# Recent Advances in Polyphenol Research

**Recent Advances in Polyphenol Research**

A series for researchers and graduate students whose work is related to plant phenols and polyphenols, as well as for individuals representing governments and industries with interest in this field. Each volume in this biennial series will focus on several important research topics in plant phenols and polyphenols, including Chemistry, Biosynthesis, Metabolic Engineering, Ecology, Physiology, Food, Nutrition, and Health.

**Volume 1 editors:**

Fouad Daayf and Vincenzo Lattanzio

# Recent Advances in Polyphenol Research

Volume 1

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## Dedication

To Michel Bourzeix and his collaborators, Carmen Bataller and Nicolas Hérédia, whose efforts and enthusiasm have been decisive for the life of the Groupe Polyphénols.

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# Preface

Plant phenolics are secondary metabolites that constitute one of the most common and widespread groups of substances in plants. They represent adaptive characters that have been subjected to natural selection during evolution, when the presence of a particular secondary metabolite has conferred a selection advantage to the plant containing it.

Polyphenols have a large and diverse array of beneficial effects on both plants and humans. For example, they are famous as antioxidants, hormones, constituents of essential oils, natural neurotransmitters, and as having many other biological activities. Their antioxidant ability is known to confer many health benefits such as reducing the risk of cardiovascular disease and cancer. They also provide antimicrobial activity for the plant's own defense against invading pathogens. The diversity of structure and activity of phenolic compounds has resulted in a multiplicity of research areas such as chemistry, biotechnology, ecology, physiology, nutrition, medicine, and cosmetics. The International Conference on Polyphenols, organized under the auspices of 'Groupe Polyphénols', is a unique opportunity for scientists in these and other fields to get together every other year and exchange their ideas and new findings. Apart from the two-page manuscripts – *Polyphenols Communications* – that comprise the proceedings of this conference, a separate volume has been concurrently published, comprising full chapters by the conference guest speakers. For the first time in the history of the conference, the board of 'Groupe Polyphénols' decided in 2005 that, starting in Winnipeg (ICP 2006), such a volume should be published by a renowned publisher such as Wiley–Blackwell, and given the series title *Recent Advances in Polyphenol Research*. The present (first) volume in the series is from the 23rd conference, which was hosted by the University of Manitoba in Winnipeg, Manitoba, Canada, from August 22 to August 25 2006, and chaired by Dr Fouad Daayf. The University of Manitoba, established in 1877, is one of the oldest universities in Canada. Its education and research programs are dedicated to many areas including agriculture, art, architecture, medicine, business, and science. The interest in polyphenols involves many departments on campus including the Departments of Plant Science, Chemistry, Food Science, Nutrition, and Human Ecology, as well as other health and functional food-related centers such as the Richardson Center for Functional Foods and Nutraceuticals.

In addition to the guest speakers' chapters, the present volume also includes full chapters from other selected speakers at the 23rd International Conference on Polyphenols, and covers five topics:

1) *Phenols and Polyphenols Chemistry*: (a) Isolation and structural elucidation; (b) Synthesis; (c) Reactivity and physico-chemical properties; (d) Biomolecular interactions.

2) *Phenols and Polyphenols Biosynthesis and Genetic Manipulation*: (a) Metabolic pathways; (b) Enzymology; (c) Biotechnology advances.
3) *Ecology and Physiology of Plant Phenolics*: (a) Biotic and abiotic stress; (b) Phenolic functions in plant development; (c) Role of phenolics in soil ecology.
4) *Food and Nutrition*: (a) Dietary intake; (b) Bioavailability; (c) Safety and toxicity; (d) Functional foods and nutraceuticals; (e) Taste.
5) *Phenolics and Health*: (a) Biological activities; (b) Drug discovery and development; (c) Cosmetics.

These topics were presented in 59 oral communications and 222 posters, and scientists had the opportunity to debate their results, and sometimes their divergent theories, in an exciting manner.

The 23rd International Conference on Polyphenols would not have been possible without the generous support of public and private donors such as the Manitoba Rural Adaptation Council and the University of Manitoba. Other sponsors include Horphag, l'Agence Universitaire de la Francophonie, Phytochemistry, Cargill, and Monsanto. Our sincere thanks go to all of our sponsors.

Fouad Daayf, Conference Chair
Vincenzo Lattanzio, President of 'Groupe Polyphénols'

Chapter 1

# Plant Phenolics – Secondary Metabolites with Diverse Functions

*Vincenzo Lattanzio, Paul A. Kroon, Stéphane Quideau and Dieter Treutter*

## 1.1 Secondary metabolism in the interactions between plants and their environment

Plant secondary metabolism constitutes a large reservoir of natural chemical diversity that encompasses an enormous range of compounds and enzymes, and a wide spectrum of mechanisms of gene regulation and of transport of metabolites and enzymes. Among the thousands of metabolites produced by plants, only a few are part of 'primary' metabolic pathways and the rest are termed 'secondary'; this term is historical and was initially associated with inessentiality. Levels of secondary metabolites in plants are both environmentally induced as well as genetically controlled.

In contrast with basic metabolism, which refers to the anabolic and catabolic processes required for cell maintenance and proliferation, secondary metabolism involves compounds present in specialized cells that are not directly essential for basic photosynthetic or respiratory metabolism, but are thought to be required for plants' survival in the environment. Secondary metabolism is considered an integral part of the developmental program of plants, and the accumulation of secondary metabolites can demarcate the onset of developmental stages. The ability to synthesize secondary compounds has been selected throughout the course of evolution in different plant lineages when such compounds addressed specific needs. Secondary metabolites apparently act as defense (against herbivores, microbes, viruses or competing plants) and signal compounds (to attract pollinating or seed dispersing animals), as well as protecting the plant from ultraviolet radiation and oxidants. Therefore, they represent adaptive characters that have been subjected to natural selection during evolution (Hättenschwiler & Vitousek, 2000; Pichersky & Gang, 2000; Kutchan, 2001; Theis & Lerdau, 2003; Wink, 2003; Kliebenstein, 2004; Kutchan & Dixon, 2005; Memelink, 2005).

This requirement for secondary metabolites to have highly diverse biological activities has led plants to accumulate a vast number of compounds. Plant genomes are variously estimated to contain 20,000–60,000 genes, and perhaps 15–20% of these encode enzymes for secondary metabolism, while the genetic complement of the fruit fly (*Drosophila melanogaster*) is substantially lower (13,601 predicted genes). One explanation for this

discrepancy in the relationship between biological and genetic complexity may lie in the differences between the ways that plants and animals protect themselves against predators, pests, diseases, and abiotic stress. Animals have developed nervous and immune systems that enable them to detect and respond to danger, and they are capable of avoiding perilous situations. By contrast, plants cannot escape from their biotic and abiotic stressors, being linked to the ground by means of their root system, and therefore they must stay and protect themselves. Plants, as sessile organisms, evolve and exploit metabolic systems to create a rich repertoire of complex natural products that hold adaptive significance for their survival in challenging ecological niches on earth. The production of chemicals that deter or kill pests and pathogens represents one mean of self-protection. The pattern of secondary metabolites in a given plant is complex; it changes in a tissue- and organ-specific way; regularly, differences can be seen between different developmental stages (e.g., organs important for survival and reproduction have the highest and most potent secondary metabolites), between individuals, and between populations (Wink, 1988; Pichersky & Gang, 2000; Osbourn *et al.*, 2003; Wink, 2003; Noel *et al.*, 2005).

Plants produce a large number of secondary metabolites, which are classified into several groups according to their biosynthetic routes and structural features. Phenolic compounds are the most widely distributed secondary metabolites, ubiquitously present in the plant kingdom: it is estimated that about 2% of all carbon photosynthesized by plants, amounting to about $1 \times 10^9$ t per annum, is converted into flavonoids or closely related compounds (Robards & Antolovich, 1997). The terms 'phenol' and 'polyphenol' can be defined chemically as substances that possess an aromatic ring bearing one (phenol) or more (polyphenol) hydroxyl substituents, but in the context of plant phenolics such a definition is not satisfactory, since it would include compounds such as the phenolic carotenoid 3-hydroxyisorenieratene or the phenolic female sex hormone estrone, which are principally terpenoid in origin (Harborne, 1989). A first comprehensive definition of 'plant polyphenols,' based on the earlier proposal of T. White, E.C. Bate-Smith & T. Swain, was given by E. Haslam, who stated that the term 'polyphenol' (*syn*. vegetable tannin) refers to water-soluble phenolic compounds having molecular masses between 500 and 3,000–4,000 Da, possessing 12–16 phenolic groups and 5–7 aromatic rings per 1000 relative molecular mass, and expressing special properties such as the ability to precipitate proteins and alkaloids (Haslam, 1998). This original definition of polyphenols has broadened out considerably over the years to include phenolics with much simpler structures. A large number of these plant phenolics are small molecules with no tanning action. As a general rule, the terms 'plant phenolics' and 'polyphenols' should refer to secondary natural metabolites arising biogenetically from either the shikimate/phenylpropanoid pathway or 'polyketide' acetate/malonate pathway, or both, producing monomeric and polymeric phenols and polyphenols, as chemically defined above, and which fulfill a very broad range of physiological roles in plants (Quideau, 2004, 2006). In fact, although the bulk of these compounds play cell wall structural roles, plant tissues synthesize a vast array of non-structural constituents that have various roles in plant growth and survival.

Unless they are completely esterified, etherified or glycosylated, plant phenolics are normally soluble in polar organic solvents. Most phenolic glycosides are water-soluble but the corresponding aglycones are usually less so. With a few exceptions, water solubil-

ity increases with the number of hydroxyl groups present. Some phenolics are solubilized by sodium hydroxide and sodium carbonate, but in alkaline media their oxidation is enhanced and therefore treatment with alkaline solvents should either be performed under $N_2$ or – preferably – be avoided. Phenolics with only a few hydroxyl groups are soluble in ether, chloroform, ethyl acetate, methanol, and ethanol. Methanol, ethanol, water, and alcohol–water mixtures are most commonly used for dissolving phenolic compounds for analytical purposes (Van Sumere, 1989). All phenolic compounds exhibit intense absorption in the UV region of the spectrum and those that are colored absorb strongly in the visible region as well. Each class of phenolic compound has distinctive absorption characteristics. For example, phenols and phenolic acids show spectral maxima in the range 250–290 nm; cinnamic acid derivatives have principal maxima in the range 290–330 nm; flavones and flavonols exhibit absorption bands of approximately the same intensity at about 250 and 350 nm; chalcones and aurones have an absorption peak of great intensity above 350 nm and a much less intense band at 250 nm; anthocyanins and betacyanins show rather similar absorption in the visible region (475–560 nm and 535–545 nm, respectively) and a subsidiary peak at about 270–275 nm (Mabry *et al.*, 1970).

Plants need phenolic compounds for pigmentation, growth, reproduction, resistance to pathogens, and many other functions. The structure of plant phenolics and polyphenols varies from simple molecules, such as phenolic acids, to highly polymerized compounds, such as proanthocyanidins, and several thousand (among them over 8,150 flavonoids) different compounds have been identified with a large range of structures. Several classes of phenolics have been categorized on the basis of their basic skeleton (Fig. 1.1): $C_6$ (simple phenols, benzoquinones), $C_6$–$C_1$ (phenolic acids), $C_6$–$C_2$ (acetophenones, phenylacetic acids), $C_6$–$C_3$ (hydroxycinnamic acids, coumarins, phenylpropenes, chromones), $C_6$–$C_4$ (naphthoquinones), $C_6$–$C_1$–$C_6$ (xanthones), $C_6$–$C_2$–$C_6$ (stilbenes, anthraquinones), $C_6$–$C_3$–$C_6$ (flavonoids, isoflavonoids), $(C_6$–$C_1)_2$ (hydrolyzable tannins), $(C_6$–$C_3)_2$ (lignans, neolignans), $(C_6$–$C_3$–$C_6)_2$ (biflavonoids), $(C_6$–$C_3)_n$ (lignins), $(C_6)_n$ (catechol melanins), $(C_6$–$C_3$–$C_6)_n$ (condensed tannins) (Harborne, 1980; Hättenschwiler & Vitousek, 2000; Iwashina, 2000).

Phenolic compounds are found throughout the plant kingdom but the type of compound present varies considerably according to the phylum under consideration. Phenolics are uncommon in bacteria, fungi, and algae and the classes of phenols recorded are few; flavonoids are almost completely absent. Bryophytes are regular producers of polyphenols including flavonoids, but it is in the vascular plants that the full range of polyphenols is found (Swain, 1975; Harborne, 1980). Phenolic compounds have been synthesized during the course of evolution by different plant species when the presence of a particular secondary metabolite has conferred a selectionary advantage on the plant containing it. As previously stated, plants synthesize a greater array of secondary compounds than animals because they cannot rely on physical mobility to escape from their predators and have therefore evolved a chemical defense against such predators. Generally, the role of phenolic compounds in defense is related to their antibiotic, antinutritional or unpalatable properties.

Besides their involvement in plant–animal and/or plant–microorganism relationships, plant phenolics also have key roles as the major red, blue, and purple pigments, as antioxidants and metal chelators, as signaling agents both above and below ground between

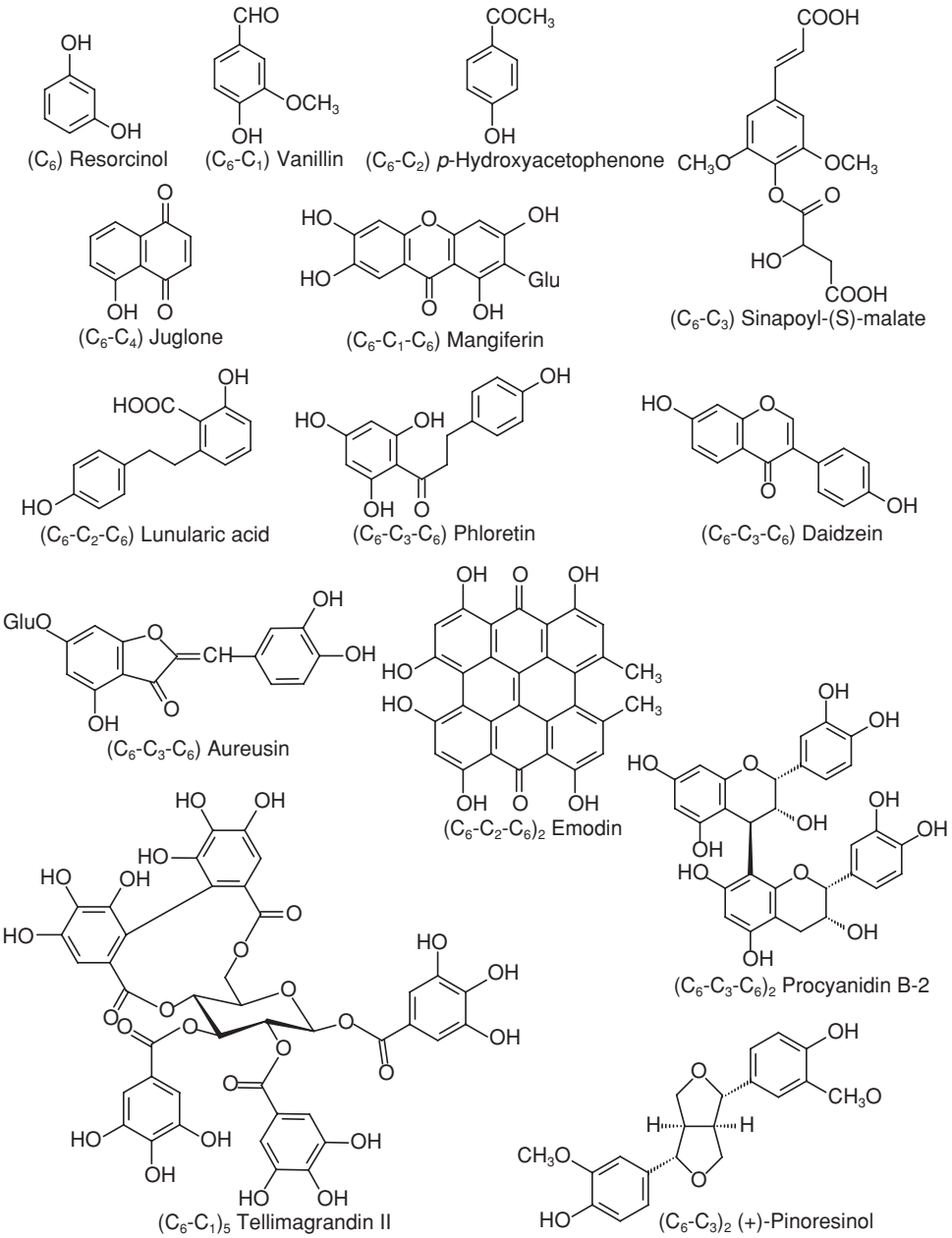


**Fig. 1.1** Some examples of phenolic structures.

plants and other organisms, and as UV light screens. This latter property has very much benefited some higher members of the Charophyceae, which are regarded as prototypes of amphibious plants that presumably preceded true land plants when they emerged from an aquatic environment onto the land; their successful adaptation to land was achieved

largely by massive formation of 'plant phenolic' compounds. Finally, some studies have shown that phenolic metabolism is not only a protective mechanism against biotic and abiotic stresses but also part of the molecular programs that contribute to normal plant growth and development (Noel *et al.*, 2005; Taylor & Grotewold, 2005).

To achieve their function plant phenolics generally accumulate in specific tissues or cell types in which subcellular localization is highly regulated. Secondary metabolites are often transported from source cells to neighboring cells, or even further to other tissues or remote organs. Several studies have indicated a high degree of compartmentalization of phenolic compounds and of the enzymes involved in their biosynthesis. Phenolics usually accumulate in the central vacuoles of guard cells and epidermal cells as well as subepidermal cells of leaves and shoots. Furthermore, some phenolics are found covalently linked to plant cell wall, while others occur in waxes or on the external surfaces of plant organs. Some findings suggest also a deposition of flavonoids in the nuclei of certain tree species; it has been suggested that a flavonoid–DNA complex provides mutual protection against oxidative damage (Wink, 1997; Sarma & Sharma, 1999; Beckman, 2000; Croteau *et al.*, 2000; Feucht *et al.*, 2004; Yazaki, 2005).

## 1.2 Function and use of plant phenolics

Plant phenolics have been considered for a long time to be waste products of primary metabolism. The defense hypothesis was not accepted by most botanists before the 20$^{th}$ century because most of them were not convinced of evolution and adaptive explanations. On the other hand, the potential value of plant secondary metabolites to taxonomy has been recognized for nearly 200 years, even if their practical application has been restricted to the 20$^{th}$ century, and predominantly to the last 40 years. The use of secondary compounds has clear advantages over the use of primary compounds in establishing phylogenetic relationships at species or infraspecific levels because differences in the complement of secondary compounds are qualitative differences whereas differences in the concentrations of primary compounds are quantitative differences, and these are subject to environmental as well as to genetic control. Flavonoids are particularly convenient for this purpose as they are widely distributed among plants and are chemically stable. The existence of a common pattern of secondary compounds may indeed provide much clearer evidence of common ancestry than morphological similarities attributable either to common ancestry or to convergent evolution. Moreover, the existence of a characteristic phenolic pattern, which taxonomists use to separate species, can also have enough adaptive value for survival through natural selection (Bell, 1980; Van Sumere & Vande Casteele, 1985; Lattanzio *et al.*, 1996; Wink, 2003).

In the past 30 years the view of plant phenolic metabolites as one of nature's meaningless waste products has been replaced by the current opinion that plant phenolics play crucial roles in plant ecology and plant physiology. In addition, biomedical research has revealed that dietary phenolics, because of their antioxidant and free radical scavenging properties, play important roles in the prevention of many of the major contemporary chronic diseases (Kutchan, 2001).

### *1.2.1 UV sunscreens*

The hypothesis of the protective role of phenolics against harmful UV rays is supported by the enhanced levels of phenolics observed in plants exposed to strong UV radiation. Plants in the field are exposed to ambient solar ultraviolet-B (UV-B) radiation (280–320 nm), which is an environmental challenge negatively affecting DNA, proteins and membranes, thus leading to altered metabolism through the generation of reactive oxygen species (ROS). Plants protect themselves from this harmful radiation by synthesizing phenolic compounds, which act as a screen inside the epidermal cell layer, and by making adjustments to the antioxidant systems at both cell and whole organism level. By means of this mechanism phenolics would prevent mutagenesis and cellular death by dimerization of thymine units in the DNA, which shows an absorption maximum at 260 nm, and possible photodestruction of coenzymes NAD or NADP, which have a maximum at 260 nm. It has been proposed that flavonoids with their high absorptivity at 250–270 and 335–360 nm act as good UV screens (Swain, 1975; Carletti *et al.*, 2003).

It is noticeable that tropical and high-altitude plants contain a higher proportion of flavonoids than temperate ones do. Several studies have demonstrated the change in flavonoid composition of plant leaves as a consequence of an excess of light or UV radiation. The activation of flavonoid biosynthetic genes by UV radiation has been shown in a number of studies (Chappell & Hahlbrock, 1984; Olsson *et al.*, 1998; Hofmann *et al.*, 2000; Logemann *et al.*, 2000; Kolb *et al.*, 2001).

The importance of flavonoids in UV protection has also been proved using mutants of *Arabidopsis* that have a block in flavonoid production and are, therefore, UV-hypersensitive phenotypes (Ryan *et al.*, 2001). These studies suggest that other phenolic compounds may be at least as important as flavonoids in UV protection. Speculating about the role of phenylpropanoids as sunscreens to absorb UV-B irradiation in various phenylpropanoid mutants of *Arabidopsis thaliana*, Kliebenstein (2004) observed that all phenylpropanoid-deficient mutants exposed to UV-B radiation were more UV-B sensitive in comparison with the wild type, but there were dramatic differences in sensitivity to UV-B between these mutants. These results have suggested that both preformed hydroxycinnamic acid sinapoyl esters and induced flavonols act as UV-B protectants and that the importance of hydroxycinnamic acids and flavonols is directly related to their relative concentrations.

### *1.2.2 Phenolics as signal compounds*

There are several reports suggesting that phenolic compounds influence the pools and fluxes of inorganic and organic soil nutrients. Polyphenols enter the soil mainly as leachates from above- and below-ground parts of plants and/or within above- and below-ground plant litter. Phenolic compounds can directly affect the composition and activity of decomposer communities thus influencing the rates of decomposition and nutrient cycling. Different types of soluble phenolics, such as ferulic acid, gallic acid or flavonoids, have been found to either stimulate or inhibit spore germination and hyphal growth of saprophytic fungi. Mycorrhizal fungi might be even more sensitive to phenolic compounds, but again different types of polyphenols can have opposite effects. Plant mycorrhizal infection, nutrient uptake and plant growth can be impaired by specific phenolics released by competitors

(Hättenschwiler & Vitousek, 2000), a process refered to as allelopathy. Commonly this term is mainly used to describe the chemical interaction between two plants. In plants, allelochemicals can be present in leaves, bark, roots, root exudates, flowers, and fruits. The delivery of allelochemicals into the rhizosphere is often thought to occur through leaching from leaves and other aerial plant parts, through volatile emission, by root exudation, and by the breakdown of bark and leaf litter. Some identified phenolic allelochemicals are: *p*-hydroxy benzoic acid and *p*-coumaric acid (present in leaves), quercetin, juglone and 2,4-dihydroxy-1,4 (2H) benzoxazin-3-one (DIBOA) (present in leaves, bark and root exudates), and (−)-catechin and sorgoleone (found in rhizosphere and root exudates) (Inderjit & Gross, 2002; Weir *et al.*, 2004). Bais *et al.* (2003) present evidence that *Centaurea maculosa*, an invasive species in the western USA, displaces native plant species by exuding the phytotoxin (−)-catechin from its roots. This allelochemical triggers a wave of reactive oxygen species initiated at the root meristem, which leads to a $Ca^{2+}$ signaling cascade triggering genome-wide changes in gene expression and, ultimately, death of the root system. Resistance to allelochemicals is largely accomplished through detoxification pathways that involve the modification, followed by the secretion or the vacuolar sequestration, of xenobiotics.

In addition to affecting the soil microorganisms responsible for nutrient mineralization, phenolic compounds can alter nitrogen availability by complexing proteins. Polyphenol–protein complexes originate either during senescence of plant tissues, when polyphenols stored in the vacuole come into contact with cytoplasmatic proteins, or in the soil, when polyphenols complex proteins from litter or extracellular enzymes from microorganisms. These complexes cause the brown coloring of senescent leaves and are resistant to most decomposing organisms, except basidiomycetes with the appropriate polyphenol oxidase activity, and earthworms, which can directly use a large proportion of nitrogen contained in the complexes (Haslam, 1998; Lattanzio, 2003; Papadopoulou & Frazier, 2004).

Plants depend on the ability of roots to communicate with microbes. The converse is also true; many bacteria and fungi are dependent on associations with plants that are often regulated by root exudates. Biological interactions that are driven by root exudates are more complex and include signal traffic between roots and soil microbes, and one-way signals that relate the nature of chemical and physical soil properties to the roots. Specific compounds identified in root exudates have been shown to play roles in these interactions. For example, isoflavonoids and flavonoids present in the root exudates of a variety of leguminous plants activate the Rhizobium genes responsible for the nodulation process, and might be responsible for vesicular–arbuscular mycorrhiza colonization. Flavonoid profiles in root exudates differ considerably among legumes, and this specificity enables mutualists and beneficial bacteria such as rhizobia to distinguish their hosts from other legumes (Bais *et al.*, 2004, 2006). Although rhizobia colonize roots in a way that is reminiscent of pathogenic microorganisms, no host plant defense reactions are triggered during successful symbioses: symbiotic interactions, by definition, are beneficial to both partners. Nevertheless, the plants obviously control the invading bacteria; failure in effective nodule formation or infections with rhizobia defective in surface polysaccharides often result in pathogenic responses. Symbiosis between leguminous plants and rhizobia involves the *de novo* development of a specialized plant organ, the root nodule. In the nodules, rhizobia fix dinitrogen into ammonia, which is assimilated by the host plant, and, in turn, rhizobia are supplied with carbon compounds.

The nodulation process in rhizobia–legume symbiosis requires a sequence of highly regulated and coordinated events, initiated by an exchange of specific signaling compounds between both partners. The prerequisite for the formation of a nitrogen-fixing nodule is the generation of flavonoid signal(s) that are secreted from the root exudates of the leguminous host. The flavonoid aglycone is presumed to diffuse into the rhizobial bacteria, perhaps through porins (Mithöfer, 2002). The flavonoids in root exudates induce, in conjunction with NodD protein [the product of the only nodulation (*nod*) gene constitutively expressed by rizobia], the transcription of an important set of Rhizobium *Nod* genes. The *Nod* genes are responsible for the synthesis of sulfated acylated tetraglucosamine glycolipids (NodRm-1), the so-called *Nod* factors, that are secreted by induced rhizobia and initiate root-hair curling and cortical cell division in the infectible zone of legume roots (Long, 1989; Brewin, 1991; Aoki *et al.*, 2000; Limpens & Bisseling, 2003; Kobayashi *et al.*, 2004). Examples of flavonoids found to be active in the induction of *Nod* gene expression are eriodictyol (3′,4′,5,7-tetrahydroxyflavanone) and apigenin-7-*O*-glucoside isolated from pea root exudate, active at a concentration lower than 50 nM, and luteolin and chrysoeriol (3′-methoxyluteolin) released from alfalfa seeds. Other flavonoid classes released naturally from legume plants to induce *nod*-gene expression in their appropriate microsymbionts are flavanones, such as naringenin and hesperetin, chalcones, and isoflavonoids, such as daidzein and genistein (Hartwig *et al.*, 1989; Mathesius *et al.*, 1998).

### *1.2.3 Phenolics as pigments*

An important role of flavonoids is to serve as visual signals by acting as pigments in fruits and flowers, firstly to attract animals as pollinators in flowers, and later to attract animals to eat the fruits and thereby help in seed dispersal. Fruit colors are primarily determined genetically, although environmental factors such as temperature, light conditions, and availability of nutrients can have an effect on flavonoid composition and on the final hue of the fruit. Concerning anthocyanins, which are mainly responsible for the bluish to purple and reddish colors in plants, several different factors can affect the final color of the fruit or flower. Delphinidin-derived anthocyanins are known to be responsible for bluish colors, whereas cyanidin- and pelargonidin-derived anthocyanins are found from mauve and reddish tissues, respectively. Anthocyanins readily form complexes with so-called co-pigments that can intensify and modify the initial color given by the pigment. Apparently, almost all polyphenols, as well as other molecules such as purines, alkaloids and metallic cations, have the ability to function as co-pigments. In addition, the temperature and pH of the vacuolar solution may affect the final color (Brouillard & Dangles, 1994; Brouillard *et al.*, 1997; Mol *et al.*, 1998; de Freitas & Mateus, 2006). Chalcones and aurones are two classes of flavonoids that contribute to yellow flower color in a number of plants; for example, the chalcone isosalipurposide is the sole yellow coloring matter of yellow carnation, while the aurone aureusidin, occurring as 6-glucoside aureusin, is the major yellow pigment in the snapdragon (*Antirrhinum majus*).

Variations in hydroxylation pattern of the five commonest flavones and flavonols (apigenin, luteolin, kaempferol, quercetin, and myricetin) produce structures that give white, yellow or ivory colors to the tissues in which they are located. For example, the insertion of a 2′-hydroxyl group into luteolin gives the flavone isoetin, which is a yellow flower