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Review article

The role of phenolic compounds in plant resistance

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Abstract: *Phenolic compounds are plant secondary metabolites playing important roles in plant resistance. Their chemical structure is based on at least one aromatic ring bonded to one or more hydroxyl groups. They are mainly synthetized from amino acid phenylalanine which is converted to cinnamic acid. Phenolics are one of the largest and most diverse groups of plant active substances. These compounds take part in the regulation of seed germination and cooperate in regulating the growth of plants, also taking part in defence responses during infection, excessive sun exposure, injuries and heavy metal stress. One of the most important features of phenolic compounds is antioxidant activity which is closely related to their chemical structure. The aim of this review is to discuss the role of phenolic compounds in the interactions of plants with various stress factors, both biotic and abiotic with special attention to their antioxidant properties.*

Keywords: *phenolic compounds, plant antioxidants, oxidative stress.*

Introduction

During the lifetime plants, like all living organisms, are exposed to pathogenic microorganisms as well as to environmental stress factors (high or low temperature, drought, mineral deficiency, increased soil salinity, or the presence of heavy metal ions). Certain species of pathogenic microorganisms attack only specific plant species in their certain growth phases. During process of evolution, plants have developed diverse defense mechanisms against stress factors. After the contact with plant's pathogenic microorganism, injury or exposure to adverse environmental factors, a cascade of biochemical changes in plant cells occurs. Immune responses, initially only local, become a signal to trigger systemic defense mechanisms embracing the entire plant body [1-3].

Plant defense mechanisms are activated in a very short time in response to harmful agents. One of the first defense reactions observed in the cells of all living organisms is the increase in the concentration of reactive oxygen species (ROS), characterized by a very high chemical reactivity [4]. The free radicals includes molecules such as superoxide anion ($O_2^{\bullet -}$) and extremely reactive hydroxyl radical ($OH^{\bullet}$) [1].

From the chemical point of view, hydrogen peroxide (H_2O_2), ozone (O_3) and singlet oxygen are not considered as oxygen free radicals as, in fact, they do not possess an unpaired electron, however, due to its high reactivity, they are customarily included to ROS [4].

Under physiological conditions, free radicals are side-products of biochemical reactions taking place in mitochondria and chloroplasts [5]. ROS naturally present in living cells at low concentration, play an important role in their functioning. They participate in the regulation of metabolism, for example in intracellular signaling. Best known signaling molecule is hydrogen peroxide being capable of diffusing into the nucleus where is directly involved in transcriptional regulation of specific defense genes [5, 6].

Oxidative burst

One of the most common defense reactions in the cells of all living organisms is the rapid increase in concentration of reactive oxygen species called oxidative burst [1, 7, 8, 9]. Due to the universality of this phenomenon, understanding the role of ROS in defense mechanisms is an important aspect of plant stress physiology research. The main place for the formation of ROS during plant stress responses are plant cell wall and membrane. It is believed that the production of ROS is a result of enzymes activity located within these structures. It has been experimentally proven that oxidative burst is absent in isolated protoplasts (plant cells without cell walls) [1]. Thus, the presence of the cell wall is necessary to initiate the oxidative burst. Also it is likely that depolarization of the cell membrane induces the activation of enzymes that catalyze the formation of ROS. The key enzyme of generating ROS is a membrane NADPH oxidase producing $O_2^{\bullet-}$, which is a homologue of the animal enzyme initiating the oxidative burst on the surface of phagocytic cells (mainly neutrophils classified into leukocytes) [10-14]. The pH-dependent peroxidase located in the cell wall is involved in the production of hydrogen peroxide. Generation of ROS within the cell wall and their release outside the cell appears to be intentional, allowing their direct toxic effect on the cells of pathogens, contributing to the reduction of infection [6, 7, 15].

Under biotic stress condition, salinity, herbicides and presence of Cd^{2+} ions in soil, large amount of $O_2^{\bullet-}$ are formed in the peroxisomes which in physiological conditions are involved in H_2O_2 detoxification [16].

Even though the high concentration of reactive oxygen species is harmful to plant cells. Prolonged exposure to environmental stress factors results in so called 'oxidative stress' and leads to the pathological condition. ROS initiate a free radical reactions that lead to oxidation of proteins, lipids and nucleic acids. Degradation of essential cell components results in impairment of their function, eventually leading to death [17]. The main source of the most toxic hydroxyl radicals is Fenton's reaction which takes place in cytoplasm. The reaction is catalyzed by transition metals include iron, copper, nickel, zinc, cobalt, manganese or chromium [4].

$$H_2O_2 + Fe^{2+} \longrightarrow OH^{\bullet} + OH^{-} + Fe^{3+}$$

The Fenton reaction is limited by free Fe^{2+} ions availability that are produced during reduction of Fe^{3+} with the participation of $O_2^{\bullet-}$ [17,18]:

$$O_2^{\bullet-} + Fe^{3+} \longrightarrow O_2 + Fe^{2+}$$

The conjunction of both equations gives Haber-Weiss reaction [19]:

$$O_2^{\bullet-} + H_2O_2 \longrightarrow OH^{\bullet-} + OH^{-} + O_2$$

The best known example of a free radical chain reaction leading to the destruction of cell components is membrane lipid peroxidation, which is understood as the oxidation of polyunsaturated fatty acids included in the phospholipids of cell membranes [17]. Lipid peroxidation is usually initiated by a hydroxyl radical. As most of free radical chain reaction, membrane lipid peroxidation consists of three major steps: initiation, propagation and termination. Products of this oxidative membrane degradation are known as lipid peroxides [4, 20]. Lipid peroxidation leads to the destruction of the membrane with consequent outflow of the cytoplasm and finally cell death [6]. Highly mutagenic malondialdehyde (MDA) is considered to be the main biochemical marker of oxidative membrane degradation [21]. Testing the lipid peroxidation by measuring the content of MDA allows to determine the level of oxidative stress [22].

Besides the destruction of biological membranes, ROS are also capable of reacting with proteins what leads to numerous modifications of amino acid residues or non-protein prosthetic groups. The most reactive hydroxyl radical may detach protons from protein molecules, resulting in the formation of protein radicals. Occurrence of non paired electrons on cysteine residues leads to the formation of disulfide bridges and, in a consequence, creation of protein dimers. The activity of ROS may lead to fragmentation or aggregation of proteins and, therefore, modified proteins lose their function [4]. Nucleic acids, as a genetic information carriers are more resistant to destabilizing activity of ROS. The indirect oxidative DNA and RNA damage might result only due to hydroxyl radical [4].

In order to protect their own tissues, plants have developed efficient antioxidant system. It includes both antioxidant enzymes, which catalyze the decomposition of ROS, as well as non-enzymatic antioxidants including well known ascorbic acid (vitamin C), tocopherols and tocotrienols (vitamin E), carotenoids, numerous of phenolic compounds, as well as tripeptide glutathione (γ-L-Glutamyl-L-cysteinyl-glycine). Maintaining high antioxidant activity allows plants to efficiently capture toxic ROS and determines their tolerance to stress [23-25]. It is worth to mention that in the model plant *Arabidopsis thaliana* nearly 300 genes responsible for the metabolism of ROS was identified [26, 27].

Phenolic compounds

Among the non-enzymatic antioxidants a special attention should be paid to phenolic compounds which are a diverse group of plant secondary metabolites. All consist of the aromatic ring (C6) bonded directly to at least one (phenol) or more (polyphenol) hydroxyl groups (-OH) and other substituents, such as methoxyl or carboxyl groups which cause the polar character of the compounds and allow dissolution in water [28].

Phenolic compounds are usually divided into two groups - simple phenols and more complex derivatives, often containing several aromatic rings linked together. The group of simple phenols include among otherse: *p*-hydroxybenzoic acid, *o*-hydroxybenzoic acid (salicylic acid), caffeic acid, gallic acid, vanillic acid, syringic acid, coumaric acid or cinnamic acid. Structure of selected simple phenols are presented at Fig.1. [1, 29].

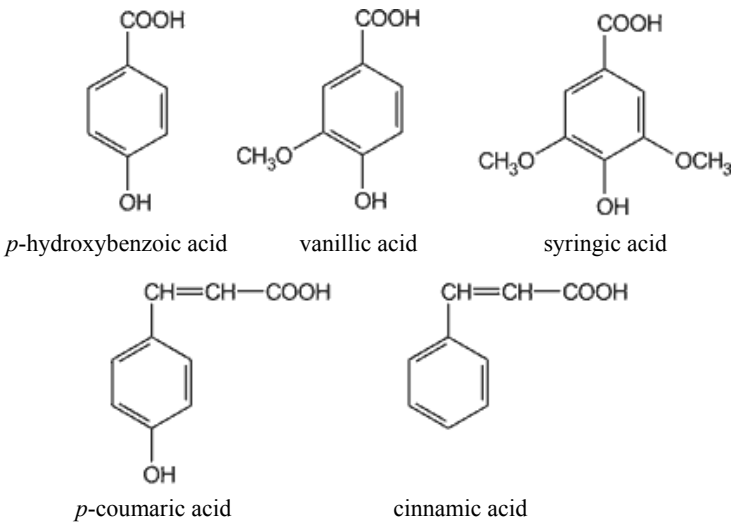
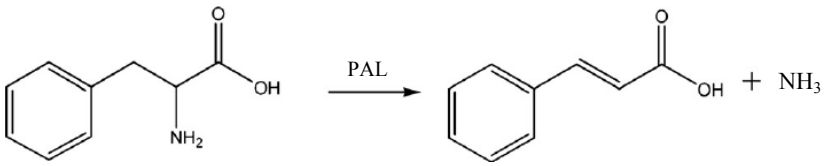


Figure 1. Simple phenolic compounds

The aromatic ring of phenolic compounds is synthesized in the shikimic acid pathway from amino acid phenylalanine - the common precursor of phenols. The initial step in the formation of all phenolic compounds is simple disengagement of the amino group $-NH_2$ from phenylalanine which converses to cinnamic acid. The reaction is catalyzed by enzyme Phenylalanine ammonia-lyase (PAL) (Fig. 2). Cinnamic acid is then used as the starting molecule for the synthesis of other phenolic compounds [1, 30, 31].



phenylalanine cinnamic acid

Figure 2. Enzymatic conversion of phenylalanine to cinnamic acid

Due to the three-carbon side chain linked to the aromatic ring, cinnamic acid and its derivatives have been described as phenylpropanoids. Phenylpropanoids and compounds formed after their transformations usually perform protective functions in plant cells. The content and rate of metabolism of phenylpropanoids is enhanced in plants under stress conditions. This phenomenon has been confirmed by independent researchers. Reyes and Cisneros-Zevallos (2003) demonstrated that mechanical damage resulted in the accumulation of phenolic compounds, total antioxidant capacity and PAL-activity in purple-flesh potatoes (*Solanum tuberosum* L.) [32]. Similar results were observed in red cabbage seedlings treated with copper by Posmyk et al. (2009). The presence of Cu initiated oxidative stress leading to the destruction of cell membranes, measured by the concentration of thiobarbituric acid reactive substances (TBARS). Authors suggested, that peroxidases and phenolic compounds were involved in defense reaction against oxidative stress mediated by copper ions [33].

Phenylpropanoids may undergo numerous transformations to form lignin and suberin that mechanically reinforce the cell walls [28]. Biosynthesis of phenolic compounds that are precursors of lignin intensifies under stress condition, e.g. in plants subjected to heavy metal stress [28]. Important role of lignin in plant resistance was confirmed in the experiment with wheat (*Triticum* L.) treated with lignin synthesis inhibitor, what resulted in a decrease resistance to a plant pathogen *Puccinia graminis* [34].

Phenylpropanoid pathway may also lead to the formation of non-polymer derivatives like salicylic acid, which lacks a side chain. Salicylic acid (SA) is an important factor involved in the induction of plant defense responses (Fig. 3.). *De novo* synthesis of SA is generally preceded by infection or certain stress factors (e.g. UV radiation). Accumulation of SA can be both local and systemic, including the entire plant. SA is the most important signaling molecule involved in systemic acquired resistance – SAR. The volatile derivative – salicylic acid methyl ester, which is spread in the air, has the ability to induce protection in other parts of the plant and to neighboring plants [29, 35, 36].

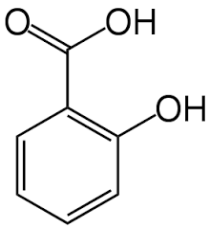


Figure 3. Salicylic acid

Another valuable property of phenolic compounds is their ability to chelate heavy metal ions. Phenols have antioxidant properties and ability to quenching of free radical reactions [37, 38]. Phenolic compounds chelate iron and copper ions due to the presence of suitable functional groups: hydroxyl and carboxyl. Plants with a high content of tannins, such as tea, are able to tolerate high concentrations of manganese in a soil, as they are protected by the direct chelation of these ions [28]. The binding of heavy metal ions by polyphenols has also been observed in the study with *Nympheae* where heavy metals (Hg, Pb, Cr) were chelating by the methanol extract rich in polyphenols [39]. Presumably, these properties of phenolic compounds result from the nature of the nucleophilic aromatic rings rather than from the presence of specific functional groups [40].

Another mechanism underlying the antioxidant properties of phenolic compounds is the inhibition of membrane lipid peroxidation by "catching" alkoxyl radicals. The activity of phenolic compounds is dependent on the structure of molecules and the number and position of hydroxyl groups. Apart from the typical antioxidant properties, phenols (flavonoids in particular) stabilize membranes by decreasing their fluidity which in turn limits the diffusion of free radicals and reduces the peroxidation of membrane lipids. Stabilization of the membrane is due to phenolics ability (especially flavonoids) to bind to some of integral membrane proteins and phospholipids [28].

Flavonoids

Flavonoids, which are derivatives of simple phenols, are one of the largest groups of plant secondary metabolites. Chemically, general structure of a molecule is based on 15-carbon skeleton, which consists of two aromatic rings connected by three-carbon bridge (C6-C3-C6). Flavonoids, as all phenolic compounds, are synthesized through the phenylpropanoid pathway leading to the formation of coumaroyl-CoA from phenylalanine (shikimic acid pathway) and malonyl-CoA (malonic acid pathway). The construction of most flavonoids is based on the flavone skeleton (Fig. 4).

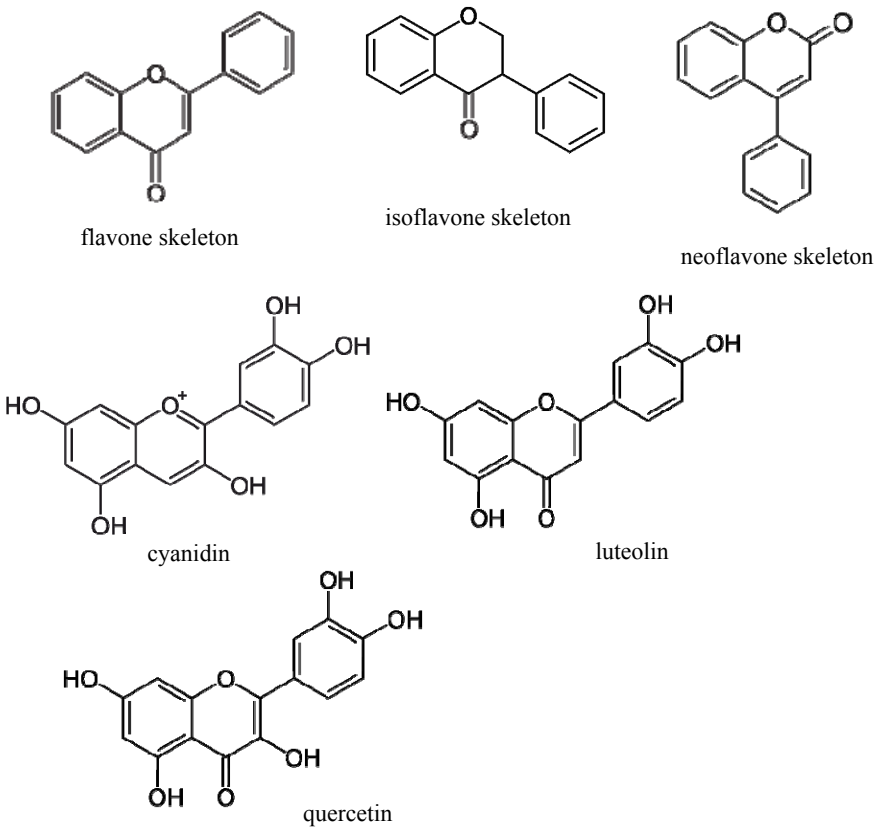


Figure 4. Structures of flavones and selected flavonoids

Flavonoids differ in the number and type of substituents. Specific properties of individual flavonoids depends on the type of substituents [29, 41]. Flavonoids have been classified into the following subgroups: chalcones, flavones, flavonols, flavandiols, proanthocyanidins and their derivatives anthocyanidins and condensed tannins [42]. Synthesis of flavonoids increases after plant microbial infection, injury, decrease in temperature and deficiency of nutrients [28]. Untill now more than 6000 flavonoid compounds have been identified [43]. Majority of flavonoids express antimicrobial activity and some are capable of the UV light excess adsorption and plant cells defense from oxidative damage. Most of flavonoids are color compounds which give plants' organs characteristic tint, most often yellow one (e.g. quercetin classified as flavonol) or shades from blue to pink (e.g. cyanidin, delphinidin, malvidin, pelargonidin belonging to anthocyanidins). Color of anthocyanidins is often dependent on the pH [44]. Plants accumulate flavonoids in vacuoles of epithelial cells in order to protect deeper-lying tissues against destructive UV radiation. The ability of flavonoids to

absorb radiation of high energy (with the maximum absorption at 250-270 nm and 335-360 nm) was confirmed in studies involving plants radiated with UV light in which increased flavonoids synthesis was observed [28, 42, 45, 46]. Particular important role of flavonoids in UV protection has also been reported by Ryan et al. (2001) who proved hypersensitiveness to UVB radiation of *Arabidopsis* mutants which lack the enzyme necessary in flavonoid synthesis pathway [47].

Certain flavonoids exhibit the ability to provide heavy metal stress protection by chelating transition metals (e.g. Fe, Cu, Ni, Zn) that generate hydroxyl radical via the Fenton's reaction [48,49]. This is confirmed by research on corn plants (*Zea mays* L.) grown on soil contaminated with aluminum ions, where high levels of catechin and quercetin in the root exudates were observed. Chelating of these metals in the soil may be an effective form of protection against the toxic effects of high concentrations of metals [50]. Kostyuk *et al.* (2004) demonstrated that metal-flavonoid chelates display superoxide dismuting activity. What is more, authors noticed that metal-flavonoid complexes exhibit significantly higher antioxidant activity when compared with parent flavonoids [51]. Kim *et al.* (1999) observed flavonoids accumulation as an answer to $CuSO_4$ treatment in cell cultures of *Ginkgo biloba* when compared with untreated cells [52]. Similarly, Bota and Deliu (2011) reported a correlation between flavonoid level and concentration of $CuSO_4$ in cell cultures of *Digitalis lanata* [53].

Phytoalexins

Compounds with typically protective properties are phytoalexins. Most of them belong to flavonoids and isoflavonoids. Some have a structure typical for terpenoids or stilbenes. Phytoalexins are synthesized *de novo* in plants in response to infection by microorganisms (bacteria, fungi, viruses) and nematodes. Phytoalexin synthesis can also be activated by abiotic stress factors, such as heavy metals, UV radiation, or mechanical damage. Flavonoid phytoalexins are characteristic for legumes (*Fabaceae*) whereas terpenoid phytoalexins are typical for *Solanaceae*. The task of phytoalexin is likely to reduce the spread of the pathogenic microorganism infection. Phytoalexins exhibit bacteriostatic properties, some even bactericidal, which function is similar to antibiotics. These compounds also limit sporulation, spore germination and hyphal growth of phytopathogenic fungi. The antimicrobial effectiveness of phytoalexins depends on the rate of synthesis and their concentration in plant tissues. However, some pathogens are tolerant to phytoalexins, posses ability to inactivate phytoalexins by demethylation or hydroxylation of aromatic rings, which makes these compounds more soluble in water and susceptible to oxidation [1].

Coumarins

Another important product of shikimic acid pathway is coumaric acid, which is produced by hydroxylation of cinnamic acid or deamination of tyrosine by the

tyrosine ammonia-lyase enzyme. Coumaric acid may then undergo cyclization leading to the formation of coumarins (Fig. 5). Coumarins are phenolic compounds exhibiting toxicity against herbivores. Dicumarol is a compound with anticoagulant properties. Its bitter taste may discourage animals from eating plants containing a large amount of these compounds [1]. Some publications also report hepatotoxic and carcinogenic properties of coumarins [54-56].

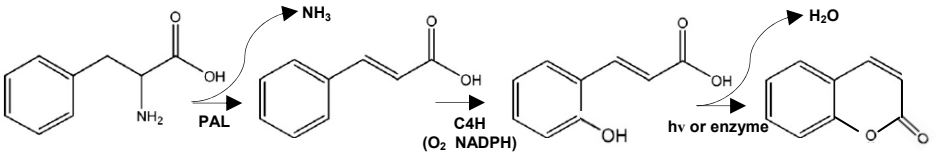


Figure 5. Biosynthesis of coumarins

Tannins

An example of phenolic compounds protecting plants from herbivores are tannins. There are two types of these compounds: easily hydrolyzable tannins (produced by polymerization of gallic acid or other phenolic acids and some sugars) and condensed tannins (created by combining multiple flavonoid units). Tannins are commonly found in relatively high concentrations in the bark of trees and in leaves. His repellent properties owe their unpleasant, bitter taste, while the toxic effect is related to their ability to bind to and denature proteins. All these features make them an excellent compounds provide plant protection against insects [57].

Conclusions

Phenolic compounds play important roles in plant growth and development, particularly in defense mechanisms. Most of the phenolic compounds has potent antioxidant properties, neutralizing the effects of oxidative stress. Some of them exhibit ability to chelate heavy metal ions. Importantly, phenolic phytoalexins exhibit antibiotic and antifungal activity. Coumarins and tannins repel herbivores, whereas phenylpropanoids are starting molecules for the synthesis of lignin and suberin, in order to strengthen cell walls.

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