BOLETIN LATINOAMERICANO Y DEL CARIBE DE PLANTAS MEDICINALES Y AROMÁTICAS
© / ISSN 0717 7917 / www.blacpma.ms-editions.cl

Articulo Original / Original Article

# Inter and intra population phenotypic variability of *Lippia integrifolia* (Verbenaceae) and its natural situation in the west-center of Argentina

[Variabilidad fenotípica inter e intra poblacional de *Lippia integrifolia* (Verbenaceae) y su situación natural en el centro-oeste de Argentina]

**Paula C. Brunetti[1], Romina Leiva[1], Ricardo Zapata[1], Lorena E. Torres[1], Ana G. Chaves[1], H. Rodolfo Juliani[2] & Marta S. Ojeda[1]**

[1]Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Córdoba, Argentina
[2]School of Environmental and Biological Sciences, Rutgers University, New Jersey, USA

**Reviewed by:**
Bladimir Diaz
Universidad de Atacama
Chile

Oscar Ariel Risso
Instituto Nacional de Tecnologia Agropecuaria
Argentina

Correspondence:
Paula C. BRUNETTI:
pbrunetti@agro.unc.edu.ar

**Section Genetic resources**

Received: 24 June 2020
Accepted: 25 Setember 2020
Accepted corrected: 12 March 2021
Published: 30 March 2022

**Citation:**
Brunetti PC, Leiva R, Zapata R, Torres LE, Chaves LG, Juliani HR, Ojeda MS
Inter and intra population phenotypic variability of *Lippia integrifolia* (Verbenaceae) and its natural situation in the west-center of Argentina
**Bol Latinoam Caribe Plant Med Aromat**
21 (2): 242 - 255 (2022)
https://doi.org/10.37360/blacpma.22.21.2.15

**Abstract:** The species *Lippia integrifolia* is an aromatic, sub-woody shrub, distributed from Northwest and Central Argentina to Bolivia. It is among the most important native aromatic species. It presents medicinal properties, some of which have been scientifically proven. As an alternative to harvesting, the process of domestication of the species was initiated. The objective of this work was to assess the phenotypic variability based on botanical-taxonomic and morpho-agronomic descriptors and the use of indicators to evaluate its natural situation in the west-center of Argentina. We worked in five wild populations, registering morphological variables, phenology, natural regeneration, herbivory and presence of diseases. *L. integrifolia* presents a wide morphological variability, which can be explained with three morphological descriptors. Natural regeneration´s rate is very low to zero, in four of the sites. The results obtained are of importance to make decisions related to the sustainable use in situ, and to begin a program of domestication of the species.

**Keywords:** *Lippia integrifolia*; Verbenaceae; Aromatic and Medicinal Plant; Morphological variability; *In situ* characterization

**Resumen:** La especie *Lippia integrifolia* es un arbusto aromático, sub-leñoso, distribuido desde el noroeste y centro de Argentina hasta Bolivia. Es una de las especies aromáticas nativas más importantes. Presenta propiedades medicinales, algunas de las cuales han sido científicamente probadas. Como alternativa a la cosecha, se inició el proceso de domesticación de la especie. El objetivo de este trabajo fue evaluar la variabilidad fenotípica basada en descriptores botánico-taxonómicos y morfo-agronómicos y el uso de indicadores para evaluar su situación natural en el centro-oeste de Argentina. Trabajamos en cinco poblaciones silvestres, registrando variables morfológicas, fenología, regeneración natural, herviboría y presencia de enfermedades. *L. integrifolia* presenta una amplia variabilidad morfológica, que puede explicarse con tres descriptores morfológicos. La tasa de regeneración natural es muy baja a cero, en cuatro de los sitios. Los resultados obtenidos son importantes para tomar decisiones relacionadas con el uso sostenible in situ y para comenzar un programa de domesticación de la especie.

**Palabras clave:** *Lippia integrifolia*; Verbenaceae; Planta aromática y medicinal; Variabilidad morfológica; Caracterización *in situ*.

## INTRODUCTION

*Lippia integrifolia* is an aromatic, leafy shrub, popularly known as "incayuyo", "pulco", "poleo", "inca yerba", "tea of the Inca", "manzanilla" and "manzanillo", whose distribution extends from the Northwest and Central Argentina to Bolivia (Denham *et al*., 2006; Zuloaga *et al*., 2008; Barboza *et al.*, 2009). This slow-growing species generally reaches up to one meter in height and blooms from early summer (Bonzani *et al*., 2003; Barboza *et al*., 2006). The fruit of this species, included in a persistent calyx, is divided into two cluses that separate at maturity, the dorsal surface of which is convex and the commissural surface flat (Barboza *et al*., 2006).

The situation in its natural environment has been scarcely relieved. According to the Instituto Nacional de Tecnología Agropecuaria reports (2017), it is among the most aromatic native species of economic importance and that are subject to high extractive pressure in the Central and Northwest Argentine Region. In the Province of Córdoba, in the Department of Minas, for dozens of years the harvest of this species has been carried out by collectors in the area, this being the most important source of income in family economies, followed by livestock (Loyola, 2008).

In traditional medicine, the decoction of the leaves and flowers is used against dyspepsia, indigestion and stomach pain, gastro-logical, diuretic, emmenagogue, antibiotic (for gonorrhea infections), febrifuge, for the treatment of cough and as a sedative (Toursarkissian, 1980; Pochettino & Martinez, 1998; Rondina *et al*., 2003; Barboza *et al*., 2009).

Scientifically, the choleretic and antispasmodic activity of aqueous extracts has been tested in rats (Gorzalczany *et al*., 2008), which shows a relationship with the popular uses made for conditions of the gastrointestinal tract. Also the traditional use of aqueous extracts for gastric inflammation seems to be rationalized: in addition to the anti-inflammatory effects on stomach cells, the non-stick properties of the extracts were identified against the main inducer of gastritis, the bacterium *Helicobacter pylori* (Marcial *et al*., 2014). Reddy *et al*. (1999), tested the anti-inflammatory activity of africanene sesquiterpene: in acute inflammation in carrageenan-induced rat edema, a live oral dose of 10 mg / kg body weight of africanene resulted in a more potent reduction than that produced by 100 mg / kg body weight of ibuprofen. Its biocidal activity has also been reported for the species in *in vitro* tests on the protozoan *Trypanosoma cruci* (in its epimastigote form), which is the cause of Chagas disease (Sülsen *et al*., 2006), either using organic or aqueous extracts from the aerial. Furthermore, its antibacterial activity has been verified by means of chloroform extracts, on bacteria of medical interest such as *Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Coronel *et al*., 2003). Studies have been carried out using the essential oil, showing larvicidal activity on *Culex quinquefasciatus* mosquito larvae (Bonino, 2009). Its antioxidant activity and the absence of genotoxicity have been reported (Ricco *et al.*, 2010).

This species is also appreciated for its flavor and aroma, being in Argentina an ingredient in some aperitif drinks, teas and "yerbas compuestas" (Juliani *et al*., 2007). It is included in the Código Alimentario Argentino (2000).

As it is an economically exploited species, used in popular medicine and for the potential that its active principles present, it is important to advance in studies that allow its domestication for its subsequent cultivation.

Plants in their natural state have an evolutionary dynamic and are continuously producing variability. This variability has been used by man to identify, study and use plant species (Franco & Hidalgo, 2003).

The Food and Agriculture Organization of the United Nations (FAO) in its Second Global Plan of Action for plant genetic resources for food and agriculture (2012), states that its rational conservation (both *in situ* and *ex situ*) begin with the study and characterization of the resources that exist in each site.

In addition, the quality of medicinal and spice plants raw materials and their products has to fulfill the requirements of safety, efficacy and stability in order to be used in food and pharmaceutical industries. To meet these claims, the use of homogeneous genotypes with well-known and stable phytochemical and biological properties is an important prerequisite (WHO, 2002).

The exploration of germplasm consists of searching and collecting materials that ensure high genetic variability (Jaramillo & Baena, 2000). In the first place, it is necessary to differentiate populations: a group of plants of the same species among which there is gene flow, which inhabit a restricted area, with relatively homogeneous ecological conditions. The breeding of medicinal and aromatic plants is efficient and successful, after only few selection steps, by exploiting the high available natural

variability (Pank, 2007).

All the variability is stored in the genome of the members of the population that constitute the species, and may or may not be expressed in characteristics that allow identification. From the point of view of its expression, the variability contained in the genome of a species can be expressed in visible characteristics that make up the phenotype, or not, being in this case the processes or internal products of the plant. It is necessary to distinguish between what may or may not be expressed visually, in order to specify what portion of the total variability of the species is being analyzed in the characterization (Franco & Hidalgo, 2003).

In relation to the phenotype, the characters that make it up correspond mainly to the morphological description of the plant and its architecture. These characters are called morphological descriptors (Cubero, 2003; Franco & Hidalgo, 2003).

Morphologically it is very similar to the "poleo" *Lippia turbinata*. Grisebach (1874) describes *L. turbinata* var. Griseb *integrifolia*., characterized by its oblong-linear, obtuse leaves and entire margin. Subsequently, Hieronymus (1882) elevated the variety to the rank of species as *L. integrifolia*, an epithet that was considered valid to the present in various floristic treatments (Troncoso & Botta, 1993; Denham *et al*., 2006; Zuloaga *et al*., 2008). Denham *et al*. (2006), have reviewed specimens of *L. integrifolia* and *Lippia boliviana* and their varieties, concluding that there are no characters that allow a clear delimitation between both species and, based on the existence of specimens with intermediate characters, they consider *L. boliviana* and varieties as synonyms for *L. integrifolia*. This shows a wide morphological variability for *Lippia integrifolia*, which could be the result of interaction with the environment and / or be genetically determined. One problem in the taxonomic revisions arises because of the fact that sometimes the specimens in herbarium collections do not cover the whole range of variation. Therefore, studying of individuals from natural populations could be more sound. Unfortunately, this is not always possible when making the taxonomic revisions and in most cases the researcher relies on incomplete set of herbarium specimens (Aneva & Zhelev, 2019).

Therefore, to begin the process of domestication, the present´s work aim is the evaluation of the phenotypic variability of *L. integrifolia*, as from botanical-taxonomic and morpho-agronomic descriptors, in sites of spontaneous growth of the center of Argentina. As well as to address characteristics that indicates the status of wild populations. Knowing the range of variability of these characters is the basis to initiate a selection and improvement program, required since it is a species of high economic interest.

## MATERIALS AND METHODS

### *Plant material*

Five sites were determined, taking as a criterion that there were geographical distances between sites wide enough to decrease the possibilities of gametic exchange, and that they were heterogeneous in terms of soil and climate conditions. These sites correspond to the locality of Talampaya, Olta and Chelcos (Province of La Rioja), Nikizanga (Province of San Juan) and San Francisco del Chañar (Province of Córdoba), representing the distribution of *Lippia integrifolia* (Griseb.) Hieron. in central-western Argentina. According to the minimum sample size established by Brunetti *et al*. (2014), at each sampling site, 40 individuals were randomly scored, at least 3 meters apart. A representative sample of each study site was herborized to be properly identified and deposited in the herbarium of the Faculty of Agricultural Sciences, National University of Córdoba:

| **Population site** | **Voucher** |
|---|---|
| Talampaya | ACOR CS 1187-1 |
| Olta | ACOR CS 1680-1 |
| Nikizanga | ACOR CS 1680-3 |
| Chelcos | ACOR CS 1681-1 |
| San Francisco del Chañar | ACOR CS 1682-1 |

***Morphological studies***

The following morphological variables were recorded:

• Higher branch height (m.): Measurement of the length of the longest branch.

• Height of the shrub (m.): Measurement of the height of the canopy.

• Larger diameter (m.): Measurement of the major axis of the canopy.

• Minor diameter (m.): Measurement of the minor axis of the canopy.

• Numbers of primary branches: count of main branches.

• Form of growth (%): erect: when more than 50% of the branches grew vertically, semi-erect: when more than 50% of the branches did not grow vertically.

From each sampled individual, a branch was herbalized to evaluate the foliar characters using the Hoja software (Verga, 2010):

• Leaf length (cm): Measured from the base of the leaf to its apex, in samples of leaves of the 5 th and 10 th knots.

• Leaf width (cm): Measured perpendicular to the length of the leaf and considering its maximum magnitude, in samples of leaves of the 5 th and 10 th knots.

• Leaf area ($cm^2$): total area of the leaf, in samples of leaves of the 5 th and 10 th knots.

• Long/Width Ratio (L/A): relationship that describes the shape of the leaf obtained by the quotient between the length and the width of the leaf, in samples of leaves of the 5 th and 10 th knots.

The samplings were carried out between the end of April and the beginning of May (2010), at which time the plants of *L. integrifolia* were finishing their cycle of growth, both vegetative and reproductive, and therefore fully developed. In this sense it was registered:

• Phenological state: despite expecting to find the plants in full fruition, this variable was recorded to detect differences between populations. It was established a range of four categories: bud, flower, flower-fruit and fruit.

• Flower color: according to floristic treatises it can be white or pink-white (Barboza *et al.*, 2006).

***Indicators of the natural situation***

In order to know the sanitary situation in which these populations are found, it was recorded:

• Goats graze.

• Presence of diseases: record of any type of disease observed by plant.

To estimate the natural regeneration of the species, it was registered:

• Number of renewals: count of new plants up to 30 cm high, within a radius of one meter around each individual sampled.

***Data analysis***

For the data analysis, the Analysis of Variance (ANOVA) was used, a procedure that decompose the total variability in the sample (total sum of squares of the observations) into components (sums of squares) each associated with a recognized source of variation. The coefficient of variation (CV) was used, because besides being a relative parameter which defines the magnitude of the variability of the characters studied, facilitates comparison of the variability of a character into two or more groups of data (eg between populations), or between characters measured on the same group. To explore the intra and inter population variability and to know the main characteristics that explain it, the data were analyzed through a Principal Components Analysis (PCA). This analysis is a useful tool to analyze the data generated from the characterization and preliminary evaluation of germplasm and allows to know the existing relationship between the quantitative variables considered and the similarity between accessions (Franco & Hidalgo, 2003). In addition, the PCA allows to select the most discriminatory quantitative variables and which of them characterize in the same direction, and thus decrease the number of characters to be relieved in subsequent characterizations. Cluster analysis was used to group elements trying to achieve maximum homogeneity in each group and the greatest difference between groups. The Infostat software (Di Rienzo *et al.*, 2014) was used for all statistical procedures performed.

## RESULTS AND DISCUSSION

***Morphological studies***

Table No. 1 presents the summary measures for 13 morphological characters registered for the species, 12 continuous quantitative and one discontinuous quantitative. These characters describe the typical architecture of the canopy and the ranges of variation that each character can take, represented by the standard deviation (SD) and by the minimum and maximum values; in addition, the magnitude of the variability is described with the CV.

Botanical descriptions of this species (Barboza *et al*., 2006; Denham *et al*., 2006) describe it as a shrub wich size is between 30 cm and one

meter in height. From the data collected here in five populations, it is important to note that this range is higher, given that individuals exceeding two meters in height were recorded. This is not only important as a botanical description of the species, but also as an indicator of the potential in biomass production and morpho-agronomic descriptor performance indicator.

**Table No. 1**
**Descriptive statistics for 13 morphological characters of *Lippia integrifolia* "incayuyo" evaluated on wild plants (*)**

| Variable | n | Mean | SD | SE | CV | Min | Max |
|---|---|---|---|---|---|---|---|
| **Height of the shrub (m)** | 180 | 0,99 | 0,32 | 0,02 | 32,81 | 0,4 | 2,1 |
| **Higher branch height (m)** | 180 | 1,04 | 0,36 | 0,03 | 34,69 | 0,4 | 2,1 |
| **Larger diameter (m)** | 180 | 0,97 | 0,37 | 0,03 | 38,44 | 0,12 | 2,3 |
| **Minor diameter (m)** | 180 | 0,77 | 0,31 | 0,02 | 39,99 | 0,2 | 1,86 |
| **Nº of primary branches** | 180 | 15,53 | 6,23 | 0,46 | 40,09 | 1 | +20 |
| **5th leaf length (cm)** | 172 | 1,84 | 0,58 | 0,04 | 31,6 | 0,6 | 5,11 |
| **5th leaf width (cm)** | 172 | 0,4 | 0,12 | 0,01 | 29,92 | 0,17 | 0,95 |
| **L/A 5th leaf** | 172 | 4,78 | 1,28 | 0,1 | 26,88 | 2,18 | 9,47 |
| **5th leaf area ($cm^2$)** | 172 | 0,52 | 0,32 | 0,02 | 61,33 | 0,11 | 3,19 |
| **10th leaf length (cm)** | 170 | 2,66 | 0,87 | 0,07 | 32,59 | 1,08 | 6,44 |
| **10th leaf width (cm)** | 170 | 0,53 | 0,19 | 0,01 | 36,4 | 0,23 | 1,29 |
| **L/A 10th leaf** | 170 | 5,25 | 1,24 | 0,09 | 23,53 | 2,44 | 9,28 |
| **10th leaf area ($cm^2$)** | 170 | 1,03 | 0,72 | 0,05 | 69,42 | 0,22 | 5,73 |

**(*)The values shown correspond to a sample composed of 5 native populations of *L. integrifolia*, with 20 to 40 individuals per population**

The habit of growth in 56.67% of cases is erect, the remaining semi-erect, with some decumbent branches that do not exceed the height of the shrub, so the average of the longest branch variable is very close to the average of the height of the bush. This last variable, and therefore the shape of the individual, is closely related, on the one hand, to the degree of predation of the specimens, whether by browse of goats or harvesting, on the other hand with environmental conditions, as only individuals associated with humid and gloomy places presented a semi-erect bearing with decumbent branches.

The diameter of the canopy has an average size of 1 m per 77 cm, which indicates the size that an adult plant reaches in width, but it must be taken into account that values greater than 2 m in diameter were recorded. This information was necessary to design subsequent experimental trials, allowing to establish the plantation framework. The character number of primary branches did not present a normal distribution, being 20 or higher in most of the individuals evaluated.

The values for the foliar characters were included in the following intervals [length (cm) x width (cm) x area ($cm^2$)], for the fifth knot leaf: 0,6-5,11 x 0,17-0,95 x 0,11-3,19; for the tenth knot leaf: 1,08-6,44 x 0,23-1,29 x 0,22-5,73. The results obtained are greater than the sizes presented by other researchers (Denham *et al.*, 2006), what evidences the variability that can present this character of high agronomic interest. The leaf length/width ratio was considered as an indicator of the shape, the higher the value the leaf was lanceolated; on average, the values obtained were 4.78 for the 5 th leaf and 5.25 for the 10t h leaf.

The color of the corolla was always white in all the populations; no white-pink inflorescences were found, as could be according to their botanical description (Barboza *et al.*, 2006).

To analyze the magnitude of how much a character can vary, it is necessary to refer to the CV. Among the 13 morphological characters analyzed, two had very high CV, these correspond to the leaf area of the 5th and 10th leaves, with CV values of 61.33 and 69.42% respectively. These results support what was reported by Denham *et al.* (2006), where researchers consider *L. boliviana* and its varieties as synonyms of *L. integrifolia*, based on the existence of specimens with intermediate characters for leaf variables such as leaf shape, leaf margin, length and

width of the leaf. It is worth mentioning that the variability reported by these authors was evaluated on 40 herbarium specimens, which represented the entire distribution area - north-west of Argentina to Bolivia - and the entire range of observed morphological variability, while in this work the evaluations consisted of registering also the intrapopulation variability.

**Table No. 2A**
**Analysis of variance for 13 morphological characters of *Lippia integrifolia* "incayuyo" evaluated on wild plants, at five collection sites**

| | Talampaya | | Olta | | Nikizanga | |
|---|---|---|---|---|---|---|
| **Variable** | **Mean** | **CV** | **Mean** | **CV** | **Mean** | **CV** |
| **Height of the shrub (m)** | 0,92 a | 29,86 | 1,11 b | 23,05 | 0,87 a | 33,85 |
| **Higher branch height (m)** | 0,92 a | 29,86 | 1,31 b | 21,87 | 0,88 a | 36,96 |
| **Larger diameter (m)** | 0,82 a | 35,88 | 1,28 c | 31,98 | 0,72 a | 36,91 |
| **Minor diameter (m)** | 0,62 a | 38,68 | 0,99 b | 32,85 | 0,57 a | 40,01 |
| **Nº of primary branches** | 16,45 b | 39,73 | 14,15 a | 42,09 | 12,95 a | 53,99 |
| **5th leaf length (cm)** | 1,55 a | 47,81 | 1,98 b | 23,52 | 2,21 c | 20,29 |
| **5th leaf width (cm)** | 0,39 a | 33,91 | 0,40 a | 26,22 | 0,42 a | 28,48 |
| **L/A 5th leaf** | 3,99 a | 24,62 | 5,15 b | 22,33 | 5,60 b | 26,88 |
| **5th leaf area ($cm^2$)** | 0,47 a | 105,57 | 0,56 a | 43,16 | 0,63 a | 34,34 |
| **10th leaf length (cm.)** | 2,58 a | 42,79 | 2,66 a | 31,46 | 2,27 a | 25,81 |
| **10th leaf width (cm)** | 0,56 b | 36,62 | 0,50 a | 39,87 | 0,42 a | 31,84 |
| **L/A 10th leaf** | 4,61 a | 23,82 | 5,56 b | 20,71 | 5,53 b | 17,52 |
| **10th leaf area ($cm^2$)** | 1,12 b | 87,55 | 1,00 b | 69,46 | 0,69 a | 50,92 |

**Table No. 2B**

| | Chelcos | | Chañar | |
|---|---|---|---|---|
| **Variable** | **Mean** | **CV** | **Mean** | **CV** |
| **Height of the shrub (m)** | 0,93 a | 42,09 | 1,06 b | 31,46 |
| **Higher branch height (m)** | 0,94 a | 42,46 | 1,07 a | 31,76 |
| **Larger diameter (m)** | 0,79 a | 35,41 | 1,11 b | 22,90 |
| **Minor diameter (m)** | 0,63 a | 34,43 | 0,96 b | 23,78 |
| **Nº of primary branches** | 14,50 a | 44,61 | 18,33 b | 23,65 |
| **5th leaf length (cm)** | 1,85 b | 30,22 | 1,80 b | 25,53 |
| **5th leaf width (cm)** | 0,44 a | 26,74 | 0,36 a | 32,06 |
| **L/A 5th leaf** | 4,32 a | 22,79 | 5,25 b | 24,68 |
| **5th leaf area ($cm^2$)** | 0,56 a | 50,79 | 0,45 a | 51,25 |
| **10th leaf length (cm.)** | 3,00 b | 27,35 | 2,55 a | 26,84 |
| **10th leaf width (cm)** | 0,60 b | 30,88 | 0,48 a | 33,93 |
| **L/A 10th leaf** | 5,12 b | 18,99 | 5,59 b | 27,83 |
| **10th leaf area ($cm^2$)** | 1,29 b | 51,56 | 0,85 a | 55,77 |

**Different letters indicate significant differences ($p \leq 0.05$), DGC comparison test**

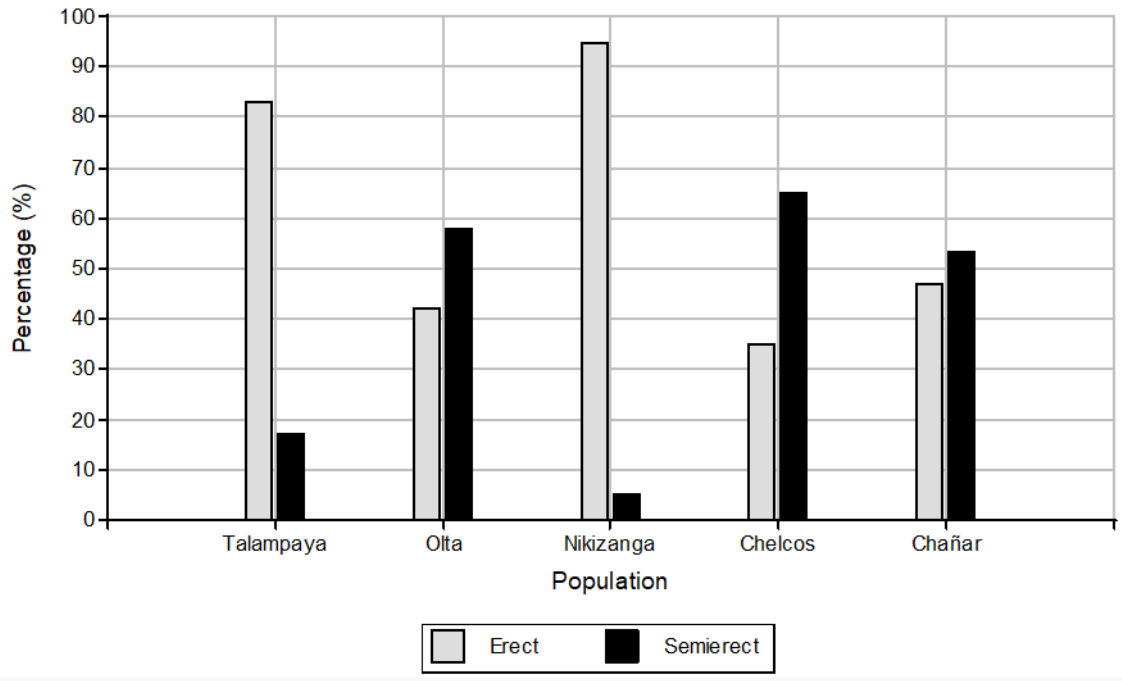
Table No. 2A and 2B presents the analysis of the variance for 13 morphological characters in 5 sampling sites of "incayuyo". If we analyze the behavior of the variables by population, not only allows us to observe which populations stand out with statistical significance for a certain character, it also reveals the populations in which a certain variable is very heterogeneous, given by a high CV.

Olta population was significantly higher in four of five variables related to the architecture of the plant - height of bush and major branch, major and minor diameter - followed by SF del Chañar that stood out in three of these variables - height of bush, smaller diameter and No. of primary branches. It should be mentioned that although Chelcos was not statistically superior in any of these 5 variables, it is in this population that individuals exceeding 2 meters in height were found, but since the range of expression of this variable in this population is so heterogeneous, the average does not stand out with the comparison test (DGC). However, it does stand out his CV being 42% for height of bush and major branch, reflecting the mentioned phenotypic heterogeneity of the architecture of the individuals.

For the variables associated with the leaflets, Chelcos was significantly higher in the four variables of the 10th node leaf, that is, this population has the largest leaves/surface. As for the 5th node lamina, Nikizanga stands out only for the length of the leaf, but no population was significantly superior in width or area. The foliar area in both studied knots, presents the highest CV of all the morphometric characters, in all the populations surveyed, which supports the variability reported for this character in "incayuyo", as mentioned previously. In this sense Talampaya was the most heterogeneous population for these two variables with CV of 105.57 and 87.55% for the area of the 5 th and 10 th knots respectively. For the L/A ratio, the population with less lanceolate leaves turned out to be Talampaya.

Regarding the predominant growth habit in each population, it is noteworthy that in Nikizanga it is mainly erect, followed by Talampaya, while in Chelcos it is mostly semierect. An intermediate situation is observed in the two remaining populations Olta and SF del Chañar (Figure No. 1).

**Figure No. 1**
**Growth habit of *Lippia integrifolia* in five wild populations**




The characterization of native phytogenetic resources of economic interest is, on the one hand, the beginning of a process of domestication and selection of the species. On the other hand, if exists the possibility of conserving material, it is a good instrument to plan the collection of germplasm; for which it is necessary to establish the representativeness of a collection and its relation to

the variability of the species in a region, or to the total variability of the species. Therefore, knowing which ones are the most heterogeneous "incayuyo" populations, allows us to decide in which site a greater effort should be made in the collection. Taking as criteria those variables whose CV exceeded 40%, Chelcos presented the highest intra-population variability for 5 characters, followed by Talampaya; while SF del Chañar was the least variable, since only the leaf surface presented a CV that exceeded 40%. This indicates that the intra-population variability observed in the species is greater in some sites, and this diversity of phenotypes could be due to different genotypes, or to a differentiated expression of the same genotype. Similar results have been found in other native populations of aromatic species of economic interest, such is the case of Hungarian populations of *Origanum vulgare*, evidencing a high degree of variability and the phenotypic response to habitat parameters (Cserháti *et al*., 2012); as well as *Aristotelia chilensis*, whose wide variability will enable to select the most suitable clones to increase production (Vogel *et al*., 2014). Leontaritou *et al*. (2020) propose that different types of morphological traits' responses to environmental parameters such as altitude, latitude and climatic type seem to be employed by different populations of Greek sage (*Salvia fruticosa* Mill.).

As it is exposed, this superior heterogeneity in some populations, at the level of morphological variables, could be due to a greater amplitude of genotypes in relation to others. For this, future studies should focus on the analysis of variability at the level of molecular markers to reinforce this hypothesis.

Figure No. 2 presents the Principal Component Analysis (PCA) performed with all morphometric characters (except for the number of primary branches, as it did not present a normal distribution), where 60% of the variability is explained by the first 2 axes or main components. The first main component explains 34.7% of the variability and the characters that contribute the most are width and area of the leaf of the 5th knot, width and area of the leaf of the 10th knot. In the second main component (25.5%) the variables that contribute the most are bush height and major branch, larger and minor canopy diameter.

From the PCA obtained, those more discriminative quantitative variables were selected, and in this way it was possible to reduce the number of characters to be analyzed in future characterizations. In this sense, others researchers (Ojeda, 2011; Chaves et al., 2014), in population studies of native species have been able to reduce the number of more discriminating variables to characterize material for subsequent selection. With this criterion, considering the value of the eigenvectors and the projection / sense in the graph, the descriptive variables selected were: the area of the 10th leaf, height of the largest branch and largest diameter of the canopy.

To determine the phenotypic similarity between populations a Cluster Analysis (Figure No. 3) was performed using average linkage algorithm with Euclidean distances between data previously standardized, considering as variables those selected from the PCA: area of the 10th leaf, height of the largest branch and largest diameter of the canopy.

From the obtained dendrogram it was possible to differentiate two groups, the first groups Olta and SF del Chañar; the second to Nikizanga together with Talampaya and Chelcos, these last two populations forming a subgroup as well. This grouping is not related to the geographical location of the populations. It could be considered that the observed phenotypes respond to environmental characteristics or to a genetic composition that has no relation with geographical distances. In the same way, it has been reported for the species Mentha cervinia, that the observed trend of morphological variation seems not to be associated with the inter-population distance. These researchers propose that the morphological variation pattern, linked with the shot plan height and stem length, suggests adaptation to the contrasting climatic conditions. Radusiene and Bagdonaite (2014) state that it can be expected that the differences observed among populations have a genetic character, as the populations from different habitats have indicated great similarity for the medicinal plant Hypericum perforatum, pointing the importance of investigations and selection of populations under uniform conditions in the field.

Another aspect of interest to initiate a process of domestication and management of the species is to know its phenology, particularly in aromatic species where the optimum time of harvest is usually in full bloom. Between the end of April and the beginning of May, when the samplings were carried out, the plants were in full fruition in Talampaya, Nikizanga and SF del Chañar; or they presented an intermediate state between flowering and fruiting, as in Olta and Chelcos (Figure No. 4).

**Figure No. 2**
**Principal Component Analysis for 12 morphometric characters evaluated on five native populations of *Lippia integrifolia***

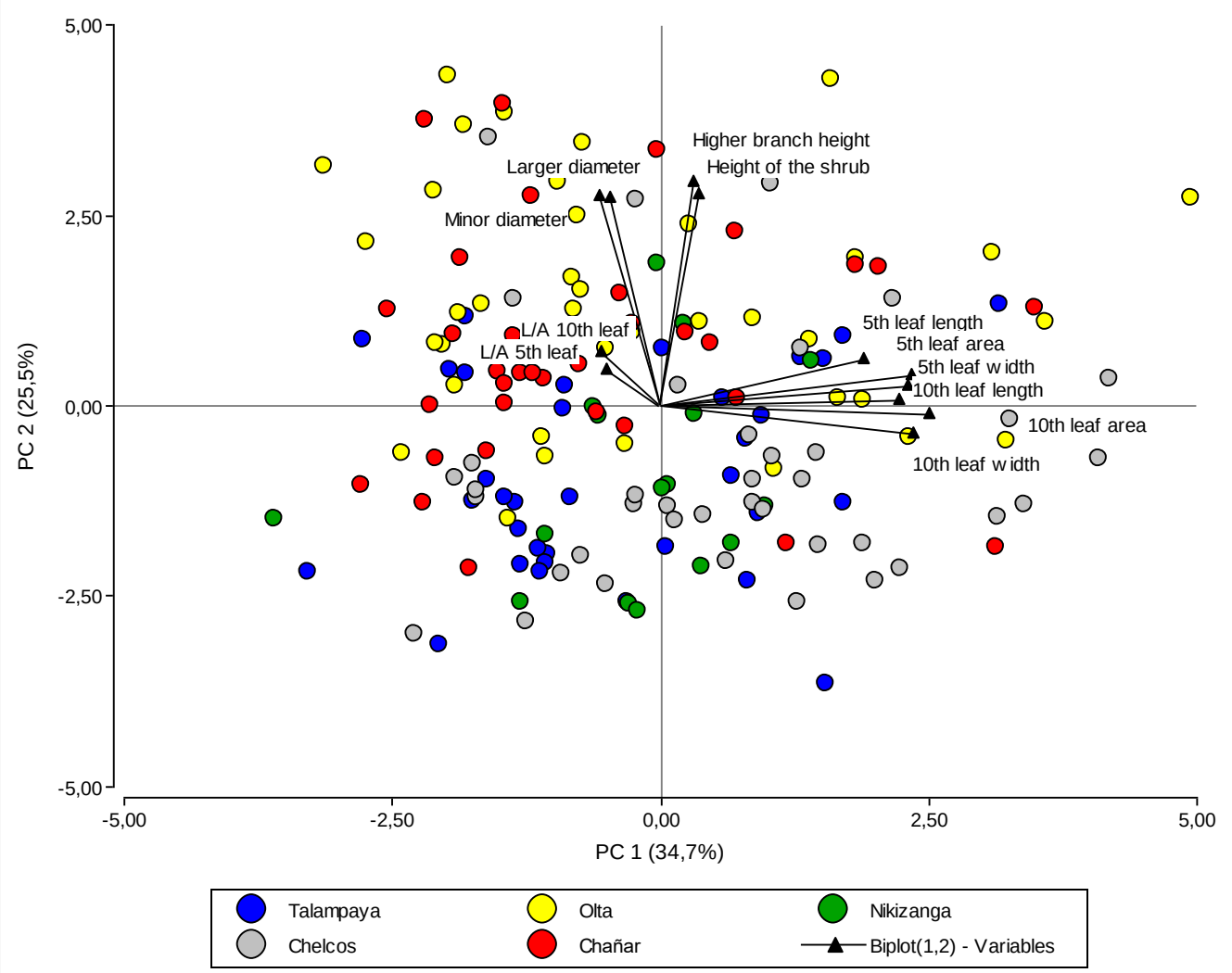


**Figure No. 3**
**Dendrogram of clustering of five native populations of *Lippia integrifolia* from the center-west of Argentina, generated from morphometric descriptive characters**

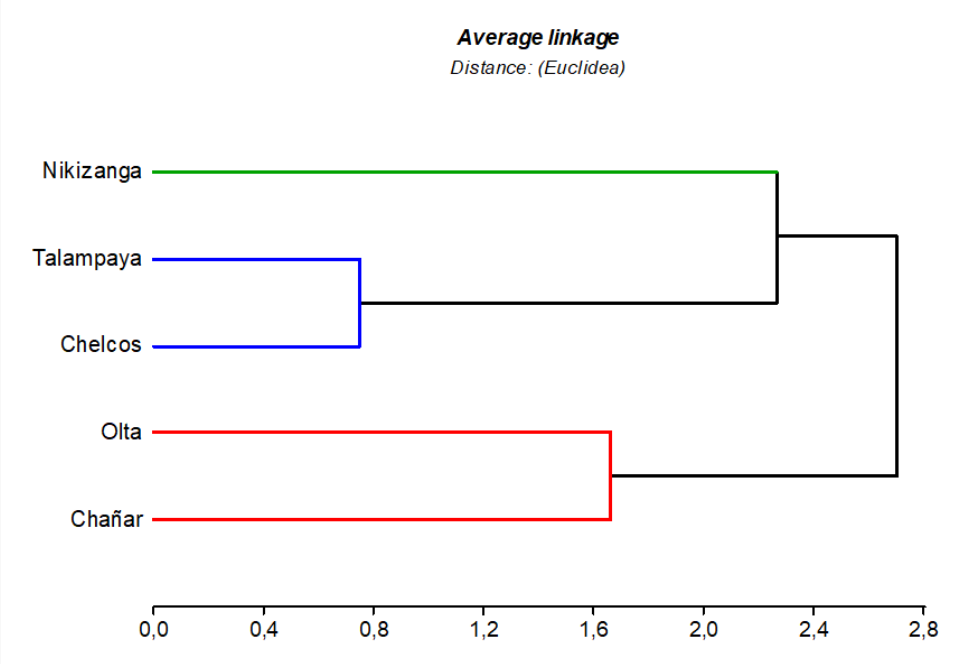
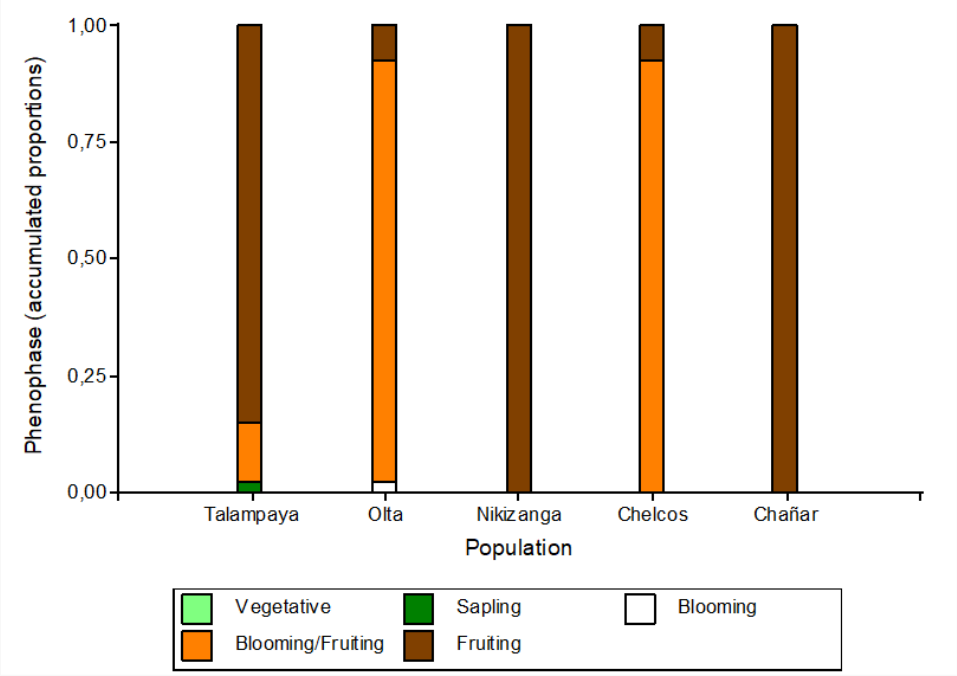



**Figure No. 4**
**Phenological stage of specimens of *Lippia integrifolia* between the end of April and the beginning of May, in five native populations of the center-west of Argentina**



It is important to consider the phenology of the species to achieve a sustainable use of germplasm *in situ*, in order to ensure the perpetuity of the resource, especially for those who find economic support in harvesting "incayuyo". Taking into account that this species blooms since early summer and completes its fruition towards the month of May, it would be appropriate that this activity was not carried out before the month of December, to allow the plants to complete their cycle with flowering and to produce fruits. Because it is a species that completes fruiting late, it would be advisable to leave a certain number of main branches without harvesting for each individual, so that the seeds achieve maturity and can be disseminated, avoiding damaging the self-regeneration of the plants and reducing the impact of genetic erosion.

***Approach to its natural situation***

There is no background for "incayuyo" about natural regeneration in environments where it grows spontaneously. This information is substantially important, given that it is an indicator that allows us to know if a population is generating new individuals, which will replace those older specimens. One way to approach this information is to track young individuals in each site. From the observations it is evident that young individuals are associated with adult plants of "incayuyo", in some cases with other species present such as "jarilla" (*Larrea divaricata* Cav.), "espinillo" (*Vachellia caven* (Mol.) Seigler & Ebinger.) and "garabato" (*Senegalia praecox* (Griseb.) Seigler & Ebinger.). Any of these species could be found fulfilling the role of nurse plant for new seedlings, providing shading and protection against high insolations common in these regions, and even against herbivory, when they are associated with spiny species. In this aspect it is necessary to make more specific studies, to advance in the knowledge of the ecology of the species.

Table No. 3 shows the average values of renewables, counted in relation to each adult plant of "incayuyo" sampled. The results show very different situations among the populations studied. In first place Talampaya is a population circumscribed within the Talampaya National Park, so its situation is of closure, it is not economically exploited, nor is there presence of animals that would put the new plants at risk. This corresponds to what was recorded, given that the largest number of renewals was found on this site, with an average of between 6-7 renewals per adult sampled. It should be noted that 66% of the renewals sampled in Talampaya were concentrated around five adult plants, for which 20 to 50 new plants were registered per individual. Regarding goat browsing, there was no evidence of it (Figure No. 5a), and about half of the plants showed signs of water stress among this population (Figure No. 5b).

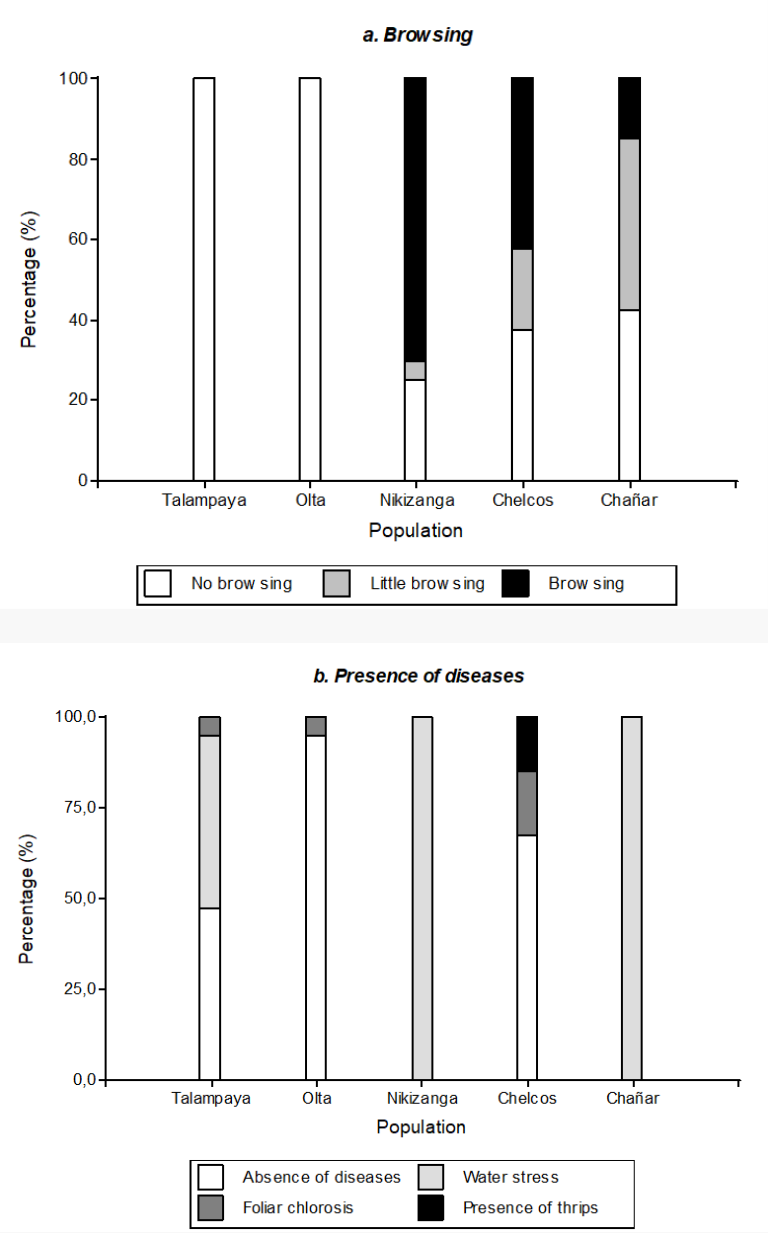
**Table No. 3**
**Summary measures for the variable number of renewals of *Lippia integrifolia*, registered by each adult individual, in five sites of spontaneous growth of the center-west of Argentina**

| Population | Adult plants | Average renewals/adult plant | Minimum | Maximum | Total of renovales |
|---|---|---|---|---|---|
| **Talampaya** | 40 | 6,65 | 0 | 51 | 266 |
| **Olta** | 40 | 0,33 | 0 | 3 | 13 |
| **Nikizanga** | 20 | 0 | 0 | 0 | 0 |
| **Chelcos** | 40 | 0,9 | 0 | 5 | 36 |
| **Chañar** | 40 | 0 | 0 | 0 | 0 |

Chelcos is the second population with the largest number of renewables, although considerably lower than the previous one. Only one new plant on average for each adult plant. Its sanitary situation is also different, more than 60% of the specimens showed signs of browse, 17.5% had chlorotic leaves and it was the only site where thrips were observed on “incayuyo” plants, which must be considered as a possible pest if the plant is brought into cultivation.

The situation of Olta is even more complicated, in relation to the 40 adult specimens only 13 new individuals were registered in total, despite the fact that the population did not show signs of browse, nor did any sick plants (only 5% of plants with some degree of chlorosis). As seen in later studies, the cause of this low natural regeneration could be linked to a low percentage of germination of the seeds collected at this site.

**Figure No. 5**
**Browsing and presence of diseases registered in *Lippia integrifolia* individuals, in five spontaneous growth sites in central-western Argentina. a) Browsing, b) Presence of diseases**




Nikizanga and SF Chañar are the two most compromised populations. No new plants of "incayuyo" associated with adult plants was recorded, nor with other accompanying species. In both populations there is evidence of goat browsing, being 75% in Nikizanga and 57% in SF Chañar. In addition, both populations showed signs of water stress in 100% of individuals.

## CONCLUSIONS

*L. integrifolia* presents a wide morphological variability, which can be explained with three morphological descriptors, such as the height of the longest branch, the largest diameter of the canopy and the leaf area of the 10th node leaf.

There are populations whose heterogeneity at the level of morphological markers is higher, for

which a greater effort of preservation *in situ* and / or *ex situ* should be made, with the aim of conserving a germplasm sample sufficiently representative of the observed variability.

The rate of natural regeneration is very low in four of the sites studied. Browsing of goats is evident, as it is a palatable species consumed in times of scarcity of food. Some populations show a high water stress, low rainfall associated with the xerophytic environments where this species inhabits.

Therefore, the results presented are substantial for making decisions related to the sustainable use of the species *in situ*, and to begin a program of domestication of this species.

**ACKNOWLEDGEMENTS**

The authors want to thank Professor Ulf Ola Karlin for his advice and support in the study of native plant species, sharing his vast and invaluable expertise in this area of knowledge. This study was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas Argentina and by the Secretaría de Ciencia y Técnica de la Universidad Nacional de Córdoba, Argentina.

**REFERENCES**

Aneva I, Zhelev P. 2019. Morphometric studies of *Sideritis scardica* Grsb. and *S. syriaca* L. in their natural populations in Bulgaria. **Bol Latinoam Caribe Plant Med Aromat** 18: 71 - 80.

Barboza GE, Cantero JJ, Nuñez CO, Ariza Espinar L. 2006. **Flora medicinal de la Provincia de Córdoba**. Ed. Museo Botánico, Universidad Nacional de Córdoba, Córdoba, Argentina.

Barboza GE, Cantero JJ, Nuñez CO, Pacciaroni A, Ariza Espinar L. 2009. Medicinal plants: A general review and a phytochemical and ethnopharmacological screening of the native Argentine Flora. **Kurtziana**. 34: 7 - 365.

Bonino M. 2009. **Bioactividad larvicida de aceites esenciales en mosquitos (Diptera: Culicidae).** Thesis, Universidad Nacional de Córdoba, Córdoba, Argentina.

Bonzani N, Filippa E, Barboza GE. 2003. Estudio Anatómico comparativo de tallo de algunas especies de Verbenaceae. **Anal Inst Biol** (Serie Botánica) 74: 31 - 45.

Brunetti PC, Bruno C, Zapata R, Torres L, Barboza GE, Ojeda M. 2014. Estimación del tamaño muestral para estudios de variabilidad morfológica *in situ* de *Lippia integrifolia* (Verbenaceae) en el Parque Nacional Talampaya, La Rioja (Argentina). **Bonplandia** 23: 15 - 23. **https://doi.org/10.30972/bon.2311234**

Chaves AG, Brunetti PC, Massuh Y, Ocaño SF, Torres LE, Ojeda MS. 2014. Variabilidad entre poblaciones silvestres *de Baccharis crispa* Spreng. de la Provincia de Córdoba, Argentina. **Phyton** 83: 4 - 11. **https://doi.org/10.32604/phyton.2014.83.145**

**Código Alimentario Argentino**. 2000. Ediciones La Rocca, Buenos Aires, Argentina.

Coronel A, Vega M, Lampasona M, Catalán C. 2003. Antibacterial activity of two *Lippia* species. **Biocell** 27: 111.

Cserhati B, Juhos K, Begyik A, Radasci P, Nemeth E, Szabo K. 2012. *In situ* morphological variability of wild marjoram (*Origanum vulgare* L.) populations in Hungary. **Acta Alimentaria** 41: 12 - 23. **https://doi.org/10.1556/aalim.41.2012.suppl.2**

Cubero J. 2003. **Introducción a la mejora genética vegetal**. Ed. Mundi-Prensa. Madrid, España.

Denham S, Múlgura de Romero M, Slanis A, Bulacio E. 2006. *Lippia integrifolia* versus *L boliviana* (Verbenaceae). **Darwiniana** 44: 363 - 374.

Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW. 2014. **InfoStat versión 2014**. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Córdoba, Argentina. **http://www.infostat.com.ar**

FAO. 2012. [Food and Agriculture Organization]. Second global plan of action, for plant genetic resources for food and agriculture. **http://www.fao.org/3/i2624e/i2624e00.pdf**

Franco TL, Hidalgo R. 2003. **Análisis estadístico de datos de caracterización morfológica de recursos fitogenéticos.** Instituto Internacional de Recursos Fitogenéticos, Cali, Colombia.

Gorzalczany S, Sülsen V, Redko F, Vescina C, Muschietti L, Martino V, Acevedo C. 2008. Choleretic and antispasmodic effects of *Lippia integrifolia* aqueous extract. **Braz J Pharmacogn** 18: 16 - 20. **https://doi.org/10.1590/s0102-695x2008000100005**

Grisebach A. 1874. Plantae Lorentziaae. **Abh Königi Ges Wiss Götyingen** 19: 196.

Hieronymus G. 1882. Plantae diaphoricae florae argentinae. **Bol Acad Nac Cienc Córdoba** 4: 199 - 531.

INTA. 2017. [Instituto Nacional de Tecnología Agropecuaria]. **Situación actual del cultivo de plantas aromáticas y medicinales en Argentina**. **Estación Experimental San Pedro.** **https://repositorio.inta.gob.ar/handle/20.500.12123/1837**

Jaramillo S, Baena M. 2000. **Material de apoyo a la capacitación en conservación *ex situ* de recursos fitogenéticos**. Instituto Internacional de Recursos Fitogenéticos, Cali, Colombia.

Juliani H R, Biurrun F, Koroch A, De Carli A, Zygadlo J. 2007. **The production of native and exotic herbs, medicinal and aromatic plants in Argentina.** In: Janick J, Whipkey A. (Eds.). Issues in new crops and new uses. ASHS Press, Alexandria, VA, USA.

Leontaritoua P, Lamari F, Papasotiropoulos V, Iatroua G. 2020. Morphological, genetic and essential oil variation of Greek sage (*Salvia fruticosa* Mill.) populations from Greece. **Ind Crops Prod** 150: 112346. **https://doi.org/10.1016/j.indcrop.2020.112346**

Loyola M. 2008. **Sustentabilidad de los recursos aromáticos medicinales: Percepciones de los pequeños productores recolectores de *Lippia integrifolia* (Griseb.) Hieron. "Incayuyo" del Depto. Minas, Provincia de Córdoba**. Thesis, Universidad Nacional de La Rioja, La Rioja, Argentina.

Marcial G, Sendker J, Brandt S, Perotti de Lampasona M, Catalán C, Font de Valdez G, Hensel A. 2014. Gastroprotection as an example: Antiadhesion against *Helicobacter pylori*, anti-inflammatory and antioxidant activities of aqueous extracts from the aerial parts of *Lippia integrifolia* Hieron. **J Ethnopharmacol** 155: 1125 - 1133. **https://doi.org/10.1016/j.jep.2014.06.039**

Ojeda MS. 2011. **La Peperina. Caracterización de poblaciones y avances en la domesticación**. Editorial Académica Española, Córdoba, Argentina.

Pank F. 2007. Use of breeding to customize characteristics of medicinal and aromatic plants to postharvest processing requirements. **Steward Postharvest Rev** 4: 1 - 9. **https://doi.org/10.2212/spr.2007.4.1**

Pochettino M, Martinez M. 1998. **Aporte al conocimiento actual de las plantas medicinales en Argentina: estudio etnobotánico en el Depto. Molinos, provincia Salta, Argentina**. En: Amat A. (coord.) Farmacobotánica y Farmacognosia en Argentina. 1980-1998. Ediciones Culturales Argentinas. La Plata, Argentina.

Radusiene J, Bagdonaite E. 2014. Phenotypic variation in *Hypericum perforatum* L. and *H. maculatum* Crantz Wild populations in Lithuania. **J Herbs Spices Med Plants** 9: 345 - 351. **https://doi.org/10.1300/j044v09n04_12**

Reddy BS, Rao V, Rao B, Dhananjaya N, Kuttan R, Babu TD. 1999. Isolation and structural determination of new sphingolipids and pharmacological activity of africanene and other metabolites from *Sinularia leptoclados*. **Chem Pharm Bull** 47: 1214 - 1220. **https://doi.org/10.1248/cpb.47.1214**

Ricco R, Wagner M, Portmann E, Reides C, Llesuy S, Gurnia A, Carballo M. 2010. Análisis de polifenoles, actividad antioxidante y genotoxicidad en especies argentinas de *Lippia* y *Aloysia* (Verbenaceae). **Bol Latinoam Caribe Plant Med Aromat** 9: 388 - 396.

Rondina R, Bandoni A, Coussio J. 2003. **Plantas silvestres argentinas con reconocidas propiedades medicinales o tóxicas.** OEA-Cyted: Buenos Aires, Argentina.

Sülsen V, Güida C, Coussio J, Paveto C, Muschietti L, Martino V. 2006. *In vitro* evaluation of trypanocidal activity in plants used in Argentine tradiotional medicine. **Parasitol Res** 98: 370 - 374. **https://doi.org/10.1007/s00436-005-0060-4**

Toursarkissian M. 1980. **Plantas Medicinales de la Argentina.** Ed. H. Sur, Buenos Aires, Argentina.

Troncoso N, Botta S. 1993. **Verbenaceae.** In: Flora de la Provincia de Jujuy. República Argentina. Parte IX. Verbenaceas a Caliceraceas. Colección Científica del INTA. Ed. Estudio Sigma SRL, Buenos Aires, Argentina.

Verga A. 2010. **Programa HOJA**. Instituto de Fisiología y Recursos Genéticos Vegetales, Centro de Investigaciones Agropecuarias, Instituto Nacional de Tecnología Agropecuaria. Cordoba, Argentina.

Vogel H, Peñailillo P, Doll Contreras G, Catenacci G, González B. 2014. Maqui (*Aristotelia chilensis*): Morpho-phenological characterizatioon to design high-yielding cultivation techniques. **J Appl Res Med Arom Plants** 1: 123 - 133. **https://doi.org/10.1016/j.jarmap.2014.09.001**

WHO, 2002. [World Health Organization]. The World Health Report. Reducing Risks, Promoting Healthy Life. Switzerland.

Zuloaga F, Morrone O, Belgrano M. 2008. Verbenacea. **Monogr Syst Bot Missouri Bot Gard** 107: 3151 - 3168.