

Mechanisms of resistance to self-produced toxic secondary metabolites in plants

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Abstract Plants produce a variety of secondary metabolites to protect themselves from pathogens and herbivores and/or to influence the growth of neighbouring plants. Some of these metabolites are toxic to the producing cells when their target sites are present in the producing organisms. Therefore, a specific self-resistance mechanism must exist in these plants. Self-resistance mechanisms, including extracellular excretion, vacuolar sequestration, vesicle transport, extracellular biosynthesis, and accumulation of the metabolite in a non-toxic form, have been proposed thus far. Recently, a new mechanism involving mutation of the target protein of the toxic metabolite has been elucidated. We review here the mechanisms that plants use to prevent self-toxicity from the following representative compounds: cannabinoids, flavonoids, diterpene sclareol, alkaloids, benzoxazinones, phenylpropanoids, cyanogenic glycosides, and glucosinolates.

Keywords Secondary metabolite · Toxicity · Self-resistance · Detoxification

Abbreviations

ABC	ATP-binding cassette
BOA	Benzoxazolin-2(3H)-one
CBCA	Cannabichromenic acid
DIBOA	2,4-Dihydroxy-2 <i>H</i> -1,4-benzoxazin-3(4 <i>H</i>)-one
DIMBOA	2,4-Dihydroxy-7-methoxy-2 <i>H</i> -1,4-benzoxazin-3(4 <i>H</i>)-one
GSH	Glutathione
GST	Glutathione S-transferase
PDR	Pleiotropic drug resistance
THC	Tetrahydrocannabinol
THCA	Tetrahydrocannabinolic acid

Introduction

Plants produce secondary metabolites for interaction with their environment. At present, more than 200,000 secondary metabolites have been identified (Hartmann et al. 2005), providing us with an enormous source of novel pharmaceutically active agents. A large number of studies have demonstrated the importance of these metabolites as plant defence compounds. These metabolites exert toxic effects on other organisms by interfering with cell protein function. A few metabolites interact with the molecules responsible

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for fundamental cellular functions, such as DNA and the proteins involved in cell division. Many of these metabolites have been used as anticancer compounds such as camptothecin, paclitaxel, vincristine, and podophyllotoxin. To avoid self-toxicity, these plants have evolved a detoxification mechanism. It is known that plants employ different mechanisms to eliminate or modify toxic compounds.

- Excretion of toxic compounds into extracellular compartments
- Sequestration of toxic compounds into vacuoles
- Biosynthesis of toxic compounds in extracellular compartments
- Modification of toxic compounds into inactive forms

In this article, we review several mechanisms that plants use to avoid self-toxicity from several important secondary compounds (Fig. 1), including cannabinoids, flavonoids, diterpene sclareol, alkaloids, benzoxazinones, phenylpropanoids, cyanogenic glycosides, and glucosinolates, in order to exploit these compounds as defence compounds.

Accumulation of cannabinoids in glandular trichomes on leaves of *Cannabis sativa*

Cannabinoids, which contain the alkylresorcinol and monoterpene groups, are unique secondary metabolites found only in *C. sativa*. Among them, tetrahydrocannabinol (THC) (Fig. 2), a well-known cannabinoid, has already been developed as a therapeutic drug (for review, see Mendizabal and Adler-Graschinsky 2007). Cannabinoids have been reported to be accumulated only in glandular trichomes (Fairbairn 1972; Sirikantaramas et al. 2005). Cannabinoid acids such as tetrahydrocannabinolic acid (THCA) (Fig. 2), the precursor of THC, are major original cannabinoids in fresh *C. sativa* (Yamauchi et al. 1967). Several cannabinoid acids (Fig. 2), including THCA, cannabigerolic acid, and cannabichromenic acid (CBCA), have the ability to induce cell death of their own cells and of the cells of other plants, such as tobacco BY-2 cells, suggesting their role as plant defence compounds (Sirikantaramas et al. 2005; Morimoto et al. 2007). Recently, Morimoto et al. (2007) have shown that CBCA and THCA can cause serious damage to mitochondria

Fig. 1 Major mechanisms of resistance to self-produced toxic secondary metabolites. The numbers indicate 7 reported mechanisms. (1)

Extracellular biosynthesis; (2) vacuolar sequestration; (3) excretion; (4) vesicular transport; (5) enzymatic detoxification; (6) target mutation; and (7) accumulation of nontoxic form. Red arrows represent enzymatic reaction. Yellow arrows represent transport across membrane

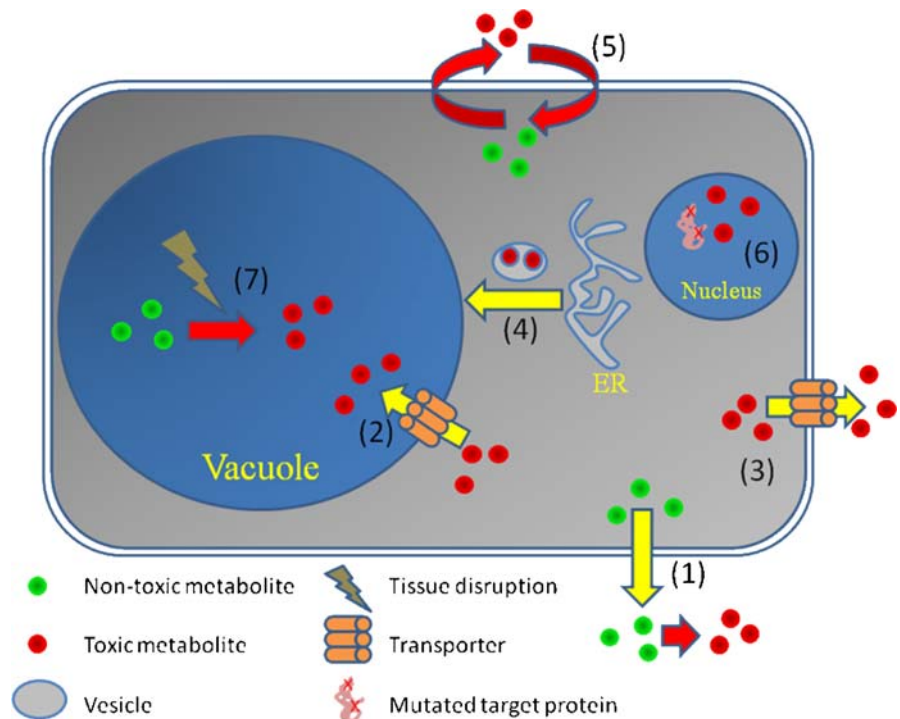
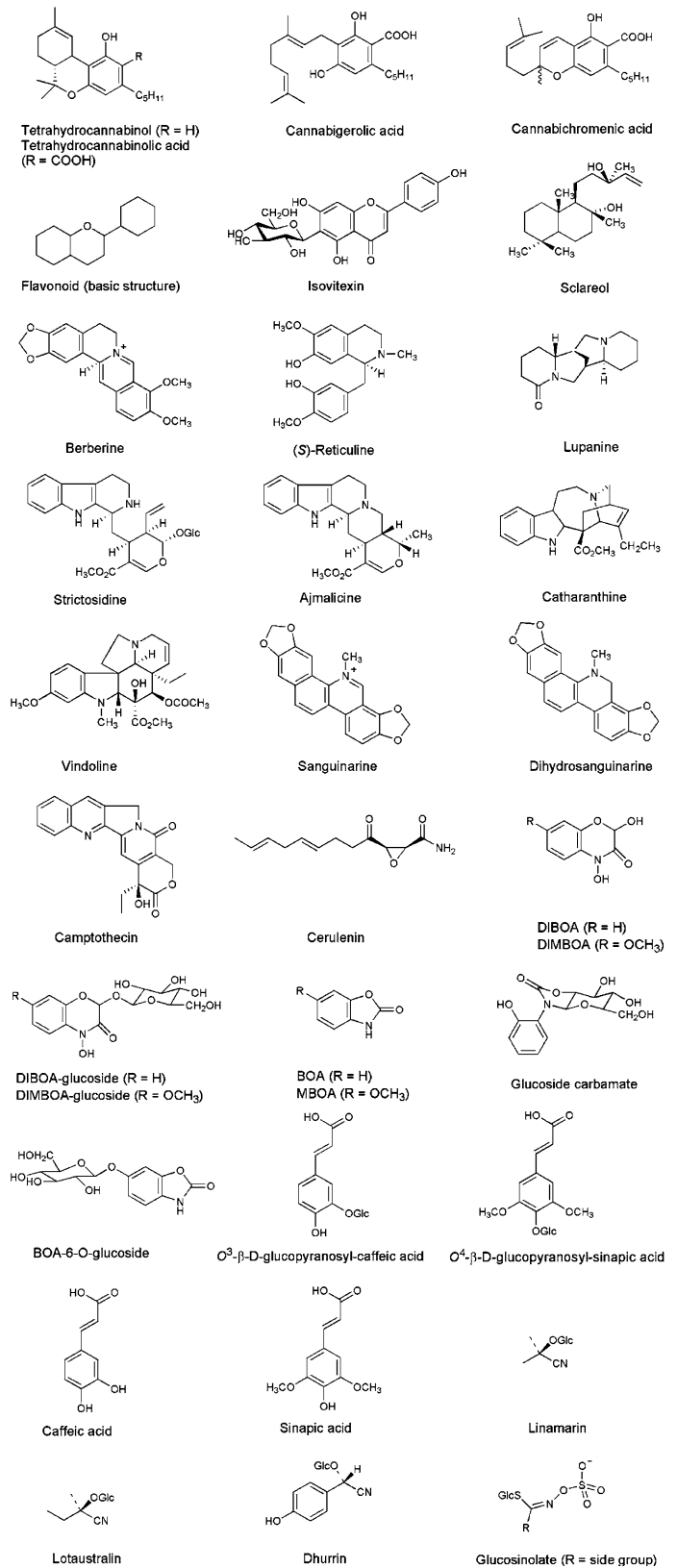


Fig. 2 Structure of the compounds mentioned in this review



through the mechanism of mitochondrial permeability transition. Because of the high toxicity of these cannabinoids, their biosyntheses must be well-regulated. Studies on the biosynthetic pathway have identified 2 secreted cannabinoid biosynthetic enzymes-THCA synthase and cannabidiolic acid synthase (Sirikantaramas et al. 2005; Taura et al. 2007; for review, see Sirikantaramas et al. 2007a). THCA synthase, which is responsible for the production of THCA, is specifically expressed in glandular trichomes (Sirikantaramas et al. 2005). An immunolocalization study of THC using the anti-THC monoclonal antibody indicated the presence of THC in the secretory cavity of glandular trichomes, in the cell wall of secretory cells facing the storage cavity, and on the surface of secretory vesicles in the storage cavity but not in the cytoplasm of secretory cells (Kim and Mahlberg 1997). These findings suggest that *C. sativa* produces the toxic THCA extracellularly rather than intracellularly in order to avoid THCA toxicity (Fig. 1(1)).

Involvement of transporters in flavonoid sequestration within vacuoles

Flavonoids (Fig. 2) are a major class of plant secondary metabolites that include flavonols, anthocyanins, proanthocyanidins, and isoflavonoids. The roles of these compounds in plants include flower pigmentation, UV protection, intracellular and extracellular signalling, male fertility, and pathogen defence (Dixon and Steele 1999; Harborne and Williams 2000). In addition, flavonoids also exhibit a vast array of medicinal properties, including antioxidation, anti-inflammatory, and antitumour activities (Harborne and Williams 2000). It has been reported that the compartmentalization of anthocyanin in vacuoles is required to limit both the mutagenic and oxidative effects of synthesis pathway intermediates (Ahmed et al. 1994; Rueff et al. 1995). Therefore, these toxic flavonoids must be excluded from the cytoplasm to prevent cellular damage. A number of studies have shown the involvement of a transporter in the vacuolar accumulation of flavonoids (for review, see Klein et al. 2006; Rea 2007) (Fig. 1(2)). Evidence from maize mutants indicates that anthocyanins are transported into vacuoles through a vacuolar ATP-binding cassette (ABC)

transporter (ZmMRP3) (Goodman et al. 2004). In *Arabidopsis*, a multidrug and toxic compound extrusion (MATE) transporter, namely, TT12, which is involved in the vacuolar deposition of flavonoids in the proanthocyanidin-synthesizing cells of the seed coat has also been identified (Debeaujon et al. 2001). Recently, a flavonoid/H⁺-antiporter function has been suggested for the TT12 protein (Marinova et al. 2007a).

In addition to the role of transporters in vacuolar sequestration, glutathione *S*-transferases (GSTs) have been known for their ability to detoxify various compounds, including toxic secondary metabolites and xenobiotic compounds. These enzymes conjugate the glutathione tripeptide (GSH) to a broad variety of substrates. As a result, the GSH-conjugated compound is transported to the vacuole via a tonoplast-located transporter. Three GSTs, namely, maize Bz2 (Marrs et al. 1995), petunia AN9 (Alfenito et al. 1998), and *Arabidopsis* TT19 (Kitamura et al. 2004), have been identified and characterized for their involvement in anthocyanin accumulation in vacuoles. Mutations in the corresponding genes caused pigment accumulation in the cytoplasm with phytotoxic effects (Marrs et al. 1995; Alfenito et al. 1998). However, Mueller et al. (2000) later showed that AN9 is a flavonoid-binding protein and is not involved in the covalent conjugation of GSH to anthocyanins. They also suggested that AN9 might serve as a cytoplasmic flavonoid carrier protein, and the formation of the GSH conjugate might not be required for transport into the vacuole (Mueller et al. 2000).

Other mechanisms for flavonoid sequestration to vacuoles have been proposed. Isovitexin (Fig. 2), a flavonoid glucoside in barley, was readily imported into isolated barley vacuoles by a Δ pH-dependent uptake mechanism (Klein et al. 1996). Marinova et al. (2007b) demonstrated that intact flavonoid biosynthesis in barley controls the activity of vacuolar flavonoid/H⁺-antiporters suggesting an association between flavonoid biosynthesis and the transport mechanism. The vacuolar uptake of flavone glucuronides non-conjugated to GSH was mediated by a multidrug resistance-associated protein (MRP)-like ABC transporter in rye mesophyll vacuoles (Klein et al. 2000). The acylation of anthocyanin was also shown to be important for the vacuolar uptake mechanism in carrot. It is interesting that vacuoles

prepared from other plant species did not import acylated anthocyanin (Hopp and Seitz 1987). These results suggest the possibility that different plants employ different mechanisms for flavonoid sequestration.

Involvement of an ABC transporter in diterpene sclareol secretion

Sclareol (Fig. 2) is a labdane-type diterpene that is found on the leaf surface of *Nicotiana* spp. It exhibits antifungal properties and also affects plant growth (Bailey et al. 1975; Cutler et al. 1977). *Nicotiana plumbaginifolia* NpPDR1—a plasma membrane pleiotropic drug resistance-type ABC transporter—has been identified to be involved in extracellular sclareol secretion (Jasinski et al. 2001; Stukkens et al. 2005) (Fig. 1(3)). The induction of NpPDR1 expression by sclareol has been demonstrated in *N. plumbaginifolia* cell culture (Jasinski et al. 2001). This suggests the presence of a regulation system that activates the expression of the transporter. This transporter pumps the toxic metabolite sclareol out of the cells before it reaches a concentration that is toxic to the cells. It has been shown that the expression of this transporter in *Arabidopsis* leads to the acquisition of resistance to sclareol (van den Brule et al. 2002). Sclareol biosynthesis has been reported to essentially occur in the trichome (Guo and Wagner 1995) where NpPDR1 is constitutively expressed (Stukkens et al. 2005). In addition, sclareol was also shown to be a possible endogenous substrate of SpTUR2, an ABC transporter in the aquaphyte *Spirodela polyrrhiza* (van den Brule et al. 2002).

Alkaloid detoxification mechanisms by sequestration

Alkaloids are generally accumulated in specific cell types because of their toxicity and their probable role in plant defence responses (Facchini and St-Pierre 2005). Several alkaloids, including berberine, (*S*)-reticuline, and lupanine, have been reported to be accumulated in vacuoles (Deus-Neumann and Zenk 1986; Mende and Wink 1987; Sato et al. 1992) (Fig. 2). Vesicular transport and vacuolar accumulation are well-known strategies for the control of

toxic alkaloids (Deus-Neumann and Zenk 1984a, b; Martinoia et al. 2000; Wink and Roberts 1998) (Fig. 1(2) and (4)).

Berberine (Fig. 2), a benzyloquinoline alkaloid, is conventionally used as an antidiarrhetic, a bitter stomachic, and an antimalarial drug (Yamamoto et al. 1993). It also shows strong cytotoxicity to plant cells that do not produce it, suggesting a species-specific detoxification mechanism (Sakai et al. 2002). Its vacuolar transport in *Coptis japonica* via an H⁺/berberine antiporter, which uses the proton gradient formed by 2 vacuolar proton pumps, namely, vacuolar H⁺-ATPase and pyrophosphatase (PPase), has been recently reported (Otani et al. 2005). In addition to the H⁺-antiporter, ABC transporters have been suggested to be involved in the vacuolar transport of several terpenoid indole alkaloids in *Catharanthus roseus*, including strictosidine, ajmalicine, catharanthine, and vindoline (Roytrakul and Verpoorte 2007). These findings suggest the involvement of various transporters in alkaloid detoxification by vacuolar accumulation.

Sanguinarine (Fig. 2), a toxic benzophenanthridine alkaloid, exhibits cytotoxic activity by interfering with the functions of DNA, tubulin, and enzymes such as Na⁺/K⁺ ATPase (Bajaj et al. 1990; Kakiuchi et al. 1987; Scheiner-Bobis 2001; Schmeller et al. 1997). At high concentrations, it strongly inhibits the growth of cultured cells of *Nicotiana* and *Arabidopsis* (Weiss et al. 2006). This compound is either accumulated in the vacuole or excreted into the cell wall and the culture medium. It is also potentially dangerous to the producing cell. Its production can be induced by both biotic and abiotic elicitors (Gundlach et al. 1992; Roos et al. 1998; Schumacher et al. 1987; Villegas et al. 2000). The transport of vacuolar-accumulated sanguinarine has been reported to be associated with the endoplasmic reticulum (ER) via a vesicle (Alcantara et al. 2005). Weiss et al. (2006) reported a unique recycling mechanism for the detoxification of excreted sanguinarine. When added to the cell suspension culture of *Eschscholzia californica*, sanguinarine rapidly disappeared from the culture medium. Concomitantly, the cellular content of dihydrosanguinarine (Fig. 2), a biosynthetic precursor of sanguinarine, increased. Sanguinarine reductase, which catalyses the reduction of sanguinarine to dihydrosanguinarine, has been identified. It was concluded that excreted

sanguinarine from the producing cell is rapidly reabsorbed and reduced to the less toxic dihydrosanguinarine that then undergoes further biosynthetic reactions (Fig. 1(5)). This mechanism would allow the producing cells to benefit from this compound without harming themselves.

Self-resistance due to the presence of the toxic metabolite-resistant target protein in camptothecin-producing plants

Camptothecin (Fig. 2), a terpenoid indole alkaloid, is found in many distantly related plants, including *Camptotheca acuminata*, *Nothapodytes foetida*, and *Ophiorrhiza pumila* (Aimi et al. 1989; Govindachari and Viswanathan 1972; Wall et al. 1966). Its semi-synthetic derivatives are currently prescribed as anticancer drugs (for review, see Sirikantaramas et al. 2007b). It has been shown that camptothecin exhibits anticancer property by inhibiting DNA topoisomerase I, a house-keeping enzyme involved in the regulation of DNA topology (Hsiang et al. 1985). Therefore, a mechanism for the well-regulated production or detoxification of this compound must exist in the producing plants. Camptothecin has been reported to be accumulated in the glandular trichomes of leaves and stems of *C. acuminata* (Li et al. 2002). Subcellular localization studies showed that camptothecin is accumulated in the vacuole, which is considered to be a site for avoiding toxicity (Pasqua et al. 2004; Sirikantaramas et al. 2007c). Interestingly, in the hairy roots of *O. pumila* (Saito et al. 2001), camptothecin is partially accumulated in the vacuole and partially excreted to the culture medium (Sirikantaramas et al. 2007c). Vesicular transport from the ER was suggested to be involved in vacuolar camptothecin accumulation (Sirikantaramas et al. 2007c). In an attempt to understand the camptothecin excretion process, Sirikantaramas et al. (2007c) found that camptothecin is excreted by passive transport depending on the concentration gradient between the intracellular and extracellular compartments. Therefore, a cytosolic camptothecin might be expected to interfere with the function of DNA topoisomerase I. A hypothesis suggests that a mutation in topoisomerase I might confer camptothecin resistance without the involvement of a transporter or vesicular transport in camptothecin-

producing plants; this has been proposed as a self-resistance mechanism in camptothecin-producing plants (Sirikantaramas et al. 2007c). This hypothesis is supported by evidence that mutations in topoisomerase I have been observed in camptothecin-resistant human cancer cells (Takatani et al. 1997; Pommier et al. 1998; Tsurutani et al. 2002). However, target mutations as a self-resistance mechanism have not been reported in plants producing toxic compounds. Recently, we have been able to confirm this hypothesis. Point mutations in topoisomerase I that confer camptothecin resistance in camptothecin-producing plants have been identified (Sirikantaramas, et al. manuscript in preparation). These results suggest a novel self-resistance mechanism through the generation of target mutations in plants (Fig. 1(6)).

Although the mutation of drug targets has contributed to drug resistance in organisms that do not produce drugs, self-resistance due to the presence of a drug-insensitive target site has been rarely observed in producing organisms. Only a few studies have reported this type of self-resistance in micro-organisms (Kawaguchi et al. 1979; Hughes et al. 1980; Glöckner and Wolf 1984; Thiara and Cundliffe 1988). For example, cerulenin (Fig. 2), an antifungal antibiotic produced by the fungus *Cephalosporium caerulens*, is a potent inhibitor of fatty acid synthase in various organisms. The producing strain possesses cerulenin-resistant fatty acid synthase (Kawaguchi et al. 1979). The homology of the condensing domains between the cerulenin-resistant type and cerulenin-sensitive type of fatty acid synthase is very low suggesting that the enzyme from *C. caerulens* might be very different (Tomoda et al. 1984; Inokoshi et al. 1994).

Modification of self-produced toxic metabolites into non-toxic forms via glycosylation

Benzoxazinoids such as 2,4-dihydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIBOA) and 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIMBOA) (Fig. 2) are secondary metabolites found mainly in Gramineae, including rye, wheat, and maize. They are stored in the vacuole as glucosides (Osborn 1996) and are considered as phytotoxins. However,

the mechanism underlying the phytotoxicity of benzoxazinoids is still unknown. It has been reported that glycosylation reduces the toxicity of benzoxazinoids as glucosides have reduced chemical reactivity (Sicker et al. 2000). Two glucosyltransferases involved in the last step of the biosynthetic pathway leading to final glucoside products, namely, DIBOA-glucoside and DIMBOA-glucoside (Fig. 2), have been identified (von Rad et al. 2001). Upon tissue disruption, the specific glucosidase accumulated in the chloroplast (Cicek and Esen 1998) is brought into contact with benzoxazinoids resulting in toxic aglycones (Fig. 1(7)). As aglycones, DIBOA and DIMBOA become unstable and thus are degraded to benzoxazolin-2(3*H*)-one (BOA) and 6-methoxybenzoxazolin-2(3*H*)-one (MBOA), respectively (Fig. 2). These compounds can also act as allelopathic compounds (Barnes et al. 1986; Sicker et al. 2000). In benzoxazinoid-producing plants, it has been shown that BOA is later detoxified by modification to nontoxic forms, mainly glucoside carbamate (Fig. 2) via *N*-glucosylation and BOA-6-*O*-glucoside (Fig. 2) via *O*-glucosylation as a minor form (Schulz and Wieland 1999; Sicker et al. 2001).

It has been suggested that reactive phenylpropanoids in the lignin biosynthetic pathway are detoxified and stored as glucoside forms (Vickery 1981) (Fig. 1(7)). Feeding experiments showed that *O*³- β -D-glucopyranosyl-caffeic acid and *O*⁴- β -D-glucopyranosyl-sinapic acid are the storage or detoxification products of caffeic acid and sinapic acid, respectively (Meyermans et al. 2000) (Fig. 2).

Cyanogenic glucosides are found in many plants, including linamarin and lotaustralin in cassava and dhurrin in sorghum (Huges 1999) (Fig. 2). It is widely accepted that plants produce these compounds as protective agents against herbivores (Riis et al. 2003; Tattersall et al. 2001). Activation of the cyanogenic glucosides is catalysed by β -glucosidases and α -hydroxynitrile lyases resulting in cyanohydrins, ketones, and toxic hydrogen cyanide (Conn 1980). Cyanogenic glucosides and 2 degrading enzymes are separately localized in different compartments and brought into contact upon tissue disruption for rapid degradation (Fig. 1(7)). In the leaves of sorghum seedlings, cyanogenic glucosides are sequestered in the vacuoles of epidermal cells, whereas the 2 degrading enzymes β -glucosidases and α -hydroxynitrile lyases are present almost exclusively in the

underlying mesophyll cells within the chloroplasts and cytosol, respectively (Kojima et al. 1979; Thayer and Conn 1981).

Glucosinolates (Fig. 2) are a unique class of thioglucosides found in the Brassicaceae and related families (Kjaer 1976; Kliebenstein et al. 2005; Windsor et al. 2005). These compounds share a common feature with the benzoxazinones and cyanogenic glycosides in the breakdown mechanism giving rise to toxic compounds. Upon tissue damage, the glucosinolates are activated by myrosinases, a specific class of β -thioglucosidases (Rask et al. 2000) (Fig. 1(7)). Different compartmentalization of glucosinolates and myrosinases has also been reported (Husebye et al. 2002; Koroleva et al. 2000).

Conclusions and future prospects

Plants have evolved various strategies, including the production of toxic secondary metabolites, for surviving in the environment. Many of these strategies often interfere with basic biological units such as DNA or housekeeping enzymes. To avoid self-toxicity, toxic metabolites should be compartmentalized or removed from sensitive areas such as the nucleus. These toxic metabolites are generally accumulated in the vacuole, the extracellular cavity of glandular trichomes, or are excreted extracellularly. Glycosylation is shown to be an essential modification for various types of secondary metabolites. A variety of transporters are also involved in the detoxification process. However, the mechanism by which plants ensure that they are not incapacitated by the toxic secondary metabolites that they produce remains to be determined. Coevolution has been used to explain the relationship between plants and insects. This concept might be able to explain the relationship between the biosynthetic pathway and detoxification mechanism in toxic secondary metabolite-producing plants.

Antibiotic self-resistance mechanisms, including drug elimination, drug modification, target modification, and drug sequestration, have been extensively studied (Biggins et al. 2003; Hopwood 2007; for review, see Cundliffe 1989). These studies have contributed substantially to the growing problem of antibiotic resistance among pathogenic bacteria. Despite a significant increase in the use of plant-

derived natural products as medicines, the study of self-resistance mechanisms has been neglected in plants. Although drug sequestration through vesicles is assumed, there is no experimental report on the mechanism employed by secondary metabolite-producing plants to avoid the toxicity of important anticancer drugs such as taxol from *Taxus brevifolia* or vinblastine from *C. roseus*. These compounds exhibit cytotoxicity by disrupting microtubule dynamics, which is a basic biological process. In human cancer cells, a number of reports have shown that the involvement of a transporter or a mutation in tubulin confers resistance to these drugs (Ueda et al. 1987; Kavallaris et al. 1997; Ranganathan et al. 1998; for review, see Gottesman et al. 2002). Understanding the mechanism underlying self-resistance to medically important toxic compounds in plants that produce such compounds would also contribute to understanding the resistance mechanism in other organisms. In addition, similar to the self-resistance mechanism achieved by target mutation observed in camptothecin-producing plants, a comparison of the targets in plants producing toxic compounds and in other organisms would provide a deeper insight into the interaction between a toxic compound and its target. This information could be exploited for use in drug design to combat drug resistance in the clinical field.

Combinatorial biosynthesis, which involves the combination of genes from different organisms to produce bioactive compounds (for review, see Julsing et al. 2006), has yielded promising results for the further diversification of natural products. Tattersall et al. (2001) have reported the transfer of the entire biosynthetic pathway of the cyanogenic glucoside dhurrin from *Sorghum bicolor* to *A. thaliana*. This study also suggested the genetic engineering of cyanogenic glucosides into acyanogenic crop plants for pest control purposes. Therefore, we believe that understanding the specific self-resistance mechanisms of certain metabolites would facilitate the development of more sophisticated methods for the implementation of the combinatorial biosynthetic approach in different plant species. Further, achieving self-resistance to self-produced toxic compounds could prove useful in metabolic engineering.

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References

- Ahmed MS, Ainley K, Parish JH, Hadi SM (1994) Free radical-induced fragmentation of proteins by quercetin. *Carcinogenesis* 15:1627–1630
- Aimi N, Nishimura M, Miwa A, Hoshino H, Sakai S, Haginiwa J (1989) Pumiloside and deoxypumiloside; plausible intermediates of camptothecin biosynthesis. *Tetrahedron Lett* 30:4991–4994
- Alcantara J, Bird DA, Franceschi VR, Facchini J (2005) Sanguinarine biosynthesis is associated with the endoplasmic reticulum in cultured opium poppy cells after elicitor treatment. *Plant Physiol* 138:173–183
- Alfenito MR, Souer E, Godman CD, Buell R, Mol J, Koes R, Walbot V (1998) Functional complementation of anthocyanins sequestration in the vacuole by widely divergent glutathione S-transferase. *Plant Cell* 10:1135–1149
- Bailey JA, Carter GA, Burden RS, Wain RL (1975) Control of rust diseases by diterpenes from *Nicotiana glutinosa*. *Nature* 255:328–329
- Bajaj NP, McLean MJ, Waring MJ, Smekal E (1990) Sequence-selective, pH-dependent binding to DNA of benzophenanthridine alkaloids. *J Mol Recognit* 3:48–54
- Barnes P, Putnam AR, Burke BA (1986) Allelopathy activity of rye (*Secale cereal* L.). In: Putnam AR, Tang CS (eds) *The science of allelopathy*. Wiley-Interscience, New York, pp 271–286
- Biggins JB, Onwueme KC, Thorson JS (2003) Resistance to enediynes antitumor antibiotics by CalC self-sacrifice. *Science* 301:1537–1541
- Cicek M, Esen A (1998) Structure and expression of a dhurrinase (beta-glucosidase) from sorghum. *Plant Physiol* 116:1469–1478
- Conn EE (1980) Cyanogenic glucosides. *Annu Rev Plant Physiol* 31:433–451
- Cundliffe E (1989) How antibiotic-producing organisms avoid suicide. *Annu Rev Microbiol* 43:207–233
- Cutler HG, Reid W, Deletang J (1977) Plant growth inhibiting properties of diterpene from tobacco. *Plant Cell Physiol* 18:711–714
- 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
- Deus-Neumann B, Zenk MH (1984) A highly selective alkaloid uptake system in vacuoles of higher plants. *Planta* 162:250–260
- Deus-Neumann B, Zenk MH (1984) Instability of indole alkaloid production in *Catharanthus roseus* cell suspension cultures. *Planta Med* 50:427–431
- Deus-Neumann B, Zenk MH (1986) Accumulation of alkaloids in plant vacuoles does not involve an ion-trap mechanism. *Planta* 167:44–53

- Dixon RA, Steele CL (1999) Flavonoids and isoflavonoids – a gold mine for metabolic engineering. *Trends Plant Sci* 4:394–400
- Facchini PJ, St-Pierre B (2005) Synthesis and trafficking of alkaloid biosynthetic enzymes. *Curr Opin Plant Biol* 8:657–666
- Fairbairn JW (1972) The trichomes and glands of *Cannabis sativa* L. *Bull Narc* 23:29–33
- Glöckner C, Wolf H (1984) Mechanism of natural resistance to kirromycin-type antibiotics in actinomycetes. *FEMS Microbiol Lett* 25:121–124
- Goodman CD, Casati P, Walbot V (2004) A multidrug resistance-associated protein involved in anthocyanin transport in *Zea mays*. *Plant Cell* 16:1812–1826
- Gottesman MM, Fojo T, Bates SE (2002) Multidrug resistance in cancer: role of ATP-dependent transporters. *Nat Rev Cancer* 2:48–58
- Govindachari TR, Viswanathan N (1972) Alkaloids of *Mappia foetida*. *Phytochemistry* 11:3529–3531
- Gundlach H, Muller MJ, Kutchan TM, Zenk MH (1992) Jasmonic acid is a signal transducer in elicitor-induced plant cell cultures. *Proc Natl Acad Sci USA* 89:2389–2393
- Guo Z, Wagner GJ (1995) Biosynthesis of labdenediol and sclareol in cell-free extracts from trichomes of *Nicotiana glutinosa*. *Planta* 197:627–632
- Harborne JB, Williams CA (2000) Advances in flavonoid research since 1992. *Phytochemistry* 55:481–504
- Hartmann T, Kutchan TM, Strack D (2005) Evolution of metabolic diversity. *Phytochemistry* 66:1198–1199
- Hopp W, Seitz HU (1987) The uptake of acylated anthocyanin into isolated vacuoles from a cell suspension culture of *Daucus carota*. *Planta* 170:74–85
- Hopwood DA (2007) How do antibiotic-producing bacteria ensure their self-resistance before antibiotic biosynthesis incapacitates them? *Mol Microbiol* 63:937–940
- Hsiang YH, Hertzberg R, Hecht S, Liu LF (1985) Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. *J Biol Chem* 260:14873–14878
- Huges MA (1999) Biosynthesis and degradation of cyanogenic glycosides. In: Barton D, Nakanishi K (eds) *Comprehensive natural products chemistry*, vol 1. Elsevier, Amsterdam, pp 881–895
- Hughes J, Mellows G, Soughton S (1980) How does *Pseudomonas fluorescens*, the producing organisms of the antibiotic pseudomonic acid A, avoid suicide? *FEBS Lett* 122:322–324
- Husebye H, Chadchawan S, Winge P, Thangstad OP, Bones AM (2002) Guard cell- and phloem idioblast-specific expression of thioglucoside glucosyltransferase 1 (myrosinase) in *Arabidopsis*. *Plant Physiol* 128:1180–1188
- Inokoshi J, Tomoda H, Hashimoto H, Watanabe A, Takeshima H, Omura S (1994) Cerulenin-resistant mutants of *Saccharomyces cerevisiae* with an altered fatty acid synthase gene. *Mol Gen Genet* 244:90–96
- Jasinski M, Stukkens Y, Degand H, Purnelle B, Marchand-Brynaert J, Boutry M (2001) A plant plasma membrane ATP binding cassette-type transporter is involved in antifungal terpenoid secretion. *Plant Cell* 13:1095–1107
- Julsing MK, Koulman A, Woerdenbag HJ, Quax WJ, Kayser O (2006) Combinatorial biosynthesis of medicinal plant secondary metabolites. *Biomol Eng* 23:265–279
- Kakiuchi N, Hattori M, Ishii H, Namba T (1987) Effect of benzo[c]phenanthridine alkaloids on reverse transcriptase and their binding property to nucleic acids. *Planta Med* 53:22–27
- Kavallaris M, Kuo DY, Burkhart CA, Regl DL, Norris MD, Haber M, Horwitz SB (1997) Taxol-resistant epithelial ovarian tumors are associated with altered expression of specific beta-tubulin isoforms. *J Clin Invest* 100:1282–1293
- Kawaguchi A, Tomoda H, Okuda S, Awaya J, Omura S (1979) Cerulenin resistance in a cerulenin-producing fungus. *Arch Biochem Biophys* 197:30–35
- Kim ES, Mahlberg PG (1997) Plastid development in disc cells of glandular trichomes of *Cannabis* (Cannabaceae). *Mol cells* 7:352–359
- Kitamura S, Shikazono N, Tanaka A (2004) TRANSPARENT TESTA 19 is involved in the accumulation of both anthocyanins and proanthocyanidins in *Arabidopsis*. *Plant J* 37:104–114
- Kjaer A (1976) Glucosinolates in the cruciferae. In: Vaughn JG, MacLeod AJ, Jones BMG (eds) *The biology and chemistry of the cruciferae*. Academic Press, London, pp 207–219
- Klein M, Weissenböck G, Dufaud A, Gaillard C, Kreuz K, Martinoia E (1996) Different energization mechanisms drive the vacuolar uptake of a flavonoid glucoside and a herbicide glucoside. *J Biol Chem* 271:29666–29671
- Klein M, Martinoia E, Hoffmann-Thoma G, Weissenböck G (2000) A membrane-potential dependent ABC-like transporter mediates the vacuolar uptake of rye flavone glucuronides: regulation of glucuronide uptake by glutathione and its conjugates. *Plant J* 21:289–304
- Klein M, Burla B, Martinoia E (2006) The multidrug resistance-associated protein (MRP/ABCC) subfamily of ATP-binding cassette transporters in plants. *FEBS Lett* 580:1112–1122
- Kliebenstein DJ, Kroymann J, Mitchell-Olds T (2005) The glucosinolate-myrosinase system in an ecological and evolutionary context. *Curr Opin Plant Biol* 8:264–271
- Kojima M, Poulton JE, Thayer SS, Conn EE (1979) Tissue distributions of dhurrin and of enzymes involved in its metabolism in leaves of *Sorghum bicolor*. *Plant Physiol* 63:1022–1028
- Koroleva OA, Davies A, Deeken R, Thorpe MR, Tomos AD, Hedrich R (2000) Identification of a new glucosinolate-rich cell type in *Arabidopsis* flower stalk. *Plant Physiol* 124:599–608
- Li S, Yi Y, Wang Y, Zhang Z, Beasley RS (2002) Camptothecin accumulation and variations in camptotheca. *Planta Med* 68:1010–1016
- Marinova K, Pourcel L, Weder B, Schwarz M, Barron D, Routaboul JM, Debeaujon I, Klein M (2007a) The *Arabidopsis* MATE transporter TT12 acts as a vacuolar flavonoid/H⁺-antiporter active in proanthocyanidin-accumulating cells of the seed coat. *Plant Cell* 19:2023–2038
- Marionova KI, Kleinschmidt K, Weissenböck G, Klein M (2007b) Flavonoid biosynthesis in barley primary leaves requires the presence of the vacuole and controls the activity of vacuolar flavonoid transport. *Plant Physiol* 144:432–444

- Marrs KA, Alfenito MR, Lloyd AM, Walbot V (1995) A glutathione S-transferase involved in vacuolar transfer encoded by the maize gene *Bronze-2*. *Nature* 375:397–400
- Martinoia E, Massonneau A, Frangne N (2000) Transport processes of solutes across the vacuolar membrane of higher plants. *Plant Cell Physiol* 41:1175–1186
- Mende P, Wink M (1987) Uptake of the quinolizidine alkaloid lupanine by protoplasts and isolated vacuoles of suspension-cultured *Lupinus polyphyllus* cell. Diffusion or carrier-mediated transport? *J Plant Physiol* 129:229–242
- Mendizabal VE, Adler-Graschinsky E (2007) Cannabinoids as therapeutic agents in cardiovascular disease: a tale of passions and illusions. *Br J Pharmacol* 151:427–440
- Meyermans H, Morreel K, Lapierre C, Pollet B, De Bruyn A, Busson R, Herdewijn P, Devreese B, Van Beeumen J, Marita JM, Ralph J, Chen C, Burggraef B, Van Montagu M, Messens E, Boerjan W (2000) Modifications in lignin and accumulation of phenolic glucosides in poplar xylem upon down-regulation of caffeoyl-coenzyme A *O*-methyltransferase, an enzyme involved in lignin biosynthesis. *J Biol Chem* 275:36899–36909
- Morimoto S, Tanaka Y, Sasaki K, Tanaka H, Fukamizu T, Shoyama Y, Shoyama Y, Taura F (2007) Identification and characterization of cannabinoids that induce cell death through mitochondrial permeability transition in *Cannabis* leaf cells. *J Biol Chem* 282:20739–20751
- Mueller LA, Goodman CD, Silady RA, Walbot V (2000) AN9, a petunia glutathione S-transferase required for anthocyanin sequestration is a flavonoid-binding protein. *Plant Physiol* 123:1561–1570
- Osborn AE (1996) Preformed antimicrobial compounds and plant defense against fungal attack. *Plant Cell* 8:1821–1831
- Otani M, Shitan N, Sakai K, Martinoia E, Sato F, Yazaki K (2005) Characterization of vacuolar transport of the endogenous alkaloid berberine in *Coptis japonica*. *Plant Physiol* 138:1939–1946
- Pasqua G, Monacelli B, Valletta A (2004) Cellular localisation of the anti-cancer drug camptothecin in *Camptotheca acuminata* Decne (Nyssaceae). *Eur J Histochem* 48:321–327
- Pommier Y, Pourquier P, Fan Y, Strumberg D (1998) Mechanism of action of eukaryotic DNA topoisomerase I and drugs targeted to the enzyme. *Biochim Biophys Acta* 1400:83–105
- Ranganathan S, Benetatos CA, Colarusso PJ, Dexter DW, Hudes GR (1998) Altered beta-tubulin isotype expression in paclitaxel-resistant human prostate carcinoma cells. *Br J Cancer* 77:562–566
- Rask L, Andreasson E, Ekblom B, Eriksson S, Pontoppidan B, Meijer J (2000) Myrosinase: gene family evolution and herbivore defense in Brassicaceae. *Plant Mol Biol* 42:93–113
- Rea PA (2007) Plant ATP-binding cassette transporters. *Annu Rev Plant Biol* 58:347–375
- Riis L, Bellotti AC, Bonierbale M, O'Brien GM (2003) Cyanogenic potential in cassava and its influence on a generalist insect herbivore *Cyrtomenus bergi* (Hemiptera: Cydnidae). *J Econ Entomol* 96:1905–1914
- Roos W, Evers S, Hieke M, Tschöpe M, Schumann B (1998) Shifts of intracellular pH distribution as a part of the signal mechanism leading to the elicitation of benzophenanthridine alkaloids. Phytoalexin biosynthesis in cultured cells of *Eschscholtzia californica*. *Plant Physiol* 118:349–364
- Roytrakul S, Verpoorte R (2007) Role of vacuolar transporter proteins in plant secondary metabolism: *Catharanthus roseus* cell culture. *Phytochem Rev* 6:383–396
- Rueff J, Gaspar J, Laires A (1995) Structural requirements for mutagenicity of flavonoids upon nitrosation. A structure-activity study. *Mutagenesis* 10:325–328
- Saito K, Sudo H, Yamazaki M, Koseki-Nakamura M, Kitajima M, Takayama H, Aimi N (2001) Feasible production of camptothecin by hairy root culture of *Ophiorrhiza pumila*. *Plant Cell Rep* 20:267–271
- Sakai K, Shitan N, Sato F, Ueda K, Yazaki K (2002) Characterization of berberine transport into *Coptis japonica* cells and the involvement of ABC protein. *J Exp Bot* 53:1879–1886
- Sato H, Taguchi G, Fukui H, Tabata M (1992) Role of malic acid in solubilizing excess berberine accumulating in vacuoles of *Coptis japonica*. *Phytochemistry* 31:3451–3454
- Scheiner-Bobis G (2001) Sanguinarine induces K⁺ outflow from yeast cells expressing mammalian sodium pumps. *N-S Arch Pharmacol* 7:1288–1289
- Schmeller T, Latz-Bruning B, Wink M (1997) Biochemical activities of berberine, palmatine and sanguinarine mediating chemical defence against microorganisms and herbivores. *Phytochemistry* 44:257–266
- Schulz M, Wieland I (1999) Variation in metabolism of BOA among species in various field communities – biochemical evidence for co-evolutionary processes in plant communities? *Chemoecology* 9:133–141
- Schumacher HM, Gundlach H, Fiedler F, Zenk MH (1987) Elicitation of benzophenanthridine alkaloid synthesis in *Eschscholzia* cell cultures. *Plant Cell Rep* 6:410–413
- Sicker D, Frey M, Schulz M, Gierl A (2000) Role of natural benzoxazinones in the survival strategy of plants. *Int Rev Cytol* 198:319–346
- Sicker D, Schneider B, Hennig L, Knop M, Schulz M (2001) Glycoside carbamates from benzoxazolin-2(3H)-one detoxification in extracts and exudates of corn roots. *Phytochemistry* 58:819–825
- Sirikantaramas S, Taura F, Tanaka Y, Ishikawa Y, Morimoto S, Shoyama Y (2005) Tetrahydrocannabinolic acid synthase, the enzyme controlling marijuana psychoactivity, is secreted into the storage cavity of the glandular trichomes. *Plant Cell Physiol* 46:1578–1588
- Sirikantaramas S, Taura F, Morimoto S, Shoyama Y (2007a) Recent advances in *Cannabis sativa* research: biosynthetic studies and its potential in biotechnology. *Curr Pharm Biotech* 8:237–243
- Sirikantaramas S, Asano T, Sudo H, Yamazaki M, Saito K (2007b) Camptothecin: therapeutic potential and biotechnology. *Curr Pharm Biotech* 8:196–202
- Sirikantaramas S, Sudo H, Asano T, Yamazaki M, Saito K (2007c) Transport of camptothecin in hairy roots of *Ophiorrhiza pumila*. *Phytochemistry* (in press)

- Stukkens Y, Bultreys A, Grec S, Trombik T, Vanham D, Boutry M (2005) NpPDR1, a pleiotropic drug resistance-type ATP-binding cassette transporter from *Nicotiana glumbaginifolia*, plays a major role in plant pathogen defense. *Plant Physiol* 139:341–352
- Takatani H, Oka M, Fukuda M, Narasaki F, Nakano R, Ikeda K, Terashi K, Kinoshita A, Soda H, Kanda T, Schneider E, Kohno S (1997) Gene mutation analysis and quantitation of DNA topoisomerase I in previously untreated non-small cell lung carcinomas. *Jpn J Cancer Res* 88:160–165
- Tattersall DB, Bak S, Jones PR, Olsen CE, Nielsen JK, Hansen ML, Hoj PB, Møller BL (2001) Resistance to an herbivore through engineered cyanogenic glucoside synthesis. *Science* 293:1826–1828
- Taura F, Sirikantaramas S, Shoyama Y, Yoshikai K, Shoyama Y, Morimoto S (2007) Cannabidiolic-acid synthase, the chemotype-determining enzyme in the fiber-type *Cannabis sativa*. *FEBS Lett* 581:2929–2934
- Thayer SS, Conn EE (1981) Subcellular localization of dhurrin beta-glucosidase and hydroxynitrile lyase in the mesophyll cells of sorghum leaf blades. *Plant Physiol* 67:617–622
- Thiara AS, Cundliffe E (1988) Cloning and characterization of a DNA gyrase B gene from *Streptomyces sphaeroides* that confers resistance to novobiocin. *EMBO J* 7:2255–2259
- Tomoda H, Kawaguchi A, Yasuhara T, Nakajima T, Omura S, Okuda S (1984) Cerulenin resistance in a cerulenin-producing fungus. III. Studies on active-site peptides of fatty acid synthetase from *Cephalosporium caerulens*. *J Biochem* 95:1713–1723
- Tsurutani J, Nitta T, Hirashima T, Komiya T, Uejima H, Tada H, Syunichi N, Tohda A, Fukuoka M, Nakagawa K (2002) Point mutations in the topoisomerase I gene in patients with non-small cell lung cancer treated with irinotecan. *Lung Cancer* 35:299–304
- Ueda K, Cardarelli C, Gottesman MM, Pastan I (1987) Expression of a full-length cDNA for the human “MDR1” gene confers resistance to colchicine, doxorubicin, and vinblastine. *Proc Natl Acad Sci USA* 84:3004–3008
- van den Brùle S, Müller A, Fleming AJ, Smart CC (2002) The ABC transporter SpTUR2 confers resistance to the anti-fungal sclareol. *Plant J* 30:649–662
- Vickery ML (1981) Sugar metabolism. In: Vickery ML, Vickery B (eds) Secondary plant metabolism. University Park Press, Baltimore, pp 32–41
- Villegas M, Sommarin M, Brodelius PE (2000) Effects of sodium orthovanadate on benzophenanthridine alkaloid formation and distribution in cell suspension cultures of *Eschscholzia californica*. *Plant Physiol Biochem* 38:233–241
- von Rad U, Huttel R, Lottspeich F, Gierl A, Frey M (2001) Two glucosyltransferases are involved in detoxification of benzoxazinoids in maize. *Plant J* 28:633–642
- Wall ME, Wani MC, Cook C, Palmer K, McPhail A, Sim G (1966) Plant antitumor agents. I. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from *Camptotheca acuminata*. *J Am Chem Soc* 88:3888–3890
- Weiss D, Baumert A, Vogel M, Roos W (2006) Sanguinarine reductase, a key enzyme of benzophenanthridine detoxification. *Plant Cell Environ* 29:291–302
- Windsor AJ, Reichelt M, Figuth A, Svatos A, Kroymann J, Kliebenstein DJ, Gershenzon J, Mitchell-Olds T (2005) Geographic and evolutionary diversification of glucosinolates among near relatives of *Arabidopsis thaliana* (Brassicaceae). *Phytochemistry* 66:1321–1333
- Wink M, Roberts MF (1998) Compartmentation of alkaloid biosynthesis, transport and storage. In: Roberts MF, Wink M (eds) Alkaloids, biochemistry, ecology and medicinal application. Plenum Press, New York, pp 239–262
- Yamamoto K, Takase H, Abe K, Saito Y, Suzuki A (1993) Pharmacological studies on antidiarrheal effects of a preparation containing berberine and geranii herba. *Nippon Yakurigaku Zasshi* 101:169–175
- Yamauchi T, Shoyama Y, Aramaki H, Azuma T, Nishioka I (1967) Tetrahydrocannabinolic acid, a genuine substance of tetrahydrocannabinol. *Chem Pharm Bull (Tokyo)* 15:1075–1076