Review

Flavonoids as Important Molecules of Plant Interactions with the Environment

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Abstract: Flavonoids are small molecular secondary metabolites synthesized by plants with various biological activities. Due to their physical and biochemical properties, they are capable of participating in plants' interactions with other organisms (microorganisms, animals and other plants) and their reactions to environmental stresses. The majority of their functions result from their strong antioxidative properties. Although an increasing number of studies focus on the application of flavonoids in medicine or the food industry, their relevance for the plants themselves also deserves extensive investigations. This review summarizes the current knowledge on the functions of flavonoids in the physiology of plants and their relations with the environment.

Keywords: secondary metabolites; flavonoids; antioxidants; plant protection

1. Introduction

Flavonoids are plant secondary metabolites, derivatives of 2-phenyl-benzyl-γ-pyrone, present ubiquitously throughout the plant kingdom. Over 9,000 compounds of this group are known [1]. Their biosynthesis pathway (part of the phenylpropanoid pathway) begins with the condensation of one p-coumaroyl-CoA molecule with three molecules of malonyl-CoA to yield chalcone (4',2',4',6'-tetrahydroxychalcone), catalyzed by chalcone synthase (CHS). The next step is

isomerization of chalcone to flavanone by chalcone isomerase (CHI). From this step onwards, the pathway branches to several different flavonoid classes, including aurones, dihydrochalcones, flavanonols (dihydroflavonols), isoflavones, flavones, flavonols, leucoanthocyanidins, anthocyanins and proanthocyanidins (Figure 1). Flavonoids undergo further modifications, for example methylation by methyltransferases and glycosylation by specific glycosyltransferases. These modifications often alter their solubility, reactivity and stability. The majority of flavonoids are present in the form of glycosides under natural conditions [2,3]. Because of their diverse chemical structure and variety resulting from the attached substituents, they have a number of important functions in plants. They participate in plant protection against biotic (herbivores, pathogens) and abiotic stresses (UV radiation, heat), and due to their antioxidative properties, they also maintain a redox state in cells. The antioxidative activity of flavonoids is connected with the structure of the molecule: the presence of conjugated double bonds and the occurrence of functional groups in the rings [4–6]. Flavonoids reduce the production of and quench reactive oxygen species (ROS) through:

1. suppression of singlet oxygen;
2. inhibition of enzymes that generate ROS (cyclooxygenase, lipoxygenase, monooxygenase, xanthine oxidase);
3. chelating ions of transition metals, which may catalyze ROS production;
4. quenching cascades of free-radical reactions in lipid peroxidation;
5. "re-cycling" of other antioxidants [4,7–11].

Due to their low redox potential, they can reduce strong free radicals (superoxides, alkyl radicals, hydroxyl radicals) [12].

2. Flavonoids in the Relations between Plants and Animals

Flavonoids are synthesized in all parts of the plant. They play a role in providing color, fragrance and taste to the fruits, flowers and seeds, which makes them attractants for insects, birds or mammals, which aid in pollen or seed transmission [13]. Plants release various chemicals both to deter and attract insects, in some cases natural predators of herbivores feeding on a plant. Flavonoids are among the chemicals that have been reported to regulate oviposition and feeding. Naringenin, hesperetin-7-*O*-rutinoside and quercetin-3-*O*-rutinoside, along with other active compounds, stimulated oviposition in swallowtail butterfly *Papilio* on young leaves of citrus plants [14]. Similar activity was found for luteolin 7-*O*-(6"-malonyl glucoside) on *Papilio polyxenes* [15] and for isorhamnetin glucoside on *Luehdorfia japonica* oviposition on the leaves of plants of the *Asarum* genus [16]. Flavonoids can also prevent insects from laying eggs, e.g., quercetin-3-*O*-rutinoside acts as a stimulant to *Danaus plexippus*, but as a deterrent to *Pieris rapae* [17,18].

It has been reported that the maize response to corn earworm, *Helicoverpa zea*, is mainly due to the presence of the C-glycosyl flavone, maysin (2"-*O*-a-L-rhamnosyl-6-C-(6-deoxy-xylo-hexos-4-ulosyl) luteolin), and the phenylpropanoid, chlorogenic acid [19]. Flavonoids, such as flavones 5-hydroxyisoderricin, 7-methoxy-8- (3-methylbutadienyl)-flavanone and 5-methoxyisoronchocarpin, and isoflavonoids (judaicin, judaicin-7-*O*-glucoside, 2-methoxyjudaicin and maackiain), were also reported as direct feeding deterrents. Flavonoids are cytotoxic and interact with different enzymes

through complexation. Both flavonoids and isoflavonoids protect the plant against insect pests by influencing their behavior, growth and development [20,21].

Figure 1. Classification of flavonoids.
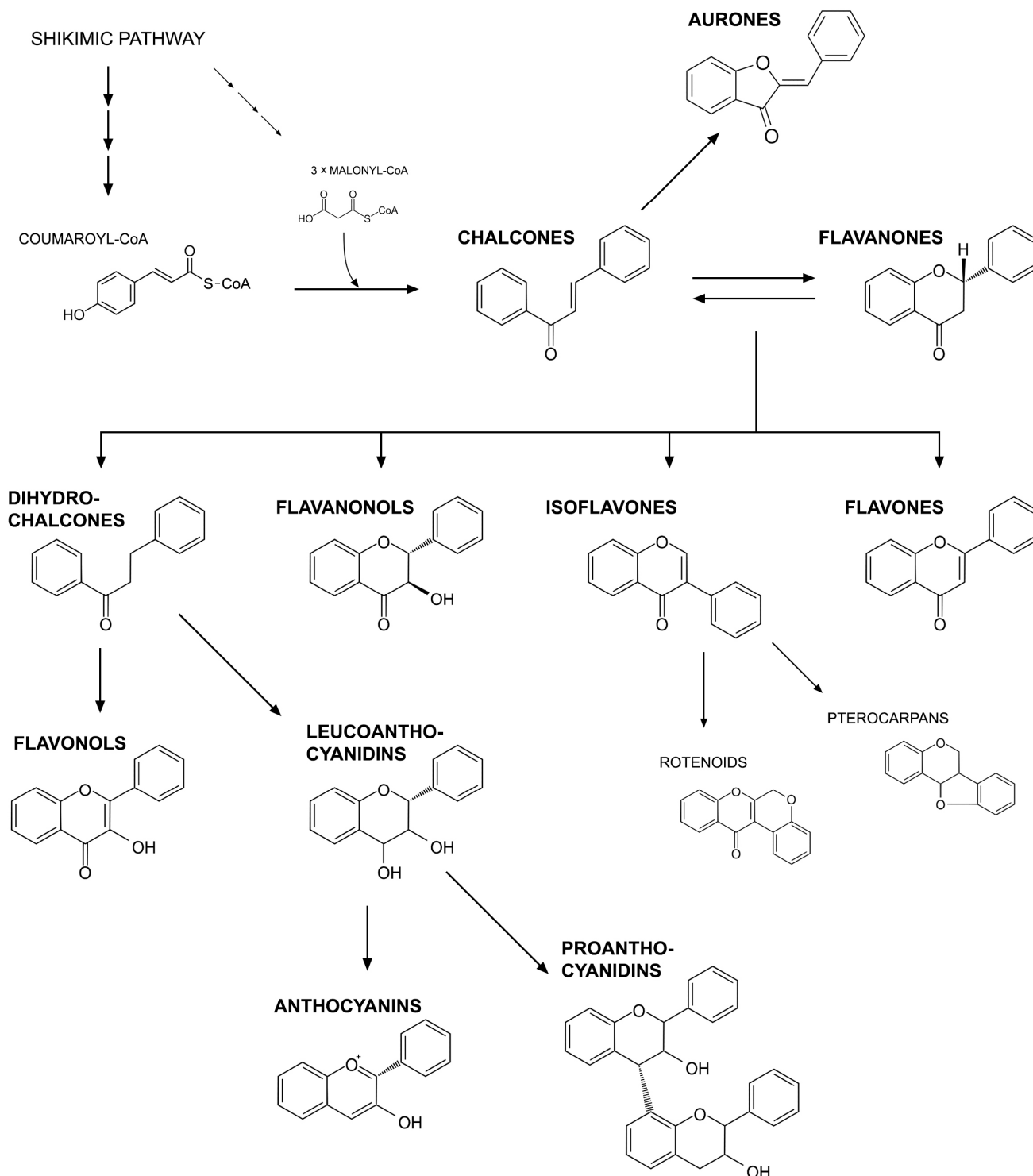
Flavonoids play an important role in the protection of plants against plant feeding insects and herbivores [8]. Their presence can alter the palatability of the plants and reduce their nutritive value, decrease digestibility or even act as toxins. A study on a mixture of flavonoids from *Cistus ladanifer* L. that contained apigenin and 3,7-di-*O*-methylkaempferol demonstrated that they can influence

calcium-dependent ATPase in the skeletal muscle sarcoplasmic reticulum and lead to deterioration of muscle relaxation [22]. A number of insect species have been shown to be sensitive to flavonoid compounds in feeding tests [23]. Rutin and quercetin-3-glucoside contained in *Pinus banksiana* inhibit the development and increase the mortality of *Lymantria dispar* [24]. Studies on peanuts revealed that the amounts of quercetin and rutin glycosides are related to increased mortality of the tobacco armyworm (*Spodoptera litura*) [25]. In rice, three flavone glucosides that inhibit digestion in insects and function as deterrent agents towards *Nilaparvata lugens* were identified [8]. Isoflavonoids and proanthocyanidins are other classes of flavonoids responsible for plant protection against insects. For example, naringenin procyanidin inhibits the development of *Aphis craccivora* [8].

Flavonoids can also have deterrent properties, with respect to feeding and physiological behavior, against soil nematodes, which feed on plants. For instance, kaempferol, quercetin and myricetin (flavonols) act as deterrents against *Radopholus similis* and *Meloidogyne incognita*, while genistein and daidzein (isoflavones) are active against *Radopholus similis*. The flavonols inhibit peristalsis of some nematodes and kaempferol, in addition to restricting their hatching [26].

Although flavonoid compounds may act as attractants or feeding/growth stimulators for certain insect species, they are of high relevance for the plant defense mechanism.

## 3. Flavonoids as Regulators of Symbiotic Interactions with Microorganisms

Flavonoids can act as specific transmitters in symbiotic relations between species, in particular, symbiotic bacteria. Low soil nitrogen concentration induces accumulation of flavonoids, which act as attractants for diazotrophs, resulting in the transport of reduced nitrogen forms to plant cells, while the bacteria utilize the plant's photosynthesis products.

Naringenin can stimulate the colonization of wheat roots by *Azorhizobium caulinodans* [27]. Flavonoids, such as luteolin and chrysin, excreted by legumes, e.g., *Medicago sativa*, act as a specific signal for *Rhizobium* bacteria to initiate symbiosis [28]. The release is strongest at root tips and the emerging root hair zone, which are the target sites for *Rhizobium* infections [29].

Flavonoids can influence, both positively and negatively, the expression level of bacterial *nod* genes, which control root nodule formation in the nitrogen-fixing bacteria [30–32]. For instance, daidzein and genistein induce *nod* genes in *Bradyrhizobium japonicum*, but inhibit their expression in *Sinorhizobium meliloti*. Similarly, naringenin stimulates Nod formation in *Rhizobium leguminosarum*, but quercetin represses its production. The mechanism of action is through binding of flavonoids to bacterial NodD proteins, members of the transcription factor LysR family [33]. These proteins respond to different flavonoid groups, and a specificity in the interaction between legumes and the nitrogen fixing bacteria can be observed [34]. Activated NodD protein binds to strongly conservative nod-box motifs in the promoter regions of *nod* genes and induces their transcription [35]. As a result, Nod factors (lipopolysaccharides) are synthesized and released by the bacteria, activating various processes in the host plant and leading to preparation of the plant for symbiosis with the bacteria. The factor is recognized by Nod factor receptors (NR) localized in the plant cell membrane. The receptors are composed of an extracellular domain with two or three lysine motifs (LysM) and an intracellular kinase domain (LysM-RLK) [36–40]. Perception of Nod factors requires dimers of LysM [41]. After binding of Nod factors, the receptor proteins start a signaling kinase cascade, in which DMI1, DMI2,

NFP and NSP proteins participate [42]. One of the earliest plant reactions in response to Nod factors is the change in intracellular calcium level and in the root hair cell cytoskeleton [33,43], leading to root cell divisions, root nodule formation and infection threads [33]. In addition, rhizobial Nod factors induce the expression of flavonoid synthesis genes. It was proposed that the flavonoids interfere subsequently with auxin transport, thereby promoting cell divisions [44] (Figure 2).

Figure 2. The role of flavonoids in the interactions between nitrogen-fixing bacteria and legume plants. Flavonoids are released by the plant bind to and activate NodD protein, which, in turn, attaches to no-box cassettes in the promoter sequences of nod genes, leading to the production of nod proteins and subsequently nod factors. These molecules bind to the nod factor receptors (NR) localized on the plant cell membrane, starting a cascade of signal transduction. This signal activates various gene expression, leading to root hair deformation, formation of infection thread, flavonoid biosynthesis, cell division and, finally, root nodule formation.
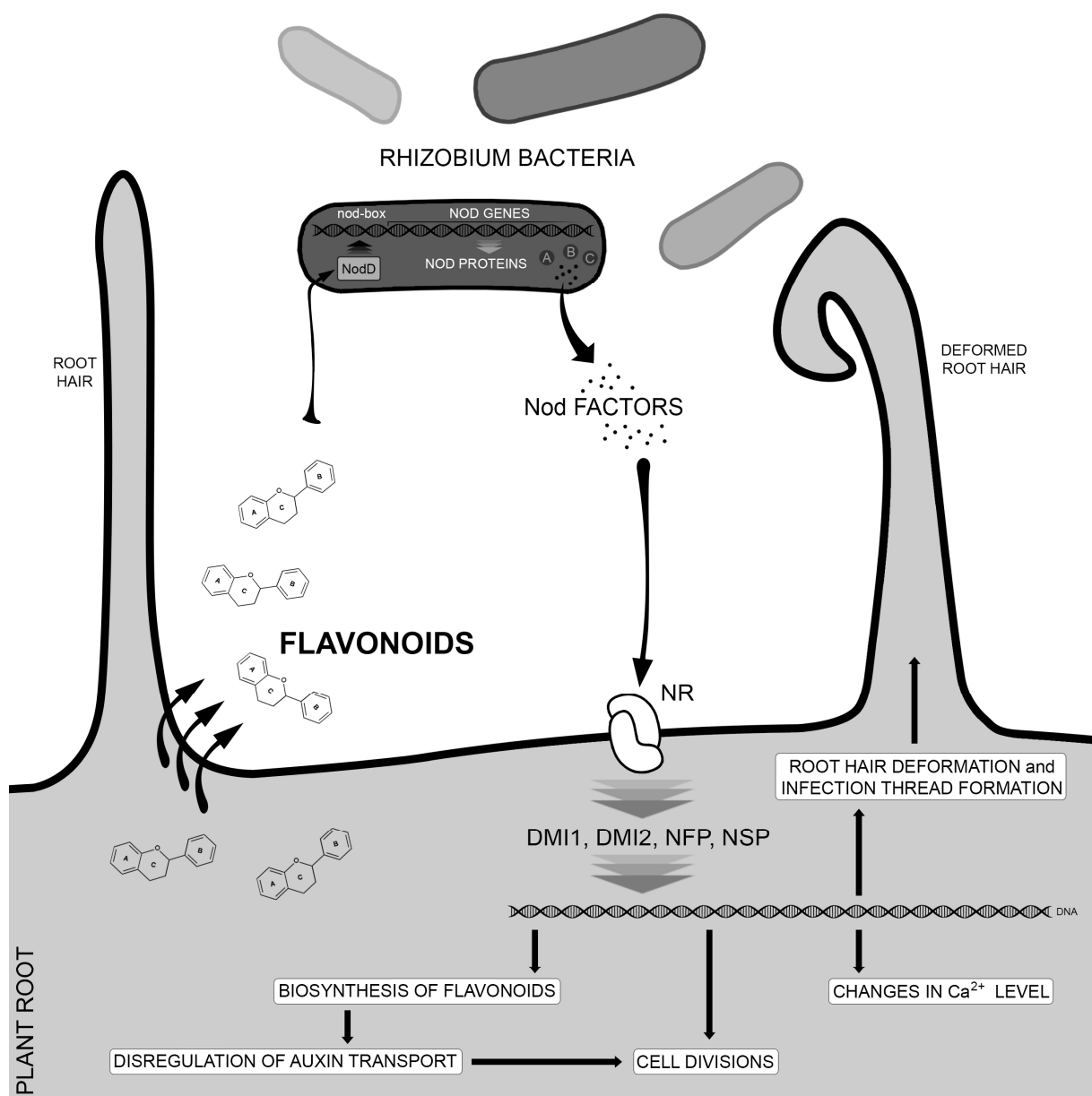
The flavonoids are also very important in the formation of mycorrhiza, which is a symbiotic relationship between a plant and soil-borne fungi that colonize the cortical tissues of roots. Though the role of flavonoids in this symbiosis has not yet been fully recognized, they can have both negative and positive effects on the mycorrhiza [45]. Elevated flavonoid biosynthesis during mycorrhiza development was found in *Trifolium repens* (white clover) [46], melon roots [47] and *Medicago truncatula* [48]. It was demonstrated that mycorrhizal formation changed the flavonoid profile in root extracts by modifying the expression of the genes involved in phenylpropanoid biosynthesis. It was also reported that flavonoids, such as quercetin, quercetin galactoside and kaempferol, have a positive effect on the growth of hyphae and spore germination of mycorrhizal fungi [49,50]. Rutin was found in *Eucalyptus globulus* spp. *bicostata* to promote hyphae of *Pisolithus* sp. [51].

4. Participation of Flavonoids in Allelopathic Interactions between Plants

Plant-plant interactions can be positive or negative and may depend on the concentrations of the flavonoids [52,53]. The negative relations are mainly based on inhibiting germination and growth of other plants' seedlings [23]. Quite often, flavonoids are excreted through roots in to the soil where they inhibit seed germination, but can be also found in leaves and even in pollen, which, after falling onto the surrounding soil, inhibits the germination of other plants [54,55]. Some examples include: catechins excreted by *Centaurea maculosa* inhibiting germination and growth of *Centaurea diffusa* and *Arabidopsis thaliana*; and flavones excreted by barley inhibiting weed seed germination [56,57]. Unfortunately, the precise mechanism by which flavonoids participate in allelopathy is still unknown. Potential ways in which they can influence allelopathy may include cell growth inhibition, ATP production disturbances and hindering the proper functioning of auxins [58]. Flavanols were reported to provoke a wave of ROS, which activates the Ca^{2+} signal cascade and root system death [59]. The significance of allelopathy gains more and more attention in agriculture, because these interactions could be employed for reducing weed growth [60–62].

5. Flavonoids and Plant Pathogen Resistance

Flavonoids are very important in plant resistance against pathogenic bacteria and fungi. Antipathogenic properties of flavonoids can be non-specific and result, in part, from their antioxidative properties. They quench ROS, which are generated both by the pathogens and the plant as a result of the infection [63,64]. Flavonoid compounds are transported to the site of infection and induce the hypersensitivity reaction, which is the earliest defense mechanism employed by the infected plants, and programmed cell death. It was shown that flavonoids are incorporated into the cell walls of necrotic and adjacent cells [63–65]. Moreover, flavonoids can contribute to tightening of the plant structures and tissues by modulating auxin (IAA) activity, which can lead to the differentiation of tissues, promotion of callus and tylose formation and closure of the vascular system to prevent pathogen infection [65]. They may also be directly involved in the inhibition of the pathogen's enzymes, especially those digesting the plant cell wall, by chelating metals required for their activity [23].

The antifungal activity is often based on the inhibition of spore development and mycelium hyphae elongation [63]. Flavonoid antipathogenic activity can also be more specific. It is suggested that the mechanism of flavonoid antibacterial activity is based on their ability to inactivate microbial adhesion

and cell envelope transport proteins [66,67]. Fat-soluble flavonoids may also disrupt microbial membranes, change their fluidity and may disturb the respiratory chain [68,69].

Moreover, the B ring of flavonoids can intercalate or form hydrogen bonds with the stacking of nucleic acid bases and further lead to inhibition of DNA and RNA synthesis in bacteria and influence DNA gyrase activity [70]. This is presumably also the basis of their antiviral properties, as they can inhibit viral polymerases and bind to the nucleic acids or capsid proteins of a virus [71].

It was shown that the anti-pathogenic effect of flavonoids depends on their structure. It was suggested that the strongest antifungal activity is demonstrated by unsubstituted flavones and unsubstituted flavanones. Hydroxyl and methyl groups in these compounds reduce their antifungal properties [72], though in some cases, methylated flavonoids reveal a stronger antifungal effect [73]. Flavonoids inhibit a number of root pathogens, especially fungal ones, and in general, isoflavones, flavanes and flavanones are acknowledged as efficient anti-microbial agents [74]. Studies on barley mutants showed that proanthocyanidins or even small amounts of dihydroquercetin are involved in the protection against *Fusarium* sp. This may result from several mechanisms of action, involving cross-linking of microbial enzymes, inhibition of pathogen cellulases, xylanases and pectinases, chelation of metal ions relevant for enzymatic activities and/or tightening of cell walls, leading to the formation of a physical barrier against pathogen attack [75]. Antimicrobial activities of various classes of flavonoids are summarized in Table 1.

Table 1. List of flavonoid compounds of anti-pathogenic activities found in plant organisms.

Plant Organism	Antimicrobial Compound	Pathogen	Reference
Dianthus caryophyllus	flavonol triglycoside of kaempferide	*Fusarium oxysporum*	[76]
Dianthus caryophyllus	kaempferol-*O*-rutinoside, kaempferol-3-*O*-b-D-glucopyranosyl	*Fusarium oxysporum*	[77]
Linum usitatissimum	isoorientin, isovitexin, vitexin	*Fusarium. oxysporum*, *Fusarium culmorum*	[78]
Triticum L. cv. Roblin	flavonoids	*Fusarium graminearum*	[79]
Lotus garcinii	catechin, epicatechin, rutin	*Fusarium graminearum*	[80]
Wheat NILs	5,6-dimethoxyflavone, 2-hydroxyisoflavanone, naringenin, naringenin 7-*O*-b-D-glucoside, 5-hydroxy-7,8-dimethoxyflavanone 5-rhamnoside, kaempferol 3-rhamnoside-7-xylosyl-(1-2)-rhamnoside	*Fusarium graminearum*	[81]
Hordeum vulgare	naringenin, kaempferol	*Gibberella zeae*	[82]

Table 1. *Cont.*

Plant Organism	Antimicrobial Compound	Pathogen	Reference
Mariscus psilostachys	chalcones	*Cladosporium cucumerinum*	[83]
Mariscus psilostachys	flavans	*Cladosporium cucumerinum*	[84]
Arabidopsis thaliana	quercetin	*Neurospora crassa*	[85]
Eucalyptus globules	flavonols	*Cytonaema* sp.	[86]
Cicer bijugum	isoflavonoids	*Botrytis cinerea*	[87]
Medicago truncatula	isoflavone	*Erysiphe pisi*	[88]
Vitis vinifera	quercetin-3-*O*-glucoside	*Plasmopara viticola*	[89]
Phaseolus vulgaris	isoflavonoid phytoalexin: genistein, daidzein, 2-hydroxygenistein, dalbergioidin, phaseollin, phaseollidin, phaseollin isoflavan, kievitone, coumestrol	*Colletotrichum lindemuthianum*	[90]
Austrian pine	flavonoids	*Diplodia pinea*	[91]
Glycine max	isoflavone	*Phytophthora sojae*	[92]
Solanum tuberosum	glucosylated forms of flavonoids	*Erwinia carotovora*	[93]
Oryza sativa	naringenin, kaempferol, quercetin, hydroxyquercetin	*Xanthomonas oryzae* pv. *oryzae*, *Pyricularia oryzae*	[94]
Brassica rapa	kaempferol glucoside	*Xanthomonas campestris* pv. *campestris*	[95]
Nicotiana tabacum, *Arabidopsis thaliana*	jaceosidin fisetin hydrate	*Pectobacterium carotovorum*, *Pseudomonas syringae*	[96]
Lycopersicon esculentum	flavonoids	*Pseudomonas syringae*	[97]
Citrus sinensis	flavonoid glycosides, polymethoxylated flavones	*Candidatus Liberibacter*	[98]

6. Flavonoids and Environmental Conditions

Due to the inability to move, plants have developed mechanisms to cope with unfavorable environmental conditions. One of those mechanisms is synthesis of secondary metabolites, including flavonoids. The levels of these compounds increase in response to various factors, such as strong light, ultraviolet (UV) radiation, low/high temperature, ozone, heavy metals, drought, *etc*. These conditions are stressful for the plant and are a source of free radicals. One of the functions of flavonoids during

oxidative stress triggered by environmental factors is the diminution of the effect caused by the presence of ROS [23,99].

Flavonoids protect plants against UV damage, which, to some extent, results from the fact that they can act as a screen absorbing UV radiation and, as they are accumulated mainly in the epidermis and hypodermis of leaves and stems, apical meristem and pollen, reducing the penetration of UV light to the vulnerable tissues or organs. Besides UV absorption, flavonoid compounds may also transfer or accept light energy to or from other molecules via sensitization [100]. The role of flavonoids in response to UV radiation is mostly due to the scavenging of ROS generated during irradiation. Flavonoids reacting to light are the dihydroxy B-ring-substituted forms, such as quercetin 3-*O* and luteolin 7-*O*-glycosides, and not the monohydroxy B-ring-substituted counterparts, such as apigenin 7-*O*-glycosides and kaempferol 3-*O*-glycosides. Dihydroxy B-ring-substituted flavonoids possess higher antioxidative properties, but lower UV absorption capacity than their monohydroxy B-ring-substituted counterparts. The ratios of quercetin to the kaempferol derivatives or luteolin to the apigenin derivatives drastically increase upon exposure to UV-B, UV-A + UV-B or photosynthetic active radiation (PAR) [101,102]. Synthesis of flavonoids and other phenolic compounds in response to increased UV radiation increases strongly [103,104]. It is probably the mechanism of the primary response of the plant to stressful conditions, which is subsequently followed by other mechanisms, such as pigment accumulations or lignification processes [105]. The regulation of flavonoid biosynthesis proceeds at the transcription level and requires the co-operation of UV-b photoreceptor [105]. Activation of the UV-b photoreceptor leads to triggering of transcription factors, which bind to specific sites on the promoters of flavonoid synthesis genes [106]. Stress factors can induce increased generation of toxic reactive oxygen species (ROS). They are also produced during normal physiological plant activity, but they are strictly controlled by the plant antioxidative system. ROS react non-specifically with lipids, proteins and nucleic acids; thus their increased generation can lead to impairment of cell structures, including cell membranes and the photosynthetic apparatus [103,104,107]. Flavonoids can undergo single electron oxidation and, thus, are capable of reducing free radicals. They act as antioxidants by direct quenching free radicals through transferring a proton from the A and/or B ring and generating less active flavonoid radicals. The capability to quench free radicals is connected with the structure of flavonoids, and the presence, position and modifications of hydroxyl groups in the A and B rings are essential (e.g., methylation or glycosylation reduces the antioxidative capacity of flavonoids, while double bond between C-2 and C-3 linked to 4-keto and 3-hydroxyl groups facilitate electron transfer) [108–110]. The spatial arrangement of the B ring can also contribute to efficient electron transfer and influence the antioxidative properties of flavonoids [111]. The ability to quench free hydroxyl radicals increases with the number of hydroxyl groups in the B ring, e.g., myricetin is a stronger antioxidant than kaempferol. One of the best ROS quenchers is quercetin, which contains a catechol group in the B ring, double bond between C2 and C3 and a hydroxyl group at C-3 [108]. Metals are involved in ROS formation in the Fenton reaction [112]. Flavonoids are able to chelate Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Al^{3+} and Mg^{2+} cations, but are unable to bind Na^+, K^+ and Ca^{2+} [113]. Metals bind to the flavonoid catechol group localized within the B ring, to the 3-hydroxyl and 4-oxo group of the heterocyclic ring and to the 4-oxo and 5-hydroxyl group between the heterocyclic and A rings [114]. In this way, they can stop lipid peroxidation dependent on Fe^{2+} and Fe^{3+} [108]. Lipid peroxidation is a particularly dangerous process, because it does not conclude with the oxidation of the

first constituent, but leads to a chain reaction. It was shown that flavonoids are located in the membrane layer between the lipid bilayer and aqueous phase and can influence both enzymatic and non-enzymatic peroxidation of lipids [115]. It was reported that flavonoids also directly interact with biological membranes, reducing their fluidity, making them more resistant to many oxidative factors and hampering diffusion of free radicals [8,11].

Flavonoids also prevent oxygen radical formation through inhibiting the activity of the enzymes involved in their generation, such as cyclooxygenase, lipoxygenase, microsomal monooxygenase, glutathione S-transferase and xanthine oxidase [8,116]. Hydroxyl groups at C-5 and C-7 and double bonds between C-2 and C-3 are responsible for the ability of flavonoids to inhibit xanthine oxidase activity. The presence of a hydroxyl group at C-3 reduces this ability. Flavanones, dihydroflavonols and flavanols, which contain this group at C-3, are unable to inhibit the activity of xanthine oxidase [111,117,118]. Flavonoids also reduce the activity of membrane NADPH oxidase, which participates in generating the superoxide anion radical [119].

Regulation of gene expression provides a complex control mechanism by which plants respond to abiotic and biotic stresses and modulate developmental processes. One of the elements facilitating gene expression is the activity of transcription factors (TF). Central to the direct regulation of flavonoid biosynthesis genes are core "MBW" regulation complexes, comprising specific members of the R2R3MYB and basic helix-loop-helix (bHLH) TF families, which are responsible for binding specific regulatory sequences and WD-repeat factor (WDR; tryptophan-aspartic acid (W-D) dipeptide repeat). Variant MBW complexes can form from different MYB and bHLH components, and these can have different target genes and vary in their activation or repression actions. The WDR protein is common to all of the variant MBW complexes. The bHLH component may also be common to MBW complexes targeting different biosynthetic pathways. However, distinct R2R3MYBs are involved in regulating the different branches of flavonoid production [120–123]. Members of the MYB transcription factor superfamily are characterized by the presence of an amino acid motif structurally and functionally related to the DNA-binding domain of the product of the retroviral oncogene v-MYB and its animal cellular counterpart, c-MYB [120,123]. MYB regulators are divided into four types: MYB1R, R2R3-MYB, R1R2R3 MYB (MYB 3R) and 4RYB [124,125]. MYB proteins have been identified in a large number of eukaryotic organisms. In many plant species, MYB proteins were reported to be capable of regulating the biosynthesis of flavonoids and to be induced by stress, exemplified by *Arabidopsis* MYB75 (PAP1), AtMYB90 (PAP2) MYB12 and MYBL2 [120,126,127], petunia AN2 and PH4 [128,129], grape MYBA1 and MYBA2 [130–133], sweet potato [134], apple MYB10/MYB1/MYBA [135,136], legume LAP1 [137] and persimmon MYB4 [138] and *Epimedium sagittatum* MYBA1 [139].

Flavonoids can modulate the plant response to stresses by controlling the process of auxin transport. Auxins are one of the most important phytohormones and influence such processes as root development, mitotic transition and gene transcription. The response to hormones is connected with specific receptors, but integration and coordination between the hormone and the signaling pathway require an integrator. Flavonoids can constitute such an integrator for auxins [140,141]. Jacobs and Rubery (1988) found that flavonoids compete for auxin transporters with 1-naphthylphthalamic acid (NPA) *in vitro* [142], which suggests that flavonoids bind to NPA-interacting proteins. Studies in *Arabidopsis* suggest that these could be either plasma membrane aminopeptidase or multi-drug

resistant (MDR) ABC type transporters. *Arabidopsis* mutants with reduced flavonoid contents show elevated auxin transport levels and phenotype changes, architectonic abnormalities and gravitropic disorders [143–146]. Two NPA-binding protein complexes were found, one with a low- and one with a high-affinity NPA binding site. The first one contained a flavonol-sensitive aminopeptidase, AtAPM1, localized at the plasma membrane [147], while the second included different proteins homologous to human MDR/ABC transporters [147,148]. Additionally, the asymmetric distribution of the PIN-FORMED 1–4 (PIN1–4) auxin efflux proteins surrounding the plasma membrane regions probably contribute to auxin polar transport [149–152]. Flavonoids are efficient inhibitors of glycoproteins of PIN and MDR, which are involved in intercellular auxin transport. The ability of flavonoids to inhibit the PIN and MDR proteins correlates with the presence of a catechol group in the flavonoid molecule scaffold. Quercetin is a stronger inhibitor of auxin polar transport than kaempferol [153,154]. A small amount of auxins is transported from cell to cell through diffusion, but the majority of auxins exist in anionic form and require specific carriers for directed transport, which is crucial for the local gradient of this phytohormone. One of the mechanisms controlling this transport involves reversible protein phosphorylation by the protein kinase, PINOID (PIN) [155]. This kinase has been found in the primordia of cotyledons, leaves and floral organs and in vascular tissue in developing organs or proximal to meristems [156]. PID belongs to AGCVIII-specific plant kinases and regulates asymmetric subcellular localization of the PIN-FORMED (PIN) auxin efflux facilitator protein responsible for the auxin gradient [157]. PID plays the role of a switch influencing the PIN protein distribution in the cell membrane [158,159]. The activity of PID is regulated by PDK1 protein kinase [160]. PID partially co-localizes with PIN. PIN and MDR glycoprotein control, separately or jointly, auxin cell-to-cell transport [161,162]. Flavonoids can regulate auxin efflux by interfering with the membrane PIN/MDR glycoproteins, PDK1 and a wide range of cell wall-associated kinases (WAKs). The WAKs allow cells to recognize the extracellular environment and react by altering the cell shape (Figure 3) [163]. Control of auxin transport by flavonoids can be important in the stress-induced morphogenetic response of plants (SIMR). Species rich in dihydroxy-flavonoids present phenotypes of strikingly different morphological features in comparison to those rich in monohydroxy-flavonoids [164,165]. Branchy phenotypes are usual in insolated environments and have a few small, thick leaves exposed directly to the sunlight, thus protecting the leaves below. In contrast, shady plants rich in kaempferol and/or apigenin derivatives have long internodes and a large, thin leaf blade [165]. SIMR can contribute to the decreased damage of tissues or organs caused by solar radiation.

Flavonoids can also regulate the activity of IAA-oxidase, with different effects depending on their chemical structure [166,167].

Besides the protective functions, flavonoids can increase the availability of nutritional elements. During the period of low nutrition availability, flavonoids are released to the soil with the help of ABC transporters, where they can bind metals necessary for plant growth [168,169]. For example, isoflavonoids excreted by roots of *Medicago sativa* L. increase the availability of iron cations and phosphate anions [170]. Genistein, quercetin and kaempferol change the availability of iron by reducing Fe^{3+} to Fe^{2+} and chelating the unavailable iron from iron oxides [171].

Figure 3. Flavonoids' interference with PIN and MDR/ABC proteins, as well as with PID, WAK and PDK1 kinases leads to impaired auxin transport.
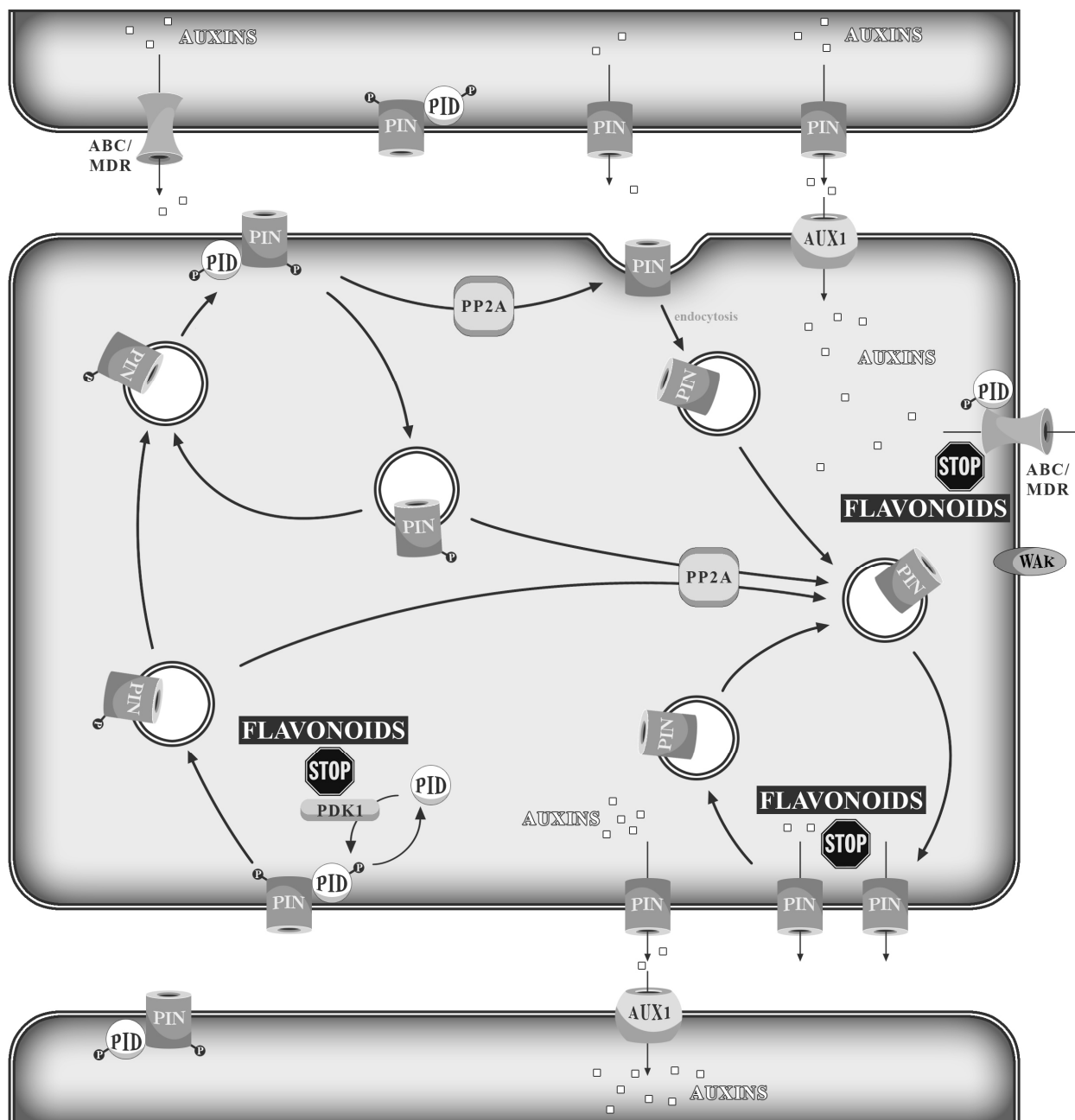
7. Prospects for the Practical Application of Flavonoids

In order to improve the efficacy of agricultural production, plant protection chemicals are widely employed. However, such chemicals are not neutral for the environment, especially for living organisms, including humans. Growing emphasis is being placed on the use of safer biopesticides. Flavonoids appear to be ideal candidates as constituents of such biopesticides. Maysin is a naturally occurring C-glycosyl flavone found in maize (*Zea mays* L.) silk tissue that confers resistance to corn earworm (*Helicoverpa zea*, Boddie). Transgenic maize with elevated quantities of this compound showed higher resistance towards larvae of the earworm. Lower body weight of the larvae and their higher mortality were observed [172]. Cespedes *et al.* found that extracts from *Calceolaria*

that contained secondary metabolites, including flavonoids, revealed insecticidal properties against *Spodoptera frugiperda* and *Drosophila melanogaster*. The authors also observed antibacterial and antifungal effects of these extracts. *Fusarium* is known to cause the greatest losses in flax cultivation. Genetic engineering, combined with traditional cross-breeding, has led to the production of new plants resistant to this pathogen [78]. Many other examples of more resistant plants overexpressing flavonoids are summarized in Table 1.

Weeds are a major problem in agriculture and are difficult to eradicate. Biopesticides based on flavonoids displaying allelopathic properties against weeds can potentially be an efficient natural defense against them. Biological trials performed by Kong *et al.* showed inhibiting activity against weeds of the species *Echinochloa crus*-galli, *Cyperus difformis* and *Cyperus iria* of 5,7,4'-trihydroxy-3',5'-dimethoxyflavone, which additionally displayed an antifungal effect [57]. Phytotoxic effects were also observed for flavonoids from *Stellera chamaejasme* L. [173]. In agriculture, flavonoids have also found application in floriculture. A number of genetic modifications of the flavonoid pathway have been described, which led to the production of new colors of flowers in such species as petunia, gerbera, rose, carnation, lisianthus and *Torenia* [174–176].

Many flavonoids are characterized by antibacterial, antifungal and antiviral activities, not only against plant pathogens, but also against the pathogens dangerous for humans. For instance, apigenin and amentoflavone display strong effects against the pathogenic fungi *C. albicans*, *S. cerevisiae* and *T. beigelii*. Kaempferol shows activity against Gram-positive and Gram-negative bacteria, as well as against the fungus *Candida glabrata* [177]. Studies also show that flavonoids can be active against antibiotic resistant strains [177]. Flavonoids, as compounds originating from plants, are part of the human diet and have many positive impacts on the human organism. They act as natural antioxidants and have an effect on many diseases. They have anti-tumor [122,178,179], anti-inflammatory [180,181], anti-allergic [182], anti-thrombotic [183], anti-diabetic [184] and anti-atherosclerotic activities [185]. A wide range of activities of flavonoid compounds is employed in cosmetology. Flavonoids improve skin hydration, restore its antibacterial barrier, smoothen its surface and induce skin cells to grow. They have protective, astringent and antiedema properties. They are also used in the treatment of acne, blackheads and dandruff, prevent baldness and wrinkles and slow down the ageing processes [186].

Transgenic plants with overproduction of flavonoids can be used in the production of plant-based medical products. Fiber, seedcake extract and oil from transgenic flax with overexpression of three genes of flavonoid synthesis became a starting point for the production of dressings for chronic wounds and ulcerations. Such wound dressings caused diminution of wound size, accelerated the wound healing process and reduced the pain connected with long lasting wounds [187].

Due to their wide range of activities, flavonoids can be used in agriculture, but also in medicine, pharmacy and cosmetology. They are an increasingly attractive group of compounds, being competitive with those used so far, mainly due to the fact that they are natural and display no adverse effects.

8. Summary

In conclusion, flavonoids are molecules displaying various biological activities with relevance to plant physiology and development. Flavonoids not only participate in protection against harmful abiotic factors, but also allow for interactions with other plants and microorganisms. Previously,

the role of flavonoids was mostly explained in terms of their antioxidative properties; however, due to recent research, they are also emerging as regulatory and signaling molecules. In particular, their influence on plant development via interaction with an auxin transport network points toward their regulatory function in view of very low concentrations needed for the activity, indeed much lower than is necessary for effective radical quenching. Their presence in the nucleus points towards their role as transcriptional regulators [188,189]. This function is already performed in regulation of microbial genes during mycorrhizal interactions. The effect of flavonoids on cellular signaling is well described in animal models where dietary flavonoids interact with many proteins of signaling cascades by directly binding to the ATP catalytic sites of protein kinases [139]. The possible interactions with MAP kinases in plants are still unexplored; however, it is likely that flavonoids may be responsible for mediating ROS-induced signaling cascades vital to cell growth and differentiation.

It is evident that flavonoids allow plants to be an integrative part of their environment by responding to biotic and abiotic stimuli. Although so much is known about the roles of these secondary metabolites, the knowledge is still incomplete and requires extensive research. Expanding knowledge on this topic and ways to manipulate flavonoid contents should allow for more practical use of these chemicals in agriculture, industry and medicine.

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Author Contributions

JM and KK–writing of the manuscript; AK–coordination of the work.

Conflicts of Interest

The authors declare no conflict of interest.

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