Review

Biosynthesis, Structural, and Functional Attributes of Tocopherols in Planta; Past, Present, and Future Perspectives

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ABSTRACT: Tocopherols are lipophilic molecules, ubiquitously synthesized in all photosynthetic organisms. Being a group of vitamin E compounds, they play an essential role in human nutrition and health. Despite their structural and functional attributes as important antioxidants in plants, it would be misleading to ignore the potential roles of tocopherols beyond their antioxidant properties in planta. Detailed characterization of mutants and transgenic plants, including *Arabidopsis* (*vte1*, *vte2*, *vte4*, and so on), maize (*sxd1*) mutants, and transgenic potato and tobacco lines altered in tocopherol biosynthesis and contents, has led to surprising outcomes regarding the additional functions of these molecules. Thus, the aim of this review is to highlight the past and present research findings on tocopherols' structural, biosynthesis, and functional properties in plants. Special emphasis is given to their suggested functions in planta, such as cell signaling, hormonal interactions, and coordinated response of tocopherols to other antioxidants under abiotic stresses. Moreover, some important questions about possible new functions of tocopherols will be discussed as future prospects to stimulate further research.
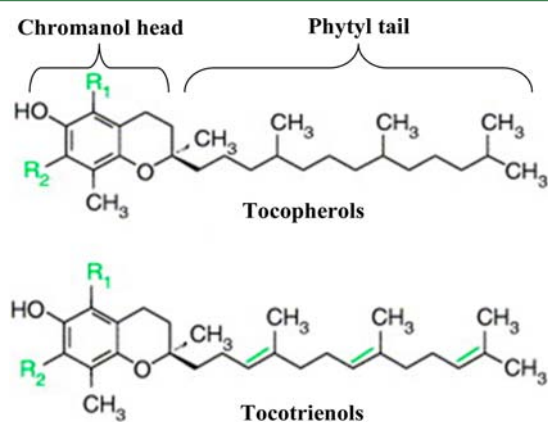
KEYWORDS: vitamin E, tocopherols, sources, tocopherols biosynthesis, antioxidants, nonantioxidants, phytohormones, ROS, abiotic stresses, cell signaling, gene expression

■ INTRODUCTION

The term "vitamin E" was first introduced by Evans and Bishop[1] in 1922 as an important nutritional factor for reproduction in rats and is a generic term used for tocochromanols, composed of closely related amphipathic molecules.[2] Tocochromanols have been divided into two groups, the tocopherols and tocotrienols (Figure 1),[3−5] each having four derivatives, alpha (α), beta (β), gamma (γ), and delta (δ). Isolation and characterization of α- and β-tocopherols was first reported in 1936 by Evans et al.[6] from wheat germ oil and those of γ- and δ-tocopherols from oil of edible plants.[7,8] These molecules consist of a polar chromanol headgroup linked to an isoprenoid-derived hydrophobic tail.[9,10] The difference

Figure 1. Generalized chemical structures of tocopherols and tocotrienols.

among α-, β-, γ-, and δ-tocochromanols is due to only the number and position of the methyl ($-CH_3$) substituent(s) on the aromatic ring (Figure 2).[11] Tocopherols (Toc) having a fully saturated phytyl tail derived from phytyl diphosphate (PDP) and tocotrienols having an unsaturated tail derived from geranylgeranyl diphosphate (GGDP)[4] contain three trans double bonds in their side chain.[12] Although both tocopherols and tocotrienols are amphipatic in nature, they differ in some biochemical properties with respect to their isoprenoid side chain (Table 1). These differences might have vital inferences for both tocopherols and tocotrienols.[13] Tocopherols are ubiquitously synthesized in all photosynthetic organisms, whereas tocotrienols are present in only certain plant groups. The presence of both types even in the same plant, but with differences in their localization in the tissues, suggests their functional differences in plants as well.[13]

In the recent past, the importance of vitamins in human health was well reviewed in terms of metabolism of vitamins in plants, successful engineering of their pathways, vitamin-related problem of quality food deficits in developing countries,[14] recent advances in the understanding of antioxidant roles of vitamins with emphasis on functions of B vitamins,[15] and tocochromanols (vitamin E) in plants with some discussion on hormone signals, which suggest a nonantioxidant function in plants.[13]

Table 1. Properties of Four Homologues of Tocopherol

				methyl groups		
homologue	molar mass (g/mol)	molecular formula	IUPAC[a] name	R1	R2	R3
α-Toc	430.71	$C_{29}H_{50}O_2$	(2R)-2,5,7,8-tetramethyl-2-[(4R,8R)-(4,8,12-trimethyltridecyl)]-6-chromanol	CH_3	CH_3	CH_3
β-Toc	416.68	$C_{28}H_{48}O_2$	(2R)-2,5,8-trimethyl-2-[(4R,8R) -(4,8,12-trimethyltridecyl)]-3,4-dihydrochromen-6-ol	CH_3	H	CH_3
γ-Toc	416.68	$C_{28}H_{48}O_2$	(2R)-2,7,8-trimethyl-2-[(4R,8R)-4,8,12-trimethyltridecyl]-6-chromanol	H	CH_3	CH_3
δ-Toc	402.65	$C_{27}H_{46}O_2$	(2R)-2,8-dimethyl-2-[(4R,8R)-4,8,12-trimethyltridecyl]-6-chromanol	H	H	CH_3

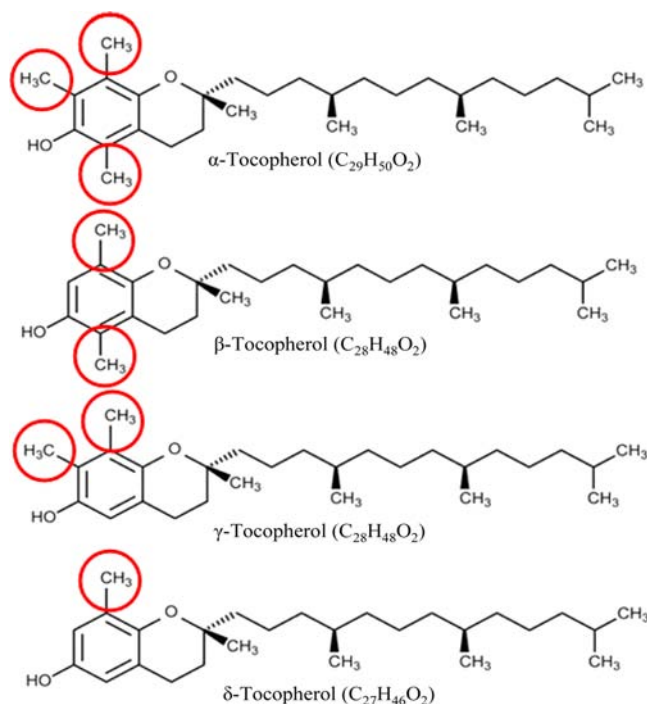[a]IUPAC, International Union of Pure and Applied Chemistry.

Figure 2. Chemical structures of tocopherol homologues showing differences only in the number and position of methyl substituents on the aromatic ring of chromanol head circled in red.

Ever since the introduction of the term tocopherol or vitamin E, the antioxidant properties were mainly focused on and reported as the most vital and primary functions of tocopherol in humans, animals, and plants. Despite the structural and functional attributes as an important antioxidant in plants, it would be misleading to ignore the potential role of tocopherol beyond the antioxidant properties in planta. Thus, the aim of this review is to unite, critically summarize, and highlight the past and present research findings on tocopherols' localization, structural, biosynthesis in plants, and functional properties in plants, humans, and animals and the importance of tocopherols in the food industry. Furthermore, special emphasis will be given to the suggested role of tocopherol in planta, such as cell signaling, hormonal interactions, and coordinated response of tocopherols to other antioxidants under abiotic stresses. Moreover, some open questions will be discussed about new functions as future prospects to stimulate further research.

The word tocopherol was taken from two Greek words "tokos" (offspring, or childbirth) and "pherein" (to carry, to bring forth), and the suffix "-ol" was added to indicate the phenolic nature (*The American Heritage Dictionary of the English Language*, 4th edition, copyright 2000, Houghton Mifflin Co.). Tocopherols are the most important and effective chain-breaking class of lipid-soluble compounds, constituting a family of antioxidants.[16,17] The presence of a methyl ($-CH_3$) group in its aromatic ring (Figure 1) makes it stable to heat, alkali, or acid. Among the four forms, if α-Toc has 100% biological activity, the rest of these vitamers have lower biological activities, that is, 30, 15, and 5% for β-, γ-, and δ-Toc, respectively.[18] Extensive studies on the importance of tocopherols indicate that α-tocopherol is the most important lipid-soluble antioxidant, as it protects cell membranes from oxidation by scavenging oxygen free radicals, lipid peroxy radicals, and singlet oxygen.[19–22] Furthermore, of the eight isomers of tocopherol, only α-tocopherol is retained at high levels in vertebrate plasma and tissues.[23] The details are described under Tocopherols Function as an Antioxidant.

PLANT SOURCES OF DIETARY TOCOPHEROLS

Tocopherol contents and composition vary with plant species and localization within plant tissues of a single species. Generally, the contents range from 10 to 50 mg Tocs/g fresh weight in green, unstressed photosynthetic plant tissues, with oil palm leaf being a notable exception.[24–27] α-Toc is predominant in photosynthetic tissues, presumably reflecting a highly conserved structural or functional role in the plastid. On the other hand, seeds have high variation in tocopherol content as well as composition. Seeds often dominate other plant parts in terms of the abundance of total Toc (T-Toc), but the contents of Toc isoforms vary from species to species. Generally, tocopherol contents in seed oils of different plant species are higher than in leaf oils of the same species and range from 300 to 2000 mg tocochromanols/kg oil.[18] Although α-Toc is the most biologically active form of vitamin E, it is synthesized in seeds in low quantity compared with other isoforms of Toc.[28–30] Among oil crops, soybean accounts for a large share of the edible oil consumption worldwide and contains 7% and 70% of α-Toc and γ-Toc, respectively, of the total tocopherol pool.[28] Other major oilseed crops including corn, canola, and cottonseed oil are good sources of tocopherol with low α- to γ-Toc ratios, whereas sunflower and palm oils have high α- to γ-Toc ratios.[29] In general, food sources with the highest concentrations of vitamin E are vegetable oils followed by nuts and seeds, including whole grains. Some of the key tocopherol (vitamin E) sources in plants are listed in Table 2.

LOCALIZATION OF TOCOPHEROLS IN PLANT TISSUES

Tocopherols are synthesized at the inner chloroplast envelope by a pathway that is conserved in higher plants and certain other photosynthetic organisms, including algae and most cyanobacteria.[31–36] In plants, Toc is generally localized in various plant tissues (mainly plastids), including chloroplasts of photosynthetic tissues or amyloplasts of seeds and tubers,

Table 2. Plant Sources of Tocopherol[a]

source	quantity as α-Toc (mg/100 g)	plant tissue
almond oil	39.2	kernel
almond	26	kernel
asparagus	1.5	young shoots
canola oil	27	seed
carrot	0.6	taproot
chestnut	1.2	nuts
coconut	1.0	seed/fruit
hazelnut	15	kernel
maize	2.0	kernel
oats	1.5	kernel
olive oil	12.0	seed
palm oil	15	kernel
peanut	17.2	kernel
safflower oil	34	kernel
soybean oil	11.6	kernel
spinach	2.0 raw, 2.1 cooked	leaf
sunflower oil	61	seeds (achene)
sunflower seed	35.17	kernels
tomatoes	0.9	raw fruit
walnut oil	20.0	fruit
wheat germ	150	germ of the kernel

[a]Source: National Institute of Health (May 4, 2009). Vitamin E fact sheet; http://www.ars.usda.gov/Services/docs.htm?docid=22114.

leucoplasts of petals, or chromoplasts of fruits.[26,37,38] Initial studies related to the intracellular distribution of Toc reveal that α-tocopherol is localized in chloroplasts.[39] However, in-depth studies reported its localization in the inner envelope membranes of chloroplasts,[37,40−42] in thylakoid membranes,[26,43] and in some cases in plastoglobuli. Plastoglobuli are regular globular plastid structures that primarily represent a reservoir for excess amounts of plastoquinone-9, α-tocopherol, and possibly other excess plant lipids[24,38,44,45] that cannot be stored in the thylakoids. Generally, in seeds localization of Toc is mainly reported in plastids, but some studies have found Toc association with cytoplasmic lipid bodies as well.[46] Commonly, leaves accumulate α-tocopherol as the predominant form, whereas seeds of many plant species are rich in γ-tocopherol, whereas β- and δ-tocopherol are usually present in significantly lower amount in most plant species.[47−49] The characterization of phytol and phytyl phosphate kinase from *Arabiodpsis thaliana*[50,51] supports the view that phytol is recycled (freed) during chlorophyll degradation and could be the possible prenyl moiety for γ-tocopherol biosynthesis in seeds.[52−54]
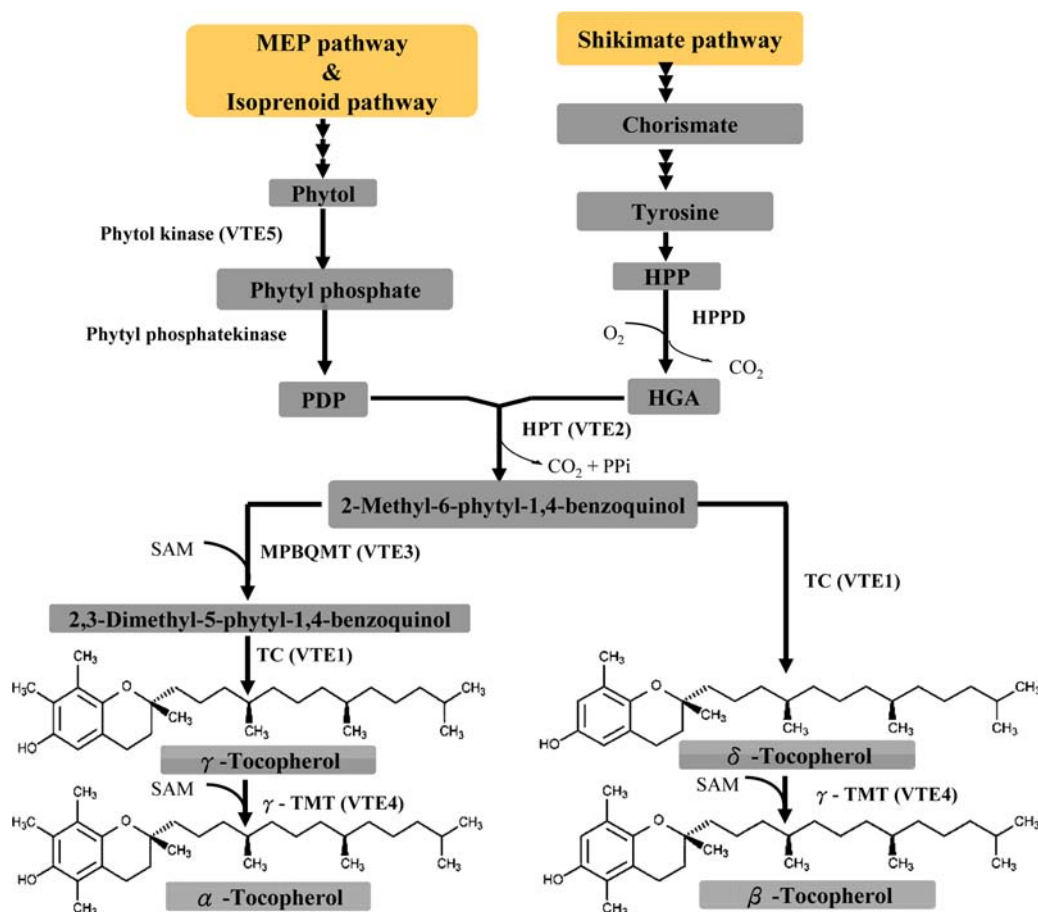
BIOSYNTHESIS OF TOCOPHEROLS

Extensive studies have been conducted to explore the biosynthesis pathway of tocochromanols in photosynthetic organisms for more than three decades, but the biosynthetic enzymes and genes of this pathway have been isolated only during the past decade.[4−12] These enzymes appear to be localized in plastids.[55−58] Increases in total tocopherol contents are observed during different plant growth and development stages, for example, during senescence,[53] during chloroplast to chromoplast transition,[58] and during seed development.[59]

Enzymes in Tocopherol Biosynthesis. Tocopherol biosynthesis is accomplished in plant photosynthetic tissues with the help of a vital group of enzymes (Table 3). In brief, homogentisate phytyltransferase (HPT) is a key enzyme that catalyzes the condensation of HGA and phytyldiphosphate (PDP) in the first step of tocopherol biosynthesis to form 2-methyl-6-phytylbenzoquinol (MPBQ), the committed intermediate of all Toc.[36] For the synthesis of α- and γ-tocopherol, the 3-position on the benzoquinol ring must be methylated. MPBQ has been localized to the chloroplast inner membrane.[60] Tocopherol cyclase (TC), the second most vital enzyme involved in tocopherol biosynthesis, has been localized to plastogloubules in chloroplast and forms the chromanol headgroup from a benzoquinol intermediate.[45,57] TC cyclizes both intermediates, mono- and dimethylated prenylbenzoquinols, involved in the biosynthesis of δ- and γ-Toc.[12] Tocopherol methyltransferase (TMT) catalyzes the final steps in the synthesis of tocopherol. On the one hand, it converts γ-tocopherol to the α form, and on the other hand, it catalyzes the conversion of δ-tocopherol to the β-isoform. The synthesis of both α- and β-isoforms occur in response to the methylation of carbon 5 on the chromanol ring by TMT (Figure 3). This enzyme is often referred to as γ-tocopherol methyltransferase (γ-TMT/VTE4) and localized to the inner member of chloroplasts.[61]

Table 3. Enzymes Involved in the Biosynthetic Pathway of Tocopherols

| enzyme	gene	locus	species	reference
p-hydroxyphenylpyruvic acid dioxygenase	HPPD/PDS1	At1g06590	*Arabidopsis*	64, 65
		slr0090	*Synechocystis*	
homogentisate phytyltransferase	VTE2, HPT1	At2g18950	*Arabidopsis*	66
		slr1736	*Synechocystis*	67, 68
2-methyl-6-phytylplastoquinol methyltransferase (MPBQMT)	VTE3	At3g63410	*Arabidopsis*	69
		sll0418	*Synechocystis*	70
tocopherol cyclase (TC)	VTE1	At4g32770	*Arabidopsis*	71
		slr1737	*Synechocystis*	72
γ-tocopherol methyltransferase (γ-TMT)	VTE4	At1g64970	*Arabidopsis*	73
		slr0089	*Synechocystis*	
phytol kinase (PK)	VTE5	At5g04490	*Arabidopsis*	51
		Slr1562	*Synechocystis*	

Figure 3. Tocopherol pathway in plants; a model from *Arabidopsis*. HPP, *p*-hydroxyphenylpyruvate; HGA, homogentisic acid/homogentisate; PDP, phytyldiphosphate; HPPD, HPP dioxygenase; HPT (VTE2), homogentisate phytyltransferase; MPBQMT (VTE3), 2-methyl-6-phytyl-1,4-benzoquinol methyltransferase; TC (VTE1), tocopherol cyclase; γ-TMT (VTE4), γ-tocopherol methyltransferase.

Phytol is an important and most abundant acyclic isoprenoid, constituting approximately one-third of the mass of chlorophyll. Phytol is also a constituent of both tocopherols[32,62] and vitamin K[63] in plants and cyanobacteria. In photosynthetic organisms, free phytol is generated during chlorophyll catabolism by chlorophyllase. Although phytol kinase (PK) activity had been described in planta,[40] there was no clear evidence until recently that the enzymatic pathway for the activation and reutilization of this free phytol in the synthesis of tocopherols exists.[50] A gene denoted VTE5 encoding PK has been recently identified and cloned by Valentin et al. using an *Arabidopsis* mutant.[51]

Tocopherol Biosynthesis Pathway. Tocopherol biosynthesis requires two substrates, homogentisate (HGA) and a C20 prenyldiphosphate (PrDP). Homogentisate, required for the formation of the aromatic ring of the chromanol headgroup, derived from tyrosine via the cytosolic shikimate pathway.[65,74−76] The deamination of tyrosine first yields *p*-hydroxyphenylpyruvate (HPP), which in turn is oxygenated to HGA by the activity of *p*-hydroxyphenylpyruvic acid dioxygenase (HPPD/PDS1). HGA is also used in plastoquinone synthesis (reviewed in ref 12). Prenyldiphosphate (PrDP), being the second substrate, is required for the formation of the tail region of tocopherols or tocotrienols. PrDP has two forms, that is, phytyldiphosphate (PDP)[77] and geranylgeranyldiphosphate (GGDP).[9] PDP and GGDP are reported to be derived from the plastidic methylerythritol phosphate (MEP) pathway.[4,5,78] Exploring further the route of

phytol synthesis revealed that GGDP can be used directly in tocotrienol synthesis or reduced to PDP for tocopherol biosynthesis.[50,51] The prenylation of HGA with PDP is catalyzed by the homogentisate phytyltransferase (HPT) encoded by slr1736 in *Synechocystis* sp. PCC 6803 (Syn-vte2) or in *Arabidopsis* by the vitamin E2 gene (VTE2), resulting in the formation of 2-methyl-6-phytylbenzoquinol (2M6PBQ), a tocopherol precursor.[66,68] The relative enzymatic activities of the tocopherol cyclase (TC) (VTE1) and the 2M6PBQ-methyltransferase (VTE3) determine the metabolic fate of VTE2 reaction. The characterization of VTE3 illustrated that it is multifunctional; that is, it not only catalyzes the methylation of the tocopherol precursors 2M6PBQ and 2M6GGBQ but also methylates 2-methyl-6-solanylbenzoquinol (2M6SBQ), the precursor of plastoquinol.[69,70,79,80] The final product of VTE3 is 2,3-dimethyl-5-benzoquinol. Thereafter, tocopherol cyclase (VTE1) uses the 2,3-dimethyl-5-benzoquinol as a substrate to form γ-tocopherol or can also utilize 2M6PBQ as a substrate to produce δ-tocopherol.[72,81] The process of biosynthesis of tocopherol and its derivatives ends with the conversion of γ-tocopherol to α-tocopherol. This final step is catalyzed by the γ-tocopherol methyltransferase (γ-TMT) encoded by VTE4 (Figure 3). In addition to this, if the substrate is δ-tocopherol, VTE4 can also methylate δ-tocopherol to form β-tocopherol.[73] Recent characterization of phytol and phytyl phosphate kinase (PK) from *A. thaliana*[50,51] elucidated that the prenyl moiety for
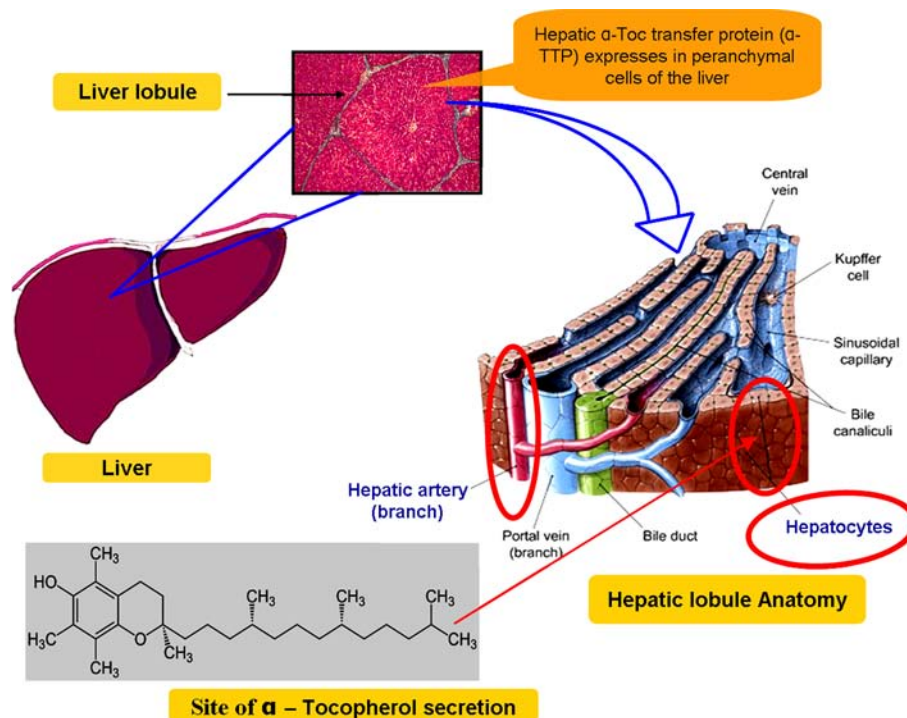
Figure 4. Anatomy of hepatic lobule, a functional unit of the liver, showing the α-tocopherol secretion sites called hepatocytes, circled in red. The hepatic α-tocopherol transfer protein (TTP) selectively binds this vitamin and facilitates its secretion from hepatocytes. The protein is expressed in parenchymal cells of the liver.

γ-tocopherol biosynthesis in seeds is derived from the free phytol, recycled as a result of chlorophyll breakdown.[54]

TOCOPHEROL VERSUS FOOD PRODUCTS

Production of reactive oxygen species (ROS) is a great threat to the quality of food and feed products. Various antioxidants are used to maintain the oxidative stability of processed foods. These antioxidants scavenge the oxygen species and can reduce microbial growth, the development of off-flavors, color and flavor changes, and nutritional losses in many food products by removing oxygen in package headspace.[82] However, due to the limited antioxidants approved for food application, it is challenging to maintain the oxidative stability of processed foods. Several strategies have been adopted to solve the problem. Tocopherol being a lipid antioxidant plays a vital role in maintaining the quality of food products and can synergistically interact with other antioxidants to inhibit lipid oxidation in foods more effectively. Panya et al. found that α-tocopherol and rosmarinic acid interactions produced an increase in antioxidant activity by promoting the conversion of rosmarinic acid into caffeic acid, thus providing a third molecule that could inhibit lipid oxidation and increasing the oxidative stability of the oil-in-water emulsions.[83] α-Tocopherol-loaded polycaprolactone nanoparticles and iron(II) chloride in an oxygen-scavenging system can be used as a heat-activated oxygen scavenger incorporated into food packaging materials.[82] Furthermore, tocopherol fingerprinting offers the potential to detect adulteration in food products.[84] One such example is that of coffee. Due to the pronounced flavor, *Coffea arabica* has a higher commercial value than *Coffea robusta*. The adulteration of coffee with cereals, coffee twigs, etc., is apparently reported in some parts of the world, for example, Brazil.[84] Moreover, tocopherols, particularly γ-tocopherol, contribute the most to the strong total antioxidant activities of both walnut and heartnut oils.[85,86]

TOCOPHEROL FUNCTIONS IN HUMANS AND ANIMALS

Being the most potent lipid-soluble antioxidant, the physiological importance of Toc (especially α-Toc) has been studied extensively in humans and animals. These studies revealed that Toc plays a vital role both in vivo[17,87,88] and in vitro.[3,89] The degree of antioxidant activities of tocopherol isomers against the lipid oxidation in vivo are reported as $\alpha > \beta > \gamma > \delta$,[16,90−92] whereas the antioxidant activities in vitro are $\delta > \gamma > \beta > \alpha$.[92] Furthermore, it is reported that the α-tocopheroxyl radical formed during antioxidant actions of α-tocopherol can be regenerated by ascorbic acid in human platelets, in low-density lipoproteins, and in solution.[93,94] Vitamin E (α-Toc in particular) has been rationalized as one of the major supplements in human diet as well. The role of Toc has been associated with the prevention of several human diseases such as coronary heart disease by decreasing vascular wall thickening through inhibition of smooth muscle cell proliferation,[95−100] atherosclerosis, diabetes, Parkinson's disease, Alzheimer's disease, vision maladies, impaired immune function,[62,101,102] and ischemic heart disease in cross-cultural epidemiology.[103] Recent papers suggest that vitamin E (tocopherol) intake, either from diet or from supplements, may reduce the risk of liver cancer.[104]

Studies in animals and artificial membranes elucidated the role of Toc such as their interaction with polyunsaturated acyl groups of lipids, stabilizing membranes, and scavenging and quenching various reactive oxygen species (ROS) and lipid-soluble byproducts of oxidative stress.[3,89] α-Tocopherol (or vitamin E) is also a lipophilic component of the membranes in muscle tissues. Several investigations have found that a high α-

tocopherol level may be critical for protecting flesh against lipid oxidation.[105,106] The coordinating role of Toc with other antioxidants (ascorbate or glutathione, etc.) has also been reported.[107] Studies in animal systems revealed that Toc, apart from their antioxidant properties, can perform roles in other significant physiological and biochemical processes, for example, the modulation of signal transduction pathways and transcriptional activation of gene expression mediated by tocopherol-binding proteins.[3,102,108−114] Moreover, α-tocopherol is mainly involved in the modulation of protein kinase C activity and eicosanoid synthesis, showing its function beyond that of an antioxidant in mammalian systems.[115−118]

In vivo, two principal processes in the liver regulate the vitamin E status: first, the degradation of the β-, γ-, and δ-tocopherols to water-soluble catabolites by the ω-hydroxylase cytochrome P450 CYP4F2;[119] second, the selective retention of α-tocopherol by the hepatic α-tocopherol transfer protein (TTP), which facilitates the secretion of this form from hepatic cells to circulating lipoproteins (Figure 4).[120] The protein is expressed in parenchymal cells of the liver and is believed to bind newly arrived α-tocopherol in the endocytic compartment. So far, TTP is supposed to be performing two activities; that is, the protein catalyzes the transfer of tocopherol between donor and acceptor membranes in vitro[121,122] and delivers the tocopherol (vitamin) to its site of secretion in the hepatocyte plasma membrane.[120,123,124] However, the molecular mechanisms underlying these activities remain poorly understood so far.

TOCOPHEROLS FUNCTIONS IN PLANTS

Tocopherols Function as an Antioxidant. Although the synthesis of Toc occurs only in photosynthetic organisms, their function in plants has been uncertain for many years. On the basis of the findings on the role of Toc in animals, it was presumed that Toc would have similar functions in plants as well. Initially, most studies focused typically on the antioxidant function of Toc in planta, mainly including (a) the protection of chloroplast membranes against ROS for regulating an optimal environment for the photosynthetic machinery[38,125] and (b) limiting the extent of lipid peroxidation in polyunsaturated fatty acids (PUFA) by reducing lipid peroxyl radicals to their corresponding hydroperoxides (Figure 5, eqs

$$LOO^\bullet + LH \longrightarrow LOOH + L^\bullet \quad [1]$$

$$^\bullet L^\bullet + O_2 \longrightarrow LOO^\bullet \quad [2]$$

$$LOO^\bullet + TOH \longrightarrow LOOH + TO^\bullet \quad [3]$$
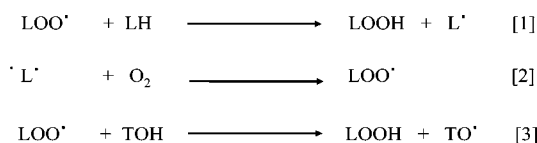
Figure 5. Antioxidant reactions of tocopherols to prevent the propagation of lipid peroxidation. LOO•, lipid peroxyl radical; LOOH, lipid hydroproxide; TO•, tocopheroxyl radical; TOH, tocopherol.

1−3).[33,92,126−128] ROS include the superoxide radical ($O_2^{\bullet-}$), hydroxyl radical (OH•), hydroperoxyl radical (HO₂•), hydrogen peroxide (H_2O_2), alkoxy radical (RO•), peroxy radical, singlet oxygen ($^1O_2$), and excited carbonyl (RO*), all of which are toxic to plants.[10,129−134] The donation of a single electron to form the resonance-stabilized tocopheroxyl radical[92,107] could be the possible mechanism responsible for scavenging and quenching ROS and lipid-soluble byproducts of oxidative stress.[3,62,102]

The in vivo antioxidant activity per molecule of each of the tocopherol isoforms revealed that α-, β-, γ-, and δ-Toc can protect up to 220, 120, 100, and 30 molecules of PUFA, respectively, against lipid oxidation[90] (Figure 5). α-Toc, having the highest vitamin E activity, is considered to be a chain-breaking antioxidant, with the ability to repair oxidizing radicals directly and thereby prevent the chain propagation step during lipid autoxidation.[123] In addition to this unique feature, Toc can also donate two electrons, which results in opening of the chromanol ring to form the corresponding tocoquinone derivative.[16,107]

Tocopherols Play a Role in Cell Signaling and Phytohormone Modulation. Recent advances in genetic dissection of biosynthetic pathway genes of tocopherol and photosynthesis revealed some functional possibility of Toc as cell signal molecules in plants.[10,56,135,136] Initially, it was presumed that the presence of the ATCTA sequence element in the promoters of genes involved in biosynthetic pathways of both Toc and photosynthesis[137] and close association between biosynthesis of Toc and plastoquinones[65] could be the functional bases of Toc in plant signaling pathways and related gene expression. Providing the initial steps toward understanding the role of Toc in plant cell signaling, Hofius et al.[56] found that Toc may affect source-sink transitions and may alter gene expression in plants (reviewed in ref 135). However, direct evidence to explain the mechanism(s) of action of Toc in regulating signal transduction and gene expression in plants has not been provided so far.[135,138] Moreover, studies on gene expression in transgenic plants such as potato[139] and tocopherol-deficient mutants of *Arabidopsis*, either single (vte4, vte2, vte1, *cad2*) or double,[34,139,140] suggest the obvious influential role of Toc in plant cell signaling, based on the fact that Toc are part of a complex signaling network modulated by ROS, antioxidants, and phytohormones.[135]

Phytohormones such as jasmonic acid (JA), salicylic acid (SA), and abscisic acid (ABA) appear to play regulatory functions in the synthesis of tocopherol in plants under environmental stresses[13,134] (Figure 6). The synthesis of JA occurs in chloroplasts, cytoplasm, and peroxisomes as a result of lipid peroxidation in chloroplasts. α-Tocopherol regulates lipid peroxidation in chloroplasts and limits the accumulation of lipid hydroperoxides required for JA synthesis, which in turn mediates stress responses and regulates the expression of tocopherol biosynthetic genes, particularly those encoding for tyrosine aminotransferase (TAT) and p-hydroxyphenylpyruvate dioxygenase, which catalyze the transamination of tyrosine to p-hydroxyphenylpyruvate and its conversion to homogentisate, respectively.[141,142] Furthermore, the possible coupling of biosyntheses of SA and α-tocopherol have been significantly correlated with each other in *Phyllirea angustifolia* plants exposed to drought stress and senescing *Salvia lanigera* leaves.[143,144] Moreover, under drought stress α-tocopherol correlated significantly higher with ABA in *Cistus creticus* compared with SA or JA.[145]

Ethylene gas, as a signal molecule, plays a vital role in plants, inducing tolerance against environmental stresses[146,147] including water and salt stresses.[148−150] The proposed pathway of ethylene signal transduction in *A. thaliana* consists of five ethylene receptors, ETR1, ERS1, ETR2, EIN4, and ERS2, the negative regulator CTR1, the downstream positive regulator EIN2, and the transcription factor EIN3 (among others), which up-regulates yet another transcription factor, ERF1.[151−153] Study on an *A. thaliana* mutant having a defect in ethylene
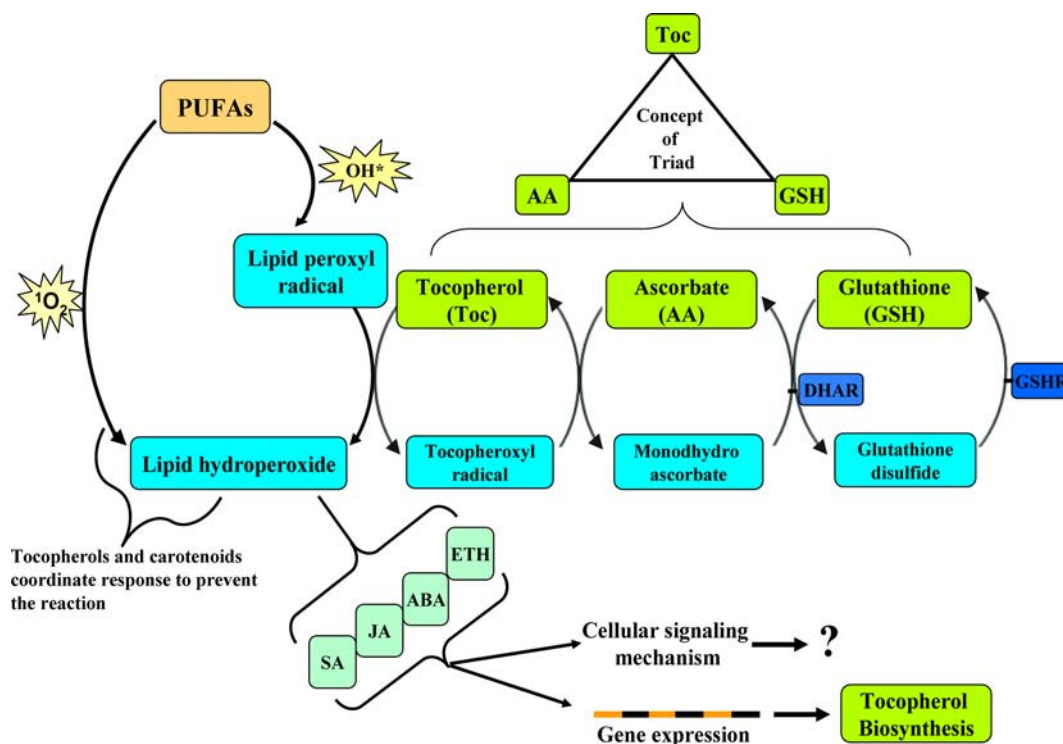
Figure 6. Model showing the functional attributes of tocopherols. Tocopherols limit the extent of lipid peroxidation by scavenging lipid peroxyl radicals and possibly prevent lipid peroxidation by reacting with reactive oxygen species (e.g., $OH^\bullet$, $^1O_2$) in coordination with carotenoids. The interactions between tocopherols, ROS, and phytohormones (JA, SA, Eth) show the intricate mechanism of cellular signaling that may directly or indirectly regulate gene expression (still unclear). The coordinate response of ascorbate and glutathione to the antioxidant activity of tocopherols gives the concept of triad, resulting in the recycling of tocopheroxyl radicals to tocopherols. PUFAs, polyunsaturated fatty acids; JA, jasmonic acid; SA, silicylic acid; ABA, abscisic acid; ETH, ethylene; DHAR, dehydroascorbate reductase; GSHR, glutathione reductase.

signaling showed alteration in tocopherol biosynthesis.[150] All of these findings are elucidating a complex functional and regulatory network of Toc and phytohormone association under various environmental stresses and need to be explored further to unravel the possible related mechanisms in cell signaling (Figure 6).

ABIOTIC STRESS EFFECTS ON TOCOPHEROL LEVELS IN PLANTS

Coordinate Roles of Tocopherol and Other Antioxidants in Plant Responses to Stress. Abiotic stresses include drought, salinity, extreme temperatures, high light intensity, chemical toxicity, and so on and are often interconnected and lead to the overproduction of ROS in plants.[154] Being highly reactive and toxic in nature, ROS can rapidly inactivate enzymes, damage vital cellular organelles in plants, and destroy membranes by inducing the degradation of pigments, proteins, lipids, and nucleic acids, which ultimately lead to irreparable metabolic dysfunction and cell death.[133,155−157] To fight against oxidative stress caused by ROS, plants possess a complex network of enzymatic and nonenzymatic antioxidant defense systems, of which the latter involves low-molecular-weight antioxidants such as tocopherol, ascorbate, and glutathione.[139,158] Among tocopherol isoforms, only α-tocopherol contributes to the antioxidant network present in chloroplasts of higher plants, along with other cellular antioxidants including ascorbate (AA) and glutathione (Figure 6). The chloroplast is considered to be the major source of ROS in plant cells. Toc, AA, and GSH are mutually dependent in mitigating the harmful effects of elevated ROS

levels in chloroplasts.[159] This defense triad can be accomplished in two ways; first, by affecting gene expression associated with abiotic stresses, altering acclimation responses; and second, by functioning as redox buffers that interact with ROS and act as a metabolic interface that modulates the appropriate induction of acclimation responses or programmed cell death.[160,161] Previous studies elucidated that various abiotic stresses activate the expression of genes responsible for the synthesis of α-Toc in higher plants[64,128,162] that ultimately increase the levels of α-Toc in plant tissues.[64,139] The reaction between α-Toc and lipid radicals including $RO^\bullet$, $ROO^\bullet$, and RO^* occurs in the membrane−water interface, resulting in the formation of $TOH^\bullet$ by donating one hydrogen atom to these radicals (Figure 5, eq 3). In the presence of other antioxidants, for example, AA, $TOH^\bullet$ can be recycled back to the corresponding α-Toc.[163,164] Ascorbate (vitamin C) is an excellent cellular redox state sensor that helps the photo- and antioxidant protection machinery by acting as a cofactor of violaxanthin de-epoxidase. AA performs the function of antioxidation by controlling the most readily diffusible ROS and hydrogen peroxide.[15] Furthermore, the regeneration of $TOH^\bullet$ back to its reduced form is presumably done by AA and GSH by recycling oxidized tocopherol.

Stress response studies in α-tocopherol-deficient mutants (*vte1*) of *Arabidopsis* revealed that Toc is negatively correlated with the content of AA and GSH. Increase in AA and GSH content was observed in vte1 mutants, whereas overexpression of α-tocopherol (VTE1) in plants led to a decline in AA and GSH contents.[139] Contrary to the negative association, in some cases, high content of Toc could elevate the levels of AA and

GSH, as reported in high-Toc-containing sunflower cell lines.[165] Moreover, *vtc1* mutants of *Arabidopsis* showed net α-Toc loss under severe water deficit caused by a deficiency of AA in chloroplasts.[166] However, increase in the levels of α-Toc and AA following triazole treatment in tomato has been observed as the membrane-protecting function against oxidative damage, contributing to chilling tolerance.[128,162] Furthermore, Giacomelli et al.[167] reported an increase in the concentrations of α-Toc, AA, and GSH in eight genotypes of *Arabidopsis* exposed to high light, whereas accumulation of more GSH was observed in four *vtc2* (ascorbate deficient) genotypes under controlled light.

Carotenoids are also an important group of lipid-soluble antioxidants, synthesized by all photosynthetic organisms as well as by some nonphotosynthetic bacteria and fungi.[168] Carotenoids have two main classes: carotenes as hydrocarbons (β-carotene and α-carotene) and xanthophylls, which are oxygenated derivatives of carotenes, such as zeaxanthin and lutein.[169] Previous studies highlighted the coordinated response of tocopherol and carotenoids to various stresses, suggesting the overlapping of their function as antioxidants in vivo. Some of the prominent reports include reduction in rapeseed Toc and chlorophyll contents occurring in response to increased carotenoids level by overexpression of phytoenne synthase;[170] accumulation of more α-Toc in young leaves of *A. thaliana* mutant *npq1* (lacks zeaxanthin) in response to high light;[171] and more zeaxanthin assimilation under high light in the *Arabidopsis vte1* mutant than in the wild type.[128] Furthermore, the mutational studies on carotenoids and Toc in other photosynthetic organisms, including *Chlamydomonas reinhardtii*[172−174] and *Synechocystis*,[33] provide some functional overlapping evidence in antioxidant activity of these two classes of molecules in vivo. However, all of these previous studies were only indicative. Recently, Li et al.[169] reported direct evidence of a functional overlapping of Toc and carotenoids in protection against photo-oxidative stress in npq1 lor1 strains with overexpressed nuclear *VTE2* gene in *C. reinhardtii*.

Functional Attributes of Tocopherols under Water and Salt Stress. Drought or water deficit is one of the major concerns to agriculture production all over the world. Under water stress, stomatal closure and over-reduction of the photosynthetic electron transport chain occur, leading to the production of active oxygen species, such as superoxide anion, hydrogen peroxide, and hydroxyl radicals, which in turn may cause lipid peroxidation and photoinhibitory damage to the photosynthetic apparatus. A positive correlation between tocopherol accumulation and water stress in several plant species,[175,176] including *A. thaliana*,[150] has been previously reported, suggesting that water stress is accompanied by increasing levels of Toc.[177] One such specific example is that of transgenic tobacco (*VTE1* overexpressed) plants with elevated Toc content, mainly α-tocopherol,[177−180] which resulted in enhanced tolerance to water stress.[181] Although the antioxidant property of tocopherol was supposed to be responsible for mitigation of the effects of ROS under drought stress,[182,183] the exact mechanism is not known.

Salt stress is another major constraint in crop production that limits growth by different mechanisms, including ion toxicity, causing osmotic unbalance and leading to oxidative stress by increasing ROS production.[156] However, there is a paucity of work about the role of Toc in protecting plants from several environmental stresses, especially salt stress. Recently, a major step to elucidate this subject was made by developing transgenic lines (RNAi) silenced for HPT and γ-TMT and

evaluating their response to salt, sorbitol stresses, and methyl viologen (paraquat; a contact herbicide) treatments in comparison to wild type plants.[58] Quite interestingly, HPT:RNAi transgenic tobacco plants with decreased total tocopherol contents showed increased sensitivity to salt stress, whereas highly increased tolerance to osmotic stress and methyl viologen was observed in γ-TMT transgenic plants, which accumulate γ-Toc instead of α-Toc.[58] Another study reported that rice mutants deficient in tocopherol cyclase (VTE1), and thus deficient in α-Toc and γ-Toc, showed less resistance to salt stress, whereas the overexpression of the same gene improved salt tolerance in rice and tobacco.[184] Furthermore, enhanced tolerance to salt stress and other abiotic stresses was reported in response to overexpression of γ-TMT in *Brassica juncea* leaves, indicating a specific role of α-tocopherol in the alleviation of various stresses.[166,185] Although molecular studies are focusing on the current subject, it is still unknown to what extent a tocopherol deficiency in these mutants can alter the plant response to water and salt stresses at the gene expression level.

■ CONCLUSION AND FUTURE PERSPECTIVE

Advances in molecular biotechnology and genetic engineering in plant science have increased the ability to determine the additional functions of Toc along with their antioxidant properties in both animals and plants.[13,21,114] In this regard, studies on tocopherol-deficient *A. thaliana* mutants have played an unprecedented role in exploring some vital functions of Toc in planta such as protection of chloroplasts from photoinhibition and photo-oxidative stress,[128] coordinating response of Toc to other antioxidants in scavenging lipid peroxyl radicals, preventing the propagation of lipid peroxidation, regulating singlet oxygen levels by quenching and scavenging,[120,167] plants adaptation to low temperature,[140,186,187] activation of seedling germination and growth,[188,189] and regulation of gene expression by modulating extra-plastidic PUFA metabolism. Currently, evaluation of tocopherol responses in plants to various environmental stresses is gaining more attention that could highlight the possible roles of tocopherols beyond that of antioxidant properties. So far, some remarkable and surprisingly robust phenotypes have been observed in tocopherol-deficient mutants of *Arabidopsis* and *Synechocystis* under several abiotic stresses,[33,66,71,72,140,190,191] leading to changing concepts and raising questions about the new functions of tocopherols. For example, the existence of compensatory mechanisms to afford adequate protection to plant physiological processes in the absence of tocopherols provides further evidence that it is the whole set of antioxidant defenses (ascorbate, glutathione, carotenoids, Toc and other isoprenoids, flavonoids, and enzymatic antioxidants) rather than a single antioxidant that helps plants to withstand environmental stress. However, some striking questions remain. For example, what are the molecular bases of these compensatory mechanisms? To what extent does tocopherol deficiency affect the plant physiology with respect to other antioxidants and non-antioxidant synthesis and regulation? Furthermore, what are the signaling cascades and mechanisms under environmental stresses that connect tocopherol levels in plastids with ion homeostatis, hormonal responses, and gene expression levels? In short, all of these aspects warrant further investigation.[192]

Although much progress has been made to understand the biosynthesis, structural, and functional dynamics of tocopherols, researchers believe that more surprises can be expected from the tocopherols regarding their antioxidant and non-

antioxidant functions and the related molecular mechanisms in plant tissues. One of the renowned plant scientists in the field of tocopherols and carotenoids, Dean Dellapenna, has pointed out the importance and scope of further research as a future perspective of these molecules: *We have at times been quite successful at pathway engineering; however, this work has also highlighted how little we understand about the integration of plant metabolism. For example, high light stress increases leaf tocopherol levels 30-fold while our best pathway engineering effort is but 4-fold. Similarly, the seed phenotypes of carotenoid mutants often cannot be predicted from leaf phenotypes. Such results indicate there are processes/genes outside the core biosynthetic pathways (e.g., transcription factors, metabolite transporters, storage and turnover pathways, cell biology components, etc.) that contribute to desired nutritional phenotypes.* Thus, further research of these aspects is needed to provide a better understanding of the role of tocopherols in plants.

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Notes

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