Heterogeneity of linalool chemotypes of *Lippia alba* (Mill.) N.E.Br., based on clonal half-sib progenies

Marcos Ribeiro Bottignon[1], Elcio Rodrigo Rufino[1], Márcia Ortiz Mayo Marques[1], Carlos Augusto Colombo[1], Joaquim Adelino de Azevedo Filho[2], André Luiz Lourenção[3], Antônio Lúcio Mello Martins[4], Walter José Siqueira[1]*

[1]*IAC/Centro de Pesquisa e Desenvolvimento de Recursos Genéticos Vegetais, C.P. 28 – 13020-902 – Campinas, SP – Brasil.*
[2]*APTA/Polo Regional Leste Paulista, C.P. 01 – 13910-000 – Monte Alegre do Sul, SP – Brasil.*
[3]*IAC/Centro de Pesquisa e Desenvolvimento de Fitossanidade, C.P. 28 – 13020-902 – Campinas, SP – Brasil.*
[4]*APTA/Polo Regional Centro Norte, C.P. 24 – 15830-000 – Pindorama, SP – Brasil.*
**Corresponding author <walterjs@iac.sp.gov.br>*
Edited by: Cláudio Lopes de Souza Jr. / Antonio Costa de Oliveira

ABSTRACT: *Lippia alba* (Mill.) N.E.Br. is an aromatic and medicinal shrub native to the American continent. Despite its potential as a source of essential oil for the pharmaceutical and cosmetics industries, few selection and genetic improvement studies have been carried out. The aim of this study was to provide genetic information on this species for breeding programs, showing its selection potential, by investigating clonal half-sib progenies. The following characteristics were evaluated per plant: leaf dry mass (LDM), total dry mass (TDM), leaf yield (LY), essential oil yield (EOY) and oil production (OP). Estimates were made for the several genetic parameters including absolute genetic gain at 30% selection intensity, correlations and relative contribution of additive and environmental effects to phenotypic correlation. Two experimental trials on 30 progenies were conducted: one in Campinas, state of São Paulo (SP), Brazil, with two harvests of the aerial part, and one in Monte Alegre do Sul, SP, Brazil, with only one harvest. The trials were conducted in a randomized block design consisting of subplots with three replications, each plot (progeny) consisting of 8 to 15 clonally-replicated plants with subplot harvesting. Variations were detected between progenies and harvests, as well as progeny/harvest interactions in the split plot experiment. High heritability and genetic gains were obtained at both sites for LDM, TDM and OP. The lowest variations among progenies were obtained for LY and EOY, highlighting selection problems. Negative additive genetic correlations were obtained for EOY × LDM, EOY × TDM, LY × TDM and LY × LDM. Selection for LDM resulted in increased oil production per plant (OP), even where there was a negative correlation between LDM × EOY.
Keywords: genetic gain, breeding, essential oils

Introduction

Lippia alba (Mill.) N.E.Br. (Verbenaceae) is a perennial shrub native to the tropical and subtropical regions of the American continent. It is a non-domesticated, outcrossing species propagated by cuttings. Because of its high essential oil content, this plant is considered to be aromatic and medicinal. Brazilian exports of plant essential oils are increasing. Citrus, eucalyptus and rosewood (*Aniba rosaeodora*) are the major oils exported, amounting to U$$ 2.29 million per year. Phytotherapeutics account for 6% of the pharmaceuticals market, with sales reaching U$$ 235.29 million per year (De la Cruz, 2005).

Several applications of *L. alba* essential oils are reported in the literature, mainly in the chemical, pharmaceutical and cosmetics industries (Barata and May, 2004; Ibrahim et al., 2001; Kishore and Mishra, 1991; Lorenzo et al., 2001; Maia et al., 2004; Rao et al., 2000; Viana et al., 2000; Zetola et al., 2002). Production of secondary metabolites is the result of complex interactions in the biosynthesis, translocation, storage and degradation of chemical compounds (Wink, 1990). Each of these processes is determined by genes and influenced by genotype, ontogenetics and environment (Harbone, 1977). However, studies carried out to date on this species rely on few genotypes, and selection or genetic breeding of *L. alba* has not yet been trialed.

Estimating genetic parameters for breeding programs is a way of initiating the selection of superior genotypes based on the population genetic structure. Determining heritability estimates provides a basis for designing selection strategies and predicting genetic gains (Fehr, 1987). The aim of this study was to evaluate the potential for selecting a base population of *L. alba*. This involved estimating parameters for clonal half-sib progenies.

Materials and Methods

We first obtained a recombinant population (or base population) from the aggregate fruits of eight *L. alba* (linalool chemotype) genotypes (IAC-1 to IAC-8) studied by Yamamoto et al. (2008), who studied 20 clones representative of five chemotypes: linalool (eight genotypes), myrcene/camphor (three genotypes), limonene/carvone (four genotypes), citral (four genotypes) and myrcene (one genotype). Since the species is outcrossing and self-incompatible, these chemotypes randomly recombined. Fruits of *L. alba* are schizocarpic, round, dry and indehiscent, measuring about 3.0 mm and composed of two mericarps, each

containing a seed of around 0.4 mm (Yamamoto et al., 2008). Since the seeds are very small, delicate and remain inside the mericarps, obtaining large quantities is not viable. Thus, for practical reasons, the fruits are planted rather than the seeds (Yamamoto, 2008). A total of 8,000 fruits were planted. Germination was irregular and slow, producing 296 seedlings after five months. Seedlings were planted in 30-L pots and kept in the shade during initial development. They were then transferred to their final positions to form the base population. In this population, 88 plants segregating to the linalool chemotype were identified by smell. Sixty-seven of them fruited to form the half-sib progenies of two recombination cycles. The fruits were planted and again germination was irregular and slow, so that few of them produced satisfactory number of seedlings (> 30). Subsequent phases of this study were carried out exclusively on the linalool chemotypes of each half-sib progeny.

Of the 67 linalool chemotype progenies identified in the base population, 30 yielded between 8 and 15 plants identified by smell as linalool chemotypes. Since this number is insufficient for studying progenies, a cloning approach was chosen, taking cuttings from each of the plants of the half-sib progenies to produce a collection of progenies.

After four months, standardized cuttings (length ~ 18 cm, diameter ~ 2.0 cm) with two buds were collected from each plant in the collection of clonal half-sib progenies. The cuttings were therefore half-siblings of each other, maintaining the genetic variability of the progenies. Thus, 30 half-sib progenies, each consisting of 8 to 15 linalool plants, were used to calculate mean values for estimating genetic parameters and to quantify the genetic variability available for selection.

Two trials were carried out on the 30 progenies. The first was set up in Campinas, characterized by Clayey Typic Endoaquent soil and tropical warm climate (22°54' S; 47°05' W, elevation 674 m). The second trial was set up in Monte Alegre do Sul, SP. The site is characterized by Clayey Typic Hapludox(22°43' S; 46°37' W, elevation 820 m).

Plants were spaced at 1.0 m × 0.50 m and irrigated by tricklers (2.4 L h^{-1}) in Campinas and by sprinklers in Monte Alegre do Sul. In both trials, plants were supported to prevent stem contact with the soil, which could stimulate unwanted root development and create clumps of mixed genotypes and increasing experimental error. Dead plants were replaced while the cuttings were bedding in. A randomized block experimental design with three replications in a split plot arrangement was adopted, a separate plot for each progeny, and harvesting subplots. Each plot (progeny) consisted of 8 to 15 plants. The second harvest was conducted when the plants in the plot rebudded. In Campinas, the first harvesting period for plant aerial parts was 14 to 16 July 2008 and the second, 13 and 15 October 2008. In Monte Alegre do Sul, aerial parts were harvested only once on 11 November 2008.

After harvesting, leaves were separated from stems and both dried at room temperature until they reached constant weight. Data were analyzed to provide mean for plants in the plot. The total dry mass of leaves and stems for each progeny was measured. Values were divided by the number of plants per progeny to obtain leaf dry mass per plant (LDM), the total (stems + leaves) dry mass per plant (TDM) and leaf yield (LY) determined as a percentage (LDM / TDM × 100).

The essential oil from the dry leaves was extracted through hydrodistillation in a Clevenger-type apparatus for 1.5 h. After separating the water from the oil, the essential oil yield was calculated as EOY (%) = (MO / LDM_{EXT}) × 100, where MO is the mass of extracted oil in grams, and LDM_{EXT} is the total mass of the leaves used for the oil extraction.

Genetic parameters were estimated based on the expectation of mean squares (EMS) obtained from two Analyses of Variance (ANOVAs): as the experimental split plot with two harvests in Campinas and in random blocks for the single-harvest experiment in Monte Alegre do Sul.

The mathematical model used in Campinas was: $Y_{ijk} = m + b_i + p_j + (bp)_{ij} + h_k + (ph)_{jk} + \varepsilon_{ijk}$, where m is the general mean; b_i is the effetct of the i^{th} block; p_j is the effect of the j^{th} progeny (plot); $(bp)_{ij}$ is the effect of the ij^{th} interaction of the block and progeny (error a); h_k is the effect of the k^{th} harvest (sub plot); $(ph)_{jk}$ is the effect of the jk^{th} interaction of the progeny and harvest (error b); ε_{ijk} is the effect of the random error. The data were obtained with average of progenies in mixed model where blocks and harvest were fixed and progenies were random (Miranda Filho, 1978). Once significant, the interaction for harvesting and progeny was extracted from the variance of progeny to obtain more realistic estimates of heritability and genetic gains.

The mathematical model used in Monte Alegre do Sul was: $Y_{ijk} = m + b_i + p_j + \varepsilon_{ijk}$, where m is the general mean; b_i is the effetct of the i^{th} block; p_j is the effect of the j^{th} progeny; ε_{ijk} is the effect of the random error. The data were obtained with average of progenies in mixed model, where blocks were fixed and progenies were random (Miranda Filho, 1978). The expectations of mean squares E(MS) obtained from two ANOVAs as the experimental split plot with two harvests in Campinas and in random blocks for the single-harvest experiment in Monte Alegre do Sul were in Table 1.

Data were analyzed using SANEST software (Machado and Zonta, 1995). Treatments (plot) consisted of the average for 30 progenies from the base population. The following genetic parameters were estimated: progeny genetic variance (σ^2_P), environmental variance (σ^2_E), genetic variation coefficient ($CV_{G\%}$), environmental variation coefficient ($CV_{E\%}$), narrow-sense heritability ($h^2_{r\%}$), b-value, absolute genetic gain (G_S) and relative genetic gain ($G_{S\%}$). The equations used in the ANOVAs and ANCOVAs (Analysis of Covariance) on progeny mean values, based on the components of the respective expected values of mean square E(MS) and mean product E(MP), are in accordance with Vencovsky (1978). A selection intensity of 30% was used to estimate the genetic gains, and the equation is taken from Cruz (2005) ($GS_{abs} = p.h_r.s_P.k_{30\%}$), with selection in both sexes ($p = 1$). Phenotypic ($r_{F\%}$), additive genetic ($r_{A\%}$) and environmental ($r_{E\%}$) correlations were calculated, taking average of the three harvests in

Table 1 – Expected values of mean square E(MS) in the analysis of variance for the split plot experiment in Campinas and randomized blocks in Monte Alegre do Sul.

Source	DF	E(MS)
Split Plot (Campinas)		
Block (b)	b - 1 = 2	$\sigma_e^2 + ph\sigma_b^2$
Progeny (p)	p - 1 = 29	$\sigma_e^2 + h\sigma_{bp}^2 + b^*h\sigma_p^2$
Error (a)	(b - 1)(p - 1) = 58	$\sigma_e^2 + h\sigma_{bp}^2$
Harvest (h)	h - 1 = 1	$\sigma_e^2 + b^*\sigma_{ph}^2 + b^*p\sigma_h^2$
Progeny × Harvest (pxc)	(p - 1)(h - 1) = 29	$\sigma_e^2 + b^*\sigma_{ph}^2$
Error (b)	p(b - 1)(h - 1) = 60	σ_e^2
Randomized blocks (Monte Alegre do Sul)		
Block (b)	b - 1 = 2	$\sigma_e^2 + p\sigma_b^2$
Progeny (p)	p - 1 = 29	$\sigma_e^2 + b\sigma_p^2$
Error	(b - 1)(p - 1) = 58	σ_e^2

DF: Degrees of freedom; E(MS): Expected values of mean square; p, h, b: number of progeny, harvest and blocks; σ_b^2: Variance of the blocks; σ_{bp}^2: Variance of the interaction blocks × progeny; σ_p^2: Variance of the progeny; σ_{ph}^2: Variance of the interaction progeny × harvest; σ_h^2: Variance of the harvest; σ_e^2: Variance of the error. For the source harvest (effect fixed), the value of the coeficient b* = b . (p/p - 1) ~b.

a set analysis for greater scope and robustness. Correlation magnitudes were analyzed based on the criterion proposed by Shimakura and Ribeiro Júnior (2009).

Since the environmental correlations were high, the relative contribution made by the additive genetic component and other non-controlled environmental or residual effects to the phenotypic correlation was calculated. The equation was proposed by Siqueira et al. (1993) based on the phenotypic correlation ($r_{F\%}$) equation in Falconer (1987):

$$r_{F(xy)} = \underbrace{\sqrt{h^2_{(x)} \cdot h^2_{(y)}} \cdot r_{A(xy)}}_{G} + \underbrace{\sqrt{(1-h^2_{(x)})(1-h^2_{(y)})} \cdot r_{E(xy)}}_{E}$$

where: $r_{F(xy)}$ = coefficient of phenotypic correlation between two characteristics (x and y); $r_{A(xy)}$ = coefficient of genetic aditive correlation; $r_{E(xy)}$ = coefficient of environmental correlation between two characteristics (x and y); $h^2_{(x)}$ and $h^2_{(y)}$ = narrow-sense heritability of x and y characteristics, respectively; G/ $r_{F(xy)}$ .100 = additive genetic component as a percentage contribution to the phenotypic correlation; E/ $r_{F(xy)}$ .100 = environmental or residual component as a percentage contribution to the phenotypic correlation.

Results and Discussion

The harvest in Campinas had the lowest mean values for all evaluated characteristics (except OP), while the Monte Alegre do Sul harvest had the highest (Table 2). In Monte Alegre do Sul, the superiority of the harvest by comparison with the Campinas site can be explained by the better soil and climate conditions in the region. Yamamoto et al. (2008) also observed that clones were superior when grown on this site.

Leaf yield (LY) showed the highest uniformity among the harvests at both sites, ranging from 36.8% (Monte Alegre do Sul) to 39.5% (Campinas). TDM yield per day during the period from experimental set-up to harvesting was 0.65 g on average for Campinas (over a period of 144.4 days) and 0.81 g for Monte Alegre do Sul (221 days), based on progeny mean values. Similarly, the mean for the two Campinas harvests was lower than the Monte Alegre do Sul mean value. Since the oil is mostly produced in the leaves of *L. alba* (Correa, 1992), it is predictable that oil yield will be higher for plants with greater leaf mass per unit planted area. Environmental (season, soil and climate) and genetic factors, together with the way they interact, could explain the phenotypic variations observed in the progenies. Oil yield varies according to plant age, harvesting season and interactions between these factors (Innecco et al., 2003; Santos and Innecco, 2004).

With the exception of LY, there were differences among the progenies in both sites. The results of the ANOVAs for split plots (Campinas) revealed differences ($p \leq 0.01$) among progenies (plots) and harvests (subplots) and harvest/progeny interaction. These significant interactions observed in the ANOVAs affected the magnitude of progeny genetic variance (σ^2_p), reducing the estimated parameter values. This suggests that, given the characteristics studied and the experimental conditions for the two harvests, the parameter estimates should take account of interaction effects in determining progeny variances. Estimated genetic parameters in cross-fertilizing plant populations based on half-sib progenies are used as input information for designing breeding programs and allow the genetic potential for recurrent or intravarietal selection over several cycles to be evaluated (Paterniani and Miranda Filho, 1978; Ramalho et al., 2004; Vencovsky, 1978).

Apart from LY and EOY, all characteristics showed high genetic variation coefficients (Table 2) at both sites (24.4 to 40.4.0%). The potential for LY variation is limited, since it requires shortening the internodes, resulting in smaller leaves. Rufino et al. (2010), when studying 62 experimental clones of *L. alba* (linalool chemotype), found only two clones with shortened internodes and no significant reduction in leaf size.

Table 2 – Genetic parameter estimates for clonal half-sib progenies based on the average values in a split plot arrangement (Campinas, SP).

Means and variances	LDM	TDM	LY	EOY	OP
	g		%		g
M	34.2254	92.3421	39.5375	61.5160	22.3420
σ_F^2	552.5174	5916.3469	93.0855	445.7236	270.6454
QM_{BxP} (Error a)	93.3134	823.9756	9.2193	72.5322	43.2349
$r\sigma^2_{HxP}$	41.6417	848.8502	3.0706	9.3892	52.3852
σ^2_P	69.5937	707.2535	13.4659	60.6337	29.1709
σ^2_E (Error b)	35.0758	304.2276	5.2829	95.1824	29.6437
Parameters					
$CV_{G\%}$	24.3745	28.7997	9.2813	12.6581	24.1742
$CV_{E\%}$	17.3043	18.8886	5.8134	15.8595	24.3694
b	1.4086	1.5247	1.5965	0.7981	0.9920
$h^2_{r\%}$	75.5745	71.7254	86.7972	81.6206	64.6696
GS	8.4054	26.1040	3.9624	8.1534	5.0339
$G_{S\%}$	24.5588	28.2683	10.0218	13.2542	22.5313

M: Mean; σ_F^2: Phenotypic variance; QM_{BxP} (error a): Mean squares of block x progeny interaction; $r\sigma^2_{HxP}$: Harvest × progeny Interaction; σ_P^2: progeny variance; σ_E^2 : Environmental variance = error b of ANOVA; $CV_{G\%}$: Genetic coeffient variation among half-sib progenies; $CV_{E\%}$: Environmental variation coefficient; h_r^2(%): narrow-sense heritability; b: Relationship $CV_{G\%}$ / $CV_{E\%}$; G_S: absolute gain under 30% of selection intensity; $G_{S\%}$: selection gain related to the mean; LDM: dry mass of leaves per plant; TDM: total dry mass per plant; LY: leaves yield; EOY: oil yield; OP: oil production per plant.

EOY had the lowest genetic variability at both sites, although variability was higher than for LY. During essential oil extraction, better experimental control is possible since Clevenger hydrodistillation procedures are well established, resulting in less residual variance σ^2_E ($CV_{E\%}$ between 15.9 and 16.3%). This contributed to a higher expression of the additive genetic effects. Due to the greater magnitudes of additive genetic variation in relation to environmental or experimental error-induced magnitudes observed at both sites, the b-values obtained were higher than 1.0 (except LY and EOY for Monte Alegre do Sul and EOY for Campinas), favoring selection even in a small population. This can be explained by the fact that *L. alba* is a cross-fertilizing, non-domesticated, rustic species, maintaining loci in heterozygosis, which is the norm for plant propagation by cuttings. Nevertheless, genetic improvement of this species in Brazil is still in its early stages (Biasi and Costa, 2003; Corrêa, 1992; Rufino et al., 2010).

Higher heritabilities were observed, mainly in Monte Alegre do Sul, except LY and EOY when compared to Campinas allowing higher genetic gains per cycle. This means that it is possible to increase essential oil production by selecting individuals with higher leaf mass or total mass. Since oil production is dependent on two characteristics, essential oil yield (EOY) and leaf dry mass (LDM), any increment in one of these characteristics would result in increased OP. The conclusion is that there is sufficient additive genetic variance in the progenies and therefore in the base population to allow superior individuals to be identified based on their LDM, TDM and OP characteristics. In Monte Alegre do Sul, the least favorable selection characteristic was LY (Table 3), which did not produce any genetic gains. Studies of leaf biomass production and harvesting periodicity could play an important role in verifying the stabilizing point for these characteristics under different environmental conditions. The variance of the evaluated characteristics (phenotype) is affected not only by genetic components, but also by the progeny interaction with environmental conditions, which could lead to overestimation of the variance components (Dudley and Moll, 1969).

Genetic variation coefficients were high for LDM, TDM and OP, resulting in b-values close to 1.0, showing that they are favorable for selection, even though high $CV_{E\%}$ values were observed at this site. No genetic variance was detected for percentage leaf yield under the experimental conditions at Monte Alegre do Sul. At the Campinas site, this characteristic exhibited the lowest $CV_{G\%}$, showing how difficult it is to select for this characteristic.

High heritability values, and therefore genetic gains, were observed in the experiments at both Campinas and Monte Alegre do Sul, showing the potential of *L. alba* for genetic improvement. Genetic gains of more than 22.5% were observed for LDM, TDM and OP and lower gains (10.0 to 13.2%) for LY and EOY in Campinas. Based on the Monte Alegre do Sul experiment, estimates for genetic gain were even higher (39 a 42.1%) for LDM, TDM and OP, suggesting that it would be viable to select half-sib progeny for increased linalool essential oil production. It also shows that there is no drop in genetic variability when a small number of progenies is studied (only 30 progenies in this study).

Observed high level of genetic variability in this species, which were also detected by Manica-Cattani et al. (2009) that studied 27 genotypes of *L. alba* through molecular markers

Table 3 – Genetic parameters estimated among clonal half-sib progenies in Monte Alegre do Sul, SP (one harvest).

Means and variances	LDM	TDM	LY	EOY	OP
	---------- g ----------		---------- % ----------		g
M	34.2254	92.3421	39.5375	61.5160	22.3420
σ^2_F	552.5174	5916.3469	93.0855	445.7236	270.6454
σ^2_P	93.3134	823.9756	9.2193	72.5322	43.2349
σ^2_E	41.6417	848.8502	3.0706	9.3892	52.3852
Parameters	69.5937	707.2535	13.4659	60.6337	29.1709
$CV_{G\%}$	35.0758	304.2276	5.2829	95.1824	29.6437
$CV_{E\%}$					
b	24.3745	28.7997	9.2813	12.6581	24.1742
$h_r^2\%$	17.3043	18.8886	5.8134	15.8595	24.3694
GS	1.4086	1.5247	1.5965	0.7981	0.9920
$G_{S\%}$	75.5745	71.7254	86.7972	81.6206	64.6696

M - Mean; σ^2_P – Genotypic variance; σ^2_E – Environmental variance; $CV_{G\%}$ - Genetic variation coefficient for half-sib progenies; $CV_{E\%}$ - Environmental variation coefficient; h_r^2(%) – Narrow-sense heritability; b – Relationship $CV_{G\%}$ / $CV_{E\%}$; G_S – Absolute gain at 30% selection intensity; $G_{S\%}$ - Selection gain related to the mean; LDM: leaf dry mass per plant; TDM: total dry mass per plant; LY: leaf yield; EOY: essential oil yield; OP: oil production per plant.

characterizing these materials according to genetic similarity and chemical composition of the accessions. Similarly, Jannuzzi et al. (2010) evaluated 16 accessions of *L. alba* and showed the existence of five distinct chemotypes, in addition to high variability in other traits.

To make correlation estimates more robust, all progenies for all three harvests were taken into account in the ANOVAs (Table 4). Since low values (significant for the T-test) are usually obtained in correlation studies, we used the classification criteria proposed by Shimakura and Ribeiro Júnior (2009). Significant but low correlation values were obtained by Yamamoto et al. (2008) in *L. alba*.

LDM and TDM characteristics were strongly correlated ($r_{A\%}$ = 98.9%), implying that selection can be based on leaf biomass or total biomass production. For the purpose of comparing different studies, essential oil is usually extracted from dry leaves (without stems, flowers, etc) to obtain yield figures (EOY), dividing the leaf mass subjected to hydrodistillation by the mass of oil obtained.

Previous study with *L. alba* carried out by Yamamoto et al. (2008) with 20 clones and by Rufino et al. (2010) with 65 clones, revealed a high correlation between wet and dry mass (96 – 99%) in several environments. Our study was therefore based on dry mass only. Apart from LDM × TDM, other positive or directly proportional additive genetic correlations were LDM × OP (92.7%: very strong), TDM × OP (91.0%: very strong) and LY × EOY (12.3%: very weak). Correlation between two characters indicates that the relationship between them is linear (Cruz, 2005). This information is important for breeding programs, suggesting the possibility of genetic gains in total oil yield based on indirect selection of plants with higher wet or dry mass so that fewer samples need to be analyzed. If we know how different characteristics are correlated, we can select for the desired characteristic based on the correlated characteristic with the highest heritability (less prone to environmental effects) and which is easier to evaluate (Cruz, 2005; Vencovsky, 1978).

Table 4 – Estimates of additive genetic correlation ($r_{A\%}$), phenotypic correlation ($r_{F\%}$) and environmental correlation ($r_{E\%}$), based on the average of 30 clonal half-sib progenies.

Trait		LDM	LY	EOY	OP
TDM	$r_{F\%}$	98.4	- 18.1	- 15.0	90.4
	$r_{A\%}$	98.9	- 36.4	- 18.1	91.0
	$r_{E\%}$	94.1	16.2	- 16.2	86.8
OP	$r_{F\%}$	92.6	- 11.3	20.5	
	$r_{A\%}$	92.7	- 28.3	18.5	
	$r_{E\%}$	92.0	20.2	33.2	
EOY	$r_{F\%}$	- 14.8	9.9		
	$r_{A\%}$	- 17.9	12.3		
	$r_{E\%}$	- 4.2	9.0		
LY	$r_{F\%}$	-12.1			
	$r_{A\%}$	- 28.8			
	$r_{E\%}$	20.7			

LDM: dry mass of leaves per plant; TDM: total dry mass per plant; LY: leaves yield; EOY: oil yield; OP: oil production per plant; $r_{A\%}$: additive genetic correlation; $r_{F\%}$: phenotypic correlation; $r_{E\%}$: environmental correlation.

Genetic correlations can reflect the mechanism of pleiotropic gene action, i.e. the degree of correlation reflects the influence of a given gene on different phenotypic traits (Falconer, 1987). Negative additive correlations were observed for LDM × LY (- 28.8%: weak), LDM × EOY and TDM × EOY (- 17.9%; - 18.1%: very weak), TDM × LY (- 36.4%: weak). The correlation between LDM × LY shows that selecting for dry leaf biomass causes a drop in leaf yield, implying that an increase in LY would affect biomass produc-

tion, which is not desirable. This can be partially explained by the fact that the only way to obtain higher leaf yield is by shortening the internodes, resulting in smaller leaves. Yamamoto et al. (2008) observed limonene/carvone chemotype clones with small leaf size and plant habit, but this was caused by a virus infection. The same authors also observed that plants with larger leaves also have longer internodes.

Environmental forces can lead to deviations from the expected phenotype, resulting positive or negative correlations (Falconer, 1987 and Ramalho et al., 2004). Cruz (2005) stated that character correlation can be a result of pleiotropic gene action or gene linkage. If genes lack strong linkage, the correlation can change in advanced generations because gene clusters are broken down by crossovers (Cruz, 2005).

We observed significant variation in environmental correlation estimates for the combinations of characteristics, as follows - positive or negative and very weak (EOY × LY: 9.0%); weak (EOY × OPP: 33.2%); strong (TDM × OPP: 86.8%); very strong (LDM × OPP: 92.0%; LDM × TDM: 94.1%). Three of the environmental correlations were negative, and weak or very weak. Intra-progeny environment variations in both traits may explain the environmental correlation observed and the presence of desigual number of individual plants by progeny (8 a 15). Siqueira et al. (1993), working with 102 carrot half-sib progenies, observed high intra-progeny environmental or residual in the ANOVAs. Based on Falconer (1987), Siqueira et al. (1993) proposed a way of proportionally quantifying the contribution of the (additive) genetic and environmental correlations to the phenotypic correlation (see equations in Material and Methods).

In the present study, taking only the very strong phenotypic correlations (LDM × TDM, LDM × OPP and TDM × OPP), the percentage additive genetic effects ($G_{\%}$) in the phenotypic correlation varied from 81.97 to 90.10%, while environmental effects accounted for 9.90 to 18.03% (Table 5). This shows that additive genetic effects played a more prominent role than environmental effects in determining of the phenotypic correlation, as observed by Siqueira et al. (1993) and Rufino et al. (2010).

Table 5 – Estimates of the contributions (%) of additive genetic (G%) and environmental (E%) components to the phenotypic correlation ($r_{F\%}$).

Trait(*)	$G_{\%}$	$E_{\%}$	$r_{F\%}$
LDM × OP	83.22	16.78	92.6
LDM × TDM	90.10	9.90	98.4
TDM × OP	81.97	18.03	90.4

*: Only for the very strong and strong phenotypic correlations $G_{\%}$ = contribution of the additive genetic component to the phenotypic correlation. $E_{\%}$ = contribution of the environmental component to the phenotypic correlation. $r_{F\%}$ = coefficient of phenotypic correlation; LDM: dry mass of leaves per plant; TDM: total dry mass per plant; OP: oil production per plant.

Conclusions

The experimental progenies have sufficient genetic variability to allow genetic improvement and selection. Although breeding programs for the species have never been set up (no selection pressure), the genetic parameter estimates are high. The progenies exhibited higher mean values for total dry mass and leaf dry mass, suggesting that inter- and intra-progeny selection for these traits is a viable proposition. Mass selection for LDM and TDM is possible to achieve gains in the total oil production. LY had lower genetic variability in the progenies studied and is therefore not well-suited to breeding programs. Despite very strong, strong and moderate environmental correlations, the environmental contribution to phenotype characteristics is low. Additive genetic effects were more significant in almost all the combinations studied.

Acknowledgments

To CNPq, Universal edict, process number 479932/2006-9, for financial support.

References

Barata, L.E.S.; May, P. 2004. Rosewood exploitation on Brazilian Amazon: options for sustainable production. Economic Botany 58: 257-265.

Biasi, L.A.; Costa, G. 2003. Vegetative propagation of *Lippia alba*. Ciência Rural 33: 455-459 (in Portuguese, with abstract in English).

Corrêa, C.B.V. 1992. Contribution to the study of *Lippia alba* (Mill.) N. E. Br. ex Britt & Wilson – lemon grass. Revista Brasileira de Farmacognosia 73: 57-64 (in Portuguese, with abstract in English).

Cruz, C.D. 2005. Principles of Quantitative Genetics. 22ed. Universidade Federal de Viçosa, Viçosa, MG, Brazil (in Portuguese).

De la Cruz, M.G. 2005. Access to herbal and medicinal plants and social inclusion: situational diagnostic pharmaceutical supply chain in the state of Mato Grosso. Available at: http://www.esalq.usp.br/siesalq/pm/diagnostico_situacional.pdf [Accessed Feb. 10, 2009].

Dudley, J.W.; Moll, R.H. 1969. Interpretation and use of estimates of heritability and genetic variances in plant breeding. Crop Science 9: 257-262.

Falconer, D.S. 1987. Introduction to Quantitative Genetics. Editora Universidade Federal de Viçosa, Viçosa, MG, Brazil (in Portuguese).

Fehr, W.R. 1987. Principles of Cultivar Development: Theory and Technique. 2ed. Macmillan, New York, NY, USA.

Harbone, J.B. 1977. The plant and its biochemical adaptation to the environment. p. 1-27. In: Introduction to ecological biochemistry. Academic Press, London, UK.

Ibrahim, M.A.; Kainulainen, P.; Aflatuni, A.; Tiilikkala, K.; Holopainen, J.K. 2001. Insecticidal, repellent, antimicrobial activity and phytotoxicity of essential oils: with special reference to limonene and its suitability for control of insect pests. Agricultural and Food Science in Finland 10: 243-259.

Innecco, R.; Cruz, G.F.; Vieira, A.V.; Mattos, S.H.; Chaves, F.C.M. 2003. Spacing, cut time and harvest number in *Mentha x villosa* Huds. Revista Ciência Agronômica 34: 247-251 (in Portuguese, with abstract in English).

Jannuzzi, H; Mattos, J.K.A.; Vieira, R.F.; Silva, D.B.; BIzzo, H.R.; Gracindo, L.A.M. 2010. Agronomic evaluation and identification of *Lippia alba* chemotypes from Distrito Federal, Brazil. Horticultura Brasileira 28: 412-417 (in Portuguese, with abstract in English).

Kishore, N.; Mishra, A.K. 1991. Effect of essential oils on sclerential germination of *Rhizoctonia solani*. National Academy Science Letters 14: 239-240.

Lorenzo, D.; Paz, D.; Davies, P.; Vilar, R.; Canigueral, S.; Delacassa, E. 2001. Composition of a new essential oil type of *Lippia alba* (Mill) N. E. Brown from Uruguay. Flavour and Fragrance Journal 16: 356-359.

Machado, A.A.; Zonta, E.P. 1995. Manual of SANEST: Statistical Analysis System for Microcomputers. Universidade Federal de Pelotas, Pelotas, RS, Brazil (in Portuguese).

Maia, N.B., Bovi, O.A.; Perecin, M.B.; Marques, M.O.M.; Granja, N.P. 2004. News crops with potential to produce essential oil with high linalool content helping preserved rosewood: an endangered Amazon species. Acta Horticulturae 629: 39-43.

Manica-Cattani, M.F.; Zacaria, J.; Pauletti, G.; Atti-Serafini, L.; Echeverrigaray, S. 2009. Genetc variation among South Brazilian acessions of *Lippia alba* Mill. (Verbenaceae) detect by ISSR and RAPD markers. Brazilian Journal Biology 69: 375-380.

Miranda Filho, J.B. 1978. Breeding populations. p. 620-650. In: Paterniani, E.; Viegas, G.P. Improvement and production of maize in Brazil, ed. Fundação Cargill, São Paulo, SP, Brazil (in Portuguese).

Paterniani, E.; Miranda Filho, J.B. 1978. Breeding populations. p. 202-256. In: Paterniani, E.; Viegas, G.P. Improvement and production of maize in Brazil. ed. Fundação Cargill, São Paulo, SP, Brazil (in Portuguese).

Ramalho, M.A.P.; Santos, J.B.; Pinto, C.A.B.P. 2004. Genetics in Agriculture. 3ed. Universidade Federal de Lavras, Lavras, MG, Brazil (in Portuguese).

Rao, G.P.; Singh, M.; Singh, P.; Singh, S.P.; Catalan, C.; Kapoor, I.P.S.; Singh, O.P.; Singh, G. 2000. Studies on chemical constituents and antifungal activity of leaf essential oil of *Lippia alba* (Mill.). Indian Journal of Chemical Technology 7: 332-335.

Rufino, E.R.; Siqueira, W.J.; Marques, M.O.M; Colombo, C.A.; Chiorato, A.F.; Azevedo Filho, J.A; Lourenção, A.L. Yamamoto, P.Y.; Martins, A.L.M. 2010. Genetic parameter estimates of three traits related to vigor of cuttings of *Lippia alba*. Bragantia: 69:779-785 (in Portuguese, with abstract in English).

Santos, M.R.A.; Innecco, R. 2004. Organic fertilization and cutting heigth of *Lippia alba*. Horticultura Brasileira 22: 182-185 (in Portuguese, with abstract in English).

Shimakura, S.E.; Ribeiro Júnior, P.J. 2007. Statistics. In: Descriptive statistics: interpretation of the correlation coefficient. Statistics Department, UFPR. Chapter 8. Available at: http://leg.ufpr.br/~paulojus/CE003/ce003/node8.html [Accessed Jan. 30, 2009] (in Portuguese).

Siqueira, W.J.; Illg, R.D.; Fornasier, J.B.; Granja, N.P.; Lisbão, R.S.; Santos, R.R. 1993. Phenotypic, additive genetic and environmental correlations in carrots. Bragantia 52: 17-26 (in Portuguese, with abstract in English).

Vencovsky, R. 1978. Quantitative inheritance. p. 122-199. In: Paterniani, E.; Viegas, G.P., ed. Improvement and production of maize in Brazil. Fundação Cargill, São Paulo, SP, Brazil (in Portuguese).

Viana, G.S.; Vale, T.G.; Silva, C.M.; Abreu-Matos, F.J. 2000. Anticonvulsant activity of essential oils and active principles from chemotypes of *Lippia alba* (Mill.) N.E. Brown. Biological & Pharmaceutical. Bulletin 23: 1314-1317.

Yamamoto, P.Y.; Colombo, C.A.; Azevedo Filho, J.A.; Lourenção, A.L.; Marques, M.O.M.; Morais, G.D.S.; Chiorato, A.F., Martins, A.L.M.; Siqueira, W.J. 2008. Performance of ginger grass (*Lippia alba*) for traits related to the production of essential oil. Scientia Agricola 65: 481-489.

Wink, M. 1990. Physiology of secondary product formation in plants. p. 23-41. In: Charlwood, B.V.; Rhodes, M.JC., eds. Secondary products from plant tissue culture Clarendon Press, Oxford, UK.

Zetola, M.; Lima, T.C.M.; Sonaglio, D.; Gonzalez, O.G.; Limberger, R.P.; Petrovick, P. R.; Bassani, V. L. 2002. CNS activities of liquid and spray-dried extracts from *Lippia alba*- Verbenaceae (Brazilian false melissa). Journal of Ethnopharmacology 82: 207-215.

Received June 25, 2009
Accepted April 08, 2011