Review TRENDS in Plant Science Vol.12 No.1

Flavonoid oxidation in plants: from biochemical properties to physiological functions

Lucille Pourcel[1], Jean-Marc Routaboul[1], Véronique Cheynier[2], Loïc Lepiniec[1] and Isabelle Debeaujon[1]

[1] Laboratoire de Biologie des Semences, UMR 204 INRA/INAPG, Institut Jean-Pierre Bourgin, INRA, route de Saint-Cyr, F-78026 Versailles, France
[2] Equipe Polyphénols, UMR Sciences pour l'Oenologie, INRA, 2 Place Viala, F-34060 Montpellier, France

Flavonoids protect plants against various biotic and abiotic stresses, and their occurrence in human diet participates in preventing degenerative diseases. Many of the biological roles of flavonoids are attributed to their potential cytotoxicity and antioxidant abilities. Flavonoid oxidation contributes to these chemical and biological properties and can lead to the formation of brown pigments in plant tissues as well as plant-derived foods and beverages. Flavonoid oxidation *in planta* is mainly catalyzed by polyphenol oxidases (catechol oxidases and laccases) and peroxidases. These activities are induced during seed and plant development, and by environmental stresses such as pathogen attacks. Their complex mode of action is regulated at several levels, involving transcriptional to post-translational mechanisms together with the differential subcellular compartmentalization of enzymes and substrates.

Flavonoids

Flavonoids are widely distributed plant secondary metabolites resulting from the addition of malonyl CoA to the phenylpropanoid molecule coumaroyl CoA (Figure 1a) [1–6]. These polyphenolic compounds are characterized by two aromatic cycles (A- and B- rings) linked by a heterocycle (C-ring) (Figure 1b). They are classified according to the oxidation degree of the C-ring, and include flavonols, anthocyanins and flavan-3-ols. These molecules can undergo modifications of their aromatic cycles, including hydroxylations, methylations, glycosylations, acylations or prenylations, which account for the diversity within a compound class [5,7]. The condensation of flavan-3-ols leads to the formation of proanthocyanidins (PAs), also called condensed tannins [4,8]. Phlobaphenes are reddish-brown pigments that are present in several plant tissues including the maize pericarp. They are poorly defined polymers hypothesized to result from non-enzymatic oxidation of colorless flavan-4-ols [9].

Flavonoids exhibit central functions in various aspects of plant life related to interactions with the environment [2,3,5,10]. For instance, they protect the plant against ultraviolet radiations. They also have antimicrobial properties and act as a deterrent for herbivores by limiting assimilation of dietary proteins and inhibiting digestive enzymes [4,8,11]. Flavonoids also play important roles in human health through consumption of plant-derived foods, by preventing degenerative diseases associated with oxidative stress [12,13].

Biological effects of flavonoids are linked to their potential cytotoxicity and their capacity to interact with enzymes through protein complexation. Furthermore, flavonoids act as scavengers of free radicals such as reactive oxygen species (ROS), and also prevent their formation by chelating metals [11,12,14,15]. In this context, it is relevant to consider how the oxidation of flavonoids affects their biological properties [16]. Interestingly, kaempferol and quercetin polymers catalyzed by polyphenol oxidases have been shown to have a stronger scavenging effect on ROS than their respective monomers in human cell lines [17]. Quinones are powerful antibiotics [18], which, apart from having the same tanning properties as their reduced counterpart, can also alkylate proteins. Quinones generated by polyphenol oxidases have been shown to limit the accessibility of alkylatable dietary proteins to plant-feeding insects, thus causing their starvation [19].

This article reviews our current understanding of flavonoid oxidation and its impact on plant physiology. The relationships between flavonoid oxidation and browning are analyzed. Moreover, the different oxidases (polyphenol oxidases and peroxidases) involved in these processes, together with the different levels of regulation that control browning reactions in plants are described. Finally, several examples illustrating the relationships between flavonoid oxidation and plant defense mechanisms are presented.

In planta flavonoid oxidation: a browning process

Flavonoids, particularly *ortho*-diphenols (*o*-diphenols), can be oxidized to their corresponding semiquinones and quinones by oxidases such as polyphenol oxidases (PPO) and peroxidases (POD) (Figure 2a). Semiquinones and quinones are highly reactive species that undergo further non-enzymatic reactions. They can spontaneously react with phenols, amino acids or proteins, yielding a complex mixture of brown products [20–22] (Figure 3a). Non-enzymatic

Corresponding author: Debeaujon, I. (debeaujo@versailles.inra.fr).
Available online 11 December 2006.

 1360-1385/$ – see front matter doi:10.1016/j.tplants.2006.11.006

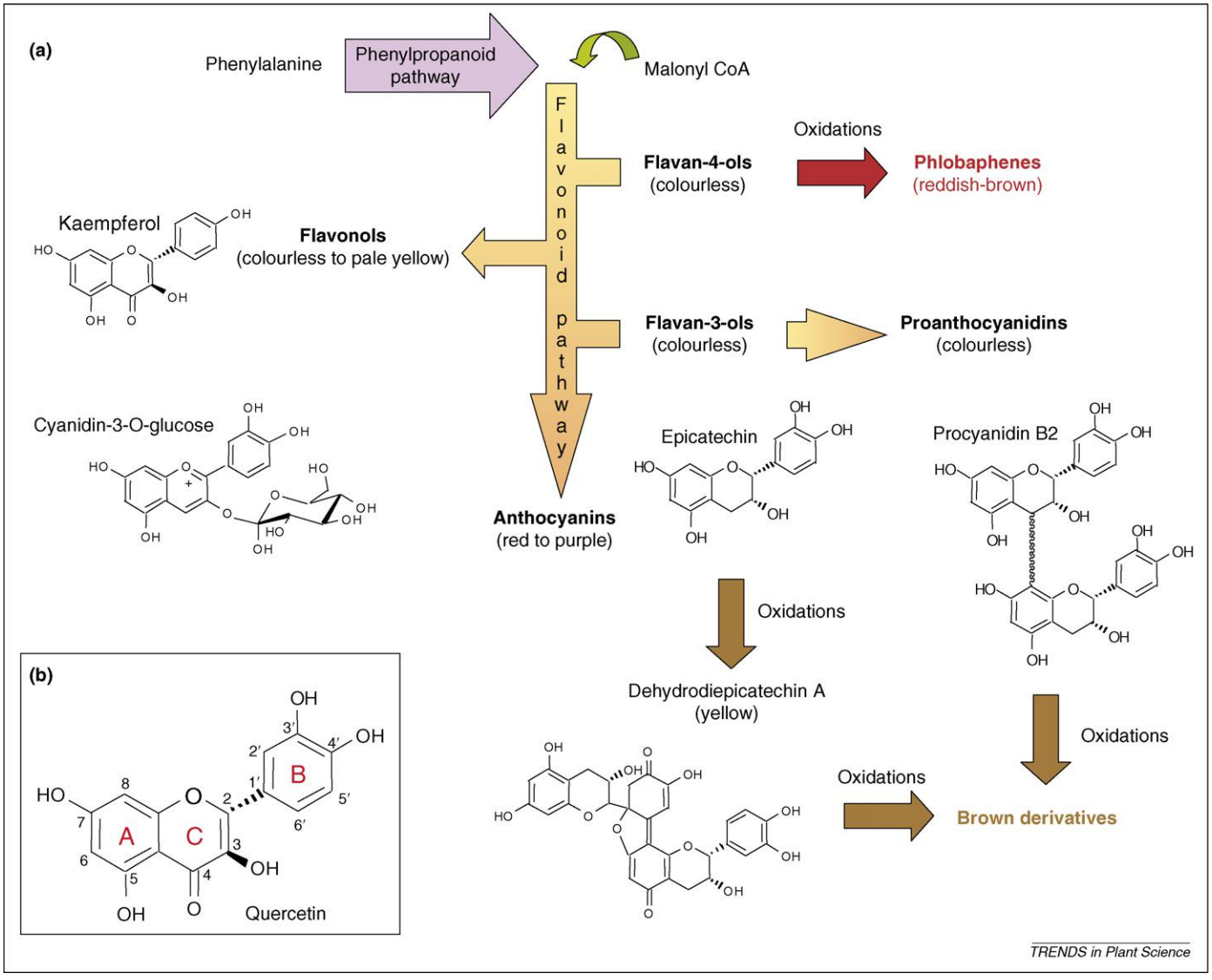

Figure 1. Structure, biosynthesis and oxidation of flavonoids. **(a)** Simplified schematic of the flavonoid pathway. The main classes of end-products are presented, and their molecular structure illustrated by one example for each class. **(b)** The structure of the flavonol quercetin is given as an example of carbon numbering. Important features influencing antioxidant potential are the di-hydroxylated B-ring, unsaturation at the C-ring and a 4-oxo function at the C-ring [13].

oxidation of flavonoids, such as autoxidation and chemical oxidation, can also lead to the formation of quinoidal compounds [21,23]. Through coupled oxidation reactions, quinones can oxidize other polyphenols that cannot be directly oxidized by the enzymes, thereby forming secondary quinones, which, in turn, contribute to the formation of heterogeneous polymers responsible for the browning reaction [24] (Figure 3b). The composition of these brown polymeric species is extremely difficult to characterize *in vivo* [20,22].

In *Arabidopsis*, seed coat browning is caused by the oxidation of epicatechin and soluble PAs by the TRANSPARENT TESTA 10 (TT10) laccase during the developmentally determined desiccation phase [6,25,26] (Figure 4). Seeds of the *tt10* null mutant are yellow at harvest, but slowly turn brown during postharvest storage until they eventually resemble wild-type seeds. They show an increase in soluble (non- or poorly oxidized) PAs and a higher ratio of quercetin-rhamnoside (QR) monomers to dimers. The contribution of QR dimers to browning is unclear. A similar enzyme could be responsible for the oxidative polymerization of flavan-4-ols leading to reddish-brown phlobaphenes in maize pericarp. Postharvest browning is also observed in pinto bean lines (*Phaseolus vulgaris* var. *Pinto*) [27]. During seed development, molecules of catechin react with kaempferol to yield heterodimers that accumulate in the seed coat. Interestingly, seed coat darkening with aging can be correlated with an increase in the amount of dimers. The browning of the peel of litchi fruits has been correlated with the rapid degradation of red anthocyanin pigments [28]. The authors suggest that to produce brown pigments, anthocyanins must become accessible to oxidation by a PPO or a POD after enzymatic removal of their sugar moieties. Other authors have shown that the major litchi anthocyanin, cyanidin 3-rutinoside, is oxidized by litchi POD and by the epicatechin quinone generated by PPO oxidation of epicatechin, through a coupled oxidation process (E. Le Roux, PhD thesis, University of Aix-Marseille, 1999) (Figure 3b).

The enzymatic oxidation of polyphenols, particularly flavonoids, also occurs during food processing of plant

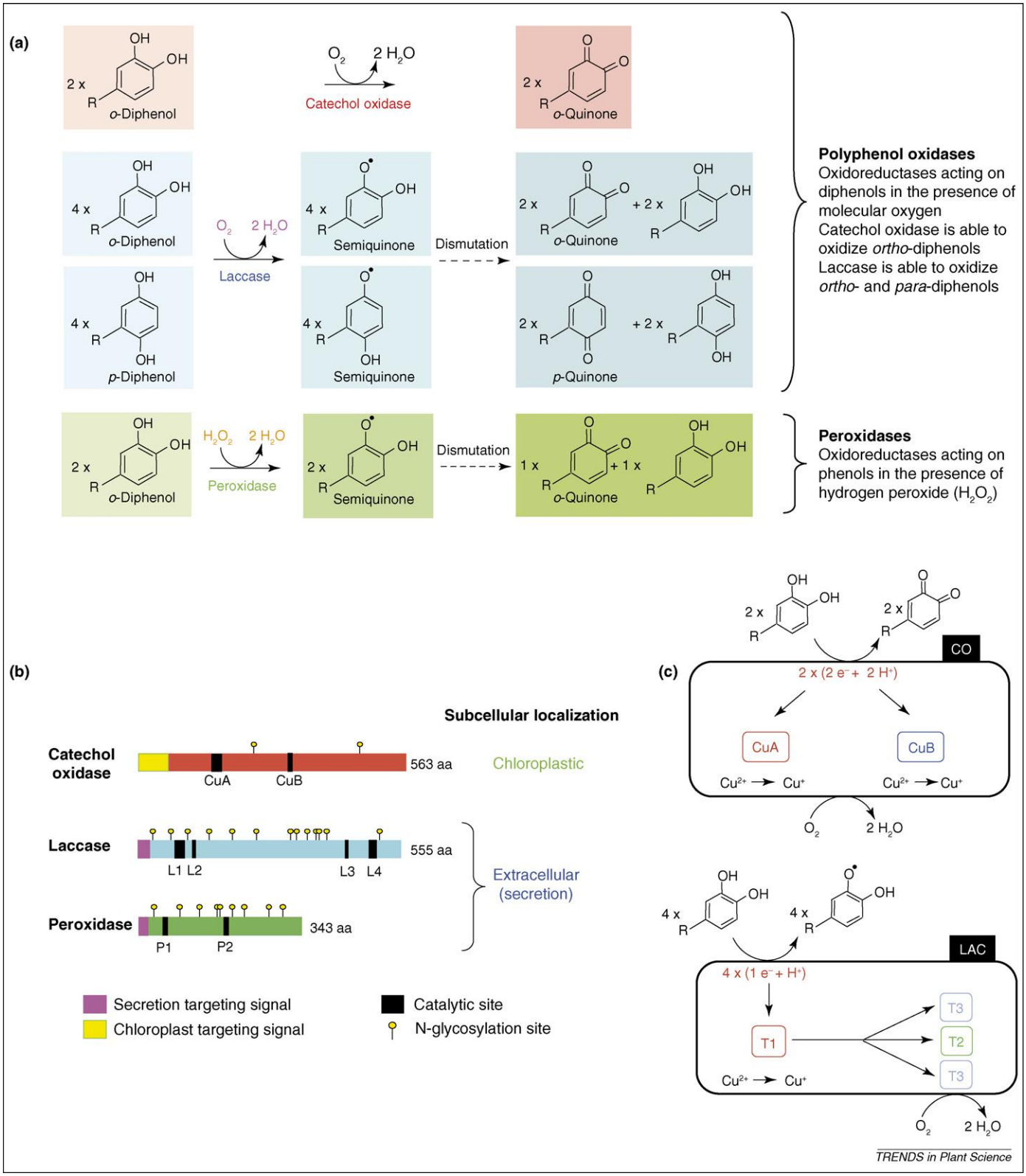

Figure 2. Three key enzymes for flavonoid oxidation in plants. **(a)** Enzymatic reactions for polyphenol oxidases (catechol oxidase and laccase) and peroxidases. **(b)** The main structural features of plant polyphenol oxidases and peroxidases are presented and compared. Poplar (*Populus* sp.) laccase (*Pe* LAC3 [33]), catechol oxidase (*Ptd* PPO1 [78]), and peroxidase (*Pt* PXP 3-4 [39]) are considered as examples. The subcellular localization was predicted by the SignalP server. The catalytic and N-glycosylation sites are PROSITE patterns. P1 and P2 stand for the active and heme-binding sites, respectively. Abbreviation: aa, amino acids. **(c)** Catalytic mechanisms for the polyphenol oxidases of the catechol oxidase and laccase types [36,37,48]. CuA, CuB and T are copper-binding sites.

material as well as during storage, when cell integrity is affected. The resulting browning is one of the main causes of quality loss [20,29]. Several techniques have been developed to inhibit browning, including physical methods (e.g. heat, modified atmosphere) and chemical inhibitors (e.g. ascorbic acid, halide ions, carboxylic acids) that can affect either the enzyme, the substrate or the product [20,22,30]. Genetic engineering also offers alternatives for producing

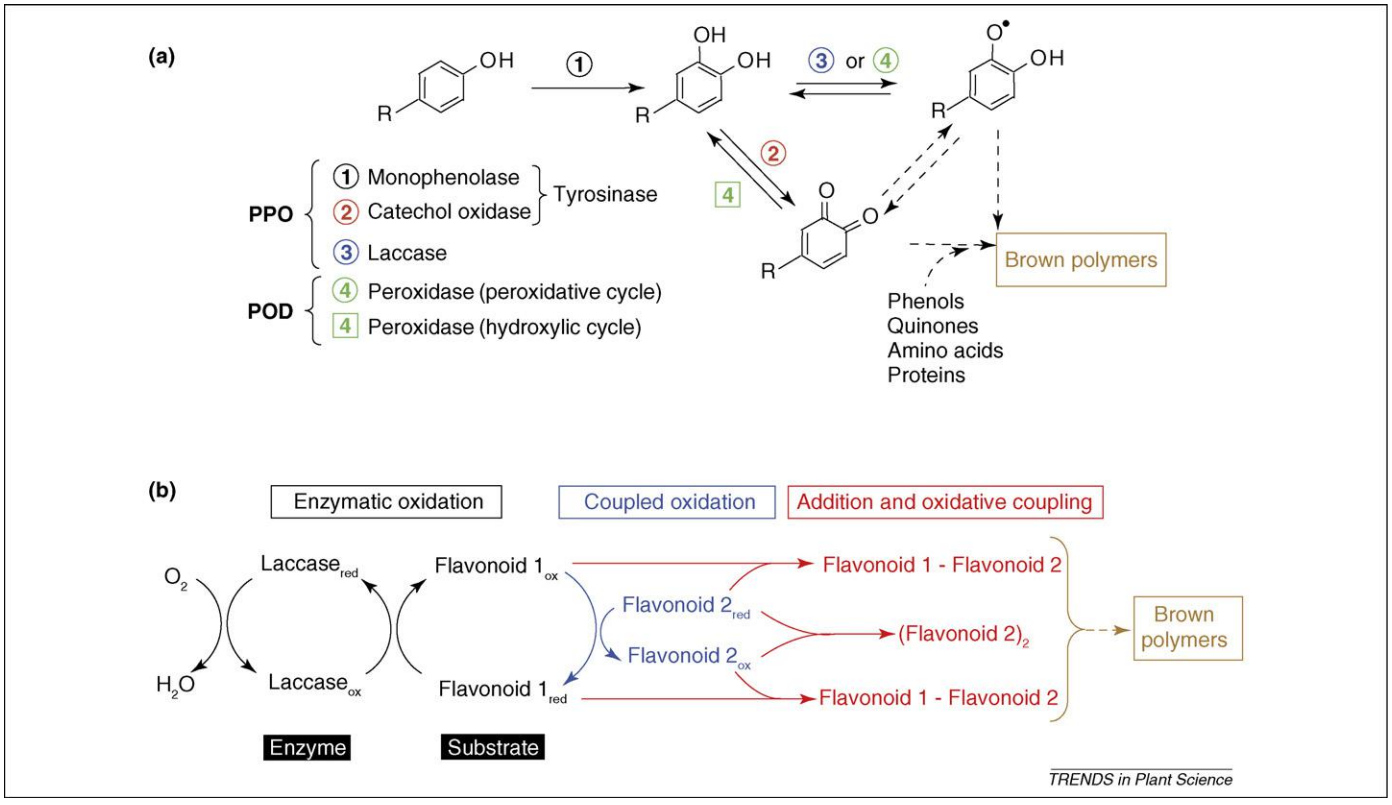

Figure 3. Mechanisms of flavonoid oxidation leading to brown polymers. **(a)** Enzymatic interactions leading to browning [20,48]. **(b)** Coupled oxidation of flavonoids [37]. As an example, the initial reaction is catalyzed here by a laccase. Broken arrows indicate unknown oxidation mechanisms. Abbreviation: R, radical.

fruits and vegetables with increased resistance to enzymatic browning. For instance, potatoes expressing sense or antisense RNA from tomato PPO show less enzymatic browning [31].

Three key enzymes

Three major enzymes, laccase (EC 1.10.3.2), catechol oxidase (EC 1.10.3.1) and peroxidase (EC 1.11.1.7), are known to be involved in flavonoid oxidation (Figure 2). Laccase and catechol oxidase belong to the PPO category.

Laccases (LAC) are *o*-diphenol and *para*(*p*)-diphenol: dioxygen oxidoreductases belonging to a group of enzymes called blue copper oxidases, which includes, among others, ascorbate oxidase and ceruloplasmin (Figure 2a). These enzymes are multi-copper glycoproteins that catalyze the oxidation of diphenolic substrates in the presence of molecular oxygen [32,33]. The catalytic sites are composed of four histidine-rich copper-binding domains (Figure 2b,c).

Catechol oxidases (CO) refer to metalloenzymes catalyzing the oxidation of *o*-diphenols to the corresponding *o*-quinones (catecholase or diphenolase activity) (Figure 2a). Generally, COs also catalyze the oxidation of monophenols to *o*-diphenols (cresolase or monophenolase activity, EC.1.14.18.1) [34] (Figure 3a). They are moderately glycosylated copper-binding proteins using molecular oxygen as a cofactor and exhibiting two copper-binding domains (Figure 2b,c). Together with laccases, they are present in eukaryotes and prokaryotes [32,34–37].

Peroxidases (POD) are hemoproteins that catalyze the oxidation of phenolic substrates through the associated reduction of hydrogen peroxide in the peroxidative cycle (Figure 2a). They are able to produce ROS such as a superoxide anion ($O_2^{-\bullet}$) or a hydroxyl radical ($OH^{\bullet}$) through the hydroxylic cycle [38,39]. Plants possess only two classes of PODs, which differ according to the subcellular localization of their members (see below).

A major issue encountered when analyzing browning processes is identifying which of the three oxidoreductase(s) is involved. Enzymatic assays can be performed with the purified enzyme in the presence of selective substrates and inhibitors. However, not all plants possess the three types of enzymes. For instance, *Arabidopsis* PPOs are represented only by laccases (no gene encoding catechol oxidase enzymes has been found in the genome) [26].

The different levels of control

From gene expression to protein activation

Most of the plant PPOs and PODs belong to multigenic families, whose members can exhibit functional redundancy when simultaneously expressed in the same tissue [26,33,40–42]. In *Arabidopsis*, the TT10 laccase appears to be the only member involved in flavonoid oxidation in seeds given that neither of the 16 other laccases, nor any POD, can compensate for the loss of its activity in the *tt10* mutant. *TT10* is mainly expressed in the seed coat (testa). The TT10 laccase colocalizes with flavonoids and is already present in tissues long before testa browning is observed [26]. Multigenic families of flavonoid oxidases, such as LACs, COs and PODs, have been maintained during evolution, suggesting that each enzyme is needed to carry out different specific functions *in planta*. In tomato, the seven members of the CO gene family are differentially induced or downregulated in various tissues and by various types of environmental stresses, suggesting that they have

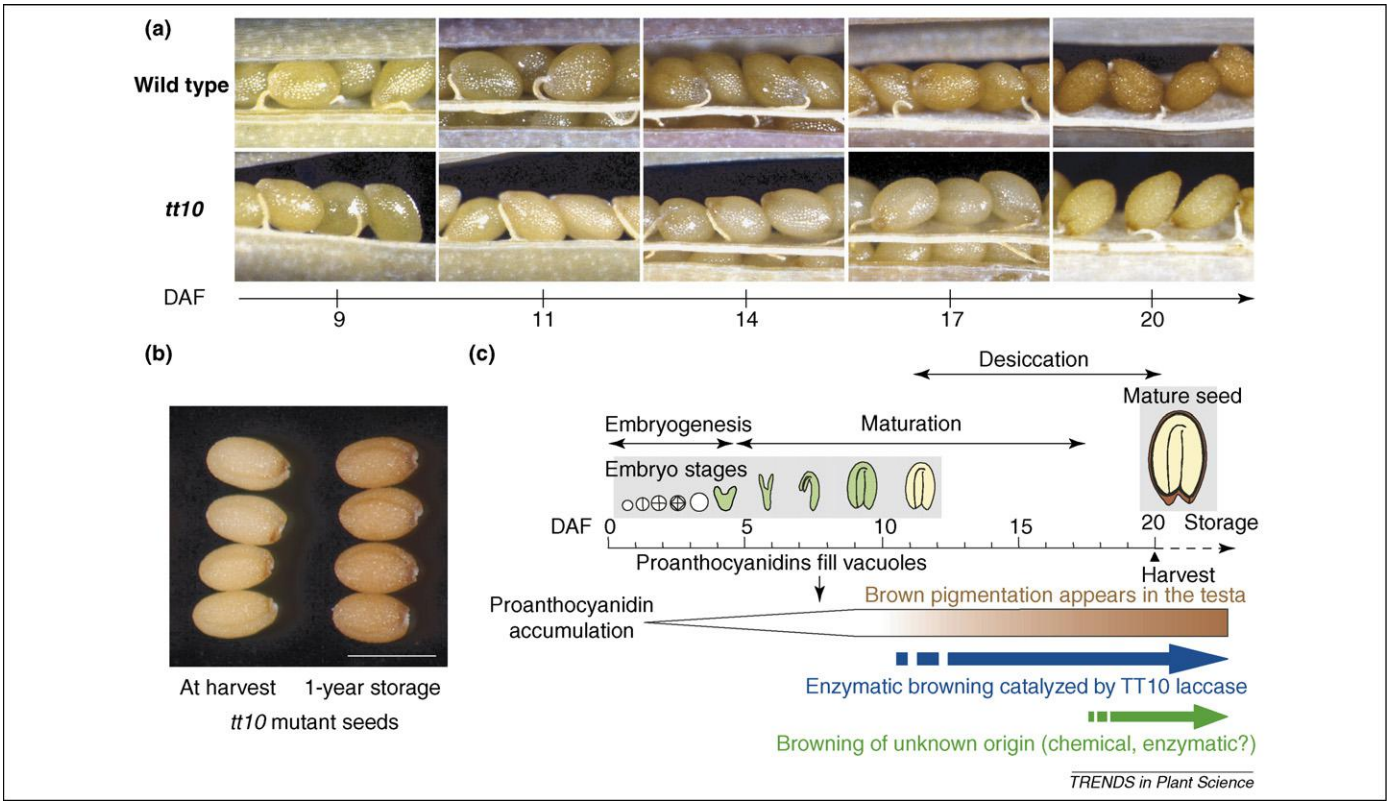

Figure 4. Seed coat pigmentation in *Arabidopsis*: illustration of a browning process. **(a)** Photographs showing the appearance of a brown pigmentation in the testa of the wild-type genotype during seed desiccation. The brown pigment is absent from the *transparent testa 10* (*tt10*) mutant defective in a laccase enzyme. **(b)** Mutant *tt10* seeds slowly become brown after harvest and eventually resemble wild-type seeds. Scale bar = 550 μm. **(c)** Schematic drawing indicating the occurrence of brown pigmentation during *Arabidopsis* seed development. Abbreviation: DAF, days after flowering.

different functions [43]. In apricot, although the amount of PPO is stable during fruit development, a peak of PPO activity is recorded at the breaker stage when no mRNA is present. This observation suggests that the protein is stable and that a post-translational control regulating PPO activity occurs [44]. Most plant PPOs are present in a latent form that requires activation [45]. For example, in apple, latent CO can be activated *in vitro* by the addition of SDS. This activation process is caused by a reorganization of the protein tertiary structure, which modifies the access of substrates to the active site of the enzyme [46,47]. *In planta*, endogenous proteases are involved in the activation of latent COs, by cleaving a 15–20 kDa C-terminus fragment, yielding an active form of the enzyme [44,48].

Differential compartmentalization of enzyme and substrate

For the browning process to occur, enzymes have to be present in the same tissues and cell compartments as the substrates and co-substrates (O_2 or H_2O_2) [20,48]. In healthy, non-senescent cells, the enzyme and its substrate(s) are distributed in different subcellular compartments or in different (but adjacent) tissues. Therefore, the oxidation reactions only occur after senescence or an environmental stress (such as pathogen attack or injury) has disorganized the cell or the tissues, and initiated decompartmentalization (i.e. the destruction of the biological barriers between the enzymes and the substrates) [20,48,49]. Vacuoles are common sites for sequestration of anthocyanins, flavan-3-ol monomers, PAs and glycosylated flavonols [3,50]. During senescence, PA polymers move to the cell wall but it is not yet clear how this happens [51].

COs are generally plastid-localized enzymes [44] (Figure 2b), with the exception of aureusidin synthase, which is vacuolar [52]. Most laccases are thought to be secreted proteins [40]. The targeting of these enzymes is conferred by an N-terminal transit peptide (Figure 2b), which is eventually cleaved by a protease, releasing the mature enzyme in the apoplast. PODs are classified according to their subcellular localization, class-I POD being intracellular, and class-III POD being secreted to the apoplast after glycosylation [38,53] (Figure 2b). Interestingly, treatment with methyl-jasmonate has been shown to enhance import and maturation of tomato CO in the chloroplast [54]. This suggests that enzyme compartmentalization is a level of control that is accessible to environmental factors. The dark-brown color in the inner part of a black walnut tree correlates with the presence of PODs and PAs [49,55]. Enzyme and substrate are localized in different cells within the living woody tissue. Therefore, PODs and PAs might interact after they have been released from their respective cell types during the progressive formation of heartwood from sapwood. In *Arabidopsis*, brown seed coat pigmentation occurs during seed desiccation, when PAs react with the TT10 laccase at the wall of dying testa cells [26] (Figure 4).

Biochemical properties of enzymes

The affinity of PPOs for their phenolic substrates depends on the stereochemistry of the substrate, together with the

position and nature of its substitutions (e.g. hydroxyl, methyl and glycosyl) [48]. In contrast to COs, which can only react with *o*-diphenols, laccases can oxidize both *o*- and *p*-diphenols, (Figure 2a). Moreover, laccases demonstrate a higher affinity for molecules with a high steric environment than COs do. Plant laccases generally have a low specificity with regard to the reducing substrate [32]. In strawberry, brown polymers resulting from (+)-catechin oxidation are formed mainly by PPOs; PODs appear to be inhibited by the reaction products [56].

Intracellular pH can also be an important factor for these enzyme activities [21,48,57]. For instance, changes in pH values during *in vitro* activities have been shown to prevent proper conformation of the active site, binding of the substrates, and/or catalysis of the reaction [48]. The oxidation of (+)-catechin in the presence of a crude grape PPO extract generates different products depending on whether the reaction occurs at pH 3 or at pH 6 [21].

Physiological roles for flavonoid oxidation

Evidence is accumulating to support the idea that flavonoid oxidation has a protection function during plant development and growth. For example, browning reactions are observed during programmed developmental events such as seed desiccation and plant senescence. Flavonoid oxidation also plays a role in defending the plant against various biotic and abiotic stresses [10,58,59] (Figure 5). Both situations are illustrated below.

Oxidations as part of normal seed and plant development

The *Arabidopsis* TT10 laccase is present in young, colorless seed coats. During the desiccation phase, oxidation of epicatechin and soluble PAs by TT10 might increase their capacity to bind to the cell wall to form, preventively, a physico-chemical protection against stresses. The activity of the TT10 promoter appears to be strongly induced in early aborted seeds, suggesting that the *TT10* gene might be transcriptionally induced by cell death [26]. During desiccation of pea, cotton or *Sida spinosa* seeds, flavonoids accumulated in seed coats are oxidized in the presence of PPOs or PODs, leading to seed coat browning and water-impermeability. The formation of quinones and insoluble polymers would explain the reinforcement of the seed coat barrier to water permeation [60–62]. A positive correlation exists between the oxidation of PAs and their cross-linking to the cell wall [8,63]. More generally, such a modification of testa characteristics might reinforce coat-imposed dormancy by increasing the physical resistance of the tissues, impeding the entrance of germination-stimulating agents such as oxygen or water, or the leakage of inhibiting substances such as abscisic acid or carbon dioxide [64–67]. Moreover, the scavenging of oxygen and hydrogen peroxide (H_2O_2) through flavonoid oxidation might protect seeds from deterioration and therefore prolong the period for which they can be stored [65,68].

Browning of onion scales during aging is caused by the autoxidation of quercetin glucosides, after their deglucosylation [69]. Quercetin autoxidation generates activated oxygen (O_2^-), which reacts with H_2O. This reaction would produce enough H_2O_2 for a POD to enhance quercetin oxidation and lead to the formation of the antifungal agent 3,4-dihydroxybenzoic acid. Finally, oxidation of exogenous flavonoids such as (−)-catechin by root-secreted laccases might play a role in allelopathic interactions between plants [70,71].

Oxidations induced by environmental stresses

The induced production of antimicrobial quinones might lead to the formation of polymeric compounds creating a protective physicochemical barrier [11,61]. Moreover, semiquinones can act as antioxidants by reacting with ROS that appear after tissue damage. Efficient

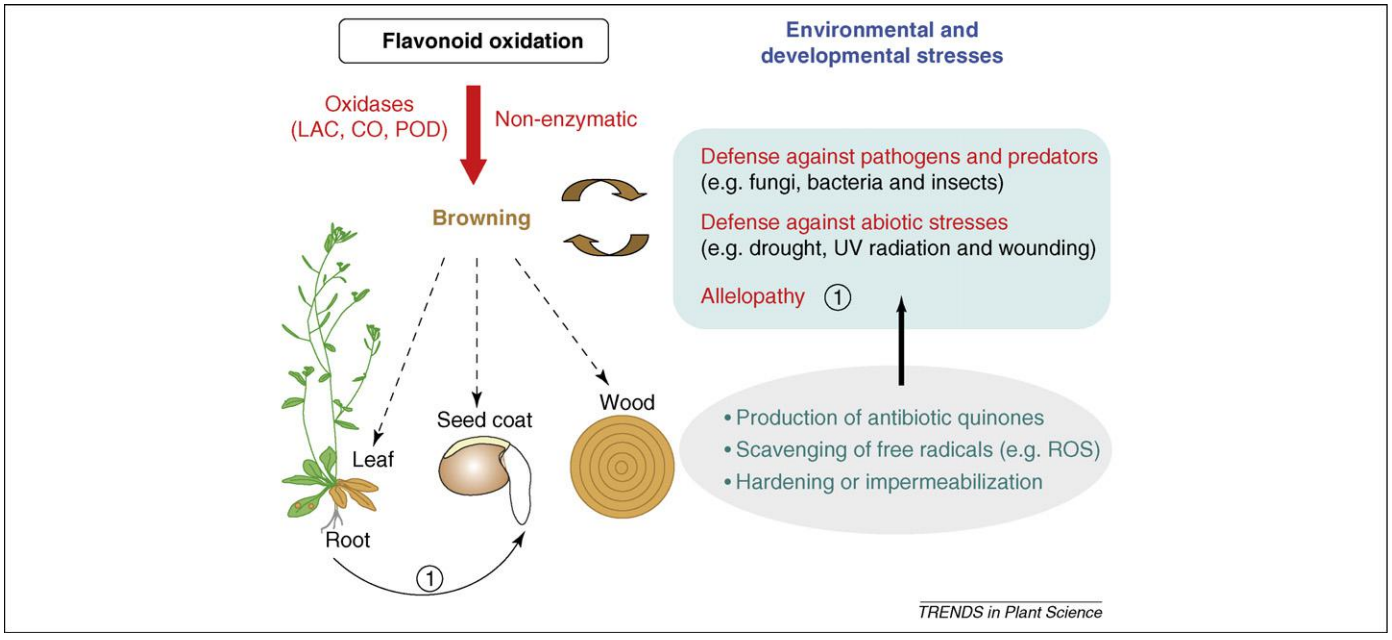

Figure 5. Flavonoid oxidation: impacts on plant physiology and mechanisms of action. The browning caused by flavonoid oxidation is initiated by developmental (seed desiccation, senescence) and environmental stresses. The main mechanisms of this plant defense reaction are highlighted in gray.

scavenging of ROS has been shown to reduce UV radiation stress [72]. In plant cells, the photosynthetic electron transport system is the major source of ROS such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^\bullet$), singlet oxygen ($^1O_2$), or perhydroxyl radical (HO_2^-). Flavonoid–POD reaction might function as a mechanism for H_2O_2-scavenging, and therefore plant cell detoxification [73].

Quinones can also behave as direct toxic compounds against pathogens [53,74]. In *Brassica oleracea*, POD activity was detected in the integumentary pigment layer, the cotyledons and the embryo axis during imbibition of seeds [75]. Activity peaked after rupture of the integuments, suggesting that quinones have a role in protecting the germinating embryo. In ginseng, reddish-brown areas develop at the root surface as a defense reaction against fungal attack [76]. They have been shown to accumulate phenolic compounds, particularly catechins, and to exhibit enhanced PPO and POD activity. In leaves of drought-stressed tea plants, the formation of epicatechin quinones negatively correlates with lipid peroxidation [77]. Wounding triggers a shift in plant metabolism towards increased biosynthesis of phenolic compounds and enzyme activation, thus providing more antioxidant and antimicrobial agents [20,78]. CO gene expression in hybrid poplar has been shown to be wound- and herbivore-induced in young leaves, suggesting that the enzyme might play a role in poplar defense by oxidizing PAs [78]. PA oxidation has been proposed to constitute an antinutritive barrier against herbivores and to protect plant polymers (lignin and cellulose) from degradation by forming complexes with them [11]. Oxidized tannins might also form covalent linkages and interfere with fungal enzymes that are necessary for plant cell invasion (e.g. pectinase, cellulase and laccase). In tomato, two of the seven CO genes are transcriptionally upregulated in abscission zones of leaf petioles in response to water stress [43]. According to the authors, this induction would generate cytotoxic quinones that facilitate cell death during abscission of old leaves. Finally, overexpression and antisense downregulation of a tomato PPO result in enhanced disease resistance and susceptibility, respectively [79,80].

Concluding remarks

Flavonoid oxidation is a complex process involving different mechanisms. Additional research is needed to understand fully the biological functions of oxidative browning in plants. Molecular genetics and the characterization of mutants affected in tissue browning should be useful to confirm and further analyze the physiological roles of flavonoid oxidation products. Finally, further research on the regulation of flavonoid oxidase biosynthesis and activity will be important to elucidate the precise functions and mechanisms of action of these enzymes, including the search for regulatory factors that induce or repress flavonoid oxidation in plants.

Acknowledgements

This work was supported by grants from CETIOM to L.P. and from the EC (FLAVO-FOOD-CT-2004-513960) to all the authors.

References

1 Grotewold, E. (2006) The genetics and biochemistry of floral pigments. *Annu. Rev. Plant Biol.* 57, 761–780
2 Winkel-Shirley, B. (2002) Biosynthesis of flavonoids and effects of stress. *Curr. Opin. Plant Biol.* 5, 218–223
3 Lepiniec, L. *et al.* (2006) Genetics and biochemistry of seed flavonoids. *Annu. Rev. Plant Biol.* 57, 405–430
4 Dixon, R.A. *et al.* (2005) Proanthocyanidins – a final frontier in flavonoid research? *New Phytol.* 165, 9–28
5 Winkel-Shirley, B.S.J. (2006) The biosynthesis of flavonoids. In *The Science of Flavonoids* (Grotewold, E., ed.), pp. 71–95, Springer
6 Routaboul, J.M. *et al.* (2006) Flavonoid diversity and biosynthesis in seed of *Arabidopsis thaliana*. *Planta* 224, 96–107
7 Schwinn, K.E. and Davies, K.M. (2004) Flavonoids. In *Plant Pigments and their Manipulation* (Vol. 14) (Davies, K.M., ed.), pp. 92–149, Blackwell
8 Marles, M.A. *et al.* (2003) New perspectives on proanthocyanidin biochemistry and molecular regulation. *Phytochemistry* 64, 367–383
9 Grotewold, E. *et al.* (1991) Alternatively spliced products of the maize *P* gene encode proteins with homology to the DNA-binding domain of myb-like transcription factors. *Proc. Natl. Acad. Sci. U. S. A.* 88, 4587–4591
10 Treutter, D. (2006) Significance of flavonoids in plant resistance: a review. *Environ. Chem. Lett.* 4, 147–157
11 Scalbert, A. (1991) Antimicrobial properties of tannins. *Phytochemistry* 12, 3875–3883
12 Santos-Buelga, C. and Scalbert, A. (2000) Proanthocyanidins and tannin-like compounds – nature, occurrence, dietary intake and effects on nutrition and health. *J. Sci. Food Agric.* 80, 1094–1117
13 Williams, R.J. *et al.* (2004) Flavonoids: antioxidants or signalling molecules? *Free Radic. Biol. Med.* 36, 838–849
14 Heim, K.E. *et al.* (2002) Flavonoid antioxidants: chemistry, metabolism and structure–activity relationships. *J. Nutr. Biochem.* 13, 572–584
15 Vaknin, H. *et al.* (2005) Active anthocyanin degradation in *Brunfelsia calycina* (yesterday-today-tomorrow) flowers. *Planta* 222, 19–26
16 Pirker, K.F. *et al.* (2006) Are the biological properties of kaempferol determined by its oxidation products? *Free Radic. Res.* 40, 513–521
17 Desentis-Mendoza, R.M. *et al.* (2006) Enzymatic polymerization of phenolic compounds using laccase and tyrosinase from *Ustilago maydis*. *Biomacromolecules* 7, 1845–1854
18 Schweigert, N. *et al.* (2001) Chemical properties of catechols and their molecular modes of toxic action in cells, from microorganisms to mammals. *Environ. Microbiol.* 3, 81–91
19 Felton, G.W. *et al.* (1992) Impact of oxidized plant phenolics on the nutritional quality of dietary protein to a noctuid herbivore, *Spodoptera exigua*. *J. Insect Physiol.* 38, 277–285
20 Walker, J.R. and Ferrar, P.H. (1998) Diphenol oxidases, enzyme-catalysed browning and plant disease resistance. *Biotechnol. Genet. Eng. Rev.* 15, 457–498
21 Guyot, S. *et al.* (1996) Structural determination of colourless and yellow dimers resulting from (+)-catechin coupling catalysed by grape polyphenoloxidase. *Phytochemistry* 42, 1279–1288
22 Nicolas, J. *et al.* (1993) Polyphenols and enzymatic browning. In *Polyphenolic Phenomena* (Scalbert, A., ed.), pp. 165–175, INRA Editions
23 Mochizuki, M. *et al.* (2002) Kinetic analysis and mechanistic aspects of autoxidation of catechins. *Biochim. Biophys. Acta.* 1569, 35–44
24 Cheynier, V. *et al.* (1994) Anthocyanin degradation in oxidizing grape musts. *J. Sci. Food Agric.* 66, 283–288
25 Debeaujon, I. *et al.* (2003) Proanthocyanidin-accumulating cells in *Arabidopsis* testa: regulation of differentiation and role in seed development. *Plant Cell* 15, 2514–2531
26 Pourcel, L. *et al.* (2005) *TRANSPARENT TESTA10* encodes a laccase-like enzyme involved in oxidative polymerization of flavonoids in *Arabidopsis* seed coat. *Plant Cell* 17, 2966–2980
27 Beninger, C.W. *et al.* (2005) Changes in polyphenols of the seed coat during the after-darkening process in pinto beans (*Phaseolus vulgaris* L.). *J. Agric. Food Chem.* 53, 7777–7782
28 Jiang, Y.M. *et al.* (2004) Advances in understanding of enzymatic browning in harvested litchi fruit. *Food Chem.* 88, 443–446
29 Cheynier, V. (2005) Polyphenols in foods are more complex than often thought. *Am. J. Clin. Nutr.* 81, 223S–229S

30 Harbowy, M.E. and Balentine, D.A. (1997) Tea chemistry. *Crit. Rev. Plant Sci.* 16, 415–480

31 Coetzer, C. *et al.* (2001) Control of enzymatic browning in potato (*Solanum tuberosum* L.) by sense and antisense RNA from tomato polyphenol oxidase. *J. Agric. Food Chem.* 49, 652–657

32 Mayer, A.M. and Staples, R.C. (2002) Laccase: new functions for an old enzyme. *Phytochemistry* 60, 551–565

33 Ranocha, P. *et al.* (1999) Biochemical characterization, molecular cloning and expression of laccases – a divergent gene family – in poplar. *Eur. J. Biochem.* 259, 485–495

34 Marusek, C.M. *et al.* (2006) Comparative analysis of polyphenol oxidase from plant and fungal species. *J. Inorg. Biochem.* 100, 108–123

35 Mayer, A.M. (2006) Polyphenol oxidases in plants and fungi: going places? A review. *Phytochemistry* 67, 2318–2331

36 Claus, H. (2004) Laccases: structure, reactions, distribution. *Micron* 35, 93–96

37 Riva, S. (2006) Laccases: blue enzymes for green chemistry. *Trends Biotechnol.* 24, 219–226

38 Passardi, F. *et al.* (2004) Performing the paradoxical: how plant peroxidases modify the cell wall. *Trends Plant Sci.* 9, 534–540

39 Christensen, J.H. *et al.* (2001) The syringaldazine-oxidizing peroxidase PXP 3-4 from poplar xylem: cDNA isolation, characterization and expression. *Plant Mol. Biol.* 47, 581–593

40 McCaig, B.C. *et al.* (2005) Gene structure and molecular analysis of the laccase-like multicopper oxidase (LMCO) gene family in *Arabidopsis thaliana*. *Planta* 221, 619–636

41 Valerio, L. *et al.* (2004) Expression analysis of the *Arabidopsis* peroxidase multigenic family. *Phytochemistry* 65, 1331–1342

42 Passardi, F. *et al.* (2004) The class III peroxidase multigenic family in rice and its evolution in land plants. *Phytochemistry* 65, 1879–1893

43 Thipyapong, P. *et al.* (2004) Suppression of polyphenol oxidases increases stress tolerance in tomato. *Plant Sci.* 167, 693–703

44 Chevalier, T. *et al.* (1999) Molecular cloning and characterization of apricot fruit polyphenol oxidase. *Plant Physiol.* 119, 1261–1269

45 Jukanti, A.K. *et al.* (2006) Molecular and biochemical characterisation of polyphenol oxidases in developing kernels and senescing leaves of wheat (*Triticum aestivum*). *Funct. Plant Biol.* 33, 685–696

46 Marques, L. *et al.* (1994) Purification of an apple polyphenoloxidase isoform resistant to SDS-proteinase K digestion. *Phytochemistry* 36, 1117–1121

47 Gandia-Herrero, F. *et al.* (2005) Differential activation of a latent polyphenol oxidase mediated by sodium dodecyl sulfate. *J. Agric. Food Chem.* 53, 6825–6830

48 Yoruk, R. and Marshall, M.R. (2003) Physicochemical properties and function of plant polyphenol oxidase: a review. *J. Food Biochem.* 27, 361–422

49 Dehon, L. *et al.* (2001) Differential compartmentation of *o*-diphenols and peroxidase activity in the inner sapwood of the *Juglans nigra* tree. *Plant Physiol. Biochem.* 39, 473–477

50 Grotewold, E. (2004) The challenges of moving chemicals within and out of cells: insights into the transport of plant natural products. *Planta* 219, 906–909

51 Kitamura, S. *et al.* (2004) *TRANSPARENT TESTA 19* is involved in the accumulation of both anthocyanins and proanthocyanidins in *Arabidopsis*. *Plant J.* 37, 104–114

52 Ono, E. *et al.* (2006) Localization of a flavonoid biosynthetic polyphenol oxidase in vacuoles. *Plant J.* 45, 133–143

53 Takahama, U. (2004) Oxidation of vacuolar and apoplastic phenolic substrates by peroxidase: physiological significance of the oxidation reactions. *Phytochem. Rev.* 3, 207–219

54 Koussevitzky, S. *et al.* (2004) Import of polyphenol oxidase by chloroplasts is enhanced by methyl jasmonate. *Planta* 219, 412–419

55 Dehon, L. *et al.* (2002) Involvement of peroxidases in the formation of the brown coloration of heartwood in *Juglans nigra*. *J. Exp. Bot.* 53, 303–311

56 Lopez-Serrano, M. and Barcelo, A.R. (2002) Comparative study of the products of the peroxidase-catalyzed and the polyphenoloxidase-catalyzed (+)-catechin oxidation. Their possible implications in strawberry (*Fragaria* × *ananassa*) browning reactions. *J. Agric. Food Chem.* 50, 1218–1224

57 Bligny, R. and Douce, R. (1983) Excretion of laccase by sycamore (*Acer pseudoplatanus* L.) cells. Purification and properties of the enzyme. *Biochem. J.* 209, 489–496

58 Buchanan-Wollaston, V. and Morris, K. (2000) Senescence and cell death in *Brassica napus* and *Arabidopsis*. In *Programmed Cell Death in Animals and Plants* (Garland, J.M., ed.), pp. 163–174, BIOS Scientific Publishers

59 Munné-Bosch, S. and Alegre, L. (2004) Die and let live: leaf senescence contributes to plant survival under drought stress. *Funct. Plant Biol.* 31, 203–216

60 Werker, E. *et al.* (1979) Relation between the anatomy of the testa, water permeability and the presence of phenolics in the genus *Pisum*. *Ann. Bot. (Lond.)* 43, 765–771

61 Halloin, J.M. (1982) Localization and changes in catechin and tannins during development and ripening of cottonseed. *New Phytol.* 90, 651–657

62 Egley, G.H. *et al.* (1983) Role of peroxidase in the development of water-impermeable seed coats in *Sida spinosa* L. *Planta* 157, 224–232

63 Renard, C.M. *et al.* (2001) Interactions between apple cell walls and native apple polyphenols: quantification and some consequences. *Int. J. Biol. Macromol.* 29, 115–125

64 Lenoir, C. *et al.* (1986) Barley (*Hordeum vulgare*) seed dormancy as related to glumella characteristics. *Physiol. Plant.* 68, 301–307

65 Debeaujon, I. *et al.* (2000) Influence of the testa on seed dormancy, germination, and longevity in *Arabidopsis*. *Plant Physiol.* 122, 403–414

66 Porter, N.G. and Wareing, P.F. (1974) The role of the oxygen permeability of the seed coat in the dormancy of seeds of *Xanthium pennsylvanicum* Wallr. *J. Exp. Bot.* 25, 583–594

67 Benech-Arnold, R.L. *et al.* (2006) Hypoxia interferes with ABA metabolism and increases ABA sensitivity in embryos of dormant barley grains. *J. Exp. Bot.* 57, 1423–1430

68 Bailly, C. (2004) Active oxygen species and antioxidants in seed biology. *Seed Sci. Res.* 14, 93–107

69 Takahama, U. and Hirota, S. (2000) Deglucosidation of quercetin glucosides to the aglycone and formation of antifungal agents by peroxidase-dependent oxidation of quercetin on browning of onion scales. *Plant Cell Physiol.* 41, 1021–1029

70 Wang, G.D. *et al.* (2004) *Ex planta* phytoremediation of trichlorophenol and phenolic allelochemicals via an engineered secretory laccase. *Nat. Biotechnol.* 22, 893–897

71 Bais, H.P. *et al.* (2003) Allelopathy and exotic plant invasion: from molecules and genes to species interactions. *Science* 301, 1377–1380

72 Jansen, M.A.K. *et al.* (2001) Phenol-oxidizing peroxidases contribute to the protection of plants from ultraviolet radiation stress. *Plant Physiol.* 126, 1012–1023

73 Yamasaki, H. *et al.* (1997) Flavonoid-peroxidase reaction as a detoxification mechanism of plant cells against H_2O_2. *Plant Physiol.* 115, 1405–1412

74 Mayer, H.A. and Harel, E. (1979) Polyphenol oxidases in plants. *Phytochemistry* 18, 193–215

75 Bellani, L.M. *et al.* (2002) Differences in the activity and distribution of peroxidases from three different portions of germinating *Brassica oleracea* seeds. *Physiol. Plant.* 114, 102–108

76 Rahman, M. and Punja, Z.K. (2005) Biochemistry of ginseng root tissues affected by rusty root symptoms. *Plant Physiol. Biochem.* 43, 1103–1114

77 Hernandez, I. *et al.* (2006) Enhanced oxidation of flavan-3-ols and proanthocyanidin accumulation in water-stressed tea plants. *Phytochemistry* 67, 1120–1126

78 Constabel, C.P. *et al.* (2000) Polyphenol oxidase from hybrid poplar. Cloning and expression in response to wounding and herbivory. *Plant Physiol.* 124, 285–295

79 Thipyapong, P. *et al.* (2004) Antisense downregulation of polyphenol oxidase results in enhanced disease susceptibility. *Planta* 220, 105–117

80 Li, L. and Steffens, J.C. (2002) Overexpression of polyphenol oxidase in transgenic tomato plants results in enhanced bacterial disease resistance. *Planta* 215, 239–247