Isolation and characterization of a potato cDNA corresponding to a 1-aminocyclopropane-1-carboxylate (ACC) oxidase gene differentially activated by stress

María Eugenia Zanetti¹, María Cecilia Terrile¹, Débora Arce¹, Andrea Verónica Godoy¹, Blanca San Segundo² and Claudia Casalongüé¹,³

¹ Instituto de Investigaciones Biológicas-Departamento de Biología, Universidad Nacional de Mar del Plata, Funes 3250, CC 1245, 7600 Mar del Plata, Argentina
² Instituto de Biología Molecular de Barcelona, Centro de Investigación y Desarrollo (CSIC), Jordi Girona 18-24, 08034 Barcelona, España

Received 22 July 2002; Accepted 27 August 2002

Abstract

1-Aminocyclopropane-1-carboxylate (ACC) oxidase enzyme catalyses the final step in ethylene biosynthesis, converting 1-aminocyclopropane-1-carboxylic acid to ethylene. A cDNA clone encoding an ACC oxidase, ST-ACO3, was isolated from potato (Solanum tuberosum L.) by differential screening of a Fusarium eumartii infected-tuber cDNA library. The deduced amino acid sequence exhibited similarity to other ACC oxidase proteins from several plant species. Northern blot analysis revealed that the ST-ACO3 mRNA level increased in potato tubers upon inoculation with F. eumartii, as well as after treatment with salicylic acid and indole-3-acetic acid, suggesting a cross-talk between different signalling pathways involved in the defence response of potato tubers against F. eumartii attack.

Key words: ACC oxidase, ethylene, Fusarium eumartii, indole-3-acetic acid, Solanum tuberosum, stress response, wounding.

Ethylene regulates a wide range of physiological processes in plant tissues. An increase in ethylene biosynthesis has been correlated with several stages of growth and development and in response to physical wounding and pathogen infection (Abeles et al., 1992). The final step in the ethylene biosynthetic pathway is catalysed by ACC (1-aminocyclopropane-1-carboxylate) oxidase, which converts 1-aminocyclopropane-1-carboxylic acid to ethylene (Kende, 1993). ACC oxidases have been identified in various plant species as a small gene family. Differential accumulation of their corresponding mRNAs has been reported in response to several stimuli including ethylene, indol-3-acetic acid and wounding treatment (Tang et al., 1993; Lasserre et al., 1996; Peck and Kende, 1995). Later on, the activation of an ACC oxidase gene promoter from melon was observed in transgenic tobacco leaves after inoculation with the bacterium Ralstonia solanacearum (Lasserre et al., 1997). Jia and Martin (1999) also reported that ACC oxidase transcript levels rapidly accumulate during incompatible interaction involving Pto-containing tomato plants and the bacterial pathogen Pseudomonas syringae pv. tomato expressing avrPto.

The isolation and expression characterization of a cDNA encoding an ACC oxidase homologue protein from potato tubers, named ST-ACO3 (Solanum tuberosum ACC oxidase 3), is described here.

ST-ACO3 cDNA clone was isolated by a differential screening of a 24 h-Fusarium eumartii infected tuber cDNA library (Godoy et al., 2000). The ST-ACO3 cDNA fragment (GeneBank Accession No. AY098939) was 1398 bp long. It contains an ORF for a protein with a predicted molecular mass of 35.6 kDa. The deduced amino acid sequence of ST-ACO3 was 63% and 60% identical to Arabidopsis thaliana ACC oxidase (GenBank Accession No. AAD10157) and Cucumis melo ACO2 (GenBank Accession No. S66175), respectively. Less conservation (40–46% identity) was found with other ACC oxidases from various plant species (Fig. 1). The polypeptide encoded by ST-ACO3 showed only 42% identity with the amino acid sequences of two previously cloned ACC oxidases from potato, ST-ACO1 (GenBank Accession No. AAK68075) and ST-ACO2 (GenBank Accession No. AAK680076). ST-ACO1 and ST-ACO2 share 79% identity each to other, indicating that ST-ACO3 is a more divergent member of gene family (Fig. 1).

Southern blot analysis was performed under highly stringent conditions. Two bands were detected in DNA samples digested with SacI or BamHI, whereas four bands with different intensity were revealed in DNA sample digested with EcoRI (Fig. 2). Since the ST-ACO3 cDNA fragment has two internal sites for Eco RI, the hybridized pattern indicated that two or more ST-ACO3 related genes might be present in the potato genome.

To investigate the expression of the ST-ACO3 gene, Northern blot analysis was conducted (Fig. 3). In this experimental system, the method used for the inoculation of potato tubers with F. eumartii involves mechanical wounding of the tissue. For this reason, the expression pattern of ST-ACO3 mRNA in wounded (W) and wounded plus F. eumartii (W+F)-infected tubers was compared. In both, a single band corresponding to a transcript of 1.5 kb was detected. At 24 h, W+F treatment resulted in a clear and reproducible increase in the ST-ACO3 mRNA level with respect to W (Fig. 3A). At 24 h after incubating tubers with a cell wall fraction from F. eumartii, used as the elicitor source, a similar increase was observed (not shown).

Salicylic acid (SA) and hydrogen peroxide (H₂O₂) have been described as signal molecules that play a central role in plant defence
responses to fungal attack (Yang et al., 1997). The effects of both molecules on the ST-ACO3 mRNA level was also investigated. SA caused a slight but reproducible increase, whereas H2O2 did not affect the ST-ACO3 mRNA level with respect to water-treated tubers (Fig. 3B).

On the other hand, it has been reported that indole-3-acetic acid (IAA) causes an increase in ACC oxidase transcript and activity levels in etiolated peas and rice seedlings (Peck and Kende, 1995; Chae et al., 2000). Accordingly, application of IAA in potato tubers caused a significant accumulation of ST-ACO3 transcript level respect to water-treated tubers (Fig. 3B).

To summarize, this is a report of the cloning of a new potato cDNA encoding a putative ACC oxidase, whose transcript level increases in potato tubers in response to F. eumartii infection, as well as after SA and IAA treatments, suggesting a cross-talk between different signalling pathways that are involved in the defence response of potato tuber against F. eumartii attack. Further studies are needed to establish if the accumulation of ST-ACO3 transcript is correlated with an increase in ethylene production during potato–F. eumartii interaction.

Acknowledgements

We thank P Heizmann (France) for providing the rDNA clone. This work was partially supported by the IFS (Sweden), CONICET, UNMDP, ANPCyT (Grant No. 01-09768), and Fundación Antorchas (Argentina). MEZ and CT were recipients of a fellowship from CONICET and UNMDP, respectively.

References


