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Production of Secondary Metabolites through Elicitors: Their Application in Agriculture

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ABSTRACT

Numerous papers have provided information on the types and role of secondary metabolites in medicine, but in the field of agriculture there are few. In this review it is explained how different biotic and abiotic elicitors are applied as effective methods in different agricultural crops to stimulate the accumulation of secondary metabolites, as well as the role that these metabolites have in agricultural production. Elicitors mean agents that stimulate plant protective responses. In addition to the types of elicitors (chemical, physical and biological), dosage and treatment regimen are the main factors determining the effects on the production of secondary metabolites. In agriculture, secondary metabolites find many applications such as protection of plants against microorganisms, and insects, as phytohormones and help decompose the soil by increasing the amount of macro and microelements.

1. Introduction

Living organisms face abiotic and biotic stresses every day. Darwin, in his study of evolution, shows that "survival of the fittest" or "natural selection" enables the strongest organism to survive and reproduce. Stronger organisms have the genetic potential to protect themselves, or to resist or avoid the consequences of stress that disrupt their physiological functions, allowing them to grow, develop, and survive [1], [2]. Plants as living organisms are constantly vulnerable to environmental stresses such as pathogens, salinity, drought, UV radiation, extreme temperatures, and nutritional imbalances [3], [4]. Plants contain secondary metabolites to protect themselves against these stresses [5]. Plant secondary metabolites are compounds that have no role in maintaining the underlying plant processes, but they do play an important role in the interaction of the plant with its environment. The production of these compounds is often low (less than 1% dry weight) and depends highly on the physiological and developmental stage of the plant [6], [7]. Secondary plant metabolites include peptides, amines, glucosides, alkaloids, terpenes, glucosinolate, cyanogenic polyacetylene, quinones, and phenolics [8]. The use of plant metabolites began as early as 2600 BC, while 4000 years later, secondary metabolites of plant origin were used for medicinal and nutritional purposes. The first natural product isolated from the opium poppy (*Papaver somniferum*) was morphine [9]. Primary metabolites affect the normal growth and development of plants, as well as serve as precursors to produce secondary metabolites which are only involved in protecting plants from various environmental stresses [10]. Plant protection against stress is enabled by the phytohormones of jasmonic acid (JA) and salicylic acid (SA), and associated pathways that interact in a complex way at transcript and protein levels. After application with JA and SA, negative effects on the survival of chewing insects (*Heliothis virescens*) and sucking insects (*Myzus persicae*) on plants were reported [11]. In Agriculture, large amounts of fertilizers, pesticides and phytohormones are used to enable the growth and development of agricultural crops and to withstand the attacks of various pathogens [12]. Nowadays, attempts are being made to reduce the use of these chemicals, due to environmental pollution and endangering human health. Secondary metabolites in plants can be used in various aspects such as pharmacy, cosmetics, food supplements, and agrochemicals (toxins, benzaquninoids, and peptides) [13].

In recent years great importance has been given to the study of secondary metabolites in plants, but the published articles are more focused on the use of secondary metabolites in medicine, pharmacy, and cosmetics and in terms of their application in agriculture there are fewer such. This review explains the production of secondary metabolites in plants using biotechnological methods as well as the role of secondary metabolites in plants and their application in agriculture.

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2. Types of Secondary Metabolites

Primary metabolites enable processes such as photosynthesis, respiration, and translocation, while secondary metabolites do not participate directly in plant growth and development. There are 3 classes of secondary metabolites of plants based on their biosynthesis pathway: 1. Phenolic group, 2. Terpenoids and steroids, 3. Nitrogen and Sulphur-containing group [14], [15] (Figure 1).

2.1. Phenolic Compounds

Phenols are compounds that have one or more aromatic rings attached to one or more hydroxyl groups. They represent the most prevalent plant secondary metabolites. There are more than 8000 phenolic structures known to date, ranging from phenolic acids to substances such as tannins [16]. Phenolic plant compounds play an important role in protecting against ultraviolet radiation, parasites, and plant pathogens; also contribute to the colors of flowers and fruits of plants [17]. Based on the number of phenol units in the molecule, phenolic compounds are divided into simple phenols and polyphenols. Based on this classification, they include simple phenols, lignans, lignins, coumarins, condensed, and hydrolyzable tannins, phenolic acids, and flavonoids [18]. While based on their structure, phenolic compounds are classified into flavonoids and non-flavonoids [19].

2.1.1. Flavonoids: Flavonoids are a large group of polyphenolic compounds that have a benzo-pyrene structure and are known about 4000 flavonoids with plant composition [20]. The chemical nature of flavonoids depends on their structural class, degree of hydroxylation, substitution and other conjugations, and degree of chemical polymerization [21]. Flavones, chalcones, flavanols, flavanones, isoflavones, and anthocyanins are the main flavonoids [15], [22]. Flavonoids play an important role in combating oxidative stress; they promote the physiological survival of the plant, protecting it from fungal pathogens and UV-B radiation. Also, flavonoids are involved in energy transfer, act as plant growth hormones, and affect respiratory control, photosynthesis morphogenesis, and sex determination [17]. Studies of *Arabidopsis* flavonoid mutants have provided new support for a role for flavonoids in auxin transport. The *transparent testa* (*tt*) mutants, which are deficient in CHS activity, have been shown to have elevated auxin transport and altered growth phenotypes that are consistent with this. These mutants also accumulate more indole acetic acid (IAA) in the upper root than do wildtype controls, and IAA appears to leak from the root tip of these mutants. Flavonoids have been shown to accumulate in the cotyledonary node, the hypocotyl–root transition zone, and the root tip of young *Arabidopsis* seedlings [23].

2.1.2. Non flavonoids: Non-flavonoids consist of phenolic acids, polyphenols, tannins, hydroxycinnamates, stilbene, and their conjugated derivatives. Phenolic acids are the most populous non-flavonoid group of plants [13]. Phenolic acids are also known as hydroxybenzoate, where the main ingredient is gallic acid. The name is derived from the French word galle, which means a swelling in the tissue of a plant after an attack by parasitic insects. Swelling comes from the accumulation of carbohydrates and other nutrients that support the growth of insect larvae [24], [25]. Phenolic acids have two main structures: i) hydroxycinnamic (derivatives include ferulic, caffeic, p-coumaric and synapic acids) and ii) hydroxybenzoic acid (derivatives consisting of gallic, vanillic, syringic and proto-acetic acids) [26]. They are produced in plants via visual acid via the phenylpropanoid pathway, as by-products of the monolignol pathway and as products of cell wall lignin and polymer degradation in vascular plants [27].

2.2. Terpenoids and Steroids

Terpenoids and steroids represent the main group of substances that are produced biosynthetically from isopentenyl diphosphate. To date, over 35,000 different terpenoids and steroids have been identified [28]. Terpenoids are synthesized through the condensation of isoprene units (C5) and are classified according to the number of five-carbon units present in the nucleus structure [29], [30]. Terpenoids have a variety of unrelated structures, while steroids have a common tetracyclic carbon skeleton and are modified terpenoids that are biosynthesized from lanosterol triterpene [28]. Terpenes are divided into monoterpene, sesquiterpene, diterpene, triterpene, and politerpene [31]. They play an important role as protectors for wood tissues, have antibacterial effects, are responsible for attracting and repelling insects, and represent the basic material for the synthesis of vegetable hormones or pigments (chlorophyll and carotenoids), and participate in the transport of mitochondrial electrons and plastoquinone [31].

2.3. Sulphur and Nitrogen-Containing Compounds

Secondary plant metabolites such as alkaloids, cyanogenic glucosides, and non-protein amino acids, contain nitrogen units in their structure and are biosynthesized from natural amino acids [13]. Alkaloids are a large group of secondary metabolites, with more than 12,000 isolated substances [32], [28]. Alkaloids are divided into three main groups based on their biosynthesis: a) True alkaloids (eg, morphine, nicotine, quinine and atropine); b) Pseudo-alkaloids (e.g., solanidine, capsaicin, and caffeine); and c) protoalkaloids (e.g., mescaline, yohimbine, and hordenine). True alkaloids and protoalkaloids are derived from amino acids, but pseudo-alkaloids are not produced from amino acids. They play an important role as a system of chemical protection of plants against the risk of predators (insects, larvae, mammals, herbivores). They act as antibiotics and pesticides preventing ingestion of plants [33]. Alkaloid toxicity to herbivores results from disruption of neuronal signal transduction and

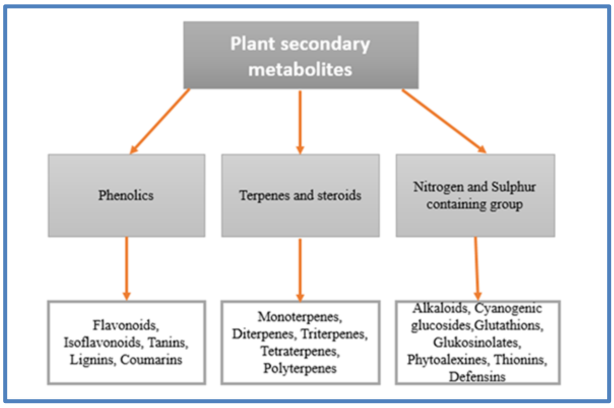

Fig. 1 The three major types of secondary metabolites [15].

interference [34]. The group of secondary metabolites containing sulphur includes GSH, GSL, phytoalexins, tions, defensins, and alinins. These elements act directly or indirectly on plant defense mechanisms against various pathogens, and are involved in several types of chemical protection, constitutive protection, promoted in a large group of plant species [35], [36], [37].

3. Production of Secondary Metabolites through Elicitors

3.1. Elicitors

Elicitors represent a group of biotic or abiotic signals which, when applied in low amounts to the living organism, stimulate or improve the biosynthesis of specific secondary metabolites, thus causing protection from various stresses [38], [39], [40]. Based on their "nature", elicitors are divided into two groups: abiotic elicitors and biotic elicitors. However, based on their "origin", we distinguish exogenous elicitors and endogenous elicitors [41]. Abiotic elicitors are composed of substances that are of non-biological origin and are distinguished by physical, chemical, and hormonal factors, while biotic elicitors are substances of biological origin and include polysaccharides originating from plant cell walls (e.g., pectin, chitin, cellulose) and microorganisms [42]. Exogenous elicitors are a group of chemicals derived from pathogens such as polysaccharides, peptides, fatty acids, polyamines, and glycoproteins, while endogenous elicitors are chemicals found within the plant cell and play a key role in the intercellular signal. They are released from the plant cell wall and intercellular signaling compounds such as MJ, SA, JA [43]. Different elicitors act as signals; and the process begins with the perception of that signal by specific receptors (elicitors present in the plant cell membrane) followed by the beginning of the signal transduction cascade, and eventually changes the level of expression of genes that regulate transcription and increased synthesis of secondary metabolites in plants [44], [43] (Figure 2).

3.1.1. Production of secondary metabolites through abiotic elicitors: Plants are regularly exposed to environmental stresses, such as temperature, salinity, drought, UV alkalinity, pathogens and herbivores, which endanger plant growth and development [3], [45]. On the other hand, plants being exposed to abiotic and biotic stresses can biosynthesize secondary metabolites to adapt and survive environmental stress [46], [47]. The synthesis of secondary metabolites from abiotic elicitors in plants is briefly discussed below.

3.1.1.1. Temperature: Although temperature stress negatively affects plant growth and development, high temperatures (heat stress) and low temperatures (cold stress) in turn increase secondary metabolite production [48], [42]. High temperatures affect leaf aging and concentrations of the secondary root metabolite in the plant *Panax quinquefolius*. By increasing the temperature to 5 °C in this plant, the ginsenoside content increased significantly [49]. Accumulation of hydroxycamptotechin has been reported to increase 6-fold in *Camptotheca acuminata* leaves during incubation at 40 °C for 2 hours [50]. *Melastoma malabathricum* cell cultures incubated at a lower temperature interval (20 ± 2 °C) grew better and had higher anthocyanin production than those grown at 26 ± 2 °C and 29 ± 2 °C [51]. The content of hyperforin and hypercine increased significantly in the shoots of *Hypericum perforatum* staying for fifteen days at 35 °C [52]. Various cultivars of *Lupinus angustifolius* exposed to high temperatures also significantly promote the accumulation of alkaloids [53]. Under conditions of temperature stress, phenylamides have reactive properties to reactive oxygen species (ROS) in bean and tobacco plants [54].

3.1.1.2. Salinity: Salinity is an abiotic stress that reduces plant growth and development, as well as altering physiological and metabolic processes [55], [56]. Salinity leads to dehydration of the cell and this causes osmotic pressure that increases or decreases the accumulation of secondary metabolites in plants. However, salt stress can also act as a stimulant of secondary metabolites to protect cells from oxidative damage caused by the accumulation of ions at cellular and subcellular levels while reducing the toxic effect of salt. These secondary metabolites include alkaloids, terpenoids, flavonoids, steroids, and phenols [57], [58]. In the plant *C. roseus* grown under salt stress showed higher levels of the vincristine alkaloid [59]. Salt stress also increased the content of diamine and polyamine in *Oryza sativa* [60]. In salt-tolerant alfalfa, proline levels increased under the influence of salt stress, and this occurred similarly in *Aegiceras corniculatum* and *Lycopersicon esculentum* [61].

3.1.1.3. Drought: Drought is one of the abiotic stresses which play a key role in plant growth and development [62]. Environmental conditions such as high temperatures as well as solar radiation cause water scarcity in the soil, which is known as drought stress [63]. Plants, to adapt to drought stress, have accumulated amounts of secondary metabolites such as terpenoid alkaloids and phenolic complexes, through the induction of osmotic stress [64]. Lack of water had increased the content of anti-inflammatory saponins in the plant *Bupleurum chinense* [65]. Also, in the plant *Salvia officinalis* the amount of terpenes increases during the lack of water [66].

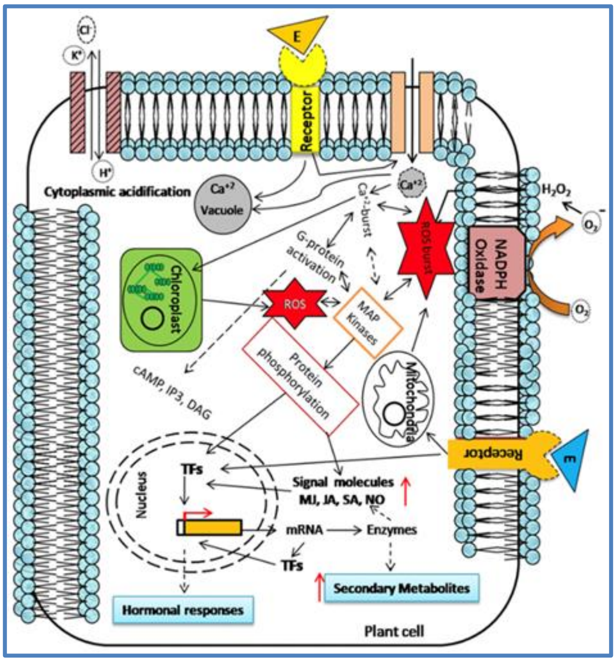

Fig. 2 Schematic representation of mode of action of elicitor in plant cell [43].

Drought causes oxidative stress, but also increases the amount of flavonoids that enable plants to protect themselves from this stress by taking on the role of antioxidant. Specifically in the tomato plant to detoxify H_2O_2 molecules quercetin and kaempferol levels increased during drought stress [67]. Also, in the plant *Hypericum brasiliense*, in the absence of water, the content of phenols and betulinic acid increased significantly [68].

3.1.1.4. Light and UV radiation: Light is an important factor in the synthesis of bioactive compounds and secondary metabolites in plants. Photoperiods, quality, and power are the components that play a role in light [12], [69]. Nowadays, the effect of UV radiation is a major concern which affects the concentrations of secondary metabolites of plants such as flavonoids, alkaloids, terpenoids, tannins, cyanogenic glycosides, and anthocyanins [70], [71]. However, UV radiation (UV-A, UV-B and UV-C) can be used as an abiotic elicitor in plants causing an increase in secondary metabolites [72], [73]. In *Pinus contorta*, it was found that the concentration of anthocyanin was lower when the plant was grown in short conditions of sunlight and its concentration was higher with long light conditions [74]. Radiation also increases the amount of secondary metabolites in plants; for example, in *Catharanthus* species the amount of catarantine and vindoline increased significantly after treatment with UV-B radiation [75].

3.1.1.5. Heavy metals: Heavy metals are important factors in the production and accumulation of secondary metabolites in plants [76], [77]. One of the heavy metals that acts as an abiotic elicitor to stimulate the production of secondary metabolites in plants is Ag^+. Application of 15 µM Ag^+ to *S. castanea Diels p. tomentosa* had increased the amount of tanshinone 1.8-fold compared to control. Also, in the wood species *Populus x canescens*, application of $CdSO_4$ amount caused an increase in soluble phenolic protective compounds in plants [78]. In general, in plants, an increased level of phenolic compounds (flavonols, hydroxycinnamic, and hydroxybenzoate), the total content of phenol and flavonoids are being treated with CuO due to the regulation of phenolic biosynthetic genes such as CHI, PAL, and FLS [79], [43].

3.1.2. Production of secondary metabolites through biotic elicitors: Biotic elicitors represent a group of organic substances that contain carbohydrates in their content and exhibit their effects in low concentrations [80]. The effect of these elicitors on the production of secondary metabolites in plants is discussed below.

3.1.2.2. Polysaccharide: Biotic elicitors, specifically polysaccharides, are widely used to increase the production of secondary metabolites in plants. In a Panax ginseng cell suspension, oligogalacturonic acid had significantly increased saponin content [81]. In S. miltiorrhiza roots, the tannin content increased approximately sevenfold (1.59 mg / g versus 0.19 mg / g) from a crude polysaccharide (designated as BPS) isolated from B. cereus cell extract [82]. Also in P. ginseng cell suspension cultures, the saponin content increased significantly when oligogalacturonic acid was used as the elicitor [81]. Similarly, seplumbagine content in Plumbago rosea increased because of treatment with chitosan [83].

3.1.2.2. Fungal elicitors: Various pathogens are used as biotic stimulants, especially to activate plant defense systems [84]. Fungi used as stimulants include pathogenic and endophytic fungi as well as non-plant-related fungi. Pathogens such as *Botrytis sp*. usually secrete toxins that kill host cells, while *Fusarium sp*. try not to kill the host cells, but to change the host's metabolism and secretory systems [85], [42]. In *C. roseus* cell suspensions, fungal cell wall fragments caused the alkaloids serpentine, indole ajmalicin, and catarantine to increase up to fivefold [86]. When fungal mycelium is used as a stimulant in *Dioscorea deltoidea*, the diosgenin content increases up to 72% in plant cells [87]. The use of fungal spores as elicitors in *Papaver somniferum* increased the content of morphine, codeine and sanguinarine by over eightfold [88], [42].

3.1.2.3. Bacterial elicitors: Bacteria are used as biotic elicitors to produce secondary metabolites in many plants. In the hairy root culture of *Scopolia parviflora*, activation of scopolamine synthesis and inhibition of H6H expression (hyoscyamine 6β hydroxylase) were observed [3]. Furthermore, in the roots of *Tavernia cuneifolia,* an increase in glycyrrhizic acid content was observed after treatment with *Rhizobium leguminosarum, Agrobacterium rhizogenes, Bacillus cereus,* and *B. aminovorans* [89]. In the seedlings of *H. perforatum,* an increase in the content of pseudohypericin and hypericin was observed after treatment with *Rhizobacterium* [90], [42].

4. Applications of Secondary Metabolites in Agriculture

Secondary metabolites find many applications in agriculture: They help protect against various microorganisms such as viruses, bacteria, fungi, herbivores, arthropods, and vertebrates [91]. Also, they participate in maintaining redox balance by clearing ROS. Secondary metabolites are found in plant root debris that can kill or inhibit herbivorous insects, subterranean microbes, and nematodes [92], [93]. Crystals of *B. thuringiensis* and spinosynat are used as bioinsecticides; Polyoxins and casugamycin are used as biopesticides; Doramectin and ivermectin are used as anthelmintics and endectocide against lice, worms, ticks, and mites; Phytohormones such as gibberellins are used as growth regulators (Demain, 1999; Thirumurugan et al., 2018). Secondary plant metabolites are also used in decomposition soil by increasing nitrogen immobilization in the soil. When the cycle of nitrogen (N) and carbon (C) is affected by tannins and terpenes [94], [93].

4. Conclusion

Nowadays there is a great interest of researchers in the production of secondary metabolites because they offer the possibility of their application in various fields such as medicine, pharmacy, and agriculture. It has been proven that these metabolites can play a key role in maintaining the health and productivity of agricultural crops, even under different conditions of environmental stress. Different forms of elicitation can be effectively used to produce secondary metabolites. This review will be a comprehensive reference for all those interested in the production of secondary metabolites through elicitation and their application in agriculture.

Declaration

Author Contribution: Conceive - M.J., S.D; Design - M.J.; Supervision - M.J., S.D.; Literature Review - M.J., S.D.; Writer - M.J., S.D.; Critical Reviews - S.D.

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