Discussion
The results of the present work demonstrate that the LOX pathway in tulip bulbs and leaves operates via the 9-LOX and AOS activities. Tulip is the second plant species known to possess predominantly 9-LOX activity in its leaves, along with recently characterized potato leaves [6].

It seems logical to assume that ketols, being the predominant LOX products in such tissues as tulip bulbs and maize seeds, must have their own physiological importance. As found recently, the γ-ketol (11E)-10-oxo-13-hydroxy-10-octadecenoic acid and a related macrolactone, (11E)-10-oxo-11-octadecen-13-olide, isolated from maize seeds, exhibit strong cytotostatic activity [1]. One might propose, in connection with this, that reduction of γ-ketols and related oxylipins by γ-ketol reductase may have physiological importance as an intracellular mechanism for detoxification of these metabolites. As reported recently [7], (12Z,15Z)-9-hydroxy-10-oxo-12,15-octadecadienoic acid (α-ketol) is an endogenous flowering factor in Lemna paucicostata.

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References

Potato tubers exhibit both homolytic and heterolytic hydroperoxide fatty
acid-cleaving activities
M.-L. Fauconnier*, J. Delcarte*, P. Hoyaux*, P. du Jardin* and M. Marlier*
*Unité de Chimie Générale et Organique, Faculté Universitaire des Sciences Agronomiques de Gembloux, Passage des Déportés, 2-B-5030 Gembloux, Belgium, and tUnité de Biologie Végétale, Faculté Universitaire des Sciences Agronomiques de Gembloux, Passage des Déportés, 2-B-5030 Gembloux, Belgium

Abstract
The action of a crude potato-tuber extract on 9- and 13-hydroperoxides of linoleic and linolenic acids was investigated. HPLC analysis revealed that 50% of the 9-hydroperoxide isomers and almost all the 13-hydroperoxide isomers were rapidly enzymically metabolized. No degradation of fatty acid hydroperoxides was observed with a thermally denatured enzymic extract. GC-MS identification of the volatiles formed by the reaction revealed that no volatiles were detected from the 9-hydroperoxide isomers, whereas 13-hydroperoxide of linolenic acid was cleaved into (Z)-3-hexenal, pentenols or dimers of pentene.

Introduction
Depending on botanical origin and on reaction conditions, plant lipoxygenases can form variable amounts of 13- or 9-hydroperoxides of linoleic or linolenic acid. Fatty acid hydroperoxides can be degraded further in a variety of compounds implicated in essential physiological roles in plants (jasmonic acid, traumatin) or responsible for the characteristic green note odour of plants and fruits (C₆ or C₈ aldehydes and alcohols) [1].

Four main fatty acid hydroperoxide-decomposing activities are described: allene oxide synthase catalyses the conversion of fatty acid hydroperoxides into allene oxides, precursors of α- and γ-ketols and of jasmonic acid (from 13-hydroperoxide of linolenic acid) [2]. Peroxygenase and epoxigenase cause epoxidation of fatty acid hydroperoxides [3,4]. Divinyl ether synthase transforms fatty acid hydroperoxides into fatty acid divinyl ethers [5,6]. Finally, hydroperoxide lyase cleaves fatty acid hydroperoxides into alde-
hydros, alkanes or alcohols and oxo-acids. We investigated here the conversion of fatty acid hydroperoxides by a crude extract of potato tubers.

**Results**

9- and 13-hydroperoxides of linoleic and linolenic acid were synthesized using respectively potato and soya bean lipoxygenases. The fatty acid hydroperoxides were extracted and purified using a C18 microcolumn. The purity, controlled by HPLC analysis, was higher than 95% for the four fatty acid hydroperoxides synthesized. Potato tubers (Bintje) were crushed in a Waring blender with a buffer containing 0.2% (w/v) Triton X-100. The extract was agitated on ice and centrifuged. The supernatant was used as the crude extract for the experiments. Crude extracts were incubated for 15 min at room temperature with, respectively, the 13- and 9-hydroperoxides of linoleic and linolenic acid. An aliquot was taken for direct HPLC analysis to determine the amount of hydroperoxides consumed. The 13-hydroperoxides of linoleic and linolenic acid were almost completely degraded by the potato extract, whereas 50% of the 9-hydroperoxides of linoleic and linolenic acid remain in the reaction medium after the reaction. The enzymic nature of the reaction was confirmed by performing the experiments in the same conditions with the crude extract thermally denatured. In those conditions, no decomposition of fatty acid hydroperoxides was observed. Volatile compounds potentially present after reaction were extracted by diethyl ether. The extract was submitted to GC-MS analysis for identification. The extract obtained from the reaction of 9-hydroperoxides of linoleic and linolenic acid with the crude extract contained no volatile compounds under our experimental conditions.

On the other hand, the following compounds were identified in the extract resulting from the action of potato extract on 13-hydroperoxide of linolenic acid: (Z)-3-hexenal, dimers of pentene (seven isomers), 2-penten-1-ol and 1-penten-3-ol. In the extract obtained with 13-hydroperoxide of linoleic acid, hexanal and pentan-1-ol were identified. No volatile compounds were identified when a thermally denatured extract was used. The identification of volatile compounds was realized on the basis of mass spectrum and by comparison with original molecules when commercially available. A crude extract of potato tubers was prepared under the conditions described above but omitting the detergent Triton X-100. The reaction of this extract with 13-hydroperoxide of linolenic acid formed dimers of pentene, 2-penten-1-ol and 1-penten-3-ol but no (Z)-3-hexenal.

**Discussion**

The results presented here show that a crude potato-tuber extract can metabolize the four isomers of fatty acid hydroperoxide; the 13-isomers are completely transformed while the 9-isomers are only partially degraded. This last result is astonishing because in potato tubers, 95% of isomers formed by lipoxygenase are 9-isomers [7]. The volatile compounds formed from the 13-isomers are typical of two different enzymic mechanisms. The first one is due to hydroperoxide lyase and furnishes (Z)-3-hexenal and hexanal, respectively, from 13-hydroperoxides of linolenic and linoleic acid. Hydroperoxide lyase is a membrane-bound enzyme and it is logical that its activity is not found in an extract obtained without detergent [8]. The formation of dimers of pentene and of C3 alcohols is due to homolytic cleavage of fatty acid hydroperoxides, but the enzymes responsible for the reaction were not clearly identified. The homolytic cleavage is usually described in mushrooms and in algae but the products have also been identified in olive oil [9] and in soya bean [10,11]. In soya bean, two hypotheses are proposed: the cleavage is either due to a soluble homolytic hydroperoxide lyase [11] or is a secondary reaction of lipoxygenase [10]. Further investigations are being undertaken to determine if lipoxygenase is responsible for the homolytic cleavage or if another enzyme is implicated. The identification of non-volatile products formed from 9-hydroperoxides is also in progress: ketols and divinyl ether fatty acids are potential metabolites formed respectively by allene oxide synthase and divinyl ether synthase.

M.-L.F. and J.D. are respectively senior research assistant and research assistant of the Fonds National de la Recherches Scientifiques de Belgique. P.H. is a research assistant of the Fonds pour la formation à la recherche dans l'Industries et dans l'Agriculture.

**References**

Characterization of a *Euphorbia lagascae* epoxide hydrolase gene that is induced 
early during germination

J. Edqvist¹ and I. Farbos
Department of Plant Biology, SLU, Box 7080, 750 07 Uppsala, Sweden

Abstract

In *Euphorbia lagascae* the major fatty acid in triacylglycerol is the epoxidated fatty acid vernolic acid (cis-12-epoxyoctadeca-cis-9-enoic acid). The enzymic reactions occurring during the catabolism of epoxidated fatty acids during germination are not known, but it seems likely that the degradation requires the activity of an epoxide hydrolase. Epoxide hydrolases are a group of functionally related enzymes that catalyse the cofactor-independent hydrolysis of epoxides to their corresponding vicinal diols by the addition of a water molecule. Here we report the cloning and characterization of an epoxide hydrolase gene from *E. lagascae*. The structure of the gene is unusual since it lacks introns. A detailed investigation of the transcription pattern of the epoxide hydrolase gene shows that the gene is induced during germination. We have used *in situ* hybridization to identify in which tissues the gene is expressed during germination. We speculate that this epoxide hydrolase enzyme is involved in the catabolism of epoxidated fatty acids during germination of *E. lagascae* seeds.

Introduction

In *Euphorbia lagascae* the major fatty acid in triacylglycerol is the epoxidated fatty acid vernolic acid (cis-12-epoxyoctadeca-cis-9-enoic acid). The enzymic reactions of epoxidated fatty acid catabolism during germination are not known, but it seems likely that the degradation requires the activity of an epoxide hydrolase (EH). EHs are a group of functionally related enzymes that catalyse the cofactor-independent hydrolysis of epoxides to their corresponding vicinal diols by the addition of a water molecule. Little is known about the actual function of EHs in plants, although they have been suggested to be involved in the biosynthesis of cutin. In potato it was shown that accumulation of the EH transcript is induced after wounding, and also in response to methyl jasmonate [1]. In the case of *Arabidopsis thaliana*, the EH transcript was found to accumulate in young plants after treatment with auxin [2]. The tobacco EH gene was shown recently to be activated during tobacco mosaic virus infection [3]. In contrast to the *A. thaliana* EH, transcription of the tobacco gene did not respond to treatment with auxin. Based on these limited data, plant EHs appear to play a role in responding to environmental stresses. Here we report the cloning and characterization of an EH gene from *E. lagascae*. The structure of the gene is unusual in that it lacks introns. A detailed investigation of the transcription pattern of the EH gene shows that the gene is induced during germination. We have used *in situ* hybridization to identify in which tissues the gene is expressed during germination. We speculate that this EH enzyme is involved in the catabolism of epoxidated fatty acids during germination of *E. lagascae* seeds.

Results and discussion

Cloning and sequencing of a cDNA encoding EH

A cDNA library was constructed of mRNA isolated from germinating seeds of *E. lagascae*. 

Key words: expressed sequence tag, gene regulation, metabolism, oxylipin.

Abbreviation used: EH, epoxide hydrolase.

¹To whom correspondence should be addressed (e-mail Johan.Edqvist@vbiol.slu.se).