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Phenolic Acid Content and Composition of Eggplant Fruit in a Germplasm Core Subset

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Abstract. Eggplant (*Solanum melongena* L.) is ranked among the top ten vegetables in terms of oxygen radical absorbance capacity due to its fruit's phenolic constituents. Several potential health promoting effects have been ascribed to plant phenolic phytochemicals. We report here a first evaluation of phenolic acid constituents in eggplant fruit from accessions in the USDA eggplant core subset. The core subset includes 101 accessions of the cultivated eggplant, *S. melongena*, and 14 accessions representing four related eggplant species, *S. aethiopicum* L., *S. anguivi* Lam., *S. incanum* L., and *S. macrocarpon* L. Significant differences in phenolic acid content and composition were evident among the five eggplant species and among genotypes within species. Fourteen compounds separated by HPLC, that were present in many but not all accessions, were identified or tentatively identified as hydroxycinnamic acid (HCA) derivatives based on HPLC elution times, UV absorbance spectra, ES$^-$–MS mass spectra, and in some cases proton NMR data. These phenolics were grouped into five classes: chlorogenic acid isomers, isochlorogenic acid isomers, hydroxycinnamic acid amide conjugates, unidentified caffeic acid conjugates, and acetylated chlorogenic acid isomers. Among *S. melongena* accessions, there was a nearly 20-fold range in total HCA content. Total HCA content in *S. aethiopicum* and *S. macrocarpon* was low relative to *S. melongena*. A *S. anguivi* accession had the highest HCA content among core subset accessions. Chlorogenic acid isomers ranged from 63.4% to 96% of total HCAs in most core accessions. Two atypical accessions, *S. anguivi* PI 319855 and *S. incanum* PI500922, exhibited strikingly different HCA conjugate profiles, which differed from those of all other core subset accessions by the presence of several unique phenolic compounds. Our findings on eggplant fruit phenolic content provide opportunities to improve eggplant fruit quality and nutritive value.

The cultivated eggplant (*Solanum melongena* L.), also known as aubergine, brinjal, or Guinea squash, is a species of considerable economic importance in many parts of the world including Asia, Africa, and the subtropics of India and Central America. It is particularly important in India, China and southeast Asia. Related eggplant allies are also grown. These include *S. aethiopicum*, *S. anomalum*, *S. macrocarpon*, *S. incanum*, *S. nigrum*, *S. gilo* and *S. duplosinuatum* grown in Africa; *S. muricatum*, *S. quitoense*, *S. piliferum* and *S. topiro* in Central and South America; and *S blumei*, *S. indicum*, *S. macrocarpon*, *S. nigrum* and *S. torvum* in southeast Asia (Swarup, 1995). Current evidence suggests that eggplant is native to India, with secondary centers of diversity in other parts of southeast Asia and China (Swarup, 1995).

Eggplant exhibits wide diversity in growth habit, vegetative characters, and floral morphology, and produces fruit with many different shapes, sizes, and colors, depending on the cultivar. The oblong to elongate-shaped purple/black eggplant is used worldwide, but other varieties that differ in color, size, and shape are also known. Eggplant genetic resources have been assessed for resistance against the most serious diseases and pests that affect crop production. Analogous detailed assessments of eggplant nutrient composition are lacking. Characterization of eggplant phenolic compounds has been limited to studies of fruit development and postharvest storage or on the role of these compounds as substrates for polyphenol oxidase in the enzymatic browning of cut or injured tissue (Esteban et al., 1989, 1992; Flick et al., 1978).

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Eggplant is ranked among the top ten vegetables in terms of oxygen radical absorbance capacity (ORAC) (Cao et al., 1996). This is attributed to the fruit's phenolic constituents. More than 4,000 phenolic phytochemicals have been identified (King and Young, 1999). Flavonoids, phenolic acids, and polyphenols are the main classes of dietary phenolics. Flavonoids, which include anthocyanins, are the largest group of plant phenols and the most studied. The black to purple pigmentation of eggplant fruit peel is attributed to anthocyanin content (Sakamura and Obata, 1961). Phenolic acids form a diverse group that includes the widely distributed hydroxybenzoic and hydroxycinnamic acids. Phenolic polymers, commonly known as tannins, are compounds of high molecular weight that are divided into two classes, hydrolyzable and condensed tannins.

Several potential health promoting effects have been ascribed to plant phenolic phytochemicals (Kinsella et al., 1993). Flavonoids and phenolic acids are very effective free radical scavengers (Nakatani et al., 2000). It has therefore been proposed that when consumed, these plant polyphenols contribute to reduced radical-mediated pathogeneses such as carcinogenesis and atherosclerosis (Ames et al., 1993; Sawa et al., 1999). Phenolic compounds extracted from eggplant fruit and administered orally to normal and cholesterol fed rats had a significant hypolipidemic action (Sudheesh et al., 1997). Vinson et al. (1998) determined that vegetables have antioxidant quality comparable to that of pure phenols and superior to that of the antioxidant vitamins A, C, and E. Using USDA per capita consumption data, this group estimated that the average per capita consumption of vegetable phenols in the United States is 218 mg·d^{-1} of catechin equivalents, which equates to three times the recommended intake of vitamins C, E and β-carotene antioxidants.

Only recently has detailed quantification of food phenolics been undertaken, and results thus far indicate high variability, even within a given food. It has been established that esters of hydroxycinnamic acids (HCAs) are the major class of phenolic compounds in egg-

Table 1. Phenolic acid content in fruit of *Solanum aethiopicum*, *S. anguivi*, *S. incanum*, *S. macrocarpon,* and *S. melongena* accessions included in the USDA eggplant core subset.

Accession (PI) [z]	Group 1 [y]	Group 2	Group 3	Group 4	Group 5	Total
			μmol/100 g dry wt (% of total)			
S. aethiopicum						
420230	955 (81.6)	19 (1.5)	23 (1.7)	16 (1.7)	139 (12.6)	1161
441895	994 (90.5)	65 (5.9)	8 (0.8)	16 (1.5)	5 (0.5)	1097
441874	882 (91.5)	40 (4.0)	95 (0.5)	24 (2.5)	5 (0.5)	966
441867	880 (92.2)	45 (4.8)	6 (0.6)	12 (1.2)	6 (0.6)	954
441856	903 (95.1)	30 (3.1)	3 (0.3)	6 (0.7)	2 (0.3)	949
441847	854 (95.1)	26 (2.9)	2 (0.3)	8 (0.9)	3 (0.4)	898
424860	767 (90.4)	39 (4.5)	5 (0.6)	11 (1.3)	5 (0.6)	850
441881	702 (91.0)	35 (4.5)	3 (0.3)	20 (2.6)	3 (0.5)	771
441839	628 (96.0)	10 (1.7)	3 (0.4)	6 (0.9)	3 (0.4)	653
441841	495 (90.7)	23 (4.2)	2 (0.4)	9 (1.6)	2 (0.3)	546
S. anguivi						
179745	6232 (79.1)	70 (0.9)	286 (3.6)	155 (1.9)	1148 (14.4)	7896
319855	1117 (32.4)	226 (6.6)	14 (0.4)	45 (1.3)	316 (9.2)	3443 [x]
S. incanum						
500922	3175 (42.4)	17 (0.2)	708 (9.4)	3 (0.04)	313 (4.2)	7495 [x]
S. macrocarpon						
441914	1010 (73.0)	2 (0.2)	110 (6.0)	3 (0.3)	254 (20.5)	1380
S. melongena						
470273	5762 (84.5)	68 (1.1)	875 (13.3)	47 (0.7)	31 (0.5)	6783
263727	6104 (92.0)	25 (0.4)	452 (6.9)	43 (0.7)	4 (0.1)	6628
593865	5451 (86.1)	24 (0.4)	789 (12.1)	51 (0.9)	35 (0.6)	6350
593875	4792 (80.5)	15 (0.2)	1099 (18.1)	53 (0.9)	17 (0.3)	5976
595220	5145 (87.4)	4 (0.1)	645 (11.0)	37 (0.7)	56 (1.0)	5887
350318	5436 (93.9)	19 (0.3)	258 (4.4)	56 (1.0)	27 (0.4)	5797
223844	5109 (89.0)	11 (0.2)	521 (8.9)	55 (0.9)	48 (0.9)	5745
593886	4843 (85.2)	42 (0.7)	717 (12.6)	56 (1.0)	26 (0.5)	5684
267104	4950 (88.5)	22 (0.4)	599 (9.1)	64 (1.2)	39 (0.8)	5675
491192	5262 (93.7)	39 (0.7)	245 (4.4)	36 (0.6)	32 (0.6)	5614
143410	4418 (83.0)	119 (2.3)	702 (13.6)	58 (1.1)	3 (0.1)	5300
181896	4559 (87.8)	1 (0.1)	499 (9.7)	36 (0.7)	95 (1.8)	5191
188816	4691 (91.7)	63 (1.4)	253 (5.2)	52 (1.1)	36 (0.6)	5096
232078	4360 (86.2)	41 (0.8)	567 (11.5)	27 (0.5)	40 (0.9)	5035
263727	4017 (82.4)	12 (0.3)	779 (16.0)	59 (1.2)	6 (0.1)	4874
222833	4144 (85.6)	2 (0.1)	607 (12.7)	59 (1.2)	18 (0.4)	4831
155511	4111 (82.9)	76 (1.6)	521 (13.6)	51 (1.0)	45 (0.9)	4807
174372	4101 (84.0)	36 (0.8)	481 (12.2)	46 (1.4)	60 (1.6)	4725
349612	4230 (89.8)	22 (0.4)	357 (7.4)	47 (1.1)	64 (1.4)	4721
169651	4371 (93.8)	47 (1.0)	132 (2.8)	48 (1.0)	60 (1.3)	4659
175914	4169 (89.9)	2 (0.1)	397 (8.5)	42 (0.9)	32 (0.7)	4641
371849	4088 (90.1)	20 (0.4)	365 (8.2)	43 (0.9)	14 (0.3)	4530
351129	4152 (94.3)	5 (0.1)	176 (4.0)	56 (1.3)	16 (0.3)	4405
321018	4032 (92.9)	32 (0.7)	191 (4.2)	65 (1.5)	29 (0.7)	4349
233916	3543 (81.8)	31 (0.8)	640 (14.8)	60 (1.4)	57 (1.3)	4331
179048	3858 (91.1)	19 (0.6)	281 (7.0)	35 (0.9)	22 (0.6)	4215
230334	3829 (92.1)	13 (0.3)	258 (5.7)	54 (1.4)	18 (0.4)	4173
358244	3918 (94.7)	19 (0.5)	133 (3.1)	45 (1.1)	27 (0.6)	4142
169666	3824 (92.7)	33 (0.8)	197 (4.8)	34 (0.8)	40 (0.9)	4128
593748	3321 (81.4)	5 (0.1)	718 (17.8)	30 (0.7)	4 (0.1)	4078
171850	3677 (91.5)	57 (1.4)	205 (4.9)	45 (1.1)	38 (1.0)	4023
419160	3575 (91.1)	2 (0.1)	297 (7.4)	37 (1.0)	22 (0.5)	3934
188816	3507 (89.4)	36 (0.9)	345 (8.6)	40 (1.0)	4 (0.1)	3933
368822	3666 (94.1)	15 (0.4)	142 (3.6)	42 (1.1)	32 (0.9)	3898
358242	3603 (94.1)	27 (0.8)	161 (4.1)	39 (1.0)	3 (0.1)	3833
593858	3121 (82.3)	52 (1.4)	584 (15.3)	39 (1.0)	1 (0.1)	3797
358232	2776 (73.8)	44 (1.2)	909 (23.7)	42 (1.2)	9 (0.2)	3782
177075	3392 (88.2)	62 (1.6)	246 (8.3)	46 (1.2)	30 (0.7)	3777
249568	3372 (91.4)	8 (0.3)	288 (6.5)	27 (0.7)	41 (1.1)	3736
452123	3085 (83.4)	16 (0.4)	576 (15.4)	17 (0.5)	9 (0.2)	3704
105346	3295 (89.1)	32 (0.9)	267 (7.5)	47 (1.3)	41 (1.2)	3683
290467	3454 (94.8)	22 (0.6)	90 (2.5)	39 (1.0)	38 (1.1)	3643
179500	5869 (79.9)	59 (1.7)	620 (16.8)	38 (1.1)	17 (0.5)	3594
234632	2483 (70.2)	42 (1.2)	973 (27.4)	26 (0.7)	17 (0.5)	3541
169659	3233 (91.5)	51 (1.5)	184 (5.2)	27 (0.8)	36 (1.0)	3533

Table 1. (continued).

Accession (PI)[z]	Group 1[y]	Group 2	Group 3	Group 4	Group 5	Total
			μmol/100 g dry wt (% of total)			
220120	2903 (82.9)	21 (0.6)	430 (12.1)	37 (1.0)	116 (3.4)	3507
169650	3200 (91.4)	45 (1.3)	205 (5.8)	23 (0.7)	31 (0.9)	3504
593744	3172 (92.1)	9 (0.3)	176 (5.2)	31 (0.9)	56 (1.6)	3444
204731	2925 (85.1)	81 (2.4)	203 (5.9)	58 (1.7)	174 (5.0)	3442
175913	2975 (87.0)	18 (0.6)	650 (10.6)	29 (0.8)	29 (0.9)	3402
23015	2982 (88.3)	2 (0.1)	344 (9.9)	43 (1.3)	14 (0.4)	3385
593885	2861 (85.5)	21 (0.7)	382 (10.5)	38 (1.1)	79 (2.2)	3381
230333	3101 (92.8)	5 (0.1)	173 (5.1)	57 (1.7)	11 (0.3)	3347
143409	2486 (74.3)	14 (0.4)	745 (23.3)	22 (0.6)	43 (1.3)	3311
169648	3015 (92.9)	20 (0.7)	143 (5.0)	46 (1.5)	25 (0.9)	3251
171851	2653 (82.7)	28 (0.9)	423 (13.2)	39 (1.2)	64 (2.0)	3208
593844	2399 (74.9)	3 (0.1)	745 (23.5)	42 (1.3)	5 (0.2)	3195
Grif1276	2268 (73.6)	43 (1.2)	274 (7.7)	52 (1.6)	481 (15.7)	3125
593820	2389 (76.6)	49 (1.6)	72 (2.3)	67 (2.2)	542 (17.2)	3120
419198	2869 (93.0)	2 (0.1)	182 (5.8)	29 (1.0)	8 (0.3)	3090
213193	2390 (77.4)	55 (1.8)	610 (19.8)	28 (0.9)	4 (0.2)	3089
102727	2842 (92.9)	2 (0.1)	171 (5.5)	22 (0.8)	21 (0.7)	3059
419158	2734 (89.2)	4 (0.1)	265 (8.8)	30 (1.0)	24 (0.8)	3057
140446	2502 (83.2)	11 (0.4)	454 (15.1)	23 (0.8)	15 (0.5)	3006
193599	2733 (90.8)	27 (0.8)	176 (6.1)	31 (1.1)	39 (1.3)	3006
181963	2436 (82.3)	23 (0.9)	413 (14.0)	29 (1.0)	49 (1.7)	2951
391649	2760 (93.7)	5 (0.2)	135 (4.7)	32 (1.1)	14 (0.5)	2945
174367	2538 (85.4)	58 (1.9)	233 (9.4)	38 (1.5)	46 (1.8)	2915
173111	2259 (77.9)	51 (1.8)	554 (19.2)	23 (0.8)	8 (0.3)	2896
176758	2600 (90.0)	2 (0.1)	222 (7.7)	30 (1.0)	36 (1.2)	2891
230335	2703 (93.5)	4 (0.1)	138 (5.0)	32 (1.1)	6 (0.2)	2883
115505	2429 (84.9)	42 (1.4)	325 (11.5)	23 (0.8)	34 (1.2)	2858
593806	2512 (88.7)	26 (0.9)	225 (7.1)	52 (2.0)	31 (1.2)	2846
561139	2695 (95.0)	5 (0.2)	92 (3.2)	30 (1.1)	12 (0.5)	2834
391647	2566 (93.7)	5 (0.2)	123 (4.4)	39 (1.5)	8 (0.3)	2741
391646	2552 (94.5)	5 (0.2)	108 (4.0)	21 (0.8)	14 (0.5)	2701
140456	2338 (87.5)	1 (0.1)	305 (11.4)	18 (0.7)	11 (0.4)	2674
199516	2116 (80.5)	67 (2.5)	413 (15.7)	29 (1.1)	5 (0.2)	2631
436680	2382 (94.0)	6 (0.2)	102 (4.0)	23 (0.9)	21 (0.8)	2533
200881	2272 (90.0)	1 (0.1)	212 (8.4)	24 (0.9)	16 (0.6)	2524
593754	2151 (85.3)	83 (3.3)	218 (8.6)	19 (0.8)	51 (2.0)	2521
251506	1950 (77.4)	48 (1.9)	451 (18.0)	44 (1.8)	22 (0.9)	2516
478390	2382 (95.0)	6 (0.2)	92 (3.6)	20 (0.8)	9 (0.4)	2509
408974	1994 (82.5)	12 (0.5)	327 (13.5)	44 (1.8)	39 (1.6)	2416
176759	1922 (79.7)	39 (1.6)	401 (16.8)	23 (0.9)	23 (0.9)	2409
560903	2216 (95.0)	4 (0.2)	91 (3.8)	22 (0.9)	4 (0.2)	2337
593816	1779 (78.8)	27 (1.4)	106 (4.3)	48 (2.1)	360 (13.4)	2320
217962	1913 (83.3)	31 (1.3)	276 (12.0)	44 (1.9)	33 (1.4)	2298
452122	1964 (87.2)	0 (0.1)	259 (11.5)	20 (0.8)	11 (0.5)	2254
593821	1701 (74.0)	1 (0.1)	201 (9.7)	24 (1.1)	286 (15.0)	2215
593827	1542 (70.8)	12 (0.5)	591 (27.4)	20 (0.9)	6 (0.3)	2171
593781	1831 (87.7)	41 (2.1)	148 (6.0)	54 (2.6)	28 (1.6)	2102
141968	1936 (94.9)	6 (0.3)	74 (3.7)	18 (0.9)	4 (0.2)	2040
249570	1663 (86.1)	49 (2.8)	104 (5.2)	44 (2.4)	75 (3.6)	1935
508503	1833 (94.8)	5 (0.2)	57 (2.9)	34 (1.8)	4 (0.2)	1934
141970	1430 (83.0)	6 (0.3)	250 (13.7)	25 (1.5)	25 (1.5)	1737
593814	1380 (79.9)	89 (5.1)	200 (11.6)	30 (1.8)	27 (1.6)	1726
413781	825 (63.4)	1 (0.1)	46 (3.6)	16 (1.3)	411 (31.7)	1300
413782	636 (87.0)	28 (3.7)	3 (0.4)	9 (1.3)	53 (7.1)	732
413784	350 (83.2)	13 (3.0)	7 (1.6)	14 (3.3)	35 (8.2)	421
413783	309 (89.2)	4 (1.1)	5 (1.4)	15 (4.6)	9 (2.8)	345

[z] PI = plant introduction.
[y] Group 1, chlorogenic acid isomers; group 2, isochlorogenic acid isomers; group 3, hydroxycinnamic acid amide conjugates; group 4, unidentified caffeic acid derivatives; and group 5, acetylated chlorogenic acid isomers.
[x] Total phenolic acids include additional compounds noted in Table 3.

plant fruit, with chlorogenic acid as the predominant constituent (Winter and Herrmann, 1986). That sole study included only two cultivars and focused on the mono-HCA quinate esters and glucosides. Whitaker and Stommel (2003) described a more diverse profile of HCA conjugates in seven commercial eggplant cultivars representing a variety of market types. Characterization of phenolic

composition and content in fruits and vegetables and knowledge of their potential health benefits is needed to establish future dietary guidelines and encourage consumption of phenolic-rich foods as modulators of disease. We report here a first evaluation of phenolic acid constituents in eggplant fruit flesh from diverse accessions in the USDA eggplant core subset.

The eggplant core subset is derived from the larger eggplant collection of 766 accessions in the genebank at the USDA, ARS, Plant Genetic Resources Conservation Unit, Griffin, Georgia. The composition of the USDA eggplant collection is predominated by *S. melongena* but also contains a number of *S. melongena* relatives. A core collection consists of a limited set of accessions derived from an existing germplasm collection, chosen to represent the genetic spectrum in the whole collection (Frankel and Brown, 1984). The USDA eggplant core subset includes 101 accessions of the cultivated eggplant, *S. melongena,* and 14 accessions representing four related eggplant species, *S. aethiopicum*, *S. anguivi*, *S. incanum*, and *S. macrocarpon*.

S. incanum, a wild species, is a progenitor of *S. melongena* and crosses freely with *S. melongena* to produce fertile hybrids (Lester and Hasan, 1991). *Solanum macrocarpon* is known as the gboma eggplant in cultivation. Hundreds of cultivars within *S. aethiopicum*, the scarlet eggplant, have been selected in Africa (Lester and Thitai, 1989). *Solanum anguivi* is considered a wild ancestor of *S. aethiopicum* (Lester and Niakan, 1986). Ideally, these accessions are chosen from the larger collection for inclusion in the core subset based upon taxonomic affinity, ecogeographic zones, and genetic characteristics (Brown, 1989). The availability of a core subset that encompasses most of the genetic diversity of the larger collection affords a logical rationale and opportunity to efficiently evaluate genetic diversity within a crop. This report describes eggplant fruit phenolic acid constituents in a core subset and provides data that will be useful for future use of this collection in genetic enhancement of eggplant fruit quality and nutritive value.

Materials and Methods

Plant material. Seed of each accession in the eggplant core subset was obtained from the USDA–ARS, Plant Genetic Resources Conservation Unit, Griffin, Ga. The core collection consisted of 101 accessions of the cultivated eggplant, *Solanum melongena,* 10 accessions representing *S. aethiopicum*, two *S. anguivi* accessions, one *S. incanum* accession, and one *S. macrocarpon* accession (Table 1). Six 7-week-old greenhouse grown plants of each accession were transplanted in a completely randomized design to field plots at the Beltsville Agricultural Research Center, Beltsville, Md., into Keyport fine loam soil (clayey, mixed, mesic Acquic Hapludult). Field grown plants were spaced at 0.45-m intervals in single rows on polyethylene covered raised beds positioned on 1.5-m centers with trickle irrigation. Pest control and fertilization regimes followed standard horticultural practices for eggplant production in Maryland (University of Maryland, 2000).

Due to the diverse nature of the germplasm and resultant variation in days to harvest, fruit market maturity of each accession was assessed visually via evaluation of fruit development among sequential fruit sets, and three marketable fruit were harvested from each of three plants for each accession. Within one day of harvest, fruit were washed, peeled, and a 2-cm wide longitudinal section from stem to blossom end was cut from the middle of the fruit. Excised tissue was quickly diced, frozen in liquid N_2, and lyophilized. The dried tissue of all three fruit from an individual plant was powdered, pooled as a single sample, and held at –80°C.

Tissue extraction and sample preparation. Phenolic acids were extracted from 0.2 g samples of lyophilized fruit tissue by sonication in two sequential 10-mL aliquots of methanol containing 0.5% butylated hydroxytoluene (BHT) as described by Whitaker and Stommel (2003). Briefly, combined extracts were filtered through Whatman no. 4 filter paper and a Whatman PTFE syringe filter (0.2 μm pore size), followed by addition of 25 μg of sesamol (3,4-methylenedioxyphenol; Sigma Chem., St. Louis, Mo.) as an internal standard (Buta and Spaulding, 1997) to a 1.5 mL aliquot of the filtered extract. Samples were dried under N_2 and the residue resuspended in 1.0 mL of 0.02% (2 mM) phosphoric acid in 1 methanol : 1 water (v/v). Insoluble BHT was pelleted by centrifugation and the supernatant stored under N_2 at –80°C prior to HPLC analysis.

HPLC analysis. Phenolic acids in eggplant fruit extracts were evaluated using a RP-HPLC method developed to achieve separation of the major HCA constituents (Whitaker and Stommel, 2003). The protocol utilized a 4.6 × 250 mm, 5μ Luna C18(2) analytical column (Phenomenex, Torrence, Calif.) with a 30 min binary mobile phase gradient of methanol in 0.01% (1 mM) aqueous phosphoric acid. Quantification was based on absorbance at 325 nm relative to the sesamol internal standard and external standards of authentic chlorogenic acid (5-CQA; Aldrich, Milwaukee, Wis.) and isochlorogenic acid isomers including 3,4-diCQA, 3,5-diCQA, and 4,5-diCQA (ICN-K&K Laboratories, Plainview, N.Y.).

LC–MS analysis. Electrospray ionization mass spectrometry (negative ion; ES^-–MS) was performed on a Quattro LC benchtop triple quadrupole mass spectrometer (Micromass Ltd., Manchester, U.K.) in the full scan mode over the 150–600 amu range as previously described (Whitaker and Stommel, 2003). Phenolic acids in sample extracts in 1 methanol : 1 water, (v/v) including 0.1% formic acid were separated using the Luna C18(2) column and mobile phase gradient described above for HPLC-UV analysis, but with 0.05% aqueous formic acid substituted for 0.01% phosphoric acid. UV spectra were acquired using a photodiode array detector over 210 to 400 nm.

Nuclear magnetic resonance spectroscopy. Tentative identification of major eggplant fruit HCA conjugates on the basis of HPLC and ES^-–MS analyses was confirmed by proton NMR spectroscopy. ^{1}H-NMR spectra of fruit HCA conjugates and caffeoylquinic acid standards in CD_3OD were acquired deuterium locked at 25 °C using a Bruker QE 300 MHz NMR spectrometer, and chemical shift values were assigned relative to the frequencies of residual nondeuterated water and methanol externally referenced to TMS.

Statistical analyses. Statistical analyses were performed using the SAS System (SAS Institute, Cary, N.C.). Analysis of variance was obtained with the SAS General Linear Models procedure with species and genotypes treated as fixed effects. SAS cluster analyses were conducted using the average linkage method.

Results and Discussion

Analysis of phenolic acids in extracts from fruit of *S. melongena*, *S. aethiopicum*, *S. anguivi*, *S incanum* and *S. macrocarpon* revealed considerable diversity in composition, as well as content (Fig. 1). Fourteen compounds separated by HPLC, that were present in many but not all accessions, were identified or tentatively identified as HCA derivatives. Identification was based on HPLC elution times, UV absorbance spectra, ES–MS mass spectra, and in some cases proton NMR data. These 14 compounds were grouped on the basis of structural relationships into five classes as follows: chlorogenic

acid isomers (group 1), isochlorogenic acid isomers (group 2), hydroxycinnamic acid amide conjugates (group 3), unidentified caffeic acid conjugates (group 4) and acetylated chlorogenic acid isomers (group 5). Criteria used for identification of individual compounds in *S. melongena* have been previously described (Whitaker and Stommel, 2003) and extended here for identification of unique compounds in related eggplant species in the core collection. Group compositions are summarized below.

Group 1 chlorogenic acid isomers included four compounds with HPLC elution times of ≈17.4, 21.8, 23.1, and 24.2 min (all evident in Fig. 1A and B). Based on their UV, ES$^-$–MS and ^{1}H-NMR spectra, they were identified as the 3-*O-trans*, 5-*O-trans*, 4-*O-trans*, and 5-*O-cis* isomers of caffeoylquinic acid (3-CQA, neochlorogenic acid; 5-CQA, chlorogenic acid; 4-CQA, cryptochlorogenic acid; and 5-(*Z*)-CQA, *cis*-chlorogenic acid), respectively. Chlorogenic acid was the predominant compound in group 1, with average levels 176-, 28.9-, and 92.5-fold higher than levels of neochlorogenic acid, cryptochlorogenic acid, and *cis*-chlorogenic acid, respectively.

Group 2 consisted of two phenolics with elution times of ≈28.4 and 28.7 min (Fig. 1B and C), both with UV spectra very similar to that of 5-CQA, and with a molecular ion minus a proton (M–1) at *m/z* 515, required for dicaffeoylquinic acid (isochlorogenic acid, diCQA; $C_{25}H_{24}O_{12}$ = 516). Elution times and mass spectra of the 28.4- and 28.7-min diCQAs were identical to those of 3,5-diCQA and 4,5-diCQA standards, respectively. Levels of the 3,5-diCQA isomer (28.4 min) were on average 11.8-fold higher than those of the 4,5-diCQA isomer (28.7 min).

Group 3, the hydroxycinnamic acid amide conjugates, included four compounds with elution times of ≈10.1-, 18.8-, 19.6-, and 20.6-min (evident in Fig. 1B and C). UV spectra of the 10.1-min peak, with first and second maxima at 317 and 292 nm, and of the 20.6-min peak, with maxima at 319 and 293 nm, were closely similar. On the basis of its LC–MS and ^{1}H-NMR spectra (Whitaker and Stommel, 2003), the 20.6-min compound was identified as *N*,*N*′-dicaffeoylspermidine ($C_{25}H_{31}N_3O_6$ = 469). The 10.1-min phenolic was tentatively identified as *N*-caffeoylputrescine ($C_{13}H_{18}N_2O_3$ = 250) on the basis of its mass spectrum, the close similarity of its UV spectrum to that of *N*,*N*′-dicaffeoylspermidine, and partial ^{1}H-NMR data that were consistent with a caffeic acid moiety exhibiting chemical shifts similar to those noted in *N*,*N*′-dicaffeoylspermidine. The nearly identical UV and LC–MS spectra of the 18.8- and 19.6-min phenolics indicated that they are structural isomers, and their molecular mass of 471 is two protons greater than that of *N*,*N*′-dicaffeoylspermidine, suggesting that they are related to this HCA amide conjugate but lack one double bond (most likely one of the caffeic acid moieties is replaced by dihydrocaffeic acid). *N*,*N*′-dicaffeoylspermidine was the predominant compound in group 3, with levels on average 2.5-, 3.8-, and 4.7–fold higher, respectively, than levels of the HCA amides eluting at 10.1-, 18.8-, and 19.6-min. Levels of the isomeric 18.8- and 19.6-min phenolics increased coincident with increased levels of *N*,*N*′-dicaffeoylspermidine.

The two compounds in group 4 had elution times ≈22.3 and 25.6 min. Their identification as caffeic acid conjugates (other than esters of quinic acid) was based solely on the close similarity of their UV spectra to those of mono- and di-*O*-caffeoylquinic acid isomers in groups 1 and 2. Because both of these compounds were typically very minor constituents and each overlapped with other

Fig. 1. HPLC chromatograms of phenolic acid derivatives extracted from eggplant fruit flesh (UV absorbance at 325 nm). Chromatograms (**A**) [accession PI-413783] and (**B**) [accession PI-155511] are representative of *S. melongena* fruit with very low and very high levels of total phenolic acids, respectively; (**C**) and (**D**) show the remarkably different phenolic acid profiles in fruit of the two *S. anguivi* accessions (PI-179745 and PI-319855, respectively); and (**E**) shows the unique phenolic acid profile of fruit from the single accession of *S. incanum* (PI-500922). Peaks highlighted in pale gray and black are, respectively, chlorogenic acid (5-CQA) and the internal standard (sesamol). Peaks in **D** and **E** highlighted in dark gray are major constituents that are absent or present at very low levels in fruit of all other accessions. Note that in order to include the last two major phenolics unique to *S. anguivi* accession PI-319855, chromatogram **D** is not aligned with **A**, **B**, **C**, and **E** but the 5-CQA and sesamol peaks serve as points of reference.

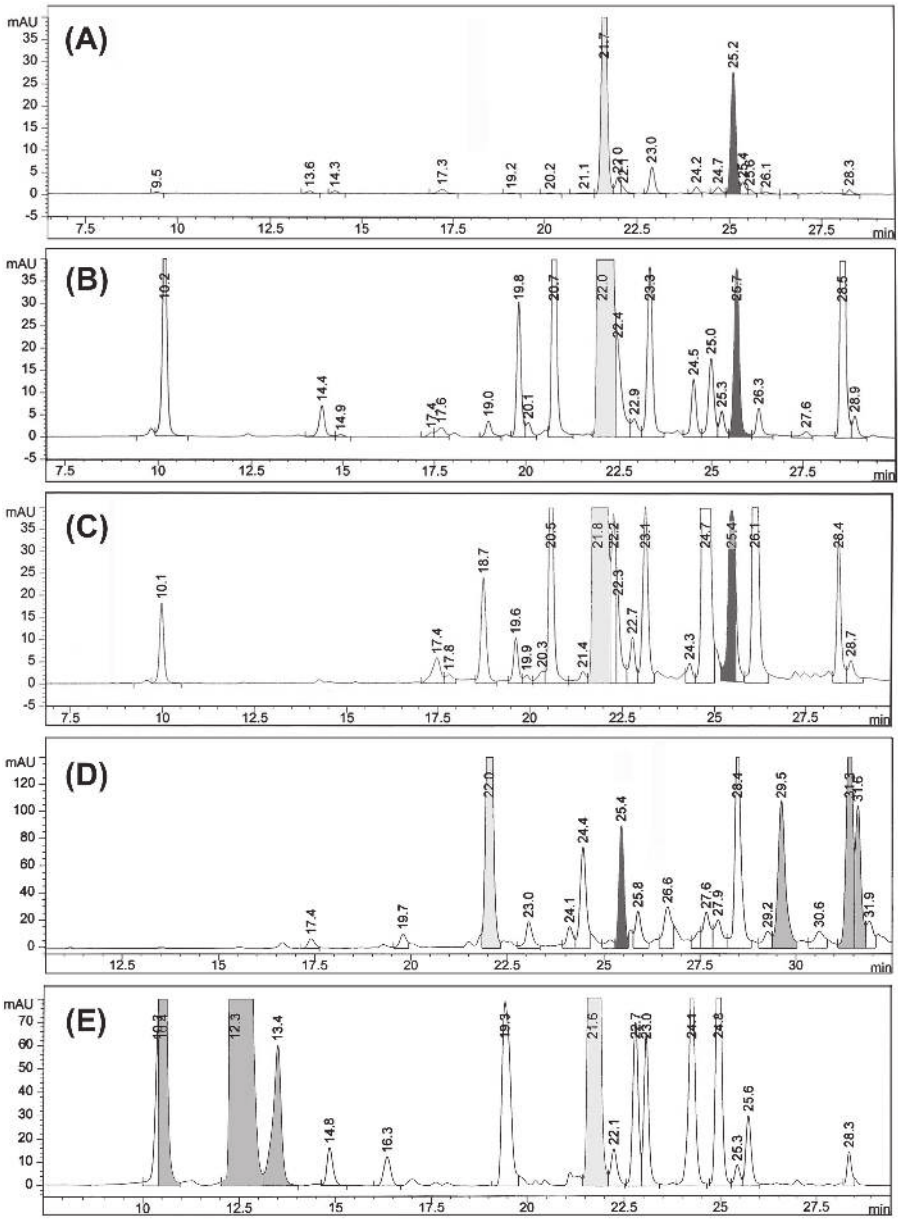

Table 2. Analysis of variance type III F values for phenolic acid content in fruit of eggplant and related wild species represented in the USDA eggplant core subset.

		Type III F-value					
Source	df	Group 1[z]	Group 2	Group 3	Group 4	Group 5	Total
Species	4	9.40***	11.47***	5.55***	16.83***	23.28***	11.01***
Genotype (species)	110	6.78***	6.59***	8.77***	3.40***	10.08***	6.57***
S. aethiopicum	9	2.66*	5.38***	2.96*	5.19**	18.61***	2.98*
S. anguivi	1	143.36***	10.52*	47.40**	8.21*	14.07*	61.39**
S. incanum[y]	0	---	---	---	---	---	---
S. macrocarpon[y]	0	---	---	---	---	---	---
S. melongena	100	6.09***	6.84***	8.62***	3.10***	14.20***	5.95***

[z]Group 1, chlorogenic acid isomers; group 2, isochlorogenic acid isomers; group 3, hydroxycinnamic acid amide conjugates; group 4, unidentified caffeic acid derivatives; and group 5, acetylated chlorogenic acid isomers.
[y]Core subset contains only one accession. F values not derived.
*, **, ***Significant at $P \leq 0.05$, 0.01 and 0.001, respectively.

Table 3. HPLC elution, UV absorbance (UVA), mass spectral, and concentration data for phenolic acids unique to fruit of *Solanum anguivi* accession PI 319855 and *S. incanum* accession PI 500922.

Genotype	HPLC elution time (min)	UVA maxima (280–330 nm)	ES$^-$–MS major ion(s)	μmol/100 g dry wt (% of total)
S. anguivi				
PI 319855	29.5	318, 300(sh)	360>367	414 (12.0)
	31.3	319, 299(sh)	381≅367	542 (15.7)
	31.6	320, 299(sh)	381>367	291 (8.5)
S. incanum				
PI 500922	10.4	317, 291	529	322 (4.3)
	12.3	319, 289	527	2569 (34.3)
	13.4	321, 297(sh)	525	170 (2.3)

more abundant compounds, it was not possible to isolate them or obtain reliable mass spectra. Levels of these minor compounds were highly variable among the accessions evaluated.

The final group of HCA derivatives, the acetylated chlorogenic acid isomers (group 5), was composed of two compounds with elution times of ≈24.7 and 26.1 min (prominent in Fig. 1, panel C). Their UV spectra differed only slightly and were similar to that of chlorogenic acid. The LC–MS and ^{1}H-NMR data for the 24.7-min compound were consistent with the structure 3-*O*-acetyl-5-*O*-caffeoylquinic acid (3-acetyl-5-CQA), $C_{18}H_{20}O_{10}$ = 396 (Whitaker and Stommel, 2003). The nearly identical UV and LC–MS spectra of the two compounds in group 5 indicated that they are isomeric. Considering the relative abundance and retention times of the 5-CQA and 4-CQA isomers in group 1, it is probable that the 26.1-min compound is 3-*O*-acetyl-4-*O*-caffeoylquinic acid (3-acetyl-4-CQA). The 3-acetyl-5-CQA isomer (24.7 min) was consistently about 2.4-fold more abundant than the putative 3-acetyl-4-CQA isomer (26.1 min).

For all five groups of phenolic acid derivatives, significant differences in content and composition were evident among the five eggplant species (Table 2). Similarly, significant differences among genotypes within species were noted. Separate analyses of genotypes within individual species were consistent with overall genotype within species effects (Table 2). Among *S. melongena* accessions, there was a nearly 20-fold difference in total HCA content, which ranged from a high of 6783 μmol/100 g dry weight in PI 470273 to a low of 345 μmol/100 g dry weight in PI 413783 (Table 1). Total HCA content in *S. aethiopicum* and the single *S. macrocarpon* accession was low relative to *S. melongena* (Table 1). *Solanum anguivi* PI 179745 contained the highest HCA content among core subset accessions and exhibited unusually high levels of acetylated chlorogenic acid isomers and unidentified caffeic acid conjugates in comparison with other accessions with very high phenolic acid content. A number of *S. melongena* accessions (e.g., PI 593820) with albeit lower total HCA content, contained high levels of these constituents on a percent of total basis. Total phenolic content has been reported to increase during the period between fruit set through postharvest maturity (Esteban et al., 1992), and to decline during extended postharvest storage (Esteban et al., 1989). Whitaker and Stommel (2003) demonstrated variation in HCA content between fruit stem- and blossom-end tissues.

Chlorogenic acid isomers ranged from 63.4% to 96% of the total HCAs in *S. melongena*, *S. aethiopicum* and *S. macrocarpon* accessions, with an 18.6-fold difference between accessions with the highest and lowest levels (Tables 1 and 3). With the exception of accessions containing relatively higher levels of acetylated chlorogenic acid isomers, the group 3 hydroxycinnamic acid amide conjugates generally accounted for most of the remaining phenolic acids. Differences among accessions in the content of group 2, group 3 and group 5 phenolics were as much as 120-, 550- and 542-fold, respectively, and were considerably greater than those noted for group 1 chlorogenic acid isomers. The unidentified caffeic acid conjugates (group 4) exhibited relatively little variation in content among core subset accessions. Cluster analysis of phenolic acid composition and content reflected levels of the monocaffeoylquinic acids in group 1, and hence total HCA content, since chlorogenic acid was the predominant HCA derivative (data not shown). A relationship between accession phenolic acid profiles and country of origin was not evident.

The content of groups 2, 3, 4, or 5 did not consistently increase or decrease with changes in the major class, group 1 (chlorogenic acid isomers). For example, the highest levels of acetylated chlorogenic acid isomers were found in accessions with intermediate to low total HCA content. Among the 10 *S. aethiopicum* accessions,

the African PI 420230 contained much higher levels of acetylated chlorogenic acid isomers than the other nine accessions that originated from South America. Higher levels of acetylated chlorogenic acid isomers were also noted in geographically diverse accessions of *S. melongena,* Grif 1276, PI 593820, PI 593816, PI 593821 and PI 413781, *S. anguivi* PI 179745 and *S. macrocarpon* PI 441914.

Two accessions, *S. anguivi* PI 319855 and *S. incanum* PI500922, exhibited strikingly different phenolic acid profiles. Chlorogenic acid constituted only 32.4% and 42.4% of total phenolic acids in PI 319855 and PI 500922, respectively. *Solanum anguivi* PI 319855 and *S. incanum* PI 500922 also contained several phenolic acid derivatives that were not present in any other core subset accession. Three unique compounds in fruit of PI 319855 that eluted after the 3,5-diCQA and 4,5-diCQA isomers (Fig. 1D) accounted for 36.2% of the total HCAs (Table 3). All three had closely similar UV spectra and yielded ES^-–MS ions at *m*/*z* 367 and/or 381, consistent with the presence of a ferulic acid and/or a 3,4-dimethoxycinnamic acid moiety, respectively. Preliminary ^{1}H-NMR data indicated that the compounds are HCA derivatives with one or more methoxy groups on the phenyl ring (strong singlet at ≈3.90 ppm). Three additional unique compounds that comprised 40.8% of the total phenolics in PI 500922 eluted between 10 and 14 min (Fig. 1E; Table 3). UV spectra of the compounds eluting at 10.4 and 12.3 min were similar to those of the 18.8- and 19.6-min putative HCA amides in group 3, whereas the UV spectrum of the compound eluting at 13.4 min closely resembled those of the 10.1- and 20.6-min caffeoylpolyamine amides in group 3. Like the phenolic acid amides in group 3, the three *S. incanum* unknowns gave ES^-–MS spectra that included only the major $[M-1]^-$ ion and minor sodium adduct ion, and the molecular masses of the three differed by two or four protons (one or two double bonds). The phenolic acid composition of additional *S. anguivi* and *S. incanum* accessions must be characterized to determine the prevalence of the unique compounds in these species. Efforts toward complete structural elucidation of these six phenolics are in progress.

Our findings on eggplant fruit phenolic content provide several opportunities to improve eggplant fruit quality and nutritive value. Phenols, sugars and ascorbic acid are important determinants of eggplant fruit flavor (Esteban et al., 1992). Because phenolic acid derivatives vary in their degree of bitterness, sensory studies are needed to determine the effect that varying phenolic acid composition may have on fruit flavor and to determine consumer group preferences. In vitro assays that assess the protective effects of phenolics against oxidative damage are valuable predictors of their disease preventive properties. However, knowledge of the bioavailability and metabolism of phenolic acids from eggplant is needed to objectively evaluate their potential contribution to improved nutrition and human health.

The inheritance of phenolic acid content and composition in eggplant has not been determined. An understanding of the genetic regulation of these parameters will facilitate breeding of new cultivars with specific phenolic acid composition and content. Most plant phenolics are products of phenylpropanoid metabolism wherein portions of the biosynthetic pathway are extremely well characterized, or conversely, poorly understood (Croteau et al., 2000). Additional information concerning the biosynthesis and molecular genetics of phenolic acids is needed to effectively manipulate these compounds using biotechnology-based approaches. Identification of unique genetic stocks, such as eggplant genotypes with divergent phenolic acid profiles, can help to clarify the genetic regulation of phenolic acid biosynthesis and accumulation.

Literature Cited

Ames, B.N., M.K. Shigenaga, and T.M. Hagen. 1993. Oxidants, antioxidants, and the degenerative diseases of aging. Proc. Natl. Acad. Sci. USA 90:7915–7922.

Brown, A.H.D. 1989. Core collections: A practical approach to genetic resources management. Genome 31:818–824.

Buta, J.G. and D.W. Spaulding. 1997. Endogenous levels of phenolics in tomato fruit during growth and maturation. J. Plant Growth Regulat. 16:43–46.

Cao, G., E. Sofic, and R.L. Prior. 1996. Antioxidant capacity of tea and common vegetables. J. Agr. Food Chem. 44:3426–3431.

Croteau, R., T.M. Kutchan, and N.G. Lewis. 2000. Natural products (secondary metabolites), p. 1250–1318. In: B.B. Buchanan, W. Gruissem, and R.L. Jones (eds.). Biochemistry and molecular biology of plants. Amer. Soc. Plant Physiol., Rockville, Md.

Esteban, R.M., E.M. Molla, L.M. Robredo, and F.J. Lopez-Andreu. 1992. Changes in the chemical composition of eggplant fruits during development and ripening. J. Agr. Food Chem. 40:998–1000.

Esteban, R.M., E. Molla, M.B. Villarroya, and F.J. Lopez-Andreu. 1989. Changes in the chemical composition of eggplant fruits during storage. Scientia Hort. 41:19–25.

Flick, G.J., F.S. Burnette, L.H. Aung, R.L. Ory, and A.J. St. Angelo. 1978. Chemical composition and biochemical properties of mirlitons (*Sechium edule*) and purple, green, and white eggplants (*Solanum melongena*). J. Agr. Food Chem. 26:1000–1005.

Frankel, O.H. and A.H.D. Brown. 1984. Current plant genetic resources — A critical appraisal, p. 1–11. In: Genetics: New frontiers. Vol. IV. Oxford and IBH Publ. Co., New Delhi.

King, A. and G. Young. 1999. Characteristics and occurrence of phenolic phytochemicals. J. Amer. Dietetic Assn. 99:213–218.

Kinsella, J.E., E. Frankel, B. German, and J. Kanner. 1993. Possible mechanisms for the protective role of antioxidants in wine and plant foods. Food Technol. 47:85–89.

Lester, R.N. and S.M.Z. Hasan. 1991. Origin, domestication of the brinjal eggplant (*Solanum melongena*) from *S. incanum* in Africa and Asia, p. 369–387. In: J.G. Hawkes, R.N. Lester, M. Nees, and N. Estrade, (eds.). Solanaceae III. Taxonomy, chemistry, and evolution. Royal Botanic Gardens, Kew.

Lester, R.N. and L. Niakan. 1986. Origin and domestication of the Scarlet Eggplant, *Solanum aethiopicum* L., from *S. anguivi*, p. 433–456. In: W.G. D'Arcy (ed.). Solanaceae: Biology and systematics. Columbia Univ. Press, New York.

Lester, R.N. and G.N.W. Thitai. 1989. Inheritance in *Solanum aethiopicum*, the scarlet eggplant. Euphytica 40:67–74.

Nakatani, N., S. Kayano, H. Kikuzaki, K. Sumino, K. Katagiri, and T. Mitani. 2000. Identification, quantitative determination, and antioxidative activities of chlorogenic acid isomers in prune (*Prunus domestica* L.). J. Agr. Food Chem. 48:5512–5516.

Sakamura, S. and Y. Obata. 1961. Anthocyanase and anthocyanins occurring in eggplant, *Solanum melongena* L. Agr. Biol. Chem. 25:75–756.

Sawa, T., M. Nakao, T. Akaike, K. Ono, and H. Maeda. 1999. Alkylperoxyl radical-scavenging activity of various flavonoids and other phenolic compounds: Implications for the anti-tumor-promoter effect of vegetables. J. Agr. Food Chem. 47:397–402.

Sudheesh, S., G. Presannakumar, S. Vijayakumar, and N.R. Vijayalakshmi. 1997. Hypolipidemic effect of flavonoids from *Solanum melongena*. Plant Foods Human Nutr. 51:321–330.

Swarup, V. 1995. Genetic resources and breeding of aubergine (*Solanum melongena* L.). Acta Hort. 412:71–79.

University of Maryland. 2000. Commercial vegetable production recommendations. Univ. Md. Coop. Ext. Serv. Bul. 236, College Park.

Vinson, J.A., Y. Hao, X. Su, and L. Zubik. 1998. Phenol antioxidant quantity and quality in foods: Vegetables. J. Agr. Food Chem. 46:3630–3634.

Whitaker, B.D. and J.R. Stommel. 2003. Distribution of hydroxycinnamic acid esters in fruit of commercial eggplant (*Solanum melongena* L.) cultivars. J. Agr. Food Chem. (in press).

Winter, M. and K. Herrmann. 1986. Esters and glucosides of hydroxycinnamic acids in vegetables. J. Agr. Food Chem. 34:616–620.