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REVIEW ARTICLE

MICROPROPAGATION OF MEDICINALLY IMPORTANT PLANT SPECIES OF FAMILY ASTERACEAE – A REVIEW

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ABSTRACT

Asteraceae is a large and wide spread family of Angiosperms with about 23,000 species belonging to 1,620 genera. It is an economically important family with plants providing products like oil seeds, sweetening agents, coffee substitutes, tea etc. and also those having medicinal importance. Since, all the plants of this family are not cultivated; those present in wild habitats are facing threat due to the overexploitation for these byproducts. So there arises the need for conservation and one of the main methods is propagation through tissue culture.

INTRODUCTION

The micropropagation techniques devised for the large scale propagation of the plants belonging to family Asteraceae 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.

***Stevia rebaudiana Bertoni*:** is sweet tasted antidiabetic herb. For its successful micropropagation a number of explants have been exploited by various workers. For the establishment of *in vitro* cultures young leaves inoculated on MS medium containing BAP and the cultures incubated in light was found to be the most effective treatment while as the cultures incubated in dark needed NAA in addition to BAP [1]. However, for the production of somatic embryo from *in vitro* raised leaves 2, 4-D was found to be most suitable growth hormone [2]. Such cultures were able to regenerate roots without the support of any growth regulator there by suggesting the endogenous capability of the somatic embryos to regenerate roots. Jain *et al* (2009) [3]cultured young primordial leaves surrounding shoot tip and leaves from field grown plants successfully on BAP and IAA supplemented MS medium. Shoot proliferation and elongation was seen best on BAP and Kn containing medium. Direct differentiation of shoot buds was also obtained from leaf explants cultured on MS medium containing BAP, IAA and $CuSO_4$. Nodal explants as a rule produced multiple shoots through axillary shoot proliferation on MS medium containing BAP and Kn in combination. IAA was found to induce highest rooting percentage [4]. However, MS medium supplemented with BAP and IAA in combination was found to be most effective in inducing bud break and growth, and in initiating multiple shoot proliferation [5]. *S. rebaudiana* has been micropropagated through callus cultures as well. For callus induction and multiplication, nodal as well as leaf segments were inoculated on MS medium containing BAP and NAA. Regenerated plants were rooted on ¼ MS medium containing IBA [6]. Anbazhagan *et al* (2010) [7]also used shoot tip, nodal segment and leaf explants for the mass propagation of *S. rebaudiana* and achieved best results on MS medium with BAP and IAA in combination. Rooting was achieved on half strength Nitsch (N6) medium supplemented with IAA. Axillary bud explants proved successful in producing maximum number of shoots on MS medium supplemented with BAP in combination with Bavastin. [8] Found that ethylene inhibitor Silver Thiosulphate favoured the shoot morphogenesis in this plant species.

Saussurea species: Medicinally important species of genus *Sassurea* that have been successfully propagated through tissue culture are:

Saussurea lappa C.B.Clarke: is an endangered medicinal perennial herb, endemic to the valley of Kashmir and western Himalayas. It is used as an ethno-medicine against a lot of diseases like pain, arthritis, head ache, ulcers, skin diseases, cough, asthma, flatulence, colic, diarrhoea, fever, general debility etc. A procedure has been devised for its *in vitro* multiplication where in shoot cultures are initiated from terminal portion of hypocotyl segments bearing the plumule. BAP and GA_3 containing MS medium was found to be the best for its multiplication. Rooting was obtained on NAA containing MS medium [9]. Another micropropagation technique for *S. lappa* was devised by culturing shoot tips of 2 week old seedlings on MS medium supplemented with thidiazuron (TDZ). Although, callus-free multiple shoots were obtained both on BAP and TDZ containing media, TDZ was seen most effective in inducing multiple shoots. Multiplication of induced shoot buds was found more effective when cultured in liquid medium than on agar-solidified medium. Shoots developed, were rooted in MS medium containing NAA [10].

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Saussurea involucrate Kar. et Kir. An efficient micropropagation system for this endangered Chinese medicinal plant was developed by inoculating leaf explants on BAP and NAA supplemented MS medium. Shoot organogenesis was further improved by pre-incubating leaf explants at low temperature [11]. Micropropagation method for this plant via direct organogenesis with adventitious buds induced from hypocotyls, cotyledons, leaves and roots on basal medium was also developed. Highest induced budding ratio was seen in MS medium containing BAP and NAA in combination and in 2, 4-D supplemented MS medium. Budding multiplication was seen highest in BAP and NAA supplemented MS medium [12].

Saussurea esthonica: is an endangered wild plant species growing in Latvia. For initiation of tissue culture seeds of this plant were used as explants. The best results on shoot proliferation were obtained using BAP containing MS medium and in case of root formation cytokinin free medium and media containing 2-ip was found to be effective [13].

Saussurea obvallata (DC.) Edgew**:** is the endangered state flower of Uttaranchal, India and is used to treat paralysis of the limbs and cerebral ischemia. A callus induction and *in vitro* plantlet regeneration system has been established after studying the explant type (root, hypocotyl, cotyledon and leaf), age and effect of different concentrations of plant growth regulators. Best results were obtained by using leaf explants and MS medium supplemented with BAP and NAA [14].

Saussurea medusa Maxim: Cell suspension cultures have been established for this valuable traditional Chinese herb. Callus production was induced in leaf explants on MS medium containing BAP and NAA. Cell suspension was produced from light-yellow calluses in liquid MS medium containing BAP and NAA [15].

Saussurea laniceps Hand.-Mazz.: One of the most famous and important medicinal herbs in China have been micropropagated by using leaf explants of the *in vitro* raised plantlets. For callusing and shoot bud regeneration, the optimal medium was found to be MS medium supplemented with NAA and BAP. Rooting was obtained on half strength MS medium containing NAA [16].

Inula species: The genus *Inula* is known for diverse biological activities viz: anticancer, antibacterial, hepatoprotective, cytotoxic, and anti-inflammatory properties The species of genus *Inula* that have been micropropagated successfully are:

Inula viscosa (L.) Aiton (sin. Dittrichia viscosa (L.) W. Greuter): An efficient micropropagation method was developed for this species of *Inula* by using nodal segments as explants. This protocol proved to be very efficient as more than 10,000 plantlets were produced in just four months time [17]. Romano (1997) [18] developed a BAP induced micropropagation method for this plant using apical shoot tips as explants.

Inula racemosa Hook.f: An *in vitro* technique for the large scale micropropagation of *I. racemosa* was developed by using shoot tips, excised from ten day old seedlings, as explants. Plantlet regeneration was obtained by a combined effect of auxins and cytokinins and it was found that Kn, IAA and Malt Extract (ME) are the most effective growth adjuvants for inducing organogenesis [19]. Jabeen *et al* (2007) [20], induced multiple shoots by using leaf and nodal segments as explants on BAP containing MS medium. Kaur *et al* (2010) [21] used aseptically grown seedlings as explants and recorded MS medium fortified with BAP as the best performing medium for *in vitro* shoot multiplication.

Inula royleana DC.**:** Stojakowska and Malarz (2004) [22] developed a micropropagation technique through axillary shoot proliferation in case of *I. royleana* using both primary explants (cotyledonary nodes) and secondary explants (node explants of in vitro regenerated shoots). MS medium supplemented with NAA and Kn proved to be the most effective medium for achieving such results.

Inula helenium L.: Stojakowska *et al* (2004) [23] established root culture of *Inula helenium* L. by using leaf, from aseptic seedlings, as explants.

Inula verbascifolia (Willd.) Hausskn. Sub species verbascifolia: Perica *et al* (2008) [24] developed a clonal propagation method for this plant by using shoots from the aseptically generated seeds for culture initiation. They achieved shoot regeneration on a BAP and GA supplemented MS medium while as rooting was achieved by the addition of either IAA or IBA in the rooting medium.

Inula japonica: Yong-Mei *et al* (2008) [25] established a micropropagation method for *Inula japonica* by using rhizome explants. They achieved callus induction on a 2,4-D supplemented MS medium; bud differentiation on BA, NAA and $Ag(NO_3)$ supplemented MS medium and rooting on NAA supplemented rooting medium.

Arnica montana L.: is endemic to Europe and is used medicinally for pharmaceutical and cosmetic use. Multiple shoots have been regenerated from shoot tips of *this plant* on MS and B_5 media supplemented with BAP and NAA. Maximum number of shoots was obtained from these explants on B_5 medium within 6 weeks time [26]. Butiuc-Keul and Deliu (2001) [27] obtained plant multiplication as well as root induction after a period of 3 weeks by culturing nodal explants on MS medium containing NAA, 2Pi and maize extract. Petrova *et al* (2011) [28], established a protocol for indirect organogenesis from leaf explants of *A. montana*. For callus induction, they cultivated two different types of explants (leaf and petiole segments) from three-months-old *in vitro* plants on MS medium supplemented with 2, 4-D. The efficiency of callus formation from leaf explants was seen more. Their results showed that shoot regeneration from leaf-derived callus was possible in MS medium supplemented with BAP and 2,4-D. however, the intensity of shoot regeneration was low. For micropropagation, maximum number of regenerated plants was cultivated on MS medium supplemented with BAP and IAA. All the micro-shoots produced normal roots on ½ MS medium containing IBA in four weeks of culture.

ATRACTYLODES SPS

Atractylodes lancea DC: a perennial medicinal herb of family Asteraceae was successfully micropropagated by using shoot tips as explants on Linsmair-Skoog medium supplemented with BAP [29].

Atractylodes macrocephala Koidz: Rapid propagation of *A. macrocephala*, an important traditional Chinese medicinal plant, was carried out through direct shoot and plant regeneration by using leaf explant. The results showed that the highest rate of direct shoot multiplication was achieved on MS+BAP+NAA. The optimal substrate for callus induction and plant regeneration from leaf was seen to be MS+KT+NAA where the highest callus introduction and proliferation were obtained. The effect of KT on

callus induction and proliferation was seen superior to BAP, whereas BAP was more efficient than KT at higher concentration on direct shoot multiplication [30]. Another efficient plant regeneration protocol was developed by Mao *et al* (2009) [31] via shoot organogenesis. They induced shoot multiplication on MS medium supplemented with various concentrations of TDZ, BAP and NAA. Rooting was induced on half-strength MS medium supplemented with NAA and IBA. They obtained maximum mean number of shoots from a single explant by the combined effect of NAA and TDZ.

ARTEMISIA SPS

This genus is known to contain terpenoids and sesquiterpenes. The aromatic leaves of many species of *Artemisia* are medicinal and some are used for flavouring. Some species that have been successfully micropropagated are:

Artemisia annua L.: An efficient protocol for *A. annua*, a medicinally important plant native to temperate Asia, has been optimized by using nodal explants from inflorescence segments. MS medium supplemented with RT vitamins, casein hydrolysate and BAP was found to be the best for the induction of shoot regeneration. Rooting of regenerated shoots was achieved on IBA containing medium [32]. Kamili *et al* (2001) [33] inoculated leaf explants on MS medium containing NAA for callus induction. They obtained shoots on NAA and Kn containing MS medium and roots on NAA containing medium. In 2004, Kaloo *et al* [34] subjected the callus, obtained on NAA containing MS medium, to extraction of phytochemicals and it was found that the callus cultures are a potential source for the production of terpenoids. Callus was induced from stem segments of *A. annua* L. on MS medium containing BAP and NAA. For rooting, success was achieved on NAA containing medium [35]. Al-Maarri and Yu-Xie (2010) [36], obtained direct organogenesis by inoculating leaf, petiole, internode and cotyledon as explants on MS medium containing TDZ.

Artemisia absinthium L.: Callus cultures of *A. absinthium*, a plant that exhibit strong antimicrobial activities, especially against Gram-positive pathogenic bacteria, were initiated by successfully micropropagating it on MS medium containing BAP/Kn in combination with IAA. Regeneration of root and shoot primordial from leaf explants on 2,4-D containing MS medium was also achieved. Furthermore, it was found that BAP and NAA containing medium facilitated both callus growth and organogenesis on some callus cultures [37].

Artemisia mutellina Vill: Mozzetti and Donato (1998) [38] initiated nodal stem cultures from apical buds of adult plants of *A. mutellina*, a plant with aromatic foliage. They obtained high multiplication rates from these cultures on MS medium supplemented with BAP.

Artemisia judaica L: An *in vitro* regeneration system for *A. judaica*, an important Egyptian medicinal plant, was devised by Liu *et al* (2003) [39]. They induced shoot organogenesis by culturing etiolated hypocotyls and intact seedlings on medium containing TDZ. For rooting, they sub-cultured shoots on IBA containing medium.

Artemisia petrosa ssp. eriantha: Pace *et al* (2004) [40] successfully micropropagated this species of *Artemisia* by using cotyledons, excised from *in vitro* raised plantlets, as explants for callus induction. They transferred callus to a medium containing NAA and BAP for shoot regeneration and for rooting, ½ strength MS medium and medium supplemented with $CaCO_3$ and IAA/IBA was proved to be best.

Artemisia scoparia: Aslam *et al* (2006) [41] devised a protocol for effective propagation of *A. scoparia* by using shoot tips, leaf and petiole explants for callus induction and shoot tips for direct organogenesis. MS medium supplemented with 2,4-D and NAA proved to be the best combination for callus induction in case of all explants, whereas, multiple shoots from shoot tip explants were induced by NAA in combination with BAP or Kn. Best root initiation was achieved in NAA supplemented media.

Artemisia vulgaris L.: Sujatha and Kumari (2007) [42] established an efficient *in vitro* micropropagation method for *A. vulgaris*, an aromatic perennial herb by using its shoot tip and nodal explants. Among the various growth regulators tested, MS medium and B_5 vitamins supplemented with BAP and Kn combination was found to yield a better response than BA or Kn alone in the medium. Rooting was highest on MS medium containing IAA. Borzabad *et al* (2010) [43] achieved callus induction on MS medium containing BAP+NAA and regeneration on MS medium with BAP+GA_3 from leaf explants. Kumar and Kumari (2010) [44] studied effect of amino acids and growth regulators on indirect organogenesis. They used cotyledonary nodes excised from 30 day old seedlings as explants. Calli were induced on MS medium containing B_5 vitamins, 2,4-D, NAA, and cysteine in combination. Maximum number of multiple shoot production was achieved on the medium containing BAP, TDZ and tyrosine. NAA, $AgNO_3$ and glutamine combination produced maximum number of roots per explant in the medium.

Artemisia amygdalina Decne: Rasool *et al* (2013) [45] studied the efficacy of different auxins and cytokinins on this plant by exploiting the morphogenetic potential of shoot tip explants and found that MS medium fortified with NAA together with BAP produced maximum number of shoots. Regenerated shoots showed maximum rooting on MS basal media.

Vicoa indica: Thulaseedharan and Vaidyanathan (1990) [46], induced callus and plant regeneration from stem and leaf explants of aseptically grown *V. indica*. Optimum callus initiation was seen in Gamborg B_5 basal medium containing NAA with Kn or BAP with NAA. They obtained shoot primordia from greenish callus on passage to B_5 basal medium containing BAP and Kn. On further subculture onto B_5 medium containing Kn, the shoot primordia developed into plantlets. Subramanian *et al* (2011) [47] developed a protocol for rooting of stem cuttings of *V. indica* on IBA containing MS medium.

Petasites hybridus Gaertn., Mey. et Scherb.: Wildi *et al* (1998) [48] developed an *in vitro* shoot regeneration protocol from sterile leaf and petiole explants and from *in situ* collected inflorescence buds of *P. hybridus*, a traditional plant used in European phytotherapy for treating migraine. MS medium with Kn and NAA in combination performed best in terms of shoot multiplication rate.

ECHINACEA SPS

***Echinacea purpurea L.*:** Choffe *et al* (2000) [49] established an *in vitro* propagation system for *E. purpurea* (purple coneflower), a medicinal plant commonly used in the treatment of colds, flu and related ailments. They incorporated an optimized minimal concentration of Plant Preservation Mixture (PPM) for seed germination in order to prevent contamination. Regeneration was induced on petiole explants from 2-month-old sterile seedlings

cultured on MS medium supplemented with BAP or TDZ in combination with IAA. They found some petiole explants exposed to BAP dedifferentiated to form calli from which *de novo* shoots arose while as other petiole explants formed somatic embryos on the epidermal layer without an intervening callus phase. Mechanda *et al* (2003) [50] carried out micropropagation of *E. purpurea* plants through direct shoot regeneration from mature leaf tissues in 30 days after culture initiation on Woody Plant Medium (WPM) supplemented with BAP. They obtained maximum shoot organogenesis with 5% coconut milk and BAP. Callus was induced using NAA and BAP. The regenerated shoots were rooted on WPM supplemented with IBA. Jones *et al* (2007) [51] investigated the development of a rapid micropropagation protocol of *Echinacea purpurea* L on a liquid medium. They observed callus development and root organogenesis on leaf explants cultured on MS medium containing 2, 4-D or dicamba, and addition of TDZ resulted in the production of regenerable callus cultures. They initiated liquid cultures from the tissue derived from TDZ treatment.

Echinacea pallida: A method for the induction of adventitious shoots of *E. pallida* was developed from its leaf tissue by using MS medium containing BAP and NAA in combination. Rooting was successful on IBA containing medium [52].

Echinacea angustifolia DC: Lucchesini *et al* (2009) [53] obtained *in vitro* cultures of *E. angustifolia* directly from sections of flower stalks of adult plants. MS medium containing BAP was seen to be effective for shoot formation. Regenerating callus was established from leaves of *in vitro* shoots cultured on BAP and IBA. Kim et al (2010) [54] established an improved method for shoot organogenesis and plant regeneration from stem cultures of *E. angustifolia.* They regenerated maximum number of shoots from stem cultures on MS medium containing BAP. The addition of IBA substantially improved the shoot regeneration of *E. angustifolia.* They found plant regeneration to be more efficient when Phytagel was used as the gelling agent. Herbage (2001) [55] micropropagated three species of *Echinacea, viz., E. angustifolia, E. pallida and E. purpurea,* from nodal segments and seed explants. He obtained shoot multiplication on BAP containing MS medium in all the three species while rooting was obtained only in case of *E. purpurea.*

HELICHRYSUM SPS

Giovannini *et al* (2001) [56] devised a protocol for tissue culture of *H. italicum* and *H. stoechas*, two important species of Asteraceae used in folk medicine, from *in vivo* germinated seedlings. They obtained shoot induction from micropropagated leaf tissue on a medium supplemented with IAA in case of *H. italicum* and on a medium with thidiazuron in case of *H. stoechas.* They also developed a system for callus production on a medium enriched with 2, 4-D and Kn.

Santolina canescens Lagaska: Shoot tips of *S. canescens*, an aromatic species producing a novel diacetylene derivative, were multiplied on MS medium supplemented with BAP and Kn in combination. The best axillary-bud proliferation was recorded on MS medium containing BAP and NAA in combination [57].

Spilanthes acmella L.: Saritha *et al* (2002) [58] induced multiple shoots in *S. acmella* by using hypocotyl segments, obtained from 1-week-old seedlings, as explants. They recorded maximum success on MS medium containing BA in combination with NAA. Haw and Keng (2003) [59], successfully micropropagated *S. acmella* by using axillary buds as explants. They achieved success within a period of five weeks on MS medium supplemented with BAP. Pandey *et al* (2011) [60] micropropagated *S. acmella* through leaf explants by using NAA and BAP supplemented MS medium while as Sahu *et al* (2011) [61] does it through axillary bud, shoot tip and leaf explants on 2,4-D, BAP and BAP+NAA supplemented MS medium.

Solidago* species*: Kalemba and Thiem (2003) [62], established *in vitro* shoot culture of four *Solidago* species, *S. virgaurea* L., *S. canadensis* L., *S. gigantea* Ait. and *S. graminifolia* (L.) Salisb from aseptically germinated seedlings. They multiplied proliferated axillary shoots on MS medium supplemented with Kn and IAA.

Pluchea lanceolata Oliver & Hiern.: Kumar *et al* (2003) [63] initiated nodular callus in *P. lanceolata* from young leaf segments on Wood and Braun medium (WB) supplemented with Kn whereas Arya *et al* (2008) [64] initiated it on NAA and BAP supplemented MS medium and obtained maximum number of shoots from the callus on BAP and Kn supplemented MS medium.

Eupatorium triplinerve Vahl: Martin (2003-2004) [65] established an effective protocol for micropropagation of the medicinal plant *E. triplinerve* through rapid axillary bud proliferation and *ex vitro* rooting. MS medium fortified with BAP and IBA proved to be best.

CALOCEPHALUS SPS

Sands *et al* (2003) [66] micropropagated two threatened Tasmanian species of *Calocephalus* viz., *C. citreus* and *C. lacteus.* They achieved initiation of aseptic cultures of *C. citreus* on MS medium supplemented with IAA and BAP while as MS medium containing NAA proved effective for *C. lacteus.* Both these species multiplied successfully on IAA and BAP containing medium. They also found that root formation was initiated on multiplication medium in case of *C. lacteus* while as, in case of *C. citreus,* optimal root induction was achieved on MS with activated charcoal.

Eclipta Alba (L.) Hassk: Baskaran and Jayabalan (2005) [67] micropropagated *E. alba* by enhanced axillary shoot proliferation from cotyledonary node segments. They found that MS medium with the synergistic combination of BAP, Kn, 2-isopentenyladenine, gibberellic acid, 5% coconut water and 3% sucrose promoted the maximum number of shoots as well as beneficial shoot length. Dhaka and Kothari (2005) [68] cultured shoot tips and nodal segments taken from *in vitro* raised plants and found that maximum shoot proliferation occurred on MS medium supplemented with BAP. Husain and Anis (2006) [69] developed an efficient protocol for rapid *in vitro* propagation of *E. Alba* L. through axillary bud multiplication. MS medium supplemented with BAP was found to be most effective in breaking bud dormancy. Maximum mean number of shoots was obtained after three subcultures. Most efficient rooting was seen on MS with IBA.

Anthemis xylopoda O. Schwarz: Erdag and Emek (2005) [70] developed a micropropagation method for *A. xylopoda,* a critically endangered endemic species of Turkey. They germinated seeds on MS medium supplemented with different concentrations of GA. Shoots obtained from these seeds were used as explants. Highest number of shoots per explant was obtained on MS medium

supplemented with TDZ and highest mean of maximum shoot length was found on MS with Kn at low concentration.

Cichorium intybus L. cv. Focus: Nandagopal and Kumari (2006) [71] achieved flowering in *C. intybus* by using its young leaves as explants. The highest percentage of callus induction and multiple shoot proliferation was observed on MS+B_5 medium containing BAP, IAA and adenine sulphate in combination. For root induction regenerated shoots were transferred to MS+B_5 medium containing IAA, IBA and NAA. *In vitro* flowers were also noticed in the *in vitro* raised plantlets in the same medium under 16h light and 8h dark condition. Abdin and Ilah (2007) [72] micropropagated this plant through somatic embryo development by nodal stem and petiole explants. Somatic embryogenesis was seen to be more intensive in MS medium with higher concentration of Kn and lower concentration of IAA supplemented with vitamin-free casein hydrolysate. Afterwards, the germination of embryoids into shoots was achieved on fresh MS medium supplemented with Kn + IAA + IBA + CH. They obtained root formation in MS medium supplemented with IBA.

Syncarpha recurvata (L.f.) B. Nord.: Swart (2006) [73] obtained an efficient propagation method for *S. recurvata*, a vulnerable (intermediate priority) species of family Asteraceae. Germinated embryos were used as explants. Success was achieved on a medium supplemented with IAA.

Arctotis arctotoides: Adebola and Afolayan (2007) [74] devised a procedure for *in vitro* plant regeneration from seed-derived callus of this plant noted for its medicinal uses among the rural people of the Eastern Cape Province of South Africa. Callus formation was best in basal MS salt supplemented with 2, 4-D after 2-weeks.

Lychnophora pinaster Mart.: De Souza *et al* (2007) [75] obtained shoot induction on MS medium containing BAP. Maximum shoot elongation before rooting occurred in the presence of gibberellic acid and microshoots were successfully rooted in the presence of NAA.

VERNONIA SPS

Khalafalla *et al* (2007) [76] induced maximum number of multiple shoots *in vitro* from the stem nodal segments of *V. amygdalina* on MS medium containing BAP alone or in combination with NAA. They rooted regenerated shoots on MS supplemented with NAA. Seetharam *et al* (2007) [77] obtained *in vitro* shoot regeneration from leaf and nodal explants of *V. cinerea* (L.) Less. on MS medium containing BAP and NAA and rhizogenesis in half strength MS medium containing IAA while as Maharajan *et al* (2010) [78] did it by culturing shoot tip explants on MS medium supplemented with BAP and obtained rooting on the medium containing IBA. Vicente *et al* (2009) [79] carried on in vitro multiplication and acclimatization of *V. condensata* by using axillary buds as explants on MS medium containing BAP.

Pentanema indicum Ling: Sivanesan and Jeong (2007) [80] investigated micropropagation and *in vitro* flowering for this medicinally important plant. They obtained maximum callus proliferation on MS medium supplemented with BAP and IBA and best shoot regeneration on BAP and IAA supplemented MS medium in five weeks. They also obtained direct multiple shoot initiation by using shoot tip and nodal explants on the same medium. Addition of adenine sulphate was found to increases the shoot multiplication.

Achyrocline flaccida (Weinm.) DC.: Bonnecarrere *et al* (2009) [81] obtained friable callus in *A. flaccida* on MS medium supplied with 2,4-D while as cell suspensions were better obtained and maintained in DKW supplied with 2,4-D.

Gynura procumbens (Lour.) Merr.: Keng *et al* (2009) [82] established a rapid micropropagation protocol for *G. procumbens*, an important medicinal plant. The nodal segments of one year old mature plants were used as the explants for the initiation of axillary branching. MS medium supplemented with BAP and NAA proved to be effective for rapid proliferation of shoots. Rooting was achieved on MS basal medium without any plant growth regulators.

Leontopodium nivale (Ten.) Heut ex Hand. - Mazz.: Pace *et al* (2009) [83] optimised an *in vitro* micropropagation method for *L. nivale*, an endemic and endangered species of the central Apennines. Callus induction was obtained from cotyledons of *in vitro* germinated seeds on MS medium containing 2, 4-D and the regeneration of shoots was readily achieved using BAP.

Carlina acaulis L.: Trejgell *et al* (2009) [84] established an efficient shoot propagation system for *C. acaulis*, a perennial plant having antibacterial carlina oxide. They used shoot tips and fragments of hypocotyls excised from 10-day-old seedlings as explants. The explants were transferred to proliferation medium supplemented with different types of cytokinins viz; BAP, Kn, and ZEA in combination with NAA. The morphogenetic response was found to be best in culture on medium supplemented with BAP and shoot organogenesis frequency was highest for shoot tips.

Wedelia chinensis*:* Agarwala *et al* (2010) [85] optimised a method for *in vitro* clonal propagation of *W. chinensis, a* medicinal plant used by traditional medicinal practitioners of Bangladesh. They used shoot tips and nodes from field grown plants as explants. Best response was seen in MS medium having BAP along with IAA. Rahman and Bhadra (2011) [86] obtained *in vitro* cultures of *W. chinensis* by using three different explants including nodal segment, shoot apex and leaf segment on MS medium with different types of cytokinins (BAP and Kn) and auxins (IAA and NAA). The maximum number of multiple shoot buds was seen in case of nodal segment cultured on MS containing BAP + IAA. But shoot apex explant was seen to produce longest shoots when only Kn was used in MS medium.

Senecio macrophyllus M. Bieb.: A protocol was optimised for *in vitro* micropropagation of *S. macrophyllus*, a rare species in Central Europe where in shoot tips and fragments of the cotyledon, hypocotyls and roots, isolated from 10-day-old sterile seedlings, were used as explants. They tested the morphological response on MS medium supplemented with different types of cytokinins: BAP, Kn and ZEA in combination with NAA, but only shoot tips were capable of shoot organogenesis. Shoot proliferation was highest for explants cultured on MS medium supplemented with BAP in combination with NAA. Rooting was achieved on full and half-strength MS medium without auxin [87].

Sphaeranthus indicus L.: Yarra *et al* (2010) [88] developed a rapid and reproducible protocol for *in vitro* regeneration of *S. indicus*, a medicinal herb. Leaf segments isolated from mature plants were used as explants and cultured on MS medium with different concentrations of BAP and Kn. Addition of IAA into BAP supplemented medium triggered the regeneration response.

They obtained maximum number of shoots with highest shoot length directly (without intervening callus phase) from the leaf explants using combination of BAP and IAA within 3-4 week of culture.

Silphium perfoliatum L., a medicinal perennial herb, was micropropagated by using apical parts of *in vitro* grown seedlings on MS medium containing BAP+NAA [89].

DISCUSSION

Medicinally important plants of family Asteraceae 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, $AgNO_3$, 2-ip, Malt Extract (ME), maize extract have also been used which proved very effective.

CONCLUSION

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 Asteraceae like, Saussurea lappa, Saussurea involucrata, Calocephalus citreus, Inula racemosa, Inula royleana, Calocephalus lacteus Anthemis xylopoda, Syncarpha recurvata, Leontopodium nivale, Senecio macrophyllus etc., 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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