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α-Tocopherol in chloroplasts: Nothing more than an antioxidant?

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Abstract

Among the eight forms of vitamin E, only tocopherols are essential compounds that are distributed throughout the entire plant kingdom, with α-tocopherol being the most predominant form in photosynthetic tissues. At the cellular level, α-tocopherol is of special relevance inside the chloroplast, where it eliminates singlet oxygen and modulates lipid peroxidation. This is of utmost relevance since tocopherols are the only antioxidants that counteract lipid peroxidation. Moreover, at the whole-plant level, α-tocopherol appears to modulate several physiological processes from germination to senescence. The antioxidant role of α-tocopherol at the cellular level can have profound effects at the whole-plant level, including the modulation of physiological processes that are apparently not related to redox processes and could be considered non-antioxidant functions. Here, we discuss whether non-antioxidant functions of α-tocopherol at the whole-plant level are mediated by its antioxidant role in chloroplasts and the regulation of redox processes at the cellular level.

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Tocopherols, Non-antioxidant functions, Redox state, Plastid retrograde signaling, Redox signaling.

Introduction

α-Tocopherol is one of the eight forms of "vitamin E″ alongside β-, γ-, and δ-tocopherol as well as the four tocotrienol homologues (α-, β-, γ-, and δ-tocotrienols). Only α-tocopherol is universally distributed in the plant kingdom, being the major form accumulating in photosynthetic tissues and, hence, in chloroplasts (Box 1). Chloroplasts are one of the main sources of reactive oxygen species (ROS). ROS can act as signaling molecules, especially when produced transiently, in many essential plant processes, being particularly relevant in chloroplast-to-nucleus communication (known as retrograde signaling). However, ROS can also act as damaging molecules. Inside chloroplasts, ROS such as the superoxide anion and the hydroxyl radicals can induce oxidative modifications of the D1 and D2 proteins, hence, damaging the photosystem II [4]. Furthermore, hydroxyl radicals and, especially, singlet oxygen, can also damage the photosystem II and they are the main cause of lipid damage when produced at high amounts and in a sustained way, leading to cell death [5,6]. The functions exerted by α-tocopherol in the chloroplasts are not only important for the resilience of the photosynthetic apparatus, but can also influence signal transduction and nuclear gene expression, with chloroplasts serving as sentinels for stress sensing and signaling in plant responses to environmental stress [7, 8].

Particular attention has been paid to the functions of α-tocopherol both in animals and plants over the past two decades. On the one hand, the whole antioxidant network, including α-tocopherol, has been shown to play a role in signal transduction and gene regulation both in animals and plants. In plants, the well-coordinated network of antioxidants modulating ROS levels in chloroplasts has been proven to have a key role in redox regulation in several physiological processes, with α-tocopherol shown to influence the production of other signaling molecules and regulate gene expression [7,8]. On the other hand, there has been a long discussion in biomedicine on whether α-tocopherol plays a role in several physiological functions in mammals through non-antioxidant activities or if all the functions of α-tocopherol are mediated by its antioxidant role [9,10]. Similarly, α-tocopherol has been related to various physiological processes in plants, particularly in functions that may not be necessarily ascribed to cellular redox processes or to any specific antioxidant function in chloroplasts, thus paving the way to assign non-antioxidant activities for α-tocopherol also in plants. Here, we will summarize the

Box 1. α-Tocopherol: the main form of “vitamin E”.

Since the discovery of vitamin E 100 years ago, several studies have been carried out to find out its role in plants and animals. In humans, α-tocopherol transfer protein (α-TTP), a hepatic protein, mainly recognizes α-tocopherol, making it practically the only one present in human plasma and tissues [1]. In plants, where “vitamin E″ is synthesized, the content and composition of the different forms of “vitamin E″ vary depending on the species, the tissue, the age, and the growing conditions. Despite this, α-tocopherol is the most abundant form in photosynthetic tissues, where it exerts an antioxidant function that was well-established two decades ago (for a review see Ref. [2]). The universality of α-tocopherol among different organisms may be explained through an evolutionary point of view. “Vitamin E″ genes arose from the endosymbiosis of an ancestral heterotrophic eukaryote with cyanobacteria. The biosynthetic pathway and the enzymes involved are homologous in cyanobacteria and plants, except for MPBQ/MSBQ-MT, which appear to represent non-orthologous functionally equivalent enzymes that are an example of convergent evolution since they evolved independently in plants and cyanobacteria [3]. Since the antioxidant activity of α-tocopherol is greater than that of the other forms, it is plausible that it has been selected by evolution, making it the predominant form in photosynthetic tissues (Figure I). Moreover, as tocopherols are an essential factor in the human diet, hence the concept of “vitamin E″, it is very likely that α-TTP also arose evolutionarily with a strong specificity for α-tocopherol in order to store the most abundant “vitamin E″ form found in vegetables. Although γ-tocopherol and, sometimes, tocotrienols are present in high amounts in seeds and fruits, α-tocopherol is by far the most predominant form in green vegetables and in some fruits and seeds. It should be noted that vitamin E has been written in quotation marks because this is correct when referring to animals, but not when dealing with plants, since it is not a vitamin in photosynthetic organisms.

major functions of α-tocopherol in plants that have been established in the literature and discuss the antioxidant and non-antioxidant functions of tocopherols at various organization levels. A special focus will be placed on analyzing whether or not the non-antioxidant functions of α-tocopherol in the regulation of various physiological processes at the whole-plant level can be explained by its antioxidant effects and involvement in cellular redox processes.

α-Tocopherol: a key player in the antioxidant network

Before tackling the possible non-antioxidant functions of α-tocopherol in plants, it is essential to examine its unique properties as an antioxidant in chloroplasts and how ROS are produced in this organelle. ROS are produced in the electron transport chain of chloroplasts by two mechanisms. In the first one, which mainly occurs in the reaction center of photosystem II (PSII) and in the antenna system, singlet oxygen ($^1O_2$) is produced via triplet chlorophyll formation. In the second one, which occurs mainly in PSI, O_2 accepts electrons through the Mehler reaction, generating superoxide anion radicals ($O_2^{\bullet-}$). $O_2^{\bullet-}$ can be dismutated into H_2O_2, which can be converted into hydroxyl radicals ($OH^\bullet$), or protonated into hydroperoxyl radicals ($HO_2^\bullet$), both of which are initiators of lipid peroxidation [11]. Lipid peroxidation can eventually destroy the integrity of membranes and, if severe, cause cell death. However, it can also generate oxylipins, which have key signaling functions during plant development and stress responses [12]. α-Tocopherol is unique among the chloroplast network of antioxidants in that it can inhibit the propagation of lipid peroxidation.

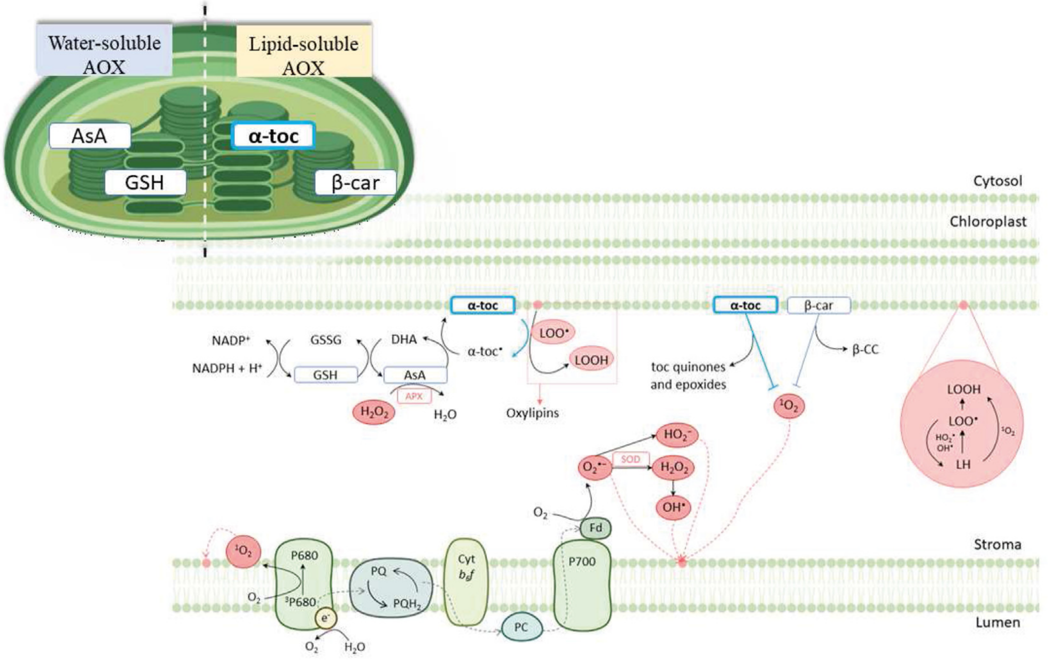
α-Tocopherol is part of a well-coordinated network of non-enzymatic antioxidants that is necessary to maintain chloroplast redox homeostasis, especially when plants are in unfavorable conditions. This network is mainly governed by two water-soluble antioxidants (ascorbate and glutathione) and two lipid-soluble antioxidants (β-carotene and α-tocopherol). Carotenoids can physically quench $^1O_2$, producing the carotenoid triplet state that ends up deactivating through thermal decay [13]. However, β-carotene can occasionally be oxidized by $^1O_2$, generating β-carotene derivatives such as β-cyclocitral (β-CC), which is able to regulate gene expression [14]. Ascorbate can directly scavenge $O_2^{\bullet-}$ and $OH^\bullet$ and, as part of the ascorbate/glutathione cycle, reduces H_2O_2 into water [5]. Glutathione by itself can scavenge a range of ROS such as H_2O_2 and $OH^\bullet$ and, as a component of the ascorbate/glutathione cycle, regenerates ascorbate using the NADPH produced in the electron transport chain [5,6]. Moreover, through this cycle, α-tocopherol can be regenerated from its tocopheroxyl radical state [15]. Therefore, α-tocopherol cooperates with β-carotene in modulating the levels of $^1O_2$ in chloroplasts, while at the same time having a unique role in inhibiting the propagation of lipid peroxidation (Figure 1).

Both β-carotene and α-tocopherol modulate membrane fluidity in chloroplasts [16,17]. Tocopherols are synthesized in chloroplasts, where they are equally distributed between the envelope and the thylakoid membranes, except under stress conditions and leaf senescence, when some of the α-tocopherol accumulates in the plastoglobuli [18]. Since α-tocopherol accumulates as a reservoir in the plastoglobuli, it is very difficult to ascertain what fraction of the α-tocopherol measured in leaves really exerts a direct antioxidant effect in chloroplasts under stress conditions. In any case, there is strong evidence in the literature showing that α-tocopherol plays a major role as an antioxidant in the plant response to several stresses (Table 1). α-Tocopherol is a (physical) quencher and a (chemical) scavenger of $^1O_2$, acting in the vicinity of PSII together with β-carotene [44]. It also reacts with the lipid peroxyl radical in thylakoid membranes, inhibiting the propagation of lipid peroxidation [45]. Furthermore, α-tocopherol stabilizes thylakoid membranes through its antioxidant effects and by providing fluidity to the lipid bilayer [46]. It should be noted that α-tocopherol exerts at least five protective direct roles against oxidative stress in chloroplasts, which has important implications for the physiology not only of photosynthetic tissues but also of several other processes at the whole-plant level (Figure 2).

Functions of α-tocopherol at the whole-plant level: do they all have a cellular redox basis?

Aside from its antioxidant function and role in plant stress tolerance, which can be attributed to its role in protecting thylakoid membranes (please see the examples in Table 1), α-tocopherol also has some intriguing roles in plant development (Table 2). These include all the essential developmental processes in the life cycle of angiosperms, such as seed longevity, dormancy, and germination, leaf senescence, as well as flower and fruit development. The effects of α-tocopherol in all these processes are not only important for better understanding the basic processes of plant biology, but also have very significant implications for agrifood biotechnology. Some of these effects are clearly associated with the antioxidant functions of α-tocopherol since some of the processes are undoubtedly related to oxidative processes (e.g., seed longevity and gemination [47–51]), but others are unexpected. The most striking effects observed in plants that may at first sight suggest that α-tocopherol has non-antioxidant roles at the whole-plant level are the altered source-sink partitioning in tocopherol-deficient plants, thus leading to alterations in various physiological processes, such as flower or fruit development [53–58] and leaf senescence [63–65].

Figure 1



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The chloroplast network of non-enzymatic antioxidants, mainly governed by two water-soluble antioxidants (ascorbic acid (AsA) and glutathione (GSH)) and two lipid-soluble antioxidants (β-carotene (β-car) and α-tocopherol (α-toc)), maintains the redox homeostasis of the organelle. The four antioxidants can efficiently counteract reactive oxygen species (ROS), an efficiency that increases with their interactions and complementation. Multiple ROS are produced in the electron transport chain that can oxidize lipid membranes, generating lipid peroxidation products such as lipid peroxyl radicals ($LOO^•$) and oxylipins, which have important roles in retrograde signaling (chloroplast-to-nucleus signaling). β-car serves an important role in eliminating $^1O_2$. However, it can occasionally be oxidized by this ROS, generating β-car derivatives such as β-cyclocitral (β-CC), a regulator of gene expression. α-Toc also removes $^1O_2$, forming α-toc quinones and epoxides. However, only α-toc can scavenge $LOO^•$, thus having a tremendous and unique relevance in protecting lipid membranes. AsA and GSH can directly scavenge ROS and, as part of the AsA/GSH cycle, regenerate α-toc from its tocopheroxyl radical (α-toc$^•$). $O_2^{•-}$, superoxide anion; $HO_2^•$, hydroperoxyl radical; $OH^•$, hydroxyl radical; H_2O_2, hydrogen peroxide; $^1O_2$, singlet oxygen; LOOH, lipid hydroperoxide. The figure was created with the help of BioRender.com.

Table 1

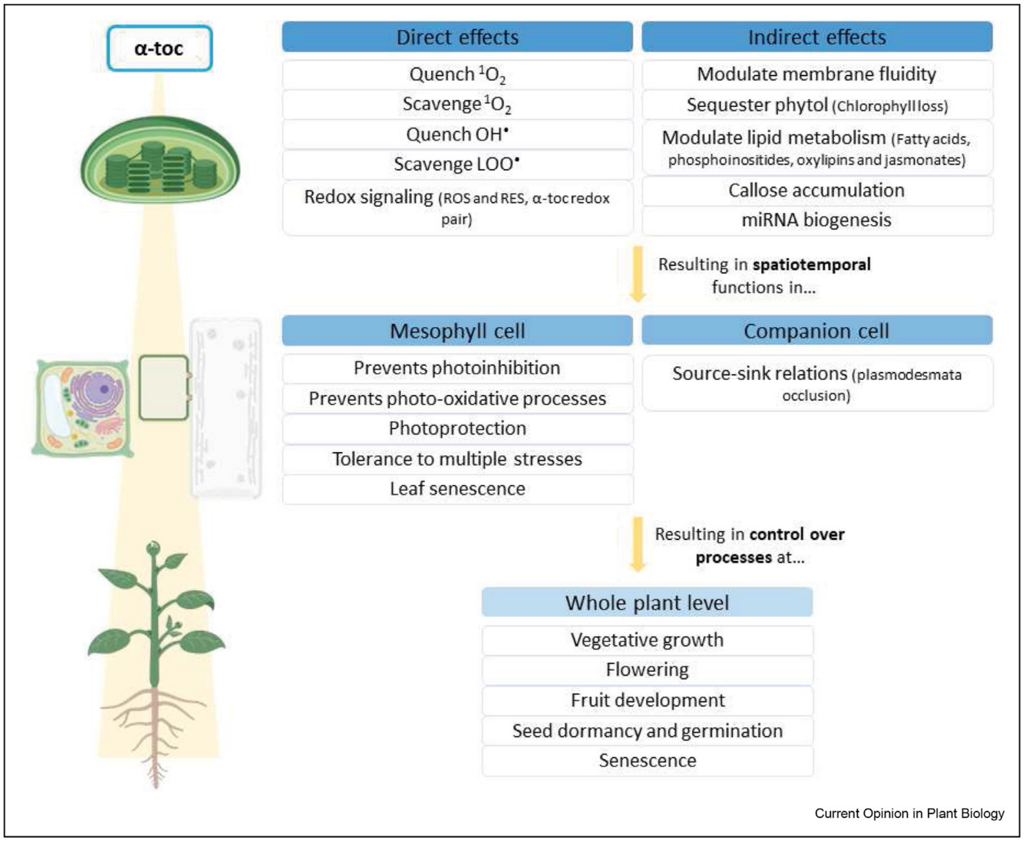
Functions of tocopherols in plant stress responses. A summary is shown of the major roles of tocopherols in plant stress responses described to date, indicating whether the mechanism is an antioxidant one or unclear. AOX, antioxidant.^{C} indicates correlative evidence only.

STRESS	FUNCTION	FORM	MODEL	MECHANISM	ROLE		REF.
					AOX	UNCLEAR	
Water deficit	Increased tolerance	α-Tocopherol	Tobacco/rosemary/oak	Reduces lipid peroxidation and protects chloroplasts from photo-oxidative stress	✓		[19–21]
	Prevents leaf desiccation	γ-Tocopherol	Heartleaf iceplant	Unknown^{C}		✓	[22]
Heavy metal ions	Alleviation of CuSO$_4$-induced stress	α-Tocopherol	Tobacco	Reduces ROS accumulation and lipid peroxidation^{C}	✓		[23]
	Increased tolerance to Cu- and Cd-induced oxidative stress	α-Tocopherol	*Arabidopsis*	Reduces lipid peroxidation^{C}	✓		[24]
	Alleviation of Ni-induced stress	α-Tocopherol	Wheat	Reduces lipid peroxidation^{C}	✓		[25]
	Alleviation of Cd-induced stress	α-Tocopherol	Rapeseed	Reduces ROS accumulation and Cd absorption by the roots^{C}	✓	✓	[26]
Salinity	Increased tolerance	α-Tocopherol	Tobacco/rice/Indian mustard	Reduces ROS accumulation and lipid peroxidation	✓		[23,27–29]
	Increased tolerance	α-Tocopherol	Potato	Allows photoassimilate export by regulating SnRK1 (sucrose non-fermenting-1-related protein kinase 1) signaling sensitivity		✓	[30]
	Increased tolerance	γ-Tocopherol	*Arabidopsis*	Protects plants as effectively as α-tocopherol by altering lipid peroxidation and hormonal modulation	✓		[31]
UV-B radiation	Increased tolerance	α-Tocopherol	*Arabidopsis*	Protects against photo-oxidative damage	✓		[32–34]
Low temperature	Increased tolerance	α-Tocopherol	*Arabidopsis*/rice/Taunton yew	Stabilizes thylakoid membranes and prevents lipid peroxidation. Allows photoassimilate export by preventing callose deposition while modulating endoplasmic reticulum fatty acid metabolism. Essential as part of the plastid retrograde signaling regulating miRNA biogenesis through 3′-phosphoadenosine 5′-phosphate modulation by COLD1-mediated calcium influx	✓	✓	[35,36]
High temperature	Increased tolerance	α-Tocopherol	*Arabidopsis*	Essential as part of the plastid retrograde signaling regulating miRNA biogenesis through 3′-phosphoadenosine 5′-phosphate modulation		✓	[7]
High light	Increased tolerance	α-Tocopherol	*Arabidopsis*/lettuce	Protects photosynthetic complexes against ROS accumulation	✓		[37–39]
High temperature and high light	Increased tolerance	α-Tocopherol	Tomato	Protects against photo-oxidative damage and reduces membrane fluidity	✓		[40]
Biotic	Increased resistance	α- and γ-tocopherol	*Arabidopsis*/drumstick tree	Allow adequate defense mobilization by modulating lipid peroxidation and membrane fatty acid composition	✓		[41,42]
	Increased resistance	α-Tocopherol	*Arabidopsis*	Inhibition of root-knot nematodes penetration		✓	[43]

A detailed analysis of the studies summarized in Table 2 revealed striking new evidence that could lead to the further understanding of the functions of α-tocopherol in plants. Carbohydrate export has been reported to be blocked in *VTE5*-deficient tomato plants that cannot synthesize tocopherols, affecting fruit quality [58]. Interestingly, the prenyllipid profile greatly differs between the source and sink organs in these tomato plants, revealing organ-specific metabolic adjustments. Furthermore, α-tocopherol has been shown to play a crucial role in low-temperature adaptation and phloem loading in *Arabidopsis* [65], confirming earlier studies performed in other species [57,66]. A defect in *VTE2*, which is essential for α-tocopherol biosynthesis, rapidly reduces photoassimilate export at low temperatures in *Arabidopsis*, coinciding with callose deposition exclusively in the phloem parenchyma transfer cell walls adjacent to the sieve element/companion cell complex [65]. Although further research is needed to better understand underlying mechanisms, it is likely that α-tocopherol deficiency affects the redox state of phloem cells with the consequent effects in the regulation of source–sink relationships and various developmental processes at the whole-plant level. Alternatively, it is also likely, and not mutually exclusive, that these effects reflect the specific interactions of α-tocopherol with enzymes, structural proteins, lipids, and transcription factors that have nothing to do with redox regulation. It is well known that, aside from its antioxidant activity, α-tocopherol can affect membrane fluidity [46], which may have profound implications in the activity of membrane enzymes and the stability of macromolecules found in the chloroplast envelope, thylakoids and plastoglobuli. Therefore, it is essential that new *VTE5* or *VTE1*-deficient tomato/*Arabidopsis* plants with inducible promoters in phloem cells or other cellular types are obtained to unravel the mechanisms underlying the functions of α-tocopherol observed at the whole-plant level.

Another aspect of special relevance that requires further research is why plants respond so strikingly to γ-tocopherol accumulation in chloroplasts. If γ-tocopherol acted as a simple precursor of α-tocopherol, its huge

Figure 2



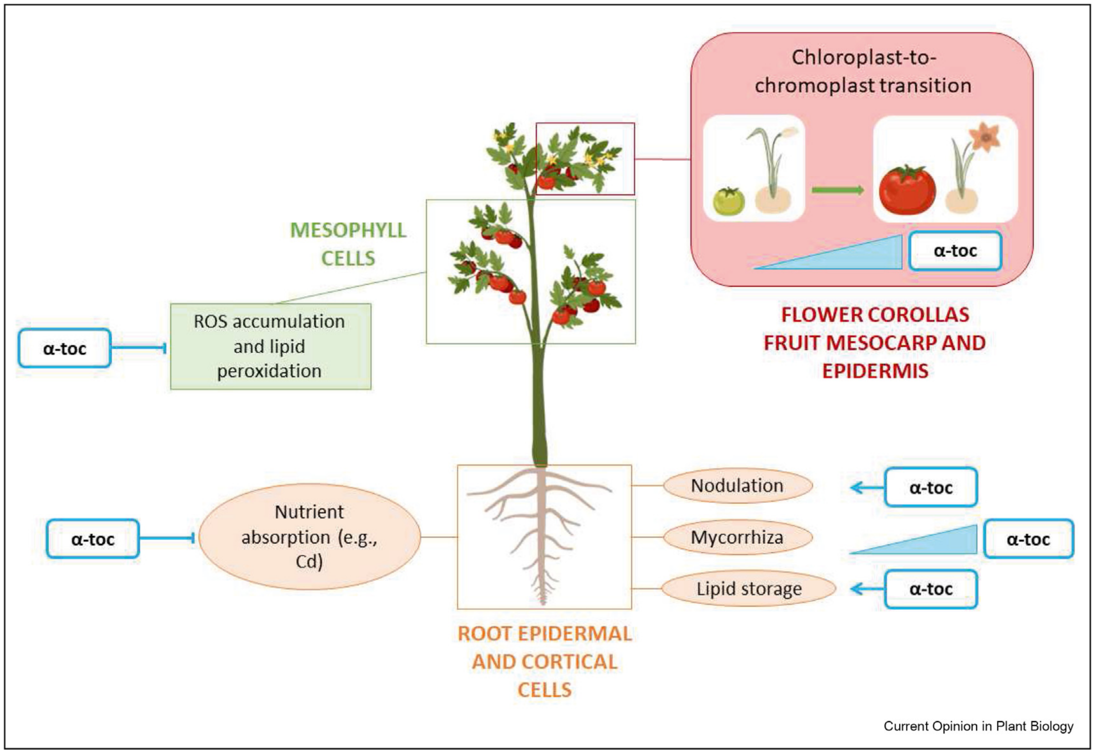
The function of α-tocopherol in chloroplasts with an impact at various organization levels. α-Tocopherol exerts key roles from the physicochemical to the organism levels, influencing various plant developmental processes. $^1O_2$, singlet oxygen; OH•, hydroxyl radical; LOO•, lipid peroxyl radical; ROS, reactive oxygen species; RES, reactive electrophile species. The figure was created with the help of BioRender.com.

Table 2

Tocopherol functions in plant developmental processes. A summary is shown of the major roles of tocopherols in plant development described to date, indicating whether the mechanism is an antioxidant one or unclear. AOX, antioxidant.^{C} indicates correlative evidence only.

PROCESS	FUNCTION	FORM	MODEL	MECHANISM	ROLE		REF.
					AOX	UNCLEAR	
Seed longevity	Protects against oxidation	α-Tocopherol	*Arabidopsis*/rice	Protects lipids and embryo from oxidation	✓		[47–49]
Germination	Protects against oxidation	α-Tocopherol	*Arabidopsis*/soybean	Protects lipids from oxidation, avoiding altered gene expression	✓		[50,51]
	Delays seedling development	γ-Tocopherol	Barley	Scavenges nitric oxide^{C}	✓		[52]
Flower development	Flower induction	α- and γ-tocopherol	*Xanthium*/*Lilium*	Increase during flowering either in the leaves or flowers^{C}		✓	[53,54]
	Flower vigor	α-Tocopherol	*Lilium*/grey-leaved cistus	Protective effect against oxidative stress and decay in flower vigor^{C}	✓		[55,56]
Tuber formation	Ensures tuber formation	α-Tocopherol	Potato	Correct carbohydrate export from source to sink tissues		✓	[57]
Fruit development and ripening	Ensures fruit development	α-Tocopherol	Tomato	Correct carbohydrate export from source to sink tissues		✓	[58]
	Protects against oxidation	α-Tocopherol	Citrus	Protects the flavedo from environmental stress	✓		[59]
	Ensure correct development and ripening	α- and γ-tocopherol	Tomato/mango/pepper	Increase during ripening^{C}		✓	[60–62]
Senescence	Photoassimilate remobilization	α-Tocopherol	*Arabidopsis*/tobacco/alfalfa/tomato	Phloem loading		✓	[63–65]

Figure 3



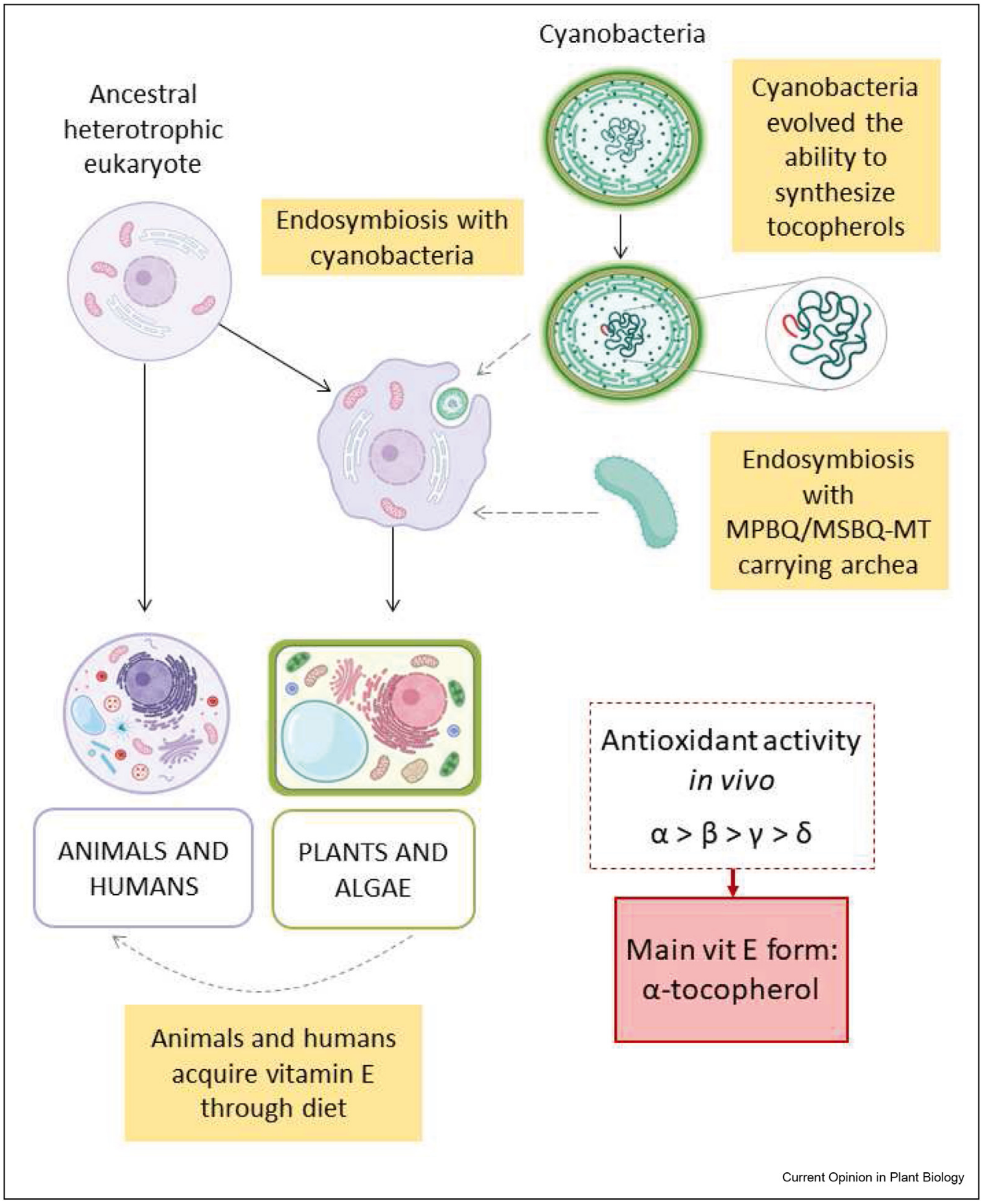
α-Tocopherol exerts essential roles in different plant tissues regulating both developmental processes and stress responses. α-Tocopherol can be found at mesophyll cells mainly inhibiting ROS accumulation and lipid peroxidation, its main antioxidant role; but also in flower corollas and fruit mesocarp and epidermis where it has been seen to increase during the chloroplast-to-chromoplast transition, an oxidative process; and in root epidermal and cortical cells where multiple roles have been described such as protecting storage lipids, improving nodules performance in legume-*Rhizobium* symbiosis, increasing its content after mycorrhization and inhibiting heavy metals absorption. Figure created with the help of BioRender.com.

accumulation would not necessarily result in contrasting phenotypes. In recent years, however, it has been shown that specific functions can be attributed to the different forms of tocopherols accumulating in chloroplasts, particularly α- and γ-tocopherol. In the *Arabidopsis vte4* mutant, which accumulates γ-tocopherol instead of α-tocopherol, multiple specific responses have been observed, such as an improved response to the osmotic stress produced by sorbitol or methyl viologen [67] or major changes in jasmonic acid and ethylene signaling in the mature leaves of plants exposed to salt stress [31]. These have led to the proposal that γ-tocopherol has specific roles in osmoprotection and gene expression. However, assigning a specific function to γ-tocopherol is difficult since different pleiotropic effects might be occurring. Furthermore, the contents of the different forms of tocopherols are highly altered in the mutants when compared to the levels found in nature. Therefore, the results obtained from these studies should be carefully analyzed and compared with other experimental approaches since a direct extrapolation to what happens in nature may lead to erroneous assumptions. In essence, we can speculate that other forms may have specific roles in nature, but, to date, α-tocopherol is the only form proven to have an antioxidant function in photosynthetic tissues.

Conclusions and future perspectives

In this review, we summarize the evidence showing that α-tocopherol plays a major role as an antioxidant in chloroplasts, not only eliminating singlet oxygen, but also inhibiting the propagation of lipid peroxidation, a function not exerted by the other antioxidants. Hence, this has important implications for understanding its protective role against photo-oxidative damage in plant responses to abiotic stress. However, new functions for α-tocopherol have been recently identified at the whole-plant level, with α-tocopherol appearing to modulate several physiological processes ranging from germination to senescence (Figure 2). The most striking phenotype observed in α-tocopherol-deficient plants that might at first sight suggest non-antioxidant functions of α-tocopherol in plants (regulation of flower and fruit development as well as leaf senescence) are the alterations in source-sink partitioning. However, it is possible that these phenotypes are mediated by the antioxidant effects of α-tocopherol through the direct or indirect regulation of the redox state of phloem companion cells. Moreover, further research on γ-tocopherol is required to better understand the unique phenotypes observed in *Arabidopsis vte4* mutants and in some plants accumulating large amounts of γ-tocopherol in the chloroplasts, taking into account the antioxidant network and possible pleiotropic effects. Of particular relevance will also be the study of the function of tocopherols in other plastid types beyond chloroplasts (Figure 3). In this regard, α-tocopherol could not only be exerting specific functions in the chloroplast-to-chromoplast transition in fruits and flowers, but also in nutrient uptake,

Figure I



The universality of α-tocopherol among different organisms may be explained through an evolutionary point of view. Vitamin E genes arose from the endosymbiosis of an ancestral heterotrophic eukaryote with vitamin E-synthesizing cyanobacteria and MPBQ/MSBQ-MT-carrying archaea. The temporal occurrence of the latter endosymbiosis is still unclear. Due to its universality, abundance, and high antioxidant activity, α-tocopherol was probably the most predominant form of vitamin E incorporated into the diet of animals, thus making α-TTP more specific for this form. The figure was created with the help of BioRender.com.

nodulation, mycorrhization and lipid storage in the root [26,68,69], aspects that warrant further investigations.

Declaration of competing interest

All the authors declare that they have no conflict of interest.

Data availability

No data was used for the research described in the article.

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