

DARWIN REVIEW

Tocochromanol functions in plants: antioxidation and beyond

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Abstract

Tocopherols and tocotrienols, collectively known as tocochromanols, are lipid-soluble molecules that belong to the group of vitamin E compounds and are essential in the human diet. Not surprisingly, most of what is known about the biological functions of tocochromanols comes from studies of mammalian systems, yet they have been shown to be synthesized only by photosynthetic organisms. The last decade has seen a radical change in the appreciation of the biological role of tocochromanols in plants thanks to a detailed characterization of mutant and transgenic plants, including several *Arabidopsis thaliana* mutants, the *sucrose export defective1 (sxd1)* maize mutant, and some transgenic potato and tobacco lines altered in tocochromanol biosynthesis. Recent findings indicate that tocopherols may play important roles in plants beyond their antioxidant function in photosynthetic membranes. Plants deficient in tocopherols show alterations in germination and export of photoassimilates, and growth, leaf senescence, and plant responses to abiotic stresses, thus suggesting that tocopherols may influence a number of physiological processes in plants. Thus, in this review not only the antioxidant function of tocochromanols in plants, but also these new emerging possible roles will be considered. Particular attention will be paid to specific roles attributed to different tocopherol homologues (particularly α - and γ -tocopherol) and the possible functions of tocotrienols, which in contrast to tocopherols are only present in a range of unrelated plant groups and are almost exclusively found in seeds and fruits.

Key words: Antioxidants, tocochromanols, tocopherols, tocotrienols, vitamin E.

Introduction

Tocopherols and tocotrienols, collectively known as tocochromanols, are lipid-soluble molecules that belong to the group of vitamin E compounds, and they play an essential role in human nutrition and health. The term ‘vitamin E’ was first introduced by Evans and Bishop (1922) to describe an important dietary factor for animal reproduction. More than 40 years passed before vitamin E was associated with an antioxidant property (Epstein *et al.*, 1966). Thereafter, the role of this vitamin was extensively studied in humans and animals, and later the function of tocochromanols was studied in plants, where they are synthesized. Although it is obvious that tocochromanols are not a dietary factor for photosynthetic organisms, they are usually referred as vitamin E in plants, which is misleading when talking about their functions in plants. Thus, the term ‘tocochromanols’

instead of ‘vitamin E’ will be used since the focus here is on the function of these compounds in plants.

Tocochromanols consist of a chromanol ring system and a polyprenyl side chain, which is saturated in tocopherols and 3-fold unsaturated in tocotrienols (Fig. 1). Both subgroups of the tocochromanols can be further classified into different homologues, that is the α -, β - or γ -, and δ -forms according to the number and position of methyl groups at the chromanol ring system (Kamal-Eldin and Appelqvist, 1996). Despite the fact that both tocopherols and tocotrienols are amphipatic molecules, they differ in some biochemical properties with respect to their isoprenoid side chain, which might have important implications for their functions, as will be discussed later. Furthermore, while tocopherols are ubiquitously found in all photosynthetic organisms, tocotrienols are only present in certain

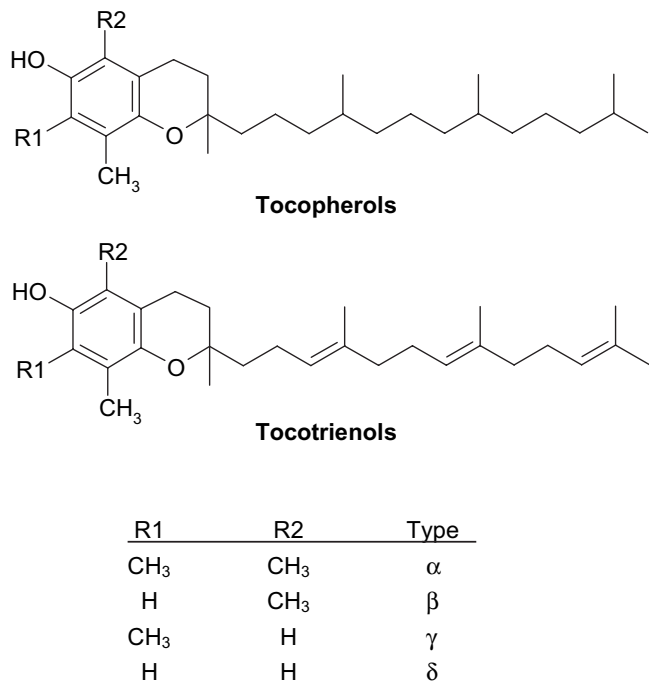


Fig. 1. Tocopherol and tocotrienol structures. The number and position of methyl groups in the chromanol ring of α -, β -, γ -, and δ -tocochromanols are indicated in the inset table.

plant groups. Even when tocopherols and tocotrienols are present in a plant, they are not necessarily found in the same tissue, which suggests different functions for tocopherols and tocotrienols in plants. Despite these compounds having generally been considered to be antioxidants in plants *in vivo*, mainly due to their potent antioxidant capacity in several *in vitro* tests, this appears not to be that simple and it would be a mistake not to consider other potential roles for tocopherols and tocotrienols in plants, as has recently been shown by using mutants and transgenic lines with altered tocochromanol levels.

Studies on the chemistry, biosynthesis, and function of tocochromanols in plants, animals, and humans have been extensively reviewed in the last decade, with a focus on their fundamental chemistry, specific location, and role in biological membranes (Wang and Quinn, 2000), the benefits of these compounds for human health (Pryor, 2000; Bell and Grochoski, 2008), biotechnological applications for their production (Ogbonna, 2009), and their biosynthesis (DellaPenna, 2005a, b; DellaPenna and Pogson, 2006) and function in plants (Munné-Bosch and Alegre, 2002a; Trebst, 2003; Munné-Bosch, 2005; Dörmann, 2007; Maeda and DellaPenna, 2007; Li *et al.*, 2008).

In this review, the current understanding of tocochromanol functions in plants will be discussed with some hints on evolutionary and ecophysiological aspects of vitamin E research, taking into account Darwin's legacy regarding natural selection and evolution. After a description of tocochromanol functions in plants, including their antioxidant function and new proposed emerging roles, the focus will be on the specific roles of different tocochromanol homologues (mainly α - and γ -tocopherol, and tocotrienols),

an aspect poorly studied to date that deserves further research.

Changing views on tocochromanol functions in plants

Different approaches can be used in the study of the functions of tocochromanol and other antioxidants in plants: (i) exogenous applications at the whole-plant or cellular level; (ii) study of the variations in the contents of these antioxidants prior to and during the physiological process under study; and (iii) the use of mutants and transgenic lines with altered tocochromanol biosynthesis and levels. Exogenous applications of antioxidants at the whole-plant level (either to roots or directly on the leaf surface) have been widely used to alleviate stresses and are extensively reported in the literature, especially for the water-soluble ascorbic acid (vitamin C), giving rise to different, sometimes contrasting, results depending on the dose, time of exposure, and plant species studied (Dolatabadian *et al.*, 2008; Athar *et al.*, 2009). In the case of tocochromanols, this approach has been particularly difficult due to their lipophilic nature that limits their application, absorption, and transport, if any, within the plant. Indeed, it has not been proven thus far that any tocopherol or tocotrienol form can be transported within the plant, and the general belief is that tocochromanols are exclusively found in plastids, where they are synthesized, despite the fact that little attention has been paid thus far to this issue and that some studies have already reported an extraplastidial localization for tocopherols. For instance, tocopherols have been found in chloroplasts, but also in vacuoles and nuclei of barley leaves (Rautenkranz *et al.*, 1994) and in both chloroplasts and mitochondria of green algae (Shigeoka *et al.*, 1986; Kusmic *et al.*, 1999). In seeds, it is generally thought that tocochromanols are exclusively present in plastids, although some studies have found tocochromanols associated with cytoplasmic lipid bodies (White *et al.*, 2006). Although studies of exogenous applications of tocochromanols have not been very successful thus far at the whole-plant level, exogenous applications of these compounds or related biosynthesis inhibitors have been extraordinarily informative when working at the cellular or subcellular levels, and these compounds have been found to have a role in the protection of thylakoids or other purified membranes from oxidative stress. For instance, the discovery of the function of α -tocopherol in protecting photosystem II from singlet oxygen using this approach (Trebst *et al.*, 2002; Kruk *et al.*, 2005) or recent studies about the protective role of tocopherols in photosynthetic (model) membranes (Hincha, 2008) are remarkable.

The second approach, very successful thanks to the development of modern chromatographic techniques (nowadays supported by mass spectrometry), has been the determination of endogenous concentrations of tocochromanols prior to and during the physiological process subject

to study. In this case, several studies have shown a relationship between several tocopherol forms and a number of physiological processes, including seed germination (Puntarulo, 1993), flowering (Battle *et al.*, 1977), leaf senescence (Peisker *et al.*, 1989; Chrost *et al.*, 1999; Munné-Bosch and Peñuelas, 2003), and plant responses to a number of abiotic stresses (reviewed by Munné-Bosch, 2005). Although limited by the possible lack of a direct cause-effect relationship, this approach is very common and is needed to unravel tocopherol function in plants, especially in combination with the third approach, that is the use of mutants and transgenic plants, which have revolutionized our understanding of the regulation of plant physiological processes, and the function of tocopherols in plants has been no exception. Indeed, the last decade has seen a radical change in the appreciation of the biological role of tocopherols in plants thanks to a detailed characterization of plants with altered biosynthesis of tocopherols and tocotrienols, including several *Arabidopsis thaliana* mutants, the *sucrose export defective1* (*sxd1*) maize mutant, and some transgenic potato and tobacco lines with altered tocopherol levels (Fig. 2).

The first studies on vitamin E mutants in higher plants were performed on the model plant *A. thaliana*. Porfirova *et al.* (2002) isolated the *vte1* mutant from this species using a TLC-based screening approach and showed that this mutant lacks all four tocopherol forms due to a deficiency in tocopherol cyclase, an enzyme later found in the plastoglobuli of chloroplasts that is essential for the formation of the chromanol ring of tocopherols (Austin *et al.*, 2006). Recently, it has also been shown that this mutant lacks not only tocopherols, but also plastochroma-

anol-8 (PC-8; Zbierzak *et al.*, 2010). PC-8 displays some antioxidant activity, comparable with that of tocopherols (Olejnik *et al.*, 1997), but PC-8 makes up only 5–10% of total tocopherols in *A. thaliana*, thus suggesting that most of the effects observed in *vte1* mutants are indeed caused by tocopherol deficiency (Zbierzak *et al.*, 2010). Growth of the *vte1* mutant, chlorophyll content, and photosynthetic quantum yield were similar to those of the wild type under low light conditions ($120 \mu\text{mol m}^{-2} \text{s}^{-1}$). After exposure for 5 d at higher light intensity ($850 \mu\text{mol m}^{-2} \text{s}^{-1}$), chlorophyll contents and photosynthetic quantum yield were only slightly reduced in the *vte1* mutant as compared with the wild type (Porfirova *et al.*, 2002). Therefore, it was concluded that the absence of tocopherols has no large impact on photosynthesis or plant viability under low light conditions and may have a limited role in photosynthesis under high light stress. Prior to this study, tocopherols were presumed to have an antioxidant function in plants similar to that found in animals and were thought to play a major role as antioxidants in photosynthesis and plant defence responses (reviewed by Fryer, 1992; Munné-Bosch and Alegre, 2002a). Many questions have therefore arisen as a consequence of these initial studies in tocopherol-deficient plants. Are tocopherols really essential for survival in plants? Do they play any major role in photoprotection?

These questions appear to be particularly relevant from an evolutionary perspective, since it is known that tocopherols are only produced by photosynthetic organisms (including algae, see Supplementary Table S1 available at *JXB* online). Indeed, it has recently been shown in an analysis of 266 species that tocopherols are present in all photosynthetic organisms, and they seem to accumulate to a higher extent in vascular plants than in ancient photosynthetic organisms (Esteban *et al.*, 2009). Why, therefore, do tocopherols accumulate in photosynthetic organisms, and particularly at high concentrations in vascular plants? Recent studies in mutant and transgenic lines have shed light on the photoprotective role of tocopherols in plants. As will be discussed later, tocopherols appear to be particularly relevant under severe photo-oxidative stress conditions (Havaux *et al.*, 2005) and, when the stress is not severe, other protection mechanisms, such as the xanthophyll cycle and other antioxidants, may partly compensate for tocopherol deficiency.

Also of significance has been the discovery of new, unexpected biological functions for tocopherols thanks to the study of transgenic lines and mutants (Fig. 3). The most striking effects observed in tocopherol-deficient plants include defects in germination and seedling growth (Sattler *et al.*, 2004, 2006), a block of carbohydrate export and anthocyanin accumulation in source leaves, which in some circumstances may be accompanied by reduced plant growth (Hofius *et al.*, 2004; Maeda *et al.*, 2006, 2008), and, finally, premature senescence (Abbasi *et al.*, 2009). Furthermore, it has recently been shown that γ -tocopherol cannot substitute for α -tocopherol under salt-induced oxidative stress, and that it improves the plant's physiological status

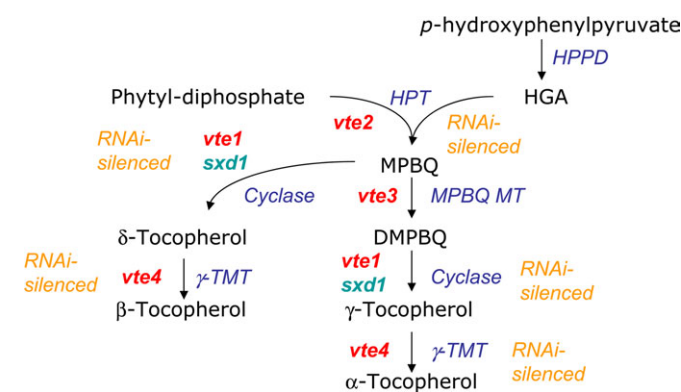


Fig. 2. Tocopherol biosynthetic pathway. Mutant and transgenic plants deficient in tocopherols have been obtained for all key steps of the tocopherol biosynthetic pathway. *Arabidopsis thaliana* and maize mutants are depicted in red and green, respectively. RNAi-silenced plants for key biosynthetic steps have also been obtained for potato and tobacco plants (marked in yellow). Cyclase, tocopherol cyclase; DMPBQ, 2,3-dimethyl-5-phytyl-1,4-benzoquinone; HGA, homogentisic acid; HPPD, *p*-hydroxyphenylpyruvate dioxygenase; HPT, homogentisate phytoltransferase; MPBQ, 2-methyl-6-phytyl-1,4-benzoquinone; MPBQ MT, MPBQ methyltransferase; γ -TMT, γ -tocopherol methyltransferase.

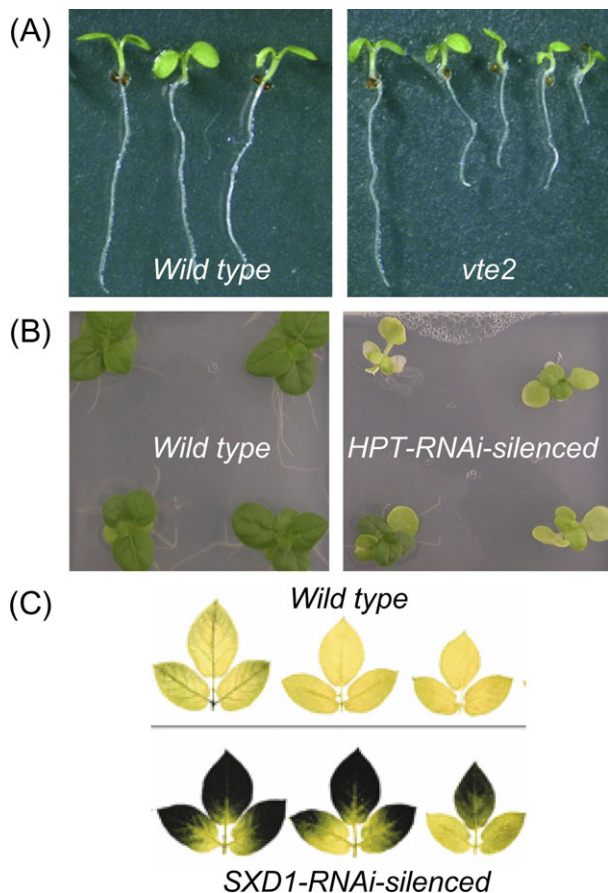


Fig. 3. Characteristic phenotypes in mutant and transgenic tocopherol-deficient plants. Depending on the plant species, plant developmental stage, magnitude of tocopherol deficiency, and growth conditions, tocopherol deficiency can be dispensable and not lead to a visible phenotype, or can lead in contrast to characteristic, dramatic phenotypes, as shown here. (A) Defects in germination and seedling growth, as has been shown in *vte2* mutants of *A. thaliana* (Sattler *et al.*, 2004). (B) Higher susceptibility to photo-oxidation under severe stress, as shown in transgenic tobacco plants constitutively silenced for homogentisate phytyl-transferase (HPT) exposed to severe salt stress (300 mM NaCl) (Abbasi *et al.*, 2007). (C) Block of photoassimilate export characterized by callose deposition and carbohydrate accumulation, as has been shown with the accumulation of starch in source leaves of potato plants by iodine (KI) staining (Hofius *et al.*, 2004). Images reproduced with permission from the Copyright Clearance Center.

under sorbitol-induced osmotic stress (Abbasi *et al.*, 2007). More recently, it has also been shown that tocotrienols can also protect photosynthetic membranes from photo-oxidative stress at low temperatures (Matringe *et al.*, 2008), and that accumulation of β -tocopherol instead of α -tocopherol improves tolerance to photo-oxidative stress in a xanthophyll-deficient strain of *Chlamydomonas reinhardtii* (Sirikhachornkit *et al.*, 2009). Why, therefore, are plants selectively accumulating tocopherols, and particularly α -tocopherol, in chloroplasts? Why are tocotrienols only present in a range of unrelated plant groups and found almost exclusively in seeds and fruits?

Tocochromanols as antioxidants: evidence or presumed role?

The biochemical properties of tocochromanols were first reviewed extensively by Kamal-Eldin and Appelqvist (1996) and they will be referred to here in brief, highlighting those points needed for the understanding of the antioxidant function of tocochromanols in plants, and with a particular emphasis on the differences between different tocochromanols. Tocochromanols are lipophilic molecules which are anchored in membranes by their polyprenyl chain, placing the chromanol moiety in the lipid–water interfacial region of the membrane (Kagan and Quinn, 1988; Gómez-Fernández *et al.*, 1989). Although generally underestimated in the literature, tocochromanols can not only play a role as lipophilic antioxidants but can also act as important membrane structure-stabilizing agents (Wang and Quinn, 1999). The excellent antioxidant activity of the tocochromanols is mainly due to the capacity of their heterocyclic chromanol ring system to donate the phenolic hydrogen to lipid-free radicals (Kamal-Eldin and Appelqvist, 1996). In homogeneous solutions the electron-releasing substitutions in the vicinity of the hydroxyl group at the chromanol ring system seem to be the main factor determining the relative antioxidant effectiveness, with α -tocochromanols being more potent as hydrogen donors than β -, γ -, and finally δ -tocochromanols. However, biological membranes consist of a complex assortment of polar lipids not distributed homogeneously and differing in polar head groups and hydrocarbon substituents. Consequently, the antioxidant potency of α -tocopherol has been shown to depend not solely on its chemistry, but also on its mobility and accessibility within the membrane (Serbinova *et al.*, 1991). While α -tocopherol and α -tocotrienol have equal antioxidant activity *in vitro* when analysed in hexane, α -tocotrienol exhibits even significantly greater peroxy radical scavenging potency when incorporated into phosphatidylcholine liposomes (Suzuki *et al.*, 1993). α -Tocopherol has been proposed not to be homogeneously distributed in membranes, but rather to be associated with the most fluid zones, placing it in the vicinity of the most unsaturated fatty acyl chains (Gómez-Fernández *et al.*, 1989). Moreover, α -tocopherol in a mixed dispersion preferentially interacts with phosphatidylethanolamine rather than with phosphatidylcholine (Wang and Quinn, 2000). Since the polyprenyl chain of tocotrienols is 3-fold unsaturated, it is expected that their three-dimensional molecular structure and behaviour in a lipid bilayer differ from those of tocopherols. It has been proposed that α -tocotrienol is located closer to the membrane surface compared with α -tocopherol and imposes more motional anisotropy on the membrane (Suzuki *et al.*, 1993). Furthermore, it has been proposed that α -tocotrienol has a higher antioxidant potency than α -tocopherol, due to the combined effects of higher recycling efficiency, a more uniform distribution in the membrane bilayer, and a stronger disordering effect on membrane lipids (Kamal-Eldin and Appelqvist, 1996). However, are all these results obtained in model membranes applicable to what happens in nature?

This question has been partly considered by Hinch (2008), who investigated the effects of α -tocopherol on vesicle stability during freezing and on lipid dynamics in liposomes resembling chloroplast membranes. In this study, it was shown that α -tocopherol had a pronounced influence on membrane dynamics and showed strong interactions in its effects on membrane stability during freezing with the cryoprotectant sucrose. Interestingly, α -tocopherol showed maximal effects at low concentrations, close to its concentration in chloroplast membranes, thus suggesting that tocopherol concentrations in photosynthetic membranes may be finely regulated to modulate membrane stability adequately. It would therefore be a mistake to assume that the more tocopherols plants accumulate the better they are protected against oxidative stress, since an overaccumulation of tocopherols can negatively affect the fluidity of photosynthetic membranes, especially when not counteracted by changes in other components of the membrane that determine its fluidity. Furthermore, the role, if any, of tocopherols as membrane-stabilizing agents in other plant systems (flowers, fruits, and seeds) where they accumulate in compartments with contrasting lipid compositions still needs to be established.

As antioxidants, the principal role of tocopherols and tocotrienols is believed to be in the scavenging of lipid peroxyl radicals, which are responsible for propagating lipid peroxidation (Liebler, 1993), which has been confirmed during the last decade in different systems. By using tocopherol-deficient mutants of the cyanobacterium *Synechocystis*, Maeda *et al.* (2005) showed that tocopherol deficiency enhances the sensitivity to linoleic or linolenic acid treatments in combination with high light, consistent with tocopherols playing a crucial role in protecting cells from lipid peroxidation. The role of tocopherols in controlling the extent of lipid peroxidation has also been confirmed in higher plants. By using the *vte1* and *vte2* mutants of *A. thaliana*, Sattler *et al.* (2004) showed that tocopherols are essential for seed longevity and for protecting lipids from oxidation during germination and early seedling growth. Moreover, in a similar approach, the same group showed that tocopherols regulate gene expression and defence responses by modulating the levels of lipid peroxidation products (Sattler *et al.*, 2006). In this study, *vte1* and *vte2* mutants exhibited higher oxylipin levels than the wild type, thereby causing major changes in gene expression in tocopherol-deficient plants. The most evident changes were observed in the *vte2* mutants, which lack both tocopherol and the biosynthetic precursor 2,3-dimethyl-6-phytyl-1,4-benzoquinone. It appears therefore that tocopherols play a crucial role in controlling the extent of lipid peroxidation during germination and early seedling growth, but the mature plants adapt very well to tocopherol deficiency via the induction of compensatory mechanisms. For example, tocopherol-deficient *vte1* *A. thaliana* mutant seedlings have high amounts of lipid peroxides, but the mature plants display only a slightly abnormal phenotype. In these mutants, tocopherol depletion results in an increase in ascorbate and glutathione, whereas accumulation of toco-

pherols in VTE1-overexpressing plants leads to a decrease in ascorbate and glutathione (Kanwischer *et al.*, 2005). Control of redox homeostasis by tocopherols, ascorbate, and glutathione therefore appears to be intimately interconnected. While tocopherols control the extent of lipid peroxidation, ascorbate and glutathione act as cellular redox buffers controlling, among others, the levels of hydrogen peroxide and protein thiol status (Foyer and Noctor, 2005). By controlling hydrogen peroxide levels, ascorbate also avoids the formation of the highly reactive hydroxyl radicals by the Fenton reaction and therefore prevents, although indirectly, lipid peroxidation (Halliwell and Gutteridge, 1999). Moreover, the ascorbate–glutathione cycle supports the antioxidant function of tocopherols by recycling tocopheroxyl radicals, which result from the reaction of tocopherols with lipid peroxyl radicals (Fig. 4). In relation to this, it has been shown that *vte1* mutants of *A. thaliana*, which are deficient in ascorbate, exhibit α -tocopherol loss under severe water deficit (Munné-Bosch and Alegre, 2002b).

Apart from their roles as membrane-stabilizing agents and scavengers of lipid peroxyl radicals, tocopherols are also excellent quenchers and scavengers of singlet oxygen. Tocopherols may therefore play an additional role as antioxidants by controlling the lifetime of reactive oxygen species. It has been estimated that, before being degraded, one molecule of α -tocopherol can deactivate up to 120 singlet oxygen molecules by resonance energy transfer (Fahrenholtz *et al.*, 1974). This will be referred to here as ‘quenching’ of singlet oxygen, since it is a physical reaction that leads to destruction of several molecules of singlet

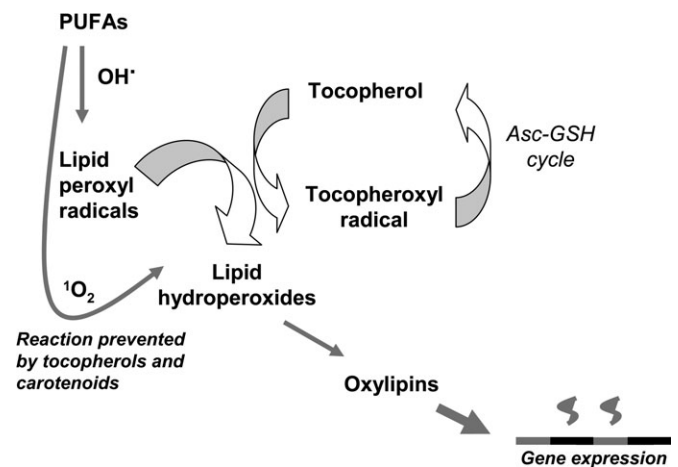


Fig. 4. Tocopherols contribute to controlling redox homeostasis and gene expression by modulating the extent of lipid peroxidation in leaves, and therefore oxylipin levels in plants. Tocopherols inhibit the propagation of lipid peroxidation by scavenging lipid peroxyl radicals and prevent lipid peroxidation by reacting with other reactive oxygen species, such as singlet oxygen, in cooperation with carotenoids. The antioxidant activity of tocopherols is supported by the ascorbate–glutathione (Asc-GSH) cycle, which allows recycling of tocopheroxyl radicals to tocopherols.

oxygen while tocopherols are consumed in very low amounts. Furthermore, tocopherols can also react chemically with singlet oxygen and are destroyed (Fukuzawa *et al.*, 1997). In this case, this is called 'scavenging' of singlet oxygen. This reaction in plant oils *in vitro* occurs through an intermediate, hydroperoxydienone, which decomposes to form tocopherol quinone, which later gives rise to tocopherol quinol and tocopherol quinone epoxides (Neely *et al.*, 1988; Murkovic *et al.*, 1997). Tocopherol quinones and tocopherol quinol have been detected in significant amounts in several plant tissues, and some studies suggest they are formed under photo-oxidative stress conditions as a consequence of the oxidation of α -tocopherol (Yamauchi and Matsushita, 1979; Munné-Bosch *et al.*, 2005; Kobayashi and DellaPenna, 2008). Interestingly, it has recently been shown that α -tocopherol quinone can be converted back to α -tocopherol in chloroplasts of *A. thaliana*, thus indicating the presence of a recycling pathway for oxidized tocopherols (Kobayashi and DellaPenna, 2008). Furthermore, a new γ -tocopherol oxidation product has been reported in plants, 5-nitro- γ -tocopherol, which is thought to be the result of the nitration of γ -tocopherol, although the biological significance of this reaction is still unclear (Desel *et al.*, 2007).

Interplay between α -tocopherol and carotenoids also appears to be particularly relevant in plant responses to photo-oxidative stress. It has been suggested that α -tocopherol and β -carotene cooperate in limiting singlet oxygen-induced damage to photosystem II in *C. reinhardtii* exposed to herbicides (Trebst *et al.*, 2002). Accumulating evidence indicates that singlet oxygen induces loss of photosystem II activity, decreases D1 protein levels, and leads to pigment bleaching during photoinhibition of photosystem II (Trebst *et al.*, 2002; Krieger-Liszkay and Trebst, 2006). Recent studies indicate that reactive oxygen species act primarily by inhibiting the repair of photodamaged photosystem II reaction centres and not by damaging them directly (Nishiyama *et al.*, 2006). Indeed, Bakasch (2008) found evidence that the repair of D1 was primarily inhibited in a tocopherol-deficient mutant of *Synechocystis* sp. PC6803 and consequently there was a lower level of the D1 protein.

Since compelling evidence suggests that tocopherols play a role in the protection of the photosynthetic apparatus (as a membrane-stabilizing agent, and controlling the extent of lipid peroxidation and the lifetime of reactive oxygen species), how is it therefore possible that early studies on *vtl* tocopherol mutants in *A. thaliana* suggested that this antioxidant may play at best a limited role in photo-protection? Indeed, studies by Porfirova *et al.* (2002) showing only subtle differences in *A. thaliana* mutants deficient in tocopherols exposed to high light were confirmed later by different research groups (Havaux *et al.*, 2005; Maeda *et al.*, 2006; Munné-Bosch *et al.*, 2007). How is this reconciled with the selection pressure leading to the accumulation of tocopherols in all photosynthetic organisms? Since the tocopherol precursor 2,3-dimethyl-5-phytyl-1,4-benzoquinone also displays some antioxidant activity (Sattler *et al.*, 2004), why has nature not selected this compound instead of tocopherol? Or, if tocotrienols can

also potentially protect thylakoids from photo-oxidation (Matringe *et al.*, 2008), why do plants not use tocotrienols instead of tocopherols for the protection of the photosynthetic apparatus?

At this point, it is important to consider the photo-protection and antioxidant defence mechanisms as a whole. There are a number of mechanisms of photoprotection in plants, but for the dissipation of excess excitation energy in chloroplasts all plants use the xanthophyll cycle-dependent energy dissipation as heat and the formation and elimination of reactive oxygen species (either the superoxide anion formed in photosystem I or singlet oxygen formed in photosystem II) (Asada, 2006). From an evolutionary point of view, nature has selected xanthophyll cycle-dependent energy dissipation as one of the most important mechanisms of photoprotection, both by its presence in all photosynthetic organisms (Esteban *et al.*, 2009) and by its importance in dissipating high amounts of excess excitation energy (Demmig-Adams and Adams, 1996). Furthermore, aside from other antioxidant mechanisms, nature has also selected and conserved the presence of tocopherols in all photosynthetic membranes (Esteban *et al.*, 2009). Tocopherols appear to be 'primary' metabolites, while tocotrienols might be considered 'secondary' metabolites in the sense that the latter are not present in all photosynthetic organisms. However, if tocopherols have been selected and conserved by nature, why do *vtl* mutants of *A. thaliana*, deficient in tocopherols, not clearly show increased sensitivity to photo-oxidation? As suggested by Sattler *et al.* (2004) a simple explanation might be that the tocopherol precursor 2,3-dimethyl-5-phytyl-1,4-benzoquinone compensates the deficiency of tocopherol, since this precursor also possesses some antioxidant activity. In addition, the ecophysiology of the model plant *A. thaliana* should be considered. All these studies have been performed with mutants in a Columbia background, which is an accession originally from temperate climates. Depending on the type and magnitude of stress imposed on plants, they show a different degree of phenotypic plasticity, which may influence the activation of adequate compensatory mechanisms for any deficiency. Since the Columbia accession naturally grows in temperate climates with occasional exposure to high light intensities, the plant may be able to acclimate more easily to this type of stress by other means, such as the activation of compensatory mechanisms. Indeed, Havaux *et al.* (2005) identified two such compensatory mechanisms under high light stress: xanthophyll cycle-dependent energy dissipation by non-photochemical quenching of photosystem II; and synthesis of zeaxanthin. Interestingly, it has been shown from an evolutionary point of view that tocopherols accumulate to a higher extent in vascular plants than they do in ancient photosynthetic organisms, while carotenoids (and particularly zeaxanthin) are more abundant in the latter (Esteban *et al.*, 2009). Also, it has been suggested that anthocyanins may, at least partly, compensate for tocopherol deficiency when *A. thaliana* plants are exposed to a combination of high light and low temperature (Munné-Bosch *et al.*, 2007). It appears therefore that α -tocopherol

may play a significant role in photoprotection that is evident when compensatory mechanisms fail. Indeed, differences in photo-oxidative stress markers were observed between tocopherol-deficient mutants and the wild type in leaf discs exposed to short-term high light stress and in entire plants exposed to prolonged severe stress combining high light with low temperatures (Havaux *et al.*, 2005). When stress is more severe, the greater therefore is the likelihood to find phenotypic differences between wild-type and tocopherol-deficient plants. Similarly, the most striking differences are observed when two or more protective mechanisms are altered in mutants. In this regard, Kanwischer *et al.* (2005) found that *vte1cad2* double mutants of *A. thaliana* are more sensitive to photo-oxidative stress than *vte1* or *cad2* single mutants, thus indicating that the simultaneous loss of tocopherols and glutathione affects the stability and the efficiency of the photosynthetic apparatus. Also, it has been shown that *A. thaliana* mutants lacking xanthophyll cycle-dependent energy dissipation accumulate higher amounts of tocopherols under high light (Golan *et al.*, 2006). Moreover, leaves of the *npq1* mutant of *A. thaliana*, which is deficient in violaxanthin de-epoxidase, accumulate higher amounts of α -tocopherol during acclimation to high light, thus suggesting interdependence between α -tocopherol and zeaxanthin in plant responses to high light stress (Havaux *et al.*, 2000). Furthermore, it has recently been shown that a triple *A. thaliana* mutant (*vte1 npq1 pdx1*) deficient in tocopherols, zeaxanthin, and vitamin B6 exhibited an increased sensitivity to high light, showing severe pigment loss and enhanced lipid peroxidation (Havaux *et al.*, 2009). This study also shows that *pdx1* mutants exposed to high light exhibit lower tocopherol levels, together with enhanced singlet oxygen production and lipid peroxidation, compared with the wild type, thus suggesting tocopherols are consumed to a greater extent in vitamin B6-deficient plants. It appears therefore that tocopherols are part of an intricate network of photoprotection mechanisms that act in concert in a plastic response to protect photosynthetic membranes from photo-oxidation, and are particularly relevant under severe stress or when other protection mechanisms are absent or fail.

New emerging roles for tocopherols

When one wants to understand the different functions of tocopherols in plants, it is very helpful to keep in mind new observations concerning the function of vitamin E in humans. Among vitamin E compounds, α -tocopherol has been shown to accumulate selectively in the human body and to be the most effective form as an antioxidant and in the regulation of cellular signalling and gene expression in animals (Brigelius-Flohé *et al.*, 2002). It is generally believed that α -tocopherol plays an important antioxidant role against reactive oxygen species and lipid peroxyl radicals generated as a result of aerobic metabolism and to help prevent human diseases associated with oxidative stress, such as cardiovascular disease, cancer, chronic inflamma-

tion, and neurological disorders (Ricciarelli *et al.*, 2002). Furthermore, studies on animal models suggest additional mechanisms of action of α -tocopherol that are independent of its antioxidant function such as the regulation of membrane-associated signalling pathways and the modulation of gene expression (Azzi *et al.*, 1998; Brigelius-Flohé *et al.*, 2002). Tocopherols may affect gene expression at the transcriptional level or regulate gene expression indirectly by modulating protein kinase C and phospholipase A₂ activities. α -Tocopherol and β -tocopherol show differential effects on gene expression, which have been attributed to a non-antioxidant mechanism of α -tocopherol in gene regulation. In addition, tocopherol-associated proteins (TAPs) translocate from the cytosol to the nucleus in animal cells, where they activate gene transcription in an α -tocopherol-dependent manner (Yamaguchi *et al.*, 2001). Finally, it is worth mentioning that Gianello *et al.* (2005) detected α -tocopheryl phosphate in biological tissues including liver and adipose tissue, suggesting a ubiquitous presence in animal and plant tissue. This observation is extremely important, since α -tocopheryl phosphate is much more water-soluble than free α -tocopherol and undetectable by standard analytical methods used to identify and quantify tocopherols. It will be of great importance, therefore, to elucidate if α -tocopheryl phosphate is also present in plants.

Some of the most unexpected discoveries related to the functions of antioxidants in plants have recently been achieved by using mutant and transgenic lines deficient in tocopherols. Studies performed during the last decade by using *vte1* and *vte2* *A. thaliana* and *sxd1* maize mutants as well as in SXD1-RNAi-silenced potato plants suggest that tocopherols may be involved in the regulation of photoassimilate export in leaves, thereby affecting carbohydrate metabolism, source-sink relationships, and growth. Sattler *et al.* (2003) provided some of the first evidence of a role for tocopherols in photoassimilate export and growth in plants, with their discovery that maize *SXD1* encodes a tocopherol cyclase similar to *A. thaliana* VTE1. The *sxd1* mutant of maize was identified originally based on a block in sucrose transport from leaves, which is caused by callose occlusion of a specific class of plasmodesmata between the bundle sheath and vascular parenchyma cells, and is characterized by overall growth reduction and source leaf-specific accumulation of anthocyanins and starch (Russin *et al.*, 1996; Botha *et al.*, 2000; Provencher *et al.*, 2001). These results were confirmed in potato plants, which, in contrast to maize, have a C3-type of photosynthetic metabolism. By using an RNA interference (RNAi) silencing approach, Hofius *et al.* (2004) showed that tocopherol deficiency leads to a photoassimilate export-deficient phenotype that is very similar to that observed in maize. These transgenic potato plants showed enhanced callose deposition in source leaves, lower photosynthetic capacity, and altered gene expression compared with the wild type. This study provided evidence that the impact of tocopherol deficiency on plasmodesmata function and carbohydrate metabolism was similar in monocot (maize) and dicot (potato) species and could not

be assigned to specific anatomical or biochemical features of C4 metabolism.

Tocopherol-deficient *A. thaliana* mutants were also shown to exhibit a phenotype that is analogous to that of *sxd1* but that it is inducible only at low temperatures. Maeda *et al.* (2006) showed that tocopherol deficiency in the *A. thaliana* *vte2* mutant, and to a lesser extent in the *vte1* mutant, leads to a photoassimilate export-deficient phenotype. This was associated with callose occlusion of plasmodesmata, but only at later time points of low temperature treatment, strongly suggesting that impaired transfer cell wall development in phloem parenchyma cells rather than plasmodesmata is the leading cause of the downstream phenotypes. Thus, the linkage between deficiency of tocopherols and reduced export of photoassimilates appeared to be conserved among all tocopherol-deficient mutants identified thus far (maize *sxd1*, potato *VTE1*-RNAi, and low-temperature-treated *A. thaliana* *vte1* and *vte2*). The mechanism by which tocopherols affect callose deposition in leaves was still poorly understood, and was complicated by the observation that this effect was observed only in specific cell types. Because callose formation is strongly correlated with oxidative damage to membrane lipids (Yamamoto *et al.*, 2001), and tocopherols function effectively in the scavenging of lipid peroxyl radicals responsible for the propagation of lipid peroxidation, it was suggested that tocopherol deficiency may indirectly affect callose synthesis by increasing the extent of lipid peroxidation in the chloroplast membranes (Hofius *et al.*, 2004). The story appears to be even more complex however. It has recently been shown that homogentisate phytyltransferase (HPT)-deficient tobacco plants exhibit a decreased photosynthetic performance but no evidence for sugar export block, thus compromising evidence obtained thus far in other plant models (Abbasi *et al.*, 2009). How is that possible? These authors suggest that the sucrose export phenotype is more clearly observed in tocopherol-deficient plants in species with a higher sink demand, such as potato or maize, and also in other species when grown at low temperature, such as *A. thaliana*. Again, the phenotype caused by tocopherol deficiency appears to be more obvious depending on the growth conditions and plant species studied.

Most importantly, do all those phenotypes associated with tocopherol deficiency indicate that tocopherols play a direct role in all these physiological processes? It is obvious that all these previous reports clearly support functions for tocopherols beyond their antioxidant activity in photosynthetic membranes, but further research is needed to unravel some of the direct cause–effect relationships attributed in previous studies using mutants and transgenic lines, since it is very likely that at least part of the observed phenotypes are simply due to pleiotropic effects caused by the inactivation of tocopherol biosynthetic genes. This might be an example of correlative variation, as discussed by Darwin (1859) to occur in nature, but induced nowadays by advances in molecular biology and applied here to the problem of understanding tocopherol functions in plants. Understanding this correlative variation, i.e. how tocopherol deficiency affects so *a priori*

unrelated, different physiological processes is one of the most important challenges in the near future. Fortunately, it is well known nowadays that such processes (photosynthesis, photoassimilate transport, growth, and senescence) are intimately connected at the whole-plant level, thus a change in the levels of an antioxidant such as tocopherols in chloroplasts can have a tremendous effect at the whole-plant level. It should also be considered that the lack of tocopherols in specific organs does not negatively affect plant function at particular developmental stages and growth conditions, but it severely limits survival in other circumstances. The adaptive plasticity of the studied species to the specific situation will determine the outcome of the physiological response observed. The window of the physiological response, and therefore the potential to observe a particular phenotype in response to tocopherol deficiency, is increased in species growing in their natural habitat or at least in conditions that simulate their natural habitat. For instance, more phenotypes in response to tocopherol deficiency are observed in *A. thaliana* grown at low temperature simply because the accession used in these studies is adapted to grow under these conditions in nature.

Finally, some recent reports suggesting specific roles for γ -tocopherol in leaves, an aspect poorly understood to date will be considered here. A recent study in transgenic tobacco plants constitutively silenced for HPT and γ -tocopherol methyltransferase (γ -TMT) activity revealed that α -tocopherol deficiency increases the sensitivity of HPT:RNAi transgenics towards methyl viologen, an inductor of photo-oxidative stress, and to severe salt and sorbitol stresses (Abbasi *et al.*, 2007). Interestingly, this study also found that γ -TMT-silenced plants, which accumulate γ -tocopherol instead of the α isoform, show a decrease in salt tolerance but an improved performance when challenged with sorbitol-induced osmotic stress (Abbasi *et al.*, 2007). This study suggests specific functions for different tocopherol homologues. Indeed, the authors postulated that the function of γ -tocopherol may be particularly relevant in desiccated tissues, such as the seeds, where this isoform is known to accumulate in significant amounts. However, γ -tocopherol also accumulates in leaves of several species together with α -tocopherol. Although this accumulation has generally been regarded as a consequence of being its immediate precursor, γ -tocopherol appears to accumulate selectively in leaves of several species at specific developmental stages (mainly in emerging, young leaves), thus suggesting an additional specific role for this homologue (Szymanska and Kruk, 2008a, b). Furthermore, it has been shown that water deficit specifically induces γ -tocopherol accumulation in leaves of the CAM plant *Aptenia cordifolia* (Cela *et al.*, 2009). Taken together, all these results obtained in different plant systems and experimental conditions suggest that aside from its potential as an antioxidant and important role as a precursor of α -tocopherol, γ -tocopherol could exert additional functions in plant resistance to osmotic stress and/or desiccation tolerance. Further research is, however, needed to unravel these possible specific functions of γ -tocopherol.

Tocotrienols: the hidden part in vitamin E research

While tocopherols are ubiquitously present in all plants and in almost all plant parts, tocotrienols are only present in a range of unrelated plant groups and are almost exclusively found in seeds and fruits. The presence and distribution of tocotrienols in seeds suggest that they fulfil specific functions which differ from the function of tocopherols in leaves. In the case of cereals the capability to produce tocotrienols could be shown to be a result of a tocotrienol-specific prenyltransferase. On the other hand, plants which normally are not accumulating tocotrienols could be shown to produce tocotrienols after transgenic manipulation of the biosynthetic pathway upstream of the prenylation step. Therefore, the presence of tocotrienols in diverse plant groups might be related to more than a single gene. So far, the function of tocotrienols in plants is not clear and will be discussed here in the context of their tissue-specific distribution in seeds.

Biochemical and molecular studies indicate that tocopherols and tocotrienols share the same biosynthetic pathway (Schultz *et al.*, 1991; Dörmann, 2003). The tocochromanol head group is derived from metabolized tyrosine (shikimic acid pathway) and the hydrophobic tail is synthesized via the plastidic 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway (DellaPenna and Last, 2006). All genes coding for enzymes of the tocochromanol biosynthetic pathway appear to be single copy genes in plants (DellaPenna, 2005a). Only recently, the capability of cereals to produce tocotrienols could be shown to be the result of a single additional homogentisate geranylgeranyltransferase (HGGT) (Cahoon *et al.*, 2003). This key enzyme condenses homogentisate derived from the shikimate pathway with geranylgeranyldiphosphate from the MEP pathway. In the case of tocopherols, phytyldiphosphate is used instead by HPT (Soll *et al.*, 1980; Collakova and DellaPenna, 2001; Savidge *et al.*, 2002). Only the HPT from *Synechocystis* sp. PC6803 could be shown to be able to use geranylgeranyldiphosphate as substrate, although with a much lower efficiency (Collakova and DellaPenna, 2001). Accordingly, the introduction of a HGGT into several plants by transgenic approaches could be shown to be sufficient to produce tocotrienol in green leaves of plants (Cahoon *et al.*, 2003). In these transgenic plants the tocochromanol content increased >10-fold while the tocopherol content was unaltered. It seems that the tocopherol pool size in those leaves was still controlled whereas the accumulation of the additional tocotrienol was not. This might indicate different regulatory mechanisms for the accumulation of tocopherols and tocotrienols in plants.

Surprisingly, plants which do not normally accumulate tocotrienols and most probably do not have a tocotrienol-specific prenyltransferase could be shown to produce tocotrienols after transgenic manipulation of steps upstream of the prenylation of homogentisate (DellaPenna, 2005a). These genetic engineering approaches have in common an increase in the flux through the shikimic acid branch of the

tocochromanol biosynthetic pathway. Usually this flux is limited by negative feedback mechanisms exerted by tyrosine (Rippert and Matringe, 2002). By introducing a gene coding for a prephenate dehydrogenase from yeast (TYR1) or bifunctional chorismate mutase/prephenate dehydrogenase (TYRA) from *Escherichia coli* in tobacco an increased production of homogentisate was achieved, bypassing the regulation by tyrosine as a negative effector (Rippert *et al.*, 2004). An increased supply of homogentisate for the biosynthesis of tocochromanols could also be achieved by the introduction of a plastid-localized tyrosine aminotransferase from rat into *A. thaliana*, a plant which usually does not produce tocotrienols either in leaves or in seeds (Herbers, 2003). Again, an accumulation of tocotrienols was observed irrespective of the natural ability of the plant to produce tocotrienols or not. In such plants, tocotrienols even accumulate in leaves, while in plants which usually are capable of tocotrienol synthesis, such as, for example, tobacco, tocotrienols are present in seeds (Falk *et al.*, 2003) and are not found in leaves even under conditions with an increased geranylgeranyldiphosphate supply (Tanaka *et al.*, 1999). In a similar transgenic approach the tocochromanol content of *A. thaliana*, canola, and soybean seeds could be dramatically increased by co-expression of up to three genes involved in the biosynthetic pathway of tocochromanols, with tocotrienols being the dominant form (Karunanandaa *et al.*, 2005). Again, no HGGT was necessary to achieve the production of tocotrienols. One can speculate that the changes in the metabolic flow of this pathway might change the substrate specificity of the HPT (Karunanandaa *et al.*, 2005). Recently, cDNAs encoding a homogentisate solanyltransferases from *A. thaliana* (Venkatesh *et al.*, 2006; Tian *et al.*, 2007) and *C. reinhardtii* (Sadre *et al.*, 2006) were identified, with properties different from those of known phytyltransferases, suggesting that a separate prenyltransferase is involved in plastoquinone-9 biosynthesis.

So far, it is not known why tocotrienols are produced when the flux through the pathway is enhanced. Besides a specific prenyltransferase other factors might have an influence on the presence of tocotrienols in plants. One could speculate that either the increased supply of homogentisate may affect the substrate specificity of the HPT (Karunanandaa *et al.*, 2005), or another prenyltransferase could be responsible for the biosynthesis of tocotrienols in these plants. A paralogue of HPT (*vte2-2*), recently identified in *A. thaliana* (Venkatesh *et al.*, 2006) and shown to be involved in the biosynthesis of plastoquinone (Sadre *et al.*, 2006; Tian *et al.*, 2007), would be another possible candidate for the occurrence of tocotrienols at least in *A. thaliana*. However, the seed-specific expression of the *vte2-2* gene resulted only in an increased tocopherol content. The broader substrate specificity of the HPT from *Synechocystis* sp. could support the first explanation, since most of the tocochromanol pathway probably originates from a cyanobacterial ancestor (DellaPenna, 2005b). Both possible explanations could hold true for the presence of tocotrienols in quite distantly related plant families. The presence of tocotrienols can be the result of a second prenyltransferase,

Table 1. List of plant species containing tocotrienols as reported in the literature

Orders	Plant family	Plant species	References
Apiales	Apiaceae	<i>Anethum graveolens</i>	Matthäus <i>et al.</i> (2003)
		<i>Carum carvi</i>	Ivanov and Aitzetmüller (1995)
		<i>Coriandrum sativum</i>	Ivanov and Aitzetmüller (1995)
		<i>Foeniculum vulgare</i>	Ivanov and Aitzetmüller (1995)
Arecales	Arecaceae	<i>Bifora</i> sp.	SOFA ^a
		<i>Attalea speciosa</i>	SOFA ^a
		<i>Elaeis guineensis</i>	Ong (1993); Choo <i>et al.</i> (2004)
		<i>Cocos nucifera</i>	Sheppard <i>et al.</i> (1993); Chun <i>et al.</i> (2006)
		<i>Maximiliana maripa</i>	Bereau <i>et al.</i> (2001)
Asterales	Asteraceae	<i>Carthamus tinctorius</i>	Firestone (1999)
		<i>Chrysanthemum coronarium</i>	Matthäus <i>et al.</i> (2003)
Brassicales	Brassicaceae	<i>Brassica napus</i>	Sheppard <i>et al.</i> (1993)
		<i>Camelina sativa</i>	Budin (1995)
		<i>Raphanus sativus</i>	Matthäus <i>et al.</i> (2003)
	Capparaceae	<i>Capparis spinosa</i>	Matthäus and Özcan (2005)
<i>Capparis ovata</i>		Matthäus and Özcan (2005)	
Bromeliales	Bromeliaceae	<i>Ananas comosus</i>	Kato <i>et al.</i> (1983)
Caryophyllales	Amaranthaceae	<i>Amaranthus</i> sp.	Lehmann <i>et al.</i> (1994); Qureshi <i>et al.</i> (1996)
Celastrales	Celastraceae	<i>Euonymus europaea</i>	Ivanov and Aitzetmüller (1998)
Cucurbitales	Cucurbitaceae	<i>Cucumis sativus</i>	Matthäus <i>et al.</i> (2003)
		<i>Cucurbita pepo</i>	Murkovic <i>et al.</i> (1996)
		<i>Momordica charantia</i>	Matthäus <i>et al.</i> (2003)
		<i>Vaccinium myrtillus</i>	Chun <i>et al.</i> (2006)
Ericales	Ericaceae	<i>Vaccinium macrocarpon</i>	Chun <i>et al.</i> (2006)
		<i>Actinidi chinensis</i>	Chun <i>et al.</i> (2006)
Fabales	Caesalpinioideae	<i>Erythrophleum fordii</i>	Matthäus <i>et al.</i> (2003)
		Fabaceae	<i>Canavalia ensiformis</i>
	<i>Cicer arietinum</i>		Krishna <i>et al.</i> (1997)
	<i>Glycine max</i>		Sheppard <i>et al.</i> (1993)
	<i>Robinia pseudoacacia</i>		Ivanov and Aitzetmüller (1998)
	<i>Vigna radiata</i>	Krishna <i>et al.</i> (1997)	
Fagales	Betulaceae	<i>Corylus avellana</i>	Crews <i>et al.</i> (2005)
Gentianales	Boraginaceae	<i>Onosma armeniacum</i>	Bağci <i>et al.</i> (2003)
		<i>Onosma polioxanthum</i>	Bağci <i>et al.</i> (2003)
		<i>Anchusa leptophylla</i>	Bağci <i>et al.</i> (2003)
		<i>Anchusa froedini</i>	Bağci <i>et al.</i> (2003)
		<i>Gnetum</i> sp.	Matthäus <i>et al.</i> , 2003
Gnetopsida	Myristicaceae	<i>Iryanthus grandis</i>	Silva <i>et al.</i> (2001)
Magnoliales		Euphorbiaceae	<i>Aleurites montana</i>
Malpighiales	<i>Hevea brasiliensis</i>		Ong (1993); Hess (1993)
	<i>Plukenetia volubilis</i>		SOFA ^a
	<i>Sapium sebiferum</i>		Aitzetmüller <i>et al.</i> (1992)
	Clusiaceae	<i>Garcinia brasiliensis</i>	Bertoli <i>et al.</i> (1998)
<i>Cratoxylum sumatranum</i>		Seo <i>et al.</i> (2002)	
<i>Calophyllum inophyllum</i>		Matthäus <i>et al.</i> (2003)	
<i>Calophyllum calaba</i>		Crane <i>et al.</i> (2005)	
<i>Bixa orellana</i>		Frega <i>et al.</i> (1998)	
Malvales	Bixaceae	<i>Adansonia</i> sp.	Firestone (1999)
	Bombacaceae	<i>Shorea</i> sp.	Soulier <i>et al.</i> (1989)
	Dipterocarpaceae	<i>Gossypium</i> sp.	Firestone (1999)
	Malvaceae	<i>Abutilon</i> sp.	SOFA ^a
Tiliaceae		<i>Tilia argentea</i>	Lehmann <i>et al.</i> (1994)
Myrtales	Onagraceae	<i>Oenothera biennis</i>	Clough (2001)
Lamiales	Oleaceae	<i>Olea europea</i>	Hassapidou and Manoukas (1993)
		<i>Jasminum fruticans</i>	Ivanov and Aitzetmüller (1998)
		<i>Orobanche</i> sp.	Velasco <i>et al.</i> (2000)
Laurales	Orobanchiaceae	<i>Persea americana</i>	Beringer and Nothdurft (1979); Chun <i>et al.</i> (2006)
	Lauraceae		

Table 1. Continued

Orders	Plant family	Plant species	References
Pinales	Cupressaceae	<i>Juniperus communis</i>	Ivanov and Aitzetmüller (1998)
	Pinaceae	<i>Picea</i> sp.	Franzen <i>et al.</i> (1991)
		<i>Pinus pinea</i> , <i>P. sylvestris</i>	Bagci and Karaagacli (2004)
Polygonales	Polygonaceae	<i>Fagopyrum esculentum</i>	Tammela <i>et al.</i> (2005)
Proteales	Proteaceae	<i>Gevuina avellana</i>	Balz <i>et al.</i> (1992)
		<i>Macadamia tetraphylla</i>	Bertoli <i>et al.</i> (1998)
Ranunculales	Ranunculaceae	<i>Delphinium ajacis</i>	Sheppard <i>et al.</i> (1993)
Rhamnales	Vitaceae	<i>Nigella sativa</i>	Matthäus <i>et al.</i> (2003)
		<i>Vitis vinifera</i>	SOFA ^a
Rosales	Rosaceae	<i>Rosa canina</i>	Sheppard <i>et al.</i> (1993); Horvath <i>et al.</i> (2006)
Sapindales	Rosaceae	<i>Prunus amygdalus</i>	Zlatanov (1999)
		<i>Prunus tenella (amygdalus nana)</i>	Slover (1971)
		<i>Prunus americana</i>	Ivanov and Aitzetmüller (1998)
	Cannabaceae	<i>Prunus domestica</i>	Sheppard <i>et al.</i> (1993)
		<i>Prunus persica</i>	Chun <i>et al.</i> (2006)
		<i>Rosa canina</i>	Chun <i>et al.</i> (2006)
	Elaeagnaceae	<i>Cannabis sativa</i>	Ivanov and Aitzetmüller (1998)
		<i>Hippophaë rhamnoides</i>	Mölleken (1999)
		<i>Anacardium occidentale</i>	Kallio <i>et al.</i> (2002)
	Anacardiaceae	<i>Mangifera indica</i>	Sheppard <i>et al.</i> (1993)
		<i>Pistacia terebinthus</i>	Matthäus <i>et al.</i> (2003)
		<i>Aesculus sinensis</i>	Matthäus <i>et al.</i> (2003)
		<i>Litchi chinensis</i>	Lehmann <i>et al.</i> (1994); Matthäus and Özcan (2006)
		<i>Delavaya toxocarpa</i>	Matthäus <i>et al.</i> (2003)
		<i>Nephelium lappaceum</i>	Matthäus <i>et al.</i> (2003)
<i>Sapindus mukorossi</i>		Matthäus <i>et al.</i> (2003)	
<i>Ribes nigrum</i>		Clough (2001)	
Saxifragales	Grossulariaceae	<i>Nicotiana tabacum</i>	Falk <i>et al.</i> (2003)
	Solanaceae	<i>Nicandra physaloides</i>	SOFA ^a
Solanales	Convolvulaceae (Cuscutaceae)	<i>Ipomoea aquatica</i>	Matthäus <i>et al.</i> (2003)
		<i>Cuscuta</i> sp.	van der Kooij (2005)

^a The database 'Seed Oil Fatty Acids' (SOFA) is no longer accessible online. For a description of the database, see Matthäus *et al.* (2002).

correlation during early kernel development (Dolde *et al.*, 1999). On the other hand, White *et al.* (2006) found tocopherols associated with oil bodies, which might direct the discussion of intracellular localizations of tocopherols in a new direction. Interestingly, it has recently been shown that tocopherols can also potentially serve as antioxidants in photosynthetic membranes (Matringe *et al.*, 2008). These authors took advantage of the strong accumulation of tocopherols in leaves of double transgenic tobacco plants that co-expressed the prephenate dehydrogenase and hydroxyphenylpyruvate dioxygenase genes to study the antioxidant function of tocopherols *in vivo*. Tocopherols increased the tolerance of transgenic plants to high light stress at low temperatures, with photosystem II photo-inhibition and lipid peroxidation being maintained at low levels relative to wild-type plants (Matringe *et al.*, 2008). However, the biological significance of these results is limited by the fact that, to our knowledge, tocopherols have not been shown to accumulate in photosynthetic membranes in any plant species thus far. However, these results illustrate the potential antioxidant function of tocopherols in plants.

The presence and distribution of tocopherols in seeds suggests that they fulfil specific functions which differ from the function of tocopherols in leaves. One could speculate that seeds have a demand for specific antioxidants during desiccation. In orthodox seeds including cereal grains significant changes of the redox status occur during development and maturation (De Gara *et al.*, 2003). Several enzymes of the antioxidative network were shown to decrease during the desiccation phase. Tocopherols and specific tocopherols might compensate for the lack of these enzymes. In contrast, in recalcitrant seeds the ascorbate system is little affected (De Tullio and Arrigoni, 2003). It could be of importance that tocopherols are accumulating in those parts of dry seeds which survive and build up the new plant, while the tocopherols are located in the endosperm and pericarp, which finally die either before or during germination, respectively. It might be casual, but seeds of *G. avellana* and *M. tetraphylla*, which accumulate α -tocopherol almost exclusively in their embryo, have a relative short viability and are desiccation sensitive (Hamilton, 1957; Halloy *et al.*, 1996). Tocopherols could be shown to be important for the longevity of the non-

endospermic seeds from *A. thaliana* since seeds of tocopherol-free mutants lost their viability very quickly (Sattler *et al.*, 2004).

It will be essential to identify the exact localization of the tocotrienols in the cells to better understand what is special about tocotrienols. If the tocotrienols are present as antioxidants it will be necessary to identify the targets they are supposed to protect. New techniques such as the use of Raman microscopy (Beattie *et al.*, 2007) might help to identify the tissue-specific or even the cellular localization of tocochromanols and therefore unravel this unsolved question

Conclusions and perspectives

The last decade has seen a radical change in the appreciation of the biological role of tocopherols in plants thanks to a detailed characterization of tocopherol-deficient plants, not only of several *A. thaliana* mutants but also of the *sxd1* maize mutant and some transgenic potato, tobacco, and rice lines deficient in tocopherols. Recent findings have demonstrated that tocopherols can affect important physiological processes, such as germination, export of photoassimilates, growth, and leaf senescence, beyond their antioxidant function in photosynthetic membranes and role in plant responses to abiotic stresses. The role of tocopherols in other important physiological processes, such as flowering or plant responses to biotic stresses remain, however, still very poorly understood. Although tocopherol deficiency may be dispensable in mature plants in some growth conditions by activation of alternative defence mechanisms, tocopherols appear to be particularly relevant in plant responses to severe photo-oxidative stress or when other protection mechanisms are absent or fail. Studies aimed at elucidating the role of specific tocopherol homologues in plants in the future should consider not only the antioxidant function of these molecules but also their role in the regulation of signal transduction and gene regulation. In addition to a significant role as part of the intricate antioxidant network in plants, tocopherols may serve important functions in signal transduction pathways and the regulation of gene expression in diverse processes, including photoassimilate export and plant defence responses. Despite really significant advances being made in vitamin E research during the last decade, simple questions such as their subcellular localization or even their distribution in the evolutionary scale still require further research, especially for tocotrienols. Tocotrienols have gained much more attention in the last few years but their functions in plants are far from being understood. It will be of importance to identify all factors responsible for the occurrence of tocotrienols in plants. It is also still unclear if tocotrienols are accumulating in specific parts because of their antioxidative properties or other as yet not understood functions. The influence of environmental factors on the tocotrienol content in plants is not covered in this review but will certainly also be an issue in the future.

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