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Author (s): Wasiq Ikram[1], Tooba Sehar[1],Muhammad Khurshid[2], Affifa Atta[1], Abdul Qadeer Wahla[3], Syed Muhammad Waqas Gillani[4], Muhammad Adil Rehman[5]

Affiliation (s):
[1]Minhaj University Lahore, Township, Lahore Punjab, Pakistan.
[2]*University of the Punjab, Lahore, Pakistan*
[3]National Institute of Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan
[4]Ministry of National Food Security and Research, Peshawar, Pakistan
[5]The Islamia University of Bahawalpur

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Plant Tissue Culture and Formation of Secondary Metabolites - A Review

Wasiq Ikram[1*], Tooba Sehar[1], Muhammad Khurshid[2], Affifa Atta[1], Abdul Qadeer Wahla[3], Syed Muhammad Waqas Gillani[4] and Muhammad Adil Rehman[5]

[1]School of Botany, Minhaj University Lahore, Township, Lahore, Pakistan
[2]School of Biochemistry and Biotechnology, University of the Punjab, Lahore, Pakistan
[3]National Institute of Biotechnology and Genetic Engineering, Faisalabad, Pakistan
[4]Pakistan Tobacco Board, Ministry of National Food Security and Research, Peshawar, Pakistan
[5]Department of Food Science and Technology, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Pakistan

ABSTRACT

Many copies of a single plant can be grown using the plant tissue culture technology. These copies have the right characteristics to satisfy medical and nutritional demands. Secondary metabolites are purposefully synthesized by using the *in vitro* technique. These metabolites act as protectors for plants during stressful conditions and offer resistance against different organisms and factors, ultimately helping the plant to survive. With the passage of time, the development of new instruments for the improved synthesis of secondary metabolites via the genetic control of biosynthetic pathways has been aided by the speedy development of recombinant DNA technology. Plants generate a wide range of secondary metabolites that have various biological functions, such as fungicide, herbicide, anti-parasitic, and anti-microbial functions. Nanotechnology has the potential to drastically alter conventional plant growing methods and bring about the synthesis of flavonoids, anthocyanin, and diosgenin by using silver nanoparticles and cadmium oxide nanoparticles (CdONPs). The technique of callus cultures is increasingly utilized to produce secondary metabolites. Hence, the main objective of the current review is to increase the synthesis of secondary metabolites.

Keywords: biosynthetic pathways, callus culture technique, *in vitro* technique, nanotechnology, recombinant DNA technology, secondary metabolites

1. INTRODUCTION

Plant tissue culture is a technique which is used to make multiple copies of a single plant from a single cell, tissue, and organ culture in the presence of mostly controlled or sterile conditions. Through this technique, many copies of a single plant with desired qualities can be generated. This process also involves the technique known as micropropagation which produces meteoric multiples form the meristematic portion of the plant, such as the nodal portion, axillary bud, soft stem, and even from a single cell [1].

*Corresponding Author: wasiq.bot@mul.edu.pk

Micropropagation plays an important role to fulfil nutritional needs and other medicinal needs all over the world through the breeding of plant species using different techniques, such as molecular biology and genetic engineering [2]. Plants produce secondary metabolites as the result of every reaction or stimuli and these can be observed by using *in vitro* techniques, such as single cell or organ culture [3]. It has been observed in recent years that the formation of secondary metabolites has increased. The formation of secondary metabolites and their use in various products including dyes, fragments, medicinal extracts, and cosmetics, have no side effects on human health and they are also inexpensive [4].

The increase in the reactivity of different particles (Cu and Zn) at atomic level (nanoparticles) promotes growth and restricts bacterial attacks. Nanoparticles cause an unpleasant effect on both harmful and beneficial microorganisms present in the soil [5]. Plants face many types of stresses such as temperature, heat, water, humidity (abiotic factors), microorganisms, animals, and carnivores plants (biotic factors), whereas, secondary metabolites act as protectors [6]. The most effective way of observing plant growth at different stages as well as the production of secondary metabolites under *in vitro* sterile conditions is the callus culture [7]. A highly maintained root culture and callus culture play a meaningful role in the production of secondary metabolites [8]. These metabolites act as resistance against pathogens, fungus, insects, and other harmful aspects and help to ensure the survival of plant species. Secondary metabolites including terpenoids, flavonoids, terpenes, alkaloids, and phenolic acid are the most important [9].

Due to the rising demand in the medical, food, commercial, and industrial sectors, the rate of synthesis of secondary metabolites has been increasing through the application of various methods of plant tissue culture. These metabolites participate indirectly in the many stages of plant development and growth and are commonly referred to as extra-compounds of plants. They are not produced by plants during any stage of growth; rather, they are produced by microorganisms belonging to a small taxonomic group that share structural similarities with secondary metabolites. The lack of secondary metabolites or their decreased production may not immediately cause a plant to die; instead, it causes a reduction in its growth rate, changes in its appearance, or physical alterations [10]. They are very important in medicine because they often offer resistance to fungi, bacteria, and malignant cells. Numerous botanists have attempted to create these substances *in vitro* under aseptic circumstances because of their numerous positive benefits [11]. The generation and preservation techniques of these beneficial compounds are influenced by a variety of factors including genetic condition and physical environment. These techniques include improved and increased creation of secondary metabolites using various plant tissue culture techniques, as well as their synthesis, utilization and future prospects [12].

2. PLANT METABOLIC ENGINEERING

The synthesis of secondary metabolites is a process which is affected by various factors including nutrients demand, temperature, oxygenation, and the acidic/basic environment [13–15] (Figure 1). In many researches, these elements have been studied, improved, and tested on an industrial scale to promote the production of secondary metabolites [16–18]. Pharmaceutical industry can potentially

benefit from the conventional mutagenesis for secondary metabolite generation and their improved synthesis. Recombinant DNA technology has improved significantly over the past several years, enabling numerous scientists to develop a variety of methods for increasing yield by manipulating the genes that control biosynthetic processes. These sophisticated tools often focus on deregulating metabolic pathways, rerouting metabolic fluxes, and expressing certain enzymes engaged in biosynthetic processes. Additionally, new attempts have been undertaken that successfully express metabolic genes in a variety of species, not only to influence production levels but also to speed up gene expression by using organisms that grow more rapidly and easily than other similar organisms [19].

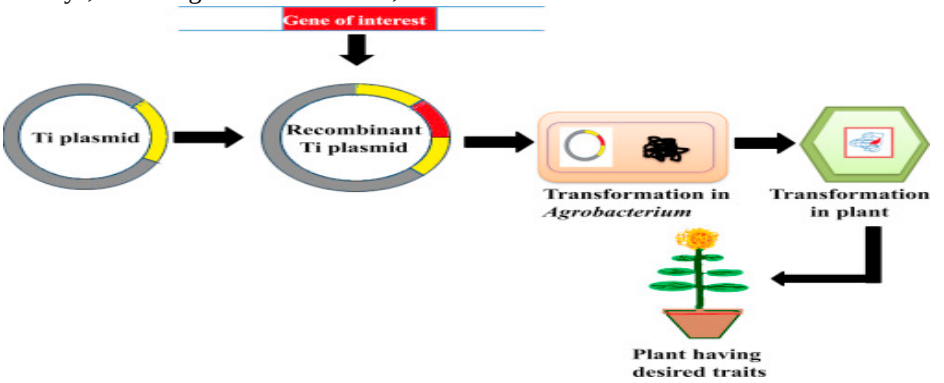

Figure 1. Metabolic Engineering of Plants [41]

3. MEDICINALLY IMPORTANT PLANTS AND THEIR SECONDARY METABOLITES

Plants can produce high quality and diverse secondary metabolites through various biological processes, such as cytotoxic, anti-microbial agents, and anti-parasitic compounds. These compounds are utilized to restrict parasitic DNA, microtubules, membrane integrity, and neuro-signal transduction. Plants take out and isolate secondary metabolites which control the growth of parasites, namely trypanosoma, trichomonas, plasmodium, intestinal worms. Many of these compound drugs still need to be proven in animal models; if successful, then in clinical trials [20–23].

4. FACTORS AFFECTING SECONDARY METABOLITES

A variety of factors including biological processes, genetic formations (which include the control of enzymes and genes), and environmental factors have a considerable impact on the production and accumulation of secondary metabolites (this includes temperature, salinity, light, and water). Numerous studies have reported recently the impact of environmental conditions on the production and accumulation of secondary metabolites in medicinal plants, as well as the elements that contribute to metabolite growth. However, they need to be systematically organized and comprehensively overviewed [24].

4.1. Secondary Metabolites and Nanoparticles

The particles ranging between 1 and 100 nm in size are known as nanoparticles

[25]. The use of nanotechnology in plant biology has the potential to drastically alter the current plant cultivation practices. According to recent studies, nanoparticles are employed to improve bacterial response resistance, bioactive molecule development, seed germination, and increased growth (Figure 2).

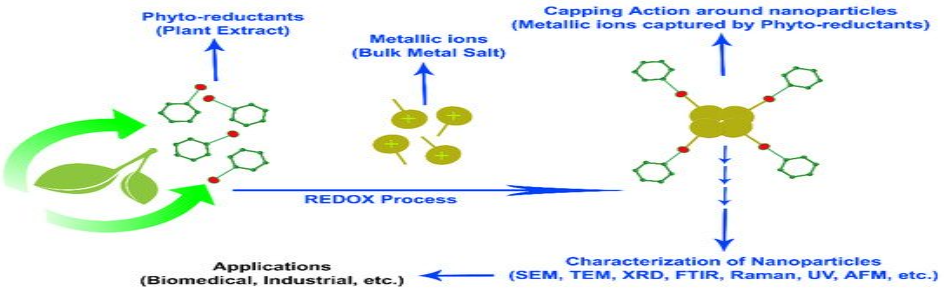

Figure 2. Secondary Metabolites in the Green Synthesis of Metallic Nanoparticles [42]

The modifications in external conditions relate the release of bioactive compounds which can be programmed to occur in certain settings [26]. Silver nanoparticles (AgNPs) have been used efficiently for bactericidal activities against many species of bacteria [27]. Metal oxide nanoparticles have been reported for improving the production of secondary metabolites, such as zinc oxide (ZnO), copper oxide (Cu_2O), iron oxide (Fe_2O_3), and titanium oxide (TiO_2) [28]. Fenugreek was used to study how silver nanoparticles affect growth and the results showed highly positive outcomes. It has been reported also that the concentration of diosgenin (a significant sapogenin) increases with the addition of silver nanoparticles. It has substantial uses in both the pharmaceutical and nutraceutical industries and remains essential to plant physiology. Diosgenin has medical uses and serves as a precursor for steroids as well. It also aids in the increased synthesis of several hormones, including testosterone and glucocorticoids [29]. In 2015, arabidopsis plants were exposed to silver and titanium oxide nanoparticles [30]. It was found that silver nanoparticles upregulated the genes involved in the production of flavonoids and anthocyanins (Figure 3).

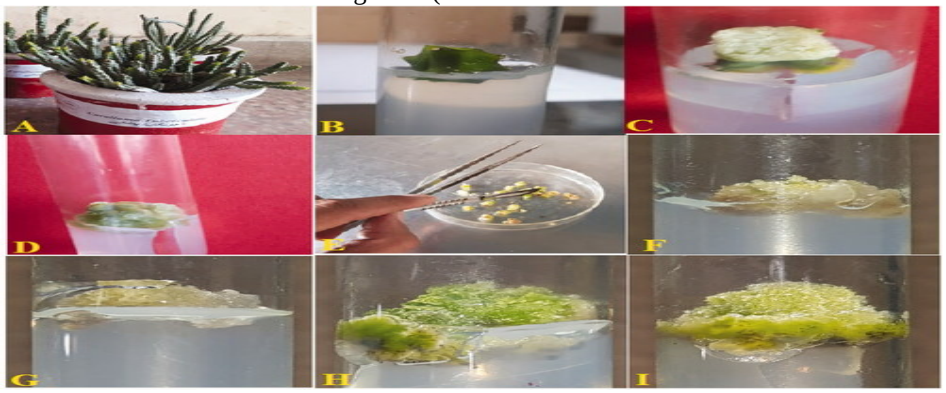

Figure 3. Production of Caralluma tuberculata Callus Using Different Concentrations of Silver Nanoparticles [43]

Hardly any plant has been noted for higher levels of isovitexin and ferulic acid. CdONPs induced alterations in the content of isovitexin and ferulic acid, which included polyphenols, when the plants were exposed to them [31].

4.2. Callus Culture and Secondary Metabolites

Medicinally significant plants yield a variety of bioactive chemicals that are employed in many different ways. Several researches have been conducted to improve the production of secondary metabolites. Callus culture and cell suspension remain the foundation of the majority of these investigations [32]. Secondary metabolites have a wide range of uses and carry out several processes. These bioactive compounds are used in a variety of products, such as cosmetics, medications, enzymes, hormones, natural insecticides, and antibiotics [33]. Plant cells are characterized, stimulated on medium, and high concentrations of auxin or a mixture of cytokinin and auxin are used to create callus cultures in *in vitro* settings [34]. Both embryogenic and non-embryogenic callus cultures are possible. Cells with embryogenic competence can be found in embryogenic calli [35]. Through a process known as somatic embryogenesis, these cells gain the capacity to regenerate whole plants. Furthermore, non-embryogenic callus cultures are utilized to produce secondary metabolites. The developed cells in callus cultures are grouped together uniformly. According to a notable study, this method is more frequently employed to produce flavonoids [36]. Research on *Vaccinium myrtillus* has been conducted to identify bioactive substances.

In a recent research, for the separation of a flavonoid secondary metabolite namely quercetin, many plants including *Chrysanthemum cinerariaefolium* were utilized. Quercetin has been used as an antiviral and anti-asthmatic agent [37–39], as shown in Figure 4.

Figure 4. Formation of Brown and Friable Callus on DK21 Medium [37].

Phenols, terpenoids, isoprenoids, and flavonoids were created as secondary metabolites. *C. cinerariaefolium* leaves were employed as explants. A combination of grown regulator and MS medium was used along with kinetin, which is a growth regulator. Prior to the experiment's initiation, sterilization was carried out. *C. cinerariaefolium* leaf fragments were planted in a vial to create a callus of 1 x 1 cm leaf size. From the commencement of the plantation to the peak of callus production, callus induction was observed. To examine the synthesis of secondary metabolites, a growth curve was created. Extraction was performed prior to the analysis. Na_2SO_4 and 95% ethanol were used. The findings of the extraction were examined by Gas Chromatography–Mass Spectrometry (GC-MS).

Another important metabolite namely *Stevia rebaudiana* is a non-caloric sweetening plant with diterpene glycosides that should be investigated for the prospects of commercialization. Secondary metabolites are in great demand in the pharmaceutical industry. These metabolites have been used in pharmaceuticals, food additives, and fine chemicals. Additionally,

they provide unique materials that are consumed by other industries. In addition to direct extraction from plants and the use of chemical synthesis to produce chemicals or derivatives, it has been established that plant cell culture is a promising alternative for generating metabolites that are challenging to obtain by chemical synthesis or plant extraction. Despite decades of work, there still remain a number of biological and biotechnological limitations to the production of secondary metabolites using plant cell culture technologies (Figure 5).

One of the main obstacles is the limited release of secondary metabolites in plant cell cultures. Commonly, secondary metabolites from plants are mainly employed to protect them from attacks by insects, herbivores, and diseases [40].

5. STRATEGIES

The following elements were the focus of the studies aimed at enhancing secondary metabolite production among plants:

1. Improvement in chemical production within plant cell cultures has been shown through enhanced chemical processing and bioreactor performance, as well as the use of elicitors, biotic stimuli, and various approaches, regardless of their specific processes.
2. Examining the signal transduction pathways that underpin a variety of efficient strategies that result in the production of secondary metabolites of plants.
3. Looking into transcription factors and their regulatory mechanisms including genetic manipulation of regulator genes to boost secondary metabolite production [40].

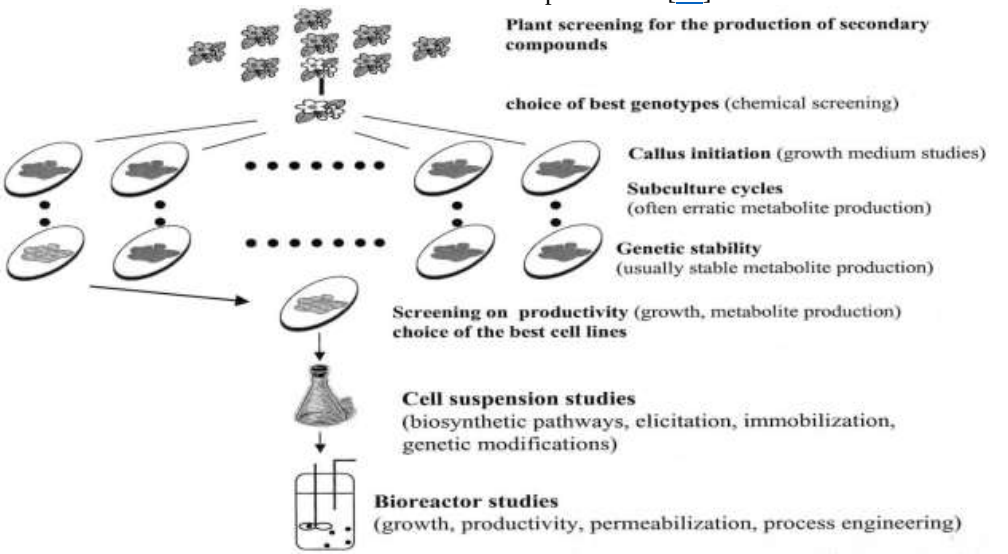

Figure 5. Production of Secondary Metabolites from Plant Cells [40]

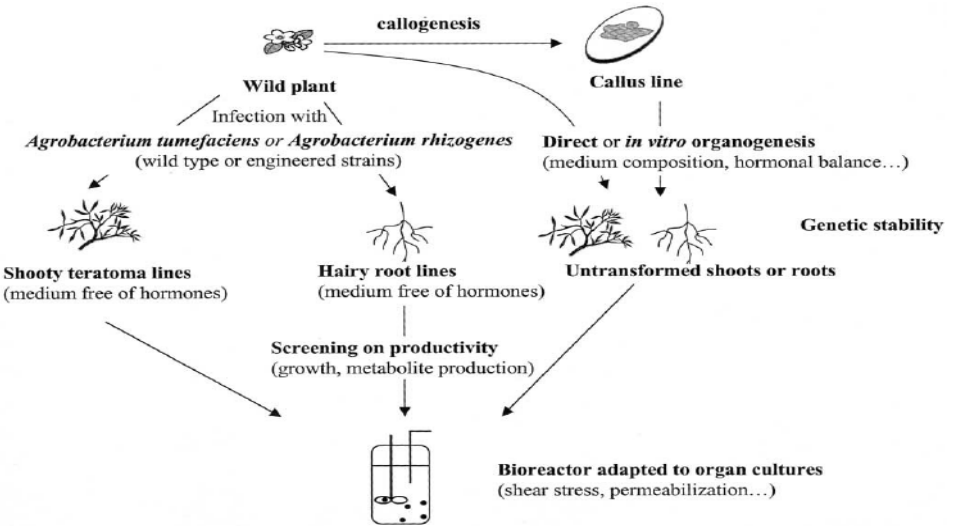

Figure 6. Technique to Improve the Production of Secondary Metabolites [44]

5.1. Addition of Precursors to Improve Secondary Metabolite Production

The use of biotic and abiotic elicitors to promote secondary metabolite production in plant cells is an effective method to enhance secondary metabolite yield in cell cultures. In previous studies, the most widely used elicitors were fungal polysaccharides, yeast extract, M, J, and chitosan. MJ, a well-known signal compound, is the most efficient elicitor of taxol production. Amino acids were found to be implicated in the biosynthesis of hyperforin and adhyperforin in H. perforatum shoot cultures. Valine and isoleucine were incorporated into the acyl side chains of hyperforin and adhyperforin, respectively after being supplied to shoot cultures. When shoot cultures were used, the production of a hyperforin was boosted 3-7 folds.

6. CONCLUSION

The production of secondary metabolites from plants using plant tissue culture methods and their benefits were succinctly outlined in this study. Secondary metabolites are crucial for both plants and a wide range of human products, thus there is a significant need for their synthesis. Plant tissue culture techniques, which can help with production, have significant importance. Secondary metabolite production may be boosted by a number of cutting-edge metabolic engineering techniques, the use of nanoparticles, callus cultures, and modern biotechnology procedures. Altering the genes involved in the production pathway and genetic engineering can help with the generation of secondary metabolites. Hence, the plant tissue culture methods and cutting-edge techniques offer promising avenues to enhance the production of valuable secondary metabolites, crucial for both plants and various human products.

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