Available online at www.sciencedirect.com
SCIENCE DIRECT
Phytochemistry 67 (2006) 1185–1195
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Differential distribution of tocopherols and tocotrienols in photosynthetic and non-photosynthetic tissues

György Horvath [a,*], Ludger Wessjohann [b], Joseph Bigirimana [c], Marcel Jansen [d], Yves Guisez [a], Roland Caubergs [a], Nele Horemans [a]

[a] Research Group of Plant Physiology, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium
[b] Leibniz-Institute of Plant Biochemistry, Weinberg 3, D-06120 Halle (Saale), Germany
[c] Research Group of Plant Pathology, University of Ghent, Coupure Links, 9000 Gent, Belgium
[d] Department of Zoology, Ecology and Plant Science, University College Cork, Butler Building, Distillery Field, North Mall, Cork, Ireland

Received 5 April 2004; received in revised form 29 March 2006
Available online 14 June 2006

Abstract

Tocopherols and tocotrienols are vitamin E compounds, differing only in the saturation state of the isoprenoid side chain. Tocopherol biosynthesis, physiology and distribution have been studied in detail. Tocopherols have been found in many different plant species, and plant tissues. In contrast, comparatively little is known about the physiology and distribution of tocotrienols. These compounds appear to be considerably less widespread in the plant kingdom. In this study 80 different plant species were analysed for the presence of tocotrienols. Twenty-four species were found to contain significant amounts of tocotrienols. No taxonomic relation was apparent among the 16 dicotyledonous species that were found to contain tocotrienol. Monocotyledonous species (eight species) belonged either to the Poaceae (six species) or the Aracaceae (two species).

A more detailed analysis of tocotrienol accumulation revealed the presence of tocotrienols in several non-photosynthetic tissues and organs, i.e. seeds, fruits and in latex, in concentrations up to 2000 ppm. No tocotrienols could be detected in mature photosynthetic tissues. However, we found the transient accumulation of low levels of tocotrienols in the young coleoptiles of plant species whose seeds contained tocotrienols. No measurable tocotrienol biosynthesis was apparent in coleoptiles, or in chloroplasts isolated from such coleoptiles. In line with these results, we found that tocotrienol accumulation in coleoptiles was not associated with chloroplasts. Based on our data, we conclude that tocotrienols may be transiently present in photosynthetically active tissues, however, it remains to be proven whether the tocotrienols are biosynthesised in such tissues, or imported from elsewhere in the plant.
© 2006 Elsevier Ltd. All rights reserved.

Keywords: Tocotrienol; Vitamin E; Tocotrienol biosynthesis; Tocotrienol distribution; Seedling development

1. Introduction

Tocopherols and tocotrienols (Fig. 1) consist of a polar chromanol group and an apolar isoprenoid side chain. The isoprenoid tail of the tocotrienols contains three unsaturated bonds, while the tail of tocopherols is fully saturated (Kamal-Eldin and Appelqvist, 1996). These amphiphilic compounds are generically known as vitamin E. Functionally, vitamins E are potent antioxidants (Serbinova, 1991; Kamal-Eldin and Appelqvist, 1996) that protect plant membranes against photoinhibiton and other photooxidative stresses (Havaux, 2005). A major physiological role of tocopherols is to prevent lipid peroxidation during seed dormancy, germination and early seedling development (Sattler et al., 2004). Little is known about the physiological role of tocotrienols. Based on the similar structures of

Abbreviations: DMT, dimethyltocol; GGDP, geranylgeranyldiphosphate; HGA, homogentisic acid; HGGT, homogentisate geranylgeranyltransferase; Nd, below the limit of detection; PDH, prephenate dehydrogenase; PhyPP, phytylpyrophosphate; VTE2, homogentisate phytyltransferase.

* Corresponding author. Tel.: +32 3 265 34 21; fax: +32 3 265 34 17.
E-mail address: gyorgy.horvath@ua.ac.be (G. Horvath).

0031-9422/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2006.04.004

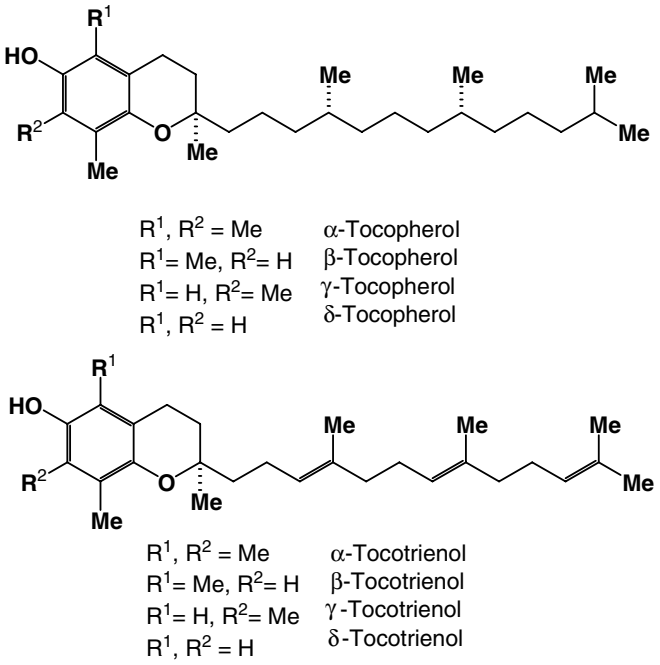

Fig. 1. Detailed chemical structures of tocopherol (top) and tocotrienol (bottom). The canonical structures are shown.

tocotrienols and tocopherols, it has often been assumed that these compounds will have comparable functional roles in the plant (Munné-Bosch and Alegre, 2002).

Tocopherols appear to be universal constituents of all higher plants (Threlfall and Whistance, 1971). Four different forms exist of both tocotrienol and tocopherol. In leaves, the most common tocopherol form is α-tocopherol whereas in other plant parts including stem, root, fruits, seeds, tubers, cotyledons, hypocotyls and flowers, γ-tocopherol is most abundant (Janiszowska and Pennock, 1976; Munné-Bosch and Alegre, 2002). Tocopherols are found in plastids, where they occur in concentrations ranging from 1 μg/g dry weight to >1 mg/g dry weight (Munné-Bosch and Alegre, 2002). Tocopherol biosynthesis takes place on the inner envelope membrane of chloroplasts (Soll et al., 1985; Schultz, 1990) and chromoplasts (Arango and Heise, 1997). Thus, tocopherols are clearly associated with plastids. Based on the structural similarities between tocotrienols and tocopherols, it has been suggested that tocotrienols are also synthesised in plastids (Munné-Bosch and Alegre, 2002).

Little is known about the distribution of tocotrienols in the plant kingdom. The limited data that are available suggest a somewhat unusual and restricted distribution. Unlike tocopherols, tocotrienols are not found in leaves of Angiosperms (Threlfall, 1971; Pennock, 1983), although the presence of low levels of tocotrienols has been reported in needles of *Picea* sp. (spruce) (Franzen et al., 1991). Tocotrienols have been found in oil extracted from cereals (Pennock, 1983; Firestone, 1999; Goffman and Bohme, 2001) and *Elaeis* sp. Jacq. (oil palm) mesocarp (Lubrano et al., 1994). Other studies report the presence of tocotrienols in the seeds of monocotyledonous (Hall and Laidman, 1967; Piironen et al., 1986; Franzen and Haass, 1991; Franzen et al., 1991; Grusak and DellaPenna, 1999; Goffman and Bohme, 2001) and dicotyledonous plants, including *Gevuina avellana* Mol. (Chilean hazelnut) (Osuna-Garcia et al., 1998), *Bixa orellana* L. (Annato) (Frega et al., 1998), *Carum carvi* L. (caraway) (Aitzetmüller, 1997), *Orobanche* sp. L. (Velasco et al., 2000), some Vietnamese plants (Matthaus et al., 2003), *Calophyllum* sp. L. (Crane, 2005), *Juglans regia* L. (Amaral et al., 2005) and *Vitis vinifera* L. (Horvath et al., 2004). Furthermore, tocotrienols have been reported to occur in specialised cells like the latex tuber of *Hevea brasiliensis* (Willd.ex.A. Juss.) Müll. Arg. (rubber tree) (Whittle et al., 1966; D'Auzac et al., 1989; Schultz, 1990).

Recently, genes were identified that encode the key enzymes of the tocochromanol biosynthesis pathway. These include homogentisate phytyl transferases (Collakova and DellaPenna, 2001; Venkatesh et al., 2006), a cyclase (Porfirova et al., 2002), and methyl transferases (Shintani et al., 2002; Cheng et al., 2003). Homogentisate geranylgeranyl transferase (HGGT) has been identified as a key enzyme in tocotrienol biosynthesis. The gene encoding HGGT was found in three monocotyledonous plants (*Oryza* sp., *Hordeum* sp. and *Triticum* sp.). HGGT was functionally expressed in a number of plant species that do not normally accumulate tocotrienol and this resulted in tocotrienol accumulation in the leaves (Cahoon et al., 2003; Karunanandaa et al., 2005). Increasing the homogentisate pool also led to the accumulation of tocotrienols in leaves of *Nicotiana tabacum* L. (Rippert et al., 2004; Matringe et al., 2005) and a shift in chromanol content from tocopherols to tocotrienols in seeds of *Arabidopsis thaliana* L., *Glycine max* and *Brassica napus* L. (Karunanandaa et al., 2005). These studies demonstrate the potential to engineer tocotrienols accumulation in leaves and seeds of species that do not normally accumulate these compounds (Valentin and Qi, 2005).

In this study, we have screened a broad range of different plant species and tissues for the presence of tocotrienols. Using our own experimental data, as well as information from the literature, we have generated a list of tocotrienol accumulating species, and analysed the distribution of tocotrienols among Angiospermae. To study the robustness of our taxanomic analysis of tocotrienol accumulation, we have analysed intraspecific variations in tocotrienol levels. Quantification of the tocotrienol levels present in the seeds of 26 different rice cultivars indicates that the ratio of tocotrienol compared to total vitamin E content fluctuates within a species. Yet, tocotrienol levels were above the detection threshold in all cultivars. The distribution of tocotrienols within the plant was studied in wheat. We found tocotrienol levels to be highest in the seed. In this paper we show for the first time that a transient peak of tocotrienols is present in the coleoptiles of young seedlings. This finding is particularly intriguing, as we found no evidence for tocotrienol biosynthesis in these coleoptiles.

Table 1
Summary of plant species and tissues that were studied

Taxon	Common name	Family	Tissue
Monocotyledonous plants			
Avena sativa L.[a]	Oat	Poaceae	s,sl,L,r
Beckmannia syzigachne (Steud.) Fernald		Poaceae	s
Briza maxima L.		Poaceae	s
Canna indica L.	Canna	Canaceae	s
Carex flacca Scherb.		Cyperaceae	s
Cenchrus ciliaris L.		Poaceae	s
Cocos nucifera L.[a]	Coconut	Arecales	s
Commelina nilagirca L.		Commelinaceae	s
Convallaria majalis L.	Lily-of-the-valley	Ruscaceae	s
Crocus vernus L. Hill		Iridaceae	s
Cyperus prolifer Lam.		Cyperaceae	s
Elaeis guineensis Jacq.[a]	Palm tree	Arecaceae	s,L
Hordeum vulgare L.[a]	Barley	Poaceae	s,sl,L,r
Iris pseudacorus L.		Iridaceae	s
Juncus effusus L.		Juncaceae	s
Lemna minor L.		Araceae	s
Lolium perenne L.	Perennial ryegrass	Poaceae	s,sL,L
Luzula luzuloïdes L.	Woodrush	Juncaceae	s
Musa Musa L.	Banana	Musaceae	s,L
Musa sapientum L.		Musaceae	s,L
Oryza sativa L.[a]	Rice	Poaceae	s,sl,L,r,fr
Pitcairnia imbratica (Brongn.) Regel		Bromeliaceae	s
Secale cereale L.[a]	Rye	Poaceae	s,sl,L,r
Tradescantia zebrina Loud.	Inchplant	Commelinaceae	s
Triticum aestivum L.[a]	Wheat	Poaceae	s,sl,L,r
Triticum dicoccum Schrank[a]	Wheat	Poaceae	s,sl,L,r
*Triticum vulgar*e Vill. [a]	Wheat	Poaceae	s,sl,L,r
Tulipa gesneriana L.		Liliaceae	s
Typha angustifolia L.	Narrow-leaf cattail	Typhaceae	s
Typha latifolia L.	Common cattail	Typhaceae	s
Vulpia myuros L.C.C. Gmel		Poaceae	s
Zea mays L.[c]	Corn	Poaceae	s,sl,L,r
Dicotyledonous plants			
Aesculus hippocastanum Hort.[a]	Horse chestnuts	Hippocastanaceae	s,L
Arabidopsis thaliana L. Heynh.	Thale cress	Brasicaceae	s,sl,L,r
Arachis hypogaea L.	Peanut	Fabaceae	s
Bixa orellana L.[a]	Lipstick tree	Malvales	s,L
Blumenbachia hieronymi (Urb.)		Loacaceae	s
Brassica napus L.	Rapeseed	Brasicaceae	s,L,
Brassica oleracea L.	Cauliflower	Brasicaceae	s
Calandrinia landiflora (Lindl.)		Portulacaceae	s
Callistephus chinensis (L.) Nees		Asteraceae	s
Cannabis sativa L.[b]	Hemp	Canabaceae	s,L,fl
Capsicum annuum L.	Pepper	Solanaceae	s
Carthamus tinctorius L.	Safflower	Asteraceae	s
Chaenorrhinum minus L.		Antirrhinaceae	s
Cnicus benedictus L.		Asteraceae	s
Cochlearia officinalis L		Brasicaceae	s
Coriandrum sativum L.[a]	Coriander	Umbeliferae	s,L
Elsholtzia ciliata (Thunb.) Hyl.		Laminaceae	s
Euphorbia helioscopa L.	Spurges	Euphorbiaceae	s
Ficus elastica Roxb. ex Hornem.	Rubber plant	Moraraceae	s,L,fl
Glycine max L. Merr.	Soybean	Fabaceae	s,L
Helianthus annuus L.	Common sunflower	Asteraceae	s,L
Hevea brasiliensis (Willd. ex A. Juss.) Müll. Arg.[a]	Rubber tree	Malpighiales	s,L,la
Humulus lupulus L.[b]	Common hop	Canabaceae	s,L
Lactuca perennis L.		Asteraceae	s
Litchi chinensis Sonn.[a]	Litchi	Sapindaceae	s
Linum usitatissimus L.	Flax	Linaceae	s
Lycopersicon esculentum Mill.	Tomato	Solanaceae	s,L
*Nasturtium officinal*e R. Br.	Water cress	Brasicaceae	s,sl,L
Nicotiana tabacum L.[c]	Tobacco	Solanaceae	s,sl,L

(*continued on next page*)

Table 1 (*continued*)

Taxon	Common name	Family	Tissue
Nicotiana tabacum L. Bright Yellow-2[b]	Tobacco	Solanaceae	Cc
Olea europea L.	Common olive	Oleaceae	s
Pharbitis purpurea (Roth.) Bojer	Morning glory	Convolvulaceae	s
Pothomorphe petala Miq.		Piperaceae	s
Rosamarinus officinales L.[a]	Rosemary	Lamiaceae	s,L
Scabiosa atropurpurea L.		Dipsacaceae	s
Spinacia oleracea L.	Spinach	Amaranthaceae	L
Theobroma cacao L.		Malvaceae	s
Tragopogon pratensis L.		Cichorioideae	s
Trigonella caerulea L.		Fabaceae	s
Vigna radiata L. R. Wilczek	Mung bean	Fabaceae	s,L
Vitis vinifera L.[a]	Grape	Rhamnales	s,sl,L,fl,fr

Full latin name, common name and plant family are given. Tocochromanol content was determined using HPLC with fluorescence detection for all the listed species mostly in seeds (s) but also in fruits (fr), leafs (L), in roots (r) in seedlings (sl), in latex (la) or cell cultures (cc). Moreover, different cultivars were often compared. Tocopherols were present in all samples.

[a] Indicates that species contained tocotrienols.

[b] Indicates that compounds coeluted with a tocotrienol form during HPLC analysis but were later rejected by another method.

[c] None of the varieties screened showed tocotrienols but literature indicates otherwise.

2. Results

2.1. Quantitative analysis of tocotrienols in Angiospermae

More than 80 plant species were analysed for their tocochromanol content and spectrum, in order to study the (chemo) taxonomic distribution of tocotrienol accumulation. We have analysed young leaves that were photosynthetically-active, as well as various non-green tissues (seeds, latex and flowers). Tested plants included species belonging to the major taxonomical groups as defined by the tree of life web project (Maddison and Schulz, 1996–2006). Table 1 shows a list of all plants tested and the taxonomical order they belong to. Whenever possible different tissues were analysed (Table 1). The majority of the plants tested, monocotyledonous or dicotyledonous, contained no tocotrienols, while all contained tocopherols.

Table 2 shows the tocochromanol content of those plant species that were found to contain significant levels of tocotrienols. In the group of the dicotyledonous plants 14 species were positively identified based on their tocotrienol content, namely: *Aesculus hippocastanum* Hort. (horse chestnuts), *Vaccinium macrocarpon* A. (cranberry), *Hevea brasiliensis* (Willd. ex. A. Juss.) Müll. Arg., *Bixa orellana* L., *Gevuina avellana* Mol., *Vitis vinifera* L. (grape), *Amaranthus hypochondriacus* L. (amaranth), *Delphinium ajacis* L. (larkspur), *Litchi chinensis* Sonn. (litchi), *Rosmarinus officinalis* L. (rosemary), *Foeniculum vulgare* L. (fennel), *Carum carvi* L. and *Coriandrum sativum* L. (coriander). These species belong to a range of different orders, including the Sapindales, Ericales, Malpighiales, Malvales, Proteales, Rhamnales, Caryophyllales, Ranunculales, Lamiales and the Apiales (Table 2). In the group of the monocotyledons eight plant species were found to contain tocotrienols, namely *Elaeis guineensis* Jacq., *Cocos nucifera* L. (coconut), *Avena sativa* L. (oat), *Triticum aestivum* L. (wheat), *Hordeum vulgare* L. (barley), *Oryza sativa* L. (rice), *Secale cereale* L. (rye) and *Zea mays* L. (maize). *Elaeis guineensis* and *Cocos nucifera* belong to the order Arecales. All other species belong to the order Poales. These data indicate that within the monocotyledons there is a high bias of tocotrienol containing species towards Poales. No such bias is apparent among the dicotyledons. Rather, we note a very broad distribution of tocotrienol accumulation trait among various orders.

The highest tocotrienol concentration (ca. 2000 μg/g) was found in the latex of *Hevea brasiliensis* Müll. Arg. In contrast, no tocotrienols were identified in the latex of several other latex producing species, including *Ficus elastica* Roxb. ex Hornem., *Ficus carica* L., and *Euphorbia helioscopa* L. These species did contain tocopherols. Tocotrienols in *Hevea brasiliensis* were extracted from both leaves and latex. However, we managed to reduce the tocotrienol level in the leaf tissue towards the lower detection limit, by carefully excluding latex veins during sample preparation. This result indicates that tocotrienols are restricted to the latex tubers, as was also previously reported (D'Auzac et al., 1989).

2.2. Distribution of tocotrienols within one species: case study rice

We did not detect any measurable quantity of tocotrienols in different cultivars of *Z. mays* L. This contrasts with work by Franzen and Haass (1991), who reported that the maize cultivars cv. Bastion and cv. A619 contained detectable levels of tocotrienols. A similar intraspecific variance was also described for *Triticum* (Hall and Laidman, 1967). To improve our understanding of intraspecific variations in tocotrienol contents, and also to assess the robustness of our taxonomic analysis of tocotrienol accumulation, we have measured the variance in tocotrienol concentration within a single species. The tocochromanol content of 26 rice cultivars was tested for this study. The

Table 2
Plant species, containing significant amounts of tocotrienol, and the families they belong to are listed

Family	Species	Common name	Tissue	Tocotrienol (μg/g tissue)				Tocopherol (μg/g tissue)			
				α	β	γ	δ	α	β	γ	δ
Eudicots											
Euphorbiaceae	*Hevea brasiliensis* Müll. Arg.	Rubber tree	la	522.4	bld	196.7	1869.6	349.8	227.0	241.6	506.3
Apiaceae	*Coriandrum sativum* L.	Coriander	s	6.0	bld	bld	tr.	5.0	1.1	4.4	36.5
Apiaceae	*Carum carvi* L.[a]	Caraway	s	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
Apiaceae	*Foeniculum vulgare* L.[a]	Fennel	s	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
Bixaceae	*Bixa orellana* L.	Lipstick tree	s	18.7	1.84	534.7	977.9	1758.2	528.1	606.5	174.7
Vitaceae	*Vitis vinifera* L.	Grape	s,(sl)	5.0	bld	25.0	bld	18.0	tr.	1.9	bld
Ranunculaceae	*Delphinium ajacis* L.[b]	Larkspur	s	566	153	Tr.	n.d.	566	153	Tr.	n.d.
Sapindaceae	*Litchi chinensis* Sonn.	Litchi	s	bld	bld	3.37	488	124.1	9.2	59.2	17.4
Hippocastanaceae	*Aesculus hippocastanum* Hort.[b]	Horse chestnuts	s	97	n.d.	626	336	97	n.d.	626	336
Solanaceae	*Nicotiana tabacum* L.[c]	Tobacco	s	tr.	tr.	30	tr.	tr.	tr.	30	tr.
Ericaceae	*Vaccinium macrocarpon* Aiton[d]	Cranberry	s	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
Amaranthaceae	*Amaranthus hypochondriacus* L.[d]	Amaranth	s	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
Proteaceae	*Gevuina avellana* Mol.[e]	Chilean hazelnut	s	0.4	bld	0.6	bld	130	1.3	0.9	0.1
Lamiaceae	*Rosmarinus officinalis* L.	Rosemary	s	560.5	300.3	109.4	tr.	2883	435.2	30.8	tr.
Clusiaceae	*Calophyllum inophyllum* L.[f]		s	2.0	bld	64.0	236.0	15.0	3.0	36.0	16.0
Juglandaceae	*Juglans regia* L.[g]	Walnut	s	bld	bld	bld	n.c.	8.7–16.6	n.c.	172–262	8.2–16.9
Monocotyledons											
Arecaceae	*Elaeis guineensis* Jacq.[h]	Palm tree	fr	4–193	0–234	0–526	0–123	4–336	14–710	0–377	98–1500
Arecaceae	*Cocos nucifera* L.	Coconut	s	104.0	26.0	36.2	204	1105.8	55.6	9.3	tr.
Poaceae	*Avena sativa* L.	Oat	s,(sl)	4.2	tr.	2.1	0.9	5.4	bld	3.2	tr.
Poaceae	*Triticum sp.*	Wheat	s,(sl)	5.0	19.6	bld	bld	15.4	5	bld	bld
Poaceae	*Secale cereale* L.	Rye	s,(sl)	1.97	0.38	0.57	bld	0.51	bld	bld	tr.
Poaceae	*Hordeum vulgare* L.	Barley	s,(sl)	0.59	tr.	tr.	tr.	0.78	tr.	tr.	tr.
Poaceae	*Oryza sativa* L.	Rice	s,(sl)	0.4–3.33	0–0.2	1.7–5.14	0–0.35	0.5–3.1	bld	0–2.23	0–0.62
Poaceae	*Zea mays* L.[i]	Maize	s,(sl)	0–239	0–450	0–20	0–709	23–573	0–356	268–2468	23–75

Only the indicated tissue contains the tocotrienols (la, latex; s, seed; fr, fruit; sl, young seedling). Tocopherol and tocotrienol content is shown in μg chromanol/g dry tissue (bld, below detection limit; tr., traces). Measurements are either from our own experiments or as indicated with a subscript letter taken from the references below (n.c. means that the author positively describes the plant as containing tocotrienols, but did not communicate the quantities). Whenever conflicting concentrations were found a range is given covering the spectrum.

[a] (Aitzetmüller, 1997).
[b] (Matthaus et al., 2003).
[c] None of the cultivars tested by us showed tocotrienols, however tocotrienols were reported in the seeds (Falk et al., 2004).
[d] Plants were not obtained but are well known to contain tocotrienols and thus were not reconfirmed.
[e] (Bertoli et al., 1998).
[f] (Crane et al., 2005).
[g] (Amaral et al., 2005).
[h] Values are in mg/kg oil (Firestone, 1999).
[i] None of the cultivars tested by us contained tocotrienols in the leaves, however tocotrienols were reported in the seeds (Franzen and Haass, 1991).

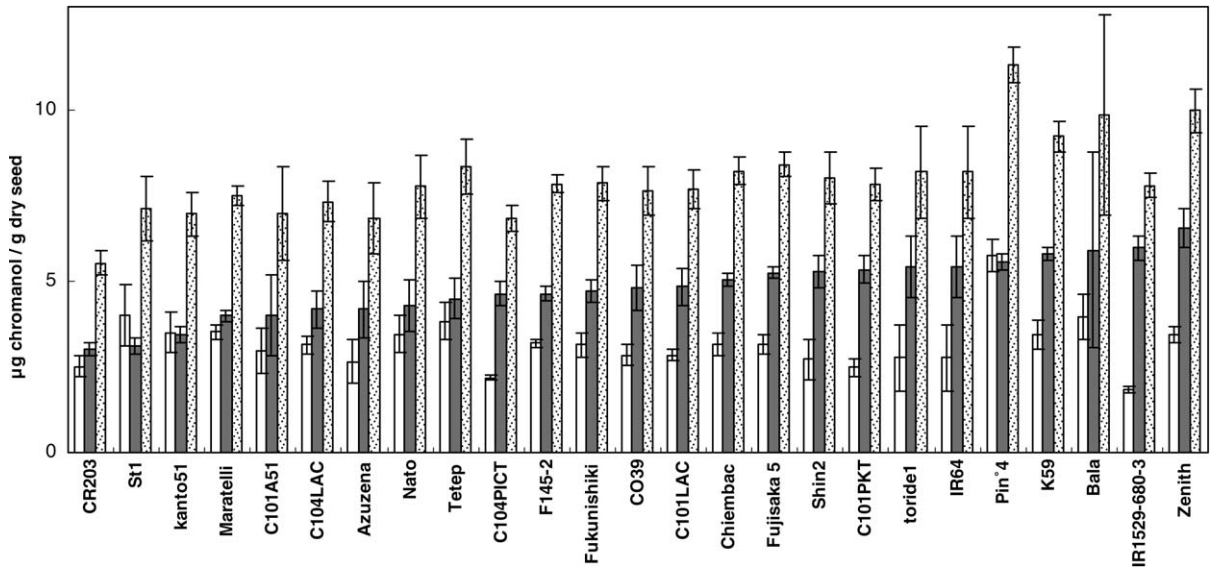

Fig. 2. Tocochromanol content of seeds of 26 rice (*Oryza sativa* L.) cultivars. White bars represent the sum of tocopherol forms (α plus β plus γ plus δ), grey bars represent the sum of tocotrienols (α plus β plus γ plus δ) in the seeds. Speckled bars are the total sum of the tocopherol and tocotrienol content. Cultivars are arranged in increasing total tocotriënol content (grey bars) ($n = 3$).

averaged tocochromanol content of all rice cultivars (sum of total tocopherols and total tocotrienols) was 7.95 ± 1.17 µg chromanol/g dry seed with an average distribution of 40% tocopherols and 60% tocotrienols. However, substantial variations were found between cultivars. In Fig. 2, cultivars are arranged in order of increasing tocotrienol content. The cultivars with the lowest tocotrienol concentrations were CR203, St1, and kanto51. The highest tocotrienol concentrations were found in IR64, PiN°4, K59, IR1529-680-3 and Zenith. Several cultivars contained equal levels of tocopherol and tocotrienol, and these include kanto51, Maratelli and Tetep. The cultivar St1 contained more tocopherols than tocotrienols (>56% tocopherol). In contrast, the cultivars IR1529-680-3 (>76% tocotrienol), C104PICT (68% tocotrienol), and C101PKT (68% tocotrienol) contained more tocotrienols than tocopherols. On average, 70% of the tocopherols was in the α-form and 30% in the γ-form. For tocotrienols the averaged distribution was 25% in the α-form and 75% in the γ-form.

2.3. Tocotrienols are not found in chloroplasts and etioplasts

We have found that tocotrienols are overwhelmingly associated with non-green plant tissues, including seeds, latex and the pericarp of fruit (Table 2). Thus far, we have identified just one case where tocotrienols were accumulated in photosynthetic tissue. The coleoptiles of young Poaceae seedlings were found to contain tocotrienols. Fig. 3 shows the changes in the tocochromanol content of *Triticum aestivum* cv. Ontop seedlings over a period of 5 days starting with imbibition. For analysis, plants were separated into the parts below and above soil, i.e. the seed together with the root tissue and the shoot containing both the coleoptile and the developing leaves. Unlike the seed, the young developing root tissue did not contain detectable levels of tocotrienols or tocopherols (data not shown). As shown in Fig. 3A, upon germination, the tocopherol content of the seeds decreased and no tocopherols could be measured after 4 days. Similarly, the tocotrienol content of the seed also gradually decreased from the moment of imbibition until the end of the experiment (Fig. 3C). A decrease of tocopherol and tocotrienol contents was also observed by Hall and Laidman (1967). Levels of tocopherols and tocotrienols increased during the first two days of shoot growth. Thereafter, tocopherol levels rapidly reached a stable level. Tocopherols were completely associated with the first leaves while the tocotrienols were associated with the coleoptile. In the seed tissue α- and β-tocopherol forms were most abundant. In contrast, in the young developing leaf tissue tocopherol appeared mostly as the β- and γ-forms. In Fig. 3D it is further shown that tocotrienols could already be detected in one day old shoots. Tocotrienol levels reached a maximum after 2 days. Tocotrienol levels rapidly decreased in 4 day old seedlings. More detailed analysis revealed that the tocotrienols in the shoot were exclusively restricted to the coleoptile of *Triticum aestivum* L. cv. Ontop. Similar transient tocotrienol peaks were found in the coleoptiles of *Orysa sativa* L. cv. Tanginbazou, *Hordeum vulgare* L., *Secale cereale* L. as well as in the first leaves of *Vitis vinifera* L. cv. Royal (results not shown).

To understand the origin of the transient tocotrienol peak in young seedlings, we have analysed the subcellular localisation of this tocochromanol. Chloroplasts were isolated from two day old shoots of *Triticum aestivum* L. cv. Ontop. At this time point, shoots contain both tocopherols and tocotrienols (as seen in Fig. 3C and D). The chloroplast fraction contained high levels of tocopherols. However, no

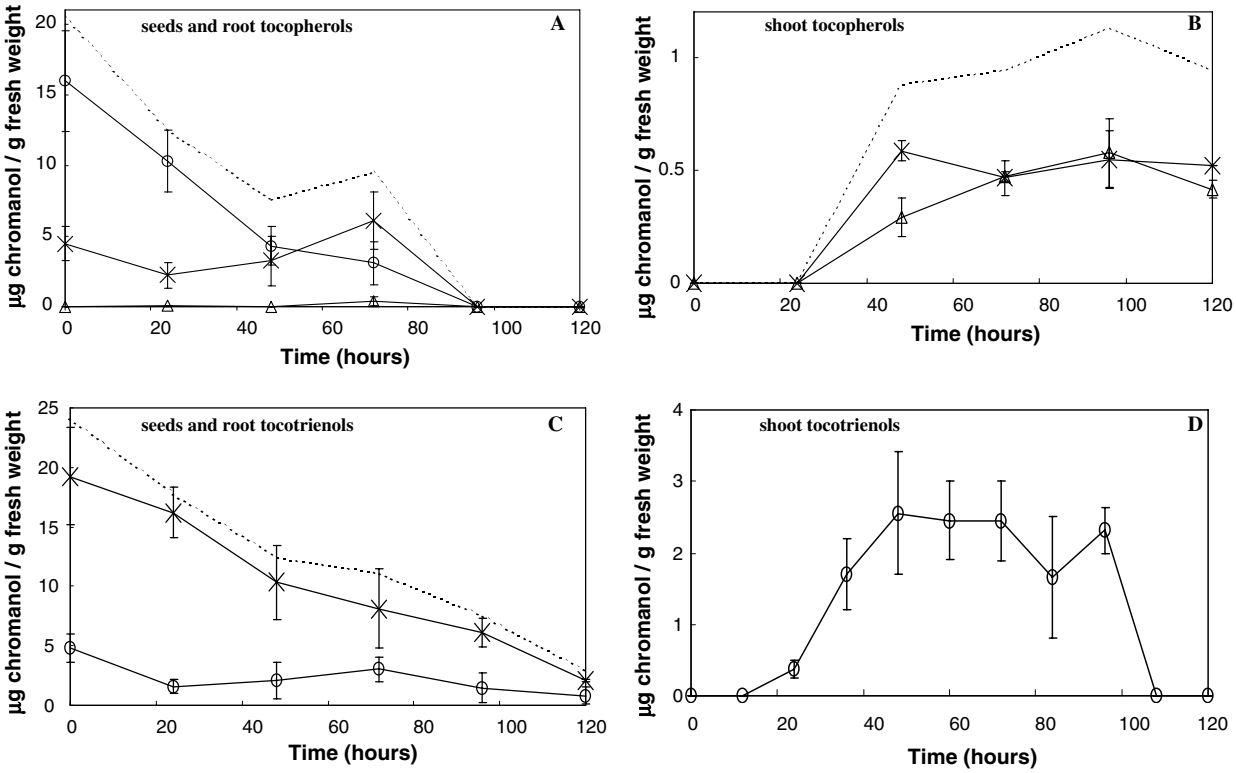

Fig. 3. Tocopherol (A and B) and tocotrienol (C and D) concentration in germinating seedlings of *Triticum aestivum* cv. Ontop L. Panel A and C give levels for the seed and root during germination whereas in panel B and D levels in the shoot are given. Total chromanol content is represented by a dashed line whereas individual forms are indicated as follows: open circles for the α-form, crosses for the β-form and open triangles for the γ-form.

tocotrienols were found in the chloroplast fraction (Table 3). To further investigate potential (inverse) links between the development of photosynthetic capacity and tocotrienol accumulation, we have analysed tocotrienol contents in three day old, etiolated *Triticum aestivum* seedlings. Tocotrienol concentrations were analysed during de-etiolation of the seedlings. A transient tocotrienol peak was observed in de-etiolating leaves (Fig. 4).

Tocotrienols were already detected in de-etiolating young seedlings within 1 h after illumination, as shown in Fig. 4. The kinetics of tocotrienol accumulation were considerably faster than those observed in continuously light grown seedlings (Fig. 3). The magnitude of the tocotrienol peak was, however, about 10-fold lower compared to the light grown seedlings. Additional experiments were performed using blue, red or far red light to de-etiolate the seedlings. The fast and transient appearance of tocotrienols observed under these conditions did not significantly differ from that observed using broad-band, non-filtered light for de-etiolation (data not shown).

Etioplasts were isolated from de-etiolating seedling 30–60 min after light induction. At this time point both tocopherols and tocotrienols were found to be present in the seedlings. However, no tocotrienols could be detected in

Table 3
The upper part of the table shows the chromanol content of *T. aestivum* shoots and of chloroplasts and etioplasts isolated from these shoots

Tissue	Tocotrienol				Tocopherol			
	α	β	γ	δ	α	β	γ	δ
Shoots of *Triticum aestivum* L. (µg/mg fr. wt)	3	bld	bld	bld	0.1	0.5	0.5	bld
Isolated chloroplasts of *T. aestivum* L. (mg/mg chlorophyll)	bld	bld	bld	bld	bld	0.23	0.12	bld
Isolated etioplasts of *Triticum aestivum* L. (mg/mg protein)	bld	bld	bld	bld	bld	0.015	0.10	bld
Radioactivity of [U-^{14}C]-homogentisate incorporated in shoots of *T. aestivum* L. after 4 days (dpm)	bld	bld	bld	bld	1364	1380	2850	15975
Radioactivity of [1-n^3H]-GGPP incorporated by chloroplasts (pmol labelled/mg chlorophyll h)	bld	bld	bld	bld	0.17 (+NADPH)	0.77 (+NADPH)	2.52 (+NADPH)	bld (+NADPH)

The lower part shows the result of labelling experiments with radioactive precursors of chromanol biosynthesis in *T. aestivum* shoots and chloroplasts isolated from these shoots (bld, below detection limit).

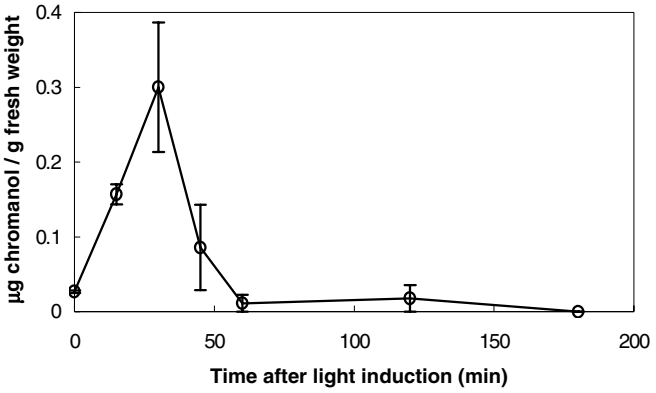

Fig. 4. Tocotrienol content ($n = 3$) in 5 day old etiolated seedlings of *Triticum aestivum* cv. Ontop L. after being put in light. Only α-tocotrienol is found, showing a transient peak 10-fold smaller in magnitude compared to the light grown seedlings (see Fig. 3). Moreover, the peak occurred within 1 h after light induction. Variation was large and some experiments had to be discarded. Seedlings continuously grown in the dark did not show this peak, not even after several (>14) days.

the plastidal (etioplast) fraction whereas tocopherols were present (Table 3). These observations indicate that tocotrienols are not associated with the plastids in the de-etiolating shoots.

2.4. Tocotrienols are not synthesised in photosynthetically active tissues

The ability of young seedlings, as well as isolated chloroplast fractions to actively synthesise tocochromanols was tested using feeding experiments with radioactive precursors of the vitamin E biosynthesis. We used [U-^{14}C]-HGA (homogentisic acid) for the aromatic moiety (Wellburn, 1969) and [^{3}H]-GGPP for the hydrophobic side chain. Seeds of *Triticum aestivum* L. were germinated with [U-^{14}C]-HGA added to the growth medium. Similar experiments have been performed on *Zea mays* L. and *Phaseolus vulgaris* L. shoots to demonstrate the function of HGA as a precursor in tocochromanol biosynthesis (Threlfall and Whistance, 1971). We found that four day old *Triticum aestivum* L. plants contained radioactively labelled tocopherols in the shoots (Table 3). However, notwithstanding the presence of tocotrienols in these seedlings (Fig. 3D), tocotrienols were not labelled. Further analysis of biosynthetic potential was performed on isolated intact and functional chloroplasts. Chloroplasts were incubated with [^{3}H]-GGPP. As shown in Table 3, no labelled tocotrienols were detected in the chloroplast fraction. In a parallel experiment, chloroplasts were incubated with [^{3}H]-GGPP as well as NADPH, in order to promote formation of PhPP from the GGPP, as described by Soll and coworkers (Soll and Schultz, 1979). In this experiment, we found labelled tocopherol, indicating functionality of the chloroplasts, and agreement with Soll et al. (1985). Our data show that neither complete shoots, nor isolated chloroplasts from young seedlings of *Triticum aestivum* L. were able to synthesise tocotrienols when fed with radioactive precursors.

3. Discussion

Tocopherols are present in virtually all plants (Threlfall and Whistance, 1971). These compounds are found in leaves but also in other plant parts (Janiszowska and Pennock, 1976; Munné-Bosch and Alegre, 2002). Tocopherols are synthesised and accumulated in plastids (Munné-Bosch and Alegre, 2002). In contrast, analysis of the tocochromanol content in more then 80 different plant species led to the identification of just 22 plant species containing tocotrienols. Tocotrienols seem to be limited to a small group of unrelated plants. The lack of a clear taxonomic pattern among dicotyledonous species may imply that the capability to synthesise tocotrienols has evolved independently in a number of families.

Tocotrienols are mostly associated with seeds, fruits and latex, rather than with mature leaves (Table 2). The presence of small quantities of tocotrienols in *Picea sp*. needles was reported (Franzen et al., 1991) but could not be confirmed by us. It is possible that these tocotrienols were present in resin ducts, rather than in leaf tissue. Indeed, we observed tocotrienols in leaf tissue of *Hevea brasiliensis*. However, careful separation of latex veins and green leaf material showed that the measured tocotrienols were contaminations of the latex containing veins. We also found a transient tocotrienol peak in shoots of germinating seeds of *Triticum aestivum* L. cv. Ontop, *Orysa sativa* L. cv. Tanginbazou, *Hordeum vulgare* L., *Secale cereale* L. In these cases, tocotrienols were restricted to the coleoptile. Even when tocotrienols were present in leaves, they were not linked to photosynthetic activities. Indeed, when chloroplasts and etioplasts were isolated from shoots of *Triticum aestivum* L. seedlings, they were found to contain tocopherols but no tocotrienols. Thus, while tocopherol synthesis and accumulation is strongly linked with plastid activity, this is not the case for tocotrienols. Our findings seem to virtually limit the presence of tocotrienols to non-photosynthetic tissues, organs and/or organelles. Nevertheless, it is clear that plants can be genetically manipulated to accumulate tocotrienols in leaves, both by overexpression of HGGT (Cahoon et al., 2003) and by increasing the HGA pool (Rippert et al., 2004). When radioactive precursors of either tocopherol or tocotrienol biosynthesis were fed to young germinating *Triticum aestivum* L. shoots or chloroplast isolated from these shoots, only radiolabelled tocopherol was accumulated. Similar experiments were performed with seeds of *V. vinifera* L. and these experiments showed that the experimental methodology was suited to study both tocopherol as well as tocotrienol biosynthesis (Horvath et al., 2004). Our results clearly showed that the shoots, and more specifically the coleoptiles of *Tricticum*, contained a transient tocotrienol peak but was not capable of synthesising tocotrienols. We hypothesise that the transient appearance of tocotrienol molecules in the newly formed leaves of germinating seedlings is due to translocation of tocotrienols from the seeds. Indeed, accumulation of tocotrienols in de-etiolating

seedlings could be measured within 15 min of light exposure (Fig. 4), a time interval that appears more in line with making existing tocotrienols bioavailable than with de novo biosynthesis. This hypothesis is also in accordance with the recent observation that a primary function of tocopherols in plants is to prevent lipid peroxidation during both seed storage and seedling development (Sattler et al., 2004). Most likely, the tocotrienols in our seedlings have identical functions. Further experiments are however necessary to confirm our hypotheses and to find an explanation for the fact that only α-tocotrienols are found in the coleoptile whereas seeds contain a mixture of both α- and β-forms. Physiologically our hypothesis would extend the function of tocotrienols from protecting the unsaturated oil in the seeds to protecting young seedlings from oxidative damage.

4. Experimental

4.1. General

[1(*n*)-^{3}H]-GGPP, triammonium salt (592 GBq/mmol) and [U-^{14}C]-tyrosine were purchased from Amersham Pharmacia Biotech UK limited. Unlabelled isoprenoids (GPP, GGPP, PhyPP) were synthesised in our laboratory or purchased from Orgentis Ltd. (www.orgentis.com). Other chemicals were purchased at VWR.

The enzymatic synthesis of [U-^{14}C]-HGA from [U-^{14}C]-tyrosine was performed as described earlier (Horvath et al., 2004).

4.2. Plant material

Plants were obtained from a variety of sources. The majority, and specifically exotic species were obtained from the botanical garden of Meise, Brussels, Belgium. Grapes of *V. vinifera* L. cv. Alphonse Lavallée (Royal), were obtained from a commercial greenhouse, Soniën wineproducers, Brusselsesteenweg 583, 3090 Overijse, Belgium. Seeds of cereals were from our private collection. Fresh seeds of all *Oryza* cultivars were obtained from the laboratory of phytopathology, University of Gent, Belgium.

For *Triticum aestivum* L., *Secale cereale* L., *Hordeum vulgare* L., *Avena sativa* L., *Vitis vinifera* L. young green seedlings were obtained after imbibition of the seeds in water for 1 h and subsequent growth in perlitte at 22 °C. Plants were maintained in an 8 h light regime in a controlled growth chamber (irradiance = 407 μmol m^{-2} s^{-1}, 75% relative humidity). Etiolated seedlings were grown in identical but completely dark conditions.

4.3. Chromanol analysis

Different extraction techniques; soxhlet extraction, grinding in liquid nitrogen, liquid liquid extraction, critical fluid extraction with CO_2 and solid phase extraction with different solvent combinations, were compared for both green leaf tissue and seeds from *Triticum aestivum* L. (wheat), *Brassica napus* L. (rape), *Nasturtium officinale* L. (water cress). Sample weight was 500 μg or lower. Extraction with hexane by grinding in liquid nitrogen typically gave the highest extraction efficiency in leaf and seed material. For isolated chloroplasts the liquid liquid extraction with hexane and ethanol showed highest extraction efficiency. In all cases 5 ppm DMT was added as internal standard prior to four subsequent extractions. The extracts were pooled, dried (Speed-Vac) and dissolved in 100 μl hexane. Separation of the different chromanol forms was achieved by a Shimadzu HPLC system (system controller SCL-10Avp), under normal phase conditions on an Alltech "Particil Pac" column (5 μm, length is 250 mm, i.d. is 4.6 mm). Elution was isocratic with a hexane – tetrahydrofuran (THF) mixture (6–12%). Flow rate was 1 ml min^{-1}. Complete baseline separation of all chromanols was obtained with 6% THF in 35 min. Higher THF concentrations gave shorter chromatography times, but resulted in lower resolution. Fig. 5, shows a chromatogram with 8% THF, giving adequate separation in just 30 min. Higher THF concentrations (up to 50%) were tested, but were mostly used for column regeneration. The column temperature was kept isothermically at 40 °C (Oven is Shimadzu, CTO-10Avp). Two on line detectors were used for chromanol detection, fluorescence detection (FL) (Shimadzu RF-10A, ex. = 295, em. = 325) because of the high sensitivity (1 ppb) and photo diode array detection (PDA) (Shimadzu SPD-M10avp, 200–600 nm) for verifying peak purity. Data were analysed with Shimadzu Class-vp 6.0 software package. Positive samples, i.e. samples containing tocotrienols, were measured at least three times independently, and peak identity was verified by spiking with tocotrienol standards. Samples showing no tocotrienols were measured only once.

4.4. Tocotrienol and tocopherol biosynthesis assay

Feeding experiments were performed on germinating seedlings of *Triticum aestivum* L. and on isolated chloroplasts and etioplasts of *Triticum aestivum* L. For the seedlings 50 seeds were sown on moist Whatmann paper in a large Petri-dish as described by Threlfall and Whistance (1971), in the presence of 20 μCi [U-14C]-HGA. The whole experiment was performed in a glass container to avoid release of radioactive labelled CO_2. After five days, when tocotrienol concentrations were maximal, the air was removed and filtered through a 20% KOH solution, leaves were harvested and immediately extracted as described above.

Chloroplasts were prepared from about 60 g of two–three day old seedlings by iso-osmotic extraction and percoll gradient centrifugation as described (Price et al., 1987). Intactness of isolated chloroplasts was typically >98% as determined by the method of Jenssen and Bassham (1966). Etioplasts were isolated by sucrose gradient

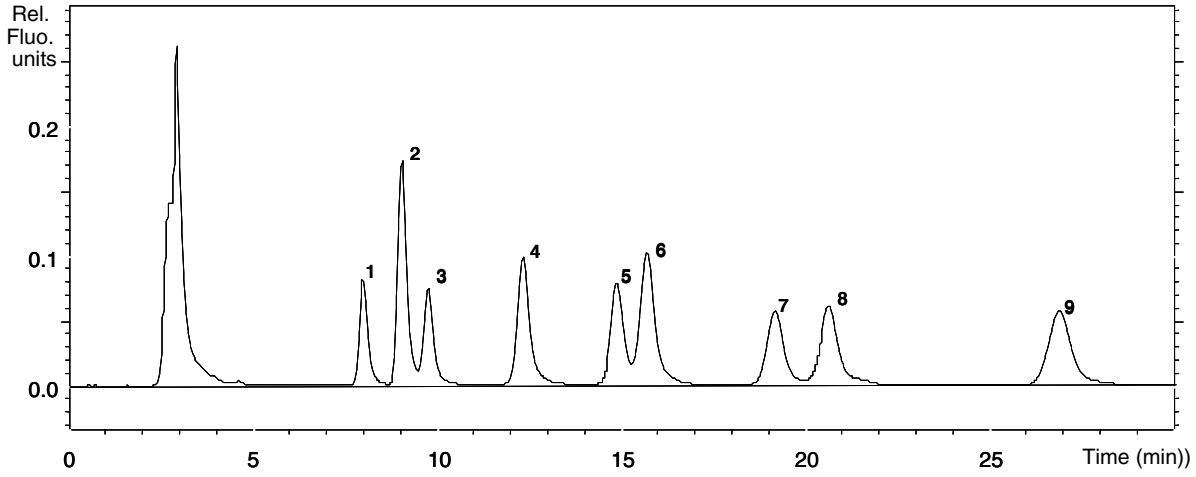

Fig. 5. HPLC chromatogram showing the different chromanol peaks after injection of 2 μM standard mixture of tocochromanols using an 8% THF in hexane mixture for isocratic elution. **1**: α-tocopherol, **2**: DMT (I.S.), **3**: α-tocotrienol, **4**: β-tocopherols, **5**: β-tocotrienol, **6**: γ-tocopherol, **7**: γ-tocotrienol, **8**: δ-tocopherol, **9**: δ-tocotrienol.

centrifugation as described (Miernyk, 1989) from etiolated *Triticum aestivum* L. seedlings. As the ferricyanide-dependent oxygen evolution used for the chloroplasts was obviously unsuitable for etioplast integrity studies the functional integrity of the etioplasts was not assessed. Structural integrity was, however, examined by phase contrast microscopy and showed mainly intact etioplasts. For biosynthesis analysis of tocopherols and tocotrienols normally 1.5 mg chlorophyll equivalent was used for the chloroplasts and 1.5 mg protein equivalent for the etioplasts. Care was taken to keep the plastidal preparation on ice. For measuring tocotrienol biosynthesis the incubation mixture was 10 mM Hepes (pH 7.6), 4 mM $MgCl_2$, 2 mM $MnCl_2$, 0.1 mM NaF, 0.5 mM ATP, 10 mM $NaHCO_3$, 70 μM *s*-adenosyl methionine, 180 μM GGPP, 180 μM HGA, 2 mM cyclodextrine and 0.2 μCi of [1 − n^3H] GGPP as radioactive precursor. In order to measure tocopherol biosynthesis, NADPH (5 mM) was added in order to promote the conversion of GGPP to PhPP – a NADPH dependent reaction (Soll and Schultz, 1981). The incubation medium was equilibrated at 30 °C. The plastidal preparation was added last, in order to ensure osmotic shock as the GGPP has limited membrane permeability. Cyclodextrine is used to prevent formation of vesicles from GGPP. Samples were taken out at 20 min intervals. Reactions were stopped by adding 5 ml EtOH and immediate extraction with 15 ml hexane. The mixture was vigorously vortexed for 5 min and three times re-extracted. The extracts were pooled and after evaporation of the solvents dissolved in 100 μl hexane and subjected to the HPLC system described above. HPLC fractions were collected every minute and each fraction measured for radioactivity in a Packard Liquid scintillation counter with OCS liquid scintillation cocktail. The three timepoints are plotted for control and the biosynthetic potential of the chromanol pathway is calculated as the amount of radioactivity incorporated in the total chromanols.

Acknowledgements

Financial support by the IWT (Instituut ter Bevordering van Wetenschappelijk Technologisch Onderzoek in Vlaanderen) is gratefully acknowledged. Eddy Biebaut is acknowledged for his skilful technical assistance and the numerous HPLC analyses. N.H. is a post-doctoral researcher supported by the Fund for Scientific Research (FWO)-Flanders.

References

Aitzetmüller, K., 1997. Antioxidative effects of *Carum* seeds. J. Am. Oil Chem. Soc. 74, 185.

Amaral, J.S., Alves, M.R., Seabra, R.M., Oliviera, B.P., 2005. Vitamin E composition of walnuts (*Juglans regia* L.): a 3-year comparative study of different cultivars. J. Agric. Food Chem. 53, 5467–5472.

Arango, Y., Heise, K.P., 1997. α-Tocopherol synthesis by *Capsicum* fruit chromoplasts. J. Plant Physiol. 150, 509–513.

Bertoli, C., Fay, L.B., Stancanelli, M., Gumy, D., Lambelet, P., 1998. Characterization of chilean hazelnut (*Gevuina avellana* Mol) seed oil. J. Am. Oil Chem. Soc. 75, 1037–1040.

Cahoon, E.B., Hall, S.E., Ripp, K.G., Ganzke, T.S., Hitz, W.D., Coughlan, S.J., 2003. Metabolic redesign of vitamin E biosynthesis in plants for tocotrienol production and increased antioxidant content. Nat. Biotechnol. 21, 1082–1087.

Cheng, Z., Sattler, S., Maeda, H., Sakuragi, Y., Bryant, D.A., DellaPenna, D., 2003. Highly divergent methyltransferases catalyze a conserved reaction in tocopherol and plastoquinone synthesis in cyanobacteria and photosynthetic eukaryotes. Plant Cell 15, 2343–2356.

Collakova, E., DellaPenna, D., 2001. Isolation and functional analysis of homogentisate phytyltransferase from *Synechocystis* sp. PCC 6803 and Arabidopsis. Plant Physiol. 127, 1113–1124.

Crane, S., Aurore, G., Joseph, H., Mouloungui, Z., Bourgeois, P., 2005. Composition of fatty acids triacylglycerols and unsaponifiable matter in *Calophyllum calaba* L. oil from Guadeloupe. Phytochemistry 66, 1825–1831.

D'Auzac, J., Jacob, J.-L., Chrestin, H., 1989. Physiology of Rubber Tree Latex. CRC Press, Inc., Boca Raton, FL.

Falk, J., Krahnstover, A., van der Kooij, T.A., Schlensog, M., Krupinska, K., 2004. Tocopherol and tocotrienol accumulaion during development of caryopses from barley (*Hordeum vulgare* L.). Phytochemistry 65, 2977–2985.

Firestone, D., 1999. Physical and Chemical Characteristics of Oils, Fats and Waxes. American Oil Chemist's Society Press, Washington.

Franzen, J., Bausch, J., Glatzle, D., Wagner, E., 1991. Distribution of vitamin E in spruce seedlings and mature tree organs, and within the genus. Phytochemistry 30, 147–151.

Franzen, J., Haass, M., 1991. Vitamin E content during development of some seedlings. Phytochemistry 30, 2911–2913.

Frega, N., Mozzon, M., Bocci, F., 1998. Identification and estimation of tocotrienols in the Annato lipid fraction. J. Am. Oil Chem. Soc. 75, 1723–1728.

Goffman, F.D., Bohme, T., 2001. Relationship between fatty acid profile and vitamin E content in maize hybrids (*Zea mays* L.). J. Agric. Food Chem. 49, 4990–4994.

Grusak, M.A., DellaPenna, D., 1999. Improving the nutrient composition of plants to enhance human nutrition and health. Annu. Rev. Plant Physiol. Mol. Biol. 50, 133–161.

Hall, G.S., Laidman, D.L., 1967. The pattern and control of isoprenoid quinone and tocopherol metabolism in the germinating grain of wheat (*Triticum vulgare*). Biochem. J. 108, 475–482.

Havaux, M., Eymery, F., Porfirova, S., Rey, P., Dormann, P., 2005. Vitamin E protects against photoinhibition and photooxidative stress in Arabidopsis thaliana. Plant Cell 17 (12), 3451–3469.

Horvath, Gy., Wessjohann, L., Guisez, Y., Biebau, E., Caubergs, R.J., Horemans, N., 2004. Seeds of grapes of *Vitis vinifera* var. Alphonse lavallee (Royal) a possible model tissue for studying tocotrienol biosynthesis. Acta Hort. 652, 415–424.

Janiszowska, W., Pennock, J.F., 1976. The biochemistry of vitamin E in plants. Vit. Horm. 34, 77–105.

Jenssen, R.G., Bassham, J.A., 1966. Photosynthesis by isolated chloroplast. Proc. Natl. Acad. Sci. 56, 1095–1101.

Kamal-Eldin, A., Appelqvist, L., 1996. The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids 31, 671–701.

Karunanandaa, B., Qi, Q., Hao, M., Baszis, S.R., Jensen, P.K., Wong, Y.H., Jing, J., Venkatramesh, M., Gruys, K.J., Moshiri, F., Post-Beittenmiller, D., Weiss, J.D., Valentin, H.E., 2005. Metabolically engineered oilseed crops with enhanced seed tocopherol. Metab. Eng. 7, 384–400.

Lubrano, C., Robin, J.R., Khaiat, A., 1994. Fatty acid and sterol and tocopherol composition of oil from the fruit mesocarp of six palm species in French Guinea. Oléagineux 49, 59–65.

Maddison, D.R., Schulz, K.S., 1996–2006. The Tree of Life Web Project. Internet address: http://tolweb.org.

Matthaus, B., Vosmann, K., Quoc Pham, L., Aitzetmüller, K., 2003. FA and tocopherol composition of Vietnamese oilseeds. J. Am. Oil Chem. Soc. 80, 1013–1020.

Matringe, M., Sailland, A., Pelissier, B., Rolland, A., Zink, O., 2005. *p*-Hydroxyphenylpyruvate dioxygenase inhibitor-resistant plants. Pest Manage. Sci. 61, 269–276.

Miernyk, J., 1989. Physiology, biochemistry, and genetics of non green plastids: leucoplast isolation. Curr. Top. Plant Physiol. 2, 15–23.

Munné-Bosch, S., Alegre, L., 2002. The function of tocopherols and tocotrienols in plants. Crit. Rev. Plant Sci. 21, 31–57.

Osuna-Garcia, J.A., Wall, M.M., Waddell, C.A., 1998. Endogenous levels of tocopherols and ascorbic acid during fruit ripening of New Mexican-type Chile (*Capsicum sativum*) cultivars. J. Agric. Food Chem. 46, 5093–5096.

Pennock, J.F., 1983. The biosynthesis of chloroplastic terpenoid quinones and chromanols. Biochem. Soc. Trans. 11, 504–510.

Piironen, G.W., Price, K.R., Rhodes, M.J.C., Wiliamsons, G., 1986. Tocopherols and tocotrienols in Finnish foods: vegetables, fruits and berries. J. Agric. Food Chem. 34, 742–746.

Porfirova, S., Bergmüller, E., Tropf, S., Lempke, R., Dörmann, P., 2002. Isolation of an Arabidopsis mutant lacking vitamin E and identification of a cyclase essential for all tocopherol biosynthesis. Proc. Natl. Acad. Sci. 99, 12495–12500.

Price, A.C., Cushmann, J.C., Mendiola-Morgenthaler, L.R., Reardon, E.M., 1987. Isolation of plastids in density gradients of percoll and other silica sols. Methods Enzymol. 148, 157–179.

Rippert, P., Scimemi, C., Dubald, M., Matringe, M., 2004. Engineering plant shikimate pathway for production of tocotrienol and improving herbicide resistance. Plant Physiol. 134, 92–100.

Sattler, S.E., Giligand, L.U., Magallanas-Lundback, M., Pollard, M., DellaPenna, D., 2004. Vitamin E is essential for seed longevity and for preventing lipid peroxidation during germination. Plant Cell 16, 1419–1432.

Schultz, G., 1990. Biosynthesis of α-tocopherol in chloroplasts of higher plants. Fat Sci. Technol. 92, 86–91.

Serbinova, E., 1991. Free radical recycling and intramembrane mobility in the antioxidant properties of α-tocopherol and α-tocotrienol. Free Radic. Biol. Med. 10, 263–275.

Shintani, D.K., Cheng, Z., DellaPenna, D., 2002. The role of 2-methyl-6-phytylbenzoquinone methyltransferase in determining tocopherol composition in *Synechocystis* sp. PCC6803. FEBS Lett. 511, 1–5.

Soll, J., Schultz, G., 1979. Comparison of geranylgeranyl and phytyl substituted methylquinols in the tocopherol synthesis of spinach chloroplasts. Biochem. Biophys. Res. Commun. 91, 715–720.

Soll, J., Schultz, G., 1981. Phytol synthesis from geranylgeraniol in spinach chloroplasts. Biochem. Biophys. Res. Commun. 99, 907–912.

Soll, J., Schultz, G., Joyard, J., Douce, R., Block, M.A., 1985. Localisation and synthesis of prenylquinones in isolated outer and inner envelope membranes from spinach chloroplasts. Arch. Biochem. Biophys. 238, 290–299.

Threlfall, D.R., 1971. The biosynthesis of vitamine E and K and related compounds. Vitam. Horm. 29, 153–200.

Threlfall, D.R., Whistance, G.R., 1971. Biosynthesis of tocopherols and biogenetically related compounds. Methods Enzymol. 18, 369–396.

Valentin, H.E., Qi, Q., 2005. Biotechnological production and application of vitamin E: current state and prospects. Appl. Microbiol. Biotechnol. 68, 436–444.

Velasco, L., Goffman, F.D., Pujadas-Salva, A.J., 2000. Fatty acids and tocochromanols in seeds of Orobanche. Phytochemistry 54, 295–300.

Venkatesh, T.V., Karunanandaa, B., Free, D.L., Rottnek, J.M., Baszis, S.R., Valentin, H.E., 2006. Identification and characterization of an Arabidopsis homogentisate phytyltransferase paralog. Planta 12, 1–11.

Wellburn, A.R., 1969. Studies on the biosynthesis of the tocopherols in higher plants. Phytochemistry 9, 743–748.

Whittle, K.J., Dunphy, P.J., Pennock, J.F., 1966. The isolation an properties of δ-tocotrienol from *Hevea* latex. Biochem. J. 100, 138–145.