**Unveiling the Potential of Plant Tissue Culture: Enhancing Phytochemical Production Using Elicitation Strategies**

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**Abstract:**
Plant tissue culture is pivotal in both the theoretical and practical aspects of plant biology. It serves as a cornerstone technique for exploring morphogenesis, embryogenesis, clonal propagation, crop enhancement, the generation of pathogen-free plants, genetic manipulation, and the production of secondary metabolites. Numerous studies have targeted the production of phytochemical extracts in in vitro plant cultures, yet the production of individual phytocompounds remains elusive due to the complexity of biosynthetic pathways and extraction procedures. Elicitation methods are commonly employed to enhance phytochemical production, often yielding higher quantities compared to non-elicited cultures. This increased production of bioactive phenolic extracts and compounds holds promise for their expanded utilization, particularly in functional foods and pharmaceuticals. Hence, this review seeks to examine how different biotic and abiotic elicitors impact the production of diverse phytoconstituents.

**Elicitors in Plant Tissue Culture**

Plants are complex organisms that play a significant role in our daily diet. Their nutritional value and component parts have been the subject of extensive research for decades. Alongside vital primary metabolites such as lipids, carbs, and amino acids, higher plants can also produce a variety of low-molecular-weight substances known as secondary metabolites. The diverse class of organic substances known as "plant secondary metabolites" are produced by plants to aid in their interactions with the biotic and abiotic environments to develop defense mechanisms (1). Elicitors are chemicals that play a role in initiating defense responses (2). There are two different kinds of elicitors: chemical and physical. One type of physical elicitor is injury. Furthermore, chemical elicitors can be classified as either biotic or abiotic depending on their natural state (3). Biotic elicitors are compounds derived from biological sources, such as microorganisms and polysaccharides derived from plant cell walls (chitin, pectin, cellulose, etc.). Abiotic elicitors are substances classified as physical, chemical, or hormonal factors that do not originate from biological sources (4).

**Abiotic elicitors:**

**Radiation:**

Radiation is a physical elicitor that includes light, UV, and ionization radiation that aids in the formation of secondary metabolites. Fluorescent lights are typically employed for in vitro plant production; however, metal halide, sodium, or incandescent lights are also occasionally utilized in commercial and research labs. These lamps' spectral range, which spans from 350 to 750 nm and incorporates mixed lights, has an impact on the development of in vitro cultured plants. LEDs, or light-emitting diodes, are a new type of light source that can be utilized in plant culture systems (5). Ocimum basilicum L. seed growth and morphology have been greatly influenced by red light. In contrast, red light has produced more dramatic results than white light. Additionally, red light enhanced the plant's production of rosmarinic acid, and in that arrangement, the plant also showed enhanced antioxidant activity (6). Additionally, phenolic compounds of medicinal and chemopreventive activity, such as ferulic, caffeic, rosmarinic, and chlorogenic acids, were accumulated in the cultures of Moluccella laevis L. The growth and development of shoots were influenced by the spectrum of LED illumination; however, this effect only occurred when plant growth regulators were supplemented (7). Likewise, the interaction of temperature and light was successful in elevating the concentration of menthol and other monoterpenes in Mentha piperita L. (8). The light period was given in between the dark period at night and maintaining a warm temperature at night (8).

**Salinity:**

Soil salinity is the presence of soluble salts in soil or soil water at levels that are detrimental to plant growth (9). Salinity has a negative impact on plants, resulting in reduced growth and development of the plants. When the levels of salinity were increased in Mexican oregano, bugleweed, and cherry skullcap, they all showed a substantial decrease in leaf area, inflorescence count, shoot count, total shoot length, and dry weight. On the other hand, the level of secondary metabolites increased (10). In general, biotic or abiotic stressors enhance the synthesis and accumulation of polyphenols. Increases in rosmarinic and caffeic acids have also been observed in O. basilicum in various tissues when salinity rises (11). The effect of salinity stress on drought led to an increase in total polyphenols by around 25% in Citrus aurantium leaves (12). It is found that soil salinity decreases plant growth and development, but it also leads to an increase in essential oil production in Thymus vulgaris and Lavandula augustifolia (13).

**Temperature:**

Since decades, it has been known that all kinds of medicinal plants are affected by biotic and abiotic stress, among which temperature is also one kind of abiotic stress. It may result in an increase or decrease in the secondary metabolites of the plants. In Thymus vulgaris, all three factors- temperature, humidity, and rainfall- affect essential oil production, but when the temperature of the room increased by 5% and 10%, increased levels of metabolites were seen (14). The essential oil composition secretion was at its maximum at 92.41% in the month of July during its flowering stage in Micromeria fruticosa (15). It was found that at the application of 40˚C, a maximum of α-caryophyllene and Cis-α-bergamontene secretion was seen in Ocimum basilicum L. and Salvia officinalis L. (16). Temperature stress also affects the levels of osmoprotectants and phenolic compounds at considerable levels, especially rosmarinic acid in Thymus lotocephalus and Lavandula viridis (17). The content of sesquiterpenes (essential oils) increased to moderate levels after the application of frost in Aloysia triphylla (18).

**Water:**

Water stress, which includes a decrease and increase in water levels, affects plant growth and development in many ways. Drought, climate change, nutritional shortages, and photodamage operating both singly and in concert may be the key factors influencing the synthesis of terpenes and other secondary metabolites as well as their evolutionary progression (19). In one study involving Salvia officinalis plants, researchers found that under 100% of field capacity (water control treatment), the essential oil concentration grew to 0.39%; under moderate (50% of field capacity) and severe (25% of field capacity) water levels, respectively, the essential oil content climbed to 1.77 and 1.01% (20). It has been proven that drought affects the amount and composition of essential oils. The genotype of Lavandula stricta possessed the maximum proportion of essential oil and especially the highest percentage of linalool under maximum levels of water deficiency (21). Under drought conditions, there was an increase in the concentration of essential oils in Satureja hortensis and Lavandula angustifolia. This could be connected to a higher density of oil glands, mostly because of the loss in leaf area caused by the stress of the water deficit (22).

**Mechanical stress:**

Mechanical stress in plants can be caused by various means, such as wind, hailstorms, rain, and animal movements. In Thymus algeriensis and Rosmarinus officinalis L., there was an increase in the accumulation of essential oil with the increase in precipitation, wind speed, and hygrometry. While there was a significant decrease in the essential oil when exposed to air humidity and aridity (23), In Lavandula augustifolia Mill., oxygenated monoterpenes (OM, 61.2–78.1%) were the most prevalent volatile organic compound (VOC), with monoterpene hydrocarbons (MH, 8.9–22.4%), sesquiterpene hydrocarbons (SH, 4.8–20.5%), and oxygenated sesquiterpenes (OS, 0.1–1.9%) following in order of abundance. Interestingly, the scent of the populations at the lowest altitudes tended to have higher concentrations of essential oils (24). Artificial damage and wounding in Minthostachys mollis resulted in the release of an abundant amount of volatile terpenoids (25).

**Chemical stress:**

In plants, chemical stress can be in the form of pollutants, heavy metals, pesticides, aerosols, gaseous toxins, and mineral salts. In Melissa officinalis L., with the increase in cadmium levels, the leaf growth and pattern reduced; however, the plant started synthesizing more bioactive compounds (26). Exposure of Salvia officinalis leaves to ozone resulted in an increase in phenolic compounds in addition to PAL and PPO activities. While the effect of ozone exposure was different for terpenoids, it led to an increase in the levels of sesquiterpenes, but it also led to a decrease in the levels of monoterpenes (27). Elevated ozone levels in Salvia officinalis have resulted in an increase in the levels of gallic acid, rosmarinic acid, catechinic acid, and caffeic acid (28). Elevated ozone and carbon dioxide levels in Melissa officinalis have also been studied, and results have shown an increase in the levels of phenols, anthocyanins, and tannins (29).

**Hormonal Elicitors:**

Plant hormones play a crucial role in signaling pathways and responses to various environmental stresses, such as biotic and abiotic stress. Exposure of plants to different plant hormones leads to changes in metabolism, gene expression, secondary metabolite production, and plant defense mechanisms (30). The interaction type and duration between the plant and the environment stress also regulate the complex signaling metabolite pathways (30). Elicitation studies with salicylic acid and methyl jasmonate have led to a significant increase in essential oil accumulation in Hyssopus officinalis, Origanum majorana, Mentha piperita, and Ocimum basilicum (31). Studies in Ajuga bracteosa have shown an increase in secondary metabolite accumulation in cell cultures when elicited with phenyl acetic acid and methyl jasmonate (32). Elicitation with salicylic acid and methyl jasmonate has been found to increase the levels of phenolics, flavonoids, and anthocyanin production in several plants and overexpression of several genes (33). Studies in Lavandula augustifolia Mill. showed that in the presence of salicylic acid and activated charcoal, a huge number of shoots were produced, and the shoots produced were taller in length than the untreated ones (34). The treated shoots also accumulated a higher number of polyphenols and carotenoids. The combination of growth hormones such as auxins and gibberellic acids has also led to an increase in the number of secondary metabolites in a few medicinal plants (35). The effect of different biotic and abiotic elicitations depends on plant species, duration of exposure, type and age of the cultures, and the specific targeted secondary metabolites in the Asteraceae family (36).

**Biotic Elicitors:**

Biotic elicitors are defined as materials added in trace amounts to a living organism to initiate the synthesis of a certain molecule that is crucial to the plant system's adaptation and ability to withstand stressful environments. Biotic elicitors are compounds with a biological origin that are further classified into two categories: microorganism-based and polysaccharide-based (37). Chitin, lignin, pectin, and cellulose are examples of polysaccharide elicitors that are extracted, isolated, and purified from the cell walls of biological creatures, whether they are obtained from plants or animals. Bacteria, fungus, and yeast cell extracts are examples of microorganism-based elicitors (38).

**Microorganism elicitors:**

This includes categories such as bacterial and fungal elicitors. Bacterial elicitors are the elicitors derived from single-celled microorganisms in the environment. Elicitation with the application of these microorganisms is known as bacterial elicitation. Fungal elicitors, both free-living and endophytic, are the most significant and commonly used in the production of commercial chemicals. The fungi and plant interact to cause hypersensitive reactions in the plant, which activate defense mechanisms and boost the production of secondary metabolites more efficiently. The hyphal tip culture is typically used to obtain pure fungal cultures (39). Studies in *Rhizobium radiobacter*, *Pseudomonas thivervalensis*, *Pseudomonas frederiksbergensis* showed significant improvement in the production of tanshinones due to the increase in the production of the major enzymes 3-hydroxy-3-methyglutary1-CoA reductase and 1-deoxy-D-xylulose-5-phosphate synthase involved in the tanshinone biosynthetic pathway in *Salvia miltiorrhiza* hairy roots (40).

**Polysaccharide elicitors:**

Signaling molecules within elicitation pathways known as polysaccharide elicitors are being researched because they can elicit a defense response in plants that is comparable to that produced by pathogen invasion. In Hypercium perforatum shoot cultures, the generation of phenylpropanoid and naphthodianthrone was influenced differently by polysaccharides such as chitin, pectin, and dextran. Few caused the CHFI activity to be reduced, which limited the formation of flavonoids, but few also caused the alternative naphthodianthrone pathway to be activated (41). Carrageenan is a polysaccharide used as an additive agent and is isolated from red seaweed (Irish moss). Studies show that after applying carrageenan treatment, basil plants showed a substantial increase in shoot length and leaf area, as well as a 26% reduction in Cuscuta campestris infection. Basil's PAL activity, phenol concentration, antioxidant capacity, and lignin content all significantly increased after being treated with carrageenan (42).

In conclusion, the utilization of plant tissue culture techniques presents a multifaceted approach to advancing our understanding and application of plant biology. Through the exploration of various biotic and abiotic elicitors, researchers have unveiled significant insights into the production of diverse phytoconstituents, ranging from secondary metabolites to essential oils. While challenges persist in isolating individual phytocompounds, elicitation methods offer promising avenues for enhancing phytochemical production, thereby expanding their potential utilization in functional foods and pharmaceuticals. Continued research in this field holds immense promise for addressing societal needs and advancing human health and well-being by harnessing the bioactive potential of plant-derived compounds.

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