ABBIOTIC ELICITATION: A TOOL FOR PRODUCING BIOACTIVE COMPOUNDS

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Abstract

Metabolism is the process by which plants produce various products and intermediates known as the ‘metabolites’ of plants. Primary metabolites such as carbohydrates, lipids and amino acids are produced. In addition to this, higher plants also synthesize various low molecular weight compounds called secondary metabolites that establish the defense mechanism in them. These secondary compounds are important sources for various industries like pharmaceuticals, food additives, flavor and fragrances, dyes and pigments, pesticides and industrially important biochemicals. Most of the drugs that are sold nowadays are modified forms of the natural compounds. This utilization of natural substances has gained much attention over the past decades, particularly in the possibility of altering the production of bioactive plant metabolites. Since ancient times these metabolites have played a significant role in the field of medicine. In Ayurveda, Unani and other traditional medical systems, plants were directly used as food or herbs to treat various diseases for many years. However, these secondary constituents are produced often very low in the plant itself and their accumulation depends on some particular conditions, or growth stage, or may be on stress. In vitro culture of plant cells respond to microbial, physical, or chemical factors called “elicitors”. This process of induced production of secondary metabolites by plants for their defence is called elicitation. “Elicitors” are termed as the chemicals (biotic or abiotic) that gets triggered by a stress induced response or stimulate the defense mechanism in plants which therefore accumulates secondary metabolites; hence it can be one of the best approaches for enhancement of production of secondary metabolites.

Keywords: Metabolites, secondary metabolites, medicines, elicitation.

Introduction

A major portion of our everyday meal constitutes plants. Researchers are intensively studying the constituents of plants and their various nutritional importance from decades. Metabolism is the process by which plants produce various products and intermediates known as the ‘metabolites’ of plants. Primary metabolites such as carbohydrates, lipids and amino acids are produced. In addition to this, higher plants also synthesize various low molecular weight compounds called secondary metabolites that establish the defense mechanism in them (Amit et al., 2014; Gupta et al., 2014; Sirohi et al., 2014a; Saraswat et al., 2014; Wang and Wu, 2013).

There are three main groups of secondary metabolites on the basis of their biosynthetic origins. They are terpenoid, flavonoid and phenolic compounds, and nitrogen-containing alkaloid and sulphur-containing compounds (Bharadwaj et al., 2018; Kaur et al., 2019; Mahmoud and Croteau, 2002; Prasad et al., 2011; Sidana et al., 2012; Saxena et al., 2016). According to their specific structure, we have glycosides, tannins and saponins as well (Prasad, 2010; Sirohi et al., 2014b).

These secondary compounds are important sources for various industries like pharmaceuticals, food additives, flavor and fragrances, dye and pigments, pesticides and industrially important biochemicals (Murthy and Paek, 2014). Most of the drugs that are sold nowadays are modified forms of the natural compounds. This utilization of natural substances has gained much attention over the past decades, particularly in the possibility of altering the production of bioactive plant metabolites (Canter and Chhikara et al., 2018a; 2018b; 2019; Ernst, 2005; Kumar et al., 2019; Pandey et al., 2016; Sidana and Joshi, 2013; Thomas et al., 2011).

Since ancient times these metabolites have played a significant role in the field of medicine. In Ayurveda, Unani and other traditional medical systems, plants were directly used as food or herbs to treat various diseases for many years (Bhat et al., 2017; Dhawan and Gupta, 2017; Dohroo et al., 2016; Fabricant and Farnsworth, 2001; Mukherjee et al., 2007; Murali et al., 2017). The discovery of many bioactive compounds such as Taxol, Morphine, Metformin etc has witnessed plants to be an important source for drug discovery. These discovered molecules have been isolated to treat diseases like obesity, cancer and diabetes (Saklani and Kutty, 2008).

The vast applications of these secondary compounds made them important for mass production. It was estimated that the international market value of naturally derived drugs was 18 billion dollars in 2005 and in 2011, it increased to more than 26 billion dollars (Saklani and Kutty, 2008). However, these secondary constituents are produced often very low in the plant itself and their accumulation depends on some particular conditions, or growth stage, or may be on stress. Also, some of these compounds are specific to a particular plant family or genus or even a species. Most of the important plants of medicinal value can only be cultivated in particular climates like tropic or subtropics regions. There are some plants that are slow in growing and some are even hard to cultivate, thus they are required to be harvested from the wild that can extinct those plant species (Affermann and Petersen, 1995; Verpoorte et al., 2002). For such reasons, an approach that uses cell or tissue cultures of plants is being preferred as an alternative method for the production of these important bioactive compounds from plants.

This project is undertaken to study, the enhancement of secondary metabolites using abiotic elicitation in holy basil (Ocimum tenuiflorum) as, not much research has been carried out.
out on the same. These plants are well known for their therapeutic potentials (Prakash et al., 2005). *O. tenuiflorum* is an herb with strong aroma cultivated throughout India. In Hindi it is called ‘Tulsi’ and in English ‘Holy Basil’. In traditional medical systems its different parts (leaves, stem, flower, root, seeds and even whole plant) were recommended for the treatment of diseases such as bronchitis, malaria, diarrhea, skin diseases, arthritis, chronic fever and insect bite and many other. *O. tenuiflorum* has also been thought to possess properties such as anticancer, anti-diabetic, anti-microbial, cardioprotective, analgesic etc. Many researchers have reported that different chemical constituents are possessed in all members (even the close ones) of the same genus * Ocimum*. Razdan (2003) has reported that *O. tenuiflorum* can cross-pollinate with different plants of the same genus due to which some plants may not be of true-type. Hence, if some genetic variations are present, there would be different chemical constituents found in the plants. Here is the situation to apply plant in vitro culture that will help to solve this problem because the tissue culturing of plants will produce the identical offspring to that of the parent plant.

For multiplication and extraction of secondary metabolite we can establish cell and tissue culturing of plants under sterile conditions from seeds or explants (leaves, stems, roots, and meristems). Further, various strategies can be used to increase the production of bioactive compounds like precursor feeding, media optimization, in situ product removal, elicitation, immobilization etc (Verpoorte et al., 1999; Zhang and Furuasaki, 1997). Among all these strategies, elicitation is proved to be one of the efficient way to enhance the production (Verpoorte et al., 1999). *In vitro* culture of plant cells respond to microbial, physical, or chemical factors called “elicitors”. This process of induced production of secondary metabolites by plants for their defence is called elicitation. “Elicitors” are termed as the chemical (biotic or abiotic) that gets triggered by a stress induced response or stimulate the defense mechanism in plants which therefore accumulates secondary metabolites (Radman et al., 2003).

Plants are vulnerable to various environmental stresses that restrict their growth and yield causing a huge loss to agricultural production across worldwide (Kapoor et al., 2018; Madakemohekar et al., 2018; Pooja and Bhupendra, 2018; Shao and Chu, 2008 Siddiqui et al., 2018). One such important factors affecting growth of plants and their secondary plant products is salt stress (Nikolova and Ivancheva, 2005). Salinity has adverse effects on plant’s physiological and metabolic activities. It results in late germination, stunted growth, poor quality and reduced productivity (Muhammad and Hussain, 2010). Also there is decrease in the rate of photosynthesis and respiration due to salt stress.

Through a process called ‘metabolism’, plants can produce different bio-active constituents. Thus they have been considered as the source for many important drugs. Plant cell carries out two major metabolisms namely, primary and secondary. The production of proteins, lipids, polysaccharides by utilizing sugars, amino acids, fatty acids and nucleotides is by primary metabolism. Plant secondary metabolism is related with the defence mechanism in plants which activates only during specific growth stages or during stress times, at the time of nutrient deficiency or microbial attacks (Yazaki et al., 2008).

**Secondary metabolites of plants**

Secondary compounds are the organic compounds that have no role in the life processes of plants that produce them but, they do provide them survival functions. These compounds are an important feature of all plants because these bioactive molecules produce defense mechanism in plants against different microbes and herbivores (Sharma et al., 2017; Stamp and Nancy, 2003). The various functions served by them are as follows:

(a) They can be used as transporting agents of metals;
(b) They act as protective agents against insects, microbes and higher animals;
(c) They can act as sexual hormones;
(d) They can also act as differentiation effectors.

Some compounds, including antibiotics helps in formation of spore and stop the germination process. Thus, the secondary compounds can perform functions like:

(a) Slowing down of spore germination till a favorable condition exists
(b) Protecting dormant spores from amoebae; and
(c) Cleansing of competitive microbe environment at the time of germination (Demain and Fang, 2000).

The extraction and purification of these molecules are difficult because they are often specific to species or genus and also produced in very low amounts. They are very high value products and have huge commercial applications in industries like food additives, biopesticides, colors, fragrances, medicines, agrochemicals, etc (Cusido et al., 2013).

**Classes of secondary metabolites**

The plant secondary metabolites can be grouped as terpenoid, alkaloid and phenolic (Verpoorte, 1998). According their specific structure, we have glycosides, tannins and saponins as part of them.

**Terpenoid**

Terpenoid is the largest group of plant secondary metabolites. They are volatile compounds. They are made of condensation of isoprene units (C5). They are classed by five carbon units found in the core structure (Mahmoud and Croteau, 2002). Isoprene is a gas released by the leaves that are produced in the chloroplasts. It protects the plants from heat.

The fragrances in the plants are due to the presence of essential oils. Some plants use the scent for the protection from herbivores and dangerous pathogens. Humans use essential oils for aromatherapy to enhance mood. It also helps in the functioning of mental and are thought to have various other benefits like oral bacteria potency, wide use in mouthwashes that are antiseptic, for skin problems and respiratory diseases.

Another type is taxol, which have great importance in the medical field to treat breast and ovarian cancers. The bark of Pacific yew tree produces taxol in very few amounts that its synthesis killed the tree. Therefore, other sources were found like a fungus on tree and needles in European yew.

Rubber is the last type which is also the largest of terpenoids because it contains 400 isoprene units. Rubbers
have a large number of applications since decades. Today, rubbers are used in shoes, tires, erasers, gloves and spandex.

**Phenol and Flavonoid**

Phenolics have one aromatic ring with hydroxyl groups’ attached with it. It present throughout the plant kingdom (Strack, 1997). Phenolics perform functions like they prevent block activity of enzyme and division of cells (Shapiro et al., 1994).

Flavonoids are the first group of phenols. They are found in vacuoles and are water-soluble. In plants, they perform functions like UV protection, pigmentation, disease resistance (Koes et al., 1994). Flavonoids are further grouped into three classes that are anthocyanin, flavone and flavonol. Anthocyanin ranges from red color to blue and purple color. The pH of environment decides its color. They can be found in grapes and berries. It can be used to treat heart diseases, diabetes and cancer. They are found in skincare products also to minimize process of aging. The other groups are flavones and flavnols that have white or yellow pigments. As a group, the phenols help in pollination of plants.

Salicylic acid is another important compound, which is obtained from bark of willow tree. It is an active ingredient in aspirin. It has been used to effectively in the treatment of aches and fevers and has cosmetic applications.

Lignin is the last compound that is present in plant structure. It provides stiffness or strength to plant cell walls. It does not allow water to enter cell wall and therefore prevent attacks by fungus.

**Nitrogen containing compounds: Alkaloids**

Plant secondary metabolites have large amounts of nitrogen in their structure. Alkaloids form part of this category which is known for its anti-herbivore property. They are of great interest because of their medicinal properties. They can also be highly toxic to the humans.

**Morphine was the first alkaloid.** Morphine can be found in plant *Papaver somniferum*, or opium poppy. It is used to treat pain and suppress cough.

**Cocaine** is another compound. It may be dangerous if taken in large amounts. It can be used as an anesthetic. Its derivatives are dangerous when consumed on regular basis and prove to be deadly.

**Caffeine** is a known compound. It protect its source plant that is tea, coffee and cocoa. Humans use it to stay alert but their high concentration is very toxic. It protect the plant by inhibiting germination of other plants in that area and in humans, caffeine is thought to minimize diabetes and heart problems.

**In vitro production of secondary compounds**

The production is often low (> 1 percent dry weight) which is dependent on geo-climatic and plant growth stage (Rao and Ravishankar, 2002). Accumulation of such metabolites is directly dependent on external and internal stresses. As a response to stress factors viz. mechanical injury, minerals in soils, microbial infections, UV radiation, and accumulation of secondary metabolites largely varies in terrestrial plants.

Most of the plants are difficult to cultivate and hence are over-harvested (Rates, 2001). Secondly, the chemical production of these active compounds is not feasible for commercial production. In this situation, *in vitro* culturing of plant cell offers a best option for secondary compound production (Jian et al., 2005).

**Defence response produced by secondary substances:**

**Elicitation concept**

The induced production of metabolites by adding trace amounts of elicitors is called Elicitation. The substances that are introduced in small amounts to generate plant defense mechanism are called elicitors (Klarzynski and Fritig, 2001). Elicitation is stated as the most preferred technique for increasing the productivity of desirable secondary substances *in vitro* (Namdeo, 2007).

**Classification of Elicitors**

Elicitors are classified as abiotic and biotic depending on their origin (Radman et al., 2003).

**Abiotic Elicitors**

Abiotic elicitors are from non-biological origin (Gorelick and Bernstein, 2014). Salts such as aluminium chloride, calcium chloride, copper chloride, potassium chloride etc. can be used to elicitation in different plant species in culture systems (Verpoorte et al., 2002).

**Biotic Elicitors**

These are substances of biological origin. They include polysaccharide originated from cell wall of the plant for example pectin and cellulose and microorganisms for example yeast, fungal extracts and bacterial extracts.

These are molecule of either pathogen or host origin which can generate defense responses in plant tissue. Biotic elicitors are recognized by unique receptors bound to the membrane of the cell. A signal transduction system transfers this stimulus, that produces changes and lead phytoalexins formation (Baenas et al., 2014).

**Effect of Abiotic Elicitors on the production of secondary compounds**

The effects of abiotic elicitors are studied in the following section. Abiotic elicitors were not much used in past years as compared to biotic elicitors (Radman et al., 2003). Studies have been done to observe the plant response towards different abiotic elicitors such as heavy metals, light, drought and many others (Rodziewicz et al., 2014).

**Physical factors**

Physical factors such as ultrasound, light, osmotic stress, salinity, drought and thermal stress can be used.

**Ultrasound**

US act as an abiotic elicitor (Yu et al., 2015). It was studied that US treatment increased the production of shikonin 2-3 times in *Lithospermum erythrorhizon* cultures (Lin and Wu, 2002). In *Panax ginseng* culture, US increased production of saponins (Lin et al., 2001).

**Light**

It was observed that light increased the production of gingerol and zingiberene in culture of *Zingiber officinale* (Anasori and Asghari, 2008). Exposure light to light increased production of artemisinin in *Artemisia annua* (Liu et al., 2002).
Osmotic stress

It is reported that osmotic stress influence growth of plants and secondary compound formation (Liu and Cheng, 2008). A study showed that in Capsicum chinensis cultures, the osmotic stress increased the productivity of capsaicin (kehie et al., 2014).

Salt stress

When plants are given salinity conditions they accumulate secondary compounds like terpene, alkaloid and phenol (Selmar, 2008). It was reported that Bacopa monnieri cultures produced bacoside A content in large amounts when given potassium chloride and calcium chloride treatments (Ahire et al., 2013). Similarly, when sodium chloride treatment was provided to Nitraria tangutorum culture, sitosterol content was increased (Ni et al., 2015).

Drought stress

Drought can limit the growth in plants as well as their reproductive development. All plant species posses different tolerance from drought stress. A study showed that when plants were provided drought conditions, it lead to accumulation of secondary substances (Al-Gabbiesh et al., 2015). Polylethylene glycol was treated with date palm callus to induce drought stress. With increase in PEG concentration there was an increase in the content of proline also (Al and Ibraheem, 2014).

Thermal stress

Extreme temperature conditions can restrict the growth in plants by affecting their metabolic processes (Xu et al., 2013). To induce callus tissues a temperature range of 17-25 degree Celsius is used (Rao and Ravishankar, 2002). A study reported that the anthocyanin yield in the culture of Perilla frutescens was maximum at temperature 25 degree Celsius (Zhong and Yoshida, 1993). Similarly, ginsenoside content was increased in Panax quinquefolius by 5 degree increase in temperature (Jochum et al., 2007).

Chemical Elicitors

Metals can be used to produce secondary substances in plants (Nasim and Dhir, 2010). It is thought to be an abiotic stress for plants due to their wide use in industries, agrotechnology (Cai et al., 2013). A study reported that metals such as nickel, silver, iron and cobalt can elicit the secondary compound production in many plants (Wang and Wu, 2013). Cobalt was used in the culture of Vitis vinifera to increase production of phenolic acid (Cai et al., 2013).

Factors which influence elicitation

Some of the important factors that can affect the elicitor-plant cell interaction and hence the elicitation responses are as follows:

Elicitor specificity

Elicitors are specific to each plant culture. If we treat a particular culture with different elicitors also we will observe the accumulation of same compounds only. The induction kinetics or level of accumulation may vary with different elicitors but, the metabolite class depends on the plant species. A study reported that elicitation by biotic or abiotic compounds in the hairy root cultures of Brugmansia candida showed differences in induction kinetics and release of hyoscyamine and scopolamine levels (Pitta-Alvarez et al., 2000).

Concentration of elicitor

The concentration of elicitor strongly affects the response intensity and dose effectiveness. The elicitor concentration may vary according to the plant species. A study reported that at a concentration of 0.5 percent sodium chloride productivity of ginseng saponin content increased 1.13 times the control value (Jeong and Park, 2007). Another report showed that yield of glycyrrhizic acid in Taverniera cuneifolia root cultures increased to 2.5 times when treated with MeJA of 100 microM concentration (Awad et al., 2014).

Treatment interval

Each plant species require different time interval for maximum metabolite accumulation. It is generally preceded by an increased metabolic enzymatic activity. A study described that the anthraquinone content was doubled when Pythium aphanidermatum was used to treat Rubia tinctorum cell cultures which was preceded by an increase in the activity of isochorismate synthase (Van Tegelen, 1999).

Growth stage

The elicitor should be added during the log phase of growth when the activity of enzyme is maximum (Vasconsuelo et al., 2003). Hence, an efficient response can be achieved.

Composition of medium

The growth factors in the medium also affect the elicitation. A study showed that if auxin is not provided in the medium, the carrot cells in the culture do not respond to elicitation (Kurosaki et al., 1985).

Light conditions

The light condition of culture also plays a major role. A study reported the production of hypericin in Hypericum perforatum L. cells showed higher cell growth under light than when incubated in the dark (Walker et al., 2002)

References


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