Journal of Plant Physiology 167 (2010) 242–245

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.de/jplph

SHORT COMMUNICATION

Metabolic acclimation to hypoxia revealed by metabolite gradients in melon fruit

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ARTICLE INFO

Article history:
Received 6 May 2009
Received in revised form
14 August 2009
Accepted 17 August 2009

Keywords:
Cucumis melo
Hypoxia
Melon fruit
Metabolite gradients
Metabolic profiling

ABSTRACT

A metabolomics approach using ^{1}H NMR and GC–MS profiling of primary metabolites and quantification of adenine nucleotides with luciferin bioluminescence was employed to investigate the spatial changes of metabolism in melon fruit. Direct ^{1}H NMR profiling of juice collected from different locations in the fruit flesh revealed several gradients of metabolites, e.g. sucrose, alanine, valine, GABA or ethanol, with increase in concentrations from the periphery to the center of the fruit. GC–MS profiling of ground samples revealed gradients for metabolites not detected using ^{1}H NMR, including pyruvic and fumaric acids. The quantification of adenine nucleotides highlighted a strong decrease in both ATP and ADP ratios and the adenylate energy charge from the periphery to the center of the fruit. These concentration patterns are consistent with an increase in ethanol fermentation due to oxygen limitation and were confirmed by observed changes in alanine and GABA concentrations, as well as other markers of hypoxia in plants. Ethanol content in melon fruit can affect organoleptic properties and consumer acceptance. Understanding how and when fermentation occurred can help to manage the culture and limit ethanol production.

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Introduction

Melon (*Cucumis melo*), one of the oldest cultivated crops, is widely cultivated across the world. According to the FAO, world production of melons in 2007 was about 26 million tons (http://www.fao.org). The numerous species differ greatly in fruit size (from a few grams to several kilograms), morphology (round to elongated shape) and organoleptic properties (bitter to sweet) (Stepansky et al., 1999). The French melon sp. Charentais is extensively consumed in Europe during the summer, as its orange flesh is freshening and sweet with a pleasant aroma. Melon fruit is also a healthy human food, providing nutrients and antioxidants such as β-carotene and vitamins (C, E and folic acid) (de Melo et al., 2000).

The most important properties of melon for fruit organoleptic quality and consumer's acceptance are the aroma profile (Kourkoutas et al., 2006) and the sucrose level (Stepansky et al., 1999b). Several aspects of sugar metabolism have therefore been studied, including the transport of stachyose and raffinose within the phloem and their translocation into the fruit (Lalonde et al., 2003), and sucrose accumulation during fruit development and ripening (Burger and Schaffer, 2007).

Few data relating to the spatial changes of metabolite concentrations in melon fruit flesh are available. However, near infra-red imaging has been used to analyze the sugar localization in melon slices (Sugiyama, 1999). Recently, a metabolomic approach using ^{1}H NMR and GC–EI–TOF–MS profiling was used to assess both the concentration and spatial localization of the main primary metabolites in various cultivars of melon fruit (Biais et al., 2009). Part of the latter study suggested the existence of several metabolite gradients within the fruit. These may be related to *in situ* hypoxia in the central part of the ripening fruit; similar results were obtained on pear fruit under low oxygen stress (Pedreschi et al., 2009). To verify this hypothesis, in parallel with quantification of primary metabolites, adenine nucleotides were quantified in melon fruit, allowing the comparison of the metabolite composition with the energy status of the tissue.

Abbreviations: ^{1}H NMR, proton nuclear magnetic resonance; AEC, adenylate energy charge; GABA, gamma amino butyric acid; GC–MS, gas chromatography–mass spectrometry; PDC, pyruvate decarboxylase; TCA, tri carboxylic acids

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doi:10.1016/j.jplph.2009.08.010

Materials and methods

Plant material

The seeds of F1 hybrid melon (*Cucumis melo* cv. Cézanne) were obtained from Clause-Tézier (France). Plants were grown in an open field in south-western France (Moissac, 44°06′17″N × 1°04′41″E) between April and July, 2007. The soil type was clay and limestone and the plant density was 9200 plants/ha. Irrigation, watering, fertilization and pathogen-pest control were performed according to standard commercial practices. Fruits were harvested at 3/4 slip maturity. Analyses were performed within 4 h after harvest.

Sampling and analyses of melon flesh pieces

One representative melon fruit was cut in half lengthwise and three slices were cut; two for metabolite analyses (^{1}H NMR and GC–MS) and the other for adenine nucleotide analyses (thickness: 1 cm). The slices were divided into five small sections (7 mm × 7 mm), cut from the skin position1, epicarp+green mesocarp) to the center of the fruit (position 5, inner orange mesocarp) according to Fig. 1, in two different areas on the equatorial plane of the slice. The refractive index (Brix %) of fresh juice was measured immediately after sampling and increased regularly from 6.3% ± 0.4 (position 1) to 15.0% ± 0.7 (position 5) (means of 2 replicates measured twice).

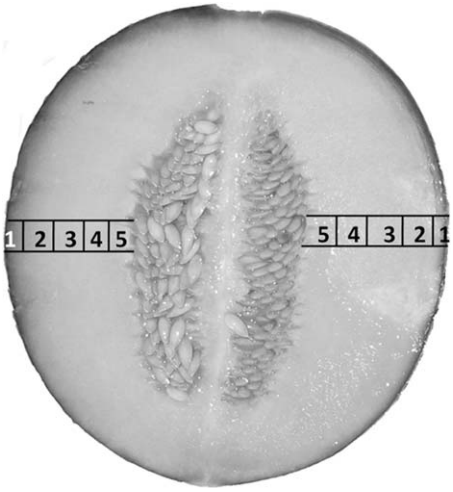

Fig. 1. Representation of the location of collected flesh pieces for ^{1}H NMR and GC–MS profiling and adenine nucleotide quantification in melon slices. Position 1 corresponds to the epicarp+green mesocarp, and positions 2–5 to the orange mesocarp.

Primary metabolites and adenine nucleotide quantification

The absolute and relative quantifications of primary metabolites in melon fruit were performed with ^{1}H NMR and GC–MS, respectively, as described by Biais et al. (2009). For ^{1}H NMR, fresh flesh pieces were squeezed and the juice was immediately flash frozen in liquid nitrogen. To stop enzymatic activities, 70% methanol-d_4 (v/v) was added prior to ^{1}H NMR acquisitions. For GC–MS, frozen ground samples were extracted with chloroform/methanol/water (1:2.5:1), derivatized, and quantification was performed using an internal standard.

Adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP) were measured in neutralized perchloric acid extracts of frozen ground samples using a luciferin–luciferase method (Napolitano and Shain, 2005) and a luminescence ATP detection kit (PerkinElmer^{TM}, Zaventem, Belgium). The extraction was performed twice for each position, to be used as technological replicates. After an ATP calibration curve, the results were expressed as pmol mg_{FW}^{-1} and calculated as the means of the values measured with four aliquot volumes of each extract, except if the saturation of the detector was observed. The adenylate energy charge (AEC) was calculated as follows: AEC=([ATP]+0.5[ADP])/([ATP]+[ADP]+[AMP]).

Data obtained for metabolites, nucleotides, the ATP/ADP ratio and AEC were analyzed using one-way analysis of variance (ANOVA) and Tukey's grouping using SAS Software v8.01 (SAS Institute, 1990).

Results and discussion

Metabolite gradients in melon fruit

The ^{1}H NMR and GC–MS profiling allowed absolute and relative quantification, respectively, of the main metabolites in melon fruit, including sugars, organic acids and amino acids. Fig. 2 presents the ^{1}H NMR concentration changes of six major sugars

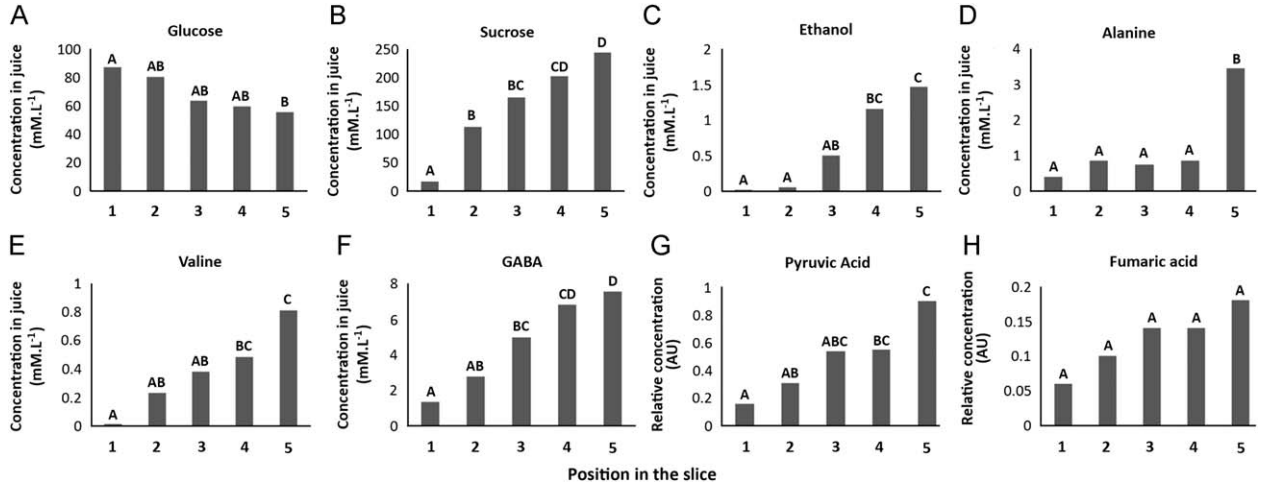

Fig. 2. Absolute concentration of some primary metabolites, determined using ^{1}H NMR profiling, depending on the location in the slice of melon fruit (Cézanne cv.). Concentrations are given in mM L^{-1} of juice. (A) Glucose, (B) sucrose, (C) ethanol, (D) alanine, (E) valine and (F) GABA. Relative concentration of organic acids determined using GC–MS profiling, depending on the location in the slice of melon fruit. (G) Pyruvic acid and (H) fumaric acid. The results are the means of 4 measurements (2 samples × 2 technical replicates). Letters above histograms correspond to Tukey's groups. The values with the same letter are not statistically different ($P < 0.05$).

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