Food Chemistry 269 (2018) 559–566

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

NMR-based global metabolomics approach to decipher the metabolic effects of three plant growth regulators on strawberry maturation

Li An[a,b], Jingwei Ma[a,b], Hong Wang[a,b], Fugen Li[c], Dongmei Qin[c], Jiakai Wu[a,b], Guangyan Zhu[c], Junfeng Zhang[a,b], Yongliang Yuan[d], Ling Zhou[a,b,*], Xujin Wu[a,b,*]

[a] Institute of Quality Standard and Testing Technology for Agro-products, Henan Academy of Agricultural Sciences, Zhengzhou 450002, China
[b] Key Laboratory of Grain Quality and Safety and Testing Henan Province, Zhengzhou 450002, China
[c] Institute for Control of Agrochemicals, Ministry of Agriculture, Beijing 100125, China
[d] Department of Pharmacy, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China

ARTICLE INFO

Keywords:
NMR
Metabolomics
Plant growth regulators
Strawberry
Maturation

ABSTRACT

Plant growth regulators (PGRs) are commonly used to regulate maturation in strawberry. Despite this, comprehensive assessments of the metabolomic effects of PGRs on strawberry maturation are lacking. In this study, a nuclear magnetic resonance-based approach, combined with multivariate and pathway analysis, was used to evaluate the regulatory effects of gibberellin, forchlorfenuron, and brassinolide, applied at two different maturation stages, on the expression of metabolites in strawberry. The results demonstrated that the PGRs differentially influenced metabolism, whether applied at the same or different maturation stages. Additionally, we also discovered that these different PGRs exhibited some similar metabolic trends when applied at the same growth period. Our findings validate the use of NMR-based metabolomics for identifying subtle changes in the expression of metabolites associated with PGR application.

1. Introduction

Strawberry is valued for both its pleasant flavour and chemical properties, including sugars, organic acids, amino acids, and some other primary metabolites (Montero, Mollá, Esteban, & López-Andréu, 1996). These metabolites are important in strawberry development and maturation (Moing et al., 2001; Pérez, Rios, Sanz, & Olías, 1992). Strawberry fruit quality is also closely associated with the above metabolites, as these are the primary contributors to its organoleptic properties, with sugars affecting the sweetness (Kallio, Hakala, Pelkkikangas, & Lapveteläinen, 2000; Skrede, 1983; Wozniak, Radajewska, Reszelska-Sieciechowicz, & Dejwor, 1997), and amino acids and organic acids influencing the umami taste or sourness (Hakala, Tahvonen, Huopalahti, & Lapveteläinen, 2000; Pérez, Olías, Luaces, & Sanz, 2002). Plant growth regulators (PGRs), such as gibberellin, forchlorfenuron, and brassinolide, play an essential role in the regulation of climacteric fruit ripening, and have been used to regulate maturation in strawberry cultivation (Given, Venis, & Gierson, 1988; Nehra, Kartha, Stushnott, & Giles, 1992). The existing research has primarily focussed on evaluating the effects of various PGRs on the yield and physicochemical characteristics of strawberry (Saima, Sharma, Umar, & Wali, 2014; Thakur, Mehta, & Sekhar, 2015). However, a global assessment of the influence of PGRs on metabolism in strawberry is lacking.

Metabolomics, a powerful systems biology tool, has been widely applied to the identification of differential changes in small molecules that arise from subtle changes in response to external or internal environmental stimuli (Nicholson, Lindon, & Holmes, 1999). Over the past decade, metabolomics has also presented itself as a promising technique in plant and natural product research (Mais et al., 2018; Warth et al., 2015). The primary goal of metabolomics, as used in the current plant metabolomics framework, is to provide a holistic view of all the metabolites present in a system (Chen et al., 2013; Tikunov et al., 2005) and to evaluate their overall effect and potential mechanisms of action under certain conditions (Bowne et al., 2012; Moradi, Ford-Lloyd, & Pritchard, 2017). Metabolomics is increasingly being applied to the field of agriculture and food, and has emerged as a valuable technology for profiling crop varieties (Huo et al., 2017; Klockmann, Reiner, Cain, & Fischer, 2017), assessing the accumulation of metabolites during plant growth and fruit maturation (Yuan et al., 2017), evaluating the natural variance in metabolite content between different plants (Souard, et al., 2018), improving the compositional quality of crops (Bernillon et al., 2013) and characterizing the metabolic response

* Corresponding authors at: Institute of Quality Standard and Testing Technology for Agro-products, Henan Academy of Agricultural Sciences, Zhengzhou 450002, China.
E-mail addresses: zlnk252@aliyun.com (L. Zhou), xujinwu2005@126.com (X. Wu).

https://doi.org/10.1016/j.foodchem.2018.07.061
Received 29 January 2018; Received in revised form 15 June 2018; Accepted 9 July 2018
Available online 10 July 2018

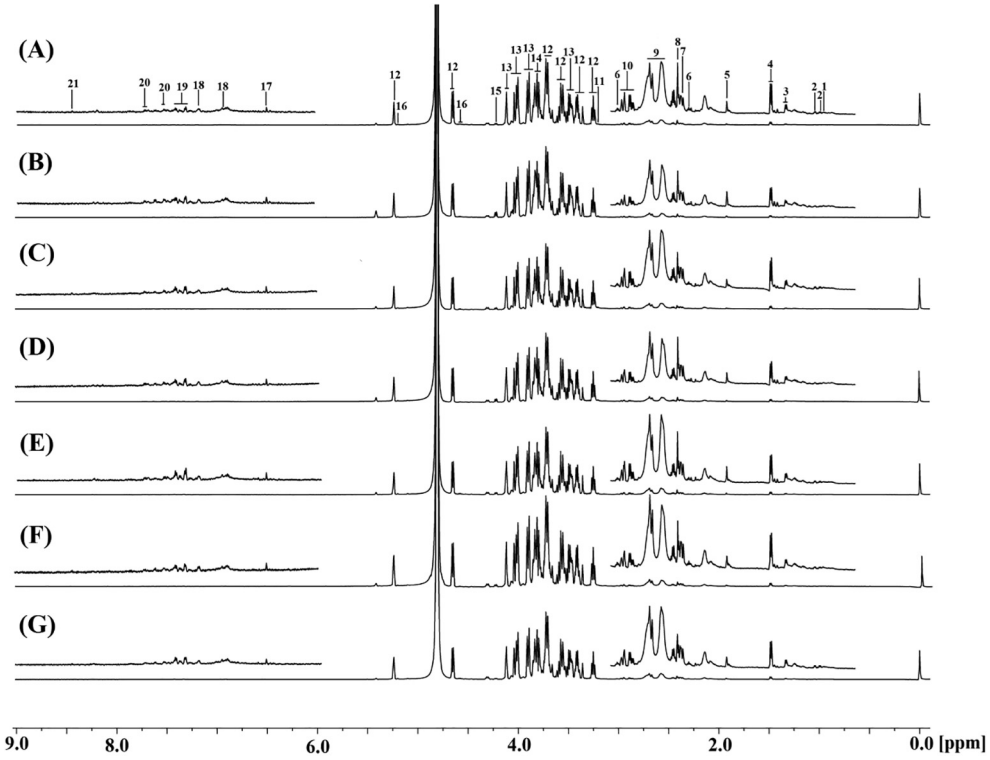

Fig. 1. ^{1}H NMR (600 MHz) spectra of strawberry associated with different PGRs. From top to bottom: (A) CG, (B) GG1, (C) GG2, (D) FG1, (E) FG2, (F) BG1, (G) BG2. Key metabolites: 1, leucine; 2, valine; 3, lactic acid; 4, alanine; 5, acetic acid; 6, gamma-aminobutanoic acid; 7, malic acid; 8, succinate; 9, citric acid; 10, asparagine; 11, choline; 12, D-glucose; 13, D-fructose; 14, overlap (primarily D-glucose, D-fructose and sucrose); 15, sucrose; 16, D-xylose; 17, fumaric acid; 18, tyrosine; 19, phenylalanine; 20, tryptophan; 21, formate.

to various biotic or abiotic stresses and exploring the associated mechanisms (Arrivault et al., 2009). Previous metabolomic approaches in strawberry have aimed to differentiate between organic and conventional non-organic strawberries (D'Urso, d'Aquino, Pizza, & Montoro, 2015), discriminate between 15 strawberry cultivars grown in Finland or Estonia (Kårlund et al., 2016); and distinguish three varieties of strawberry cultivars and characterize the metabolomic changes associated with different environmental and agronomic conditions (Akhatou, González-Domínguez, & Fernández-Recamales, 2016). Nuclear magnetic resonance (NMR)-based metabolomics has been widely applied in food and agriculture research, and constitutes a highly sensitive, specialized, and powerful tool, possessing the unique advantages of rapid, non–destructive, and relatively simple sample preparation (Forino, Tartaglione, Dell'Aversano, & Ciminiello, 2016). In addition, chemical structure information of metabolites also can be directly obtained from NMR spectroscopy, which renders NMR an ideal tool for identifying differential metabolites and further evaluating their potential mechanisms.

In this study, an ^{1}H NMR-based metabolomics approach was used to evaluate the global metabolic influences of three different PGRs, including gibberellin, forchlorfenuron, and brassinolide, on strawberry maturation. The PGRs were applied at two maturation stages in order to explore whether the PGRs elicit different metabolomic responses at these stages. The differential metabolites associated with the different PGRs at each maturation phase were also evaluated in order to gain insight into the underlying regulatory mechanisms of the three PGRs on strawberry maturation.

2. Materials and methods

2.1. Plant materials

Strawberry (*Fragaria* × *ananassa* Duch. cv. Fengxiang) plants were grown in a field plot inside a greenhouse in Jiyuan city, Henan Province. The temperature inside the greenhouse ranged from 15 to 23 °C, and the relative humidity was 60–70%. The BBCH-scale was used to identify the phenological development stages (Meier, 2001), with principal growth stage 8 being associated with fruit maturation. During this growth stage, strawberry maturation is associated with three phases, namely BBCH-81, in which most of the fruits are white in colour, BBCH-85, when the fruits develop their cultivar-specific colour and BBCH-87, when the fruits have developed their colour and are ripe. The field plot was divided into four equal experimental treatment groups: untreated control group (CG), gibberellin group (GG), forchlorfenuron group (FG) and brassinolide group (BG). An aqueous solution of each PGR, namely gibberellin (30 ppm), forchlorfenuron (50 ppm), and brassinolide (100 ppm), was lightly sprayed onto the plants in their respective field plots. These solutions were applied at the BBCH-85 or BBCH-81 growth stages, and were respectively labelled GG1, FG1, and BG1, and GG2, FG2, and BG2. Each of these constituted a separate treatment group. After spraying the PGRs just once, the strawberries in each group were harvested when they reached BBCH-87, following which they were immediately cleaned, freeze-dried, powdered, and stored at −80 °C until extraction.

2.2. Chemicals

Disodium phosphate (Na_2HPO_4) and monosodium phosphate (NaH_2PO_4) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Deuterium oxide (D_2O, 99.9% D, containing 0.05 wt % 3-(trimethylsilyl) propionic-2,2,3, 3-d 4 acid sodium salt [TSP], an internal standard) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Phosphate buffer (Na_2HPO_4/NaH_2PO_4, 0.2 M, pH 7.0), prepared in D_2O, was used for the NMR sample preparation. Phosphate buffer was used to minimise the NMR shift variation; TSP acted as a chemical shift reference (CH_3, δ 0.0) and D_2O was used for a lock signal.

2.3. Sample preparation and NMR analysis

Forty milligrammes of powdered strawberry sample were placed in a 2 ml Eppendorf tube and extracted with 800 µl of phosphate buffer. The solution was vortexed for 2 min at room temperature and centrifuged for 10 min at 10,000 rpm, after which 600 µl of the supernatant were transferred to a 5 mm NMR tube. ^{1}H NMR spectra were acquired