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Genetic Diversity of Basil (*Ocimum* spp.) Based on RAPD Markers

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Abstract. Molecular markers were used to assess genetic diversity in basil (*Ocimum* L. spp., Lamiaceae). Using randomly amplified polymorphic DNA (RAPD) analysis, 11 primers generated 98 polymorphic bands, ranging from 300 to 2,000 base pairs, that discriminated among 37 accessions across nine *Ocimum* spp. Means of genetic similarities within *Ocimum* spp. showed that the domesticated species, *O. minimum* L. (0.887), *O. basilicum* L. (0.769), and *O.* ×*citriodorum* Vis. (0.711) had highest similarity indices within species, while the nondomesticated, *O. americanum* L. (0.580), *O. gratissimum* L. (0.408), and *O. kilimandscharicum* Guerke (0.559) showed the lowest similarity. RAPD results indicated that *O. minimum* should not be considered a distinct species but rather a variety of *O. basilicum*. Consistent clusters among all but one of the *O.* ×*citriodorum* spp., all containing citral as the major constituent, were identified using bootstrap analysis. RAPD analysis was useful in discriminating among *Ocimum* spp., although within species resolution will require a higher number of polymorphic bands.

The genus *Ocimum* (Lamiaceae) consists of about 30 species distributed in the tropics and subtropics of the Old and New Worlds, with some species cultivated in temperate areas (Paton, 1992). The main center of diversity of *Ocimum* spp. appears to be Africa (Hedge, 1992), with a secondary center in South America (Brazil) and Asia (India) (Sobti and Pushpangadan, 1982, Vieira and Simon, 2000).

Among *Ocimum* spp., common basil (*O. basilicum*) is the most important economically. The aromatic leaves of basil are used fresh and dried as flavorings or spices in a wide variety of foods. Volatile oils of basil are used to flavor foods, in dental and oral products, and in fragrances. Basil and other closely related *Ocimum* spp., all referred to collectively as basil, are also used in traditional ceremonial rituals and as medicines, and contain biologically active constituents that are insecticidal, nematicidal, fungistatic, or antimicrobial (Simon et al., 1990).

The genus *Ocimum* is divided into three sections: 1) section *Ocimum*, with appendiculate posterior stamens, which is subdivided, based on calix morphology, into subsection *Ocimum* (containing the most economically important species, such as *O. basilicum, O. americanum, O. kilimandscharicum, O. minimum*, and *O.* ×*citriodorum*), and subsection *gratissima* (*O. gratissimum*); 2) section *Hierocymum*, with fascicles of hairs at the base of the posterior stamens (*O. selloi* Benth. and *O. tenuiflorum* L.), and 3) section *Gymnocymum*, with glabrous posterior stamens (*O. campechianum* Mill.) (Paton, 1992).

Interspecific hybridization and polyploidy, common occurrences within this genus (Harley and Heywood, 1992), have created taxonomic confusion and challenges to our understanding of the genetic relationships among the multitude of basils, highlighting a need to revise the taxonomy of the genus (Tucker, 1986). Taxonomy of *O. basilicum* is also complicated by existence of numerous botanical varieties, cultivars, and chemotypes within the species that do not differ significantly in morphology (Simon et al., 1990). Morphological differences between species can be tenuous, and attempts to solve these problems with only morphology are innocuous. Taxonomic issues in this genus based on morphology and chromosome number were reviewed recently and addressed partially by Paton and Putievisky (1996).

Assays based on polymerase chain reaction (PCR), such as random amplified polymorphic DNA (RAPD) (Williams et al., 1990), provide an efficient means to produce large number of molecular markers. These can be used to estimate genetic relatedness among plants and breeding materials, to distinguish cultivars, and allow reexamination of the classification of important species delimitation (Chalmers et al., 1994, Tingey and del Tufo, 1993). A major advantage of RAPD markers over some other DNA-based markers is that they require no prior sequence information, and no prior knowledge about any particular gene in a target taxon (Palumbi, 1996). Therefore, RAPD markers can be used in the systematic study of wild plants and new crops. PCR methods require lower amounts of genomic DNA, are nonradioactive, relatively low cost, and can be developed rapidly. These characteristics facilitate screening of large populations of plants.

Several studies have highlighted the benefits of RAPD markers to assess genetic diversity in crops, including blueberry (*Vaccinium* L. spp.) (Levi and Rowland, 1997), *Brassica* L. spp. (Demeke et al., 1992; dos Santos et al., 1994), *Chrysanthemum* L.

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spp. (Scott et al., 1996), cranberry (*Vaccinium macrocarpon* (Ait.) Pursh) (Novy et al., 1994), grain amaranth (*Amaranthus* spp., Adams) (Transue et al., 1994), palms (*Elaeis guineensis* A. Cheval) (Shah et al., 1994), and white potato (*Solanum tuberosum* L.) (Demeke et al., 1996). RAPD markers have also been used successfully for taxonomic purposes. They have been used in discrimination of aromatic plants in *Juniperus* L. spp. to compare junipers (*Juniperus excelsa* M. Bieb.) from Saudi Arabia with *J. excelsa* M. Bieb. from Greece and *J. procera* Hochst ex Endl. from Ethiopia. Both RAPD markers and leaf terpenoids enabled identification of the Saudi Arabia junipers as *J. procera* (Adams et al., 1993). Most recently, RAPD markers have been used successfully in differentiating chemotypes of *O. gratissimum* (Vieira et al., 2001). They have also been reported useful in classification of *Brassica* spp. (Demeke et al., 1992) at the taxonomic level ranging from individuals, to cultivars and species; in coffee (*Coffea* L. spp) (Orozco-Castill et al., 1996); and cocoa (*Theobroma* L. spp.) (Figueira et al., 1994). When used in concert with other plant characteristics such as morphology, chromosome number, chemical constituents, and other molecular markers, RAPD markers can provide a robust classification criteria that could be useful in species separation and systematic. Therefore, the objective of this research was to characterize genetic diversity within *Ocimum*, comparing accessions from nine species and chemotypes within *Ocimum*, using RAPD markers.

Materials and Methods

Plant material. Thirty-seven accessions in nine *Ocimum* spp. (Table 1) obtained from Richters Seed Co., Goodwood, Ontario, Canada (12 accessions), Companion Plants, Athens, Ohio (seven accessions), Purdue University, West Lafayette, Ind., (four accessions), Embrapa, Brasília, Brazil (five accessions), U.S. Department of Agriculture (USDA) (four accessions), Kew Garden, United Kingdom (four accessions), and Nichols Garden, Albany, Oregon (one accession), were grown in the field at the Purdue University Research Station, West Lafayette. Tissue samples of fresh young leaves of the 37 accessions were collected, and placed in Eppendorf tubes, the tubes were placed immediately in liquid

Table 1. Species key number, accession name, and origin of *Ocimum* spp. used in RAPD analysis.

Ocimum spp.	Key no.[z]	Accession name	Origin
O. americanum L. var. *americanum*	ot32	Bra60	Embrapa, Brazil
O. americanum L. var. *americanum*	ot5	Kew90157	Kew Garden, U.K.
O. americanum L. var. *pilosum* (Wild) Paton	ot21	PI 414204	USDA, U.S.
O. americanum L. var. *pilosum* (Wild) Paton	ot75	PI 500953	USDA, U.S.
O. basilicum L. 'Dark Opal'	ot38	'Dark Opal'	Richters Co., Canada
O. basilicum L. 'Purple Ruffles'	ot40	'Purple Ruffles'	Richters Co., Canada
O. basilicum L.	ot2	Kew7711	Kew Garden, U.K.
O. basilicum L.	ot43	'Mammoth'	Richters Co., Canada
O. basilicum L.	ot44	'Sweet Basil'	Richters Co., Canada
O. basilicum L.	ot49	'Cinnamon'	Richters Co., Canada
O. basilicum L.	ot69	'Comoro'	Purdue Univ., U.S.
O. basilicum L.	ot71	Methylcinnamate	Purdue Univ., U.S.
O. basilicum L.	ot72	Linalool	Purdue Univ., U.S.
O. basilicum L. [y]	ot70	'Sweet Dani'	Purdue Univ., U.S.
O. basilicum L. [y]	ot61	'Lemon Mrs. Burns'	Companion Plants, U.S.
O. basilicum L. var . *difforme*	ot41	'Green Ruffles'	Richters Co., Canada
O. basilicum L. var. *thyrsiflorum*	ot56	'Thai'	Richters Co., Canada
O. basilicum L. var. *thyrsiflorum*	ot57	'Thai Siam Queen'	Richters Co., Canada
O. campechianum Mill.	ot30	Lbb710	Embrapa, Brazil
O. gratissimum L.	ot26	Bra43	Embrapa, Brazil
O. gratissimum L.	ot27	Bra78	Embrapa, Brazil
O. gratissimum L.	ot63	'Tree'	Companion Plants, U.S.
O. gratissimum L.	ot65	'Green'	Companion Plants, U.S.
O. gratissimum L.	ot80	PI 500952	USDA, U.S.
O. gratissimum L.	ot84	Pu-geraniol	Purdue Univ., U.S.
O. kilimandscharicum Guerke	ot79	PI 500950	USDA, U.S.
O. kilimandscharicum Guerke	ot3	Kew12290	Kew Garden, U.K.
O. kilimandscharicum Guerke	ot50	'African Blue'	Richters Co., Canada
O. minimum L.	ot34	'Bush'	Richters Co., Canada
O. minimum L.	ot35	'Spicy Globe'	Richters Co., Canada
O. minimum L.	ot60	'Dwarf Opal'	Companion Plants, U.S.
O. selloi Benth.	ot73	Lin1	Embrapa, Brazil
O. tenuiflorum L.	ot67	'Sacred'	Nichols Garden, U.S.
O. ×*citriodorum* Vis.	ot53	'Lemon'	Richters Co., Canada
O. ×*citriodorum* Vis.	ot58	'Lemon'	Companion Plants, U.S.
O. ×*citriodorum* Vis.	ot62	'New Guinea'	Companion Plants, U.S.
O. ×*citriodorum* Vis.	ot1	Kew365	Kew Garden, U.K.

[z]ot = *Ocimum* trial. Key number used in a double blind assay.
[y]Accessions identical morphologically to *O. basilicum*, but with strong influence of *O.* ×*citriodorum.*

nitrogen, and maintained at –80 °C until used. Voucher specimens of each accession included in this research were collected, dried, and deposited at the Ralph Kriebel Herbarium, Purdue Univ. Vouchers were also sent to the Royal Botanical Garden, Kew, United Kingdom, Delaware State University, Dover, Del., and Embrapa Recursos Genéticos e Biotecnologia herbaria, Brasilia, for permanent collection and repository. Taxonomic identification was conducted in collaboration with A. Paton, Royal Botanical Gardens, Kew, United Kingdom; A. Tucker, Delaware State University, and U. Albuquerque, Federal University of Pernambuco, Recife, PE, Brazil.

DNA EXTRACTION. The DNA extraction protocol used was that of Doyle and Doyle (1987). Samples were ground with liquid nitrogen, and transferred quickly to another Eppendorf tube. Hexadecyltrimethylammonium bromide (CTAB) extraction buffer (700 mL) was added and the mixture was incubated for 60 min at 65 °C. An equal volume of 24 chloroform:1 isoamyl alcohol (v/v) was added to each sample. Samples were centrifuged for 8 min at 13,000 g_n and 600 mL of the aqueous layer supernatant was removed to a clean test tube. Cold (–20 °C) isopropanol (700 mL) was then added, and samples were placed in a freezer at –80 °C for 1 h, centrifuged for 8 min at 13,000 g_n to collect the precipitated nucleic acids, and the supernatant discarded. The pellet was washed with 1 mL of 70% ethanol, centrifuged for 8 min at 13,000 g_n, and the supernatant removed carefully. Pellets were then dried, resuspended in 200 mL Tris-EDTA, and treated with RNAse for 30 min. The concentration of DNA was estimated by agarose gel electrophoresis and comparison with a DNA ladder of known concentration and using spectrophotometry with absorbance at 260 nm. Working stocks of DNA were then prepared based on both estimates.

PCR AMPLIFICATION. Nineteen arbitrary decamer oligonucleotide primers (Operon Technologies, Inc., Alameda, Calif.) were tested and 11 were selected and then used for PCR amplification following the procedures of Williams et al. (1990). Amplifications were performed in 25-mL volumes containing 50 to 200 ng of template DNA, PCR buffer (50 mM KCl, 10 mM Tris-HCl pH 8.8, 1.5 mM $MgCl_2$, and 0.1% Triton X-100), 0.1 mM of each dNTP, 0.2 mM primer, and 1 unit *Taq* DNA polymerase . All reactions were overlaid with 1 mL of mineral oil before amplification. Amplifications were performed in a TwinBlock thermocycler (Ericomp, San Diego) programmed for 45 cycles of denaturing for 20 s at 94 °C; annealing for 40 s at 37 °C; and extension for 1 min at 72 °C. PCR products were electrophoresed on a 1.5% agarose gel at 100 V for 3 h, stained with ethidium bromide, and visualized under ultraviolet light. A negative control lacking template DNA was included in each set of amplification reactions.

DATA ANALYSIS. The presence of a specific band of amplified DNA was scored as 1 or 0 if absent, for all prominent bands within the fingerprint. Dice's index (Hillis et al., 1996) of similarity was used, as follows: d(J,K) = 2 N j,k / Nj+Nk; where J and K are two different individuals; $N_{j,k}$ is the number of shared fragments; and N_j and N_k are the total number of fragments of plants J and K, respectively. A phenogram was constructed using the unweighted pair-group method with arithmetical average (UPGMA). Numerical taxonomic system (NTSYS) was used to perform the cluster analysis (Rholf, 1995). Bootstrap analysis was performed using Winboot software (Yap and Nelson, 1996).

Results and Discussion

Among 19 primers tested, 11 generated 98 polymorphic fragments ranging from 300 to 2000 base pairs (bp), in the accessions examined as follows: OPC-01 (TTCGAGCCAG) with five fragments of 500–1300 bp; OPC-06 (GAACGGACTC) with nine fragments of 300–2000 bp; OPC-08 (TGGACCGGTG) with eight fragments of 500–1300 bp; OPC-13 (AAGCCTCGTC) with six fragments of 800–2000 bp; OPG-02 (GGCACTGAGG) with 11 fragments of 300–1500 bp; OPG-04 (AGCGTGTCTG) with eight fragments of 300–1500 bp; OPG-05 (CTGAGACGGA) with seven fragments of 550–1500 bp; OPG-10 (AGGGCCGTCT) with 10 fragments of 500–1600 bp; OPG-13 (CTCTCCGCCA) with 17 fragments of 350–1350 bp; OPG-14 (GGATGAGACC) with 10 fragments of 500–1400 bp; and OPG-19 (GTCAGGGCAA) with seven fragments of 500–1500 bp. Typical reaction products with primer OPG-13 are illustrated in Fig. 1.

Means of genetic similarities within *Ocimum* spp., as revealed by RAPD analysis indicated that *O. minimum* (0.887), *O. basilicum* (0.769), and *O.* ×*citriodorum* (0.711) have the highest within species similarity indices (Table 2). In contrast, *O. americanum* (0.580), *O. gratissimum* (0.408), and *O. kilimandscharicum* (0.559) showed lower similarity within species (Table 2). These results indicate that within the cultivated species (*O. basilicum, O. minimum*, and *O.* ×*citriodorum*), the accessions are more homogeneous. A higher level of polymorphism is indicative of the heterogeneity in nondomesticated species, such as *O. selloi* and *O. tenuiflorum*.

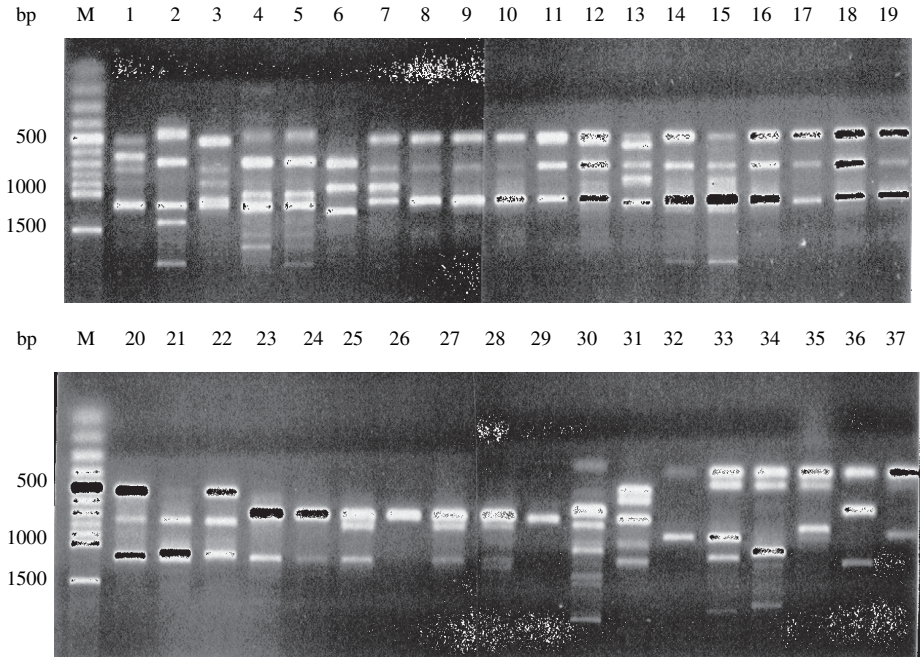

Fig. 1. RAPD profile of *Ocimum* spp. generated by primer OPG-13. M = molecular weight marker in bp; lanes 1–2 = *O. americanum* var. *americanum*, lanes 3–4 = *O. americanum* var. *pilosum*, lanes 5–7 = *O. kilimandscharicum,* lanes 8–10 = *O. minimum,* lanes 11–22 = *O. basilicum,* lanes 23–28 = *O.* ×*citriodorum,* lane 29 = *O. campechianum,* lane 30 = *O. selloi,* lane 31 = *O. tenuflorum*, and lanes 32–37 = *O. gratissimum.*

Table 2. Means of similarity values between and within *Ocimum* spp. based on RAPD markers.

Section/subsection	Species[z]										Within
and species	ame	pil	bas	x cit	kil	min	gra	sel	ten	cam	species
Ocimum/Ocimum											
Ocimum americanum var. *americanum*	1.000										0.580[y]
Ocimum americanum var. *pilosum*	0.578	1.000									
Ocimum basilicum	0.530	0.671	1.000								0.769
Ocimum x *citriodorum*	0.494	0.582	0.630	1.000							0.711
Ocimum kilimandscharicum	0.429	0.485	0.433	0.438	1.000						0.559
Ocimum minimum	0.502	0.663	0.802	0.649	0.438	1.000					0.887
Ocimum/Gratissima											
Ocimum gratissimum	0.300	0.325	0.374	0.333	0.251	0.370	1.000				0.408
Hierocymum/Foliosa											
Ocimum selloi	0.421	0.445	0.531	0.442	0.375	0.580	0.348	1.000			---[x]
Ocimum tenuiflorum	0.233	0.244	0.212	0.186	0.285	0.264	0.286	0.320	1.000		---
Gymnocymum											
Ocimum campechianum	0.311	0.554	0.356	0.390	0.258	0.374	0.243	0.263	0.178	1.000	---

[z]ame = *O. americanum* var. *americanum*; pil = *O. americanum* var. *pilosum*; bas = *O. basilicum*; xcit = *O. x citriodorum*; kil = *O. kilimandscharicum*; min = *O. minimum*; gra = *O. gratisissimum*; sel = *O. selloi*; ten = *O. tenuiflorum;* and cam = *O. campechianum*.
[y]Mean represents both varieties of *O. americanum.*
[x]Only one accession.

The cluster of *O. basilicum* showed some specific relationships between cultivated basil varieties (Table 1 and Fig. 2). 'Purple Ruffles' (ot40), a cross between 'Dark Opal' (ot38) and 'Green Ruffles' (ot41), and 'Dark Opal' (ot38) clustered very closely (0.945). 'Purple Ruffles' showed an index of similarity of 0.815 with 'Green Ruffles', and 'Dark Opal' 0.783. The closest genetic similarity (0.960) was observed between accessions ot56 ('Thai') and ot57 ('Thai Siam Queen'), and these appeared to be morphologically and chemically similar varieties of *O. basilicum*.

'Mammoth' (ot43), 'Sweet Basil' (ot44) and 'Cinnamon' (ot49) form another cluster within *O. basilicum* spp. 'Mammoth' and 'Sweet Basil' are both green basil types with linalool and methylchavicol as major oil constituents, while 'Cinnamon' basil has some purple pigmentation in stem and leaf veins, and has methyl (E)-cinnamate as a major constituent. An improved line (ot71) of the aromatic phenylpropanoid methyl (E)-cinnamate that was bred by Simon and Morales (unpublished) clustered apart from the generic commercial 'Cinnamon' basil (0.732) indicating that it is genetically less similar from the other commercial source of methyl (E)-cinnamate. Methyl (E)-cinnamate-rich plants have been reported for O. *basilicum, O. americanum*, and *O. kilimandscharicum*.

Ocimum basilicum group showed a high similarity index (Table 2) when compared to *O. minimum* (0.802), which some taxonomists consider a variety of *O. basilicum* (Darrah, 1980; Sobti and Pushpangadan, 1982). Paton and Putievsky (1996) considered it partially isolated reproductively from *O. basilicum*. *Ocimum minimum* is also relatively close to *O. americanum* var. *pilosum* (0.663) and *O.* ×*citriodorum* (0.649).

Although results obtained by cluster analysis do not show a high consensus tree for all accessions, some clusters can be discussed as they show an interesting relationship with morphological taxonomy. An arbitrary threshold of 70% similarity index places *O. minimum* inside *O. basilicum* species cluster, suggesting they are closely related. *Ocimum minimum* is considered by some taxonomists to be its own species, while others consider it a variety of *O. basilicum*. Our RAPD marker results strongly suggest that it should not be considered a distinct species, but rather a variety of *O. basilicum*.

Ocimum ×*citriodorum*, considered to be a natural hybrid between *O. basilicum* and *O. americanum* (Paton and Putievsky, 1996) showed a high similarity index when compared to *O. basilicum* (0.630) and *O. minimum* (0.649). 'Comoro' basil (ot69), which was considered by Paton and Tucker (personal communication) as *O. basilicum*, showed a high similarity with the *O.* ×*citriodorum* accessions, being very close to ot70 ('Sweet Dani') (0.889), ot58 (lemon basil from Companion Plants) (0.857), and ot61 ('Lemon Mrs. Burns') (0.808). 'Sweet Dani', a citral-rich cultivar developed at Purdue University by Morales and Simon (1997) showed a very high similarity index with the *O.* ×*citriodorum* accessions, although it was denominated by Paton as *O. basilicum* with strong morphological influence of *O.* ×*citriodorum*. 'Sweet Dani' was developed from an *O. basilicum* population (Morales and Simon, 1997) originally from an accession rich in linalool and geraniol (Simon et al. 1990). The same situation was observed in 'Lemon Mrs. Burns', also considered morphologically as *O. basilicum*.

According to DeBaggio and Belsinger (1996), lemon basil was first introduced into the United States from Thailand by the USDA in 1940. The lemon basil introduced from Thailand appears to be a true *O.* ×*citriodorum*, while 'Lemon Mrs. Burns' appears to be a segregant population from *O. basilicum* that was selected naturally for its citral aroma. 'Sweet Dani' was selected from an *O. basilicum* population originally from Yugoslavia (Morales and Simon, 1997), though results obtained with RAPD markers suggest it should be classified as *O.* ×*citriodorum*. The morphological characters of 'Sweet Dani' (ot70) resemble those of *O.* ×*citriodorum*, as the cultivar produces copious amounts of citral and it clustered with *O.* ×*citriodorum* in our analysis. These observations when grouped together provide strong evidence that its original taxonomic assignment in *O. basilicum* is questionable on genetic grounds though it was reported originally as *O. basilicum*. Both *O. basilicum* lemon cultivars ('Sweet Dani' = ot70 and 'Lemon Mrs. Burns' = ot61) appear consistently (bootstrap value = 73.3) closer to each other than to the generic lemon basil (a truly natural hybrid *O.* ×*citriodorum*).

Comparing species from section *Ocimum* subsection *Ocimum*, *O. americanum* var. *americanum* showed low similarity index

(0.578) when compared to *O. americanum* var. *pilosum*. However, *O. americanum* var. *pilosum* appears to have higher genetic similarity to *O. basilicum* (0.671) and *O. minimum* (0.663). Paton (1992) considered *O. americanum* very closely related to, and difficult to distinguish morphologically from, *O. basilicum*. However, in this study using RAPD markers, *O. basilicum* was clearly distinguishable from both *O. americanum* varieties using RAPD markers (Fig. 2).

Accession ot32 (*O. americanum* var. *americanum*) is a methyl (E)-cinnamate-rich accession (Vieira and Simon, 2000), and has a low similarity index (0.528) compared to the other *O. americanum* variety (ot5), an anisole-rich chemotype. *Ocimum americanum* is found in several different countries, and distinct chemotypes have been reported, such as a citral type in India (Sarin et al., 1992), a camphor type from Africa (Demissew, 1993), and a methyl (E)-cinnamate chemotype from Brazil (Vieira and Simon, 2000). We found ot5 to be close to ot75 (0.755), an accession of *O. americanum* var. *pilosum*, which is rich in 1,8-cineole and camphor (Simon et al., 1990). This large variability in chemotypes might explain the cluster separation of *O. americanum* from other species. Considering the average of similarity indices within each species of subsection *Ocimum*, *O. kilimanscharicum* was the least similar genetically (0.559) (Table 2). *Ocimum kilimandscharicum* is a perennial shrub, originally from Africa (Uganda, Kenya, Tanzania, and Rwanda) (Paton, 1992), growing naturally in the grassland, and disturbed ground. *Ocimum kilimandscharicum* contains mostly large amounts of camphor, although an African blue, a hybrid between *O. basilicum* 'Dark Opal' and *O. kilimandscharicum* have shown large amounts of linalool. The chemical and morphological heterogeneity of accessions might explain the low similarity indices presented.

In the section *Ocimum* subsection *gratissima, O. gratissimum* had the lowest similarity index (0.408) compared to all other within species indices (Table 2), clustering in a separated group, which we observed to be quite heterogeneous (0.408 similarity index among all *O. gratissimum* accessions). *Ocimum gratissimum* species were divided into two subclusters, consisting of thymol (ot27 and ot63) and eugenol (ot26 and ot65) types, in agreement with Vieira et al. (2001). The two other accessions of *O. gratissimum*, ot84 and ot80, were the most dissimilar being placed far away from the main group. The first is a geraniol type of *O. gratissimum* developed from a commercial source of tree basil (Charles and Simon, 1992), while the other (ot80) appears to be a hybrid between *O. lamiifolium* and either *O. americanum* or *O. basilicum* (A.O.Tucker, personal communication), although Paton classifies it as *O. gratissimum* (A. Paton, personal communication).

The species belonging to the section *Hierocymum* and *Gymnocymum* were separated and in isolation from all other species (Fig. 2), for *O. tenuiflorum*, *O. selloi*, and *O. campechianum*. Although *O. tenuiflorum* and *O. selloi* belong to the same section, they exhibited only 32% similarity. Also, *O. selloi* appears to be genetically more related to the section *Ocimum* than to section *Hierocymum* (*O. tenuiflorum*), though additional species from both sections would need to be analyzed before reevaluating their current systematic position.

Cluster analysis of the genetic similarity values based on RAPD generated a phenogram showing genetic relationships within and between *Ocimum* spp. (Fig. 2). The number at the fork shows the percentage of times the group consisting of the species that are to the right of that fork occurred. Bootstrap analysis (2000 times) showed robust clusters (>70%) between accessions belonging to the citral group, ot69/ot58, ot70/ot61, and ot53/ot1. Accessions of *O. americanum* var. *americanum* (ot5) and var. *pilosum* (ot75) showed 72.1% consistency. The cluster between accessions ot50 and ot3, the first a hybrid between *O. basilicum* and *O. kilimandscharium*, and the second, a true *O. kilimandscharicum*, showed 86.2% consensus. *O. gratissimum* accessions, ot65 and ot26, showed a consensus cluster of 98.6%, both rich in eugenol.

RAPD analysis associated with volatile oils and flavonoids has been reported for *O. gratissimum* (Vieira et al., 2001), where it was shown to be an effective fingerprinting technique for that *Ocimum* spp. to detect intra- and interspecific variation. In this study, RAPD analysis enabled us to construct a phenogram showing the genetic similarity and cluster of a wide collection of *Ocimum* spp. (Table 1, Fig. 2). Analyses of the RAPD markers showed that most *Ocimum* spp. are distinguishable. High polymorphism was detected between species, which can be explained by the large genetic and geographic distance between each species, such as *O. selloi* and *O. campechianum*, originally from South America, and *O. tenuiflorum*, considered to originate in India.

The present investigation has demonstrated that RAPD markers can be applied to discriminate among *Ocimum* spp. The large number of polymorphic bands indicates there is large genetic

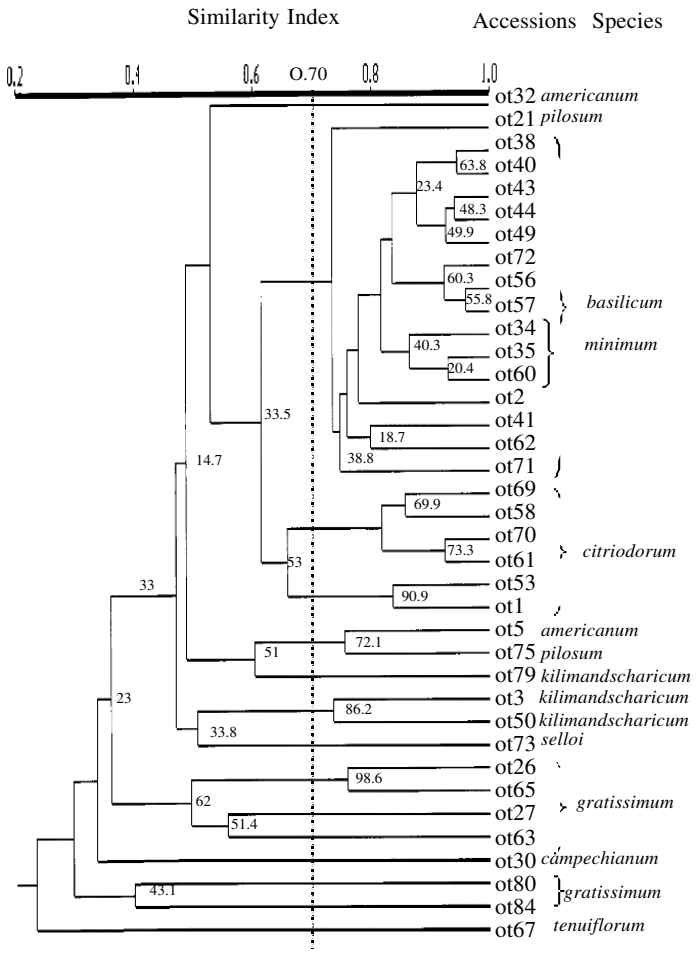

Fig. 2. Phenogram derived from 37 accessions of *Ocimum* and 98 RAPD bands using Dice similarity index (Hillis et al., 1996) and UPGMA. Dotted line equals arbitrary line of 70% similarity. Fork number indicates the percentage of times the cluster occurred in a bootstrap analysis.

variation within *Ocimum*. This was expected given the wide morphological, chemical characteristics, and ploidy levels. However, within *O. basilicum* cultivars, the RAPD markers showed narrow genetic variation.

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