JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Phytochemical Diversity in Tubers of Potato Cultivars and Landraces Using a GC-MS Metabolomics Approach

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Phytochemical diversity with respect to a range of polar (including amino acids, organic acids, sugars, and sugar alcohols) and nonpolar (including fatty acids, alkanols, and sterols) metabolites was examined within tubers from a total of 29 genetically diverse potato cultivars and Chilean landraces using a metabolomics approach by gas chromatography−mass spectrometry. From principal component analysis of the polar and nonpolar metabolite data there was insufficient variation to differentiate the majority of cultivars and landraces. Analysis of all polar metabolite profiles revealed separation of two cultivars (Glenna and Morag) from the other cultivars and landraces and a separate cluster of one landrace line, largely due to higher levels of sugars. Pentland Javelin was distinct in containing high levels of many amino acids. The two *Solanum tuberosum* group phureja cultivars (Inca Sun and Mayan Gold) were not particularly similar and were not separated from the *S. tuberosum* group tuberosum cultivars. Analysis of the nonpolar metabolite data revealed partial separation of two landrace lines and, on the basis of some minor fatty acids, Mayan Gold was distinct. The differences in metabolite profiles are considered in terms of the taxonomy and breeding history of the cultivars and possible influences from other factors such as developmental stage of the tuber. With a view to exploring biosynthetic links between metabolites, a pairwise correlation analysis was performed on all metabolites. The significance of high correlations between many amino acids and between several nonpolar metabolites is discussed.

KEYWORDS: Gas chromatography−mass spectrometry; metabolic profiling; metabolomics; potato; *Solanum tuberosum*; tubers

INTRODUCTION

Potato is the crop with the fourth highest production in the world (*1*), and the level of production is increasing annually. In potato, considerable effort is being put into improving the nutritional value and organoleptic properties such as texture and taste (*2, 3*). In addition, the issues of sustainability and climate change are increasingly becoming overarching factors that affect breeding programs and therefore fundamental potato research (*4, 5*). Thus, our evaluation of phytochemical diversity was intended to provide data relevant to potato quality that could be utilized in breeding programs.

Using targeted analyses, a wealth of information has been accumulated on selected metabolites, including amino acids (*6*), ascorbic acid (*7*), carotenoids (*8, 9*), fatty acids (*10*), glycoalkaloids (*11*), and sugars (*12, 13*), in tubers of a range of cultivars and genotypes. Analytical technology and the associated software have advanced significantly in the past decade, which means that the ability now exists to simultaneously analyze multiple, non- (biochemical) class specific metabolites (metabolomics), thereby facilitating a more comprehensive overview of global metabolite changes. The adoption of this approach to crops could be the key to addressing issues such as functionality, disease resistance, and climate adaptation.

Metabolomics involves the application of broad-spectrum analytical technologies, notably proton nuclear magnetic resonance (^{1}H NMR) spectroscopy (*14*), gas chromatography−mass spectrometry [GC-MS (*15, 16*)], and liquid chromatography−electrospray ionization−mass spectrometry [LC-ESI-MS; (*17*)]. These can be applied to a variety of plant tissue and cell types (*18, 19*).

Potato has been the subject of many metabolomic studies (*14*−*16, 20*−*25*). A GC-MS method was developed for analyzing polar metabolites (amino acids, aromatic amines, organic

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10.1021/jf801370b CCC: $40.75
Published on Web 10/21/2008

acids, sugars, and sugar alcohols) in potato tubers (*15*) and was applied to study the effects of environment and genetic modifications in sucrose and starch metabolism (*15, 23*). A similar approach was used to study the effects in potato plants with genetic modifications in fructokinase activity (*21*) and in 14-3-3 protein levels (*24*), for determining whether tubers from genetically modified plants were comparable with conventional cultivars (*20*) and for examining diurnal metabolite patterns in potato leaves (*25*). The effects of genetic modifications on tuber metabolites have also been examined by ^{1}H NMR, high-performance liquid chromatography with ultraviolet detection (*14*), and flow injection electrospray ionization−mass spectrometry (FIE-MS) (*20, 22*). Recently, we evaluated the advantages and limitations of a GC-MS method for measuring metabolites in freeze-dried tuber material (*16*).

In this study the aim was to use GC-MS to explore phytochemical diversity of low molecular weight polar and nonpolar metabolites in tubers from a diverse range of potato germplasm. Modern and old European cultivars, mainly from *Solanum tuberosum* group Tuberosum but also from *S. tuberosum* group Phureja, and Chilean landraces were included. Apart from providing fundamental information, it is hoped that the data will be of use to identify germplasm with suitable levels of specific metabolites relevant to potato quality that can be utilized in breeding programs. A secondary aim was to use the data to explore tuber biochemistry, with a view to detecting any unexpected close linkages between metabolites, by applying correlation analysis. A final objective, to be the subject of a future publication, is to use the data to form the basis for the background against which unintended effects due to genetic modifications can be judged, to establish whether the levels of metabolites of modified plants fall within the range of conventional cultivars.

MATERIALS AND METHODS

Plant Material. A total of 27 tetraploid cultivars and landraces, representative of *S. tuberosum* group Tuberosum, were included in the study. There were 16 modern cultivars, included for their agronomic traits such as high yield and disease resistance, often with genes introgressed from wild species by deliberate breeding (Anya, Barbara, Brodick, Cara, Desiree, Eden, Glenna, Maris Piper, Morag, Pentland Crown, Pentland Dell, Pentland Javelin, Record, Shelagh, Stirling, and the breeder's line 91 MT 46 E 15). Four others were cultivars registered at least 100 years ago and have no disease resistance deliberately introgressed from wild species (Fortyfold, Golden Wonder, Lumpers, and Pink Fir Apple). A total of seven Chilean landraces, comprising three lines [CPC 3369(1), CPC 3369(3), and CPC 3369(4)], which had origins as seedlings from accession no. CPC 3369, three lines [CPC 5646(1), CPC 5646 (2), and CPC 5646(4)] from CPC 5646, and one line [CPC 3302(2)] from CPC 3302, were included. As each of these Commonwealth Potato Collection (CPC) lines was created by self-pollination of a single clone and maintained by intercrossing seedlings, each is a genetically unique clone but related by descent to those with the same accession number. Diploid cultivars (Inca Sun and Mayan Gold) of *S. tuberosum* group Phureja, derived from accessions of the CPC and selected to yield well in the U.K. environment, were also included because their appearance and flavor are distinct from those of Tuberosum cultivars (*2*). The Desiree reference material was grown at a different time from the material grown for the present study and was the same material described in an earlier publication (*16*).

Lines were planted on April 23, 2002, in four replicate plots in a randomized block design using standard agronomic practices at SCRI. Within the field there were four plots, each containing all lines that were randomized so that the orders were different in the four plots. Each line was grown in a single drill containing five plants. The tubers from the five plants were bulked to represent a single replicate, resulting in a total of four replicates for each line. Tubers from all lines were harvested at maturity on September 19, 2002, following standard industrial practices: at 2 and 3 weeks prior to harvest, sulfuric acid was applied to burn down foliage and, after harvest, tubers were stored at ambient temperature (ca. 8−12 °C) for 2 weeks.

Samples were freeze-dried and ground in a laboratory mill fitted with a 1 mm screen, and the resulting powdered samples were stored in the dark at −20 °C until used for metabolite analysis.

Chemicals. Standards and reagents were purchased from Sigma-Aldrich Co. Ltd. (Poole, U.K.). Solvents were of Distol grade and were supplied by Fischer Scientific U.K. (Loughborough, U.K.).

Extraction of Polar and Nonpolar Metabolites from Freeze-Dried Powder. Internal standards (IS) for polar metabolites (100 μL of aqueous ribitol, 2 mg mL^{-1}) and nonpolar metabolites (100 μL of methanolic methyl nonadecanoate, 0.2 mg mL^{-1}) were added to powdered freeze-dried potato tuber (100 mg) in a glass culture tube (125 × 16 mm). Methanol (3 mL) was added, and the mixture was shaken vigorously on a vortex shaker at 30 °C for 30 min. Water (0.75 mL) and chloroform (6 mL) were added sequentially, and after each addition, the mixture was shaken at 30 °C for a further 30 min. Finally, more water (1.5 mL) was added, and the mixture was shaken by hand and then separated by centrifugation into upper (polar) and lower (nonpolar) fractions. Samples were stored at −20 °C pending further processing. Polar fractions were stored directly, whereas nonpolar fractions were first evaporated to dryness and dissolved in isohexane containing 50 ppm of 2,6-di-*tert*-butyl-4-methylphenol.

Derivatization of Polar Extracts. An aliquot (250 μL) of the polar fraction was evaporated to dryness using a centrifugal evaporator and oximated with methoxylamine hydrochloride (20 mg mL^{-1}) in anhydrous pyridine (80 μL) at 100 °C for 45 min. Samples were then silylated at 37 °C for 30 min with 80 μL of *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA). A subsample (40 μL) was taken and added to an autosampler vial containing a mixture of *n*-alkanes (undecane, tridecane, hexadecane, eicosane, tetracosane, triacontane, tetratriacontane, and octatriacontane) to serve as retention index (RI) markers. The sample was diluted with pyridine (1:1) and analyzed by GC-MS.

Derivatization of Nonpolar Extracts. The entire nonpolar fraction was evaporated to dryness and transesterified at 50 °C overnight with 1% (v/v) methanolic sulfuric acid (2 mL). Sodium chloride (5 mL, 5% w/v) and chloroform (3 mL) were added, and the mixture was shaken and left to separate into two layers. The upper aqueous layer was discarded, and the lower chloroform layer was shaken with 2% (w/v) potassium bicarbonate (3 mL). The lower chloroform layer was dried over anhydrous sodium sulfate and then evaporated to dryness. The extract was solubilized in chloroform (50 μL), pyridine (10 μL) was added, and silylation was achieved with MSTFA (80 μL) at 37 °C for 30 min. A subsample (40 μL) was prepared for analysis by GC-MS as described for the polar fraction.

Analysis of Tuber Metabolites by GC−Time-of-Flight (TOF)-MS. The polar and nonpolar samples were analyzed similarly using a Thermo Finnigan Tempus GC-(TOF)-MS system (Thermo, UK). Samples were analyzed in sequences each containing blank controls (subjected to extraction and derivatization but without sample) and reference samples, derived from the same bulked Desiree freeze-dried material, freshly prepared for each sequence of samples. Samples (1 μL) were injected into a programmable temperature vaporizing (PTV) injector with a split of 167:1. The PTV conditions were as follows: injection temperature, 132 °C for 1 min; transfer rate, 14.5 °C s^{-1}; transfer temperature, 320 °C for 1 min; clean rate, 14.5 °C s^{-1}; and clean temperature, 400 °C for 2 min. Chromatography was effected on a DB5-MS column (15 m × 0.25 mm × 0.25 μm; J&W, Folsom, CA) using helium at 1.5 mL min^{-1} (constant flow). The GC temperatures were 100 °C for 2.1 min, increased at 25 °C min^{-1} to 320 °C, and then isothermal for 3.5 min. The GC-MS interface temperature was 250 °C. MS acquisition conditions were electron impact (EI) ionization at 70 eV, solvent delay of 1.3 min, source temperature of 200 °C, and mass range of 35−900 amu at 4 spectra s^{-1}. Acquisition rates were set to give approximately 10 data points across a chromatographic peak. Data were acquired using the Xcalibur software package V. 1.2.

Table 1. Relative Mean Levels of Polar Metabolites[a]

compound[b]	m/z[c]	Rri[d]	CPC 3369(1)	CPC 3369(3)	CPC 3369(4)	CPC 5646(1)	CPC 5646(2)	CPC 5646(4)	CPC 3302(2)	91 MT 46 E 15	Anya	Barbara	Brodick	Cara	Desiree	Eden
amino acids																
alanine $(TMS)_2$	116	1095	0.38	0.52	0.31	0.16	0.15	0.43	0.46	0.24	0.74	0.60	0.73	0.44	0.36	0.64
valine $(TMS)_2$	144	1216	0.72	0.69	0.86	0.46	0.60	0.85	0.58	0.37	0.89	0.63	0.75	0.69	0.57	0.56
leucine $(TMS)_2$	158	1270	1.64	0.92	0.78	0.48	1.22	1.83	0.75	0.36	1.11	0.48	0.97	0.46	0.46	0.61
isoleucine $(TMS)_2$	158	1290	1.10	0.66	0.79	0.46	0.70	1.09	0.55	0.33	0.85	0.58	0.74	0.58	0.57	0.48
proline $(TMS)_2$	142	1293	2.09	1.24	1.87	0.63	2.39	1.71	1.05	0.73	0.66	1.07	1.02	0.60	0.69	0.80
glycine $(TMS)_2$	174	1300	0.79	0.83	0.66	0.41	0.34	0.43	0.56	0.41	1.09	1.00	0.88	1.05	0.81	0.89
serine $(TMS)_3$	204	1366	1.53	0.69	1.00	0.50	0.64	1.01	0.83	0.39	1.53	0.84	1.05	0.64	0.69	0.76
threonine $(TMS)_3$	218	1393	1.91	0.61	0.97	0.41	0.43	0.53	0.48	0.33	1.12	0.61	0.53	0.50	0.64	0.47
β-alanine $(TMS)_3$	248	1438	1.64	1.29	1.43	0.58	0.38	0.75	0.73	0.55	0.90	1.13	0.83	1.07	0.95	1.45
methionine $(TMS)_2$	176	1524	1.09	0.66	0.57	0.54	0.91	1.10	0.79	0.37	1.29	0.80	1.19	0.70	0.74	0.80
oxoproline $(TMS)_2$[e]	156	1525	0.76	0.46	0.59	0.64	0.79	0.57	0.48	0.56	0.66	0.71	0.72	0.53	0.61	0.42
aspartic-acid $(TMS)_3$	232	1527	1.57	0.76	1.23	0.78	1.18	1.20	0.99	0.92	1.12	0.69	0.68	0.49	0.68	0.60
γ-aminobutyric acid $(TMS)_3$	174	1535	1.04	0.87	0.86	0.53	0.55	0.64	0.55	0.60	0.63	0.68	0.57	0.57	0.57	0.88
glutamic acid $(TMS)_3$	246	1618	1.28	0.77	0.84	0.64	0.76	0.57	0.64	0.73	1.04	0.53	0.64	0.37	0.61	0.52
phenylalanine $(TMS)_2$	218	1623	0.59	0.40	0.22	0.25	0.54	0.62	0.32	0.32	1.07	0.55	0.67	0.39	0.51	0.40
asparagine $(TMS)_3$	116	1670	1.30	0.23	0.50	0.32	0.61	0.41	0.36	0.54	1.15	0.48	0.44	0.32	0.46	0.22
lysine $(TMS)_4$	174	1923	1.56	0.63	0.75	0.47	1.04	1.13	0.48	0.39	1.08	0.69	0.58	0.45	0.63	0.44
tyrosine $(TMS)_3$	218	1939	0.51	0.25	0.23	0.20	0.36	0.58	0.31	0.11	0.69	0.40	0.36	0.34	0.50	0.25
organic acids																
2,3-dihydroxypropanoic acid $(TMS)_3$	189	1331	0.76	0.55	0.92	1.06	1.61	1.48	1.13	0.82	0.67	0.83	0.94	0.92	0.87	1.19
fumaric acid $(TMS)_2$	245	1356	4.91	2.13	3.66	2.80	2.93	2.13	2.42	2.12	1.33	1.37	3.25	2.75	0.89	4.39
2-piperidinecarboxylic acid $(TMS)_2$	156	1368	0.24	0.21	0.60	0.68	0.57	1.16	0.57	0.41	0.46	0.22	0.25	0.75	0.31	0.25
malic acid $(TMS)_3$	233	1497	0.90	0.90	1.25	2.76	4.03	2.47	1.90	0.45	0.53	0.98	0.72	3.10	1.10	1.77
2,3,4-trihydroxybutyric acid (threonic acid) $(TMS)_4$	292	1562	0.89	0.72	0.90	1.74	1.63	2.56	2.35	0.79	1.35	0.68	1.38	2.73	0.60	1.86
citric acid $(TMS)_4$	273	1824	0.94	1.00	1.15	0.78	0.91	0.85	0.86	0.87	0.91	0.96	0.60	0.90	0.85	0.68
caffeic acid $(TMS)_3$	396	2138	0.77	0.44	0.57	1.03	2.21	2.18	1.72	0.59	1.52	1.35	0.76	0.71	1.36	1.42
sugars																
dehydroaldohexonic acid $MEOX^f$ $(TMS)_5$	103	1657	0.99	0.57	1.54	1.56	1.32	1.01	1.01	1.21	0.81	3.87	1.28	1.03	1.25	4.45
carbohydrate A	217	1702	1.48	1.26	2.47	1.42	1.68	0.92	0.99	1.00	1.26	0.94	0.82	0.67	1.13	0.54
fructose MEOX $(TMS)_5$	103	1873	0.57	0.49	0.56	0.68	1.11	0.61	0.56	0.39	0.60	1.40	0.32	2.83	1.23	0.86
glucose MEOX $(TMS)_5$	160	1914	0.97	1.08	1.20	1.61	1.55	0.93	1.28	0.69	1.12	3.52	0.54	6.31	1.81	6.92
carbohydrate B	204	2019	1.51	0.99	2.02	2.40	1.90	1.24	1.24	1.08	1.21	3.05	1.05	4.07	3.11	8.31
carbohydrate C	185	2022	0.17	0.82	1.51	1.52	3.32	2.29	0.80	0.53	0.33	0.82	0.20	1.11	0.68	0.60
glucaric or galactaric acid $(TMS)_6$	292	2036	0.96	2.08	2.15	2.51	1.76	1.88	1.89	1.25	1.36	1.37	0.84	1.45	0.10	1.72
carbohydrate D	205	2105	1.87	1.42	1.69	1.97	1.54	1.24	1.35	1.10	1.16	1.76	0.83	2.13	1.45	1.93
carbohydrate E	217	2366	1.57	1.12	1.72	1.85	1.76	1.93	1.80	0.72	1.37	1.26	0.49	1.10	1.14	1.45
polysaccharide F	204	2973	0.98	0.85	2.08	1.34	3.33	2.67	1.65	0.43	0.41	0.81	0.50	0.72	0.72	0.35
polysaccharide G	204	3114	1.36	1.12	2.89	1.80	2.47	2.72	1.93	0.67	1.33	1.64	1.15	1.43	1.63	1.78
sugar alcohols																
glycerol $(TMS)_3$	205	1274	0.62	0.83	1.24	1.11	1.56	1.32	1.09	1.01	0.77	0.98	1.26	1.08	1.01	0.95
inositol $(TMS)_6$	217	2086	1.04	0.97	1.19	1.82	1.26	1.36	1.25	0.89	1.56	1.46	1.81	1.28	1.35	1.54
others																
urea $(TMS)_2$	189	1244	1.81	0.96	1.22	1.31	1.07	1.02	1.23	1.67	1.10	1.16	2.19	1.71	1.30	1.51
phosphate $(TMS)_3$	299	1269	1.00	1.40	1.99	1.52	1.58	1.58	1.61	1.25	0.98	1.17	1.13	1.39	1.25	0.95
dihydroxydihydrofuranone $(TMS)_2$	247	1378	1.09	0.62	1.08	2.30	1.68	2.65	1.75	1.37	1.32	0.91	1.85	2.56	0.59	1.84
putrescine $(TMS)_4$	174	1742	1.55	1.26	1.30	1.28	1.44	1.55	1.03	0.83	1.43	0.80	0.95	1.60	1.14	1.08
α-glycerophosphate $(TMS)_4$	299	1767	0.89	0.81	1.59	1.44	1.54	1.76	1.34	0.83	1.03	1.02	1.15	0.54	1.46	1.03
allantoin $(TMS)_4$	331	1885	1.20	0.65	0.72	0.77	1.73	0.88	0.77	0.84	1.16	0.62	0.64	0.45	0.48	0.68
unidentified																
P1	138	1411	1.18	1.59	4.34	1.88	1.70	0.94	1.26	1.29	1.13	1.21	0.84	1.25	1.79	1.65
P2	292	1751	0.59	0.64	0.59	0.57	2.19	0.99	0.68	2.08	1.22	0.99	0.40	0.69	0.89	0.62
P3	142	1758	0.88	0.43	0.80	0.74	1.05	0.79	0.67	0.50	0.92	1.42	0.32	1.44	1.86	2.48
P4	167	1815	0.25	0.86	1.58	1.68	3.64	2.24	0.92	0.61	0.40	0.74	0.34	0.75	0.67	0.52
P5	174	1858	1.26	1.29	1.39	1.26	1.10	1.36	1.18	1.03	0.95	0.99	3.22	1.57	1.33	0.96
P6	188	1871	1.56	0.54	1.16	1.41	1.18	0.95	0.56	0.74	1.42	1.07	1.66	1.67	2.99	0.78
P7	205	1950	0.76	0.62	0.98	1.21	0.98	0.94	0.88	0.55	0.56	1.77	0.60	2.65	1.46	2.12
P8	174	2121	1.02	0.69	0.92	1.05	0.60	0.63	0.77	0.79	0.80	0.94	0.29	1.67	1.31	1.80
P9	245	2420	1.33	0.66	1.21	1.12	1.88	1.07	0.78	0.61	0.97	1.23	0.51	1.42	1.09	1.22

Data Analysis. A number of Xcalibur raw GC-MS data files were selected as being representative examples for both polar and nonpolar metabolites. These files were used with the AMDIS software package to verify the presence of individual analytes and to deconvolute coeluting peaks. Specific ions characteristic of each metabolite were selected to be used for compound detection in processing methods created using Xcalibur. For each component, including the appropriate IS, a time window was defined relative to an adjacent RI standard, and a selected ion chromatogram (SIC) was generated for each metabolite within the appropriate time window. Response ratios were automatically calculated for each analyte relative to the IS using the calculated SIC areas for both components and were expressed relative to the mean response ratio for Desiree "line 50" (the parental line used for production of genetically modified (GM) material in our laboratory and having a cultivation history different from the Desiree in **Tables 1** and **2**). For any one metabolite, the values in **Tables 1** and **2** represent the relative levels among different lines. However, the relative levels between different metabolites could not be deduced. Processed data were checked and corrected for incorrect integration or assignment of the position of the selected ion before being subjected to further data analysis. Compounds were identified by analysis of standards, comparison with MS libraries and literature data, and extrapolation from data for known compounds. Processed data were subjected to appropriate statistical treatment including analysis of variance (ANOVA) allowing for the block effects, principal component analysis (PCA), and correlation

Table 1 Continued

Fortyfold	Glenna	Golden Wonder	Lumpers	Maris Piper	Morag	Pentland Crown	Pentland Dell	Pentland Javelin	Pink Fir Apple	Record	Shelagh	Stirling	Inca Sun	Mayan Gold	LSD ($P = 0.05$)
0.33	0.31	0.12	1.08	0.64	0.72	0.39	1.54	2.27	0.42	0.78	0.36	0.22	1.12	0.10	0.462
0.39	0.73	0.23	0.62	0.90	0.59	0.54	0.67	1.33	0.58	0.59	0.50	0.60	0.96	0.36	0.320
0.37	0.34	0.22	0.82	1.10	0.81	0.41	0.75	2.13	0.68	0.58	0.32	0.47	1.48	0.44	0.538
0.39	0.48	0.20	0.70	1.02	0.54	0.37	0.58	1.67	0.53	0.49	0.44	0.51	1.08	0.40	0.394
0.49	0.73	0.59	0.73	0.88	0.92	0.52	1.48	1.78	0.54	0.79	0.70	0.62	1.27	0.54	0.472
0.53	0.76	0.26	1.15	1.37	0.71	0.63	1.34	1.80	0.81	0.90	0.85	0.61	0.96	0.27	0.460
0.56	0.64	0.25	1.07	1.50	0.76	0.51	1.50	1.95	0.72	0.91	0.67	0.51	1.94	0.54	0.441
0.43	0.61	0.22	0.48	0.71	0.42	0.44	0.81	1.16	0.57	0.73	0.50	0.33	0.86	0.28	0.351
0.75	1.17	0.47	1.79	1.12	1.39	0.55	1.81	2.15	1.41	1.13	0.98	0.56	0.98	0.21	0.445
0.54	0.69	0.21	0.87	2.03	0.95	0.67	1.22	2.48	0.44	1.00	0.81	0.92	2.17	0.74	0.538
0.39	0.63	0.28	0.75	0.86	0.50	0.72	0.93	1.43	0.56	0.66	0.70	0.71	1.22	0.54	0.392
0.64	0.53	0.60	0.98	0.87	0.56	0.75	1.10	1.11	0.82	0.78	0.62	0.68	0.94	0.54	0.250
0.52	0.80	0.36	1.19	0.77	0.87	0.39	0.80	0.99	0.70	0.86	0.87	0.36	0.77	0.25	0.305
0.63	0.22	0.58	0.77	0.79	0.36	0.38	0.76	0.67	0.79	0.57	0.39	0.30	0.77	0.85	0.375
0.43	0.44	0.21	0.63	0.91	0.47	0.39	0.81	1.27	0.32	0.50	0.38	0.63	1.06	0.48	0.357
0.21	0.29	0.28	0.49	0.60	0.27	0.33	0.66	0.85	0.44	0.36	0.33	0.26	0.76	0.21	0.403
0.40	0.33	0.24	0.56	1.21	0.50	0.64	1.23	2.16	0.49	0.64	0.35	0.46	1.67	0.54	0.640
0.23	0.20	0.09	0.37	0.39	0.28	0.27	0.41	1.04	0.36	0.28	0.20	0.35	0.77	0.23	0.268
0.78	1.28	0.55	0.92	0.98	1.97	0.74	0.85	1.07	0.68	0.75	0.92	1.03	1.63	1.34	0.455
2.58	2.24	1.38	1.97	6.34	4.05	2.22	2.10	1.46	1.48	2.41	2.79	3.58	3.15	2.47	1.390
0.31	0.35	0.25	0.23	0.16	0.22	0.17	0.21	0.23	1.04	0.21	0.38	0.29	1.34	0.39	0.398
1.14	1.77	0.87	0.99	2.27	1.79	2.70	1.67	0.94	0.86	1.12	1.79	2.23	1.01	1.23	0.590
0.70	1.36	0.75	0.97	2.86	2.28	1.19	1.38	3.02	0.94	1.25	1.36	1.41	1.93	0.59	0.782
0.69	0.79	0.91	0.99	0.98	0.68	0.75	0.68	0.99	0.97	0.79	0.85	0.68	0.91	1.07	0.199
1.33	1.44	1.21	0.82	1.47	1.26	0.68	0.85	1.34	2.13	1.49	2.38	1.01	0.69	0.32	0.693
0.97	5.82	0.82	1.57	1.26	1.64	2.83	1.14	2.82	1.94	1.00	1.80	3.16	1.03	1.40	2.483
0.98	0.80	0.90	1.16	0.85	0.72	0.93	1.31	1.00	1.80	1.02	1.18	0.77	0.84	0.65	0.640
0.37	2.02	0.51	1.23	0.96	0.77	0.93	1.19	0.61	0.68	0.65	2.29	2.35	0.43	0.55	0.599
1.04	16.43	0.90	1.70	1.45	11.38	3.67	2.27	1.49	1.28	1.21	5.24	4.73	0.87	0.89	4.010
1.09	15.27	1.25	1.89	1.72	10.88	2.89	2.31	2.47	1.77	1.30	4.91	4.55	1.24	1.59	4.765
0.75	0.46	0.86	1.49	0.30	0.32	0.84	0.83	0.62	1.03	0.45	0.75	0.62	0.67	0.34	0.660
1.39	2.78	1.49	1.24	2.05	1.35	1.05	1.21	2.00	1.86	1.48	1.37	2.09	1.79	1.51	0.716
1.70	2.48	1.23	1.27	1.81	1.29	1.87	1.31	1.42	2.19	1.93	2.98	2.20	1.74	1.98	0.676
0.85	1.20	0.92	1.31	1.08	1.79	0.72	1.05	1.01	1.23	0.85	1.09	1.19	0.88	0.52	0.437
0.70	1.05	1.12	1.03	0.26	0.26	0.77	0.55	0.42	0.40	0.81	0.49	0.58	0.34	0.24	0.690
1.32	1.61	1.31	1.61	1.35	2.54	1.60	1.33	1.67	2.13	0.94	1.40	1.54	1.83	1.09	0.730
0.99	1.04	0.89	1.13	0.82	1.25	0.80	1.31	0.98	1.44	0.71	0.85	0.86	1.58	0.87	0.493
0.78	1.31	1.34	0.98	1.56	1.94	1.84	1.86	1.64	2.03	1.14	2.00	1.32	0.92	0.47	0.450
1.28	0.77	0.82	1.11	1.23	1.42	1.02	2.16	1.70	1.17	0.91	1.67	1.84	1.00	0.75	0.570
0.91	1.05	0.94	1.17	1.57	1.11	1.12	1.00	1.06	1.00	0.93	1.47	1.28	1.09	1.40	0.361
0.95	2.13	0.76	1.18	2.44	2.03	3.15	1.92	3.51	1.49	0.91	1.75	1.74	2.84	0.96	0.769
1.23	0.67	0.82	1.39	1.07	0.91	1.87	1.54	1.36	1.33	1.09	1.67	1.31	1.08	0.81	0.385
0.73	0.91	0.93	1.18	1.04	1.44	0.95	0.94	1.00	1.59	0.75	0.96	1.13	1.06	0.65	0.387
0.50	1.18	0.65	0.58	0.92	0.82	0.45	0.80	0.98	0.48	0.85	0.58	0.56	1.01	0.50	0.373
1.21	4.52	1.01	1.24	0.88	8.30	1.68	2.07	1.63	1.98	1.33	2.18	1.47	0.79	0.87	4.312
0.46	1.55	2.04	0.43	0.78	0.62	0.39	0.51	0.56	0.77	1.09	1.94	0.81	1.11	0.80	0.459
0.36	5.19	0.45	1.00	0.95	3.27	1.27	1.29	2.06	0.64	0.65	1.43	1.64	0.79	0.54	1.308
0.74	0.39	0.94	1.02	0.32	0.31	0.94	0.87	0.58	1.10	0.46	0.70	0.52	0.74	0.44	0.639
0.67	0.96	0.57	1.91	1.36	0.79	1.57	1.14	1.84	1.07	0.77	0.72	0.87	1.92	2.00	1.457
0.47	1.37	0.68	1.18	0.98	0.82	0.71	1.12	1.57	1.01	0.61	1.32	1.40	0.88	1.10	0.949
0.61	4.27	0.54	1.24	0.90	2.60	1.66	1.33	1.06	1.29	0.51	2.06	3.07	0.70	0.96	1.112
0.65	5.59	0.59	0.69	0.58	6.19	1.99	1.53	1.24	0.68	0.86	2.18	1.86	0.71	0.62	2.128
0.86	1.35	0.82	1.57	0.98	1.38	0.79	1.27	1.15	1.28	0.85	1.69	1.13	1.23	0.62	0.506

[a] Values are response ratios relative to Desiree ("line 50"). [b] MEOX, methyloxime; TMS, trimethylsilyl. [c] Masses shown are those of the ion(s) selected for identification and quantification of individual derivatized metabolites. [d] Values shown are relative retention indices (RRi) based on linear interpolation of retention times between alkane retention standards, *n*-triacontane, for example, having a RRi value of 3000. [e] Oxoproline is derived from glutamic acid during the methyloximation step. [f] Probably 2-ketogluconic acid but not confirmed.

analysis. All statistical analyses were carried out using Genstat for Windows, 10th ed.

RESULTS AND DISCUSSION

Polar Metabolites. Twenty-six of the 79 polar metabolites were omitted due to high variability in their measurement. The majority of these metabolites were unidentified, but included glutamine, tryptophan, oxalic acid, and sucrose. Some metabolites such as fructose and glucose methyloximes produced two anomeric peaks, and asparagine produced two peaks corresponding to tri- and tetramethylsilyl derivatives, but only one peak was included in the study (the two peaks were always highly correlated). An initial analysis of the response ratio data by PCA revealed a separate group of 17 samples, all of which belonged to the same GC-MS run sequence (data not shown). Subsequent manipulations of the data omitted these 17 samples, leaving an average of 3 replicates per cultivar or landrace.

Table 2. Relative Mean Levels of Nonpolar Metabolites[a]

compound[b]	*m/z*[c]	Rri[d]	CPC 3369(1)	CPC 3369(3)	CPC 3369(4)	CPC 5646(1)	CPC 5646(2)	CPC 5646(4)	CPC 3302(2)	91 MT 46 E 15	Anya	Barbara	Brodick	Cara	Desiree	Eden
fatty acids																
n-14:0 ME	242	1734	0.74	0.40	0.76	0.85	0.53	0.48	0.63	0.36	0.98	0.56	0.39	0.50	0.50	0.43
n-15:0 ME	256	1836	1.43	1.24	1.52	1.05	1.51	1.05	0.76	0.88	0.94	0.76	1.31	0.95	0.69	0.83
n-16:0 ME	74	1932	1.14	0.72	1.46	1.51	1.53	1.28	1.31	0.84	1.04	0.93	0.82	0.93	0.80	0.81
n-17:0 ME	74	2028	0.84	0.67	0.81	0.73	0.83	0.60	0.50	0.55	0.49	0.55	0.55	0.65	0.56	0.56
n-18:0 ME	298	2132	1.19	0.85	1.19	1.08	1.04	1.09	0.85	1.08	0.60	1.06	0.72	1.52	0.82	0.85
n-20:0 ME	326	2331	1.38	0.92	1.56	1.21	1.19	1.06	0.73	0.95	0.88	0.74	0.67	1.30	0.96	0.85
n-21:0 ME	340	2427	2.01	1.05	2.16	1.24	2.02	1.49	0.94	0.82	1.22	0.65	0.56	0.76	0.73	0.78
n-22:0 ME	354	2537	1.46	0.62	1.87	0.90	1.05	0.84	0.60	0.63	0.71	0.55	0.43	0.82	0.70	0.61
n-23:0 ME	368	2641	2.04	0.84	2.21	1.12	1.44	1.18	0.73	0.77	1.06	0.70	0.56	0.93	0.91	0.74
n-24:0 ME	382	2742	1.47	0.78	1.82	1.22	0.94	0.77	0.55	0.65	0.90	0.65	0.60	1.32	0.86	0.73
n-25:0 ME	396	2842	1.42	0.86	1.75	1.31	1.02	0.79	0.48	0.64	1.16	0.76	0.59	1.24	0.92	0.68
n-26:0 ME	410	2933	0.99	0.76	1.42	2.38	1.54	1.06	0.63	0.91	0.89	0.56	0.90	1.14	0.70	0.97
n-28:0 ME	438	3136	0.57	0.54	0.67	1.10	1.07	0.72	0.37	0.44	1.01	0.41	0.54	0.84	0.62	0.65
n-29:0 ME	452	3237	0.77	0.38	0.64	0.50	0.67	0.59	0.22	0.25	0.69	0.49	0.42	0.34	0.58	0.59
br-15:0 ME	256	1807	0.68	1.45	0.61	1.62	2.54	1.35	0.17	1.28	1.12	0.85	0.75	1.04	0.75	0.99
iso-17 ME	74	1989	0.29	0.91	0.36	1.21	1.20	0.72	0.16	0.83	0.82	0.48	0.90	0.52	0.66	0.53
15:1 ME	222	1818	1.87	1.65	1.26	1.45	1.76	1.50	0.84	1.18	1.02	0.74	0.87	0.41	0.94	1.11
16:1 ME	236	1909	1.39	0.69	1.24	0.99	1.34	0.87	0.61	0.64	0.80	0.91	1.07	0.87	0.79	0.81
18:1 ME	264	2110	1.01	0.70	1.07	1.20	1.17	0.88	0.52	0.64	0.89	0.66	0.64	0.73	0.66	0.59
19:1 ME	278	2168	0.27	0.96	0.35	1.07	1.93	0.48	0.12	1.22	0.82	0.61	1.05	0.47	0.66	0.46
18:2(*n*-6) ME	294	2099	1.70	1.33	1.43	2.07	1.83	1.63	0.94	1.44	1.38	1.20	0.98	1.73	0.83	0.70
20:2 ME	322	2301	2.61	1.72	2.43	3.37	4.93	4.07	2.76	5.21	1.82	2.82	1.66	4.23	2.29	2.23
18:3(*n*-3) ME	292	2105	2.37	2.01	1.50	3.39	3.53	3.00	1.63	1.67	1.93	1.27	0.98	2.34	0.97	0.95
2-hydroxy-16:0 ME (TMS)	343	2127	0.94	1.01	1.62	1.33	1.53	1.43	0.67	1.04	1.53	0.68	0.60	0.80	0.70	0.69
2-hydroxy-24:0 ME (TMS)	411	2909	0.46	0.30	0.87	0.94	0.83	0.73	0.40	0.36	0.52	0.35	0.33	0.38	0.65	0.33
alkanols																
n-21:0 (TMS)	369	2450	0.36	0.19	0.50	0.65	1.16	0.57	0.69	0.40	1.02	0.20	0.26	0.72	0.55	0.33
n-22:0 (TMS)	383	2558	0.74	0.42	0.76	1.07	1.10	0.77	0.75	0.72	0.82	0.39	0.39	0.74	0.52	0.47
n-23:0 (TMS)	397	2658	1.52	0.69	1.15	1.70	2.97	1.54	1.44	1.59	1.53	0.48	0.82	1.26	0.68	0.93
n-24:0 (TMS)	411	2759	1.76	0.78	1.72	1.69	2.04	1.72	1.24	1.37	1.09	0.63	0.87	1.00	0.73	0.95
n-26:0 (TMS)	439	2943	1.45	0.90	1.46	1.80	2.28	1.51	0.78	1.01	1.16	0.60	0.82	0.80	0.74	0.81
n-27:0 (TMS)	453	3039	1.21	1.09	1.21	0.76	1.35	0.97	0.25	0.39	0.91	0.72	0.53	0.38	0.86	0.60
n-28:0 (TMS)	467	3140	0.85	0.71	0.62	0.66	1.22	0.59	0.28	0.49	1.50	0.58	0.52	0.70	1.03	0.51
n-29:0 (TMS)	481	3237	0.92	0.79	0.53	0.38	0.75	0.47	0.14	0.33	0.85	0.79	0.48	0.23	1.02	0.52
sterols																
stigmasterol (TMS)	484	3276	1.57	0.65	2.64	1.03	1.53	1.91	1.01	0.45	1.25	0.74	0.49	0.49	0.92	0.59
fucosterol (TMS)[e]	386	3330	0.78	0.60	0.81	1.19	1.45	2.04	0.70	0.80	1.17	0.44	0.65	0.68	0.65	0.73
β-sitosterol (TMS)	357	3334	0.88	0.88	2.15	1.68	2.36	1.57	0.49	0.89	1.77	0.76	0.45	0.92	1.00	0.81
Δ5-avenasterol (TMS)	386	3346	1.09	0.82	0.88	1.17	1.06	1.18	0.83	0.81	1.05	0.56	0.87	1.62	1.08	1.06
Δ5,24(25)-stigmastadienol (TMS)	386	3369	0.35	0.22	0.73	0.96	1.33	2.58	0.78	0.65	1.08	0.34	0.54	0.56	0.36	0.72
others																
solanid-5-en-ol (TMS)	469	3198	0.28	0.68	0.58	1.19	3.29	1.69	0.45	1.41	1.10	0.48	0.61	0.31	0.53	0.77
3- or 4-Methoxy-4- or 3-hydroxy cinnamic acid ME (TMS) (1)	280	1832	3.67	3.16	2.57	4.89	5.68	4.49	3.57	3.65	5.29	2.14	2.08	3.01	2.12	2.17
3- or 4-methoxy-4- or 3-hydroxy cinnamic acid ME (TMS) (2)	250	1950	1.76	1.28	1.36	2.86	3.37	2.21	2.02	1.64	2.81	1.34	1.21	1.76	1.15	1.15
unidentified																
NP1	314	1655	1.42	1.90	2.07	6.14	4.34	2.36	1.37	1.11	2.45	0.91	1.21	1.67	1.24	1.78
NP2	239	1764	1.59	1.60	1.89	3.40	2.47	2.49	1.54	1.49	2.89	1.54	1.38	1.81	1.45	1.90
NP3	239	1845	1.92	1.48	1.22	2.36	2.41	1.40	1.44	1.35	2.45	1.12	1.00	1.69	1.59	1.19
NP4	259	1895	0.85	0.14	1.09	0.77	0.65	0.75	1.03	0.44	0.80	0.58	0.39	0.53	1.09	0.78
NP5	259	2504	0.52	0.29	1.30	0.55	0.83	0.71	1.11	0.55	0.75	0.63	0.95	0.38	1.20	1.30

After this curation, the polar metabolite response ratios for all remaining samples were analyzed by PCA. Within the plot of the first two scores (**Figure 1A**), representing 39% of the variation, although the replicates of some cultivars and landraces were grouped closely, whereas others were more widespread, the intracultivar variation (plot to plot plus analytical variation) between replicates was considerably less than the overall variation. Indeed, with the first 10 scores, accounting for nearly 80% of the variation, there was no separation of samples according to the four different plots.

The most obvious cluster was that of Glenna and Morag (one of the four replicates did not cluster with the others), which had high positive values in PCA score 2 (PC2). Glucose, carbohydrate B, and P8 were significantly ($P < 0.05$) higher in both Morag and Glenna (**Table 1**) than in the other cultivars (carbohydrate B was not significantly higher in Morag than in Eden) and landraces. Glenna was different from Morag in containing significantly higher levels of a dehydro aldohexonic acid, fructose, glucose, glucaric/ galactaric acid, carbohydrate D, polysaccharide F, and unidentified compounds P2, P3, and P7, whereas Morag was significantly higher in 2,3-dihydroxypropanoic acid, fumaric acid, threonic acid, carbohydrate E, polysaccharide G, inositol, urea, and α-glycerophosphate.

Within the Chilean landraces, an examination of the plot of the first two scores revealed, as expected, that there was generally greater variability between the three different accessions than between the clones of any one accession (**Figure 1A**). Within the PC1 versus PC2 plot there was some evidence of separation of two [CPC 5646(2) and CPC 5646(4)] of the three clones of landrace line CPC 5646 from the other cultivars and landraces, but all three clones formed a distinctive group in PC3 [**Figure 1B**; the separation was less evident for CPC 5646(1)]. In CPC 5646(2) and CPC 5646(4), but not CPC 5646(1), carbohydrate C, polysaccharide F, and unidentified compound P4 were significantly ($P < 0.05$) higher than in all of the cultivars and almost all of the other landraces, but only

Table 2 Continued

Fortyfold	Glenna	Golden Wonder	Lumpers	Maris Piper	Morag	Pentland Crown	Pentland Dell	Pentland Javelin	Pink Fir Apple	Record	Shelagh	Stirling	Inca Sun	Mayan Gold	LSD ($P = 0.05$)
0.52	0.82	0.39	0.87	0.38	0.45	0.46	0.46	0.28	0.59	0.44	0.26	0.67	0.52	0.80	0.275
1.00	1.71	0.90	1.42	0.69	0.63	0.72	0.97	0.84	0.61	1.21	0.66	1.13	0.85	1.04	0.507
0.83	1.37	0.76	1.35	0.79	0.90	0.85	0.66	0.82	1.20	0.68	0.65	0.87	1.24	0.89	0.415
0.66	0.59	0.63	0.67	0.51	0.48	0.55	0.64	0.58	0.45	0.69	0.50	0.63	0.63	0.55	0.253
1.11	0.55	0.96	1.09	0.93	0.78	0.91	0.92	0.81	0.72	0.82	0.73	0.73	1.00	0.70	0.287
0.87	0.86	0.68	1.01	0.93	0.73	0.86	0.84	1.07	0.96	0.93	0.91	0.74	0.87	0.79	0.389
0.91	0.65	0.78	1.08	0.58	0.64	0.71	0.80	0.74	1.07	0.97	0.64	0.63	0.86	0.81	0.490
0.76	0.54	0.64	0.73	0.58	0.56	0.60	0.65	0.55	0.89	0.63	0.60	0.66	0.54	0.54	0.420
0.97	0.76	0.82	1.01	0.65	0.61	0.83	0.92	0.62	1.24	0.89	0.80	0.82	0.72	0.83	0.510
0.91	0.87	1.06	0.84	0.83	0.63	0.93	0.86	0.56	1.29	0.80	1.26	0.89	0.60	0.51	0.419
0.90	1.15	1.01	0.78	0.91	0.56	1.00	0.88	0.53	1.43	0.85	1.56	0.84	0.61	0.59	0.435
1.06	0.96	2.63	1.08	1.09	0.86	0.97	0.86	0.65	1.34	0.91	2.18	1.05	0.90	0.63	0.591
0.60	0.58	0.58	0.64	0.55	0.54	0.81	0.70	0.52	1.15	0.64	1.07	0.52	0.65	0.33	0.299
0.40	0.62	0.20	0.73	0.58	0.36	0.68	0.23	0.25	0.63	0.54	0.82	0.22	0.44	0.14	0.323
1.77	2.57	0.77	0.71	1.49	0.31	0.45	0.87	1.57	1.64	1.48	0.57	0.91	1.57	5.52	1.013
1.03	1.17	0.75	0.71	0.59	0.27	0.48	0.74	1.35	1.13	0.82	0.32	0.85	1.01	2.85	0.583
1.70	1.73	1.47	1.19	1.51	1.03	0.74	0.61	1.00	1.50	1.51	0.67	0.53	1.71	2.85	0.736
0.89	1.55	0.61	1.16	0.70	0.73	0.86	0.84	0.92	0.98	0.68	0.63	0.85	0.95	0.66	0.399
0.87	0.69	0.54	0.94	0.62	0.54	0.77	0.58	0.62	1.02	0.62	0.47	0.47	0.87	0.72	0.318
1.36	1.18	0.40	0.22	0.70	0.12	0.25	0.94	1.75	0.60	1.13	0.32	0.31	1.26	5.01	0.877
1.11	1.50	0.74	1.81	1.14	0.58	1.28	0.39	0.66	1.08	1.10	0.88	0.42	1.26	1.59	0.599
2.00	2.23	2.97	1.68	2.75	1.56	2.35	1.25	1.45	2.67	0.96	3.41	1.98	2.12	3.34	2.162
2.06	1.96	1.11	2.88	1.24	0.60	1.54	0.44	0.85	1.84	1.60	0.97	0.62	1.37	2.23	0.917
1.17	1.11	0.79	1.54	0.78	0.68	0.84	0.76	1.05	0.83	0.97	0.75	0.76	1.40	1.16	0.610
0.44	0.38	0.37	0.51	0.33	0.33	0.30	0.34	0.38	0.57	0.35	0.45	0.39	0.53	0.51	0.289
0.27	0.22	0.41	0.55	0.15	0.41	0.29	0.40	0.19	0.60	0.21	0.21	0.24	0.44	0.34	0.255
0.53	0.52	0.70	0.62	0.32	0.50	0.46	0.55	0.35	0.68	0.46	0.47	0.48	0.51	0.38	0.350
1.09	0.84	1.46	0.92	0.98	1.02	0.49	2.35	0.34	0.91	0.89	1.22	1.41	0.92	1.39	0.546
1.33	0.81	1.79	1.01	0.81	1.04	0.69	1.35	0.59	0.98	0.81	1.23	1.30	0.87	0.82	0.518
1.40	0.59	1.85	1.07	0.85	0.84	0.72	1.04	0.60	1.23	0.93	1.58	1.20	1.03	0.96	0.483
0.99	0.67	0.48	0.92	0.71	0.48	1.05	0.24	0.53	0.71	1.17	1.24	0.68	0.67	0.34	0.458
0.53	0.48	0.32	0.63	0.47	0.48	0.79	0.63	0.65	0.91	0.71	0.59	0.45	0.70	0.43	0.338
0.40	0.82	0.16	0.69	0.69	0.34	0.82	0.19	0.37	0.58	0.90	0.51	0.21	0.49	0.16	0.358
0.90	0.62	0.45	1.01	0.55	0.44	1.00	0.48	0.79	1.06	0.77	0.61	0.63	0.74	0.41	0.606
1.27	1.03	0.61	1.83	0.67	0.75	0.65	0.57	0.90	0.68	0.57	0.67	0.70	1.31	1.19	0.581
1.30	1.68	0.82	2.06	1.13	0.46	0.75	1.18	0.83	1.62	0.84	0.78	0.72	0.92	0.76	0.530
0.76	0.93	0.71	1.29	1.07	0.89	0.97	0.82	1.28	1.08	0.71	1.14	0.85	0.92	1.09	0.391
1.18	0.83	0.68	1.98	0.59	0.59	0.54	0.41	0.86	0.59	0.20	0.53	0.50	0.58	0.94	0.604
1.57	1.95	0.59	0.68	0.19	1.24	0.68	1.00	0.71	1.18	0.75	0.72	1.05	0.87	0.47	0.828
3.44	3.17	4.04	4.66	1.97	1.84	2.59	2.60	1.72	3.26	2.82	1.75	2.16	2.36	3.40	1.833
1.60	1.98	2.26	2.12	0.96	1.27	1.18	1.25	0.85	2.00	1.13	1.06	1.52	2.02	1.72	0.959
3.36	0.36	1.49	1.72	1.73	1.47	2.33	2.18	0.85	0.49	2.22	2.42	2.33	2.50	0.78	2.994
1.77	3.21	3.07	2.38	1.41	1.43	1.90	1.28	2.10	3.20	1.67	2.36	1.64	2.18	0.55	1.154
1.19	1.49	1.74	1.65	0.94	0.95	1.34	1.51	0.87	1.59	1.36	0.99	1.15	1.20	1.44	0.752
0.65	0.16	0.54	0.58	0.66	0.81	0.41	1.47	0.76	0.98	0.42	0.55	1.00	0.90	0.22	0.621
0.50	0.52	0.84	1.22	0.52	2.00	0.53	1.11	0.76	1.74	0.59	0.82	1.50	0.77	0.32	0.500

[a] Values are response ratios relative to Desiree ("line 50"). [b] ME, methyl ester; TMS, trimethylsilyl; *n*, normal (straight chain); *br*, methyl branch in undetermined position; *iso*, *iso* methyl branch; 16:1, e.g., = hexadecenoic acid. [c] Masses shown are those of the ion(s) selected for identification and quantification of individual derivatized metabolites. [d] Values shown are relative retention indices (RRi) based on linear interpolation of retention times between alkane retention standards, *n*-triacontane, for example, having a RRi value of 3000. [e] Probably formed by acid-catalyzed isomerization of Δ5-avenasterol during transesterification.

CPC 5646(2) was significantly higher in proline, malic acid, and allantoin. Within the CPC 5646 grouping, all three clones could also be distinguished by PCA (**Figure 1B**), which is not surprising considering that although the three clones are related, they are genetically distinct. Considering the three clones of CPC 5646 only, CPC 5646(1) contained significantly the lowest levels of some amino acids (leucine, proline, and aspartic acid), caffeic acid, carbohydrate C, and polysaccharide F and the highest level of inositol. CPC 5646(2) was significantly the highest in proline, malic acid, carbohydrate C, allantoin, P2, P4, and P9, and CPC 5646(4) was the highest in 2-piperidenecarboxylic acid and threonic acid.

There was less evidence for the distinctiveness of CPC 3369, but again the three clones were different. Two clones [CPC 3369(1) and CPC 3369(3)] clustered with the majority of the cultivars, whereas the other [CPC 3369(4)] tended to cluster with the CPC 5646 clones (**Figure 1B**). CPC 3369(4) had significantly ($P < 0.05$) higher levels of carbohydrate A and phosphate and, together with CPC 5646(2) and CPC 5646(4), had higher levels of polysaccharides F and G than almost all of the other cultivars and landraces. CPC 3369(4) and CPC 3369(1) had higher levels of proline, but only CPC 3369(1) had higher levels of other amino acids (threonine, aspartic acid, glutamic acid, and asparagine). Within the three clones of CPC 3369, CPC 3369(1) was significantly the highest in some amino acids (leucine, serine, threonine, aspartic acid, glutamic acid, asparagine, and lysine), fumaric acid, urea, and allantoin and lowest in glucaric/ galactaric acid, and phosphate. CPC 3369(3) was

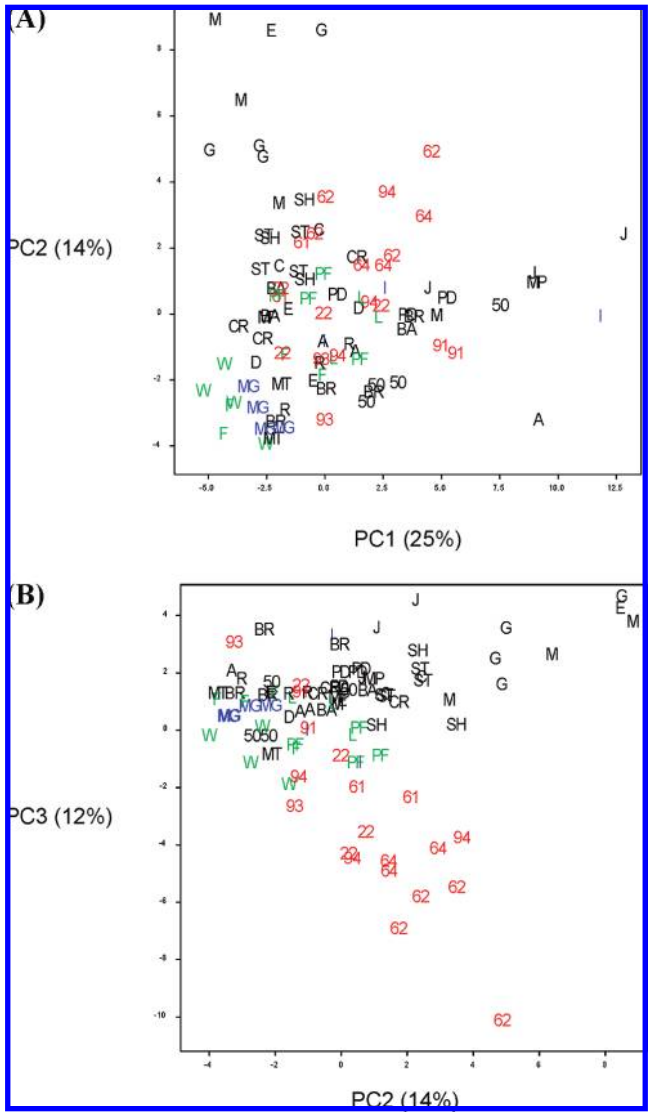

Figure 1. Principal component score plots of (**A**) PC1 vs PC2 and (**B**) PC2 vs PC3 of response ratios of polar metabolites of all *S. tuberosum* cultivars and landraces. Modern *S. tuberosum* group Tuberosum cultivars (black): 50, Desiree ("line 50"); MT, 91 MT 46 E 15; A, Anya; BA, Barbara; BR, Brodick; C, Cara; D, Desiree; E, Eden; G, Glenna; MP, Maris Piper; M, Morag; CR, Pentland Crown; PD, Pentland Dell; J, Pentland Javelin; R, Record; SH, Shelagh; ST, Stirling. Old *S. tuberosum* group Tuberosum cultivars (green): F, Fortyfold; W, Golden Wonder; L, Lumpers; PF, Pink Fir Apple. *S. tuberosum* group Phureja cultivars (blue): I, Inca Sun; MG, Mayan Gold. Chilean landraces (red): 22, CPC 3302(2); 91, CPC 3369(1); 93, CPC 3369(3); 94, CPC 3369(4); 61, CPC 5646(1); 62, CPC 5646(2); 64, CPC 5646(4). The numbers in parentheses refer to different clones of the respective accession numbers.

lowest in proline, threonine, aspartic acid, carbohydrate E, and P9, and CPC 3369(4) was highest in α-glycerophosphate, carbohydrates A and C, polysaccharides F and G, phosphate, and P4. The remaining landrace line, CPC 3302, represented by a single clone [CPC 3302(2)], did not separate as a distinct cluster by PCA.

PC1 of all the samples (**Figure 1A**) suggested variation between the other cultivars (other than Morag and Glenna), but distinct groupings of cultivars was not evident. PCA of all cultivars, excluding Morag and Glenna (and Eden outlier in **Figure 1**), was performed in attempt to show further separations (**Figure 2**). There was relatively little variation within some cultivars, for example, Golden Wonder, Mayan Gold, and

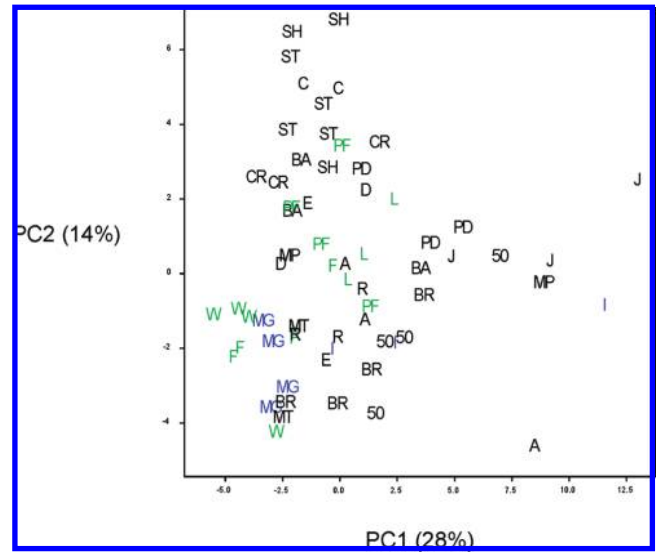

Figure 2. Principal component score plot (PC1 vs PC2) of response ratios of polar metabolites of all cultivars except Glenna and Morag and Eden outlier. Key to abbreviations and color coding of cultivars is as in **Figure 1**.

Stirling, compared to others, for example, Anya, Pentland Javelin, Inca Sun, Maris Piper, and Pink Fir Apple. In PC1 Pentland Javelin was distinct, and a loose cluster of Barbara, Cara, Pentland Crown, Shelagh, and Stirling was evident in PC2.

Pentland Javelin was distinctive in containing high levels of many amino acids. Some (alanine, valine, isoleucine, and tyrosine) were significantly ($P < 0.05$) higher than in all other cultivars and landraces, and others (leucine, glycine, oxoproline, β-alanine, methionine, phenylalanine, and lysine) were significantly higher than in all but one or two cultivars. Dihydroxydihydrofuranone was also high in Pentland Javelin. In contrast, cultivars such as Fortyfold, Golden Wonder, and Mayan Gold, with highly negative scores in PC1, had low levels of many metabolites including amino acids, although the levels were not usually significantly different from many other cultivars and landraces. Fructose, glucose, carbohydrate B, and P7 tended to be higher in Barbara, Cara, Pentland Crown, Shelagh, and Stirling compared to the other cultivars (not including Glenna and Morag), although the differences were usually not significant.

In contrast to Phureja cultivar Mayan Gold, the replicates of the other Phureja cultivar, Inca Sun, exhibited considerable variation in metabolite profiles. These two cultivars were not separated by PCA from the Tuberosum cultivars and shared little similarity to one another, each sharing more similarity with different Tuberosum cultivars (e.g., Mayan Gold clustered with Golden Wonder). There were no metabolites that were significantly different in either Inca Sun or Mayan Gold compared to the other cultivars and landraces, although Inca Sun tended to be high in amino acids. Indeed, Inca Sun had significantly ($P < 0.05$) higher levels of all amino acids (with the exception of glutamic acid), piperidine carboxylic acid, threonic acid, polysaccharide G, glycerol, inositol, dihydroxydihydrofuranone, α-glycerophosphate, allantoin, and P9 than Mayan Gold.

Nonpolar Metabolites. Considering the potato reference materials analyzed with each set of samples, of 52 nonpolar metabolites, the measured levels of 6 metabolites, all unidentified with the exception of *n*-tricosane, were highly variable and were excluded from the study. Using the response ratios of the remaining 46 nonpolar metabolites, all samples were analyzed by PCA. As for the polar metabolites, in the first 10 scores,

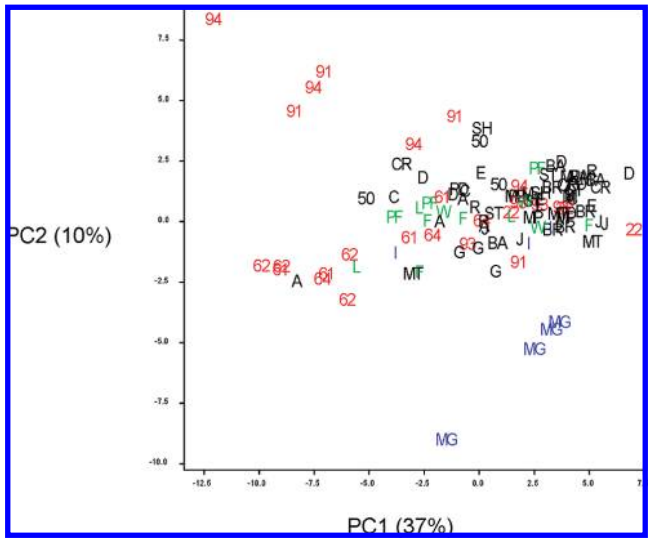

Figure 3. Principal component score plots (PC1 vs PC2) of response ratios of nonpolar metabolites of all cultivars and landraces. Key to abbreviations and color coding of cultivars and landraces is as in **Figure 1**.

accounting for 82% of the total variation, there was no separation of samples according to the four different plots.

The majority of cultivars did not separate by PCA in PC1, PC2 (**Figure 3**), or other scores. Mayan Gold, however, was clearly separated in PC2 and had significantly ($P < 0.05$) higher levels of some minor fatty acids (*br*15:0, *iso*-17:0, 15:1, and 19:1) than all other cultivars and landraces. In a comparative study of the fatty acids of tubers of *S. tuberosum*, *iso*-17 was found to be higher in Phureja cultivars (the study included Mayan Gold but not Inca Sun) than in Tuberosum (*10*). Thus, in contrast to the polar metabolite data, the nonpolar metabolite data highlight the distinctiveness of this Phureja cultivar from the Tuberosum cultivars and landraces. However, the other Phureja cultivar, Inca Sun, was not distinguished.

Within a plot of the first two scores, representing 47% of the variation, there was partial separation of landraces CPC 5646 and CPC 3369 from each other and from the cultivars, but CPC 3302 did not separate from the cultivars. There was considerable variation between the landrace replicates, often making it difficult to differentiate between individual clones. Within CPC 5646, all of the replicates of CPC 5646(2), but only some of CPC 5646(1) and CPC 5646(4), formed a cluster mainly due to PC1. The loadings for PC1 indicated that many metabolites were responsible for the separations in this score. In all CPC 5646 clones the mean replicate values of some fatty acids and alkanols were higher than in the majority of cultivars and landraces, but only a few significant ($P < 0.05$) differences were observed; *n*-21:0 and 18:3(*n*-3) fatty acids, and *n*-21:0−*n*-26:0 and *n*-28:0 alkanols in CPC 5646(2), and 18:3(*n*-3) and 2-hydroxy-24:0 fatty acids in CPC 5646(1). CPC 5646(2) was also significantly higher than most cultivars and landraces in solanid-5-en-ol, 3- or 4-methoxy-4 or -3-hydroxycinnamic acid (2), and β-sitosterol, CPC 5646(4) was elevated in other sterols [fucosterol, stigmasterol, Δ5,24(25)-stigmastadienol], and NP1 was high in CPC 5646(1). Considering only the CPC 5646 clones, CPC 5646(1) was significantly the highest in *n*-14:0 and *n*-26:0 fatty acids, and CPC 5646(2) was highest in *n*-21:0 fatty acid, some alkanols (*n*-21:0, *n*-23:0, *n*-26:0, and *n*-28:0), β-sitosterol, and solanid-5-en-ol. CPC 5646(4) was highest in some sterols [fucosterol and Δ5,24(25)-stigmastadienol)] and lowest in *n*-28:0 fatty acid and NP3.

Two [CPC 3369(1) and CPC 3369(4)] of the three CPC 3369 clones separated out in the plot of PC1 versus PC2 (**Figure 3**), but they were different from the remaining clone [CPC 3369(3)] that clustered with the majority of cultivars. *n*-21:0−*n*-23:0 fatty acids were significantly ($P < 0.05$) elevated in CPC 3369(1) and CPC 3369(4) compared to almost all of the other cultivars and landraces. Among the CPC 3369 clones, CPC 3369(3) was significantly the lowest in many fatty acids (*n*-14:0, *n*-16:0, *n*-18:0− *n*-25:0, 16:1), *n*-24:0 and *n*-26:0 alkanols, stigmasterol, and NP4. CPC 3369(4) was the highest in both hydroxy fatty acids, β-sitosterol, stigmasterol, and NP5.

Further examination of the data revealed little separation of the landraces and cultivars. One exception was Anya, which separated in higher scores and contained significantly ($P < 0.05$) higher levels of two *n*-alkanols (*n*-21:0 and *n*-28:0) than most cultivars and landraces.

Interpreting Differences in Metabolic Profiles among Cultivars and Landraces. The variation in metabolic profile between replicates was greater for some cultivars and landraces than others, indicating that the growth environment affected the profile to various degrees. Although the variation between the replicates of any one line was considerably less than the overall variation, there was insufficient variation to differentiate the majority of lines either singularly or as groups. When groupings were observed, for example, the loose grouping of Barbara, Cara, Stirling, Shelagh, and Pentland Crown or that of Fortyfold, Golden Wonder, and Mayan Gold, they could usually not be rationalized in terms of cultivar pedigree. However, Morag and Glenna, separated from the other cultivars by their polar metabolite profile, are genetically related and both have resistance (derived from the South American wild species *S. vernei*) to the golden (*Globodera rostochienesis*) and white (*G. pallida*) potato cyst nematodes (*26*).

Although they both come from the relatively narrow genepool of Phureja, Inca Sun and Mayan Gold are not related closely by pedigree, which was borne out by the polar and nonpolar metabolite data. Mayan Gold was distinguished from all other cultivars and landraces on the basis of some minor fatty acids. However, the closeness of both cultivars to different Tuberosum cultivars, on the basis of polar metabolite profile (and nonpolar metabolite profile for Inca Sun), is perhaps surprising because Phureja is distinct in many respects. It is diploid, comes from low-altitude sites in the central Andes, and is adapted to multiple cropping in the more humid, milder climate in which it originally grows (*27*). The two Phureja cultivars have yellow flesh, due to high carotenoid levels, a trait less common in Tuberosum, and have distinctive mouthfeel characteristics and intense favorable flavor attributes (*2*).

The polar metabolite profile of Pentland Javelin was distinct, particularly in containing high levels of amino acids. In terms of pedigree there is no obvious reason the metabolite profile of Pentland Javelin should be different; it shares a common parent with Pentland Crown. The metabolite profile will be influenced not only by the genetic distance between cultivars but also by physiological factors such as differences in the stage of maturity, resulting from differences in the rate of tuber development and inherent dormancy between cultivars. Because the tubers of Pentland Javelin develop early (it is a "First Early" cultivar) and because all cultivars were harvested at the same time, the distinct profile may reflect a more advanced development stage of the tubers. It is possible that the tubers from some other cultivars used in this study may have been harvested at different developmental stages, although the majority would have been at a roughly comparable mature stage of development.

The high levels of glucose and carbohydrate B in Morag and Glenna were important in separating these cultivars from the others, but there is no obvious reason for the elevated levels. Reducing sugar levels generally decrease with increasing tuber maturity, often increasing at the end of the season, and they increase during cold storage, the extent of which is cultivar dependent (*28, 29*). Morag and Glenna are early varieties, so they would have been fully mature at harvest, and they were not harvested late in the season. Both cultivars are prone to low-temperature sweetening, but it is unlikely that reducing sugars would have increased for the short time (2 weeks) that the tubers were stored at 8−12 °C.

The different Chilean landrace accessions could be partially distinguished from one another and from the cultivars on the basis of metabolic profile; CPC 5646 was particularly distinct from the cultivars on the basis of polar metabolites. It is difficult to predict the degree of similarity expected between the landraces and cultivars. Although distinct, Chilean landraces have contributed to the genepool in European cultivars (*30*). The different clones within each accession could also be sometimes distinguished; notably, the polar metabolic profile differentiated the three clones of CPC 5646 (**Figure 1B**). This indicates that even between highly related lines the metabolic differences are still under genetic control and that metabolic profiling not only is a powerful technique for separating genetically related plants but also has potential for understanding the underlying biochemical differences between groups of clones.

In a study of the proteomes of cultivars and landraces similar to those included in this study, it is interesting that Glenna, Morag, and Pentland Javelin (as well as Maris Piper) were also distinct from the other cultivars (*31*). However, the major separation was between Phureja, including Inca Sun and Mayan Gold, and Tuberosum cultivars. Similar to metabolomics, only a small percentage of the proteome was examined, but in this case it seems that, fortuitously, proteins that discriminated between the two groups were included. Also in contrast to the present metabolic profiling data, proteome analysis separated Chilean landrace CPC 3302 from the cultivars, but CPC 5646 was not distinct.

Correlation of Metabolite Levels. Using all samples, pairwise correlation analysis was performed on the response ratios of all metabolites. This approach can identify both biosynthetically related (*32*) and coordinately regulated metabolites. Ninety-nine polar and nonpolar metabolites were examined, giving a total of 4851 correlation coefficient values. Two metabolites were considered to be highly correlated if the coefficient had a value of ≥0.7, and on this basis there were 102 highly positively correlated pairs of metabolites. Of these, 70 correlations were between polar metabolites; 14 involved unidentified metabolites, and 56 were between amino acids (**Figure 4A**). The remaining 32 correlations involved nonpolar metabolites, mainly fatty acids, alkanols, and sterols (**Figure 4B**). None of the metabolites were highly negatively correlated, and there were no strong correlations between polar and nonpolar metabolites.

The most striking feature of the data was the extent of correlation within the amino acids. In addition to the 56 correlations with values of ≥0.7, there were a further 20 correlations between 0.6 and 0.7 (**Figure 4A**); that is, over half of the 136 possible correlations between the 17 amino acids studied had values of >0.6. Although many correlations were predictable (e.g., between serine and glycine, phenylalanine and tyrosine, and β-alanine and alanine), others were between amino acids (e.g., serine and lysine, phenylalanine and isoleucine, and

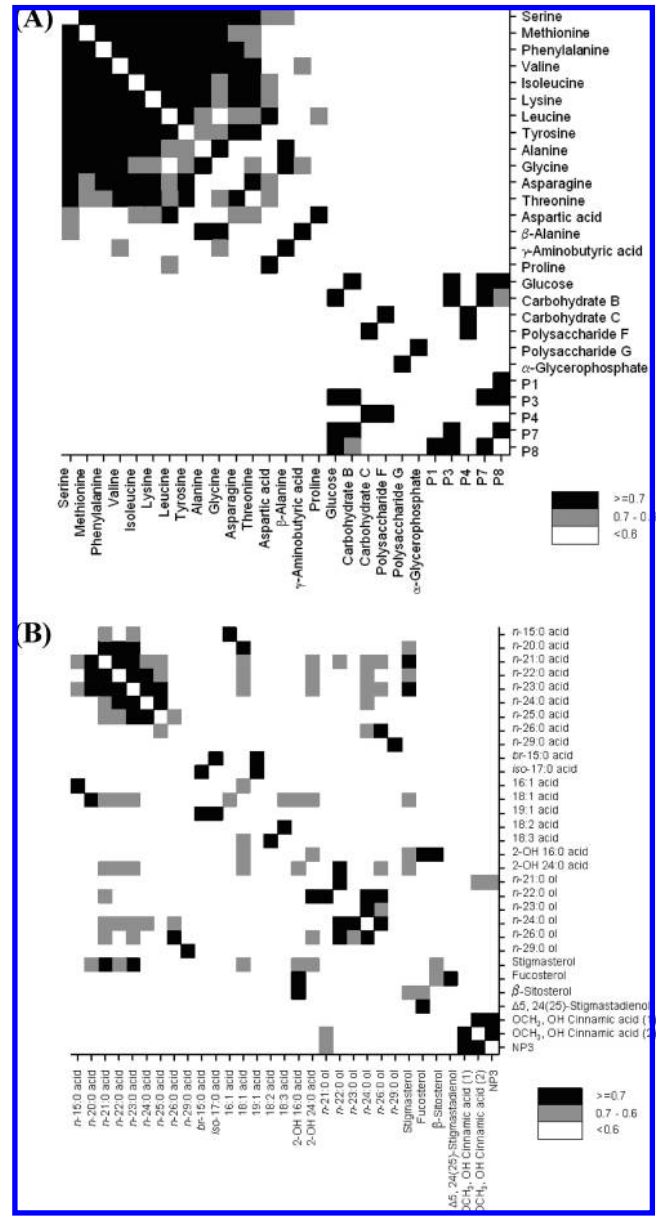

Figure 4. Correlation matrices, based on all cultivars and landraces, of (**A**) polar and (**B**) nonpolar metabolites with high correlation coefficients. Only metabolites that have a correlation coefficient of ≥0.7 with any other metabolite are included. For these metabolites, correlations between 0.6 and 0.7 are also shown.

β-alanine and γ-aminobutyric acid) that are not closely linked biosynthetically. Unexpected correlations between amino acids were previously noted in metabolic profiling studies of potato tubers from different lines modified in sucrose catabolism (*23*) and from identical genotypes grown under uniform and controlled conditions (*32*). It is interesting that in the present study high correlations between amino acids were still evident even when the data set was derived from relatively genetically diverse cultivars and landraces.

Explanations have been sought for the high correlations between amino acids. The high correlation of leucine and isoleucine (also observed in the present study) was proposed by Roessner et al. (*23*) to be due to the sharing of the same terminal enzyme activity (branched-chain amino acid transaminase) and the same cofactor (glutamate) in the biosynthesis of both amino acids. The same authors obtained a hyperbolic curve for the plot of lysine and methionine (*23*), and it was suggested

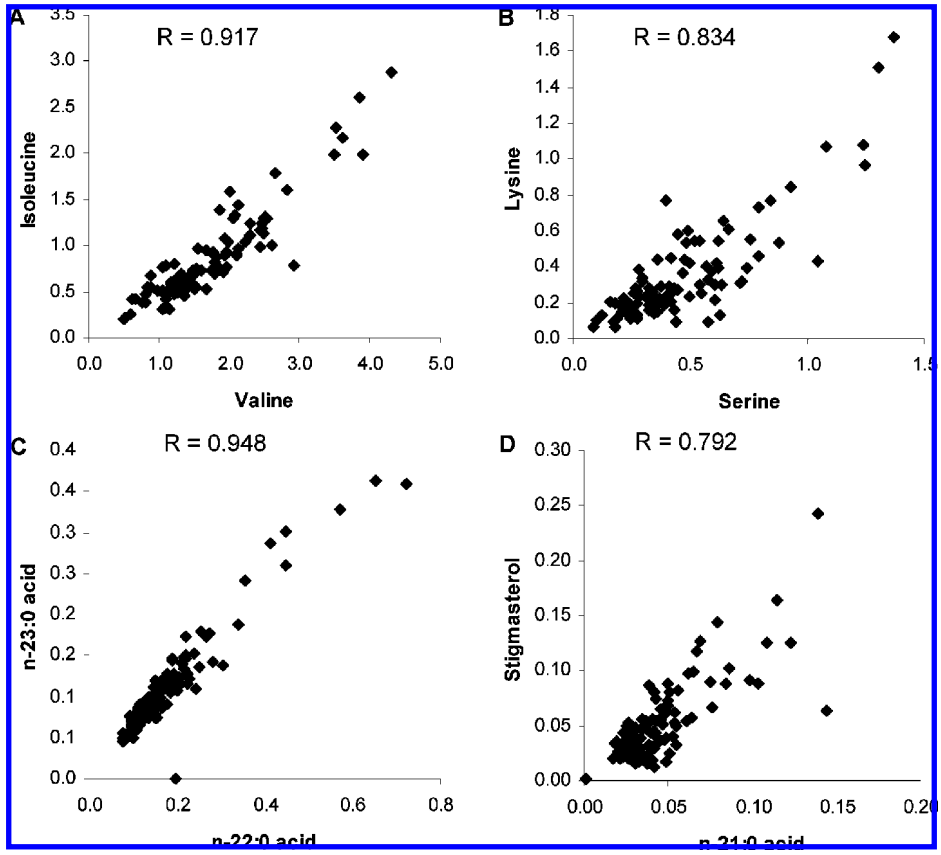

Figure 5. Selected plots of response ratios of highly correlated metabolites.

to be in agreement with a model of feedback regulation under conditions of high flux (*33*). In the present study, a linear relationship was obtained for this correlation. Although the majority of plots showed a linear relationship (e.g., **Figure 5A**), some plots of amino acids gave nonlinear relationships, as observed for lysine and serine (**Figure 5B**).

The high correlations between amino acids support the existence of the controversial mechanism of general amino acid control in plants (*34*). In a study of leaves from crops, including potato, grown under different photosynthetic conditions, the "minor amino acids", especially the branched-chain (isoleucine, leucine, and valine) and aromatic amino acids (phenylalanine and tyrosine), were highly correlated (*35*), as was the case in the present study. Interestingly, in contrast to the present study, methionine was not highly correlated to other amino acids.

Although the majority of amino acids were highly correlated to each other, β-alanine, γ-aminobutyric acid, aspartic acid, glutamic acid, and proline were not strongly correlated with most other amino acids. It is interesting that γ-aminobutyric acid, glutamic acid, and proline are all biosynthetically related as members of the glutamate family.

When the correlations were performed with only the cultivars (omitting the Chilean landraces), the results (data not shown) were similar except that there were even stronger correlations between some amino acids, notably between aspartic acid or proline and other amino acids. The effect of removing the landraces would be to narrow the genetic diversity and, therefore, presumably increase the biochemical similarity of lines within the data set. This might explain the observation.

Within the nonpolar metabolites there were high correlations between saturated fatty acids from C_{20} to C_{25} (**Figure 4B**). There were expected correlations between fatty acids with even carbon numbers and between those with odd carbon numbers, the members of each series being biosynthesized sequentially from the same starting unit by addition of a C_2 unit from malonyl-CoA (*36*). Correlations between the fatty acids, usually differing by only one carbon (e.g., between *n*-22:0 and *n*-23:0; **Figure 5C**), of both series were more surprising because each series uses different acyl coenzyme A starting units. It is interesting that fatty acids of shorter chain length than C_{20} were not highly correlated, neither were those greater than C_{25}; the biosynthesis of fatty acids of C_{18} or less involves different enzymes to the elongases utilized for those of longer chain length, and those above C_{25} may be components of surface waxes rather than membrane lipids. The high correlation of 18:2(*n*-6) and 18:3(*n*-3), but surprisingly less so between these fatty acids and 18:1, reflects the adjacent biosynthetic positions of these fatty acids. The high correlation of pairs such as *n*-15:0/16:1 was less easy to understand, considering that monounsaturated fatty acids are derived from the unsaturated acids of the same carbon chain length.

Alkan-1-ols are formed, via the aldehyde, from the corresponding acyl-CoA of the same chain length, and therefore relationships between fatty acids and alkan-1-ols of the same chain length (*n*-26:0 and *n*-29:0) were not unexpected. Additionally, there were some correlations between different alkan-1-ols from C_{21} to C_{26} but not to the same extent as for correlations between fatty acids.

The high correlations of *n*-20:0−*n*-23:0 fatty acids with stigmasterol (**Figures 4B** and **5D**) and between 2-OH 16:0 and other sterols is unexpected considering that fatty acids and sterols are biosynthetically unrelated. In tubers, such saturated fatty acids are minor moieties of glyceroglycolipids and glycerophospholipids, and 2-hydroxy fatty acids (biosynthesized from the corresponding saturated fatty acid, with which there was not a high correlation) are the major moieties of ceramides

and cerebrosides (*37*). Glyceroglycolipids, glycerophospholipids, cerebrosides/ceramides, and sterols are all components of cell membranes, and the correlations may reflect control mechanisms to regulate the balance of these constituents in cell membranes. It is interesting that there was not a high correlation between sterols and the major saturated (*n*-16:0 and *n*-18:0) or unsaturated [18:2(*n*-6) and 18:3(*n*-3)] fatty acids, all of which are also constituents of membrane lipids.

SUMMARY

In summary, it is evident that, although the variation among the cultivars and landraces was not great, and there was sometimes considerable variation among field replicates, the GC-MS-based metabolomics approach adopted in this study was useful for exploring phytochemical diversity in potato tubers. Differences between cultivars and landraces were observed even down to different clones of the same accession. The possible impact of development stage of the tuber on the metabolic profile should be appreciated, and currently we are examining this in selected Tuberosum and Phureja cultivars. The usefulness of applying correlation analysis for supporting known biochemical links between metabolites and for giving insights into unexpected linkages has also been established.

We are currently using the data set to assess whether the levels of metabolites in the tubers of GM plants fall within the range of conventional cultivars. When this issue was addressed in an earlier study of genetically modified potatoes, using FIE-MS followed by more detailed analysis by LC-ESI-MS and GC-MS (*20*), it was concluded that, apart from the expected changes, the metabolite levels in GM plants fell within the range for the cultivars. The authors stressed the importance of including a range of cultivars, and not just the parental line.

ABBREVIATIONS USED

ANOVA, analysis of variance; br, methyl branch; CPC, Commonwealth Potato Collection; EI, electron impact; FIE-MS, flow injection electrospray ionization−mass spectrometry; GC-MS, gas chromatography−mass spectrometry; GM, genetically modified; ^{1}H NMR, proton nuclear magnetic resonance; IS, internal standard; *iso*, *iso* methyl branch; LC-ESI-MS, liquid chromatography−electrospray ionization−mass spectrometry; ME, methyl ester; MEOX, methyloxime; MSTFA, *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide; n, normal (straight chain); PCA, principal component analysis; PTV, programmable temperature vaporising; RI, retention index; RRi, relative retention index; SIC, selected ion chromatogram; TOF, time-of-flight; TMS, trimethylsilyl.

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Received for review May 2, 2008. Revised manuscript received August 26, 2008. Accepted August 28, 2008. We acknowledge financial support of the Food Standards Agency (Project G02001) and the Rural and Envioronment Research Analysis Directorate of the Scottish government.

JF801370B