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α-Tocopherol: A Multifaceted Molecule in Plants

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α-Tocopherol, which belongs to the vitamin E group of compounds, is a lipophilic antioxidant that has a number of functions in plants. Synthesized from homogentisic acid and isopentenyl diphosphate in the chloroplast envelope, α-tocopherol is essential to maintain the integrity of photosynthetic membranes and plays a major role in photo- and antioxidant protection. α-Tocopherol scavenges lipid peroxy radicals, thereby preventing the propagation of lipid peroxidation, and protects lipids and other membrane components by physically quenching and reacting chemically with singlet oxygen. Moreover, given that α-tocopherol increases membrane rigidity,

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its concentration, together with that of the other membrane components, may be regulated to afford adequate fluidity for membrane function. Furthermore, recent studies on tocopherol-deficient plants indicate that α-tocopherol may affect cellular signaling in plants. Evidence thus far indicates that the effects of this compound in plant cellular signaling may be linked to the control of redox homeostasis. α-Tocopherol may influence cellular signaling by controlling the propagation of lipid peroxidation in chloroplasts, therefore modulating the formation of oxylipins such as the phytohormone jasmonic acid.

I. 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 the vitamin E was associated with an antioxidant property (Epstein *et al.*, 1966). Thereafter, and especially during the last 30 years, the roles of this vitamin and other antioxidants have been extensively studied in humans. Reviews of several studies on tocopherols and tocotrienols during this period, which had distinct objectives but which were complementary, have been made. Most of the research into tocopherols and tocotrienols has focused on the fundamental chemistry that explains their antioxidant properties (Kamal-Eldin and Appelqvist, 1996), their specific location, and their role in biological membranes (Wang and Quinn, 2000), and particularly on the benefits of these compounds for human health (Fuchs, 1998; Pryor, 2000). Consequently, many studies have been carried out to obtain tocopherols and tocotrienols from plant extracts, chemically or via microalgal culture (Vandamme, 1992). Moreover, advances in the molecular biology of plants have provided new insights into the manipulation of the synthesis of α-tocopherol to increase the amount of vitamin E in food and thereby prevent nutrient deficiencies (DellaPenna, 1999, 2005; Grusak and DellaPenna, 1999). However, although plants are the sole source of vitamin E, fewer studies have focused on the function of tocopherols and tocotrienols in these organisms. In the 1990s, the function of α-tocopherol in plants was thought to be associated with only its antioxidant activity in the maintenance of membrane integrity (Fryer, 1992). It has been proposed that beside its photo- and antioxidant protective function α-tocopherol could play a role in cellular signaling in plants (Munné-Bosch, 2005; Munné-Bosch and Alegre, 2002; Munné-Bosch and Falk, 2004). Recent evidence obtained in tocopherol-deficient plants demonstrates that

α-tocopherol actually plays a role in cellular signaling in plants, thus shedding new light on the multiple functions this compound has in plants. This chapter will focus on the functions of α-tocopherol in plants and will emphasize advances in research over the last few years.

II. OCCURRENCE AND ANTIOXIDANT FUNCTION OF α-TOCOPHEROL IN PLANTS

All vitamin E compounds (tocopherols and tocotrienols) are formed by a chromanol head group and a phytyl side chain. They are amphipatic molecules in which the hydrophobic phytyl tail associates with membrane lipids, and the polar chromanol head groups are exposed to the membrane surface. Tocopherols differ from tocotrienols only in the degree of saturation of their hydrophobic tails. Tocopherols have been found in photosynthetic bacteria, fungi, algae, plants, and animals, despite the inability of the latter to synthesize them (Grusak and DellaPenna, 1999; Lichtenthaler, 1968; Singh *et al.*, 1990). These compounds have been detected in all the photosynthetic organisms examined (Lichtenthaler, 1968), except in the cyanobacterium *Anacystis nidulans* (Omata and Murata, 1984). Tocopherols have been found in seeds and fruits, flowers (e.g., sepals and petals), roots, tubers, cotyledons, hypocotyls, stems, and particularly in leaves of higher plants. In leaves, they predominate in the α-tocopherol form, though in some cases significant amounts of its precursor, γ-tocopherol, have also been found. Plant tissues vary enormously in their total tocopherol content with total concentrations ranging from extremely low levels in potato tuber (<1-μg/g dry weight) to very high levels in leaves and seeds (>1-mg/g dry weight) (Munné-Bosch and Alegre, 2002).

α-Tocopherol is found in the chloroplasts of leaves. α-Tocopherol is synthesized in the chloroplast envelope from homogentisic acid and isopentenyl diphosphate (Arango and Heise, 1998; Soll *et al.*, 1985). Furthermore, α-tocopherol is found in plastoglobuli of the chloroplast stroma, where it is stored (Grumbach, 1983; Lichtenthaler *et al.*, 1981). Plastoglobuli are lipoprotein particles inside chloroplasts. During oxidative stress and senescence, they increase in number and form linkage groups that are attached to each other and remain continuous with the thylakoid membrane by extensions of the half-lipid bilayer (Austin *et al.*, 2006). Finally, α-tocopherol is found in thylakoid membranes, where it exerts its functions (Fryer, 1992; Havaux, 1998). Most of the α-tocopherol synthesized is partitioned between the chloroplastic envelope and the thylakoids, and is stored in plastoglobuli only in some cases, particularly during periods of oxidative stress and senescence. In spinach chloroplasts, one-third of the total α-tocopherol is located in envelope membranes, and the remaining two-thirds in thylakoids (Wise and Naylor, 1987). Within the membranes, tocopherols are thought to

be restricted to the lipid matrix of thylakoids and chloroplastic envelope membranes, rather than to protein domains (Havaux, 1998; Havaux *et al.*, 2000).

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 tocopherol-deficient mutants were also more susceptible to high-light treatment in the presence of sublethal levels of norflurazon, an inhibitor of carotenoid synthesis, suggesting carotenoids and tocopherols functionally interact in protecting *Synechocystis* from lipid peroxidation and high-light stress.

The principal role of tocopherols as antioxidants is believed to be in the scavenging of lipid peroxy radicals, which are responsible for propagating lipid peroxidation (Liebler, 1993). Ingold *et al.* (1986, 1990) showed that the antioxidant activity of tocopherols as free radical scavengers is associated with the ability to donate its phenolic hydrogen to lipid-free radicals and with specific requirements of the molecule, that is (1) the degree of methylation in the aromatic ring ($\alpha > \beta = \gamma > \delta$), (2) the size of the heterocyclic ring, (3) the stereochemistry at position 2, and (4) the length of the phytyl chain (optimum between 11 and 13 carbons). It is generally agreed that the antioxidant activities of tocopherols against lipid peroxidation *in vivo* are $\alpha > \beta > \gamma > \delta$ (Kamal-Eldin and Appelqvist, 1996).

Lipid peroxidation can be divided into three phases: initiation, propagation, and termination. It begins by generating a radical, generally an alkyl radical from a (poly)unsaturated fatty acid (PUFA) by the action of an initiator, which could be various reactive oxygen species (ROS), lipoxygenase, heat, light, and/or trace metals. The alkyl radical formed during initiation is highly reactive and combines with oxygen to form peroxy radicals. These, in turn, abstract the hydrogen from PUFAs and give rise to lipid hydroperoxides and new alkyl radicals, which propagate the reaction chain (Fig. 1). Alternatively, singlet oxygen ($^1O_2$) can react with PUFAs and give rise to lipid hydroperoxides. Lipid hydroperoxides can be converted, among other products, to (1) alcohols by hydroperoxide glutathione peroxidase or 2-Cys peroxiredoxin, which are localized in chloroplasts (Baier and Dietz, 1999); (2) jasmonic acid by allene oxide synthase and other enzymes found in chloroplasts, cytoplasm, and peroxisomes (Schaller, 2001); or (3) *n*-hexanal, traumatic acid, and other compounds by the action of hydroperoxide lyase and other enzymes (Matsui *et al.*, 1996, 1999). Tocopherols scavenge the lipid peroxy radical before it can abstract hydrogen from the target lipids. The chromanol heads of tocopherols lose a hydrogen atom that is given to the lipid peroxy radical, and tocopheroxyl radicals are then formed (Fig. 1). The ascorbate–glutathione cycle recycles tocopheroxyl radicals to tocopherols (Smirnoff and Wheeler, 2000).

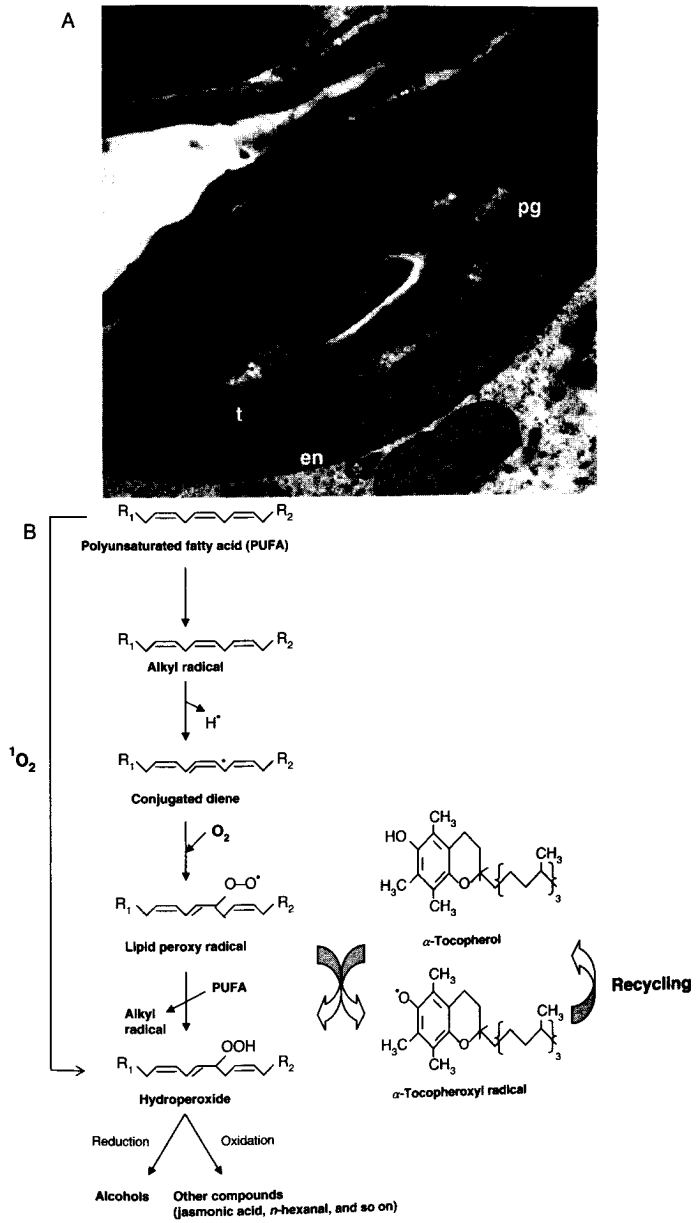

FIGURE 1. (A) Detail of a chloroplast, where α-tocopherol is located in the envelope (en), thylakoids (t), and plastoglobuli (pg). (B) α-Tocopherol prevents the propagation of lipid peroxidation in photosynthetic thylakoid membranes by scavenging lipid peroxy radicals. Photograph is a courtesy from Tana Jubany.

Tocopherols also play a key role as antioxidants because they physically quench or chemically scavenge $^1O_2$. Production of $^1O_2$ in thylakoids occurs because of the interaction of triplet excited reaction center chlorophyll ($^3P680^*$) with $^3O_2$. $^1O_2$ production is enhanced when photon energy is in excess of the CO_2 assimilation and the intersystem electron carriers Q_A, Q_B, or plastoquinone of photosystem II occur at the reduced state (Hideg *et al.*, 2000; Melis, 1999). Alternatively, $^1O_2$ has also been proposed as a by-product of membrane lipid peroxidation, arising from disproportionation of peroxy radicals (Cadenas, 1989; Halliwell, 1981). $^1O_2$ can oxidize membrane lipid, protein, amino acids, nucleic acids, nucleotides, pyridine nucleotides, carbohydrates, and thiols (Halliwell and Gutteridge, 1999; Straight and Spikes, 1985). α-Tocopherol can physically quench and therefore deactivate $^1O_2$ in chloroplasts. During quenching, $^1O_2$ is deactivated to $^3O_2$ through a charge transfer mechanism. An electron is lost from the tocopherol and is donated to the electron-deficient $^1O_2$, thereby forming a charge transfer exciplex, which subsequently undergoes intersystem crossing and then dissociates into α-tocopherol and $^3O_2$ (Thomas and Foote, 1978; Yamauchi and Matsushita, 1977). It has been estimated that, before being degraded, one molecule of α-tocopherol can deactivate up to 120 $^1O_2$ molecules by resonance energy transfer (Fahrenholtz *et al.*, 1974). Furthermore, tocopherols also react chemically with $^1O_2$ and are destroyed (Fukuzawa *et al.*, 1997). The reaction occurs through an intermediate hydroperoxydienone, which decomposes to form tocopherol quinone and tocopherol quinone epoxides (Murkovic *et al.*, 1997; Neely *et al.*, 1988) (Fig. 2). α-Tocopherol quinone can be enzymatically converted to α-tocopherol quinol in an NADH- or NADPH-dependent reaction (Kruk and Strzalka, 1995). Experiments using model membranes suggest that α-tocopherol quinol is a potent antioxidant comparable to α-tocopherol (Bindoli *et al.*, 1985; Kruk *et al.*, 1997a; Mukai *et al.*, 1992). However, further experiments on α-tocopherol quinol are required to confirm its antioxidant activity *in vivo*.

III. PHOTOPROTECTIVE FUNCTION OF α-TOCOPHEROL IN PLANTS

The advent of photosynthesis is a central event in the early development of life on Earth. Photosynthesis is all about the collection of solar energy and its conversion into chemical energy. As with other natural light processors such as the human eye, photosynthesis would have not been possible without a safety valve that dissipates excess excitation energy in a harmless way. Photosynthesis in nature thus operates in a constantly shifting balance between efficient capture of solar energy and quick loss of that energy when it is captured in excess. Under optimal growth conditions, plants use most of the absorbed energy for photosynthesis, and photoprotection

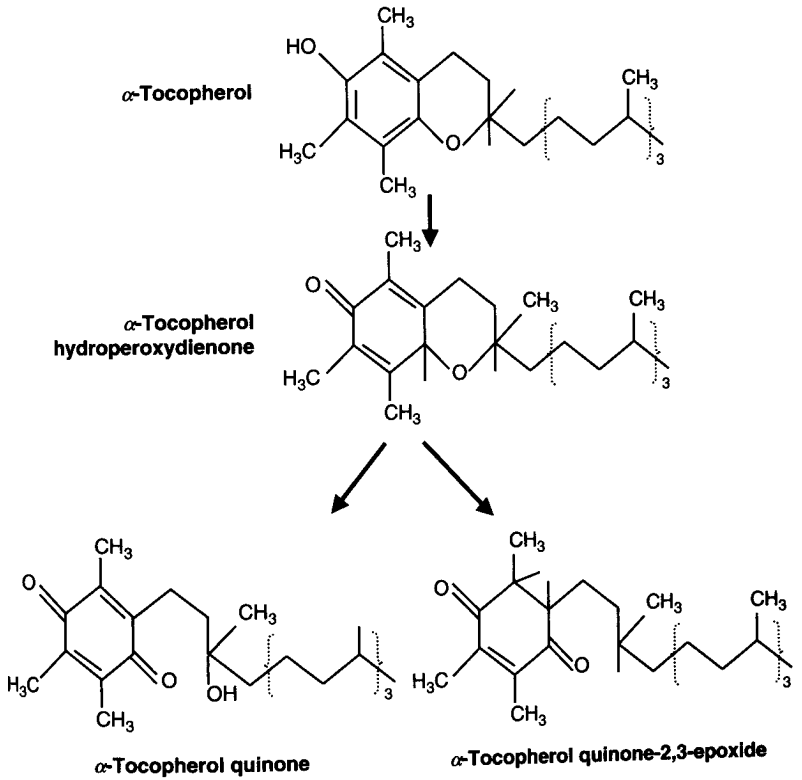

FIGURE 2. Scavenging of singlet oxygen by α-tocopherol and formation of α-tocopherol quinone.

mechanisms are hardly needed. On the other hand, when plants are exposed to adverse environmental conditions (drought, extreme temperatures, nutrient deficit, air pollutants, or pathogen attack), photosynthesis is reduced and photoprotection mechanisms are needed to allow safe dissipation of excess energy.

Plants have evolved several mechanisms to avoid or to get rid of excess energy in photosynthetic membranes. These mechanisms include, among others, alterations in leaf structure (Terashima *et al.*, 2001; Weston *et al.*, 2000), leaf and chloroplast movements (Kasahara *et al.*, 2002; Satter and Galston, 1981), changes in antenna size and pigment composition (Demmig-Adams and Adams, 2002; Havaux, 1998), light attenuation by anthocyanins or other compounds (Steyn *et al.*, 2002), and the regulation of light energy utilization and dissipation (Asada, 1999; Munné-Bosch *et al.*, 2005; Niyogi, 1999). The formation of ROS and its consequent detoxification by antioxidants represents an efficient mechanism of dissipation of excess energy in chloroplasts

(Asada, 2006). Consequently, the detoxification of $^1O_2$ and other ROS by tocopherols afford protection to the photosynthetic apparatus and it serves to dissipate excess energy in chloroplasts.

The formation of α-tocopherol quinone can play an additional role in photoprotection. Using lyophilized, petroleum ether-extracted thylakoid membranes that preserve the structure of thylakoids, Kruk *et al.* (1998) found that α-tocopherol and α-tocopherol quinone interact with photosynthetic electron transport. It was suggested that cyclic electron transport around photosystem II was inhibited by α-tocopherol and stimulated by α-tocopherol quinone (Kruk *et al.*, 1997b). Later Kruk and Strzalka (2001) confirmed that α-tocopherol quinone efficiently oxidizes the reduced cytochrome b559, and thus play a role in cyclic electron flow around photosystem II when the photosynthetic electron transport chain is over-reduced. Therefore, this compound contributes to the dissipation of excess energy in thylakoids, thereby conferring photoprotection onto photosynthetic apparatus.

Furthermore, it has been suggested that the presence of α-tocopherol in thylakoids could reduce the permeability of these membranes to ions. It could therefore affect the maintenance of the light-generated transmembrane proton gradient (Fryer, 1992), which is responsible for ATP synthesis, and the conversion of violaxanthin to zeaxanthin in the xanthophyll cycle, which is involved in the harmless dissipation of excess excitation energy in thylakoids (Demmig-Adams and Adams, 1992; Eskling *et al.*, 1997; Horton *et al.*, 1996). Therefore, an accumulation of α-tocopherol in thylakoids could afford photoprotection by (1) scavenging $^1O_2$ and other ROS, (2) activating cyclic electron flow around photosystem II through the formation of α-tocopherol quinone, and (3) reducing the permeability of thylakoid membranes to protons, which would favor the acidification of the thylakoid lumen in high light and activate violaxanthin de-epoxidase.

By using tocopherol-deficient mutants, Havaux *et al.* (2005) showed that tocopherols protect *Arabidopsis thaliana* plants against photooxidative stress. Leaf disks of two tocopherol mutants, a tocopherol cyclase mutant (*vte1*) and a homogentisate phytyl transferase mutant (*vte2*), were exposed to high-light stress at low temperatures, which resulted in bleaching and lipid photodestruction. However, this was not observed in whole plants exposed to long-term high-light stress, unless the stress conditions were very severe, suggesting compensatory mechanisms for tocopherol deficiency under physiological conditions. These authors identified two such compensatory mechanisms: xanthophyll cycle-dependent energy dissipation by nonphotochemical quenching of photosystem II and synthesis of zeaxanthin. 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). It appears therefore that tocopherols are part of an intricate network of photoprotection mechanisms that act in concert to protect photosynthetic membranes from photooxidation. Munné-Bosch and Cela (2006) showed

that xanthophyll cycle-dependent excess energy dissipation precedes oxidation of α-tocopherol to its quinone in water-stressed sage plants, thus indicating that the formation of ROS and its consequent detoxification by α-tocopherol become relevant when violaxanthin is completely de-epoxidized to zeaxanthin and xanthophyll cycle-dependent energy dissipation cannot increase further.

IV. α-TOCOPHEROL AND THE STABILITY OF PHOTOSYNTHETIC MEMBRANES

α-Tocopherol strongly interacts with membrane lipids and increases the rigidity of the membrane. Thus, its presence at high amounts in photosynthetic membranes during specific periods of plant development or stress could be detrimental to plant function in terms of membrane stability. However, plants show changes not only in α-tocopherol content but also in the lipid composition of the membrane, and in the concentrations of β-carotene and other lipophilic antioxidants, which also affect membrane fluidity and counteract the rigidifying effects of α-tocoperol (Munné-Bosch and Alegre, 2000; Quartacci *et al.*, 1997). The degree of lipid peroxidation in membranes has been shown to depend not only on the amount of ROS and antioxidants but also on the composition of the membrane (McKersie *et al.*, 1990). Increased fatty acid unsaturation in the membranes, which tends to maintain the liquid crystalline phase, has been correlated with increased stress tolerance in plants (McKersie *et al.*, 1988).

V. ROLE OF α-TOCOPHEROL IN CELLULAR SIGNALING

Recent studies in mutant and transgenic plants have made a significant contribution to furthering our understanding of the role of antioxidants in plants. In particular, it has been shown that antioxidants such as ascorbic acid and glutathione regulate signal transduction and gene expression, particularly in plant responses to stress (Ball *et al.*, 2004; Chen and Gallie, 2004). Furthermore, given that α-tocopherol affects oxidative stress and the extent of lipid peroxidation in chloroplasts, it was proposed that this compound could also affect intracellular signaling in plants (Munné-Bosch, 2005; Munné-Bosch and Alegre, 2002; Munné-Bosch and Falk, 2004). Recent evidence has now emerged indicating that tocopherols participate in cellular signaling in plants, which has several implications for our understanding of the role of tocopherols in plant development and stress tolerance.

The first indication toward identifying a role of tocopherols in cellular signaling came from studies using the *sxd1* mutant of maize (C4 plant). This mutant carries a defect in the *sxd1* gene, which encodes for tocopherol

cyclase (SXD1), an enzyme that is essential for the formation of the chromanol ring of tocopherols. An overall growth reduction and source leaf-specific accumulation of anthocyanins and starch characterize this mutant, which is deficient in tocopherols. In addition, minor veins of maturating leaf blades exhibit ultrastructural alterations and callose occlusion of a specific class of plasmodesmata between bundle sheath and vascular parenchyma cells of this mutant, thus leading to a blocking of sucrose transport into the phloem (Botha *et al.*, 2000; Russin *et al.*, 1996).

These results were confirmed in potato plants, which, in contrast to maize, have a C3 type of photosynthetic metabolism. By using an 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 show enhanced callose deposition in source leaves, lower photosynthetic capacity, and altered gene expression compared to the wild type. The transcription of the photosynthesis-related *rbcS* and *cab* genes is reduced, while transcripts of the defense-related proteinase inhibitor II (*pin2*) and of proline (*p5cs*), and jasmonic acid (*aoc*) biosyntheses are induced in source leaves. This study provides evidence that the impact of tocopherol deficiency in plasmodesmata function and carbohydrate metabolism is similar in monocot (maize) and dicot (potato) species and cannot be assigned to specific anatomical or biochemical features of C4 metabolism.

A similar phenotype has been observed in *vte1* mutants of *A. thaliana*. This mutant was discovered during a screen for altered tocopherol content, and it has been shown that VTE1 and SXD1 are single-copy orthologues, both encoding an enzyme with tocopherol cyclase activity (Porfirova *et al.*, 2002). Tocopherol deficiency in the *vte1* mutant of *A. thaliana* leads not only to a slightly reduced growth and enhanced susceptibility to photooxidative stress (Porfirova *et al.*, 2002) but also to a photoassimilate export-deficient phenotype characterized by anthocyanin accumulation at low temperatures (Maeda *et al.*, 2006). Similar low-temperature-induced accumulation of anthocyanins has been observed in a regulatory *vte1 A. thaliana* mutant, which has an insertion in the promoter region of the gene-encoding tocopherol cyclase (Munné-Bosch *et al.*, 2007). In these mutants, it is shown additionally that this enhanced accumulation of anthocyanins is triggered by a transient accumulation of jasmonic acid in tocopherol-deficient plants. This study demonstrates that tocopherols may play a role in cellular signaling by altering phytohormone levels in plants. Furthermore, it indicates that tocopherols exert effects on cellular signaling by modulating jasmonic acid levels in plants, rather than directly regulating gene expression. By controlling ROS levels, the extent of lipid peroxidation, and thus hydroperoxide contents in chloroplasts, tocopherols may not only indirectly regulate the amounts of jasmonic acid in leaves but also affect jasmonic acid-dependent gene expression in the nucleus.

Enhanced callose synthesis, and the consequent occlusion of specific plasmodesmata, is the most significant characteristic of the photoassimilate export-deficient phenotype in tocopherol-deficient plants. Callose synthesis is a specific plant response to distinct abiotic and biotic stress factors to control the size exclusion limits of plasmodesmata, and therefore the type and amount of molecules transported from cell to cell. Callose formation is strongly correlated with oxidative damage to membrane lipids and changes in intracellular calcium homeostasis, indicating a mechanistic link between lipid peroxidation and callose synthesis. By controlling the extent of lipid peroxidation, and therefore hydroperoxide content in chloroplasts, toco pherols may indirectly regulate the amounts of jasmonic acid in leaves and affect jasmonic acid-dependent gene expression involved, for instance, in wound stress response. In addition to enhanced callose synthesis, tocopherol-deficient plants show increased accumulation of jasmonic acid-responsive *Pin2* transcripts and upregulation of the jasmonic acid biosynthetic gene *AOC* (Hofius *et al.*, 2004), which supports this contention. Jasmonic acid also regulates the expression of anthocyanin biosynthetic genes (Creelman and Mullet, 1997), which explains the relationship between tocopherol deficiency and anthocyanin accumulation in the source leaves of plants with the photoassimilate export-deficient phenotype.

However, this photoassimilate export-deficient phenotype has not been observed in the *vte1* mutants of *A. thaliana* grown at 22°C under low-light conditions (Sattler *et al.*, 2003), thus indicating that the phenotype observed in tocopherol-deficient plants is strongly dependent on climatic growth conditions. Compelling evidence indicates that the phenotypes described thus far for tocopherol deficiency, characterized by reduced growth, photoassimilate export deficiency, and higher susceptibility to photooxidation, (1) are species-specific, (2) depend on the extent of tocopherol deficiency, (3) depend on growth conditions (light intensity, photoperiod, temperature, and so on) and plant developmental stage, and (4) are more evident as growth conditions become more stressful (Hofius *et al.*, 2004; Munné-Bosch and Falk, 2004; Porfirova *et al.*, 2002).

VI. HAVE THE FUNCTIONS OF TOCOPHEROLS BEEN EVOLUTIONARY CONSERVED?

Among tocopherols, α-tocopherol has been shown to accumulate selectively in the human body and to be the most effective form as an antioxidant and in regulating cell signaling in animals (Brigelius-Flohé *et al.*, 2002). Since α-tocopherol is also the major form in the embryos of seeds and photosynthetic tissues of plants, it appears that plants and animals have converged in selectively using α-tocopherol among all tocopherols.

The antioxidant activity of tocopherols seems to be highly conserved throughout evolution. It has been shown that both plants and animals use tocopherols to reduce the levels of $^1O_2$ and other ROS and to inhibit the propagation of lipid peroxidation within the cell. The aspects related to this antioxidant activity at the membrane level seem similar, although the cells and tissues bearing such membranes are different. The most evident difference is that tocopherols protect photosynthetic membranes in plants, thus it seems that compared to animals, plants will need higher amounts of tocopherols (or additional antioxidants) to cope with potentially higher amount of photogenerated $^1O_2$.

The mechanisms of action of tocopherols in cellular signaling are much less understood in plants than in animals. Specific lipoxygenases, responsible for the initiation of lipid peroxidation, are inhibited by α-tocopherol, at least in part, by efficiently reducing the active site Fe^{3+} of the enzyme to the inactive Fe^{2+} (Cucurou *et al.*, 1991). This study shows that soybean and potato lipoxygenases are inhibited by α-tocopherol, which supports the contention that, in conjunction with the scavenging of lipid peroxyl radicals which propagate lipid peroxidation, α-tocopherol suppresses hydroperoxide formation by inactivation of lipoxygenases. Oxilipins such as jasmonic acid and other lipid peroxidation products activate many genes in plants, especially those involved in plant responses to environmental stress (Weber *et al.*, 2004). Thus, the regulation of lipid peroxidation by α-tocopherol may strongly modulate signal transduction and gene expression not only in animals but also in plants.

Studies on animal models suggest additional mechanisms of action of tocopherols that are independent of their antioxidant functions in the regulation of gene expression (Azzi *et al.*, 1998; Brigelius-Flohé *et al.*, 2002). The inhibition of protein kinase C by tocopherols is one of the best-studied effects of these antioxidants in cellular signaling in animals. Tocopherols may inhibit protein kinase C as a result of the dephosphorylation of the enzyme via activation of protein phosphatase 2A (Clement *et al.*, 1997; Ricciarelli *et al.*, 1998). Alternatively, it has also been proposed that the inhibition of protein kinase C is caused by the activation of diacylglycerol kinase by α-tocopherol, consequently decreasing diacylglycerol and leading to protein kinase C inhibition (Koya *et al.*, 1997). Several studies have examined the effects of α-tocopherol on phospholipase A_2 activity and have shown that a degree of inhibition occurs both *in vitro* and in animal systems when the concentration of α-tocopherol is increased. Grau and Ortiz (1998) suggest that tocopherols inhibit phospholipase A_2 activity by altering the physical properties of the membrane. Chandra *et al.* (2002) have provided the first structural evidence of a specific inhibition of phospholipase A_2 by α-tocopherol. Furthermore, some genes are affected by α-tocopherol at the transcriptional level in animal cells (Azzi *et al.*, 1998; Carlberg, 1999). α- and β-tocopherols show differential effects, which have been attributed to a nonantioxidant mechanism of α-tocopherol in gene regulation. Tocopherol-associated proteins (TAPs) translocate from the cytosol to

the nucleus in animal cells, where they may activate gene transcription in an α-tocopherol-dependent manner (Yamaguchi *et al.*, 2001).

Evidence of nonantioxidant functions of tocopherols in higher plants is still limited, although it has been proposed that tocopherols may exert nonantioxidant functions and control photosynthesis and nutrient homeostasis in the cyanobacterium *Synechocystis* sp. PCC 6803 (Sakuragi *et al.*, 2006). Evidence obtained thus far in higher plants, yet limited to the model plant *A. thaliana*, maize, and potato plants, tend to favor a role of tocopherols in regulating the cell redox homeostasis and exert effects on cell signaling by modulating oxylipins levels in plants, rather than directly regulating gene expression. By controlling ROS levels, the extent of lipid peroxidation, and thus hydroperoxide contents in chloroplasts, tocopherols may not only indirectly regulate the amounts of oxylipins, such as jasmonic acid in leaves, but also affect jasmonic acid-dependent gene expression in the nucleus. Jasmonic acid is known as a growth inhibitor and regulates the expression of anthocyanin biosynthetic genes (Creelman and Mullet, 1997), thereby supporting the relationship between tocopherol deficiency, reduced growth, and anthocyanin accumulation described in previous studies (Hofius *et al.*, 2004; Munné-Bosch *et al.*, 2007). Tocopherols therefore influence cellular signaling in plants and may modulate gene expression in the nucleus by affecting lipid peroxidation, and therefore the levels of oxylipins such as jasmonic acid.

VII. FUTURE PERSPECTIVES

The recent findings indicating the involvement of tocopherols in cellular signaling open a new field of research to explore the effects of tocopherols in plant development and stress responses. Studies aimed at elucidating the role of tocopherols in plants should not only consider the photo- and antioxidant protective function of these molecules but also their role in the regulation of signal transduction and gene regulation. Although nonantioxidant functions of tocopherols have not been demonstrated thus far in higher plants, the presence of TAPs and tocopherol-specific motifs in the promoters of genes in plants should be investigated. The elucidation of signal transduction pathways and gene expression regulated by tocopherols may probably provide some of the most exciting discoveries awaiting plant biologists in the near future.

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