

## **A Review on Micropropagation of Some Medicinally Important Plant Species of Family Solanaceae**

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### **Abstract**

The Solanaceae or nightshades are an economically important family of flowering plants. The family ranges from annual and perennial herbs to vines, lianas, epiphytes, shrubs, and trees, and includes a number of important agricultural crops, medicinal plants, spices, weeds, and ornamentals. Many members of the family contain potent alkaloids, and some are highly toxic, but many cultures eat nightshades, in some cases as staple foods. The family belongs to the order Solanales, in the asteroid group dicotyledons (Magnoliopsida). The Solanaceae consists of about 98 genera and some 2,700 species with a great diversity of habitats, morphology and ecology. The family has a worldwide distribution, being present on all continents except Antarctica. The greatest diversity in species is found in South America and Central America.

**Keywords:** Solanaceae, 98 genera, worldwide distribution, medicinal plants, spices, alkaloids

### **Introduction**

The micropropagation techniques devised for the large scale propagation of the plants belonging to family Solanaceae are mainly explant based, although, the combination of growth regulators used by various workers varies. Some medicinally important plant species that have been successfully micropropagated at large scale are discussed below.

### ***Atropa* species**

*Atropa* is a genus of flowering plants in the nightshade family, Solanaceae: tall, calcicole, herbaceous perennials (rhizomatous hemi cryptophytes), bearing large leaves and glossy berries particularly dangerous to children, due to their combination of an attractive, cherry-like appearance with a high toxicity.

***Atropa acuminata* Royle:** It is a perennial plant commonly known as indian belladonna. The rhizome of this plant have been traditionally used as a sedative, antidote in cases of mushroom or toadstool poisoning, analgesic, antispasmodic, hallucinogenic, mydriatic, narcotic, diuretic and anodyne, arthritis related inflammatory disorders, muscle and joint pain, muscle spasms, sore throat, ulcerative colitis. An efficient protocol was developed for *Atropa acuminata* by using leaf explants (Akram *et al.*, 1994). Callus was produced when cultured on MS medium supplemented with 2, 4- D. Root initiation from the midrib of some explants was also observed. Roots also regenerated from 1-year old callus cultures on MS medium supplemented with Kn. An efficient micropropagation protocol for *Atropa acuminata* was developed using shoot tips and nodal cuttings (Ahuja *et al.*, 2002). Induction of shoot proliferation from shoot tips and nodal explants on MS medium supplemented with BAP and IBA. Shoot tip explants show better response than the nodal explant. Maximum shoot production (5-6 per culture) was obtained on MS medium supplemented with BAP (1mg/l) and IBA (1mg/l) after 40 days. Percent shoot development as

well as number of shoots per node retained highest value 90% i.e 5-6 shoots per node. Rooting was obtained on full strength RT medium enriched with IBA (1mg/l).

***Atropa belladonna*:** *Atropa belladonna*, commonly known as belladonna or deadly nightshade, is a perennial herbaceous plant in the Nightshade family Solanaceae, native to Europe, North Africa and Western Asia. Belladonna has been used in herbal medicine for centuries as a pain reliever, muscle relaxer, and anti-inflammatory, and to treat menstrual problems, peptic ulcer disease, histaminic reaction, and motion sickness.

An efficient protocol was developed for root, callus and cell suspension cultures from seedlings of *Atropa belladonna*, L. and *Atropa belladonna*, cultivar *lutea* Doll. Callus cultures transferred to auxin (NAA) free medium initiated roots and shoots. Excised root cultures have been established from such roots and plants from such shoots (Bhandary *et al.*, 1969). A protocol was devised for tissue cultures from leaves of anther-derived haploid plant of *Atropa belladonna* L. Regenerants obtained from callus cultures were transferred to soil and reared to maturity (Eapen *et al.*, 1978). An efficient protocol was optimized for multiple shoot cultures of *Atropa belladonna* L. by using shoot tip and axillary meristem as explant on MS liquid medium supplemented with BAP (Benjamin *et al.*, 1987).

***Atropa baetica*:** An *in vitro* propagation protocol was achieved in *Atropa baetica* by using axillary buds as explant. They obtained multiple shoot induction on MS medium supplemented with BAP (0.75 and 1.25mg/l) and sucrose (3%). Rooting was obtained on MS basal medium (Zarate *et al.*, 1997). A standardized protocol was developed for *in vitro* germplasm conservation of *Atropa baetica* by cold storage at 4<sup>0</sup>C in darkness. Cold stored germplasm was studied on MS medium supplemented with BAP (0.66 mg/l), NAA (0.24mg/l), inositol (100mg/l), thiamine (1mg/l) and sucrose (3%) (Cantos *et al.*, 1998).

### ***Anisodus species***

***Anisodus tanguticus*:** is a species of flowering plant in the family Solanaceae which includes many important agricultural plants. It is mostly found growing in the Qinghai-Tibetan Plateau. It is collected and used mostly for its medicinal effects thought to be derived from the plants biologically active nicotine and tropane alkaloids. It has a significant impact in China as one of the 50 traditional herbs used in traditional Chinese medicine.

A tissue culture studies was carried on *Anisodus tanguticus*, a Tibetan medicinal plant. Callus induction and rooting was obtained from dormant buds and young leaves on MS medium supplemented with different hormones. MS medium supplemented with 2, 4-D (2.0mg/l), NAA (0.2mg/l) and BAP (0.2mg/l) was suitable for the induction and subculture of callus. MS medium supplemented with NAA and sucrose (30g) was suitable for the induction of roots (Xu *et al.*, 2008).

An *in vitro* protocol was developed for efficient shoot multiplication of *Anisodus tanguticus*, an endangered medicinal plant in the Qinghai-Tibet Plateau. Multiple shoots regenerated from shoot tips on MS medium supplemented with BA and Kn or in combination with NAA. The presence of BA was more effective than Kn on shoot multiplication. Addition of NAA (0.5 or 1.0mg/l) to BA-containing medium promoted callus formation and reduced shoot multiplication. Shoots cultured on MS medium supplemented with IAA (0.5mg/l) induced rooting (He *et al.*, 2011).

### ***Brugmansia species***

*Brugmansia* is a genus of seven species of flowering plants in the family Solanaceae. They are woody trees or shrubs, with pendulous flowers, and have no spines on their fruit. Their large, fragrant flowers give them their common name of angel's trumpets.

***Brugmansia suaveolens***: is a large perennial bush with woody stems and large, smooth, oval leaves. *B. suaveolens* leaves are applied externally to treat wounds, rashes, and ulcers. An efficient tissue culture protocol was devised for *Brugmansia suaveolens* Humb. and Bonpl. ex Wild. Fresh sprouts were used as explant and cultured on MS medium supplemented with BAP (2.0mg/l). Maximum numbers of shoot developed on MS medium augmented with BAP and Kn (0.5mg/l). Both IAA and IBA were equally effective for root induction (Shekhawat, 2012)

***Brunfelsia* species**: *Brunfelsia* is a genus of about 40 species of shrubs and small trees native to Brazil.

***Brunfelsia calycina***: *B. calycina* have been cultivated as ornamentals. It has become a popular garden and pot plant due to its large blue flowers and pleasant fragrance. An efficient protocol was developed for plant regeneration of Neotropical shrub *Brunfelsia calycina*. Shoot formation was obtained from young and mature leaves on MS medium supplemented with BAP (4.44 $\mu$ M), IAA (2.85 $\mu$ M), sucrose (30g/l) and agar (7.5g/l). Rooting was obtained on MS medium supplemented with IBA (1.23-2.46 $\mu$ M) (Lieberman *et al.*, 2010).

***Brunfelsia latifolia***: A protocol was standardised for callus induction in *Brunfelsia latifolia* using different explants viz. petal, leaves and stem segments. MS medium supplemented with BAP (1.0mg/l) and 2, 4-D (1.0mg/l). Among the three explants, young leaves produced maximum callus as compared to stem and petal explants (Yuan and Fang-Qing, 2010)

### ***Capsicum* species**

*Capsicum* is a genus of flowering plants in the nightshade family Solanaceae. Its species are native to the Americas, where they have been cultivated for thousands of years. Following the Columbian exchange has become cultivated worldwide, and it has also become a key element in many cuisines. In addition to use as spices and food vegetables, *Capsicum* species have also been used as medicines and lachrymatory agents.

***Capsicum annum***: is a species of the plant genus *Capsicum* native to Southern North America and northern South America. An *in vitro* plant regeneration protocol was developed for three varieties of *Capsicum* by using cotyledon and hypocotyl explants excised from aseptically germinated seedlings. They achieved regeneration of shoot buds on MS medium supplemented with IAA and BAP. They also obtained rooting on MS medium enriched with IAA or NAA (Gunay and Rao, 1978). An *in vitro* regeneration protocol was developed for *Capsicum annum* L. Immature zygotic embryo explants produced somatic embryos directly without any callusing phase when cultured on MS medium supplemented with adjuvants such as high sucrose, coconut water and 2, 4-D (Harini and Sita, 1993). A protocol was developed for induction of somatic embryogenesis and plant regeneration in *Capsicum annum* from immature zygotic embryos via direct somatic embryogenesis. Somatic embryos formed directly, without any intervening callus, on the zygotic embryo apex, embryo axis and cotyledons on MS medium supplemented with 2, 4-D (418 $\mu$ M), TDZ (10 $\mu$ M) and a high concentration of sucrose (6-10%). The best response was observed on MS medium supplemented with 2, 4-D (9 $\mu$ M), coconut water (10%) and high sucrose (8%) (Binzel *et al.*, 1996) An efficient plant regeneration protocol was achieved in *Capsicum annum* L. cv. Pusa Jwala. Multiple shoots were induced by culturing nodal explants excised from 1-month-old aseptic seedlings on MS medium supplemented with TDZ (0.1-10 $\mu$ M). Adventitious roots were induced two weeks after transfer of shoots on MS medium supplemented with IAA, IBA or NAA. Optimum root formation was obtained on MS medium containing IBA (1.0 $\mu$ M). *Ex-vitro* rooting was also achieved by pulse treatment with 300  $\mu$ M IBA for 10 min (Ahmad *et al.*, 2006). An *in vitro* regeneration protocols for direct somatic embryogenesis of two *Capsicum annum* L. genotypes from hypocotyl explants cultured on MS medium supplemented with different

concentrations of TDZ and 2, 4-D. Two different media were used, WPM and MS and varying sucrose concentrations were examined for induction of direct somatic embryogenesis. WPM induced more effective formation of somatic embryos as compared with that of MS results (Aboshama, 2011). An efficient protocol was optimized for shoot organogenesis and plant regeneration of *Capsicum annuum* L. cv, an economically important crop plant used as spice and vegetable. Callus was obtained from internodal segments on MS medium supplemented with 2, 4-D (10 $\mu$ M) and BAP (2.0 $\mu$ M). Shoot differentiation was achieved from the surface of callus when transferred on shoot induction medium containing BA and TDZ alone or in combination. The individual elongated shoots were rooted on MS medium supplemented with IBA (1.0 $\mu$ M) (Khan *et al.*, 2011). An effective micropropagation protocol for *Capsicum annuum* L. of Kandhari variety, a traditional medicinal plant and nutritionally important spice crop. They obtained proliferation of shoots on MS medium supplemented with BAP (0.1-1mg/l), sucrose (15gm/l), and agar (8gm/l). Rooting was achieved on MS medium enriched with IAA (1.0mg/l) (Robinson and Maheshwari, 2013).

***Capsicum* species:** A protocol was developed for *in vitro* plant regeneration of *Capsicum* species viz, *C. praetermissum*, *C. baccatum* and *C. annuum* cvs. Shoot regeneration was induced in hypocotyl, cotyledon and leaf explants on MS medium supplemented with IAA (5.7 $\mu$ M) and BA (13.3 $\mu$ M). Rooting of regenerated shoots was obtained on MS medium fortified with IAA (5.7 $\mu$ M) (Christopher and Rajam, 1996). A procedure was developed for *in vitro* plant propagation of *Capsicum* species viz, *Capsicum annuum* cv CA960, *C. baccatum*, *C. frutescens* and *C. praetermissum* by culturing shoot meristems explants. MS medium along with RT medium supplemented with BA, Kn and TDZ. TDZ was most effective in the regeneration of maximum number of shoots in all three species. Rooting of regenerated shoots was achieved on MS medium supplemented with IAA (5.71 $\mu$ M) (Peddaboina *et al.*, 2006).

***Capsicum frutescens*:** An efficient and highly reproducible protocol for micropropagation of *Capsicum frutescens*. Shoot tips and nodes were used as explants for shoot regeneration and inoculated on MS medium fortified with BAP (0.5-3.0mg/l), 2-iP, Kn, IAA (0.5-2.0mg/l), IBA and activated charcoal (1g/l) (Gururaj *et al.*, 2004). A novel micropropagation technique was developed for *Capsicum frutescens* L. cv. 'Uchithi', a pungent chilli cultivar using axillary shoot segments as explant. Multiple shoots were obtained on MS medium supplemented with BAP (44.4 $\mu$ M) and Kn (9.3-46.7 $\mu$ M). The separated shoots rooted and elongated on MS medium fortified with IAA (2.8 $\mu$ M) or IBA (2.4 $\mu$ M) (Sanatombi and Sharma, 2007).

***Capsicum baccatum*:** An efficient protocol was developed for callus regeneration of *Capsicum baccatum* by using the radicle-side half-seed as explant. Callus was induced on semisolid MS medium supplemented with BAP (5mg/l), IAA (1mg/l) and GA<sub>3</sub> (2mg/l) (Montero and Phillips, 2005).

***Capsicum chinense*:** An efficient protocol was achieved for multiple shoot production of *Capsicum chinense* Jacq. Nodes and stem segments were used as explants and cultured on MS medium supplemented with Kn, BAP, and TDZ. TDZ was the key growth regulator in the process which induced seven to eight shoots at (3.4 $\mu$ M) that developed into healthy plants (Buzzy *et al.*, 2005). A highly efficient protocol for somatic embryogenesis of *Capsicum chinense* Jacq. Somatic embryos were produced from cotyledons, zygotic embryos, germinated zygotic embryos, hypocotyls and cotyledonary leaves. Explants were cultured on MS medium supplemented with 2, 4-D (9.05 $\mu$ M) (Lopez-Puc *et al.*, 2006). A protocol was developed for somatic embryogenesis in *Capsicum chinense* Jacq. using different types of explants (node, internode, hypocotyl, half seeds and fruit segments). They used MS

medium supplemented with 2, 4-D (9.5 $\mu$ M), sucrose (3%), gelrite (0.8%) and TDZ (3.4 $\mu$ M) and got results over a period of 30 days (Zapata-Castillo *et al.*, 2007).

#### **Cyphomandra species**

**Cyphomandra betacea:** An efficient protocol was developed for micropropagation of *Cyphomandra betacea*. Explants from axillary and flower buds of two mature tamarillo plants were cultured on MS medium supplemented with BA (0.1-4.0mg/l), Kn (0.25-4.0mg/l) and NAA (0.25-4.0mg/l). In anther culture, pollen sacs were cultured on MS medium supplemented with BA (0.1 or 1.0mg/l), with or without NAA or IBA (0.3mg/l) (Barghchi, 1998). An efficient protocol for rapid propagation of *Cyphomandra betacea* through *in vitro* nodal culture. The nodal explants were cultured on MS media supplemented with BAP, 2iP, Kn, myo-inositol (100mg/l), sucrose (3%) and gelrite (0.3%). Micro shoots developed from nodal explants on MS medium fortified with BAP (40 $\mu$ M) were found to be the most effective for inducing bud break and multiple shoot production. The micro shoots were able to root without addition of an exogenous auxin (Waweru *et al.*, 2011).

#### **Datura species**

*Datura* is a genus of nine species of poisonous vespertine flowering plants belonging to the family Solanaceae. They are commonly known as daturas, but also known as devil's trumpets.

**Datura insignis:** An *in vitro* protocol for shoot multiplication and elongation of *Datura insignis* Barb. Rodr. using nodal explants. They used MS medium supplemented with BA alone or in combination with 2, 4-D or IAA. Best results were obtained on MS medium fortified with BA (1.0mg/l) (Santos *et al.*, 1990). A standardized protocol for micropropagation of *Datura insignis* by using leaf and stem explants on MS medium supplemented with NAA and BAP. Callus was obtained from the stem explants on MS medium fortified with BAP and NAA (1mg/l). Bud formation was observed from nodal segments cultured on MS medium supplemented with NAA (0.06mg/l). The shoots were rooted and elongated on B5 medium without growth regulators and supplemented with activated charcoal (Figueiredo and Esquibel, 1991).

**Datura metel:** An efficient protocol for *in vitro* plant regeneration of *Datura metel* by using nodal explants collected from both *in vitro* germinated seedlings and field grown plants (*in vivo*). Explants were cultured on MS medium supplemented with BAP (0.5-3.0mg/l) and NAA (0.5mg/l). The nodal explants isolated from *in vivo* source exhibited higher number of healthy multiple shoots than *in vitro* sources. Regeneration of shoot lets was optimal on MS medium supplemented with BAP (3mg/l) and NAA (0.5mg/l) (Muthukumar *et al.*, 2004). An *in vitro* micropropagation protocol was developed for *Datura metel* L. Roots were used as explant. A large number of somatic embryos developed from roots on MS supplemented with BAP (4.0mg/l). Shoot elongation and root growth was achieved on MS medium supplemented with BAP (2mg/l), GA<sub>3</sub> (1mg/l) and IBA (1mg/l) (Nithiya and Arockiasamy, 2007).

**Datura innoxia:** A protocol for large scale *in vitro* production of plantlets through shoot tip culture of *Datura innoxia*. They obtained *in vitro* multiple shoots from the nodal segments and shoot tips when cultured on MS medium supplemented with BAP alone or in combination with NAA and Kn. High frequency of micro shoots were also obtained from the explants cultured on MS medium supplemented with BAP (0.1-1.0mg/l) along with NAA (Ashwini *et al.*, 2013).

#### **Duboisia species**

**Duboisia myoporoides:** An efficient protocol was developed for plant regeneration of *Duboisia myoporoides* from nodal explant cultures. They obtained optimal growth of the axillary buds and their multiplication on MS medium fortified with IAA (1.0mg/l) and Kn or BAP (2.0mg/l). Rooting was obtained on semi-solid MS medium supplemented with NAA (0.5mg/l) (Mathur and Kukreja, 1985). An efficient protocol was devised for

organogenesis and callus regeneration of *Duboisia* hybrid (*D. leichhardtii* x *D. myoporoides*) by culturing shoot tips and young seed as explants on MS medium fortified with NAA (54 $\mu$ M) and BAP (1 $\mu$ M). They also obtained bud formation from callus when cultured on MS medium supplemented with BA (22 $\mu$ M). The buds formed shoots when transferred on MS medium supplemented with BA (5 $\mu$ M) and NAA (0.5 $\mu$ M). These shoot regenerated roots when cultured on MS medium containing IBA (25 $\mu$ M) (Lin and Griffin, 1992).

### ***Hyoscyamus* species**

***Hyoscyamus niger***: *Hyoscyamus niger*, commonly known as henbane, black henbane or stinking nightshade, is a poisonous plant in the family *Solanaceae*

An efficient protocol for induction of adventitious roots of *Hyoscyamus niger* L. from 2-year-old suspension cultures of cultured roots grown on LS medium supplemented with BAP (10<sup>-8</sup>M) and sucrose (3%) (Hashimoto and Yamada, 1983).

An efficient protocol for shoot regeneration of *Hyoscyamus niger* L. Hypocotyl, cotyledon and stem cultures were used as explants. MS medium supplemented with TDZ (16 $\mu$ M) was the most effective medium inducing 100% regeneration frequency. Plantlets were rooted on MS medium supplemented with IBA and NAA (Uranbey, 2005).

***Hyoscyamus muticus***: An *in vitro* propagation protocol was developed for plant regeneration of *Hyoscyamus muticus* L. Cotyledonary leaf segments were used as explant. Embryogenic callus was induced from cotyledonary leaf explants on MS medium supplemented with 2, 4-D (1.13-4.52 $\mu$ M) or NAA (0.26-2.64 $\mu$ M) and BAP (2.22 $\mu$ M) (Verma and Chand, 2009).

### ***Nicotiana* species**

*Nicotiana* is a genus of herbaceous plants and shrubs that is indigenous to the Americas, Australia, South West Africa and the South Pacific. Various *Nicotiana* species commonly referred to as tobacco plants, are cultivated as ornamental garden plants. *N. tabaccum* is grown worldwide for production of tobacco leaf for cigarettes and other tobacco products.

***Nicotiana rustica***: An *in vitro* propagation protocol was obtained for callus cultures from seed, root and leaf explants of *Nicotiana rustica* L. var. Brasilia. Callus initiation was optimum on MS medium supplemented with 2, 4-D (1 $\mu$ M) and Kn ( $\mu$ M) (Tabata and Hiraoka, 1976).

***Nicotiana tabaccum***: Tissue culture studies was carried out on *Nicotiana tabaccum* by culturing leaf lamina explant excised from 30-35 days old seedling on MS medium supplemented with BA and NAA at varying concentration. Multiple shoots regenerated on MS medium fortified with BA (0.5mg/l) and NAA at a concentration of (1.0mg/l) (Kaeochanit and Phromchan, 1999).

An *in vitro* propagation protocol achieved callus and plant regeneration in two cultivars of *Nicotiana tabacum* L. (K-399 and SPTG-172) from leaf explant cultured on MS medium supplemented with NAA and BAP. Callus was regenerated in 32% cultures with average of 2.14 shoots per calli after 14days of inoculation. Rooting was obtained on MS medium in both cultivars (Ali *et al.*, 2007).

**Lycium species**

***Lycium barbarum***: An efficient protocol was developed for plant regeneration protocol of *Lycium barbarum* L. from leaf tissue and callus culture. Regeneration of shoots was obtained from leaf segments on B5 medium supplemented with BAP (1.5mg/l) and NAA (0.5mg/l). Callus was obtained from leaf and inter node tissues on MS medium fortified with 2, 4-D (0.4mg/l). Shoots were rooted on MS medium supplemented with NAA (0.1mg/l) (Ratushnyak et al., 1990).

A micropropagation protocol was developed in *Lycium barbarum* (L.) by using root explants. Callus was produced from root explants cultured on MS medium supplemented with 2, 4-D (0.2mg dm<sup>-3</sup>). After three subcultures on the same medium, calli was then transferred onto the MS medium supplemented with lactalbumin hydrolysate to induce somatic embryogenesis (Hu *et al.*, 2008). A rapid micropropagation protocol for induction of callus and adventitious buds in *Lycium barbarum* L. using leaf explants. MS medium supplemented with NAA (0.2mg/l) and BAP (1.0mg/l) showed maximum callus induction and adventitious bud differentiation. Rooting was obtained on half strength MS medium supplemented with IBA (0.6mg/l) and NAA (0.2mg/l) (Guo-qin and Jin-feng, 2012).

**Lycopersicon species**

***Lycopersicon cheesmanii***: An efficient protocol was developed for *in vitro* shoot regeneration of *Lycopersicon cheesmanii* from leaflets, petioles, cotyledon explants and through callus regeneration on MS medium supplemented with BA, 2-iP and Kn (3-4mg/l) in association with auxins (Dorion *et al.*, 1999).

***Lycopersicon esculentum***: An effective protocol was developed for plant regeneration of *Lycopersicon esculentum* Millcv. PKM, from hypocotyl explants cultured on MS medium supplemented with different concentrations of auxins and cytokinins. Callus induction was achieved on MS medium supplemented with NAA (1.0g/l) and Kn (0.1mg/l). BAP was found to be more suitable for shoot bud differentiation as well as multiple shoot induction as compared to Kn. Rooting of the regenerated shoots was induced on half strength MS medium supplemented with IBA (0.1-0.5mg/l) (Venkatachalam et al., 2000). An *in vitro* regeneration protocol of *Lycopersicum esculentum* Mill. Callus was obtained from aseptic explants on MS medium supplemented with 2,4-D. Regeneration of callus was achieved on MS medium supplemented with BAP, IAA and NAA (Aishwarya and Robinson, 2013).

***Lycopersicon pennellii***: A micropropagation protocol was developed for *Lycopersicon pennellii* from the leaf explants cultured on MS medium supplemented with different hormone concentrations. Callus induction and adventitious bud differentiation was obtained on MS medium supplemented with BA and IAA. Rooting was obtained on MS medium supplemented with IAA (Chun *et al.*, 2011).

**Petunia species**

***Petunia hybrida***: is an ornamental of high economic importance in global horticulture. An optimized protocol was developed for callus induction of *Petunia hybrida*. Shoot tips, stem segments and young leaves were used as explants and cultured on MS medium supplemented with different combinations of plant growth regulators. Callus was induced on MS medium enriched with BA (1mg/l) and NAA (0.1mg/l). The calli were then transferred to MS medium supplemented with BA (1mg/l) and NAA (0.1mg/l) for shoot differentiation (Su-ping, 2001)). An effective protocol was developed for shoot regeneration of two varieties of *Petunia hybrida* using stem explant. Shoots regenerated on MS medium supplemented with BAP (1mg/l) in both the varieties. The two varieties responded positively and gave over 50% of regeneration (Clapa and Canter, 2006). A standardized protocol was developed for direct *in vitro* regeneration of *Petunia hybrida*. Direct shoot regeneration was achieved from leaf

explants on MS medium supplemented with BAP (2mg/l) and NAA (0.1mg/l). Micro shoots cultured on MS medium supplemented with BAP produced the maximum number of shoots. Roots were induced on half-strength MS medium supplemented with IBA (1mg/l) and activated charcoal (2g/l) (Rao *et al.*, 2006).

### ***Solanum* species**

*Solanum* is a large and diverse genus of flowering plants, which include two food crops of high economic importance, the potato and the tomato. It also contains the nightshades and horse nettles, as well as numerous plants cultivated for their ornamental flowers and fruit.

### ***Solanum melongena*:**

Eggplant (*Solanum melongena*), or aubergine, is a species of nightshade grown for its edible fruit. The fruit is widely used in cooking. An efficient protocol was developed for shoot organogenesis of *Solanum melongena* L. variety (F-100) by using leaves and cotyledons as explant. They obtained optimal shoot bud induction on MS medium supplemented with TDZ (0.2 $\mu$ M). Rooting was induced on half strength MS medium supplemented with IAA (0.6 $\mu$ M) (Magioli *et al.*, 1997). An optimized protocol for callus induction and plant regeneration of *Solanum Melongena* L. Stem and root explants were cultured on MS medium supplemented with BAP (2.0, 3.0, 4.0mg/l) and NAA (0.1, 0.5, 1.0mg/l). In stem explants maximum callus was produced (48.66%) on MS medium enriched with BAP (2.0mg/l) and NAA (0.5mg/l) from stem (Ray *et al.*, 2011 ). A rapid and an efficient *in vitro* regeneration protocol of two varieties of *Solanum melongena* L. var. Mattu Gulla (MG) and var. Perampalli Gulla (PG) cultivated in Udupi, Karnataka State. Callus induction and multiple shoot regeneration was achieved from leaf, cotyledon and hypocotyl explants on MS medium supplemented with 2, 4-D, BAP and IAA. The hypocotyl explants showed better callus induction and multiple shoot regeneration. High frequency of shoot initiation was achieved from hypocotyl derived calli on MS medium supplemented with BAP and IAA in MG and PG. The *in vitro* regenerated shoots produced healthy roots when cultured on MS medium supplemented with IBA (Muthusamy, *et al.*, 2014). An efficient protocol for plant regeneration of *Solanum melongena* L. Cotyledonary leaf segments were used as explants. Explants from two varieties of *Solanum melongena* Pusa purple long (PPL) and Black beauty (BB) were cultured on MS medium supplemented with BAP, Kn, TDZ and Zeatin. Highest number of shoots was obtained on MS medium supplemented with BAP (2.0mg/l) and Kn (0.5mg/l). Shoots were then excised from shoot clumps and transferred to rooting medium supplemented with IBA (3.0mg/l) (Shivaraj and Rao, 2011). An *in vitro* propagation system for *Solanum melongena* L. by using tender shoot tip, hypocotyl, leaf and stem segments as explants. Multiple shooting was obtained on MS medium supplemented with BAP, Kn, NAA and IAA. The successful rooting was recorded on MS medium supplemented with IBA (0.4mg/l) (Robinson and Saranya, 2013).

***Solanum trilobatum*:** A rapid micropropagation protocol of *Solanum trilobatum* L. Shoot tips and nodes were used as explants and inoculated them on MS medium supplemented with BAP (2.22 - 13.32 $\mu$ M) and Kn (2.32 - 13.82 $\mu$ M). The highest rate of multiple shoot proliferation was observed on MS medium fortified with BAP (8.88 $\mu$ M) and Kn (9.28 $\mu$ M). Shoots were rooted on MS medium supplemented with IBA (9.48  $\mu$ M) (Jawahar *et al.*, 2004).

***Solanum nigrum*:** European black nightshade (*Solanum nigrum*) or locally just black nightshade, duscle, garden nightshade, garden huckleberry, hound's berry, petty morel, wonder berry, small-fruited black nightshade, or popolo).



A protocol was developed for *in vitro* regeneration and flower induction in *Solanum nigrum* L. Compact green callus and multiple shoot induction were achieved on MS medium supplemented with BAP (0.5mg/l) and IAA (2.0mg/l). For *in vitro* flowering nodal explants were cultured on MS medium supplemented with BAP (2.0-7.0mg/l) and NAA (0.5mg/l). Rooting was obtained on MS medium supplemented with IBA (0.5mg/l) (Kolar *et al.*, 2008)). An efficient procedure for rapid callus induction of *Solanum nigrum* Linn. using young leaves as explant. They obtained callus initiation on MS medium supplemented with IAA (1-3mg/l) or NAA (1-3mg/l) and BAP (0.5mg/l) (Yogananth *et al.*, 2009). A procedure for *in vitro* plant regeneration of *Solanum nigrum*. Shoot formation was achieved from leaf explants on MS medium supplemented with BAP (2.0mg dm<sup>-3</sup>) and Kn (1.5mg dm<sup>-3</sup>) without any callusing stage. Hundred percent rooting was achieved on MS medium supplemented with IBA (2.0mg dm<sup>-3</sup>) (Bhat *et al.*, 2010). An efficient and reliable protocol for the *in vitro* propagation of *Solanum nigrum* L. from nodal tips. Shoot multiplication was obtained on MS medium supplemented with Kn (15mg/l). After 3-4 weeks, shoots formed secondary shoots and roots on solid MS medium supplemented with Kn (37.5mg/l) and IAA (25mg/l) (Wilfred *et al.*, 2010). An *in vitro* protocol for shoot proliferation of *Solanum nigrum* L. Nodal explants were cultured on MS medium supplemented with BAP and Kn. Highest rate of shoot proliferation (100%) was obtained on MS medium supplemented with BAP (10-15µM) and Kn (10-15µM). Rooting was obtained on MS medium supplemented with IBA (10-15µM) and 2, 4-D (10-15µM) (Padmapriya *et al.*, 2011).

***Solanum tuberosum***: An efficient protocol was developed to investigate the effect of TDZ on *in vitro* propagation of *Solanum tuberosum* L. cvs. Desiree and Cardinal. Shoot apices from both the cultivars were separately inoculated on full strength MS basal medium as well as on MS full strength medium supplemented with different concentrations of TDZ (10<sup>10</sup>, 10<sup>-9</sup> or 10<sup>-8</sup> M). Results were recorded for shoot length, shoot number, root length, root number, number of nodes, fresh and dry weight of plants after 30 days of inoculation. MS full strength medium was found to be the best for *in vitro* propagation (Sajid and Aftab, 2009).

***Solanum torvum***: A protocol for regeneration of *Solanum torvum* Sw. Nodal segments excised from germinated seeds and cultured on MS medium supplemented with GA<sub>3</sub> and grown in different concentrations of IAA, IAA + BAP and NAA + BAP (Moreira *et al.*, 2010).

***Solanum hainanense***: An *in vitro* protocol for shoot regeneration of *Solanum hainanense* Hance, a valuable medicinal plant. Leaf discs were cultured on MS medium supplemented with IBA (0.1mg/l), Kn (1.8mg/l) and BAP (3.8mg/l). Rooting was induced on MS medium supplemented with sucrose (3%), Agar (0.8%) and IBA (0.5mg/l) (Loc and Kiet, 2011).

***Solanum lycopersicon***: An *in vitro* regeneration protocol of *Solanum Lycopersicon*. Hypocotyl and cotyledons excised from germinated seedlings were used as explants. Callus was obtained on MS medium supplemented with 2, 4-D and BAP. Shoot bud regeneration was achieved on MS medium enriched with BAP (2.0mg/l). Rooting was obtained on MS medium supplemented with IBA (0.5mg/l) (Chandra *et al.*, 2013).

***Solanum xanthocarpum***: Tissue culture studies carried out on *Solanum xanthocarpum*, an important medicinal herb by using nodes as explant. Multiple shoot induction was obtained on MS medium fortified with BAP (0.3-3.0mg/l) and Kn (0.3-3.0mg/l). Rooting was obtained on MS medium supplemented with IBA (0.5-5.0mg/l) (Kumari *et al.*, 2013).

***Solanum stramoenifolium***: An *in vitro* protocol for direct and indirect shoot organogenesis of *Solanum stramoenifolium*, a medicinal plant used in dropsy and rheumatism. Nodal explants inoculated on MS medium supplemented with BAP (4 $\mu$ M) produced maximum number of shoots (19.5 $\pm$ 0.16) (Manjunatha *et al.*, 2013).

***Solanum americanum***: *Solanum americanum*, commonly known as American black nightshade, small-flowered nightshade or glossy nightshade is a herbaceous flowering plant of wide though uncertain native range.

A protocol was devised for direct plant regeneration of *Solanum americanum* using *in vitro* leaf explants. Shoot induction was achieved on MS solid medium supplemented with Zeatin riboside and NAA or BAP and NAA. Plantlets were efficiently rooted on half-strength MS basal medium supplemented with sucrose (58.5mM) (Connor-Sanchez *et al.*, 2010). A protocol for *in vitro* flower induction and shoot regeneration of *Solanum americanum*. Multiple shoot induction was achieved from nodal explants cultured on MS medium supplemented with BAP (3.0mg/l), 2, 4-D (0.5mg/l) and GA<sub>3</sub> (2.0mg/l). They also observed *in vitro* flower induction and multiple shoot induction from leaf explants on MS medium supplemented with BAP (2.0mg/l), 2, 4-D (1.0mg/l) and IAA (1.0mg/l). The regenerated shoots were transferred on MS medium fortified with NAA, IBA and IAA for root induction (Ramar *et al.*, 2014).

#### ***Withania species***

***Withania somnifera***: commonly known as *ashwagandha*, Indian ginseng, poison gooseberry, or winter cherry.

An efficient large-scale clonal propagation protocol devised for *Withania somnifera* (L.) Dunal, a valuable medicinal plant, by using cotyledonary nodes derived from axenic seedlings as explants. MS medium supplemented with BAP (1.0mg/l) was found to be optimum for production of multiple shoots (100% shoot proliferation). They also achieved multiple shoot proliferation from nodal segments, derived from *invitro* raised shoots, on MS medium fortified with BAP (1.0mg/l). Regenerated shoots were best rooted on half-strength MS medium supplemented with IBA (1.0mg/l) (Nayak *et al.*, 1962).

An efficient protocol for direct shoot bud regeneration of *Withania somnifera* by culturing node, internode, hypocotyl and embryo explants on MS medium supplemented with BAP and TDZ. Nodal explants formed multiple shoots both from pre-existing and *de novo* buds on MS medium supplemented with BAP (0.1–5.0mg/l) and a ring of *de novo* shoot buds on MS medium fortified with TDZ (0.2 and 0.3mg/l). Internodal explants formed shoot buds on MS medium supplemented with BAP (1.0 and 5.0mg/l) while the hypocotyl explants gave rise to multiple shoots on MS medium supplemented with BAP (0.5mg/l). Isolated embryos gave rise to shoot buds on MS medium supplemented with TDZ (0.2 and 0.3mg/l). The shoot buds elongated and rooted either on MS medium supplemented with BAP (0.01mg/l) or half salt strength MS medium (Kulkarni *et al.*, 2000).

An *in vitro* propagation protocol of *Withania somnifera* L. using shoot-tips from aseptically germinated seedlings as explants. Culture conditions were optimized using different plant growth regulators which gave rise to 120 shoots from a single bud. The plantlets were then transferred to pots and maintained in the greenhouse for 4 months. 90% of these *in vitro* propagated plantlets survived and showed normal growth (Furmanowa *et al.*, 2001). An efficient protocol for callus induction of *Withania somnifera* (L.) Dunal using hypocotyl, root and cotyledonary leaf segments as explants. Maximum callusing (100%) was obtained from root and cotyledonary leaf segments on MS medium supplemented with a combination of 2, 4-D (2mg/l) and Kn (0.2mg/l). Hypocotyl segments used as explants showed 91% callus induction. Shoot regeneration was obtained on MS medium supplemented with BA (2mg/l), 2, 4-D (2mg/l) and Kn (0.2mg/l). These shoots were rooted on MS medium supplemented with IBA

(2mg/l) (Rani *et al.*, 2003). A rapid micropropagation protocol of *Withania somnifera*, an antitumor medicinal plant by using axillary bud explants. Multiple shoots were induced on MS medium fortified with BAP (2mg/l) and NAA (0.1mg/l). The regenerated plantlets were found to form tiny green floral buds after 4–6 weeks of culture on MS medium supplemented with Kn (0.5-0.4mg/l) and IAA (0.1mg/l) (Saritha and Naidu, 2007).

An efficient and reproducible procedure for direct shoot regeneration of *Withania somnifera* L. Petioles and leaves were used as explants. A large number of shoots regenerated on MS medium supplemented with BA (2mg/l) alone or with NAA (0.1mg/l). Rooting was achieved on MS medium supplemented with IBA (0.1mg/l) (Ghimire *et al.*, 2010). An efficient protocol for *in vitro* regeneration of *Withania somnifera* (L) Dunal via direct adventitious shoot proliferation from leaf explants. MS medium supplemented with BAP (1.5mg/l) and IAA (1.5mg/l) was found to be effective in inducing 100% shooting. Rooting was achieved on MS medium fortified with GA<sub>3</sub> (0.15mg/l) and IBA (5mg/l) (Kumar *et al.*, 2013).

Tissue culture studies and plant regeneration of *Withania somnifera* L. Dunal using leaves as explant. Callus was induced on MS medium supplemented with 2, 4-D (1.0mg/l) and Kn (0.1mg/l). Rooting was obtained on half strength MS medium supplemented with NAA (Luo *et al.*, 2012).

An effective, rapid and improved *in vitro* plant regeneration protocol of *Withania somnifera* L. using shoot tip and nodes as explant excised from 15 day old aseptic seedlings. MS medium supplemented with BAP, Kn and 2iP was found to be the most effective growth hormones in proliferating apical and axillarybuds. Multiple shoots were obtained from nodal segments on MSmedium fortified with BAP (2.5µM) and NAA (0.5µM). Rooting was achieved on half-strength MS medium supplemented with NAA (0.5µM) (Fatima and Anis, 2012). A protocol for *in vitro* regeneration and multiplication of *Withania somnifera* (L) Dunal. They cultured cotyledons explants on MS medium fortified with BAP and Kn (0.5-3.0mg/l). They also recorded maximum number of shoots (57.4±0.59 and 50.0±0.40) per explant on MS medium supplemented with BAP (2.0mg/l) and IAA (1.5mg/l) respectively. Rooting was induced on MS medium supplemented with IBA (5mg/l) (Kumar *et al.*, 2013). An efficient protocol for induction and proliferation of callus in *Withania somnifera* L. Callus was obtained from stem explants on MS medium supplemented with BAP, NAA, 2, 4-D and Kn. The callus was compact and yellowish brown in color. MS medium supplemented with BAP (0.5mg/l) + NAA (1.5mg/l); followed by MS medium supplemented with BAP (0.5mg/l) + NAA (0.5mg/l), BAP (0.5mg/l) + NAA (2.0mg/l) and BAP (1.0mg/l) + NAA (1.5mg/l) showed maximum callus growth (Adhikari and Pant, 2013). A rapid and highly effective protocol for the micropropagation of *Withania somnifera* an endangered medicinal plant by using shoot tips as explant. Shoot cultures were initiated on MS medium supplemented with BAP (0.5-2.0mg/l), NAA (0.2-0.5mg/l), agar (4.5gm/l) and commercial sugar (3%) (Baba *et al.*, 2017).

***Withiana coagulans***: also known as (Ashutoshbooti) are economically significant. The berries contain a rennet-like protease that can be used to clot milk for cheese production.

An efficient micropropagation protocol of *Withania coagulans*, a highly endangered medicinal herb and an important natural source of withanolides. Prolific multiplication of axillary buds was obtained from the nodal segments and shoot tips on MS medium enriched with BAP (0.5mg/l), Kn (0.5mg/l) and PG (0.5mg/l). Rooting was obtained on MS medium supplemented with IBA (0.25mg/l), PAA (0.5mg/l) and CC (2mg/l) (Jain *et al.*, 2009). A micropropagation protocol for adventitious shoot regeneration of *Withania coagulans* Dunal. using leaf explant. Green compact nodular organogenic callus was developed on MS medium supplemented with Kn and

BA. While multiple adventitious shoot bud differentiation occurred on MS medium fortified with Kn and BAP. Shoots were rooted on MS medium supplemented with CC, PG and IBA (Jain *et al.*, 2011). An efficient micropropagation protocol of *Withania coagulans* Dunal. Multiple shoot buds were developed from nodal explant cultured on MS medium fortified with BAP and IBA. Root formation was induced in *in vitro* proliferated shoots by culturing them on half salt strength MS medium supplemented with sucrose (2%), IBA, IAA and Kn (Valizadeh and Valizadeh, 2011). A simple, rapid and effective *in vitro* micropropagation protocol for *Withania coagulans*, popularly called vegetable rennet, a critically endangered and highly valued medicinal plant. Nodal segments were used for multiple shoot bud induction on MS medium supplemented with BAP (8.88 $\mu$ M), IAA (0.57 $\mu$ M), agar (0.8%) and additives (L-ascorbic acid (100mg/l), citric acid (25mg/l), adenine sulphate and L-arginine). The micro shoots were rooted both *in vitro* and *ex vitro* on agar gelled half strength MS medium supplemented with IBA and activated charcoal (200mg/l) (Rathore *et al.*, 2012)..

### Discussion and conclusion

Medicinally important plants of family Solanaceae have been successfully micropropagated through tissue culture. In most of the cases, MS medium with different concentrations of auxins and cytokinins either alone or in different combinations was used. In some cases, other growth media like, Gamborg B5, Braun medium (WB), Nitsch (N6) medium, Linsmair-Skoog medium and growth adjuvants like TDZ, Zeatin (ZEA), casein hydrolysate (CH), tyrosine, glutamine, AgNO<sub>3</sub>, 2-ip, Malt Extract (ME), maize extract have also been used which proved very effective. Plant tissue culture is an important technique for propagating and conserving the medicinal and aromatic plants which are either threatened or at a risk of becoming threatened in near future due to overexploitation of their important bi- products. There are many plants of family Solanaceae which are facing threat one way or the other. Plant tissue culture has helped not only in micropropagating these plants at large scale but also in making them available for future generation.

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