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Chapter

# Effect of Biotic and Abiotic Stresses on Plant Metabolic Pathways

*Venkanna Banothu and Addepally Uma*

## Abstract

Plants are prone to encounter some environmental stresses that include both biotic and abiotic. Plants in response to these stress conditions alter their metabolism at the genetic level with consequential effects at the metabolite production. Phenolic compounds, which are secondary metabolites are one such chemical entity which plays a significant role in various physiological processes of the plant. They are mainly formed by three different types of metabolic pathways that produce phenyl propanoid derivatives, flavonoids, terpenoids based on the needs of the plant and the rate of their production is solely dictated by the type of stress condition. A number of phenolic compounds like phytoalexins, phytoanticipins and nematicides exhibit negative response to biotic stress against several soil borne pathogens and nematodes. But some of the phenolic compounds like acetosyringone, umbelliferone, vanillyl alcohol, *p*-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, apigenin and luteolin are found to exhibit beneficial effects to plants by encouraging rhizosphere formation particularly in Leguminosae family. Some of the ROS produced in various stress conditions are effectively dealt by various phenolics with antioxidant activity like hydroxyl benzoic acids and hydroxyl cinnamic acids. As the in vivo production of phenolics in plants is influenced by external factors it can certainly provide information for the adoption of agronomic practices to yield the full befits of commercial exploitation. As the in vivo production of phenolics in plants is influenced by external factors it can certainly provide information for the adoption of agronomic practices to yield the full befits of commercial exploitation.

**Keywords:** Phenolics, Plant Secondary metabolites, Plant physiology, Natural bioactive molecules, Antioxidant activities

## 1. Introduction

Every living organism shares some basic features like order, sensitivity or response to the environment, reproduction, growth and development, regulation, energy processing, and evolution with adaptation [1]. A basic concept in classical genetics emphasizes that the phenotype of the organism is based on the interaction of genotype with the environment. The emergence of specific natural products is dependent on the highly ordered interaction between plants with the biotic and abiotic environments around them [2]. Plants are sessile organisms and respond to the stress conditions by changing the expression levels of certain genes involved in the production of metabolites which are secreted in response to its interaction with its environment [3]. The various

metabolic pathways produce different types of metabolites and based on the pathway they are classified as primary and secondary, although a strict demarcation is difficult to draw between them. The primary metabolites are essential to the cellular growth and reproduction whereas the secondary metabolites although not required directly for the same, these are the compounds that are synthesized in response to any biotic or abiotic stress which may be exogenous or endogenous the cell [3].

The production of secondary metabolites is infact influenced by primary metabolites. Some of the C and N fluxes can be diverted for the production of secondary metabolites during the stressful conditions and there is always a dynamic balance maintained between the two based on the cellular needs. In comparison with the primary metabolites the concentration of secondary metabolites is low and the type of secreted plant secondary metabolite (PSM) is based on the type of stressful physiology induced by biotic and abiotic stress condition. Some of the secondary metabolites are acts as regulators of development, growth and defense. Some of these compounds can be reintegrated into plant primary metabolism [4] *senu lato*.

The regulation of production of PSM involves extensive cross talk and signaling pathways with the key roles played by molecules like salicylic and jasmonic acids, calcium, abscisic acid, spolyamines and nitric oxides [5–8].

Over 2,14,000 types of secondary metabolites are known and are commonly classified according to their structure, function and biosynthetic pathway. Plant secondary metabolites can be classified into four major classes: i) Terpenoids, ii) Phenolic compounds, iii) Alkaloids and iv) Sulfur-containing compounds [9].

## 2. Types of stresses

The environment around the plant can influence the physiological condition of the plant and any disturbance in the external environment including physical factors and biological factors can influence the metabolic pathways in the cell. Accordingly, the types of stress conditions are defined as biotic and abiotic stresses, such as pathogen infection, water deprivation, salinization, high/low temperature stress, heavy metal toxicity, nutrient deficiency, atmospheric pollution and UV irradiation [10]. In addition to this endogenously generated stress also can influence the production of PSM which are consequential effect of external factors or molecules generated due to various physiological activities within the plant like reactive oxygen and nitrogen species (RONS) burden [10].

## 3. Phenolic compounds as secondary metabolites

The adoption of plants from aquatic habitat to terrestrial occurred at about 480–300 million years ago and to cope up with this change they adopted protective UV screens called phenolic compounds [11]. They constitute an important set of secondary metabolites which are ubiquitously spread in plant kingdom and the type of phenolic compounds differs in different genre of plant kingdom. Polyphenols are one of the important classes of specialized metabolites that play crucial physiological roles throughout the plant life cycle including responses to stress [12]. Therefore, as an adaptive response to adverse environmental conditions, phenolics are accumulated in various plant tissues which confers evolutionary fitness to the plant. Plants are continuously exposed to various biotic and abiotic stresses like intense light, low temperature nutrient deficiency, microbial infections with

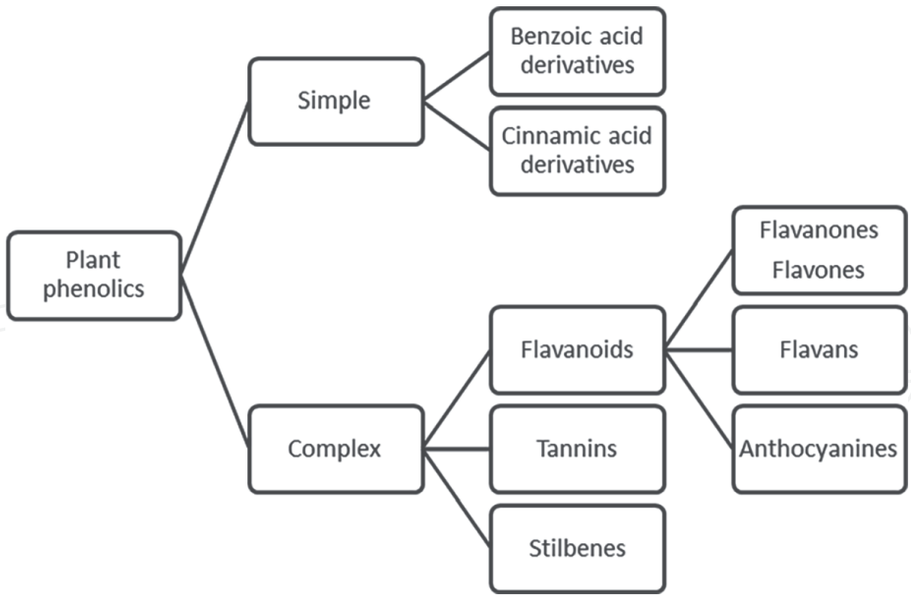



**Figure 1.**
*Classifications of the plant phenols based on their structure.*

increased free radical and other oxidative species and plant phenolics are compounds which play a defense role by scavenging these high reactive species [13]. Plants adapt themselves their phenolic patterns to a changing environment through the emergence of new genes brought about by gene duplication and mutation and subsequent recruitment for adaptation to specific functions [14]. Many of the genes related to secondary metabolism are duplicated in plant genome and many of these secondary metabolites production demands change in the amounts of precursors supplied by primary metabolism to balance the perturbations in chemical ecology [15, 16]. The induction of secondary metabolism gene expression by biotic and abiotic stress is often mediated by integrating signaling molecules such as salicylic acid, jasmonic acid, and their derivatives [17]. Flavonoids, stilbenes and proanthocyanidins are collectively grouped in polyphenols, the name indicating both the compounds with a second aromatic ring and those arising from the polymerization of flavonoidic/catechin units. The main structure in flavonoids is the flavan nucleus consisting of 15 carbon atoms which are arranged into three rings with two benzene rings (A & B) connected by an oxygen containing pyran ring (C). Various flavonoids differ in the level of oxidation and saturation of the C ring and accordingly are classified into flavanones, flavones, flavanals, flavanols and anthocyanidins. The individual compounds in a particular class of flavonoids differ in the sunstitution pattern of the A and B rings [18]. Due to the heterogeneous structures of these phenolic acids which range from low molecular weight single aromatic ring structure to high molecular weight polymeric compounds, they can be broadly classified into simple and complex phenolics (**Figure 1**).

## 4. Metabolic pathways for the formation of phenolic secondary metabolites

As discussed above both primary and secondary metabolites share some of the precursor compounds and there is delicate balance in the production of these two metabolites. Accordingly carbon fluxes are diverted between the pathways.

Phenolics are formed by three different biosynthetic pathways:

I. The **shikimate** is also termed as chorizmate or succinylbenzoate pathway, This produces the phenyl propanoid derivatives ($C_6$–$C_3$);

II. The **acetate** is also termed as malonate or polyketide pathway, this produces the phenyl propanoids with side-chain-elongates including the large group of flavonoids ($C_6$–$C_3$–$C_6$) and some quinones; and

III. The **acetate** is also named as mevalonate pathway which produces the aromatic terpenoids mainly monoterpenes.

The addition of hydroxyl groups to the phenyl ring plays the major role, which involved in the biosynthesis of phenolic acids [19].

I. Shkimate/Phenylpropanoids Pathway

The precursor compound for phenyl propanoid pathway is phenylalanine which can form various types of phenolics which range in the number of aromatic rings from 1 to 6 which also differ in the substitution pattern (**Figure 2**). Hydroxycinnamic acids (HCAs) ($C_6$–$C_3$) which include caffeic, ferulic, *p*-coumaric and sinapic acids varies in the degrees of hydroxylation and methylation at C6 position. The cleavage of aliphatic side chain of P-coumaric acid can lead to the formation of hydroxybenzoic acids like salicylic, vanillic, gallic and syringic acids.
The synthesis of chorismic acid from the precursors phosphoenol pyruvate and erythrose-4-phosphate acts as a precursor for the synthesis of cinnamic acid derivatives. Various derivatives of benzoic acids are formed from chorismic acid via oxidative and non oxidative pathways and the precursor for protocatechuic acid is isochorismic acid.
The condensation of 3 $C_2$ residues with an activated hydroxycinnamic acid products are two classes of metabolites with a second aromatic ring linked to the phenylpropanoid moiety, the flavonoids ($C_6$–$C_3$–$C_6$) and the stilbenes ($C_6$–$C_2$–$C_6$).

II. Acetate/Malonate or Polyketide Pathway

The acetate-malonate pathway is the fatty acids both those $1^o$ metabolites which arise universally and the more infrequent compounds with a limited distribution. This pathway also makes an important contribution to plant aliphatic and aromatic compounds; these are biosynthesised through the formation of polyketides.
Acetyl coenzyme-A is the precursor of the acetate-malonate pathway. This is a metabolite of very importance in both $1^o$ and $2^o$ metabolism. The metabolic pool of acetyl CoA is incessantly replenished by glycolysis and the catabolism of fatty acids and amino acids, which depletes for the synthesis of fatty acids, steroids, polyketides, terpenoids, aromatic compounds and acetyl esters and amides (**Figure 3**).

III. Acetate - Mevalonate Pathway

Mevalonic acid is the 1° precursor of steroids with phenolic ring biosynthesised by plants. It is consequent from acetyl CoA through the transitional formation of acetoacetyl CoA and 3-hydroxy-3-methylglutaryl CoA (HMG CoA) these reactions being catalyzed by acetyl CoA acetyltransferase and HMG CoA synthase respectively (**Figure 4**).

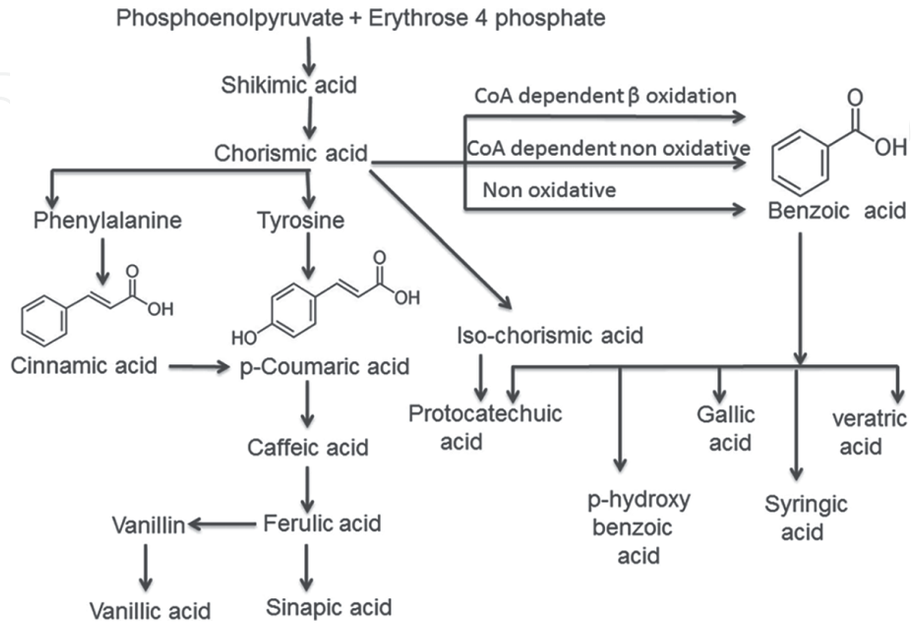



**Figure 2.**
*Synthesis of plant phenolic compounds by shikimate pathway.*

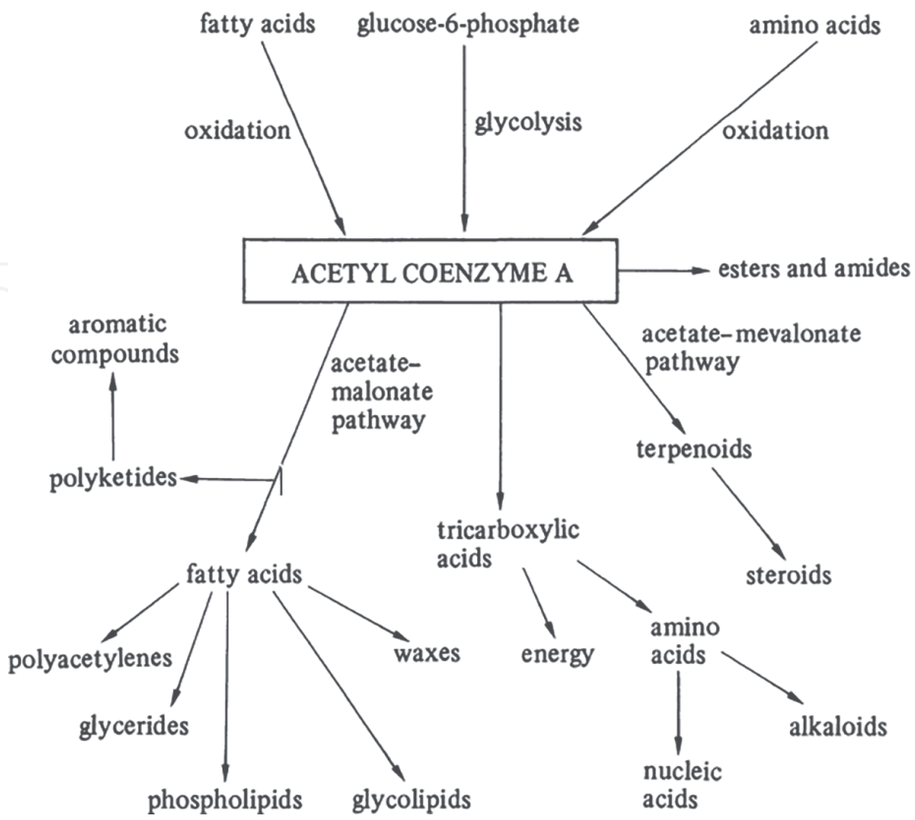



**Figure 3.**
*The acetyl coenzyme a metabolie process.*

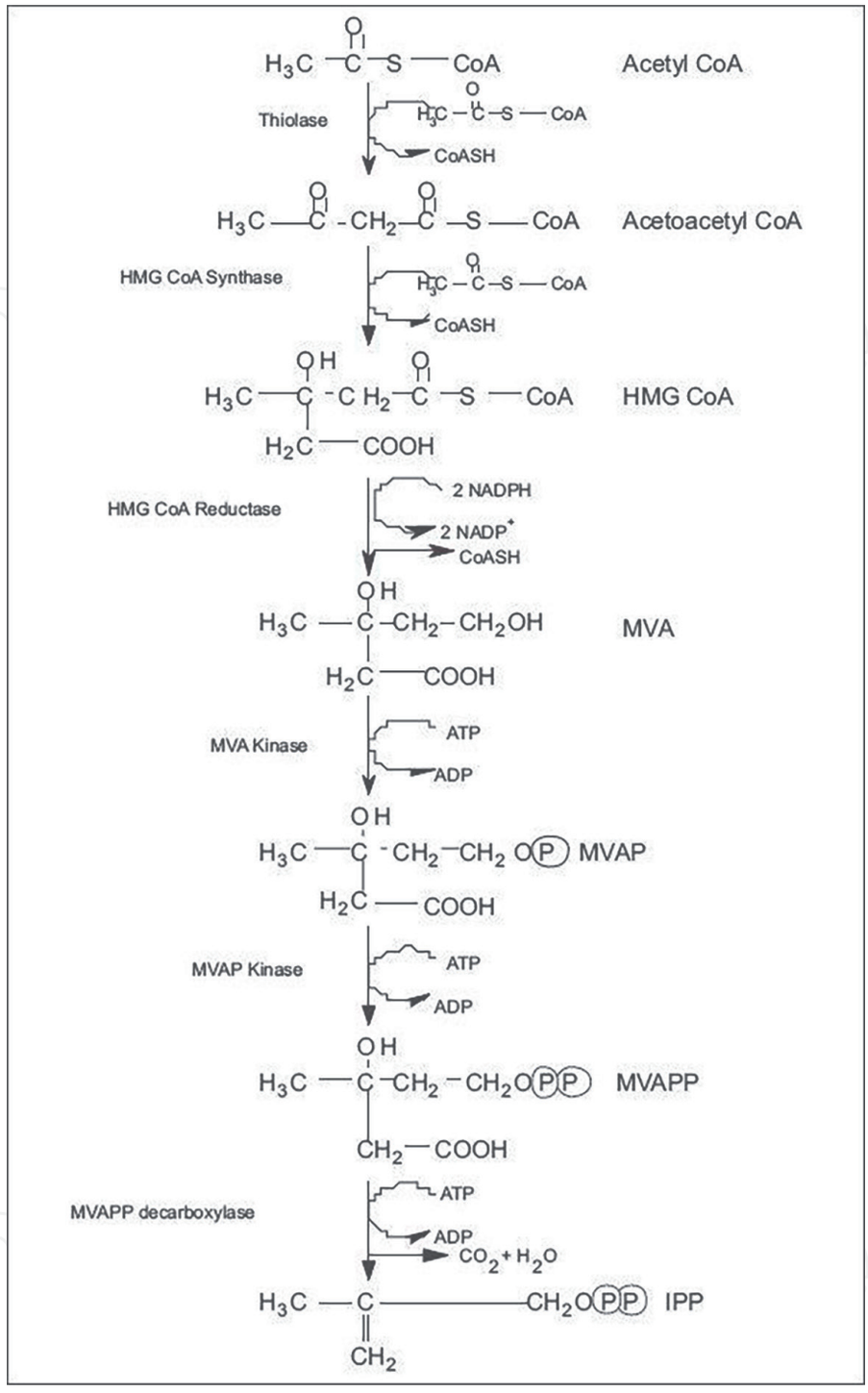



**Figure 4.**
*The acetate/mevalonate pathway for the formation of IPP, the basic five-carbon unit of terpenoid biosynthesis. Synthesis of each IPP unit needs three molecules of acetyl-CoA.*

## 5. Distribution of carbon fluxes between primary and secondary metabolism during stress conditions and regulation

The influence of exogenous/endogenous biotic and abiotic stresses influence the plant to make a trade off between growth & reproduction with defense mechanism intended to protect the plant. This feature in plants attracts special focus in plant ecophysiology. Primary metabolism which provides carbon skletons for the synthesis of PSMs including phenolics requires large amounts of available resources. The stress

condition leads to several biochemical and molecular mechanism triggering the adaptive response. As a part of this strategy cells try to divert the available carbon fluxes between primary and secondary metabolism and other limited resources. A considerable quantity of photosynthates are diverted to the production of phenolics and other PSMs. Lattanzio *et al.*, (2015) [20] proposed that there is a link between primary and secondary metabolism couples the accumulation of proline, a stress metabolite with energy transfer towards phenyl propanoid biosynthesis via the oxidative pentose phosphate pathway. Accordingly, some of the transduction pathways that involve a) proline redox cycle, b) pentose phosphate pathway are biased towards PSMs synthesis.

Phosphoenol pyruvate (PEP), a metabolite from glycolytic pathway is shared by four different metabolic routes leading to the formation of 1) TCA cycle, ATP generation and amino acid synthesis, 2) Methyl erythritol 4-phosphate pathway for the formation of isoprene units, 3) Shikimate-phenyl propanoid pathway for the formation of phenolic compounds, 4) Another anaplerotic route to refill PEP with PEP carboxylase. The stress conditions which favors phenolic formation increases the gene expression of shikimate dehydrogenase, phenyl ammonia lyase, chalcone synthase and PEP carboxylase specific enzymes involved in their production [21].

Biotic stress induced by *Amphibolis michoacaensis* induces gall formation in *Quercus castanea* wherein there is upregulation of phenolic related genes Phenylalanine ammonia lyase (PAL) at the intermediate and late growth stages; Phenyl propanoid genes at the intermediate stage and lignin genes at late stage. There is differential regulation of molecular switches related to secondary metabolites production during different stages of gall formation [22].

Infestation of rice by brown planthopper (BPH) in rice is observed to increase expression of *OsPAL6* and *OsPAL8* for the synthesis of phenylalanine ammonia lyase (PAL) through direct up-regulated by OsMYB30, an R2R3 MYB transcription factors [23].

Plants possess an effective immune system to combat most microbial attackers . There is an analogous immune system in plants like in animals to combat the microbial infestation. Salicylic acid is one of the hormone which is triggered in response to biotic stress. The immune response elicited leads to massive transcriptional reprogramming, cell wall strengthening, production of secondary metabolites and antimicrobial proteins [24].

Another mode of immune response to stress conditions is to influence epigenetic modifications in the development of stress memory. This is particularly of significance in high temperature heat shock stress. These modifications in turn can activate heat shock responsive genes and transcriptional factors by providing conceptual frame work for understanding molecular mechanisms behind the 'transcriptional stress memory' as potential memory tools in the regulation of plant heat stress response (HSR) [25].

## 6. Phenolics as antioxidants

Aerobic metabolism induces the formation of reactive oxygen and nitrogen species (RONS) radicals whose levels are expected to increase in various types of stress conditions. Plant phenolics are powerful antioxidants that can mediate scavenging of harmful reactive oxygen species (ROS). The antioxidant activity of phenolics is based on number of hydroxyl groups, the presence of alkyl chains and the number of unpaired electrons. Phenolic acids form stable phenoxyl radicals in reaction with radical molecules. Ellagic acid is a powerful antioxidant as it is high in hydrogen bonds so that they can act as electron acceptors and hydrogen donors. Hydroxycinnamic acids (HCAs) are

more effective antioxidants than hydroxybenzoic acids (HBAs). Some of the compounds like ferulic acid not only act as antioxidants but also inhibit enzymes involved in free radical generation and activate other scavenging enzymes [26, 27]. The type of antioxidants and their quantity is dependent on the type of endogenous or exogenous stress and to exploit these antioxidants for human use and commercialisation induction can be achieved by exposing the plants to the selected exogenous stress conditions.

## 7. Conclusions

(Poly)phenols are plant secondary metabolites that constitute one of the most common and widespread groups of substances in plants. Plants adapt themselves their phenolic patterns to a changing environment through the emergence of new genes brought about by gene duplication and mutation and subsequent recruitment for adaptation to specific functions. Central metabolism requires high levels of limited plant resources, and during intense growth, the synthesis of phenolic metabolites may be substrate- and/or energy-limited. On the other hand, either abiotic or biotic stresses divert substantial amounts of substrates from primary metabolism into secondary defensive product formation, and this may lead to constraints on growth. The allocation pattern of a plant defines its ecological roles and is therefore an important factor in understanding plant distribution and adaptation. On the other hand, as far as the development of a new strategy to enable the production of useful secondary metabolites on a commercial scale is concerned, any progress made in the basic understanding of metabolic pathways and regulatory mechanisms may be addressed to exploit the plant cell and tissue culture potentials to produce food additives, such as antioxidant phenolics for specific recommendation for industrial and pharmaceuticals applications.

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## Availability of data and materials

Not applicable.

## Abbreviations

| | |
|---|---|
| ASA | Ascorbic acid |
| BA | Benzoic acid |
| BPH | Brown plant hopper |
| CA | Cinnamic acid |
| CGA | Chlorogenic acid |
| CHS | Chalcone synthase |
| HBA | Hydroxybenzoic acid |
| HCA | Hydroxycinnamic acid |
| HMG CoA | 3-hydroxy-3-methylglutaryl CoA |
| HSR | Heat stress response |
| $NADP^+$ | Nicotinamide adenine dinucleotide phosphate |
| ROS | Reactive oxygen species |
| RONS | Reactive oxygen and nitrogen species |
| SA | Salicylic acid |
| PCs | Phenolic compounds |
| PAL | Phenylalanine ammonia lyase |
| PEP | Phosphoenol pyruvate |
| PSM | Plant secondary metabolite |
| UV | Ultraviolet |

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