Available online at www.sciencedirect.com
ScienceDirect
Postharvest Biology and Technology 42 (2006) 31–40
Postharvest Biology and Technology
www.elsevier.com/locate/postharvbio

A comparison between intact fruit and fruit explants to study the effect of polyamines and aminoethoxyvinylglycine (AVG) on fruit ripening in peach and nectarine (*Prunus persica* L. Batch)

Anna Maria Bregoli [a], Vanina Ziosi [b], Stefania Biondi [b], Bonghi Claudio [c], Guglielmo Costa [a], Patrizia Torrigiani [b,*]

[a] *Dip. di Colture Arboree, Università di Bologna, Via Filippo Re 6, 40126 Bologna, Italy*
[b] *Dip. di Biologia e.s., Università di Bologna, Via Irnerio 42, 40126 Bologna, Italy*
[c] *Dip. di Agronomia Ambientale e Produzioni Vegetali, Agripolis, Università di Padova, Padova, Italy*

Received 1 February 2006; received in revised form 13 April 2006; accepted 31 May 2006

Abstract

In order to establish whether *in vitro* model systems are suitable to study the reciprocal relationships between ethylene and polyamines (PAs) in peach fruit, whole detached fruit and fruit explants from "Redhaven" peaches and "Stark Red Gold" nectarines at two different ripening stages were subjected to *in vitro* treatments with 10 mM putrescine (Pu), 1 mM spermidine (Sd) or 0.32 mM aminoethoxyvinylglycine (AVG) in the presence or in the absence of labelled Pu or methionine. Labelled Pu uptake studies showed that, in the short-term, much more label was recovered in intact nectarines than in peaches. In fact, in the former, ethylene production was strongly impaired by Pu and Sd at both stages, while it was substantially unaffected in the latter. In treated fruit, flesh firmness, soluble solids content and fresh weight were only sporadically affected. Under the same experimental conditions, AVG almost totally inhibited ethylene production although fruit quality was practically unaltered. In explants obtained from fruit at the firmer ripening stage, Pu and Sd did not alter and even enhanced methionine incorporation into ethylene, while in those from softer fruit only Sd was able to counteract ethylene biosynthesis. Also in this case, AVG dramatically reduced ethylene biosynthesis. Short-term treatments of fruit explants showed that only Sd and AVG counteracted ripening. Comparing results from intact fruit and fruit explants indicates that Pu and Sd exert a differential effect on ethylene and fruit quality, depending upon ripening stage and cultivar.

Keywords: 1-Aminocyclopropane-1-carboxylate oxidase (ACO); Aminoethoxyvinylglycine (AVG); Ethylene biosynthesis; Peach fruit; Polyamines; *Prunus persica*

1. Introduction

Field experiments have shown that pre-harvest polyamine (PA) applications to on-tree fruit strongly interfere with ethylene biosynthesis and perception, and with fruit quality (flesh firmness, soluble solids concentration, fresh weight, etc.) resulting in a rejuvenating effect/ripening delay both in peach and apricot (Paksasorn et al., 1995; Bregoli et al., 2002; Torrigiani et al., 2004). In fact, the aliphatic biogenic PAs, putrescine, spermidine and spermine, are required for plant growth and differentiation; in addition, they contrast stress and senescence (Bagni and Torrigiani, 1992). Although their mechanism of action has not yet been clarified, because of their polycationic nature, PAs form weak bindings with most negatively charged biological macromolecules thereby modulating their activity (Cohen, 1998; Messiaen and Van Cutsem, 1999). Moreover, PAs covalently bind and cross-link proteins which regulates their function (Della Mea et al., 2004), and form conjugates with phenolic compounds to yield metabolic endproducts (Martin-Tanguy, 1985). Their synthesis starts from the amino acids arginine or ornithine which lead, via specific decarboxylases, to the diamine putrescine; the latter is the precursor, together with methionine, of the higher PAs, the triamine spermidine and the tetramine spermine. As in other growth processes, also during fruit set,

* Corresponding author. Tel.: +39 051 2091291; fax: +39 051 242576.
E-mail address: patrizia.torrigiani@unibo.it (P. Torrigiani).

0925-5214/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.postharvbio.2006.05.009

development and ripening, endogenous PA titres undergo conspicuous changes which are related to the inherent rate of cell division and/or differentiation (Biasi et al., 1991; Kushad, 1998; Ziosi et al., 2003a,b).

Based on the fact that, possibly due to competition for their common precursor *S*-adenosylmethionine, PAs and ethylene exert opposite biological functions, their reciprocal relationships during ripening have been investigated in several species using either detached fruit, fruit explants or field experiments, as reviewed in Valero et al. (2002). In most cases, results show an inverse relationship between the levels of PAs and ethylene. *In vitro* models proved to be useful for several climacteric and non-climacteric fruit (tomato, strawberry, orange, pear, kiwifruit, apple and plum); in particular, peach fruit explants were successfully used in studies on IAA and ethylene metabolism (Tonutti et al., 1991; Ohmiya, 2000). The aim of the present work was to establish whether an *in vitro* peach fruit model system was suitable to investigate the biochemical consequences of PA action on ethylene production and fruit quality and to evaluate whether results were comparable with those from intact fruit. Whole detached fruit and fruit explants from "Redhaven" peaches and "Stark Red Gold" nectarines, at two different ripening stages (commercial harvest and tree-ripe), were subjected to treatment with 10 mM putrescine or spermidine. Intact fruit were analysed for PA uptake, ethylene production and quality parameters, while fruit explants were analysed for ethylene biosynthesis, PA uptake and metabolism, and sucrose accumulation. In all cases, the well-known inhibitor of aminocyclopropane-1-carboxylate synthase (ACS) activity, aminoethoxyvinylglycine (AVG; Huai et al., 2001) was used as a positive control, *viz*. to check that the explant model system, despite its being artificial, functions properly at least in terms of a well-established phenomenon such as AVG inhibition of ethylene production.

2. Materials and methods

2.1. Plant material and experimental design

Peach fruit (*Prunus persica* L. Batsch) were harvested from orchards grown at the experimental farm of the Faculty of Agriculture, University of Bologna, Italy. "Stark Red Gold" (SRG) nectarines were collected from 6-year-old trees, grafted on seedling rootstock and trained to a Y-shape. "Redhaven" (RH) peaches were harvested from 3-year-old trees grafted on seedlings and trained to a free open-vase. Fruit at two ripening (firmness) stages were selected for position and size uniformity. Peaches and nectarines were harvested at 36 and 42 N mean firmness, respectively ("commercial" harvest), and at 14 and 16 N, respectively ("tree-ripe"). Experiments were performed utilising either whole detached fruit or fruit explants: in the former, [^{14}C]-labelled putrescine uptake was measured, and ripening parameters (ethylene production, flesh firmness and soluble solids content) were determined after incubation with 10 mM putrescine (Pu), 1 mM spermidine (Sd) or ReTain® (ABG 3178, Valent Biosciences Corporation, Libertyville, IL, USA), a commercial product containing 15% (w/w) AVG a.i., at a dose corresponding to 0.32 mM (62.5 ppm) AVG. Such concentrations were chosen on the basis of previous work which showed that they were able to modulate fruit ripening *in planta* (Bregoli et al., 2002; Torrigiani et al., 2004). Fruit explants were used for the evaluation of ethylene biosynthesis and of PA and sucrose levels.

2.2. Labelled putrescine uptake experiments

Peach or nectarine fruit, harvested at the tree-ripe stage, were submerged in beakers containing 250 ml 10 mM Tris–HCl buffer, pH 7, and 37 kBq [1,4-^{14}C]-putrescine (4.03 TBq mol^{-1}, Amersham Pharmacia Biotech Italia, Milan, Italy) for 60 s or 0.5, 1, 2, and 4 h at room temperature. Following treatment, fruit were left to dry for 2 h; a cylinder of pericarp was obtained using a cork-borer (10-mm diameter) and then cut into three portions: epicarp, outer (OM) and inner (IM) mesocarp (IM). Tissues were separately weighed, put in scintillation cocktail (Ultima Gold, Beckman Analytical, Milan, Italy), and counted for radioactivity in a beta counter (Beckman LS 6500).

2.3. Ethylene production and quality parameter determination

Peaches or nectarines (10 for each treatment and time of incubation) were submerged for 60 s in 50 mM Tris–HCl buffer, pH 7 (Sigma–Aldrich, Milan, Italy) containing or not (controls) 10 mM Pu, 1 mM Sd or 0.32 mM AVG. Fruit were then left to dry at room temperature for 2 h. Ethylene production was measured by placing fruit in a 1.7-l jar sealed with an air-tight lid equipped with a rubber stopper, and left at room temperature for 1 h after which a 10-ml gas sample was taken and injected into a Dani HT 86.01 (Dani, Milan, Italy) packed-gas chromatograph as described previously (Bregoli et al., 2002). Flesh firmness (FF) was measured using a pressure tester, fitted with an 8-mm plunger (EFFE.GI, Ravenna, Italy), and soluble solids concentration (SSC) was measured with an Atago digital refractometer (Optolab, Modena, Italy), as described in Bregoli et al. (2002). Sucrose concentration was determined in fruit explants as described in Miller (1959) with minor modifications.

2.4. RNA extraction and northern analysis

Total RNA was extracted from the mesocarp of PA- and AVG-treated tree-ripe nectarines using the method described by Bonghi et al. (1998). RNA (18 μg/track) was size-fractionated and blotted onto nylon membranes (Hybond-N, Amersham Pharmacia Biotech Italia) according to standard methods (Sambrook et al., 1989) and hybridised with the homologous ACO1 probe.