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Article

Relation of chlorophyll and foliar nitrogen of *Gmelina arborea* Roxb. at the nursery and in the field

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Abstract

The fundamental role that *Gmelina arborea* (*melina*) has reached to guarantee the supply of raw material for the forest industry, in the tropical and sub-tropical areas, highlights the need to find superior genotypes; for this, the parameter (s) that will be used as a reference in a possible selection at early stages, must necessarily be associated to the development of the material in the field. The objective of the present investigation was to identify differences between *G. arborea* clones in their chlorophyll content, obtained in the laboratory and through the portable SPAD meter and thus determine correlations with their performance in a one-year-old plantation, and their potential for the early selection of superior genotypes of this species. Clones 8 and 3 recorded the extreme values in the chlorophyll content. The value of the correlation between SPAD and total chlorophyll in the laboratory was 0.52, which is considered moderate for forest species. Clones 10 and 12 reached the highest correlation figures between SPAD-DAP and SPAD-total volume, which opens the possibility of being able to select clones that will have better performance in the field from the chlorophyll reading in the nursery stage, obtained by the portable meter. However, it is necessary to develop and validate a methodology for obtaining chlorophyll content at early ages for the species.

Key words: Clones, chlorophyll, Costa Rica, vegetal physiology *Gmelina arborea* Roxb., forest nursery.

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Introduction

Gmelina arborea Roxb. is native to the moist deciduous forests of Southeast Asia (Osorio, 2004; Wee *et al*., 2012). At present, the species is considered as a promising option to guarantee the supply of raw material for the forest industry in the tropics and sub-tropics. This is mainly due to its easy handling, low establishment cost, rapid growth in its first stages and high biomass production in short cycles (Kumar, 2007; Adebisi *et al*., 2011; Rojas *et al*., 2012).

It is the second most important forest *taxon* in Costa Rica with more than 18 000 ha, which stand for 20.5 % of the total area established for timber purposes (INEC, 2015). Its position in the forestry sector is due to the promotion of clonal genetic improvement programs, initiated in the nineties, from which excellent results were obtained in characteristics of commercial interest; the aim was to increase the productivity and quality of wood, by selecting outstanding genotypes for specific genetic characteristics (Balcorta and Vargas, 2004).

A key strategy in clonal production is to select or exclude genotypes that have desirable traits, starting at phases prior to their establishment in the field (Puttonen, 1989, Mattsson, 1996). This is crucial for reducing costs and, therefore, increasing the efficiency of the productive system, by decreasing the timing of the selection processes of superior genetic materials. To achieve this objective, criteria, parameters or variables that can be used in their early selection must be identified. Necessarily, they should be able to be associated to the development of the forest mass, so that morphological and physiological variables in particular become a main option (Puttonen, 1989; Mattsson, 1996; Rakocevic *et al.,* 2005; Ávila, 2013; Ávila *et al.,* 2016).

In this way, correlating physiological parameters evaluated in the nursery stage with the field development of the genotypes, through the evaluation of formally established clonal assays, offers the possibility of identifying genotypes with optimal growth for areas of continuous forest production, which in turn, they serve as an

instrument for this selection of superior clones (Puttonen, 1989; Goswami and Tewari, 2011; Codesido *et al*., 2012; Ávila *et al*., 2016); such is the condition of the experimental site studied here.

In spite of the importance of knowing in detail the behavior of different types of variables, for the genetic materials that are currently used for the production of wood, and in each of its stages of life, the existing and necessary information to make scientifically based decisions is extremely limited at the international level, and almost non-existent for Costa Rica (Gonçalves *et al.,* 2008; Silla *et al*., 2010).

It is because of this need for knowledge that photosynthesis has been studied extensively, a vital physiological process in plants, and in relation to photosynthetic capacity and efficiency in particular (Puttonen, 1989). The content and type, both chlorophyll and carotenoids, influence the capture of light and the transfer of that energy to the reaction centers. In addition, chlorophyll is involved in the synthesis of cell growth molecules, which is a key indicator of the physical state of the plant, reflected in its photosynthetic capacity, productivity and stress level, among other aspects (Puttonen, 1989; Mattsson, 1996; Richardson *et al.,* 2002; Umazi *et al*., 2016).

An alternative to the above to indirectly estimate the chlorophyll content quickly and non-destructively, is by means of a portable field chlorophyll meter Minolta SPAD™ 502 (Soil Plant Analysis Development), which calculates the greenness index that is directly related to the chlorophyll content in the leaf (Rodríguez *et al.,* 1998; Rincón and Ligarreto, 2010).

Several authors suggest that the use of the portable meter can help in the preliminary discrimination of genotypes with high and low chlorophyll content; it could eventually be used in the early selection of superior genotypes, once the chlorophyll reference values have been identified (Neufeled *et al*., 2006; Barrios *et al*., 2011; Díaz, 2014), and also to associate them with their growth in the field (Puttonen, 1989; Mattsson, 1996; Avila *et al*., 2016).

By assuming possible differences between clones in the chlorophyll content in the nursery stage and in the field, a relationship between both can be established, which would allow genotypes to be identified as superior from early stages in the

nursery (Mattsson, 1996; Codesido *et al.,* 2012). Thus, technical-scientific information would be available to decide the incorporation or exclusion of genotypes to clonal assemblages that are part of genetic improvement programs (Mattsson, 1996), and thus, increase the precision of the information that sustains forestry packages of continuous and intensive production.

In this regard, the *Instituto de Investigación y Servicios Forestales de la Universidad Nacional de Costa Rica* (INISEFOR-UNA) (Institute of Forest Research and Services of the National University (INISEFOR-UNA) of Costa Rica) carries out research projects aimed at generating knowledge that contribute to improve the productivity of forest plantations. In this context, the objective of this research was to identify possible differences between clones of *G. arborea* or *melina*, which make up their genetic collection in terms of chlorophyll content, and to associate this variable with their performance in the field in a year, to finally determine the potential of early selection of superior genotypes based on the possible nursery-field correlation, between chlorophyll and mensuration variables.

Materials and Methods

This study was carried out jointly between INISEFOR-UNA and the School of Biology of the University of Costa Rica. The experimental material was asexually produced in the INISEFOR clonal nursery, in the *La Palma* locality, *Puerto Jiménez* district, *Golfito* canton, *Puntarenas* province, southern zone of Costa Rica (83.39655° N, 8.57701° W). The site is located at an altitude of 20 m, records an annual rainfall of 3 500 to 4 000 mm and average annual temperature of 24 to 28 °C (Kappelle *et al.,* 2002). The region is classified as very wet premontane forest transition to basal (Holdridge, 1967).

Experimental material

The research was carried out with 14 genotypes of *melina*, which belong to the genetic collection of INISEFOR. Ten plants of each clone were evaluated, for a total of 140. The genotypes were reproduced from clonal gardens of the genetic collection of the same institution.

Determination amount of chlorophyll and nitrogen

The amount of chlorophyll was estimated by two methods:

1) Determination by means of a portable meter: the readings were made with the Minolta SPAD 502 meter on a sheet of each of the 140 plants evaluated, on the first new pair with full development that did not exhibit any damage. Five readings were taken from each leaf in different parts, one in the center and the remaining four in its edge, in such a way that it covered most of the leaf area (10 cm^2 on average); these measurements are automatically averaged by the equipment, with which only one data per sheet was recorded. The measurements were made during the day.

2) Determination in the laboratory: the sheets on which the portable meter was read were marked for identification and sectioned from the plants. From each one, a 1 cm diameter disc with an average weight of 0.6 ± 0.01 mg was extracted, which was subsequently macerated in a mortar with the help of a pistil and 5 mL of 90 % ethanol. The content of the mortar was transferred to 50 mL tubes, which were centrifuged at 2000 rpm for 5 minutes. From the supernatant 1.5 mL were taken to measure the absorbances in the Implen P300 nanophotometer with the following wavelengths: 430, 470, 620, 665, 645, 647, 663, and 750. Finally, the chlorophyll *a* variables were determined, by total, and carotenoids (mg L^{-1})

according to Lichtenthaler (1987); and foliar nitrogen using the Kjeldahl method (Horneck and Miller, 1998).

Chl a: Chlorophyll a: (13.36 × A664) – (5.19 × A649)

Chl b: Chlorophyll b: (27.43 × A649) – (8.12 × A664)

Chl T: Total clorophyll: Chl a + Chl b

Statistic analysis

In order to statistically calculate significant differences for the variables evaluated between the genotypes, a variance analysis and a Tukey means test ($p > 0.05$) were performed for the database from the laboratory and the portable meter.

A Pearson correlation test was applied between the chlorophyll readings obtained from the portable meter (SPAD units) and those determined in the laboratory (mg L^{-1}), both chlorophyll and nitrogen, for all individuals ($n = 140$) and for each of the clones ($n = 10$). Subsequently, the results were grouped into three clonal subsets according to their level of correlation in high (≥ 0.61), medium ($0.31 \leq r \leq 0.60$) and low ($r \leq 0.30$).

Finally, a Pearson correlation test was carried out between the chlorophyll in nursery and the mensuration variables of growth of the genotypes sampled in the field at one year of age, planted in a genetic test where the 14 genotypes are present. The mensuration variables measured in this investigation were the following:

Diameter at Breast Height (DBH) = the normal diameter (at 1.3 m) was determined for each tree by a *Forestry Suppliers* diametric tape

Total Height (HT) = from the reading of the *Suunto* Pm5/15-20 m inclinometer

Total Volume (VT) = ((DAP / 100) 2) * 0.7854 * HT * 0.65

The statistical program InfoStat version 2015 was used for the statistical analyzes (Balzarini *et al.,* 2008*;* InfoStat, 2015).

Results and Discussion

Evaluation of chlorophyll determined by portable and laboratory meter

The chlorophyll calculated by the portable meter recorded an average for the species of 21.51 units. Ávila (2013), in the same region and age of the individuals, registered a general chlorophyll average of 23.57 SPAD units for clonal *melina* material, which is a data that supports the results obtained (Table 1).

Table 1. Average value of the assessed parameters for the *melina* individuals (n = 140) and for every one of the genotypes (n = 10) generated by SPAD and in the laboratory.

Clone	*SPAD* (*SPAD* units)	Chl-T (mg L^{-1})	Chl-a (mg L^{-1})	Chl-b (mg L^{-1})	N (%)
1	18.11 de	2.25 cde	1.64 de	0.61 b	2.00 d
2	23.38 abc	3.84 ab	1.60 de	2.23 a	3.20 ab
3	17.32 e	1.60 e	1.20 e	0.40 b	2.18 cd
4	25.31 a	3.15 abcd	2.52 a	0.63 b	3.22 ab
5	25.45 a	4.40 a	2.19 abc	2.21 a	3.05 ab
6	19.86 bcde	2.04 cde	1.43 de	0.61 b	2.60 bcd
8	23.72 ab	2.51 bcde	1.91 bcd	0.60 b	3.02 ab
9	19.34 cde	2.01 de	1.53 de	0.48 b	3.27 ab
10	21.81 abcd	2.33 cde	1.66 de	0.67 b	3.08 ab
11	24.50 a	4.04 a	2.33 ab	1.71 ab	3.42 a
12	22.18 abcd	2.10 cde	1.59 de	0.51 b	3.64 a
13	21.43 abcde	3.45 abca	1.93 bcd	1.52 ab	3.60 a
15	21.46 abcde	2.16 cde	1.64 de	0.53 b	2.95 abc
16	21.29 abcde	2.27 cde	1.74 cd	0.53 b	3.47 a
Average	21.51	2.63	1.73	0.89	3.01
Min	12.5	1.18	0.51	-1.1	1.6
Max	29.4	1.28	3.27	4.85	4.95
CV(%)	15.97	43.21	25.11	115.13	21.89

Chl-T= Total chlorophyll, Chl-a= Chlorophyll a, Chl-b= Chlorophyll b, N= Foliar nitrogen; CV = Variation coefficient. Means with different letter in a line are statistically different (Tukey, $p \leq 0.05$).

The previous results (Table 1) reveal a potential 10: 1 relationship between the average chlorophyll value of the portable meter and its similar from the laboratory (SPAD units *vs* mg L^{-1}). Therefore, it is feasible to predict the chlorophyll content revealed by the laboratory analysis from the reading in the SPAD units, although more studies are needed to corroborate this. This possibility has been recorded by several authors for species such as *Acer saccharum* Marshall (Van den Berg and Perkins, 2004), *Betula pendula* Roth (Uddling *et al*., 2007), *Lindera melisifolia* (Walter) Blume (Hawkins, 2009), *Bombacopsis macrocalyx* (Ducke) A. Robyns., *Eugenia* cumini (L.) Druce, *Iryanthera macrophylla* (Benth.) Warb. and *Senna reticulata* (Willd.) H. S. Irwin & Barneby (Gonçalves *et al*., 2008), *Platanus occidentalis* L., *Liquidambar styraciflua* L., *Fraxinus pennsylvanica* Marshall and *Populus heterophylla* L. (Chang and Robinson, 2003).

Clone 5 recorded the most outstanding value of total chlorophyll (Chl-T) in laboratory and field (SPAD) (Table 1) and high results of chlorophyll a (Chl-a) and chlorophyll b (Chl-b). This suggests a better ability to capture light energy, which could maximize the photosynthetic process and eventually express it with faster growth under natural conditions (Richardson, 2002; Rakocevic, 2005; Ávila, 2013; Díaz, 2014; Ávila *et al*., 2015). Additionally, according to Umazi *et al.* (2016), the trees with the highest chlorophyll foliar content could show better growth, which would increase their resistance to weathering.

The opposite case was verified with clone 3, which reached the lowest average value of chlorophyll SPAD, Chl-T, Chl-a and Chl-b, which would represent a disadvantage for its development in the field, since the amount of solar radiation absorbed by a leaf is largely a function of the foliar concentrations of photosynthetic pigments; therefore, under this condition, the photosynthetic potential can be directly limited and, with it, the primary production (Umazi *et al*., 2016).

In the same way, clonal differences in the foliar concentration of N in which clone 12 had the highest average (3.64 %), clone 1 recorded the lowest value (2.00 %). The concentration of nitrogen has been directly associated with variations in the photosynthetic

capacity of the leaf and, finally, the productivity and performance of the genotypes (Mattsson, 1996; Richardson, 2002; Corcuera *et al*., 2005; Ávila *et al.,* 2015).

Beyond the genetic aspect, the contrasts found could be explained based on different factors such as the calibration of the equipment and the degree of maturity of the sampled leaf; despite always evaluating the first pair of leaves, they were not always of the same size or development because of dissimilarities in stem elongation between clones. In this regard, Silla *et al*. (2010) reported significant interactions between the readings generated with a portable meter and the leaf stage for several *Quercus* species; therefore, the interpretation of the readings should be limited to samples of leaves with similar appearance and conditions (Puttonen, 1989), and then, it is convenient to homogenize the material that will be used to the maximum.

On the other hand, the calibration of the chlorophyll meters is linked to the differences in the optical and anatomical properties of the leaves. The meter is sensitive to characteristics such as leaf veins, thickness and moisture content, so it is advised to make multiple evaluations to the same leaf (Chang *et al*., 2003; Pinkard, 2006; Wang *et al*., 2009).

The reading depends not only on the chlorophyll content but also on its distribution in the leaf, which in turn is co-determined by the arrangement of the chloroplast in the cells, which depends on the light conditions and their variation during the day. As the portable meter measures the transmittance of light through the leaf, the movement of the chloroplasts within it also influences the readings (Naus *et al.,* 2010). Therefore, the importance of having a standardized methodology for the use of the portable meter in obtaining a reliable chlorophyll value for the species in question is ratified.

A difference was verified between the amount of Chl-a and Chl-b for each of the clones evaluated, with a very close ratio of 3: 1, which coincides with values for other higher plants (Mustafa *et al*., 2015). According to Chazdon and Montgomery (2002), the proportion of the different types of chlorophyll constitutes an adaptation

to the amount of light received. The heliophyte species contain higher content of chlorophyll "a" than "b". *Melina* is located within such species, which gives validity to the result obtained in the present investigation.

Differences between genotypes for SPAD and laboratory chlorophyll

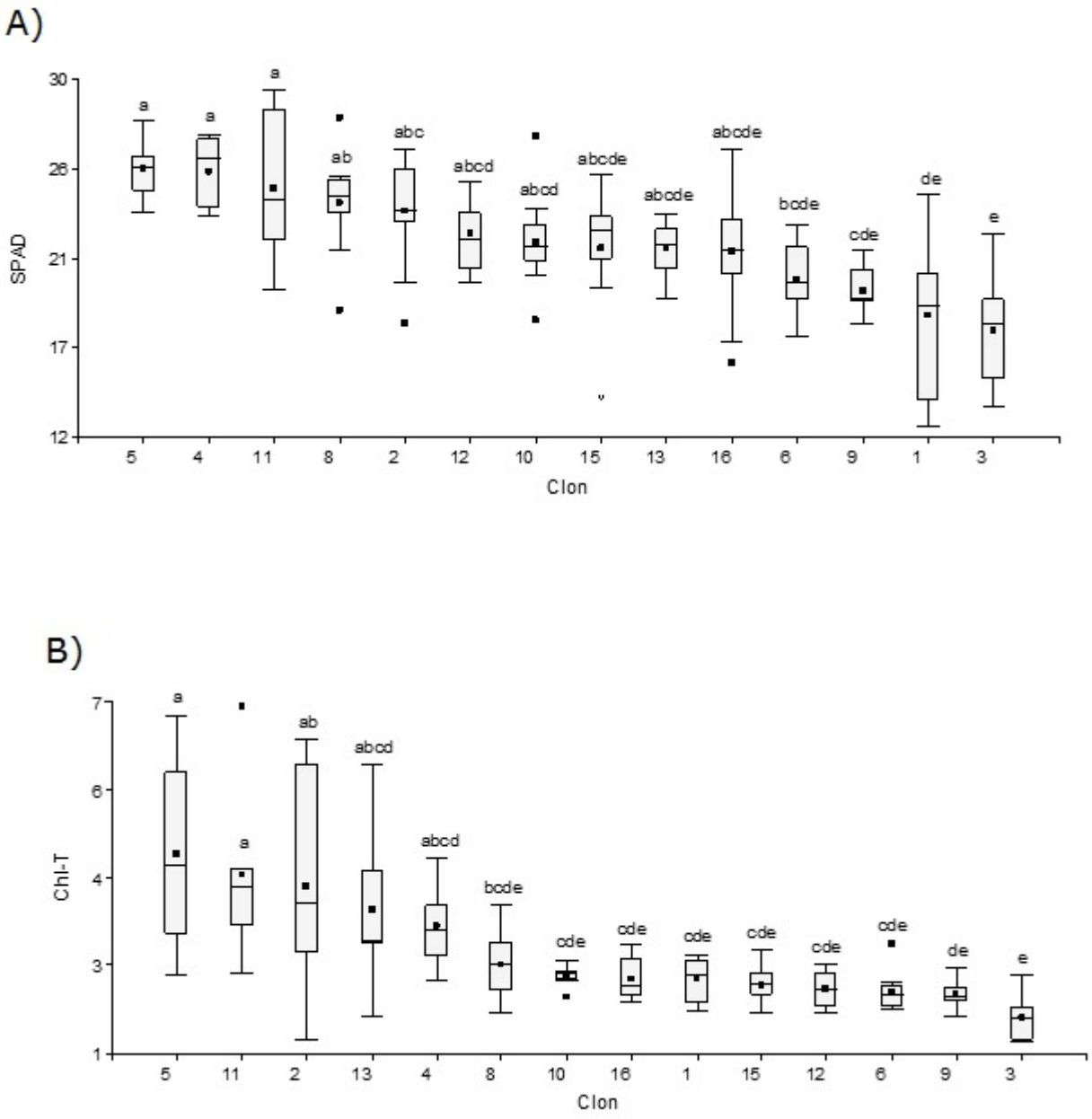
There were statistically significant differences between clones for all the variables evaluated ($p < 0.05$) (Table 1). The chlorophyll obtained by the portable meter showed the highest number of them among clones, contrary to chlorophyll b. Clones 3 and 5 reached extreme averages (17.32 and 25.45 SPAD units, respectively).

For all the values of chlorophyll and nitrogen obtained, both in the laboratory and with the portable meter, a pattern of three groups was configured. The first with the superior genotypes; a second broad group that shares intermediate values with each other, and the third group formed by the clones with the lowest average values for the variables.

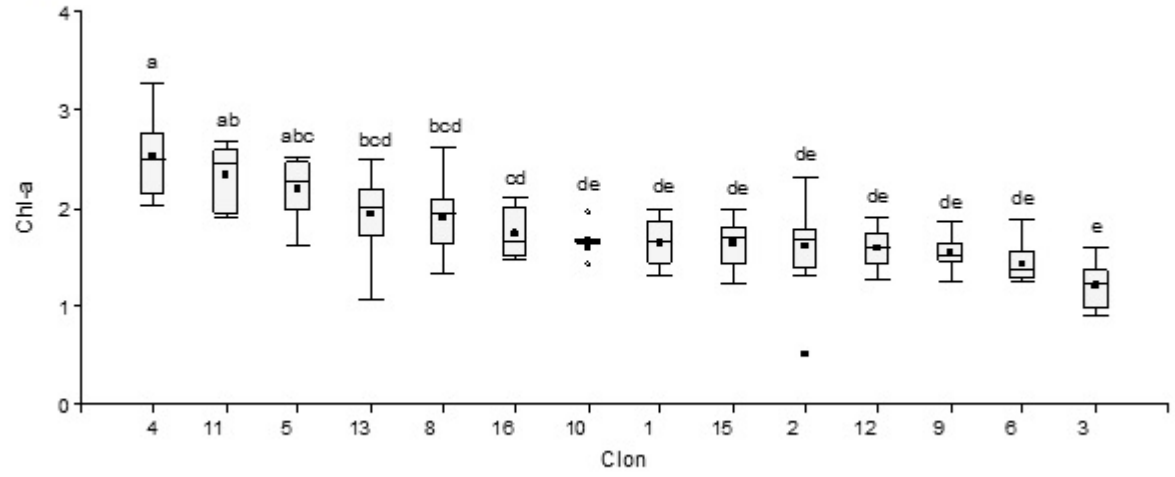
Such a group pattern is the result of the same variability among genotypes; nevertheless, the one made up of clones 3 and 9 with such reduced pigment contents draws attention, despite the growths in the field without statistically significant differences compared to the other genotypes. For this particular case, the values of chlorophyll could be due, in the first instance, to that in them were evaluated leaves with different degree of maturity than the others, which is explained because all the genotypes do not have the same speed in the development and maturation of its leaves (Silla *et al.,* 2010). This is a methodological aspect that should be taken into account in future studies; and in the second instance, an affectation by optical factors, which could have directly influenced the contents of the pigments.

The differences between clones for each of the evaluated variables are illustrated in Figure 1. As can be deduced from Table 1, the chlorophyll reading with SPAD

exhibits the greatest number of differences among clones; Clones 3 and 9 are located to the right of the chlorophyll charts with the lowest values.



C)


Chl-a
Clon
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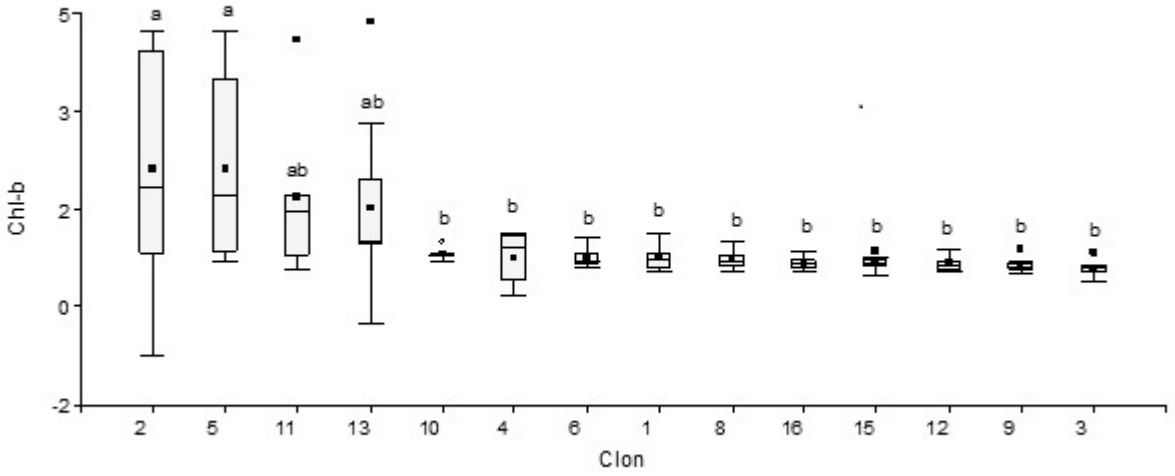
Chl-b
Clon
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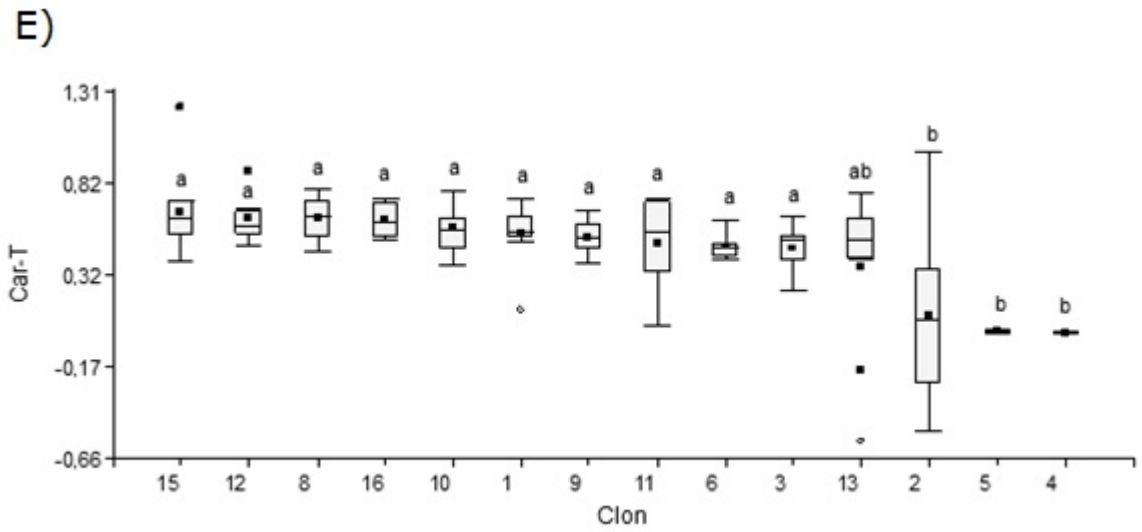
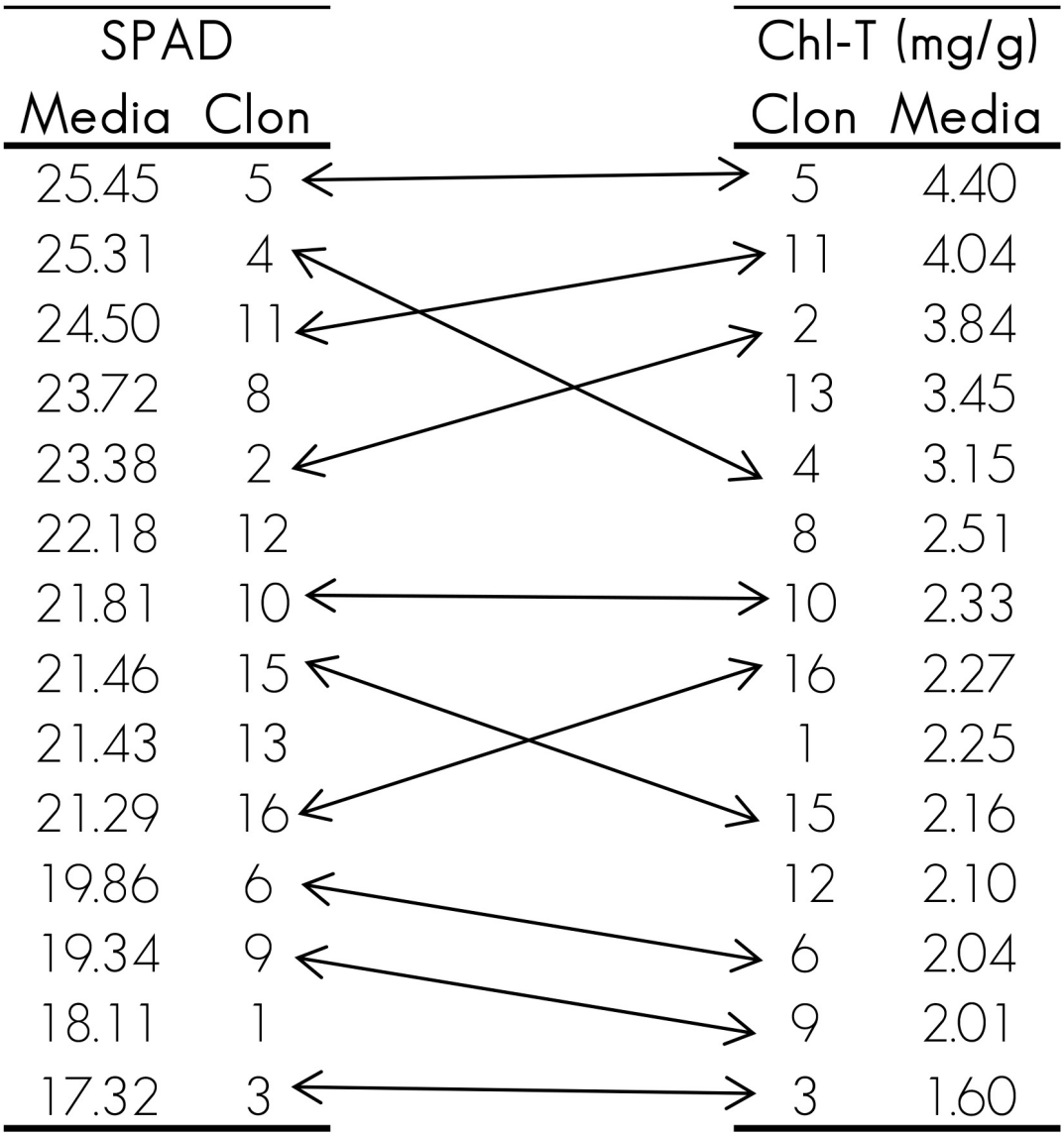


A = SPAD chlorophyll; (B), Chl-T= total chlorophyll (C), Chl-a= chlorophyll a; (D), Chl-b= chlorophyll b; N= foliar nitrogen and (E) = Car-T. *Clon* = Clone

Figure 1. Average values of the chlorophyll and carotene content in clones of *Gmelina arborea* Roxb.

Trying to predict the chlorophyll content of plants through simple evaluations necessarily implies reliability in the measurements made on the predictor variables; that is why it is important to observe the position occupied by each clone for each variable and the possible correlation between them. Identifying the subsets of genotypes evidences their genetic variability and the possibility of generating predictive models.

Ten genotypes maintained a stable trend regarding their position for both variables (Figure 2), which suggests that the measurement with SPAD could have a linear, or directly proportional, relationship with the chlorophyll data generated in the laboratory, having the same (or very similar) sorting based on its average.



Media = Average; *Clon* = Clone

Figure 2. Position of the clones by the average of total chlorophyll obtained with SPAD *vs* laboratory.

The identification of these three subsets suggests two hypotheses. The first is that the use of certain genotypes could be ruled out due to their low photosynthetic

pigment values, and the second, that *melina* chlorophyll readings by portable meters could be reliable.

Possibility of prediction in the reading of chlorophyll in the laboratory from the SPAD

A reliable reading of the amount of chlorophyll from a simple measurement such as that made with the SPAD™ equipment, would be very useful in research programs and forest genetic improvement. For the actual investigation, almost all the correlations obtained were positive and higher than 0.50, except for the one between SPAD and Chl-b (due to the difficulty of generating the value of Chl-b, explained above) (Table 2). With respect to Chl-b, other authors have described a similar pattern; thus, Gonçalves *et al*. (2008) recorded a lower correlation between Chl-b and Chl-T compared to those obtained between Chl-a and Chl-T. Table 3 lists the correlation values between the variables evaluated by each genotype.

Table 2. Pearson correlation matrix for the chlorophyll type and its average absolute value for *Gmelina arborea* Roxb. genotypes.

	SPAD	**Chl-T**	**Chl-a**	**Chl-b**
Chl-T	0.53	-	-	-
Chl-a	0.65	0.96	-	-
Chl-b	0.31	0.92	0.64	-
N	0.42	0.26	0.27	0.18

Chl-T = Total chlorophyll; Chl-a = Chlorophyll a; Chl-b = Chlorophyll b; N = Nitrogen. Significant: $p \leq 0.05$.

Table 3. Correlation between genotypes of *Gmelina arborea* Roxb. and the evaluated chlorophyll and nitrogen variables.

		Clone													
		8	**10**	**3**	**11**	**9**	**5**	**15**	**6**	**4**	**2**	**1**	**13**	**12**	**16**
SPAD	Chl-T	0.81	0.75	0.67	0.66	0.65	0.59	0.49	0.44	0.37	0.33	0.17	0.12	0.09	0.02
	Chl-a	0.80	0.86	0.72	0.47	0.42	0.51	0.63	0.51	0.59	0.75	0.36	0.16	0.05	0.17
	Chl-b	0.78	0.19	0.48	0.59	0.79	0.47	0.11	0.28	0.02	0.12	-0.15	0.05	0.13	-0.29
	N	0.78	-0.36	-0.07	0.70	0.19	0.89	0.14	0.43	0.37	0.02	0.30	-0.48	-0.15	0.59
Chl-T	Chl-a	0.99	0.83	0.91	0.34	0.92	0.03	0.97	0.94	0.83	-0.27	0.85	-0.70	0.94	0.98
	Chl-b	0.96	0.67	0.91	0.98	0.85	0.98	0.87	0.89	0.82	0.97	0.75	0.98	0.90	0.93
	N	0.50	-0.78	-0.22	0.64	0.34	0.53	-0.53	0.58	0.08	0.17	-0.29	0.18	0.24	-0.61
Chl-a	Chl-b	0.92	0.14	0.78	0.12	0.57	-0.18	0.72	0.68	0.36	-0.49	0.29	-0.83	0.70	0.85
	N	0.51	-0.49	-0.24	0.94	0.46	0.26	-0.42	0.63	0.49	0.06	-0.35	-0.15	0.30	-0.52
Chl-b	N	0.44	-0.83	-0.15	0.14	0.12	0.46	-0.68	0.40	-0.36	0.14	-0.09	0.18	0.12	-0.73

Significant = ($p \leq 0.05$)

Based on the results of all the plants evaluated for the species, the chlorophyll content determined by SPAD had a correlation of 0.53 with the total chlorophyll obtained in the laboratory, which is considered moderate for forest species. In their study with *Acer saccharum*, Van den Berg and Perkins (2004) calculated a correlation of 0.76 between reading chlorophyll with SPAD and total chlorophyll. In the same way Silla *et al.* (2010), for three *Quercus* species, recorded correlations of 0.58 to 0.83 between these same variables. However, none of the above species exhibits total heliophyte behavior similar to that of *melina*.

The chlorophyll reading of the SPAD had a correlation with Chl-a higher than Chl-T, which suggests that the low correlation between SPAD and Chl-b (0.31) significantly affected the SPAD-Chl-T correlation. This result confirms that the laboratory determination of Chl-b values for the species must be fine-tuned, since it shows an important variability that warrants a more thorough reading of this pigment. This identification of significant correlations between variables is vital as an additional aspect in the possibility of early

predicting their performance in the field, which is the final objective of this research. In the same way, this information would act as an indicator of the health and physiological performance of different plant materials (Neufeld *et al.,* 2006; Gonçalves *et al.*, 2008; Wang *et al*., 2009; Silla *et al*., 2010; Díaz, 2014; Umazi *et al*., 2016). All of the above would allow to identify the genotypes that should be used in intensive forest production systems (Mattsson, 1996; Ávila, 2013).

Clone 8 recorded the highest correlation values in most of the variables; otherwise, clone 16, the lowest. A homogeneous behavior was obtained for clone 3, with correlations considered high (r≤0.60) for all the assessed variables (Table 3). This information allows establishing a baseline for comparison, which could be a reference point in the search for parameters for the possible early selection of superior genotypes; however, these variables are only some of the possible ones to select.

The clones were classified into three groups, based on the values of individual correlations (Table 4).

Table 4. Recorded correlation between Chl-T and SPAD for every set of the *Gmelina arborea* Roxb. clones.

Set	**Correlation Chl-T–SPAD**
High	0.73
Moderate	0.55
Low	0.11

Significant = (p ≤ 0.05)

Clones 8, 10, 3, 11 and 9 were correlated, mainly between SPAD and CHL-T, cataloged as high, while for 1, 12, 13 and 16 they were low (<0.30). The identification of these groups suggests that there is a marked variability between genotypes, which at the beginning can be explained by differences in the degree of maturity and sheet in the evaluated leaves of each plant at the time of taking the measurement. Calvo *et al*. (2008), Crespo *et al*. (2011), Rojas *et al*. (2012) and Umazi *et al*. (2016) agree about attributing the variability in the content of chlorophylls and / or carotenoids to situations of water stress; however, this would not be extensive to the present investigation, since homogeneity was guaranteed in the experimental conditions in which all the genotypes were developed during the evaluation period (Puttonen, 1989; Mattsson, 1996; Rojas *et al*., 2012).

Chlorophyll types correlation *vs* field development

Young - adult correlations, that is, those based on variables evaluated at early ages and in the field, are a very valuable tool for the selection of plant materials that could express desirable characteristics in plantation at the productive level. The analysis made to all the individuals indicates that the correlations between Chl-T and / or SPAD with the mensuration variables (of growth) in field at one year of age, turned out to be very low, without exceeding 0.19 (Table 5). However, despite not being able to statistically link field performance from chlorophyll types, this result is of great importance as it is a first attempt to explain the variability of field parameters very early, from these levels in the plants in nursery conditions.

Table 5. Pearson Correlation Matrix for the amount and type of average chlorophyll and the mensuration variables in one-year-old plantation, for ten clones of *Gmelina arborea* Roxb.

	Chl-T	**SPAD**	**Chl-a**	**Chl-b**	**Car-T**	**D**	**Ht**
SPAD	0.52	-	-	-	-	-	-
Chl-a	0.96	0.6	-	-	-	-	-
Chl-b	0.84	0.25	0.64	-	-	-	-
Car-T	0.55	0.51	0.66	0.2	-	-	-
DAP	0.02	0.08	-0.03	0.11	0.03	-	-
HT	0.09	0.19	0.09	0.07	0.15	0.41	-
VT	0.04	0.11	1.00E-03	0.11	0.07	0.97	0.6

DAP= Diameter at Breast Height; HT = Total height; VT= Total volume.
Significant = $p \leq 0.05$.

Similar results have been obtained in retrospective studies for early selection, generally with very low correlations (Harfouche, 2003; Ávila *et al*., 2016). These authors point out several reasons to explain this situation. From the genotype-environment interaction, the chlorophyll contents can be affected by temperature, solar irradiation and water stress.

Jansson (1998) adds to the previous explanations, that different sets of genes regulate growth at different ages, so that the age of the seedlings used in nursery measurement may not have been the necessary to generate the inference nursery-field. Therefore is ratified by different authors, concluding that seedlings must reach a minimum development stage so that juvenile traits become a reliable selection criterion (Erickson *et al.,* 1993; Rojas *et al*., 2012). The amount of material evaluated has also been pointed out in the literature as a possible reason that could

affect juvenile-adult correlations (Puttonen, 1989). All the above must be taken into account in the methodological approach of future investigations.

As in the previous evaluations, clones with very low correlations were identified (Table 6), which finally could affect the general database and could explain the low correlations of chlorophyll and growth in general found for the species.

Table 6. Pearson correlation matrix between the chlorophyll reading of the SPAD and the mensuration variables in a one- year old plantation for each of the evaluated clones of *Gmelina arborea* Roxb.

		Clone									
		1	**2**	**3**	**6**	**8**	**9**	**10**	**12**	**15**	**16**
SPAD	DAP	0.39	-0.12	-0.04	-0.21	0.34	0.17	0.69	0.80	-0.12	-0.26
	HT	0.62	0.31	0.28	0.65	0.81	0.14	0.01	0.62	-0.18	0.12
	VT	0.49	0.01	0.03	-0.03	0.50	0.18	0.62	0.81	-0.18	-0.21

= High correlations (x>0.60); = Medium correlations (0.30>x<0.60); = Low correlations (x< 0.30).

DAP = Diameter at Breast Height; HT = Total height; VT = Total volume.
Significant = $p \leq 0.05$.

Clones 12 and 10 achieved the highest correlations between SPAD - DAP and SPAD - HT; clones 1 and 8 showed mean values for the same correlations, and the remaining genotypes, very low values between the SPAD reading and all the mensuration variables (<0.18).

This new genotype grouping pattern led to a stratification according to the level of correlation between SPAD and Total Volume (VT), high (x> 0.60), medium (0.30> x <0.60) and low (x <0.30). The identified pattern suggests that the genotypes behave with a group tendency. The results of the correlation test, separately for each of the groups determined above, are in Table 7.

Table 7. Pearson correlations for each of the sets formed from the correlation between SPAD and total volume (VT) of *Gmelina arborea* Roxb.

		High (10-12)	Medium (8-1)	Low (2-3-6-9-15-16)
SPAD	DAP	0.65	0.30	-0.16
	HT	0.25	0.75	0.0012
	VT	0.63	0.45	-0.14

DAP = Diameter at Breast Height; HT = Total height; VT = Total volume. Significant = $p \leq 0.05$.

Clones with high correlation of SPAD with DAP and VT (clones 10 and 12) were recorded. This result suggests the possibility of finding a relationship between the chlorophyll content in the nursery and the growth variables in the field, which contribute as an aspect in the early selection of clones. However, care must be taken in the interpretation of these correlations since it is necessary to establish specific parameters for each species (Mattsson, 1996).

With the exception of the third group of clones, the value was always higher than 0.30 for the correlations between chlorophyll and the mensuration variables; the low values of correlations can be explained by the weak genetic expression at seedling level in nursery *vs* its performance in plantation (Ávila, 2013). The number of clones evaluated also influenced the estimation of the correlations, as well as what was mentioned regarding the genotype - environment interaction, including at the micro - site level (Puttonen, 1989, Mattsson, 1996). Some studies were conducted in controlled environment conditions (Mattsson, 1996; Karisson *et al*., 2002; Jansson *et al.,* 2005), as well as in combinations of juvenile features in the nursery measurement (Jansson *et al.,* 2005), which could increase the correlations with the development in the field. On the other hand, Jansson *et al.* (1998) suggest the need for genomic studies to identify the genes that regulate the growth of juveniles and adults. This

confirms the complexity of trying to predict the behavior of genotypes in the field, first identifying variables - parameters - reliable indicators (Mattsson, 1996).

Conclusions

The reading of chlorophyll with the portable meter recorded the lowest coefficient of variation of all the variables studied, which suggests that it could provide consistent data. Clones 8 and 3 reached extreme values in the chlorophyll content and showed a more homogeneous behavior both in chlorophyll levels and in the correlation between SPAD-Chl-T. On average, for all genotypes of assessed *Gmelina arborea*, the SPAD-Chl-T correlation was 0.52, which is considered moderate for forest species.

The possibility of predicting laboratory chlorophyll content from portable meter readings is latent, using a linear regression model mainly for clones 8 and 10. Clones 10 and 12 achieved the highest correlations between SPAD with DAP and VT, which leaves open the possibility of being field performance variables that can be used to select early superior genotypes, from easily measured variables such as the chlorophyll determined with the portable meter in the nursery stage.

Finally, it is necessary to develop and validate a standardized methodology for obtaining chlorophyll content at early ages in *melina* clones, mainly due to the characteristics of the experimental material that is to be evaluated.

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Conflict of interest

The authors declare no conflict of interests.

Contribution by author

Katherine Barrantes Madrigal: Chlorophyll data processing, writing of the manuscript, design of tables and figures; Carlos Ávila Arias: production of plants for their assessment, collection and analysis of information on growth of the species, writing of the manuscript and design of tables; Rafael Murillo Cruz: production of plants for evaluation, collection and analysis of information on growth of the species and writing of the manuscript; Laura Solís Ramos: sample collection and processing, generation of chlorophyll data and writing of the manuscript; Romano Porras Murillo: sample collection and processing, generation of chlorophyll data and writing of the manuscript; Pablo Herrera Vargas: sample collection and processing, generation of nitrogen data and writing of the manuscript.