DIFFERENTIAL EFFECTS OF LOW TEMPERATURE INHIBITION ON
KIWIFRUIT RIPENING AND ETHYLENE PRODUCTION

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1. Abstract

Our previous studies [7] have shown that there is an inhibition of propylene-induced ethylene production in kiwifruit below a critical temperature range of 11-14.8°C. The aim of this research was to identify the biochemical basis of inhibition of the propylene-induced ethylene production in kiwifruit, below the above mentioned critical temperature range. “Hayward” kiwifruit were treated with 130µl/l propylene or air free of propylene and ethylene at 10°C and 20°C. Ethylene production as well as ACC synthase and ACC oxidase activities were measured during a period of 312 hours. Changes in soluble solid content (SSC) and flesh firmness were also monitored during the same time-course period. RNA blot hybridisations using specific probes for ACC synthase and ACC oxidase were performed with total RNA from untreated fruit as well as from those that had received 192 hours of propylene treatment, at 10°C and 20°C. We propose that the main reasons for the inhibition of the propylene-induced (autocatalytic) ethylene production in kiwifruit at low temperature (<11°C) are: a) primarily the inhibition of the expression of the propylene-induced ACC synthase gene and b) the possible post-transcriptional modification(s) of ACC oxidase, since expression of the propylene-induced ACC oxidase gene existed at the low temperature storage.

2. Introduction

Most of the factors influencing ethylene production, act primarily by enhancing endogenous levels of ACC via de novo synthesis of ACC synthase [1]. In kiwifruit, low temperature (< 11° -14.8°C) blocks initiation of autocatalytic ethylene production.
(induced by propylene) but not ripening [8]. The rate limiting factor was found to be ACC production rather than ACC oxidase activity.

The aim of this research was to identify the biochemical basis of inhibition of the propylene-induced ethylene production in kiwifruit, below the above mentioned critical temperature range.

3. Material and Methods

Kiwifruit (cv. Hayward) were placed at 10° and 20°C in 5-litre jars into which a continuous humidified air stream with or without 130µl/l propylene was passed at a rate of 100ml/min. At periodical intervals, fruits of each treatment were removed from storage and used for analysis.

ACC synthase was extracted and assayed as described previously [2]. One unit of ACC synthase activity is defined as the formation of 1 nmol of ACC/2hrs at 30°C. ACC oxidase was measured in vivo by infiltrating flesh disks with 1mM ACC under vacuum as described elsewhere [5].

Total RNA was isolated from flesh tissue without seeds based on the method of Slater et al. [6]. RNAs were transferred to nylon membrane and hybridised with radiolabelled specific probes cDNA MEL1 [3] for ACC Oxidase and KWACC1 [4] for ACC Synthase. Total RNA extraction and Northern blotting were performed 192 hours after the commencement of the experiment.

4. Results and Discussion

Kiwifruit treated at 20°C with propylene, resulted in induced ripening (Fig. 1A, B) and ethylene production (Fig. 1C). Ripening progressed immediately after propylene treatment, while autocatalysis of ethylene production had a lag period of 72 hours. The latter event was attributed to the delay found in the induction of ACC synthase activity (Fig. 1D). In contrast, propylene treatment induced ACC oxidase activity with no lag period (Fig. 1E). Moreover, accumulation of ACC synthase and ACC oxidase transcripts was only evident (Fig. 1F) in ethylene-producing kiwifruit at 20°C (Fig. 1C).

In contrast, kiwifruit treated at 10°C with propylene, resulted in a strong inhibition of ethylene production (Fig. 1C), which was attributed to the low found activities of both ACC synthase and ACC oxidase (Fig. 1D, E). Interestingly, propylene at 10°C induced the appearance of mRNA of ACC oxidase but not of ACC synthase (Fig. 1F). However, propylene induced ripening of that fruit with almost the same rate found for the propylene-treated fruit at 20°C (Fig. 1A, B). It should be noted that during the whole experimental period (312 hours) the control fruit (treated with air free of propylene) showed no ripening, ACC synthase or ACC oxidase activities or ethylene production at either 10 or 20°C (Fig. 1A, B, C, D, E).

Although decreased temperature (10°C) reduces ACC oxidase activity, the fact that at low temperature mRNA of the propylene-induced ACC oxidase gene is still present, led us to propose that the main reasons for the inhibition of the propylene-induced (autocatalytic) ethylene production in kiwifruit at low temperature (<11 °C) are: a)