JIPB Journal of Integrative Plant Biology

Journal of Integrative Plant Biology 2010, **52** (1): 98–111

Invited Expert Review

Flavonoids: New Roles for Old Molecules

Charles S. Buer*, **Nijat Imin and Michael A. Djordjevic**
Genomic Interactions Group, ARC Centre of Excellence for Integrative Legume Research, Research School of Biology, College of Medicine, Biology, and Environment, The Australian National University, Canberra ACT 2601, Australia
*Corresponding author
Tel: +61 2 6125 3087; Fax: +61 2 6125 8525; E-mail: charles.buer@anu.edu.au
Available online on 6 January 2010 at www.jipb.net and www.interscience.wiley.com/journal/jipb
doi: 10.1111/j.1744-7909.2010.00905.x

Charles S. Buer
(Corresponding author)

Abstract

Flavonoids are ubiquitous in the plant kingdom and have many diverse functions including defense, UV protection, auxin transport inhibition, allelopathy, and flower coloring. Interestingly, these compounds also have considerable biological activity in plant, animal and bacterial systems – such broad activity is accomplished by few compounds. Yet, for all the research over the last three decades, many of the cellular targets of these secondary metabolites are unknown. The many mutants available in model plant species such as *Arabidopsis thaliana* and *Medicago truncatula* are enabling the intricacies of the physiology of these compounds to be deduced. In the present review, we cover recent advances in flavonoid research, discuss deficiencies in our understanding of the physiological processes, and suggest approaches to identify the cellular targets of flavonoids.

Buer CS, Imin N, Djordjevic MA (2010) Flavonoids: new roles for old molecules. *J. Integr. Plant Biol.* **52**(1), 98–111.

Introduction

Flavonoids are low molecular weight secondary metabolites (**Figure 1**) that, unlike primary metabolites, are not essential for plant survival. Nevertheless, they are bioactive across kingdoms with over 9 000 structural variants known (Williams and Grayer 2004). The chemical diversity, size, three-dimensional shape, and physical and biochemical properties of flavonoids allow them to interact with targets in different subcellular locations to influence biological activity in plants, animals, and microbes (Taylor and Grotewold 2005; Peer and Murphy 2007).

Flavonoids have roles in many facets of plant physiology. One of their most important roles is to influence the transport of the plant hormone, auxin (Peer and Murphy 2007). Other roles include defense (Treutter 2005), allelopathy (Bais et al. 2006), and modulating the levels of reactive oxygen species (ROS) (Taylor and Grotewold 2005; Bais et al. 2006). Flavonoids also provide flower coloring to attract pollinators (Mol et al. 1998), and in many species are required for pollen viability (Coe et al. 1981; Mo et al. 1992; Taylor and Jorgensen 1992). Unlike other plant species, the pollen of *Arabidopsis thaliana* mutants lacking flavonoids are not sterile (Burbulis et al. 1996), and this role is probably compensated for by other molecules. As flavonoids absorb UV light, flavonoid-deficient mutants are susceptible to UV irradiation, but some can compensate by the over-production of sinapate esters (Li et al. 1993; Sheahan 1996).

Recent evidence has shown that flavonoids have evolved particular roles in legumes. They are not only required to signal symbiotic bacteria in the legume-bacterium symbiosis (Redmond et al. 1986; Djordjevic et al. 1987; Wasson et al. 2006) but also play important direct roles in root nodule organogenesis (Wasson et al. 2006; Zhang et al. 2009). Flavonoids also accumulate in the progenitor cells for different legume organs (Mathesius et al. 1998a; Morris and Djordjevic 2006)

© 2010 Institute of Botany, Chinese Academy of Sciences

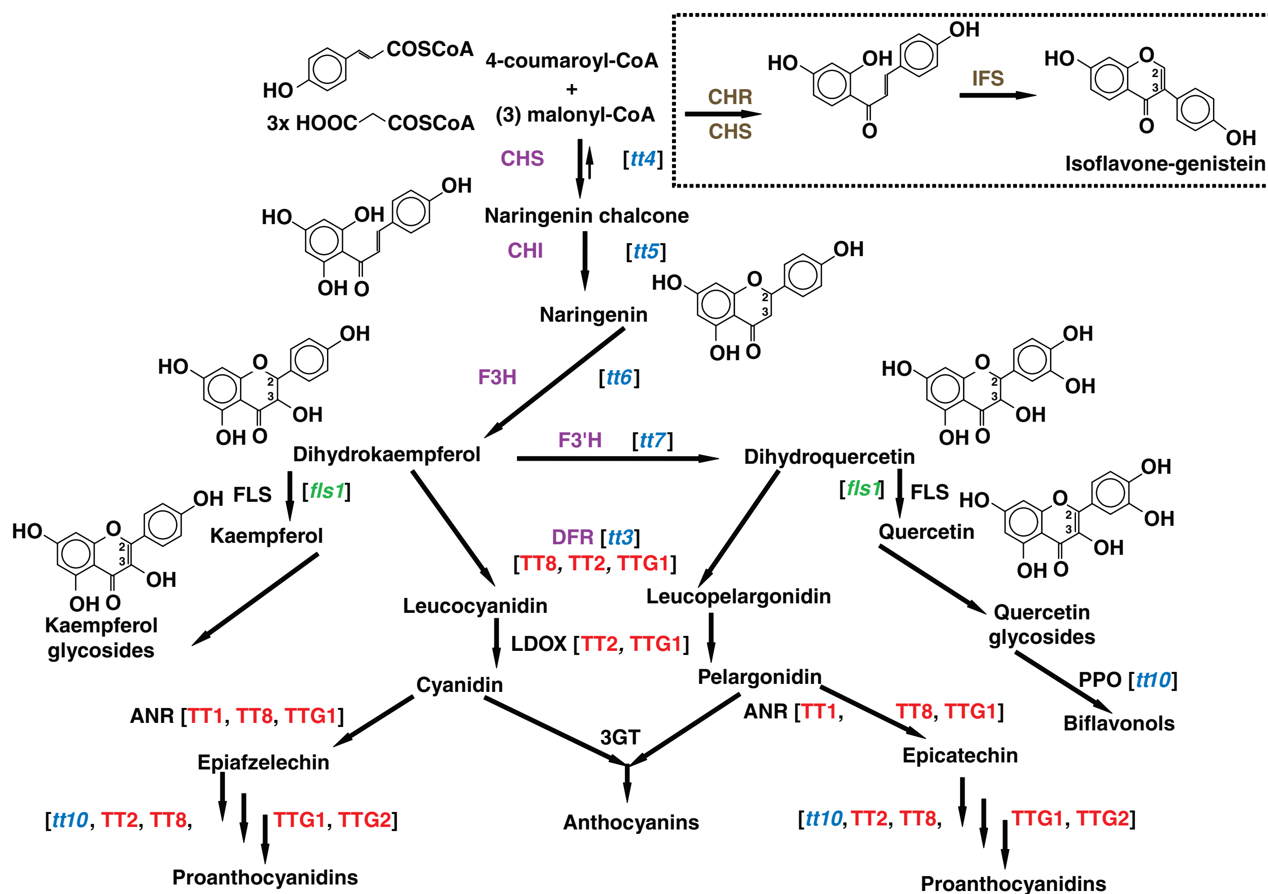


Figure 1. The flavonoid branch of the phenylpropanoid pathway.

Known locations of *Arabidopsis transparent testa* (*tt*) mutations (in parentheses) are indicated on the phenylpropanoid pathway. *Arabidopsis* does not have chalcone reductase (CHR) nor isoflavone synthase (IFS) and, thus, does not produce any isoflavonoids (dashed box); this branch of the pathway is mostly restricted to legumes. The chemical structures of the aglycones produced during the early steps of the pathway are shown. Mutations *tt1*, *tt2*, *tt8*, *ttg1*, and *ttg2* are in regulatory genes involved in controlling several points of the pathway. The gene products affected by these regulatory mutations are TT2, an R2R3 repeat MYB transcription factor; TT8, a bHLH transcription factor; and TTG1, which encodes a WD40 repeat gene. These proteins act as a complex to induce anthocyanidin reductase (BANYLUS) expression. Anthocyanidin reductase catalyses the formation of anthocyanins. TT1 is a WIP family zinc finger transcription factor and TTG2 is a WRKY type transcription factor that acts downstream of *TTG1*. The mutated structural genes and the affected products are TT3 (DFR: dihydroflavonol reductase); TT4 (CHS: chalcone synthase); TT5 (CHI: chalcone isomerase); TT6 (F3H: flavonol 3-hydroxylase); TT7 (F3′H: flavonol 3′-hydroxylase); and TT10 (PPO: polyphenol oxidase) an enzyme for biflavonol conversion and oxidizing procyanidins to proanthocyanidins in the seed testa. Other genes involved in the pathway are 3GT (anthocyanidin 3-*O*-glycosyltransferase), ANR (anthocyanidin reductase), and LDOX (leucoanthocyanidin dioxygenase).

and influence the development of *in vitro* root formation (Imin et al. 2007).

Apart from being important dietary components, many therapeutic benefits of flavonoids are known in animal systems. Flavonoids have anti-oxidant, anti-proliferative, anti-tumor, anti-inflammatory, and pro-apoptotic activities, and some molecular targets have been identified (Williams et al. 2004; Taylor and Grotewold 2005; Garcia-Mediavilla et al. 2007; Pandey et al. 2007; Sung et al. 2007; Kim et al. 2008; Singh et al. 2008). The health-promoting effects of flavonoids may relate to interactions with key enzymes, signaling cascades involving cytokines and transcription factors, or antioxidant systems (Polya 2003).

The present review will focus on advances in our knowledge pertaining to flavonoid biosynthesis, differential *in situ* accumulation of flavonoids in various flavonoid pathway mutants

as well as flavonoid uptake and movement within the plant. We will then discuss the roles played by flavonoids in plant physiology and development, including efforts to define the molecular targets for flavonoids. Readers are directed to recent reviews that cover auxin modulation and other developmental processes (Taylor and Grotewold 2005; Boudet 2007; Peer and Murphy 2007).

What is New in Flavonoid Biosynthesis?

Flavonoids are synthesized in plants via the flavonoid branch of the phenylpropanoid and acetate-malonate metabolic pathway (**Figure 1**). Unlike legumes, *Arabidopsis* lacks chalcone reductase and isoflavone synthase enzymes, and subsequently it cannot produce isoflavonoids (Aoki et al. 2000) (**Figure 1**, box). The flavonoid pathway is well-described in *Arabidopsis*, and mutants are available with defects in genes encoding for each enzymatic step (**Table 1**) and within regulatory genes encoding transcription factors that affect the pathway. These mutants were selected based on the seed testa color. A wide range of color, from tan to yellow, result due to different levels of proanthocyanidins in the flavonoid pathway mutants (Debeaujon et al. 2000) (**Figure 2**). In *Arabidopsis*, most flavonoid pathway genes occur in single copies, which greatly simplifies experimentation (Winkel-Shirley 2001); whereas in legumes, for example, most genes occur in multigene families (Arioli et al. 1994; Lawson et al. 1994; McKhann and Hirsch 1994; Mathesius et al. 2001). Interestingly, an *Arabidopsis* flavonol synthase (FLS) locus with putative redundancy also proved to be a single functioning gene, as the other FLS genes were found to be non-functional (Owens et al. 2008a).

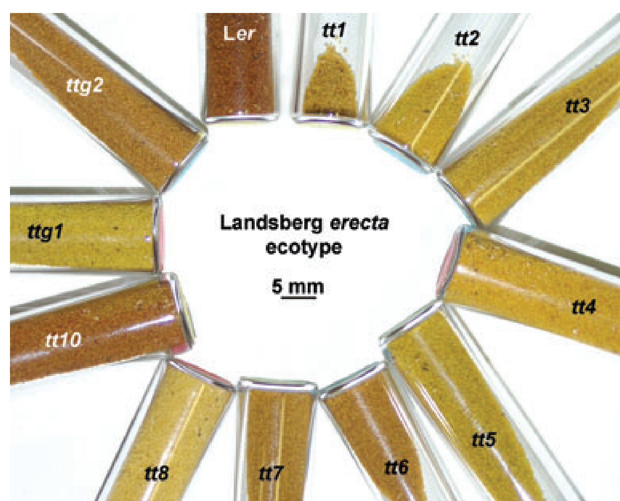


Figure 2. The *transparent testa* mutants of *Arabidopsis* have light colored seed testa compared with the wild type. The absence of testa color gave these mutants their name and provided a means for their identification and isolation from mutagenized libraries.

Recent studies have characterized the TT6 locus, which encodes flavanone 3-hydroxylase (F3H) (Owens et al. 2008b). This enzyme is a member of the 2-oxoglutarate dependent (2-OG) dioxygenase super family of enzymes. The *tt6* mutation was presumed to be leaky, as the seed testa gradually darkens, but subsequent research showed that the enzyme encoded by this mutation is catalytically inactive (Owens et al. 2008b). It is thought that other 2-OG dioxygenases (FLS or anthocyanidin synthase (ANS)) can partially complement for F3H activity.

Table 1. Selected *transparent testa* mutants from *Arabidopsis*

Line	Enzyme lesion	Locus[a]	Mutagen	Reference
Ler	Wild type	–	–	–
tt1	WIP zinc finger protein	At1G34790	X-rays	(Sagasser et al. 2002)
tt2	MYB domain protein 123	At5G35550	X-rays	(Nesi et al. 2001)
tt3	Dihydroflavonol 4-reductase	At5G42800	X-rays	(Shirley et al. 1995)
tt4	Chalcone synthase	At5G13930	EMS	(Koornneef 1990)
tt5	Chalcone isomerase	At3G55120	Fast neutrons	(Shirley et al. 1995)
tt6	Flavonone 3-hydroxylase	At3G51240	EMS	(Pelletier and Shirley 1996)
fls1	Flavonol synthase	At5G51240	T-DNA	(Owens et al. 2008a)
tt7	Flavonoid 3′-hydroxylase	At5G07990	EMS	(Schoenbohm et al. 2000)
tt8	bHLH domain protein	At4G09820	Unknown	(Nesi et al. 2000)
tt10	Polyphenol oxidase (laccase-like protein)	At5G48100	EMS	(Pourcel et al. 2005)
ttg1	WD40 repeat protein	At5G24520	EMS	(Walker et al. 1999)
ttg2	WRKY TF	At2G37260	Unknown	(Ishida et al. 2007)

[a]Locus locations are from TAIR (The *Arabidopsis* Information Resource: http://www.arabidopsis.org).
EMS, ethylmethane sulfonate; TF, transcription factor.

The *tt6* mutant has a previously unrecognized long hypocotyl phenotype (**Figure 3**) (Buer and Djordjevic 2009), which can also be clearly seen from examining the images in other publications (Owens et al. 2008b). This phenotype is similar to the *Arabidopsis hy5* mutation which has flavonoid-deficient roots and elevated auxin transport (Sibout et al. 2006). Attempting to complement the *tt6* phenotype by adding dihydrokaempferol (DHK) unexpectedly caused the hypocotyls to further elongate rather than restoring normal hypocotyl growth (Buer and Djordjevic 2009). This mutant clearly requires further investigation.

Differential *in situ* Accumulation of Flavonoids in *transparent testa* Mutants

A useful property of flavonoids is that they can be visualized locally, *in planta*, due to their fluorescence properties. This is best imaged after the addition of exogenous diphenylboric acid 2-amino ethyl ester (DPBA), which complexes with flavonoids and greatly enhances their fluorescence properties (Buer et al. 2007) (**Figure 4**). Excitation at 488 nm produces an easily observable fluorescence (**Figure 4C**). UV excitation also works, but autofluorescence from callose interferes with flavonoid fluorescence (light blue color in **Figure 4B,D**) and the overall fluorescence is subsequently weaker. The DPBA enhancement of fluorescence depends on the structure of the flavonoid. Quercetin (Q)-DPBA (gold) and kaempferol (K)-DPBA (green) complexes fluoresce strongly. Q-DPBA fluoresces 400 times more strongly than DPBA complexes with dihydroquercetin (DHQ), dihydrokaempferol (DHK) or naringenin (N), (Buer et al. 2007). Specific flavonoid-DPBA complexes also occur in legumes (Djordjevic et al. 1997; Mathesius et al. 1998a, 2000).

Arabidopsis flavonoid mutants have differential DPBA-flavonoid fluorescence determined by which compound accumulates due to the specific mutation (**Figure 5**). In some mutants, Q accumulates strongly in the distal elongation zone where gravitropic bending, auxin accumulation, and auxin synthesis occur (Blancaflor and Masson 2003). Given the role that certain flavonoids, including Q, have in affecting auxin transport (Jacobs and Rubery 1988), the effects of Q accumulation in this region requires careful examination.

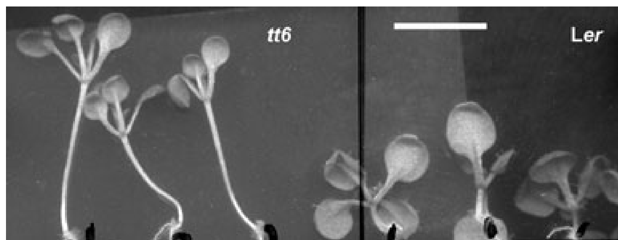


Figure 3. The long hypocotyl phenotype of the *tt6* mutant. Seedlings were grown on Murishige and Skoog medium without sucrose for 6 d. Plates were scanned on a flatbed scanner through the medium. A cover slip presses the seedlings onto the plane of the medium. Bar, 7 mm.

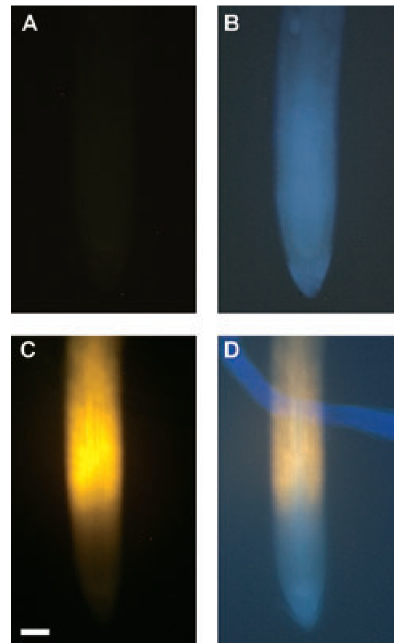


Figure 4. Excitation at 488 nm provides excellent visualization of diphenylboric acid 2-amino ethyl ester (DPBA)-flavonoid complexes.

(A) Negative control micrograph of Landsberg *erecta* root tip without DPBA stain and 488 nm excitation.

(B) The same root with UV (4′6′-diamidino-2-phenylindole dihydrochloride (DAPI)) excitation without DBPA staining.

(C) 488 nm excitation of a L*er* root stained with DPBA following the protocol of (Buer et al. 2007).

(D) UV excitation of the same root.

Bar, 50 μm. The golden fluorescence is predominately from quercetin.

Although the *in situ* concentrations of flavonoid compounds in root tips remain unknown, experimentation provides some clues. The application of a range of naringenin concentrations to the root tips of the flavonoid-less *tt4* mutant showed that, after 1 h, a weak fluorescent signal could be detected in the presence of 1 nM naringenin (**Figure 6**). This offers a remarkable sensitivity for detecting flavonoids *in situ* in individual plants. By comparison, the detection level of flavonoids using high performance liquid chromatography (HPLC) is around 1 μM (**Table 2**), and requires extraction from over 1 000 roots (approximately 300 mg fresh weight).

Flavonoid Uptake and Movement

Using *Arabidopsis* flavonoid-pathway mutants, two new features for flavonoids were recently demonstrated. Selective uptake systems exist for flavonoids, and flavonoids are capable of long-distance movement (Buer et al. 2007, 2008). This was best demonstrated using the *tt4* mutant, which is defective in flavonoid biosynthesis, due to a lesion in chalcone synthase (CHS) but retains the downstream enzymatic machinery in the pathway. Adding the downstream aglycones (N, DHK, DHQ, K, or Q) to the *tt4* mutant allowed uptake to be assessed, in real time, within different tissues through the analysis of flavonoid fluorescence. The uptake systems in *Arabidopsis* were highly selective preferring the non-planar flavonoids (N, DHQ, and DHK), which are unsaturated at the 2,3 position, but not the planar Q or K (**Figure 1**).

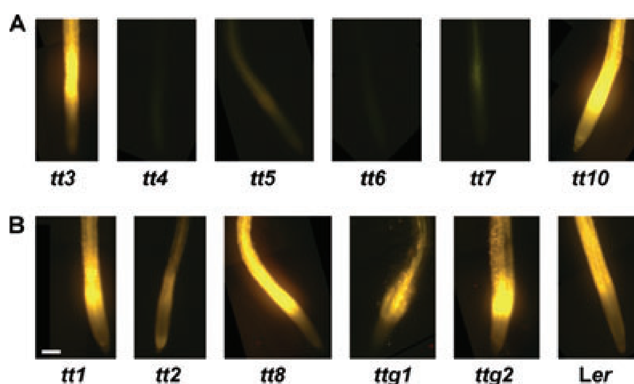


Figure 5. Flavonoid fluorescence in the root tip region of *Arabidopsis tt* mutants. The diphenylboric acid 2-amino ethyl ester (DPBA)-flavonoid fluorescence in the distal elongation zone varies according to the aglycone that accumulates in this region.

The distal elongation zone is where gravitropic bending occurs. The mutants in (**A**) possess lesions in the structural enzymes of the pathway, whereas those in (**B**) are in the regulatory genes, except L*er*, which is the wild-type control. Many seedlings accumulate quercetin, whose fluorescent signal is a bright golden yellow when complexed with DPBA. Mutants *tt4* and *tt6* have very low fluorescent signal resulting from background sinapate esters and naringenin, respectively. Naringenin-DPBA fluoresces over 400 times less strongly than quercetin- or kaempferol-DPBA complexes (Buer et al. 2007). The *tt7* mutant shows low fluorescent signal from dihydroquercetin- and kaempferol-DPBA complexes. The *tt5* mutant has low Q-DPBA fluorescence resulting from the spontaneous conversion of naringenin chalcone to naringenin and the subsequent formation of downstream products. Seedlings were analyzed for fluorescence 5-d post germination. Seedlings were grown without sucrose in the medium. Bar, 100 μm.

Table 2. Minimum detection levels of flavonoid standards using high performance liquid chromatography (HPLC)

Flavonoid compound	Minimum detection level
N	10 μM
DHK	5 μM
DHQ	5 μM
K	1 μM
Q	5 μM

Flavonoid standards were serially diluted and injected onto a C-18 column using the protocol from (Buer et al. 2007). The level shown is where the compounds were no longer detectable from the noise of the trace. DHK, dihydrokaempferol; DHQ, dihydroquercetin; K, kaempferol; N, naringenin; Q, quercetin.

Different plant tissues were capable of flavonoid uptake. Once taken up, the flavonoids were converted to downstream products and transported long distances in the plant. This was confirmed using grafting (Buer et al. 2008), where flavonoid competent roots could "complement" flavonoid production in a *tt4* shoot scion and vice versa (**Figure 7**). Movement in the root-to-shoot direction appears to be symplastic, and shoot-to-root movement appears to be limited to the vascular tissue (**Figure 8**) – but the precise conducting tissue was not defined.

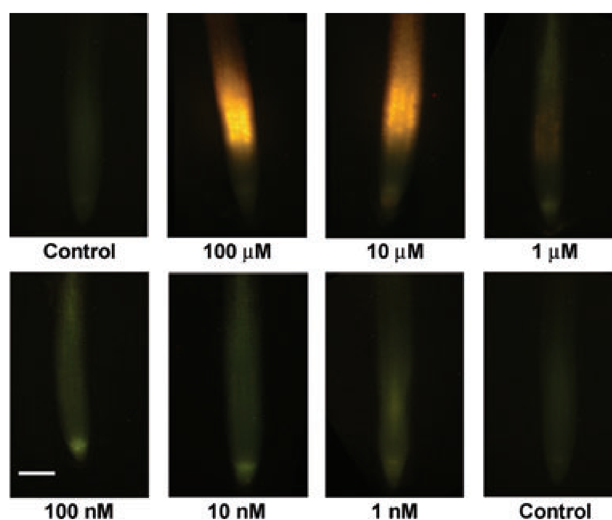


Figure 6. The detection of diphenylboric acid 2-amino ethyl ester (DPBA)-flavonoid complexes in *tt4* plants after the addition of different naringenin concentrations.

Titration of naringenin addition to *tt4* indicates that 1 nM is sufficient to visualize flavonoid fluorescence in the *Arabidopsis* root tip. Naringenin at the indicated concentration was added to root tips for 1 h, seedlings were stained with DPBA, and viewed with 488 nm excitation. Control roots were incubated with agar devoid of flavonoids. Bar, 100 μm.

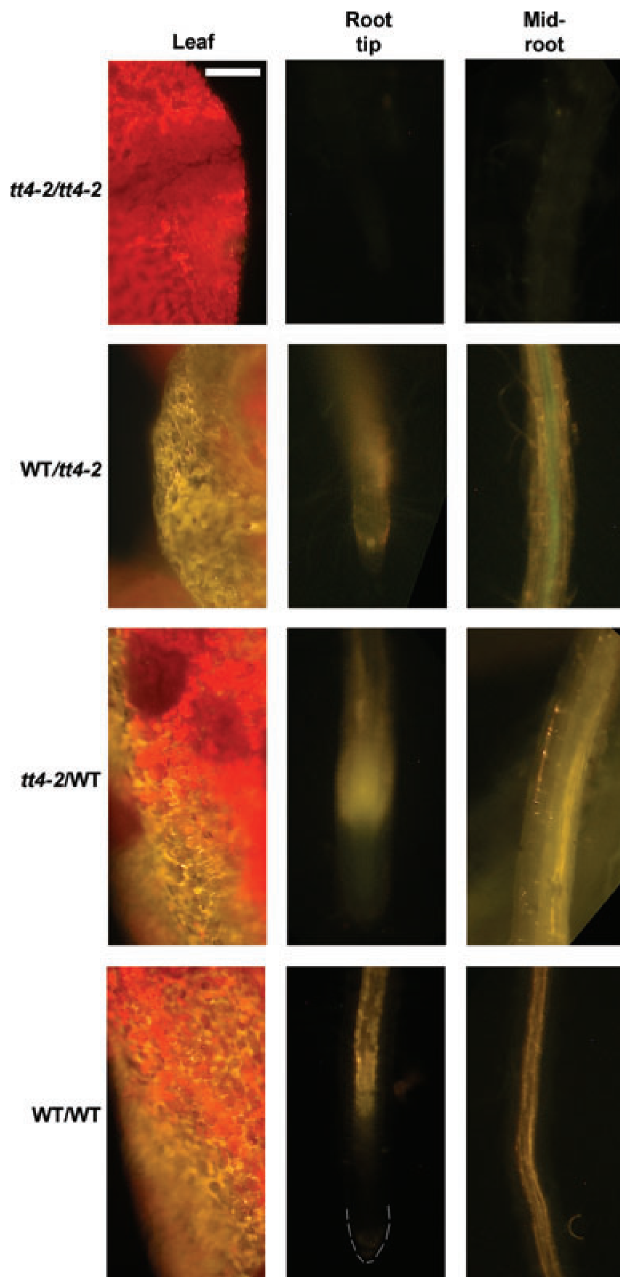


Figure 7. Grafting confirms flavonoid movement from shoot-to-root and root-to-shoot in *Arabidopsis*.

The different combinations of reciprocal grafts are represented. The first symbol indicates the top of the graft and the second symbol the bottom of the graft, thus, WT/WT indicates that a wild type shoot was grafted to a wild type root. Grafts were done following the protocol of (Turnbull et al. 2002), except the substrate used to mount the seedlings was filter paper. A wild type shoot can provide flavonoids to a *tt4* root system and a wild type root can provide flavonoids to the leaves of *tt4* shoots. The dotted line in WT/WT shows the root tip location. Bar, 50 μm.

Figure 8. Vascular tissue localization of diphenylboric acid 2-amino ethyl ester (DPBA)-flavonoid fluorescence following N application to *tt4*.

Shown are false-color confocal laser scanning micrographs.
(A) Z-stack of DPBA fluorescence in vascular tissue distal to the application site following mid-root application of N to *tt4* root for 1 h.
(B) Cross section of A showing the localization of DPBA fluorescence in the vascular tissue following N addition to *tt4* mid-root.
Bar, 50 μm.

The physiological relevance of selective flavonoid uptake and movement requires further investigation. Flavonoid uptake could have implications for allelopathic interactions between root systems of different plants. As the roots of *Arabidopsis* seedlings grown in the dark do not produce flavonoids, a possible role for shoot-to-root flavonoid movement could be to provide flavonoids to the roots of seedlings to control root growth and gravitropic responses.

Deciphering the players involved in flavonoid movement is required to understand the mechanism. Unlike auxin, aglycones are uncharged molecules and therefore lipophilic, and likely to associate with plant membranes. Therefore, directional movement from one membrane system to the next would require a transport mechanism. The multi-drug and toxin efflux (MATE) superfamily has been identified recently as a membrane protein family (Brown et al. 1999) with 9 to 14 membrane-spanning domains (Schwacke et al. 2003). These proteins are possible candidates for mediating the short and long distance movement of compounds within plants as well as for intracellular metabolite trafficking. There are 56 genes

belonging to the MATE family in *Arabidopsis*, and only a few of these proteins have been studied to date. Biochemical data on transport properties and functional aspects of family members are scarce, as is the identification of functional conserved signatures or domains. The MATE transporters encoded by these genes may cover a diverse range of functions in plant growth and development, in particular in the transport of important compounds. Their characterization, however, is consistent with them also transporting small, organic compounds. For example, the TRANSPARENT TESTA12 (TT12) protein is thought to localize to the vacuolar membrane in developing seeds, where it controls the sequestration of flavonoids (Debeaujon et al. 2001). ABERRANT LATERAL ROOT FORMATION5 (ALF5), which is expressed strongly in the root epidermis, is required for resistance to a number of compounds (Diener et al. 2001). ENHANCED DISEASE SUSEPTIBILITY5 (EDS5) might transport salicylic acid or a precursor required for its synthesis (Nawrath et al. 2002). *A. thaliana* DETOXIFICATION1 (AtDTX1) is capable of effluxing plant-derived alkaloids such as berberine and palmatine (Li et al. 2002), and FERRIC REDUCTASE DEFECTIVE3 (FRD3) acts as a proton-coupled citrate exporter on the plasma membrane, extruding citrate into the xylem or the rhizosphere (Durrett et al. 2007).

We used the gene expression profiling tool Genevestigator (Grennan 2006) to demonstrate the expression of the MATE gene family (**Figure 9A**) and it is clear from this analysis that the expression of these genes is quite diverse. While several family members show wide expression in many tissue types (such as At3G21690; At4G25640, At1G64820, At1G66780, At2G34360, At5G10420, At2G38330, At1G47530, At4G21900, and At4G21900), many others showed specific expression in a particular tissue (At4G00350, At4G29140, At5G19700, At1G71870, At5G17700, At3G26590, FRD3, ALF5, TT12, At4G22790, At1G11670, At3G23550, At1G61890, and At1G58340). For instance, transcripts At3G26590, FRD3, At4G22790, At5G52450, At1G47530, At2G38330, At2G34360, At3G21690, At4G38380, and At4G23030 express in the xylem. These expression analyses were confirmed by the "electronic fluorescent pictograph" browser available at http://bbc.botany.utoronto.ca/efp/cgi-bin/efpWeb.cgi (Winter et al. 2007). To examine the expression of MATE genes in roots, we extracted the transcriptome data for MATE genes from the root expression map database (www.arexdb.org) (Birnbaum et al. 2003; Brady et al. 2007). The expression patterns of some of the MATE genes (including FRD3 and ALF5) are presented in **Figure 9B**. Many of the transcripts (EDS5, FRD3, At1G47530, and At3G21690) showed very tissue-specific expression patterns while others showed more general expression (ALF5, At1G11670, At1G61890, and At3G23550). In summary, the analysis of expression patterns of the members of the MATE family exhibit distinct, yet overlapping, expression patterns, which may support their potential involvement in the transport of a broad range of compounds including flavonoids in a variety of plant cells and tissues. A further functional characterization of MATE genes may shed light on their involvement in flavonoid transport.

A method to determine the genes responsible for transport could be to re-mutagenize *tt4* mutant seeds using ethylmethane sulfonate (EMS) and screen for subsequent mutants that cannot fluoresce after supplying N to the roots. Alternatively, identified transport mutants could be genetically crossed with *tt4* to screen for the lack of flavonoid uptake. There is precedence for considering these proteins as candidates, as flavonoid interactions have been shown with the MATE-type ABCB proteins (Lewis et al. 2007) and glutathione S-transferase (Smith et al. 2003). Also, glybenclamide, which inhibits ABCC transporters, inhibited flavonoid movement in *Arabidopsis* (Buer et al. 2007). Active movement is also supported by the tissue-specific localization of flavonoids, their cell-to-cell movement, and their unidirectional movement when applied mid-root. If the movement was diffusion mediated, the flavonoid distribution should be less localized and movement bidirectional. Interestingly, ABC transporters are also involved in auxin transport (Geisler et al. 2005; Terasaka et al. 2005; Lewis et al. 2009), and p-glycoproteins are inhibited by some flavonoids (Zhang and Morris 2003).

Flavonoids Have Important Roles in Plant Physiology and Development

Architectural phenotypes

A long-held belief was that flavonoids influence plant architecture (Taylor and Grotewold 2005; Buer and Djordjevic 2009), particularly through their effects upon auxin transport. However, some of the best supporting evidence for flavonoid roles in shoot branching (Brown et al. 2001) was refuted recently when this phenotype in the *tt4* mutant was found to be due to a background *max* mutation (Bennett et al. 2006), which affects the branching hormone, strigolactone (Gomez-Roldan et al. 2008). Nevertheless, recent studies have reaffirmed the link between flavonoids and plant architecture by showing that flavonoid-defective mutants display a wide range of alterations to root and shoot development (Buer and Djordjevic 2009). Here, flavonoid mutants compromised at different steps in the flavonoid pathway were affected, to varying degrees, in a myriad of developmental processes. Traits affected included alterations to root growth, lateral root density, root hair development and length, shoot/flower organ number, overall architecture and stature, and seed organ density. The roots of *tt4* have a delayed gravitropic response, a conditional increased in lateral root density, a root looping phenotype when grown on hard slanted agar (**Figure 10A**), and produce unusual root organs more rapidly than wild-type plants when grown in agar (Buer and Djordjevic 2009). Importantly, normal root architecture is restored in some

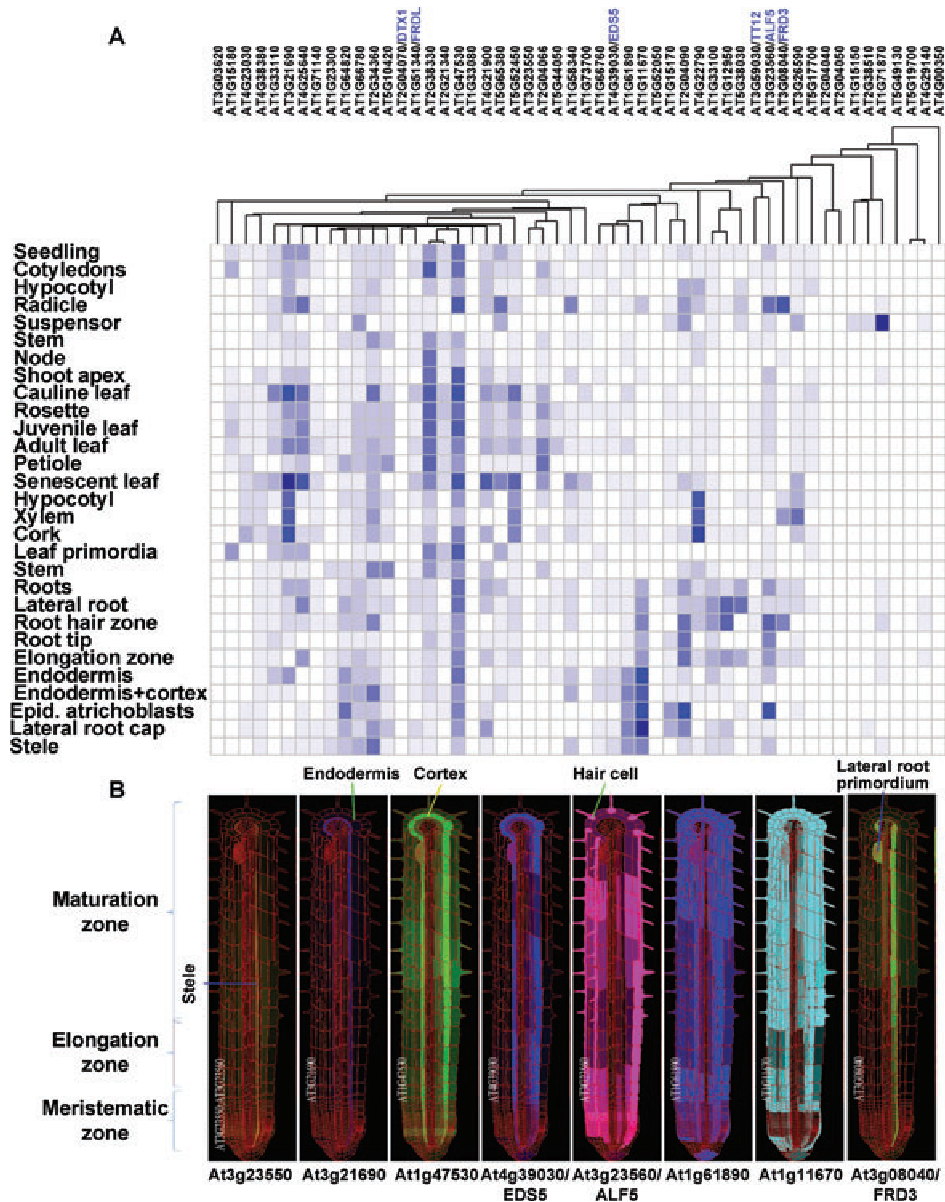


Figure 9. Multi-drug and toxin efflux (MATE) genes may be involved in flavonoid transport in *Arabidopsis*.

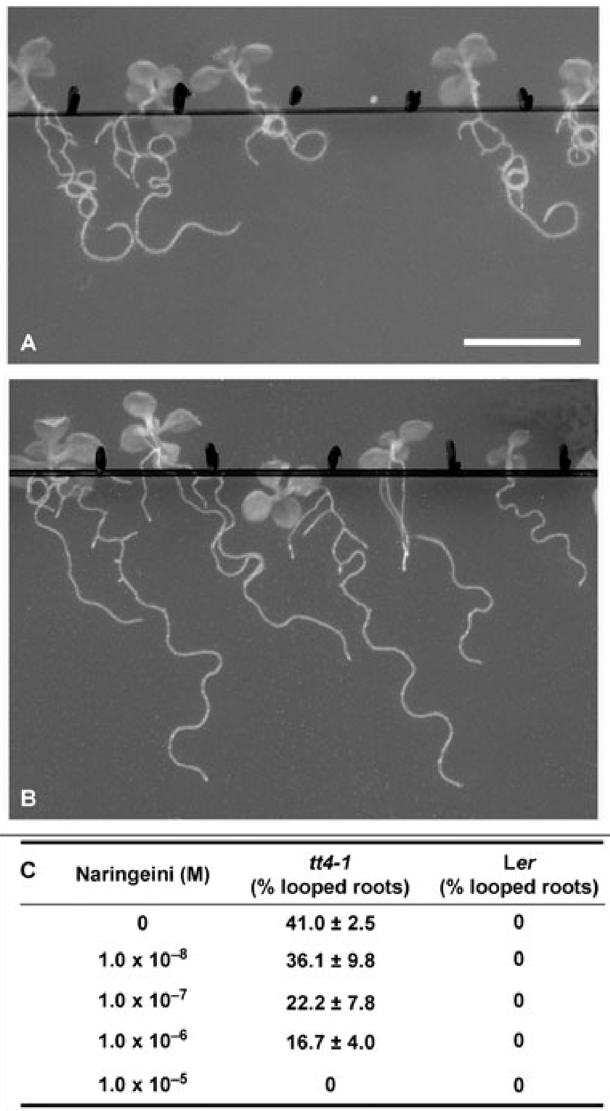
(A) Hierarchical clustering analysis of the expression of selected *Arabidopsis* MATE genes (includes characterized members TT12, DTX1, EDS5, ALF5, FRD3, and FRD3-like [FRDL]). The clustering was carried out using the clustering tool integrated into Genevestigator version 3 (www.genevestigator.ethz.ch) after selection of the ATH1 22k chip type and the anatomy profile (Distance measure: Pearson correlation). Increasing intensity of the blue coloration relates to an increase in expression signal strength.

(B) Root digital *in situ* analyses of MATE genes in *Arabidopsis*.

The cell type specific expression data of roots (Birnbaum et al. 2003) were obtained from databases of AREX (the *Arabidopsis* gene expression database: www.arexdb.org).

mutants by direct flavonoid supplementation. For example, adding naringenin to the media restores the looping *tt4* root phenotype to that of the wild type (**Figure 10B**) and is titratable (**Figure 10C**).

Further investigation is required for several interesting *tt* mutants. The branching phenotype discovered in *tt8*, *tt10*, and *ttg1* (Buer and Djordjevic 2009) that leads to multiple influorescences may be important for increasing seed yield



C Naringeini (M)	*tt4-1* (% looped roots)	Ler (% looped roots)
0	41.0 ± 2.5	0
1.0 x 10^{-8}	36.1 ± 9.8	0
1.0 x 10^{-7}	22.2 ± 7.8	0
1.0 x 10^{-6}	16.7 ± 4.0	0
1.0 x 10^{-5}	0	0

Figure 10. Chemical complementation of root architecture by naringenin addition to the medium.

(A) Root coiling/looping phenotype of *Arabidopsis tt4* mutant plants. Seedlings were grown on Murishige and Skoog medium without sucrose and plates were wrapped with Nescofilm to prevent ethylene build up. The medium was solidified with 1.5% agar to prevent root penetration and plates were placed at a 45° angle in continuous light.

(B) Addition of 10 μM naringenin to the culture medium abolished the root coiling/looping phenotype. Bar, 7 mm.

(C) The root coiling/looping phenotype is titrated by increasing the level of exogenous naringenin.

in agricultural plants, as increased numbers of inflorescences should produce plants with more seeds. Are these transcription factors (*tt8* and *ttg1*) or enzymes (*tt10*) mediating these branching effects by regulating auxin transport directly or indirectly, or do they have other roles in regulating branching? Are there strigolactone-independent branching pathways? TT10 is interesting in this regard as it may have roles in multiple biochemical pathways: it functions as an enzyme for biflavonol production and has a role in oxidizing procyanidins to the brown proanthocyanidins (**Figure 1**).

The flavonoid-mediated architectural changes discovered in plants are likely to have broad implications. For example, some mutants possessed changes that could be important for yield (e.g. increased flower number and increased seed organ production), or provide more resilient responses to environmental stress (increased lateral root and root hair density). However, this requires further exploration as the mechanisms through which the perturbations in flavonoid levels mediate these changes are not well understood. Three likely mechanistic possibilities are: direct effects of flavonoids on unidentified molecular targets, indirect effects mediated by the ability of flavonoids to modulate the levels of auxin, or through ROS regulation.

Flavonoids play distinct roles in root nodule formation

Indeterminate nitrogen-fixing nodules form on the roots of the model legume, *Medicago truncatula* due to the symbiosis established with the bacterium, *Sinorhizobium meliloti*. Flavonoids play several roles in establishing this symbiosis through a precise molecular dialogue. First, Nod factors (chitolipooligosaccharides) are produced by *S. meliloti* in response to specific flavonoids released by the plant roots into the rhizosphere. This is thought to be due to a precise recognition of particular flavonoids by the bacterial nodD protein (Redmond et al. 1986; Djordjevic et al. 1987; McIver et al. 1989) but more than 20 years on from this discovery, this has not been formally demonstrated (Cooper 2007). The Nod factors produced are required to trigger the nodule organogenesis program in the plant (Denarie et al. 1996).

Recent studies showed that different flavonoid classes also play distinct roles in the plant in establishing root nodules (Wasson et al. 2006; Subramanian et al. 2007; Zhang et al. 2009). These compounds, however, are not required for the formation of other root organs, such as lateral roots or galls formed by root knot nematodes (Wasson et al. 2009) even though flavonoids accumulate during the formation of these organs. Hairy root transformation was used to silence different genes in the *Medicago* flavonoid pathway and to alter flavonoid accumulation patterns (Wasson et al. 2006; Zhang et al. 2009). These plants were then used in conjunction with direct flavonoid complementation and specific *S. meliloti* constructs to dissect the distinct roles of flavonoids in root nodule formation. These elegant *in vivo* studies first confirmed that specific flavones, such as 7,4′-dihydroxyflavone, were required to stimulate the production of Nod factor synthesis in Sinorhizobia in the

rhizosphere and *in planta.* Second, these findings suggested that certain flavonols such as kaempferol play direct roles in orchestrating plant organogenesis. This most likely occurs via the ability for kaempferol to induce localized inhibition of auxin transport, thus establishing more favorable hormonal gradients to enable root nodule formation from differentiated root cells. A third finding was that isoflavones appear to act as anti-inducers *in planta* to presumably modulate flavone-dependent Nod factor synthesis (Zhang et al. 2009).

These *in planta* studies confirm and support conclusions made using *in vitro* studies conducted over many years (Redmond et al. 1986; Djordjevic et al. 1987, 1997; Mathesius et al. 1998a, 1998b). During the formation of determinate root nodules, in plants such as soybean, flavonoid-regulated auxin transport inhibition was not a requirement for nodule organogenesis, but flavonoids were still required to orchestrate the symbiotic interaction between the plant and the microbial symbiont both in the rhizosphere and *in planta* (Subramanian et al. 2007). Collectively, these studies have provided direct evidence for the essential roles that flavonoids play in root nodule formation. It remains to be determined if the induction of flavonoid synthesis in the direct vicinity of root nodule initiation, and the accumulation of specific flavone classes in the cells and nuclei of nodule cells induced early in the ontogeny process play specific roles in nodule ontogeny.

Gravity responses and the auxin connection

Flavonoids also negatively regulate polar auxin transport in plants (Mathesius et al. 1998b; Brown et al. 2001; Buer and Muday 2004). Jacobs and Rubery (1988) showed that flavonoids compete, *in vitro*, for auxin transporters with the auxin efflux inhibitor, 1-napthyphthalmic acid (NPA). These experiments suggest that flavonoids bind to NPA-interacting proteins: either a plasma membrane aminopeptidase or the multi-drug resistant ABCD type transporters, but this remains to be proven. Consistent with flavonoids modulating auxin transport in plants, mutants of *Arabidopsis* devoid of flavonoids (CHS defective *tt4*) show elevated levels of auxin transport and gravitropic and architectural abnormalities (Brown et al. 2001; Buer and Muday 2004; Lewis et al. 2007; Buer and Djordjevic 2009). Some of these abnormalities can be complemented by the external application of naringenin, which is taken up by the *tt4* mutant, converted to downstream flavonoid compounds, and distributed throughout the plant (Buer et al. 2007). These *tt4* mutants have a delayed gravity response, but although devoid of flavonoids, they can still respond to gravity stimulation. Thus, flavonoids are not absolutely required for gravitropic responses in *Arabidopsis*.

One way to further examine the role of flavonoids in the gravity response is to test those *transparent testa* mutants that accumulate more quercetin in the distal elongation zone compared with the wild-type plants (**Figure 3**). As quercetin is a potent inhibitor of auxin transport (Jacobs and Rubery 1988), these mutants would be expected to have different gravity responses and possibly greater inhibition of auxin transport. The influence of other flavonoid intermediates (N, DHK, DHQ, and K) on auxin transport has not been assessed. The availability of mutants that accumulate these intermediates could be used to assess the individual contributions of particular flavonoids to auxin transport levels. Therefore, although N can complement several defects in flavonoid-less plants, the specific flavonoid product(s) and target(s) responsible for restoration of gravitropic and architectural defects remain to be defined.

Recent advances have shed some light on how flavonoids may operate in plants. Flavonoids appear to modulate MDR4, a P-glycoprotein (Lewis et al. 2007), now called ABCB (Verrier et al. 2008), and either interact with PIN2 (Santelia et al. 2008) or affect the distribution of PIN proteins (Peer et al. 2004). Recently, flavonoids were shown to bind to mammalian actin and affect its rate of polymerization (Böhl et al. 2007). Other experiments showed that auxin and actin interact, and this interaction had a subsequent effect on gravitropism in rice (Nick et al. 2009). If flavonoids negatively affect actin function in plants, it is possible that this could explain the mislocalization of PIN proteins mediating auxin transport, and the diverse effects of flavonoids on auxin transport given the role of actin in vesicular trafficking and recycling. The activity of plant peroxidases that destroy auxin is also affected by flavonoids–at least using *in vitro* studies (Mathesius 2001). However, translating *in vitro* observations to *in vivo* function remains enigmatic. Chemical biology approaches, such as photoaffinty tagging and chemical genetics, are needed to identify specific flavonoid interacting partners.

Defining the Molecular Targets for Flavonoids

Animal studies

Flavonoids have diverse bioactivities in plants and animals. Surprisingly, a better understanding of the interacting partners for flavonoids exists in animals. Flavonoids interact with plasma membrane and cytosolic targets, and some similarities exist between plant and mammalian molecular targets. For example, a potential pharmacological outcome for flavonoids is their ability to inhibit the P-glycoprotein MDR1 (Conseil et al. 1998), an integral membrane protein in mammals with similarity to the plant auxin efflux protein, PIN. MDR proteins render tumors resistant to anti-tumor drugs. Other membrane targets for flavonoids include cyclo-oxygenase 2 (COX2), nitric oxide synthase (NOS) (Garcia-Mediavilla et al. 2007; Kim et al. 2008) and the membrane receptor, GLUT4, that mediates insulin-dependent glucose uptake (Strobel et al. 2005).

Plant studies

Flavonoids have broad biological activity, but the molecular mechanisms by which flavonoids interfere with the relevant signal chains and their molecular targets remain elusive. Pollen fertility is flavonoid-dependent in a wide range of species (angiosperms, gymnosperms, and mono- and dicotyledonous plants) (Coe et al. 1981; Mo et al. 1992; Taylor and Grotewold 2005). Flavonoid-less pollen fails to produce a functional pollen tube, and this defect can be complemented rapidly by the external addition of specific flavonols, which restores pollen tube growth (Mo et al. 1992). It is not known if this flavonoid-dependent fertility is mediated through auxin changes in the pollen, or if other novel targets exist for flavonoids, and thus this presents a good experimental system to investigate flavonoid interacting proteins.

Conclusions and Future Perspectives

The diversity, ubiquity, and bioactivity of flavonoids make these compounds of interest to a wide variety of research. It is still unknown exactly how these compounds exert their influence on growth, auxin transport, and other biologically relevant interactions in the plant kingdom. It is obvious that considerably more research is required to dissect out the complexities involved with flavonoid functions in the plant and animal kingdoms. Research on mechanisms of bioactivity in animals, and the use of modern molecular tools in model plant systems should enable mechanisms and interacting molecules to be determined in the near future.

Acknowledgements

The Australian Research Council Centre of Excellence for Integrated Legume Research is thanked for support (CEO348212). The colleagues of the Genomic Interactions Group are thanked for their valuable input, especially Jeremy Weinman for editing advice.

Received 14 Oct. 2009 Accepted 25 Oct. 2009

References

Aoki T, Akashi T, Ayabe S (2000) Flavonoids of leguminous plants: structure, biological activity, and biosynthesis. *J. Plant Res.* **113**, 475–488.

Arioli T, Howles PA, Weinman JJ, Rolfe BG (1994) In *Trifolium subterranium*, chalcone synthase is encoded by a multigene family. *Gene* **138**, 79–86.

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu. Rev. Plant Biol.* **57**, 233–266.

Bennett T, Sieberer T, Willett B, Booker J, Luschnig C, Leyser O (2006) The *Arabidopsis* MAX pathway controls shoot branching by regulating auxin transport. *Curr. Biol.* **16**, 553–563.

Birnbaum K, Shasha DE, Wang JY, Jung JW, Lambert GM, Galbraith DW, Benfey PN (2003) A gene expression map of the *Arabidopsis* root. *Science* **302**, 1956–1960.

Blancaflor EB, Masson PH (2003) Plant gravitropism. Unraveling the ups and downs of a complex process. *Plant Physiol.* **133**, 1677–1690.

Böhl M, Tietze S, Sokoll A, Madathil S, Pfennig F, Apostolakis J, Fahmy K, Gutzeit HO (2007) Flavonoids affect actin functions in cytoplasm and nucleus. *Biophys. J.* **93**, 2767–2780.

Boudet AM (2007) Evolution and current status of research in phenolic compounds. *Phytochemistry* **68**, 2722–2735.

Brady SM, Orlando DA, Lee JY, Wang JY, Koch J, Dinneny JR, Mace D, Ohler U, Benfey PN (2007) A high-resolution root spatiotemporal map reveals dominant expression patterns. *Science* **318**, 801–806.

Brown DE, Rashotte AM, Murphy AS, Normanly J, Tague BW, Peer WA, Taiz L, Muday GK (2001) Flavonoids act as negative regulators of auxin transport *in vivo* in *Arabidopsis*. *Plant Physiol.* **126**, 524–535.

Brown MH, Paulsen IT, Skurray RA (1999) The multidrug efflux protein NorM is a prototype of a new family of transporters. *Mol. Microbiol.* **31**, 394–395.

Buer CS, Djordjevic MA (2009) Architectural phenotypes in the *transparent testa* mutants of *Arabidopsis thaliana*. *J. Exp. Bot.* **60**, 751–763.

Buer CS, Muday GK (2004) The *transparent testa4* mutation prevents flavonoid synthesis and alters auxin transport and the response of *Arabidopsis* roots to gravity and light. *Plant Cell* **16**, 1191–1205.

Buer CS, Muday GK, Djordjevic MA (2007) Flavonoids are differentially taken up and transported long distances in *Arabidopsis*. *Plant Physiol.* **145**, 478–490.

Buer CS, Muday GK, Djordjevic MA (2008) Implications of long-distance flavonoid movement in *Arabidopsis thaliana*. *Plant Signal. Behav.* **3**, 415–417.

Burbulis IE, Iacobucci M, Shirley BW (1996) A null mutation in the first enzyme of flavonoid biosynthesis does not affect male fertility in *Arabidopsis*. *Plant Cell* **8**, 1013–1025.

Coe EH, McCormick SM, Modena SA (1981) White pollen in maize. *J. Hered.* **72**, 318–320.

Conseil G, Baubichon-Cortay H, Dayan G, Jault JM, Barron D, Di Pietro A (1998) Flavonoids: a class of modulators with bifunctional interactions at vicinal ATP- and steroid-binding sites on mouse P-glycoprotein. *Proc. Natl. Acad. Sci. USA* **95**, 9831–9836.

Cooper JE (2007) Early interactions between legumes and rhizobia: disclosing complexity in a molecular dialogue. *J. Appl. Microbiol.* **103**, 1355–1365.

Debeaujon I, Leon-Kloosterziel KM, Koornneef M (2000) Influence of the testa on seed dormancy, germination, and longevity in *Arabidopsis*. *Plant Physiol.* **122**, 403–413.

Debeaujon I, Peeters AJ, Leon-Kloosterziel KM, Koornneef M (2001) The TRANSPARENT TESTA12 gene of *Arabidopsis* encodes a multidrug secondary transporter-like protein required for flavonoid sequestration in vacuoles of the seed coat endothelium. *Plant Cell* **13**, 853–871.

Denarie J, Debelle F, Prome JC (1996) Rhizobium lipo-chitooligosaccharide nodulation factors: signaling molecules mediating recognition and morphogenesis. *Annu. Rev. Biochem.* **65**, 503–535.

Diener AC, Gaxiola RA, Fink GR (2001) *Arabidopsis* ALF5, a multidrug efflux transporter gene family member, confers resistance to toxins. *Plant Cell* **13**, 1625–1638.

Djordjevic MA, Mathesius U, Arioli T, Weinman JJ, Gartner E (1997) Chalcone synthase gene expression in transgenic subterranean clover correlates with localized accumulation of flavonoids. *Aust. J. Plant Physiol.* **24**, 119–132.

Djordjevic MA, Redmond JW, Batley M, Rolfe BG (1987) Clovers secrete specific phenolic-compounds which either stimulate or repress Nod gene-expression in *Rhizobium trifolii*. *Embo J.* **6**, 1173–1179.

Durrett TP, Gassmann W, Rogers EE (2007) The FRD3-mediated efflux of citrate into the root vasculature is necessary for efficient iron translocation. *Plant Physiol.* **144**, 197–205.

Garcia-Mediavilla V, Crespo I, Collado PS, Esteller A, Sanchez-Campos S, Tunon MJ, Gonzalez-Gallego J (2007) The anti-inflammatory flavones quercetin and kaempferol cause inhibition of inducible nitric oxide synthase, cyclooxygenase-2 and reactive C-protein, and down-regulation of the nuclear factor kappaB pathway in Chang Liver cells. *Eur. J. Pharmacol.* **557**, 221–229.

Geisler M, Blakeslee JJ, Bouchard R, Lee OR, Vincenzetti V, Bandyopadhyay A, Titapiwatanakun B, Peer WA, Bailly A, Richards EL, Ejenda KFK, Smith AP, Baroux C, Grossniklaus U, Muller A, Hrycyna CA, Dudler R, Murphy AS, Martinoia E (2005) Cellular efflux of auxin catalyzed by the *Arabidopsis* MDR/PGP transporter AtPGP1. *Plant J.* **44**, 179–194.

Gomez-Roldan V, Fermas S, Brewer PB, Puech-Pages V, Dun EA, Pillot JP, Letisse F, Matusova R, Danoun S, Portais JC, Bouwmeester H, Becard G, Beveridge CA, Rameau C, Rochange SF (2008) Strigolactone inhibition of shoot branching. *Nature* **455**, 189–194.

Grennan AK (2006) Genevestigator. Facilitating web-based gene-expression analysis. *Plant Physiol.* **141**, 1164–1166.

Imin N, Nizamidin M, Wu T, Rolfe BG (2007) Factors involved in root formation in *Medicago truncatula*. *J. Exp. Bot.* **58**, 439–451.

Ishida T, Hattori S, Sano R, Inoue K, Shirano Y, Hayashi H, Shibata D, Sato S, Kato T, Tabata S, Okada K, Wada T (2007) *Arabidopsis* TRANSPARENT TESTA GLABRA2 is directly regulated by R2R3 MYB transcription factors and is involved in regulation of GLABRA2 transcription in epidermal differentiation. *Plant Cell* **19**, 2531–2543.

Jacobs M, Rubery PH (1988) Naturally-occurring auxin transport regulators. *Science* **241**, 346–349.

Kim JY, Park SJ, Yun KJ, Cho YW, Park HJ, Lee KT (2008) Isoliquiritigenin isolated from the roots of *Glycyrrhiza uralensis* inhibits LPS-induced NOS and COX-2 expression via the attenuation of NF-kappa B in RAW 264.7 macrophages. *Eur. J. Pharmacol.* **584**, 175–184.

Koornneef M (1990) Mutations affecting the testa color in *Arabidopsis*. *Arabidopsis Information Services* **27**, 1–4.

Lawson CGR, Djordjevic MA, Weinman JJ, Rolfe BG (1994) Rhizobium inoculation and physical wounding result in the rapid induction of the same chalcone synthase copy in *Trifolum subterraneum*. *Mol. Plant-Microbe Interact.* **7**, 498–507.

Lewis DR, Miller ND, Splitt BL, Wu GS, Spalding EP (2007) Separating the roles of acropetal and basipetal auxin transport on gravitropism with mutations in two *Arabidopsis* Multidrug Resistance-Like ABC transporter genes. *Plant Cell* **19**, 1838–1850.

Lewis DR, Wu GS, Ljung K, Spalding EP (2009) Auxin transport into cotyledons and cotyledon growth depend similarly on the ABCB19 Multidrug Resistance-like transporter. *Plant J.* **60**, 91–101.

Li JY, Oulee TM, Raba R, Amundson RG, Last RL (1993) *Arabidopsis* flavonoid mutants are hypersensitive to UV-B irradiation. *Plant Cell* **5**, 171–179.

Li L, He Z, Pandey GK, Tsuchiya T, Luan S (2002) Functional cloning and characterization of a plant efflux carrier for multidrug and heavy metal detoxification. *J. Biol. Chem.* **277**, 5360–5368.

Mathesius U (2001) Flavonoids induced in cells undergoing nodule organogenesis in white clover are regulators of auxin breakdown by peroxidase. *J. Exp. Bot.* **52**, 419–426.

Mathesius U, Bayliss C, Weinman JJ, Schlaman HRM, Spaink HP, Rolfe BG, McCully ME, Djordjevic MA (1998a) Flavonoids synthesized in cortical cells during nodule initiation are early developmental markers in white clover. *Mol. Plant-Microbe Interact.* **11**, 1223–1232.

Mathesius U, Keijzers G, Natera SHA, Weinman JJ, Djordjevic MA, Rolfe BG (2001) Establishment of a root proteome reference map for the model legume *Medicago truncatula* using the expressed sequence tag database for peptide mass fingerprinting. *Proteomics* **1**, 1424–1440.

Mathesius U, Schlaman HRM, Spaink HP, Sautter C, Rolfe BG, Djordjevic MA (1998b) Auxin transport inhibition precedes root nodule formation in white clover roots and is regulated by flavonoids and derivatives of chitin oligosaccharides. *Plant J.* **14**, 23–34.

Mathesius U, Weinman JJ, Rolfe BG, Djordjevic MA (2000) Rhizobia can induce nodules in white clover by “hijacking” mature cortical cells activated during lateral root development. *Mol. Plant-Microbe Interact.* **13**, 170–182.

McIver J, Djordjevic MA, Weinman JJ, Rolfe BG (1989) Extension of host range of *Rhizobium leguminosarum* bv. *trifolii* caused by point mutations in *nodD* that result in alterations in regulatory function and recognition of inducer molecules. *Mol. Plant-Microbe Interact.* **2**, 97–106.

McKhann HI, Hirsch AM (1994) Isolation of chalcone synthase and chalcone isomerase cDNAs from alfalfa (*Medicago sativa* L.) – highest transcript levels occur in young roots and root tips. *Plant Mol. Biol.* **24**, 767–777.

Mo YY, Nagel C, Taylor LP (1992) Biochemical complementation of chalcone synthase mutants defines a role for flavonols in functional pollen. *Proc. Natl. Acad. Sci. USA* **89**, 7213–7217.

Mol J, Grotewold E, Koes R (1998) How genes paint flowers and seeds. *Trends Plant Sci.* **3**, 212–217.

Morris AC, Djordjevic MA (2006) The *Rhizobium leguminosarum* biovar *trifolii* ANU794 induces novel developmental responses on the subterranean clover cultivar Woogenellup. *Mol. Plant-Microbe Interact.* **19**, 471–479.

Nawrath C, Heck S, Parinthawong N, Metraux JP (2002) EDS5, an essential component of salicylic acid-dependent signaling for disease resistance in *Arabidopsis*, is a member of the MATE transporter family. *Plant Cell* **14**, 275–286.

Nesi N, Debeaujon I, Jond C, Pelletier G, Caboche M, Lepiniec L (2000) The TT8 gene encodes a basic helix-loop-helix domain protein required for expression of DFR and BAN genes in *Arabidopsis* siliques. *Plant Cell* **12**, 1863–1878.

Nesi N, Jond C, Debeaujon I, Caboche M, Lepiniec L (2001) The *Arabidopsis* TT2 gene encodes an R2R3 MYB domain protein that acts as a key determinant for proanthocyanidin accumulation in developing seed. *Plant Cell* **13**, 2099–2114.

Nick P, Han MJ, An G (2009) Auxin stimulates its own transport by shaping actin filaments. *Plant Physiol.* **151**, 155–167.

Owens DK, Alerding AB, Crosby KC, Bandara AB, Westwood JH, Winkel BSJ (2008a) Functional analysis of a predicted flavonol synthase gene family in *Arabidopsis*. *Plant Physiol.* **147**, 1046–1061.

Owens DK, Crosby KC, Runac J, Howard BA, Winkel BSJ (2008b) Biochemical and genetic characterization of *Arabidopsis* flavanone 3 beta-hydroxylase. *Plant Physiol. Biochem.* **46**, 833–843.

Pandey MK, Sandur SK, Sung B, Sethi G, Kunnumakkara AB, Aggarwal BB (2007) Butein, a tetrahydroxychalcone, inhibits nuclear factor (NF)-kappa B and NF-kappa B-regulated gene expression through direct inhibition of I kappa B alpha kinase beta on cysteine 179 residue. *J. Biol. Chem.* **282**, 17340–17350.

Peer WA, Bandyopadhyay A, Blakeslee JJ, Makam SI, Chen RJ, Masson PH, Murphy AS (2004) Variation in expression and protein localization of the PIN family of auxin efflux facilitator proteins in flavonoid mutants with altered auxin transport in *Arabidopsis thaliana*. *Plant Cell* **16**, 1898–1911.

Peer WA, Murphy AS (2007) Flavonoids and auxin transport: modulators or regulators? *Trends Plant Sci.* **12**, 556–563.

Pelletier MK, Shirley BW (1996) Analysis of flavanone 3-hydroxylase in *Arabidopsis* seedlings–Coordinate regulation with chalcone synthase and chalcone isomerase. *Plant Physiol.* **111**, 339–345.

Polya GM (2003) *Biochemical Targets of Plant Bioactive Compounds: A Pharmacological Reference Guide to Sites of Action and Biological Effects*. Taylor & Francis, London. New York.

Pourcel L, Routaboul JM, Kerhoas L, Caboche M, Lepiniec L, Debeaujon I (2005) TRANSPARENT TESTA10 encodes a laccase-like enzyme involved in oxidative polymerization of flavonoids in *Arabidopsis* seed coat. *Plant Cell* **17**, 2966–2980.

Redmond JW, Batley M, Djordjevic MA, Innes RW, Kuempel PL, Rolfe BG (1986) Flavones induce expression of nodulation genes in *Rhizobium*. *Nature* **323**, 632–635.

Sagasser M, Lu GH, Hahlbrock K, Weisshaar B (2002) *A. thaliana* TRANSPARENT TESTA 1 is involved in seed coat development and defines the WIP subfamily of plant zinc finger proteins. *Genes Dev.* **16**, 138–149.

Santelia D, Henrichs S, Vincenzetti V, Sauer M, Bigler L, Klein M, Bailly A, Lee Y, Friml J, Geisler M, Martinoia E (2008) Flavonoids redirect PIN-mediated polar auxin fluxes during root gravitropic responses. *J. Biol. Chem.* **283**, 31218–31226.

Schoenbohm C, Martens S, Eder C, Forkmann G, Weisshaar B (2000) Identification of the *Arabidopsis thaliana* flavonoid 3′-hydroxylase gene and functional expression of the encoded P450 enzyme. *Biol. Chem.* **381**, 749–753.

Schwacke R, Schneider A, Van Der Graaff E, Fischer K, Catoni E, Desimone M, Frommer WB, Flugge UI, Kunze R (2003) ARAMEMNON, a novel database for *Arabidopsis* integral membrane proteins. *Plant Physiol.* **131**, 16–26.

Sheahan JJ (1996) Sinapate esters provide greater UV-B attenuation than flavonoids in *Arabidopsis thaliana* (Brassicaceae). *Am. J. Bot.* **83**, 679–686.

Shirley BW, Kubasek WL, Storz G, Bruggemann E, Koornneef M, Ausubel FM, Goodman HM (1995) Analysis of *Arabidopsis* mutants deficient in flavonoid biosynthesis. *Plant J.* **8**, 659–671.

Sibout R, Sukumar P, Hettiarachchi C, Holm M, Muday GK, Hardtke CS (2006) Opposite root growth phenotypes of *hy5* versus *hy5 hyh* mutants correlate with increased constitutive auxin signaling. *PLoS Genet.* **2**, 1898–1911.

Singh RP, Gu M, Agarwal R (2008) Silibinin inhibits colorectal cancer growth by inhibiting tumor cell proliferation and angiogenesis. *Cancer Res.* **68**, 2043–2050.

Smith AP, Nourizadeh SD, Peer WA, Xu JH, Bandyopadhyay A, Murphy AS, Goldsbrough PB (2003) *Arabidopsis* AtGSTF2 is regulated by ethylene and auxin, and encodes a glutathione S-transferase that interacts with flavonoids. *Plant J.* **36**, 433–442.

Strobel P, Allard C, Perez-Acle T, Calderon R, Aldunate R, Leighton F (2005) Myricetin, quercetin and catechin-gallate inhibit glucose uptake in isolated rat adipocytes. *Biochem. J.* **386**, 471–478.

Subramanian S, Stacey G, Yu O (2007) Distinct, crucial roles of flavonoids during legume nodulation. *Trends Plant Sci.* **12**, 282–285.

Sung B, Pandey MK, Aggarwal BB (2007) Fisetin, an inhibitor of cyclin-dependent kinase 6, down-regulates nuclear factor-kappa B-regulated cell proliferation, antiapoptotic and metastatic gene products through the suppression of TAK-1 and receptor-interacting protein-regulated I kappa B alpha kinase activation. *Mol. Pharmacol.* **71**, 1703–1714.

Taylor LP, Grotewold E (2005) Flavonoids as developmental regulators. *Curr. Opin. Plant Biol.* **8**, 317–323.

Taylor LP, Jorgensen R (1992) Conditional male-fertility in chalcone synthase-deficient petunia. *J. Hered.* **83**, 11–17.

Terasaka K, Blakeslee JJ, Titapiwatanakun B, Peer WA, Bandyopadhyay A, Makam SN, Lee OR, Richards EL, Murphy AS, Sato F, Yazaki K (2005) PGP4, an ATP binding cassette P-glycoprotein, catalyzes auxin transport in *Arabidopsis thaliana* roots. *Plant Cell* **17**, 2922–2939.

Treutter D (2005) Significance of flavonoids in plant resistance and enhancement of their biosynthesis. *Plant Biol.* **7**, 581–591.

Turnbull CGN, Booker JP, Leyser HMO (2002) Micrografting techniques for testing long-distance signalling in *Arabidopsis*. *Plant J.* **32**, 255–262.

Verrier PJ, Bird D, Buria B, Dassa E, Forestier C, Geisler M, Klein M, Kolukisaoglu U, Lee Y, Martinoia E, Murphy A, Rea PA, Samuels L, Schulz B, Spalding EJ, Yazaki K, Theodoulou FL (2008) Plant ABC proteins – a unified nomenclature and updated inventory. *Trends Plant Sci.* **13**, 151–159.

Walker AR, Davison PA, Bolognesi-Winfield AC, James CM, Srinivasan N, Blundell TL, Esch JJ, **Marks MD**, **Gray JC** (1999) The TRANSPARENT TESTA GLABRA1 locus, which regulates trichome differentiation and anthocyanin biosynthesis in *Arabidopsis*, encodes a WD40 repeat protein. *Plant Cell* **11**, 1337–1349.

Wasson AP, Pellerone FI, Mathesius U (2006) Silencing the flavonoid pathway in *Medicago truncatula* inhibits root nodule formation and prevents auxin transport regulation by rhizobia. *Plant Cell* **18**, 1617–1629.

Wasson AP, Ramsay K, Jones MGK, Mathesius U (2009) Differing requirements for flavonoids during the formation of lateral roots, nodules and root knot nematode galls in *Medicago truncatula*. *New Phytol.* **183**, 167–179.

Williams CA, Grayer RJ (2004) Anthocyanins and other flavonoids. *Nat. Prod. Rep.* **21**, 539–573.

Williams RJ, Spencer JPE, Rice-Evans C (2004) Flavonoids: antioxidants or signalling molecules? *Free Radic. Biol. Med.* **36**, 838–849.

Winkel-Shirley B (2001) It takes a garden. How work on diverse plant species has contributed to an understanding of flavonoid metabolism. *Plant Physiol.* **127**, 1399–1404.

Winter D, Vinegar B, Nahal H, Ammar R, Wilson GV, Provart NJ (2007) An "electronic fluorescent pictograph" browser for exploring and analyzing large-scale biological data sets. *PLoS One* **2**, e718.

Zhang J, Subramanian S, Stacey G, Yu O (2009) Flavones and flavonols play distinct critical roles during nodulation of *Medicago truncatula* by *Sinorhizobium meliloti*. *Plant J.* **57**, 171–183.

Zhang SH, Morris ME (2003) Effects of the flavonoids biochanin A, morin, phloretin, and silymarin on P-glycoprotein-mediated transport. *J. Pharmacol. Exp. Ther.* **304**, 1258–1267.

(Co-Editor: William J. Lucas)