Flavonoids (Antioxidants Systems) in Higher Plants and Their Response to Stresses

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Abstract Flavonoids are a diverse group of secondary metabolites with a wide range of roles in mechanisms relating to UV protection, insect attraction, pathogen defense, symbiosis, variation of flower color, male fertility, pollination, allelopathy and auxin transport. Except bryophytes and pteridophytes, flavonoids are found only in higher plants. Flavonoids act as an antioxidative agent and scavenge reactive oxygen species (ROS), which are generated in plants during biotic and abiotic stresses. The ROS prevention by flavonoids is achieved through the inhibition of ROS-generating enzymes, the recycling of other antioxidants and the chelation of transition metal ions. Flavonoids are considered to be a secondary antioxidant system since they complement the function of other ROS scavenging systems when the reduction in the activities of antioxidant enzymes. This chapter describes the role of flavonoids in response to various stresses in higher plants.

Keywords Flavonoids · Reactive oxygen species · Abiotic stress
Biotic stress · Antioxidant system

1 Introduction

Flavonoids are a diverse group of secondary metabolites consisting of >10,000 structures present in various natural resources such as vegetables, fruits, bark, stem, flowers, roots, tea and wine (Middleton 1998). In addition to bryophytes and pteridophytes, the sole natural source of flavonoids is higher plants (Rausher 2006). These aromatic molecules are derived from phenylalanine and malonyl-coenzyme A via the fatty acid pathway and include six major subgroups: chalcones, flavones,

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flavonols, flavandiols, anthocyanins and the condensed tannins (or proanthocyanadins). All of these subgroups exist in most of the higher plants and the seventh group, the aurones, has been found to be widespread, but not ubiquitous. Flavonoids are well known for their associated plant tissue colors, plant fitness, and development and can be seen in various parts of plants. They perform a variety of physiological and biological functions including UV protection, insect attraction, pathogen defense, symbiosis and variation of flower color, male fertility, signaling during nodulation, pollination, allelopathy and auxin transport (Winkel-Shirley 2001). Upon exposure to various stress conditions, the flavonoid biosynthetic genes are induced, thereby increasing the flavonoid levels, especially during wounding, drought, metal toxicity and nutrient deprivation. These environmental conditions result in the production and accumulation of various reactive oxygen species (ROS), which can damage the cellular components, such as DNA, lipids, proteins and sugars. To combat these conditions, plants have evolved an elaborate machinery of antioxidants for protection against oxidative stress. Flavonoids are one among the major non-enzymatic antioxidants produced in the stressed plant and are involved in the suppression of generation of ROS, as well as reducing the ROS once formed. ROS molecules such as superoxide, hydroxyl radicals and hydrogen peroxide (H_2O_2) were scavenged by the action of flavonoids. The prevention of ROS generation by flavonoids was carried out through their capacity to chelate transition metal ions, namely Fe and Cu. Flavonoids contain the functional hydroxyl groups that mediate the antioxidant activity by scavenging free radicals as well as chelating metal ions. The metal chelating capability is essential for the impediment of radical regeneration (Kumar and Pandey 2013). Some recent studies have shown a huge accumulation of flavonoids in mesophyll cells in the vacuole as well as in the chloroplasts, which indicated their putative role as ROS quenchers. The flavonoids produced in the chloroplasts efficiently quenched singlet oxygen ($^1O_2$) and stabilized the chloroplast outer envelope membrane (Agati et al. 2012 and Goff et al. 1990). Furthermore, flavonoids act as substrates for the class III peroxidases to reduce H_2O_2 and play a crucial role in maintaining the concentration of H_2O_2 at a sub-lethal level. Under severe stress conditions, the H_2O_2, which is able to freely escape from the chloroplasts, was significantly reduced by the flavonoids that accumulated in the vacuoles of epidermal cells (Yamasaki et al. 1997). In cuticles and epicuticular waxes, flavonoids serve as an antioxidant barrier in protecting cellular components against oxidizing pollutants such as ozone (O_3) and sulfur dioxide (SO_2). Polyphenols act as antioxidants through the hydrogen-donating capacity of their phenolic groups. The metal-chelating potential also plays a role in the protection against iron and copper-induced free radical reactions. Based on the *in planta* antioxidant assay, the antioxidant capacity of flavonoids is several times higher than those of vitamin C and E due to their enhanced capacity to donate electrons or hydrogen atoms (Hernandez et al. 2009). Ascorbate has been proposed to be involved in recycling oxidized flavonoids. In chloroplasts, flavonoids oxidized by ROS molecules such as $O_2^{\cdot -}$ and H_2O_2 are recycled back to the reduced form by ascorbate. A recent study reported that the pool of vacuolar ascorbate increased dramatically because of excess light stress, and it may be speculated to be involved

in the peroxidase-catalyzed reduction of H_2O_2 using flavonoids as substrates (Zechmann et al. 2011). Flavonoid is of great interest to human health for its potential in preventing degenerative disease associated with oxidative stress through the consumption of plant-derived food. In this chapter, the biosynthesis of flavonoids in higher plants and their response to various stresses are described briefly.

2 Biosynthesis of Flavonoids

The flavonoid biosynthesis is one of the most extensively studied areas of secondary metabolites, and their identification and characterization came from enzymatic and genetic studies. For example, the enzyme chalcone synthase was isolated from irradiated parsley cells and it was the first flavonoid gene used for gene cloning experiments. Cytosol is the site of synthesis of most of the flavonoid biosynthetic enzymes and some are loosely bound with the endoplasmic reticulum and transported to the vacuole for storage. The biosynthesis of flavonoids is represented in Fig. 1.

Flavonoid biosynthesis starts with the enzyme chalcone synthase (CHS) catalyzing the condensation of three molecules of malonyl-CoA with one molecule of 4-coumaroyl-CoA to synthesis the substrate naringenin chalcone (the common intermediate for all the flavonoids (Miranda et al. 2012). Chalcone reductase (*CHR*) co-acts with *CHS* and leads to the generation of 6′-deoxychalcone, the precursor of 5-deoxyflavonoids. Chalcone isomerase (*CHI*) catalyzes the cyclization of chalcones into the corresponding (2*S*)-flavanones, the branchpoint intermediates used for isoflavonoid synthesis. Flavone synthase (*FNS*) makes the conversion of (2*S*)-flavanones to flavones. Then the flavanone 3-hydroxylase converts the (2*S*)-flavanones to their respective (2*R*, 3*R*)-dihydroflavonols, which are an intermediate in the biosynthesis of flavonols, catechins and anthocyanins. Flavonol synthase (*FLS*) catalyzes the flavonols and dihydroflavonol 4-reductase (*DFR*), which catalyzes the reduction of dihdroflavonols to leucocyanidins (Miranda et al. 2012). Leucoantho cyanidins reductase (*LAR*) and anthocyanidin reductase (*ANR*) converts the 2,3-cis-flavan-3-ols ((+)-catechin) and 2,3-cis-flavan-3-ols ((−)-epicatechin), respectively, and can be found in an outer layer of the berry skin and inner layer of the seed coat in grape berries. Anthocyanin synthase (*ANS*) involved in the biosynthesis of both anthocyanins and proanthocyanidins and is localized in grape berries, stem and leaves of the grapevine. The unknown enzyme involved in the condensation of catechin and epicatechin to form proanthocyanidins or condensed tannins is an important branch of the pathway leading to the formation of isoflavones and pterocarpans. Isoflavone synthase (*IFS*) catalyzes the conversion of (2S)-naringenin to isoflavone genistein with the formation of 2-hydroxyisoflavanone. 2-hydoxyisoflavo none dehydratase (*IFD*) catalyzes the formation of isoflavone from 2-hydroxyiso

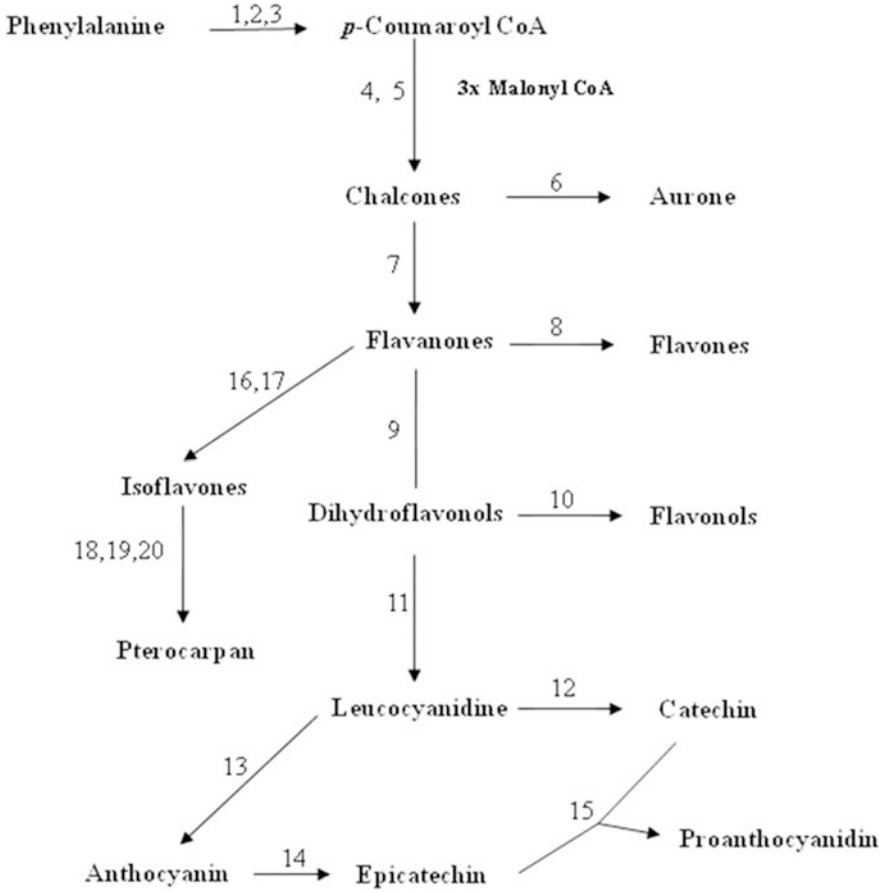

Fig. 1 Outline showing the biosynthesis of flavonoids (Miranda et al. 2012): 1: Phenylalanine ammonia lyase (*PAL*); 2: Cinnamate-4-hydroxylase (C4H); 3: 4-Coumarate:CoA ligase (*4CL*); 4: Chalcone synthase (*CHS*); 5: Chalcone reductase (*CHR*); 6: Aureusidin synthase (*AS*); 7: Chalcone isomerase (*CHI*); 8: Flavone synthase I and II (*FNS I & II*); 9: Flavanone 3-hydroxylase (*F3H*); 10: Flavonol synthase (*FLS*); 11: Dihydroflavonol 4-reductase (*DFR*); 12: Leucoanthocyanidin reductase (*LAR*); 13: Anthocyanidin synthase (*ANS*); 14: Anthocyanidin reductase (*ANR*); 15: Unknown condensing enzyme (*CON*); 16: 2-hydroxyflavonone synthase (*IFS*); 17: 2-hydroxyisoflavonone dehydratase (*IFD*); 18: Isoflavone 2′-hydroxylase (*IF2′H*); 19: Isolflavone reductase (*IFR*); 20: Pterocarpan synthase (*PTS*)

flavanone. Isoflavone reductase (*IFR*) converts the 5-deoxy-2′-hydroxyisoflavones to their 3R-isoflavonone derivatives. Finally, pterocarpan synthase (*PTS*) mediates the conversion of 2′-hydro-xyisoflavanones to their corresponding 3,9-dihydroxyptero carpans (Miranda et al. 2012).

3 Regulation of Flavonoids

In order to respond towards various environmental stress situations, plants have a controlled mechanism to regulate their gene expression in a spatiotemporal manner mediated by transcription factors (TFs). These TFs help plants to switch on and off their activity against these environmental conditions and help to adjust the physiology and metabolism according to the situation, thereby protecting against injury or death. MYB, WD40, and bHLH transcription factors together regulate the flavonoid pathway genes (Hichri et al. 2010).

The MYB family of proteins is large, functionally diverse and involved in a variety of crucial functions including controlling developmental regulations, metabolism and biotic and abiotic stress responses. There are several MYB proteins are involved in the regulation of flavonoids in various plants such as Arabidopsis (MYB75 (PAP1), MYB90 (PAP2), MYB12 and MYBL2), petunia (AN2, PH4), grape (MYBA1, MYBA2), sweet potato, apple (MYB10/MYB1/MYBA), legume (LAP1) and persimmon (MYB4) and *Epimedium sagittatum* (*MYBA1*) (reviewed in Mierziak et al. 2014). Most of the MYB TFs positively regulate the flavonoid biosynthesis whereas, some acts as a suppressor of flavonoid biosynthesis. For example, R3, AtMYBL2 and AtMYB60 act as anthocyanin repressors and inhibit anthocyanin production. Recent research in Arabidopsis and grapevine suggest that MYB regulates the flavonol pathway.

WD40 repeat proteins are a very abundant protein family in eukaryotes and have a role in providing a rigid network for the interaction of proteins with other cellular components, which helps in controlling the signaling cascades, cellular transport and apoptosis by influencing transcription. TTG1, a WD40 protein regulating the flavonoid pathway, is able to control pigmentation in seed coat and formation of trichome in leaves (Dressel and Hemleben 2009).

bHLH is widely distributed in plants and regulates processes like the development of floral organs, photomorphogenesis, hormone response, and so on. *ZmLc,* a bHLH family TF, regulates anthocyanin production in maize (Goff et al. 1990). Transient overexpression of *GL3* in *Matthiola incana* leads to the higher accumulation of anthocyanin (Ramsay et al. 2003). All these transcription factors require an additional partner and complex formation for their regulation mechanism to fulfill a different biological function.

4 Flavonoids and Stress Responses

Plants often face a stressful environment that affects their normal growth and developmental process. These adverse effects including biotic stress (pathogen infection and herbivore attack), and abiotic stress (drought, cold, heat, salt, nutrient deficiency, heavy metal toxicity, flooding, and pollution) disturb the geographical distribution of plants in nature, reduce plant yield in agriculture and threaten global

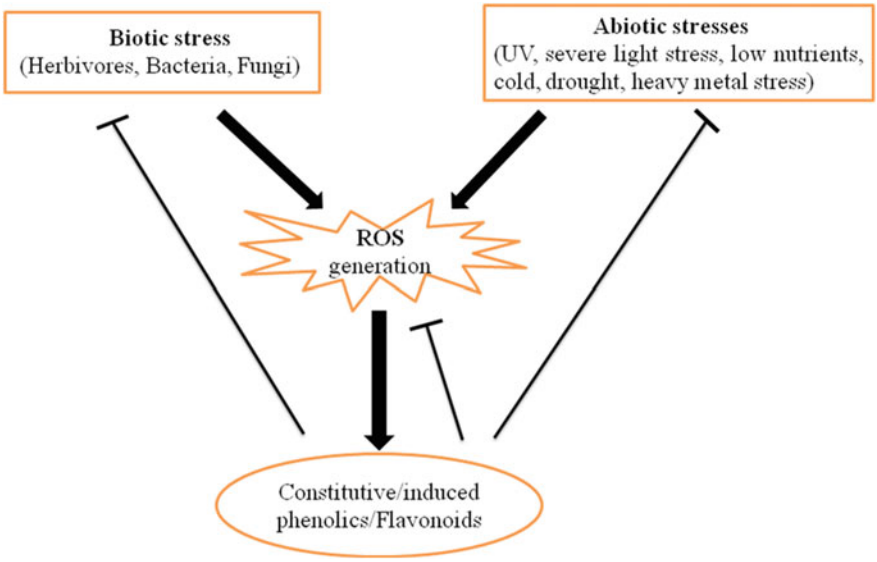

Fig. 2 Effects of flavonoids in response to biotic and abiotic stresses in higher plants

food security. ROS molecules are also generated through various metabolic pathways as byproducts in plants. Under normal conditions, these molecules are detoxified by different antioxidative components restricted to particular compartments. The imposition of both biotic and abiotic stress leads to increases in ROS levels and can cause extensive damage to normal cellular functioning. Therefore, the survival of plants depends on the severity and duration of the stress level and the capacity of plants to quickly adapt to changing conditions. The detoxification of ROS in plants is carried out through the highly evolved ROS scavenging systems including anti-oxidants and anti-oxidative enzymes. The prevention of ROS generation by the flavonoids (antioxidants) is achieved through: suppression of singlet oxygen; inhibition of ROS-generating enzymes including cyclooxygenase, lipoxygenase, monooxygenase and xanthine oxidase; the chelation of transition metal ions such as Fe and Cu; and the recycling of other antioxidants (Mierziak et al. 2014). The response of flavonoids to biotic and abiotic stresses was shown in Fig. 2. Here we briefly described the response of flavonoids to various stresses in plants.

4.1 UV and Light Stress

The energy source for plants comes from sunlight, in a process known as photosynthesis. UV radiation is also present in the sunlight; plants sense and respond to this radiation, which is known to cause damage to plant processes. Tolerance to UV

radiation depends on both the repair and acclimation response of the plants. Flavonoids act as a screen for absorbing UV radiation and scavenging the ROS generated during radiation, which in turn, protects plants against UV-induced damage. The UV-B photoreceptor activation triggers the TFs, which in turn activates the transcription of the flavonoid biosynthesis genes (Greenberg et al. 1997). Jenkins (2013) exposed *Arabidopsis thaliana* wild type, and *uvr8-1* (UV-B photoreceptor mutants) plants under a lower dose of UV-B radiation for 4 h and found that the levels of *CHS* were stimulated compared with that of unexposed control plants. The increased level of flavonoids and higher antioxidant capacity was found in blueberries after illumination with UV-C radiation (Wang et al. 2009). When *Ulva fasciata* was exposed to an elevated dose of UV-B radiation, it led to the enhanced production of flavonoids, whereas the activity of catalase (CAT) and (ascorbate peroxidase) APX (antioxidant enzymes) was greatly reduced (Aguilera et al. 2002; Shiu and Lee 2005). Soitamo et al. (2008) reported the elevated expression of genes related to the biosynthesis and conjugation of flavonoids when exposed to severe light stress. However, the excess light treatment did not affect the SOD activity. Huang et al. (2016) reported that upon UV-B stress, the hairy root cultures of *Fagopyrum tataricum* showed a higher accumulation of rutin (4.11 times higher) compared with that of the non-transformed culture. In addition, a dramatic change in transcription of flavonoid biosynthetic genes was also observed. In various plant species, UV-induced enhancement of the ratio of dihydroxy to monohydroxy B-ring-substituted flavonoid glycosides (i.e., luteolin to apigenin or quercetin to kaempferol ratios) was reported previously (reviewed in di Ferdinando et al. 2012). Interestingly, the luteolin and quercetin glycosides prevent ROS generation by effectively chelating the Fe and Cu ions (Brown et al. 1998; Melidou et al. 2005). UV-B radiation exposure induced the production of quercetin derivatives in grape leaves (Berli et al. 2010). Flavonoids are considered to be one of the secondary antioxidant systems due to their upregulation in stress conditions and they also contribute to detoxification of ROS molecules. All these results suggest the role of flavonoids in the UV-mediated stress response in higher plants.

4.2 Water and Salt Stress

When plants are exposed to drought or salt stress, this leads to osmotic stress and ROS accumulation, which in turn negatively affects the cellular structure and metabolism. Plants can adapt to these unfavorable conditions, through a reduction in growth, accumulation of compatible solutes, increased level of antioxidants and so on. Hernández et al. (2004) analyzed drought-induced changes in *Cistus clusii* grown under field conditions and showed that epigallocatechin gallate (flavonoids), ascorbic acid and α-tocopherol (low molecular weight antioxidants) increased by 2.8-, 2.6- and 3.3-fold, respectively, after 50 days of drought treatment. Shojaie et al. (2016) studied the flavonoid pattern in drought-induced *A. thaliana* seedlings, and they observed that both flavonols (quercetin and kaemferol) and total flavonoids

were greater in roots than in shoots. Lama et al. (2016) reported that there were increased concentrations of flavonoids in *Jatropha* seedlings under oxidative stress in simulated higher drought (200 mm $year^{-1}$) and artificial damage (50%) conditions. Furthermore, they suggested that the protection against oxidative damage and photodamage in *J. curcas* leaves is performed by flavonoids. Vasquez-Robinet et al. (2008) showed that severe drought stress significantly enhanced the expression of flavonoid biosynthetic genes (*CHS* and *GST*), indicating the protective role of flavonoids against water stress. Genes involved in flavonoid biosynthesis and their transport were effectively induced following water stress in grape berries (Castellarin et al. 2007a, b). Similarly, the water stress and sunlight irradiance in leaves of *Ligustrum vulgare* caused the increased biosynthesis of flavonoids.

The salt stress imposed by NaCl induced the flavonoid biosynthetic genes. The upregulation of *F3'H,* which leads to the biosynthesis of antioxidant flavonoids (ortho-dihydroxylated B-ring), was higher in the salt-sensitive genotype than in the salt-tolerant genotype rice (Walia et al. 2005). A positive correlation was found between the elevated level of flavonoid biosynthesis and the increased glutathione S-transferase (involved in the transportation of flavonoids to the vacuole) (Zhao and Dixon 2009). The enhanced carbon allocated to the two flavonoid anti-oxidants, such as myricetin and quercetin glycosides, was higher in the salt-sensitive *Myrtus communis* compared with the salt-tolerant *Pistacia lentiscus* and participates in the peroxidase-mediated reduction of H_2O_2 (Tattini et al. 2006). Salinity and UV radiation significantly enhanced the biosynthesis of luteolin 7-O-glycosides (Agati et al. 2011). Abdallah et al. (2016) investigated the effect of salt stress using the seedlings of *Solanum nigrum* and showed a reduction in the dry biomass of roots and leaves followed by a higher accumulation of total flavonoid, as well as induced transcription of flavonoid genes associated with a higher salt concentration. Martinez et al. (2016) analyzed the phenylpropanoid metabolism at the gene and enzyme level in the tomato plants exposed to heat, salinity or a combination of both stresses. Their results indicated that the oxidative damage was lower when flavonols accumulated over as compared with the level of hydroxycinnamic acids. Taken together, all of these results suggested the antioxidant role of flavonoids in response to drought and salt stress in higher plants.

4.3 Ozone

O_3 is a strong oxidizing secondary pollutant formed in the troposphere, which are ready to interact with the biomacromolecule (Mustafa 1990). O_3 treatment induced the aromatic secondary metabolism, such as flavonoid and shikimate biosynthesis (reviewed in di Ferdinando et al. 2012). Interestingly, *PAL* transcripts were induced within 3 h of O_3 treatment in Arabidopsis, whereas the transcripts of other antioxidant enzymes were induced after 12 h of treatment (Sharma and Davis 1994). *PAL* and *GST* expression were induced within 2–3 h of O_3 treatment, which led to a two-fold higher concentration of flavone glycoside (reviewed in di

Ferdinando et al. 2012). Furthermore, O_3 fumigation lead to the upregulation of flavonoid biosynthetic genes such as chalcone synthase (*CHS*) and chalcone isomerase (*CHI*) in several plant species (Kangasjarvi et al. 1994; Paolacci et al. 2001). The enhancement of kaempferol 3-O-glycoside was observed in beeches treated with O_3 (Betz et al. 2009). He et al. (2009) reported that the O_3 treatment reduced the level of total phenolics, whereas, it increased the biosynthesis of quercetin derivatives in the leaves of *Ginkgo biloba*. In another study, a mild O_3 stress specifically induced the flavonoid biosynthesis, which in turn was involved in the counteraction of damage imposed by O_3 (Saviranta et al. 2010). These results clearly indicate the strong responsiveness of flavonoids in response to O_3 stress in plants.

4.4 Nitrogen Deficiency and Cold

Nutrient deprivation and cold stress were also shown to influence the level of flavonoids in many plant species. Flavonoids were shown to accumulate in response to low temperature (19–11 °C) in the epidermal cells of a diverse plant species and this enhancement was observed when plants were exposed to light irradiance (Bilger et al. 2007). Similarly, in *A. thaliana* accessions, a positive correlation between the levels of flavonoid and the cold tolerance was observed by Korn et al. (2008). The biosynthetic genes of di-hydroxylated B-ring flavonols (*CHS*, *CHI*, *DFR*, *FLS1*, and *F3'H*) were strongly expressed in cold-tolerant plants compared with cold-sensitive plants, which also directly correlates with the accumulation of quercetin derivatives and anthocyanins (Hannah et al. 2006). The nitrogen depletion and low-temperature treatment significantly enhanced the production of quercetin as compared with kaempferol. Kaempferol glycosides showed less responsiveness to low nitrogen treatment (Olsen et al. 2009). Therefore, low nitrogen and low-temperature treatment regulate the flavonoid biosynthesis and favor the biosynthesis of quercetin derivative (an antioxidant) as compared with the corresponding monohydroxy B-ring-counterparts—namely, kaempferol glycosides.

4.5 Heavy Metals and Other Stress Stimuli

Heavy metals and elicitors also induced the production of ROS, which in turn are scavenged by the action of flavonoids. Izbianska et al. (2014) found that treating yellow lupin with lead at a concentration of 150 mg l^{-1} increases the total flavonoid content to 67% in cotyledons compared with root, which increases to 54% total flavonoid content. Babu et al. (2003) used the aquatic plant *Lemna gibba* treated with metal copper; this lead to the accumulation of ROS and induced the synthesis of flavonoids. The increased concentration of flavonoids and caffeic acid was found in the leaves of high Ni^{2+} treated *Matricharia camomilla* plants, whereas, the

concentrations of coumaric acid derivatives and phenolic acids was not altered (Kováčik et al. 2009). Root suspension culture treated with excess Cu^{2+} leads to increased flavonoid content and enhanced ROS scavenging activity. Moreover, the excess Cu^{2+} ions were reported to induce the biosynthesisof flavonoids (mostly luteolin glycosides) in the absence of UV irradiance (Ali et al. 2006). Flavonoids provide a beneficial activity on Cd^{2+} stress through influencing the auxin transport and therefore tightly control the root architecture (Potters et al. 2007). Methyl jasmonate (MeJa) treatment did not affect the flavonoid concentration in different commercially available broccoli florets but varied significantly among cultivars and growing seasons (Ku and Juvik 2013). *Nicotiana tabacum* and *Lemna gibba* plants were grown in flavonoid (quercetin)-supplemented medium and the authors observed that the quercetin counteracted with paraquat and retained the chlorophyll level (Kurepa et al. 2016). Gondor et al. (2016) reported that the exogenous treatment of salicylic acid (SA) in maize caused increased levels of oxidative stress in leaves, which in turn induced the expression of genes involved in flavonoid metabolism. The enhanced stress tolerance induced by SA treatment in wheat is achieved through the increased expression of flavonoid metabolism-related genes and the enhanced level of non-enzymatic antioxidant compounds (e.g., quercetin and ortho-hydroxy-cinnamic acid) (Gondor et al. 2016).

5 Flavonoids in the Biotic Stress Response

The damage induced by bacteria, fungi, nematodes, protists, insects, viruses and viroids in plants are known as biotic stresses, which is a primary concern in terms of crop losses in agriculture (Baskar et al. 2012). Antioxidants play a role in the detoxification of ROS induced during abiotic and biotic stresses in plants. Among the different antioxidants, flavonoids also play an essential role in the ROS scavenging mechanism. Beckman (2000) described that phenolic compounds, including flavonoids, were stored in specialized tissues and were dislocated to the infected site (i.e., xylem vessels) when pathogen infestation occurred. In the infected plants, flavonoids accumulated at the site of infection in order to induce the hypersensitivity reaction and programmed cell death (PCD). In general, the phenolic infusion took place at the earlier stage of infection. The pathogen invasion was blocked by the formation of callus and tylose and was mediated through the modulation of IAA by flavonoids.

In higher plants, many flavonoids have been reported to be antifungal phenolic compounds (Grayer and Harborne 1994). Spore development and hyphae elongation in fungal infection are inhibited by the flavonoids (Blount et al. 1992). The antipathogenic activity of flavonoids is specific in nature. The antibacterial activity is mediated through the inactivation of microbial adhesion and cell envelope transport proteins (Plaper et al. 2003; Naoumkina et al. 2010). The disruption of microbial membranes and the alteration of their fluidity by the fat-soluble flavonoids which results in the disruption of respiratory chain (Haraguchi et al. 1998; Mishra et al. 2009). Usually, when a pathogen or pest attacks, plants induce the

production of flavonoids (Barry et al. 2002; Gallet et al. 2004). The anthocyanin accumulation in the epidermal tissues of cotton leaves is a sign of resistance against the *Xanthomonas campestris* pv. *malvacearum* (Kangatharalingam et al. 2002). Skadhauge et al. (1997) reported that the dihydroquercetin are involved in the defense activity against *Fusarium s*pecies in barley mutants. This is mainly through the cross-linking of microbial enzymes and the inhibition of microbial enzymes (e.g., cellulases, xylanases and pectinases) by the chelation of metal ions, which are crucial for the enzyme activity and act as a hard physical barrier against pathogen attack. Padmavati et al. (1997) reported that the growth inhibitory activity of naringenin was higher followed by kaempferol, quercetin and dihydroquercetin against the fungal blast pathogen *Pyricularia oryzae*. However, except naringenin, all others were ineffective against the bacterial blight pathogen *Xanthomonas oryzae* pv. *Oryzae.* Moreover, these flavonoids were not significantly affected the growth of *Rhizoctonia solani* (fungal sheath blight of rice).

Benzylaminopurine and Brotomax have been used for inducing resistance in various crops such as olive, grape and *Citrus* spp (del Rio et al. 2000, 2003; Gonzalez et al. 2001). Anti-fungal sakuranetin and other phytoalexins were enhanced in the rice plants treated with chitosan (Agrawal et al. 2002). Yogendra et al. (2015) reported that the resistance against late blight disease (*Phytopthora infestans*) in potato plants is correlated with the cell wall thickening due to the deposition of hydroxycinnamic acid amides, flavonoids and alkaloids. Furthermore, flavonoids play a crucial role in the post-harvest disease resistance in fruits and vegetables (Lattanzio et al. 1994; Lattanzio 2003). The presence of the elevated level of flavonoids in fruits is mostly correlated with the reduced prevalence of pathogens. Industrial methods such as light, UV radiation, temperature, humidity and phytoregulators can also alter the production of flavonoids in citrus peel (Arcas et al. 2000). The levels of flavonoids such as naringin, tangeretin, sinensetin and nobiletin have been increased in the peels of *Citrus aurantium* fruits upon UV treatment (Arcas et al. 2000). This treatment also decreased the damage imposed by *Penicillium digitatum*. Zhang and Quantick (1997) reported that litchi fruits coated with the chitosan increased the flavonoid content as well as resistance to browning and post-harvest decay. Similarly, the plant extract, Milsana® induced the production of flavonoids at the infection site of cucumber leaves and decreased the incidence of powdery mildew (Fofana et al. 2002; McNally et al. 2003). Flavonoids have been shown to be located in the haustorial complex of the pathogen *Podosphaera xanthii,* where they may contribute to the destruction of the pathogen.

6 Concluding Perspectives

Flavonoids are major secondary metabolites with multifunctional bioactivities and they are widely distributed in various parts of the plants. They are structurally diverse metabolites, and more than 10,000 structures have been reported. Flavonoids performed a variety of physiological and biological functions and

mainly act as an antioxidant to prevent damage from the ROS generated during stress conditions. The plethora of functions of flavonoids is due to their locations in different cells and sub-cellular compartments, as well as their diversified chemical structures. Flavonoids are induced by various stresses such as abiotic and biotic stresses, and in turn prevent the oxidative damage caused by these stresses in plants. Moreover, as compared with other antioxidants such as ascorbate and tocopherols, the antioxidant role of flavonoids in plants has been less studied. Detailed studies should be carried out to explore the relevance of stress-induced flavonoids in an *in planta* environment. Several studies have indicated the antioxidant, antiproliferative, antitumor, anti-inflammatory and proapoptotic activities of flavonoids in animal systems. Most of the studies emphasized the antioxidant role of flavonoids against various stresses in plants. The interaction of flavonoids with the cellular signaling systems and their molecular targets remains elusive. Moreover, detailed molecular studies investigating the mechanisms behind the roles of flavonoids in response to biotic and abiotic stresses in plants should be carried out in order to uncover the potential usefulness of these compounds.

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