Chapter

# Biosynthesis Pathways of Vitamin E and Its Derivatives in Plants

*Makhlouf Chaalal and Siham Ydjedd*


## Abstract

Naturally occurring vitamin E, comprised of four forms each of tocopherols and tocotrienols, are synthesized solely by photosynthetic organisms and function primarily as antioxidants. The structural motifs of the vitamin E family and specifically the chroman moiety, are amenable to various modifications in order to improve their bioactivities towards numerous therapeutic targets. Tocopherols are lipophilic antioxidants and together with tocotrienols belong to the vitamin-E family. These lipid-soluble compounds are potent antioxidants that protect polyunsaturated fatty acids from lipid peroxidation. Biosynthetic pathways of plants producing a diverse array of natural products that are important for plant function, agriculture, and human nutrition. Edible plant-derived products, notably seed oils, are the main sources of vitamin E in the human diet. The biosynthesis of tocopherols takes place mainly in plastids of higher plants from precursors derived from two metabolic pathways: homogentisic acid, an intermediate of degradation of aromatic amino acids, and phytyldiphosphate, which arises from methylerythritol phosphate pathway. Tocopherols and tocotrienols play an important roles in the oxidative stability of vegetable oils and in the nutritional quality of crop plants for human and livestock diets. Here, we review major biosynthetic pathways, including common precursors and competitive pathways of the vitamin E and its derivatives in plants.


**Keywords:** Vitamin E, Biosynthetic pathways, Tocopherols, Tocochromanol, Shikimate pathway, Methylerythritol pathway

## 1. Introduction

Under biotic and abiotic stresses conditions, including pathogens, temperature, drought, salt, and high light, the reactive oxygen species (ROS) resulting the oxidation of cellular components, as proteins, chlorophyll, and lipids [1]. To defend against oxidative stress, the plants have developed two general protective mechanisms, enzymatic and non-enzymatic detoxification, of which the latter involves vitamin E [2].

Plants are a major source of vitamins in the human diet. Due to their significance for human health and development, research has been initiated to understand the biosynthesis of vitamins in plants [3]. Vitamin E is thought to be involved in many essential processes in animals and plants. The function of vitamin E in plants is far from being clear. Likewise, in animal cells, the vitamin E acts as an antioxidant, thus it protects the plant from oxygen toxicity.

Four different forms of tocopherols and tocotrienols occur in nature and differ by the numbers and positions of methyl groups on the aromatic portion of the chromanol head group (**Figure 1**).

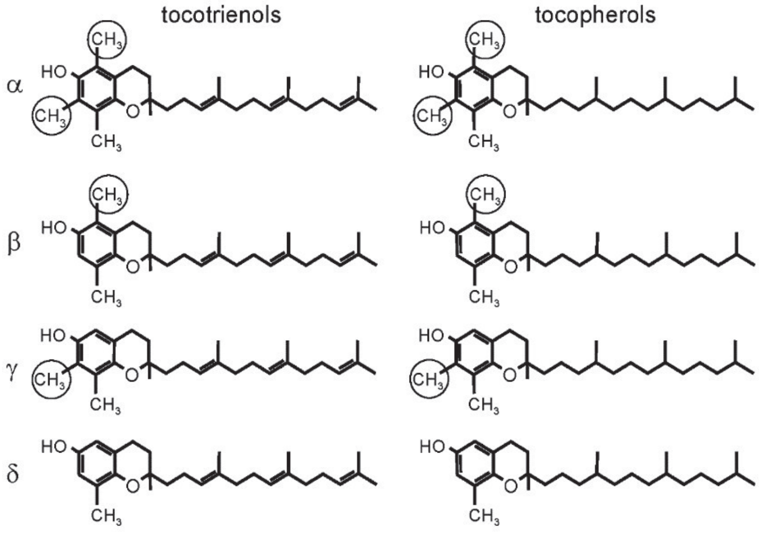


**Figure 1.**
*The eight forms of naturally occurring vitamin E (or tocochromanols) [4].*

| Sources | Plant organs | Usable Products | Vitamin E contents (g/kg) |
|---|---|---|---|
| Wheat | Kerne | Germ | 1500 |
| Sunflower | Seed | Oil | 610 |
| Sunflower | Seed | Kernel | 351 |
| Almond | Kernel | Oil | 392 |
| Safflower | Kernel | Oil | 450 |
| Canola | Seed | Oil | 270 |
| Walnut | Fruit | Oil | 200 |
| Peanut | Seed | Edible nut | 172 |
| Palm | Kernel | Oil | 150 |
| Olive | Seed | Oil | 120 |
| Soybean | Kernel | Oil | 116 |
| Maize | Seed | Entire grain | 20 |
| Oat | Seed | Kernel | 15 |
| Coconut | Seed/fruit | Oil | 10 |
| Asparagus | Shoot Young | shoot | 15 |
| Spinach | Leaf | Raw leaf | 20 |
| Spinach | Leaf | Cooked leaf | 21 |
| Tomato | Fruit | Raw fruit | 9 |
| Carrot | Root | Taproot | 6 |
| Tobacco | Leaf | Young leaf | 57 |
| Tobacco | Leaf | Old leaf | 180 |

**Table 1.**
*Vitamin E content in different cultivated plant species (reported by Has).*

Only plants and some cyanobacteria are able to synthesise vitamin E. α-Tocopherol is the predominant form of vitamin E green parts of higher plants, and is synthesized and localised mainly in plastids, whereas generally in non-photosynthetic tissues, γ-tocopherol is the major form [5].

The accumulation of vitamin E was varied in a number of plant species and in different plant parts. Generally, their content was ranged between 100 and 500 mg/kg fresh weight of normal plants with some exceptions. Oil-yielding plants present a higher vitamin E amount. Likewise, the seeds showed a highest total vitamin E content compared to other plant parts. **Table 1** indicates the amount of α-tocopherol in different plant species. In seeds, the vitamins were localized in plastids; however, in some cases it was also observed in cytoplasmic lipid bodies [6]. Commonly, α-Tocopherol was the major form of vitamin E in leaves, while many plants seeds contain γ-Tocopherol. Heowever, β-tocophenrol and δ-Tocophenrol are uncommon in plants [1, 7]. Thus, this work complements highlighted the biosynthetic origins of vitamin E biosynthetic precursors in plants.

## 2. Biosynthesis of vitamins in plants

The biosynthesis of different vitamins in plants has been carried out generally by bacterial pathways, except in the case of vitamin C, which is synthesized exclusively by eukaryotes. The biosynthesis of some vitamins is limited to the compartment as carotenoids (pro-vitamin A), vitamins E and K and water-soluble riboflavin are produced in the plastids of plants [8, 9]. However, some enzymes of phylloquinone biosynthesis have been found in peroxisomes [10] and riboflavin is further converted to flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) in the cytosol, plastids or mitochondria [11]. Furthermore, the biosynthesis of the water soluble vitamins is split between different compartments, including the mitochondria [12] (**Figure 2**).

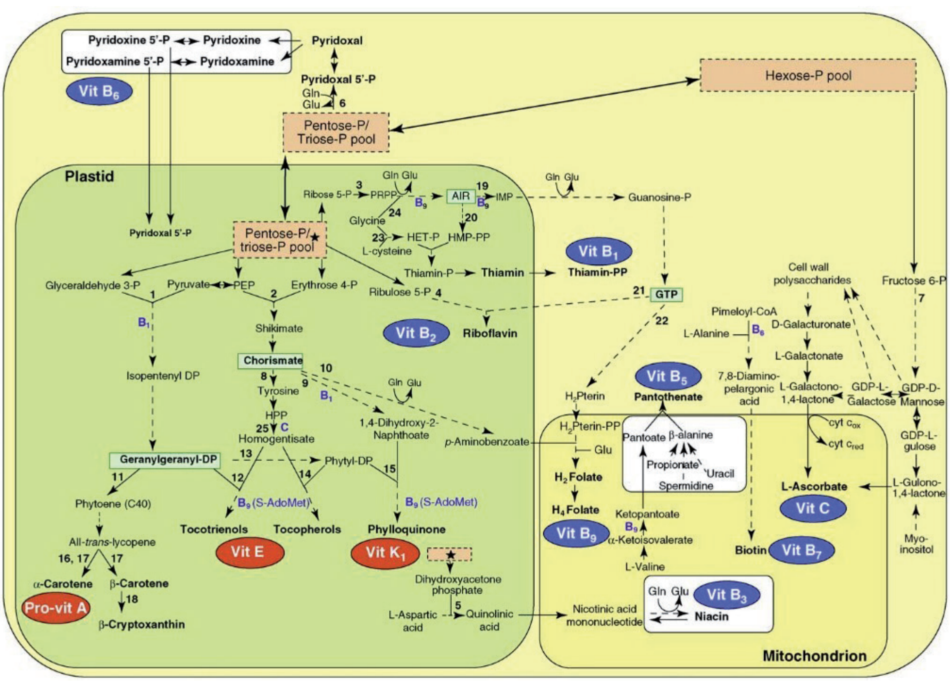


**Figure 2.**
*Cross-points on the biosynthetic pathways of vitamins in plants [13].*

The vitamins precursors were coming from carbohydrate metabolism, which regulates the pools of hexoses, pentoses and trioses in the plastids and the cytosol. The pentose and triose pool in the plastids provides: (a) erythrose-P and phosphoenolpyruvate for the synthesis of chorismate, the common intermediary in the biosynthesis of tocochromanols [14, 15]; (b) glyceraldehyde 3-P and pyruvate (from phosphoenolpyruvate), which are required for the synthesis of geranylgeranyl-PP, a key shared precursor of lipid-soluble vitamins [8, 14].

## 3. Vitamin E structures and biosynthesis

Plants synthesize eight different molecules with vitamin E antioxidant activity, including α-, β-, γ-, and δ-tocopherols and the corresponding four tocotrienols. These forms were different with respect the number and position of the methyl groups on their chromanol ring. The tocotrienols have an unsaturated tail containing three double bonds, while the four tocopherols have a phytyl tail.

Two main pathways of vitamin E biosynthesis are occurs at the inner envelope of plastids. The shikimate pathway gives rise to the chromanol ring from homogentisate (HGA). While, the methylerytrithol phosphate (MEP) pathway provides the prenyl tail from geranylgeranyl diphosphate (GGDP) for the synthesis of tocotrienol and phytyl diphosphate (phytyl-DP) for the synthesis of tocopherol (**Figure 3**). Furthermore, an additional pathway for phytyl-DP production from chlorophyll degradation, also known as the phytol recycling pathway (**Figure 4**). Seeds and leaves showed 80% and 65% reductions in total tocopherol content, respectively, compared to other plant parts. Chlorophyll synthase and geranylgeranyl diphosphate reductase (GGDR) are also involved in

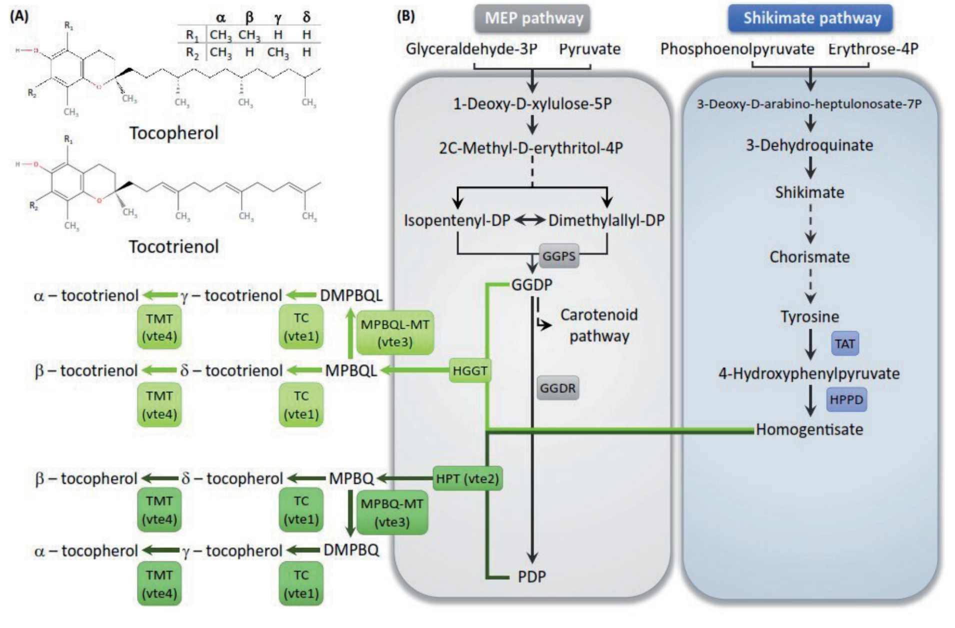


**Figure 3.**
*Vitamin E Chemical Structure and Biosynthesis in Plants [16]. (A) Vitamin E chemical structure. A chromanol head and a prenyl tail constitute the chemical structure of tocopherols and tocotrienols. While tocopherols have a saturated tail, tocochromonals have three unsaturations (orange lines), at 3′, 7′, and 11′. (B) Biosynthesis of tocopherols and tocotrienols in plants. Tocopherols and tocotrienols are formed from the combination of the methylerythritol phosphate and shikimate pathways.*

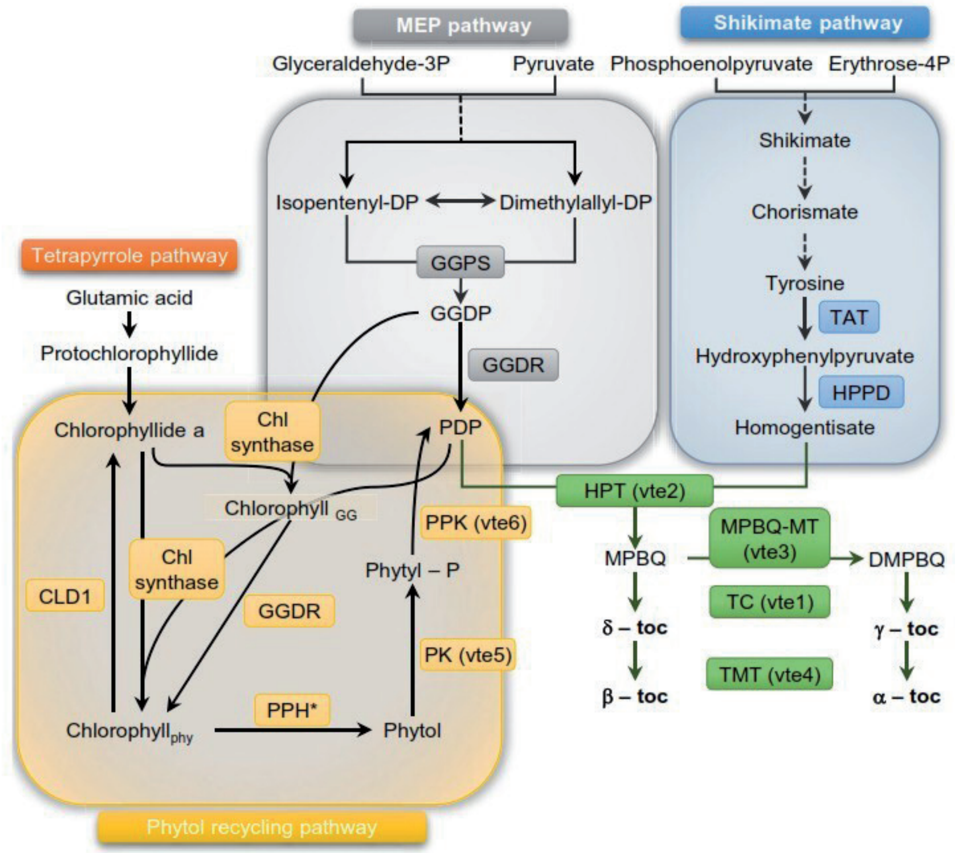


**Figure 4.**
*Tocopherol Biosynthesis with Chlorophyll Degradation in Plants [16].*

vitamin E biosynthesis [17]. The identity of the enzymes involved in chlorophyll dephytylation is less clear and the hydrolases such as CLD1 may allow phytol remobilization during fruit ripening and seed maturation [18, 19].

## 4. Tocopherols biosynthetic pathway

Tocopherols are found in higher plants, in algae, and in some nonphotosynthetic plants, such as yeasts and mushrooms [20]. Tocopherol biosynthesis was carried out via the condensation of homogentisate, derived from the shikimate pathway, and phytyl pyrophosphate (phytyl-PP), derived from the non-mevalonate pathway, through the action of the homogentisate prenyltransferase (HPT) (**Figure 5**). Subsequent ring cyclization and methylation reactions result in the formation of the four major tocopherol derivatives. The final methylation reaction resulting inα - and β-tocopherol, respectively, is expected to be catalysed by the same methyltransferase (γ-TMT) [21]. Theγ -TMT gene was isolated from the putative 10-gene tocopherol biosynthetic operon in *Synechocystis* sp.

### 4.1 Shikimate pathway

The shikimate pathway has been found in plants and in some microorganisms serves as a biosynthetic way of aromatic amino acids (phenylalanine (Phe),

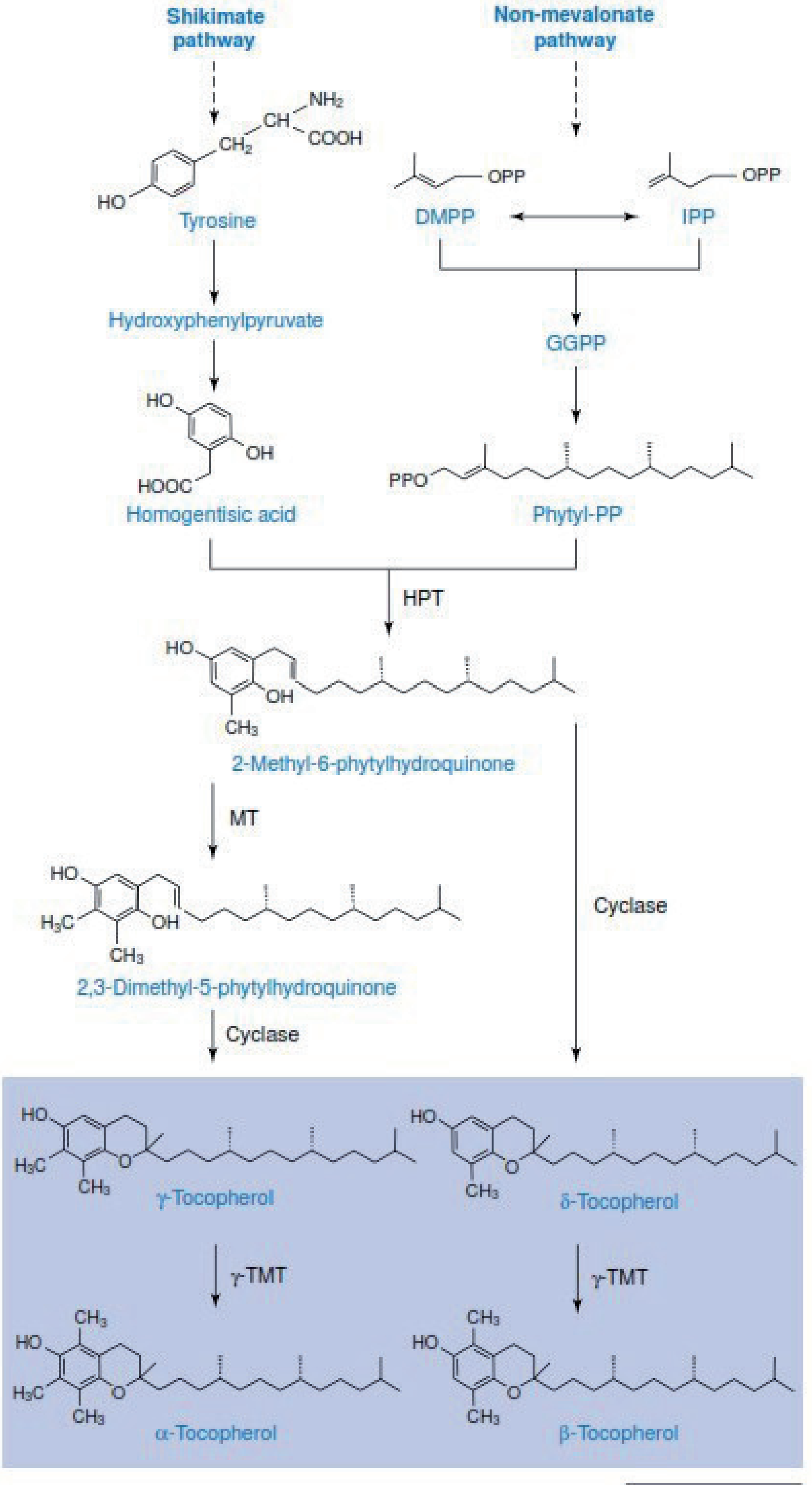


**Figure 5.**
*Vitamin E biosynthetic pathway [21]. The blue box highlights the four naturally occurring tocopherol derivatives in plants.*

tyrosine (Tyr) and tryptophan (Trp)), and as precursors for many secondary metabolites, such as pigments, vitamins, etc. [22]. It consists of seven steps where the glycolytic intermediate phosphoenol pyruvate and the pentose phosphate pathway intermediate erythrose-4-phosphate are converted in chorismate (**Figure 6**). Numerous synthases, dehydratases and kinases are involved in this pathway, but their participation in tocopherols biosynthesis is not clear. The limitation step in the shikimate pathway are the reversible formation of

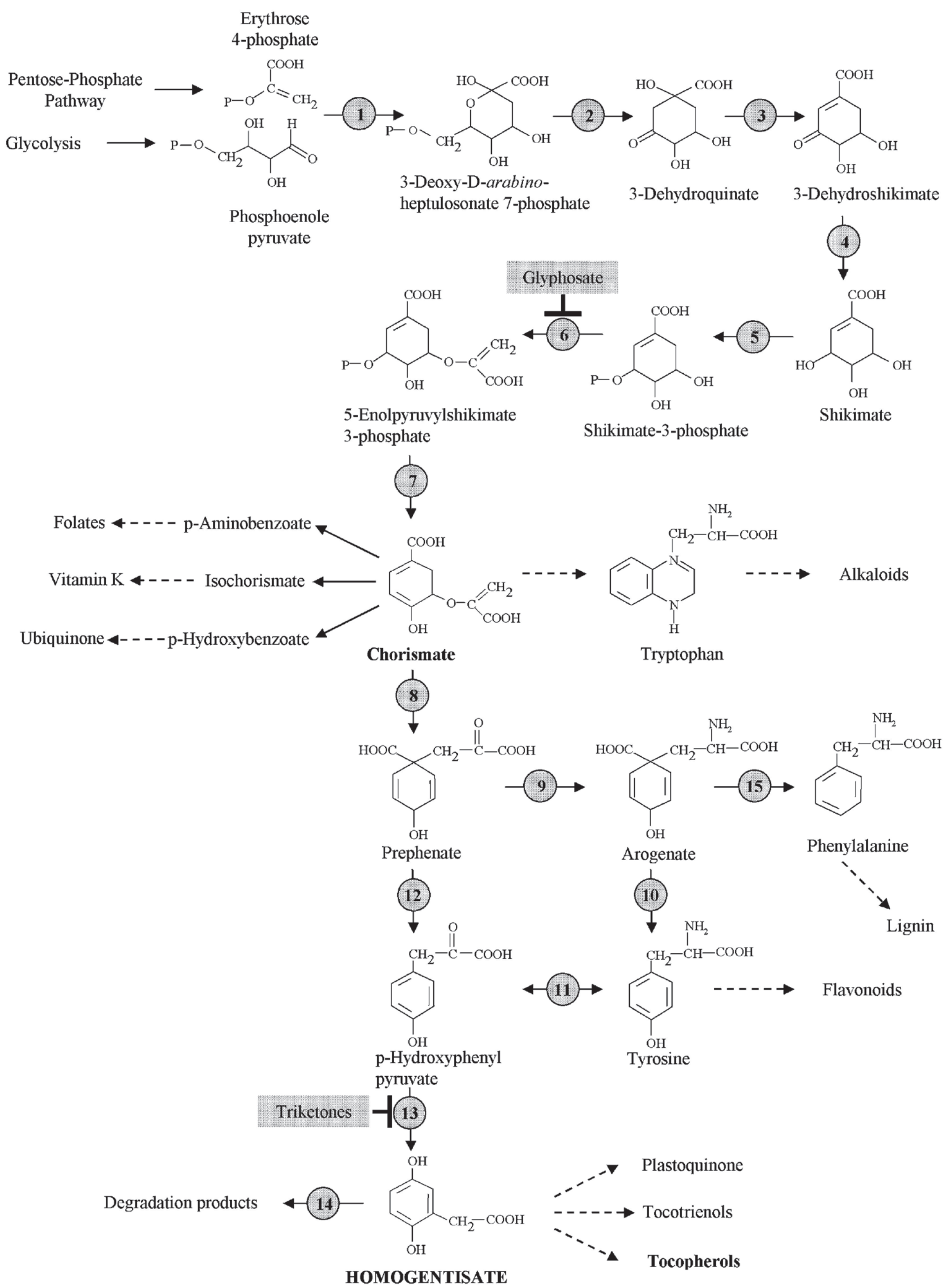


**Figure 6.**
*The shikimate pathway of homogentisate biosynthesis in photosynthetic organisms [23].*

5-enolpyruvylshikimate 3-phosphate (EPSP) and inorganic phosphate from shikimate 3-phosphate and phosphoenolpyruvate. Likewise, the reaction is catalyzed by EPSP synthase (EC 2.5.1.19), which is the unique target for herbicide glyphosate (N-phosphonomethylglycine) [24]. Glyphosate interacts with the binding site of phosphoenolpyruvate and forms a stable ternary complex with the enzyme and shikimate 3-phosphate Likewise, Chorismate is the end product of the shikimate pathway and, at the same time, is a precursor for many primary and secondary metabolites, such as vitamin-K, folates, alkaloids,

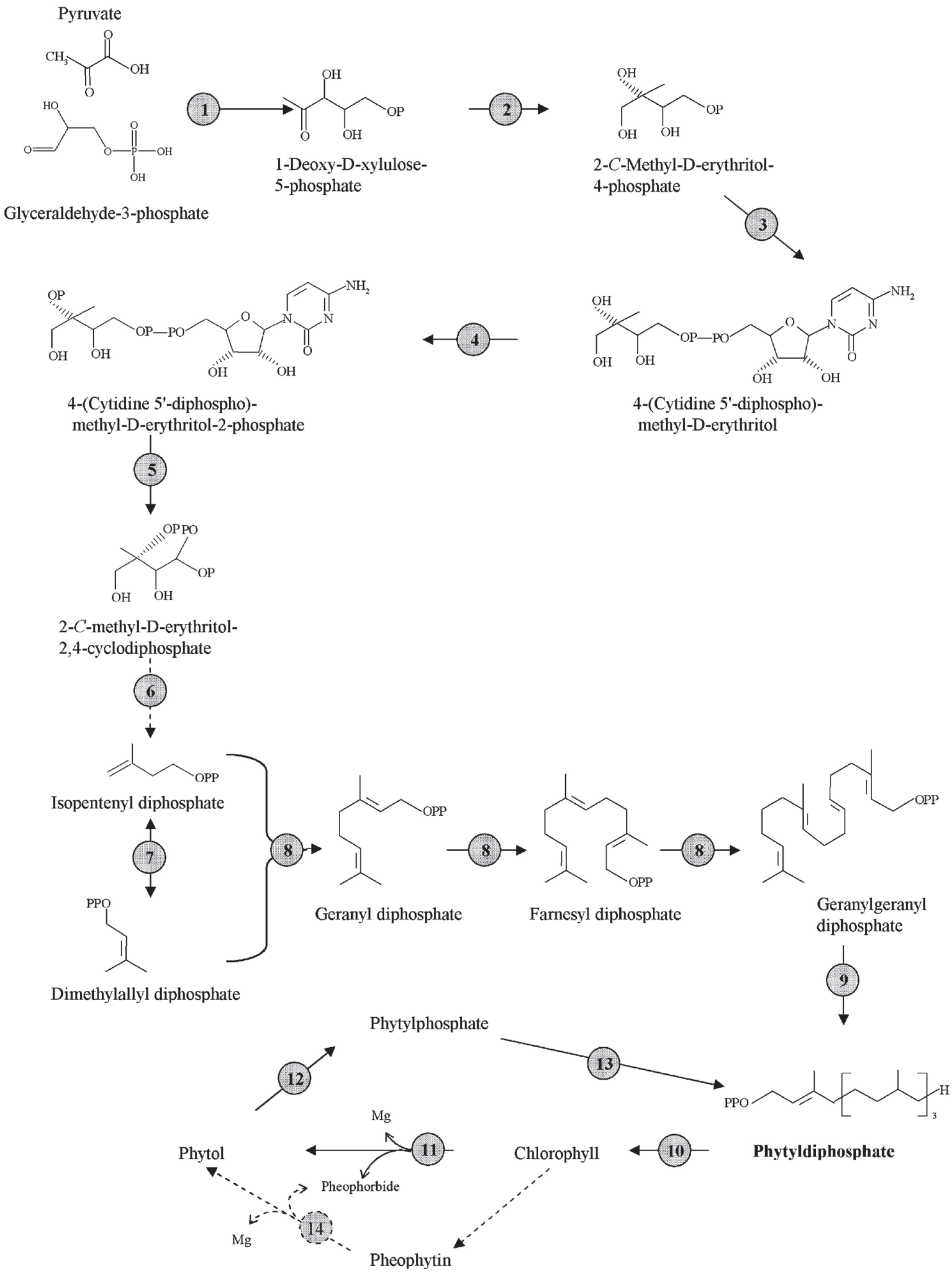


**Figure 7.**
*Methylerythritol pathway of phytyl diphosphate biosynthesis in the plastids of higher plants [23].*

quinones, tocopherols and three aromatic amino acids (Phe, Tyr and Trp) [23]. *p*-Hydroxyphenylpyruvate (HPP) is the first intermediate in tocopherol biosynthesis. Different ways of HPP synthesis exist in photosynthetic organisms. In higher plants, it is formed from prephenate via arogenate and tyrosine. A portion of fixed carbon is incorporated into Tyr used for biosynthesis of HPP and homogentisate, a tocochromanol (tocopherols and tocotrienols) precursor [25].

The formation of homogentisate from HPP occurs in the reaction catalyzed by HPPD. Homogentisate may either enter the prenylquinone biosynthesis pathway or be metabolized by homogentisate dioxygenase (EC 1.13.11.5) to yield maleylacetoacetate, which further is catabolized to fumarate and acetyl-CoA [23].

### 4.2 Methyl erythritol phosphate (MEP) synthesis

The plastidic 2C-methyl-D-erythritol 4-phosphate (MEP) pathway produces isopentenyl diphosphate (IPP) that is used for the biosynthesis of isoprenes, monoterpenes ($C_{10}$), diterpenes ($C_{20}$), carotenoids, plastoquinones, and phytol conjugates such as chlorophylls and tocopherols.

The first step in the MEP pathway involves a transketolase-type condensation reaction of pyruvate and glyceraldehyde 3-phosphate to form 1-deoxy-D-xylulose-5-phosphate (DOXP) (**Figure 7**), which is also an intermediate in the biosynthesis of thiamin and pyridoxol [26–28]. The formed isopentenyl diphosphate is further isomerized to DMAPP. However, the IPP is suggested to be the final production of the MEP pathway in higher plants [26]. The chlorophyll-derived phytol may be a precursor for the biosynthesis of tocopherols, because, the accumulation of tocopherol negatively correlated with chlorophyll content in some plant species during leaf senescence [29].

## 5. Tocochromanol biosynthetic pathway

According to the degree of methylation of the chromanol ring, four different forms of tocochromanol was obtained (**Figure 8**). Four forms of tocopherol, tocotrienol, and tocomonoenol have been identified in wild-type plant extracts, only the solanesyl-derived tocochromanol PC-8form is exists in the nature [30].

It has been assumed for a long time that tocochromanol biosynthesis was the exclusive appanage of plants, algae, and some cyanobacteria that are all photosynthetic organisms. Tocochromanol biosynthesis is initiated by the condensation of the polar aromatic head HGA with various lipophilic polyprenyl pyrophosphates that determine the type of tocochromanol. The condensation reaction is catalyzed by three types of HGA prenyltransferases that possess each their substrate specificities. Tocopherol synthesis is initiated by HGA phytyltransferases (HPTs) that condense HGA and PPP. The condensation between HGA and polyprenyl pyrophosphates produces 2-methyl-6-phytyl-1,4-benzoquinol (MPBQ), 2-methyl-6-geranylgeranyl- 1,4-benzoquinol (MGGBQ), 2-methyl-6-solanesyl-1,4-benzoquinol (MSBQ), and 2-methyl-6-tetrahydrogeranylgeranyl-1,4-benzoquinol (MTHGGBQ) for tocopherols, tocotrienols, PC-8, and for tocomonoenols, respectively (**Figure 8**). Finally, tocochromanol biosynthesis consists of the methylation of γ- and δ-tocochromanols intoα - and β-tocochromanols, respectively [31, 32]. In Arabidopsis leaves and seeds, VTE4 converts γ anjdδ -tocopherols intoα - and β-tocopherol, respectively [32].

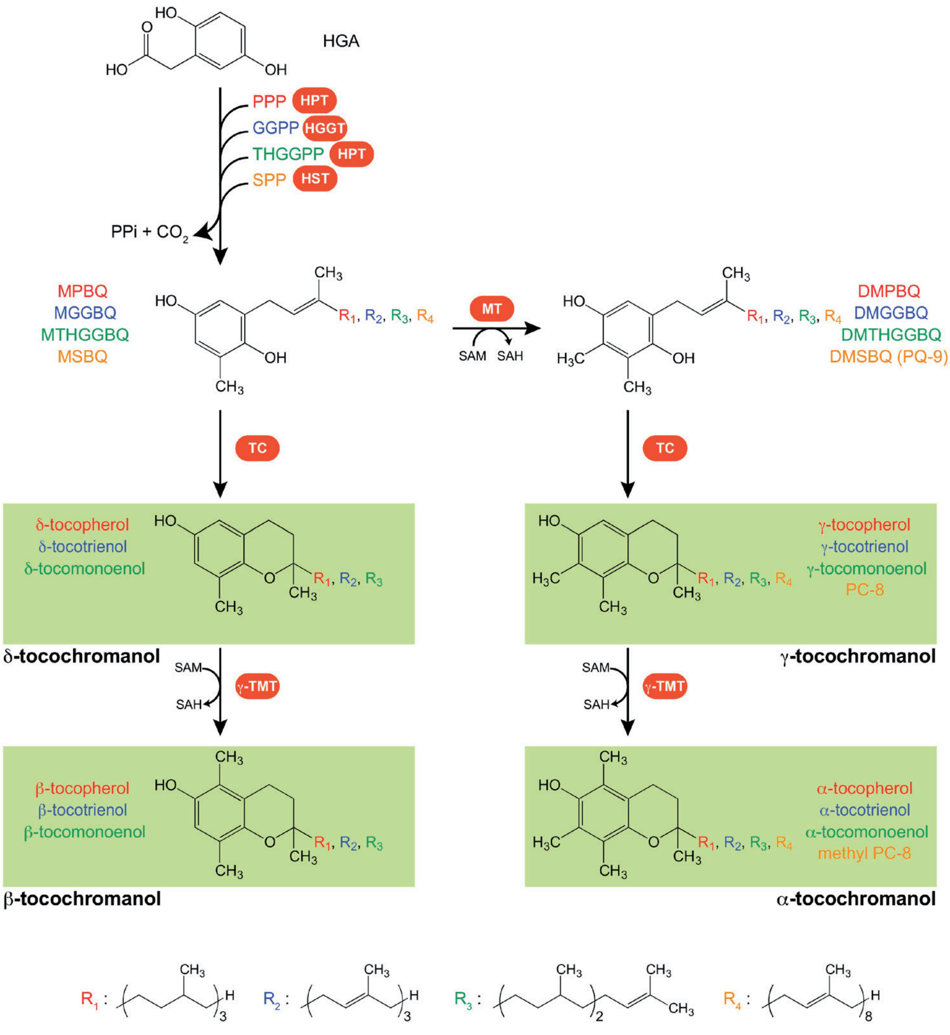


**Figure 8.**
*Tocochromanol (tocopherol, tocotrienol, tocomonoenol, and methyl PC-8) biosynthetic pathways in plants [30].*

In addition, transgenic Arabidopsis lines overexpressing the barley HGGT gene notably produceα -tocotrienol [33].

## 6. Conclusion

Vitamin E biosynthesis mobilizes two distinct biosynthetic pathways, the shikimate pathway and the MEP pathway. Indeed, the shikimate pathway gives rise to the chromanol ring from homogentisate (HGA). While, the methylerytrithol phosphate (MEP) pathway provides the prenyl tail from geranylgeranyl diphosphate (GGDP) and phytyl diphosphate (phytyl-DP) for the synthesis of tocotrienol and tocopherol, respectively. An additional pathway for phytyl-DP production from chlorophyll degradation, known as the phytol recycling pathway. Understanding the regulation of vitamin E biosynthesis will imply that we take up the challenges to understand the regulation of each of these numerous events. The fundamental role of this vitamin in human reproduction and its benefit in current widespread diseases such as high cholesterol and neurodegenerative pathologies makes it a candidate of choice to improve human health.

## Acronyms and abbreviations

| | |
|---|---|
| AIR | 5-aminoimidazole ribonucleotide |
| CLD1 | chlorophyll dephytylase 1 |
| DMGGBQ | dimethylgeranylgeranylbenzoquinol |
| DMPBQ | dimethylphytylbenzoquinol |
| DMPP | dimethylallyl pyro-phosphate |
| GGDP | geranylgeranyl diphosphate |
| GGDR | geranylgeranyl diphosphate reductase |
| GGPP | geranylgeranyl pyrophosphate |
| GGPS | geranylgeranyl diphosphate synthase |
| HET-P | 4-methyl-5-b-hydroxyethyl thiazole phosphate |
| HGGT | homogentisate geranylgeranyl transferase |
| HMP-PP | 2-methyl-4-amino-5-hydroxymethylpyrimidine diphosphate |
| HPP | hydroxyphenylpyruvate |
| HPPD | hydroxyphenylpyruvate dioxygenase |
| HPT | homogentisate phytyl transferase |
| IMP | inosine monophosphate |
| IPP | isopentenyl pyrophosphate |
| MEP | methylerythritol phosphate |
| MGGBQ | methylgeranylgeranylbenzoquinol |
| MPBQ | methylphytylbenzoquinol |
| MPBQ-MT | MPBQ methyltransferase |
| MT | 2-methyl-6-phytylhydroquinone methyltransferase |
| PDP | phytyl diphosphate |
| phytyl-PP | phytyl pyrophosphate |
| PPRP | 5-phosphoribosyl-1pyrophosphate |
| S-AdoMet | S-adenosylmethionine. |
| TAT | tyrosine aminotransferase |
| TC | tocopherol cyclase |
| TMT | tocopherol methyltransferase. |

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