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Protocols of micropropagation of some medicinal plants

Majid Abdulhameed Ibrahim[a*] | **Haider Sabeh Shanow Al-Jabir**[a] | **Ahmed Yousef Lafta**[a]

[a]Department of Horticulture and Landscape Design, College of Agriculture, University of Basrah, Iraq.

*Corresponding author: majid.abdulhameedl@uobasrah.edu.iq

Abstract The plant produces large numbers of chemical compounds with diverse physiological roles. The plant takes advantage of cheap and available natural resources in its surroundings of air, water, mineral elements, and solar energy to produce these chemical compounds. These compounds are called phytochemicals, in which biotechnologists are concerned with the mass production of economically important compounds. The compounds produced by the plant are generally divided into two groups, primary metabolites and secondary metabolites. In this paper, we will review what researchers have reached in the field of accurate propagation of some important medicinal plants through a presentation of several protocols used to propagate some medicinal plants that aim to mass micropropagation them or expose them to treatments aimed at increasing the production of secondary compounds that have a role when extracted in the manufacture of medicines and pharmaceuticals.

Keywords: nutrition, prevention, sun exposure, supplementation

1. Introduction

The plant produces large numbers of chemical compounds with diverse physiological roles. The plant takes advantage of cheap and available natural resources in its surroundings of air, water, mineral elements, and solar energy to produce these chemical compounds. These compounds are called phytochemicals, in which biotechnologists are concerned with the mass production of economically important compounds. The compounds produced by the plant are generally divided into two groups, primary metabolites and secondary metabolites. The compounds of primary metabolites are necessary for growth and development, they are similar in properties, have limited genetic heterogeneity, and can be produced in large quantities and inexpensively. Their relatively simple structures can be manufactured in the laboratory, whereas, secondary metabolites are compounds that plants do not need for growth and development, which differ in their properties and have a broad genetic diversity. They are produced in small quantities and are expensive and have complex structures that are difficult to manufacture in a laboratory. Secondary metabolites are also called natural products, as there are believed to be more than one hundred thousand secondary compounds in different structures produced by living organisms in an amount estimated at about 10^9 million tons annually. About 80% of it is found in plants and used as a pharmaceutical, nutritional and health material.

The secondary products are believed to have the primary function of eliminating the toxic effect resulting from accumulating primary metabolites. These compounds defend the plant when it is exposed to biotic and abiotic stresses, as well as antibacterial, fungi and viruses that inhibit its growth. It also helps the plant in competition with other plants, such by inhibiting the germination of its seeds and the growth of its seedlings. Secondary compounds have an important role in protecting plants from the harmful effects of ultraviolet rays. Secondary compounds are used as a source of pharmaceutical compounds, from which industrial preparations are prepared. According to the reports of the World Health Organization, approximately 70 - 80% of the world population depends on alternative medicine from herbal plants. In other words, complex chemical compounds that are difficult to produce industrially can be produced by plants. Specialty phytochemical products are of an industrial and marketing value worth billions of dollars annually. If the opportunities provided by plant tissue and cell culture are invested in this field, the world will witness a significant decrease in the prices of medicines, due to the possibility of producing them in bioreactors with capacities of up to thousands of liters, if used the modern technologies represented by the plant tissues, organ, and cell culture compared to the old traditional methods. The importance of in vitro propagating medicinal plants in order to extract the secondary products is the possibility of producing them under optimal conditions controlled throughout the year without being restricted to the growing season and geographical location, also, the micro-propagation environment is free from microbial contaminants, pests, and weeds. The use of biotechnology methods for micro-propagation encourages crops to produce secondary compounds and increase their quantities. Tissue culture is also an important source of secondary metabolites in plants that are difficult to grow in field conditions. The production of secondary compounds with tissue culture technology takes less time, effort, and labour compared to traditional methods of cultivation

and production. In this paper, we will review what researchers have reached in the field of accurate propagation of some important medicinal plants through a presentation of several protocols used to propagate some medicinal plants that aim to mass micropropagation them or expose them to treatments aimed at increasing the production of secondary compounds that have a role when extracted in the manufacture of medicines and pharmaceuticals.

2. Moringa plant (*Moringa olivera*)

The Moringa plant is called the miracle tree, which belongs to the family Moringaceae (Poteet 2006), almost all parts of this plant have nutritional and medicinal benefits. Its leaves are used as a food supplement for people with immunodeficiency because it contains large amounts of vitamins, carbohydrates, amino acids, β-carotene, iron, potassium, phosphorus, zinc, selenium and antioxidants (Farooq et al 2012; Yadav and Srivastava 2016). This plant is rich in zeatin which is an anti-ageing compound that stimulates cell division and prolongs the juvenile phase in the plant. This plant also contains many other compounds of medicinal importance such as β-sitosterol, caffeoylquinic acid, quercitin, and kaempferol. As the last two compounds work to reduce the incidence of cancer and some diseases of the heart and the circulatory system, as well as their importance as antioxidants and vitality against bacteria and fungi pathogenic to humans (Makonnen et al 1997; Abdulkarim et al 2005).

Ibrahim and Ameen (2017) developed a protocol to stimulate callus induction from hypocotyl explants in moringa plants with the aim of increasing the production of the active secondary compounds represented by zeatin, quercitin and kaempferol by exposing the induced callus to different concentrations of sucrose (30, 60, 90 and 120 g L-1). 1) and polyethene glycol, PEG (0, 25, 50 and 100 g L-1). The nutrient medium on which the callus cultures were induced was MS medium (Murashige and Skoog, 1962), supplied separately with 2.0 mg L-1 2, 4-dichloro phenoxy acetic acid (2, 4-D) and 0.1 mg L-1 naphthalene acetic acid, NAA. The researchers showed from this protocol that a higher concentration of 120 g L-1 sucrose or 100 g L-1 PEG recorded the highest content of zeatin, quercitin, and kaempferol in induced callus that were 103.4, 1324.6, and 966.5 g g-1 dry weight, respectively, at a concentration of 120 g. L-1 sucrose, and 92.01, 3528.0, and 931.0 g g-1 dry weight, respectively, at 100 mg L-1 PEG. It was concluded from this protocol that the possibility of increasing the production of the secondary active compounds in induced callus by subjecting it to stress with high concentrations of sucrose and PEG with the possibility of isolating and purifying them for use as natural product sources in the pharmaceutical industry.

3. Vinca rosea (*Catharanthus roseus*)

Vinca rosea belongs to the family Apocynaceae and is a perennial herbaceous evergreen plant (Al-Zarkani 2003). This plant is considered one of the most important medicinal plants because it contains many alkaloids used to treat heart disease and blood pressure (Al-Shahat 2000), contains more than 50 alkaloids, which have a fast pharmacological effect in treating some severe diseases. The aqueous extracts of the vinca rosea plant are used in the treatment of gingivitis, dental pain and decay. These extracts are also useful in treating stomach and intestinal ulcers. Recently, it has been known the biological effect of some alkaloids of this plant in treating chronic constipation. Forty years ago, it was proven that many alkaloids exist in this plant that has an effective effect in treating some serious cancers, such as vincristine, vinblastine, ajmalicine and serpentine, in addition to hypertension, which plays an important role in treating high blood pressure diseases (Leveque et al 1996; Van Der Heijden et al 2004).

Al-Memari et al (2018) found, the callus is induced after 28 days of culture through the protocol of leaf segments, nodal segments, and internode segments as explants of vinca rosea cultured on an MS medium supplied with 0.1 mg L^{-1} naphthalene acetic acid led. The weights of the induced callus from these explants reached 0.612, 0.639, and 0.835 g, respectively. The explants, leaf segments, nodal segments, and internode segments, which were cultured on MS medium supplied with 0.1, 0.3 or 0.5 mg L^{-1} benzyl adenine and 0.1 mg L^{-1} naphthalene acetic acid, led to callus induction after 50 days of culture (Figure 1). The alkaloid compounds ajmalicine, vindoline, vincristine, and vinblastine were diagnosed using a High-Performance Liquid Chromatography (HPLC) instrument. The highest absorbance value of vinblastine in the callus of leaf segments cultured on MS medium supplemented with 0.3 mg L^{-1} BA and 0.1 mg L^{-1} NAA. The highest absorbance value of vincristine in the callus of internode segments cultured on MS medium supplemented with 0.5 mg L^{-1}BA and 0.1 mg L^{-1} NAA. The highest absorbance value of vindoline in the callus of leaf segments cultured on MS medium supplemented with 0.5 mg L^{-1}BA and 0.1 mg L^{-1} NAA. The highest absorbance value of ajmalicine in the callus of nodal segments cultured on MS medium supplemented with 0.1 mg L^{-1}BA and 0.1 mg L^{-1} NAA.

4. Pot marigold (*Calendula offcinalis*)

Pot marigold is one of the plants belonging to the Asteraceae family, which is one of the most important medicinal annuals. The original home of this plant is the Mediterranean basin and grows wild in southern and central Europe and northern Africa (John and Harold 2004). The pot marigold plant has multiple uses in the field of folk medicine in India, as its flowers are used in the manufacture of ointments to treat wound sores and purify the blood. The leaves are used as a paste to treat varicose veins externally. In Britain, boiled flowers are used to treat smallpox and measles, and as an antispasmodic. Flower

juice, it is used to treat jaundice and constipation and to quell menstrual bleeding (Itcolor and Major 1993; Khare, 2004). Pure extracts from the dry parts of pot marigold have been shown to have therapeutic and medicinal activities as they are antioxidants containing carotenoids, polyphenols, and flavonoids (Preethi et al 2006; Bernatoniene et al 2011). Pharmaceutical studies prove the efficacy of the pure extracts of this plant as an anti-inflammatory, anti-viral, and anti-genotoxic (Medina et al 2006).

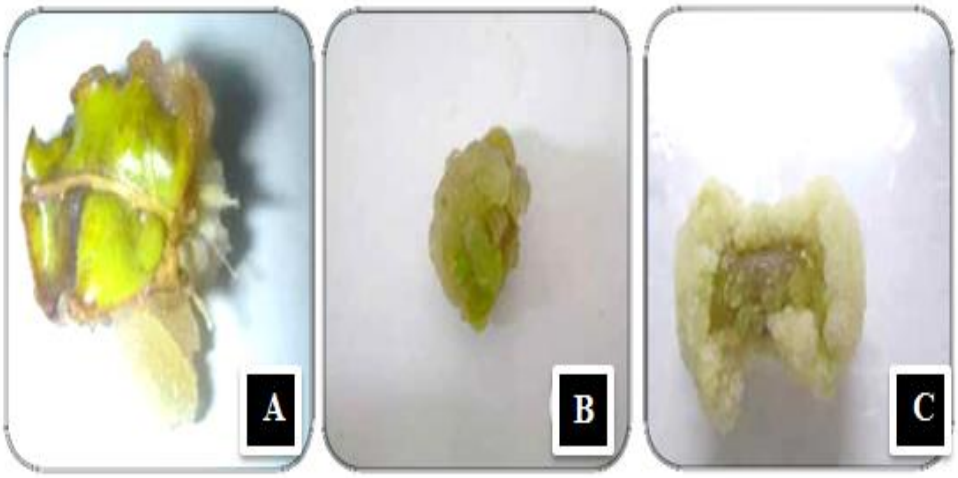

Figure 1 Callus induction of Catharanthus roseus from leaf segment (A), nodal segment (B) and internode segment explant (C); (Al-Memari et al 2018).

The extracts taken from the roots, leaves, and flowers work as anti-bacterial for some bacteria species and have medicinal activity in treating HIV and hemorrhagic diseases (Muley et al 2009; Amoian et al 2010; Bissa and Bohra, 2011). The reason for the pharmacokinetic and pharmacological effectiveness of vinca rosea extracts is that they contain effective chemical compounds, including terpenes, such as the well-known compound calenduladiol-3-myristate, as well as flavonoids, comarine, narcissin, and rutin.

Leaf segments of young and flowering plants of pot marigold were cultured on MS medium supplied with 0.0, 0.1, 0.2 and 0.5 mg L^{-1} 2,4 -D with the aim of callus induction (Bashi and Al-Noah, 2018). The leaf segments of young plants that were grown on the MS medium supplied with 0.1 mg L^{-1} 2, 4-D recorded the highest callus fresh weight of 1.14 g after three weeks of culturing. Then callus induced from the treatment, 0.1 mg L^{-1} 2,4 -D, was cultured on the MS medium supplied with 0.0, 0.5, 1.0, 1.5, and 2.0 mg L^{-1} NAA for the purpose of stimulating the production of salicylic acid and resorcinol, which were diagnosed by the HPLC instrument. The induced callus of young plants that cultured on MS medium supplied with 1.0 mg L^{-1} NAA and 0.1 mg L^{-1} BA recorded the highest callus fresh weight reached 13.04 g after nine weeks of culturing. The induced callus of flowering plants that cultured on MS medium supplied with 1.5 mg L^{-1} NAA and 0.1 mg L^{-1} BA recorded the highest callus fresh weight reached 20.50 g after nine weeks of culturing. The induced callus of young plants that cultured on MS medium supplied with 1.5 mg L^{-1} NAA and 0.1 mg L^{-1} BA recorded the highest amount of salicylic acid reached 30.02 mg g^{-1} dry weight, after six weeks of culturing. The induced callus of flowering plants that cultured on MS medium supplied with 2.0 mg L^{-1} NAA and 0.1 mg L^{-1} BA recorded the highest amount of resorcinol reached 139.28 mg g^{-1} dry weight after six weeks of culturing.

5. Chamomile (*Matricaria chmomilla* L.)

Chamomile belongs to the Compositae family, which includes 2,500 species (Abu Zaid 2001). This annual herbaceous plant is one of the most important medicinal and aromatic plants, and it is a major source of the active compounds found in volatile oils. From the flowers of the chamomile plant, volatile essential oil is extracted from 0.5-1.5% of its dry weight (Attar Bashi, 2004). This plant is included in the pharmaceutical industry as it is a major source of effective plant compounds (Abbas and Al-Shamaa 2009). Chamomile plant extracts are used to improve the appearance of the skin and protect it from rashes and some types of skin allergies. Its flowers are also used in the treatment of wounds and acne (Zaila 1975). The flowers of this plant are used in the treatment of colds, colds, rheumatism, stomach and intestinal ulcers, and as an anti-inflammatory, anti-inflammatory, mouth and tongue ulcers, and tonsillitis. As a result, its flowers contain the active compounds bisablol, hernairine and coumarins (Al-Naimi 2005).

A protocol for the embryogenesis from the culturing of capitulum explants of chamomile flowering was developed by Ahmad (2015). Ahmad (2015) found that MS medium supplied with 26.8 µM NAA and 11.5 µM kinetin led to somatic embryo formation from capitulum explants after 2-4 weeks of culture. The new plantlets were obtained from the germination of the obtained somatic embryos. The researcher also indicated the possibility of increasing the production of the active compound chamazulene through micropropagation of chamomile plants using capitulum as explants.

6. Miswak (*Silvadora persica* L.)

The miswak (*Silvadora persica* L.) plant is one of the evergreen shrubs belonging to the Silvadoraceae family that grows in desert areas. This plant propagates by means of seeds, but its germination rate is very weak, as well as plants growing from seeds are genetically variable (Phulwaria et al 2011; Kumar et al 2012). The miswak stick is commonly used in the regions of the Arabian Peninsula, the Islamic world and some different countries of the world. The toothpick is used as a traditional brush to clean the teeth in those areas because it contains effective compounds that are useful and strong affecting mouth bacteria and gum disease (Bergstorm et al 2006).

A protocol was developed by Emhamed (2015) to micropropagation the miswak plant, as it is one of the endangered plants. The shoot tips, leaf segments, nodal stems, flowers, immature seeds, axillary buds, apical meristems, and leaf petioles were used as explants that were cultured on the MS medium supplied with different concentrations of auxins and cytokinins. Shoot tip explants recorded the best results in micropropagation of miswak plants compared to the other explants. The MS medium supplied with 0.5 mg L^{-1} NAA and 1.0 mg L^{-1} BA recorded the highest shoot multiplication after five weeks of culturing. The apical meristem explants that were cultured on an MS medium supplied with 0.5 mg L^{-1} zeatin (cytokinin) and 0.5 mg L^{-1} activated charcoal recorded the highest number of shoots, reaching 48 shoots (Figure 2A). While the shoots that were cultured on MS medium supplied with 0.5 mg L^{-1} NAA and 1.0 mg L^{-1} BA recorded the highest response to callus induction after several callus subcultures (Figure 2B).

7. Roja (*Hypericum triquetrifolium*)

The Roja is a medicinal herbaceous plant that belongs to the Hypericaceae family (Mohammed and Kheravii 2012). The Roja plant is used in traditional Arabic medicine to treat various infections and diseases and as an analgesic, astringent, and antispasmodic, for intestine and bile disorder and poisoning (Couladis et al 2002; Saad et al 2011; Mohammed and Kheravii, 2012). This plant is one of the most important medicinal herbal plants that contain many effective compounds such as acylphoroglucinols, triquetriborin, triquetrireboudin, and volatile and essential oils. It also contains phenolic compounds such as chlorogenic acid, pcoumaroylquinic aid, caffeoylquinic acid, rutin and epicatechin. The Roja plant contains 0.013 0.001 w/w% hyperforin and 0.020± 0.001 w/w% hypericin. Pharmacological studies have shown that Roja has antimicrobial, anti-inflammatory, analgesic, anti-cytotoxic, antioxidant and vasorelaxant effects (Al-Snafi 2018).

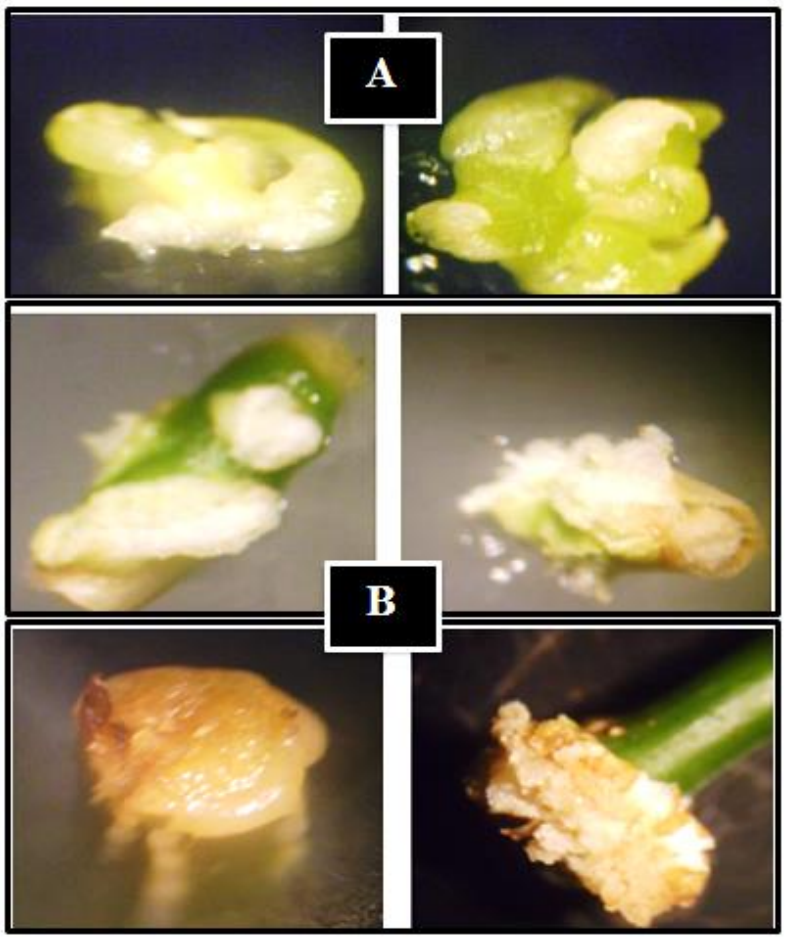

Figure 2 Micropropagation of Miswak (*Silvadora persica* L.) plant. Shoot formation from apical meristem explants (A); Callus induction from explants (B); (Emhamed 2015).

A protocol was developed by Abdulrazzaq et al (2014) on stimulating the production of secondary products in the tissues of Roja plants by using some physical treatments. The secondary products, catchin, hypersoid, hypericin, pseudohypericin, hyperforin and prenylated phloglucin were estimated by HPLC instrument. The leaf segment explants that culturing on the MS medium supplied with 0.1 mg L^{-1} 2, 4- D and 0.5 mg L^{-1} BA led to callus induction. The study indicated the possibility of increasing the secondary products catchin, hypersoid, hypericin, pseudohypericin, hyperforin and prenylated phloglucin in Roja callus when exposed to 12 hours of light day^{-1} or ultraviolet radiation with a wavelength of 100-280 nm and energy of 3.43-12.4 volts for 10 or 20 minutes.

8. Moonflower (*Datura stramonium*)

The moonflower plant is a herbal medicinal plant that belongs to the Solanaceae family, which includes 96 genera and 3000 species. The original home of this plant is Europe (Al-Khatib et al 2006). Moonflower is one of the most important medicinal plants in the world because it contains many biologically active compounds called alkaloids compounds (Al-Mallah and Awab 2001; Al-Khalidi 2005). These alkaloids are among the best drugs for neurological pain and are used in the treatment of Parkinson's disease (Roddick, 1991). In 1762, the scientist Stoerck was able to produce from the highly toxic moonflower plant some important treatments in treating point disease, convulsions and mental disorders. The method of tissue culture is one of the important methods in the propagation of medicinal plants such as moonflower with the aim of producing effective compounds in optimum conditions far away from many different weather factors, insects, diseases, and external influences after they were taken from local wild sources that have become threatened with depletion for their indiscriminate use in traditional medicine (Saidon 2008).

El-Sawaf et al (2015) established a protocol for the accurate propagation of moonflower by *in vitro* culture technique. Moonflower seeds were taken after sterilizing their external surfaces to the culture room with the aim of producing a sufficient number of plants in the initiation stage by culturing them on the MS medium. After that, the explants were taken and cultured on the MS medium supplied with different concentrations of cytokinin, BA or kinetin and auxin, NAA or IBA at 0.5, 1.0, 1.5 and 2.0 mg L^{-1} for each of them to the purpose of shoot multiplication. Then the shoots were rooted by culturing them in an MS medium with different concentrations of indole butyric acid IBA at 0.1, 0.5, 1.0 and 2.0 mg L^{-1}. The results of the study showed that 0.5% sodium hypochlorite concentration was significantly superior in the surface sterilization of moonflower seeds compared to the other concentrations, it recorded 28% seed germination. The two treatments of MS medium that supplied 1.5 or 2.0 mg L^{-1} BA and 1.0 mg L^{-1} IBA were significantly superior to the other treatments in the number of shoots and leaves, which reached 3.43 and 3.53 shoots $explant^{-1}$, 12.91 and 14.64 leaves $shoot^{-1}$ respectively. The MS medium supplied with 2.0 mg L^{-1} IBA was significantly superior compared to the other media in the percentage of rooting, which was recorded at 75%.

9. Digitalis (*Digitalis purpurea*)

Digitalis belongs to the Plantaginaceae family and is an annual herbaceous plant that grows naturally in Western Europe (Anonymous, 2003). This medicinal plant is used in treating heart diseases because its leaves contain compounds like Digoxin and Digitoxin (Bruneton 1995; Harris 2000). Reddy (2010) also noted that these two compounds are used in the treatment of weak heart diseases, as they increase the effectiveness of the muscle tissue of the heart, and also have a role in regulating blood circulation and arrhythmia. Several researchers have found that cytokinins play an important role in the micropropagation of the digitalis plant. Vela et al (1991) indicated that the shoot tips that were cultured on the MS medium supplied with 1.1 or 4.4 mg L^{-1} BA led to the highest number of shoots, which reached 36.1 and 32.3 shoots per explant, respectively. Lapena et al (1992) also indicated that the shoot tips of the digitalis plant that were cultured on the MS medium supplied with 1.0 mg L^{-1} BA and 0.1 mg L^{-1} IAA led to the recording of the highest number of shoots, which reached 17.4 shoots for each explant. Ghanem et al. (2010) found that the shoot tips of the digitalis plant that were cultured on the MS medium that provided 0.1 mg L^{-1} Kinetin and 0.5 mg L^{-1} NAA had led to the highest percentage of response to shooting multiplication, which reached 100% after three weeks of culturing. Pe´rez-Alonso et al (2009) showed that shoot tips of digitalis plant that cultured on MS medium supplied with 0.1 mg L^{-1} BA and 0.1 mg L^{-1} IAA recorded the highest value in the number of shoots reached 6.03 shoot per explant after four weeks of culturing.

Alabasi and Bashi (2013) established a protocol for the micropropagation of digitalis plants by culturing the shoot tip and nodal segment explants that were taken from the seedlings in the MS medium supplied with different concentrations of BA and kinetin for shoot multiplication. The results study showed that nodal segments were the best explants compared with shoot tips. The explants that were treated with BA had the highest response to shoot multiplication compared to the explant treated with kinetin. The nodal segment explants that were cultured on MS medium supplemented with 0.75 mg L^{-1} BA recorded the highest value in the number of shoots reaching 30 shoots per explant (Figure 3, A). While these explants recorded 13.4 shoots per explant when they were cultured on an MS medium supplied with 4.0 mg L^{-1} kinetin (Figure 3B). The results of the study showed the shoots that were cultured on half strength of MS medium without hormones or supplied with different concentrations of IBA led to recording the highest response to rooting reaching 100%. The shoots that were cultured on the

MS medium supplied with 0.1 mg L^{-1} IBA were recorded as the highest in number and length of roots after four weeks of culturing.

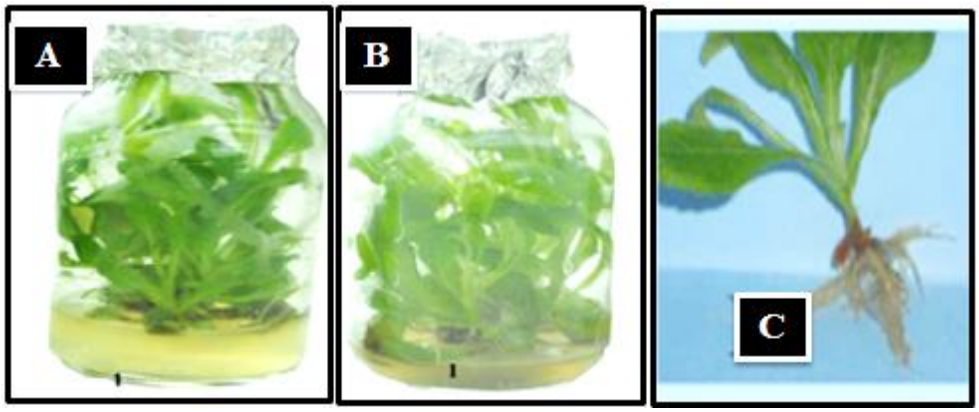

Figure 3 Micropropagation of Digitalis (*Digitalis purpurea*) plant. Shoot multiplication on MS medium supplemented with 0.75 mg L^{-1} BA (A); Shoot multiplication on MS medium supplied with 4.0 mg L^{-1} kinetin (B); Rooting shoots on MS medium supplied with 0.1 mg L^{-1} IBA (C); (Alabasi and Bashi 2013).

10. Ginger (*Zingiber officinale* Roscoe)

The ginger is a perennial herbaceous plant that belongs to the Zingiberaceae family and is cultivated in China, India, Japan, Australia, Nigeria and Indonesia (Haghighi et al 2005). This plant is used in spices and as a remedy in folk medicine in those countries. The ginger plant contains the most important active compounds such as paradols, gingerols, and shogaols (Stoner 2013). Several studies have shown that the active compounds in the ginger extract have the ability to prevent many diseases such as cardiovascular disease (Akinyemi et al 2015), obesity (Suk et al 2017), neurodegenerative diseases (Ho et al 2013), diabetes mellitus (Wei et al 2017), respiratory disorders (Townsend et al 2013), and nausea resulting from chemotherapy (Walstab et al 2013). The active compounds in this plant also act as anti-microbial (Kumar et al 2014), anti-inflammatory (Zhang et al 2016), antioxidant (Nile and Park 2015) and anti-cancerous agents (Citronberg et al 2013). There are many studies conducted with the aim of micropropagation of the ginger plant from the study conducted by Villamor (2010) that confirmed the possibility of micropropagation of the ginger plant by tissue culture technique when culturing the explants on the MS medium prepared with different concentrations of NAA and BA growth regulators. David et al (2016) also obtained the same results when culturing the ginger explants on the same nutrient medium and growth regulators that were used in the above study. Kambaska and Santilata (2009) succeeded in micro-propagation of the ginger plant when culturing the buds that were removed from the root rhyzomes in the MS medium with different concentrations of growth regulators IBA and NAA.

Keer et al (2019) devised a protocol for the micro-propagation of the ginger plant by germinating rhizomes in dark conditions at room temperature in order to obtain sprouts. Then the sprouts were taken after two months of germination, which were sterilized with ethyl alcohol at a concentration of 70% for two minutes, and then soaked with a solution of sodium hypochlorite at a concentration of 2.5% for 20 minutes. Then they were washed with sterile distilled water three times. In this protocol, the MS nutrient medium supplemented with 0.0, 2.0 mg L^{-1} BA, 0.5 mg L^{-1} NAA+ 2.0 mg L^{-1} BA or 0.5 mg L^{-1} NAA+ 2.0 mg L^{-1} BA+ 2.0 mg L^{-1} activated charcoal were used. The sprouts that were cultured on the MS nutrient medium supplied with 2.0 mg L^{-1} BA recorded the highest number of shoots formed from multiplication (Figure 4A). These shoots that were cultured on the MS medium supplied with 2.0 mg L^{-1} BA recorded the highest percentage of response to rooting, which was 95%. The plantlets were acclimatized in plastic pods (Figure 4B).

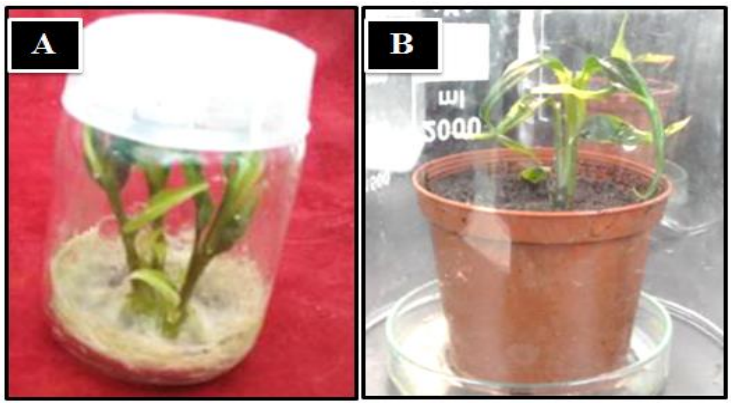

Figure 4 Micropropagation of Ginger (*Zingiber officinale* Roscoe) plant; Shoot multiplication (A); Acclimatized plant (B); (Keer et al 2019).

11. Ziziphora (*Ziziphora canescens*)

Ziziphora is a perennial herbaceous plant belonging to the Lamiaceae family that is widely spread throughout the world. This plant is found in grasslands, forests, coastal, arid and mountainous regions. Ziziphora is used as flavouring or flavouring in various foods, as well as used in folk medicine to treat digestive and viral diseases (Khodaparast et al 2007). Ziziphora is an edible medicinal plant whose stems, leaves and flowers are added to give flavour to food (Zargari 1995). The dried leaves of this plant are used for treating colds and coughs (Verdian-rizi 2008), as well as for digestive disorders and infections (Zargari, 1997; Naghibi et al 2005). Several studies have demonstrated the effect of ziziphora as an antibacterial (Salehi et al 2005; Sonboli et al 2006), antifungal (Behravan et al 2007), antioxidant (Meral et al 2002; Salehi et al 2005) and anti-inflammatory (Ghafari et al 2006). Studies have also indicated that ziziphora species (*Ziziphora clinopodioides* Lam.) contains highly active compounds such as pulegone, iso menthone, menthol, menthone 1,8-cineole, thymol, p-cymene, carvacrol, terpinen-4-01 and linalool (Kivanc and Akguel 1986; Baser et al 1991).

Dakah et al (2015) developed a protocol for the micro-propagation of ziziphora species (*Ziziphora canescens* Lam) using an in vitro culture technique. The terminal and axillary buds were used as explants, which were cultured on an MS nutrient medium with different concentrations of plant growth regulators. The treatment of explants with a surface sterilization solution, mercury chloride, which was applied at a concentration of 0.1% for two minutes, resulted in reducing contamination and obtaining uncontaminated explants by 80%. The buds that were cultured on MS medium supplemented with 0.1 mg L^{-1} BA or kinetin and 0.1 mg L^{-1} IBA recorded the highest mean number of shoots resulting from the multiplication of 14.0 and 14.3 shoots per explant, respectively. The shoots that were cultured on MS nutrient medium supplied with 1.0 mg L^{-1} NAA recorded the highest percentage of rooting response and the number of roots with 95% and 7.1 roots per shoot, respectively. Whereas, the shoots that were cultured on MS nutrient medium supplied with 3.0 mg L^{-1} IBA recorded a low response rate to rooting and fewer roots which amounted to 90% and 5.8 roots per shoot, respectively. The survival rate of plants that exceeded the acclimatization stages was about 90%.

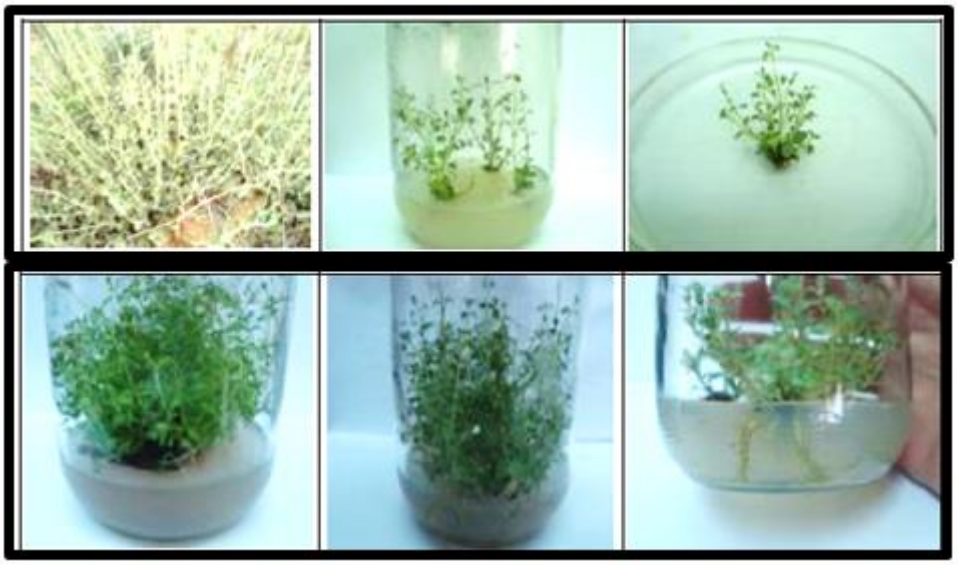

Figure 5 Micropropagation of Ziziphora (*Ziziphora canescens*) plant by in vitro culture technique; (Dakah et al 2015).

12. Neem (*Azadirachta indica*)

Neem is a perennial evergreen tree that belongs to the Meliaceae family. The original home of the neem tree is India and spread in tropical and sub-tropical regions (Hashmat et al 2012). These trees are among the oldest plants that have been used in folk medicine in most countries of the world. The neem tree is used as a treatment for some diseases and disorders such as tooth decay, ulcers, malaria and bed bugs (John 2001). Studies have proven that the seeds, bark and leaves of the neem plant act as anti-viral, anti-breast cancer, anti-microbial, anti-fungal, anti-ulcer and antipyretic (Ahmed 2008). The parts of the neem tree contain many important active compounds such as alkaloids, phenolic compounds, flavonoids, steroids, triterpenoids, ketones and carotenoids. The most effective compound in the neem plant is azadirachtin, which is a mixture of the aforementioned seven compounds called azadirachtin A-G and azadirachtin E. The most effective compound in the neem plant is azadirachtin, which is a mixture of the aforementioned seven compounds called azadirachtin A-G and azadirachtin E (Verkerk and Wright 1993). There are other compounds in the neem plant that are bioactive, such as salannin, volatile oils, meliantriol and nimbin (NRCN 1992).

Ahmed (2008) developed a protocol to produce callus cells from leaf segments of neem trees using the in vitro culture technique, extracting effective compounds from callus and using them in biological control and comparing them with extracts of the neem plant that grows in nature. The MS supplied with 1.0 mg L^{-1} IBA resulted in the induction of callus from leaf segments one week after culturing. The largest amount of greenish-coloured callus was formed after five weeks of culturing on MS nutrient medium 1.0 mg L^{-1} IBA. The results showed that callus extract at 20 mg ml^{-1} inhibited the growth of the pathogenic bacteria under study represented by *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. The neem plant callus extract at 20 mg ml^{-1} also proved to be effective in inhibiting the growth of the pathogenic fungi *Drechslera roslratii*, *Fusarium oxysporum* and *Alterneria alternata*, by 84% after 72 hours of treatment. While the extract of the leaves of the neem plant is naturally growing at 20 mg ml^{-1}, it inhibited bacterial and fungal growth by 75%.

13. Belladonna (*Atropa belladonna*)

The belladonna plant is one of the most important perennial herbal medicinal plants in the Solanaceae family (Rita and Animesh 2011). The original home of the belladonna plant is Middle and Southern Europe, from which it spread to Middle and Western Asia (Chevalio 2010). The word belladonna in the Italian language means beautiful lady. The leaves and fruits of this plant are highly toxic, containing tropane alkaloids, which include scopolamine and hyoscyamine which cause hallucinations and bizarre delirium. The species of plant is the source of the alkaloid atropine that has proven to be the mainstay in the studies of autonomic pharmacology (Lee 2007; Rita and Animesh 2011). Belladonna is important for use in medicine and cosmetics. The balance between auxin and cytokinin stimulates the explant that was cultured in the nutrient medium to callus induction (Hamad and Jassem 2011). Numerous studies have shown that the explants represented by the shoot tips and the cotyledon leaves have a high response in the induction and formation of callus (Iranbakahsh and Riazi, 2000; Zaid and Wink 2004; Ibrahim et al 2009).

Hamad and Jassem (2011) developed a protocol for micro-propagation for the explants of the medicinal plant belladonna by cultivating shoot tips, cotyledon leaves and hypocotyl segments on two types of nutrient medium, MS and B5 (Gamborg and Eveleigh 1968). Two different types of auxin were added to the food medium, NAA and 2, 4-D, with different concentrations of 0.0, 1.5, 3.0 and 4.5 mg L^{-1} for each of them. The results of the study showed that no contamination appeared in the explants when their surfaces were sterilized with a 4.5% sodium hypochlorite solution for 15 minutes. The results showed the significant superiority of the nutrient medium MS supplied with 1.5 mg L^{-1} 2, 4-D and 0.4 mg L^{-1} BA in recording the highest dry and fresh weight of callus induced from the shoot tips, which were 177.5 and 18.9 mg, respectively, compared to the same concentration 2, 4-D in the nutrient medium B5 which recorded 110.0 mg fresh weight and 12.3 mg dry weight. The induced callus of hypocotyl segments cultured on the MS medium supplied with 3.0 mg L^{-1} 2, 4-D recorded the highest fresh and dry weight of callus reaching 44.3 and 5.0 mg, respectively, while the same nutrient medium supplied with 3.0 mg L^{-1} NAA had a fresh and dry weight of 18.9 and 2.5 mg, respectively. The shoot tips were significantly superior in recording the highest fresh and dry weight of callus, reaching 45.74 and 5.0 mg, respectively, compared to the cotyledon leaves and hypocotyl segments which recorded 38.98 and 17.54 mg fresh weight and 3.78 and 2.04 mg dry weight of callus, respectively. The results showed that the best nutrient medium for induction and formation of callus from explants of the belladonna plant is MS compared to B5 and that the best growth regulator for callus induction is 2, 4-D compared to NAA. The best explants for induction of callus were the shoot tips compared to the cotyledon leaves and hypocotyl segments.

14. Strawberry (*Fragaria ananassa* Duch.)

The Strawberry plant is a perennial herbaceous plant of the Rosales and Rosaceae family. The fruits of this plant are considered to be of high nutritional and therapeutic value (Al-Saeedi 2000). Biotechnologies are used in the agricultural, industrial and medical fields in the production of effective and useful compounds under controlled environmental conditions for the purpose of extracting compounds of medicinal uses from the callus of plants throughout the year without being restricted to the growing season of this plant (Ramawat 2004; Park et al 2008). The obtaining of effective compounds with medicinal uses in the plant tissue culture technique is done by controlling the metabolic pathways of plant cells that are difficult to induce in plants growing under natural environmental conditions (Purohit 1999; Alzubaidi 2004). It was found in many studies the possibility of isolating more than 200 active compounds from cell suspension cultures in the strawberry plant, as Hong et al (1990) was able to extract butanol and α-ketovalerate as other examples to obtain aromatic compounds from cell cultures as well as extracting acetyl aldehyde and ethanol from tissue cultures of the strawberry plant (Drawert and Berger 1983). Phenolic compounds are among the most important compounds that are used in medical fields, such as resistance to cancerous tumours (Galati and O'Brien 2004). The phenolic compounds were extracted from the leaves, flowers and fruits (Aharoni et al 2002; Maatta-Riihinen et al 2004; Aaby et al 2007; Hukkanen et al 2007; Hanhineva et al 2008).

Obaid et al (2018) devised a protocol for inducing and forming callus from in vitro culture of strawberry plant and producing phenolic compounds from it. The results showed that the leaf segments that were cultured on the MS nutrient medium supplied with 0.5 mg L^{-1} 2, 4-D led to the induction and formation of callus with a high response rate and a dry weight of callus of 70% and 0.039 g, respectively (Figure 6A and 6C). The MS medium supplied with 1.0 mg L^{-1} Kinetin and 1.0 mg L^{-1} 2,

4-D recorded the highest value in fresh weight of callus reaching 2.560 g (Figure 6D). The results showed that MS medium supplied with 1.5 mg L^{-1} kinetin and 1.0 mg L^{-1} 2, 4-D recorded the highest values in fresh and dry weight of callus reaching 2.107 and 0.127 g, respectively. The results showed that the differentiated tissues contained higher levels of phenolic compounds compared with the (undifferentiated) callus tissues. Strawberry leaves recorded the highest levels of meryctin, caffic acid, and Gallic acid compounds. The flowers recorded the highest levels of alpha penine, comarins and quercetin compounds. The root of strawberry recorded the highest levels of P-hydroxy benzoic acid and ferulic acid compounds. While, the callus tissue of strawberries recorded the highest levels of champene, ellagic acid and catachin compounds.

15. Black nightshade (*Solanum nigrum*)

The black nightshade is a perennial herbal medicinal plant that belongs to the Solanaceae family. It grows naturally as a weed plant in tropical and warm regions (Särkinen et al 2018). This plant is an important source for extracting effective compounds that are used in pharmaceutical and medical preparations, as well as being an important source of human food. The leaves of this plant contain amino acids, protein, vitamin C and A, phosphorous, iron, calcium, and fiber, and it is rich in the amino acid methionine, which other vegetable crops lack (Ikeda et al 2000). Numerous studies have shown that the black nightshade plant has succeeded in working in anti-cancerous diseases, which it has high effectiveness against hepatocellular carcinoma cells, human endometrial carcinoma, human ovarian carcinoma cells and human colorectal carcinoma cells (Tai et al 2013; Wang et al 2015).

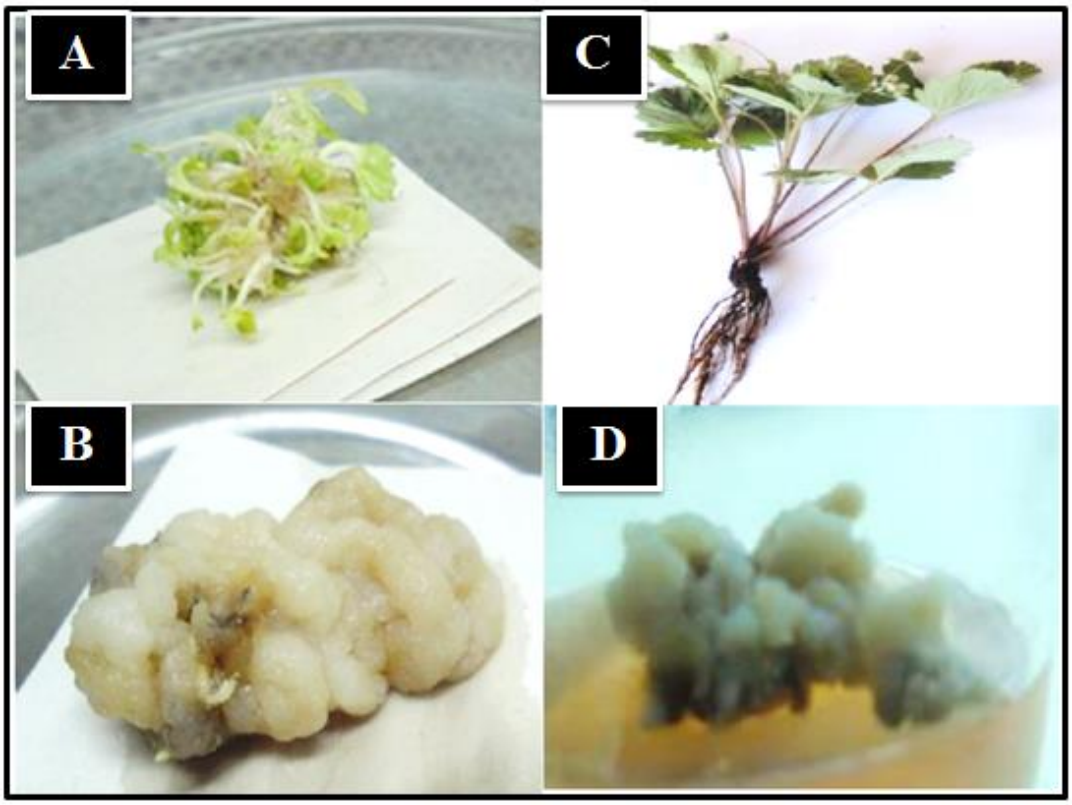

Figure 6 Callus induction and formation of Strawberry (*Fragaria ananassa* Duch.) plant. Shoot multiplication (A); Callus formation on MS medium supplied with 0.5 mg L^{-1} 2, 4-D (B); Strawberry plant (C); Callus formation on MS medium supplied with 1.0 mg L^{-1} kinetin and 1.0 mg L^{-1} 2, 4-D (D); (Obaid et al 2018).

Younes et al. (2019) developed a protocol for the micropropagation of black nightshade plants by culturing the nodal segments on the MS nutrient medium provided with BA, thidiazuron (TDZ), or kinetin at different concentrations of 0.0, 0.5, 1.0 and 1.5 mg L^{-1} + 0.1 mg L^{-1} NAA, for the purpose of shoot multiplication. The shoots were cultured on the MS nutrient medium that was supplemented with IBA, NAA or IAA at different concentrations of 0.0, 0.5, 1.0 and 1.5 mg L^{-1} for the purpose of rooting. The MS medium supplied with 1.5 mg L^{-1} TDZ recorded the highest values in shoot number and length, reaching 15.0 shoots per explant and 2.06 cm, respectively (Figure 7A). The shoots cultured on MS medium with growth regulator-free recorded the best values in the number of roots reaching 6.7 roots per shoot. The shoots that were cultured on MS medium supplemented with 1.5 mg L^{-1} IBA recorded the highest response to rooting, number and length of roots reached 80%, 4.90 roots per shoot and 1.95 cm, respectively (Figure 7B). The black nightshade plants were successfully acclimatized, and a 100% survival percentage was recorded in all plants four weeks after being transplanted to the greenhouse (Figures 7C and 7D).

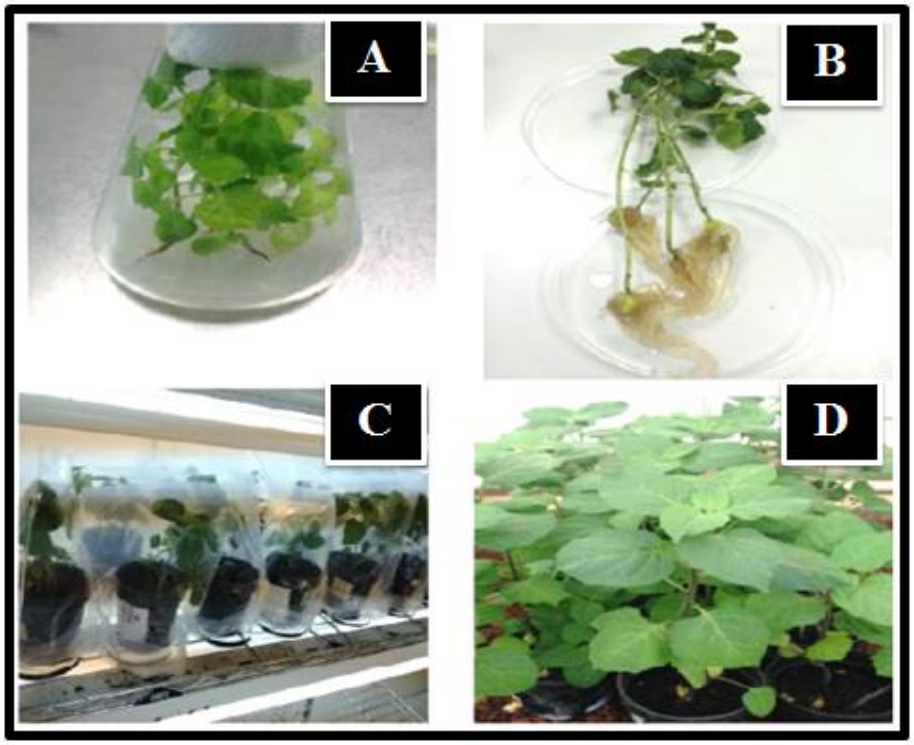

Figure 7 Micropropagation of Black nightshade (*Solanum nigrum*) plant. Shoot multiplication (A); Root formation (B); Acclimatized plants (C, D); (Younes et al 2019).

16. Turmeric (*Curcuma longa* Linn.)

Turmeric is a monocotyledon herbaceous plant that belongs to the Zingiberaceae family. This plant reproduces by the asexual (vegetative) method by rhizomes. Turmeric is one of the most important medicinal plants that have been commonly used in traditional medicine in India as well as a spicy plant. The turmeric rhizome is bitter; maturant, carminative, diuretic; useful in urinary discharges, liver problems, bruises, and scabies (Kiritikar and Basu 1996). It improves the complexion and is useful in leucoderma, blood diseases, inflammations, bad taste in the mouth, dyspepsia, biliousness, ulcer, snake-bite, elephantiasis, smallpox, boils, swellings and sprains (Arora et al 1971; Satoskar et al 1986; Thamlikitkul et al 1989; Kositchaiwat et al 1993; Kiritikar and Basu 1996; Van Dau et al 1998).

Nasirujjaman et al (2005) developed a protocol for accurate propagation of turmeric plants by culturing the rhizome buds on the woody plant nutrient medium (Lloyd and McCown 1981) that supplied with different concentrations of BA alone or combined with different concentrations of NAA. The explants that were cultured on WP medium supplied with 4.0 mg L^{-1} BA and 1.0 mg L^{-1} NAA recorded the highest response to shoot multiplication which reached 95% after 8-10 days of culturing (Figures 8A and 8B). This treatment recorded the highest number of shoots which reached 6.70 shoots per explant. The results also indicated that the shoots were rooted spontaneous in all transactions and combinations. All acclimatized plants have survived after being transplanted to the field soil.

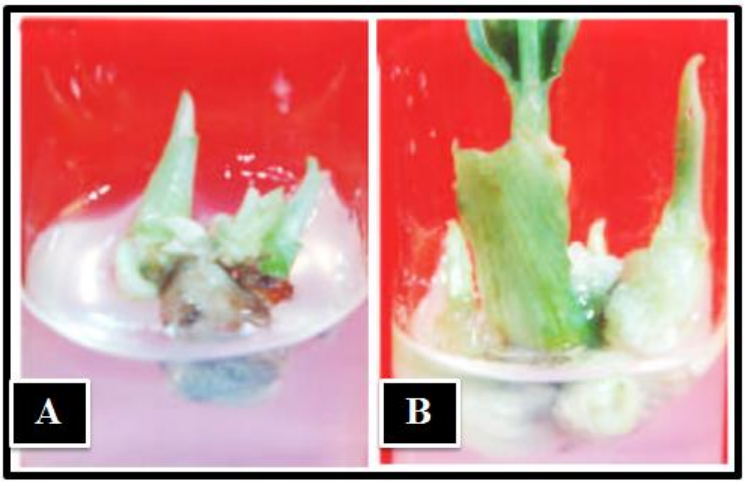

Figure 8 Micropropagation of Turmeric (*Curcuma longa* Linn.) plant. Shoot multiplication (A and B); (Nasirujjaman et al 2005).

17. Toothache plant (*Spilanthes acmella* (L.) Murry)

The toothache is a medicinal herbal plant that belongs to the Asteraceae family. This plant is considered a versatile herb in the field of folk medicine in India. This plant grows in tropical and subtropical regions (Tiwari et al 2011). This plant is a rich source of the active compound alkamide splanthol, which is used in the manufacture of pharmaceuticals and therapeutics. The toothache plant is effective as a larvicide for *Anopheles*, *Aedes aegypti* and *Culex mosquitoes*, and can be used as an antimalarial agent. It is also used in the traditional field as a spice and medicinally as an antiseptic, anti-bacterial and anti-fungal, treatment for toothache, throat pains, stomatitis, anti-inflammatory, immune-modulatory, analgesic, antipyretic, aphrodisiac, anti-microbial, antioxidant, anti-nociception, vasorelaxant, diuretic, tuberculosis and insecticidal (Di Stasi et al 1994; Akah and Ekekwe 1995; Storey and Salem 1997; Rios-Chavez et al 2003).

Joshi et al (2015) developed a protocol for toothache plant propagation plant tissue culture technique through culturing nodal segment explants on MS nutrient medium prepared with different concentrations of cytokinin (Figure 9A). The results of the study revealed that the MS nutrient medium supplied with 0.5 mg L^{-1} BA recorded the maximum growth of buds in nodal segments. The liquid MS nutrient medium provided with 1.0 mg L^{-1} kinetin recorded the highest values in the number and length of shoots, which were 6.20±0.45 shoots per explant and 8.01 ±0.87 cm, respectively, as compared to the semi-liquid MS medium treatment (Figure 9B and 9E). The roots were formed in all treatment treatments without auxins, but the best treatment for rooting was when 1.0 mg L^{-1} IBA was added to the MS medium (Figure 9, F). The plants were irrigated with a half-strength solution of MS salts during the acclimatization stage led to 100% success (Figure 9G and 9H).

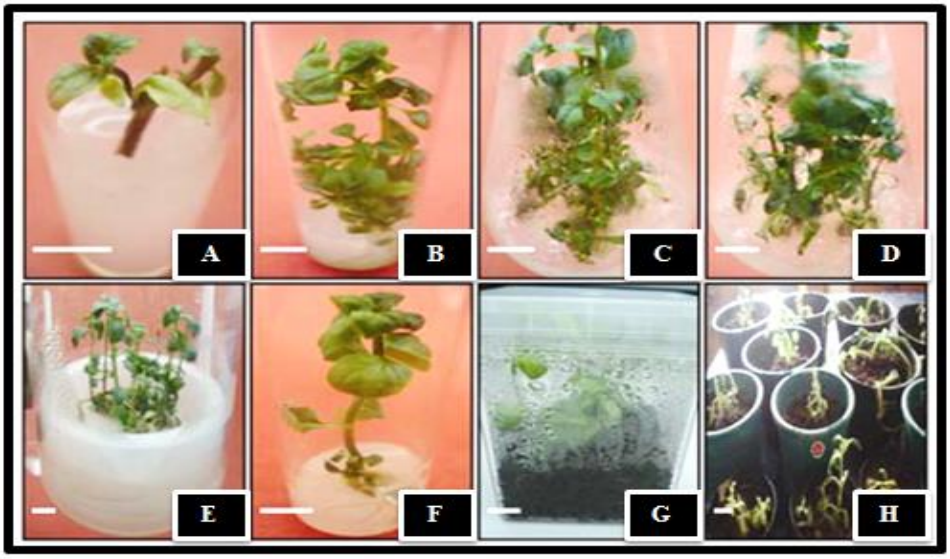

Figure 9 Micropropagation of Toothache plant (*Spilanthes acmella* (L.) Murry) plant. Nodal segment explant (A); Shoot multiplication (B-E); Root formation (F); Acclimatized plants (G-H); (Joshi et al 2015).

18. Final considerations

It is concluded from reviewing the protocols for producing bioactive compounds at high levels using the tissue culture technique of explants for some important medicinal plants, economically and medically by adding chemical or natural stimulators to the culture media. The possibility of extracting bioactive compounds from cultures in vitro with high purity with a safe and healthy technology can be used to manufacture medical drugs and pharmaceutical preparations. The possibility of producing secondary compounds from some medicinal and aromatic plants in vitro culture without resorting to cultivating them in the fields, especially medicinal plants that cannot be grown due to the lack of appropriate conditions for their growth and productivity in that region.

Conflict of Interest

We declare no conflicts of interest.

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