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ENHANCEMENT OF PLANT SECONDARY METABOLITES USING TISSUE CULTURE APPROACHES: A REVIEW

Vishal N. Patil
Vidyabharti College Seloo Dist. Wardha (MS) India-442104
Email: vnpatil85@gmail.com

ABSTRACT: Plant cell and tissue culture techniques appear as environmentally friendly alternative methods for the production of secondary metabolites. Secondary metabolites are organic compounds produced by living organisms viz. bacteria, fungi or plants. They are not directly involved in the normal growth, development or reproduction of the organism. Plants are a remarkable source of high-value secondary metabolites with applications in various fields. Secondary metabolites play an important role in plant defense against herbivory and other interspecies defenses. Humans use secondary metabolites as medicines, flavourings, pigments and recreational drugs. When natural supply is limited or chemical synthesis is unviable, the plant tissue culture is one of the important techniques which appear as environmentally friendly alternatives for the production of secondary metabolites. In the present review, the main advantages of using plant tissue culture techniques for the production of plant secondary metabolites are presented as well as the different plant biotechnological approaches available to improve their production. The study reviewed shows that undifferentiated cells are the preferred culture system used for the production of high-value secondary metabolites in vitro although there are many examples reporting the production in differentiated tissues particularly in root hair cultures. Efforts have been made to extent the production and several strategies have been successfully applied to increase the production.

Key words: - secondary metabolites, enhancement, tissue culture.

INTRODUCTION:

Recently, the production of secondary metabolites using plant cells has been the subject of extended research. Plants, animals and minerals have been used as medicines by man, since prehistoric time. Especially, the plants have provided a large variety of potent drugs to cure diseases and also to prevent them. Man has realized these curative and preventive properties of plant parts. Today, developing as well as the developed counties shows a swing towards the herbals (Raghunathan and Mitra 1982) as a safer and alternative option against the synthetic medicines.

The knowledge of plant metabolic pathways is still very limited. On the basis of biosynthetic origin, metabolites can be structurally divided into polyketides, isoprenoids, alkaloids, phenylpropanoids and flavonoids (Oksmann-Caldentey and Inze 2004). It is well accepted that, secondary metabolites are associated with survival of plant in its ecosystem. They show antimicrobial and anti-insect properties, deter potential redatos, discourage competing plant species and attract pollinators and symbionts (Dixon 2001). Plant cell and tissue culture techniques appear as environmentally friendly alternative methods for the production of secondary metabolites (Kolewe et al. 2008; Khani et al. 2012). Secondary metabolites assist a host in important functions such as protection, competition and species interactions. Research also shows that secondary metabolites can affect different species in varying ways (Pichersky and Gang 2000).

Usually, secondary metabolites are specific to an individual species, though there is considerable evidence that horizontal transfer across species or genera of entire pathways plays an important role in bacterial (and likely fungal) evolution (Pichersky and Gang 2000; Juhas et al. 2009). The most studied classes of plant secondary metabolites using plant cell and tissue culture production systems are alkaloids and the

anticancer drug (Isah et al. 2018). Over the past decades, efforts have been directed at the extraction, structure elucidation and evaluation of biological activity of many plant secondary metabolites. About 25–28% of modern medicines are derived from higher plants (Samuelsson 2004) and over 60% of anticancer drugs are directly or indirectly derived from plants (Cragg and Newman 2005). In the last decades, considerable progress has been made concerning the production of secondary metabolites by using plant tissue culture techniques. Plant tissue culture techniques were approved by Food and Agriculture Organization as safe for the production of compounds for food application (Dias et al. 2016). The efforts were taken to discuss the main advantages in this review is to improve the plant secondary metabolites production by using plant cell and tissue culture.

Plant tissue culture: An alternative method for secondary metabolite production

The rapid growth of the international market of plant based drugs has emphasized the establishment of more environmentally suitable and economically viable production strategies for consistent production of high quality products. Synthetic drug often act in the body as irritants and toxins, upsetting the balance of whole system and producing side effects that be lethal. This may results in differences in therapeutic effect and adverse effect in terms of benefit to risk ratio. Thus, pharmacologist considers drug products that consist of different stereo isomers as different drugs rather than different formulations of the same drug. For these reasons, plant drugs can provide better natural bioactive medicine than synthetic drugs without harmful side effects (Sivakumar 2006).

Strategies such as field cultivation and green house cultivation were studies as alternate for production of natural products. Field cultivation is the least expensive mode of production of natural products. But an infestation, diseases and application of pesticides additionally decrease the quality of plant material. The evolving commercial interest in natural products resulted in assessment of possible manipulation of secondary metabolites by using plant cell cultures. The possibility of in vitro plant cell cultures for the production of plant pharmaceuticals was studied in detail as an alternate and complimentary method to whole plant extraction (Dornenburg and Knorr 1955; Dicosmo and Misawa 1995; Bourgaud et al. 2001). A plant cell culture offers an additional advantage for production of secondary metabolites such as (i) independent of seasonal variation, climate and soil conditions, (ii) efficient production system ensuring continuous and reliable supply of high yield of quality products, (iii) optimization of product accumulation through physical and chemical parameters, (iv) genetic modification through introduction of heterologous genes to manipulate accumulation of desired compounds (Luczkiewicz and Kokotkiewicz 2005b).

Advantages of plant tissue culture techniques for the production of secondary metabolites:

Tissue culture technique for a number of medicinal plants has been established and this enables the analyses of callus and cell suspension for the presence of various secondary metabolites. Now, it is possible to achieve the production of secondary metabolites similar to that of naturally grown plants or otherwise on large scale by application of biotechnological approaches to the plant tissue and cell culture technique (Komaraiah et al. 2003, Patil and Deokule 2020). Secondary metabolites have a huge number of natural compounds with a wide diversity in chemical structure. These compounds are used as pharmaceuticals, flavours, fragrances, insecticides, dyes, food additives, toxins, etc. indiscriminate harvesting of

the plants can lead to its extinction from nature. Plant tissue and cell culture techniques are considered to be excellent producers of a broad variety of chemical compounds. Some compounds can be obtained from naturally grown plants, but sometimes there are regional and environmental restrictions, which can limit the commercial production (Yue et al. 2016). Therefore, production of secondary metabolites by cultivation of plants and chemical synthesis are important agronomic and industrial objectives. As a promising alternative to produce plant secondary metabolites, plant cellular technology has many advantages over traditional field cultivation and chemical synthesis, particularly for many natural compounds that are either derived from slow growing plants or difficult to be synthesized with chemical methods (Zarate and Verpoorte 2007).

Also, traditional cultivation of some plant species is difficult or takes several years. In this context, plant cell and tissue culture techniques appear as environmentally friendly alternative methods for the production of secondary metabolites when natural supply is limited and traditional methods are unfeasible. The mass propagation of plants in aseptic and environmental controlled conditions and the large-scale production of secondary metabolites in a year-round system without seasonal constraints are some of the advantages of plant tissue culture techniques (Isah, 2018). Moreover, cultures can be established in any part of the world independently of the plant growth requisites and are free of microbes and insects avoiding the use of pesticides and herbicides (Murthy et al. 2014; Ochoa-Villarreal et al. 2016). Plant tissue culture techniques provide a reliable and predictable method for isolating the secondary metabolites at a high efficiency within a short time when compared to the extraction from wild plant populations (Verpoorte et al. 2002). Also, the simplicity in the extraction of the metabolites from in vitro-produced tissues makes the method appellative for commercial application (Kolewe et al. 2008).

Products from in vitro cultures can still be used as models of whole plants, and cell cultures can be radiolabeled so that secondary products can be traced metabolically (Khani et al. 2012). In vitro propagation through plant tissue culture techniques allows the large-scale multiplication of true-to-type plants within a short span of time and without a negative impact on the natural resources (Verpoorte et al. 2002). This method is particularly valuable for plants difficult to propagate by conventional techniques or with slow propagation rates. In this context, in the last years, there has been an increased interest on the use of these methodologies for the propagation and conservation of medicinal plants.

Culture systems

The production of secondary metabolites by in vitro cultures usually occurs in a two-step process, biomass accumulation and secondary metabolites synthesis, in which both steps need to be optimized independently (Isah et al. 2018; Murthy et al. 2014). The production of callus by division of differentiated tissues or excised plant cells, tissues, organs and plant parts is known as callogenesis. The main benefit of this technology is that, it may provide potential, renewable, year round and reliable source for large scale production and extraction of secondary metabolites (Murthy et al. 2014).

Among differentiated tissues, hairy roots culture offers new opportunities for the in vitro production of plant-valuable compounds (Chandra and Chandra 2011). Hairy roots are induced by the infection of plants with Agrobacterium rhizogenes, a Gram-negative soil bacterium. Although there are many studies reporting the production of secondary metabolites using callus cultures and differentiated tissues (Murthy et al. 2014; Isah et

al. 2018) in most cases, undifferentiated cells are the preferred culture system (Yue et al. 2016).

Cell suspension culture

Earlier it was thought that undifferentiated cells, such as callus or cell suspension cultures were not able to produce secondary compounds (Krikorian and Steward 1969). But Zenk (1991) experimentally demonstrated, as he could observe dedifferentiated cell culture. It is the most suitable system easily amenable for improvement of culture yield using various strategies such as media manipulation, elicitation, precursor feeding and immobilization (Dicosmo and Misawa 1995).

Callus and suspension cell cultures have showed potential of accumulating wide range of secondary metabolites (Bais et al. 2002; Wu et al. 2003; Narayan et al. 2005). Plants are known to produce secondary metabolites when challenged by compounds of pathogenic origin and other chemical stress like salt, heavy metals, etc (Rajendran et al. 1992) these compounds are called elicitors. Elicitors have been effective in enhancing the production of many secondary metabolites (Ciddi et al. 1995).

Approaches to improve the production of secondary metabolites

The production of secondary metabolites in plants is genotype dependent. Thus, the first step to initiate cell or organ cultures is the choice of the parent plant containing higher contents of the secondary product of interest for callus or organ induction and the selection of high-producing cell/organ lines (Murthy et al. 2014). Callus and suspension cell cultures have collectively showed potential of accumulating wide range of secondary metabolites. Elicitors are the compounds which upon contact with higher plant cells triggered the increased production of pigments, flavones, phytoalexins and other defense related compounds (Savitha et al. 2006).

Elicitors from fungal origin have been widely employed to increase natural product formation in plant cell cultures and this strategy has been affective in stimulating the production of many chemical classes of secondary metabolites such as flavonoids (Tamari et al. 1995), coumarin derivatives (Conrath et al. 1989), alkaloids (Godoy-Hernandez et al. 2000), phenylethanoid glycosides (Lu and Mei 2003), carotenoids (Wang et al. 2006), terpenoids (Chakraborty and Chattopadhya 2008) and sesquiterpense (Ma 2008). Till now, employment of pathogenic and non- pathogenic fungal preparations has become one of the most important strategies to improve secondary metabolite production in in vitro conditions.

Traditional strategies

Traditional practices are involved the cultivation and maintenance of plants in the field. Moreover, some plants do not survive throughout the year and remain dormant for most part of the year. Such plants require the special attention to maintain them throughout the year. There are several factors that can be optimizing to improve the growth and metabolites production of the in vitro cultures (Murthy et al. 2014; Ochoa-Villarreal et al. 2016; Isah et al. 2018). The culture medium strongly affects the biomass and metabolites productivity and thus the selection of the suitable culture medium formulation is an imperative step. It must be selected according to the physiological requirements of the plant species and there are several parameters that can be optimized viz. nutrients composition, salt strength, nitrate and phosphate levels, plant growth regulators type and concentration, carbon source, etc. For instance, carbon source plays significant roles in the signal transduction systems through regulating gene expression and developmental processes (Isah et al. 2018)

Elicitation

Elicitation of plant cells represents a useful biotechnological tool to improve the production of secondary metabolites. Elicitors are defined as chemicals from various biotic, abiotic and physical sources that can trigger a response in living organism leading to increase the accumulation of secondary metabolites (Vasconsuelo and Boland 2007). The capacity for plant cell, tissue and organ cultures to produce and accumulate many of the same valuable chemical compounds as the parent plant in nature has been recognized almost since the inception of in vitro technology. In addition, plant cell cultures of several species have been utilized successfully as models to study the biochemical changes related to plant defense responses against biotic and abiotic stresses (Ochoa-Villarreal et al. 2016).

Each elicitor on the basis of its characteristics can induce specific responses depending on interaction between elicitors and plants. Elicitor influences secondary metabolism by modulating biosynthetic rate, accumulation and /or vacuolar transport, turnover and degradation (Barz et al. 1990). Elicitors have the ability to control an array of cellular activities at the biochemical and molecular level since they induce the upregulation of genes (Zhao et al. 2005). The combination of some elicitors with physical factors (e.g., UV light, temperature regime, and pulsed electric field) yielded good results for secondary metabolite production (Saw et al. 2012). As reviewed by Giri and Zaheer (2016), cell suspension culture is the most used culture system for elicitation treatment and secondary metabolites production. Due to its inherent characteristics of hormone autotrophy, uncontrolled growth, biosynthetic, and genetic stability distinctiveness, hairy root cultures have proved to be also a valuable culture system for elicitation experiments. In addition, there are some secondary metabolites that are synthesized only in the roots (Murthy et al. 2014; Zaheer et al. 2016). Nutrient and precursor feeding are also used to improve the yields of secondary metabolites production (Murthy et al. 2014). Thus, the treatment of elicitors can lead to increase in the production of secondary metabolites. Addition of precursors externally in the culture medium results in increased the production of end product i.e. secondary metabolites (Thorpe 1981).

Metabolic engineering

Metabolic engineering approach also uses the inhibition of competitive pathways to increase metabolic flux of targeted biosynthetic pathway intermediates for a higher production through a variety of approaches. The understanding of phenylpropanoyl biosynthetic pathway that is involved in the biosynthesis of several plant secondary metabolites is the most successful and recent application (Nanda et al. 2016).

Metabolic engineering offers a new perspective to understand the expression of genes involved in the biosynthesis of secondary metabolites through over expression studies allowing the alteration of biosynthetic pathways (O'Connor 2015). This involves the study of enzymatic reactions and biosynthetic processes at gene, transcriptomic and proteomic levels, and the manipulation of the genes encoding the critical and rate limiting enzymes in the biosynthetic pathways (Cusido 2014; Lu et al. 2016).

Gene transfer techniques are currently being used for genetic modification of medicinally important character and also the structure and composition of active metabolites in medicinal plants. An efficient in vitro plant regeneration system is a basic necessity for such approach. As an alternative, the culture of callus tissue provided an important technique that can be preliminary to the regeneration of whole plant (Thorpe 1981; Robert and Dennis 2000).

Scale up production

Production of secondary metabolites is an important aspect, efforts have been made to study the feasibility of their production at the industrial scale. This is not always a simple process because plant cells have a relatively unstable productivity, high shear sensitivity, a slow growth rate and low oxygen requirements (Murthy et al. 2014). The scale-up involves the use of bioreactors of varying sizes and features and cell suspension culture is the better culture system having several advantages. The simplicity, predictability, and high efficiency at which the metabolites can be isolated from biomass or cultivation media are some of these advantages (Park and Paek 2014).

Several factors should be considered in scaling up the production of secondary metabolites using bioreactors, namely the optimization of culture conditions, biomass production measurement (especially with tissue and organ cultures) (Steingroewer et al. 2013). On the other hand, airlift bioreactors are suitable for not highly shear sensitive cells and for hairy and adventitious root cultures (Murthy et al. 2014). The interested researchers could be find more important details about the scale-up process in the works by Murthy et al. (2014), Yue et al. (2016) and Isah et al. (2018). There are several plant secondary metabolites including among others alkaloids, terpenes, flavonoids and glycosides which can be produced by plant tissue culture techniques using different strategies (Isah et al. 2018; Yue et al. 2016; Murthy et al. 2014).

Conclusions and Future prospects

Plant cell and tissue culture techniques appear as environmentally friendly alternative methods for the production of secondary metabolites. This is an attractive system for the cultivation of a broad range of secondary metabolites, including important alkaloids with anticancer properties and bioactive phenolics. This alternative provides a continuous, sustainable, economical and viable production of secondary metabolites, independent of geographic and climatic conditions. Though there are great progresses in this area from last decades, production occurs at very low yields, in some cases and there are many difficulties in scaling up the production. The limited the improvement of the production yields is because of incomplete knowledge about the biosynthetic pathways of bioactive molecules. The R&D of production of secondary metabolites through biotechnology has made a beginning and is expected to provide disease remedial or disease preventive molecules at affordable costs for the benefit of mankind.

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