the world. The Western Ghats are one of the most important biogeographical zones in Peninsular India. They are very rich in endemic flora (Mac Kinnon and MacKinnon, 1986), due to their varied topography and bioclimate. Among the 600 taxa considered to be rare or threatened in the flora of the peninsular India, about 90% species are in the Western Ghats (Sastry and Sharma 1991).

The southern part (80 – 100 30 N) which corresponds to Travancore and the hills of south Palghat gap comprises 87% of the total endemic tree species of the Western Ghats, while 37% of the total area restricted to this area alone. It constitutes the main center of endemism in India. Many medicinal species occurring in the Western Ghats of peninsular India have become rare and threatened (few have been included in the endangered list).

The southern part of Western Ghats pertaining to Kerala and Tamil Nadu is a rich biome with the tropical rainforests where the vegetation in much varied due to the variation in altitude and climatic conditions. It is a pathetic fact that many valuable
members of flora of this region are on the verge of varnishing and even some are in the there at of extinction. Among them include highly potential medicinal plants too.

**Distribution**

*H. adakodien* is reported that the plant is distributed in the hills, west wards to the lower slopes of the Ghats and coast of south Travancore. It is restricted to certain areas, localized and form small colonies. By the bulk collection of root tubers, these populations are found to be diminishing. Moreover, herbivorous mammals also eat the delicious stem and leaves. Seed setting and natural dispersal of this plant are thus prevented (Mathew Dan and Shanavakshan, 1991). Cultivation of the plant can be an effective method to fulfill the demand and to conserve the natural wealth.

It is a vulnerable medicinal plant of Munnar forest region (Bhat and Padmaja, 1991) and threatened in Kerala (Sasidharan, 1991). It is in the vulnerable status in India (Nayar and Sastry, 1987; Rajasekharan and Ganeshan, 2002). In Kanyakumari District, it in present in the upper Kodayar region of Kalkulam Taluk, and its distribution status in Endemic, Endangered and Rare in the sacred groves (Sukumaran, 2002). It is present in the Thiruvattar region.

The entire plant of *Holostemma adakodien* is used to improve body strength by the Paniya tribes of Wayanadu District of Kerala (Raj and Raveendran, 2011). The tuberous roots of the plant is shade dried, bark is powdered and administered with milk or hot water in doses of 3-5gm a day works as an aphrodisiac by the ethnic people of Chittoor District of Andhra Pradesh (Jyothi 2011). Root paste of the plant mixed with equal amount of garlic paste, about 20 to 30 gm, administered orally for two months daily in the morning and evening relieves gonorrhea. External application of latex of
stem cures mouth ulcers as used by the tribal people of Raipur forest division of Nellore District, Andhra Pradesh (Neelima et al., 2011). The rural folks and the Kani tribals of Kanyakumari District, Tamil Nadu, apply the root paste of *H. adakodien* for ophthalmia. Dried powdered root with equal quantity roots of *Ceiba petandra* is given to spermatorrhoea (Sukumaran, 2002).

RFLP markers have also been used to analyze the relationship of closely related taxa. (Miller and Tanksley, 1990), for diversity studies (De breuit et al., 1996), as tools for finger printing (Fang 1997) and for hybridization studies and introgression, including studies of gene flow between crops and weeds (Brubaker and Wender, 1994; Clausen and Spooner, 1998).

The application of DNA technology in agricultural research has progressed rapidly over the last twenty years, especially in the area of cultivar identification and characterization (Nybom, 1990) as well as determination of population diversity in many plant species (Lei, 2006; Chen and Yang, 2004; Nan et al., 2003; Ipek and Madison, 2001; Muluvi et al., 1999; Cardoso et al., 2000). Recently global interest in oriental medicine, production of those plants has grown even more over the following years. Since many species and varieties exist, development of molecular markers would be important for quality assessment in the medicinal industry (Sang-Bok Lee, 2000). During the last two decades several novel DNA markers (RAPD, RFLP, SSR, ISSR, etc.) have been rapidly integrated into the tools available for genome analysis. Salimah et al., (1995), has been used for DNA finger printing and assessing genetic diversity. Presence or absence of DNA bands in the gel may be used as RAPD markers to study close genetic relationships (Sang-Bok Lee et al., 2000), for the identification of specific genes (Paran, 1991) and to study the gene expression pattern (Valle et al., 2000).
Markers in plant breeding

Many kinds of molecular markers, e.g. Restriction Fragment Length Polymorphism (RFLP), Polymerase Chain Reaction (PCR), Simple Sequence Repeats (SSR) and Amplified Fragment Length Polymorphism (AFLP) are being used in present day breeding programs (Karp, 1997). Among all these markers, Randomly Amplified Polymorphic DNA (RAPD) is especially useful for users friendly as it does not require any sequence information (Link et al., 1995). RAPD have been widely used to study genetic diversity, genome structure and gene tagging.

Antibacterial Activity

The use of plant extracts and phytochemicals both with known antimicrobial properties can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency (Al magboul et al., 1985; Artizzu et al., 1995; Ikram et al., 1984; Kubo et al., 1993; Shapovol et al., 1994). Over the last three centuries, intensive efforts have been made to discover clinically useful antimicrobial drugs (Werner et al., 1999). Kareem et al., (2008) conducted the study on the antimicrobial activities of Calotropis procera on E.coli, S.aureus, S.albus, P.aeruginosa, S.pyrogens and S.pneumoniae. Guleria and Kumar (2006) found out that leaf extract of Thuja orientalis produced a compound which showed antifungal activity. Martinez et,al., (1996), studied the antimicrobial properties of compounds obtained from Parthenium argentatum against Candida albicans, Torulopis, Hansemula, Klebsiella pneumoniae and Pseudomonas aeruginosa.

Antimicrobial drugs are used to treat AIDS and sexually transmitted diseases (Vermani and Garg, 2002). The antimicrobial activity of methanolic extracts
of all the parts of plants including fruits stem and leaves of *Tribulus terrestris*, L. was made evident (Ody, 2000). Essential oil have been shown to possess antibacterial, antifungal, antiviral and insecticidal properties (Burt, 2004). Chaturvedi *et al.*, (1983) found an overall effectiveness of *Andrographis paniculata* (Burm.f.) Wall exNees leaves on bacterial dysentery and diarrhea. Wilson *et al.*, (2005) studied the antimicrobial activity of extracts of *C. zedoaria* (Christm.) Roscoe. Antifungal and antibacterial activity of *Curcuma longa*, *C. zeodaria*, *C. aromatica* and *C. amada* has been reported (Apisariyakul *et al.*, 1995; Negi *et al.*, 1999; Mujumdar *et al.*, 2000).

Methanol extracts of *Cryptolepis buchanani* inhibited the growth of *Staphylococcus aureus* and *Salmonella typhi* (Yogesh, Mahinda and Mohan, 2007). Latex of *Calotropis procera* exhibited strong inhibitory effect on the *E. coli*, *Staphylococcus aureus*, *Streptococcus pyrogens* and *S. pneumoniae* (Kareem *et al.*, 2008).

The antimicrobial properties of compounds obtained from *Parthenium argentatum* against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were detected (Mertinez *et al.*, 1994, 1996). The antibacterial activity of the leaf extract of *Andrographis paniculata* against *Salmonella typhimurium*, *Salmonella* sp. *Escherichia coli* and *Pasteurella multocida* was studied by Tipakorn *et al.*, (2004). Thomas *et al.*, (1999) studied the antibacterial activity of *Adhatoda vasica* extract against gram positive and gram negative bacteria.

Basil is being studied as a source of antimicrobial agents that could be used in antimicrobial packaging for food preservation (Suppakul *et al.*, 2003). Alzorekey and Nakahara (2002) screened the extracts of 26 species of edible plants
from China, Japan, Thailand and Yemen for their antibacterial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* and *Salmonella infantis*. Palombo and Semple (2001) examined a panel of plant extracts commonly used by Australian aboriginals and found approximately 20% of the samples tested were able to inhibit bacterial growth.

**Micropropagation**

Most widely used *in vivo* methods of cloning the plant species includes cutting of vegetative parts, layering, grafting, budding etc., but *in vivo* clonal propagation of plants is often difficult, expensive and unsuccessful. Tissue culture methods offer an alternative means of plant propagation. Clonal propagation through tissue culture popularly called micropropagation can be achieved in a short time and space. Tissue culture techniques have been applied for the propagation of approximately 20% of 7000 known Ayurvedic plant species (Rajendra and D.Souza, 1999).

Micropropagation protocols are available for endemic and endangered plants throughout India. Protocols for micropropagation are available for *Woodfordia fruticosa* (Krishnan and Seeni, 1994), *Eremostachys superba* *Decalepis hamiltoni* (Obul et al., 2001), *Psoralea corylifolia*, *Agave victoriareginae*, *Saussurea obvallata* (Joshi and Dhar 2003) and *Anemopaegma arvense*.

**Explant Source**

All parts of the plant have been used as the source for micropropagation. Nodal segments of healthy plants have been however used in most cases. Saba (1991) reported the use of nodal segments in *Ammi majus* L., Nodal explants of *Bacopa monnieri* were propagated in
vitro using liquid shake cultures (Tiwari et al., 2000). Tiwari et al., (2000) reported the use of nodal segments for clonal propagation of Centella asiatica. Shoot tip, nodal and internodal segments were used as explants in Phyllanthus amarus (Ghanti et al., 2004). The morphogenetic potential of node, internode and leaf explants of Bacopa monnieri was investigated to develop reliable protocols for shoot regeneration and somatic embryogenesis (Tiwari et al., 1998).

Regeneration from callus cultures of Centella using stem and leaf explants from greenhouse grown mother plant was done by Patra (1998.) Shoot tips, terminal and basal nodes of Holostemma adakodien were used as explants for its in vitro propagation (Sudha et al., 1998). In vitro adventitious root formation from leaf explants was carried out for the secondary metabolite production in Leptadenia reticulate (Bhatt et al., 2002). Reports were there on the somatic embryogenesis from the leaf explants of this plant (Martin, 2004). For somatic embryogenesis in Tylophora indica, one year old mature leaf explants were used (Chandrasekhar et al., 2006). Nodal segments were used as explants in the regeneration of Hemidesmus indicus (Siddique et al., 2003).

The most commonly used explants are shoot tips, nodal buds and root tips. Large explants can increase chances of contamination and small explants like meristems can sometimes show less growth (Fowler 1993; Staba and Seabrook 1980). Sudha (2000) described plant regeneration from chlorophyllous root segments derived from in vitro rooted plants of Holostemma adakodien. Reddy (1998) used nodal explants of Gymnema sylvestre for in vitro multiplication.
Rhizomes were used as explants in the *in vitro* propagation of *Geodorum densiflorum* (Sheelavanthmath *et al.*, 2000) and *Kaempferia galanga* (Shiri 2000)

**Sterilization treatment**

Surface sterilization in the most important step before inoculation of explants. Different steps have been employed for treatment of explants. Shrivastava and Rajani (1999) have described sterilization treatment of *Bacopa*, which includes use of 0.1% mercuric chloride (W/V) for 2 mins. followed by rinsing thoroughly with sterile distilled water. Different sterilization treatment was followed by Mathur and Kumar (1998) in which leaves and stem explants were shaken for 10 mins in Tween 20 (Ranbaxy) and Savlon (Johnson & Johnson) in water for 10 minutes, rinsed in running water for 30 minutes, treated with 0.1% Mercuric chloride for 3-4 minutes and washed several times with sterile water.

Tiwari *et al.*, (2000) suggested that for micropropagation of *Centella asiatica*, plants were washed thoroughly for 30 mins under running tap water followed by removal of leaves, which was followed by soaking in the mixture of 1% cetrimide solution containing 150 mg/l Bavistin and 50 mg/l. trimethoprim for 25-30 mins. The explants were finally treated with 0.1% mercuric chloride for 3-4 mins followed by rinsing in sterile distilled water for 4-5 times.
In *Holostemma adakodien* surface decontamination of the microcuttings was performed by immersion in 15% (v/v) sterile commercial bleach (Combi Organic Chem. (p) Ltd., New Delhi) for 7-10 mins for microcuttings and 12-15 min for basal nodes. The explants were then immersed in 0.1% (Wt/Vol) HgCl2 for 5-7 min in the case of the microcuttings and 8-10 min for the basal nodes. Each step of sterilization was followed by five rinses in sterile distilled water (Sudha *et al*., 1998).

In *Leptadenia reticulata* the intermodal explants were thoroughly washed in running tap water (30 min), then in 5% lab wash (Merck) and again in running tap water (10 min). The washed explants were surface sterilized for 6 min with the mixture of 0.1% Mercuric chloride + 0.1% Bavistin + 0.1% Cetrimide and rinsed five times with sterile distilled water to remove the traces of sterilants (Sathyanarayana, 2008). Chandrasekhar *et al*., (2006) washed the leaf explants of *Tylophora indica* with distilled water for two times, rinsed with 1% (v/v) detergent teepol for 5 min, later were surface sterilized with 0.1% (w/v) aqueous solution of HgCl2 for 5 min followed by 4-5 rinses in sterilized ddH2O.

Gururaj *et al*., (2007) washed the single bud explants of *Tinospora cordifolia* with 1% sodium hypochlorite solution (v/v) for 5 min followed by thorough washing under running tap water for 15 min. The explants were surface sterilized with 0.15% (w/v) mercuric chloride for 3-5 min and later rinsed 4 or 5 times with sterile distilled water. A considerable
decrease in bacterial contamination was seen by using ultrasonic sonicator (Garro-Monge, 2008).

**Multiple shoot induction**

Murashige and Skoog medium (1962) supplemented with different combination of growth regulators has been used for growth of herbaceous species. Multiple shoots were obtained from root tips derived from field-grown plants of *Bacopa moinnieri* within 6 days of culture whereas in the case of *Paederia foetida* and *Centella asiatica* multiple shoots were obtained from field-grown plants in MS Medium supplemented with 1.0 mg BAP / litre within 7 days of culture (Singh *et al.*, 1999).

Tiwari *et al.*, (2000) proposed an efficient and rapid method using liquid shake cultures for in vitro propagation of *Bacopa*, a medicinally important herb. This was achieved by culturing nodal explants on liquid MS medium with or without 6–benzyladenine. Compared to single axillary shoot proliferation on a growth regulator free agarified medium, the respective liquid medium included 4 or 5 shoots per nodal explant, 4 weeks after culture. Addition of 6–benzylandenine (0.01 – 0.1 mg/l) resulted in the increase in morphogenetic response (number of shoots mean shoot length and number of roots per node explants) in both type of culture media. Tiwari *et al.*, (2001) reported the use of cytokinins for multiple shoot induction for *Bacopa*. Best shoot induction
was observed in *Aloe barbadensis* on MS medium supplemented with BAP and NAA (Baksha 2005).

In *Baliospermum montanum* maximum shoot induction from shoot tip and nodal buds was on MS medium supplemented with BAP 2.0 mg/l (Sasikumar *et al.*, 2009). Maximum shoot induction and increase in shoot number and height from the shoot tip explants of *Woodfordia fruticosa* were noticed on SH medium augmented with BAP and NAA (Krishnan and Seeni 1994). In *Vitis thunbergii* WPM (Woody Plant Medium) supplemented with BA proved highly efficient for length and shoot proliferation (Lu, 2005). MS medium fortified with BA and NAA showed better response in producing maximum shoot induction, shoot numbers and height from the shoot tip explants of *Withania somnifera* (Saritha and Naidu, 2007). Most number and longest shoots from the nodal bud explants of *Hoslundia opposita* was observed on MS medium supplemented with 4.4 um BA (Prakash and Van Staden, 2007).

Maximum response and maximum shoot length (6.0 – 6.5 cm) from the nodal explants of *Holostemma adakodien* were noticed on MS medium supplemented with 8.86 um BA and 0.25 um NAA (Sudha 1998). The combination treatment of (0.25mg/l BA along with 0.25mg/l kn) was found to exhibit highest frequency of shoot multiplication (90%) in *Leptadenia reticulata* (Sudipta *et al.*, 2011). Maximum proliferation of shoot buds in *Hemidemus indicus* was observed in MS medium supplemented with Kn in combination with NAA (Siddique 2003).
An excellent protocol for *Solanum trilobatum* on MS medium fortified with 11.1µM Kn reported the production of highest number of multiple shoots. A maximum of 3-4 shoots were produced from nodal explants of *Tinospora cordifolia* (100% response) on MS medium containing 13.94uM Kinetin (Gururaj *et al.*, 2007).

**Rooting and Hardening**

In medicinal plants, rooting of micro shoots have been obtained in MS medium with IAA, IBA, NAA used singly or in combinations or when transferred to hormone free medium. Root induction in *Paederia foetida* and *Centella asiatica* was observed within 12 – 21 days of culture when single shoots of both the plant species were cultured on MS media supplemented with 0.25mg/l BAP + 0.5mg/l BA and 0.5mg/l BAP + 1.5 mg/l NAA respectively (Singh 1999).

Root induction has also been reported in *Phyllanthus amarus* (Ghanti *et al.*, 2004) using MS medium supplemented with a concentration of 0.5mh/l of IBA. Rooted shoots were hardened on basal liquid medium and subsequently in sterile soil + vermiculite (1: 1). In the case of *Withania somnifera* (L.) Dunal. shoots rooted best (87%) on MS medium containing 2mg/l indole butyric acid (IBA). The plantlets were transferred to the field after acclimatization and showed 60% survival (Rani *et al.*, 2003).

In *Centella asiatica* shoots regenerated from the stem and leaf callus were rooted within 11 days in ½ strength basal salt supplemented with 0.5mg/l indole – 3 – acetic acid and 2% (w.v) sucrose. About 85% of rooted plantlets were acclimatized and transferred to green house (Patra 1998). Tiwari *et al.*, (2000) tried
rooting on different media in *Bacopa*, i.e. MS media with or without hormones and found that rooting was highest (90%) on full strength MS medium containing 2.46 µm IBA.

In *Tinospora cordifolia* when the shoots were cultured on an MS medium supplemented with IBA 0.4 µM, 100% shoots showed rooting after 15 to 20 days. The rooted plants were transplanted *ex vitro* and raised in pots under house conditions followed by field transfer and they showed 70 – 80% survival (Gururaj *et al.*, 2007). In MS medium supplemented with NAA 70% rooting was noted at the cut ends of *Abrus laevigatus* microshoots within two weeks of culture. Shoots with strong and stout root system were acclimatized outside growth chamber for a week. These juvenile plants were transferred in earthen pots, which were placed in natural environment containing mixture of soil and manure (1:1), 75% plants survived (Narayan Padhure 2010).

It has already been found that rooted plants of *Azadirachta indica* showed 90% survival when transferred to pots containing soil mixture (Quarishi 2004). Maximum rooting responses (85%) in *Tylophora indica* were obtained by the subculture of regenerated shoots on half strength MS medium supplemented with IBA (0.5mg/l) after 15 days of incubation. The complete plantlets were taken out from the culture vials transferred to polycups containing vermicompost and sterile soil (1:3). These plantlets were again transferred to earthen pots followed by transplanting in natural environment conditions, where they showed 75% survival (Roop Narayan Verma *et al.*, 2010). In *Wattakaka volubilis*, maximum number of rooting was observed in MS medium supplemented with 1.0mg/l IBA (John Peter Arulandandam 2011). A protocol for *Tridax procumbens* in which excellent rooting on the excised shoots raised
from secondary cultures on half strength MS medium having 1mg/l IBA was developed (Malik and Wadhwani, 2009).

On the medium containing BAP 13.32 uM/l + IBA 0.49uM/l to 1.23 uM/l the microshoots rooted best in *Ceropgia pulsilla*. Microshoots with well developed root system were transferred to small pots containing sand vermiculite and coco peat (1:1) rejuvenated growth within 20 days and the rate of survival is 80% (Kondamudi *et al.*, 2010).

Patnaik *et al.*, (1996) used MS medium fortified with IBA and Kn for root induction in *Hemidesmus indicus*. In *Hemidesmus indicus* Siddique *et al.*, (2003) attained 80% of root regeneration in MS medium augmented with IBA 4mg/l + km 1 mg/l.

**Callus culture**

Callus formation occurs from revered process of cell differentiation, known as dedifferentiation or redifferentiation (Fowler *et al.*, 1993). New plants can be successfully regenerated from callus through organogenesis. Callus was induced from leaves of *Rauwolfia serpentina*, on WCR supplemented with coconut milk (CM), extra nitrogen source solution, biotin (10mg/l) BAP (2mg/l), NDA (0.8 mg/l) and GA3 (0.0001 mg/l). Callus, subcultured on WRC supplemented with coconut milk (CM),BAP (0.1mg/l) and NAA(0.1mg/l), grew well and differentiated roots. Differentiated roots dedifferentiated into callus, which subsequently redifferentiated into roots. This cycle of differentiation was observed repeatedly on further subculture.
Calli derived from nodal explants of *Bacopa* cultured on MS medium containing 0.5 mg/l 2,4-Dichlorophenoxy acetic acid (2,4-D) when subcultured on MS medium containing 0.1 or 0.5 mg/l BA or 0.2 mg/l 2,4-D + 0.1 or 0.5 mg/l kinetin, developed somatic embryos. The somatic embryos germinated either on the same media or on MS basal medium and the resulting plantlets were successfully transplanted to soil (Tiwari *et al.*, 1998).

Rani *et al.*, (2003), reported that callus induction in *Withania somnifera* was observed from hypocotyls, root and cotyledonary leaf segments, grown on MS medium supplemented with various concentrations and combinations of 2,4-D and Kn. Maximum callusing (100%) was obtained from root and cotyledonary leaf segments grown on MS medium supplemented with combination of 2 mg/l 2,4-D and 0.2 mg/l Kn. When hypocotyl segments were used as explants, callus induction was noticed in 91% of cultures, which showed shoot regeneration on MS medium supplemented with 2 mg/l 2,4-D and 0.2 mg/l Kn.