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Review

Adventitious Root Culture—An Alternative Strategy for Secondary Metabolite Production: A Review

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Abstract: Medicinal plants are valuable sources of botanical drugs, extracts and pure compounds. Although several species can be propagated or collected, the access to herbal material is limited in certain cases. The protection of natural habitats and biodiversity demands new sources to provide plant secondary metabolites of medical importance. Adventitious root culture is used to harvest the secondary metabolites from the medicinally important plants, thereby offering an alternative to collection and propagation of medicinal plants. In this review, we comprehensively summarize the previously published data on the use of adventitious root cultures for numerous therapeutic plants. Adventitious roots showed elevated growth rates and production of pharmaceutically important metabolites under sterilized condition with optimized plant-growth regulators in culture media. In the present study, major influencing factors, such as the stages involved in the process of adventitious root formation, medium composition and type of growth regulators, specifically the effect of different auxins on the initiation and formation of roots, are discussed. Elicitation strategies using biotic (yeast extracts, chitosan and pectin) and abiotic factors (MJ, SA, $CuSO_4$, $AgNO_3$, NaCl) that affect the in vitro growth of adventitious roots and the role of bioreactors, which are new advancements in the scale-up process, are also highlighted. The development of adventitious root cultures for the production of secondary metabolites of medicinal importance is a perspective that is advantageous from ecological and economical aspects as well.

Keywords: auxins; bioreactors; elicitors; micropropagation; secondary metabolites

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1. Introduction

The biodiversity on Earth is the result of a complex meshwork of species. Each species has a specific and essential role in the maintenance of this network. Plants, being producers are vital for the survival of life and help other living organisms to regulate the shape of ecosystem, maintain the quality of air and water, and provide raw material for food and pharmaceuticals industries [1]. Nearly 80% of the plants that are used as drugs are harvested from forests and other natural resources [2]. The incessant and un-organized collection and utilization of plants has caused many plants to become rare and some species have even became extinct. To overcome this problem, several strategies have been undertaken—the in situ and ex situ conservations are the chief methods. In situ methods

show less versatility as these are encountered with problems, such as fragmented habitat, climate change, pathogen attack and invasive species. Additionally, the ex situ approach is more pronounced, effective and scientifically established [3].

The pharmaceutical and food industries require a large quantity of medicinal plants. Almost 65% of the cancer drugs are either directly derived or are a variation of the natural molecule [4]. Drugs of plant origin have an outstanding contribution to modern medicine. Vincristine, vinblastine and ajmalicine isolated from *Catharanthus roseus* [5] and Taxol obtained from *Taxus brevifolia* are known to possess antineoplastic properties [6]. Serpentine derived from the roots of *Rauvolfia serpentina* revolutionized the treatment of hypertension and low blood pressure [7]. Quinine obtained from *Cinchona officinalis* is antimalarial [8], morphine (*Papaver somniferum*) is analgesic [9] and atropine (*Atropa belladonna*) is a muscle relaxant [10]. Nicotine (*Nicotiana tabacum*) and azadirachtin (*Azadiracta indica*) show insecticidal properties [11], while some other compounds, such as shikonin, anthraquinone and steviosides have varied commercial importance.

Plant secondary metabolites are typically low-molecular-weight organic compounds produced by numerous complex biosynthetic pathways and do not have any recognized role in the growth, development or reproduction of the plants. Secondary metabolites are extremely diverse in their structures; several thousand metabolites have been identified in various classes, e.g., alkaloids, essential oil components, phenolics and terpenoids [12,13]. Although these compounds are not essential for survival, they have numerous secondary functions, such as protection, competition or interaction with the environment. These compounds are characteristic to certain taxa and are often produced in low quantities. The qualitative and quantitative compositions of secondary metabolites depend on the physiological and developmental stage of the plants and exogenous factors as well [14].

Plant tissue culture is a promising biotechnological tool useful in various applied plant-science studies having commercial applications as well. Plant tissue cultures are widely used for large-scale vegetative propagation of plants. It involves the growth and multiplication of cells, tissues and organs under a controlled environment, which also provides an alternative method for the rapid vegetative propagation of plants by to generate clonal plants without altering the genetic makeup [15]. Auxin and cytokinin are the important plant-growth regulators for determining the fate of plant cells during organogenesis [16]. This discovery led to the foundation of the science of various tissue-culture systems. Cell or organ cultures provide an alternative method for the enhanced production of complex secondary metabolites under controlled environmental condition with reduced costs. It takes less time and is independent of seasonal variations with a high storage ability and convenient transportability using less energy and space, when compared to the conventional ways. The development of cell suspension, adventitious or excised root culture, hairy root culture and shoot culture of a number of plant species to obtain a high yield in biomass production provides complementary aid for the pharmaceutical industry for the extraction of secondary metabolites [17,18].

As per the available literature, the synthesis of metabolites is mostly higher in differentiated plant tissues and thus several protocols were developed to cultivate whole plant organs, i.e., shoots or roots under in vitro conditions with an objective to yield pharmaceutically important compounds. Shoot culture is considered as an alternative method of callus or cell suspension culture for the production of secondary metabolites [19,20]. Due to their genetic stability and better capacities for secondary metabolite production, shoot cultures represent a practical alternative to cell cultures for secondary metabolite production, particularly when metabolites are confined to the shoot region of the plant [21–24]. Numerous plant species, such as *Allamanda cathartica* [25–27], *Aegle marmelos* [28], *Croton floribundus* [29], *Decalepis hamiltonii* [30] and *Decalepis arayalpathra* [31], have been used and demanded at a large scale by the pharmaceutical industry.

At a commercial level, the production of important secondary metabolites is mainly performed in root cultures [32–34]. The roots of many medicinally important plants serve as an origin of various bioactive molecules that consist of a diversity of metabolites, proteins,

agrochemicals, flavors, fragrances and dyes [35,36]. There are few studies that report the development and scaling up of the hairy root culture of *numerous plant species* [37,38]. However, hairy roots are not preferred by the pharmaceutical industry for metabolite production as they are genetically transformed with *Agrobacterium rhizogenes* and may also produce some opine-like substrates that might be hazardous to mammalian cells [32]. The extraction and purification of opine-like substrates might be very costly, which makes this technique inefficient [39]. Using adventitious root cultures seems to be more optimal to obtain high biomass production, which later provides raw materials for the isolation of medicinally important compounds.

Adventitious root culture offers the possibility of year-round production of biomass with reduced cost and time. Considering increasing industrial needs for bioactive secondary metabolites, the optimization of metabolite production is of primary importance. The main objective of this review is to compile the reports available on the effect of plant-growth regulators, specifically auxins, on the induction of adventitious roots. It also covers the effect of various biotic and abiotic elicitors that affect the root biomass and facilitate the elicitation of bioactive compounds in various medicinal plant species. The large-scale production of the enhanced metabolite content using bioreactors is also discussed. This review might be helpful to many researchers, as well as the pharmaceutical, food and agrochemical industries, who wish to conduct research or work on adventitious root culture.

2. Adventitious Root Culture

2.1. Formation of Adventitious Roots in Plants

In folk medicine, the roots of some plants are used and harvested by local people. Roots are an abundant source of much valued secondary metabolites, which can be beneficial to mankind for combating various ailments [35]. Roots that arise from any plant organ, other than the plant root itself, are known to be adventitious roots. Several complex molecular processes, such as endogenous and exogenous physiological factors, are involved in the formation of adventitious roots [40]. These roots are formed during normal growth and development or either in the reaction to wounding, nutrient deficiency or various kinds of environmental stresses. They generally develop from the leaves, petioles, nodes and internodes and help plants to survive in environmentally adverse conditions [41]. It has been reported that the formation of adventitious roots involves four stages [42]:

- The root pre-emergence: this phase includes alterations in the molecular and biochemical process prior to cytological development until the occurrence of root primordia;
- Root development;
- Root growth;
- Root configuration.

Under aseptic conditions, the initiation and differentiation of the physiological stages of adventitious rooting can be initiated by variations in the endogenous level of auxins in the explants, and the addition of extrinsic auxins on a suitable medium [43]. Adventitious roots can be formed via direct organogenesis from cambium cells and indirect organogenesis from callus tissues. [44,45] (Figure 1A–D). When compared to undifferentiated tissues, the differentiated ones, similar to adventitious roots, are comparatively more established, stable and can store a high quantity of secondary metabolites [46]. Various endogenous and exogenous factors are involved in the complex formation of adventitious roots [40]. The roots grown from inoculum cultured in a plant-growth-regulator-amended medium are highly stable in nature and synthesize ample amounts of plant metabolites in the intercellular spaces, which can easily be extracted further [47].

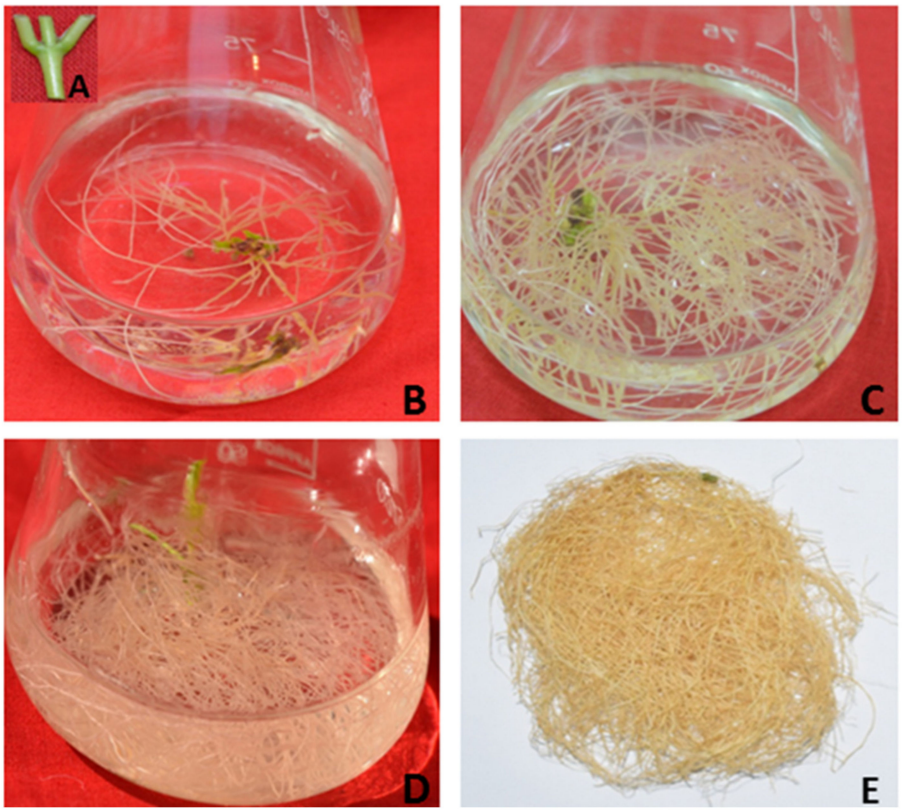

Figure 1. Establishment of adventitious root culture: (**A**): Nodal explant from *Allamanda cathartica*; (**B**): initiation of roots from nodal segments; (**C**): growth of root cultures; and (**D**,**E**): escalation of root biomass.

2.2. Media Properties and Culture Conditions

The growth, development and morphogenic response of plant tissues may vary among the plant species because all plants need different nutritional requirements. During the culture condition, there are several factors, including type, strength and concentration of the media used, which influence the establishment and proliferation of adventitious root cultures. Some other major factors, which also influence the culture condition, include salt strength, carbon source, pH adjustment of the media and type of explant used with its inoculum density. Some studies reported that MS medium has been the widely accepted medium for the initiation and growth of adventitious root culture in therapeutic plants, such as *Allamanda cathartica* [45], *Boesenbergia rotunda* [48], *Vernonia amygdalina* [49], *Camellia sinensis* [50] and *Echinacea purpurea* [51]. However, for the abovementioned plant species, the optimal growth was achieved with variable concentrations of MS medium. Half-strength MS medium showed optimal growth in cultures of *Allamanda cathartica*, *Camellia sinensis* and *Echinacea purpurea*, while full-strength MS medium was the most suitable for *Boesenbergia rotunda* and *Vernonia amygdalina*. In the case of *Aloe vera*, among the three tested mediums for adventitious root proliferation, B5 media was found to be optimum for culture establishment than MS and SH media [52,53]. Therefore, by studying various plant species for adventitious root-culture development, it was concluded that the selection of basal media was strongly influenced by individual plant species.

2.3. Effect of Auxins on Adventitious Root Formation

Auxins have an important role in rhizogenesis, as they facilitate the initiation, differentiation and establishment of adventitious roots by stimulating the cells at wounding sites, which triggers the formation of meristemoids and, ultimately, the development of adventitious roots [54,55]. Externally provided auxin enters through the wounded sites and may be potentially taken up by the cells both through a pH-trapping mechanism [56]

and influx carriers [57]. However, by the application of exogenous auxins, the level of endogenous auxins might also change, which is also associated with the physiological stages of rooting [58]. Auxin canalization to target cells is controlled *via*. auxin transporters, where auxin acts mainly through selective proteolysis and cell-wall loosening via. their receptor proteins TIR1 (transport inhibitor response 1) and ABP1 (auxin-binding proteins 1). A complex microRNA circuitry is involved in the control of auxin-response factors essential for gene expression in adventitious roots. New hormonal controls occur during the growth process after the establishment of the roots, with auxins being required at lower concentrations for root meristem maintenance and cytokinins needed for root-tissue differentiation. Furthermore, it has been reported that the efficacy of numerous auxins for the stimulation and proliferation of adventitious roots varies from species to species [59,60].

There are two alternative methods for the production of roots: hairy root culture and adventitious root culture. Hairy roots are genetically transformed with *Agrobacterium rhizogenes*, and transformed roots produced by the bacterium are not generally preferred by the pharmaceutical industry as a secondary metabolite source, due to changing the genetic composition of the plant cell; hence, the secondary metabolite profile may alter further. Excised or adventitious roots grow vigorously in medium supplemented with phytohormones and have the potential to accumulate valuable secondary metabolites with a pattern similar to the wildly growing plants [46]. Auxins are the main class of phytohormones that are directly involved in adventitious rooting. The establishment of adventitious root cultures is a complex procedure that contains uninterrupted developmental phases demanding different hormonal signals and other factors [61].

Adventitious root cultures are ideal for increasing biomass production to provide raw materials for the pharmaceutical industry and also for compound isolation. Plant-tissue culture, especially organ culture, provides an alternative means for the production of adventitious roots in a short time span. The formation of adventitious roots occurs through direct organogenesis from cambium cells or indirectly from callus tissues due to mechanical damage. Adventitious root culture shows a high rate of proliferation and active metabolism [44]. This technique has recently been applied to numerous medicinally and economically important plants, resulting in the rapid growth of roots and stable secondary metabolite production for the pharmaceutical industries [46]. Generally, field-grown plant materials are used for the commercial production of secondary metabolites, but, due to the unsuitable environmental conditions, the genotype of the plants are affected, and therefore the quality of the metabolites and the level of phyto-constituents are also hampered. The uniformity of the metabolites under stressed environmental conditions is maintained through in vitro cultures, so that it can provide an alternative method to the conventional production of medicinally valuable compounds. The synthesis of bioactive compounds in plants is altered by numerous biotic and abiotic stress conditions. Thus, by exposure to different conditions, the bioactive compounds present in the roots of the plant can be altered and controlled in the adventitious root-culture system.

For adventitious root regeneration, numerous types of explants are used. Explants can be collected from mature field-grown plant or from in vitro raised plants. Leaf, root, stem, hypocotyl, nodes and internodes are used as explants. The ex vitro- and in vitro-raised explants respond differently to various plant-growth regulators (PGRs) due to their endogenous and exogenous factors. Leaf explants have served as major sources of adventitious root-culture systems in recent years. Some of the important attempts made towards the extraction of secondary metabolites from different explants are listed in Table 1 [62].

Table 1. Role of PGRs in secondary metabolite production in various plants.

Plant Species	Explants Used	Hormones Used	Metabolites	References
Allamanda cathartica	Nodal segment	1/2 MS + IBA (0.5 µM)	Iridoid glycosides	[45]
Andrographis paniculata	Leaf	MS + NAA (2.7 µM)	Andrographolide and diterpenoids	[43]
Artemisia vulgaris	Leaf and roots	MS + IAA (11.4 µM) + IBA (4.9 µM)	Coumarins, sesquiterpene lactones, volatile oils and inulin	[63]
Astragalus membranaceus	Seedling-derived roots	B_5 + IBA (2.0 mg/L)	Polysaccharides, saponins and flavonoids	[64]
Boerhaavia diffusa	Leaf	MS + (1.0 mg/L) NAA	Punarnavine	[65]
Echinacea purpurea	Root	MS + IBA (2.0 mg/L)	Chichoric acid, chlorogenic acid and caftaric acid	[51,66]
Eurycoma longifolia	Leaf	1/2 MS + NAA (3.0 mg/L)	Quassinoids and cathine-6-one Alkaloids	[67]
Glycyrrhiza uralensis	In vitro root	1/2 MS + IBA (6.5 g/L)	Glycyrrhetinic acid, flavonoids and polysaccharides	[68]
Gynura procumbens	Leaf	MS + NAA (3 mg/L) + IBA (1 mg/L)	Caffeic acid, chlorogenic acid and 3, 5-di-O-caffeoylquinic acid	[69]
Hypericum perforatum	Leaf and stem	1/2 MS + Kn (0.1 mg/L) + IBA (1 mg/L)	Flavonols, napthodianthrones phloroglucinols and xanthones	[70]
Hypericum perforatum	Adventitious root	MS + IBA (1.25 mg/L)	Hypericin	[71]
Morinda citrifolia	Leaf	1/4 MS + IBA (5.00 mg/L) + 5% Sucrose; MS + IBA (5.00 mg/L)	Anthraquinones, phenolics and flavonoids	[34,72,73]
Orthosiphon stamineus	leaf, root and stem	MS + IAA (3.00 mg/L)	Rosmarinic acid, oxygenated diterpenes and sinensitin	[74]
Panax ginseng	Leaf and root calluses	MS without NH_4NO_3 + NAA (2 mg/L); MS without NH_4NO_3 + IBA (25 µM); MS+ 2,4-D (4.5 µM)	Ginsenosides	[75–77]
Panax notoginseng	Leaf stalk, leaves and lateral roots	1/2 MS + IBA (3.0 mg/L)	Protopanaxatriol saponins	[78]
Plumbago rosea	Leaf	MS + IAA (1.50 mg/L) + IBA (1.00 mg/L)	Plumbagin	[79]
Podophyllum hexandrum	Root	1/2 MS + IBA (3 mg/L) + sucrose (2%)	Podophyllotoxin	[80]
Polygonum multiflorum	Root	1/2 MS + IBA (9.84 µM) + sucrose (50 g/L)	Anthraquinones, stilbenes, flavonoids, tannins and phospholipids	[81]
Prunella vulgaris	Leaf-derived callus	MS + NAA (1.00 mg/L)	Phenolics and flavonoids	[82]
Psoralea corylifolia	Hypocotyl	MS + IBA (3 µM)	Psoralen	[60]
Raphanus sativus	Seedling-derived roots	1/2 MS + IBA (0.5 mg/L)	Anthocyanin	[83]
Rhus javanica	Root	LS + NH_4^+ (30 mM) + NO_3^- (30 mM) + IBA (10^{-6} M)	Galloylglucoses (gallotannins), anthocyanidin and riccionidin A	[84]
Rumex crispus	Leaf	MS + NAA (5.0 µM) + Kn (0.5 µM)	Flavonoids	[85,86]
Stevia rebaudiana	Root tip	MS + NAA (10.7 µM)	Stevioside and rebaudioside	[87]
Withania somnifera	Leaf, leaf callus, cotyledon and internode	1/2 MS + IAA (2.85 µM) + IBA (9.85 µM); 1/2 MS + IBA (0.5 mg/L); 1/2 MS + IBA (0.5 mg/L) + IAA (0.1 mg/L)	Withanolides, withaferin A and withanone	[88–91]

2.4. Effect of Sucrose on Adventitious Root Cultures

Carbohydrates serve as a respiratory substrate that has role in the synthetic pathways of many compounds. Simple carbohydrates possess various essential functions in developmental processes and are the building blocks of macromolecules [92,93]. In plant tissue culture medium, sucrose is commonly used as a carbon source because, in angiosperms, it is the most common carbohydrate, which is present in the phloem sap [94]. In the nutrient medium, invertase is released by the explant, which splits sucrose into glucose and fructose [95]. Therefore, the explant uses a mixture of sucrose, glucose and fructose as a carbon source. De Klerk and Gerrits [96] reported that there are many effects of sucrose, such as the fact that it leads to dormancy development, storage organ formation and maturation of somatic embryos in the plant cell and tissue culture system. Generally, sucrose is actively transported in plant cells, but sometimes through the passive mode [97]. The mobilization of carbohydrates is stimulated with the help of auxin [98–100].

There are studies available on the effect of carbohydrates on adventitious organ formation. Lazzeri et al. [101] reported the interactive effect of sucrose and auxin on soybean somatic embryogenesis. In *Centella asiatica*, the role of sucrose is emphasized in adventitious rooting [102]. Khanam et al. [45] reported the positive effect of sucrose on adventitious rooting from a nodal explant in *Allamanda cathartica*. Sucrose, in culture media, induces osmotic stress above a certain concentration, which leads to an increased accumulation of secondary metabolites in plant cells. The modulation of metabolism and transport or action of auxin is affected by the extreme concentrations of sucrose, which helps in the formation of organs [103]. Wilson et al. [104] estimated that sucrose acts similar to a signal molecule to increase vascular regeneration in lettuce pith.

2.5. Effect of Abiotic Elicitors on Adventitious Root Cultures

Numerous stress agents enhance the production of secondary metabolites in particular cells, tissues and organs because of their role in defense mechanisms. Biotic and abiotic elicitors are extensively used because they have shown a great impact on indole alkaloid biosynthesis [105]. Various types of abiotic stressors act differently on medicinal plants [106]. In the case of agricultural or field crops, the applied stress proves to be harmful as it reduces the total biomass and yield of the plant. Salinity alters the plant growth and development and a wide range of physiological and metabolic processes [107,108], while salt stress often enhances the level of these valued therapeutic compounds in plant cells. Plant cells develop mechanisms for the adaptation to osmotic, ionic and oxidative stresses induced by salt stress [109,110]. Brachet and Cosson [111] reported that salt treatment increases the total tropane alkaloid content in young leaves. In *Triticum aestivum*, the levels of betaine and diamine increased under salinity conditions [112]; whereas, in *Trifolium alexandrinum*, betaine content increased under salt stress [113].

A significant increment in the concentration of anthocyanin was observed under salinity exposure in two species of *Grevillea*: *G. ilicifolia* (salt-tolerant) and *G. arenaria* (salt sensitive) [114]. Jacob and Malpathak [115,116] optimized the culture conditions, such as light, temperature, CO_2 concentration and proportion, of major and minor salts in the medium for enhancing the solasodine content in hairy root cultures of *Solanum khasianum*. Misra and Gupta [117] reported that *Catharanthus roseus* grown under salt stress showed increased levels of the alkaloid vincristine accumulation in plant cells. The 100 mM NaCl treatment improved the nutritional value and germination of radish sprouts, and therefore enhanced the health-promoting compounds in the plant [118]. Valifard et al. [119] observed that the moderate level of salt stress increased the synthesis of phenolics and some important volatile components in *Salvia mirzayanii*. Fatima et al. [120] reported that NaCl enhanced the synthesis of vinblastine and vincristine in the embryogenic callus tissues of *Catharanthus roseus*. Chattoi et al. [121] observed that a low concentration of NaCl (100 mg/L) produced healthy and thick roots in the *Musa* species, but the length of the roots was reduced. In *Allamanda cathartica*, the effect of NaCl in culture media resulted in the enhanced production of root biomass and metabolite content from nodal explants [45].

The growth and development of medicinal plants are affected by various important stress-causing factors, which are directly involved in the accumulation of secondary metabolites (Figure 2). To adapt these adverse effects, the plant starts to produce and accumulate certain metabolites. Stress factors that boost the biosynthesis of a specific secondary metabolite, when applied in small quantities, are called elicitors. The most practical and feasible strategy among several biotechnological approaches to enhance the desired compound is through elicitation. Some abiotic elicitors, such as methyl jasmonate (MJ), salicylic acid (SA), $CuSO_4$, $AgNO_3$, sorbitol, caffeic acid, oxalic acid, phenyl acetic acid (PAA) and ethephon, have been applied to improve the biosynthesis and accumulation of secondary metabolites produced by the adventitious root culture of medicinal plants [41].

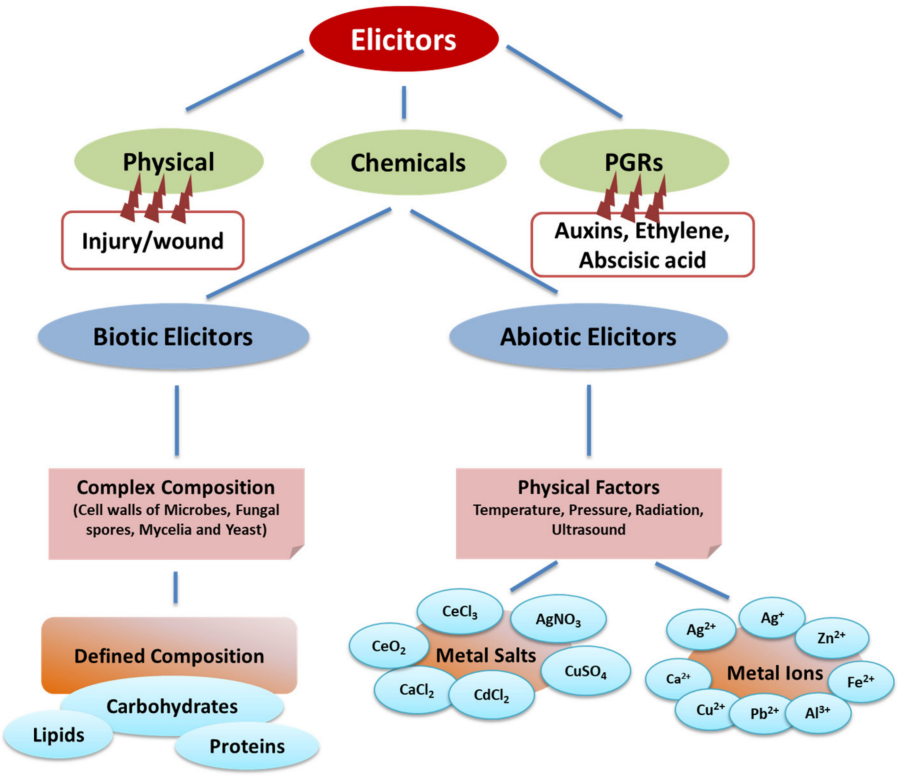

Figure 2. Strategies to improve phyto-constituents in adventitious root cultures.

A wide variety of elicitors were employed to alter cell metabolism in order to enhance the production of secondary metabolism in plant-cell cultures. Bis-carboxyethyl germanium sesquioxide was used as an elicitor to enhance the biomass and ginsenoside productions [122]. The effects of methyl jasmonate (MJ) and salicylic acid (SA) on the production of tropane alkaloids and the expression of putrescine *N*-methyltransferase and hyoscyamine 6β-hydroxylase were studied in adventitious root cultures of *Scopolia parviflora* [123]. The effects of $CuCl_2$ and glutathione on phytochelatin (class III methallothioneins) induction and the increased production of anthraquinone pigments were studied in *Rubia tinctorum* [124]. Methyl jasmonate and salicylic acid as an elicitor improved the productivity of withanolides in the adventitious root culture of *Withania somnifera* [125] (Table 2).

Table 2. List of medicinal plant species with applied elicitors in adventitious root cultures.

S.No.	Plant Species	Secondary Metabolites	Elicitors Applied	References
1	*Aloe vera*	Aloe-emodin and chrysophanol	SA, MJ, ethephon	[53]
2	*Ajuga bracteosa*	Flavonoids, phenolics	MJ, PAA	[126]
3	*Fagonia indica*	Apigenin, rutin	MJ, PAA	[127]
4	*Gynura procumbens*	kaempferol and quercetin	*Saccharomyces cereviciae*, $CuSO_4$	[128]
5	*Hypericum perforatum*	Phenolics	MJ, SA, lactabumin hydrolysate	[129]
6	*Morinda citrifolia*	Antrhaquinones, phenolics, flavonoids	Chitosan, pectin	[72]
7	*Oldenlandia umbellata*	Anthroquinones	Yeast extract, pectin, xylan,	[130]
8	*Panax ginseng*	Ginsenoside	MJ	[131]
9	*Panax ginseng*	Ginsenoside	*Mesorhizobium amorphae*	[132]
10	*Panax quinquefolium*	Ginsenoside	MJ	[133]
11	*Perovskia abrotanoides*	Tanshinone	MJ, $AgNO_3$, sorbitol, yeast extract	[134]
12	*Polygonum multiforum*	Phenolics	MJ, SA, yeast extract, chitosan	[81]
13	*Psammosilene tunicoides*	Triterpenoid saponins	oxalic acid	[135]
14	*Rubia tinctorum*	Antrhaquinones	MJ and caffeic acid	[136]
15	*Scopolia parviflora*	Scopolamine	MJ, SA, Bacteria	[123,137]
16	*Stevia rebaudiana*	Steviol glycosides	MJ, PAA	[138]
17	*Withania somnifera*	Withanolide, withaferin	Aluminium chloride, chitosan	[89]

MJ: methyl jasmonate, SA: salicylic acid, and PAA: phenyl acetic acid.

2.6. Effect of Biotic Elicitors on Adventitious Root Cultures

The substances that originate from living organisms are known as biotic elicitors (Figure 2). These include polysaccharides originated from plants (chitin, pectin and cellulose), yeasts, fungi or bacteria [137]. These elicitors bind with the receptors and act by activating or deactivating ion channels or enzymes, therefore leading to the increased or decreased production of secondary metabolites [88]. According to several reports, yeast extracts, chitosan, pectin and different species of bacteria increased metabolite production in adventitious root cultures of medicinal plants. In plant cells, yeast stimulated defense mechanisms, which ultimately resulted in the enhanced biosynthesis of secondary metabolites. Reports are available for the enhanced production of quercetin (1.9 fold) in *Gynura procumbens* by the application of *Saccharomyces cereviceae* extract [128]. Similar results are documented for cryptotanshinone production (3.63 fold) in *Perovskia abrotanoides* [134]. Likewise, chitosan and pectin increased the secondary metabolite production in the adventitious root culture of *Morinda citrifolia* [72]. In *Withania somnifera*, chitosan also enhanced withanolide production in adventitious root cultures [89]. Plant growth-promoting rhizobacteria (PGPR) enhanced the defense mechanism of plants to produce bioactive metabolites in adventitious root cultures. In addition, PGPR also promoted the biosynthesis of endogenous jasmonic acid, which then acted as a transducer for elicitor signaling pathways, which can increase the production of secondary metabolites [137] (Table 2).

3. Adventitious Root Culture in Bioreactors

Recently, the industrial-scale production of active constituents from therapeutic plants using plant cells, tissues and organ cultures has been a major challenge for biotechnology. The need for these medicinal compounds has been increasing, and to meet the requirements of the global market, it is necessary to yield these compounds in bulk quantities. Several medicinal plant species have been utilized to scale up the production of active constituents using bioreactor technology. This technology is more cost effective compared to conventional methods, because the complete process occurs in a bioreactor system, which can be controlled to produce high-grade yields in bulk quantities [21] (Figure 3).

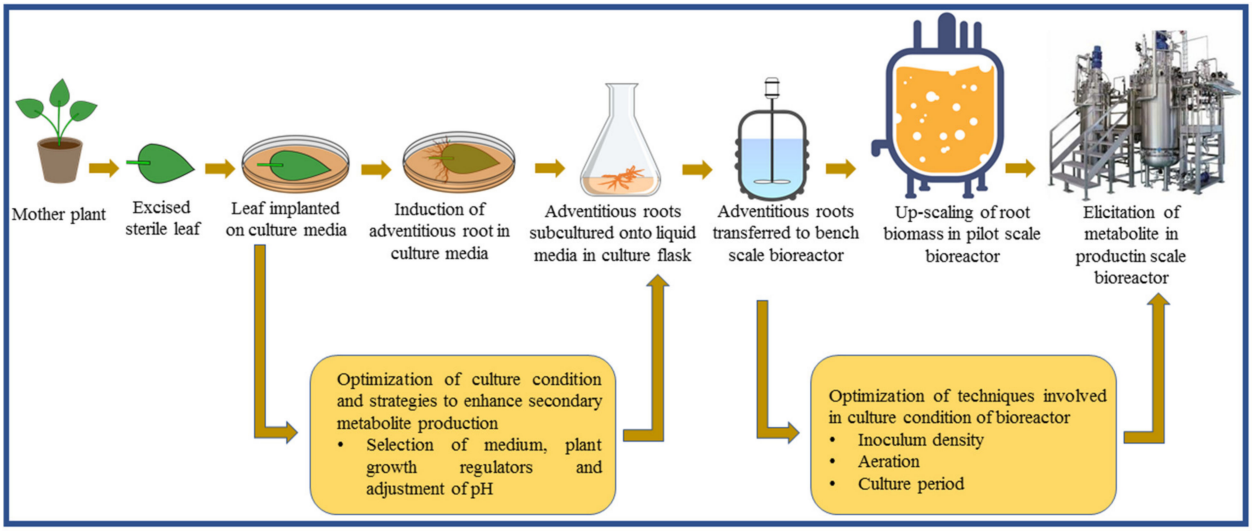

Figure 3. Illustrative representation of adventitious root-production strategy with the help of bioreactors.

Various engineering parameters for bioreactors consisting of pH, fluid mixing, temperature, aeration, shear sensitivity, carbon dioxide evolution rate and dissolved oxygen must be considered for the successful cultivation of plant cells, organs or tissues. Specific bioreactor systems for higher productivity have been established for culturing plant cells up to the volumes of 75,000 L [139]. Culture of plant cells in a bioreactor is a significant step towards the industrialization and commercial production of secondary metabolites by plant biotechnology. Several reports are available for the improved biosynthesis and accumulation of metabolites using bioreactors (e.g., *Polygonum multiflorum* [81] and *Hypericum perforatum* [71]).

Moreover, recently, Fan et al. [140] showed the enhanced production of eurycomanone and polysaccharides in an adventitious root culture of *Eurycoma longifolia* using the bioreactor system. In *Gynura procumbens*, the production of root biomass was compared to different liquid cultures (shake flask, balloon-type bubble bioreactor and temporary-immersion bioreactor). It has been reported that some problems in liquid culture, such as asphyxia and hyperhydricity, were observed during the low oxygen and submerged conditions in the medium [141]. However, to overcome this problem, a temporary immersion system bioreactor and balloon-type bubble bioreactor was applied. Temporary immersion system (TIS) bioreactors help to eliminate hyperhydricity and asphyxia by adjusting its immersion time [142]. Additionally, aeration systems are used in balloon-type bubbles, which can stimulate the growth rates of the culture. The bubbles of the balloon-type bubble bioreactor (BTBB) lessen the shear forces; therefore, this kind of bioreactor is suitable for culturing plant organs. However, the study conducted by Manuhara et al. [141] showed that the highest biomass production (fresh weight) was found in the balloon-type bubble bioreactor (13.1-fold higher than the initial explant), followed by that in the temporary immersion system bioreactor (5.12-fold higher than the initial explant) and shake flask (3.9-fold higher than the initial explant), respectively [141].

For the establishment of a large-scale adventitious root culture of *Hypericum perforatum*, a bioreactor system was adopted. A leaf explant was cultured on MS solid medium in different types of bioreactors. The results show that the pattern of adventitious root growth in the larger bioreactors is similar to that in smaller bioreactors [143]. In the case of *Polygonum multiforum*, the scale-up production of adventitious root cultures was established for phenolic compound production using small and large bioreactors and also a pilot production system. All the analysis revealed that the root biomass outcome was lower in the pilot production system, but the formation and synthesis of secondary metabolites were the same as in the small and large bioreactors [144].

4. Advantages of Adventitious Root Cultures

Adventitious root culture has several advantages over the conventional method of root growth, as it helps to enhance the production of important active constituents, such as alkaloids, glycosides, phenolics, flavonoids or terpenoids [145]. The advantage of using plant cell, tissue and organ cultures for the formation of root biomass is that the rate of growth is higher, less time consuming, a low amount of inoculum is required in culture medium and the metabolite production is stable [47,146]. It generally shows an enhanced biosynthetic capacity when compared to cell-suspension culture, which produces a relatively lower yield of bioactive metabolites [147]. Several authors reported that the cultured adventitious roots of medicinally important plants, such as *Andrographis paniculata* [43], *Ophiorrhiza mungos* [148] or *Polygonum multiflorum* [81], displayed enhanced root multiplication and a higher accumulation of secondary metabolites, which was ultimately found to be advantageous for pharmaceutical industries. Therefore, this technique is beneficial for the establishment and production of adventitious roots, as it is genetically stable and free from any interference with microorganisms, and the production of active metabolites is elevated.

5. Challenges to Use Adventitious Root Culture

Adventitious root culture has several positive aspects for the production of crude biomass, but, additionally, there are few challenges, which has to be kept on record for future consideration. The process itself is laborious and time consuming; skilled personnel are required for the optimization of media and culture conditions and it is a technique that is more expensive than conventional propagation methods.

6. Future Prospects

Modern plant biotechnological techniques, such as plant cell, tissue and organ culture, DNA manipulation and biochemical engineering have major impacts on agriculture. These methods affect various parameters, such as plant growth, the activation of defense mechanisms against biotic stress and the production of pharmaceutically important compounds. New achievements in plant biotechnology have led to innovative techniques, which ultimately help in the conservation of biodiversity [149]. In vitro propagation methods provide the development of pathogen-free plants, safe exchange of germplasm, establishment of extensive collections of germplasms in a relatively smaller space and supply of material for the recovery of wild populations [150,151].

These techniques ensure limitless opportunities for the production of high-yield crops and sustainability for future use. Biotechnology offers tools and techniques that may revolutionize, to a greater extent, the industries of agriculture, horticulture, pharmaceuticals and food. Biotechnological methods in agriculture include tissue culture, molecular breeding, marker-assisted selection, genetic engineering, production of GM crops, variety development through genetic variability and somaclonal variation, development of haploid, diploid and polyploid plants and hybrid and cybrid development.

The effective initiation, growth and development of adventitious root culture for the medicinally valuable metabolites on a large scale needs the suitable range of bioreactors and the process needs to be improved accordingly by increasing the size of the bioreactor or by parallelizing the bioreactor. Different types of bioreactors are available according to the requirements of plant cell or tissue culture studies; bioreactors for adventitious root culture may greatly differ from those used for culturing animal cells or microorganisms. The integrated bioreactor technology, metabolic and bioreactor engineering, two-phase and two-stage culture systems, genetic transformation and precursor feeding techniques can be used to increase the production of secondary metabolites [34,72]. In the near future, the elicitation, characterization and production of plant-based metabolites will be of prime importance; therefore, the optimization and the conservation of plant genetic resources for elite germplasm is necessary for future developments. To date, there are more cost-efficient technologies, which consume less time and provide higher production values for

the adventitious root culture and biosynthesis of metabolites. Genomics will help studies involved in complex developmental processes at the gene level, such as organogenesis, somatic embryogenesis and the occurrence of somaclonal variation. The adventitious root cultures or plantlets propagated from tissue cultures may be used further for the extraction and characterization of natural novel compounds with the help of metabolomics; these techniques will help the pharmaceutical industries.

7. Conclusions

The development in the field of biotechnology provides a light for a better future and it also contributes to sustainable industrial development. The use of modern biotechnological tools and techniques with a multidisciplinary approach to solve various complex problems in the field of science has recently gained interest. In vitro methods in the field of medicinal plants, especially micropropagation and secondary metabolite production, have extraordinary importance, as these are essential for the sustainable production of secondary metabolites and for the protection of natural habitats. The use of adventitious root cultures may help to formulate alternative protocols for mass propagation and root biomass production using tissue culture technology. These protocols may help the pharmaceutical industry to obtain metabolites without hampering the natural habitat of plant species, and, ultimately, may help in environmental conservation. The use of plant-growth regulators, elicitors and bioreactors will trigger further research to improve the commercial production of metabolites from root cultures. The optimized use of these methods will result in increased biomass productivity and bioactive compound accumulation.

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