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Methodology for the initial screening tests of *Lippia multiflora* Moldenke: Necessary number of repetition of the same genotype for the estimation of heritability in the broad sense

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Abstract

The aim of this work was to determine the repetition of vegetative material that was necessary to evaluate some agronomic quantitative traits and genetics parameters of *Lippia multiflora*. Two hundred fourty accessions of *Lippia multiflora* were used. Each accession was fragmented into two parts and was transplanted in the experimental farm (1 m x 0, 8m). About one year after, some quantitative traits were taken. Then parameters like correlation, covariance, error- type and broad sense heritability were graphiquilly analysed according to the number of the individuals represented the same accession in the farm. It was noticed that the number of vegetative material varied according to the traits submitted. But the analysis also revealed that a more precision of the narrow heritability and a best reduction of the area effect could be obtained generally by using a number between 3 or 5 of individuals of the same accession, vegetative material or "clone". Indeed that number could express about 60 % of that heritability and about 70 % of the error-type diminution. In over side the analysis demonstrated that the traits like (NFNE) and (TEMF) were strongly heritable (h^2_{sl} = 0, 77 and h^2_{sl} = 0, 95). The study showed that *Lippia multiflora* obtained from stump regeneration needs a period of 235± 4 and 281 ± 2, 73 days before producing flowers.

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Introduction

Lippia multiflora is savanna plant (Bouquet, 1967; Bouquet, 1969; Adjanahoun, 1983 Demissew, 1993) widely known and esteemed by tropical communities (Gautier, 1992; FAO, 1996) because of its food uses, medicinal and its delicate scent (Adjanohoun et al., 1988; Herzog, 1992; Valentin et al., 1995; Abena *et al*., 2003). Nowadays, it occupies an important place in the economic activities (Spore, 2007; and Yao N'Guessan-Kouamé, 2010) and scientific (Yao-Kouamé *et a*l, 2008, Yao-Kouamé *et a*l., 2009; Amyaw 2009) in West Africa. It's agro-morphological characterization revealed the existence of different populations based on the accession's provenance (Adou *et al*, 2011). Chemists of various origins (Menut *et al.,* 2005) showed the existence of several chemotypes of this plant. Kanko *et al* (1990) have shown that the composition of the essential oil extracted from the leaves also varies according to their place of collection in Côte d'Ivoire. However, it is well known that the extension of plant material with high productivity and an acceptable level of performance for a good domestication demand a lot of technical knowledge and equipment. That is why the search for information by testing for the determination of a suitable methodology for the evaluation of the selection criteria remains an important agenda for *Lippia multiflora* still considered as wild heritage. However if we consider the fact that species may include genetic heterogeneity, one of the necessary conditions to fulfill, to carry out this study is how to deal with each accession within this species. Indeed, if the experiments are conducted on every single strain taken at random in nature without repetitions, it will be difficult and even impossible to distinguish the part of genetic heterogeneity or variability due to individuals within species which is related to study environment (Gallais, 1990). This is why; in the objective of present work this study was determine the number of individuals vegetative repetition from of the same accession, necessary for plot accurate to evaluate genetic parameters useful for *Lippia multiflora* varietal selection.

Material and methods

Plant materials

The plant material evaluated consists of 240 plants collected in Côte d'Ivoire since the "V Baoulé" to the north of Côte d'Ivoire, from Bondoukou to Touba. These vegetative material of *Lippia multiflora* was transplanted (Amyaw, 2009) in Dimbokro. Each strain collected at random in nature has been fragmented (Margarat 1986, Hamilton, 2002) in two and transplanted into the experimental plot. And observations focused on accessions genotypes repeated (Gallais, 1990 Baradat 1986, Harlan, 1995). The units of observation in this study thus were composed by the combination of the two individuals representing the same accession. Analyses were made on their harmonic mean (n = 240).

Method

The study took place in a town Dimbokro (6 ° 70 N, 4 ° 70W) in east-central Côte d'Ivoire. The area is a transition between forest and savanna. It is characterized by woody vegetation; baouléen climate and rainfall annual average is 1000 mm. Temperatures range between 22 ° C and 37 ° C. The region is a vast plateau at an altitude between 200 m and 500 m.

Experimental device

The experimental plot were Planted in completely randomized single tree divided into two blocks, with a border line overall with 2 repetitions of each accession. The lines were separated from each other by a distance of 1 m with 0.8 m between adjacent bushes on the same line. This device should be particularly shrubs in general, because it allows better discrimination of objects under test (Lotodé and Lachenaud 1988; Charmetant and Leroy, 1990) and especially to estimate the value of genetics and heredity in the broad sense of characters from phenotypic values (Kremer, 1986; Gallais, 1990). The plants were until flower formation (Heller et al. 1993), were followed individually, and were identified in the field by their position on the line in the block, and serial number on the line. The elementary block has 40 rows of 6 trees.

Agronomics traits

Agronomic traits of 240 accessions were observed and measured on adult plant of *Lippia multiflora*. Activities focused on quantitative traits following:

Height (HPLT) expressed in centimeters from the neck up to the top (or termination of the inflorescence) of the plant. Number of primary branches (NBRP) counting the number of branches present on the main stem. Number of secondary branches (NBRS): Indicates the number of branches that appear on the primary branches. Number of greedy (NBRG): This number was taken just after the regeneration of the accession transplanted. Number of nodes (NBNE) obtained by counting the number of nodes on the stem. Number of inter-nodes (NBEN) is the number of nodes found on the stem (NBNE-1); length of nodes (LOEN) represents the average length between nodes (HPLT / NBEN). Number of days from transplanting to flowering in plot (TEMF). Number of leaves at each node (NFNE) is the number of sheets found at each node of the plant. 3: When the three stems of the plant has three leaves at each node, 2 when at least one of the 3 door 2 leaf stems on each node, 4 if at least one of the rods to door 4 leaf nodes (Figure 1). Leaf length (LONF) and leaf width (LARF) are the measures of the 7th centimeter sheet taking as reference the sheet located just below the inflorescence. Because leaves at that node seems to have less variation. These values are used to deduce the following parameter:

FOFE = (LARF / LONF) about which the shape of the sheet ("round, elongated").

Parameters analyzed

Broad sense of the heritability

For a repetition J of an individual i, it is possible to write this phenotypic value: Pij = Gi + eij (Gallais, 1990)

Where Gi is the value of the genotype desired and ei the micro-environment effect, j coincided with the repetition of the genotype i. The genotypic value G can then be defined as the expected phenotypic value of the genotype considered, that is to say the phenotypic values average when the number of repetition is very large:

Gi = Ej (Pij) (Comstock and Moll, 1963; Gallais, 1990).

For the considered genotype i, the variance σ^2 ei of these values represents the variance of the effects of micro-environment. For all the genotypes in this being test, from the same species, it is possible to define the variance σ^2 G of the units studied. A variance σ^2 e can be associated with each genotype i. Under the hypothesis of independence the effects of micro-environment x genotype (randomized monoarbre device) at the population of genotypes (accessions), the variance of phenotypic values can be written:

$\sigma^2 p = \sigma^2 G + \sigma^2 e$ (Kremer, 1986; Gallais, 1990).

The covariance between two repetitions of a genotype can so be written like this: covPijPij = σ^2 G (Gallais, 1990)

the correlation between two repetitions of the same genotype which is given by: $h^2 = \sigma^2 G_{SL} / \sigma^2 P$ is defined as the broad sense of the heritability (Gallais, 1990).

Number of repetitions necessary to genotype evaluation of agro morphological traits

It's about how to determine the number of vegetative plants Plot to really catch the genetic value that can give an average of broad sense heritability close as possible to the value of 1 (100%). In the present activity this determination was made graphically from the following functions:

F1 (n) = $h^2 = \sigma$ sl 2G / [σ^2 + 2G σe^2 / n] (Gallais, 1990) where, n = number was the number of repetitions. 2G σ^2 = variance due to genetic factor. σe^2 = residual variance. This function is defined for σ 2G different from zero. It is increasing and tends to 1, the maximum value of heritability, when n tends to infinite. In terms of graphics, the evolution curve of F1 (n) = h^2 sl tend asymptotically towards the value 1 when n grows. Its value we seek to define for each trait will be studied that from which the evolution curve of heritability tend toward the landing. The purpose of this section is to determine, by studying the graph, the number n of repetition for family value (size of individuals regenerated from a strain of

Lippia multiflora) that allows to estimate for each of the trait value as close as possible to its maximum heritability (100%). It does not find the value of the asymptote, but one from which the slope of the curve begins its trend towards this asymptote.

We set ourselves the hypothesis that the maximum size that can be considered for individuals in comparison in terms of human capacity for experimentation followed by shrub by shrub of these genotypes for 4 years (study duration of the project) of 10 individuals. The accession number is collected on average equal to 200; the number of trees would test 2000. The hypothesis of 10 "clones" by accession is certainly exaggerated, but it is perfectly valid for this study because in reality, if the environmental conditions are not very volatile, 2-5 clones sufficient to evaluate (Heller, 1983).

The study of the function F2, eliminating theoretical confidence interval threshold at the risk of α = 5% depending on the number of repetitions is also associated with this experiment: F2 = [t x √ (var/n)]/n] (Fenelon, 1981 ; Dagnelie, 1975 ; Hamilton *et al*, 2002).

Results

Covariance within accessions and environmental variance

Covariances obtained varied from 0, 56 (FOMF) at 253, 35 number of days the plant before flowering (TEMF). The variances caused by the environment for the same traits are equal to 0, 30 and 232, 65. The trait with the highest variance with a value of 4015, 68 was expressed by plant height (HPLT)

Broad sense of heritability

The heritability in broad-sense was found in this interval [0, 09; 0, 95]. The lowest values were calculated for the trait plant height at maturity (HPLT), number of secondary branches (NBRS) and number of primary branches (NBRP) with the following values respectively 0,09, 0,12 and 0,26. Others had broad heritability with medium to high numbers ranging from 0, 47 for the number of nodes (NBNE) to 0, 95 (NFNE) related to the character sheet number found at each node (Table 1).

Evolution of broad sense heritability based on the number of repetition

The study of the function of broad sense heritability $h^2 = F1\ sl\ (n)^1$ as defined above gives the graphs of figure 2. The calculated values of the heritability from our study population are given in Table II. The number n used for these calculations was the harmonic mean of individuals obtained by vegetative propagation of accessions (n = 2). The table below shows the different values of broad-sense heritability for effective family ranging from 1 to 10 individuals, and each of these estimates, it represents the percentage compared to the maximum reference value for the 10 members "family." We note that this estimated maximum value never reaches 1, the theoretical maximum value of any heritability, whatever the character in question. The observed values were between 0.24 and 0.98, respectively 12, 72% and 100% of the theoretical maximum. These heritabilities were changing very quickly depending on the number n of repetition, to see already reach more than 50% of their maximum observed with 10 individuals per family for TEMF, NBNE, NBEN, and LOEN. It does not reach the 50% with at least 5 individuals / families to plant height (HPLT), the number of primary branches (NBRP) and the number of secondary branches (NBRS). A number of shrubs in the family, an estimated 92%, 79%, 83%, 68%, 68%, 59% and 98% of the number of days before flowering (TEMF), the number of nodes (NBNE) the number of nodes (NBEN) the length between nodes (LOEN), number of primary branches (NBRP), number of secondary branches (NBRS) and the number of nodes sheet (NFNE). These rates are respectively 95%, 66%, 89%, 89%, 77%, 69% and 99% with 6 shrubs "family."

Table 1. Variance and residual variance within accession characters of *Lippia multiflora*.

	Phénotypes								
Traits	TEMF	HPLT	NBNE	NBEN	LOEN	NBRP	NBRS	NFNE	NBRG
covariance	253,75	127,01	21,69	25,88	5,30	2,20	3,34	106,26	3,89
variance	232,65	4015,68	72,66	84,97	13,55	18,66	70,63	16,82	3,87

	Phénotypes		
Traits	LONF	LARF	FOMF
covariance	9,55	3,96	0,56
résiduelle	89,27	71,15	0,30

Table 2. Evolution of the broad heritability of Lippia Multiflora characters according to the number of repetition.

n	h²bs TEMF	%	h² bs HPLT	%	h² bs NBNE	%	h²bs NBEN	%	h²bs LOEN	%	h²bs NBRP		h² bs NBRS	%	h²bs NFNE	%
1	0,52	56,95	0,03	12,76	0,23	30,69	0,28	35,31	0,28	35,31	0,11	19,49	0,05	14,06	0,86	87,70
2	0,69	74,85	0,06	24,76	0,37	49,91	0,44	55,11	0,44	55,11	0,19	35,26	0,09	26,91	0,93	94,13
3	0,77	83,61	0,09	36,07	0,47	63,07	0,54	67,79	0,54	67,79	0,26	48,29	0,12	38,70	0,95	96,49
4	0,81	88,81	0,11	46,74	0,54	72,65	0,61	76,60	0,61	76,60	0,32	59,23	0,16	49,54	0,96	97,72
5	0,85	92,25	0,14	56,83	0,60	79,94	0,66	83,08	0,66	83,08	0,37	68,54	0,19	59,56	0,97	98,47
6	0,87	94,70	0,16	66,38	0,64	85,67	0,70	88,05	0,70	88,05	0,41	76,57	0,22	68,84	0,97	98,97
7	0,88	96,53	0,18	75,44	0,68	90,29	0,73	91,97	0,73	91,97	0,45	83,56	0,25	77,46	0,98	99,34
8	0,90	97,94	0,20	84,04	0,70	94,10	0,76	95,16	0,76	95,16	0,49	89,71	0,27	85,49	0,98	99,61
9	0,91	99,08	0,22	92,22	0,73	97,29	0,78	97,79	0,78	97,79	0,51	95,15	0,30	92,99	0,98	99,83
10	0,92	100,00	0,24	100,00	0,75	100,00	0,80	100,00	0,80	100,00	0,54	100,00	0,32	100,00	0,98	100,00

Table 3. Evolution of errors based on the number of repeated characters of *Lippia multiflora*.

n	TEMF	%	HPLT	%	NBNE	%	NBEN	%	LOEN	%	NBRP	%	NBRS	%	NFNE	%
1	0,58	0,00	99	0,00	0,35	0,00	0,14	0,00	0,14	0,00	0,16	0	0,03	0,00	0,32	0
2	0,29	50,00	1,2	98,79	0,18	48,57	0,07	50,00	0,07	50,00	0,08	50	0,02	33,33	0,16	50
3	0,19	67,24	0,8	99,19	0,12	65,71	0,05	64,29	0,05	64,29	0,05	68,75	0,01	66,67	0,11	65,625
4	0,14	75,86	0,6	99,39	0,09	74,29	0,04	71,43	0,04	71,43	0,04	75	0,01	66,67	0,08	75
5	0,12	79,31	0,48	99,52	0,07	80,00	0,03	78,57	0,03	78,57	0,03	81,25	0,01	66,67	0,06	81,25
6	0,1	82,76	0,4	99,60	0,06	82,86	0,02	85,71	0,02	85,71	0,03	81,25	0,01	66,67	0,05	84,375
7	0,08	86,21	0,34	99,66	0,05	85,71	0,02	85,71	0,02	85,71	0,02	87,5	0	100,00	0,05	84,375
8	0,07	87,93	0,3	99,70	0,04	88,57	0,02	85,71	0,02	85,71	0,02	87,5	0	100,00	0,04	87,5
9	0,06	89,66	0,27	99,73	0,04	88,57	0,02	85,71	0,02	85,71	0,02	87,5	0	100,00	0,04	87,5
10	0,06	89,66	0,24	99,76	0,04	88,57	0,01	92,86	0,01	92,86	0,02	87,5	0	100,00	0	100

Evolution of the error characteristics of Lippia multiflora in function of the number of a repeat individual

The study of the function F2 (n)³gave values between 0 and 99 which correspond to a decrease from 100% to 0% of the standard error of the theoretical maximum value. These decreases were changing very quickly depending on the number of repeats "family" to quickly reach or exceed 50% for all variables with only two individuals from the vegetative propagation of the same accession of their maximum value observed with 10 individuals. The number of nodes (NBNE) and the number of secondary branches (NBRS) had their standard errors decreased by 49% and 34% with this number. The theoretical value of 50% reduction of the error for all characters was obtained with a repeat number greater than or equal to 3 individuals per "family" (table III). They were respectively 68%, 99%, 66%, 65%, 65%, 69%, 67% and 66% for the number of days before flowering, plant height, number of node, between the number of nodes, the length between nodes and the number primary branches, number of secondary branches and number of leaves at nodes.

Fig. 1. Young plants of *Lippia multiflora* with 2, 3 or 4 leaves at their node.

Discussion

Covariance, variance and heritability in the broad sense

The covariance measures the degree of similarity between members of a family (Gallais, 1990) we identified by individuals obtained from each accession after "break." And variables with less fluctuation in accession experimental plot were TEMF, NFNE, NBRG and FOMF. Indeed the characteristics of the obtained covariance represent at least one or even seven times the variance environment. So these are traits that were more stable during the study. This was also noticed by Beninga (2009) in millet. Indeed he showed that this species has a high heritability for early maturity and parameters related to architecture. Those who have been much influenced by the micro-environment of this study were plant height (HPLT), the number of secondary branches (NBRS), width (LONF) of the sheet. The percentage of variance and their covariance gave values of 32% and 9% respectively for plant height and leaf length (Table I). This goes in the same direction as the results Adou *et al* (2011) have demonstrated a high variability of this parameter within the population have *Lippia multiflora*. These are indeed characters and generally considered by many authors such as Ford (1972) as very plastic because of the high number of genes that govern them. With a broad sense heritability of 0.95 the number of leaves at each node was the most stable character in the context of this activity. That he should be made a high intensity work. However, this character within the meaning of Gallais (1990) could be a good predictor of the length and width of leaves because they are not only significantly related but more importantly it had a broad sense heritability stronger. Other characters, more or less stable heritabilities were broadly weaker and require more attention and especially a greater number of individuals to understand the limits of variability.

Repetitions of the same accession necessary in a plot

The present study showed that individuals of an accession were many repetitions of the average value of the accession; it was the same genotypic values individual and intra species. The genotypic values are not directly accessible; they have been predicted from phenotypic values. The accuracy of the estimate of mean phenotypic values accessions are based on this number of repetitions (Gallais, 1990), they influenced the quality of this prediction (Fig. 1a and 1b) geno-phenotype, ie heritability (Falconer, 1974; Nyquist, 1991; Gallais, 1990). The heritability for a given trait was even better than the estimated size of the individuals studied was large (figure 2). Here this number has been defined by studying the evolution of heritability knowing the components of genetic variance (covariance intra-accession and residual).

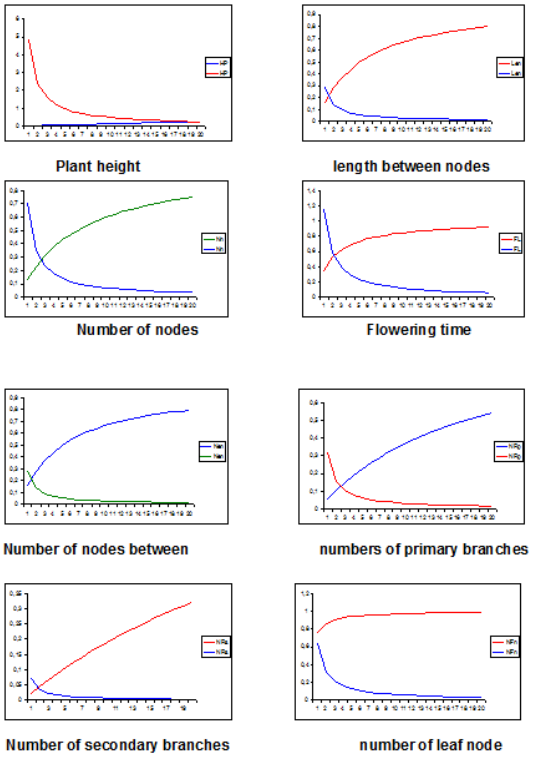

Fig. 2. changes in the broad sense heritability and standard error based on the repetition of traits of *Lippia multiflora* (*[1] F1 is represented by the grown graphics; [3] F2 is represented by the decreased graphics).*

The study of the evolution of the number of individuals allowed to observe two rehearsals with an estimated 50% heritability than half of the studied traits (TEMF; NBEN; LOEN; NFNE) while others have percentage that is remain below the average. Four individuals theoretically estimated more than half of the heritability. The study of the rate of decrease of the error showed that two replicates were obtained more than 50% reduction for all traits. With such a number was estimated at only 26%, 48% and 34% respectively broad heritability of plant height (HPLT) number of nodes (NBNE) and number of secondary branches (NBRS). Choosing the one hand, plant height (HPLT) and the number of secondary branches (NBRS), because of their significant correlations with each other, as the two traits of target selection we saw that we must up to 4 people per family to meet both of these criteria. With this theoretical number of individuals, it was estimated 57% and 59% of the heritability of each of these two characters. This number allows a reduction of the standard error of 99% and 67%. This number seems therefore a good compromise compared to the target initially. Such a number per family, compared to 12 or 15 currently shrubs recommended respectively by Adou, (2001) in connection with the study of narrow heritability of half-brother of common female parent (ALF-SIB) of the species *Coffea canephora* or Marchenay and Lagarde (1986) for the collection of strains of rhizome or stem of a species, would reduce at least 67% or 75% of the current size of the trials (15 plants / family and 200 families on average), 134 or 200 other plants that could be converted into a hundred additional families to assess, for the same density of work.

Conclusion

It appears from this study that:

Plant height of *Lippia multiflora* is greatly influenced by the culture environment. The number of days before flowering (h^2_{bs} = 0, 95) and the port of the node (h^2_{bs} = 0, 77) have strong heritability in the broad sense. Moreover, the study of the evolution of the agronomic traits' parameters based on the number of individuals from the vegetative propagation of the same plant material showed that it is necessary, plot, at least 4 repetitions for a same genotype. This allows obtaining at least, 60% of the broad sense heritability and reducing at least 70% of the standard error.

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