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Reduction of divinyl ether-containing polyunsaturated fatty acids in transgenic potato plants

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Abstract

Oxygenated polyunsaturated fatty acids synthesized via the lipoxygenase pathway play a role in plant responses to pathogen attack. In solanaceous plants, the preferential stimulation of the 9-lipoxygenase pathway in response to pathogen infection leads to the formation of the divinyl ether-containing polyunsaturated fatty acids colneleic and colnelenic acid, as well as hydroxy and trihydroxy polyunsaturated fatty acids. To functionally assess the role of divinyl ethers, transgenic potato plants were generated which express an RNA interference construct directed against the pathogen-inducible 9-divinyl ether synthase. Efficient reduction of 9-divinyl ether synthase transcript accumulation correlated with reduced levels of colneleic and colnelenic acid. However, in response to infection with virulent *Phytophthora infestans*, the causal agent of late blight disease, no significant differences in pathogen biomass could be detected suggesting that the levels of antimicrobial divinyl ethers are not critical for defense against *Phytophthora infestans* in a compatible interaction.

Keywords: *Solanum tuberosum*; *Phytophthora infestans*; RNA interference; Oxylipin; Lipoxygenase pathway

1. Introduction

As signaling molecules and as antimicrobial secondary metabolites, oxylipins play a role in pathogen defense in plants (Rosahl and Feussner, 2004). Oxylipins arise as a consequence of autoxidation (Mueller, 2004) or are synthesized enzymatically *via* α-dioxygenases or the lipoxygenase (LOX) pathway (Feussner and Wasternack, 2002). Introduction of molecular oxygen into polyunsaturated fatty acids (PUFAs) such as linoleic (LA) and α-linolenic acid (LnA) by either 9- or 13-LOXs leads to the formation of 9-/13-hydroperoxy octadecadienoic acid (9-/13-HPOD) and 9-/13-hydroperoxy octadecatrienoic acid (9-/13-HPOT), respectively. The hydroperoxides are substrates for at least seven enzymes which catalyze the formation of epoxides, aldehydes, divinyl ethers or hydroxy PUFAs (Feussner and Wasternack, 2002).

Products of the LOX pathway have been implicated to play a role in pathogen defense as signaling molecules and as antimicrobial compounds. Jasmonic acid (JA), a product of the 13-LOX pathway, as well as its biosynthetic precursor 12-oxophytodienoic acid (OPDA) both act as

Abbreviations: CA, colneleic acid; CnA, colnelenic acid; CP-HPLC, chiral phase-HPLC; DES, divinyl ether synthase; GC, gas chromatography; HODE, hydroxy octadecadienoic acid; HOTE, hydroxy octadecatrienoic acid; HPODE, hydroperoxy octadecadienoic acid; HPOTE, hydroperoxy octadecatrienoic acid; JA, jasmonic acid; LOX, lipoxygenase; LA, linoleic acid; LnA, α-linolenic acid; PUFA, polyunsaturated fatty acid; OPDA, 12-oxophytodienoic acid; RP-HPLC, reversed phase-HPLC; SP-HPLC, straight phase-HPLC.

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signaling molecules (Blechert et al., 1999; Kramell et al., 2000; Stintzi et al., 2001; Taki et al., 2005) and were shown to be essential for effective defense against a subset of pathogens and insects (Vijayan et al., 1998; McConn et al., 1997; Stintzi et al., 2001). An additional role for LOXs in membrane lipid peroxidation was postulated based on the observation that most of the hydroperoxides detected during the hypersensitive cell death were generated enzymatically by 9-LOXs (Rustérucci et al., 1999). However, in transgenic potato plants depleted of 9-LOX activity, enhanced autoxidation is observed and hypersensitive cell death appears unaltered (Göbel et al., 2003).

In potato, pathogen infection or elicitor treatment leads to the preferential stimulation of the 9-LOX pathway (Göbel et al., 2001, 2002). Apart from 9-hydroxy octadecadi(tri)enoic acid (9-HOD/T), products of the epoxy alcohol synthase pathway, 9,10,11- and 9,12,13-trihydroxy octadeca(di)enoic acid, and of the 9-divinyl ether synthase (9-DES) pathway (Fig. 1a), colneleic (CA) and colnelenic acid (CnA), accumulate in response to pathogen attack. Several lines of evidence suggest an important role of CA and CnA for the plant's response to pathogens. Firstly, antimicrobial activity of the 9-LOX-derived divinyl ethers has been demonstrated (Weber et al., 1999; Prost et al., 2005). This appears to be a specific effect since spore germination is inhibited to higher extents by treatment with CnA than CA (Prost et al., 2005). Secondly, accumulation of CA and CnA occurs earlier and to higher extents in potato cultivars with higher resistance against *Phytophthora infestans*, the causal agent of late blight disease of potato (Weber et al., 1999). Finally, application of CA reduces infection of barley by the powdery mildew *Blumeria graminis* f. sp. *hordei* (Cowley and Walters, 2005).

To address the role of CA and CnA in a functional manner, we modulated the levels of divinyl ether containing PUFAs in potato by expressing an RNA interference (RNAi) construct directed against the pathogen-inducible *9-DES* of potato. Interestingly, despite reduced levels of CA and CnA in the transgenic potato plants, growth of *P. infestans* was not affected. Thus, accumulation of divinyl ethers to high levels is apparently not required for basal resistance in potato.

2. Results and discussion

The divinyl ethers CA and CnA are synthesized from LA and LnA, respectively, by the action of a 9-LOX and a 9-DES (Itoh and Howe, 2001; Stumpe et al., 2001; Fig. 1a). In order to specifically reduce the levels of CA and CnA in potato plants, an RNAi construct was generated using a 348 bp fragment of the coding region of potato *9-DES* (Stumpe et al., 2001). The fragment was cloned in antisense orientation upstream, and in sense orientation downstream, of a truncated GUS gene behind the 35S pro-

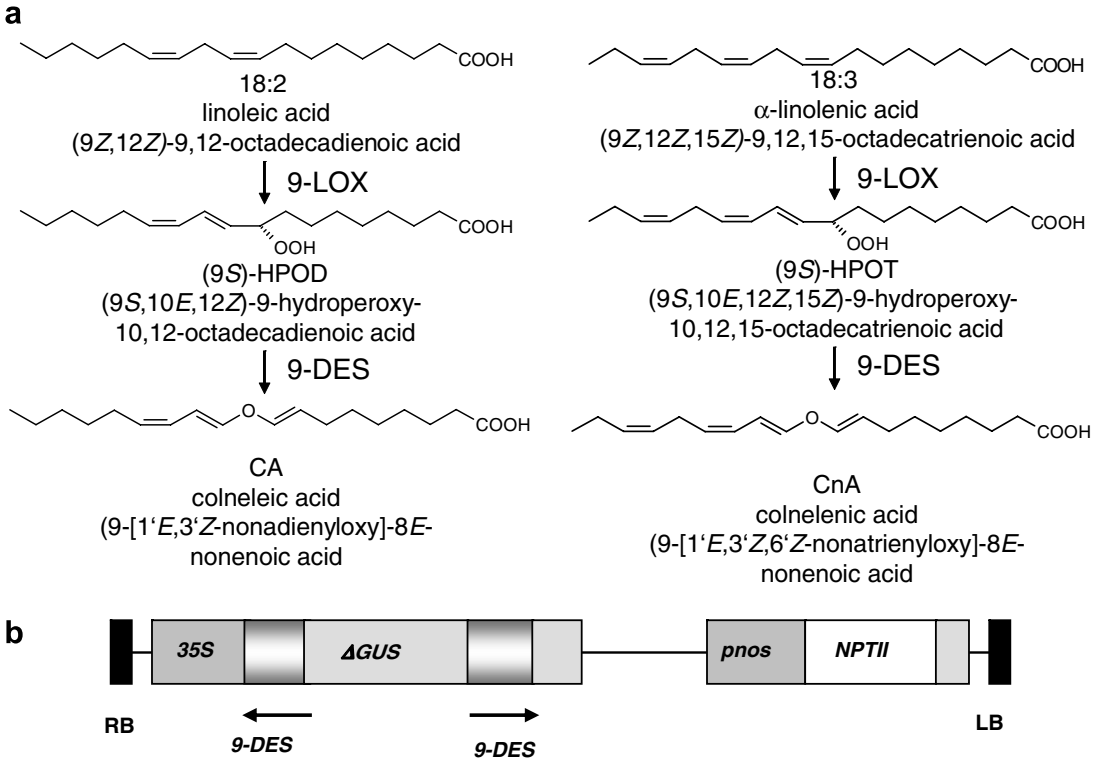

Fig. 1. (a) Schematic presentation of the synthesis of CA and CnA in plants. (b) Structure of the *9-DES*-RNAi construct. 35S: 35S promoter, *GUS*: coding region of the ß-glucuronidase, LB: left border of the T-DNA, *NPTII*: neomycin phosphotransferase, pnos: nopaline synthase promoter, RB: right border of the T-DNA.