DIFFERENTIAL EXPRESSION OF (+)δ-CADINENE SYNTHASES IN COTTON CELLS

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Abstract

Recently two (+)δ-cadinene synthases have been cloned from *G. arboreum* L. cv. Nangking cells and heterologously expressed. Both enzymes catalyze the same reaction but differ in amino acid sequence (80% identity), pH optimum and kinetic parameters. To answer the question why two identical enzymes are required for the catalysis of the rate limiting reaction in the biosynthetic pathway(s) leading to cotton tissue phytoalexins, the differential induction of the mRNA for the two enzymes was measured after elicitation, using quantitative reverse transcription polymerase chain reactions. The two enzymes, CAD A and CAD C, are differentially expressed in cotton roots and leaves. Transcription of CAD A is preferentially induced by the phytopathogenic fungus *Verticillium dahliae* in root tissue of four weeks old *G. arboreum* seedlings. Whereas both CAD A and C expression is induced in leaf tissue by the phytopathogenic bacterium *Xanthomonas campestris* pv. *malvacearum.*

Introduction

Stressed cotton tissue produces phytoalexins. Infection of *Gossypium arboreum* L. cv Nanking cell suspension cultures with heat denatured *Verticillium dahliae* conidia resulted in a twenty fold induction of the synthesis of hemigossypol, gossypol and derivative (Heinstein, 1985).

Similar results are obtained when a 65 kD protein, an elicitor purified from *V. dahliae* T9, was used to stimulate *G. arboreum* and a variety of *G. hirsutum* cell suspension cultures (Davis, *et al.* 1993). This induction of the sesquiterpenoid biosynthetic pathway leading to these phytoalexins does occur *in vivo*, since *G. hirsutum* or *G. arboreum* plantlets can be inoculated with *V. dahliae* viable conidia and a subsequent increase in aromatic sesquiterpene aldehydes can be demonstrated.

A similar elicitation - response mechanism has been demonstrated in cotton leaves. Inoculation of leaves of *G. hirsutum* Acala 44 with *Xathomonas campestris* pv. *malvacearum* resulted in the induction of the synthesis of the phytoalexins 2,7-dihydroxycadalene, lacinilene C, and lacinilene C7 methyl ether in localized quantities to inhibit bacterial colonization (Essenberg, *et al.* 1982).

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In both cases an induction of the sesquiterpene biosynthetic pathway can be inferred. The first and rate limiting enzyme in this pathway has been shown to be a farnesyl diphosphate cyclase in tobacco cell suspension cultures (Chappell and Nable, 1987; Chappell 1995). In cotton tissue two (+)δ-cadinene synthases have been cloned which catalyze the first committed step in the sesquiterpene biosynthetic pathway (Chen, *et al.* 1995). Both enzymes are inducible in *G. arboreum* and *G. hirsutum* cell suspension suspension cultures upon fungal or bacterial elicitation. We describe herein the differential expression of the two (+)δ-cadinene synthases (CAD A and CAD C) in cotton tissues.

Materials and Methods

Four weeks old *G. arboreum* L. cv Nanking plants were inoculated immediately below the internode with $5x10^5$ conidia of *Verticillium dahliae* T9. Leaves of plants were infiltrated with one ml of *Xanthomonas campestris* pv. *malvacearum* (~ 10^6 cfu/ml) (Essenberg *et al.* 1990). Roots and leaves were harvested 8, 12, 24 and 48 hrs after inoculation. Control samples were obtained from double distilled water inoculated plants 8 hrs after inoculation. Total RNA was extracted from the sample tissues with the TRIzol™ reagent (Life Technologies, GIBCO BRL). Routinely, ~50 mg of total RNA/500 mg root tissue and ~100 mg of total RNA/500 mg leaves was obtained. Aliquots of the isolated RNA was subjected to reverse transcription (RT) using AMV reverse transcriptase. Aliquots of this preparation were used as templates in quantitative polymerase chain reactions (QtPCR) (Gilliland *et al.* 1990; Vanden Heuvel *et al.* 1994). The competitive templates, used as internal standards for quantitation, were two genomic DNA fragments, 246 bp for CAD A and 193 bp for CAD C, containing introns. These DNA fragments were prepared from *G. arboreum* genomic DNA (Chen *et al.* 1995). Primers specific for CAD A and CAD C were as described previously (Chen *et al.* 1995). PCR mixtures were analyzed by agarose gel electrophoresis and quantitated using pdi laser gel densitometry of the ethidium bromide stained agarose gels (Vanden Heuvel *et al.* 1994).

Results and Discussion

The rather surprising results that at least two (+)δ-cadinene synthases exist in cotton tissue, which catalyze identical reactions, raised the question of a plausible reason for this observation. Possible explanations are that the two enzymes are tissue specific or that one enzyme controls the first committed step in the biosynthetic pathway leading to hemigossypol and derivatives whereas the other enzyme controls the pathway leading to 2,7-dihydroxycadalene and derivatives. In an attempt to answer these questions we studied the induction of the two gene products, CAD A and CAD C, in leaves and roots inoculated with two phytopathogens, *V. dahliae* and *X. campestris* pv.

malvacearum. To differentiate between two very similar gene products, quantitative RT-PCR was used.

As documented in Table I, both CAD A and C are expressed in leaf tissue of *G. arboreum* when inoculated with *X. campestris* pv. *malvacearum*. Induction of CAD A was 3.8 fold and CAD C was 5 fold. Induction of CAD A and C in root tissue by *X. campestris* pv. *malvacearum* was 0 and 2.5 fold, respectively. However, only the expression of CAD A was induced by *V. dahliae* T9 in roots. Induction was 16 fold. In leaves, CAD A and C expression was 2 fold higher in *V. dahliae* inoculated plants, which is not significant from controls.

These results demonstrate that the two (+)δ-cadinene synthases are active in different pathways, one leading to aromatic sesquiterpene aldehydes (hemigossypol and derivatives) in root tissue and a second pathway leading to cadalene derivatives in leaf tissue. In addition, these result infer differential expression of the two enzymes upon elicitation by phytopathogens.

Table 1. Estimation of mRNA of Gene CAD A and CAD C in Cotton Tissue

Tissue	Gene	Elicitor	Treatment hrs.	mRNA x10^5 molecules/ µg total RNA
Leaf	CAD C	VD[a]	control	0.5
			8	1.2
			24	0.8
			48	0.7
Leaf	CAD A	Vd[a]	control	0.6
			8	1.2
			24	0.6
			48	0.3
Leaf	CAD C	Xcm[b]	control	0.9
			12	5.9
			24	1.6
Leaf	CAD A	Xcm[b]	control	0.5
			12	4.3
			24	1.6
Root	CAD C	Vd[a]	control	3.0
			8	3.6
			24	3.4
			48	3.2
Root	CAD C	Xcm[b]	8	8.3
Root	CAD A	Vd[a]	control	3.5
			8	55.4
			24	3.3
			48	2.7
Root	CAD A	Xcm[b]	8	3.3

a: The *Verticillium dahliae* T9 (Vd) induced samples are the average from three separate experiments. Each sample was subjected to quantitative reverse transcription PCR in triplicate.

b: The *Xanthomonas campestris* pv. *malvacearum* (Xcm) induction was done once and each sample subjected to quantitative reverse transcription PCR in triplicate.

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