SUPPORTING INFORMATION FOR PUBLICATION

Comparative proteome analysis of the tuberous roots of six cassava (*Manihot esculenta*) varieties reveals proteins related to phenotypic traits

Gabriela Justamante Händel Schmitz [a], Jonathan de Magalhães Andrade [b], Teresa Losada Valle [c], Carlos Alberto Labate [d], João Roberto Oliveira do Nascimento [a, e, f, *]

[a] University of São Paulo, Department of Food Science and Experimental Nutrition, FCF, São Paulo, Brazil.

[b] University of Guelph, Department of Food Science, Guelph, Canada.

[c] Agronomic Institute of the State of São Paulo, Campinas, Brazil.

[d] University of São Paulo, Department of Genetics, Piracicaba, Brazil.

[e] Food and Nutrition Research Center (NAPAN), University of São Paulo, São Paulo, Brazil.

[f] Food Research Center (FoRC–CEPID), São Paulo, Brazil

[*]Corresponding author: Departamento de Alimentos e Nutrição Experimental, FCF, Universidade de São Paulo, Av. Lineu Prestes 580, Bloco 14, CEP 05508-000, São Paulo — SP, Brazil, Tel.: +55 11 30913647; fax: +55 11 38154410.Tel: +55-11-30213647.

E-mail: jronasci@usp.br (João R.O. Nascimento)

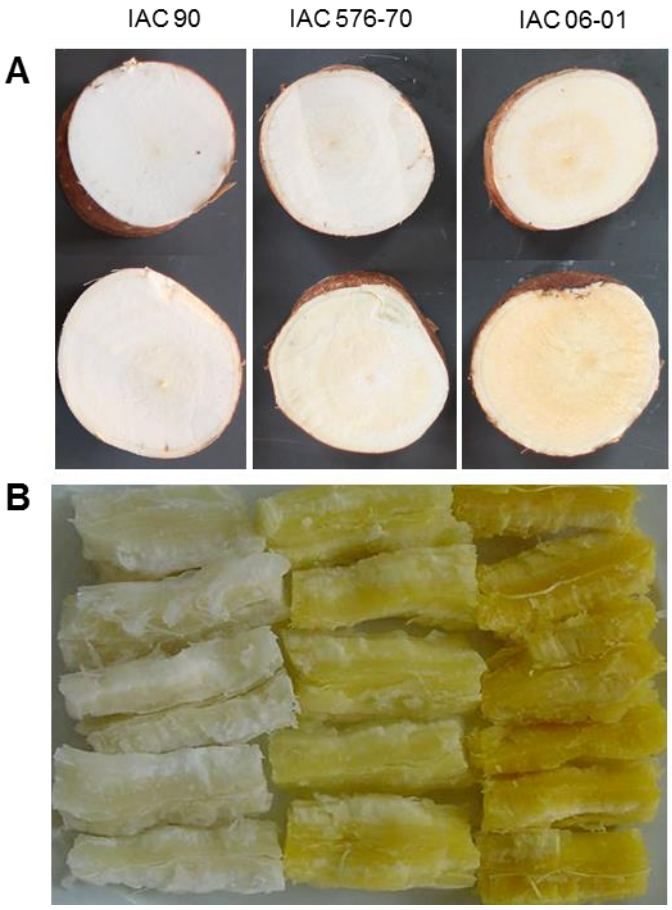

Figure S1. Appearance of the pulp of the tuberous roots of white (IAC-90), white/cream (IAC 576-70) and yellow (IAC 06-01) cassava varieties. (A) Cross sections of the fresh raw tuberous roots. (B) Longitudinal sections of cooked tuberous roots.

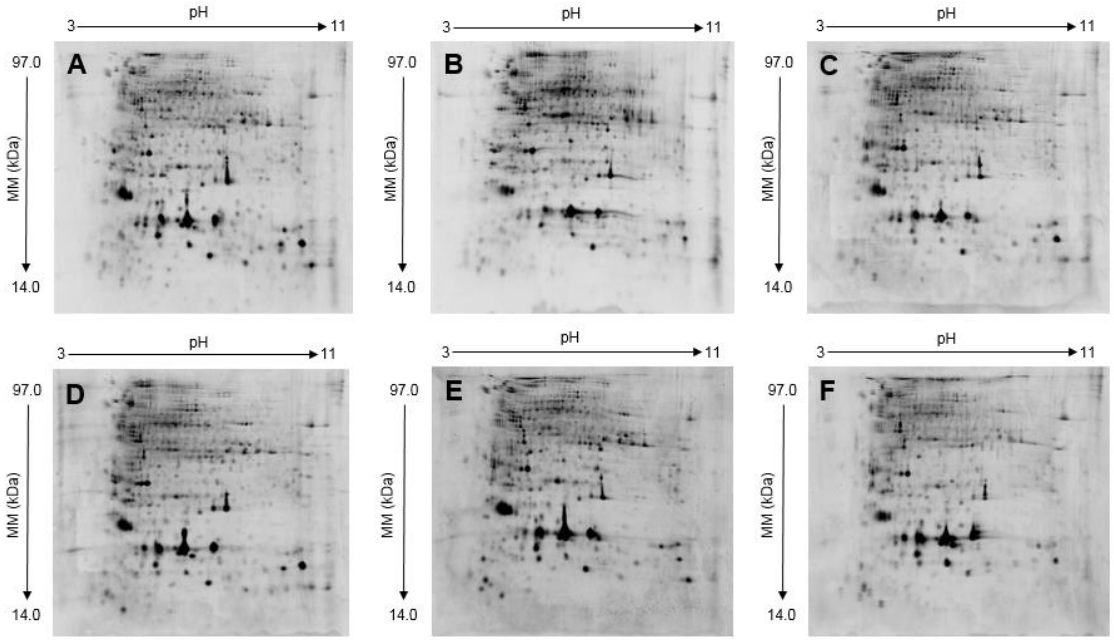

Figure S2. 2-D map of cassava proteome revealed by SYPRO® Ruby staining of 100 µg of protein extract from cultivar Cigana Preta (A), Vassourinha Paulista (B), IAC 12 (C), IAC 576-70 (D), IAC 90 (E), IAC 06-01 (F) separated in pH 3-11 NL strip and 12.5% SDS-PAGE. The molecular mass (MM) standard for SDS electrophoresis is on the left and the pH gradient is indicated above the gel.

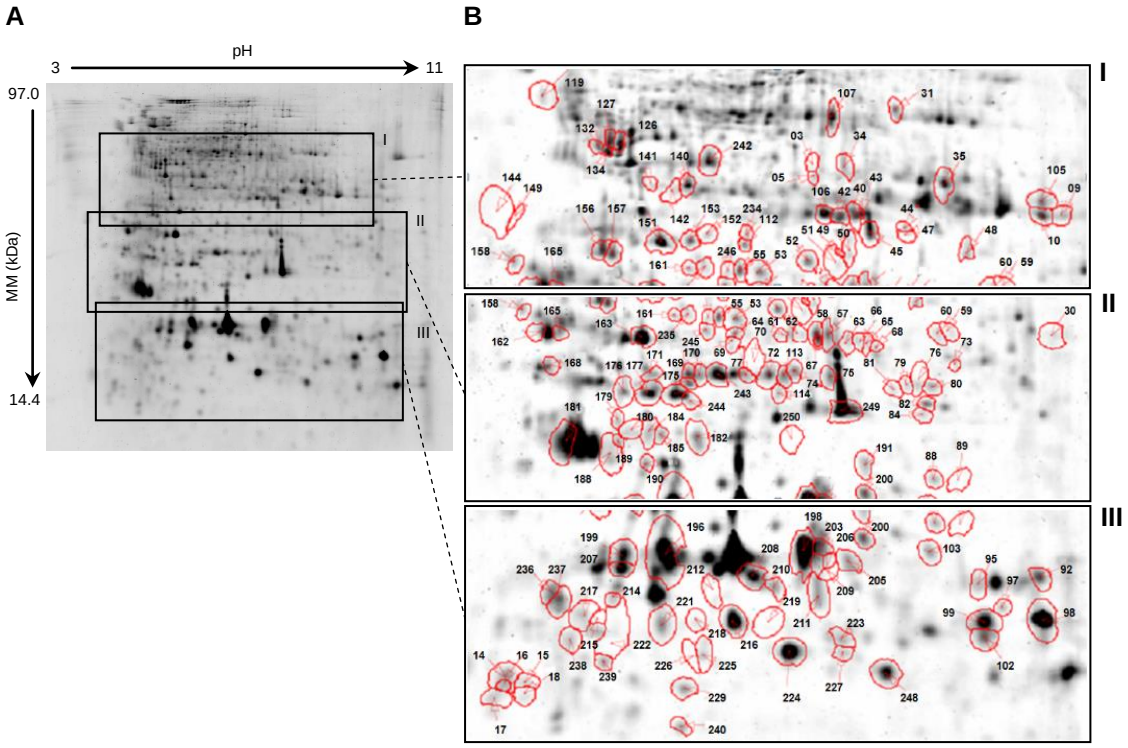

Figure S3. A 2-D map of the cassava tuberous root proteome. (A) Representative protein map revealed by SYPRO® Ruby staining of 100 µg of protein extracts from the cultivar Cigana Preta separated in a pH 3-11 NL strip and 12.5% SDS-PAGE. The position of the molecular mass (MM) standard for SDS electrophoresis is on the left and the pH gradient is indicated above the gel. The areas of the gel with 146 differential spots are boxed and numbered (I, II and III). (B) Differential protein spots of the six cassava varieties revealed by Delta2D 4.2 (DECODON) software are marked by the red line and the identification number. The areas I, II and III from part A were enlarged to enable a better visualization of the spots.

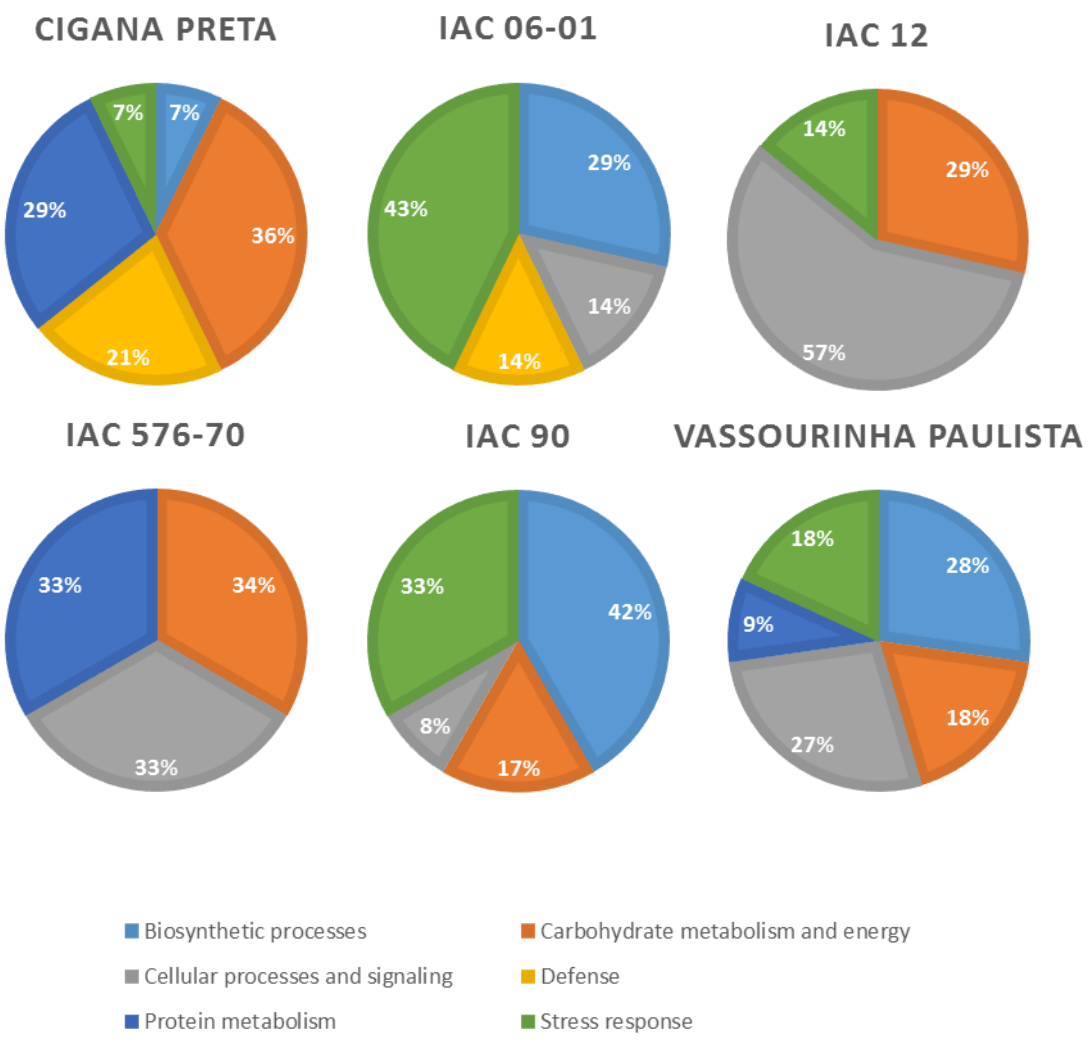

Figure S4. Distribution of the 54 differential proteins from the cassava varieties Cigana Preta, IAC 06-01, IAC 12, IAC 576-70, IAC 90 and Vassourinha Paulista into the following functional categories: stress response, protein metabolism, defense, cellular processes and signaling, carbohydrate metabolism and energy, and biosynthetic processes.

Table S1. Differential proteins from the 2-D map of the six cassava varieties with Mascot scores < 58.

Spot	Protein name	Accession	Specie	Score[a]	MM (kDa)[b]	pI[b]	Variety[c]
Byosynthetic processes							
47	GroES-like zinc-binding dehydrogenase family protein isoform 3	cassava4.1_018148m	*Manihot esculenta*	34	17.10	8.94	IAC 576-70
250	Aldolase-type TIM barrel family protein	cassava4.1_012402m	*Manihot esculenta*	34	33.40	6.26	IAC 06-01
Carbohydrate metabolism and Energy							
149	Glycosyl hydrolase family protein with chitinase insertion domain	cassava4.1_010113m	*Manihot esculenta*	35	40.87	9.60	IAC 576-70
Cellular processes and signaling							
31	U5 small nuclear ribonucleoprotein helicase, putative	cassava4.1_000124m	*Manihot esculenta*	45	197.48	5.77	Vassourinha Paulista
Defense							
152	Malate dehydrogenase	cassava4.1_008657m	*Manihot esculenta*	38	43.53	5.90	IAC 90
214	Cytosolic class II small heat-shock protein HSP17.5	gb|ABO84842.1|	*Rosa hybrid cultivar*	49	17.57	5.95	IAC 90
217	Cytochrome P450, family 71, subfamily B, polypeptide 37	cassava4.1_031263m	*Manihot esculenta*	39	56.84	8.27	IAC 576-70
Protein metabolism							
175	N-terminal nucleophile aminohydrolases (Ntn hydrolases) superfamily protein	cassava4.1_015183m	*Manihot esculenta*	39	37.36	4.64	IAC 576-70
Stress response							
34	Dehydration-responsive element-binding protein 2A	cassava4.1_012655m	*Manihot esculenta*	31	33.85	5.85	IAC 06-01

a) Mascot score for the most significant hits ($p<0.05$).
b) Theoretical molecular weight (MM) and isoelectric point (pI) of identified proteins.
c) Cassava variety in which the spot was more abundant.

Table S2. Medium intensity of pixels density of differential proteins from the 2-D map of the six cassava varieties with significant Mascot scores.

Spot	Variety					
	Vassourinha	Cigana	IAC 12	IAC 576-70	IAC 90	IAC 06-01
10	0.09965	0.28110 *	0.13895	0.14627	0.15461	0.18384
18	0.06065	0.06562	0.11484	0.12511	0.12266	0.19475 *
35	0.62437 *	0.43988	0.44111	0.44392	0.51551	0.28261
42	0.29977	0.28504	0.23951	0.26624	0.23748	0.39875 *
43	0.05409	0.11861 *	0.05931	0.05589	0.06099	0.05967
44	0.02079	0.02931	0.03837	0.12417 *	0.00905	0.11458
45	0.36467 *	0.32190	0.34566	0.25984	0.36242	0.11943
53	0.45800 *	0.16657	0.14830	0.06513	0.27570	0.09712
57	0.26133	0.24695	0.18399	0.11719	0.33743 *	0.23120
72	0.40670	0.41107	0.31261	0.46201	0.52137 *	0.40673
73	0.08530 *	0.04976	0.05375	0.01901	0.03900	0.03390
74	0.24082 *	0.23760	0.22965	0.16280	0.16570	0.14682
75	0.29512	0.27073	0.33711 *	0.25208	0.15207	0.24481
82	0.12711	0.13106 *	0.12069	0.10343	0.07053	0.08478
84	0.11142 *	0.07285	0.08820	0.03643	0.03884	0.03278
92	0.54009	0.34251	0.63763 *	0.47560	0.46657	0.33749
95	0.18982	0.14975	0.13218	0.09009	0.23553 *	0.21116
97	0.09011	0.10842 *	0.09759	0.03494	0.04070	0.04327
98	1.01377	1.90144	1.64455	2.24547 *	0.81283	1.08632
99	0.50527	0.86737 *	0.57180	0.72962	0.27560	0.27044
106	0.32513	0.40786 *	0.25041	0.30940	0.22132	0.27694
107	0.35804	0.39170 *	0.30203	0.36307	0.14322	0.15302
112	0.06826	0.05489	0.06039	0.12111 *	0.05735	0.04695
114	0.11113	0.13160 *	0.06515	0.09143	0.11583	0.07468
127	0.17622	0.22407	0.22844 *	0.18770	0.20173	0.07524
134	0.17189	0.20807	0.05812	0.22554	0.23991 *	0.05424
140	0.54615 *	0.22715	0.30137	0.20249	0.21057	0.25438
151	0.27906	0.50554	0.25043	0.10523	0.66501 *	0.16471
153	0.19944	0.13948	0.22328	0.16119	0.17483	0.30120 *
156	0.14444	0.31687 *	0.16981	0.15847	0.14454	0.17797
157	0.08376	0.28324 *	0.11849	0.12901	0.07169	0.14595
162	0.29546 *	0.11538	0.29380	0.25058	0.20074	0.22767
165	0.26875	0.18762	0.34619 *	0.19194	0.16874	0.12831
168	0.22972	0.12135	0.40546 *	0.25382	0.16992	0.34408
171	0.18089 *	0.15512	0.13569	0.10508	0.12084	0.08399
179	0.08726 *	0.07145	0.05797	0.02504	0.05757	0.03571
181	1.26296	1.54619	1.72017 *	1.54330	1.64191	0.81057
182	0.42085	0.33300	0.30145	0.39884	0.48564 *	0.26027
184	0.21323	0.20075	0.15490	0.08029	0.27061 *	0.12821
188	0.23397	0.26893	0.20374	0.32177	0.72972 *	0.27689

Table S2 (Continued). Medium intensity of pixels density of differential proteins from the 2-D map of the six cassava varieties with significant Mascot scores.

Spot	Variety					
	Vassourinha	**Cigana**	**IAC 12**	**IAC 576-70**	**IAC 90**	**IAC 06-01**
190	0.07274	0.12357	0.02778	0.02934	0.14039 *	0.13771
191	0.05886	0.12506 *	0.02680	0.03977	0.00714	0.03894
198	2.51441	2.46805	2.26433	2.85296	3.03222	3.09064 *
207	0.56836	0.65711	0.76193	0.49296	0.36760	0.94366 *
208	0.99499	1.17169	0.96572	1.04187	0.60699	2.13306 *
211	0.22101	0.28656	0.33633	0.36373	0.34497	0.49539 *
212	0.07577	0.05441	0.05819	0.07975	0.24394 *	0.05988
224	1.26200	1.17235	1.24664	1.17788	1.28193 *	0.92240
225	0.13794	0.09992	0.15356	0.18071	0.21330 *	0.12458
242	0.28961	0.51713 *	0.23727	0.45713	0.38401	0.19940
245	0.06121	0.09849	0.10491 *	0.06135	0.04588	0.04232
246	0.09620 *	0.03207	0.02477	0.01161	0.08892	0.01115
248	0.33544	0.61616 *	0.45465	0.54546	0.30480	0.27331
249	1.88253	1.97428 *	1.30577	1.92693	1.24291	1.23236

***** Cassava variety in which the spot was more abundant.
One-way ANOVA ($p < 0.01$) was applied to compare the varieties and to reveal the significantly different spots.