Metabolomics (2013) 9:57–77
DOI 10.1007/s11306-012-0429-1

ORIGINAL ARTICLE

Metabolomic and elemental profiling of melon fruit quality as affected by genotype and environment

Stéphane Bernillon · Benoît Biais · Catherine Deborde · Mickaël Maucourt · Cécile Cabasson · Yves Gibon · Thomas H. Hansen · Søren Husted · Ric C. H. de Vos · Roland Mumm · Harry Jonker · Jane L. Ward · Sonia J. Miller · John M. Baker · Joseph Burger · Ya'akov Tadmor · Michael H. Beale · Jan K. Schjoerring · Arthur A. Schaffer · Dominique Rolin · Robert D. Hall · Annick Moing

Received: 17 February 2012 / Accepted: 17 April 2012 / Published online: 7 June 2012

Abstract Melon (*Cucumis melo* L.) is a global crop in terms of economic importance and nutritional quality. The aim of this study was to explore the variability in metabolite and elemental composition of several commercial varieties of melon in various environmental conditions. Volatile and non-volatile metabolites as well as mineral elements were profiled in the flesh of mature fruit, employing a range of complementary analytical technologies. More than 1,000 metabolite signatures and 19 mineral elements were determined. Data analyses revealed variations related to factors such as variety, growing season, contrasting agricultural management practices (greenhouse vs. field with or without fruit thinning) and planting date. Two hundred and ninety-one analytes discriminated two contrasting varieties, one from the var. inodorous group and the other from the var. cantaloupensis group. Two hundred and eighty analytes discriminated a short shelf-life from a mid-shelf-life variety within the var. cantaloupensis group. Three hundred and twenty-seven analytes discriminated two seasons, and two hundred and fifty-two analytes discriminated two contrasting agricultural management practices. The affected compound families greatly depended on the factor studied. The compositional variability of identified or partially identified compounds was used to study metabolite and mineral element co-regulation using correlation networks. The results confirm that metabolome

Electronic supplementary material The online version of this article (doi:10.1007/s11306-012-0429-1) contains supplementary material, which is available to authorized users.

S. Bernillon · B. Biais · C. Deborde · Y. Gibon · A. Moing (✉)
INRA, UMR1332 Fruit Biology and Pathology, Centre INRA de Bordeaux, 33140 Villenave d'Ornon, France
e-mail: moing@bordeaux.inra.fr

S. Bernillon · C. Deborde · M. Maucourt · C. Cabasson · Y. Gibon · A. Moing
Metabolome Facility of Bordeaux Functional Genomics Center, IBVM, Centre INRA de Bordeaux, 33140 Villenave d'Ornon, France

M. Maucourt · C. Cabasson · D. Rolin
Université Bordeaux, UMR1332 Fruit Biology and Pathology, Centre INRA de Bordeaux, 33140 Villenave d'Ornon, France

T. H. Hansen · S. Husted · J. K. Schjoerring
Plant and Soil Science Laboratory, Department of Agriculture and Ecology, Faculty of Science, University of Copenhagen, Frederiksberg C, 1871 Copenhagen, Denmark

R. C. H. de Vos · R. Mumm · H. Jonker · R. D. Hall
Plant Research International, P. O. Box 16, 6700 AA Wageningen, The Netherlands

R. C. H. de Vos · H. Jonker · R. D. Hall
Netherlands Metabolomics Centre, Einsteinweg 55, 2333 CC Leiden, The Netherlands

R. C. H. de Vos · R. Mumm · H. Jonker · R. D. Hall
Centre for BioSystems Genomics, P. O. Box 98, 6700 AB Wageningen, The Netherlands

J. L. Ward · S. J. Miller · J. M. Baker · M. H. Beale
The National Centre for Plant and Microbial Metabolomics, Rothamsted Research, West Common, Harpenden, Hertfordshire AL5 2JQ, UK

J. Burger · Y. Tadmor
Newe Yaar Research Center, ARO, P. O. Box 1021, 30095 Ramat Yishay, Israel

A. A. Schaffer
Volcani Center, ARO, P. O. Box 6, 50250 Bet-Dagan, Israel

and mineral element profiling are useful diagnostic tools to characterize the quality of fruits cultivated under commercial conditions. They can also provide knowledge on fruit metabolism and the mechanisms of plant response to environmental modifications, thereby paving the way for metabolomics-guided improvement of cultural practices for better fruit quality.

Keywords *Cucumis melo* · Fruit quality · ^{1}H-NMR · MS · Metabolomics · Mineral elements

1 Introduction

Melon is one of the most important fresh fruits in terms of economic importance and consumption with a world production of about 25 Mt in 2010 (http://faostat.fao.org/). The organoleptic quality of melon fruit (Lester 2006), including sweetness, flesh color and aroma, directly depends on its composition in primary metabolites, isoprenoids and volatiles, which has largely been described using a series of targeted analyses (Aubert and Pitrat 2006; Lester 2008). The nutritional quality of melon fruit involves mineral elements, isoprenoids and other non-volatile secondary metabolites (Lester 2006). For instance, melon fruits with orange flesh are a rich source of dietary carotenes, those with white or green flesh are sources of lutein. At maturity, the biochemical composition of melon fruits as determined using classical targeted analytical methods has been shown to vary according to genotype (Burger et al. 2006; Portnoy et al. 2008; Stepansky et al. 1999), growth conditions (Demiral and Koseoglu 2005; Dufault et al. 2006; Feigin 1990; Lester 2005) and post-harvest treatment (Lester and Crosby 2002; Lester et al. 2010). The effect of several environmental factors, including light, temperature and nitrogen fertilization, on the vitamin and secondary metabolite content as determined using targeted methods has recently been reviewed for a range of fruits and vegetables (Poiroux-Gonord et al. 2010). Strikingly, this review reveals that the environmental effect on metabolism of cucurbits has been relatively poorly investigated so far.

The science of metabolomics is being increasingly used to gain insight into the biochemical composition of crop plant organs (Davies et al. 2010; Harrigan et al. 2007; Stewart et al. 2011), including melon (Allwood et al. 2009; Biais et al. 2009). Experimental cases include temporal changes in the metabolome of fruits related to developmental stage and variations over the growing season as well as between years (Carrari et al. 2006; Moco et al. 2007; Pereira et al. 2006). There has also been some focus on the influence of biotic or abiotic stress on the metabolome (Guy et al. 2008; Jahangir et al. 2008; Shulaev et al. 2008). In contrast, the effects of cultivation practices on fruit metabolomes (Johnson et al. 2003; Pereira et al. 2006) received less attention, although their study may provide diagnostic tools and relevant knowledge on the mechanisms of plant response to environmental modifications.

Two types of analytical strategies are widely used in metabolomics, the first based on mass-spectrometry (MS) coupled to liquid or gas chromatography (LC or GC) and the second based on nuclear magnetic resonance spectroscopy (NMR). The advantages, drawbacks and complementarities of these strategies have been widely described (Hall 2011; Krishnan et al. 2005; Sumner et al. 2003). In melon, a combination of MS and NMR based analyses has recently been used to study fruit physiology and metabolism using a single variety and growth condition (Biais et al. 2009; Moing et al. 2011).

The aim of the present study was to investigate the effect of several environmental conditions such as agricultural management practices, growing season and planting date on fruit metabolism for several contrasting melon varieties. The biochemical changes were determined using flow injection electrospray mass spectrometry (FIE-MS) fingerprinting of polar extracts, untargeted quantitative proton nuclear magnetic resonance (^{1}H-NMR) profiling of polar extracts, liquid chromatography coupled to QTOF mass spectrometry (LC-QTOF-MS) of semi-polar compounds, gas chromatography coupled to mass spectrometry (GC-MS) of volatiles and inductively coupled plasma mass spectrometry (ICP-MS) of mineral elements. This allowed comparing the potential of these analytical technologies for diagnosis of quality parameters and highlighted several discriminant metabolites or mineral elements for variety, year, growth conditions or season. In addition, the data of identified compounds have provided new knowledge on the relationships between metabolites mutually, and between metabolites and mineral elements.

2 Materials and methods

2.1 Plant material

Three different experiments were performed in the present study on melon (*Cucumis melo* L.) fruit from different varieties and after cultivation under contrasting growth conditions in Israel or France.

For Experiment 1, the fruits were grown and the samples were prepared in Israel. The two varieties chosen represent two distinct groups of sweet melon: "Noy Yizre'el" variety, a climacteric type, aromatic, green fleshed with a fleshy fruit texture, of the *C. melo* var. cantaloupensis group, and "Tam Dew" variety, a non-climacteric, weakly aromatic, pale fleshed variety with a more firm and crunchy flesh, of the *C. melo* var. inodorous group. These two

varieties were cultivated under two different growth conditions in two successive growing seasons. The first crop was grown in the fall-winter season in a heated greenhouse and the second crop was grown in the open field during the Spring–Summer season. The crops were also cultivated under differing agricultural management practices. The greenhouse crop (20,000 plants/ha density) was trained to a single-stem and yield was limited to a single fruit per plant. The field crop (10,000 plants/ha density) was grown untrained on the ground with no control of fruit number. Irrigation, fertilizer management (Table 1) and pathogen-pest control were performed according to standard commercial practices. Irrigation in the greenhouse was 250 ml water per plant per day, and irrigation in the field was about 250 m^3 per ha once a week corresponding to 25 l per plant per week. Three replications each consisting of a minimum of 10 ripe fruits at commercial maturity harvested in the morning of the same day were prepared for each variety in each growing season. Samples of fruit flesh from the 10 fruits were cubed and combined, immediately frozen in liquid nitrogen and ground to a fine powder while still frozen (D3V10 grinder, Hsiangtai Machinery Industry Co., Taiwan). Approximately 30 g of frozen powder was quickly placed in 50 ml screw cap tubes and stored at −80 °C until shipment to all analytical partners.

For Experiments 2 and 3, the fruits were grown and the samples were prepared in France. Seeds were obtained from Clause-Tézier (Portes-lès-Valence, FR). Plants were grown in an open field in Moissac (South-West France). The plant density was 9,200 plants/ha. Irrigation, fertilisation (Table 1) and pathogen-pest control were performed according to standard commercial practices. For Experiment 2, the varieties studied were "Cézanne" and "Escrito" of the *C. melo* var. cantaloupensis group. Cézanne is an aromatic variety with short shelf-life and Escrito is a mid shelf-life variety. The plants were grown between April and August in 2007 and 2008. For Experiment 3, the variety studied was "Hugo" of the *C. melo* var. cantaloupensis group. One plantation date was chosen according to standard commercial practices (May) and two other plantation dates were used (April and June). Therefore plants were grown during April–July, May–August or June–September in 2007. For Experiments 2 and 3, the environmental changes were monitored for each variety using basic meteorological data to calculate the duration of insolation (duration of direct irradiance from the sun of at least 120 W m^{-2}) and degree-days (accumulated product of time and temperature between the lower and upper developmental thresholds for each day) from plantation to harvest (Table 1). Fruits at commercial maturity were

Table 1 Summary of some of the environmental changes for experiment 1 of melon varieties cultivated in a greenhouse or an open field in Israel in 2006 and 2007, and for experiments 2 and 3 of melon varieties cultivated in an open field in South-West France in 2007 or 2008

Variety	Year/condition	Mineral fertilization	Insolation duration from plantation to harvest (h)	Degree-days from plantation to harvest (°C days)[a]
Noy Yize'el	2006	6 %-6 %-6 %	nd	nd
Tam Dew	Greenhouse	N-P-K, 15 l ha^{-1}		
Noy Yize'el	2007	16 % Ammonium nitrate 300 l ha^{-1}	nd	nd
Tam Dew	Field	85 % phosphate acid 60 l ha^{-1}		
Cézanne	2007	143-118-102	508	963
	April–July	N-P-K, kg ha^{-1}		
Cézanne	2008	139-158-108	616	941
	April–July	N-P-K, kg ha^{-1}		
Escrito	2007	110-117-68	554	1,031
	May–August	N-P-K, kg ha^{-1}		
Escrito	2008	139-158-108	642	1,120
	May–August	N-P-K, kg ha^{-1}		
Hugo	2007	143-164-102	587	1,119
	April–July	N-P-K, kg ha^{-1}		
Hugo	2007	110-117-68	554	1,031
	May–August	N-P-K, kg ha^{-1}		
Hugo	2007	110-117-68	579	996
	June–September	N-P-K, kg ha^{-1}		

nd not determined

[a] Degree-days was calculated with a lower threshold of 6 °C and an upper threshold of 30 °C

harvested in the morning and transported within two hours of harvest to the laboratory in insulated boxes and processed immediately. For each harvest, 36 melons were selected in order to make six homogeneous biological replicates of six fruits each. Samples of fruit flesh from the six fruits were cubed, combined and immediately deep frozen in liquid nitrogen and stored at −80 °C. Frozen flesh cubes were kept for the elemental analyses and the rest of the samples were ground in liquid nitrogen (knife grinder UMC5, Stephan™, Lognes, France). Aliquots of ~20 g of frozen powder were quickly placed in 50 ml screw cap tubes and stored at −80 °C until shipment.

For all experiments, the fresh-frozen powdered samples or cubes were stored at −80 °C and transported using dry-ice to the different analytical laboratories. Fresh-frozen samples were used for LC-QTOF-S and GC-MS analyses and lyophilised samples for FIE-MS, ^{1}H-NMR and ICP-MS analyses.

2.2 Flow injection electrospray mass spectrometry (FIE-MS) fingerprinting

Replicate aliquots (3 × 15 mg) of freeze-dried powder were prepared using the protocol described previously for combined NMR-FIE-MS (Ward et al. 2010). Samples were extracted with an 80:20 D_2O:CD_3OD mixture (1 ml) and 50 µl of the extraction solution was transferred to a glass autosampler vial and diluted with a mixture of H_2O:CH_3OH (80:20; 950 µl) prior to injection into the mass spectrometer to replace all exchangeable deuterons with protons. One hundred µl of each sample was infused into the spectrometer (Esquire 3000, Bruker Daltonics, Coventry, UK) by flow injection using an Agilent 1100 series HPLC system with degasser, quartenary pump and autosampler. The flow rate was 100 µl min^{-1} of 20 % methanol in water. Mass spectra were recorded from 1.7 to 4.2 min after the sample had entered the flow. Spectra were recorded in both positive and negative ion mode on the same sample via an alternating sequence of the two ionisation modes, each time recording the average of 25 scans. The spectra were recorded over an *m/z* range of 50–1,000 using the "smart tuning" function (Bruker Daltonics, Coventry, UK) with a target mass of *m/z* 300, a trap-drive and stability of 100 % and a scan speed of 13,000 (*m/z*) s^{-1}. The spectra were recorded using ion charge control with a maximum accumulation time of 40 ms for 20,000 (negative mode) or 50,000 (positive mode) ions. The nebuliser pressure was 20 psi and the dry gas 6 l min^{-1} at 350 °C. A series of "tracker" samples were also injected into the mass spectrometer throughout the experiment to serve as QA samples. Combination of all the recorded spectra was carried out to give a single positive-mode and negative mode spectrum and data were exported as ASCII files containing mass-intensity pairs. The ASCII files from all samples were automatically reduced, using AMIX software version 3.9.11 (Bruker Biospin, Coventry, UK), to a single CSV file for each ionization mode, containing integrated regions, named 'buckets', of equal width (*m/z* width = 1). Individual bucket intensities were scaled to the total intensity. These scaled variables will be referred to as "nominal *m/z* buckets".

2.3 ^{1}H-NMR analysis of polar compounds

For NMR analysis, polar metabolites were extracted from freeze-dried powder (50 mg) using a hot ethanol/water series and quantified by ^{1}H-NMR as previously described (Biais et al. 2009). For the preparation of extracts and NMR acquisition parameters, special care was taken to allow absolute quantification of individual metabolites through addition of ethylene diamine tetraacetic acid (EDTA) sodium salt solution to improve the resolution and quantification of organic acids such as malic and citric acids, and adequate choice of the NMR acquisition parameters. Quantitative ^{1}H-NMR spectra were recorded at 500.162 MHz and 300 K on a Bruker Avance spectrometer (Wissembourg, France) with a 5-mm inverse probe using a 90° pulse angle with a 2.73 s acquisition time and a 25 s recycle delay, and an electronic reference for quantification. Two technical replicates were extracted and analyzed for each biological replicate. Unknown metabolites, named using the mid value of the chemical shift and the multiplicity of the corresponding resonance group (e.g. unknown D7.87 for a doublet at 7.87 ppm), were quantified in arbitrary units. The ^{1}H-NMR spectra of all varieties and growth conditions were converted into JCAMP-DX format and have been deposited, with associated metadata, into the Metabolomics Repository of Bordeaux MeRy-B (Ferry-Dumazet et al. 2011) (http://www.cbib.u-bordeaux2.fr/MERYB/view/project/M06003 for Experiment 1, http://www.cbib.u-bordeaux2.fr/MERYB/view/project/M06002 for Experiments 2 and 3). The assignments of metabolites in the NMR spectra were made by comparing the proton chemical shifts with literature (Biais et al. 2009; Fan 1996; Mounet et al. 2007) or database values (MeRy-B 2010, http://bit.ly/meryb; HMDB, http://www.hmdb.ca/), by comparison with spectra of authentic compounds recorded in the same solvent conditions (own local library) and by spiking the samples. For assignment verification, ^{1}H-^{1}H COSY NMR experiments were acquired for selected samples. This resulted in a total of 30 identified compounds and 12 unknown compounds annotated according to MSI recommendations (Sumner et al. 2007) and listed in Online Resource 1.

2.4 LC-QTOF-MS profiling of semi-polar compounds

LC-QTOF-MS profiling of aqueous-methanol extracts from fresh frozen powder (500 mg per 2 ml) was performed as

previously described (De Vos et al. 2007). In short, an Alliance 2975 HT HPLC (Waters Corporation, Milford, USA) was used to separate metabolites on a reversed phase C18 column (150 × 2.1 mm, 3 µm; Phenomenex, Torrance, USA) using a 45 min linear gradient from 5 to 35 % acetonitrile, acidified with 0.1 % formic acid. Metabolites were detected using both a photodiode array detector (Waters 2996 PDA, Waters Corporation, Milford, USA) coupled to a high resolution quadrupole time-of-flight (QTOF) Ultima MS (Waters Corporation, Milford, USA). Electrospray ionization in negative mode was used to ionize the compounds. Scan rate for ions at *m/z* range 100–1,500 was fixed at 1 scan per second. Raw data were subsequently processed in an untargeted manner using Metalign software (Lommen 2009). From the total of 1,794 mass signals with signal/noise >3, the data set was filtered for being present in at least three samples and with a minimum signal to noise ratio of five in at least one of these samples. This left 1,205 mass signals. The noise values were subsequently randomised between 80 and 100 % of the noise. The 1,205 peaks were then clustered according to their correlations over samples and retention time, resulting in 107 clusters (called centrotypes, i.e. reconstructed metabolites) using MSClust software (Tikunov et al. 2005, 2012) of which some were annotated according to reference compounds or published melon data using corresponding accurate mass (Moing et al. 2011). Some known single mass signals were similarly annotated and added (these single masses are so low that their natural ^{13}C isotope was below detection limit, i.e. no other mass detected to enable clustering into a centrotype), resulting in a total of 94 unidentified and 21 identified or putatively identified compounds annotated according to MSI recommendations (Sumner et al. 2007) and listed in Online Resource 2.

2.5 GC-MS analysis of volatile compounds

Headspace volatiles were collected by solid phase micro extraction (SPME) of fresh frozen powder (200 mg) using a 65-mm polydimethylsiloxane–divinylbenzene fiber (Supelco, Bellefonte, USA) and analyzed as described previously (Verhoeven et al. 2012). In short, the melon samples were mixed in 4 ml of 4.6 M $CaCl_2$ with 5 mM EDTA (to stop enzymatic reactions) in a 10 ml headspace vial closed with a silicon/Teflon septum. Volatile compounds were released from the solvent into its headspace by pre-heating the vial at 50 °C for 10 min with agitation, using a CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland). The volatiles released were subsequently trapped by inserting the SPME fiber into the headspace for 20 min at 50 °C, again with agitation. Volatiles trapped on the fiber were thermally desorbed at 250 °C by inserting the fiber for 1 min into the GC injection port (GC 8000, Fisons Instruments, Milan, Italy). The released compounds were transferred on the analytical column (HP-5, 30 m × 0.25 mm ID, 1.05 µm-film thickness, Hewlett Packard, USA) in splitless mode. The temperature program started at 45 °C (2-min hold) and rose 5 °C min^{-1} to 250 °C (5-min hold). The column effluent was ionised by electron impact (EI) ionisation at 70 eV (MD800 electron impact MS, Fisons Instruments, Milan, Italy). Mass scanning was done from 35 to 400 *m/z* with a scan time of 2.8 scans s^{-1}. An untargeted metabolomics approach was applied to process the raw GC-MS data (Tikunov et al. 2005, 2012). Metalign software (Lommen 2009) was used to extract and align all mass signals (s/n ≥ 3). Absent values were randomized between 0.1 and 3 times the noise. Mass signals that were present in ≤6 samples were discarded, signal redundancy per metabolite was removed by means of clustering and mass spectra were reconstructed using MsClust software (Tikunov et al. 2005, 2012). Metabolites were putatively identified by matching the mass spectra of obtained metabolites to authentic reference standards and the NIST08 (National Institute of Standards and Technology, Gaithersburg, MD, USA), Wiley, and Wageningen Natural compounds spectral libraries and by comparison with retention indices of the literature calculated using a series of alkanes and fitted with a third order polynomial function (Strehmel et al. 2008). A total of 101 volatile compounds putatively identified and annotated according to MSI recommendations (Sumner et al. 2007) are listed in Online Resource 3.

2.6 Multi-elemental profiling by ICP-MS

For Experiment 1, frozen milled melon samples were freeze-dried followed by digestion of 200–250 mg material in 100 ml closed vessels using a microwave oven (Multiwave 3000 software version 1.24, Anton Paar, Graz, Austria) for 50 min at 210 °C with a maximum pressure of 40 bar. The digestion medium consisted of 5 ml 65 % HNO_3 and 5 ml 15 % H_2O_2 (Hansen et al. 2009). After digestion, the samples were diluted to a final concentration of 3.5 % HNO_3. For Experiments 2 and 3, fresh-frozen flesh cubes were freeze-dried and 200–250 mg digested as indicated above. In connection with Experiments 2 and 3, a pre-experiment was performed in which samples milled with the Stephan knife grinder were compared with chopped samples for the same fruit lots, the latter cut with a ceramic knife. No significant difference in element contents between the two kinds of samples were seen for 16 out of 20 elements (paired Student's t test, $P > 0.05$, data not shown). Only S, Zn, Se and Ba were significantly increased by milling but the mean increase was less than 20 % for S and Zn.

Multi-elemental analysis was performed using ICP-MS (Agilent 7500ce, Agilent Technologies, Wokingham, UK).

The ICP-MS was tuned in standard mode (no reaction/collision gas used) to achieve a sensitivity higher than 18,000, 36,000 and 18,000 cps ppb^{-1} on the masses ^{7}Li, ^{89}Y and ^{205}Tl, respectively, while at the same time ensuring that the oxide ratio (*m/z* 156/140) was below 0.5 %. The plasma power was operated at 1,500 W and the Ar carrier and make-up gases were set at 0.83 and 0.17 l min^{-1}, respectively. Sample uptake was maintained at approximately 0.6 ml min^{-1} by a micro-flow nebulizer (Agilent Technologies, Wokingham, UK). The impacts of spectral interferences on analytes were reduced using an octopole ion guide, pressurized with He or H_2 using principles described by Laursen et al. (2009). For each series of 50 melon samples, seven replicates of certified reference material (NIST 1515, apple leaves, particle size <75 µm; National Institute of Standards and Technology, Gaithersburg, MD, USA) were included. The NIST apple leaf reference material was selected because it best matched the melon samples in terms of digestability and concentrations levels of the major part of the elements included in the study. Only elements deviating less than ±10 % from the certified reference values were included in this study. For data extraction and analysis, ChemStation software version B.03.07 was used (Agilent Technologies, Wokingham, UK).

2.7 Chemicals

For NMR analysis, Methanol-d_4 (99.8 %) was purchased from Eurisotop (Gif sur Yvette, France) and TSP (98 %) from Aldrich (Saint Quentin Fallavier, France). For LC-QTOF-MS analyses, standards were purchased from Sigma (Zwijndrecht, Netherlands) and ExtraSynthese (Genay, France). For GC-MS analyses of volatiles, chemicals were as described in Verhoeven et al. (2012) and alkanes were purchased from Sigma-Aldrich (Zwijndrecht, Netherlands).

2.8 Data analysis

The data issued from each analytical technology were log-transformed to improve normality. Then for each experiment and technology the data were submitted to an analysis of variance (ANOVA), with a two factor and interaction model and *P* values estimated using 1,000 permutations, to select significant variables using MEV software v4.6.1 (Saeed et al. 2006).

For each experiment, to explore the metabolite multidimensional data set, both an unsupervised and a supervised multivariate statistical method were used on mean-centered data scaled to unit variance: principal component analysis (PCA) and partial least square discriminant analysis (PLS-DA) (Lindon et al. 2001), using SIMCA-P+ software v12.0.1.0 (Umetrics, Umeå, Sweden). PCA was performed, after the ANOVA screen ($P < 0.05$), separately for each experiment and analytical technology to visualize the clustering of the different samples without any knowledge of their group membership. PLS-DA was performed for each experiment on the combined data of all analytical technologies that passed the ANOVA screen. PLS-DA allows calculating synthetic variables, called latent variables, along which the scores are maximized to discriminate the groups of samples according to the studied discriminating factor, i.e. variety or environment factor. The predictability of the model (Q^2) was estimated by a sevenfold partial cross validation. A variable importance (VIP) score higher than 1.2 was chosen to select the most discriminant variables. To facilitate reading of the PLS-DA figures, only variables with a VIP score higher than 1.5 were annotated on the loadings plots.

Since for Experiments 1 and 2, absolute quantification data (^{1}H-NMR and ICP-MS) were acquired under exactly the same conditions, and relative quantification data (GC-MS and LC-QTOF-MS) were acquired in the same analytical batches for each analytical technique, Experiments 1 and 2 data could be combined. For identified or partially identified compounds quantified in both Experiments 1 and 2 that passed the ANOVA screen, analyte relationships were studied using correlation networks (Schauer et al. 2006; Weckwerth et al. 2004) with distances based on 1-the absolute value of Spearman correlation coefficients (r) calculated using MatLab version 7.4.0 (The MathWorks, Inc, Natick, MA, USA). Bonferroni correction was used for the r significance threshold ($P < 0.001$). These relationships were visualized for all discriminant analytes using network cartography with Cytoscape software, version 6.2 with the spring-embedding algorithm (Shannon et al. 2002, http://www.cytoscape.org/).

3 Results

3.1 Two melon types and agricultural management practices in Israel strongly and differentially modified the fruit metabolome and elemental profiles

For Experiment 1, FIE-MS allowed the relative quantification of 931 nominal *m/z* buckets in positive mode and 931 in negative mode. ^{1}H-NMR provided the absolute quantification for 39 metabolites. LC-QTOF-MS and GC-MS provided the relative quantification of 115 semi-polar analytes and 107 volatiles, respectively. ICP-MS allowed the absolute quantification of 19 mineral elements. An ANOVA was performed to identify the analytes that were affected by the variety, management practice and the interaction between these two factors. Depending on the

analytical technology, 23 to 84 % of the analytes were affected by the management practice, 14 to 58 % by the variety and 8 to 49 % by their interaction. However, overall, 28 % of the analytes were affected by the management practice, 22 % by the variety and 12 % by their interaction. In order to get an overview of sample distribution, a PCA was first performed for each analytical technology separately, using analyte data that passed the ANOVA screen. The corresponding scores plot (Fig. 1) showed that each analytical technology clearly separated the varieties and agricultural management practices. For FIE-MS (Fig. 1a, b), ^{1}H-NMR (Fig. 1c), LC-QTOF-MS (Fig. 1d) and ICP-MS (Fig. 1f), the first principal component (PC1), explaining from 37 to 58 % of total variability,

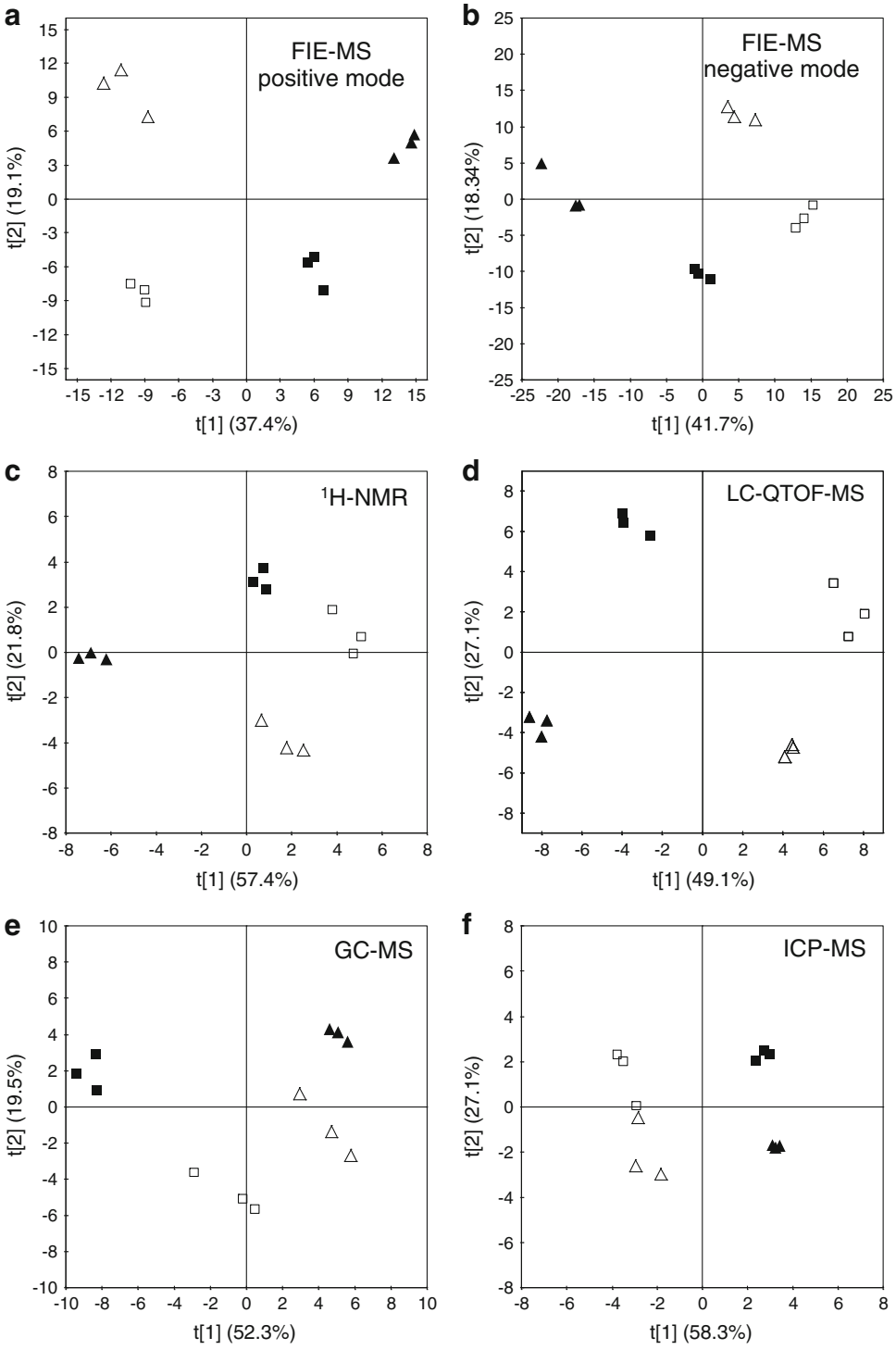

Fig. 1 Principal component analysis of absolute or relative content of analytes, selected using ANOVA, in melon flesh of mature fruit of Noy Yizre'el (*squares*) and Tam Dew (*triangles)* varieties cultivated under two different agricultural management practices in Israel, high-yield in field (*closed symbols*) and low-yield in greenhouse (*open symbols*). PCA scores plot of **a** FIE-MS in positive mode (327 variables), **b** FIE-MS in negative mode (387 variables), **c** ^{1}H-NMR (34 variables), **d** LC-QTOF-MS in negative mode (84 variables), **e** GC-MS (66 variables) of metabolite and **f** ICP-MS (17 variables) of mineral element data. All samples followed the Hotelling's T2 ($P < 0.05$) for each analytical technology (*ellipse* not shown)

separated the agricultural management practices and the second principal component (PC2), explaining from 18 to 27 % of total variability, separated the two varieties. Interestingly, for GC-MS of volatiles (Fig. 1e), PC1 explaining 52 % of total variability separated the two varieties and PC2 explaining 20 % of total variability, separated the two management practices.

In order to highlight discriminant analytes, the matrix containing the combined data of the 915 variables that passed the ANOVA screen was explored using PLS-DA separately for each factor (Fig. 2). First, a PLS-DA was performed for the effect of agricultural management practice (Fig. 2a, b). As expected, the PLS-DA scores plot separated the two management practices along the first component (Fig. 2a) with the greenhouse management practice on the positive side and the field management practice on the negative side. Examination of the first two latent variable loadings plot (Fig. 2b) and VIP values (Online Resource 4) showed that the most discriminant variables included 190 nominal *m/z* buckets determined by FIE-MS, 12 metabolites determined by ^{1}H-NMR, 37 semi-polar metabolites determined by LC-QTOF-MS, four volatiles and nine mineral elements. Among the analytes with VIP >1.2, the following could be identified on the positive

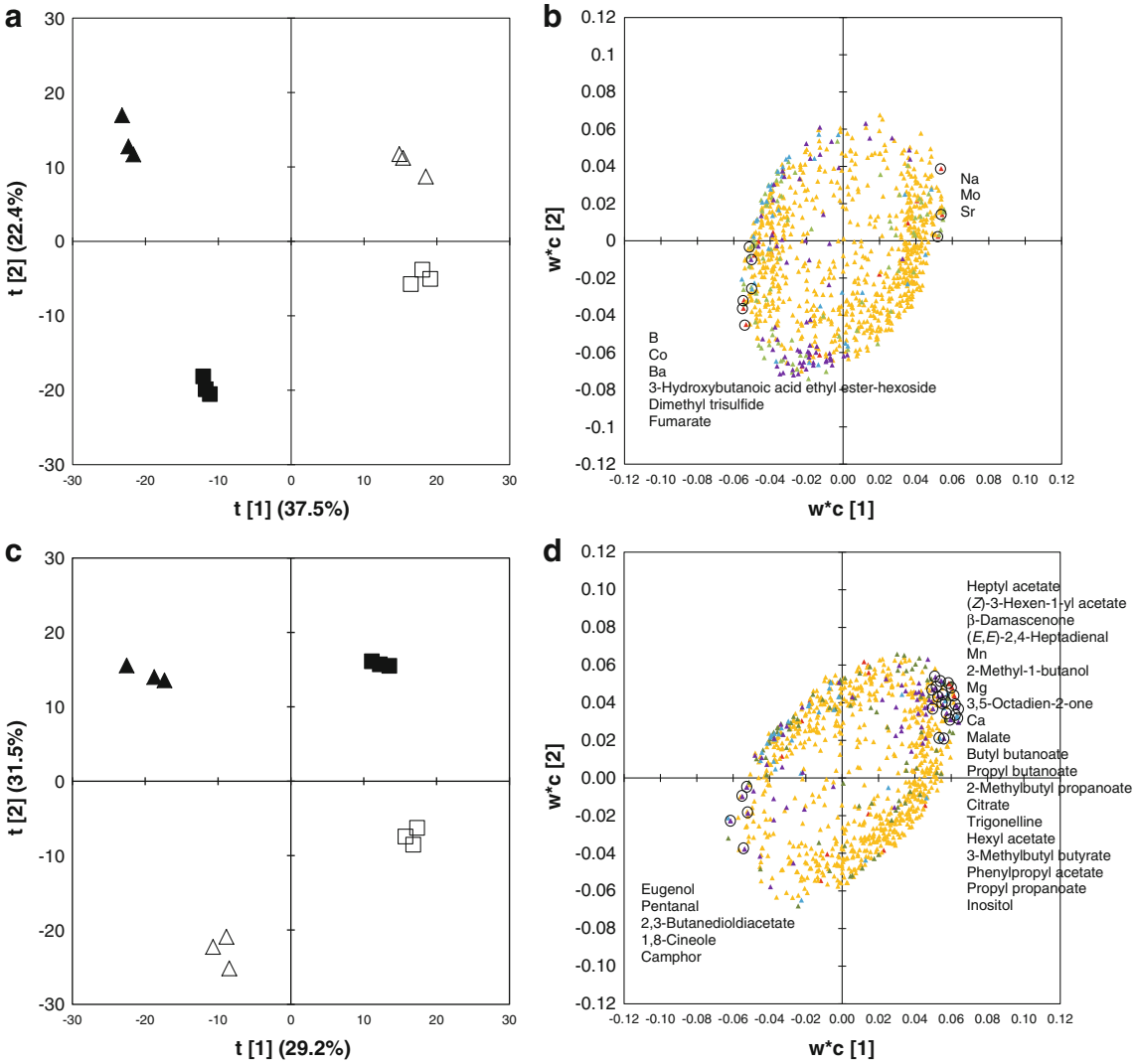

Fig. 2 PLS-DA of absolute or relative content of 915 analytes, selected using ANOVA, issued from FIE-MS, ^{1}H-NMR, LC-QTOF-MS, GC-MS and ICP-MS in melon flesh of mature fruit of Noy Yizre'el (squares) and Tam Dew (triangles) cultivated in Israel under two agricultural management practices, high-yield in field (*closed symbols*) and low-yield in greenhouse (*open symbols*). For Components 1 and 2, loadings are colored according to the analytical technology (*yellow* FIEMS, *blue* ^{1}H-NMR, *green* LC-QTOF-MS in negative mode, *purple* GC-MS, *red* ICP-MS) and Component 1 loadings with VIP scores >1.5 are *circled* and indexed with the corresponding analyte name for identified analytes. **a**, **b** Discrimination of the two management practices. **a** PLS-DA scores plot. **b** PLS-DA loadings plot. $R^2Y[1] = 0.948$, $Q^2[1] = 0.898$. **c**, **d** Discrimination of the two varieties. **c** PLS-DA scores plot. **d** PLS-DA loadings plot. $R^2Y[1] = 0.924$, $Q^2[1] = 0.870$ (Color figure online)

side of the first component, i.e. having higher levels under the greenhouse management practice: three primary metabolites (fructose, glucose, isocitrate), and four mineral elements (Cr, Mo, Na, Sr). Among the analytes with VIP >1.2, the following could be identified on the negative side of the first component, i.e. with higher levels under the field management practice: 10 primary metabolites (sucrose, UDP-glucose, fumarate, alanine, choline, GABA, glutamate, glutamine, phenylalanine, valine), three secondary non-volatile metabolites (3-hydroxybutanoic acid ethyl ester-hexoside, pantothenic acid-hexose, vanillic acid-neohesperidoside), four volatiles (dimethyl trisulfide, phenylethanol, hexyl formate, 1-pentanol), five mineral elements (Ba, B, Co, Ni, Zn). Surprisingly, among identified compounds, the highest VIP value was for B. Several of the FIE-MS ions contributing to the first component could potentially be produced from discriminant metabolites identified using ^{1}H-NMR: *m/z* 145 [glutamine−H]$^{-}$, 146 [glutamic acid−H]$^{-}$, 179 [hexose−H]$^{-}$ and 341 [sucrose–H]$^{-}$ in the negative mode, and *m/z* 104 [GABA+H]$^{+}$ or [choline]$^{+}$, 169 [glutamine+Na]$^{+}$, 204 [phenylalanine+K]$^{+}$ and 203 [hexose+Na]$^{+}$ in the positive mode. However, accurate mass measurements or MS-MS analyses would be required to confirm this.

Second, a PLS-DA was performed for variety effect (Fig. 2c, d). As expected, the PLS-DA scores plot separated the two varieties along the first component (Fig. 2c) with Noy Yizre'el on the positive side and Tam Dew on the negative side. Examination of the first component loadings (Fig. 2d) and VIP values (Online Resource 5) showed that the major discriminant variables included 207 nominal *m/z* buckets determined by FIE-MS, 12 metabolites determined by ^{1}H-NMR, 29 semi-polar metabolites determined by LC-QTOF-MS, 39 volatiles determined by GC-MS and four mineral elements. Among the analytes with VIP >1.2, the following could be identified on the positive side of the first component, i.e. with higher levels in Noy Yizre'el: eight primary metabolites (galactose, inositol, sucrose, UDP-glucose, acetic acid, citric acid, malic acid, trigonelline), 28 volatiles (four alcohols, nine acetate esters, six butanoate esters, four propanoate esters, two ketones, one aldehyde, one alkane, one thiol compound) and three mineral elements (Ca, Mg, Mn, P). On the negative side of the first component, the identified discriminant metabolites with VIP >1.2 were three primary metabolites (isoleucine, tryptophan, tyrosine), one non-volatile secondary metabolite (a benzylalcohol-hexose-pentose), and 10 volatiles (eugenol, benzylalcohol, 2,3-butanedioldiacetate, 3-octen-2-one, camphor, pentanal, phenylacetaldehyde, decane, 2-methylthioethanol, 1,8-cineole), that tended to have higher levels in Tam Dew. Among the identified compounds, the highest VIP value was for 3-methylbutyl butanoate. Several of the FIE-MS ions contributing to the first component could possibly arise from discriminant metabolites identified using ^{1}H-NMR or LC-QTOF-MS: *m/z* 138 [trigonelline]$^{+}$, 132 [isoleucine+H]$^{+}$ and 193 [citric acid+H]$^{+}$ in the positive mode, and *m/z* 191 [citric acid−H]$^{-}$ and 565 [UDP-glucose−H]$^{-}$ in the negative mode, although confirmation is needed.

Overall, in this experiment involving two contrasting melon types in two contrasting agricultural management practices, nearly as many analytes were affected by the variety as by the management practice (291 vs. 252 analytes), but only 20 analytes were shared by these two factors. Both factors greatly affected polar and semi-polar compounds. However, the agricultural management practice also greatly affected mineral elements, whereas the variety greatly affected a number of volatile compounds including nine acetate esters and six butanoate esters.

3.2 Two close varieties clearly differed for fruit metabolome and elemental profiles in relation with shelf-life and showed common differences between years

For Experiment 2, FIE-MS allowed the relative quantification of 949 nominal *m/z* buckets in positive mode and 118 in negative mode. ^{1}H-NMR provided the absolute quantification of 36 metabolites. LC-QTOF-MS and GC-MS provided the relative quantification of 115 analytes and 107 volatiles, respectively, and ICP-MS allowed the absolute quantification of 19 mineral elements. An ANOVA showed that depending on the analytical technology, 26–78 % of the analytes were affected by the year, 23–81 % by the variety and 17–67 % by their interaction. However, overall, 63 % of the analytes were affected by the year, 40 % by the variety and 30 % by the year × variety interaction. PCAs performed for each analytical technology separately for the analytes that passed the ANOVA screen (scores plot Fig. 3a–f), showed that each analytical technology clearly separated the years on the PC1 × PC2 plot, except GC-MS of volatiles. The separation between varieties for a given year depended on the analytical technology. For FIE-MS (Fig. 3a–b), the first principal component (PC1), explaining 49 and 21 % of total variability, separated the 2 years and the second principal component (PC2), explaining 8 and 14 % of total variability, separated the two varieties in 2007 only. For ^{1}H-NMR (Fig. 3c), LC-QTOF-MS (Fig. 3d) and ICP-MS (Fig. 3f), the first two principal components explaining 63 to 80 % of total variability, separated the years and varieties. For ^{1}H-NMR (Fig. 3c) and LC-QTOF-MS (Fig. 3d), PC1 separated Cézanne in 2007 from all the other samples and PC2 clearly separated the 2 years for each variety. For ICP-MS (Fig. 3f), PC1 separated the varieties in each year and PC2 clearly separated Cézanne samples in 2007 from

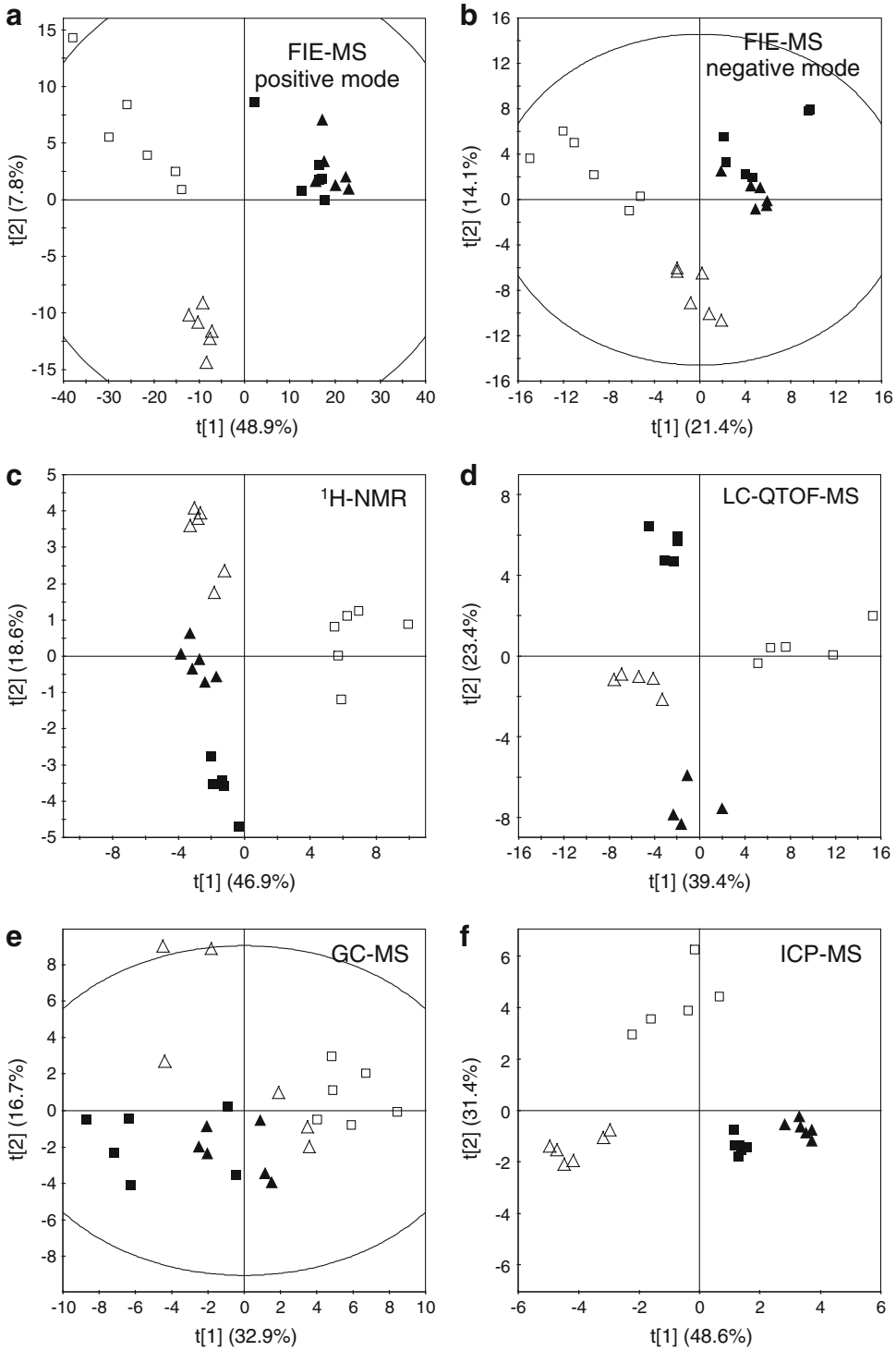

Fig. 3 Principal component analysis of absolute or relative content of analytes, selected using ANOVA, in melon flesh of mature fruit of Cézanne (*squares*) and Escrito (*triangles*) varieties cultivated in field in France for 2 years, 2007 (*open symbols*), 2008 (*closed symbols*). PCA scores plot of **a** FIE-MS in positive mode (713 variables), **b** FIE-MS in negative mode (210 variables), **c** ^{1}H-NMR (36 variables), **d** LC-QTOF-MS in negative mode (96 variables), **e** GC-MS (68 variables) of metabolite and **f** ICP-MS (18 variables) of mineral element data. The *ellipse* indicates the Hotelling's T2 ($P < 0.05$) for each analytical technology (the ellipse is not shown when all samples follow the Hotelling's T2)

the other samples. For GC-MS of volatiles (Fig. 3e), the separation between sample groups was less clear along the first two principal components explaining 50 % of total variability. However, for Cézanne samples, the years were clearly separated along PC1.

The data matrix containing the combined data of the 1141 variables that passed the ANOVA screen was explored using PLS-DA (Fig. 4). First, a PLS-DA was performed for year effect (Fig. 4a, b). As expected, the PLS-DA scores plot clearly separated the 2 years along the first component (Fig. 4a) with 2007 on the positive side and 2008 on the negative side. Examination of the first component loadings (Fig. 4b) and VIP values (Online Resource 6) showed that the major discriminant variables included 308 nominal *m/z* buckets determined by FIE-MS, eight metabolites determined by ^{1}H-NMR, two volatiles and nine mineral

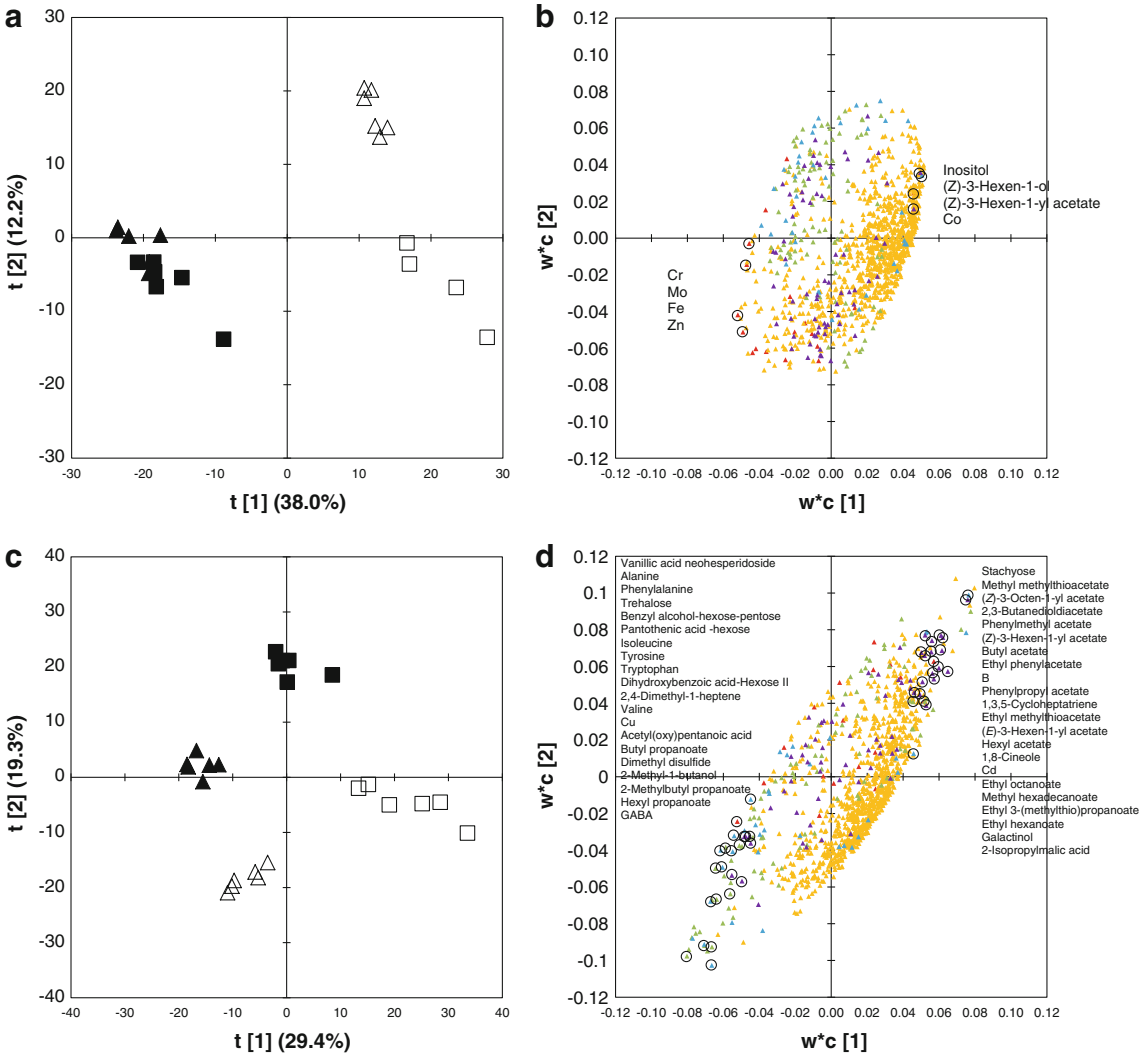

Fig. 4 PLS-DA analysis of absolute or relative content of 1,141 analytes, selected using ANOVA, issued from FIE-MS, ^{1}H-NMR, LC-QTOF-MS in negative mode, GC-MS and ICP-MS in melon flesh of mature fruit of Cézanne (*squares*) and Escrito (*triangles*) varieties cultivated in field in France for 2 years, 2007 (*open symbols*), 2008 (*closed symbols*). For Components 1 and 2, loadings are colored according to the analytical technology (*yellow* FIE-MS, *blue* ^{1}H-NMR, *green* LC-QTOF-MS, *purple* GC-MS, *red* ICP-MS) and Component 1 loadings with VIP scores >1.5 are circled and indexed with the corresponding analyte name for identified analytes. **a**, **b** Discrimination of years. **a** PLS-DA scores plot. **b** PLS-DA loadings plot. $R^2Y[1] = 0.886$, $Q^2[1] = 0.850$. **c**, **d** Discrimination of varieties. **c** PLS-DA scores plot. **d** PLS-DA loadings plot. $R^2Y[1] = 0.611$, $Q^2[1] = 0.499$ (Color figure online)

elements. Among the analytes with VIP >1.2, the following could be identified: glucose, galactose, inositol, galactinol, malate, glutamate, choline, (*Z*)-3-hexen-1-ol, (*Z*)-3-hexen-1-yl acetate and Co on the positive side, i.e. with higher contents in 2007, and citrulline, Ba, Cr, Fe, Mo, Ni, P, S and Zn on the negative side, i.e. with higher contents in 2008. Surprisingly, among identified compounds, the highest VIP value was for Cr. Several of the FIE-MS ions contributing to the first component could potentially be produced from discriminant metabolites identified using ^{1}H-NMR: *m/z* 104 $[choline]^+$, 219 $[hexose+K]^+$, 379 $[galactinol+H]^+$, 401 $[galactinol+Na]^+$ and 417 $[galactinol+K]^+$ in the positive mode. However, confirmation is needed.

Second, a PLS-DA was performed for variety effect (Fig. 4c, d). As expected, the PLS-DA scores plot separated the two varieties along the first component (Fig. 4c) with Cézanne mostly on the positive side and Escrito on the negative side. Examination of the first component loadings (Fig. 4d) and VIP values (Online Resource 7) showed that the major discriminant variables included 171 nominal *m/z* buckets determined by FIE-MS, 23 metabolites determined by ^{1}H-NMR, 51 semi-polar metabolites determined by LC-QTOF-MS, 30 volatiles and five mineral elements. Among the analytes with VIP > 1.2, the following could be identified on the positive side of the first component: five polar compounds (galactinol, galactose, citrate,

adenosine-like, stachyose), three semi-polar compounds (2-isopropylmalic acid, a benzylalcohol-hexose-pentose, a methylbutanol-hexose-pentose), 23 volatiles (including 11 acetate esters, three other esters, six thiol compounds, one aldehyde, one alkane) and three mineral elements (Li, Cd, B), that tended to have higher levels in Cézanne. Among the analytes with VIP >1.2, the following could be identified on the negative side: 13 polar compounds including 10 amino compounds (trehalose, fumarate, alanine, asparagine, GABA, glutamine, isoleucine, phenylalanine, tryptophan, tyrosine, valine, trigonelline, choline), eight semi-polar compounds (acetyl(oxy)pentanoic acid, dihydroxybenzoic acid-hexose I, dihydroxybenzoic acid-hexose II, pantothenic acid -hexose, vanillic acid-neohesperidoside, *p*-hydroxybenzoic acid-hexose, 3-hydroxybutanoic acid ethyl ester-hexoside, a benzylalcohol-hexose-pentose), seven volatiles (methyl 2-methyl-butanoate, butyl propanoate, 2-methylbutyl propanoate, hexyl propanoate, dimethyl disulfide, 2-methyl-1-butanol, 2,4-dimethyl-1-heptene) and two mineral elements (Ni, Cu), that tended to have higher levels in Escrito. Among identified compounds, the highest VIP value was for vanillic acid-neohesperoside. Several FIE-MS ions contributing to the first component could potentially be produced from discriminant metabolites identified using ^{1}H-NMR: *m/z* 164 $[\text{phenylalanine}-\text{H}]^{-}$ in the negative mode, and *m/z* 166 $[\text{phenylalanine}+\text{H}]^{+}$, 215 $[\text{citrate}+\text{Na}]^{+}$, 689 $[\text{stachyose}+\text{Na}]^{+}$, 705 $[\text{stachyose}+\text{K}]^{+}$ in the positive mode. However, confirmation is needed.

In this experiment involving two varieties within the var. cantaloupensis group over two cultivation years, globally more analytes were affected by year than variety. However, when looking at all analytes except the nominal *m/z* buckets determined by FIE-MS, it appears that a larger number of primary metabolites, semi-polar compounds, and volatiles were affected by variety than by year, while a larger number of mineral element were affected by year than by variety. Both year and variety affected polar compounds but the variety also affected a number of semi-polar compounds and a number of volatile compounds including 11 acetate esters and six thiol compounds.

3.3 Even the growing period of one variety in one year in France modified the fruit metabolome and elemental profiles

For Experiment 3, FIE-MS allowed the relative quantification of 931 metabolite nominal *m/z* buckets in positive mode and 931 in negative mode. ^{1}H-NMR provided the absolute quantification of 36 polar metabolites. LC-QTOF-MS and GC-MS provided the relative quantification of 115 analytes and 63 volatiles, respectively, and ICP-MS allowed the absolute quantification of 19 mineral elements. An ANOVA showed that depending on the analytical technology, 11–69 % of the analytes were affected by the growing period. Overall, 17 % of the analytes were affected by the growing period. For FIE-MS (Fig. 5a, b), ^{1}H-NMR (Fig. 5c), LC-QTOF-MS (Fig. 5d) and GC-MS of volatiles (Fig. 5e), the first two PCs, explaining from 49 to 76 % of total variability, separated the three growing periods corresponding to three different plantation dates. It was only with volatiles determined by GC-MS that the early and late growth periods were not clearly separated. May–August is the usual growing period for Hugo in South-West France. For non-volatile compounds, examination of the loadings plots (data not shown) showed that an earlier growing period tended to increase the content in galactinol, citrate, isocitrate, a methyl butanol hexose-pentose, Ca, Ba, K, Mg, Sr, and decrease the content in glutamine, citrulline and choline. A later growing period tended to increase the content in galactose, inositol, UDP-glucose-like compound, malate, GABA, pantothenic acid-hexose and decrease the content in sucrose, trehalose and tyrosine for instance. Surprisingly for volatiles, examination of the loadings plots (data not shown) showed that both the earlier and later growing periods tended to decrease the content in 1-pentanol, 2(3H)-furanone 5-methyl-5-vinyl and eugenol, and increase the content in a number of other volatiles including nine esters and three thiol compounds. However, the significance of these tendencies remains to be verified using post hoc univariate analyses.

Overall, in this experiment involving one variety in one year, the environmental modifications related to the growing period resulted in a lower number of analytes affected than in Experiments 1 or 2, but the growth periods could nevertheless be separated using metabolome and elemental profiles.

3.4 A combined analysis of discriminant analytes from Experiments 1 and 2 reveals unexpected relationships between metabolites and mineral elements

The absolute and relative quantification data obtained within the same analytical series for each analytical technique (exactly same conditions for absolute quantification and gathered acquisitions for relative quantification) for Experiments 1 and 2 were combined for the identified or partially identified compounds that passed the ANOVA screen in both experiments. This provided a matrix of 166 analytes in 33 samples, issued from all the analytical technologies except FIE-MS. We took the opportunity to use the variability of this dataset to study the inter-analyte associations. To visualize the inter-analyte associations, we employed Spearman correlation coefficients (r) between the 166 discriminant analytes. At $P < 0.001$ significance threshold after Bonferroni correction, when eliminating the isolated sub-networks of less than five

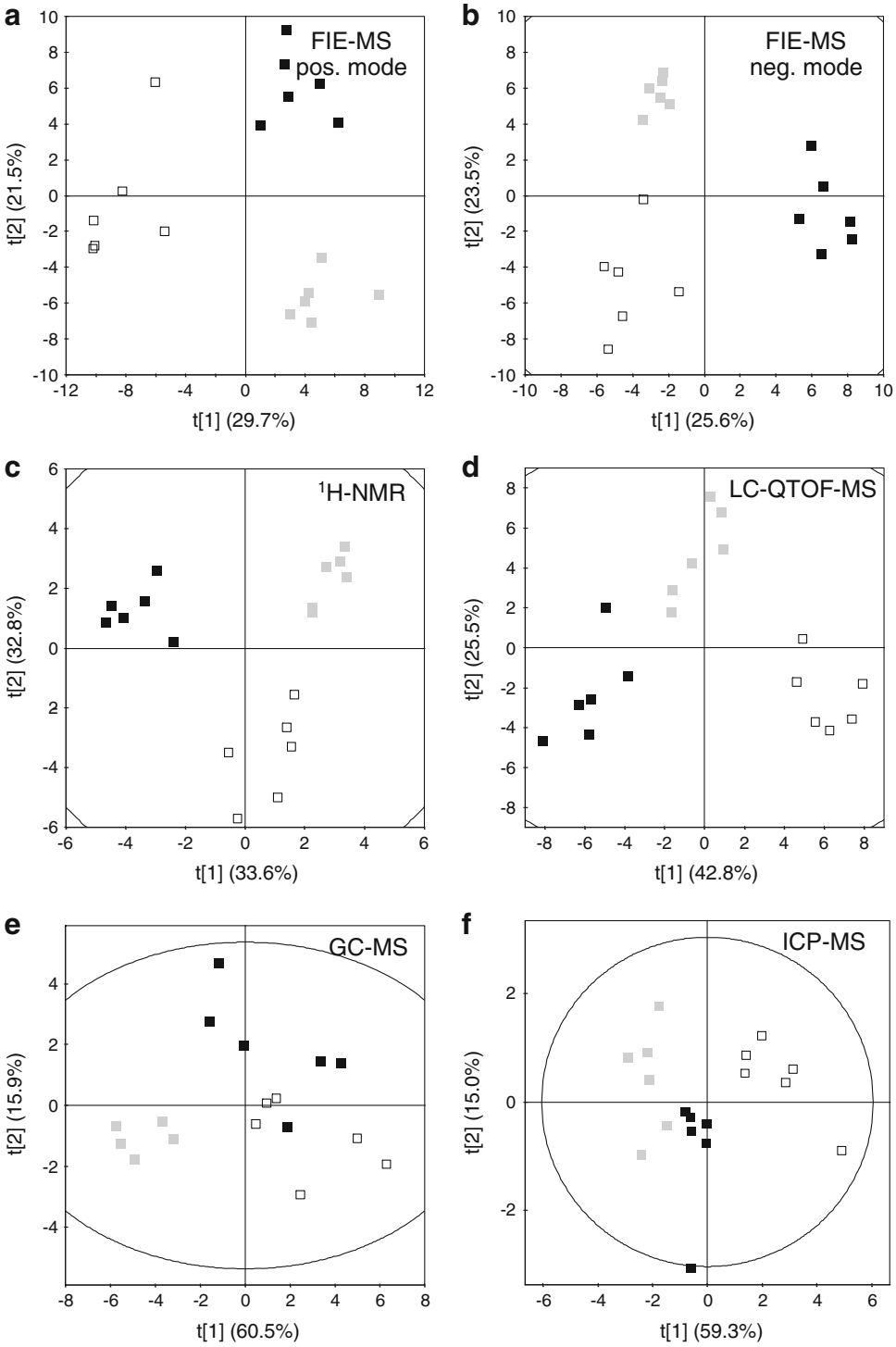

Fig. 5 Principal component analysis of absolute or relative content of analytes, selected using ANOVA, in melon flesh of mature fruit of Hugo variety cultivated in field in France in 1 year for three growing periods (*open square*, April–July; *grey square*, May–August; *closed square*, June–September). PCA scores plot of **a** FIE-MS in positive mode (138 variables), **b** FIE-MS in negative mode (102 variables), **c** ^{1}H-NMR (25 variables), **d** LC-QTOF-MS in negative mode (62 variables), **e** GC-MS (23 variables) of metabolite and **f** ICP-MS (8 variables) of mineral element data. All samples followed the Hotelling's T2 ($P < 0.05$) for a given analytical technology when the corresponding *ellipse* is not shown

compounds, this resulted into two networks: a small network of three metabolites (galactose, inositol, choline) and two mineral elements (Cr, Mo) and a large network containing 98 metabolites and seven mineral elements with a total of 724 connections (Fig. 6a). Two parts of the large network were very dense and seemed to correspond to highly connected modules. The densest part of the network is presented in Fig. 6b. It shows tight connections between primary metabolites, semi-polar compounds, volatiles and mineral elements. In the large network, 13 identified compounds were connected to more than 20 other analytes and could be considered as network hubs (Table 2). These hubs included seven amino acids (alanine, glutamine, isoleucine, phenylalanine, tryptophan, tyrosine, valine and citrulline), two secondary metabolites (ethyl hexanoate, a hydroxybutanoic acid ethyl ester hexoside) and three mineral elements (Cu, Sr and Zn).

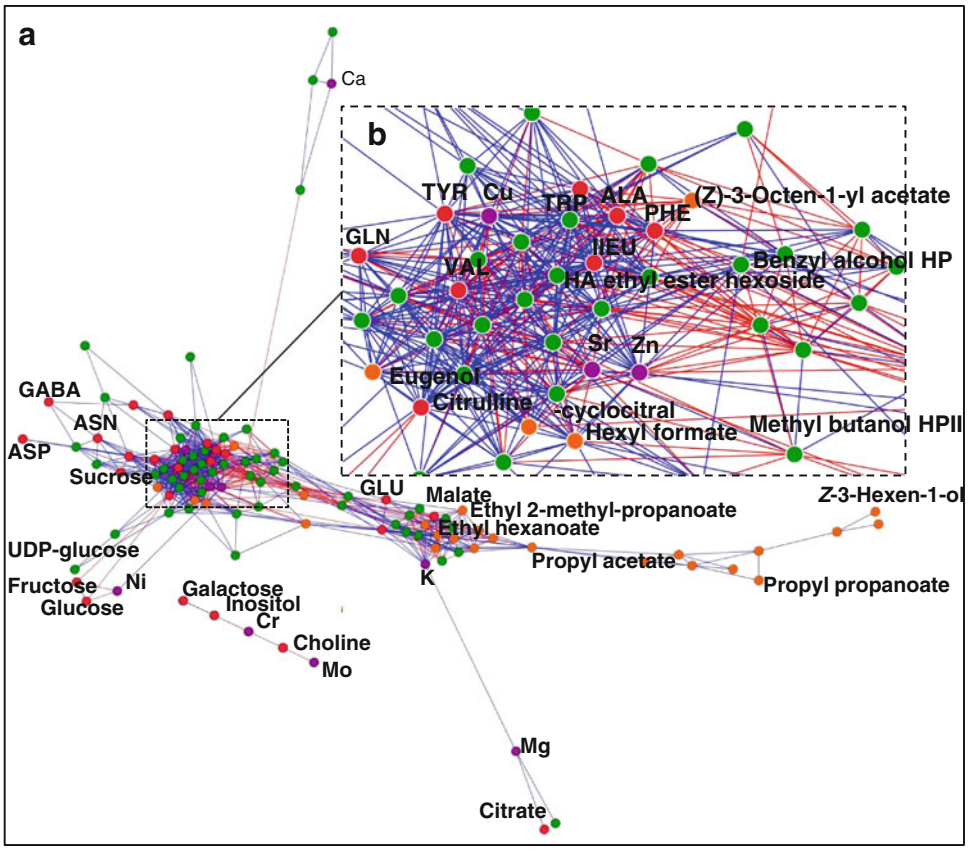

Fig. 6 Melon flesh correlation network based on Spearman r coefficients for 101 identified or partially characterized metabolites and 9 elements, corresponding to analytes significant in both Experiments 1 and 2 and having correlation coefficients with a probability threshold of $P < 10^{-3}$ after Bonferroni correction. (**a**) The analyte × analyte correlation network was visualized using Cytoscape software. Vertices are colored according to the analytical technique: *red* ^{1}H-NMR, *green* LC-QTOF-MS, *blue* GC-MS of volatiles and *purple* ICP-MS. For the edges, *blue color* corresponds to positive correlations and *red color* to negative correlations. **b** A zoom in on a dense cluster. The most highly connected analytes (hubs) are listed in Table 2**.** *HA* hydroxybutanoic acid, *HP* hexose-pentose (Color figure online)

They are all listed along with their immediate neighbors when identified in Table 2. Twelve of these analytes are part of the module presented in Fig. 6b. Several common amino acids and citrulline were highly correlated. Citrulline was also connected to hexoses and two secondary volatile metabolites. Ethyl hexanoate was connected to glutamate, malate, K and nine volatiles. A hydroxybutanoic acid ethyl ester hexoside was connected to seven amino acids and three mineral elements. Cu was connected to eight amino acids (seven common amino acids and citrulline), two volatiles and Zn. Sr was connected to four amino acids, three non-volatile secondary metabolites (hydroxybutanoic acid ethyl ester-hexoside, methylbutanol-hexose-pentoseII, benzylalcohol-hexose-pentose) and five volatiles. Zn was connected to six amino acids including citrulline, hydroxybutanoic acid ethyl ester-hexoside and two other mineral elements.

4 Discussion

4.1 Varieties cultivated in the same or contrasting conditions showed both expected and unexpected compositional differences

As a proof of concept, Tam Dew and Noy Yizre'el, two contrasting varieties each from a different marketing type (Honey Dew/Ha'Ogen) and taxonomic group (Inodorus/Cantalupensis), could readily be distinguished based on their metabolic profiles only. Using univariate and multivariate analysis, the discrimination between Tam Dew and Noy Yizre'el involved 24 unidentified semi-polar metabolites and 54 identified or potentially identified compounds (Fig. 2c, d; Online Resource 5) including 40 volatiles. The major contribution of volatiles in discriminating Tam Dew from Noy Yizre'el can be related to their non-climacteric versus climacteric behavior. Noy Yizre'el, the climacteric variety, tended to have higher levels of 28 volatiles including nine acetate derivatives and ethyl butyrate, whereas Tam Dew tended to have higher concentrations of 12 volatiles including pentanal. This is in general agreement with previous data on climactericity (Obando-Ulloa et al. 2008) and/or shelf-life (Aubert and Bourger 2004). Seventeen esters tended to be predominant in Noy Yizre'el whereas two aldehydes tended to be higher in Tam Dew. Again this is in general agreement with previous comparisons of climacteric and non-climacteric melon varieties (Gonda et al. 2010; Obando-Ulloa et al. 2008). The two genotypes also differed for sucrose, citric acid and malic acid, with higher levels observed in Noy Yizre'el. For sucrose, this was in general agreement with the tendency observed on a broad spectrum of *C. melo* genotypes, including several Cantalupensis and Inodorus varieties

Table 2 List of the 13 identified hub metabolites or mineral elements in the correlation network presented Fig. 6

Identified metabolite	Number of connections	Identified compounds connected
Alanine	24	Glutamate, glutamine, isoleucine, phenylalanine, tyrosine, tryptophan, valine, hydroxybutanoic acid ethyl ester-hexoside, 2-octanone, Cu, Sr
Citrulline	24	Fructose, glucose, glutamine, isoleucine, phenylalanine, tryptophan, tyrosine, valine, hexyl formate, eugenol, Cu, Zn
Glutamine	24	Alanine, asparagine, isoleucine, pyroglutamate, tyrosine, citrulline, valine, hydroxybutanoic acid ethyl ester-hexoside, eugenol, Cu, Sr
Isoleucine	32	Alanine, citrulline, glutamine, phenylalanine, tryptophan, tyrosine, valine, hydroxybutanoic acid ethyl ester-hexoside, (Z)-3-octen-1-yl acetate, Cu, Zn
Phenylalanine	25	Alanine, citrulline, isoleucine, sucrose, phenylalanine, tryptophan, tyrosine, valine, hydroxybutanoic acid ethyl ester-hexoside, (Z)-3-octen-1-yl acetate, Cu, Zn
Tryptophan	22	Citrulline, isoleucine, phenylalanine, tyrosine, valine, hydroxybutanoic acid ethyl ester-hexoside, (Z)-3-octen-1-yl acetate, Cu, Zn
Tyrosine	30	Alanine, citrulline, isoleucine, glutamine, phenylalanine, pyroglutamate, tryptophan, valine, hydroxybutanoic acid ethyl ester-hexoside, eugenol, (Z)-3-octen-1-yl acetate, Cu, Zn
Valine	33	Alanine, citrulline, isoleucine, glutamine, phenylalanine, pyroglutamate, sucrose, tyrosine, eugenol, tryptophan, β-cyclocitral, hexyl formate, Cu, Sr, Zn
Ethyl Hexanoate	21	Glutamate, malate, propyl acetate, ethyl 2-methyl-butanoate, ethyl butanoate, dimethyl disulfide, ethylmethylthioacetate, ethyl 2-methyl-propanoate, ethyl propanoate, ethyl 3-methylthio-propanoate, 2,3-butanedioldiacetate, K
Hydroxybutanoic acid ethyl ester hexoside	27	Alanine, glutamine, isoleucine, phenylalanine, tryptophan, tyrosine, valine, Cu, Sr, Zn
Cu	25	Alanine, citrulline, glutamine, isoleucine, phenylalanine, tryptophan, tyrosine, valine, eugenol, (Z)-3-octen-1-yl acetate, Zn
Sr	26	Alanine, glutamine, pyroglutamate, valine, hydroxybutanoic acid ethyl ester-hexoside, methylbutanol-hexose-pentoseII, benzylalcohol-hexose-pentose, β-cyclocitral, 2.3-butandioldiacetate, hexyl formate, 2-octanone, eugenol
Zn	23	Citrulline, isoleucine, phenylalanine, tryptophan, tyrosine, valine, hydroxybutanoic acid ethyl ester-hexoside, Cu, Ni

Compounds quantified using ^{1}H-NMR, LC-MS, GC-MS or ICP-MS with a number of connections higher than 20 for the chosen threshold of $P < 0.001$ (after Bonferroni correction) are listed with their direct neighbours when identified

(Burger et al. 2006; Portnoy et al. 2008; Stepansky et al. 1999). All this agrees with a better flavor of Noy Yizre'el fruits.

Surprisingly, the two varieties also differed for four mineral macroelements tending to have higher content in Noy Yizre'el, the climacteric variety: Ca, Mg, Mn and P. Ca is important for stabilization of cell walls. Genotypic differences may reflect the abundance of Ca binding sites in the cell walls, primarily associated with polygalacturonic acids (Lester and Grusak 1999). This seems to be in agreement with the difference in fruit texture observed between the two varieties: Noy Yizre'el is fleshier whereas Tam Dew is crunchier. Mg constitutes an important counter ion for organic and inorganic anions stored in vacuoles. Genotypic differences in Mg content may be related to difference in the accumulation of organic anions in fruit mesocarp vacuoles. Indeed, Noy Yizre'el had higher content than Tam Dew for both Mg and citrate. Mg also regulates activities of major enzymes in mitochondria and cytosol, in particular it strongly affects respiratory ATP synthesis (Igamberdiev and Kleczkowski 2006). P has a central role in energy turnover and P nutrition has been shown to affect fruit respiratory metabolism during the climacteric rise and post-harvest (Knowles et al. 2001; Rowan et al. 1969). Mn is an activator of numerous enzymes including malic enzyme and isocitrate dehydrogenase involved in TCA cycle (Hansch and Mendel 2009). Therefore, P, Mg and Mn levels may be regulated in relation to the climacteric rise in respiration. However, studies using additional climacteric and non-climacteric varieties are needed to confirm if all these macroelements are directly or indirectly related to climactericity.

Two genotypes of the same group (Cantalupensis), Cézanne and Escrito cultivated under identical conditions in France could also be differentiated based on their metabolic profiles. According to FIE-MS and ICP-MS, the two varieties differed more in 2007 that in 2008. However, when considering the data from both years (Fig. 4c, d; Online Resource 7), Cézanne tended to have higher levels of 23 volatiles compared to Escrito, while vice versa

Escrito contained higher levels than Cézanne of nine amino acids including isoleucine, valine and phenylalanine which have been recently proven to be precursors of volatiles in melon (Gonda et al. 2010). Such a separation between the two varieties was expected for volatiles and amino acids that are precursors of volatiles since Cézanne is a classic aromatic variety with short shelf-life and Escrito is a mid shelf-life variety. More than fifty semi-polar metabolites also differentiated the two varieties but only 11 of them were identified, including 2-isopropylmalic acid with a higher content in Cézanne. This compound is an intermediate of leucine biosynthesis (Hagelstein and Schultz 1993). The higher level of 2-isopropylmalic acid in the aromatic variety may be related to the synthesis of volatiles from leucine (Gonda et al. 2010). Among the 49 semi-polar compounds other than amino acids differentiating the two varieties, 34 tended to have higher levels in Escrito. Some of the latter compounds that have been putatively identified (3-hydroxybutanoic acid ethyl ester-hexoside, benzylalcohol-hexose-pentose, dihydroxybenzoic acid-hexose I, dihydroxybenzoic acid-hexose II, *p*-hydroxybenzoic acid-hexose, vanillic acid-neohesperidoside) might be glycosidic aroma precursors of volatiles (Ortiz-Serrano and Gil 2009; Sarry and Ganata 2004). As 26 out of these 34 compounds remained unidentified so far, further identification is needed to reinforce this hypothesis. Such a non-targeted profiling approach of several compound classes in varieties differing for fruit aroma should help to provide hypotheses on the biosynthetic pathways of volatiles.

4.2 All profiling technologies revealed compositional changes from contrasting agricultural management practices and more subtle environmental differences

In our study conducted in Israel (Experiment 1), two contrasting agricultural management practices could be differentiated with each analytical technology highlighting common responses in two contrasting melon varieties (Fig. 2a, b; Online Resource 4). The effect of individual environmental factors on the content of primary metabolites in fruit has been described for a number of species and has even been modeled in eco-physiological approaches for several, including tomato (Liu et al. 2007). The source-sink relationships in the field-grown crop (high irradiance, no fruit thinning, high yield) were highly different from those of the greenhouse-grown crop (low irradiance, one fruit per plant, low yield). Sucrose content was higher whereas glucose and fructose contents were lower in the high-yield management practice which is likely related to the higher irradiance levels in the spring-summer field crop. The content of several amino acids including glutamine, glutamate, GABA and phenylalanine were also higher in the field management practice. This is likely due to the combination of better nitrogen nutrition with efficient photosynthesis for the field-grown crop.

The effect of various environmental factors on the content of micronutrients in fruit or vegetables has been demonstrated in a number of cases (Poiroux-Gonord et al. 2010) with a special interest for temperature and irradiance effects, e.g. in tomato (Gautier et al. 2008). Unfortunately, the temperature, photoperiod duration and light intensity were not recorded for the two management practices in our experiment conducted in Israel. Nevertheless, the two management practices produced clear differences in secondary metabolites. The higher levels of 26 semi-polar metabolites determined by LC-QTOF-MS in the field management practice could potentially be related to the generally positive response of phenolics to light intensity, through enhanced photooxidative stress and/or increased photosynthesis (Poiroux-Gonord et al. 2010). On the contrary, for volatiles, the management practice had limited effects that were common to the two varieties, possibly because these two varieties are much contrasted for volatile profiles in a given management practice.

A clear effect of the management practice also appeared for several mineral elements, with for instance, higher Na levels in the greenhouse and lower B levels under field conditions. This likely reflects differences in the mineral composition between the soil and irrigation water that may cause primary effects related to contrasting supplies. The differences in soil and water mineral composition may also cause secondary effects related to ionic antagonisms, e.g. between Cl^- and NO_3^- (Feigin et al. 1987) that may have contributed to the differences in amino acid contents mentioned above.

In Experiment 2 conducted in France, the composition of Cézanne and Escrito varieties differed significantly between the experimental years. The year 2008 was characterized by higher duration of insolation from plantation to harvest. The fruits harvested in 2008 had lower contents in several sugars or sugar-alcohols including galactinol, malate, glutamate, as well as Co, and higher contents in citrulline and eight mineral elements (Ba, Cr, Fe, Mo, Ni, P, S, Zn) compared with fruits harvested in 2007 (Fig. 4a, b; Online Resource 6). Galactinol can be produced by alkaline β-galactosidases involved in raffinose and stachyose phloem unloading into the fruit (Gao and Schaffer 1999). A lower content in galactinol in the warmer year could be linked to a decrease in the activity of alkaline β-galactosidases above 40 °C as was shown in vitro (Gao and Schaffer 1999). A lower malate content in the warmer year in melon is in agreement with several previous findings on grapes (Sweetman et al. 2009). It may be linked to an increased malate degradation through a shift between developmentally-regulated malate dehydrogenase isoforms

contributing to fuel mitochondrial respiratory, with different temperature sensitivities as has been proposed for grape berries (Taureilles-Saurel et al. 1995). Citrulline was discovered in water-melon in 1930 (Wada 1930), and recently the content of citrulline has been demonstrated to be influenced by year environmental factor and cropping region (Fish and Bruton 2010). The higher citrulline level may be related to its important role in nitrogen transport and storage and a higher rate of nitrogen assimilation during the warmer year (Macduff et al. 1987). The higher level of two macroelements (P and S) and six microelements (Ba, Cr, Fe, Mo, Ni, Zn) may be related to increased transpiration during the warmer year, leading to a greater supply of elements via the xylem to the low-transpiring melon fruits.

Within-season compositional differences have already been shown in melon for different commercial fields for soluble solid content measured in Brix degrees and relative volatile content (Beaulieu 2005). In Experiment 3 conducted in France with Hugo variety, different plantation dates at a unique location, corresponding to different growing periods within a given year, induced compositional differences. These compositional differences did not result from immature harvested fruit for the late plantation as special care was taken to select the fruits at harvest. According to the ANOVA data, the environmental changes linked to the growing period in a given year in Experiment 3 affected a lower number of analytes (about half) compared to the environmental changes in Experiments 1 and 2 that were linked to contrasted management practices or different years.

Therefore, the present experiments, using a combination of several analytical technologies for biochemical phenotyping, showed compositional changes between contrasting agricultural management practices, as testing the concept, but also revealed much more subtle compositional differences along a growing season in the same location with the same grower related to limited changes in temperature and irradiance. The link between these subtle compositional differences and modifications of fruit flavor remains to be verified. This could be done in further studies on fruit through a combination of sensory tests performed by a panel of experts and metabolome analyses.

4.3 The genotype x environment interaction was clearly highlighted by FIE–MS in the experiment conducted in France with two varieties over 2 years

In Experiment 1 conducted in Israel with Tam Dew and Noy Yizre'el, the two contrasting varieties globally responded in the same way to the effect of agricultural management practice for each compound class. However, in Experiment 2 conducted in France with Cézanne and Escrito varieties, the yearly changes in mineral element profiles determined by ICP–MS, semi-polar compounds determined by LC-QTOF-MS, and polar compounds determined by FIE-MS were variety specific (Fig. 3). Therefore, such untargeted biochemical phenotyping could be used to select varieties adapted to a given environmental condition (e.g. greenhouse versus field) or on the contrary with a larger adaptation capacity. The possibility to use high-throughput metabolite fingerprinting with FIE-MS seems promising for this purpose as this implies that an enormous number of samples have to be characterized. High-throughput targeted profiling with MS or other technologies (Gibon et al. 2012) could also be used if variety and growth conditions have to be selected for a higher content of a few micronutrients of particular interest for a specific purpose, or after a first step of untargeted screening.

4.4 The compositional variability induced by variety and environment can be used to study melon flesh metabolism

As discussed above, the genotypes and growth conditions studied showed clear compositional differences for all compound classes including mineral elements. Therefore we used this variability to study metabolite and mineral element co-regulation in melon flesh. Analyte relationships were visualized and studied using correlation networks (Weckwerth et al. 2004, Schauer et al. 2006). In the networks, analytes (vertices) were connected with a link (edge) in a two-dimensional plane, such that their pair-wise distances reflected their pair-wise correlation coefficients when these coefficients exceeded a given threshold as indicated in Methods. We expected that correlation networks in melon would reveal analyte clusters, and also several so-called 'hub' analytes closely associated to a multitude of other analytes. These hub analytes may be crucial to understanding as yet unknown metabolic regulations.

In the present work, correlation networks revealed a cluster of metabolites comprising branched-chain and aromatic amino acids and several volatiles or possible volatile precursors in agreement with the involvement of amino acid transaminases for the catabolism of amino acids into aroma volatiles as recently shown for melon (Gonda et al. 2010). The coregulation networks also revealed metabolites or mineral element hubs and significant links between metabolites and minerals elements. Among the 13 metabolites or mineral element hubs revealed, three aromatic amino acids and two branched-chain amino acids are precursors of melon aroma compounds (Gonda et al. 2010). Therefore the correlation networks revealed five hubs directly linking primary and secondary metabolism. Citrulline, that has long been identified in several organs of

melon (Rimando and Perkins-Veazie 2005; Wada 1930), was another hub. This compound may have roles in both nitrogen metabolism and ripening regulation. In muskmelon, citrulline, glutamate and glutamine are the three major amino compounds transported in the phloem sap from leaves to fruits (Mitchell et al. 1992). Therefore after its import into the fruit flesh, citrulline metabolism is a key part of melon fruit nitrogen metabolism. In addition, citrulline synthesis or resynthesis may participate in the regulation of fruit maturation since citrulline and nitric oxide can both be produced from arginine, and nitric oxide has been suggested to be a regulator of maturation in fruit (Leshem et al. 1998). Citrulline also seems to have several bioactive properties for human health (Tarazona-Díaz et al. 2010). Therefore melon genotypes or growth conditions producing higher levels of citrulline may be of interest. However, it may prove difficult to significantly increase the content of a cross-road (hub) metabolite.

For two of the hub metabolites identified, their relations in biochemical pathways or with plant physiology are yet unknown. The volatile compound identified as a hub, ethyl hexanoate, was directly connected to nine other volatiles (including three volatiles derived from methionine, one derived from valine and one derived from isoleucine according to Gonda et al. 2010) and also to glutamate and malate. This is in favour of the study of the metabolism of this ester in melon. The semi-polar compound identified as a hub, hydroxybutanoic acid ethyl ester hexoside, was connected to seven amino acids and three mineral elements. This calls for the complete identification of the structure of this compound and the parallel targeted study of its potential volatile product ethyl hydroxybutanoate.

Surprisingly, three mineral elements were also identified as hubs: Cu, Sr and Zn. Cu was correlated with 25 compounds including phenylalanine, the precursor of most phenylpropanoids. This may be linked with the activation role of Cu for enzymes of the phenylpropanoid biosynthetic pathway that are involved in cell wall biosynthesis (Femandes and Henriques 1991). The positive correlation observed here between Cu and Zn is not surprising and probably reflects links via a joint transporter, e.g. a P-type ATPase (Hussain et al. 2004) or via binding to specific chelators such as phytochelatins or metallothioneins, promoting their translocation in the phloem (Husted et al. 2011). The connection of Zn to six amino acids and a secondary metabolite may reflect its structural and activating role in many enzymes involved in carbohydrate metabolism and protein synthesis (Broadley et al. 2007). Sr, a non-essential microelement for plants, was directly connected to four amino acids and eight identified secondary metabolites. Despite the physico-chemical similarities of Sr to Ca, Sr effects on plant metabolism remains poorly characterized (Moyen and Roblin 2009). A small network linked Cr to choline and inositol, and Mo to choline in melon flesh. In humans, Cr has essential functions in the metabolism of glucose and lipids, and/or a beneficial effect on diabetes resulting from inadequate synthesis of insulin or insulin resistance (Ghosh et al. 2002; Vincent 2003). In plants, there are few data on the regulatory role of Cr on metabolism although Cr interferes with several metabolic processes including respiration, photosynthesis, water relations and nutrient uptake (Arun et al. 2005).

The co-regulations highlighted here between metabolites and mineral elements do not prove direct effects, but call for the re-evaluation of the roles these minerals have in fruit primary and secondary metabolisms. Moreover, further analyses of the metabolite-element or metabolite-metabolite co-regulations should be addressed from a biochemical pathway perspective.

5 Concluding Remarks

Here we have explored the metabolite and mineral element compositional variability of several commercial varieties of melon grown under different environmental conditions. Combining a range of advanced complementary analytical technologies, together with univariate and multivariate analyses of the data, highlighted analytes varying according to variety, growing season, agricultural management practice and growing period. Following the number of analytes affected, and their distribution among the different compound families, allowed a comparison of the effects of the different factors studied and their specificity. The co-regulation between metabolites, and between metabolites and mineral elements, were also used to generate hypotheses on metabolic regulation. Therefore, metabolome and mineral element profiling appear to be useful diagnostic tools to characterize the quality of melons cultivated under commercial conditions. The metabolomics approach, complemented with mineral element analysis, also provided new knowledge on fruit flesh metabolism and on the plant response to environmental modifications and fluctuations. Besides tomato (De Vos et al. 2011; Ezura 2009), melon is now confirmed as an alternative model plant for elucidating fleshy fruit ripening and more generally fleshy fruit metabolism and development (Ezura and Fukino 2009).

References

Allwood, J. W., Erban, A., de Koning, S., et al. (2009). Inter-laboratory reproducibility of fast gas chromatography-electron impact-time of flight mass spectrometry (GC-EI-TOF/MS) based plant metabolomics. *Metabolomics, 5*, 479–496.

Arun, K., Shankera, T., Cervantes, C., Loza-Taverac, H., & Avudainayagam, S. (2005). Chromium toxicity in plants. *Environment International, 31*, 739–753.

Aubert, C., & Bourger, N. (2004). Investigation of volatiles in Charentais Cantaloupe melons (*Cucumis melo* Var. cantalupensis). Characterization of aroma constituents in some cultivars. *Journal of Agricultural and Food Chemistry, 52*, 4522–4528.

Aubert, C., & Pitrat, M. (2006). Volatile compounds in the skin and pulp of Queen Anne's pocket melon. *Journal of Agricultural and Food Chemistry, 54*, 8177–8182.

Beaulieu, J. C. (2005). Within-season volatile and quality differences in stored fresh-cut cantaloupe cultivars. *Journal of Agricultural and Food Chemistry, 53*, 8679–8687.

Biais, B., Allwood, J. W., Deborde, C., et al. (2009). 1H-NMR, GC-EI-TOF-MS and data set correlation for fruit metabolomics, application to spatial metabolite analysis in melon. *Analytical Chemistry, 81*, 2884–2894.

Broadley, M. R., White, P. J., Hammond, J. P., Zelko, I., & Lux, A. (2007). Zinc in plants. *New Phytologist, 173*, 677–702.

Burger, Y., Sa'ar, U., Paris, H. S., et al. (2006). Genetic variability for valuable fruit quality traits in *Cucumis melo*. *Israel Journal of Plant Sciences, 54*, 233–242.

Carrari, F., Baxter, C., Usadel, B., et al. (2006). Integrated analysis of metabolite and transcript levels reveals the metabolic shifts that underlie tomato fruit development and highlight regulatory aspects of metabolic network behavior. *Plant Physiology, 142*, 1380–1396.

Davies, H. V., Shepherd, L. V. T., Stewart, D., et al. (2010). Metabolome variability in crop plant species—When, where, how much and so what? *Regulatory Toxicology and Pharmacology, 58*, S54–S61.

De Vos, R. C. H., Hall, R., & Moing, A. (2011). Metabolomics of a model fruit: tomato. In R. Hall (Ed.), *Biology of plant metabolomics* (pp. 109–155). Oxford: Wiley-Blackwell Ltd.

De Vos, R. C. H., Moco, S., Lommen, A., et al. (2007). Untargeted large-scale plant metabolomics using liquid chromatography coupled to mass spectrometry. *Nature Protocols, 2*, 778–791.

Demiral, M. A., & Koseoglu, A. T. (2005). Effect of potassium on yield, fruit quality, and chemical composition of greenhouse-grown galia melon. *Journal of Plant Nutrition, 28*, 93–100.

Dufault, R. J., Korkmaz, A., Ward, B. K., & Hassell, R. L. (2006). Planting date and cultivar affect melon quality and productivity. *HortScience, 41*, 1559–1564.

Ezura, H. (2009). Tomato is a next-generation model plant for research and development. *Journal of the Japanese Society for Horticultural Science, 78*, 1–2.

Ezura, H., & Fukino, N. (2009). Research tools for functional genomics in melon (*Cucumis melo* L.): Current status and prospects. *Plant Biotechnology, 26*, 359–368.

Fan, T. W. M. (1996). Metabolite profiling by one- and two-dimensional NMR analysis of complex mixtures. *Progress in Nuclear Magnetic Resonance Spectroscopy, 28*, 161–219.

Feigin, A. (1990). Interactive effects of salinity and ammonium/nitrate ratio on growth and chemical composition of melon plants. *Journal of Plant Nutrition, 13*, 1257–1269.

Feigin, A., Rylski, I., Meiri, A., & Shalhevet, J. (1987). Response of melon and tomato plants to chloride–nitrate ratio in saline nutrient solutions. *Journal of Plant Nutrition, 10*, 1787–1794.

Femandes, J. C., & Henriques, F. S. (1991). Biochemical, physiological, and structural effects of excess copper in plants. *The Botanical Review, 57*, 246–273.

Ferry-Dumazet, H., Gil, L., Deborde, C., et al. (2011). MeRy-B: A web knowledgebase for the storage, visualization, analysis and annotation of plant ^{1}H-NMR metabolomic profiles. *BMC Plant Biology, 11*, 104.

Fish, W. W. & Bruton, B. D. (2010). Quantification of L-citrulline and other physiologic amino acids in watermelon and selected cucurbits. In *Cucurbitacae 2010*, Charleston, SC (pp. 152–154).

Gao, Z., & Schaffer, A. A. (1999). A novel alkaline alpha-galactosidase from melon fruit with a substrate preference for raffinose. *Plant Physiology, 119*, 979–987.

Gautier, H., Diakou-Verdin, V., Bénard, C., et al. (2008). How does tomato quality (sugar, acid, and nutritional quality) vary with ripening stage, temperature, and irradiance? *Journal of Agricultural and Food Chemistry, 56*, 1241–1250.

Ghosh, D., Bhattacharya, B., Mukherjee, B., et al. (2002). Role of chromium supplementation in Indians with type 2 diabetes mellitus. *Journal of Nutritional Biochemistry, 13*, 690–697.

Gibon, Y., Rolin, D., Deborde, C., Bernillon, S. & Moing, A. (2012). New opportunities in metabolomics and biochemical phenotyping for plant systems biology. In U. Roessner (Ed.), *Metabolomics*: InTech. http://www.intechopen.com/articles/show/title/new-opportunities-in-metabolomics-and-biochemical-phenotyping-for-plant-systems-biology.

Gonda, I., Bar, E., Portnoy, V., et al. (2010). Branched-chain and aromatic amino acid catabolism into aroma volatiles in *Cucumis melo* L. fruit. *Journal of Experimental Botany, 61*, 1111–1123.

Guy, C., Kaplan, F., Kopka, J., Selbig, J., & Hincha, D. K. (2008). Metabolomics of temperature stress. *Physiologia Plantarum, 132*, 220–235.

Hagelstein, P., & Schultz, G. (1993). Leucine synthesis in spinach-chloroplasts: Partial characterization of 2-isopropylmalate synthase. *Biological Chemistry Hoppe-Seyler, 374*, 1105–1108.

Hall, R. D. (2011). Plant metabolomics in a nutshell: Potential and future challenges. In R. D. Hall (Ed.), *Biology of plant metabolomics* (pp. 1–24). Chichester: Wiley-Blackwell.

Hansch, R., & Mendel, R. R. (2009). Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl). *Current Opinion in Plant Biology, 12*, 259–266.

Hansen, T. H., Laursen, K. H., Persson, D. P., et al. (2009). Micro-scaled high-throughput digestion of plant tissue samples for multi-elemental analysis. *Plant Methods, 5*. doi:10.1186/1746-4811-1185-1112.

Harrigan, G., Martino-Catt, S., & Glenn, K. (2007). Metabolomics, metabolic diversity and genetic variation in crops. *Metabolomics, 3*, 259–272.

Hussain, D., Haydon, M. J., Wang, Y., et al. (2004). P-type ATPase heavy metal transporters with roles in essential zinc homeostasis in Arabidopsis. *Plant Cell, 16*, 1327–1339.

Husted, S., Persson, D. P., Laursen, K. H., et al. (2011). The role of atomic spectrometry in plant science. *Journal of Analytical Atomic Spectrometry, 26*, 52–79.

Igamberdiev, A. U., & Kleczkowski, L. A. (2006). Equilibration of adenylates in the mitochondrial intermembrane space maintains respiration and regulates cytosolic metabolism. *Journal of Experimental Botany, 57*, 2133–2141.

Jahangir, M., Kim, H. K., Choi, Y. H., & Verpoorte, R. (2008). Metabolomic response of *Brassica rapa* submitted to pre-harvest bacterial contamination. *Food Chemistry, 107*, 362–368.

Johnson, H. E., Broadhurst, D., Goodacre, R., & Smith, A. R. (2003). Metabolic fingerprinting of salt-stressed tomatoes. *Phytochemistry, 62*, 919–928.

Knowles, L., Trimble, M. R., & Knowles, N. R. (2001). Phosphorus status affects postharvest respiration, membrane permeability and lipid chemistry of European seedless cucumber fruit (*Cucumis sativus* L.). *Postharvest Biology and Technology, 21*, 179–188.

Krishnan, P., Kruger, N. J., & Ratcliffe, R. G. (2005). Metabolite fingerprinting and profiling in plants using NMR. *Journal of Experimental Botany, 56*, 255–265.

Laursen, K. H., Hansen, T. H., Persson, D. P., Schjoerring, J. K., & Husted, S. (2009). Multi-elemental fingerprinting of plant tissue by semi-quantitative ICP-MS and chemometrics. *Journal of Analytical Atomic Spectrometry, 24*, 1198–1207.

Leshem, Y.a. Y, Wills, R. B. H., & Ku, V. V.-V. (1998). Evidence for the function of the free radical gas—nitric oxide ($NO^{\bullet}$)—as an endogenous maturation and senescence regulating factor in higher plants. *Plant Physiology and Biochemistry, 36*, 825–833.

Lester, G. E. (2005). Whole plant applied potassium: effects on cantaloupe fruit sugar content and related human wellness compounds. In *Proceedings of the fifth international postharvest symposium* (pp. 487–492) Verona, Italy, 6–11 June, 2004. Leuven: International Society for Horticultural Science (ISHS).

Lester, G. (2006). Consumer preference quality attributes of melon fruits. In *Proceedings of the IVth international conference on managing quality in chains MQUIC 2006*: *Integrated view on fruits and vegetables quality* (Vol. 1, pp. 175–181), Bangkok, Thailand, 7–10 August 2006.

Lester, G. E. (2008). Antioxidant, sugar, mineral, and phytonutrient concentrations across edible fruit tissues of orange-fleshed honeydew melon (*Cucumis melo* L.). *Journal of Agricultural and Food Chemistry, 56*, 3694–3698.

Lester, G. E., & Crosby, K. M. (2002). Ascorbic acid, folic acid, and potassium content in postharvest green-flesh honeydew muskmelons: influence of cultivar, fruit size, soil type, and year. *Journal of the American Society for Horticultural Science, 127*, 843–847.

Lester, G. E., & Grusak, M. A. (1999). Postharvest application of calcium and magnesium to honeydew and netted muskmelons: effects on tissue ion concentrations, quality, and senescence. *Journal of the American Society for Horticultural Science, 124*, 545–552.

Lester, G. E., Jifon, J. L., & Makus, D. J. (2010). Impact of potassium nutrition on postharvest fruit quality: Melon (*Cucumis melo* L.) case study. *Plant and Soil, 335*, 117–131.

Lindon, J. C., Holmes, E., & Nicholson, J. K. (2001). Pattern recognition methods and applications in biomedical magnetic resonance. *Progress in Nuclear Magnetic Resonance Spectroscopy, 39*, 1–40.

Liu, H.-F., Génard, M., Guichard, S., & Bertin, N. (2007). Model-assisted analysis of tomato fruit growth in relation to carbon and water fluxes. *Journal of Experimental Botany, 58*, 3567–3580.

Lommen, A. (2009). MetAlign: Interface-driven, versatile metabolomics tool for hyphenated full-scan mass spectrometry data preprocessing. *Analytical Chemistry, 81*, 3079–3086.

Macduff, J. H., Hopper, M. J., Wild, A., & Trim, F. E. (1987). Comparison of the effects of root temperature on nitrate and ammonium nutrition of oilseed rape (*Brassica napus* L.) in flowing solution culture. *Journal of Experimental Botany, 38*, 1104–1120.

Mitchell, D. E., Gadus, M. V., & Madore, M. A. (1992). Patterns of assimilate production and translocation in muskmelon (*Cucumis melo* L.). 1. Diurnal patterns. *Plant Physiology, 99*, 959–965.

Moco, S., Capanoglu, E., Tikunov, Y., et al. (2007). Tissue specialization at the metabolite level is perceived during the development of tomato fruit. *Journal of Experimental Botany, 58*, 4131–4146.

Moing, A., Aharoni, A., Biais, B., et al. (2011). Extensive metabolic cross talk in melon fruit revealed by spatial and developmental combinatorial metabolomics. *New Phytologist, 190*, 683–696.

Mounet, F., Lemaire-Chamley, M., Maucourt, M., et al. (2007). Quantitative metabolic profiles of tomato flesh and seeds during fruit development: Complementary analysis with ANN and PCA. *Metabolomics, 3*, 273–288.

Moyen, C., & Roblin, G. (2009). Uptake and translocation of strontium in hydroponically grown maize plants, and subsequent effects on tissue ion content, growth and chlorophyll *a*/*b* ratio: comparison with Ca effects. *Environmental and Experimental Botany, 68*, 247–257.

Obando-Ulloa, J. M., Moreno, E., Garcia-Mas, J., et al. (2008). Climacteric or non-climacteric behavior in melon fruit. 1. Aroma volatiles. *Postharvest Biology and Technology, 49*, 27–37.

Ortiz-Serrano, P., & Gil, J. V. (2009). Quantitative comparison of free and bound volatiles of two commercial tomato cultivars (*Solanum lycopersicum* L.) during ripening. *Journal of Agricultural and Food Chemistry, 58*, 1106–1114.

Pereira, G. E., Gaudillere, J. P., van Leeuven, C., et al. (2006). ^{1}H NMR metabolic fingerprinting of grape berry: comparison of vintage and soil effects in Bordeaux grapevine growing areas. *Analytica Chimica Acta, 563*, 346–352.

Poiroux-Gonord, F., Bidel, L. P. R., Fanciullino, A. L., et al. (2010). Health benefits of vitamins and secondary metabolites of fruits and vegetables and prospects to increase their concentrations by agronomic approaches. *Journal of Agricultural and Food Chemistry, 58*, 12065–12082.

Portnoy, V., Benyamini, Y., Bar, E., et al. (2008). The molecular and biochemical basis for varietal variation in sesquiterpene content in melon (*Cucumis melo* L.) rinds. *Plant Molecular Biology, 66*, 647–661.

Rimando, A. M., & Perkins-Veazie, P. M. (2005). Determination of citrulline in watermelon rind. *Journal of Chromatography A, 1078*, 196–200.

Rowan, K. S., McGlasson, W. B., & Pratt, H. K. (1969). Changes in adenosine pyrophosphates in Cantaloupe fruit ripening normally and after treatment with ethylene. *Journal of Experimental Botany, 20*, 145–155.

Saeed, A. I., Bhagabati, N. K., Braisted, J. C., et al. (2006). TM4 microarray software suite. In A. R. Kimmel & B. Oliver (Eds.), *Methods in enzymology. DNA microarrays, part B: Databases and statistics* (pp. 134–193). Oxford: Academic Press.

Sarry, J.-E., & Ganata, Z. (2004). Plant and microbial glycoside hydrolases: Volatile release from glycosidic aroma precursors. *Food Chemistry, 87*, 509–521.

Schauer, N., Semel, Y., Roessner, U., et al. (2006). Comprehensive metabolic profiling and phenotyping of interspecific introgression lines for tomato improvement. *Nature Biotechnology, 24*, 447–454.

Shannon, P., Markiel, A., Ozier, O., et al. 2002. Cytoscape: A software environment for integrated models of biomolecular interaction networks. In *Proceedings of the 3rd international conference on systems biology, ICSB 2002* (pp. 2498–2504), Stockholm, Sweden.

Shulaev, V., Cortes, D., Miller, G., & Mittler, R. (2008). Metabolomics for plant stress response. *Physiologia Plantarum, 132*, 199–208.

Stepansky, A., Kovalski, I., Schaffer, A. A., & Perl-Treves, R. (1999). Variation in sugar levels and invertase activity in mature fruit representing a broad spectrum of *Cucumis melo* genotypes. *Genetic Resources and Crop Evolution, 46*, 53–62.

Stewart, D., Sheperd, L. V. T., Hall, R. D., & Fraser, P. D. (2011). Crops and tasty, nutritious food: How can metabolomics help? In R. D. Hall (Ed.), *Biology of plant metabolomics* (pp. 181–217). Chichester: Wiley-Blackwell.

Strehmel, N., Hummel, J., Erban, A., Strassburg, K., & Kopka, J. (2008). Retention index thresholds for compound matching in GC-MS metabolite profiling. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences, 871*, 182–190.

Sumner, L. W., Amberg, A., Barrett, D., et al. (2007). Proposed minimum reporting standards for chemical analysis. *Metabolomics, 3*, 211–221.

Sumner, L. W., Mendes, P., & Dixon, R. A. (2003). Plant metabolomics: large-scale phytochemistry in the functional genomics era. *Phytochemistry, 62*, 817–836.

Sweetman, C., Deluc, L. G., Cramer, G. R., Ford, C. M., & Soole, K. L. (2009). Regulation of malate metabolism in grape berry and other developing fruits. *Phytochemistry, 70*, 1329–1344.

Tarazona-Díaz, M. P., Viegas, J., Moldao-Martins, M., & Aguayo, E. (2010). Bioactive compounds from flesh and by-product of fresh-cut watermelon cultivars. *Journal of the Science of Food and Agriculture, 91*, 805–812.

Taureilles-Saurel, C., Romieu, C. G., Robin, J.-P., & Flanzy, C. (1995). Grape (*Vitis vinifera* L.) malate dehydrogenase. II. Characterization of the major mitochondrial and cytosolic isoforms and their role in ripening. *American Journal of Enology and Viticulture, 46*, 29–36.

Tikunov, Y., Laptenok, S., Hall, R., Bovy, A., & de Vos, R. (2012). MSClust: A tool for unsupervised mass spectra extraction of chromatography–mass spectrometry ion-wise aligned data. *Metabolomics*. doi:10.1007/s11306-011-0368-2.

Tikunov, Y., Lommen, A., de Vos, C. H. R., et al. (2005). A novel approach for nontargeted data analysis for metabolomics. Large-scale profiling of tomato fruit volatiles. *Plant Physiology, 139*, 1125–1137.

Verhoeven, H. A., Jonker, H., de Vos, R. C. H., & Hall, R. D. (2012). Solid-phase micro-extraction (SPME) GC-MS analysis of natural volatile components in melon and rice. In N. G. Hardy & R. D. Hall (Eds.), *Plant metabolomics methods*. Ithaca: Humana Press.

Vincent, J. B. (2003). Recent advances in the biochemistry of chromium(III). *Journal of Trace Elements in Experimental Medicine, 16*, 227–236.

Wada, M. (1930). Über citrullin, eine neue aminosäure im presssaft der wassermelone. *Citrullus vulgaris Schrad. Biochemische Zeitschrift, 224*, 420.

Ward, J. L., Forcat, S., Beckmann, M., et al. (2010). The metabolic transition during disease following infection of *Arabidopsis thaliana* by *Pseudomonas syringae* pv. tomato. *Plant Journal, 63*, 443–457.

Weckwerth, W., Loureiro, M. E., Wenzel, K., & Fiehn, O. (2004). Differential metabolic networks unravel the effects of silent plant phenotypes. *Proceedings of the National academy of Sciences of the United States of America, 101*, 7809–7814.