Research Article

Field Evaluation of Twelve Clones of Oregano Grown in the Main Production Areas of Argentina: Identification of Quantitative Trait with the Highest Discriminant Value

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In Argentina, oregano (*Origanum* spp.) is one of the most important aromatic species. Leaves and flowering tops are used as seasoning, targeting the retail consumer, industrial and less to export. Local production has low-average yields due to the variability of cultivated material, the vegetative propagation methods used, and the lack of knowledge and adaptive experimentation on advanced cultivation practices. Clones of oregano grown in the country were collected in order to characterize the germplasm used in Argentina. Twelve oregano clones, sanitized by meristems culture and micropropagated *in vitro*, were field evaluated in three different growing locations. Regardless of growing site, the quantitative variables with more discriminating value were essential oils yield, internode length, length of the longest branch, fresh weight, dry weight of leaf and stem, leaf/stem ratio, and leaf area. Based on the quantitative traits, oregano clones can be classified into four groups. From the observations based on botanical characteristics, it was determined that the evaluated clones belong to three different taxa: *Origanum vulgare* ssp. *vulgare*, *Origanum vulgare* ssp. *hirtum*, and *Origanum x majoricum* (hybrid). Within each group, the clones belong to the same taxon.

1. Introduction

The genus *Origanum* belongs to the family Lamiaceae and includes more than 70 species, subspecies, varieties, and hybrids that are commonly found as wild plants in the Mediterranean areas [1]. This aromatic herb, mainly used as condiment, in liquors formulations, tomato sauces, baked goods, and salad dressing [2–10], is one of the most important aromatic spices cultivated in Argentina from the point of view of demand and acreage (80%) [11]. The main production areas are located in the provinces of Mendoza, Córdoba, and San Juan [12] although there are smaller production areas in the northwestern regions of the provinces of Salta and Jujuy [10]. The local production of oregano is mainly for domestic consumption; however, in recent years a surplus production for export was registered.

Despite this surplus production, the cultivation of oregano in our country is still affected by several problems. The oregano produced often does not have the best qualities to be considered a premium product for food because it is grown regardless of the variety, health, and the harvest and postharvest practices; in this situation, it is not possible to conserve a product free of contamination. Besides there is little taxonomic identification of the plant material used for cultivation; in fact, in our country registered cultivars and/or varieties of oregano do not exist [12], and the fantasy names used to designate the different materials are local names assigned by the producers, who include in those names phenotypic characteristics of the material or its place of origin [13]; even more, those fantasy names are often given to materials that are genetically equivalent [10].

TABLE 1: Twelve clones of oregano collected from the main production areas of Argentina.

Provenance	Germplasm
Mendoza	Criollo (Cr-Mza)
	Compacto (Cm-Mza)
	Mendocino (Me-Mza)
	Cordobés (Cd-Mza)
Córdoba	Clon 1 (C ₁ -FCA)
	Clon 2 (C ₂ -FCA)
	Clon 3 (C ₃ -FCA)
	Clon 8 (C ₈ -FCA)
	Negrito (NB-TS)
	Mendocino (Me-TS)
	Verde Limón (VL-TS)
San Juan	Negrito (Ne-SJ)

Mza: Mendoza; FCA: Facultad de Ciencias Agropecuarias; TS: Traslasierra Valley; SJ: San Juan.

Regarding to the agronomic evaluation of oregano cultivated in Argentina, in addition, the agronomic performance assessment of the growing materials [14–18], the study of different types of irrigation [19, 20], different methods of drying and storage [21–25], and the characterization of essential oils (yield and composition) [12, 26–28] was carried out. Besides, Fariás et al. [10] generated a list of 41 descriptors of oregano, with the aim of facilitating the identification, and characterization of different clones.

From the characterization of genotypes of oregano grown in the country, in terms of agronomic performance, morphology, botanical identification and study of essential oils, it is possible to plan the competitive development of this aromatic herb of great commercial value.

According to the above, the aims of this study were (1) to evaluate and characterize twelve genotypes of oregano grown in major production areas of the country through agronomically important variables, (2) to establish traits with the highest discriminant value to distinguish the genotypes of oregano, and (3) to carry out the taxonomic identification of these clones.

2. Materials and Methods

2.1. Plant Material. During spring and summer season of 2006/2007, plants belonging to twelve oregano clones coming from different collection sites were sanitized by meristem culture and micropropagated *in vitro* in order to use them as mother plants (Table 1). During autumn season of 2008, those mother plants were placed in pots containing vermiculite, irrigated with a nutrient and fungicidal solution, and maintained under the greenhouse environmental conditions. A month after transplanting, these mother plants were multiplied by binodal cuttings to get the number of plants needed to carry out the field essays. Plants grown from cuttings binodal were maintained under controlled environmental conditions until transplanting to the field in spring of 2008.

TABLE 2: Weather characteristics from the three culture locations where the comparative essays were implanted.

Locations	Altitude (mosl)	JaMT (°C)	JuMT (°C)	MAP (mm)
LC-Mza	954	21.8	6.2	212.5
PG-Cba	810	21.3	9.9	723.3
CE-Cba	370	22.8	9.7	606.5

mosl: meters over sea level; JaMT: January mean temperature; JuMT: July mean temperature; MAP: mean annual precipitation. LC-Mza: La Consulta (Mendoza); PG-Cba: Potrero de Garay (Córdoba); CE-Cba: Campo Escuela FCA-UNC (Córdoba).

2.2. Field Essays. In spring season of 2008, three field essays were conducted in order to evaluate the agronomic performance of twelve genotypes of oregano. They were implanted in three different locations: the Agricultural Experiment Station La Consulta: INTA (33°29' S, 69°04' O, San Carlos, Mendoza), a private farm in Potrero de Garay (31°47' S, 64°33' O, Santa María, Córdoba) and the Experimental Farm of the Faculty of Agricultural Sciences at the Universidad Nacional de Córdoba, (31°29' S, 64°00' O, Capilla de los Remedios road, 15.5 Km, Córdoba). The weather characteristics from the three culture locations are described in Table 2.

In every location, the oregano plants were transplanted following a completely randomized design, where each of the 12 clones was represented by 10 plants. The plants were distributed in lines spaced at 0.70 m apart with a distance of 0.25 m between plants on the line. The essays were watered twice a week, especially during the summer, and the cultural labors required for the proper development of the culture (as weeding) were also carried out.

The different genotypes were evaluated based on their morphological, phonological, and yield characteristics. Along the growing season, the longest branch length (LBL*) (cm) was measured. Three months after planting, the first harvest was carried out (all plants were harvested individually and correctly identified) and internode length (IL) (cm), fresh weight (FW*) (g plant⁻¹), dry weight (DW*) (g plant⁻¹), leave dry weight (LDW*) (g plant⁻¹), and stem dry weight (SDW*) (g plant⁻¹) were also measured. To determine the leaf area (LA) (cm²), fully expanded leaves located in the middle third of each branch were taken and scanned; for the image processing, software ImageJ (ver. X.XX, NIH, USA) was used. In order to estimate the essential oil content (EO) (%), from each one of the clones under study, three samples of dry leaves (5 g each) were taken. The oil extraction was carried out for 45 minutes, by hydrodistillation in a modified Clevenger equipment; subsequently, oil content was expressed in mL per 100 g of dry material. Besides, the ratio leave dry weight/stem dry weight (L/S*), water content (WC) (%) and fiber content (FC) (%) were estimated. One year after planting, a second harvest was carried out and the longest branch length (LBL**) (cm), number of branches (n° plant⁻¹) fresh weight (FW**) (g plant⁻¹), and dry weight (DW**) (g plant⁻¹) were registered.

TABLE 3: Quantitative trait mean values for the twelve clones of oregano evaluated in La Consulta (San Carlos, Mendoza).

Clon	LBL ₁ (cm)	IL (cm)	LA (cm ²)	FW* (g pl ⁻¹)	DW* (g pl ⁻¹)	LDW* (g pl ⁻¹)	SDW* (g pl ⁻¹)	L/S*	EO (%)	WC* (%)	FC* (%)	LBL ₂ (cm)	Branch (N°)	FW** (g pl ⁻¹)	DW** (g pl ⁻¹)
Me-TS	19.02a	2.27b	0.36a	60.61a	18.73a	14.34a	4.39a	3.31a	2.00d	69.15b	30.90a	47.34a	94a	272a	107.5a
VL-TS	23.42a	2.53c	0.53c	52.80a	16.79a	11.32a	5.48a	2.02a	0.72b	68.41b	31.80a	54.86c	112a	240a	106.0a
NB-TS	29.59b	2.16b	0.57c	73.72a	23.08a	17.75b	5.33a	4.07b	0.83b	68.17b	31.30a	56.01c	97a	282a	105.1a
Ne-SJ	30.93b	1.82a	0.84d	87.31b	25.51a	20.65b	4.86a	5.29b	0.35a	70.38b	29.21a	46.04a	101a	322a	108.5a
Me-Mza	28.56b	2.59c	0.49b	63.10a	19.07a	13.56a	5.51a	2.52a	2.36e	69.94b	30.22a	52.56b	96a	268a	101.0a
Cm-Mza	30.02b	2.17b	0.53c	80.41a	22.94a	18.63b	4.30a	4.48b	0.91b	71.17b	28.53a	56.77c	118a	314a	118.5a
Cd-Mza	32.86b	1.66a	0.40a	62.70a	21.08a	14.52a	6.56a	2.28a	1.42c	65.96a	33.62b	46.33a	109a	266a	110.0a
Cr-Mza	34.49b	2.13b	0.49b	50.87a	17.94a	11.81a	6.13a	1.85a	1.35c	64.42a	35.27b	57.21c	115a	353a	128.9a
C ₁ -FCA	38.86b	1.94b	0.45b	66.97a	22.39a	15.66a	6.73a	2.33a	1.42c	66.53a	33.43b	57.28c	123a	368a	131.0a
C ₂ -FCA	37.72b	2.04b	0.40a	74.47a	25.56a	17.73b	7.83b	2.30a	1.55c	65.76a	34.32b	57.74c	119a	334a	142.5a
C ₃ -FCA	23.04a	1.68a	0.47b	94.12b	28.58a	20.33b	8.25b	2.78a	0.50a	69.72b	30.37a	51.68b	102a	544b	191.1b
C ₈ -FCA	35.99b	1.75a	0.49b	98.89b	31.50b	23.66b	7.84b	3.29a	0.66b	68.24b	31.85a	51.82b	103a	544b	192.0b

C₁-FCA: clone 1, C₂-FCA: clone 2, C₃-FCA: clone 3, C₈-FCA: clone 8, Me-Mza: clone "Mendocino," Cm-Mza: clone "Compacto," Cd-Mza: clone "Cordobés," Cr-Mza: clone "Criollo," Me-TS: clone "Mendocino," NB-TS: clone "Negrito B," VL-TS: clone "Verde Limón," Ne-SJ: clone "Negrito," LBL: longest branch length; IL: internode length, LA: leaf area, FW: fresh weight, DW: dry weight, LDW: leave dry weight, SDW: stem dry weight, L/S: ratio LDW/SDW, EO: essential oil content, WC: water content, FC: fiber content, * measured at 1st harvest, ** measured at 2nd harvest, ¹three month after sowing, ²one year after sowing. Different letters indicate significant differences ($P \leq 0.05$).

2.3. Taxonomic Identification. In order to determine the taxonomic name of the twelve evaluated clones, one flowering branch belonging to every clone was collected, herborized and conserved in the Museo Botánico del Instituto Multidisciplinario de Biología Vegetal Herbarium (IMBIV). The botanical characterization, mainly based in floral characters, was carried out using the identification keys of Ietswaart [29], Xifreda [2], and Rouquaud and Videla [3].

2.4. Statistical Analysis. All results obtained for each of the measured variables were analyzed using the software INFOSTAT [30].

The differences among clones and growing locations for each one of the evaluated variables were estimated using an ANOVA according to the following model:

$$Y_{ijk} = \mu + C_i + L_j + L_j > R_k + C * L_{ij} + \varepsilon_{ijk}, \quad (1)$$

where Y_{ijk} represents the average value of each variable of the i th clone (C_i) evaluated at the j th location (L_j), R_k is the number of repetitions of each clone in every location, $C * L_{ij}$ is the interaction between the i th clone, and j th location and ε_{ijk} with $\sim N(0, S^2)$ is the random error term associated with observation Y_{ijk} . Differences between mean values of each clone and location were determined using the posteriori test for comparison of means DGC [31].

By using the principal component analysis (PCA) that was assessed the potentiality of quantitative traits measured was assessed, in order to discriminate the different clones in each growing environment. Finally, a Generalized Procrustes Analysis (GPA) was conducted in order to determine the level of consensus between the configurations obtained for all evaluated characters in each growing location, in other words to determine whether the variables have the same discriminant value in all growing environment.

3. Results and Discussion

Significant differences among the twelve evaluated clones were observed in most of quantitative traits measured in all cultivation locations (Tables 3, 4, and 5). The agronomic performance of 12 clones evaluated in each cultivation sites is described below.

3.1. La Consulta INTA Essay. Clones Me-TS, VL-TS, and C₃-FCA showed the lower mean value for longest branch length, when this trait was measured three months after sowing. One year after sowing, the clones with the lower longest branch length mean value were Me-TS, Ne-SJ, and Cd-Mza.

Regarding the fresh weight yield of the first harvest, clones C₈-FCA, C₃-FCA, and Ne-SJ stood out for their higher production of fresh biomass, followed by clones Cm-Mza and NB-TS. In relation to dry weight, again C₈-FCA clone differed significantly from the other clones because of its greater dry biomass yields, followed by clones C₃-FCA, C₂-FCA, Ne-SJ, Cm-Mza and NB-TS. The production of fresh and dry biomass can be related to the length of the internodes and leaf area; clones C₃-FCA, C₂-FCA, Ne-SJ, Cm-Mza, and NB-TS also showed shorter internodes and greater area leaf (Table 3). In the second harvest, clones C₃-FCA and C₈-FCA significantly surpassed the rest of the material for both fresh and dry weight. Regardless of the clone, the fresh and dry weight yields observed in both harvests are generally similar to those reported by Droushiotis and Della [32] and Weglarz and Geszprych [33].

With regard to the percentage of water that is lost during the drying process, significant differences were observed among the clones both in the first as in the second harvest. In the first harvest, the clones with the lowest content of water were Cr-Mza, C₂-FCA, Cd-Mza, and C₁-FCA, which in turn showed the highest percentage of fibers (66.47%, and 34.16%

TABLE 4: Quantitative trait mean values for the twelve clones of oregano evaluated in Potreto de Garay (Santa María, Córdoba).

Clon	LBL ₁ (cm)	IL (cm)	LA (cm ²)	FW* (g pl ⁻¹)	DW* (g pl ⁻¹)	LDW* (g pl ⁻¹)	SDW* (g pl ⁻¹)	L/S*	EO (%)	WC* (%)	FC* (%)	LBL ₂ (cm)	Branch (N°)	FW** (g pl ⁻¹)	DW** (g pl ⁻¹)
Me-TS	28.04a	0.96a	0.68b	8.90a	5.53a	2.51a	3.02a	0.73a	3.11c	39.39a	62.13b	21.43a	30b	31.97a	13.76a
VL-TS	32.25a	1.08b	0.36a	36.50b	16.22b	10.64b	5.58a	1.68b	0.71a	49.58b	44.44a	24.16a	30b	37.79a	15.80a
NB-TS	28.75a	1.17b	1.06d	54.85c	22.47c	15.99c	6.48a	2.58c	0.43a	57.26c	40.97a	31.12b	68d	145.73c	56.30c
Ne-SJ	41.54b	1.14b	0.65b	61.70c	25.11c	18.62c	6.49a	3.30d	0.36a	58.89c	40.70a	36.41b	83d	194.51d	64.94c
Me-Mza	33.18a	0.91a	0.53b	8.55a	5.05a	1.86a	3.19a	0.52a	4.63d	38.07a	59.06b	20.76a	17a	19.42a	8.75a
Cm-Mza	26.90a	1.14b	0.92c	60.30c	25.11c	17.60c	7.51a	2.54c	0.41a	57.34c	41.64a	32.50b	76d	144.86c	57.19c
Cd-Mza	29.17a	1.09b	0.55b	21.35a	9.91b	5.90b	4.01a	1.45b	1.46b	53.94c	46.42a	31.43b	38b	66.30b	28.28b
Cr-Mza	33.60a	1.35c	0.61b	31.35b	13.21b	8.03b	5.17a	1.48b	1.19b	55.72c	42.14a	35.53b	45b	86.06b	37.23b
C ₁ -FCA	35.42a	1.17b	0.56b	35.85b	16.80b	10.42b	6.38a	1.43b	1.49b	47.44b	46.86a	35.14b	57c	109.65b	46.49b
C ₂ -FCA	33.27a	1.11b	0.61b	35.75b	16.81b	10.70b	6.11a	1.75b	1.56b	53.03c	47.02a	34.42b	54c	95.23b	40.07b
C ₃ -FCA	36.91a	1.22b	0.44a	42.79b	18.28b	12.21b	7.58a	1.66b	1.00b	55.33c	42.72a	47.10d	53c	143.99c	57.38c
C ₈ -FCA	38.16a	1.22b	0.41a	68.50c	29.54c	16.33c	13.20b	1.72b	1.01b	56.39c	43.12a	41.88c	61c	135.78c	55.02c

C₁-FCA: clone 1, C₂-FCA: clone 2, C₃-FCA: clone 3, C₈-FCA: clone 8, Me-Mza: clone "Mendocino," Cm-Mza: clone "Compacto," Cd-Mza: clone "Cordobés," Cr-Mza: clone "Criollo," Me-TS: clone "Mendocino," NB-TS: clone "Negrito B," VL-TS: clone "Verde Limón," Ne-SJ: clone "Negrito," LBL: longest branch length, IL: internode length, LA: leaf area, FW: fresh weight, DW: dry weight, LDW: leave dry weight, SDW: stem dry weight, L/S: ratio LDW/SDW, EO: essential oil content, WC: water content, FC: fiber content, * measured at 1st harvest, ** measured at 2nd harvest, ¹three month after sowing, ²one year after sowing. Different letters indicate significant differences ($P \leq 0.05$).

TABLE 5: Quantitative trait mean values for the twelve clones of oregano evaluated in Capilla de los Remedios (Río Segundo, Córdoba).

Clon	LBL ₁ (cm)	IL (cm)	LA (cm ²)	FW* (g pl ⁻¹)	DW* (g pl ⁻¹)	LDW* (g pl ⁻¹)	SDW* (g pl ⁻¹)	L/S*	EO (%)	WC* (%)	FC* (%)	LBL ₂ (cm)	Branch (N°)	FW** (g pl ⁻¹)	DW** (g pl ⁻¹)
Me-TS	26.01b	1.24b	0.53a	113.55a	35.12a	25.94a	9.18a	3.22b	1.91b	69.58a	30.93b	22.91b	86a	62.04a	29.06a
VL-TS	14.03a	0.75a	0.48a	113.48a	31.85a	20.07a	11.78a	2.09a	0.52a	72.04a	28.07b	17.30a	97a	28.36a	12.55a
NB-TS	17.04a	0.90a	0.71b	227.26b	63.99b	45.92b	18.07a	3.16b	0.80a	72.03a	28.16b	25.78b	188b	134.77b	53.88b
Ne-SJ	21.22a	0.86a	0.96c	275.49b	84.81b	53.99b	30.82b	2.27a	0.41a	69.45a	30.79b	13.37a	74a	50.01a	16.75a
Me-Mza	31.86b	1.29b	0.57a	143.58a	43.60a	31.10a	12.50a	2.70a	2.38c	70.16a	30.37b	31.96b	76a	93.35b	42.15b
Cm-Mza	16.26a	0.87a	0.74b	211.80b	62.86b	43.67b	19.19a	3.60b	0.76a	70.70a	29.68b	28.51b	147a	112.26b	44.74b
Cd-Mza	26.59b	1.38b	0.62a	140.52a	41.04a	24.20a	16.84a	1.77a	1.11a	70.88a	29.21b	39.77c	125a	116.37b	55.77b
Cr-Mza	26.49b	1.34b	0.60a	145.49a	40.27a	23.91a	16.36a	1.54a	0.88a	72.24a	27.68b	40.55c	157b	162.36c	77.14c
C ₁ -FCA	31.67b	1.22b	0.52a	125.22a	37.81a	22.60a	15.21a	1.65a	0.73a	70.03a	30.19b	29.46b	126a	109.79b	47.33b
C ₂ -FCA	25.96b	1.24b	0.52a	132.50a	36.57a	21.85a	14.71a	1.73a	0.82a	72.43a	27.60b	34.24b	142b	119.16b	57.39b
C ₃ -FCA	19.71a	0.87a	0.69b	240.43b	67.35b	44.27b	23.07b	2.28a	0.33a	72.17a	28.01b	22.51b	102a	86.87b	31.89a
C ₈ -FCA	16.32a	0.82a	0.61a	188.87b	45.98a	32.18a	13.80a	2.79a	0.52a	75.12b	24.34a	24.12b	110a	80.98b	31.43a

C₁-FCA: clone 1, C₂-FCA: clone 2, C₃-FCA: clone 3, C₈-FCA: clone 8, Me-Mza: clone "Mendocino," Cm-Mza: clone "Compacto," Cd-Mza: clone "Cordobés," Cr-Mza: clone "Criollo," Me-TS: clone "Mendocino," NB-TS: clone "Negrito B," VL-TS: clone "Verde Limón," Ne-SJ: clone "Negrito," LBL: longest branch length, IL: internode length, LA: leaf area, FW: fresh weight, DW: dry weight, LDW: leave dry weight, SDW: stem dry weight, L/S: ratio LDW/SDW, EO: essential oil content, WC: water content, FC: fiber content, * measured at 1st harvest, ** measured at 2nd harvest, ¹three month after sowing, ²one year after sowing. Different letters indicate significant differences ($P \leq 0.05$).

resp.). In the second harvest, the clones with lowest water content and higher fiber content were Cd-Mza, VL-TS, and C₂-FCA, with an average percentage of 54.68% of water and 45.32% fiber. The percentage of water found in the second harvest was similar to that reported by Leto and Salamone [34], who studied 62 biotypes of oregano coming from 17 different locations. Higher fiber content (or less water loss) is equivalent to a higher dry matter yield. That is, on average, those clones with higher fiber content showed a dry matter yield 7.62% higher than the rest of the clones in the first crop and 15.85% in the second harvest. This is important when estimating production volumes of dry matter.

Significant differences were also observed among the clones in terms of dry weight of leaves and stems and leaf/stem ratio (Table 3), being this latter variable very important when estimating the returns as are leaves the part of the plant that is consumed [35]. Clones that showed the highest values for leaf/stem ratio were Ne-SJ, Cm-Mza, and NB-TS, these clones had short internodes, a greater number of nodes (and therefore more foliage), and greater leaf area.

Based on the essential oil yield, it was possible to distinguish four groups of oregano. The first group showed an average essential oil content of 2.18% which includes the clones Me-Mza and Me-TS. The second group, including

FIGURE 1: Biplot plane formed by the two first principal components (CP1 and CP2), where the dots represent the 12 clones of oregano evaluated and the vectors, quantitative characteristics measured in La Consulta (Mendoza). C₁-FCA: clone 1, C₂-FCA: clone 2, C₃-FCA: clone 3, C₈-FCA: clone 8, Me-Mza: clone "Mendocino," Cm-Mza: clone "Compacto," Cd-Mza: clone "Cordobés," Cr-Mza: clone "Criollo," Me-TS: clone "Mendocino," NB-TS: clone "Negrito B," VL-TS: clone "Verde Limón," Ne-SJ: clone "Negrito," LBL: longest branch length, IL: internode length, LA: leaf area, FW: fresh weight, LDW: leave dry weight, SDW: stem dry weight, L/S: ratio LDW/SDW, EO: essential oil content, *measured at 1st harvest (three month after sowing); **measured at 2nd harvest (one year after sowing).

the clones C₂-FCA, C₁-FCA, Cd-Mza and Cr-Mza, showed an average essential oil content of 1.44%, while the third group, involving the clones Cm-Mza, NB-TS, VL-TS y C₈-FCA, showed an average oil percentage of 0.78%. Finally, the group with the lowest essential oil content was constituted by clones C₃-FCA and Ne-SJ, which showed an average oil yield of 0.43%. The average essential oil yield observed for the first and second group surpass in 67.7% and 10.8%, respectively, the percentages of oil reported by Weglarz and Geszprych [33], whereas the essential oil content observed in the third and fourth group was in the range reported by these authors (0.30–1.30%).

Finally, the number of branches per plant ranged from 94 (Me-TS) to 123 (C₁-FCA); however, no significant differences were observed among the twelve evaluated clones.

Principal component analysis (PCA) showed that 73.1% of the total variability is explained by the first factorial plane (PC1 and PC2). At the level of the first axis (PC1), which explains 42.2% of the total variability, the variables with greater weight were the leave dry weight, fresh weight of the second harvest, internode length, and essential oil content, whereas the second axis (PC2) explains 30.9% of total variability, and the variables with greater inertia are the stem dry weight, leaf/stem ratio, and leaf area (Figure 1).

3.2. Potrero de Garay Essay. With regard to the length of the longest branch, measured three months after planting, clone Ne-SJ differed significantly from the other clones,

showing the longest branch length mean value, while no differences were observed among the 11 remaining clones (whose branch length ranged between 26.90 and 38.16 cm) (Table 4). Considering the same variable, measured one year after sowing, the clones Me-Mza, Me-TS, and VL-TS differed from the other clones by their shorter length (22.12 cm on average), and the clones C₈-FCA (41.88 cm) and C₃-FCA (47.10 cm) were distinguished by their greater length, while no differences were observed between the remaining clones, with an average branch length of 33.79 cm.

Regarding the fresh weight yield of the first harvest, clones C₈-FCA, Ne-SJ, Cm-Mza, and NB-TS were those that stood out for their higher production of fresh biomass, with an average fresh weight of 61.34 g plant⁻¹, while the clones Me-Mza and Me-TS differed from the rest of the clones by their poor performance in fresh matter (on average 8.73 g plant⁻¹), followed by the clone Cd-Mza with a fresh biomass yield of 21.35 g plant⁻¹. In relation to dry weight, clones C₈-FCA, Ne-SJ, Cm-Mza, and NB-TS showed dry biomass yields significantly superior from those observed in the remaining clones, followed by clones C₃-FCA, C₂-FCA, C₁-FCA, VL-TS, Cr-Mza, and Cd-Mza, with an average dry matter yield of 15.21 g plant⁻¹. Being the clones Me-Mza and Me-TS those showed the lowest yield in dry weight (Table 4).

The higher production of fresh and dry biomass observed in clones C₈-FCA, Ne-SJ, Cm-Mza and NB-TS can be associated with the largest number of branches per plant (more than 60 branches per plant), with a longer branch (C₈-FCA and Ne-SJ) and greater leaf area (Cm-Mza and NB-TS) (Table 4). The low yields observed in the first harvest for fresh and dry weight, lower than those values reported by Droushiotis and Della [32] and Weglarz and Geszprych [33], can be attributed to the dehydration suffered by the plants during the week before the harvest, being the clones Me-Mza and Me-TS the clones that most resented the lack of water.

In the second harvest, clones Ne-SJ, NB-TS, Cm-Mza, C₃-FCA, and C₈-FCA showed a significantly superior yield, both in fresh as in dry biomass, with an average fresh weight of 152.97 g plant⁻¹ and an average dry weight of 58.17 g plant⁻¹. Clones C₁-FCA, C₂-FCA, Cr-Mza, and Cd-Mza showed average yields in fresh and dry matter of 89.31 g plant⁻¹, and 38.02 g plant⁻¹ respectively; while the clones Me-Mza and Me-TS were those with the lowest yield both in fresh weight (25.70 g plant⁻¹) as in dry weight (11.26 g plant⁻¹), as they were in the first harvest, followed by the clone VL-TS.

As regards the percentage of water that is lost during the drying process, significant differences were observed between the clones both in the first as in the second harvest. In both harvests, the clones Me-Mza and Me-TS showed the lowest water content (and the highest percentage of fibers) (Table 4). At this point, it is important to note that in the week preceding the first harvest, Potrero de Garay field was exposed to water stress, so the percentage of water lost during the drying process was lower than in La Consulta and Capilla de los Remedios. For this same reason, the percentage of fibers of the different clones, measured in the first harvest, was overestimated; however, it was possible to differentiate the oregano clones based on these variables. It is reasonable

to assume that a high fiber content (equivalent to less water loss) results in a higher dry weight yield. Even though clones Me-Mza and Me-TS had higher fiber content, their biomass production was so low that they fail to align their yields with those clones that have higher percentages of water.

In addition, significant differences were found among the clones in terms of the dry weight of the leaves and the stems and leaf/stem ratio (Table 4), being this latter variable of major economic importance when estimating amount of production since it is the leaves that are used for consumption. The clones Ne-SJ, NB-TS, and Cm-Mza were those that showed the greatest values for leaf/stem ratio, coinciding with the greatest number of branches, longest branch, and greatest leaf area (Table 4).

Concerning the content of essential oils, the clones Me-Mza and Me-TS showed an essential oil yield significantly higher than the rest of the clones, with an average of 3.87% of oil, followed by the clones C₂-FCA, C₁-FCA, Cd-Mza, Cr-Mza, C₈-FCA, and C₃-FCA, which showed an average oil content of 1.29% and the clones VL-TS, NB-TS, Cm-Mza and Ne-SJ (clones with the lowest performance in oils, producing on average 0.48%). The essential oil yield observed for the clones Me-Mza and Me-TS was similar the one reported for *Origanum dubium* (3.30 to 5.85%) [32]. These percentages were three times higher than those reported by Weglarz & Geszprych [33] and 32.5% higher than the yield observed in Turkish oregano (*Origanum onites*) [36], one of the species of greater content of essential oils.

The principal component analysis (PCA) showed that 82.6% of the total variability was explained by the first factorial plane (CP1 and CP2). On the level of CP1, component that by itself explains 63.8% of the total variability, the variables with higher weight were the dry weight of leaf, the fresh weight measured at the second harvest, and the content of essential oils, while, at the level of CP2, the variables with greater inertia were leaf area and length of the longest branch (measured at 400 days after planting) (Figure 2).

3.3. Capilla De Los Remedios Essay. Regarding to the length of the longest branch, measured 85 days after seeding, the clones VL-TS, Cm-Mza, NB-TS, C₈-FCA, C₃-FCA, and Ne-SJ differed significantly from the rest, recording the lowest values for this variable (range 14.03 and 21.22 cm) (Table 5). When this variable was determined 400 days after sowing, Ne-SJ and VL-TS differed from the other clones for its smaller branch length (15.34 cm on average), while Cd-Mza and Cr-Mza were stood out for their greater length (40.16 cm). No differences were observed between the remaining genotypes, whose average branch length was 27.44 cm (Table 5).

With regard to fresh weight yield of the first harvest, two significantly different groups were distinguished. The clones C₈-FCA, Cm-Mza, NB-TS, C₃-FCA, and Ne-SJ comprise the higher group, with an average production of fresh biomass of 228.77 g plant⁻¹, exceeding 75.14% the group of lower values for the variable formed by the seven remaining clones, which had an average yield of fresh weight of 130.62 g plant⁻¹. The

FIGURE 2: Biplot plane formed by the two first principal components (CP1 and CP2), where the dots represent the 12 clones of oregano evaluated and the vectors, quantitative characteristics measured in Potrero de Garay (Córdoba). C₁-FCA: clone 1, C₂-FCA: clone 2, C₃-FCA: clone 3, C₈-FCA: clone 8; Me-Mza: clone "Mendocino," Cm-Mza: clone "Compacto," Cd-Mza: clone "Cordobés," Cr-Mza: clone "Criollo," Me-TS: clone "Mendocino," NB-TS: clone "Negrito B," VL-TS: clone "Verde Limón," Ne-SJ: clone "Negrito," LBL: longest branch length, IL: internode length, LA: leaf area, FW: fresh weight, LDW: leave dry weight, SDW: stem dry weight, L/S: ratio LDW/SDW, EO: essential oil content, *measured at 1st harvest (three month after sowing), **measured at 2nd harvest (one year after sowing).

same trend was observed in relation to dry weight, where the clones Cm-Mza, NB-TS, C₈-FCA and Ne-SJ differed significantly from the rest by its higher values which averaged 69.75 g plant⁻¹ (Table 5). As in the essays of La Consulta and Potrero de Garay, clones with higher production of fresh and dry biomass are generally characterized by the presence of a greater number of branches per plant (more than 70 branches per plant), shorter internodes, and greater leaf area. Once again, the observed values for the variables fresh weight and dry weight are within the range reported by Droushiotis and Della [32] and Weglarz and Geszprych [33].

Although in the second harvest the clones differed from one to another both in fresh as dry weight (Table 5), due to the strong predation caused by ants, the recorded yields did not reflect the real potential of each genotype. Although all the clones were targeted by these insects, Ne-SJ, VL-TS, Me-TS, C₈-FCA, and C₃-FCA were the most affected, being Ne-SJ the clone that showed the most important performance reduction for fresh and dry biomass (50.01 g plant⁻¹ and 16.75 g plant⁻¹, resp.). Taking into account the agronomic performance observed for the same clone (Ne-SJ) both in the essays conducted in La Consulta and Potrero de Garay as in the first harvest of this essay, it can be assumed that in normal conditions (without predation) the yield of this clone would be far higher. Furthermore, it is reasonable to assume that without ants, the second harvest would be maintained the trend observed in the first one for all the clones. Regardless of the insect damage, the clones VL-TS

and Me-TS were usually the lowest performing group both in fresh as in dry biomass yield, following the same trend observed in La Consulta and Potrero de Garay. The clones NB-TS, Cm-Mza, C₃-FCA, and C₈-FCA (which were the top performers in the first harvest) and the clone Me-Mza (low performance in the first harvest) did not differ significantly from clones C₁-FCA, C₂-FCA, and Cd-Mza, with an average fresh weight yield of 106.69 g plant⁻¹ while the clone Cr-Mza had a biomass yield significantly higher than the rest of the clones, with an average fresh weight of 162.36 g plant⁻¹. In terms of dry weight, the clones VL-TS, Ne-SJ, Me-TS, C₈-FCA, and C₃-FCA had an average yield of 24.34 g plant⁻¹; the clones Me-Mza, Cm-Mza, C₁-FCA, NB-TS, Cd-Mza, and C₂-FCA yielded an average of 50.21 g plant⁻¹ while Cr-Mza was significantly higher than the rest with a fresh biomass production averaged 77.14 g plant⁻¹ (Table 5).

Respecting to the percentage of water lost during the drying process, significant differences were seen among the clones both in the first as in the second harvest. In the first harvest, C₈-FCA differed significantly from the rest of the clones, with a water content exceeding 5.71% of the 11 remaining clones and being also the genotype with the lowest fiber content (Table 5). In the second harvest, the clone with highest water content was Ne-SJ (65.46%), a value that was on average 7.58% higher than that observed for clones. Regarding the fiber content in the second harvest, the clones Cd-Mza, C₂-FCA, Cr-Mza, Me-TS, VL-TS, and Me-Mza had the highest value for this variable, containing average of 45.54% of fiber (Table 5). As already noted above it can be assumed that, at the same fresh weight yield, a higher fiber content is equivalent to a higher dry matter yield.

Regarding the leaves and stems dry weight and leaf/stem ratio, significant differences were also observed between genotypes. The clones mainly characterized by shorter internodes, high number of branches, and the greater leaf area (clones NB-TS and Cm-Mza) and the low stems weight (clone Me-TS) were those who showed the highest values for leaf/stem ratio (Table 5).

Concerning the content of essential oils, Me-Mza and Me-TS differed significantly from the rest of clones, with an average essential oil content of 2.15%, while no differences were observed among the 10 remaining clones (which oil content ranged from 0.33% to 1.11%). According to Kokkini [37], based on performance of essential oils, it is possible to categorize the different genotypes of oregano into three groups: (a) those producing less than 0.05% ("poor" in oils), (b) those with essential oil content between 0.05 and 2%, and (c) those with an essential oil yield over 2% ("rich" in oil). Both in this essay as in those conducted in La Consulta and Potrero de Garay, clones of oregano belonging to the three groups were distinguished. The clones called "Mendocino" (Me-Mza and Me-TS) belong to the rich-oil group, with a performance that in the three locations exceeds 2%, while the clones called "Compacto" (Cm-Mza) and "Negritos" (NB-TS and Ne-SJ) belong to the group poor-oil group. These results are consistent with those observed by De Mastro [38]. Franz & Novak [35] propose to use differential production of oregano based on the essential oil yield, destinating for use as dried herb those materials having 1 to 3% of oil, and

FIGURE 3: Biplot plane formed by the two first principal components (CP1 and CP2), where the dots represent the 12 clones of oregano evaluated and the vectors, quantitative characteristics measured in Capilla de los Remedios (Córdoba). C₁-FCA: clone 1 C₂-FCA: clone 2, C₃-FCA: clone 3, C₈-FCA: clone 8; Me-Mza: clone "Mendocino," Cm-Mza: clone "Compacto," Cd-Mza: clone "Cordobés," Cr-Mza: clone "Criollo," Me-TS: clone "Mendocino," NB-TS: clone "Negrito B," VL-TS: clone "Verde Limón," Ne-SJ: clone "Negrito," LBL: longest branch length, IL: internode length, LA: leaf area, FW: fresh weight, LDW: leave dry weight, SDW: stem dry weight, L/S: ratio LDW/SDW, EO: essential oil content, *measured at 1st harvest (three month after sowing); **measured at 2nd harvest (one year after sowing).

for essences production those clones containing levels of oil exceeding 3%. Oregano production intended for obtaining essential oils becomes more important if we consider that these essences and/or its components represent a potentially important source of pharmacologically active compounds [39] or agrochemicals (as herbicides) [40]. Particularly, it has been tested that the essential oil of oregano has a strong antioxidant activity in foods with high-fat content and fried-salted peanuts [41, 42].

The principal component analysis (PCA) showed that 73% of the total variability is explained by the first factorial plane formed by the two first principal components. On the level of CP1, that by itself explains 50.1% of the total variability, the more discriminating variables were leaf dry weight, leaf area, dry weight of stems, the length of internodes and the essential oil content, while on the level of CP2 (which accounts for 22.9% of the total variability observed) were fresh weight measured in the second harvest, leaf/stem ratio, and the length of the longest branch. The PCA showed that all 12 clones of oregano evaluated tend to cluster in a manner similar to those observed in La Consulta and Potrero de Garay (Figure 3).

With regard to generalized procrustes analysis (GPA), combining the information obtained for quantitative traits in clones of oregano grown in the three locations, the eigenvalues indicate that the first two axes explain 100% of the variability. Figure 4 shows the consensus configuration between the rankings generated by the principal components

TABLE 6: Taxonomic identification of 12 clones of oregano from the main cultivation areas.

Clone	Common name	Origin	Herbarium number	Taxa
Me-TS	“Mendocino”	Córdoba (Valle de Traslasierras)	Torres 0901 (CORD)	<i>Origanum x majoricum</i> Cambessedes
VL-TS	“Verde Limón”	Córdoba (Valle de Traslasierras)	Torres 0909 (CORD)	<i>Origanum vulgare</i> ssp. <i>hirtum</i> (Link) Iestwaart
NB-TS	“Negrito”	Córdoba (Valle de Traslasierras)	Torres 0910 (CORD)	<i>Origanum vulgare</i> ssp. <i>vulgare</i>
Ne-SJ	“Negrito”	San Juan (Santa Lucía)	Torres 0911 (CORD)	<i>Origanum vulgare</i> ssp. <i>vulgare</i>
Me-Mza	“Mendocino”	Mendoza (La Consulta)	Torres 0902 (CORD)	<i>Origanum x majoricum</i> Cambessedes
Cm-Mza	“Compacto”	Mendoza (La Consulta)	Torres 0903 (CORD)	<i>Origanum vulgare</i> ssp. <i>vulgare</i>
Cd-Mza	“Cordobés”	Mendoza (La Consulta)	Torres 0905 (CORD)	<i>Origanum vulgare</i> ssp. <i>hirtum</i> (Link) Iestwaart
Cr-Mza	“Criollo”	Mendoza (La Consulta)	Torres 0904 (CORD)	<i>Origanum vulgare</i> ssp. <i>hirtum</i> (Link) Iestwaart
C ₁ -FCA	—	Córdoba (FCA-UNC)	Torres 0912 (CORD)	<i>Origanum vulgare</i> ssp. <i>hirtum</i> (Link) Iestwaart
C ₂ -FCA	“Criollo”	Córdoba (FCA-UNC)	Torres 0906 (CORD)	<i>Origanum vulgare</i> ssp. <i>hirtum</i> (Link) Iestwaart
C ₃ -FCA	—	Córdoba (FCA-UNC)	Torres 0907 (CORD)	<i>Origanum vulgare</i> ssp. <i>hirtum</i> (Link) Iestwaart
C ₈ -FCA	—	Córdoba (FCA-UNC)	Torres 0908 (CORD)	<i>Origanum vulgare</i> ssp. <i>hirtum</i> (Link) Iestwaart

FIGURE 4: Arrangement the 12 clones of oregano on the plane formed by first two axes of a PGA (with minimum tree trail), considering quantitative characteristics evaluated at the three locations of cultivation. C₁-FCA: clone 1, C₂-FCA: clone 2, C₃-FCA: clone 3, C₈-FCA: clone 8, Me-Mza: clone “Mendocino,” Cm-Mza: clone “Compacto,” Cd-Mza: clone “Cordobés,” Cr-Mza: clone “Criollo,” Me-TS: clone “Mendocino,” NB-TS: clone “Negrito B,” VL-TS: clone “Verde Limón,” Ne-SJ: clone “Negrito.”

of the quantitative characters measured in La Consulta, Potrero de Garay, and Capilla de los Remedios. From the ratio between the consensus and the total sum of squares, it can be concluded that there is a 77.3% of consensus between the configurations produced by the quantitative traits measured in each one of the growing locations. From the GPA it can be said that, regardless of their agronomic performance, the 12 clones of oregano evaluated tend to be clustered in a similar way in the three locations where the essays were conducted; moreover four groups of oregano can be clearly distinguished (Figure 4).

3.4. Taxonomic Identification of the Evaluated Clones. The taxonomic identification of the material evaluated at field was conducted using the keys of Iestwaart [29], Xifreda [2], and Rouquaud & Videla [3]. In accordance with those keys, the classification of the different clones of oregano was largely based on floral characteristics such as shape of the calyx, the length of the bracts, the length of the staminal filaments, and the color of bracts and flowers. From the observations made, it was determined that 10 of the 12 clones of oregano evaluated belong to the genus *Origanum*, section *Origanum*, and two of them are hybrids (Table 6).

In our country, Rouquaud & Videla [3] reported the existence of “oregano” belonging to *Origanum vulgare* ssp. *vulgare* and the hybrid *Origanum x majoricum*, while the subspecies *Origanum vulgare* ssp. *hirtum* is reported for the first in this paper. In addition to the taxa mentioned, specimens belonging to the species *Origanum dictamnus* and *Origanum vulgare* ssp. *viride* [2], *Origanum vulgare* ssp. *virens* [3], *Origanum majorana* and the hybrid *Origanum x applii* [2, 3] were described in the country. Since the “oregano” includes a group of cross-pollinated species, the genetic variability within and between them is very high [32]. Thus the variability observed between clones belonging to the same species can be attributed to genetic variation originated by recombination processes inherent in all species whose members reproduce sexually [43].

4. Conclusions

The quantitative traits analyzed allowed the characterization of the 12 clones of oregano studied, establishing four clearly distinguishable groups. Regardless of the cultivation site, most discriminate quantitative traits were essential oil content, internodes length, longest branch length (400 days), fresh weight (2nd harvest), leave dry weight, stem dry weight, ratio H/T, and foliar area. The 12 evaluated clones can be clustered as follow: (Group 1) clones Me-TS and Me-Mza; (Group 2) clones Cd-Mza, Cr-Mza, C₁-FCA, and C₂-FCA; (Group 3) clones Cm-Mza, NB-TS, and Ne-SJ (Group 4) clones C₃-FCA and C₈-FCA. The clone VL-TS is a particular

case since if it shares characteristic of different groups such as long branches and long internodes (group 2) and low essential oil content (less than 1%) (group 4).

Otherwise, the 12 clones were identified taxonomically, confirming that two of these clones correspond to the hybrid *Origanum x majoricum* Cambessedes, three of the clones belong to the species *Origanum vulgare* ssp. *vulgare* and the remaining 7 clones belong to the species *Origanum vulgare* ssp. *hirtum* (Link) Iestwaart. Regardless of the growing location, clones were clustered in the same way, and these groups are consistent with their taxonomic determination. Differences observed among the clones pertaining to the same species can be attributed to the variability generated in the recombination processes.

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