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Research article

Regulatory mechanisms of ethylene biosynthesis in response to various stimuli during maturation and ripening in fig fruit (*Ficus carica* L.)

W.O. Owino[a,*], Y. Manabe[b], F.M. Mathooko[a], Y. Kubo[b], A. Inaba[b]

[a] *Department of Food Science and Technology, Faculty of Agriculture, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000, Nairobi, Kenya*
[b] *Laboratory of Postharvest Horticulture, Faculty of Agriculture, Okayama University, Tsushima, Okayama 700-8530, Japan*

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Abstract

In order to obtain a greater uniformity of maturation, the growth of the fig fruit (*Ficus carica* L.) can be stimulated by the application of either olive oil, ethrel/ethephon or auxin. The three treatments induce ethylene production in figs. In this study, we investigated the regulatory mechanisms responsible for oil, auxin and ethylene induced ethylene production in figs. The ethylene production in response to olive oil, auxin, and propylene treatments and during ripening were all induced by 1-methylcyclopropene (1-MCP) and inhibited by propylene indicating a negative feedback regulation mechanism. Three 1-aminocyclopropane-1-carboxylic acid (ACC) synthase genes (*Fc-ACS1*, *Fc-ACS2* and *Fc-ACS3*) and one ACC oxidase gene (*Fc-ACO1*) were isolated and their expression patterns in response to either oil, propylene or auxin treatment in figs determined. The expression patterns of *Fc-ACS1* and *Fc-ACO1* were clearly inhibited by 1-MCP and induced by propylene in oil treated and ripe fruits indicating positive regulation by ethylene, whereas *Fc-ACS2* gene expression was induced by 1-MCP and inhibited by propylene indicating negative regulation by ethylene. The *Fc-ACS3* mRNA showed high level accumulation in the auxin treated fruit. The inhibition of *Fc-ACS3* gene by 1-MCP in oil treated and in ripe fruits suggests that auxin and ethylene modulate the expression of this gene by multi-responsive signal transduction pathway mechanisms. We further report that the olive oil-induced ethylene in figs involves the ACC-dependent pathway and that multiple ethylene regulatory pathways are involved during maturation and ripening in figs and each specific pathway depends on the inducer/stimulus.

Keywords: ACC oxidase; ACC synthase; Auxin; Ethylene; Fig fruit; Propylene; Olive oil

Nucleotide sequence data reported in this study appear in the Genbank data base under accession numbers DQ 269492 (*Fc-ACS1*), DQ 269493 (*Fc-ACS2*), DQ 269494 (*Fc-ACS3*) and DQ 269495 (*Fc-ACO*).

1. Introduction

The gaseous plant hormone ethylene regulates many processes during plant growth and development and is also an important mediator of plant responses to biotic and abiotic stresses [32]. The pathway of ethylene synthesis is well established in higher plants and regulatory control is achieved at two steps: the formation of 1-aminocyclopropane-1-carboxylic acid (ACC) from *S*-adenosyl-L-methionine and the conversion of ACC to ethylene [11]. The first is catalyzed by the enzyme ACC synthase (ACS) and the second by ACC oxidase (ACO). In higher plants both of the enzymes are encoded by multigene families generating the option of multiple control points at which ethylene synthesis may be regulated. These genes have been isolated and structurally characterized, and are differentially expressed in various tissues at different stages of development and in response to internal or external stimuli such as ripening, senescence, wounding, and auxin [11].

The fig fruit is a highly perishable climacteric fruit and has been referred to as the oldest species of the fruit tree having been cultivated by humans for over 5000 years [6]. Cumulative growth in diameter of the fig fruit is portrayed by a double

Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; DIG, digoxigenin; 1-MCP, 1-methylcyclopropene; 2-(2,4,5-TP), 2-(2,4,5-trichlorophenoxy)-propionic acid; RT-PCR, reverse transcription-polymerase chain reaction.

* Corresponding author. Present address: Gene Research Center, University of Tsukuba, Tennodai 1-1-1, Tsukuba City, 305-8572 Ibaraki, Japan. Tel./fax: +81 298 537 734.
E-mail address: willis@gene.tsukuba.ac.jp (W.O. Owino).

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sigmoid curve. In the first stage, of intense cell division and differentiation (period I), growth is rapid. A long period of stasis follows (period II) during which mitotic activity ceases, then finally a second phase of rapid growth (period III), in which cell expansion and a change of color and texture are observed, besides aroma enrichment (Fig. 1) [6]. The practice of stimulating growth to obtain greater uniformity of maturation has been the subject of several studies. It has been known since the 3rd Century BC that a drop of olive oil applied to the ostiole (a process termed as Oleification) of the fig fruit stimulates growth and leads to uniform ripening of fruits [6]. The application of several vegetable oils such as rape seed oil, sesame oil, soybean oil, camellia oil and linseed oil also had similar effects as the olive oil whereas animal oil stimulated fruit ripening but the effect was less than that of the vegetable oils [12]. It was also observed that refined olive oil was more effective in accelerating maturation of the fig fruit as compared to unrefined olive oil or other vegetable oils. Treatment of fig fruit with linolenic acid induced rapid production of ethylene [13]. In order to determine which fatty acid was more effective in hastening maturation of figs, various fatty acids such as linolenic acid, oleic and stearic acid were tested and linolenic acid was found to be the most effective [14]. However to date, how and why oleification stimulates maturation and ethylene production in figs is not known.

Accelerated fig fruit growth, and much earlier ripening than in non-treated fruits can also be obtained by auxin application and the action of auxin stimulated the production of ethylene in fruits and leaves [21]. Similar results were also obtained by using products which release ethylene such as ethephon [8]. Ethylene application to fruit on the tree showed that ripening occurs only when physiological conditions are right for autocatalytic ethylene production [20]. The aim of the present study was to investigate the regulatory mechanisms responsible for oil, auxin and ethylene induced ethylene production during the final stages of growth and ripening in fig fruit.

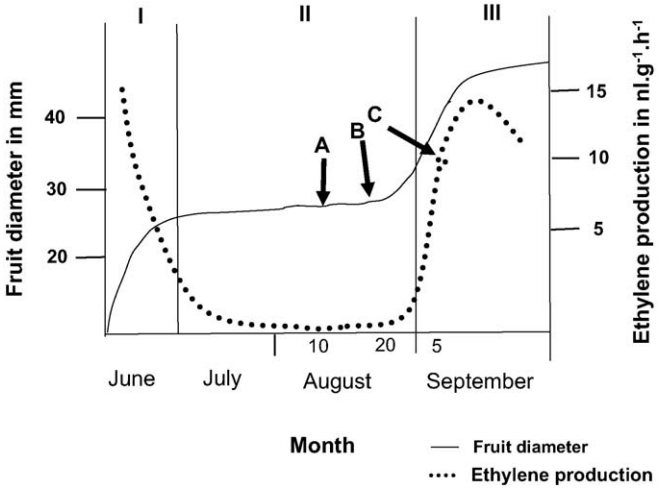

Fig. 1. Double sigmoid growth curve of the fig showing the progression in fruit diameter and ethylene evolution during the three phases of fruit growth. I–III indicates the three phases of fruit growth and development. Point A indicates the date of on-tree treatment, whereas points B and C indicate the harvest dates for off-tree treated fruit in the present study.

2. Results

2.1. Isolation and sequence analyses of the isolated ACS and ACO clones

To identify ACS and ACO genes expressed during maturation and ripening of the figs, total RNA was isolated from ripe fig fruit tissues and used for reverse transcriptase-PCR reactions, together with degenerate primers based on deduced amino acid domains conserved between ACS and ACOs. The cDNAs isolated were designated *Fc-ACS1*, *Fc-ACS2* and *Fc-ACS3* and *Fc-ACO1* for ACS and ACO genes, respectively. The percentage homologies at the amino acid level are shown in Table 1.

The isolated partial length *Fc-ACS1* showed 77% amino acid identity with the peach *ACSI* (Accession number, AAF61235). The *Fc-ACS2* showed 72% identity with the stress induced ACS synthases such as the chilling induced ACS2 of *Citrus sinensis* (Accession number, CAB60831), and the ozone induced ACS2 of birch plant (Accession number, AAM80889). *Fc-ACS3* showed 77% amino acid identity with the IAA-induced *VR-ACS7* of mung bean (Accession number, AAD41083). The Fc-ACO1 showed 69% identity with the Japanese persimmon, *DK-ACO2* (Accession number, BAB89352).

2.2. Genomic organization of the ACS and ACO genes

Southern blot analyses were performed to determine the genomic organization of the isolated ACSs and ACO genes. Specific primers were used for PCR amplifications of cDNAs corresponding to specific regions of the isolated *Fc-ACS1*, *Fc-ACS2*, *Fc-ACS3* and *Fc-ACO1* gene and used to probe the genomic gel-blots (Fig. 2). These results indicate that *Fc-ACS1*, *Fc-ACS2* and *Fc-ACO1* genes exist as single copy genes in the fig fruit genome. The presence of weak bands in the *Fc-ACS2* blot may represent distantly related single genes. The cDNA used to generate the *Fc-ACS3* probe contains an *Eco*RV site and this may explain the two hybridizing bands detected in *Eco*RV digest. However the presence of two hybridizing bands detected in the *Xba*I digests suggest that this gene may exist as either a single or two copy genes in the fig fruit genome. It was apparent that the probes generated from the three ACSs did not cross-react under our hybridizing and washing conditions, indicating that these probes were specific for each gene family member.

Table 1
Percentage sequence identity between ACS genes isolated from fig fruit

cDNA clone	*Fc-ACS1*	*Fc-ACS2*
Fc-ACS2	71.2	
Fc-ACS3	48	50