ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Biotechnology

ISSN 1682-296X
DOI: 10.3923/biotech.2023.1.17

Review Article
In vitro Production of Plant Nutraceuticals

[1]Sahwky Bekheet and [2]Valbona Sota

[1]Department of Plant Biotechnology, National Research Centre, 33 El Buhouth Street, Dokki 12622, Giza, Egypt
[2]Department of Biotechnology, Faculty of Natural Sciences, University of Tirana, Tirana, Albania

Abstract

Biotechnology methods particularly tissue culture techniques offer new approaches for the *in vitro* production of valuable plant nutraceuticals. The large-scale production of secondary metabolites in a year-round system without seasonal constraints is one of the advantages of plant tissue culture techniques. Furthermore, enhancement of *in vitro* nutraceuticals production can be realized by a selection of high-producing cell lines and medium optimization. In this respect, several techniques have been adopted to improve the production of plant-derived nutraceuticals such as mutation, elicitation, precursor feeding, genetic transformation and metabolic engineering. *In vitro* mutagenesis of cultured cells and tissues by irradiation or chemical mutagens represents a feasible method for the improvement of nutraceuticals production. Likewise, transformation is another strategy that is used to enhance nutraceuticals in plant cells or organ tissues. At this point, hairy root culture induced by *Agrobacterium rhizogenes* is the most promising transformation technique used for *in vitro* production of valuable plant compounds. The benefits of hairy roots are high metabolite productivity, high growth rates and inherent genetic stability. On the other hand, due to its effectiveness and practical feasibility, elicitation is considered the most applied strategy for enhancing the production of desired compounds in plant biotechnology. Biotic (biological origin) and abiotic (non-biological origin) elicitors are used to increase the production of plant metabolites by different effects on the cellular processes in the plant system. Furthermore, bioreactors developed for *in vitro* industrial-scale production of desired compounds can be used for continuous and scaling-up plant nutraceuticals production. This article discusses different aspects of biotechnology used for the selection and enhancement production of nutraceuticals from plant cultures grown cells, *in vitro*.

Key words: Nutraceuticals, *in vitro* culture, mutation, transformation, hairy roots, elicitation, bioreactors

Citation: Bekheet, S. and V. Sota, 2023. *In vitro* production of plant nutraceuticals. Biotechnology, 22: 1-17.

Corresponding Author: Sahwky Bekheet, Department of Plant Biotechnology, National Research Centre, El Centre, 33 El Buhouth Street, Dokki 12622, Giza, Egypt

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nutraceutical is referred to as nutrient compounds that have medicinal properties. Nutraceuticals include antioxidants, vitamins, amino acids and dietary substances[1,2]. Nutraceuticals can be concentrated in the form of pills, tablets, liquids, or powders for direct consumption or use as ingredients in functional foods. Bioactive compounds which provide health benefits are also referred to as nutraceuticals, a term that reflects their existence in the human diet and the biological activity[3]. The term nutraceuticals, itself is a wide class that includes many categories and subcategories under it. In this respect, nutraceuticals can be classified on their natural sources, pharmacological conditions, as well as the chemical constitution of the products.

The contribution of nutraceuticals to public health is considered one of the most concerned approaches in the healthcare system. Due to their supposed safety and possible therapeutic effects, nutraceuticals have attracted considerable interest over the years. Recently, they have been explored as sustainable alternatives for the fight and reducing a large number of diseases. In this respect, nutraceuticals are used to prevent chronic diseases and postpone the ageing process. Moreover, nutraceuticals are beneficial in fighting life-threatening diseases as well as different infections[4]. Furthermore, research has indicated the involvement of nutraceuticals in the treatment of insomnia, digestion problems, cancer, blood pressure abnormalities, cold and cough, depression and coronary heart disease[5]. Otherwise, some plant-derived bioactive and therapeutics play a preventive role against the incidence of certain diseases such as cancer, ageing, inflammation and cardiovascular disorders[6-8]. Continuing demand from the consumer for beneficial foods leads to the exploration and recognition of new and emerging sources of nutraceuticals and functional foods. At this point, higher plants are considered a biochemical factory to produce secondary metabolites used as nutraceuticals such as flavonoids, polyphenols, alkaloids, terpenes and glycosides.

Difficulties in cultivation practices, seasonal variation in productivity and economic cost are the major hinders to large scale-production of phytochemicals from field-grown plants. To search for alternatives, biotechnological methods especially *in vitro* culture technique represent a potential renewable source of such compounds. Plant tissue cultures are established from different explants for the accumulation and gathering of secondary compounds such as nutraceuticals. The advantages of tissue culture technology are: (1) Desired compounds can be obtained independent of cultivation season, (2) Cultured cells will be free of contaminations (3) Automated control of cell growth of metabolite processes would reduce labour costs, (4) Isolation of the phytochemical can be rapid and efficient when compared with extraction from whole plants and (5) Biosynthetic of these phytochemicals can be *in vitro* regulated to maximize yields. In this respect, various biotechnological strategies such as *in vitro* mutagenesis, selection of high yielding lines, optimization of culture media composition and physical parameters, precursor feeding, elicitation, large scale cultivation in a bioreactor system, hairy root culture, metabolic engineering, plant cell immobilization, biotransformation, etc., have been studied for their effectiveness towards enhancement of phytochemicals production utilizing *in vitro* of culture different plant varieties[9].

Classification of nutraceuticals: Nutraceuticals are the compounds that are found in foods, dietary supplements and herbal products and have health benefits and medicinal properties. Based on their source, chemical composition and nutrients, nutraceuticals are grouped into several classes. Also, they can be classified as traditional and nontraditional nutraceuticals. Traditional nutraceuticals are the components that deliver benefits beyond the basic nutrition of natural foods. While non-traditional nutraceuticals are the fortified components prepared by adding new characteristics for the well-being of humans. Recombinant nutraceuticals are food with added nutrients and others that are produced with the help of biotechnology. Otherwise, nutraceuticals are classified into seven different groups based on the source Table 1.

Phytochemicals as nutraceuticals: Phytochemicals have been given more attention as human nutrition because of their pharmaceutical effects. There are more than 4,000 phytochemicals catalogued and are mostly classified based on their biological activity, physical characteristics and chemical characteristics[20,21]. Three major classes of plant secondary metabolites are involved in these phytochemicals alkaloids, terpenes and phenolics. Moreover, there are other many phytochemicals classes recognized with health benefits such as phytosterols, thiosulfonates etc.

Terpenoids (terpenes): Plant terpenoids, the large class of natural organic chemicals are used for their aromatic qualities and play a role in traditional herbal remedies. Terpenoids contribute to the scent of eucalyptus, the flavours of cinnamon, cloves and ginger, the yellow colour in sunflowers and the red colour in tomatoes. Meanwhile, terpenoids are important for plants due to their ability to fix carbon through

Table 1: Source and health benefits of the main nutraceuticals classes

Nutraceuticals	Sources	Health benefits
Dietary fibre	Dietary fibre is a plant origin substance that includes polysaccharides, oligosaccharides, lignin and associated plant substances. Fibre is found in grains, pulses, fruits and vegetables	Functionally, fibres promote beneficial physiological effects including laxation and/or blood cholesterol attenuation and/or blood glucose attenuation[10]. The research results proved that people who eat fibre rich diets tend to have a reduced risk of certain cancers, coronary heart disease and obesity[11]
Probiotics	The foods that contain probiotics are curd, beverages, yoghurt, cheese and pickles. Lactobacillus is the most probiotic found in yoghurt and other fermented foods	Probiotics are effective in diarrhoeal diseases, irritable bowel syndrome, stomach infections and colic diseases[12]
Prebiotics	Lactulose, lactitol oligofructose, inulin, fructooligosaccharides and galacto-oligosaccharides are prebiotics	Prebiotics supports the probiotic growth of gut bacteria and potentially enhance digestion and metabolism[13]
Polyunsaturated fatty acids	Polyunsaturated fatty acids contain omega-3 (essential fatty acids) and omega-6 fatty acids. Fish oils, nuts, salmon, tuna, groundnuts, oysters, flaxseeds are rich sources of essential fatty acids. However, omega 6-fatty acid is found in sunflower, soybean and corn	Polyunsaturated fatty acids are important for nerve function, blood clotting, brain health and muscle strength. Moreover, polyunsaturated fats can help reduce cholesterol levels and the risk of heart disease and stroke[14]
Antioxidants	Vitamin C (ascorbic acid), vitamin E (tocopherols and tocotrienols) and carotenoids are considered antioxidant vitamins. Also, red wine, tea (green, black), tea (black beverage) and cocoa are other antioxidant polyphenols	Antioxidant vitamins prevent oxidation of cellular organelles, membranes, biochemical pathways leading to several degenerative diseases including cancer, cardiovascular diseases and cataracts[15]. Also, phenolic antioxidants decrease oxidative cell injuries and inflammatory reactions improving the brain's health[16]
Polyphenols	Flavonoids polyphenols are widely found in fruits, vegetables, wines, teas and cocoa as glycosides, dimers and polymers. Non-flavonoid phenolic compounds i.e., resveratrol, caffeic acid and curcumin are found in coffee beans, potatoes, apples and olive oil[18]	Polyphenols have been reported to have antioxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic activities[17]
Spices	Among the traditional spices, there is a growing interest in wasabi, horseradish, turmeric and ginger whose antioxidant properties	Spices have antioxidative, chemopreventive, anti-mutagenic, anti-inflammatory, immune-modulatory effects for the benefit of human health[19]

photosynthetic reactions using photosensitizing pigments like chlorophyll and carotene. They are also well recognized for their role in stress response or defence mechanisms[22]. On the other hand, terpenoids have a wide range of biological functions and have been used in the preparation of functional foods, flavourings, bio-colourants pharmaceuticals, cosmetics, disinfectants and agrichemicals. From a healthy point of view, several terpenoids have been studied and used as pharmacological agents to benefit human health. In this regard, certain diseases such as chronic damage and growth dysregulation were protected using terpenoids compounds[23]. Tocopherol, α-d-tocopherol (vitamin E) has been extensively reviewed for its antioxidant potential and is also reported effective apoptotic inducer for human breast cancer cells[24]. Carotenoids, another important class of terpenoids are coloured (yellow, orange and red-pigmented) compounds and are present in various fruits and vegetables with a very rich antioxidant profile. More than 600 carotenoids have been found in plants and they comprise two types of molecules, carotenes and xanthophylls. Carotenoids have a tissue-specific biological activity and have been reported to protect against uterine, prostate, breast, colorectal and lung cancers[25].

Polyphenolics: Polyphenols are natural compounds synthesized by plants with chemical features related to phenolic substances. Phenolic compounds can be divided into flavonoids, flavonols (quercetin), flavones (apigenin), flavanols (catechin and its derivatives, proanthocyanidins), anthocyanidins (anthocyanins), flavanones (hesperidin), isoflavones (genistein, daidzein) and non-flavonoids, stilbenes (resveratrol), phenolic acids (gallic acid, hydroxybenzoic acid, hydroxycinnamic acid, chlorogenic acid, caffeic acid, ferulic acid). There are approximately 8,000 different sub-classes of polyphenols. The most important are flavonols, flavones, flavan-3-ols, flavanones and anthocyanin[18]. The main sources of polyphenols are fruits, vegetables, black tea, green tea, coffee, chocolate, red wine, olives and extra virgin olive oil. Nuts, spices, herbs and algae are also potentially significant for supplying certain polyphenols[26]. Recognition of the antioxidant activities of many polyphenols has established a correlation with the health benefits of such compounds[27]. In this respect, flavonoids, the major active phenolic compounds have long been recognized to possess anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral and anticarcinogenic activities. The main representatives of flavonoids polyphenols are quercetin, catechin and kaempferol. Generally, phenolic compounds have beneficial effects on several diseases including cancer, cardiovascular disease, myocardial damage and neurodegenerative disorders[28]. These compounds can defend the body against

cancer by protecting DNA from radical damage and avert cardiovascular disease by preventing the oxidation of lipids and cholesterol[29].

Alkaloids: Alkaloids are a class of naturally occurring organic compounds that contain at least one nitrogen atom. It was found that 20% of plant species contain alkaloids and the major source of the alkaloid is the flowering of the plants. Alkaloids are medicinally known as local anaesthetic and stimulants, psychedelics, anticancer drugs, cholinomimetic, spasmolysis agents, vasodilators, antiarrhythmics, antiasthma therapeutics, etc[30]. Alkaloids are, being present in several economically relevant plant families. Among alkaloids, glucosinolates contain sulfur and nitrogen and are derived from glucose and amino acid. Glucosinolates present in cruciferous vegetables such as broccoli, cauliflower and cabbage are widely consumed and their beneficial health-promoting effects are well established[23]. The current interest in glucosinolates is focused on their ability to protect against cancer since studies have shown an inverse relationship between the consumption of vegetables containing glucosinolates and the risk of cancer[31]. Otherwise, purine alkaloids, including caffeine (coffee), theophylline (antiasthma drug), theobromine (chocolate) and other methyl-xanthines, play a significant role in pharmacology and food chemistry[32]. The main effects of moderate consumption of caffeine by humans are an increase in attention, memory performance, physical performance, muscle recovery and intraocular pressure and a possible decrease in risk of heart disease.

Phytosterols: Phytosterols, a generic term that refers to both plant sterols and stanols, are a group of lipophilic steroid alcohols that naturally occur in plants. To date, more than 250 phytosterols have been identified and successfully isolated from various plants[33]. The best dietary sources of phytosterols are unrefined vegetable oils, seeds, cereals, nuts and legumes (sterols) and corn, wheat, rye and rice (stanols)[34]. Concerning the health benefits, phytosterols are reported to lower cholesterol[33] and cancer protection[35]. It was proved that dietary supplementation with plant sterols, stanols and their esters reduces intestinal cholesterol absorption[36]. Meanwhile, studies have proved the evidence that phytosterols have a role in protecting against the development of various cancers (ovarian, breast, stomach, prostate and lung cancer)[37]. This has been attributed to the effect of phytosterols on the membrane structure and function of tumour and host tissue and stopping the growth and spread of cancer cells.

Thiosulfonates: Thiosulfonates are organic sulfur-containing compounds isolated from plants i.e., garlic, onion, cabbage and cauliflower. Propyl-propane thiosulfonate, isolated from garlic, affects enteropathogens, such as subspecies *Salmonella*, *Campylobacter jejuni*, *Clostridium perfringens* and *Escherichia coli*[38]. Thiosulfonates reduce blood pressure and have anticancer and antimicrobial properties[39]. The antimicrobial activity of thiosulfate acids is closely related to their ability to block the normal metabolism of microorganisms through sulfenylation of thiol groups of their enzymes[40]. Moreover, organosulfur compounds found in garlic and onions are known, as anticarcinogenic agents. In this respect, Rao *et al.*[41] mentioned that diallyl sulfide (DAS), a major component of garlic oil, is an inhibitor of tumorigenesis by various metabolically activated carcinogens.

***In vitro* production of plant nutraceuticals:** The recent advances in biotechnology particularly methods of plant tissue cultures offer new means for the *in vitro* production of valuable plant phytochemicals. In this respect, different tissue culture techniques such as cell, callus and organ cultures have been developed as an alternative for the production of economically important valuable phytochemicals from field-grown plants. The greatest advantage of the plant tissue culture technique has been its ability to provide a continuous, sustainable, economical and viable production of natural compounds, regardless of the geo-climatic conditions and under a microenvironment regime highly controlled[42]. Moreover, cell suspension cultures and bioreactor techniques could be used to regulate metabolic processes to maximize yields[43]. Otherwise, hairy root cultures provide instrumental in enhancing the production of valuable plant metabolites[44]. For improving the production of phytochemicals of interest by plant cell cultures, different steps should be achieved: (1) Identification of plant starting materials that contain the largest amount of the desired compound, (2) Optimization of culture medium and culture conditions, (3) Addition precursors or elicitor to *in vitro* cultures that can drive and speed up the metabolic flow leading to the phytochemical of interest, (4) Selection of the most productive plant cell lines and (5) Scale-up through the use of appropriate bioreactors.

For improvement phytochemicals production, mutagenesis methods such as irradiation and chemical mutagens have been applied to induce mutations in plants. Mutagenesis of cultured explants, cells and tissue cultures represents a feasible method for induction of genetic variability. *In vitro* mutagenesis is a genotypic change in a culture and the derived population can be maintained via rigorous subculturing. This mutagenesis can be achieved by mutation-induced by radiations or chemicals. The advantages

include low exposure levels and a wide variation of mutation. At the present, elicitation is the most effective biotechnological tool for enhanced biosynthesis as well as accumulation of nutraceuticals *in vitro* plant cultures. The application of elicitors reduced the period of cultivation and yielded a high level of product. In this respect, various types of elicitors have been used to modify plant cells' metabolism to enhance the accumulation of desired compounds. However, various parameters, such as elicitor type, concentrations, duration of exposure, treatment schedule, culture type, cell line, medium composition, presence or absence of growth regulation and age or stage of the culture at the time of elicitor treatment are important factors that can determine the effectiveness of any elicitation strategy on plant nutraceuticals production[45]. Otherwise, genetic engineering can be used to manipulate the metabolic pathways of plant nutraceuticals. In this context, hairy root cultures have become an important method for producing secondary metabolites in plant tissues because of their stability and high productivity in hormone-free culture conditions[46]. Therefore, the productivities of hairy root cultures have been reported to be enhanced through adopting strategies in plant metabolite production enhancement[47]. Recently, nanoparticles have been extensively used in plant tissue culture to induce various growth parameters as well as bioactive metabolites improvement[48]. All of the studies mentioned above confirm the possibility of nanoparticles being employed as successful and promising bioactive compound elicitors in plant cell and organ cultures. Meanwhile, bioreactors are utilized in bioprocessing industries, wherein the optimum environmental conditions are maintained to achieve the required biological products on a large scale. The advantages of bioreactors include a better rate of product multiplication, lesser time for multiplication and a minimum cost[49].

In vitro culture was found more efficient than the whole plant for the production of different bioactive secondary metabolites since the level of ajmalicine, ajmaline, anthraquinones, benzylisoquinoline alkaloids, berberine, bisoclaurine, coniferin, diosgenin, ginseng, ginsenoside, glutathione, nicotine, rosmarinic acid, raucaffricine, shikonin, taxol, turpentine, triptolide, ubiquinone-10, etc., were high in *in vitro* culture compared to whole plant production following agronomic method[50]. In this respect, callus induction of globe artichoke was achieved as a method of *in vitro* production of caffeoylquinic acids and phenolic compounds[51]. Likewise, the production of total phenolic and total flavonoids of chicory from adventitious root cultures was reported by Ibrahim *et al.*[52]. Callus cultures of *Lepidium sativum* L., were established for *in vitro* production of glucosinolate compounds[53]. Furthermore, different abiotic stresses were used for enhancement of *in vitro* production of phenolics[54], anthraquinone[55], flavonoids[56] and anthocyanin[57]. On the other hand, fatty acids were enhanced in callus cultures of jojoba by gamma irradiation treatment[58].

Enhancement of nutraceuticals production by mutation: The mutation is reported as an important factor in improving nutritional quality in plant species. Physical or chemical mutagenic agents are used to induce mutations in different planting materials. Regarding physical mutagens, ionizing radiations have been used widely for inducing hereditary aberrations and more than 70% of mutant varieties were developed using physical mutagenesis[59]. The major advantage of using physical mutagenesis compared to chemical mutagenesis is the degree of accuracy and sufficient reproducibility, particularly for gamma rays, which have a uniform penetrating power in the tissue[60]. In the mutation-breeding programme, *in vitro* cultured explants provide a wider choice of controlled selection following mutagenic treatment as compared to treatments of *in vivo* material. Moreover, *in vitro* culture techniques are particularly relevant for mutagenesis as totipotent plant cells are cultured proliferated in large volumes and can be induced into the regeneration of complete plants. Among the different *in vitro* methods, somatic embryogenesis is the most useful tool for mutagenesis as somatic embryos usually originate from single cells. Furthermore, haploid cell and protoplast cultures have advantages in studies on mutant selection *in vitro*, since mutations particularly recessive in nature can easily be detected in subsequent generations. In this respect, gamma rays are known to influence plant growth and development by inducing cytological, genetic, biochemical, physiological and morphogenetic changes in cells and tissue. The effects of gamma radiation on changes in plant photosynthetic pigments, the composition of chemical, cellular structure and crude oil yield were investigated[61]. To improve the oil content of jojoba, El-Shabrawi *et al.*[58], exposed embryonic callus to different doses of gamma radiation. They found that oil content was increased by 1.41% by exposing it to 5 Kr gamma radiations. Increasing in ginsenoside production in calli cultures of *Panax ginseng* was also observed by exposure to gamma radiations (10-100 Gy)[62,63]. A dosage of 30 Gy was selected as the adequate dose and via HPLC and TLC analysis, it was confirmed that there was an increase in ginsenoside production in the mutant lines. To enhance phenolic compounds and flavonoids in *Artemisia annua*, callus cultures were exposed to different doses of gamma irradiation[64]. Irradiation dose of 15 Gy showed the highest amounts of

phenols and flavonoid content as compared to the control. Likewise, the low doses (16 Gy) of gamma irradiation stimulated the production of shikonin derivatives in callus cultures of *Lithospermum erythrorhizon* but higher doses than 32 Gy did not enhance the shikonin derivatives[65]. Also, El-Beltagi *et al*.[66] found that low doses of gamma irradiation (15 and 20 Gy) enhanced the total phenolic and flavonoid accumulation in rosemary (*Rosmarinus officinalis* L.) callus cultures. Similarly, a 15 Gy dose of gamma irradiation slightly enhanced stevioside content over the control of *Stevia rebaudiana* callus cultures[67]. Gamma irradiation was also used for promoting some phytochemicals such as ascorbate, photosynthetic pigments and some antioxidant enzymes activity in red pepper (*Capsicum annuum*) when treated with gamma rays ranging from 2-16 Gy[68].

On the other hand, chemical mutagens are applied as an alternative to induce mutations where no physical is available. An advantage of chemical mutagenic agents is that they can be applied without complicated equipment or facilities. But, the chemical mutagens are extremely toxic compared with physical mutagens and require more care in their application. Chemical mutagens belonging to the class of alkylating agents are mostly used such as ethyl methanesulfonate (EMS), diethyl sulfate (dES), ethylamine (EI), ethyl nitroso urethane (ENU), ethyl nitrosourea (ENH) and methyl nitrosourea (MNH). Among these mutagens, EMS has been used in several crops such as urd bean[69] and barley[70]. Mostafa[71] stated that the efficiency of mutant induced by chemical mutagens is influenced by pH, soaking into water, temperature, concentration and treatment duration. Particularly, *in vitro* mutagenesis depends on the establishment of reproducible *in vitro* plant regeneration procedures, optimization of mutagenic treatments and screening of the mutagenized populations for desired variations. To develop reproducible protocol mutagenesis of *Hyoscyamus niger* targeting putrescine N-methyltransferase (PMT) and 6β-hydroxy hyoscyamine (H_6H) genes of alkaloid biosynthetic pathway, callus was treated with different concentrations (0.01-0.1%) of ethyl methane sulfonate (EMS)[72]. The results depicted that EMS has an intense effect on PMT and H6H gene expression and metabolite accumulation. The mutagenic potential of EMS was also recorded on biochemical components, cytological features and morphology in *Coriandrum sativum* L.[73]. At this point, the induction of polyploidy or artificial chromosome doubling is implemented for enhancing the biomass of a plant and, thus consequently amplifying the metabolite profile as well. The superiority of secondary products of polyploidy compared to diploid might be the expression increasing of crucial biosynthesis genes in polyploidy. In this respect, colchicines have been widely used in increasing the level of ploidy. In this context, adventitious roots of *Panax ginseng* were treated with 100 mg L^{-1} colchicines over 60 hrs to obtain octoploid genotypes. HPLC analysis, of the resultant regenerated octoploid plantlets, demonstrated that the chromosome doubling can enhance biomass and ginsenoside accumulation, simultaneously[74]. Likewise, autotetraploid of *Bacopa monnieri* was obtained *in vitro* by exposure nodal segments to colchicine (0.5%) for 48 hrs[75]. The maximum total bacoside content was obtained from an autotetraploid plant, which was 2.3-fold higher than the level in diploid plants. Similarly, polyploidization enhances flavonoids in *Fagopyrum tataricum* as compared to diploid plants[76].

Enhancement of nutraceuticals production by transformation: Transformation is another strategy that can be used to produce high-value-chemical products in plant cells or organ tissues. Manipulation of plant metabolic signalling pathways has been intensively used to enhance the production of valuable compounds in plants and *in vitro* systems[77]. The transformation was found to be useful in those cases where different methods commonly used to increase secondary metabolite production (cell selection, elicitor treatments and addition of a biosynthetic precursor) only slightly enhance cell productivity. Results of research prove that the transfer of some DNA (T-DNA) oncogenes, such as the *rolB* and *rolC* genes of *Agrobacterium rhizogenes* and the *6b* gene of *Agrobacterium tumefaciens*, affect the biosynthesis of secondary metabolites in transformed plant cells[78,79]. Hairy root culture is the most promising transformation technique used for *in vitro* production of valuable plant compounds. Hairy roots are unique in their genetic and biosynthetic stability, faster in growth and more easily maintained. Furthermore, a hairy root can be transformed to produce more than one secondary metabolite and this unique ability makes them very economical for commercial exploitation[80]. These roots are induced on aseptic, wounded parts of plants by inoculating them with *Agrobacterium rhizogenes*. Sevón and Oksman-Caldentey[81] mentioned that the secondary metabolites produced by hairy roots arising from the infection of plant material by *Agrobacterium rhizogenes* are the same as those usually synthesized in intact parent roots, with similar or higher yields. During the infection process, *Agrobacterium rhizogenes* transfers a part of the DNA (transferred DNA, T-DNA) located in the root-inducing plasmid Ri to plant cells and the genes contained in this segment are expressed in the same way as the endogenous genes of the plant cells. Moreover, the auxin content of the transformed root is comparatively higher than

the non-transformed roots which play an important role in their growth behaviour[82]. In this context, several studies have demonstrated that *Agrobacterium rhizogenes*-mediated transformation with root locus (rol) genes enhances secondary metabolite biosynthesis in transgenic roots by activating biosynthetic genes[79,83,84]. Hairy root cultures of many plant species have been widely studied for the production of secondary metabolites useful as pharmaceuticals, cosmetics and food additives[83,85,86]. Furthermore, elicitation of hairy root promotes phytochemical production and also arrests feedback inhibition, preventing degradation of metabolites in the culture medium[87]. In this respect, Ismail *et al.*[88], found that total flavonoid and phenolic contents were higher in hairy root cultures of *Lactuca sativa* as compared to the non-transformed ones. Hairy root cultures of *Momordica charantia* were elicited with jasmonic acid and salicylic acid to enhance biomass accumulation and phenolic compound production[89]. The *rolB* gene was introduced in *Vitis amurensis* cells and the *rolB*-transformed calli are able of producing up to 3.15% dry weight of resveratrol[90]. This ability is tightly correlated with the abundance of *rolB* mRNA transcripts. In their investigation of *in vitro* production of phenolic compounds from hairy root culture of Tartary buckwheat, Kim *et al.*[91], found that the concentration of phenolic compounds in the hairy roots was several-fold higher compared with wild type roots of the same species.

Genetic engineering is used to improve minerals and vitamins such as carotenoids in food that can be useful in the treatment of some cancers, heart disease and blindness[92]. Also, genetic engineering can be used to increase the levels of unsaturated fatty acids in some commonly used oils such as canola, soybean, sunflower and peanuts[93]. Genetic modification leads to oilseed crops with unusual fatty acids such as short-, medium- and long-chain fatty acids and those with double bonds at unusual positions, or those that carry hydroxyl or epoxy groups. On this point, the strategies for enhancing the production of plant nutraceuticals *in vitro* are built on the manipulation of existing metabolic pathways by overexpressing or silencing selected elements involved in their biosynthesis. One of the successful examples of approaches to of enhancement plant nutraceuticals using genetic engineering is the development of "Golden Rice" involving the transfer of the genes necessary for the accumulation of carotenoids (vitamin A precursors) in the endosperm that are not available in the rice gene pool[94-96]. Potato tubers were biofortified by three bacterial genes encoding phytoene synthase, phytoene desaturase and lycopene β-cyclase to increase β-carotene[97]. The transformation of the PrLeg gene into the potato, which contains low amounts of sulfur-containing amino acids, was found to enhance Met content in the tubers[98]. In tomatoes, increased content of flavanols in fruits was achieved by engineering tomato plants with transcription factor (LC and C1) genes. The ectopic expression of these two genes allowed the upregulation of flavonoid pathway genes and the accumulation in berry flesh of these antioxidant compounds[99]. The increase of anthocyanins in "purple" tomato was achieved by expressing two genes of *Antirrhinum majus* en coding the transcription factors *Delila* and *Rosea 1*[100]. Likewise, excessive expression of the *Petunia* CHI gene caused a 78-fold increase in flavonoids in tomato peel[101]. Shi *et al.*[102] reported the development of canola transgenic with change in fatty acids compositions, using *B. napus* cultivar "CY2" as the transgenic recipient of BnFAE1, a fragment involved in the synthesis of very-long-chain fatty acids. Gold kiwifruit was genetically modified for a high level of ascorbic acid, carotenoids and lutein and zeaxanthin[103]. Vereshchagina *et al.*[104] transformed artichoke with the *rolC* gene, which is a known inducer of secondary metabolism. Analysis revealed that the predominant metabolites synthesized in the transgenic calli were 1,5-dicaffeoylquinic acid, 3,4-dicaffeoylquinic acid and chlorogenic acid. The overall production of these metabolites was three times higher than that of the corresponding control calli. In another study, stilbene synthase genes isolated from *Vitis vinifera* were transferred to *Lactuca sativa*, resulting in transgenic red lettuce capable of producing a high amount of resveratrol[105].

Enhancement of plant nutraceuticals production by elicitation: Elicitation is one of the most important strategies for enhancing the *in vitro* production of plant phytochemical compounds. Elicitors are stress factors that trigger the inducible defence changes in a plant system that results in induction or expansion of biosynthesis of fine chemicals[106]. Elicitors have been widely used to increase the production of plant metabolites in *in vitro* plant cell cultures. Hairy root cultures are preferred for the application of elicitation due to their genetic and biosynthetic stability and high growth rate in growth regulator-free media. Depending on their nature, elicitors are classified as biotic (biological origin) and abiotic (non-biological origin). Biotic elicitors include compounds released by microorganisms and other pathogens or formed by the action of plant enzymes on microbial cell walls, microbial enzymes, fungal and bacterial lysates, yeast extracts and polysaccharides from microorganism cell walls. On the other hand, chemical substances, e.g., mineral salts, heavy metals, or physical factors such as light (UV-B, UV-C radiation), temperature and osmotic stress represent abiotic elicitor's

factors. Several parameters such as elicitor concentration and selectivity and duration of elicitor exposure are influencing the successful elicitation. A high dosage of elicitor has been reported to induce hypersensitive response leading to cell death, whereas an optimum level was required for induction[107]. Otherwise, the different elicitors require a different duration of time to elicit the plant cell culture. Moreover, the composition of the medium or selection of medium also played a vital role in the elicitation process.

Enhancement by abiotic elicitors: The abiotic elicitors enhance the biosynthesis of plant secondary metabolites by triggering the defense- or stress-induced responses[108]. In this respect, Naik and Al-Khayri[109] mentioned that abiotic elicitors have different effects on the plant cellular processes such as carbon partitioning, carbohydrate and lipid metabolism, osmotic homeostasis, protein synthesis and gene expression. Their effect depends on the concentration of the elicitor, the growth stage of the culture, the period of contact and the time course of the elicitation. The research results proved that salt stress increases terpenes, phenols and alkaloids compounds in plant cells[110]. Also, saltation of the culture medium with NaCl enhanced the total ginseng and saponin in hairy root cultures of *Panax ginseng*[111]. In response to salt stress, anthocyanins are reported to increase in the non-sensitive species[112]. In red peppers, total phenolic content increased with a moderately saline level in culture medium[113]. Hussein and Aqlan[114] found that salt stress induced by the lower NaCl concentration increased total phenolics, total flavonoids and total tannins in callus cultures of Fenugreek (*Trigonella foenum-graecum* L.). Salinity also increased the diamine and polyamine content in *Oryza sativa*[115]. Likewise, light can enhance several metabolites in certain plants as a physical factor[116-118]. In this respect, anthocyanins and flavones increased in response to high visible light levels[119]. Similarly, UV–B irradiation stimulates flavonoid synthesis by enhancing the phenylpropanoid pathway in *Kalanchoe pinnata*[120]. Also, an increase in astragaloside biosynthesis was observed in hairy root cultures of *Astragalus membranaceus* when exposed to UV-B[121]. Yu *et al.*[122] studied the effect of different light sources on both biomass and ginsenosides biosynthesis in ginseng hairy root cultures and they found root growth was stimulated by red light in comparison to dark treatment. These results suggest that it is possible to manipulate secondary metabolite accumulation by varying the light and dark regimes.

In terms of chemicals elicitation, salicylic acid and jasmonic acid have been used to induce the production of phenolic compounds by *in vitro* cultures, being signalling molecules of different pathways[123]. When exogenously applied to plant cell cultures of a variety of species, methyl jasmonate positively stimulates the workflow of secondary biosynthetic pathways, leading to increased production of terpenoids, flavonoids and alkaloids and phenylpropanoids[124,125]. Jasmonic acid was used for enhancing the phenolic compound production and flavonoid contents in hairy root cultures of *Momordica charantia* (Cucurbitaceae)[92]. Salicylic acid (SA) also affects nutraceuticals metabolism in plants. It induced accumulation of the triterpenoids ginsenosides in ginseng and glycyrrhizin in liquorice[126,127]. Evidence demonstrated that suitable concentrations of SA can also promote monoterpene production[128]. Likewise, salicylic acid exhibits improving anthocyanin content in calli and cell cultures of *Vitis vinifera* L. cv. Gamay Fréaux[123,129]. Capsaicin was improved in suspension cultures of *Capsicum frutescens* by supplying isocaproic acid as a precursor[130]. Regarding the application of hormonal compounds, the effect of different concentrations of ethephon on cynarin accumulation in callus cultures of globe artichoke was evaluated[131]. High cynarin content of the callus cultures was observed by the addition of 90 µL ethephon into the culture medium for 9 and 12 days. On the other hand, metals like Ni, Ag, Fe and Co have been shown to elicit the production of secondary metabolites in several plants. For instance, copper was used to enhancing the production of phenolic compounds in the root culture of *Panax ginseng*, increasing up to 76% of the production of phenolics and flavonoids[126]. Increasing four times more anthocyanin accumulation in the cell culture of *Vitis vinifera* cv., Gamay Red was obtained by the application of magnesium to the culture medium[132]. The combination of $CuSO_4$ and Dimethyl Sulfoxide (DMSO) increase the grindelic acid production in callus and cell suspension cultures of *Grindelia pulchella*[133].

Enhancement by biotic elicitors: Biotic elicitors are extracts or products derived from biological origins such as fungal, bacterial, yeast or plant. The most commonly biotic elicitors are polysaccharides, glycoproteins, inactivated enzymes, chitosan, pectin, chitin, alginate, curdlan, xanthan, elicitin[134]. In this respect, chitosan the biotic elicitor polysaccharide was used to improve the yield of many useful compounds in tissues and whole plants. The effectiveness of polysaccharides, among which chitosan, as active elicitors, is dependent on their molecular structures. In general, polysaccharides with a high number of side chains are more active than those with a lower number. Chitosan treatments have been shown to act as an elicitor in plants, to enhance phytochemical contents of cells and tissues of different varieties[135,136]. In the basil plant, chitosan increased the total amount of phenolic and terpenic compounds, especially rosmarinic acid and eugenol which

have strong antioxidant, antiviral, antibacterial, anti-inflammatory, antiseptic and anaesthetic properties and show application in perfumery and flavouring[137]. High levels of alkaloids coumarins and fluoroquinolone were found in shoot cultures of *Ruta graveolens* by supplementation of culture medium with chitin or chitosan[138]. In a study on elicitation of *Morinda citrifolia* plant, Purwianingsih *et al.*[139], found that the addition of chitosan into medium amended with 2,4-D and kinetin increases anthraquinone in callus cultures. Likewise, chitosan enhanced the production of trans-resveratrol and vinifera in the cell system of *V. vinifera*[140]. On the other hand, *Aspergillus niger* is used as a fungal elicitor to enhance the production of valuable compounds in plants. The positive influence of *Aspergillus niger* in combination with methyl-jasmonate on *in vitro* aggregation of total phenolic and peroxides from date palm was reported by Taha *et al.*[43]. Also, augmentation of culture medium with 1.5% of *Aspergillus niger* in combination with 100 µM of methyl jasmonate increased capsaicin accumulation in *Capsicum annum* cell culture[141]. Cai *et al.*[142] reported that the number of flavonoids in the *Avena sativa* L. was markedly increased by the fermentation by the *Aspergillus niger*. In this context, different concentrations of a root endophytic fungus *Piriformospora indica* cell homogenates were utilized as an abiotic elicitor in *Withania somnifera* hairy root for different periods[143]. When the hairy root cultures of *Withania somnifera* were treated with 3% cell homogenates of *Piriformospora indica* for 48 hrs, it enhanced the biomass and the production of withanolides, viz., withaferin A, withanolide A, withanoside V and withanoside IV as compared to control (untreated hairy root).

Elicitation by nanoparticles: The application of nanotechnology is a new sustainable generation of *in vitro* cultures for the improvement of the plant's active constituent production. Nanoparticles have the promise to be used as novel effective elicitors in plant biotechnology. In this context, the elicitation of nanoparticles was found to increase the amounts of bioactive compounds accumulated in various plants[144-150]. The effects of metal oxide nanoparticles such as: titanium oxide, zinc oxide, iron oxide and copper oxide have been indicated for facilitating plant growth and production of secondary metabolites[151,152]. The total phenols and flavonoid contents of *Cucumis anguria* hairy root cultures were significantly increased by silver nanoparticles elicitation[153]. Likewise, zinc oxide nanoparticles have an obvious effect on the accumulation of plant secondary metabolites such as flavonoids and phenols. The influence of copper oxide nanoparticles (CuO NPs) on the aggregation of glucosinolates and phenolic compounds in hairy root cultures of Chinese cabbage was investigated[154]. Glucosinolates (gluconasturtiin, glucobrassicin, 4-methoxyglucobrassicin, neoglucobrassicin, 4-hydroxyglucobrassicin, glucoallysin, glucobrassicanapin, sinigrin, progoitrin and gluconapin) and transcript (*MYB34, MYB122*, *MYB28* and *MYB29*) levels were considerably escalated in CuO NPs-elicited hairy root cultures compared to non-elicited cultures. Moreover, phenolic compounds (flavonols, hydroxybenzoic and hydroxycinnamic acids) were significantly enriched in CuO NPs-elicited hairy root cultures. The presence of TiO_2 nanoparticles (4.5 or 6.0 mg L^{-1}) significantly increased the content of gallic acid, chlorogenic acid, o-coumaric acid, tannic acid and cinnamic acid in embryonic calli of *Cicer arietinum*[151]. The accumulation of steviol glycosides in shoot cultures of *Stevia rebaudiana* was significantly enhanced on MS medium fortified with 1 mg L^{-1} CuO nanoparticles[149]. In addition, the total flavonoid and phenolic content also increased with ZnO nanoparticles treatment. However, higher concentrations of ZnO nanoparticles led to decreased secondary metabolite production due to the phytotoxic effects of ZnO nanoparticles. Concerning Ag NPs, Bhat and Bhat[155] found that 3 mg L^{-1} to *Capsicum frutescens* cell cultures, increased the content of capsaicin about 2-fold.

Scaling up and using of bioreactor for production of plant nutraceuticals: With the increasing demand for nutraceuticals derived from plants, *in vitro* culture became a reliable technique for the mass production of such compounds. In practice, *in vitro* cultures provide a source of homogeneous highly active cells that allow overcoming some plant limits such as the slow growth, seasonal and environmental variations and disease susceptibility. Adventitious roots or non-transgenic roots can be utilized for producing plant natural products, especially those that are linked to root differentiation. Moreover, the cell culture technique could be used for the large-scale production of desired compounds from different plant species. In this respect, a bioreactor system is employed for the large-scale production of valuable bioactive compounds from plant cells. Many physical factors, like the intensity, stress and operation conditions should be optimized as a first step in scaling up the process through bioreactors. The scaling-up from shake flasks to bioreactors is typically not straightforward due to changes in the cell growth environment. By improving bioreactor design and optimizing key parameters of the culture, some of these limitations can be overcome. In this context, different varieties either old or new designs of bioreactors are used for large-scale culturing of plant cells or other microorganisms[156-158]. Due to the easy

scaling-up, good fluid mixing and oxygen transferability, the conventional stirred tank reactor (STR) bioreactor is most widely exploited. Moreover, this type of bioreactor is recommended for optimal production of the target Phyto molecule by elicitation[159]. For the large-scale harvesting of phytochemicals, it is possible to use two kinds of procedures. When the target compound accumulates inside the cells, the final biomass is harvested from the tank of the bioreactor and the target compound is extracted from the cells. On the contrary, when the product is released from the plant cells, part of the culture medium is removed from the tank and replaced by the fresh medium to rejuvenate the cell biomass.

Scaling-up of hairy roots and their phytochemical compounds to an industrial scale using bioreactors has developed[160,161]. In this respect, a bioreactor system has been utilized for the production of different compounds from cell and hairy root cultures of many species. Enhancing ginsenoside production in adventitious/hairy roots of ginseng in various levels of liquid-phase airlift bioreactors was achieved by optimizing nutrient growth and elicitors[162]. The accumulation of phenolic compounds: Phenolic acids and flavonoids in the micro shoots of *Schisandra chinensis* grown in different types of the bioreactor was evaluated[163]. The maximum phenolic acids content was recorded in the biomass maintained in the cone-type bioreactor for 30 days. While the highest total content of flavonoids was found in the micro shoots maintained in the nutrient sprinkle bioreactor for 30 days. Large-scale production of steviol glycosides (low-calorie glucoside sweeteners) in *Stevia rebaudiana* cultures was achieved in a temporary immersion bioreactor[164]. *In vitro* aggregation of phenolic and peroxides compounds from cells of date, palm cv. Zaghlool using stirred tank reactor was reported[43]. The maximum content of these compounds was obtained by elicitation of modified MS-medium with *Aspergillus niger* extract at 0.1% combined with methyl-jasmonate (100 μM), after 10 days of cultivation.

Plants are considered biochemical factories to produce nutraceuticals such as alkaloids, flavonoids and polyphenols. *In vitro* culture technique, in which plant cells, tissues and organs offer alternatives for producing the important plant nutraceuticals. Moreover, biotechnological approaches such as mutagenesis, transformation and elicitation can be employed to enhance the nutraceuticals production utilizing *in vitro* techniques. Although it needs complicated facilities, physical mutagenesis produces sufficient reproducibility compared to chemical mutagenic agents. On the other hand, transformation using *Agrobacterium rhizogenes* is used to enhance nutraceuticals in plant cells or organ tissues. Meanwhile, elicitation is widely used to increase the biothensis of plant metabolites in plant cell cultures. The elicitor's effects depend on the concentration of the elicitor, the growth stage of the culture and the exposure period. On the other hand, a bioreactor system has been developed for the large-scale production of desired compounds from plant cell suspension and organ cultures.

CONCLUSION

Plant tissue culture techniques offer a perpetual source for the continuous production of plant nutraceuticals under controlled conditions. Moreover, mutagenesis, transformation and elicitation can be used to enhance the plant nutraceuticals production. To induce mutations, planting materials are exposed to physical i.e., irradiation or chemical mutagenic agents such as methanesulfonate, diethyl sulfate, ethylamine, ethyl nitroso urethane ethyl nitrosourea and methyl nitrosourea. Transformation can be used for the manipulation of plant metabolic pathways leading to enhancing the production of valuable compounds in plants. In this respect, hairy root culture induced by *Agrobacterium rhizogenes* was found the most promising transformation technique used for *in vitro* enhanced production of valuable plant compounds since the *rolB* and *rolC* genes of *Agrobacterium rhizogenes* positively affect the biosynthesis of secondary metabolites in transformed plant cells. Otherwise, biotic and abiotic elicitors enhance the plant nutraceuticals by acting on their biosynthesis enzymes. On the other hand, different designs of bioreactors can be used for the large-scale production of nutraceuticals from plant cells. The conventional stirred tank reactor (STR) is the most common bioreactor used for plant cells.

SIGNIFICANCE STATEMENT

Although the healthcare has advanced techniques to treat different diseases, still nutraceuticals represent great importance because of the power it holds to treat diseases through diet. This review is focused on the various biotechnological approaches used for the production of plant nutraceuticals as well as their contribution to public health. In this respect, various techniques of *in vitro* culture have been used for the production of plant nutraceuticals under aspects conditions. The main advantage of *in vitro* culture over the conventional cultivation of whole plants is that nutraceuticals can be produced under controlled conditions independent of climatic changes. In this regard, the cell culture technique in combination with elicitation now represents an efficient way

to produce the plant nutraceuticals in suspension cultures. Otherwise, hairy roots cultures, induced by infection of *Agrobacterium rhizogenes* proved to be an important technique not only for *in vitro* production but also for biosynthesis enhancement of such plant compounds. Meanwhile, such compounds can be commercially manufactured using a bioreactor which offers the opportunity for large amounts of production with the maintenance of genetic integrity of plant cell lines. Generally, this article will help to recognize an applicable method of *in vitro* production of plant nutraceuticals using different types of techniques and enhancement factors.

REFERENCES

1. Hardy, G., 2000. Nutraceuticals and functional foods: Introduction and meaning. Nutrition, 16: 688-689.
2. Kwak, N.S. and D.J. Jukes, 2001. Functional foods. Part 2: The impact on current regulatory terminology. Food Control, 12: 109-117.
3. Biesalski, H.K., L.O. Dragsted, I. Elmadfa, R. Grossklaus and M. Müller *et al*., 2009. Bioactive compounds: Definition and assessment of activity. Nutrition, 25: 1202-1205.
4. Nasri, H., A. Baradaran, H. Shirzad and M. Rafieian-Kopaei, 2014. New concepts in nutraceuticals as alternative for pharmaceuticals. Int. J. Preventive Med., 5: 1487-1499.
5. Elless, M.P., M.J. Blaylock, J.W. Huang and C.D. Gussman, 2000. Plants as a natural source of concentrated mineral nutritional supplements. Food Chem., 71: 181-188.
6. Liu, H., N. Qiu, H. Ding and R. Yao, 2008. Polyphenols contents and antioxidant capacity of 68 Chinese herbals suitable for medical or food uses. Food Res. Int., 41: 363-370.
7. Lucock, M., 2004. Is folic acid the ultimate functional food component for disease prevention? BMJ, 328: 211-214.
8. Siddhuraju, P. and K. Becker, 2007. The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) Walp.) seed extracts. Food Chem., 101: 10-19.
9. Halder, M., D. Roychowdhury and S. Jha, 2018. A Critical Review on Biotechnological Interventions for Production and Yield Enhancement of Secondary Metabolites in Hairy Root Cultures. In: Hairy Roots: An Effective Tool of Plant Biotechnology, Srivastava, V., S. Mehrotra and S. Mishra (Eds.), Springer, Singapore, ISBN: 978-981-13-2561-8, pp: 21-44.
10. Buttriss, J.L. and C.S. Stokes, 2008. Dietary fibre and health: An overview. Nutr. Bull., 33: 186-200.
11. Bingham, S.A., N.E. Day, R. Luben, P. Ferrari and N. Slimani *et al*., 2003. Dietary fibre in food and protection against colorectal cancer in the European prospective investigation into cancer and nutrition (EPIC): An observational study. Lancet, 361: 1496-1501.
12. Rodiño-Janeiro, B.K., M. Vicario, C. Alonso-Cotoner, R. Pascua-García and J. Santos, 2018. A review of microbiota and irritable bowel syndrome: Future in therapies. Adv. Ther., 35: 289-310.
13. Delgado, G.T.C., W.M. da Silva Cunha Tamashiro, M.R. Marostica Jr., Y.M.F. Moreno and G.M. Pastore, 2011. The putative effects of prebiotics as immunomodulatory agents. Food Res. Int., 44: 3167-3173.
14. Simopoulos, A.P., 2016. An increase in the omega-6/omega-3 fatty acid ratio increases the risk for obesity. Nutrients, Vol. 8. 10.3390/nu8030128.
15. Kurutas, E.B., 2015. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: Current state. Nutr. J., Vol. 15. 10.1186/s12937-016-0186-5.
16. Youdim, K.A. and J.A. Joseph, 2001. A possible emerging role of phytochemicals in improving age-related neurological dysfunctions: A multiplicity of effects. Free Radical Biol. Med., 30: 583-594.
17. Dzialo, M., J. Mierziak, U. Korzun, M. Preisner, J. Szopa and A. Kulma, 2016. The potential of plant phenolics in prevention and therapy of skin disorders. Int. J. Mol. Sci., Vol. 17. 10.3390/ijms17020160.
18. Panche, A.N., A.D. Diwan and S.R. Chandra, 2016. Flavonoids: An overview. J. Nutr. Sci., Vol. 5. 10.1017/jns.2016.41.
19. Kaefer, C.M. and J.A. Milner, 2008. The role of herbs and spices in cancer prevention. J. Nutr. Biochem., 19: 347-361.
20. Thakur, A. and R. Sharma, 2018. Health promoting phytochemicals in vegetables: A mini review. Int. J. Food Ferment. Technol., 8: 107-117.
21. Das, L., E. Bhaumik, U. Raychaudhuri and R. Chakraborty, 2012. Role of nutraceuticals in human health. J. Food Sci. Technol., 49: 173-183.
22. Tholl, D., 2006. Terpene synthases and the regulation, diversity and biological roles of terpene metabolism. Curr. Opin. Plant Biol., 9: 297-304.
23. Dillard, C.J. and J.B. German, 2000. Phytochemicals: Nutraceuticals and human health. J. Sci. Food Agric., 80: 1744-1756.
24. Kline, K., W. Yu and B.G. Sanders, 2004. Vitamin E and breast cancer. J. Nutr., 134: 3458S-3462S.
25. Milani, A., M. Basirnejad, S. Shahbazi and A. Bolhassani, 2017. Carotenoids: Biochemistry, pharmacology and treatment. Br. J. Pharmacol., 174: 1290-1324.
26. D'Archivio, M., C. Filesi, R. Vari, B. Scazzocchio and R. Masella, 2010. Bioavailability of the polyphenols: Status and controversies. Int. J. Mol. Sci., 11: 1321-1342.
27. Crozier, A., I.B. Jaganath and M.N. Clifford, 2009. Dietary phenolics: Chemistry, bioavailability and effects on health. Nat. Prod. Rep., 26: 1001-1043.

28. Yao, L.H., Y.M. Jiang, J. Shi, F.A.Tomás-Barberán, N. Datta, R. Singanusong and S.S. Chen, 2004. Flavonoids in food and their health benefits. Plant Foods Hum. Nutr., 59: 113-122.
29. Escarpa, A. and M.C. Gonzalez, 2001. Approach to the content of total extractable phenolic compounds from different food samples by comparison of chromatographic and spectrophotometric methods. Anal. Chim. Acta, 427: 119-127.
30. Kuete, V., H. Fouotsa, A.T. Mbaveng, B. Wiench, A.E. Nkengfack and T. Efferth, 2015. Cytotoxicity of a naturally occurring furoquinoline alkaloid and four acridone alkaloids towards multi-factorial drug-resistant cancer cells. Phytomedicine, 22: 946-951.
31. Traka, M. and R. Mithen, 2009. Glucosinolates, isothiocyanates and human health. Phytochem. Rev., 8: 269-282.
32. Ashihara, H., H. Sano and A. Crozier, 2008. Caffeine and related purine alkaloids: Biosynthesis, catabolism, function and genetic engineering. Phytochemistry, 69: 841-856.
33. Brufaua, G., M.A. Canelab and M. Rafecasa, 2008. Phytosterols: Physiologic and metabolic aspects related to cholesterol-lowering properties. Nutr. Res., 28: 217-225.
34. Sharma, R., S. Kumar, V. Kumar and A. Thakur, 2019. Comprehensive review on nutraceutical significance of phytochemicals as functional food ingredients for human health management. J. Pharmacogn. Phytochem., 8: 385-395.
35. Grattan, B.J., 2013. Plant sterols as anticancer nutrients: Evidence for their role in breast cancer. Nutrients, 5: 359-387.
36. Kamal-Eldin, A. and A. Moazzami, 2009. Plant sterols and stanols as cholesterol-lowering ingredients in functional foods. Recent Pat. Food Nutr. Agric., 1: 1-14.
37. Woyengo, T.A., V.R. Ramprasath and P.J.H. Jones, 2009. Anticancer effects of phytosterols. Eur. J. Clin. Nutr., 63: 813-820.
38. Peinado, M.J., R. Ruiz, A. Echávarri and L.A. Rubio, 2012. Garlic derivative propyl propane thiosulfonate is effective against broiler enteropathogens *in vivo*. Poult. Sci., 91: 2148-2157.
39. Torres-Palazzolo, C., D. Ramirez, D. Locatelli, W. Manucha, C. Castro and A. Camargo, 2018. Bioaccessibility and permeability of bioactive compounds in raw and cooked garlic. J. Food Compos. Anal., 70: 49-53.
40. Pylypets, A.Z., R.Y. Iskra, V.V. Havryliak, A.V. Nakonechna, V.P. Novikov and V.I. Lubenets, 2017. Effects of thiosulfonates on the lipid composition of rat tissues. Ukr. Biochem. J., 89: 56-62.
41. Rao, P.S.S., N.M. Midde, D.D. Miller, S. Chauhan, A. Kumar and S. Kumar, 2015. Diallyl sulfide: Potential use in novel therapeutic interventions in alcohol, drugs, and disease mediated cellular toxicity by targeting cytochrome P450 2E1. Curr. Drug Metab., 16: 486-503.
42. Karuppusamy, S., 2009. A review on trends in production of secondary metabolites from higher plants by *in vitro* tissue, organ and cell cultures. J. Med. Plants Res., 3: 1222-1239.
43. Taha, H.S., S.A. Bekheet and M.K. El-Bahr, 2012. A new concept for production and scaling up of bioactive compounds from Egyptian date palm (Zaghlool) cultivar using bioreactor. Emir. J. Food Agric., 24: 425-433.
44. Gabr, A.M.M., H. Ghareeb, H.M. El Shabrawi, I. Smetanska and S.A. Bekheet, 2016. Enhancement of silymarin and phenolic compound accumulation in tissue culture of milk thistle using elicitor feeding and hairy root cultures. J. Genet. Eng. Biotechnol., 14: 327-333.
45. Dhiman, N., V. Patial and A. Bhattacharya, 2018. The Current Status and Future Applications of Hairy Root Cultures. In: Biotechnological Approaches for Medicinal and Aromatic Plants, Kumar, N. (Ed.), Springer, Singapore, ISBN 978-981-13-0534-4, pp: 87-155.
46. Hidalgo, D., R. Sanchez, L. Lalaleo, M. Bonfill, P. Corchete and J. Palazon, 2018. Biotechnological production of pharmaceuticals and biopharmaceuticals in plant cell and organ cultures. Curr. Med. Chem., 25: 3577-3596.
47. Gutierrez-Valdes, N., S.T. Häkkinen, C. Lemasson, M. Guillet, K.M. Oksman-Caldentey, A. Ritala and F. Cardon, 2020. Hairy root cultures-A versatile tool with multiple applications. Front. Plant Sci., Vol. 11. 10.3389/fpls.2020.00033.
48. Wang, P., E. Lombi, F.J. Zhao and P.M. Kopittke, 2016. Nanotechnology: A new opportunity in plant sciences. Trends Plant Sci., 21: 699-712.
49. Almusawi, A.H.A., A.J. Sayegh, A.M. Alshanaw and J.L. Griffis, 2017. Plantform Bioreactor for Mass Micropropagation of Date Palm. In: Date Palm Biotechnology Protocols, Al-Khayri, J.M., S.M. Jain and D.V. Johnson (Eds.), Humana Press, New York, US, ISBN: 978-1-4939-7156-5, pp: 251-265.
50. Alamgir, A.N.M., 2017. Therapeutic Use of Medicinal Plants and Their Extracts: Volume 1. 1st Edn., Springer, Cham, Switzerland, ISBN: 978-3-319-63862-1, Pages: 546.
51. Menin, B., A. Moglia, C. Comino, J.C. Hakkert, S. Lanteri and J. Beekwilder, 2013. *In vitro* callus-induction in globe artichoke (*Cynara cardunculus* L. var. *scolymus*) as a system for the production of caffeoylquinic acids. J. Hortic. Sci. Biotechnol., 88: 537-542.
52. Ibrahim, M.M., M.K. El-Bahr and M.R. Rady, 2019. *In-vitro* adventitious root production of *Cichorium endivia* L. and antioxidants, total phenolic, and total flavonoids assessments. Egypt. Pharm. J., 18: 216-227.
53. Ibrahim, M.M., M.M. Mounier and S.A. Bekheet, 2019. Glucosinolates constituents and cytotoxic activities of *Lepidium sativum* L. callus cultures. J. Environ. Sci. Technol., 12: 138-148.
54. Grace, S.C. and B.A. Logan, 2000. Energy dissipation and radical scavenging by the plant phenylpropanoid pathway. Philos. Trans. R. Soc. B. Biol. Sci., 355: 1499-1510.
55. Nazif, N.M., M.R. Rady and M.S. El-Nasr, 2000. Stimulation of anthraquinone production in suspension cultures of *Cassia acutifolia* by salt stress. Fitoterapia, 71: 34-40.

56. Chutipaijit, S., S. Cha-Um and K. Sompornpailin, 2009. Differential accumulations of praline and flavonoids in indica rice varieties against salinity. Pak. J. Bot., 41: 2497-2506.
57. Chan, L.K., S.S. Koay, P.L. Boey and A. Bhatt, 2010. Effects of abiotic stress on biomass and anthocyanin production in cell cultures of *Melastoma malabathricum*. Biol. Res., 43: 127-135.
58. El-Shabrawi, H.M., H.E. Wahba, A.M. Gabr, S.I. El-Morsy, M.A. Saber and S.A. Bekheet, 2019. Improvement of wax oil content of embryonic callus of jojoba using gamma radiation. Plant Tissue Cult. Biotechnol., 29: 207-217.
59. Mba, C., 2013. Induced mutations unleash the potentials of plant genetic resources for food and agriculture. Agronomy, 3: 200-231.
60. Yamaguchi, H., S. Nagatomi, T. Morishita, K. Degi, A. Tanaka, N. Shikazono and Y. Hase, 2003. Mutation induced with ion beam irradiation in rose. Nucl. Instrum. Methods Phys. Res., Sect. B: Beam Interact. Mater. Atoms, 206: 561-564.
61. Wi, S.G., B.Y. Chung, J.H. Kim, M.H. Baek, D.H. Yang, J.W. Lee and J.S. Kim, 2005. Ultrastructural changes of cell organelles in *Arabidopsis* stems after gamma irradation. J. Plant Biol., 48: 195-200.
62. Kim, D.S., S.Y. Kim, I.Y. Jeong, J.B. Kim, G.J. Lee, S.Y. Kang and W. Kim, 2009. Improvement of ginsenoside production by *Panax ginseng* adventitious roots induced by γ-irradiation. Biol. Plant. 53: 408-414.
63. Kim, D.S., M. Song, S.H. Kim, D.S. Jang and J.B. Kim *et al*., 2013. The improvement of ginsenoside accumulation in *Panax ginseng* as a result of γ-irradiation. J. Ginseng Res., 37: 332-340.
64. Patil, A.S., P. Suryavanshi and D. Fulzele, 2018. Evaluation of effect of gamma radiation on total phenolic content, flavonoid and antioxidant activity of *in vitro* callus culture of *Artemisia annua*. Nat. Prod. Chem. Res., Vol. 6. 10.4172/2329-6836.1000345.
65. Chung, B.Y., Y.B. Lee, M.H. Baek, J.H. Kim, S.G. Wi and J.S. Kim, 2006. Effects of low-dose gamma-irradiation on production of shikonin derivatives in callus cultures of *Lithospermum erythrorhizon* S. Radiat. Phys. Chem., 75: 1018-1023.
66. El-Beltagi, H.S., O.K. Ahmed and W. El-Desouky, 2011. Effect of low doses γ-irradiation on oxidative stress and secondary metabolites production of rosemary (*Rosmarinus officinalis* L.) callus culture. Radiat. Phys. Chem., 80: 968-976.
67. Khalil, S.A., N. Ahmad and R. Zamir, 2015. Gamma radiation induced variation in growth characteristics and production of bioactive compounds during callogenesis in *Stevia rebaudiana* (Bert.) New Neg. Plant Sci., 1-2: 1-5.
68. Kim, J.H., B.Y. Chung, J.S. Kim and S.G. Wi, 2005. Effects of *in Planta* gamma-irradiation on growth, photosynthesis and antioxidative capacity of red pepper (*Capsicum annuum* L.) plants. J. Plant Biol., 48: 47-56.
69. Goyal, S., M.R. Wani and S. Khan, 2019. Frequency and spectrum of chlorophyll mutations induced by single and combination treatments of gamma rays and EMS in urdbean. Asian J. Biol. Sci., 12: 156-163.
70. Nicoloff, H., 1974. Effects of sodium acetate and sodium chloride on EMS-induced chlorophyll mutations in barley. Mutat. Res. Fundam. Mol. Mech. Mutagen., 23: 57-62.
71. Mostafa, G.G., 2015. Effect of some chemical mutagens on the growth, phytochemical composition and induction of mutations in *Khaya senegalensis*. Int. J. Plant Breed. Genet., 9: 57-67.
72. Shah, D., A.N. Kamili, A.A. Wani, U. Majeed, Z.A. Wani, N. Sajjad and P. Ahmad, 2020. Promoting the accumulation of scopolamine and hyoscyamine in *Hyoscyamus niger* L. through EMS based mutagenesis. PLoS ONE, Vol. 15. 10.1371/journal.pone.0231355.
73. Kumar, G. and A. Pandey, 2019. Ethyl methane sulphonate induced changes in cyto-morphological and biochemical aspects of *Coriandrum sativum* L. J. Saudi Soc. Agric. Sci., 18: 469-475.
74. Kim, Y.S., E.J. Hahn, H.N. Murthy and K.Y. Paek, 2004. Adventitious root growth and ginsenoside accumulation in *Panax ginseng* cultures as affected by methyl jasmonate. Biotechnol. Lett., 26: 1619-1622.
75. Inthima, P. and K. Sujipuli, 2019. Improvement of growth and bacoside production in *Bacopa monnieri* through induced autotetraploidy with colchicine. Peer J., Vol. 7. 10.7717/peerj.7966.
76. Wang, L.J., M.Y. Sheng, P.C. Wen and J.Y. Du, 2017. Morphological, physiological, cytological and phytochemical studies in diploid and colchicine-induced tetraploid plants of *Fagopyrum tataricum* (L.) Gaertn. Bot. Stud., Vol. 58. 10.1186/s40529-016-0157-3.
77. Hidalgo, D., M. Georgiev, A. Marchev, R. Bru-Martínez, R.M. Cusido, P. Corchete and J. Palazon, 2017. Tailoring tobacco hairy root metabolism for the production of stilbenes. Sci. Rep., Vol. 7. 10.1038/s41598-017-18330-w.
78. Gális, I., Y. Kakiuchi, P. Simek and H. Wabiko, 2004. *Agrobacterium tumefaciens* AK-*6b* gene modulates phenolic compound metabolism in tobacco. Phytochemistry, 65: 169-179.
79. Bulgakov, V.P., 2008. Functions of rol genes in plant secondary metabolism. Biotechnol. Adv., 26: 318-324.
80. Srivastava, S. and A.K. Srivastava, 2007. Hairy root culture for mass-production of high-value secondary metabolites. Crit. Rev. Biotechnol., 27: 29-43.
81. Sevón, N. and K.M. Oksman-Caldentey, 2002. *Agrobacterium rhizogenes*-mediated transformation: Root cultures as a source of alkaloids. Planta Med., 68: 859-868.
82. Veena, V. and C.G. Taylor, 2007. *Agrobacterium rhizogenes*: Recent developments and promising applications. *In vitro* Cell. Dev. Biol. Plant, 43: 383-403.

83. Vojin, T., M. Snežana, C. Aleksandar, P. Marija and T. Milana *et al*., 2014. Production of hairy root cultures of lettuce (*Lactuca sativa* L.). Cent. Eur. J. Biol., 9: 1196-1205.
84. Ismail, H., E. Dilshad, M.T. Waheed, M. Sajid, W.K. Kayani and B. Mirza, 2016. Transformation of *Lactuca sativa* L. with *rol C* gene results in increased antioxidant potential and enhanced analgesic, anti-inflammatory and antidepressant activities *in vivo*. 3 Biotech, Vol. 6. 10.1007/s13205-016-0533-4.
85. Christey, M.C. and R.H. Braun, 2005. Production of Hairy Root Cultures and Transgenic Plants by *Agrobacterium rhizogenes*-Mediated Transformation. In: Transgenic Plants: Methods and Protocols. Methods in Molecular Biology, Peña, L. (Ed.), Humana Press, United States, ISBN: 978-1-59259-827-4, pp: 47-60.
86. Georgiev, M.I., A.I. Pavlov and T. Bley, 2007. Hairy root type plant *in vitro* systems as sources of bioactive substances. Appl. Microbiol. Biotechnol., 74: 1175-1185.
87. Chandra, S. and R. Chandra, 2011. Engineering secondary metabolite production in hairy roots. Phytochem. Rev., 10: 371-395.
88. Ismail, H., E. Dilshad, M.T. Waheed and B. Mirza, 2017. Transformation of lettuce with *rol ABC* genes: Extracts show enhanced antioxidant, analgesic, anti-inflammatory, antidepressant, and anticoagulant activities in rats. Appl. Biochem. Biotechnol., 181: 1179-1198.
89. Chung, I.M., M. Thiruvengadam, K. Rekha and G. Rajakumar, 2016. Elicitation enhanced the production of phenolic compounds and biological activities in hairy root cultures of bitter melon (*Momordica charantia* L.). Braz. Arch. Biol. Technol., Vol. 59. 10.1590/1678-4324-2016160393.
90. Kiselev, K.V., A.S. Dubrovina, M.V. Veselova, V.P. Bulgakov, S.A. Fedoreyev and Y.N. Zhuravlev, 2007. The *rolB* gene-induced overproduction of resveratrol in *Vitis amurensis* transformed cells. J. Biotechnol., 128: 681-692.
91. Kim, Y.K., X. Li, H. Xu, N.I. Park, M.R. Uddin, J.Y. Pyon and S.U. Park, 2009. Production of phenolic compounds in hairy root culture of tartary buckwheat (*Fagopyrum tataricum* Gaertn). J. Crop Sci. Biotechnol., 12: 53-57.
92. Zhu, C., S. Naqvi, S. Gomez-Galera, A.M. Pelacho, T. Capell and P. Christou, 2007. Transgenic strategies for the nutritional enhancement of plants. Trends Plant Sci., 12: 548-555.
93. Ruiz-Lopez, N., O. Sayanova, J.A. Napier and R.P. Haslam, 2012. Metabolic engineering of the omega-3 long chain polyunsaturated fatty acid biosynthetic pathway into transgenic plants. J. Exp. Bot., 63: 2397-2410.
94. Tang, G., J. Qin, G.G. Dolnikowski, R.M. Russel and M.A. Grusak, 2009. Golden rice is an effective source of vitamin A. Am. J. Clin. Nutr., 89: 1776-1783.
95. Tang, G., Y. Hu, S.A. Yin, Y. Wang, G.E. Dallal, M.A. Grusak and R.M. Russell, 2012. Retracted: β-carotene in golden rice is as good as β-carotene in oil at providing vitamin A to children. Am. J. Clin. Nutr., 96: 658-664.
96. Ye, X., S. Al-Babili, A. Klöti, J. Zhang, P. Lucca, P. Beyer and I. Potrykus, 2000. Engineering the provitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science, 287: 303-305.
97. Diretto, G., S. Al-Babili, R. Tavazza, V. Papacchioli, P. Beyer and G. Giuliano, 2007. Metabolic engineering of potato carotenoid content through tuber-specific over expression of a bacterial mini-pathway. PLoS ONE, Vol. 2. 10.1371/journal.pone.0000350.
98. Goo, Y.M., T.W. Kim, M.K. Lee and S.W. Lee, 2013. Accumulation of PrLeg, a perilla legumin protein in potato tuber results in enhanced level of sulphur-containing amino acids. C. R. Biol., 336: 433-439.
99. Bovy, A., R. de Vos, M. Kemper, E. Schijlen and M.A. Pertejo *et al*., 2002. High-flavonol tomatoes resulting from the heterologous expression of the maize transcription factor genes *LC* and *C1*. Plant Cell, 14: 2509-2526.
100. Butelli, E., L. Titta, M. Giorgio, H.P. Mock and A. Matros *et al*., 2008. Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors. Nat. Biotechnol., 26: 1301-1308.
101. Muir, S.R., G.J. Collins, S. Robinson, S. Hughes and A. Bovy *et al*., 2001. Overexpression of petunia chalcone isomerase in tomato results in fruit containing increased levels of flavonols. Nat. Biotechnol., 19: 470-474.
102. Shi, J., C. Lang, X. Wu, R. Liu and T. Zheng *et al*., 2015. RNAi knockdown of *fatty acid elongase1* alters fatty acid composition in *Brassica napus*. Biochem. Biophys. Res. Commun., 466: 518-522.
103. Beck, K., C.A. Conlon, R. Kruger, J. Coad and W. Stonehouse, 2011. Gold kiwifruit consumed with an iron-fortified breakfast cereal meal improves iron status in women with low iron stores: A 16-week randomised controlled trial. Br. J. Nutr., 105: 101-109.
104. Vereshchagina, Y.V., V.P. Bulgakov, V.P. Grigorchuk, V.G. Rybin and G.N. Veremeichik *et al*., 2014. The *rolC* gene increases caffeoylquinic acid production in transformed artichoke cells. Appl. Microbiol. Biotechnol., 98: 7773-7780.
105. Liu, S., Y. Hu, X. Wang, J. Zhong and Z. Lin, 2006. High content of resveratrol in lettuce transformed with a stilbene synthase gene of *Parthenocissus henryana*. J. Agric. Food Chem., 54: 8082-8085.
106. Goel, M.K., S. Mehrotra and A.K. Kikreja, 2011. Elictor-induced cellular and molecular events are responsible for productivity enhancement in hairy root cultures: An insight study. Appl. Biochem. Biotechnol., 165: 1342-1355.
107. Qiao, J.J., Y.J. Yuan, H. Zhao, J.C. Wu and A.P. Zeng, 2003. Apoptotic cell death in suspension cultures of *Taxus cuspidate* co-treated with salicylic acid and hydrogen peroxide. Biotechnol. Lett., 25: 387-390.
108. Mishra, A.K., K. Sharma and R.S. Misra, 2012. Elicitor recognition, signal transduction and induced resistance in plants. J. Plant Interact., 7: 95-120.

109. Naik, P.M. and J.M. Al-Khayri, 2016. Impact of abiotic elicitors on *in vitro* production of plant secondary metabolites: A review. J. Adv. Res. Biotechnol., Vol. 1. 10.15226/2475-4714/1/2/00102.
110. Yang, L., K.S. Wen, X. Ruan, Y.X. Zhao, F. Wei and Q. Wang, 2018. Response of plant secondary metabolites to environmental factors. Molecules, Vol. 23. 10.3390/molecules23040762.
111. Jeong, G.T. and D.H. Park, 2006. Enhanced Secondary Metabolite Biosynthesis by Elicitation in Transformed Plant Root System. In: Twenty-Seventh Symposium on Biotechnology for Fuels and Chemicals, McMillan, J.D., W.S. Adney, J.R. Mielenz and K.T. Klasson (Eds.), Humana Press, United States, ISBN: 978-1-59745-268-7, pp: 436-446.
112. Parida, A.K. and A.B. Das, 2005. Salt tolerance and salinity effects on plants: A review. Ecotoxicol. Environ. Saf., 60: 324-349.
113. Navarro, J.M., P. Flores, C. Garrido and V. Martinez, 2006. Changes in the contents of antioxidant compounds in pepper fruits at different ripening stages, as affected by salinity. Food Chem., 96: 66-73.
114. Hussein, E.A. and E.M. Aqlan, 2011. Effect of mannitol and sodium chloride on some total secondary metabolites of fenugreek calli cultured *in vitro*. Plant Tissue Cult. Biotechnol., 21: 35-43.
115. Abhilash, J.E., V.V. Radhakrishnan and K.V. Mohanan, 2016. Variation in total polyamine content in some native rice cultivars of North Kerala, India in response to salinity stress. Int. J. Agric. Environ. Biotechnol., 9: 731-738.
116. Liu, C.Z., C. Guo, Y.C. Wang and F. Ouyang, 2002. Effect of light irradiation on hairy root growth and artemisinin biosynthesis of *Artemisia annua* L. Process Biochem., 38: 581-585.
117. Hemm, M.R., S.D. Rider, J. Ogas, D.J. Murry and C. Chapple, 2004. Light induces phenylpropanoid metabolism in *Arabidopsis* roots. Plant J., 38: 765-778.
118. Jaakola, L. and A. Hohtola, 2010. Effect of latitude on flavonoid biosynthesis in plants. Plant Cell Environ., 33: 1239-1247.
119. Dębski, H., W. Wiczkowski, D. Szawara-Nowak, N. Bączek, M. Szwed and M. Horbowicz, 2017. Enhanced light intensity increases flavonol and anthocyanin concentrations but reduces flavone levels in the cotyledons of common buckwheat seedlings. Cereal Res. Commun., 45: 225-233.
120. Ferrari, S., 2010. Biological Elicitors of Plant Secondary Metabolites: Mode of Action and Use in the Production of Nutraceutics. In: Bio-Farms for Nutraceuticals: Functional Food and Safety Control by Biosensors, Giardi, M.T., G. Rea and B. Berra (Eds.), Springer, Boston, United States, ISBN: 978-1-4419-7347-4, pp: 152-166.
121. Gai, Q.Y., J. Jiao, M. Luo, W. Wang, C.J. Zhao, Y.J. Fu and W. Ma, 2016. UV elicitation for promoting astragaloside production in *Astragalus membranaceus* hairy root cultures with transcriptional expression of biosynthetic genes. Ind. Crops Prod., 84: 350-357.
122. Yu, K.W., H.N. Murthy, E.J. Hahn and K.Y. Paek, 2005. Ginsenoside production by hairy root cultures of *Panax ginseng*: Influence of temperature and light quality. Biochem. Eng. J., 23: 53-56.
123. Mewis, I., I.M. Smetanska, C.T. Müller and C. Ulrichs, 2011. Specific poly-phenolic compounds in cell culture of *Vitis vinifera* L. cv. gamay fréaux. Appl. Biochem. Biotechnol., 164: 148-161.
124. Uppalapati, S.R., P. Ayoubi, H. Weng, D.A. Palmer, R.E. Mitchell, W. Jones and C.L. Bender, 2005. The phytotoxin coronatine and methyl jasmonate impact multiple phytohormone pathways in tomato. Plant J., 42: 201-217.
125. Wasternack, C. and B. Hause, 2013. Jasmonates: Biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in annals of botany. Ann. Bot., 111: 1021-1058.
126. Ali, M.B., K.W. Yu, E.J. Hahn and K.Y. Paek, 2006. Methyl jasmonate and salicylic acid elicitation induces ginsenosides accumulation, enzymatic and non-enzymatic antioxidant in suspension culture *Panax ginseng* roots in bioreactors. Plant Cell Rep., 25: 613-620.
127. Shabani, L., A.A. Ehsanpour, G. Asghari and J. Emami, 2009. Glycyrrhizin production by *in vitro* cultured *Glycyrrhiza glabra* elicited by methyl jasmonate and salicylic acid. Russ. J. Plant Physiol., 56: 621-626.
128. Xu, Y.W., S.S. Lv, D. Zhao, J.W. Chen, W.T. Yang and W. Wu, 2012. Effects of salicylic acid on monoterpene production and antioxidant systems in *Houttuynia cordata*. Afr. J. Biotechnol., 11: 1364-1372.
129. Saw, N.M.M.T., H. Riedel, O. Kutuk, K. Ravichandran and I. Smetanska, 2010. Effect of elicitors and precursors on the synthesis of anthocyanin in grape *Vitis vinifera* cell cultures. Energy Res. J., 1: 189-192.
130. Sharma, A., V. Kumar, P. Giridhar and G.A. Ravishankar, 2008. Induction of *in vitro* flowering in *Capsicum frutescens* under the influence of silver nitrate and cobalt chloride and pollen transformation. Electron. J. Biotechnol., 11: 84-89.
131. El-Bahr, M.K., S.A.E.H. Bekheet, A.M.M. Gabr, R. El-Shenawy and Y.S. El Abd, 2018. Accumulation of cynarin, the hepatoprotective compound, in ethephon treated callus cultures of globe artichoke (*Cynara scolymus* L.). J. Biol. Sci., 18: 243-250.
132. Sinilal, B., R. Ovadia, A. Nissim-Levi, A. Perl, M. Carmeli-Weissberg and M. Oren-Shamir, 2011. Increased accumulation and decreased catabolism of anthocyanins in red grape cell suspension culture following magnesium treatment. Planta, 234: 61-71.
133. Hernandez, X.E., A.A. Orden, O.S. Giordano and M. Kurina, 2005. Effects of elicitor and copper sulfate on grindelic acid production in submerged cultures of *Grindelia pulchella*. Electron. J. Biotechnol., 8: 276-283.

134. Vasconsuelo, A. and R. Boland, 2007. Molecular aspects of the early stages of elicitation of secondary metabolites in plants. Plant Sci., 172: 861-875.
135. Yu, L.J., W.Z. Lan, W.M. Qin, W.W. Jin and H.B. Xu, 2002. Oxidative stress and taxol production induced by fungal elicitor in cell suspension cultures of *Taxus chinensis*. Biol. Plant., 45: 459-461.
136. Putalun, W., W. Luealon, W. De-Eknamkul, H. Tanaka and Y. Shoyama, 2007. Improvement of artemisinin production by chitosan in hairy root cultures of *Artemisia annua* L. Biotechnol. Lett., 29: 1143-1146.
137. Kim, H.J., F. Chen, X. Wang and N.C. Rajapakse, 2005. Effect of chitosan on the biological properties of sweet basil (*Ocimum basilicum* L.). J. Agric. Food Chem., 53: 3696-3701.
138. Orlita, A., M. Sidwa-Gorycka, M. Paszkiewicz, E. Malinski and J. Kumirska *et al*., 2008. Application of chitin and chitosan as elicitors of coumarins and furoquinolone alkaloids in *Ruta graveolens* L. (common rue). Biotechnol. Appl. Biochem., 51: 91-96.
139. Purwianingsih, W., R.Y. Hidayat and A. Rahmat, 2019. Increasing anthraquinone compounds on callus leaf *Morinda citrifolia* (L.) by elicitation method using chitosan shell of shrimps (*Penaeus monodon*). J. Phys. Conf. Ser., Vol. 1280. 10.1088/1742-6596/1280/2/022001.
140. Taurino, M., I. Ingrosso, L. D'amico, S. de Domenico and I. Nicoletti *et al*., 2015. Jasmonates elicit different sets of stilbenes in *Vitis vinifera* cv. Negramaro cell cultures. SpringerPlus, Vol. 4. 10.1186/s40064-015-0831-z.
141. Taha, H.S. and H.M.A. El-Ghit, 2018. Implement of biotic and abiotic stress for enhancement and production of capsaicin in suspension cultures of *Capsicum annuam* spp. Pak. J. Biol. Sci., 21: 292-299.
142. Cai, S., O. Wang, W. Wu, S. Zhu and F. Zhou *et al*., 2012. Comparative study of the effects of solid-state fermentation with three filamentous fungi on the total phenolics content (TPC), flavonoids, and antioxidant activities of subfractions from oats (*Avena sativa* L.). J. Agric. Food Chem., 60: 507-513.
143. Saxena, P., S. Ahlawat, A. Ali, S. Khan and M.Z. Abdin, 2017. Gene expression analysis of the withanolide biosynthetic pathway in hairy root cultures of *Withania somnifera* elicited with methyl jasmonate and the fungus *Piriformospora indica*. Symbiosis, 71: 143-154.
144. Zhang, B., L.P. Zheng, W.Y. Li and J.W. Wang, 2013. Stimulation of artemisinin production in *Artemisia annua* hairy roots by Ag-SiO_2 core-shell nanoparticles. Curr. Nanosci., 9: 363-370.
145. Ghasemi, B., R. Hosseini and F.D. Nayeri, 2015. Effects of cobalt nanoparticles on artemisinin production and gene expression in *Artemisia annua*. Turk. J. Bot., 39: 769-777.
146. Shakeran, Z., M. Keyhanfar, G. Asghari and M. Ghanadian, 2015. Improvement of atropine production by different biotic and abiotic elicitors in hairy root cultures of *Datura metel*. Turk. J. Biol., 39: 111-118.
147. Jamshidi, M., F. Ghanati, A. Rezaei and E. Bemani, 2016. Change of antioxidant enzymes activity of hazel (*Corylus avellana* L.) cells by AgNPs. Cytotechnology, 68: 525-530.
148. Moharrami, F., B. Hosseini, A. Sharafi and M. Farjaminezhad, 2017. Enhanced production of hyoscyamine and scopolamine from genetically transformed root culture of *Hyoscyamus reticulatus* L. elicited by iron oxide nanoparticles. *In vitro* Cell. Dev. Biol. Plant, 53: 104-111.
149. Javed, R., A. Mohamed, B. Yücesan, E. Gürel, R. Kausar and M. Zia, 2017. CuO nanoparticles significantly influence *in vitro* culture, steviol glycosides, and antioxidant activities of *Stevia rebaudiana* Bertoni. Plant Cell Tissue Organ Cult., 131: 611-620.
150. Javed, S.B., A.A. Alatar, R. Basahi, M. Anis, M. Faisal and F.M. Husain, 2017. Copper induced suppression of systemic microbial contamination in *Erythrina variegata* L. during *in vitro* culture. Plant Cell Tissue Organ Cult., 128: 249-258.
151. Kim, D.H., J. Gopal and I. Sivanesan, 2017. Nanomaterials in plant tissue culture: The disclosed and undisclosed. RSC Adv., 7: 36492-36505.
152. Marslin, G., C.J. Sheeba and G. Franklin, 2017. Nanoparticles alter secondary metabolism in plants via ROS burst. Front. Plant Sci., Vol. 8. 10.3389/fpls.2017.00832.
153. Chung, I.M., G. Rajakumar and M. Thiruvengadam, 2018. Effect of silver nanoparticles on phenolic compounds production and biological activities in hairy root cultures of *Cucumis anguria*. Acta Biol. Hung., 69: 97-109.
154. Chung, I.M., K. Rekha, G. Rajakumar and M. Thiruvengadam, 2018. Production of bioactive compounds and gene expression alterations in hairy root cultures of Chinese cabbage elicited by copper oxide nanoparticles. Plant Cell Tissue Organ Cult., 134: 95-106.
155. Bhat, P. and A. Bhat, 2016. Silver nanoparticles for enhancement of accumulation of capsaicn in suspension culture of *Capsicum* sp. J. Exp. Sci., 7: 1-6.
156. Georgiev, M.I., J. Weber and A. Maciuk, 2009. Bioprocessing of plant cell cultures for mass production of targeted compounds. Appl. Microbiol. Biotechnol., 83: 809-823.
157. Georgiev, M.I., R. Eibl and J.J. Zhong, 2013. Hosting the plant cells *in vitro*: Recent trends in bioreactors. Appl. Microbiol. Biotechnol., 97: 3787-3800.
158. Huang, T.K. and K.A. McDonald, 2012. Bioreactor systems for *in vitro* production of foreign proteins using plant cell cultures. Biotechnol. Adv., 30: 398-409.
159. Garcia-Ochoa, F. and E. Gomez, 2009. Bioreactor scale-up and oxygen transfer rate in microbial processes: An overview. Biotechnol. Adv., 27: 153-176.
160. Wu, C.H., H.N. Murthy, E.J. Hahn and K.Y. Paek, 2007. Large-scale cultivation of adventitious roots of *Echinacea purpurea* in airlift bioreactors for the production of chichoric acid, chlorogenic acid and caftaric acid. Biotechnol. Lett., 29: 1179-1182.

161. Kusakari, K., M. Yokoyama, S. Inomata, Y. Gozu, C. Katagiri and Y. Sugimoto, 2012. Large-scale production of saikosaponins through root culturing of *Bupleurum falcatum* L. using modified airlift reactors. J. Biosci. Bioeng., 113: 99-105.
162. Sivakumar, G., K.W. Yu and K.Y. Paek, 2005. Production of biomass and ginsenosides from adventitious roots of *Panax ginseng* in bioreactor cultures. Eng. Life Sci., 5: 333-342.
163. Szopa, A., A. Kokotkiewicz, M. Bednarz, K. Jafernik, M. Luczkiewicz and H. Ekiert, 2019. Bioreactor type affects the accumulation of phenolic acids and flavonoids in microshoot cultures of *Schisandra chinensis* (Turcz.) Baill. Plant Cell Tissue Organ Cult., 139: 199-206.
164. Vives, K., I. Andújar, J.C. Lorenzo, O. Concepción, M. Hernández and M. Escalona, 2017. Comparison of different *in vitro* micropropagation methods of *Stevia rebaudiana* B. including temporary immersion bioreactor (BIT®). Plant Cell Tissue Organ Cult., 131: 195-199.