

Chemical Characterization of Basil (*Ocimum basilicum* L.) Found in Local Accessions and Used in Traditional Medicines in Iran

JAMAL JAVANMARDI,^{†,‡} A. KHALIGHI,[†] A. KASHI,[†] H. P. BAIS,[‡] AND
J. M. VIVANCO^{*,‡}

Department of Horticulture, Faculty of Agriculture, Tehran University, Karaj, Iran, and
Department of Horticulture and Landscape Architecture, Colorado State University,
Fort Collins, Colorado 80523-1173

Ocimum species are used in traditional Iranian medicine, as a culinary herb, and as a well-known source of flavoring principles. Horticultural characteristics, including quantitative and qualitative traits along with the chemical variation of phenolic acids, of 23 accessions of basil (*Ocimum basilicum* L.) from Iran were studied. Morphological studies of accessions showed a high level of variability in recorded traits. Quantification of phenolic acids was determined using high-performance liquid chromatography and showed drastic variations between accessions. Chemical studies revealed that rosmarinic acid is the predominant phenolic acid present in both flower and leaf tissues. Unusual basil accessions were identified that can serve as genetic sources of phenolic acids for crop improvement.

KEYWORDS: Lamiaceae; basil; *Ocimum basilicum*; phenolic acids

INTRODUCTION

Basil (*Ocimum basilicum* L.), a member of the Lamiaceae family, is used both as a culinary and ornamental herb (1–3). The genus *Ocimum* contains between 50 and 150 species of herbs and shrubs found in the tropical regions of Asia, Africa, and Central and South America (4, 5). Traditionally basil has been used as a medicinal plant in the treatment of headaches, coughs, diarrhea, constipation, warts, worms, and kidney malfunctions (6). Externally, basil can be used as an ointment for insect bites, and its oil is applied directly to the skin to treat acne (7). Natural components from basil have long been used to flavor foods and dental and oral products (6, 8). Iranian basil accessions are used to treat fevers, throat congestions, and stomachache (9).

Rosmarinic acid (RA) (α -*O*-caffeoyl-3-4-dihydroxyphenyl-lactic acid) is one of the most abundant caffeic acid esters present in *Ocimum* spp. (10). RA and its derivatives have been reported to have antioxidant, anti-HIV (11), and anti-inflammatory or cyclooxygenase inhibitory activity, comparable to ibuprofen, naproxen, and aspirin (12). Similar to RA, lithospermic acid B (LAB) is known to be a common phenolic constituent in most members of the Lamiaceae family (3, 13) and exhibits endothelium-dependent vasodilator and hypotensive effects (14–16). In an earlier communication, we demonstrated

that RA may serve as a constitutively accumulated and induced defense compound against various plant pathogens and *Pseudomonas aeruginosa* (10).

In this paper, we report on the examination of 23 Iranian basil accessions and their secondary metabolite content. The phenolic acid (PhA) constituents were examined in 23 *O. basilicum* accessions. As the environmental conditions can significantly modify the secondary metabolite content in basil (17), we collected *O. basilicum* accession seeds, which are cultured traditionally in small farms and home gardens, and raised them to maturity at Tehran University Experimental Research Station. This allowed us to comparatively examine RA, LAB, caffeic acid, and free phenolic acid contents without environmental influences.

MATERIALS AND METHODS

Plant Material. Seeds of 23 accessions of basil (*O. basilicum* L.) were collected from small farmers, home gardens, and open markets in Iran and were grown to flowering stage at Tehran University Experimental Research Station, Iran (Table 1). This collection represents most of the traditional basil plants used for culinary and medicinal purposes in Iran. Seeds of all *O. basilicum* accessions were grown in a sterilized soil mix. Three seedlings of each accession were transplanted into 8 L pots and arranged in randomized complete block design and in three replicates. Greenhouse plants were irrigated to pot capacity daily and maintained at day/night temperatures of 26–30 and 18–21 °C, respectively. Four quantitative characters (days to flowering, plant length, branch numbers, and inflorescence length) were measured before harvesting. Six qualitative or phenotypic characters (leaf shape, leaf margin, leaf color, leaf surface, stem color, and flower color) were

* Corresponding author. Jorge M. Vivanco, Department of Horticulture and Landscape Architecture, Colorado State University, Fort Collins, CO, 80523. E-mail: jvivanco@lamar.colostate.edu.

[†] Tehran University.

[‡] Colorado State University.

Table 1. Different Basil (*O. basilicum* L.) Accessions from Iran, Depicting Variable Phenotypic Traits^a

accession no.	origin (city)	leaf shape	leaf surface	leaf color	leaf margin	stem color	bract color	flower color	branch no.	plant weight ^b (g)	plant height (cm)	inflorescence length (cm)	days to flowering
140-05	Babol	O-L	flat	green	entire	green	green	white	15.11	64.33	100.22	20.00	39
143-01	Birjand	ovate	undulate	green	entire	green	green	white	14.44	79.56	91.67	23.67	49
133-15	Brujerd	O-L	flat	green	entire	green	green	white	14.44	108.89	103.67	21.22	42
125-17	Dezful I	O-L	flat	green	entire	green	green	white	14.44	80.00	104.67	19.00	42
125-12	Dezful II	O-L	undulate	green	entire	green	green	white	13.78	60.89	84.00	20.11	39
133-15	Isfahan	O-L	undulate	green	entire	green	green	white	14.33	69.33	100.33	19.78	43
133-19	Isfahan (green)	ovate	flat	green	entire	green	green	white	17.56	47.11	104.67	11.00	49
133-12	Isfahan (purple)	lanceolate	flat	purple-green	serrate	red	purple	white	16.22	70.00	99.22	12.11	39
170-13	Kerman	ovate	flat	green	entire	green	green	white	15.67	60.78	103.44	18.00	49
171-04	Kermanshah	O-L	undulate	green	entire	green	green	white	14.78	99.33	104.00	21.78	40
151-11	Khorram abad	ovate	undulate	purple-green	entire	green	pale purple	white	13.33	87.33	100.89	15.56	39
155-08	Khuzestan	O-L	flat	green	entire	green	pale purple	white	12.00	66.67	104.89	27.33	42
174-07	Mahallat	lanceolate	flat	green	entire	green	green	white	14.67	105.56	85.11	22.33	39
178-14	Malavi	lanceolate	flat	purple	serrate	purple	purple	pink	14.67	54.11	84.22	19.78	46
116-21	Orumieh	lanceolate	flat	green	serrate	red	purple	white	15.44	96.44	100.56	18.44	47
168-08	Qazvin	O-L	flat	purple-green	serrate	red	purple	white	13.33	51.11	105.56	10.78	39
163-11	Qom	O-L	flat	purple	entire	green	green	white	13.22	46.67	96.78	21.33	42
157-06	Sanandaj	O-L	flat	green	entire	green	green	white	14.67	80.78	103.78	18.11	49
148-16	Shahr rey	lanceolate	flat	purple-green	serrate	purple	purple	pink	13.78	40.44	101.22	22.78	39
183-27	Yazd	lanceolate	flat	purple-green	serrate	purple	purple	white	10.22	39.22	91.33	19.22	39
121-12	Zabol I	ovate	flat	green	entire	green	green	white	18.00	79.78	115.33	19.89	42
121-18	Zabol II	O-L	flat	green	entire	green	green	white	15.67	62.67	94.44	19.89	42
121-14	Zabol III	ovate	undulate	green	entire	green	green	white	18.56	127.67	111.11	21.33	45
LSD ^c ($p \leq 0.05$)									3.06	41.18	8.97	7.03	
LSD ^c ($p \leq 0.01$)									4.09	55.01	11.99	9.39	

^a Different accessions were scaled on the basis of their origin and morphological characteristics such as leaf shape, leaf margin, stem color, bract color, number of shoots, yield parameters, and days to flowering. All basil accessions were grown to flowering stage at Tehran University Experimental Research Station, Iran. ^b On a fresh weight basis. ^c Least significant differences.

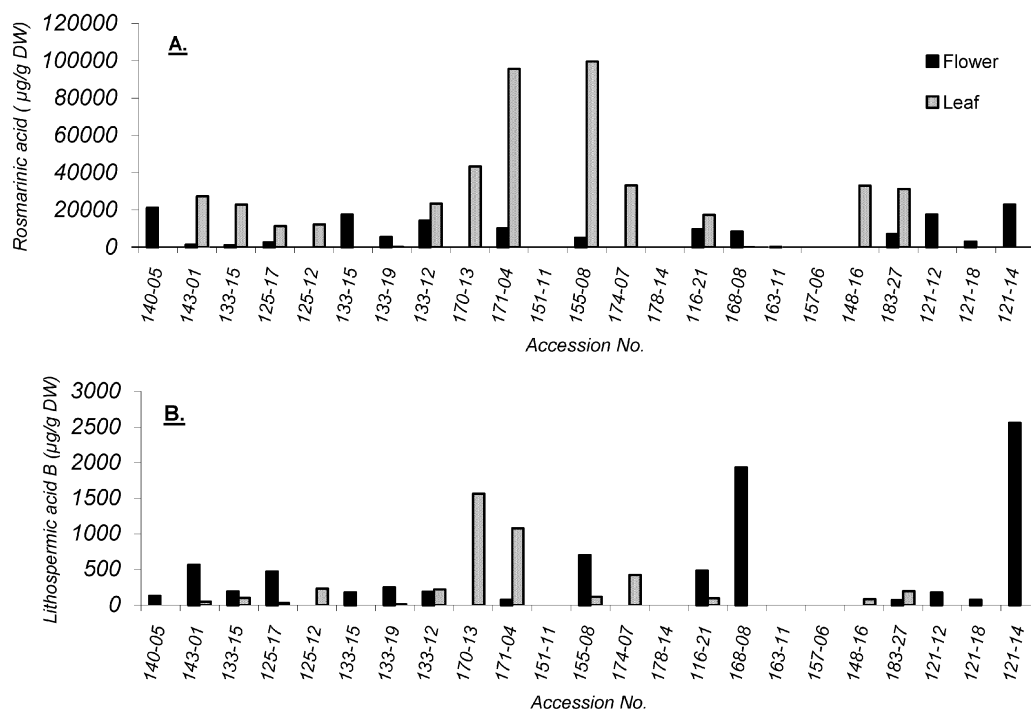


Figure 1. Chemical characterization of 23 accessions of basil (*O. basilicum* L.) from Iran based on endogenous titers of rosmarinic acid (A) and lithospermic acid B (B). Rosmarinic acid and lithospermic acid B were quantified using HPLC. Identification and quantification of each compound was calculated by the comparison of retention time and peak area of respective standards.

also recorded. The above-ground biomass of each plant was harvested at full bloom, weighed, bulked, placed in a paper bag, and dried in a forced-air drier at 32 °C for 15 days for chemical analyses.

Voucher specimens of each accession were also collected, dried, and stored at the Tehran University Herbarium, Iran (Table 1). Taxonomic identification of each accession was conducted by T. Masumi (Plant Taxonomist at Tehran University).

Extraction of Phenolic Compounds. For each accession, 500 mg of dried leaf and flower head material were pulverized separately in a

mortar, suspended in 5 mL of absolute methanol, and left overnight at 4 °C under dark conditions. All supernatants were decanted and filtered using a Whatman syringe filter with a 0.45 µm pore size.

Phenolic Compound Standards. RA used as standard was purchased from ICN (Costa Mesa, CA). Other free phenolic acids including LAB, vanillic acid, *p*-coumaric acid, hydroxybenzoic acid, syringic acid, ferulic acid, protocatechuic acid, caffeic acid, and gentisic acid were obtained from Sigma Co. (St. Louis, MO). All other chemicals used were of analytical and HPLC grade.

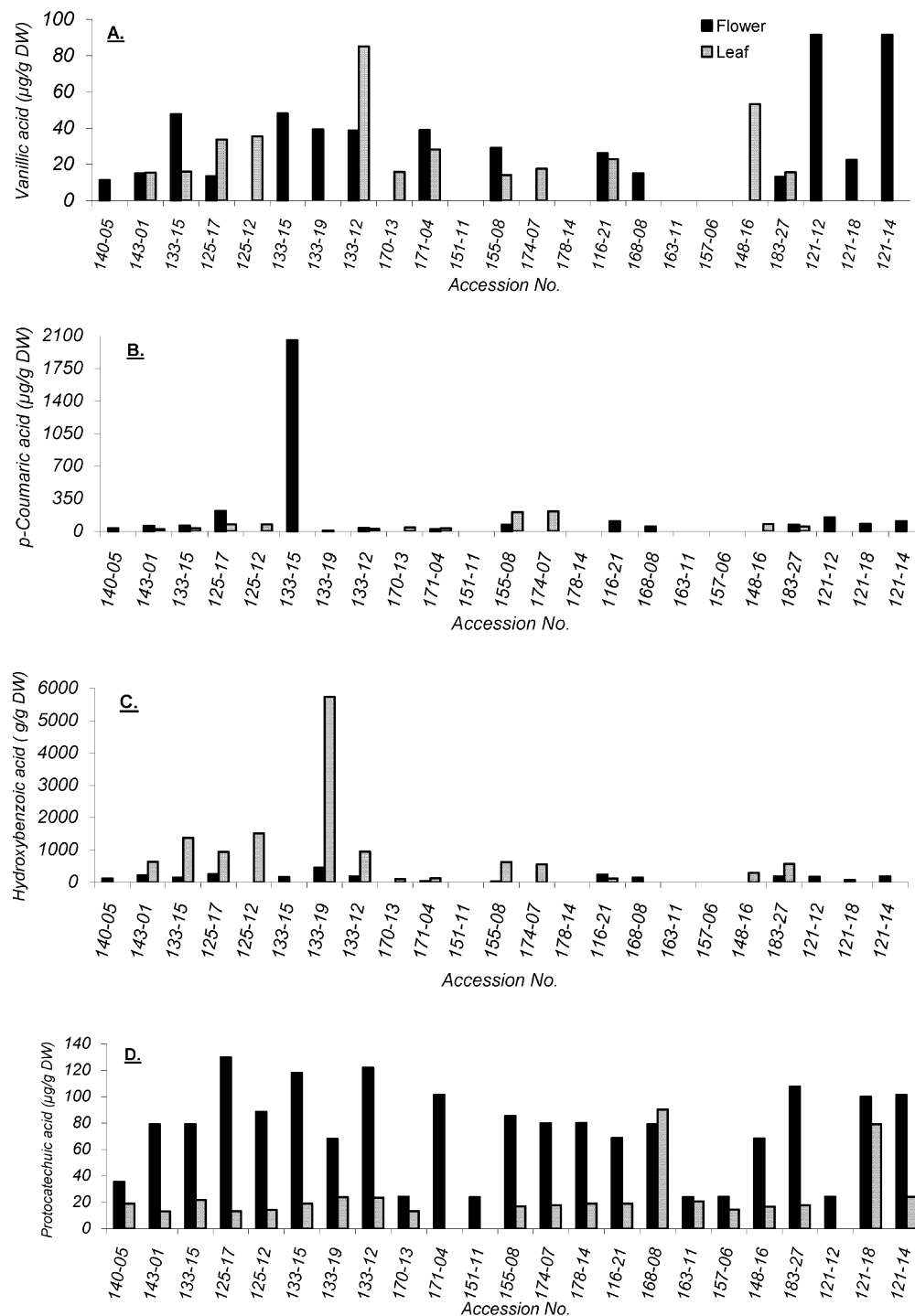


Figure 2. Chemical characterization of 23 accessions of basil (*O. basilicum* L.) from Iran based on endogenous titers of vanillic acid (A), *p*-coumaric acid (B), hydroxybenzoic acid (C), and protocatechuic acid (D) using HPLC. Identification and quantification of each compound was calculated by the comparison of retention time and peak area of respective standards.

High-Performance Liquid Chromatography (HPLC) Analysis of Phenolic Compounds. The HPLC system consisted of a P580 pump (Dionex Co., Sunnyvale, CA), connected to an ASI-100 automated sample injector. A reverse phase C_{18} column ($5 \mu\text{m}$ particle size, $25 \text{ cm} \times 4.6 \text{ mm}$) was used. The absorbance at 280 nm was measured by a PDA-100 Photodiode array variable UV/vis detector (Dionex Co.). Mobile phase solution A consisted of 0.1% TFA (trifluoroacetic acid) in water, and absolute acetonitrile was used as solution B (Fisher Co., USA). A multistep gradient was used for all separations with an initial injection volume of $15 \mu\text{L}$ and a flow rate of 1 mL min^{-1} . The multistep gradient was as follows: 10% B, 0–5 min; 35% B, 5–20 min; 70%

B, 20–40 min; 90% B, 40–41 min; 50% B, 41–42 min; 25% B, 42–43 min; 5% B, 43–44 min; 100% A, 45 min. Phenolic acids in each sample were identified using retention time and were further quantified by comparison of peak area of the standard runs.

Statistical Analysis. The data of phytochemical analysis were used to compute a similarity distance matrix. Clustering of accessions and correlation analysis among PhAs were obtained by hierarchical clustering Ward's method and the Pearson correlation method, respectively. Analysis of variance (ANOVA), least significant differences (LSD), clustering, and correlation were all performed using a SPSS 8 (SPSS Inc., Chicago, IL) statistical computer package.

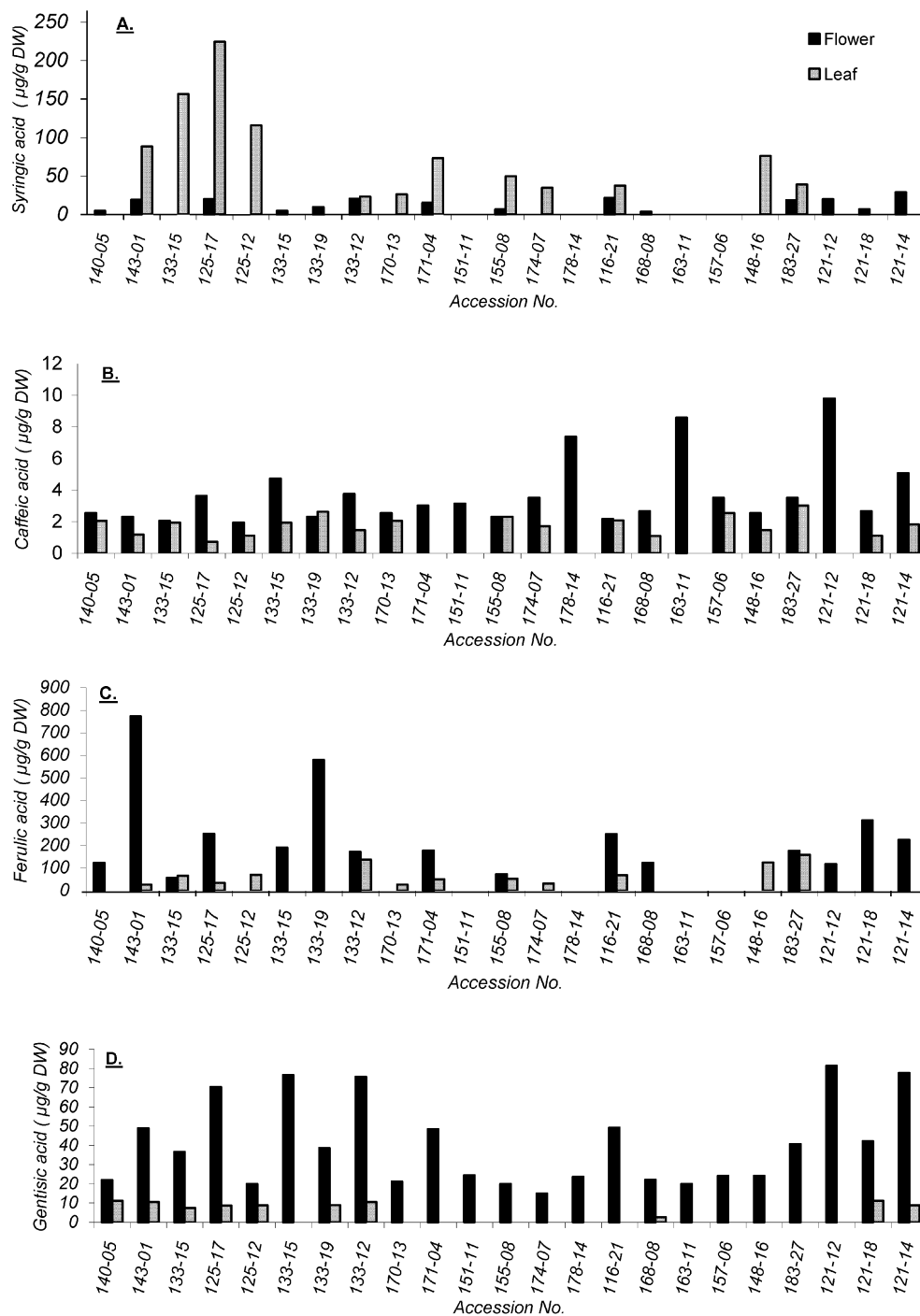


Figure 3. Chemical characterization of 23 accessions of basil (*O. basilicum* L.) from Iran based on endogenous titers of syringic acid (A), caffeic acid (B), ferulic acid (C), and gentisic acid (D) using HPLC. Identification and quantification of each compound was calculated by the comparison of retention time and peak area of respective standards.

RESULTS AND DISCUSSION

Morphological Analysis. The examined basil accessions showed variability in observed phenotypic characteristics including leaf shape, leaf margin, leaf color, leaf surface, stem color, and flower color (**Table 1**). The same trend was observed for plant height and inflorescence length (**Table 1**). Interspecific hybridization and polyploidy are common occurrences within the *Ocimum* genus, which has led to taxonomic confusion and challenges, yet very little has been published on *Ocimum* taxonomy. The morphological diversity within basil species has been accentuated by centuries of cultivation and is characterized by great variation in pigmentation and leaf shape and size (18).

Taxonomy is further complicated by the existence of chemotypes or chemical races within species that do not differ significantly in morphology (2).

Phytochemical Analysis. RA, LAB, vanillic acid, *p*-coumaric acid, hydroxybenzoic acid, syringic acid, ferulic acid, protocatechuic acid, caffeic acid, and gentisic acid were identified in various concentrations in flower and leaf tissues of the 23 examined accessions (**Figures 1–3**). RA was the predominant phenolic compound found in these basil accessions. The highest concentration of RA was observed in the leaves of accession 155-08 (99.62 mg/g DW) (**Figure 1**). It is possible that a dry-warm condition of the geographical origin of accession 155-08

Table 2. Pearson Correlations between Phenolic Acids in Leaf and Flowers of *O. basilicum* Based on 23 Iranian Accessions

PhAs ^a	RA-L	LAB-L	VA-L	PCA-L	HBA-L	SA-L	FA-L	PC-L	CA-L	GA-L	RA-F	LAB-F	VA-F	PCA-F	HBA-F	SA-F	FA-F	PC-F	CA-F	GA-F
RA-L	1.000	0.576 ^b	0.354	0.627 ^b	-0.049	0.288	0.421 ^c	-0.282	0.095	-0.276	-0.126	-0.102	-0.032	-0.158	-0.239	0.066	-0.058	0.216	-0.333	-0.155
LAB-L		1.000	0.224	0.209	-0.086	0.089	0.160	-0.235	0.016	-0.254	-0.153	-0.214	-0.147	-0.139	-0.295	-0.093	-0.186	-0.085	-0.217	-0.162
VA-L			1.000	0.297	0.072	0.447 ^c	0.783 ^b	-0.178	0.023	0.190	-0.067	-0.188	-0.101	-0.155	0.005	0.216	-0.053	0.434 ^c	-0.281	0.192
PCA-L				1.000	0.044	0.347	0.324	-0.168	0.212	-0.189	-0.311	-0.104	-0.227	-0.136	-0.287	-0.168	-0.231	0.225	-0.263	-0.320
HBA-L					1.000	0.149	0.055	-0.038	0.333	0.372	-0.135	-0.088	0.091	-0.118	0.636 ^b	0.025	0.458 ^c	0.128	-0.282	-0.012
SA-L						1.000	0.372	-0.225	-0.047	0.271	-0.332	-0.096	-0.106	-0.095	0.119	0.116	0.091	0.418 ^c	-0.339	0.100
FA-L							1.000	-0.170	0.314	-0.027	-0.088	-0.208	-0.114	-0.164	0.021	0.218	-0.087	0.410	-0.316	0.055
PC-L								1.000	0.098	0.282	0.012	0.419 ^c	-0.061	-0.019	0.095	-0.117	0.124	0.227	-0.199	-0.130
CA-L									1.000	0.121	0.124	0.104	0.012	0.120	0.329	0.055	0.169	0.135	-0.537 ^c	-0.080
GA-L										1.000	0.215	0.216	0.184	-0.142	0.465 ^c	0.298	0.540 ^b	0.347	-0.325	0.266
RA-F											1.000	0.459 ^c	0.731 ^b	0.369	0.397	0.587 ^b	0.168	0.196	0.218	0.644 ^b
LAB-F												1.000	0.474 ^c	-0.008	0.300	0.459 ^c	0.218	0.253	-0.030	0.287
VA-F													1.000	0.266	0.483 ^c	0.635 ^b	0.269	0.220	0.322	0.775 ^b
PCA-F														1.000	0.162	0.004	0.079	0.330	0.122	0.447 ^c
HBA-F															1.000	0.621 ^b	0.757 ^b	0.338	-0.116	0.567 ^b
SA-F																1.000	0.556 ^b	0.435 ^c	0.112	0.790 ^b
FA-F																	1.000	0.309	-0.232	0.406
PC-F																		1.000	-0.252	0.468 ^c
CA-F																			1.000	0.306
GA-F																				1.000

^a RA, rosmarinic acid; LAB, lithospermic acid B; VA, vanillic acid; PCA, *p*-coumaric acid; HBA, hydroxybenzoic acid; SA, syringic acid; FA, ferulic acid; PC, protocatechuic acid; CA, caffeic acid; GA, gentisic acid. L = leaf; F = flower. ^b Correlation is significant at the 0.01 level. ^c Correlation is significant at the 0.05 level.

may be the cause of high titers of RA in leaf tissues, which may protect the plant against the fluctuating environmental conditions. Accordingly, it has been reported that production of caffeic acids including RA is highly dependent on climatic conditions (19). RA is a known antioxidant and anti-inflammatory and immunostimulating agent (20); thus this accession is characterized by its high RA content, which may be useful in breeding programs.

The highest amounts of LAB were observed in the flowers of accession 121-14 (~2500 $\mu\text{g/g}$ DW) (Figure 1). Vanillic acid in flowers of accessions 121-12 and 121-14 was in the range of ~90–100 $\mu\text{g/g}$ DW (Figure 2). In contrast, Zgorzka and Glowniak (21) have reported 8 $\mu\text{g/g}$ DW of vanillic acid in basil accessions from Poland. This striking variation in the vanillic acid content in flowers may be related to a survival strategy in these plants for producing seeds. Vanillic acid, *p*-coumaric acid, protocatechuic acid, syringic acid, ferulic acid, and gentisic acid in most of the examined accessions were in the range of 10–250 $\mu\text{g/g}$ DW (Figures 2 and 3). Exceptionally, it was observed that the flowers of accession 133-15 accumulated >2000 $\mu\text{g/g}$ DW of *p*-coumaric acid compared to other examined accessions (Figure 2). *p*-Coumaric acid is also reported as an abundant plant phenolic acid (7) and has dietary chemoprotectant and antioxidant activity (22). It has been shown that ferulic acid may have potential as a preventative agent against colon tumor development (23). It was observed that accession 133-19 harbored the maximum content of hydroxybenzoic acid in leaves (>5700 $\mu\text{g/g}$ DW) compared to other accessions (Figure 2). The harsh climatic and environmental conditions of the origin of accessions 133-15 and 133-19 seem to affect this basil's seed accessions to produce a high amount of *p*-coumaric acid and hydroxybenzoic acid (Figure 2).

In the present study, caffeic acid was present in the lowest concentration among analyzed PhA compounds in both flower and leaf extracts (<10 $\mu\text{g/g}$ DW) (Figure 3). In contrast, Zgorzka and Glowniak (21) observed higher endogenous titers of caffeic acid (>2500 $\mu\text{g/g}$ DW). This result could be due to the changes in the biosynthetic pathway of PhAs connected to environmental stress conditions during maturation. The decrease in the total concentration of free phenolics during maturation has been shown in other fruits such as apple and pepper (19, 24).

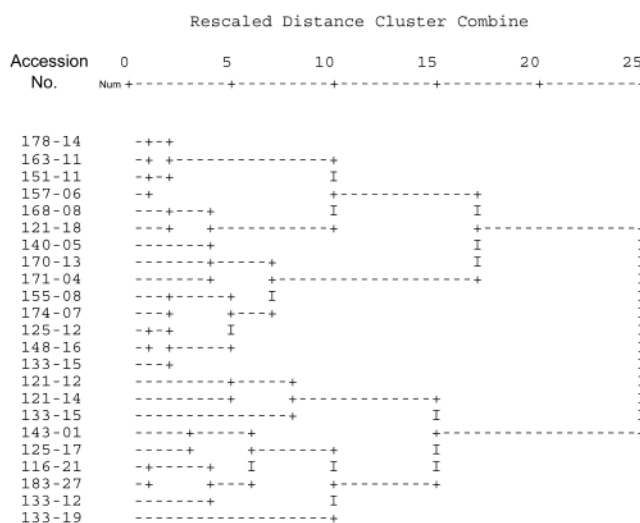


Figure 4. Clustering of 23 Iranian basil accessions based on total phenolic acid constituents in leaf and flower tissues. The data were standardized with Z-score, and the dendrogram was drawn using Ward's method in the SPSS 8 statistical computer package.

Statistical Analysis. The Pearson correlation analysis showed that there are highly significant correlations between concentrations of certain phenolic acids in leaves and flowers of *O. basilicum* (more than 0.6 at the 0.01 level) (Table 2). In the case of leaves, there were positive correlations between RA and *p*-coumaric acid, and vanillic acid and ferulic acid (Table 2). A highly significant correlation between flower and leaf tissues was observed for hydroxybenzoic acid with its occurrence in minimum endogenous concentrations in both leaf and flower tissues in all the tested accessions (Table 2).

On the basis of cluster analysis of PhA content, four groups were chemically distinguished (Figure 4). The first group belonged to north and northwest Iran and was low in PhAs in both leaves and flowers; the second group, from the western provinces, was high in PhAs, especially RA and LAB, in leaves, but most of these accessions did not show any PhAs in flowers (data not shown). The third group, from the eastern part of Iran, did not show a considerable amount of PhAs in leaves but had high levels of endogenous RA in the flower tissues (Figure 1).

Eastern and western Iran are characterized by arid and semiarid regions with extended dry periods (25), and it appears that basil accessions in these places tend to produce more PhAs in flower tissues in response to severe environmental conditions. The north and northwest of Iran are characterized by humid and semihumid cold regions (25); basil plants from this area showed low to moderate amounts of PhAs in flowers and leaves. Since PhAs are stress-related compounds produced under harsh environmental conditions (10), it seems that in humid and semihumid conditions there is no need to produce these compounds.

ACKNOWLEDGMENT

We thank T. R. Masumi for his collaboration in the taxonomical identification and M. R. Nagahvi, M. Fazel, and S. Rezaei, Tehran University, for their assistance.

LITERATURE CITED

- (1) Morales, M. R.; Simon, J. E. New basil selections with compact inflorescences for the ornamental market. In *Progress in new crops*; Janick, J., Ed.; ASHS Press: Arlington, VA, 1996; pp 543–546.
- (2) Simon, J. E.; Morales, M. R.; Phippen, W. B.; Vieira, R. F.; Hao, Z. Basil: A source of aroma compounds and a popular culinary and ornamental herb. In *Perspectives on new crops and new uses*; Janick, J., Ed.; ASHS Press: Alexandria, VA, 1999; pp 499–505.
- (3) Tada, H.; Murakami, Y.; Omoto, T.; Shimomura, K.; Ishimura, K. Rosmarinic acid and related phenolics in hairy root culture of *Ocimum basilicum*. *Phytochemistry* **1996**, *42* (2), 431–434.
- (4) Bailey, L. H. *Manual of cultivated plants*; MacMillan: New York, 1924.
- (5) Darrah, H. H. *The cultivated basil*; Buckeye Printing: Independence, MO, 1980.
- (6) Simon, J. E.; Chadwick, A. F.; Craker, L. E. *Herbs: an indexed bibliography, 1971–1980. The scientific literature on selected herbs, and aromatic and medicinal plants of the temperate zone*. Archon Books: Hamden, CT, 1984.
- (7) Waltz, L. *The Herbal Encyclopedia*; <http://www.wic.net/waltzark/herbenc.htm> (1996).
- (8) Simon, J. E. *New Crop Fact Sheet Basil*; <http://www.hort Purdue.edu/newcrop/CropFactSheet/basil.html> (1995).
- (9) Omidbeigi, R. *Production and processing of medicinal plants*; Astan Ghods Razavi Press: Tehran, Iran, 2000; Vol. I, pp 99–104.
- (10) Bais, H. P.; Walker, T. S.; Schweizer, H. P.; Vivanco, J. M. Root specific elicitation and antimicrobial activity of rosmarinic acid in hairy root cultures of sweet basil (*Ocimum basilicum* L.). *Plant Physiol. Biochem.*, in press.
- (11) Mazumder, A.; Neamati, N.; Sunder, S.; Schulz, J.; Pertz, H.; Eich, E.; Pommier, Y. Curcumin analogues with altered potencies against HIV-1 integrase as probes for biochemical mechanisms of drug action. *J. Med. Chem.* **1997**, *40*, 3057–3063.
- (12) Kelm, M. A.; Nair, M. G.; Strasburg, G. M.; DeWitt, D. L. Antioxidant and cyclooxygenase inhibitory phenolic compounds from *Ocimum sanctum* Linn. *Phytomedicine* **2000**, *7* (1), 7–13.
- (13) Tanaka, T.; Morimoto, S.; Nonaka, G.; Nishioka, I.; Yokozawa, T.; Chung, H. Y.; Oura, H. Magnesium and ammonium-potassium lithospermates B, the active principles having a uremia-preventive effect from *Salvia miltiorrhiza*. *Chem. Pharm. Bull.* **1989**, *37*, 340–344.
- (14) Hase, K.; Kasimu, R.; Basnet, P.; Kadota, S.; Namba, T. Preventive effect of lithospermate B from *Salvia miltiorrhiza* on experimental hepatitis induced by carbon tetrachloride or D-galactosamine: lipopolysaccharide. *Planta Med.* **1997**, *63*, 22–26.
- (15) Kamata, K.; Iizuka, T.; Nagai, M.; Kasuya, Y. Endothelium-dependent vasodilator effects of the extract from *Salvia miltiorrhizae Radix*. A study on the identification of lithospermic acid B in the extracts. *Gen. Pharmacol.* **1993**, *24*, 977–981.
- (16) Kamata, K.; Noguchi, M.; Nagai, M. Hypotensive effects of lithospermic acid B isolated from the extract of *Salvia miltiorrhiza Radix* in the rat. *Gen. Pharmacol.* **1994**, *25*, 69–73.
- (17) Vieira, R. F.; Simon, J. E. Chemical characterization of basil (*Ocimum* spp.) found in the markets and used in traditional medicine in Brazil. *Econ. Bot.* **2000**, *54* (2), 207–216.
- (18) Simon, J. E.; Quinn, J.; Murray, R. G. Basil: A source of essential oils. In *Advances in new crops*; Janick, J., Simon, J. E., Eds.; Timber Press: Portland, OR, 1990; pp 484–489.
- (19) Burda, S.; Oleszek, W.; Lee, C. Y. Phenolic compounds and their changes in apple during maturation and cold storage. *J. Agric. Food Chem.* **1990**, *38*, 945–948.
- (20) Simpol, L. R.; Otsuka, H.; Ohtani, K.; Kasai, R.; Yamasaki, K. Nitrile glycosides and rosmarinic acid, the histamine inhibitor from *Ehretia philippinensis*. *Phytochemistry* **1994**, *36*, 91–95.
- (21) Zgorka, G.; Glowniak, K. Variation of free phenolic acids in medicinal plants belonging to the Lamiaceae family. *J. Pharm. Biomed. Anal.* **2001**, *26* (1) 79–87.
- (22) Torres, Y.; Torres, J. L.; Rosazza, J. P. Reactions of *p*-coumaric acid with nitrite: product isolation and mechanism studies. *J. Agric. Food Chem.* **2001**, *49* (3), 1486–1492.
- (23) Han, B. S.; Park, C. B.; Takasuka, N.; Naito, A.; Sekine, K.; Nomura, E.; Taniguchi, H.; Tsuno, T.; Tsuda, H. A ferulic acid derivative, ethyl 3-(4'-geranyloxy-3-methoxyphenyl)-2-propenoate, as a new candidate chemopreventive agent for colon carcinogenesis in the rat. *Jpn. J. Cancer Res.* **2001**, *92* (4), 404–409.
- (24) Berta, E.; Bernal, M. A.; Díaz, J.; Pomar, F.; Merino, F. Fruit development in *Capsicum annuum*: changes in capsaicin, lignin, free phenolics, and peroxidase patterns. *J. Agric. Food Chem.* **2000**, *48* (12), 6234–6239.
- (25) *National CBD Reports*. Current status of biodiversity conservation and sustainable development of Iran. <http://www.caspianenvironment.org/biodiversity/iran/first.htm> (2000).

Received for review April 25, 2002. Revised manuscript received July 26, 2002. Accepted July 30, 2002. The Colorado State University Agricultural Experiment Station (J.M.V.) supported work reported in this communication. J.M.V. is a NSF Early Career Development Faculty Fellow. J.J. acknowledges the support from Tehran University, Iran.

JF020487Q