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MARSCHNER REVIEW

# Release of plant-borne flavonoids into the rhizosphere and their role in plant nutrition

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**Abstract** Plants release a multitude of organic compounds into the rhizosphere, some of which are flavonoids. These products of secondary metabolism are mainly studied for their antioxidant properties and for their role in the establishment of rhizobium-legume symbiosis; however, it has been recently demonstrated that flavonoids can also affect nutrient availability through soil chemical changes. This review will give an overview of the types and amounts of flavonoids released by roots of different plant species, as well as summarize the available knowledge on root exudation mechanisms. Subsequently, factors influencing their release will be reported, and the methodological approaches used in the literature will be critically described. Finally, the direct contribution of plant-borne flavonoids on the nitrogen, phosphorous and iron availability into the rhizosphere will be discussed.

**Keywords** Nutrient availability · Nitrogen · Phosphorous · Iron · Root exudates · Transmembrane transport

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## Introduction

The term "rhizosphere", defined for the first time by Hiltner (1904), describes the volume of soil surrounding roots and affected by their presence. A multitude of compounds are released into the rhizosphere of soil-grown plants; most of these are organic compounds and plant constituents derived from photosynthesis and secondary metabolism. Within root exudates, phenolic compounds (for review, see Neumann and Römheld 2007), and especially flavonoids have been detected. Flavonoids consist of various groups of plant metabolites including flavones, flavanones, isoflavones and flavanols. The structures of flavonoids cited in this chapter are reported in Figs. 1 and 2.

Soil chemical changes related to the presence of these compounds also influence nutrient availability for plant acquisition. In this chapter, the flavonoids identified in root exudates and the mechanisms involved in their release are described, and the role of these organic compounds in plant nutrient acquisition from the rhizosphere is discussed.

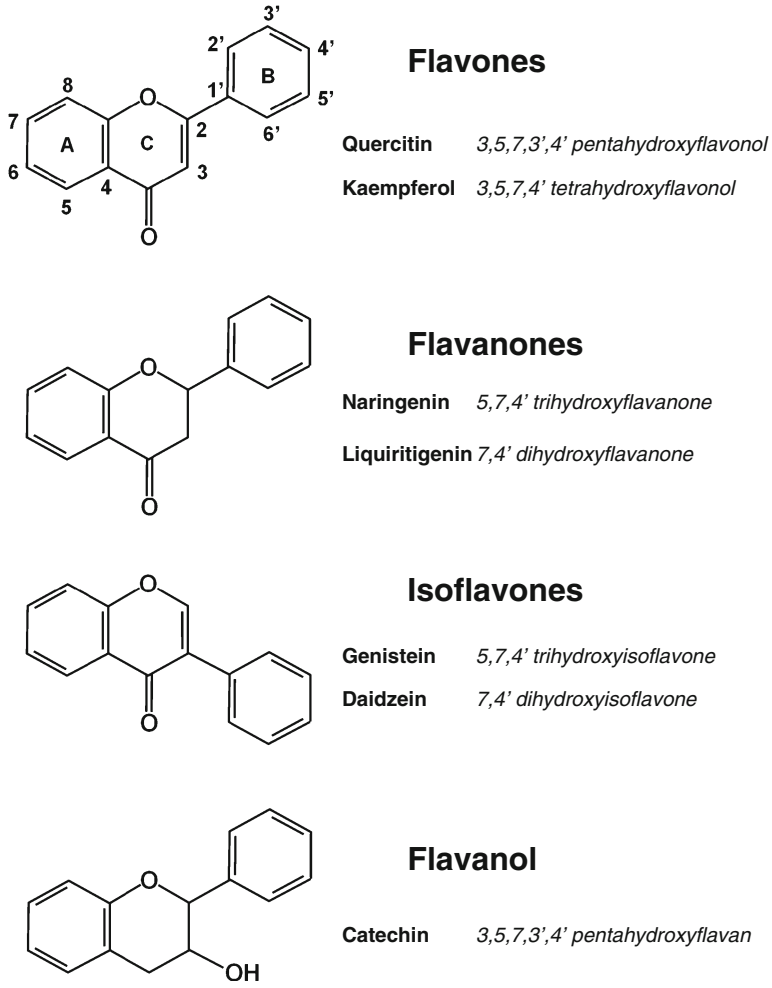


**Fig. 1** Structures of some flavonoids (modified after Mira et al. 2002)

## Flavonoids released by roots

### *Types and concentrations*

That plant roots secrete phenolic compounds into the rhizosphere is well established, but information on which compounds and what amounts are actually released has remained scarce. Further data is available on root contents (for review, see Rao 1990). We focus here on studies analyzing flavonoid exudation, rather than those in which only root tissue contents were investigated.

Most of the available literature on root flavonoid exudation has been focused on the response of plants to the presence of microorganisms and/or to biotic or abiotic challenges. We aim to summarize the available knowledge on flavonoid exudation by plant roots that are not submitted to a particular biotic or abiotic stress (with the exception of P and N deficiency, which will be included). The types and amounts of flavonoids exuded from roots of various plant species are summarized in Table 1. Only studies in which the chemical structure of the flavonoids was investigated are included in the table.

### *Flavonoids in the root exudates of non-leguminous plants*

Most of the efforts devoted to the study of root flavonoid exudation have been directed to leguminous plants. However, there are a couple of non-leguminous species for which flavonoid exudation has been investigated. Firstly, Kidd et al. (2001) quantified the secretion of quercetin from the roots of

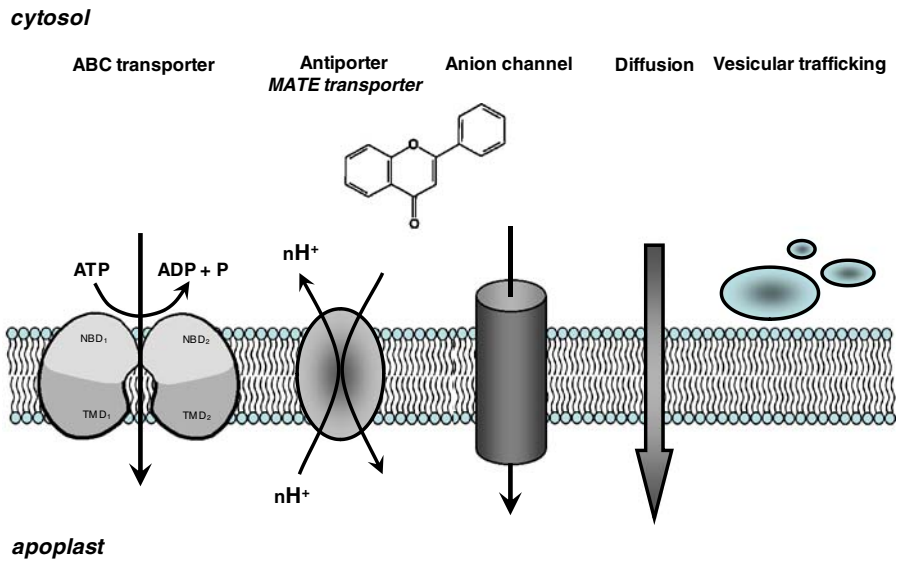


**Fig. 2** Proposed mechanism of flavonoid exudation from roots (modified from Jasinski et al. 2003; Badri and Vivanco 2009)

maize (*Zea mays* L. Iltis) cultivars, by monitoring changes occurring in root exudation upon aluminum stress (after pre-treatment with silicon). Only nanomolar quantities of quercetin were detected in control plants (Table 1). However, phenolic secretion drastically increased when plants were pretreated with silicon and/or challenged with aluminum. Some of the tested cultivars also released catechin and, in much lower concentrations, quercetin. The second report of root-exuded flavonoids in a non-leguminous plant concerns, unsurprisingly, *Arabidopsis thaliana* (L.) Heynh. Narasimhan et al. (2003) used a metabolomic approach to analyze the root exudates of *A. thaliana* (Landsberg erecta accession) and found that flavonoids accounted for 37% of all secondary metabolites. Quercetin was the most abundant flavonoid, but glycosylated conjugates were also found, albeit in lower quantities (Table 1). In a later study, Badri and co-workers (2008a) revealed the presence of flavonoid diglucosides (kaempferol-3, 7-*O*-bisrhamnoside and kaempferol-3-*O*-ß-d-glucopyranoside-7-*O*-α-1-rhamnoside) in the Columbia accession of *A. thaliana*. Finally, the root exudates of *Alnus glutinosa* (L.) Gaertn were also investigated, and quercetin and kaempferol identified as the major compounds (Hughes et al. 1999). The authors observed that light-stimulated roots released increased quantities of both flavonoids, which in turn reduced the capacity of *Frankia* strains to establish nitrogen-fixing symbiosis.

*Flavonoids in the root exudates of leguminous plants*

Root exudation of flavonoids has been studied most extensively for leguminous species, and especially for crops with agronomical interest. Here we describe four of them: 1) soybean, 2) french bean, 3) alfalfa, and 4) white lupin.

The flavonoids released by the roots of soybean (*Glycine max* L. Merr) were analyzed in six different cultivars: *G. max* cv. Amsoy 71 (d'Arcy-Lameta 1986), *G. max* cv. Williams (Graham 1991), *G. max* cv. Maple Arrow (Kape et al. 1992a), *G. max* cv. Preston (Schmidt et al. 1994), and *G. max* cv. Peking cv. McCall (Pueppke et al. 1998). Daidzein was the only compound identified in all cultivars. Genistein and coumestrol were found in all of them except in *G. max* cv. Amsoy 71 (genistein) and in *G. max* cv. Williams (coumestrol). Quantitative measurements were not performed for *G. max* cv. Amsoy71 and *G. max* cv. Maple Arrow, however, the concentrations of exuded flavonoids in the other cultivars were very similar (in the picomolar range), with the exception of *G. max* cv. Williams, where concentrations were approximately 1,000 times higher (Table 1). This discrepancy might be due to a different exudation physiology of *G. max* cv. Williams compared with the others, or more likely to the cultivation and collection systems employed, which differed from the other studies. While authors generally grew soybean and

**Table 1** Types and amounts of flavonoids released by roots of various plant species

| Plant species | Cultivar/ variety | Trivial name | Chemical name | Amounts | Units | Age (days) | Cultivation system | Collection medium | Reference |
|---|---|---|---|---|---|---|---|---|---|
| *Alnus glutinosa* | Gaertn | quercetin | 3,5,7,3′,4′-pentahydroxyflavonol | | | | | | Hughes et al. 1999 |
| | | kaempferol | 3,5,7,4′-tetrahydroxyflavonol | | | | | | |
| *Arabidopsis thaliana* | Landsberg erecta | quercetin<br>quercetin glucoside<br>quercetin rhamnoside<br>quercetin rhamnosyl glucoside<br>kaempferol rhamnoside<br>kaempferol,3-*O*-galactoside<br>cyanidin glucoside | 3,5,7,3′,4′-pentahydroxyflavonol | nd | | 20 | Water agar | Water | Narasimhan et al. 2003 |
| | Columbia | kaempferol-3-*O*-ß-D-glucopyranoside-7-*O*-α-L-rhamnoside<br>kaempferol-3,7-*O*-bisrhamnoside<br>kaempferol-3-*O*-α-L-rhamnoside | | nd | | 7 | MS | MS | Badri et al. 2008a |
| *Cajanus cajan* | BDN2 | naringenin | 5,7,4′-trihydroxyflavanone | nd | | 2 | HC (nutrient solution) | Nutrient solution | Pandya et al. 1999 |
| *Cicer arietinum* | nd | biochanin A | 5,7-dihydroxy-4′-metoxyisoflavone | 20–30 | IA. $g^{-1}$ FW. $h^{-1}$ | 3 | Vermiculite (nutrient solution) | Phosphate buffer | Armero et al. 2001 |
| | | medicarpin | 3-hydroxy-9-methoxypterocarpan | 20–30 | | | | | |
| | | formononetin | 7-hydroxy-4′-methoxyisoflavone | 10–20 | | | | | |
| | | maackiain | 3-hydroxy-8,9-methylenedioxy pterocarpan | 1–10 | | | | | |
| *Desmodium uncinatum* | nd | genistein<br>uncinanone A | 5,7,4′-trihydroxyisoflavone<br>5,7,2′,4′-tetrahydroxy-6-(3-methylbut-2-enyl) isoflavanone | | | nd | HC (nutrient solution) | Nutrient solution | Tsanuo et al. 2003 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | uncinanone B | 4″,5″-dihydro-5,2′,4′-trihydroxy-5″-isopropenylfurano-(2″,3″;7,6)-isoflavanone | | | | | | |
| | | uncinanone C | 4″,5″-dihydro-2′-methoxy-5,4′-dihydroxy-5″-isopropenylfurano-(2″,3″;7,6)-isoflavanone | | | | | | |
| *Fagopyrum esculentum* | Pyra | vitexin | apigenin-8-C-glucoside | 50–100 | ng. plant$^{-1}$. 12d$^{-1}$ | 12 | Water agar | Agar extraction | Kalinova et al. 2007 |
| | | epicatechin | 3,5,7,4′,5′-pentahydroxy flavan | 10–50 | | | | | |
| | | quercetin (derivative) | 3,5,7,3′,4′-pentahydroxyflavonol (derivative) | 10–50 | | | | | |
| *Galega orientalis* | Nd | apigenin | 5,7,4′-trihydroxyflavone | | | 4 | HC (nutrient solution) | Nutrient solution | Suominen et al. 2003 |
| | | luteolin | 5,7,3′,4′-tetrahydroxyflavone | | | | | | |
| *Glycine max* | Amsoy 71 | daidzein | 4′,7-dihydroxyisoflavone | ndnd | | 15 | Sand | Water | D'Arcy-Lameta 1986 |
| | | coumestrol | 3,9-dihydroxy coumestan | | | | | | |
| | Williams | | 6″-O-malonyl-7-O-β-D-glucosyldaidzein | 900–1,000 | nmol. seedling$^{-1}$. 24 h$^{-1}$ | 7 | AC (water) | Cotton in root contact | Graham 1991 |
| | | | 6″-O-malonyl-7-O-β-D-glucosylgenistein | 600–700 | | | | | |
| | | daidzein | 4′,7-dihydroxyisoflavone | 500–600 | | | | | |
| | | genistein | 5,7,4′-trihydroxyisoflavone | 300–400 | | | | | |
| | Maple Arrow | coumestrol | 3,9-dihydroxy coumestan | nd | | 7 | MES-$CaCl_2$ | MES-$CaCl_2$ | Kape et al. 1992a |
| | | daidzein | 4′,7-dihydroxyisoflavone | nd | | | | | |
| | | genistein | 5,7,4′-trihydroxyisoflavone | nd | | | | | |
| | | isoliquiritigenin | 2,4′,4-trihydroxychalcone | nd | | | | | |
| | Preston | daidzein | 4′,7-dihydroxyisoflavone | 300–500 | pmol. plant$^{-1}$. 18 h$^{-1}$ | 2 | MES | MES | Schmidt et al. 1994 |
| | | coumestrol | 3,9-dihydroxy coumestan | 40–60 | | | | | |
| | | genistein | 5,7,4′-trihydroxyisoflavone | 20–30 | | | | | |
| | Peking | daidzein | 4′,7-dihydroxyisoflavone | 1,000–1,500 | pmol. root$^{-1}$. 2d$^{-1}$ | 2 | MES-$CaCl_2$ | MES-$CaCl_2$ | Pueppke et al. 1998 |
| | | glycitein | 4′,7-Dihydroxy-6-methoxyisoflavone | 300–400 | | | | | |
| | | genistein | 5,7,4′-trihydroxyisoflavone | 150–250 | | | | | |
| | | coumestrol | 3,9-dihydroxy coumestan | 50–100 | | | | | |
| | McCall | daidzein | 4′,7-dihydroxyisoflavone | 700–800 | pmol. root$^{-1}$. 2d$^{-1}$ | | | | |
| | | glycitein | 4′,7-Dihydroxy-6-methoxyisoflavone | 150–250 | | | | | |
| | | genistein | 5,7,4′-trihydroxyisoflavone | 100–200 | | | | | |
| | | coumestrol | 3,9-dihydroxy coumestan | 20–40 | | | | | |
| *Lens culinaris Medicus* | Anicia | | 4′,7-dihydroxyflavone | nd | | 15 | Sand | Water | D'Arcy-Lameta 1986 |
| | | | 3′,4′,7-trihydroxyflavone | nd | | | | | |

**Table 1** (continued)

| Plant species | Cultivar/ variety | Trivial name | Chemical name | Amounts | Units | Age (days) | Cultivation system | Collection medium | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | geraldone | 4′,7-dihydroxy 3′-methoxyflavone | nd | | | | | |
| *Lotus pedunculatus* | G4705 | catechin | 3,5,7,3′,4′-pentahydroxy flavan | nd | | 6 | HC (N-free) | Nutrient solution | Steele et al. 1999 |
| | | hersperidin (hersperitin glycoside) | glycoside of 3′,5,7-trihydroxy-4′-methoxy flavanone | nd | | | | | |
| | | isorhoifolin | apigenin-7-O-rutinoside | nd | | | | | |
| | | kaempferol | 3,5,7,4′-tetrahydroxyflavonol | nd | | | | | |
| | | naringenin | 5,7,4′-trihydroxyflavanone | nd | | | | | |
| | | quercetin aglycone | 3,5,7,3′,4′-pentahydroxyflavonol | nd | | | | | |
| | | quercetin glycoside | glycoside of 3,5,7,3′,4′-pentahydroxyflavonol | nd | | | | | |
| | | rhamnetin | 3,5,3′,4′-tetrahydroxy-7-methoxyflavone | nd | | | | | |
| *Lupinus albus* | Kievskij | wighteone | 5,7-dihydroxy-3-(4-hydroxyphenyl)-6-(3-methylbut-2-enyl)chromen-4-one | nd | | 7 | HC (nutrient solution) | Nutrient solution | Gagnon and Ibrahim 1997 |
| | | isowighteone | 5,7-dihydroxy-3-(4-hydroxyphenyl)-3-(3-methylbut-2-enyl)chromen-4-one | nd | | | | | |
| | | lupiwighteone | 5,7,4′-trihydroxy-8(3,3-dimethyl allyl) isoflavone | nd | | | | | |
| | | luteone | 5,7,2′,4′-tetrahydroxy-6(3,3-dimethly allyl) isoflavone | nd | | | | | |
| | | licoisoflavone A | 5,7,2′,4′-tetrahydroxy-3(3,3-dimethy allyl) isoflavone | nd | | | | | |
| | | lupalbigenin | 6,3′-diprenyl-5,7,4′-trihydroxyisoflavone | nd | | | | | |
| | | 2-hydroxylupalbigenin | 6,3′-diprenyl-5,7,2′,4′-tetrahydroxyisoflavone | nd | | | | | |
| | Amiga (cluster roots) | genistein | 5,7,4′-trihydroxyisoflavone | 30–60 | µg. g root $FW^{-1}$. $h^{-1}$ | 35 | HC (P-free) | Water | Weisskopf et al. 2006a |
| | | | genistein 6′-O-malonyl-diglucoside | 10–20 | | | | | |
| | | | genistein 7-0-diglucoside | 1–5 | | | | | |
| | | | genistein 6′-O-malonyl-O glucoside | 1–5 | | | | | |
| | | | genistein 4-O-glucoside | nd | | | | | |
| | | | genistein 7-O-glucoside | nd | | | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | genistein 6′-O-malonyl-glucoside 4-O glucoside | nd | | | | | | |
| | | hydroxygenistein | 2′,4′,5,7-tetrahydroxyisoflavone | nd | | | | | | |
| | | | hydroxygenistein 7-O-glucoside | nd | | | | | | |
| | | | hydroxygenistein 7-O-diglucoside | nd | | | | | | |
| | | | hydroxygenistein 6′-O-malonyl-glucoside | nd | | | | | | |
| | | | hydroxygenistein-6′-O-malonyl-glycoside | nd | | | | | | |
| | Amiga (noncluster roots) | genistein | genistein 6′-O-malonyl-diglucoside | | 5–15 | µg. g root $FW^{-1}$. $h^{-1}$ | 35 | HC (P-free) | Water | |
| | | | 5,7,4′-trihydroxyisoflavone | | 1–10 | | | | | |
| | | | genistein 7-0-diglucoside | | 1–5 | | | | | |
| | | | genistein 6′-O-malonyl-O glucoside | | 0.2–2 | | | | | |
| | | | genistein 4-O-glucoside | nd | | | | | | |
| | | | genistein 7-O-glucoside | nd | | | | | | |
| | | | genistein 6′-O-malonyl-glucoside 4-O glucoside | nd | | | | | | |
| | | hydroxygenistein | 2′,4′,5,7-tetrahydroxyisoflavone | nd | | | | | | |
| | | | hydroxygenistein 7-O-glucoside | nd | | | | | | |
| | | | hydroxygenistein 7-O-diglucoside | nd | | | | | | |
| | | | hydroxygenistein 6′-O-malonyl-glucoside | nd | | | | | | |
| | | | hydroxygenistein-6′-O-malonyl-glycoside | nd | | | | | | |
| | Bac | genistein | 5,7,4′-trihydroxyisoflavone | | 1–5 | µg. g root $FW^{-1}$. $d^{-1}$ | 14 | HC (nutrient solution) | Water | Pislewska et al. 2002 |
| | | luteone | 5,7,2′,4′-tetrahydroxy-6(3,3-dimethly allyl) isoflavone | | 1–5 | | | | | |
| | | wighteone | 5,7-dihydroxy-3-(4-hydroxyphenyl)-6-(3-methylbut-2-enyl)chromen-4-one | | 0.1–1 | | | | | |
| | | hydroxygenistein | | | 0.1–1 | | | | | |
| *Lupinus luteus* | Nd | genistein | 5,7,4′-trihydroxyisoflavone | | 1–10 | µg. g root $FW^{-1}$. $h^{-1}$ | 21 | HC (nutrient solution) | Water | Kneer et al. 1999 |
| *Medicao sativa* | Moapa 69 | | 7,4′-dihydroxyflavone | | 1,800–2,000 | pmol. $g^{-1}$ FW. 8 $h^{-1}$ | 3 | HC (N-free) | Nutrient solution | Maxwell and Phillips 1990 |
| | | equinatin | 4,4′-dihydroxy-2′-methoxychalcone | | 500–600 | | | | | |
| | | liquiritigenin | 4′, 7-dihydroxyflavanone | | 300–400 | | | | | |

**Table 1** (continued)

| Plant species | Cultivar/ variety | Trivial name | Chemical name | Amounts | Units | Age (days) | Cultivation system | Collection medium | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | subsp. varia cv. A2 | coumestrol | 3,9-dihydroxy coumestan | nd | | 15 | HC (N-limited) | Nutrient solution | Zuanazzi et al. 1998 |
| | | | 7,4′-dihydroxyflavone | nd | | | | | |
| | | medicarpin | 3-hydroxy-9-methoxypterocarpan | nd | | | | | |
| *Phaseolus vulgaris* | PI 165426CS | naringenin | 5,7,4′-trihydroxyflavanone | 300–400 | nmol. plant$^{-1}$. d$^{-1}$ | 9 | HC (N-free) | Nutrient solution | Hungria et al. 1991 |
| | | eriodictyol | 5,7,3′,4′-tetrahydroxyflavanone | 200–300 | | | | | |
| | | genistein (glycoside) | 7′-O-glycoside of 5,7,4′-trihydroxyisoflavone | 20–50 | | | | | |
| | Rab39 | coumestrol | 3,9-dihydroxy coumestan | 1,000–1,200 | pmol. seedling$^{-1}$. 22 h$^{-1}$ | 3 | HC (MES) | MES | Bolaños-Vásquez and Werner 1997 |
| | | daidzein | 4′,7-dihydroxyisoflavone | 700–800 | | | | | |
| | | naringenin | 5,7,4′-trihydroxyflavanone | 500–600 | | | | | |
| | | liquiritigenin | 4′, 7-dihydroxyflavanone | 400–500 | | | | | |
| | | isoliquiritigenin | 2′,4′,4-trihydroxychalcone | 200–300 | | | | | |
| | | genistein | 5,7,4′-trihydroxyisoflavone | nd | | | | | |
| | Celina | daidzein | 4′,7-dihydroxyisoflavone | 400–500 | ng. m root length$^{-1}$. 14 h$^{-1}$ | 30 | Soil | $CaSO_4$ with antibiotics | Isobe et al. 2001 |
| | | genistein | 5,7,4′-trihydroxyisoflavone | 10–20 | | | | | |
| | Hilds Maxi GS | coumestrol | 3,9-dihydroxy coumestan | 50–100 | µmol. g root DW$^{-1}$. 6d$^{-1}$ | 6 | CaSO4 (on filter papers) | Filter extraction | Haase et al. 2007 |
| | | genistein | 5,7,4′-trihydroxyisoflavone | 1–5 | | | | | |
| | | daidzein | 4′,7-dihydroxyisoflavone | 1–10 | | | | | |
| | | isoliquiritigenin | 2′,4′,4-trihydroxychalcone | 1–10 | | | | | |
| | | coumestrol | 3,9-dihydroxy coumestan | 50–100 | µmol. g root DW$^{-1}$. 6d$^{-1}$ | 12 | | | |
| | | genistein | 5,7,4′-trihydroxyisoflavone | 1–5 | | | | | |
| | | daidzein | 4′,7-dihydroxyisoflavone | 1–5 | | | | | |
| | | isoliquiritigenin | 2′,4′,4-trihydroxychalcone | 1–5 | | | | | |
| *Trifolium repens* | Nd | | 4′, 7-dihydroxyflavone | nd | | nd | nd | nd | Redmond et al. 1986 |
| | | geraldone | 3′-methoxy apigenin | nd | | | | | |
| | | | 4′hydroxy-7-methoxyflavone | nd | | | | | |
| *Zea mays* | Sikuani | quercetin | 3,5,7,3′,4′-pentahydroxyflavonol | 1–5 | nmol. root tip$^{-1}$. h$^{-1}$ | 4 | HC (nutrient solution) | Nutrient solution | Kidd et al. 2001 |
| | Clavito | quercetin | 3,5,7,3′,4′-pentahydroxyflavonol | 10–20 | nmol. root tip$^{-1}$. h$^{-1}$ | | | | |

*nd* not determined; *IA* integration area; *HC* hydroponic culture; *AC* aeroponic culture; *MES* 2-(N-morpholino)ethanesulfonic acid

collected the exudates in MES (2-(N-morpholino) ethanesulfonic acid) buffer, Graham (1991) used an aeroponic system and collected flavonoids by direct application of cotton sticks to the roots.

The flavonoid composition of french bean (*Phaseolus vulgaris* L.) root exudates has been assessed in four studies: Hungria et al. (1991) used *P. vulgaris* var. PI165426CS, Bolaños-Vásquez and Werner (1997) studied *P. vulgaris* var. Rab39, Isobe et al. (2001) analyzed *P. vulgaris* cv. Celina, and Haase et al. (2007) investigated *P. vulgaris* cv. Hilds Maxi GS. Isobe and co-workers cultivated plants in soil, while all other studies were carried out either in a hydroponic system (PI 165426CS and Rab39) or on filter paper (Hilds Maxi GS). Only daidzein and genistein were recovered from exudates of *P. vulgaris* cv. Celina, possibly due to the difficulty of detecting low-abundance compounds in the exudates of soil-grown plants. Genistein was found in all cultivars, whereas daidzein was observed in the exudates of all cultivars, except *P. vulgaris* var. PI165426CS. Coumestrol was the main compound exuded from the roots of two cultivars (Rab39 and Hilds Maxi GS), but it was not detected in the two others (var. PI 165426CS and Celina). These differences in the exudates recovered from different cultivars of the same species could also be due to the fact that differing cultivation and extraction procedures were applied in the various studies. Furthermore, plant age varied from 3 to 30 days which makes direct comparisons difficult, even if Haase et al. (2007) did not observe any significant change in exudate composition between 6- and 12 day-old seedlings. As for quantities, Hungria et al. (1991) measured about 500-fold higher concentrations of flavonoids than Bolaños-Vasquez and Werner (1997) for seedlings of 9 and 3 days old, respectively. As mentioned above, seedling age did not affect flavonoid exudation in *P. vulgaris* cv. Hilds maxi GS, and this 500-fold difference most likely results from the presence or absence of nitrogen in the nutrient solution. Hungria et al. (1991) used N-free nutrient solution, while Bolaños-Vasquez and Werner (1997) supplied the plants with all nutrients required for growth.

Despite the large abundance of data available on alfalfa (*Medicago sativa* L.) root flavonoid contents or seed exudates, surprisingly few studies assessed the flavonoid composition of the root exudates in this model plant. Maxwell and Phillips (1990) analyzed *M. sativa* cv. Moapa 69, while Zuanazzi et al. (1998) investigated *M. sativa* var. A2. Both studies used N-limited (or N-free) hydroponic cultures. One compound, 7,4′-dihydroxyflavone, was found in both cultivars and was the most abundant flavonoid in the exudates of *M. sativa* Moapa 69. 7,4′-dihydroxyflavone was also found in the exudates of *Lens culinaris* Medik and of *Trifolium pratense* L. (Table 1). Plant age differed greatly between the two studies, with 3-day-old seedlings in Maxwell and Phillips (1990) and 15 day-old seedlings for *M. sativa* var. A2. With the exception of the common 7,4′-dihydroxyflavone, the two cultivars exuded different types of flavonoids: a chalcone (equinatin) and a flavanone (liquiritigenin) for *M. sativa* cv. Moapa 69, and a coumestan (coumestrol) and a pterocarpan (medicarpin) for *M. sativa* var. A2.

Three independent studies investigated the flavonoids exuded from the roots of white lupin (*Lupinus albus* L.). White lupin has often been used as a model owing to its capacity to survive on soils in which concentrations of available phosphorus are very low. When grown under P-deficient conditions, white lupin develops root structures known as “cluster roots” or “proteoid roots” (Dinkelaker et al. 1995; Neumann and Martinoia 2002). These cluster roots exude large amounts of organic acids and concomitantly acidify the rhizosphere (Gardner et al. 1982, 1983; Gerke et al. 1994; Neumann et al. 2000; Tomasi et al. 2009). However, these particular roots of white lupin were only investigated in one of three studies (Weisskopf et al. 2006a); in the other cases, plants were grown in P-sufficient conditions and thus most likely did not form cluster roots. In each study, a different cultivar was used. Gagnon and Ibrahim (1997) analyzed *L. albus* cv. Kievskij, Weisskopf et al. (2006a) investigated *L. albus* cv. Amiga, and Pislewska et al. (2002) studied *L. albus* cv. Bac. The age of the plants was also different, with 1-week-old seedlings in Gagnon and Ibrahim (1997), 2-week-old plants in Pislewska et al. (2002) and 5-week-old plants in Weisskopf et al. (2006a). The two latter studies observed similar concentrations of flavonoids in the root exudates of non-cluster roots of white lupin, despite the age differences. Genistein and hydroxygenistein were found in both cases. Weisskopf et al. (2006a) recovered, in addition, many glycosylated conjugates of both genistein and hydroxygenistein, while Pislewska et al. (2002) found two additional aglycones, luteone and wighteone. These two isofla-

vonoids were also present in the exudates of *L. albus* cv. Kievskij, in addition to prenylated isoflavonoids, which were specific for this cultivar (Gagnon and Ibrahim 1997). Moreover, comparing cluster and non-cluster roots of the same plants revealed that similar isoflavonoids were exuded from both root types, but in higher quantities from cluster roots than from non-cluster roots. This indicates that the rapid exudation from cluster roots is not restricted to organic acids, but also includes phenolic compounds (Weisskopf et al. 2006a, b). White lupin cluster roots exuded a lower quantity of flavonoids (nanomolar range, Weisskopf et al. 2006a) than of organic acids (micromolar range, Massonneau et al. 2001) and the exudation of phenolics took place before the exudation of organic acids. This suggested a role of these compounds in inhibiting the rhizosphere microorganisms (Weisskopf et al. 2006b; Tomasi et al. 2008).

*Specificity of root-exuded flavonoids*

From the comparisons of cultivars listed above, one might conclude that the pattern of exuded flavonoids is strongly cultivar-specific, especially for white lupin and alfalfa (Table 1). Caution should, however, be applied when comparing results of independent studies: flavonoids, as secondary metabolites, might vary greatly depending on cultivation systems and growth conditions. Extraction procedures also often differ between studies, and this can affect the pattern of retrieved flavonoids. Taking these aspects into account, the only situation where cultivars can really be compared is the case of *G. max* cv. Peking and cv. McCall, where both cultivars were analyzed in the same study and under the same conditions (Pueppke et al. 1998). The same compounds were observed in the exudates of both cultivars, but slight quantitative differences were found, one cultivar releasing generally more flavonoids than the other. Further studies are needed to establish the specificity of the flavonoids exuded by different cultivars of the same species, or by different species of the same genus. As can be seen in Table 1, the exuded flavonoids differed greatly between the different plant genera. Most compounds (29) were found in only one genus, seven were present in two genera and three (naringenin, coumestrol and 4′,7-dihydroxyflavone) were retrieved from three genera. The most widespread compound was genistein, which was retrieved from 4 out of 14 genera (*Desmodium*, *Glycine*, *Lupinus* and *Phaseolus*).

*Concentrations of root-exuded flavonoids*

Large variations in the concentrations of root-exuded flavonoids were observed, e.g. from nanograms per plant in 12 days for *Fagopyrum esculentum* Moench to microgram per gram of root in 1 h for *Lupinus albus*. Here again, the type of cultivation, the extraction procedure, as well as the sensitivity of the detection method might partly account for this large variation, in addition to the species differences. Moreover, not only the age of plants, but also the root age, zone and developmental stage might have a major influence on the amounts of flavonoid exuded. Graham (1991) noted that flavonoid exudation occurring at the root tip was 10 times higher than that from the zone situated 1 cm behind it. Similarly, Weisskopf et al. (2006a) compared the amounts of flavonoids released from roots of different ages and found that the exudation rate was higher in young, developing roots than in older roots. Beyond these differences in root age, biotic and abiotic challenges likely cause the highest quantitative changes in flavonoid exudation.

## Methodological approaches

According to the available literature, there is great variation in the quantities of flavonoids released from plant roots (Table 1). This clearly depends on plant species, plant developmental stage and growing conditions, and also, most probably, on the techniques employed for exudate collection and analysis. However, understanding the possible functions of flavonoids in the rhizosphere also requires detailed knowledge of the real concentrations of these compounds in the rhizosphere soil and in the rhizosphere soil solution. This holds particularly true when studying the possible role of flavonoids in nutrient mobilization, and also when investigating the functions of flavonoids as antibiotics or as signal molecules.

Most frequently, root exudation of flavonoids is assessed in plants grown in hydroponic culture by immersion of the root systems in trap solutions (e.g., water, nutrient solution, buffered aqueous solutions) for a certain period (Fig. 3a; Isobe et al. 2001;

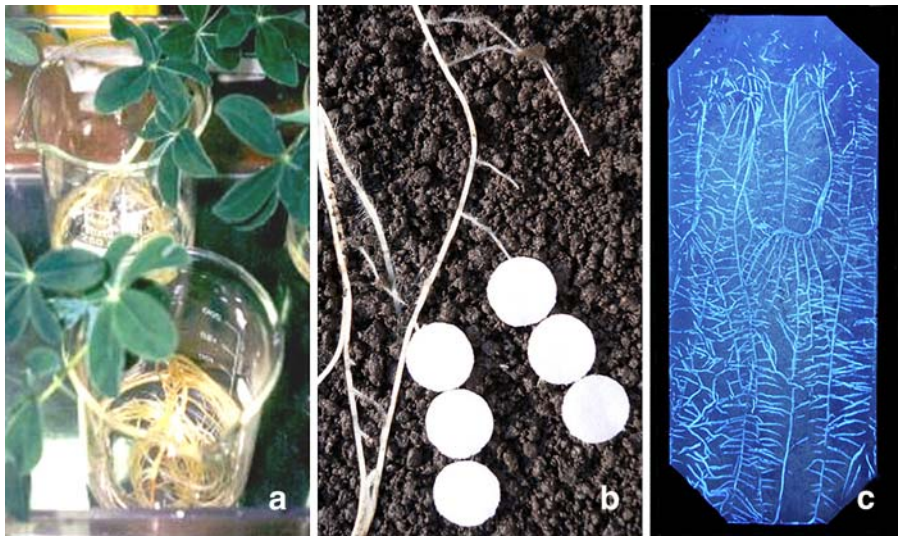


**Fig. 3** Techniques for collection of flavonoids released from roots. **a** Collection by immersion of the root systems of plants grown in hydroponic culture (*Lupinus albus* L.) into trap solutions; **b** use of sorption filters (chromatography paper) placed onto the root surface of soil-grown plants (*Phaseolus vulgaris* L.), cultivated in rhizoboxes equipped with root-observation windows; **c** blue-fluorescent coumestrol released along the whole root system of *Phaseolus vulgaris* grown in rhizoboxes. Collection by application of a nylon membrane onto the root surface over a period of 16 hrs (adapted from Neumann 2006; Haase et al. 2007)

Hofmann et al. 2009). This allows a rapid evaluation of flavonoid patterns and changes in exudation rates dependant on growing conditions, which can be controlled easily in hydroponic systems. However, flavonoids are only moderately water-soluble and are readily adsorbed to organic polymers such as cellulose, cellulose-acetate or nylon (Neumann 2006), and also to cationic binding sites, due to their ability to complex metals. This implies a comparatively low mobility of flavonoids in the rhizosphere of soil-grown plants. However, even in hydroponics, collection techniques using aqueous trap solutions are associated with a high risk of incomplete recovery of flavonoids. This occurs owing to adsorption to the cell wall, which limits the reliability of quantitative measurements. Overestimation of exudation might also take place due to membrane damage caused by rough handling or, in cases of exudate collection over extended time periods, by lack of calcium for membrane stabilization in trap solutions. Cakmak and Marschner (1988) demonstrated a two-fold increase in release of phenolic compounds from cotton seedlings when the roots were incubated for 6 h in distilled water without addition of calcium. An increased release of amino acids and $K^+$ was also observed, indicating a general increase of membrane permeability under these conditions.

Exudate collection is usually performed in aerated trap solutions to avoid limitation of oxygen to the roots. During extended collection periods, this technique increases the risk of oxidation and microbial degradation of flavonoids released into the trap solution. To minimize microbial degradation, Tang and Young (1982) used cartridges filled with XAD resin for adsorption of hydrophobic compounds. The nutrient solution was continuously percolated through the cartridges, and the adsorbed phenolics were protected to some extent from microbial degradation.

Another limitation of exudate collection by immersion of whole root systems into trap solutions is that when using this method, one cannot assess spatial variability of flavonoid exudation along the roots. Apart from investigations with isolated root segments (Weisskopf et al. 2006b), where wounding effects can never be completely excluded, some researchers used various adsorption media with a high binding potential for flavonoids, such as cellulose-acetate membranes (Kape et al. 1992b; Dinkelaker et al. 1997), cotton sticks (Graham 1991), nylon membranes or filter paper (Neumann 2006; Haase et al. 2007), placed on the root surface (Fig. 3b). These studies demonstrate that root exudation of flavonoids exhibits a high qualitative and quantitative spatial variability

along the root. *Nod* gene-inducing flavonoids are usually released preferentially from sub-apical root zones with developing root hairs as infection sites for rhizobia (Kape et al. 1992b). However, the exudation of flavonoids potentially involved in nutrient mobilization and protection of nutrient-mobilizing carboxylates is mainly confined to distinct developmental stages of cluster roots in members of the Proteaceae (Dinkelaker et al. 1997) and in *Lupinus albus* (Neumann et al. 2000; Weisskopf et al. 2006b; Tomasi et al. 2008). These represent plant species with a high potential for chemical mobilization of poorly soluble nutrients in soils. In contrast, the exudation of coumestrol, a blue-fluorescent flavonoid with putative antibiotic functions occurs over the whole root system in soil-grown *Phaseolus vulgaris* (Fig. 3c; Haase et al. 2007). For future studies, the use of adsorption media for exudate collection from the roots of soil-grown plants in culture systems with planar root-observation windows such as rhizoboxes (Engels et al. 2000; Neumann 2006) or even root windows in the field (Smit et al. 2000; Neumann et al. 2009) may allow estimation of flavonoid concentrations in the rhizosphere under more realistic conditions. Adsorption of flavonoids to sorption media placed onto the root surface resembles the behavior of these compounds in the soil; moreover, adsorbed flavonoids are protected to some extent against microbial degradation during the collection period.

Another problem related within the sampling of root exudates is that collection techniques based on root washings with trap solutions often yield very diluted solutions of flavonoids, necessitating further sample concentration. Simple vacuum-evaporation techniques are usually not suitable for this purpose, since flavonoids can undergo substantial structural changes during extended evaporation times at elevated temperatures. In addition to lyophilisation, solid-phase extraction by passing the aqueous samples over cartridges containing a silica filling with hydrophobic surface modifications (C8/C18) is probably the best strategy for further sample concentration. Hydrophobic and moderately hydrophilic compounds such as flavonoids are bound by these matrices. Subsequent elution with organic solvents such as methanol or ethylacetate serves as a pre-purification step, and the solvents can be rapidly evaporated for further concentration, thereby minimizing the risk of structural modifications of flavonoids. In samples collected with sorption media applied to distinct root zones, low absolute amounts of flavonoids are frequently a problem, requiring sensitive analytical facilities such as HPLC-MS or capillary electrophoresis. Owing to the extremely high heterogeneity of flavonoid structures and the comparatively limited availability of commercial standards, structure elucidation is frequently difficult, and usually requires LC-MS and NMR facilities. Recently Lu et al. (2005) published a method for covalent immobilization of dibromobutane-derivatized flavonoids at the surface of glass beads. This approach may be helpful in affinity binding studies for the identification of putative flavonoid receptors involved in plant-microbial interactions.

## Mechanisms of root exudation

Although it has been known for decades that flavonoids are released from plant roots, the mechanisms of root exudation have remained unclear until very recently. ABC transporters (Klein et al. 2000, 2006) seem good candidates for the mediation of flavonoid exudation, since they have been shown to transport these compounds in plant cells of different species (for a review of secondary metabolite transporters, please refer to Yazaki 2005): the first hints came in 2002, when Frangne et al. discovered that an ABC-transporter mediated the vacuolar uptake of saponarin (apigenin 6-C-glucosyl-7-*O*-glucoside) in *Arabidopsis thaliana*. In barley (*Hordeum vulgare* L.) however, which in contrast to *Arabidopsis* produces saponarin, the uptake involved a different transport mechanism ($H^+$/antiport system), showing that the same molecule can be transported by different transporters in different plant species. Goodman and coworkers (2004) later identified a MRP (Multidrug Resistance Protein) required for vacuolar uptake of anthocyanins in maize. In addition to their role in intracellular flavonoid transport, recent work indicates that ABC transporters might be involved in root exudation of flavonoids: In 2007, Loyola-Vargas and co-workers subjected *A. thaliana* to a collection of transporter inhibitors and compared the profiles of root-exuded phenolics following different treatments. They found drastic changes in phenolic exudation, implying an energy-driven transport mechanism. More specifical-

ly, they observed that the ABC-transporter inhibitor sodium orthovanadate led to dose-dependent changes in the exuded phenolic compounds. However, no direct link between a specific transporter and the export of flavonoids from the roots could be established in this study. The first biochemical evidence for the involvement of an ABC-transporter in the exudation of genistein from soybean roots was obtained by Sugiyama et al. (2007). Using membrane vesicles isolated from soybean roots, they showed that genistein was transported in an ATP-dependent manner. The transport activity was inhibited by sodium orthovanadate, but not by other transporter inhibitors, suggesting that an ABC-transporter was responsible for the transport. Furthermore, they demonstrated that the transporter activity was constitutive, and, surprisingly, was not induced as a response to nitrogen deficiency: the transport activity in vesicles of N-starved roots was only slightly enhanced in comparison with the vesicles of N-sufficient roots. The transporter was shown to mediate the transport of daidzein in addition to genistein, the two major isoflavonoids exuded from soybean roots (see also Table 1). Finally, competitive transport experiments indicated that the transporter had a strong preference for aglycones, even though genistin (7′-O-glucoside of genistein) weakly inhibited the transport of genistein in a competitive transport assay. This was not surprising, as it has long been postulated that flavonoids are mainly exuded as aglycones, while the more hydrophilic glycosylated flavonoids are thought to mainly represent storage forms in vacuoles. This was observed by d'Arcy-Lameta in 1986, who recovered only aglycones from soybean and lentil exudates, while mainly glycosylated flavonoids were found in the root tissues of both plants. Likewise, most of the studies analyzing root flavonoid exudates recovered solely or mostly aglycones (Table 1). However, glycosylated flavonoids were also recovered from root exudates in some of the studies (Hungria et al. 1991; Kalinova et al. 2007; Graham 1991; Steele et al. 1999; Weisskopf et al. 2006a). Moreover, Leon-Barrios and co-workers (1993) reported the presence of formonetin-7-O-glucoside in soil, suggesting that the glycosylated flavonoids are indeed released into the rhizosphere, and that their recovery in root exudates is not an artifact caused by artificial cultivation systems or severe extraction procedures. The question of whether the sugar moiety is cleaved from the aglycone backbone after root exudation, or whether the flavonoids display biological activity in their glycosylated forms, are points that require further study. Moreover, the transporters themselves remain to be identified and characterized at the molecular as well as biochemical level. In the case of the genistein transporter described by Sugiyama et al. (2007), a member of the Pleiotropic Drug Resistance (PDR) subfamily seems the most likely candidate, as many PDR genes are expressed in soybean roots. A later study from Badri and co-workers (2008b) corroborated the role of ABC transporters in root exudation of secondary metabolites: they analyzed the root exudates of seven ABC-transporter mutants in *A. thaliana*. They obtained significant differences in the profiles of the phytochemicals retrieved from the different genotypes, and especially three non polar compounds that were exuded from the wild-type plants but missing in the exudates of specific ABC-transporter mutants.

In addition to ABC transporters, multidrug and toxic compound extrusion (MATE) transporters (Brown et al. 1999) may also have a function in root exudation of secondary metabolites such as flavonoids (Walker et al. 2003). Accordingly, expression of ESTs with homology to MATE transporters has been detected in cluster roots of *Lupinus albus* (Uhde-Stone et al. 2003) which release huge amounts of flavonoids and citrate into the rhizosphere. Also root exudation of citrate in response to aluminum toxicity in *Zea mays* (L.) Iltis and *Hordeum vulgare* L. has been linked with MATE transporters (Delhaize et al. 2007). In cluster roots *of Lupinus albus* electrophysiological experiments suggest the activation of anion channels for citrate exudation (Zhang et al. 2004). However, a second transporter system seems to be involved in this process (Tomasi et al. 2009). Therefore, the observed expression of MATE transporter genes in cluster roots could be related both to citrate and flavonoid exudation. Moreover, transport experiments with yeast vesicles expressing two grapevine transporters of the MATE family revealed that these two proteins mediated the uptake of acylated anthocyanins (Gomez et al. 2009). The involvement of MATE transporters in vacuolar uptake of flavonoid glucosides has been recently confirmed in the cases of *Arabidopsis* (TT12) and *Medicago truncatula* (MATE1) by Marinova et al. (2007) and Zhao and Dixon (2009). While it seems evident that MATE transporters play a role in intracellular trans-

port of flavonoids, their involvement in root exudation processes still needs to be assessed.

Finally, since biosynthesis of secondary metabolites in higher plants occurs in close association with the endoplasmic reticulum with subsequent compartmentation of the frequently cytotoxic products in vacuoles and vesicles, also vesicle transport has been discussed as a possible release mechanism (Gagnon et al. 1992).

Despite their importance in plant-microbe interactions and especially in the establishment of nitrogen-fixing symbiosis, surprisingly little information is available on the mechanisms of exudation of flavonoids by plant roots. A major effort is needed to identify the transporters involved, to characterize them with respect to their regulation, and, to determine their transport affinity and specificity. A mechanism of flavonoid exudation by roots is summarized in Fig. 2.

## Role in plant nutrient acquisition

Various stress factors, such as nutrient deficiencies (e.g. P, N, Fe), wounding, pathogen infection, oxidative stress, and UV-irradiation, are associated with up-regulation of phenylpropanoid metabolism, and thereby frequently with increased biosynthesis and release of flavonoids into the rhizosphere. While in some cases a controlled exudation acts as an adaptive response to the respective stress factor, in others the release may simply represent leaking as a consequence of stress-induced impaired membrane integrity (Cakmak and Marschner 1988; Neumann and Römheld 2007). From a methodological point of view, it is not always easy to separate these processes.

Flavonoids can exert various protective functions in plants, comprising roles in feeding deterrence, allelopathic growth inhibition of competitors, or suppression of pathogens (for detailed reviews see: Iwashina 2003; Treutter 2005). These functions have an indirect impact on nutrient acquisition.

Together with other secondary plant metabolites such as terpenoids and alkaloids, functions in feeding deterrence have been reported for various flavonoids belonging to the flavonols, flavones, protoanthocyanidins, flavan-3-ols, flavanones, flavans and isoflavonoids (Iwashina 2003). The respective compounds are localised in root and shoot tissues. Examples comprise nine isoflavones isolated from the roots of *Lupinus angustifolius* (L.) with deterrent activity against larvae of various pasture scarabs (Lane et al. 1987). These isoflavonoids (e.g. genistein) were also found in lupin root exudates (Weisskopf et al. 2006a, b), and at least some of them (e.g. luteone and wighteone) exhibit antifungal activity (Lane et al. 1987). Other flavonoids produced as phytoalexins in response to fungal or bacterial pathogen attack have been identified as aurones, pterocarpans, flavanones, flavonols, flavans, chalcones, some anthocyanins and isoflavonoids (Iwashina 2003). Allelopathic activity against plant competitors has been reported for various chalcones, flavanones, dihydroflavonols, flavonols and isoflavones (Iwashina 2003). Isoflavonoids, such as biochanin A, genistein, daidzein, formononetin and their glycosides have been implicated in autoallelopathy of *Trifolium pratense* L. (Tamura et al. 1969; Chang et al. 1969) and similar effects are discussed e.g. for Phloridzin released from apple roots (Hofmann et al. 2009). However, care must be taken when drawing conclusions from biotests to demonstrate antibiotic activity of flavonoids and other secondary metabolites. It is necessary to show that the respective compounds are also present in the rhizosphere soil solution in concentrations sufficiently high to exert antibiotic effects, as recently exemplified in the controversial discussion on the putative allelopathic potential of (±)-catechin, released from roots of *Centaurea stoebe* L. (Chobot et al. 2009).

Apart from these indirect effects of flavonoids on nutrient acquisition, there is also evidence for a direct involvement of flavonoids in adaptive mechanisms to nutrient limitation. The most important examples are summarized in the following chapters.

### Nitrogen

Nitrogen (N) is an essential macronutrient that is required in great quantities by plants. It is very often poorly available in soils due to its high immobilization/mineralization ratios (Tinker and Nye 2000). Plants can directly acquire nitrogen from different molecules: nitrate, ammonium and some small organic compounds like urea and amino acids. Moreover, leguminous plants can also obtain nitrogen symbiotically, via the formation of $N_2$-fixing nodules. This latter case is currently the best investigated contribu-

tion of flavonoids in root exudates to nutrient acquisition, due to their role as signal compounds in the establishment of the $N_2$-fixing symbiosis with rhizobia. These flavonoids are released from germinating seeds and roots of members of the Fabaceae, for example *Phasolus vulgaris* (L.), *Glycine max* (L.), *Medicago sativa* (L.) and *Lotus japonicus* (L.), which are among the most intensively studied (Cooper 2007; Werner 2007). Flavonoid exudation is influenced by the form and level of external N supply, with inhibitory effects induced particularly by high levels of nitrate fertilization (Wojtaszek et al. 1993). Flavonoids in seed and root exudates can act as chemo-attractants and growth stimulants for rhizobia in the rhizosphere. This similarly holds true for simple phenolic acids, occurring in the rhizosphere also as breakdown products of flavonoid catabolism in rhizobia. These metabolites comprise, for example, p-coumaric, caffeic, protocatechuic, p-hydroxybenzoic and phenyllactic acids (Werner 2001; Cooper 2007). Another putative function of the flavonoid metabolites is suppression of rhizosphere microorganisms competing with rhizobia for colonization of the rhizosphere. Flavonoids such as the isoflavones genistein and daidzein released as root exudates can induce rhizobial resistance to phytoalexins produced by plant roots as a defense mechanism against infections of the root by microbial pathogens (Werner 2001). In the subapical root zones with developing root hairs as preferential infection sites for rhizobia, certain flavonoids (e.g. luteoline, daidzein, naringenin and trigonellin as best studied compounds) are released which act as transcriptional activators for the expression of *nod*-genes in rhizobia (Cooper 2007). These genes are responsible for the production of Nod factors, a special group of lipochito-oligosaccharides, triggering early plant responses involved in root infection and nodule formation (Fig. 4). *Nod* gene-inducing flavonoids in the rhizosphere are active in nanomolar to low micromolar concentrations. In some cases the flavonoid signals act simultaneously to non-flavonoid co-inducers, such as various betaines (*Medicago* species), aldonic acids (*Lupinus* species), jasmonic acid or simple phenolic acids. Different flavonoids in root exudates can act synergistically as *nod* gene inducers, but also in an antagonistic manner as anti-inducers (Werner 2007; Cooper 2007). Inducers in one species or strain of rhizobium are frequently anti-inducers in another species; thus, one type of flavonoid can have opposing effects on different bacteria, and both

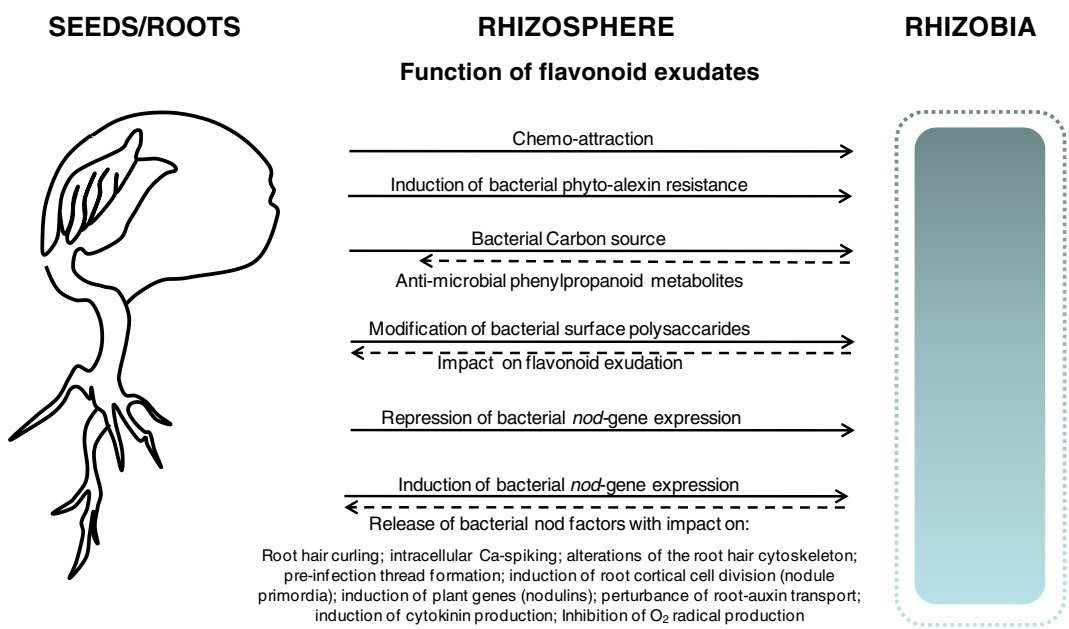


**Fig. 4** Schematic summary of the involvement of flavonoid root exudates in the establishment of rhizobium-legume symbiosis (modified after Cooper 2007; Werner 2001, 2007)

functions (induction or anti-induction) can co-occur in the exudates of the same plant. Exudation patterns can change during plant development. The ratio of inducers to anti-inducers in root exudates may be involved in determination of host recognition. Differential release in special root zones (Kape et al. 1992b) may characterize the optimal sites for infection by rhizosphere bacteria.

Apart from chemo-attraction, resistance, and *nod*-gene induction, flavonoids are also involved in the modification of bacterial surface polysaccharides, which in turn can influence flavonoid exudation of the host plant. Endogenous root flavonoids may have an impact on regulation of auxin transport during nodule development and differentiation (Eckhardt 2006). In addition to the case of $N_2$-fixation with rhizobiae, there is also evidence that flavonoids are involved in signaling for the establishment of symbiosis between various tree species and the filamentous $N_2$-fixing *Actinobacteria* of the genus *Frankia* (Perry et al. 2007).

The importance of flavonoid signals exuded from roots in the establishment of ectomycorrhizae has been discussed. Ectomycorrhizae are characteristic for forest ecosystems in Eurasia and North America, and largely contribute to plant acquisition of organic and inorganic N forms in soils (Martin et al. 2001; Smith and Read 2008). Flavonoids and phenylpropanoid metabolites have been implicated in spore germination, promotion of hyphal growth, hyphal branching and root infection of ectomycorrhizal fungi (Perry et al. 2007).

## Phosphorus

Phosphorus (P) is quantitatively the second most important macronutrient for plant mineral nutrition, as it is needed for many important molecules, such as nucleic acids, ATP and phospholipids. P is often in low availability in soils, because of its poor solubility and low diffusion rates (Marschner 1995). Moreover, a large portion of the P present in the soil is immobilized in organic compounds, which must be hydrolyzed by plant or microbial phosphatases before plants can take it up. Therefore, in 40% of arable land, limited P availability causes P deficiency (Vance 2001), which usually leads to a stimulation of the biosynthesis of phenolic compounds, including flavonoids. This process has been discussed in the context of the induction of Pi-independent metabolic bypass reactions, to achieve a more economic internal P utilization under conditions of P limitation (Plaxton 1998). There are also numerous reports on increased exudation of flavonoids in the roots of P-deficient plants (see Neumann and Römheld 2007 for review). This has prompted investigations on putative contributions of flavonoids to P acquisition in higher plants.

In natural ecosystems, ectomycorrhizal symbioses and particularly arbuscular mycorrhizae (AM) are involved in the acquisition of soil phosphorus by plants (Marschner 1995; Martin et al. 2001; Smith and Read 2008). Numerous reports are available on the potential functions of flavonoids as signals inducing spore germination, hyphal growth and hyphal branching during establishment of mycorrhizal interactions (Perry et al. 2007). AM colonization can, in turn, differentially alter flavonoid exudation of the host plant, depending on the developmental stage of the symbiosis (Larose et al. 2002). Elevated $CO_2$ concentrations, a typical root-induced modification of the rhizosphere, have been reported to induce hyphal growth stimulation by flavonols (Bécard et al. 1992). Recently, strigolactones have been identified as non-flavonoid branching factors for AM fungi (Akiyama et al. 2005), however a specific role for flavonoids, at least during the early stages of mycorrhizal establishment, is still under debate (Steinkellner et al. 2007). Arguments against a specific function of flavonoids as signals in the establishment of mycorrhizal associations are based on the analysis of a *Zea mays* mutant lacking flavonoid biosynthesis due to a defect of the chalcone-synthase gene, encoding the key enzyme for flavonoid biosynthesis. The mutant revealed normal hyphal branching and mycorrhization (Bécard et al. 1995; Buee et al. 2000). Moreover, flavonoids with strong stimulatory effects on AM fungi, such as quercetin, myricetin, and kaempferol, have been detected in significant amounts in root exudates of non-mycorrhizal plants (Buee et al. 2000). However, these are not necessarily conclusive arguments against a specific role of flavonoids in mycorrhizal establishment. Members of different chemical classes could act as signals, either simultaneously or as single compounds, even in exudates of the same host plant, as has been observed in *nod*-gene induction (previously described). Therefore, the absence of one compound does not necessarily imply the absence of responses of the micro-symbiont. Similar to the case of *nod*-

gene induction, it is probably not simply the absence or presence of a specific compound in root exudates which determines the signal properties, but rather a tightly controlled time-, site-, and concentration-specific release pattern of inducers and anti-inducers.

Since flavonoids can also act as metal chelators, a putative function for chemical mobilization of sparingly soluble soil-P forms has been discussed. Similar to metal-chelating carboxylates detected in root exudates of P-deficient plants, the proposed mechanisms comprise (1) exchange chelation at the cationic P-binding sites (e.g., Fe, Al, Ca), (2) occupation of P-binding sites, and (3) iron-phosphate splitting by Fe reduction due to reducing properties of flavonoids (Neumann and Römheld 2007). Mobilization of sufficient amounts of P as a macro-nutrient is expected to require a high level of accumulation of the respective metal chelators in the rhizosphere, as previously reported for P-mobilizing carboxylates (Amann and Amberger 1988; Gerke et al. 2000a, b; Neumann and Martinoia 2002; Neumann and Römheld 2007). This aspect has been overlooked in many earlier studies and currently the only example in which a significant contribution to P mobilization can be expected, is the release of huge amounts of isoflavonoids in cluster roots of *Lupinus albus* (Weisskopf et al. 2006a, b). In these studies, release of the isoflavonoid genistein at rates up to 3 μmol $h^{-1} g^{-1}$ root fresh weight has been reported for cluster roots. This is comparable with the release rates of citrate and malate reported for cluster rooted plant species with a proven ability for mobilization of poorly soluble soil P sources (Dinkelaker et al. 1995; Neumann and Martinoia 2002). However, studies attempting to demonstrate mobilization of P and micronutrients by flavonoids in root exudates must also consider the physicochemical conditions within the rhizosphere, which include pH, soil buffering capacity, redox conditions and the presence of other organic ligands, as factors determining the complex stability of the respective flavonoid with metal ligands, and thereby efficiency in nutrient mobilization. These aspects are currently largely undetermined.

Another putative contribution of flavonoids in P mobilization within soils is in the protection of simultaneously released P-mobilizing carboxylates, such as citrate, against microbial degradation. Accordingly, isoflavonoids released from cluster roots of *Lupinus albus* prior to a pulse of intense citrate exudation (Neumann et al. 2000) are able to reduce microbial respiration and to induce reproductive growth of fungal populations in the rhizosphere, which are associated with a decline in microbial C consumption (Weisskopf et al. 2006b; Tomasi et al. 2008).

## Iron

Iron (Fe) is an essential micronutrient for major metabolic processes in plants such as nitrogen and sulfur metabolism, and the energy-yielding electron transfer reactions of respiration and photosynthesis (Guerinot and Ying 1994). Although being the fourth most abundant element of the Earth's crust, at pH values compatible with plant growth and in oxic environments, Fe(III) is mostly precipitated as hydroxides, oxyhydroxides, and oxides, while Fe(II) is predominantly included in the crystal lattice of a range of primary and secondary ferromagnesian silicates (Cornell and Schwertmann 2003). Because of the poor solubility of Fe oxide/hydroxide pools, the concentration of free Fe within the soil solution is extremely low (Lindsay 1979; Marschner 1995). In the rhizosphere, this concentration is even lower due to the Fe uptake by both roots and microbes, and the concentration of Fe(III) species are generally far below those required for optimal growth of plants ($10^{-4}$–$10^{-9}$ M; Loper and Buyer 1991). To acquire this essential element, in spite of its low availability, two types of strategies for Fe uptake have been developed by plants, as first proposed by Römheld and Marschner (1986). Strategy I is carried out by all higher plants, except *Gramineae*, and involves physiological changes, such as increased excretion of protons (Bienfait 1985; Guerinot and Ying 1994; see Ma 2005 as review) and organic compounds (Marschner 1995), enhanced Fe (III)-reducing capacity mainly dependent on a NAD(P) H-ferric chelate reductase (Robinson et al. 1999; Yi and Guerinot 1996), and associated morphological changes such as the development of root hairs and transfer cells (Schmidt 2003). Strategy II, found in graminaceous monocots, involves the synthesis of phytosiderophores, which form complexes with Fe(III) (von Wiren et al. 2000) and the uptake by roots of the Fe (III)-phytosiderophore complex by a specific transporter (von Wiren et al. 1994; Curie et al. 2001).

In non-graminaceous monocots and dicots (Strategy I plants), phenolic compounds are frequently reported to be the main components of root exudates

in response to Fe-deficiency (Olsen et al. 1981; Römheld and Marschner 1986; Treeby and Uren 1993; El-Baz et al. 2004). Römheld and Marschner (1986) have identified caffeic acid as the main reducing substance released by roots of Fe-deficient peanut (*Arachis hypogaea* L.; >85% of exudates). Moreover, the excretion of riboflavin 3′- and 5′-sulfate has also been described in Fe-deficient sugar beet (*Beta vulgaris* L.) plants (Susin et al. 1993, 1994). Cluster roots of white lupin, which release large amounts of flavonoids (identified as mainly genistein- and hydroxygenistein-derivatives (Weisskopf et al. 2006a)) under P-deficient conditions (Neumann et al. 1999, 2000), become yellow, as does the remainder of the root system, in Fe-deficient plants, as a consequence of phenolic accumulation (Hagström et al. 2001). This accumulation is a pre-requisite for the root-release of these compounds into the rhizosphere. Furthermore, Malinowski et al. (1998) demonstrated that plants of *Festuca arundinacea* released more Fe-reductive exudates, presumably phenolic compounds, when they were infected by a fungal endophyte (*Neotyphodium coenophialum*). Although the release of reductants by roots of Fe-deficient plants is well documented, a detailed analysis of the reducing compounds as well as the flavonoids within them is still scarce. An isoflavonoid, which intensively dissolved ferric phosphate making Fe and P available for plant utilization, has been identified as 2-(3′,5′-dihydroxyphenyl)-5,6-dihydroben zofuran by HPLC analysis in root exudates collected from Fe-deficient alfalfa (*Medicago sativa* L.) plants (Masaoka et al. 1993).

Considering the mechanisms by which phenolic compounds can regulate the mobility of Fe in the rhizosphere, it is widely accepted that the reducing and complexing properties play an important role. In cases of Fe deficiency, the contribution of reductants to the total root activity of Fe(III) reduction was estimated to be less than 7% (Zheng et al. 2003). However, Schwab and Lindsay (1989) upheld the idea that the release of electrons near actively absorbing roots is a major mechanism by which Fe-stressed plants are able to increase the solubility and availability of Fe to meet plant nutritional needs. When oxidizing conditions prevail, which is the case in most agricultural soils, the activity of Fe(II) species is very low; however, the $Fe^{2+}$ concentration will increase ten-fold when the soil pH drops by one unit (Robin et al. 2008). In addition, when reduction of Fe occurs in soils (i.e. when electrons are supplied by reductants), the dissolution of Fe(III)-bearing minerals such as goethite takes place (Hinsinger et al. 2003).

Olsen et al. (1981) showed that caffeic acid, released as a response to stress conditions caused by low Fe availability, triggers a redox reaction where Fe (III) is reduced to Fe(II). In in vitro experiments, we observed that pure flavonoids (genistein, quercetin and kaempferol) can reduce Fe(III). The magnitude of this capacity was higher when the iron was supplied as Fe(III)-EDTA rather than as Fe(III)-hydroxide (Table 2). The reducing capacity of flavonoids has been chemically characterized, demonstrating that this property is determined by the B-ring (Fig. 1). The presence of an ortho-catechol group was shown to be the most important feature for a high antioxidant capacity (Bors et al. 1990). For flavonoids lacking a catechol group, the basic structure becomes important, i.e. the presence of both 2,3-double bonds and 3-OH group is of fundamental importance for a high antioxidant activity.

Altogether, the close contact of phenolics exuded from roots with Fe-containing soil particles, as found in the rhizosphere, helps to efficiently release Fe from mineral particles by reduction and complexation mechanisms, which in turn increases the micronutrient availability.

Phenolic compounds can also affect the availability of Fe in the rhizosphere through the formation of Fe complexes/chelates. According to van Hees and Lundström (2000), more than 95% of Fe in soil solution is likely to be chelated. The chelating capacities of phenolic compounds could allow them to dissolve Fe-oxides and/or poorly soluble Fe

**Table 2** Fe(III)-reducing capacity of pure flavonoids. Data, expressed per mg of flavonoid, are means ± SD of two independent experiments carried out in quadruplicate. Different letters indicate significant differences ($P<0.05$) within each column. Experimental procedure was as described in Tomasi et al. (2008)

| Source of Fe | $Fe^{III}$-EDTA<br>μmol $Fe^{II}$ $mg^{-1}$ $h^{-1}$ | $Fe(OH)_3$ |
|---|---|---|
| Genistein | 0.820±0.042 b | 0.811±0.025 c |
| Quercetin | 1.451±0.373 a | 2.585±0.221 a |
| Kaempferol | 1.242±0.252 a | 1.189±0.167 b |

minerals, such as goethite, by enhancing the rate of ligand-promoted dissolution of these minerals, as proposed for phytosiderophores released by *Gramineae* (Reichard et al. 2005; Kraemer et al. 2006). Table 3 shows that the incongruent dissolution of a $^{59}$Fe-vivianite suspension, leading to a solubilization of $^{59}$Fe and P, was enhanced when pure flavonoids (genistein, quercetin or kaempferol) were added to the medium. Mira et al. (2002) showed that two flavones, myricetin and quercetin, interact strongly with $Fe^{3+}$ with complexation occurring between the 5-hydroxyl and 4-oxo groups. Also the oxidation products of caffeic acid, which is exuded by Fe-deficient tomato plants and which solubilizes Fe from insoluble Fe sources (Olsen et al. 1981), interact with both Fe(III) and Fe(II) resulting in the formation of soluble and insoluble complexes (Deiana et al. 2003). Because of the high content of negatively charged sites in the apoplast, it has been suggested that root apoplastic Fe comprises an important storage pool used by plants (Bienfait et al. 1985). Jin et al. (2007) have clearly demonstrated a direct involvement of secreted phenolics in the remobilization and re-utilization of apoplastic root Fe. They have suggested chelation- and reduction-based effects mediated by phenolics on Fe bound to the cell wall.

Although Tomasi and co-workers (2008) clearly demonstrated that microbial respiration was reduced in the presence of flavonoids, it cannot be discounted that flavonoids might also be used as C sources by some microorganisms. In this case, the $CO_2$ produced could increase the level of Fe available within the rhizosphere. When $pCO_2$ increases in soil, this should also increase concentration of carbonic acid ($H_2CO_3$), causing significant acidification due to its dissociation in alkaline conditions (pKa $H_2CO_3$ = 6.36). Kraemer et al. (2006) have calculated that the concentration of dissolved Fe increased from $10^{-10}$M, at ambient atmospheric $CO_2$ concentration, up to $2\times10^{-9}$M, at a $CO_2$ concentration of 0.2 mol mol$^{-1}$ which is a common value in the rhizosphere (Gollany et al. 1993).

A proposed mechanism for flavonoid contribution to increased iron availability in the rhizosphere is summarized in Fig. 5.

**Table 3** Solubilization of $^{59}$Fe or Pi from $Fe_3(PO_4)_2.8H_2O$ by flavonoids. Data, expressed per mg of flavonoid, are means ± SD of two independent experiments carried out in quadruplicate. Different letters indicate significant differences ($P<0.05$) within each column. Data of P or $^{59}$Fe mobilization were calculated as differences between the mobilization level of each treatment (150 mg of flavonoid) and its control (P: 0.675±0.05; $^{59}$Fe: 0.91±0.04 μmol h$^{-1}$). Experimental procedure as described in Tomasi et al. (2008)

| | Pi mmol g$^{-1}$ h$^{-1}$ | $^{59}$Fe |
|---|---|---|
| Genistein | 0.50±0.13 c | 0.20±0.07 c |
| Quercetin | 1.46±0.32 b | 0.68±0.10 b |
| Kaempferol | 3.08±0.38 a | 1.55±0.28 a |

## Other micronutrients

Manganese (Mn) solubility in soils can be limited by changes in redox level and pH, which have a profound effect on the solubility of Mn–containing minerals (Lindsay 1991). Only the reduced form ($Mn^{2+}$) is available to plants; the oxidized form ($Mn^{4+}$) is unavailable. Under Mn-deficient conditions, which pertain to millions of hectares of arable land worldwide (Welch 1995), the roots of Mn-efficient wheat (*Triticum aestivum* L.; Rengel et al. 1996) and oat (*Avena sativa* L.; Timonin 1946) release increased amounts of Mn-reducing compounds into the rhizosphere compared to plants which are less Mn-efficient. These results show the contribution of phenolic-containing exudates to increased Mn availability through a mechanism based on chemical reduction, as proposed by Marschner (1988), however, clear evidence of the involvement of flavonoids in this phenomenon is still lacking.

Most of the copper (Cu) found in soils is associated with organic matter, but it can also be held on cation-exchange surfaces of soil colloids or bound in Fe-Cu oxides (Hartley et al. 2007), making Cu relatively immobile. Cu is a transition element, and can, like Fe, form stable complexes. It can also easily transfer electrons. The divalent form is readily reduced to monovalent copper, which is unstable. Although Cu reduction and its complexation by flavonoids has been clearly demonstrated and chemically characterized (El Hajji et al. 2006) and evidence for Cu binding by extracellular-root exudates of tall fescue has been reported (Malinowski et al. 2004), the contribution of this phenomenon to Cu availability in the rhizosphere, and to plant acquisition of Cu, has not yet been evaluated.

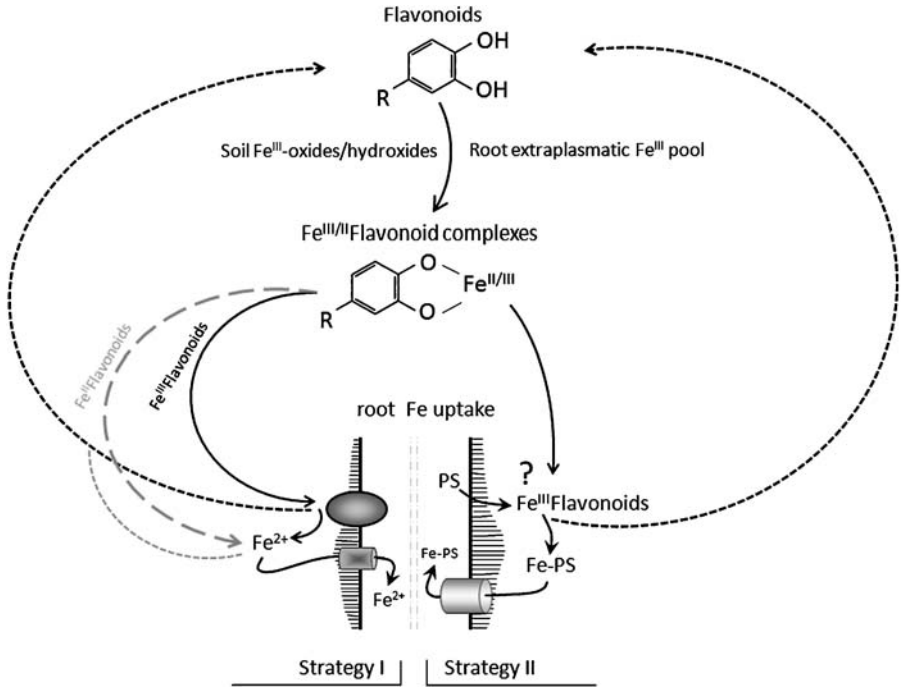


**Fig. 5** Proposed mechanisms of the contribution of flavonoids to iron availability in the rhizosphere, and its acquisition by plants

## Concluding remarks and perspectives

In this chapter, the flavonoids identified so far in root exudates have been described, as well as the mechanisms involved in their release. Moreover, the roles of these compounds in plant nutrient acquisition from the rhizosphere have been discussed. Based on the present knowledge, it can be expected that many more so far unknown functions of flavonoids with regard to plant nutrient acquisition will be discovered in the future. The well-studied example of the role of flavonoids in the establishment of nitrogen-fixing symbiosis underlines the importance of flavonoids as chemical signals in the rhizosphere. The enormous structural variability of flavonoids perfectly fits with a signaling role, and one can speculate that these compounds are involved in many more plant-microbial communication processes than are currently known (for symbiotic or pathogen interactions). Moreover, the high variability between the patterns of exuded flavonoids from different cultivars or ecotypes of the same species underlines the complexity of the root exudation of phenolics into the rhizosphere.

Despite the well-investigated role of flavonoids in the establishment of symbiosis with rhizobia, surprisingly little is known concerning the release mechanisms from plant roots. The highly specialized release patterns in space and time suggest the presence of tightly controlled mechanisms for the regulation of these processes. A more detailed knowledge concerning these aspects of flavonoid exudation will not only improve the understanding of plant microbial interactions but may also offer prospects for improvement of plant nutrient acquisition by direct modifications of the rhizosphere management.

Reducing and chelating properties are other interesting features of flavonoids with a potential role for plant nutrient acquisition. However, although many model studies are available dealing with the behavior of pure flavonoids in soils, only very limited information exists on the real rhizosphere conditions of soil-grown plants. The interactions of flavonoids with surface-reactive particles such as clay minerals (for hydrophilic flavonoids) and hydrophobic humic molecules (for hydrophobic flavonoids) can indeed affect the behavior of flavonoids once they reach the soil environment. Knowledge of such interactions and of flavonoid concentrations within the rhizosphere is a prerequisite for understanding the potential involvement of these multifaceted molecules in plant nutrient acquisition.

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