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Chemical characterization of the essential oil from leaves of basil genotypes cultivated in different seasons

[Caracterización química del aceite esencial de hojas genotipos de albahaca cultivados en diferentes estaciones del año]

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Abstract: The aim of this study was to evaluate the concentration and chemical composition of the essential oil the leaves of basil cultivars and hybrids cultivated in different cropping seasons: dry season and rainy season. The variables evaluated were the content and composition of essential oils in the two seasons. The essential oil content ranged from 0.66% to 3.21% in the dry season and from 0.80% to 4.20% in the rainy season. The major compounds found among the genotypes were linalool, methyl chavicol, neral, geranial, eugenol, and methyl (E)-cinnamate, defining the formation of five groups in each season, classified in the following chemotypes: methyl chavicol (Group 1), citral (neral+geranial) (Group 2), methyl cinnamate (Group 3), linalool (Group 4), and intermediate linalool (Group 5). All the traits evaluated had heritability (h^2) greater than 95% and high CVg/CVe ratio values. The cropping season affected the content and chemical compositions of basil essential oil.

Keywords: *Ocimum basilicum*; Seasonal variation; Chemical diversity; Essential oils.

Resumen: El objetivo de este estudio fue evaluar la concentración y composición química del aceite esencial las hojas de cultivares de albahaca e híbridos cultivados en diferentes temporadas de cultivo: estación seca y estación lluviosa. Las variables evaluadas fueron la contenido y la composición de los aceites esenciales en las dos estaciones. La contenido de aceite esencial varió de 0.66% a 3.21% en la estación seca y de 0.80% a 4.20% en la estación lluviosa. Los principales compuestos encontrados entre los genotipos fueron linalool, metilchavicol, neral, geranial, eugenol y metil (E)-cinamato, definiendo la formación de cinco grupos en cada estación, clasificados en los siguientes quimiotipos: metil chavicol (Grupo 1), citral (neral + geranial) (Grupo 2), cinamato de metilo (Grupo 3), linalool (Grupo 4) y linalol intermedio (Grupo 5). Todos los rasgos evaluados mostraron una heredabilidad (h^2) mayor que el 95% y altos valores de relación CVg/CVe. La temporada de cultivo afectó la contenido y las composiciones químicas del aceite esencial de albahaca.

Palabras clave: *Ocimum basilicum*; Variación estacional; Diversidad química; Aceites esenciales.

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INTRODUCTION

Ocimum basilicum L., popularly known as basil, belongs to the *Ocimum* genus of the Lamiaceae family. It is a species native to Asia (India, Pakistan, Iran, Thailand, and other countries) and grows in a spontaneous manner in tropical and sub-tropical regions (Khair-ul-Bariyah *et al*., 2012). Basil is known for its wide chemical diversity, and these variations are mainly derived from its locations of origin and growth. This varied composition allows expansion of sources of raw materials to industries (Costa *et al*., 2015; Costa *et al*., 2016).

The composition of essential oils extracted from basil leaves and apices with inflorescences varies according to the species and geographic location. The essential oils can be classified in four chemotypes according to the main components: linalool-methyl chavicol, methyl chavicol, methyl cinnamate, and eugenol (Martins *et al*., 2010). There is variability of chemical composition since different chemotypes have been found in diverse regions of the world (Hassanpouraghdam *et al*., 2010), and they are classified according to their aroma in categories, such as sweet, lemon, cinnamon, camphor, anise, and cloves (Costa *et al*., 2014). The presence of essential oils with distinct constituents determines the specific aroma and flavor of basil and, consequently, its industrial potential.

Studies confirm that the variability of chemical composition in basil (Fischer *et al*., 2011; Costa *et al*., 2015) can be improved through use of hybrid combinations and consequent choice of genotypes for future plant breeding programs and cultivar development. Diverse abiotic factors, such as temperature, the intensity of light radiation, nutrition, water availability, and season can affect plant growth and development (Cabello *et al*., 2014), as well as production and chemical composition of essential oils (Selmar and Kleinwächter, 2013). The season in which a plant is gathered is one of the most relevant factors because the quantity and even the nature of the constituents is not constant throughout the year (Paulus *et al*., 2013). Given the need for information in respect to the varieties of basil and production of essential oil, the aim of this study was to evaluate the concentration and chemical composition of the essential oil from leaves of 24 genotypes of basil (*Ocimum basilicum* L.) in two cropping seasons: dry season and rainy season.

MATERIALS AND METHODS

Plant material and experimental design

The trials of the dry season (Oct.-Dec./2015) and rainy season (Apr.-Jun./2016) were conducted on the Research Farm "Campus Rural da UFS", located in the municipality of São Cristóvão, SE, Brazil, in the central part of the physiographic region of the coast of the state of Sergipe, 15 km from Aracaju, at the geographic coordinates: 10° 55' 27" S, 37° 12' 01" W, and altitude of 46 m. The region is characterized by type As climate (tropical rainy with dry summer, according to the Köppen classification) and mean annual temperature of 25.2°C (Santos, 2006). The average lowest and highest temperature were 23,4°C and 29,4°C in October 2015, 23,7°C and 29,8°C in November 2015, 23,7°C and 30,0°C in December 2015, 23,6°C and 30,1°C in May 2016, 22,5°C and 29,1°C in June 2016, and 22,3°C and 28,6°C in May 2016, respectively. The average mean temperatures in the rainy and dry season were 26,1 and 26,7°C, respectively. Commercial cultivars and experimental hybrids from the Basil Genetic Breeding Program of UFS were evaluated. The trial included 24 genotypes, 20 cultivars (Anise, Ararat, Edwina, Dark Opal, Genovese, Green Globe, Italian Large Leaf - Richters, Magical Michael, Mrs. Burns, Napoletano, Nufar F1, Osmin, Purple Ruffles, Sweet Dani, Grecco a Palla, Italian Large Leaf - ISLA, Italian Large Red Leaf, Red Rubin Purple Leaf, Limoncino, and Maria Bonita) and 4 hybrids (Cinnamon x Maria Bonita, Genovese x Maria Bonita, Sweet Dani x Cinnamon, and Sweet Dani x Genovese), and was implemented in a randomized block experimental design, with three replications. Each plot consisted of two rows of three plants, for a total of six plants per plot. The spacing used was 0.60 m between rows and 0.50 m between plants. The plant beds were covered with mulch (Pinto *et al*., 2018).

Seedlings were produced using seeds and a substrate composed of soil from the "Campus Rural da UFS", earthworm casting, and coconut coir fiber (2:1:1), with the addition of 1 g of limestone and 6 g of the fertilizer Hortosafra® 6-24-12 for each liter of substrate. The seedlings were produced in 162-cell polyethylene trays. The experimental area was disked and 2 t ha^{-1} of limestone was applied at 30 days before planting, according to soil analysis. The basil was managed in accordance with the study of Blank

et al. (2004). Basil was harvested after the first flowers of 50% of the plants had opened in each cropping season evaluated (Pinto *et al*., 2018).

Extraction and analysis of essential oil

The leaves collected in each cropping season were dried in a laboratory oven at 40ºC with forced air circulation for five days. Essential oil was extracted by hydrodistillation in a modified Clevenger type apparatus. Samples of 50 g of dried leaves were distilled for 140 minutes (Ehlert *et al*., 2006). After extraction, the essential oil was collected and stored in amber bottles at -20°C up to the time of analysis of chemical composition. To calculate the content (%) obtained in each samples, the following equation was used:

$$Content = \left(\frac{v}{m}\right) x\ 100$$

in which
v = volume of essential oil extracted from the sample;
m = leaf dry matter of the sample.

The chemical composition of the essential oils was analyzed using a GC-MS/FID (QP2010 Ultra, Shimadzu Corporation, Kyoto, Japan) equipped with an autosampler AOC-20i (Shimadzu). The MS and FID data were simultaneously acquired using a Detector Splitting System; the split flow ratio was 4:1 (MS:FID). Quantification of each constituent was estimated by FID peak-area normalization (%). Compound concentrations were calculated from the GC peak areas, and they were arranged in order of GC elution. The retention index (van den Dool & Kratz, 1963) was obtained by injecting a C_7-C_{30} linear hydrocarbon mixture under these same conditions, and identification of constituents was made on the basis of comparison of the retention index and MS with those in the literature (Adams, 2007), as well as by computerized matching of the acquired mass spectra with those stored in the NIST21, NIST107, and WILEY8 mass spectral libraries of the GC-MS data system.

Statistical analysis

The data on essential oil content were subjected to individual and combined analysis of variance, and the means were grouped by the Scott-Knott test at $p \leq 0.05$ probability, using the Sisvar® software.

Combined analysis of variance followed the model proposed by Vencovsky and Barriga (1992). The mathematical model used was:

$$Y_{ijk} = m + B/A_{jk} + G_i + A_j + GA_{ij} + E_{ijk}.$$

in which
Yijk is the value observed of the i-th genotype in the k-th block within the j-th cropping season;
m is the overall mean;
B/Ajk is the effect of the k-th block within the j-th cropping season;
Gi is the effect of the i-th genotype;
Aj is the effect of the j-th cropping season;
GAij is the effect of the interaction of the i-th genotype with the j-th cropping season;
Eijk is the experimental error.

The estimates of the genetic and phenotypic parameters, such as heritability (h^2), coefficient of variation (CV%), and phenotypic and genotypic correlations, were carried out by the GENES program (Computational Application in Genetics and Statistics) version 2006.4.1 (Cruz, 2005). From the expected values of mean squares, the components of genetic variance (σ^2_g) and environmental variance (σ^2_e) were estimated for the main characteristics evaluated. The genetic parameters, such as coefficients of heritability (h^2) and CV_g/CV_e ratio, were also estimated.

$$\sigma_f^2 = \sigma_g^2 + \sigma_e^2 + \sigma_{ge}^2$$

$$\sigma_g^2 = \frac{QM_G - QM_{GA}}{ar}$$

$$\sigma_{ge}^2 = \frac{QM_{GA} - QM_R}{r} \times \frac{g-1}{g}$$

$$h^2 = \frac{\sigma_g^2}{GM_G/ar}$$

$$CV_g = \frac{100\sqrt{\sigma_g^2}}{m}$$

From the data of analyses of the chemical constituents of the essential oils from each cropping season, two multivariate analyses were performed, cluster analysis and principal component analysis (PCA), using the Statistica software. The dissimilarity matrix based on Euclidean distances was simplified with dendrograms using the Ward clustering method. The means of the concentrations of the chemical constituents were obtained with the software Graph Pad Prism®.

RESULTS AND DISCUSSION

Differences were observed among the treatments (α=0.05) in essential oil content (Table No. 1). The essential oil content ranged from 0.66% to 3.21% in the dry season and from 0.80% to 4.20% in the rainy season. The genotype with the highest content in the dry season was the cultivar Maria Bonita (3.21%) and in the rainy season, highest contents were in the cultivars Mrs. Burns and Maria Bonita (4.20% and 3.93%).

Answers in the essential oil content between the harvest seasons suggest an origin from the influence of environmental factors of temperature and rainfall. The means of minimum and maximum temperature in the dry season ranged from 23.4°C to 30.0°C, with rainfall of 26.83 mm. In the rainy season, the mean temperatures were from 25.6°C to 28.3°C and 159.33 mm of rainfall during this period. In general, most of the genotypes were higher or equal in essential oil content between the growing seasons, but the rainy season (1.77%) provided an average content higher than the dry season (1.32%). The concentration of secondary metabolites in the essential oil of the plants depends on genetic control and also on the genotype x environment interactions, which may be due to abiotic stress conditions, i.e., excess or deficiency of some environmental factor, such as light, temperature, or nutrients, among others (Luz *et al.*, 2014).

The essential oil concentrations found by Smitha & Tripathy (2016) in different species of Ocimum grown in India responded in a manner similar to the present study, with variation in the amount between two seasons. This was also observed in Pogostemon cablin, with contents in genotypes ranging from 2.60% to 1.18% between the rainy season and dry season (Blank *et al.*, 2011). In contrast, Botrel *et al*. (2010), working with Hyptis marrubioides, found higher mean values in the summer (0.42%) compared to the winter (0.27%) for essential oil content. Among the analyzed compounds, the phenylpropanoids methyl chavicol and eugenol were the ones that had the greatest influence from the planting season (Table No. 1). Cultivar Genovese presented higher methyl chavicol content in the rainy season (27.43%) when compared to the dry season (2.13%). For eugenol content an opposite behavior was observed, with plants producing essential oil with 29.69% of eugenol in the dry season and without this constituent the rainy season (Table No. 1). Such difference observed in these constituents in relation to the terpenes, is possibly due to the biosynthesis pathway of these compounds. While terpenes are formed through the mevalonate pathway, the phenylpropanoids are produced by the shikimic acid pathway, in which the enzyme phenylalanine ammonia-lyase is involved. The activation of this enzyme is influenced by a large number of factors, biotic and abiotic, being thus highly sensitive to environmental conditions (Mandoulakani *et al.*, 2017).

Table No. 1

Contents (%) of essential oil and of the principal chemical constituents of 24 basil genotypes, consisting of 20 cultivars and 4 hybrids, grown in dry and rainy seasons in the municipality of São Cristóvão, Sergipe, Brazil.

Genotype	Compounds																Essential Oil (%)
	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	
IRRo	1015	1080	1161	1183	1209	1224	1234	1253	1338	1369	1374	1415	1467	1482	1573	1626	
IRRl	1026	1096	1174	1195	1227	1235	1249	1264	1356	1376	1389	1432	1484	1500	1582	1638	
Chemical composition in dry season																	
Anise	8.41	-	-	82.23	-	-	-	-	-	-	0.51	0.47	-	-	-	2.07	1.63dB
Ararat	2.69	27.90	-	54.51	-	-	-	-	-	-	1.29	0.87	-	0.21	1.4	2.20	0.86fA
Edwina	8.03	60.79	0.11	-	-	-	-	-	9.88	-	1.38	6.92	-	-	-	3.31	0.80fB
Dark Opal	15.42	53.96	0.16	-	-	-	-	-	6.58	-	1.61	8.82	-	0.47	-	2.15	0.68gA
Genovese	8.19	49.57	-	2.13	-	-	-	-	29.69	-	0.41	2.20	-	0.28	-	2.14	0.88fB
Green Globe	2.73	17.24	-	65.05	-	-	-	-	-	-	0.99	-	-	-	-	3.54	1.13eA
Italian Large Leaf	7.82	65.70	-	-	-	-	-	-	7.72	-	1.11	4.87	-	0.28	-	4.44	0.87fB
Magical Michael	-	65.20	-	-	-	-	-	-	9.06	-	4.01	1.68	-	-	-	8.38	1.20dB
Mrs Burns	0.79	38.52	4.62	-	1.21	19.89	0.27	25.43	-	-	-	2.68	0.87	-	0.46	-	2.73bB
Napoletano	11.1	50.10	1.48	6.05	-	-	-	-	4.86	-	1.74	7.51	-	0.26	-	4.43	0.86fB
Nufar F1	5.42	44.73	-	39.32	-	-	-	-	-	-	1.39	0.08	1.98	1.09	-	1.76	1.20eA
Osmin	7.10	68.46	-	-	-	-	-	-	1.38	-	2.70	8.99	-	0.52	-	2.82	0.58gA
Purple Ruffles	9.28	20.12	-	54.4	-	-	-	-	-	-	1.35	6.14	-	0.39	-	1.43	1.88cA
Sweet Dani	-	-	-	-	5.19	35.28	2.25	45.59	-	-	-	0.81	0.78	-	2.02	-	0.90fB
Grecco a Palla	3.08	28.98	8.14	-	-	-	-	-	14.94	-	4.68	8.54	3.10	2.25	-	9.43	1.10eA
Italian Large Leaf (Isla)	10.04	57.96	-	-	-	-	-	-	12.59	-	0.96	5.72	-	0.24	-	3.44	1.00eB
Italian Large Red Leaf	10.92	60.77	0.13	-	-	-	-	-	8.22	-	1.10	4.93	1.16	0.21	-	3.47	0.86fB
Red Rubin Purple Leaf	9.65	65.69	-	-	-	-	-	-	1.69	-	2.15	9.65	-	0.53	-	2.77	0.66gA
Limoncino	-	4.69	-	-	6.51	32.8	2.28	41.24	-	-	-	1.03	-	-	5.40	-	1.20eA
Maria Bonita	5.11	77.23	-	-	-	-	13.28	-	-	-	0.17	1.32	-	-	-	0.83	3.21aB
Cinnamon x Maria Bonita	2.02	29.04	-	-	-	-	-	-	-	43.37	3.71	1.68	-	-	1.08	3.78	2.00cA
Genovese x Maria Bonita	4.00	67.07	-	-	1.02	6.22	2.05	6.58	-	-	0.18	0.96	-	-	0.65	1.69	1.67dA
Sweet Dani x Cinnamon	5.56	17.04	-	1.03	0.85	1.09	1.75	0.95	-	60.71	0.98	1.02	-	-	-	0.71	2.00cB
Sweet Dani x Genovese	3.09	64.53	-	14.05	0.38	1.37	0.77	1.76	-	-	0.95	1.77	-	-	0.36	2.36	1.80cA
Chemical composition in rainy season																	
Anise	7.78	-	-	81.02	-	-	-	-	-	-	0.75	0.32	-	-	-	1.74	2.67bA
Ararat	3.15	15.67	-	67.69	-	-	-	-	-	-	2.00	1.01	-	0.38	-	2.82	1.00eA
Edwina	6.93	72.76	-	-	-	-	-	-	5.66	-	0.83	4.54	-	-	-	2.42	1.40dA
Dark Opal	17.97	54.64	0.12	-	-	-	-	-	-	-	1.73	10.98	-	0.29	-	2.56	0.80eA
Genovese	6.35	57.33	0.24	27.43	-	-	-	-	-	-	0.81	0.93	-	0.33	-	2.23	2.07cA
Green Globe	5.14	22.95	0.16	58.40	-	-	-	-	-	-	0.27	0.33	-	-	-	4.50	1.33dA
Italian Large Leaf	8.80	63.80	-	-	-	-	-	-	11.29	-	1.01	3.35	-	0.20	-	3.33	1.53dA
Magical Michael	0.65	63.78	-	-	-	-	-	-	20.13	-	1.94	0.99	-	0.19	-	4.30	2.07cA
Mrs Burns	0.62	38.31	3.22	-	1.88	22.21	0.84	27.35	-	-	-	1.22	0.55	-	0.18	-	4.20aA
Napoletano	6.45	26.13	1.54	54.48	-	-	-	-	-	-	0.72	2.73	1.02	0.23	-	2.59	1.27dA
Nufar F1	6.51	66.25	-	11.86	-	-	-	-	-	-	1.79	-	2.34	1.07	-	3.90	1.40dA
Osmin	15.61	58.44	-	-	-	-	0.39	-	8.41	-	1.54	3.25	1.19	0.35	-	1.91	0.73eA
Purple Ruffles	10.54	18.37	-	57.46	0.23	-	-	-	-	-	1.03	4.77	0.67	0.31	-	0.92	1.87cA
Sweet Dani	-	0.36	-	1.40	4.00	37.03	0.58	49.07	-	-	-	0.41	0.78	-	0.86	-	1.80cA
Grecco a Palla	4.13	24.10	6.30	-	-	-	-	-	29.85	-	3.61	5.77	2.21	1.50	-	6.65	1.40dA
Italian Large Leaf (Isla)	12.33	61.15	-	-	-	-	-	-	7.54	-	0.92	3.87	-	-	-	3.71	1.40dA
Italian Large Red Leaf	10.49	64.31	-	-	-	-	-	-	12.57	-	0.70	2.30	0.79	-	-	2.34	1.53dA
Red Rubin Purple Leaf	13.80	60.95	-	-	-	-	-	-	5.76	-	1.30	7.27	-	0.35	-	2.04	0.80eA
Limoncino	-	8.54	-	-	17.74	21.68	5.53	27.59	-	-	-	1.77	-	-	7.44	-	1.07eA
Maria Bonita	4.76	78.10	-	-	-	-	13.62	-	-	-	0.15	0.95	-	-	-	0.65	3.93aA
Cinnamon x Maria Bonita	2.37	33.73	-	-	-	-	-	-	-	38.76	0.63	1.89	0.66	-	1.28	4.45	2.07cA
Genovese x Maria Bonita	5.02	67.65	-	-	0.59	9.16	0.35	11.45	-	-	0.25	1.17	-	-	0.16	0.91	1.87cA
Sweet Dani x Cinnamon	5.40	16.90	-	0.99	0.85	1.09	1.75	0.95	-	61.54	0.98	1.05	-	-	-	0.71	2.40bA
Sweet Dani x Genovese	6.16	58.72	-	16.62	-	4.07	-	4.98	-	-	0.66	1.64	1.25	-	0.09	1.48	1.80cA

Means followed by the same lowercase letters in the column and uppercase letters in the cropping seasons do not differ among themselves by the Scott-Knott test (p≤0.05). Compounds: C1: 1,8-cineole; C2: linalool; C3: terpinen-4-ol; C4: methyl chavicol; C5: nerol; C6: neral; C7: geraniol; C8: geranial; C9: eugenol; C10: methyl (E)-cinnamate; C11: β-elemene; C12: α-(E)-bergamotene; C13: germacrene-D; C14: bicyclogermacrene; C15: caryophyllene; C16: *epi*-α-cadinol. IRRo: Relative Retention Index - calculated; IRRl: Relative Retention Index- literature (Adams, 2007).

In chemical analysis of the dry and rainy seasons, 16 principal constituents in the essential oils of the basil genotypes evaluated were observed (Table No. 1). The compounds found in greatest amounts among the genotypes were linalool, methyl chavicol, neral, geranial, eugenol, and methyl (E)-cinnamate, which defined the formation of five groups according to cluster analysis in each cropping season (Figure No. 1A & Figure No. 1B).

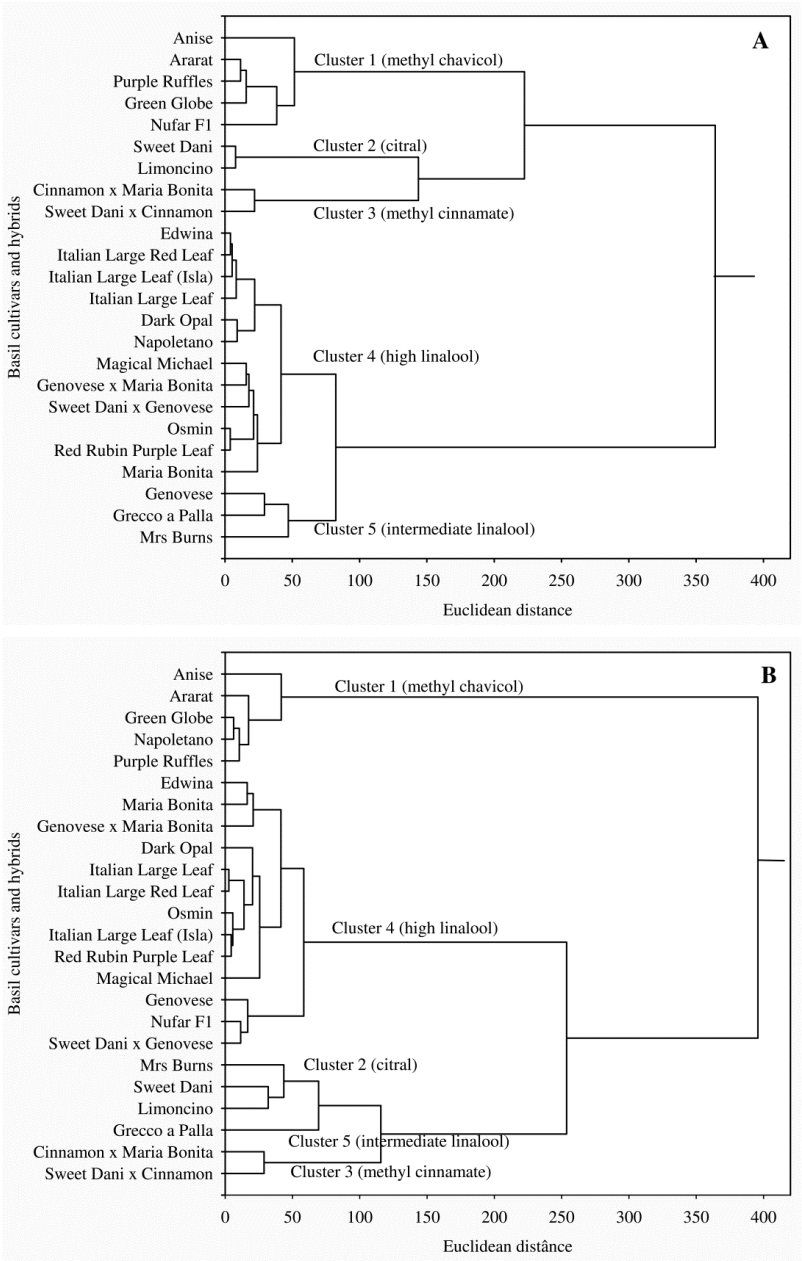

Figure No. 1
Bidimensional dendrogram representing the similarity between 24 basil cultivars and hybrids for the chemical composition of the essential oils from plants cultivated in the dry (A) and rainy (B) seasons.

Considering the similarities of the principal chemical constituents found in the essential oils of the 24 genotypes of basil in the dry season, the groups were characterized as Group 1: Anise, Ararat, Purple Ruffles, Green Globe, and Nufar F1, with methyl chavicol (39.32%-82.23%) as the main compound; Group 2: Sweet Dani and Limoncino, with geranial (41.24%-45.59%) and neral (32.80%-35.28%) as main compounds; Group 3: Cinnamon x Maria Bonita and Sweet Dani x Cinnamon hybrids, with methyl (E)-cinnamate (43.37%-60.71%) as the main compound; Group 4: Edwina, Italian Large Red Leaf, Italian Large Leaf (Isla), Italian Large Leaf, Magical Michael, Osmin, Red Rubin Purple Leaf, Dark Opal, Napoletano, Maria Bonita, and the Genovese x Maria Bonita and Sweet Dani x Genovese hybrids, with linalool (50.10%-77.23%) as the main compound; and Group 5: Genovese, Grecco a Palla, and Mrs. Burns, with intermediate linalool

(28.98%-49.57%) as the main compound (Figure no. 2).

In relation to the rainy season, the main chemical constituents found in the essential oils of basil cultivars and hybrids were characterized as Group 1: Anise, Ararat, Green Globe, Napoletano, and Purple Ruffles, with methyl chavicol (54.48%-81.02%) as the main compound; Group 2: Mrs. Burns, Sweet Dani, and Limoncino, with geranial (27.35%-49.07%) and neral (21.68%-37.03%) as main compounds; Group 3: Cinnamon x Maria Bonita and Sweet Dani x Cinnamon hybrids, with methyl (E)-cinnamate (38.76%-61.54%) as the main compound; Group 4: Edwina, Maria Bonita, Dark Opal, Italian Large Leaf, Italian Large Red Leaf, Osmin, Italian Large Leaf (Isla), Red Rubin Purple Leaf, Magical Michael, Genovese, Nufar F1, and the Genovese x Maria Bonita and Sweet Dani x Genovese hybrids, with linalool (54.63%-78.10%) as the main compound; and Group 5: Grecco a Palla, with intermediate linalool (24.09%) and eugenol (29.85%) as the main compounds (Figure No. 3).

The formation of five distinct groups (Figures No. 1A & B) of the chemical composition of essential oil of 24 genotypes of basil in two cropping seasons under study showed the chemical diversity that exists in the species, and this is especially affected by genetic control. Giachino *et al*. (2014), characterizing the chemical diversity of *O. basilicum*, identified seven chemotypes: linalool, methyl chavicol, citral/methyl chavicol, eugenol, methyl cinnamate/linalool, linalool/methyl eugenol, and methyl chavicol/linalool. In a similar study with the same species, five chemotypes were found: linalool/eugenol (30%-35% and 12%-20%), linalool (52%-66%), methyl chavicol/linalool (22%-48% and 21%-37%), methyl cinnamate (19%-38%), and methyl chavicol (38%-95%) (Liber *et al*., 2011).

Considering the five groups formed, the cultivars Nufar F1, Napoletano, Genovese, and Mrs. Burns did not remain in the same groups in the two different cropping seasons. The cultivar Nufar F1 migrated from Group 1 (methyl chavicol) in the dry season to Group 2 (linalool) in the rainy season. The change in group was probably due to the variation from 39.32% to 11.86% of methyl chavicol and from 44.73% to 60.25% of linalool between the dry and rainy seasons. The same variation between these compounds was observed for the cultivar Napoletano, which migrated from Group 2 (linalool) in the dry season to Group 1 (methyl chavicol) in the rainy season. The cultivar Genovese migrated from Group 5 (intermediate linalool) in the dry season to Group 4 (linalool) in the rainy season. This change may be related to the increase in the concentration of the linalool compound, which changed from 49.57% to 57.33%. Knowledge regarding the occurrence of seasonal variability in the production of active ingredients is one of the main parameters to be considered in planning the harvest of medicinal and aromatic plants when the objective is the quality of the raw material and the presence of active ingredients of interest.

According to the principal component analysis of the dry season, the primary principal component represented 34.60% of the total variation and was positively related to the compound nerol (r=0.93), neral (r=0.95), geranial (r=0.95), and caryophyllene oxide (r=0.84). The secondary principal component represented 19.56% of the total variation and was positively related to terpinen-4-ol (r=0.84), germacrene-D (r=0.82), bicyclogermacrene (r=0.88), and epi-α-cadinol (r=0.71) (Figure No. 4A).

In principal component analysis for the rainy season, the primary principal component represented 32.67% of the total variation and was positively related to the compound 1,8-cineole (r=0.71) and negatively with the compounds nerol (r=-0.81), neral (r=-0.88), geranial (r=-0.88), and caryophyllene oxide (r=-0.73). The secondary principal component represented 18.24% of the total variation and was negatively related to terpinen-4-ol (r=-0.80), eugenol (r=-0.75), β-elemene (r=-0.81), germacrene-D (r=-0.71), and bicyclogermacrene (r=-0.89) (Figure No. 4B).

According to analysis of variance (Table No. 2), genetic variability was observed between the means of the genotypes obtained based on the various replications and on the two cropping seasons analyzed. It was observed that all the sources of variation (genotypes, environments, and genotype x environment interaction) were significant at 5% probability by the F test for all the traits (Table No. 2).

All the traits evaluated had genetic variances greater than the residual variances (Table No. 2). A CV_g/CV_e ratio greater than 1 was also observed for the traits. The estimates of heritability (h^2) were above 95% for all the traits.

All the variables had a high coefficient of heritability (>60%) (Table No. 2). Individuals selected in these environments have the tendency of transferring their levels of productivity to the next generations. These traits can be exploited in breeding

programs for the species.

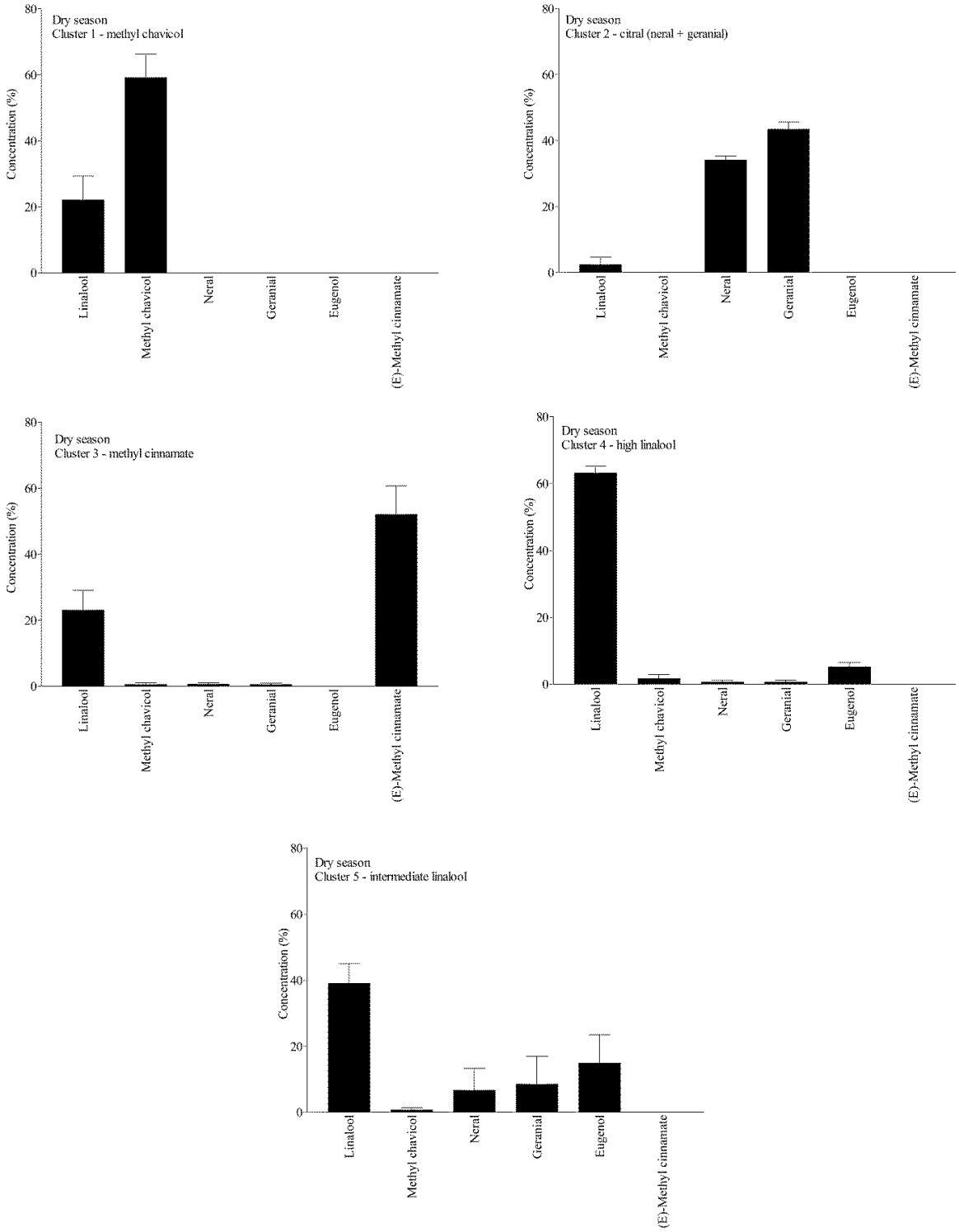

Figure No. 2
Means of the chemical compounds of the essential oils of 24 basil cultivars and hybrids cultivated in the dry season, clusters 1 to 5.

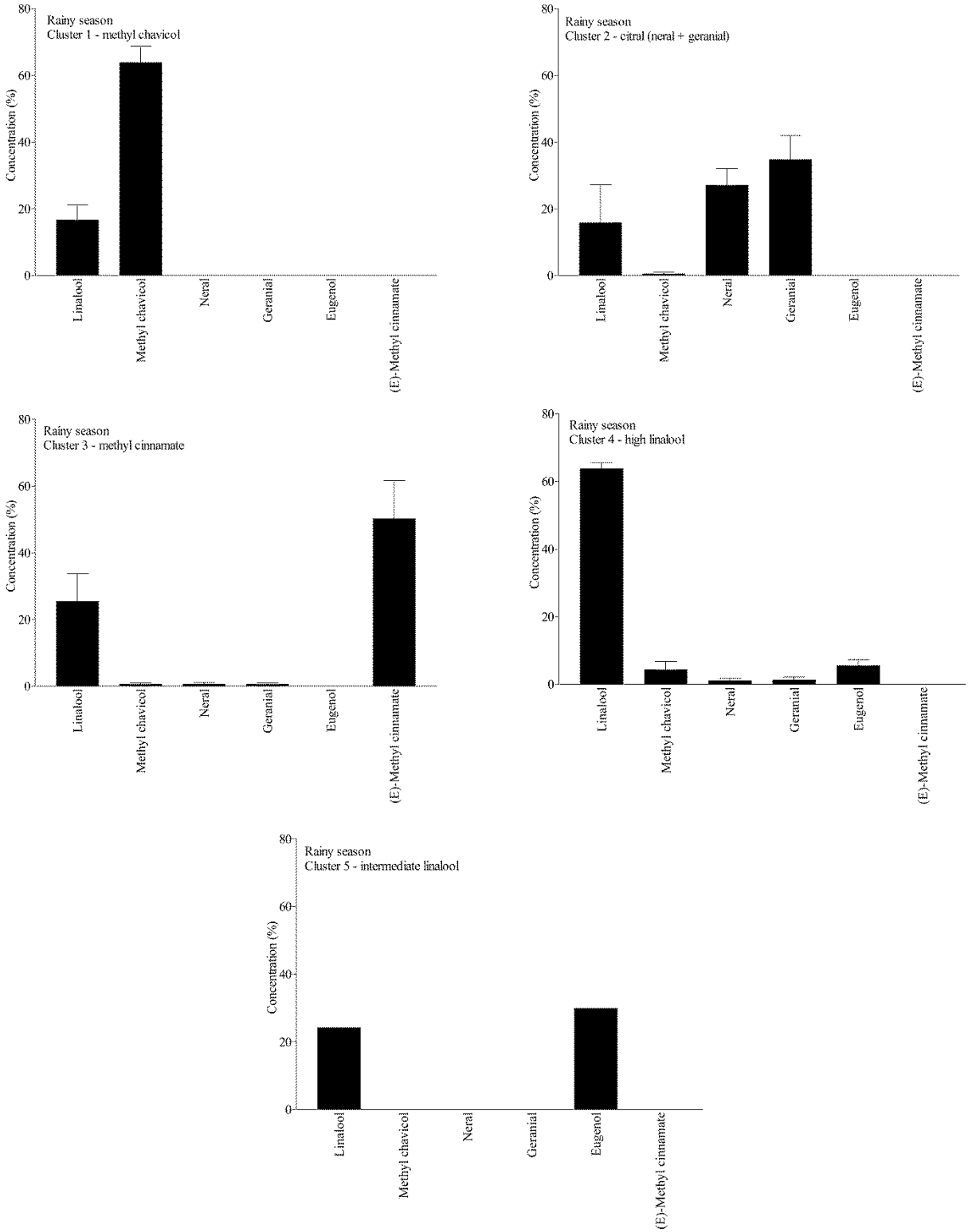

Figure No. 3
Means of the chemical compounds of the essential oils of 24 basil cultivars and hybrids cultivated in the rainy season, clusters 1 to 5.

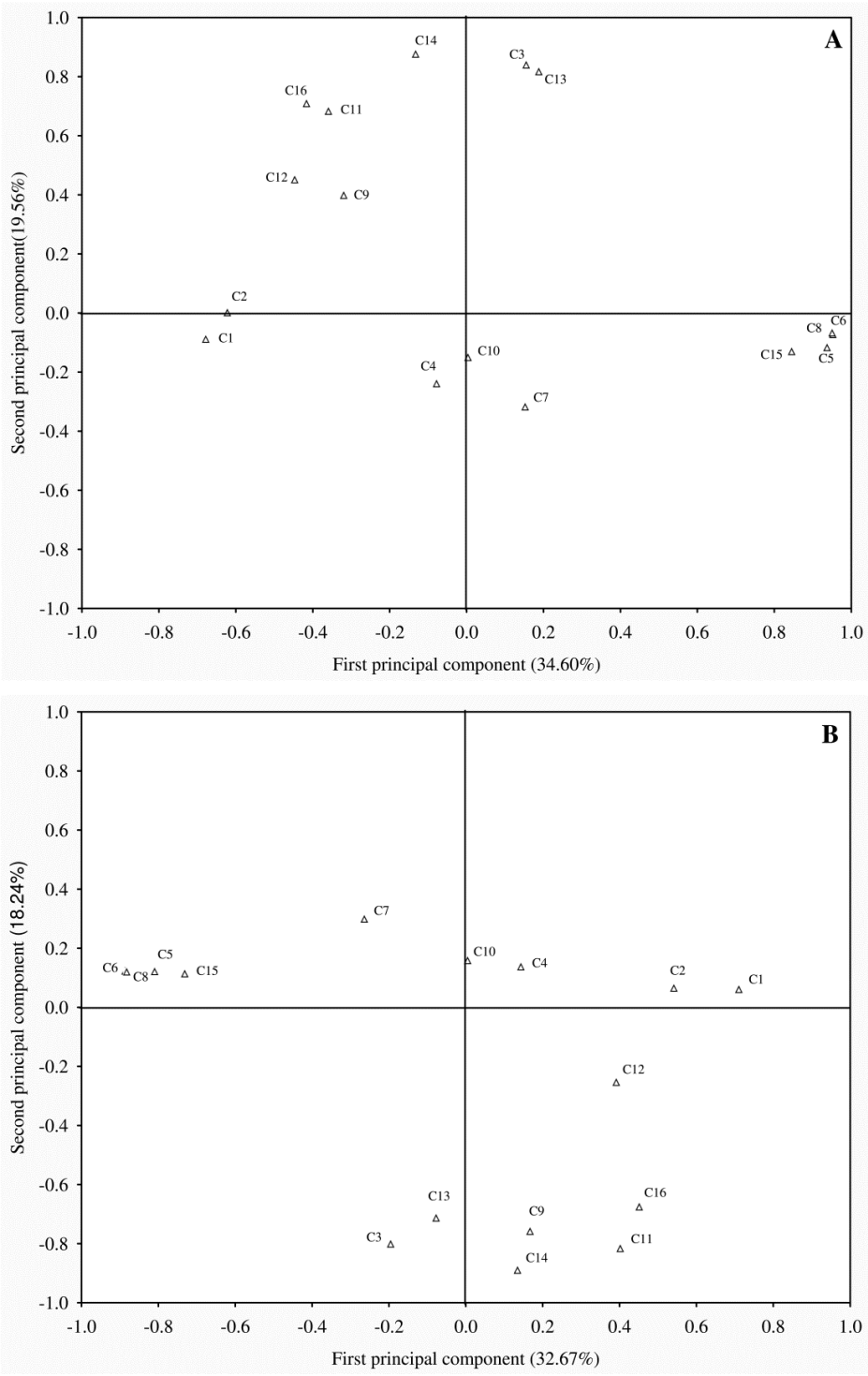

Figure No. 4

Distribution of the chemical compounds of the essential oil of 24 basil cultivars and hybrids, cultivated in the dry (A) and rainy (B) seasons, in relation to the two principal components through the principal component analysis (PCA). Compounds: C1: 1,8-cineole; C2: linalool; C3: terpinen-4-ol; C4: methyl chavicol; C5: nerol; C6: neral; C7: geraniol; C8: geranial; C9: eugenol; C10: methyl (E)-cinnamate; C11: β-elemene; C12: α-(E)-bergamotene; C13: germacrene-D; C14: bicyclogermacrene; C15: caryophyllene oxide; C16: epi-α-cadinol.

Table 2
Summary of combined analysis of variance and genetic parameters for the main chemical compounds and essential oil concentration of basil cultivars and hybrids, grown in two cropping seasons

		Mean Square						
SV	DF	linalool	methyl chavicol	neral	geranial	eugenol	methyl (E)-cinnamate	Essential oil
Blocks/Seasons	4	0.10	0.00	0.00	0.01	0.05	0.02	0.17
Genotypes (G)	23	3442.44*	3733.62*	567.75*	939.14*	234.72*	1301.06*	3.26*
Seasons (S)	1	0.35*	214.55*	0.12*	0.01*	1.82*	0.89*	4.94*
G x S	23	109.16*	250.06*	9.65*	15.40*	93.45*	1.39*	0.31*
Error	92	0.07	0.01	0.00	0.01	0.02	0.03	0.06
CV (%)		0.64	0.72	1.16	2.24	3.73	4.19	15.65
Genetic parameters								
Genetic variance		555.54	580.59	93.01	153.95	23.54	216.61	0.49
Residual variance		0.07	0.01	0.00	0.01	0.02	0.03	0.05
h^2 % (mean)		96.82	93.30	98.29	98.35	60.18	99.89	90.53
CV_g (%)		54.70	166.14	241.24	245.13	112.07	345.65	46.36
CV_e (%)		0.64	0.72	1.17	2.24	3.73	4.19	15.66
CV_g/ CV_e ratio		85.46	230.75	206.20	109.43	30.04	82.49	2.96

***Significant at 5% by the F test**
h^2: mean heritability
CV_g: coefficient of genetic variation
CV_e: coefficient of environmental variation

The estimates of heritability confirm that the traits under study undergo little environmental influence, as can be observed for the compounds linalool, methyl chavicol, and the citral group in the two cropping seasons (Table No. 2). The high values of the CV_g/CV_e ratio for all the traits studied confirm that breeding for these traits is feasible.

Heritability expresses the proportion of genetic variance in phenotypic variance, calculated in terms of estimate (h^2); it can vary under different conditions that may involve genotype, year, and location (genotype X environment interaction) (Blank *et al.,* 2010). The CV_g/CV_e ratio is a parameter that indicates success in selection of superior genotypes. Values of this ratio greater than or equal to 1.0 indicate that the genetic variation available is most responsible for the CV and is little influenced by environmental variation (Faluba *et al.,* 2010).

CONCLUSION

The results show that the cropping season influences the essential oil concentration of basil, and the characterization of chemical diversity shows a great variety of compounds found in essential oils that will be able to assist in breeding programs for the purpose of developing new cultivars with new aromas and fragrances, as well as studies involving potential pharmacological, medicinal, and biological activities.

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