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Partial Leaf Chemical Profiles of a Desert Watermelon Species (*Citrullus colocynthis*) and Heirloom Watermelon Cultivars (*Citrullus lanatus* var. *lanatus*)

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Abstract. **Whiteflies [*Bemisia tabaci* (Gennadius)] and aphids [*Aphis gossypii* Glover and *Myzus persicae* (Sulzer)] are serious threats to watermelon by direct feeding and by transmitting viruses of important virus diseases. The desert watermelon *Citrullus colocynthis* (L.) has been shown to exhibit resistance to these insect pests and could be a useful source for breeding resistance into watermelon [*Citrullus lanatus* var. *lanatus* (Thunbs) Matsum & Nakai]. Using high-performance liquid chromatography (HPLC), we found differences among the chemical profiles of two U.S. PIs of *C. colocynthis,* one PI of C. *lanatus* var. *citroides,* and two heirloom watermelon (*C. lanatus* var. *lanatus*) cultivars ('Charleston Gray' and 'Mickey Lee'). Flavonoid and caffeic acid derivatives were identified in the leaf extracts by a combination of ultraviolet (UV) and mass spectrometry (MS) spectral analyses. Four phenolic derivatives of caffeic and/or ferulic acid were found to be essentially unique to *C. colocynthis*. Total flavonoid content was found to be approximately four to 18 times higher in *C. colocynthis* accessions and seven to nine times higher in *C. lanatus* var. *citroides* as compared with watermelon cultivars. Caffeoylglucose was also identified in the leaves of watermelon cultivars for the first time. Leaf sugar concentrations (198 to 211 mg·dL^{-1}), read from a glucometer, were statistically the same among the various germplasm entries. These results will help in the development of pest-resistant watermelon.**

Watermelon [*C. lanatus* var. *lanatus* (Thunbs) Matsum & Nakai] is an important crop globally. The origin of *Citrullus* spp. is in central or southern Africa (Jarret et al., 1997; Mujaju et al., 2010). On that continent, a wide variation of watermelon populations exists in diverse geographical regions and the fruit is considered a vital source of water and food for the native people and animals. As a result of many years of cultivation and selection for desirable qualities, a large number of the American heirloom watermelon cultivars shares a narrow genetic base and is susceptible to diseases and pests (Levi et al., 2001a; Simmons and Levi, 2002). On the other hand, the *Citrullus* spp. germplasm collected mainly in central and southern Africa shows a wide phenotypic and genetic diversity (Levi et al., 2001b).

The genus *Citrullus* includes several species or subspecies. Among them is the bitter watermelon *C. colocynthis* (L.) Schrad that thrives in the deserts of North Africa, the Middle East, and Asia. It has distinct morphological and biochemical features such as thick leaves, fairly small fruits, and a bitter odor that repels insects (Simmons and Levi, 2002). Additional species, found in southern Africa, are *C. ecirrhosus* Cogn. and *C. rehmii* De Winter (Robinson and Decker-Walters, 1997). *C. lanatus* var. *lanatus* is considered the progenitor of cultivated watermelon. The *C. lanatus* also includes the citron watermelon, C. *lanatus* (Thunbs) Matsum & Nakai var. *citroides* (L.H. Bailey), which thrives in the deserts of southern Africa. It is known as the "Citron Watermelon," "Cow Watermelon," or "Tzama" (Jarret et al., 1997; Mujaju et al., 2010) and is considered a valuable germplasm source because different accessions of this subspecies contain resistance to diseases or pests (Levi et al., 2001b; Thies and Levi., 2007). The *Citrullus* germplasm collection maintained by the USDA-ARS Plant Genetic Resources and Conservation Unit, Griffin, GA (http://www.ars-grin.gov) includes over 1800 U.S. PIs. These PIs have been useful sources of germplasm for identifying disease or pest resistance that through intensive breeding programs could be incorporated into elite watermelon cultivars.

Whiteflies [*Bemisia tabaci* (Gennadius)] and aphids [*Aphis gossypii* Glover and *Myzus persicae* (Sulzer)] are major pests that feed on and transmit viruses to watermelon plants (Simmons et al., 2010; Simmons and Levi, 2002). However, several *C. colocynthis* PIs possess resistance to the sweetpotato whitefly, *B. tabaci* (Simmons and Levi, 2002), the two-spotted spider mite, *Tetranychus urtichae* Koch (Lopez et al., 2005), and aphids (Simmons, unpublished data). These sources of germplasm should be useful for incorporating pest resistance into watermelon cultivars.

Very little work has been reported on the flavonoids and phenolics of watermelon, especially in the leaves. Most previous investigations of flavonoid or phenolic content of watermelon have been limited to determination by colorimetric methods (Asyaz et al., 2010; Chopra et al., 1974; Ibrahim et al., 2010; Tlili et al., 2011; Venkataramaiah and Narayana, 1983). Others have used acid hydrolysis before analysis to measure individual aglycone flavonoids (Harsh and Nag, 1988; Lugasi and Hovari, 2002; Meena and Patni, 2008) or phenolic acids (Das et al., 1967; Venkataramaiah and Narayana, 1983). Delazar et al. (2006) determined a number of flavonone-C-glycosides in *Citrullus colocynths* fruits. To our knowledge, only the report of Maatooq et al. (1997) reports specific and novel hydroxybenzyl-flavonoids in the leaves of watermelon (*Citrullus colocynthis*) and only that of Chopra et al. (1974) relates phenolic content to resistance and susceptibility to disease (*Alternaria cucumerina*). Furthermore, there is no sufficient information on the chemical profile and compounds that may lure or repel insect pests and affect their feeding habits and reproduction on plants of watermelon cultivars vs. *C. colocynthis* PIs.

The objective of this study was to determine if differences exist in the chemical profiles of leaves of *C. colocynthis* PIs that showed whitefly resistance (Simmons and Levi, 2002) vs. those of susceptible watermelon cultivars (*C. lanatus* var. *lanatus*) and in a representative PI of the citron watermelon (*C. lanatus* var. *citroides*).

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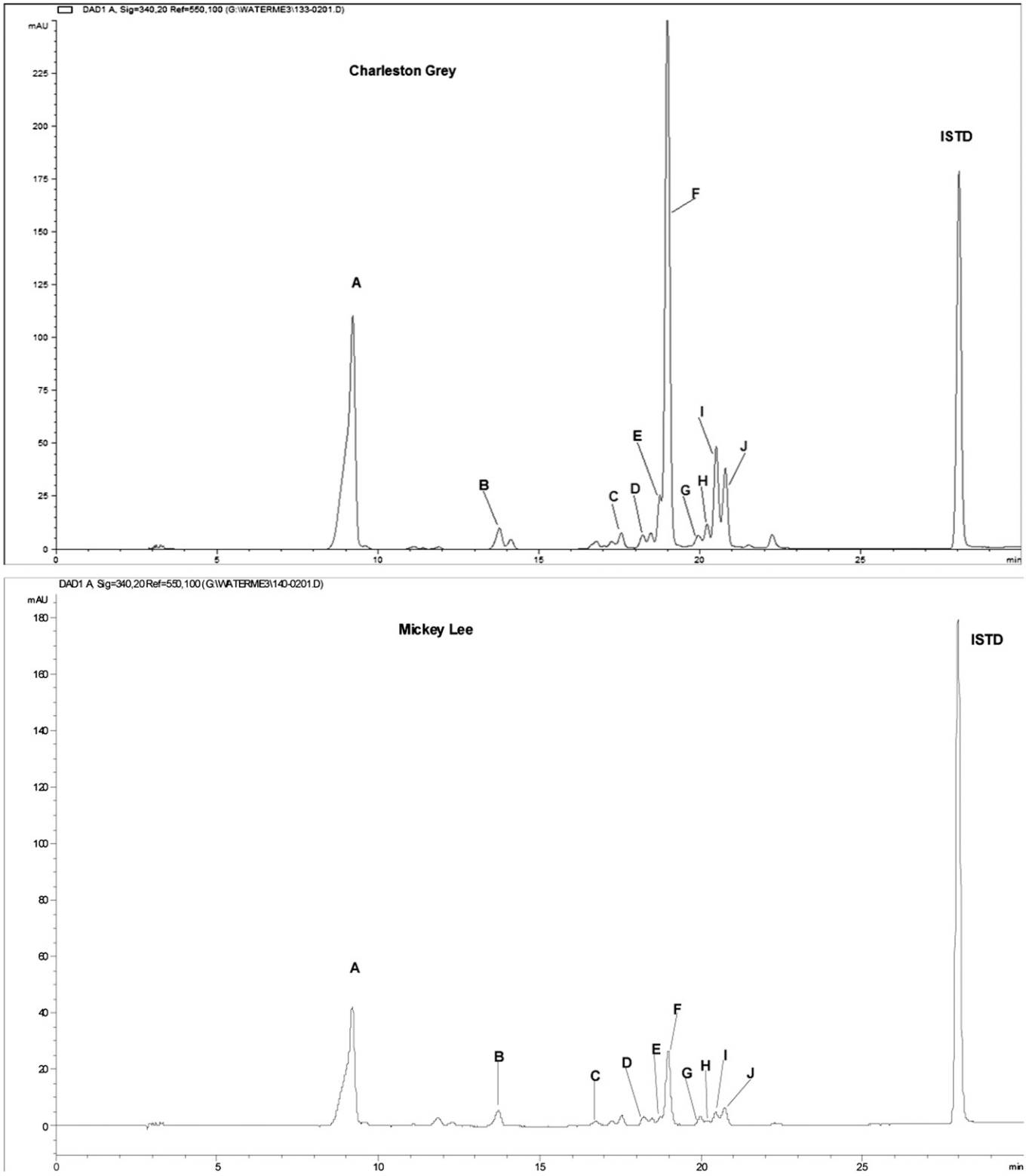

Fig. 1. High-performance liquid chromatography. Spectral patterns of flavonoids and caffeoyl-quinic acid (CQA) derivatives found in 'Charleston Gray' and 'Mickey Lee' watermelon, *Citrullus lanatus* var. *lanatus*. Refer to Table 1 for the meaning of the letters representing various peaks. ISTD = Chrysin.

Materials and Methods

Plant material. The plants in this study included the watermelon cultivars Charleston Gray and Mickey Lee (*C. lanatus* var. *lanatus*), the *C.* var. *citroides* PI 500354, and the *C. colocynthis* PI 386015 and PI 432337. The plants were grown in pots in the greenhouse using a standard watering regime.

Methanolic extract of fresh leaves of each *Citrullus* accession was made by clipping five healthy leaves from 7-week-old plants of each accession and cutting them into pieces with a pair of scissors. Three-gram portions of the leaf samples of each watermelon accession was then placed in separate 14 × 7-cm, 118.5-mL glass bottles (a Teflon-lined cap was used) and 100 mL of methanol was added to each bottle. Chrysin (Sigma-Aldrich, Milwaukee, WI; recrystallized from amyl alcohol) was used as an internal standard. Three

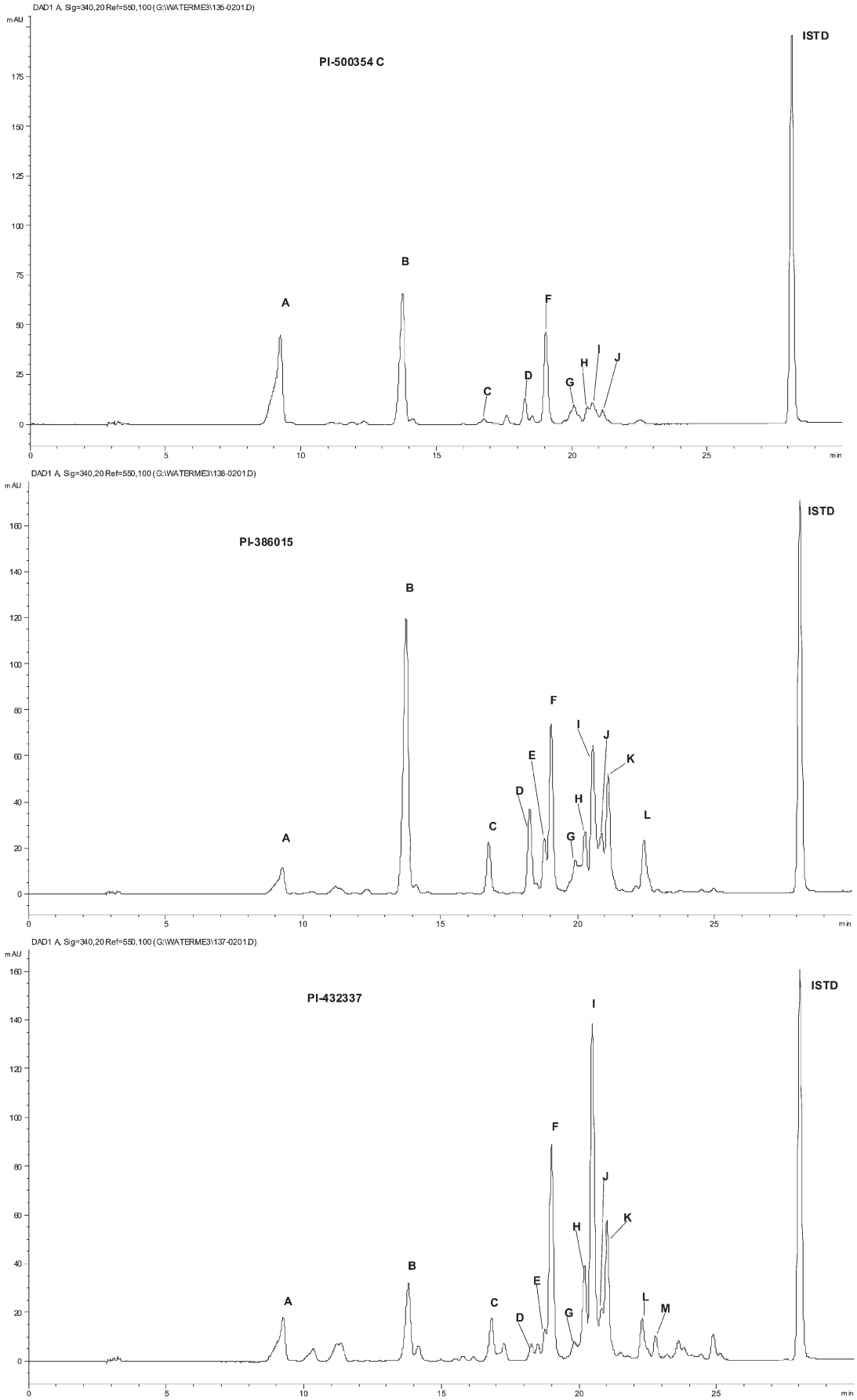

Fig. 2. High-performance liquid chromatography. Spectral patterns of flavonoids and caffeoyl-quinic acid (CQA) derivatives found in accessions PI 500354 (*Citrullus citroides*), PI 386015 (C*itrullus lanatus* var. *colocynthis*), and PI 432337 (C*itrullus lanatus* var. *colocynthis*). Letter C after PI 500354 represents the in-house designation assigned to this sample. Refer to Table 1 for the meaning of the letters representing peaks. ISTD = Chrysin.

milliliters of a methanolic solution (4.8 mg chrysin/3 mL) was added to each leaf extract and the solution mixed. The submerged leaves were subsequently further cut into smaller pieces with scissors and ground for ≈1 min with a polytron (Kinematic-PCU-2; Brinkmann Instruments, Inc., Westbury, NY) equipped with a 6-mm diameter sawtooth grinder type of tissue cutter. The solutions were filtered through 0.45-µm nylon-66 filters in preparation for HPLC analysis.

High-performance liquid chromatography analysis. Extracts were analyzed once by reversed-phase HPLC using a H_2O/MeOH linear gradient from 10% to 100% MeOH in 35 min, a flow rate of 1 mL·min^{-1}, and detection at 340 nm. Each solvent contained 0.1% H_3PO_4. Analyses were performed with a Beckman Ultrasphere C18, 5 micron (4.6 × 250 mm; Beckman Instruments, Norcross, GA) column using a Hewlett-Packard 1050 diode array HPLC (Palo Alto, CA). Quantitation was performed by using chrysin's response factor. In addition to chrysin, other standards used were apigenin, caffeic acid, chlorogenic acid for tuning the HPLC-Mass Spectrophotometer (Sigma-Aldrich, St. Louis, MO), and isoorientin (Indofine Chemical Co., Belle Mead, NJ).

Identification. Preliminary identifications of compounds were by ultraviolet spectra and retention time correlations with standards. Also, mass spectra were obtained with a Thermo-Finnigan LCQ HPLC/MS. For HPLC/MS, chlorogenic acid was used for tuning. Spectra were obtained in the negative ion mode. Gas chromatographic (GC) analyses were performed with a Hewlett-Packard 5890 GC fitted with a DB-5 capillary column (30 m × 0.25 mm i.d., 1-mL·min^{-1} flow rate); injector, 250 °C; flame ionization detector, 350 °C; linear temperature program, 100–320 °C at 8 °C·min^{-1}; splitless mode. Compounds were analyzed as their silylated derivatives.

Peaks A and B were isolated from the methanolic leaf extract of PI-500354 after concentration by rotary evaporation to remove methanol. The solution was then placed on a preparative liquid chromatography column packed with PrePAK-500 C18 packing (Waters Millipore Corp., Milford, MA; washed with MeOH and recycled to water, 20 psi nitrogen pressure-aided flow). The column was eluted with MeOH/water solutions and Peak A was eluted with 10%, whereas Peak B was eluted with 30% MeOH/water.

The HPLC peaks were labeled alphabetically based on the retention times (the retention times were based on 340 nm milliabsorption units) at which the respective chemical compounds were eluted and detected (Figs. 1 and 2). The internal standard (chrysin) eluted consistently at 28 min. The mathematical area occupied by each peak correlated with the amount of the compound and was either directly provided by the software or was mathematically derived from similar (or neighboring) peaks (Table 1).

Leaf sugar concentration. Extracts of 0.5 mL volume from leaves representing three groups of germplasm (Mickey Lee, PI 500354, and PI 386015) were obtained by leaf grinding and diluted with equal amounts (0.5 mL) of distilled water and centrifuged for 4 min at 1000 rpm. A drop of each of the diluents was applied to a clinitest strip, and the sugar content was read from a glucometer. Data on sugar content were analyzed for any statistically significant variance using SAS statistical software (SAS Institute, 2003). Significant differences were determined at $P < 0.05$.

Results and Discussion

Noticeable differences exist in the HPLC profiles between the *C. colocynthis* and *C. lanatus* var. *lanatus* or *C. lanatus* var. *citroides* accessions (Tables 1 and 2; Figs. 1 and 2). The flavonoid compounds (Peaks B and D; Table 1; Figs. 1 and 2) are low in the watermelon cultivars ('Charleston Gray' and 'Mickey Lee') but are significantly higher in the *C. lanatus* var. *citroides* (PI 500354) or the *C. colocynthis* accessions (PI 386015 and PI 432337). Peaks G and H showed similar results. Peaks C, E, K, and L are unique to the *C. colocynthis* PI 386015 and PI 432337 (Fig. 2). A clear peak M was observed only in PI 432337, whereas peaks A, J, and F occurred in sufficient amounts in all accessions analyzed (Table 1; Figs. 1 and 2). The consistent elution of chrysin at 28 min for all analyzed samples suggests an overall high accuracy of the results.

A higher number of compounds was seen in chromatograms with *C. colocynthis* accessions as compared with those of *C. lanatus* var. *lanatus* or *C. lanatus* var. *citroides* accessions (Table 1; Figs. 1 and 2). Ultraviolet spectra and chromatography elution points are only indicators of what may be in a peak. It is possible that one or several of these compounds could be associated with the relative resistance of *C. colocynthis* to fluid-feeding pests. This could be possible for phenolic compounds of peaks K, L, or M. Although chlorogenic acid compounds are found in certain plants (Harrison et al., 2008), chlorogenic acid is unique to *C. colocynthis* accessions in *Citrullus* (Wu, 2007). The spectral analysis indicated that Peak A was caffeoyl-glucose: MS: 341 (M-H); MS^2: 179 (M-caffeoyl), 161 (M-glucose). Acid hydrolysis yielded caffeic acid (confirmed by HPLC and GC retention time and MS) and glucose (confirmed by GC retention time). Peak B represented Isovitexin-2″-O-glucoside: MS: 593 (M-H); MS^2: 503 (M-H-90), 473 (M-H-120). Ultraviolet analysis indicated an apigenin or kaempferol aglycone. After isolation, this flavonoid (Peak B) appeared to lose a glucose moiety on acid hydrolysis and had a molecular mass of 594, suggesting that it might be either apigenin-diglucoside or kaempferol-rhamnosyl-glucoside. After hydrolysis, isovitexin (MS:431; M-H) was identified by HPLC/MS and liberated glucose by GC retention time. Mass fragmentation yielded ions of masses 503, 473, and 311. The abundance of the M-H-90 ion in the MS^2 spectrum showed that the C-bound sugar was attached to the C-6-position, indicating an isovitexin structure. The data are in agreement with literature (Qimin et al., 1991) for 6-C-glucosyl-O-glycosyl-apigenin (glucosyl-isovitexin). Peak C represented Isoorientin: MS: 447 (M-H); MS^2: 357 (M-H-90), 327 (M-H-120). Ultraviolet spectra indicated a luteolin aglycone base.

Peaks D to M had broad ultraviolet maximum near 310 nm, which indicated they were phenolic in nature, but HPLC/MS was inconclusive and requires further study.

Leaf sugar content was tested to investigate if sugar may play a direct role in the feeding preference on *Citrullus* accessions by pests such as whiteflies and aphids. Overall means for sugar concentrations were not significantly different among the genotypes ($P < 0.05$) (mean = 198 to 211 mg·dL^{-1}). This suggests that leaf sugar content plays no role in insect preference of one *Citrullus* accession over another. Sugar specificity was not assayed. Hence, it is not known what types of sugars were involved.

Table 1. High-performance liquid chromatography analysis showing the amount (µg·g^{-1}) of each chemical compound (lettered A to M) detected in the various *Citrullus* accessions.

	Chemical compounds (µg·g^{-1})												
Entry	A[z]	B[y]	C[x]	D[w]	E	F	G	H	I	J	K	L	M
Charleston Gray	2438	91	22	46	—	1582	44	59	240	266	—	—	—
Mickey Lee	759	66	17	36	33	281	31	16	47	56	—	—	—
PI 500354	1044	803	—	102	—	424	123	90	68	26	—	—	—
PI 386015	203	1458	247	416	213	748	274	275	747	268	576	279	—
PI 432337	334	384	214	69	147	1053	88	414	1498	284	767	200	88

[z]Caffeoyl-glucose.
[y]Isovitexin-2″-O-glucoside.
[x]Isoorientin.
[w]Peaks D to M = unknown phenolic derivatives of caffeic and/or ferulic acid.
— = Undetected.

Table 2. Total flavonoid content (µg·g^{-1} fresh leaf weight) of various *Citrullus* accessions.[z]

Entry	No.	Total flavonoid content (µg·g^{-1}) ± SEM[y]
C. lanatus var. *lanatus* (Charleston Gray)	2	171.7 ± 7.8
C. lanatus var. *lanatus* (Mickey Lee)	1	102.7
C. lanatus var. *citroides* (PI 500354)	3	905.7 ± 109.5
C. colocynthis (PI 386015)	1	1872.0
C. colocynthis (PI 432337)	1	452.9

[z]Data obtained by high-performance liquid chromatography.
[y]SEM available for two entries.

The *C. colocynthis* thrives in the deserts of northern Africa, the Middle East, and Asia, and a relatively wide genetic diversity exists among accessions of this species collected in these locations (Levi et al., 2001b). A wide genetic distance exists between *C. colocynthis* and watermelon cultivars (*C. lanatus* var. *lanatus*) (Jarret et al., 1997; Levi et al., 2001b). Still, the *C. colocynthis* should be a valuable germplasm source to improve resistance of watermelon cultivars to insect pests. A recent study (Hadizadeh et al., 2009) identified *C. colocynthis* as having antifungal activities. The HPLC analysis in this study identified several compounds unique to *C. colocynthis* (Table 1; Figs. 1 and 2). However, further analysis is needed to determine if these differences exist in a large number of accessions representing the *Citrullus* species or subspecies and to determine what role these compounds may have in pest resistance. We have constructed genetic populations derived from crosses between *C. colocynthis* and *C. lanatus* var. *lanatus* or *C. lanatus* var. *citroides* PIs that will be further evaluated for the presence or absence of the compounds identified in this study and determine if any of these compounds are associated with resistance to whiteflies, aphids, or other pests.

Literature Cited

Asyaz, S., I. Hussain, F. Khan, A. Muran, and I.U. Khan. 2010. Evaluation of chemical analysis profile of *Citrullus coloynthis* growing in southeastern area of Khyber Pukhtunkhwa Pakistan. World Appl. Sci. J. 10:402–405.

Chopra, B., J.S. Jhooty, and K.L. Bajaj. 1974. Biochemical differences between two varieties of watermelon resistant and susceptible to *Alternaria cucunerina*. Phytopathol. Zeitscrift 79:47–52.

Das, V.S.R., J.V.S. Rao, and K.N. Rao. 1967. Levels of phenolic acids during fruit growth in *Citrullus colocynthis*. Intl. J. Plant Physiol. 56:466–468.

Delazar, A., S. Gibbons, A.R. Kosari, H. Nazemiyeh, M. Modarresi, L. Nahar, and S.D. Sarker. 2006. Flavone-C-glycosides and cucurbitacin glycosides from *Citrullus colocynthis*. Daru, J. Faculty Pharmacy, Tehran Univ. Med. Sci. 14:109–114.

Hadizadeh, I., B. Peivastegan, and M. Kolahi. 2009. Antifungal activity of nettle, colocynth, oleander and konar extracts on plant pathogenic fungi. Pak. J. Biol. Sci. 12:58–63.

Harsh, M.L. and T.N. Nag. 1988. Flavonoids with antimicrobial activities of arid zone plants. Geobios (Jodhpur, India) 15:32–35.

Harrison, H.F., Jr., T.R. Mitchell, J.K. Peterson, W.P. Wechter, G.R. Majetich, and M.E. Snook. 2008. Contents of caffeoylquinic acid compounds in the storage roots of sixteen sweetpotato genotypes and their potential biological activity. J. Amer. Soc. Hort. Sci. 133:492–500.

Ibrahim, T.A., H.M. El-Hefnawy, and A.A. El-Hela. 2010. Antioxidant potential and phenolic acid content of certain cucurbitaceous plants cultivated in Egypt. Nat. Prod. Res. 24:1537–1545.

Jarret, R.L., L.C. Merrick, T. Holms, J. Evans, and M.K. Aradhya. 1997. Simple sequence repeats in watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai]. Genome 40:433–441.

Levi, A., C.E. Thomas, A.P. Keinath, and T.C. Wehner. 2001a. Genetic diversity among watermelon (*Citrullus lanatus* and *Citrullus colocynthis*) accessions. Genet. Resources Crop Evol. 48:559–566.

Levi, A., C.E. Thomas, T.C. Wehner, and X. Zhang. 2001b. Low genetic diversity indicates the need to broaden the genetic base of cultivated watermelon. HortScience 36:1096–1101.

Lopez, R., A. Levi, B.M. Shepard, A.M. Simmons, and D.M. Jackson. 2005. Sources of resistance to two-spotted spider mite (Acari: Tetranychidae) in *Citrullus* spp. HortScience 40:1661–1663.

Lugasi, A. and J. Hovari. 2002. Flavonoid aglycons in foods of plant origin. II. Fresh and dried fruits. Acta Aliment. 31:63–71.

Maatooq, G.T., S.H. El-Sharkawy, M.S. Afifi, and J.P.N. Rosazza. 1997. C-p-hydroxybenzylglycoflavones from *Citrullus colocynthis*. Phytochem. 44:187–190.

Meena, M.C. and V. Patni. 2008. Isolation and identification of flavonoid 'quercetin' from *Citrullus coloynthis* (Linn.). Schrad. Asian J. Exp. Sci. 22:137–142.

Mujaju, C., J. Sehic, G. Werlemark, L. Garkava-Gustavsson, M. Fatih, and H. Nybom. 2010. Genetic diversity in watermelon (*Citrullus lanatus*) landraces from Zimbabwe revealed by RAPD and SSR markers. Hereditas 147: 142–153.

Qimin, L., H. Van Den Heuvel, O. Delorenzo, J. Corthout, L.A.C. Pieters, A.J. Vlietinck, and M. Claeys. 1991. Mass spectral characterizations of C-glycosidic flavonoids isolated from a medicinal plant (*Passiflora incarnata*). J. Chromatogr. B Biomed. Sci. Appl. 562: 435–446.

Robinson, R.W. and D.S. Decker-Walters. 1997. Cucurbits. CAB International Publishing, Oxon, UK.

SAS Institute. 2003. SAS/STAT user's guide. SAS Institute, Cary, NC.

Simmons, A.M., C.S. Kousik, and A. Levi. 2010. Combining reflective mulch and host plant resistance for sweetpotato whitefly (Hemiptera: Aleyrodidae) management in watermelon. Crop Prot. 29:898–902.

Simmons, A.M. and A. Levi. 2002. Sources of whitefly (Homoptera:Aleyrodidae) resistance in *Citrullus* for the improvement of cultivated watermelon. HortScience 37:581–584.

Thies, J.A. and A. Levi. 2007. Characterization of watermelon (*Citrullus lanatus* var. *citroides*) germplasm for resistance to root-knot nematodes. HortScience 42:1530–1533.

Tlili, I., C. Hdider, M.S. Lenucci, I. Riadh, H. Jebari, and G. Dalessandro. 2011. Bioactive compounds and antioxidant activities of different watermelon [*Citullus lanatus* (Thunb.) Mansfeld] cultivars as affected by fruit sampling area. J. Food Compost. Anal. 24:307–314.

Venkataramaiah, C. and R.K. Narayana. 1983. Studies on indoyl-3-acetic acid oxidase and phenolic acid pattern in Cucurbitaceous fruits. Intl. J. Plant Physiol. 111:459–463.

Wu, L. 2007. Effect of chlorogenic acid on antioxidant activity of *Flos Lonicerae* extracts. J. Zhejiang Univ. Sci. B 8:673–679.