

## Evaluation of micropropagation for the introduction into cultivation and conservation of *Lippia junelliana*, an endemic aromatic plant from Argentina

H. Rodolfo Juliani<sup>a,\*</sup>, Adolfin R. Koroch<sup>b</sup>, Julio A. Zygodlo<sup>c</sup>, Victorio S. Trippi<sup>d</sup>

<sup>a</sup> New Use Agriculture and Natural Plant Products Program, Department of Plant Biology & Plant Pathology, Foran Hall, 59 Dudley Road, Rutgers University, New Brunswick, NJ 08901, USA

<sup>b</sup> CUNY, Borough Manhattan Community Coll, Dept Sci, New York, NY 10007, USA

<sup>c</sup> Cátedra de Química Orgánica-Productos Naturales, Facultad de Ciencias Exactas Físicas y Naturales, Universidad Nacional de Córdoba, IMBIV-CONICET-ICTA, Av. Velez Sarsfield 1611, Córdoba, Argentina

<sup>d</sup> Instituto de Fitopatología y Fisiología Vegetal (INTA/IFFIVE), Camino a 60 cuadras s/n. 5000, Córdoba, Argentina

### ARTICLE INFO

#### Article history:

Received 7 April 2010

Received in revised form 1 December 2010

Accepted 8 December 2010

Available online 11 January 2011

#### Keywords:

*Lippia junelliana*

Verbenaceae

Salvia lora

Essential oil

Cultivation

Wild populations

Collecting

Aromatic

Medicinal plant

Monoterpenes

### ABSTRACT

The aims of this work were to introduce *Lippia junelliana* into cultivation, to compare the essential oil accumulation between cultivated and wild plants, and to reintroduce micropropagated plants in the location of the original population. The leaves and inflorescences of cultivated plants accumulated, on a dry weight basis, higher amounts of essential oil than their wild counterparts. Thus, total essential oil accumulation of cultivated plant parts was also significantly higher than that of wild counterparts. The cultivated plants showed the same essential oil profile than the wild plants. This work demonstrates that cultivation can be a more efficient vehicle to both preserve and exploit *L. junelliana*, than collection from the wild, because higher yields of biomass and oil accumulation can be achieved, while essential oil composition is less affected by the different treatments. The reintroduction of new plants into the species' original location has proved to be a viable alternative for their *in situ* preservation or enrichment planting. This model of introduction of aromatic plants into cultivation through micropropagation could be a useful technique to recover valuable chemotypes from the wild in the search for new alternatives in the agriculture and for the preservation of natural resources for future generations.

© 2010 Elsevier B.V. All rights reserved.

### 1. Introduction

Medicinal and aromatic plants constitute an important group among the economic plants. In Argentina, most medicinal plant species are collected from the wild to supply the increasing demand of the pharmaceutical, food and flavor industries. As a result, the natural populations of medicinal plants have seriously declined and in some cases become endangered (Koroch et al., 1997). Loss of habitat due to urbanization, deforestation, overgrazing, and fire are additional causes of decreasing medicinal plant populations.

Cultivation of medicinal plants permits production of uniform and high quality raw material compared to the collection from the wild. However, studies on the agronomic aspects of medicinal plants are few (Palevitch, 1991). Therefore, the movement toward domestication as a vehicle for genetic preservation and conservation is an important strategy (Franz, 1993). Several arguments have

been mentioned to promote conservation of plants, they include the economic value of plants as resources for humanity both now and in the future, the scientific value of plants, the role of plants in maintaining stable environments, in inspiring people and in transforming their values (Given, 1994). The reintroduction of species and restoration programs that enhance, restore or recreate native or semi-natural habitats are now essential tools in conservation's armory. Nowadays, the trend in plant conservation is to combine *ex situ* and *in situ* species within an integrated conservation program including the collaboration among scientific or plant breeding organizations (Akeroyd, 2006).

Micropropagation has been used for the rapid multiplication of many Argentinean medicinal and aromatic plants including *Hedeoma multiflorum* (Koroch et al., 1997), *Mintostachys mollis* (Chebel et al., 1998) and *Lippia junelliana* (Juliani et al., 1999). This technique offers a way to multiply plants possessing a special phenotypic character directly and rapidly, thereby shortening the time needed for the introduction of a new plant variety into the marketplace (Schumacher, 1991) and providing an effective technique for repopulation of the original locations of the plants (Socorro et al., 1998).

\* Corresponding author. Tel.: +1 732 932 9711.

E-mail address: [hjuliiani@rci.rutgers.edu](mailto:hjuliani@rci.rutgers.edu) (H. Rodolfo Juliani).

The genus *Lippia* belongs to the *Verbenaceae* family and includes approximately 200 species in central and south America and tropical Africa (Terblanché and Kornelius, 1996). Argentina is particularly rich in a large number of these species. *L. junelliana* (Mold.) Tronc. is an endemic perennial aromatic shrub of central Argentina, which reaches 1.5 m in height and can be found in the hilly regions of Córdoba, La Rioja, San Luis, Tucuman, Jujuy, Catamarca, Salta and Santiago del Estero provinces (Zuloaga and Morrone, 1999). Locally known as “salvialora” or “salvia morada (purple sage)”, its leaves are claimed to have medicinal properties used for abdominal complaints (Juliani et al., 1994). The therapeutic effects of this plant have been partially attributed to its essential oil (Zygodlo and Juliani, 2000).

The objectives of this study were to introduce wild varieties of *L. junelliana* into cultivation, to compare the biomass partition and essential oil accumulation between cultivated and wild plants, and to reintroduce micropropagated plants into the species' original location.

## 2. Materials and methods

The first objective of this study was to introduce a single chemotype (Juliani et al., 1994) of *L. junelliana* into cultivation. Seeds of *L. junelliana* were collected from wild plants growing in the hilly region of San Luis province, city of Merlo (Piedras Blancas I, PB I), Rep. Argentina (lat: 32° 19', long west 64° 59' 960 m asl, mean annual rainfall 650 mm, with sandy soils not adequate for agriculture). Seeds were germinated under aseptic conditions. One plantlet, out of many, was selected due to its better *in vitro* growth and then clonally micropropagated, acclimatized and transferred to a greenhouse (Juliani, 1998; Juliani et al., 1999). The micropropagated plantlets (three-month-old) were transferred at the beginning of spring (September, 1996) to three 1 m<sup>2</sup> plots, row spacing of 70 cm, and plants separated by 70 cm within each row (9 plants per m<sup>2</sup>). The plots with soils very suitable for agriculture were located in the research fields of “Facultad de Ciencias Agropecuarias” National University of Córdoba (Córdoba province, Argentina, 31 29 00.93 S, 64° 00 23 W 370 m asl, mean annual rainfall 750 mm). The plots were watered once a week and weed control was carried out manually as necessary.

Wild and cultivated plants were harvested in mid-autumn (April 1997), six plants of a similar size (1 m in height) to the cultivated ones were selected. Ten micropropagated plants were randomly distributed in the original location of the wild plants at the beginning of the spring (September 1999) and six of these re-introduced plants were later harvested (April 2000). The above ground biomass of each plant was harvested and separated into leaves, stems and inflorescences and dried at ambient temperature in the shade over a 10-day period. The essential oil (EO) content of the plant parts was measured for the three types of plants.

Each group of plant parts was steam distilled (90 min) using a Clevenger-type apparatus. The stem oils were extracted from the distillation water with butyl methyl ether and then the solvent removed under reduce pressured. Essential oil content was calculated as the percentage of sample dry weights (g EO/100 g organ

dry weight). The oils were dried over anhydrous sodium sulfate and stored at –20 °C in the dark until analysis. The leaf essential oils were analyzed by gas chromatography (Juliani et al., 2002). All the compounds were listed in order of elution on SE30 column (Tables 3 and 5).

A second objective of this study sought to recover the variability of *L. junelliana* chemotypes identified in a previous study (Juliani et al., 2002). Seeds of *L. junelliana* from different chemotypes were collected from the populations of Piedras Blancas (II) (dihydrocarvone chemotype, San Luis province), Arroyo San Antonio (ASA, ocimene), Altautina (ALT, limonene-piperitenone) and Los Chañaritos N° 2 (LC, limonene-piperitenone oxide) (Cordoba Province) (Juliani et al., 2002). Seeds were germinated under aseptic conditions and clonally micropropagated, acclimatized and transferred to a greenhouse as described above (Juliani et al., 1999). Six plants of the four chemotypes were transferred to three plots with the same plant separation (3 rows and plants separated 70 cm) as listed above and growing in the same research station. The plots were watered once a week. The plants were transferred early September 1999. The four chemotypes were distributed randomly within each plot. Two plants were harvested (early April 2000) per plot and variety (6 in total) and the above ground biomass of plants was used in the study. The same plants growing in the wild that served as the sources of seeds were also harvested at the same time (early April 2000). All the plants were dried, separated into leaves, stems and inflorescences and the essential oil extracted and analyzed as described above. Voucher for all specimens were deposited (Juliani et al., 2002).

The experimental design was fully randomized. Data presented correspond to the mean of the values at least in triplicate. Data were analyzed statistically by analysis of variance (ANOVA). The differences between the means was compared using LSD test (least significant differences) with the level of significance set at  $p = 0.05$ .

## 3. Results and discussion

In the first study, it was observed a variation in the biomass of the wild, cultivated, and reintroduced plants, and also in the way in which the plants partitioned the biomass (Table 1). The biomass of the wild plants (115.5 g) showed no significant differences when compared with the cultivated plants (107.7 g), while the biomass of reintroduced plants (88.2 g) was significantly lower (Table 1). The cultivated plants accumulated more biomass in the leaves (43.3%) and inflorescences (3.0%), than the wild (33.5% on the leaves and 0.9% on inflorescences), and in the reintroduced plants (31.1% for leaves, and 0.7% for inflorescences).

The inflorescence biomass was much higher for cultivated plants (3.2 g) than for either of the other plants (less than 1 g) (Table 1), it was observed that in these plants 93.2% of the oil accumulated by the whole plant was produced in the leaves and 6.1% in the inflorescences. In comparison, wild and reintroduced plants accumulated 96.7% and 97.1%, respectively of their essential oils in the leaves and only 2.1% and 1.8%, respectively in the inflorescences (Table 1).

The leaves and inflorescences of cultivated plants accumulated, on a dry weight basis (3.8% and 3.5%, respectively) (Table 2), higher

**Table 1**  
Biomass and essential oil partition of wild (W), cultivated (C) and reintroduced (R) populations of *Lippia junelliana* in different sites of Central Argentina.

	Biomass (g)			Biomass accumulation (%)			Essential oil partition (%)		
	W	C	R	W	C	R	W	C	R
Leaves	37.3 ± 1.2cd*	46.7 ± 4.0bc	27.6 ± 2.3d	33.5 ± 3.5d	43.3 ± 0.5c	31.1 ± 1.8d	96.7 ± 0.2a	93.2 ± 0.9b	97.1 ± 0.5a
Inflorescences	1.0 ± 0.2f	3.2 ± 0.2e	0.7 ± 0.1f	0.9 ± 0.2e	3.0 ± 0.2e	0.7 ± 0.1e	2.1 ± 0.4d	6.1 ± 0.9c	1.8 ± 0.4de
Stems	77.2 ± 12.1a	57.8 ± 5.0ab	59.9 ± 3.5ab	65.6 ± 3.7a	53.7 ± 0.5b	68.2 ± 1.9a	1.3 ± 0.3de	0.6 ± 0.1e	1.1 ± 0.1ed
Total	115.5 ± 11.0a	107.7 ± 9.1ab	88.2 ± 4.9b						

\* Mean ± standard error; letters in the same parameter do not differ statistically according to LSD test ( $p = 0.05$ ).

**Table 2**  
Essential oil content of wild (W), cultivated (C) and reintroduced (R) populations of *Lippia junelliana* in different sites of Central Argentina.

	EO content (%) <sup>a</sup>			EO per plant part (g)		
	W <sup>a</sup>	C	R	W	C	R
Leaves	3.0 ± 0.2bc*	3.8 ± 0.17a	2.8 ± 0.2c	1.1 ± 0.1b	1.8 ± 0.2a	0.8 ± 0.1b
Inflorescences	2.6 ± 0.17c	3.5 ± 0.3ab	1.9 ± 0.3d	0.03 ± <0.01c	0.1 ± 0.03d	0.01 ± <0.01ef
Stems	0.02 ± <0.01e	0.02 ± <0.01e	0.02 ± <0.01e	0.01 ± <0.01e	0.012 ± <0.01ef	0.008 ± <0.01f
Total essential oil content				1.2 ± 0.1 <sup>a</sup>	1.9 ± 0.2b	0.8 ± 0.1a

<sup>a</sup> g essential oil (EO)/100 g dry weight.\* Mean ± standard error; letters in the same parameter do not differ statistically according to LSD test ( $p=0.05$ ).**Table 3**  
Essential oil percent composition of wild, cultivated and reintroduced populations of *Lippia junelliana* in different sites of Central Argentina.

RI <sup>a</sup>	Compounds	Wild	Cultivated	Reintroduced
937	α-Pinene	0.2 ± 0.0a*	0.6 ± 0.1b	0.5 ± 0.1b
953	Camphene	1.6 ± 0.1a	1.8 ± 0.3a	1.7 ± 0.3a
980	β-Pinene	0.6 ± 0.2a	0.3 ± 0.1a	0.8 ± 0.1a
986	Myrcene	0.2 ± 0.1a	0.2 ± 0.1a	0.2 ± 0.1a
1031	Limonene	32.4 ± 4.0a	33.4 ± 5.1a	27.3 ± 5.3a
1040	cis-Ocimene	0.2 ± 0.1a	0.2 ± 0.1ab	0.2 ± 0.1b
1114	Camphor	3.0 ± 0.3a	3.5 ± 0.3a	3.4 ± 0.3a
1146	Borneol	1.7 ± 0.3a	2.3 ± 0.3a	1.5 ± 0.1b
1201	Estragal	0.2 ± 0.1a	0.3 ± 0.1a	0.2 ± 0.1a
1260	Geranial	15.8 ± 3.2ab	18.3 ± 3.9a	17.6 ± 5.1b
1218	Carvone	0.8 ± 0.3a	0.5 ± 0.2a	0.8 ± 0.3a
1304	Piperitenone	31.6 ± 4.1ab	27.7 ± 3.4b	35.6 ± 4.0a
1352	Piperitenone oxide	1.7 ± 0.6a	1.4 ± 0.4a	1.0 ± 0.2a
1371	Methyleugenol	2.1 ± 0.2a	1.5 ± 0.5a	2.0 ± 0.2a
1419	β-Caryophyllene	2.3 ± 0.3a	1.9 ± 0.4a	1.6 ± 0.3b
1452	α-Humulene	0.4 ± 0.1a	0.3 ± 0.1a	0.3 ± 0.1a
1556	Germacrene B	0.8 ± 0.2a	0.5 ± 0.1ab	0.2 ± 0.1b
1576	Spathulenol	0.6 ± 0.1a	0.3 ± 0.1a	0.5 ± 0.2b

<sup>a</sup> Retention index.\* Mean (relative percentage of oil components) ± standard error; letters in the same component do not differ statistically according to LSD test ( $p=0.05$ ).

amounts of essential oils than their wild counterparts (3% for leaves and 2.6% for inflorescences), and for reintroduced plants 2.8% for leaves, and 1.9% inflorescences. These results showed that the organs of the cultivated plants were more efficient in accumulating essential oils (Table 2). The cultivated plants produced more biomass in the leaves (43.3%) than the wild and re-introduced plants (33.5 and 33.1%, respectively). In contrast, the wild plants accumulated greater stem weight (Table 2), the stems accumulated small quantities of essential oils in comparison with the leaves and inflorescences.

Our previous study (Juliani et al., 1994) reported low essential oil accumulation by the stems. Accordingly, the total essential oil accumulation of cultivated plants (1.9 g) was significantly higher than the wild (1.2 g) and reintroduced plants (0.8 g) (Table 2). The oil content varied in the three types of plants while the essential oil profile of the cultivated plants was quite similar to the wild and re-introduced plants (Table 3). All plants showed high levels of limonene (32.4%, 33.4% and 27.3% for the wild, cultivated and reintroduced plants, respectively), geranial (15.8%, 18.3%

and 17.6%) and piperitenone (31.6, 27.7, and 35.6%) as the main monoterpenes. Minor significant differences were observed for geranial and piperitenone (Table 3). With all the plants showing lower and significantly similar levels of camphene (1.6–1.8%), camphor (3.0–3.5%), methyleugenol (1.5–2.1%) and β-caryophyllene (1.6–2.3%) (Table 3).

In the second study, the wild plants accumulated significantly higher levels of total biomass in the aerial parts (Table 4) when compared with the cultivated plants (Piedras Blancas varieties, 148 g and 37.9 g, respectively; Arroyo San Antonio, 272 g and 51.3 g, respectively; Altautina, 111 g and 102 g; Los Chañaritos, 117.7 and 72.4 g, respectively). The cultivated plants partitioned more biomass into the leaves than the wild varieties (Fig. 1), the highest percentage was observed in the Piedras Blancas variety (48% for cultivated, 11% for wild, respectively). Arroyo San Antonio (46%, 3.2%), Altautina (41%, 17%), Los Chañaritos (42%, 25%), and both type of plants partitioned a small amount of biomass into the inflorescences (<1.6%) (Fig. 1). The cultivated plants partitioned similar amounts into the stems, ranging from 50 to 59%. While the wild plants showed the highest relative amount of stems, the highest amount was observed in Arroyo San Antonio varieties (97%), Piedras Blancas (87%), Altautina (82%) and Los Chañaritos (75%) (Fig. 1).

The plants growing in the wild showed higher contents of essential oils in the leaves when compared with the cultivated plants (Table 4). The higher oil content was observed in the wild plants from Piedras Blancas (4.8%) and lower content in the cultivated plants (3.8%), a similar trend was observed in Arroyo San Antonio (3.7%, 2.6%, respectively), Los Chañaritos (2.7% and 2.4%), and no significantly different essential oil content was observed in Altautina (2.9% and 2.7%). The cultivated plants accumulated higher total amounts of oils, Arroyo San Antonio cultivated varieties accumulated 0.6 g of oils per plant, while the wild varieties 0.4 g, in Altautina 1 g and 0.5 g, Los Chañaritos 0.7 g and 0.5 g, respectively. While the Piedras Blancas varieties showed similar values (0.7–0.8 g, respectively). The wild plants had a different physiological age (woody shrub perennials having grown in the wild for several years) (Table 4), while the cultivated plants grew during one season.

The chemical composition of the wild varieties also showed the same profile of the cultivated plants (Table 5). In the Piedras Blancas variety the wild and cultivated plants were dominated by dihydrocarvone (79.3%, 85.4%), with lower

**Table 4**  
Biomass partition and essential oil content of wild and cultivated populations of *Lippia junelliana* in different sites of Central Argentina.

	Location							
	Piedras Blancas II		Arroyo San Antonio		Altautina		Los Chañaritos	
	Wild	Cultivated	Wild	Cultivated	Wild	Cultivated	Wild	Cultivated
Biomass	148 ± 19a*	37.9 ± 7b	272 ± 19a	51.3 ± 6b	111 ± 27a	102 ± 11b	117.7 ± 31a	72.4 ± 9b
EO content	4.8 ± 0.1b	3.8 ± 0.1a	3.7 ± 0.3b	2.6 ± 0.1a	2.9 ± 0.1a	2.7 ± 0.1a	2.7 ± 0.2a	2.4 ± 0.1a
EO in leaves	0.8 ± 0.1a	0.7 ± 0.1a	0.4 ± 0.1b	0.6 ± 0.1a	0.5 ± 0.1b	1.0 ± 0.1a	0.5 ± 0.1b	0.7 ± 0.1a

\* Mean ± standard error; letters in the same parameter and variety do not differ statistically according to LSD test ( $p=0.05$ ).

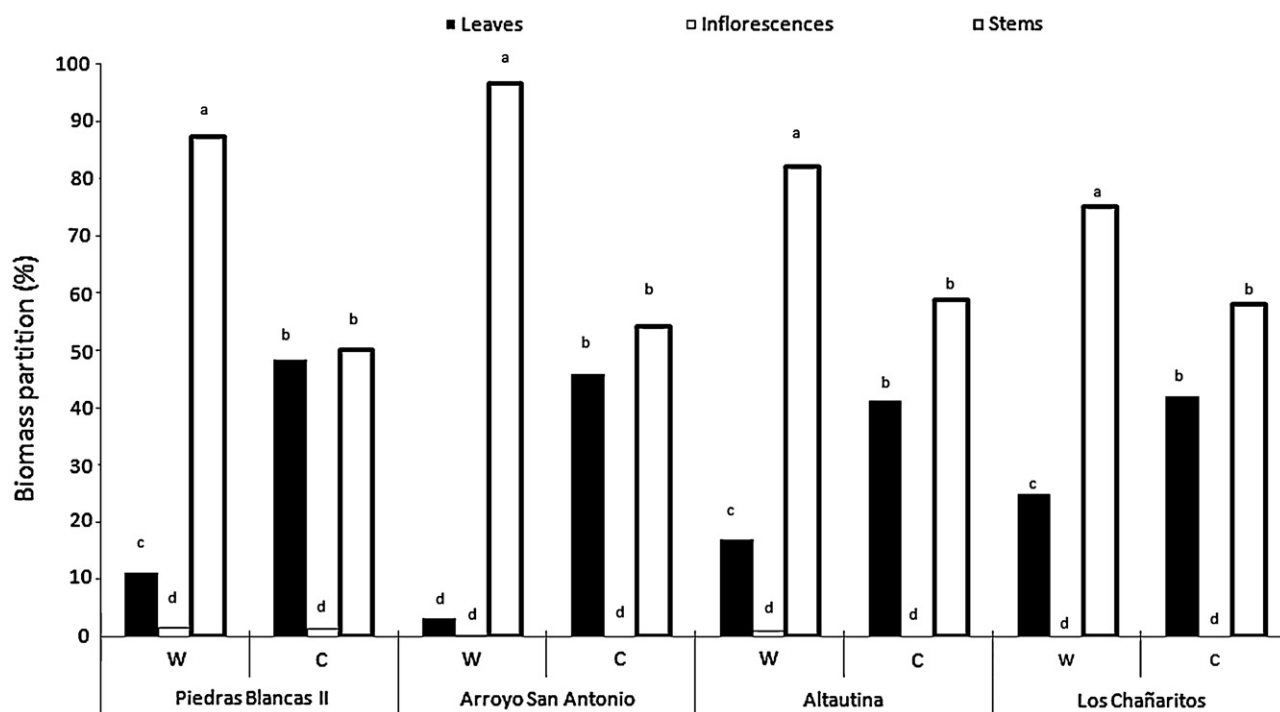


Fig. 1. Biomass percent partition of leaves, inflorescences and stems in wild (W) and cultivated (C) populations of *Lippia junelliana* in different sites of Central Argentina. Values followed by the same letters do not differ statistically according to LSD test ( $p=0.05$ ).

amounts of limonene (5.3–8.7%),  $\beta$ -caryophyllene (4.1–2.2%) and camphene (3.2–1.1%, respectively). The Arroyo San Antonio varieties were dominated by ocimenone (64.7–69.1%) with lower amounts of myrcene (10.8–11.6%) and piperitenone (4.1–7.9%, respectively). The Altautina varieties were dominated by limonene (28.2–23.1%) and piperitenone (28.9–45.6%, respectively). The Los Chañaritos variety was dominated by limonene (36.7–42.3%), piperitenone oxide (29–20.5%) with lower amounts of camphor (8.2–9.0%), (E) caryophyllene (3.0–4.6%) and germacrene B (3.7–4.9%, respectively) (Table 5). In this latter variety, significant differences were found in the main component piperitenone oxide. While the main components

from the other sites did not show any significant differences (Table 5).

These results suggest that the chemical composition of the oil was less affected by the environments in which the plants were grown, since the cultivated plants derived from seeds from the wild plants and were growing under different environmental conditions. Since the first generation growing in different places maintained the essential oil profile of the parent plant, the cultivated clone could be considered a stable chemotype. It was also observed in *L. junelliana* that the essential oil content showed a remarkable variability year round whereas the composition was less affected (Juliani et al., 1998). Therefore, the essential oil content is more

Table 5  
Essential oil percent composition of wild (W) and cultivated (C) populations of *Lippia junelliana* in different sites of Central Argentina.

RI <sup>a</sup>	Location component	Piedras Blancas II		Arroyo San Antonio		Altautina		Los Chañaritos	
		W	C	W	C	W	C	W	C
937	$\alpha$ -Pinene			1.3 $\pm$ 0.2a	1.6 $\pm$ 0.1a	0.6 $\pm$ 0.2a	0.4 $\pm$ 0.0a	0.5 $\pm$ 0.0a	0.9 $\pm$ 0.0b
953	Camphene	3.2 $\pm$ 0.4a*	1.1 $\pm$ 0.2b			4.5 $\pm$ 0.9a	2.7 $\pm$ 0.1a	3.4 $\pm$ 0.1a	5.6 $\pm$ 0.2b
986	Myrcene			10.8 $\pm$ 0.1a	11.6 $\pm$ 0.5a				
1031	Limonene	5.3 $\pm$ 1.2a	8.7 $\pm$ 0.2b			28.2 $\pm$ 4a	23.1 $\pm$ 1a	36.7 $\pm$ 1a	42.3 $\pm$ 1.8a
1040	<i>cis</i> - $\beta$ -Ocimene					1.4 $\pm$ 0.4a	0.3 $\pm$ 0.0a	0.2 $\pm$ 0.0a	0.2 $\pm$ 0.0a
1096	Mircenone			0.9 $\pm$ 0.0a	0.6 $\pm$ 0.0b				
1114	Camphor			0.5 $\pm$ 0.0a	0.6 $\pm$ 0.0a	12.0 $\pm$ 2a	6.8 $\pm$ 0.3a	8.2 $\pm$ 0.2a	9.0 $\pm$ 0.4b
1132	Z-Tagetone			1.6 $\pm$ 0.1a	1.2 $\pm$ 0.0a				
1146	Borneol							0.8 $\pm$ 0.2a	0.5 $\pm$ 0.0a
1172	(Z+E) Dihydrocarvone	79.3 $\pm$ 3a	85.4 $\pm$ 1a					0.9 $\pm$ 0.2a	3.7 $\pm$ 0.7a
1209	(Z+E) Ocimenone			64.7 $\pm$ 1a	69.1 $\pm$ 2a				
1218	Carvone			3.4 $\pm$ 0.0a	2.0 $\pm$ 0.1b				
1223	Piperitone					1.2 $\pm$ 0.2a	3.2 $\pm$ 0.1b		
1304	Piperitenone			4.1 $\pm$ 0.6a	7.9 $\pm$ 0.2a	28.9 $\pm$ 6a	45.6 $\pm$ 1a	2.3 $\pm$ 0.2a	1.1 $\pm$ 0.1b
1352	Piperitenone oxide							29 $\pm$ 0.2a	20.5 $\pm$ 1.7b
1371	Methyleugenol			1.8 $\pm$ 0.0a	0.9 $\pm$ 0.1a				
1419	(E)-Caryophyllene	4.1 $\pm$ 0.0a	2.2 $\pm$ 0.1b	3.0 $\pm$ 0.0a	1.3 $\pm$ 0.0b	2.0 $\pm$ 0.3a	3.1 $\pm$ 0.2a	3.0 $\pm$ 0.2a	4.6 $\pm$ 0.0b
1452	$\alpha$ -Humulene					0.8 $\pm$ 0.0a	1.0 $\pm$ 0.1a	0.8 $\pm$ 0.0a	1.5 $\pm$ 0.0b
1556	Germacrene B			0.7 $\pm$ 0.1a	1.4 $\pm$ 0.1b	1.8 $\pm$ 0.5a	1.4 $\pm$ 0.2a	3.7 $\pm$ 0.1a	4.9 $\pm$ 0.0b
1576	Spathulenol			2.7 $\pm$ 0.3a	1.5 $\pm$ 0.1a	2.6 $\pm$ 0.5a	1.1 $\pm$ 0.2a	0.5 $\pm$ 0.0a	1.0 $\pm$ 0.2a

<sup>a</sup> RI, retention index.

\* Mean (relative percentage of oil components)  $\pm$  standard error. Letters in the same component and site do not differ statistically according to LSD test ( $p=0.05$ ).

influenced by the environmental conditions than the oil composition.

In *Rosmarinus officinallis*, drought reduced the essential oil accumulation (Ross and Sombrero, 1991). It was reported that with an optimum (adequate) water supply, biomass production tends to be higher than at sites where nutrients or water are scarce (Lambers et al., 1989). The wild and re-introduced plants accumulated less biomass and essential oils, as these plants were not as well cared for as the cultivated ones. Therefore, periodic watering and the absence of weeds in the plots, may explain the higher essential oil accumulation of the cultivated plants. In mint, the application of mulch significantly increased biomass accumulation and essential oil yields, while in the non-mulch treatment, the presence of weeds decreased biomass accumulation and reduced oil yields (Singh and Saini, 2008). In *Carum carvi*, the oil content of wild and cultivated populations was almost equal (Galambosi and Peura, 1996), but in *Tagetes minuta* accessions, the essential oil accumulation was higher in the cultivated plants (Gil et al., 2000). In 20 varieties of *Origanum vulgare*, most of the cultivated varieties (85%) showed higher or similar content of essential oils when compared with the wild ones. Both cultivated and wild varieties showed a similar composition in their essential oils (Esen et al., 2007). While in fruits of *Bunium persicum*, the essential oil content of the wild plants (9%) was higher as compared with the cultivated plants (5–6%), and the oil composition of the wild and cultivated plants was also similar (Azizi et al., 2009). The present work supports the conclusion of Palevitch (1991), who stated that the introduction of medicinal and aromatic plants from the wild into cultivation can lead to a more uniform, higher yielding and more consistent products.

#### 4. Conclusions

This study has demonstrated that wild plants have a potential that is not fully expressed under *in situ* conditions. Cultural practices seem to improve biomass and essential oil production while leaving the essential oil composition less affected. Cultivation of *L. junelliana* may serve as an efficient method to exploit this medicinal plant in a more sustainable manner, and contribute to the preservation of wild populations. The reintroduction of new plants into the species' original location has proved to be a viable alternative for their *in situ* preservation or for enrichment planting in sustainable collection programs. This technique of introduction of aromatic plants into cultivation through micropropagation could be a useful technique to recover valuable chemotypes from the wild in the search for new alternatives in the agriculture and for the preservation of natural resources for future generations.

#### Acknowledgements

This work was supported by the Research Council of Córdoba (CONICOR), the National University of Córdoba (SECYT/UNC) and "Academia Nacional de Agronomía y Veterinaria".

#### References

- Azizi, M.A., Davareenejad, G., Bos, R., Woerdenbag, H.J., Kayser, O., 2009. Essential oil content and constituents of Black Zira (*Bunium persicum*) from Iran during field cultivation (domestication). *J. Essent. Oil Res.* 21, 78–82.
- Akeroyd, J.R., 2006. Plant taxonomy and reintroduction. In: Leadley, E., Jury, S. (Eds.), *Taxonomy and Plant Preservation*. University Press, Cambridge, UK, pp. 221–227.
- Chebel, A.V., Koroch, A.R., Juliani Jr, H.R., Juliani, H.R., Trippi, V.S., 1998. Micropropagation of *Minthostachys mollis* (H.B.K.) Griesb. and essential oil composition of clonally propagated plants. *In Vitro Plant Cell. Dev. Biol.* 34 (3), 249–251.
- Esen, G., Azaz, A.D., Kurkcuoglu, M., Can Baser, K.H., Tinmaz, A., 2007. Essential oil and antimicrobial activity of wild and cultivated *Origanum vulgare* L. subsp. *hirtum* (Link) letsuaert from the Marmara region, Turkey. *Flavour Fragr. J.* 22, 371–376.
- Franz, C., 1993. Domestication of wild growing medicinal plants. *Plant Res. Dev.* 37, 99–111.
- Galambosi, B., Peura, P., 1996. Agrobotanical features and oil content of wild and cultivated forms of Caraway (*Carum carvi* L.). *J. Essent. Oil Res.* 8, 389–397.
- Gil, A., Ghersa, C.M., Leicach, S., 2000. Essential oil yield and composition of *Tagetes minuta* accessions from Argentina. *Biochem. Syst. Ecol.* 28, 261–274.
- Given, D.R., 1994. Principles and Practices of Plant Conservation. Timber Press, Portland, OR, 3 pp.
- Juliani Jr, H.R., Trippi, V.S., Juliani, H.R., Ariza-Espinar, L., 1994. Essential oils from various plant parts of *Lippia junelliana*. *An. Soc. Quim. Argent.* 82, 53–55.
- Juliani Jr, H.R., Koroch, A.R., Juliani, H.R., Zygadlo, J.A., Trippi, V.S., 2002. Intraspecific variation in leaf oils of *Lippia junelliana* (Mold.) Tronc. *Biochem. Syst. Ecol.* 30, 163–170.
- Juliani Jr, H.R., Koroch, A.R., Juliani, H.R., Trippi, V.S., 1999. Micropropagation of *Lippia junelliana* (Mold.) Tronc. *Plant Cell Tiss. Org.* 59, 175–179.
- Juliani, H.R., 1998. Domestication of *Lippia junelliana* and the regulation of essential oil accumulation. Doctoral Thesis. (National University of Córdoba), Argentina, pp. 132–138.
- Juliani, H.R., Koroch, A.R., Juliani, H.R., Trippi, V.S., 1998. Variación estacional de la acumulación de aceites esenciales en *Lippia junelliana*. *An. Soc. Quim. Argent.* 86 (3/6), 193–196.
- Koroch, A.R., Juliani Jr, H.R., Juliani, H.R., Trippi, V.S., 1997. Micropropagation and acclimatization of *Hedeoma multiflorum* Benth. *Plant Cell Tiss. Org.* 48 (3), 213–217.
- Lambers, H., Freijsen, N., Poorter, H., Hirose, T., Van Der Wef, A., 1989. Analyses of growth bases on net assimilation rate and nitrogen productivity. Their physiological background. In: Lambers, H., Cambridge, M.L., Konings, H., Pons, T.L. (Eds.), *Causes and Consequences of Variation in Growth Rate and Productivity of Higher Plants*. SPB Academic Publishing B.V., The Hague, The Netherlands, pp. 1–17.
- Palevitch, D., 1991. Agronomy applied to medicinal plant conservation. In: Akerele, O.V., Heywood, G., Synge, H. (Eds.), *Conservation of Medicinal Plants*. Cambridge University Press, Cambridge, UK, pp. 167–168.
- Ross, J.D., Sombrero, C., 1991. Environmental control of essential oil production in Mediterranean plants. In: Harborne, J.B., Tomas-Barberan, F.A. (Eds.), *Ecological Chemistry and Biochemistry of Plant Terpenoids*. Clarendon Press, Oxford, UK, pp. 83–94.
- Schumacher, H.M., 1991. Biotechnology in the production and conservation of medicinal plants. In: Akerele, O., Heywood, V., Synge, H. (Eds.), *Conservation of Medicinal Plants*. Cambridge University Press, Cambridge, UK, pp. 179–198.
- Singh, M.K., Saini, S.S., 2008. Planting date, mulch, and herbicide rate effects on the growth, yield, and physicochemical properties of menthol mint (*Mentha Arvensis*). *Weed Technol.* 22, 691–698.
- Socorro, O., Tárrega, I., Rivas, F., 1998. Essential oils from wild and micropropagated plants of *Origanum bastetanum*. *Phytochemistry* 48 (8), 1347–1349.
- Terblanché, F.C., Kornelius, G., 1996. Essential oil constituents of the genus *Lippia* (Verbenaceae)—a literature review. *J. Essent. Oil Res.* 8, 471–485.
- Zuloaga, F.O., Morrone, O., 1999. Catálogo de plantas vasculares de la República Argentina II. Missouri Botanical Garden Press, Saint Louis, MI, pp. 161–1542.
- Zygadlo, J.A., Juliani Jr, H.R., 2000. Bioactivity of essential oil components. *Curr. Top. Phytochem.* 3, 203–214.