Early Selection Potential of *Gmelina arborea* Roxb. Clones Based on Physiological Correlations of Their Nursery-Field behavior

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Abstract

The development of superior genetic materials points out the need to identify parameters for early selection of superior genotypes. The aim of this research was to identify possible correlations between young-adult materials, based on their photosynthetic capacity determined in five genotypes evaluated at nursery and 15-months-old field stages. Light response curves and gas exchange were generated. Physiological behaviors of five clones were assessed, both at rooted mini-cuttings stage and at 15-months old field stage, where 5 trees (ramets) per clone in each of both stages were evaluated. The water use efficiency in nursery (WUE-N) reported positive and moderate to strong correlations for all genotypes, with respect to P_n*-F (net photosynthesis in field) and WUE-F. The same parameter in the field (WUE-F) correlated moderately and directly with P n-F (r = 0.5), whereas* G_s*-F and E-F was inversely and strongly correlated (r = -0.66 and r = -0.82 respectively). The chlorophyll content in rooted mini-cuttings leaves (Cloro-N) correlated directly with* P_n*-F, which suggests that a measurement of chlorophyll content could identify genotypes with a higher net photosynthesis later on in the field. These results evidence the possibility of early selection of G. arborea superior genotypes using physiological parameters.*

Keywords: *Gmelina arborea*, early selection, young-adult correlations, photosynthetic capacity, Costa Rica

Resumen

La selección de materiales genéticos superiores puntualiza la necesidad de identificar parámetros con posibilidad de selección temprana. El objetivo de la investigación fue identificar posibles correlaciones juvenil-adulto en cinco clones de Gmelina arborea, basadas en la evaluación de su capacidad fotosintética en estadío de vivero y a los 15 meses de edad en campo. Se generaron curvas de respuesta a la luz e intercambio de gases. El comportamiento fisiológico de los cinco clones fue evaluado en el estado de mini-estaquilla enraizada en vivero, así como como en campo a los 15 meses de edad, donde 5 individuos (rametos) de cada clon en cada estadío fueron evaluados. La eficiencia en la utilización del agua en vivero (WUE-V) reportó correlaciones positivas de moderadas a fuertes, para todos los genotipos, entre P_n*-C (fotosíntesis neta en campo) y WUE-C. El parámetro WUE-C a los 15 meses en campo correlacionó moderada y directamente con* P_n*-C (r = 0,5) así como con* G_s*-C (conductancia estomática en campo) y E-C (transpiración en campo)(correlación inversa y fuerte r = -0,66 yr = -0,82 respectivamente). El contenido de clorofila en las hojas de las mini-estaquillas enraizadas en vivero (Cloro-V) correlacionó directamente con* P_n*-C. Lo que sugiere la posibilidad de que con una medición del contenido de clorofila, podría ayudar en la identificación temprana de genotipos que podrían presentar altas tasas fotosintéticas en campo. Los resultados obtenidos evidencian la posibilidad de identificar parámetros fisiológicos con los cuales se realice selección temprana de genotipos superiores en esta especie.*

Keywords: *Gmelina arborea, selección temprana, correlaciones joven-adulto, capacidad fotosintética, Costa Rica*

Introduction

The development of superior genetic material points out the need to identify the criteria for early selection of genotypes with fast growth at an early age. Morphological and physiological parameters correlate primarily with growth rates in field have been viewed and analyzed as early indicators of development in the field of superior genotypes. The development of systems for early selection may be useful to shorten improvement cycles and thereby increase the gain per unit time (Zas et al., 2005), without sacrificing genetic gain (Adams et al., 2001). The main utility of selection at early age is to remove genotypes that register values in the desirable parameters below acceptable levels, before the establishment of field trials, so that the cost is reduced and efficiency is statistically increased (Adams et al., 2001).

To Zas et al. (2005), comparing the juvenile behavior in nursery and adult behavior of genotypes in field by testing pre-installed, ie retrospective studies, becomes the main alternative in the search for appropriate juvenile characters that maximize genetic correlation nursery-field.

However, it is noted that the correlation between morphological and physiological parameters and growth in the field are generally poor (Blake and Bevilacqua, 1990). The youth-adult correlations based on physiological parameters are generally very low, highly variable between sites and even negative (Zas et al., 2005), mainly in young plantations since the genotype has not expressed some of his traits as a result of genotype-environment interaction at such early ages (Adams et al., 2001;Codesido et al., 2012). Among the main reasons offered to explain this behavior found in previous works (Blake & Bevilacqua, 1990; Adams et al., 2001; Flood et al., 2011;Aspinwall et al., 2011) involves the evaluation of materials genetic extreme greenhouse conditions, the small number of evaluated genotypes, small experimental samples and the strong interaction genotype x environment primarily of trees in the field. Retuerto et al. (2003) justifies small samples in most technical difficulty in collecting data on physiological processes, so most studies have focused primarily on morphological characters based components. However, one of the main mistakes people make is to try to extrapolate results from small time scales to large time scales (Retuerto et al., 2003).

Moreover, results from retrospective studies in which the performance of families in early assessment compared with the same trial previously established field, have been reported for conifers (several authors cited by Adams et al., 2001), usually showing weak to moderate correlations. Therefore, the question of interest is: what is the magnitude of nursery-field, or juvenile-adult correlation to expect and what variables are more appropriate for retrospective analysis?

The objective of this research was to identify possible correlations on youth – adult individuals, based on the photosynthetic capacity of five genotypes evaluated in greenhouse and the field, as a potential tool for early selection of genotypes of *Gmelina arborea*. Five genotypes were evaluated in both greenhouse and field at 15 months of age by a retrospective analysis.

Materials and Methods

Experimental Material

The plant material used in this study to evaluate the photosynthetic behavior, both in the nursery and in the field, consisted of five genotypes of *Gmelina arborea*, which form part of the collection of genetic improvement program of the National Forest Research Institute of National University (INISEFOR-UNA) in Costa Rica.

Evaluation at nursery stage

The production of *Gmelina arborea* materials were conducted in the nursery of vegetative propagation of INISEFOR-UNA, south Pacific of the country. These were reproduced from clonal gardens, following usual protocols used by INISEFOR-UNA (Abrahams & Vassart, 2011). The mini-cuttings were evaluated in their first growth period, that is ready to leave the mini-tunnels rooting acclimation to the acclimation area (semi-open nursery).

Field evaluation

Trees assessed were derived from a clonal trial established by the INISEFOR-UNA in September 6, 2011 The trial is an experimental design of randomized complete block, where 15 clones were tested are part of the genetic collection INISEFOR-UNA. Six individuals were established for each clone by block, in pairs (three pairs of each clone per block and repeated in six blocks). An individual of each of the five genotypes in each of blocks 1 to 5, for a total of 25 trees were evaluated. Trees were evaluated when they turned 15 months old.

Gas exchange measurement

Measurements were done on clear days with an air temperature of 30 °C within the cuvette. In both stages investigated (rooted mini-cuttings in nursery and plantation at 15 months) assessment of gas exchange was performed using a portable photosynthesis system (CIRAS-2, PP Systems, USA) (Figure 3) with a cuvette at a concentration PLC6 366.16 ± 7.05 ppm CO_2 and light intensity of 1000 µmol m^{-2} s^{-1}, to prevent photo inhibition (Evans & Poorter, 2001). The physiological parameters obtained for the determination of possible correlations between the two stages were: net photosynthesis (A_n = µmol CO_2 m^{-2} s^{-1}), stomatal conductance (G_s = mmol H_2O m^{-2} s^{-1}), transpiration (E = µmol H_2O m^{-2} s^{-1}) and water use efficiency (WUE = µmol H_2O m^{-2} s^{-1}). In the case of nursery rooted mini-cuttings, measurements were performed in the first new pair of leaves with full development of each plant (Evans & Poorter 2001), these were labeled for assessment five times at different times of day. For the stage of plantation at 15 months of age the gas exchange measurements were performed on a fully expanded leaf, at the third pair of a branch located in the top tier of the crown (Evans & Poorter, 2001; Rojas et al., 2012). In both stages of development of the clones five ramets per genotype were analyzed.

Determining the amount of chlorophyll

Chlorophyll measurement was performed using the SPAD-502, in the first new set of blades with full development of each plant. Five ramets were evaluated for each genotype and the average was reported.

Determination of the percentage of nitrogen in leaves

The percentage of nitrogen in rooted mini-cuttings in nursery (% N-N) was obtained as follows:
% N-N: digesting of 0.1 grams in five leaves from different plants of each genotype was performed. Subsequently each of the samples were allowed to cool and water were added. Finally these were dubbed with H_2SO_4 and the based on mm of sulfuric acid the % of nitrogen is calculated.
Gas exchange and total height for the rooted mini-cuttings (H_t-N) were measured parallely; height was measured from the base to the apex using a ruler.

Growth parameters evaluated in field

A census of all genotypes of the field test was performed, from which an average for each of the five genotypes in this study was obtained, namely the average value of each of the parameters detailed below, for each block and for each genotype:

- Diameter breast height (DBH) was measured normal diameter (1.3 m) for each tree using diameter tape (cm).
- Overall height (H_t) was estimated by using the clinometer, getting the angle to the base of the tree and the other to the apex thereof, from a distance of 10 m for all observations. In office, using a mathematical formula that height is obtained (m).
- Total volume with bark (V_t) was estimated by the function (m^3 $tree^{-1}$):
  $V_t = (DBH/100)^2 * 0.7854 * H_t * 0.65$

The parameters evaluated for each genotype in both conditions (nursery/field), from which the correlations were determined nursery are presented in table 1.

Table 1: **Evaluated variables at nursery and field to determine correlations.**

Nursery variables		**Field variables**	
Acronym	**Definition**	**Acronym**	**Definition**
P_n-N	Nursery Net photosynthesis	P_n-F	Filed Net photosynthesis
G_s-N	Nursery Stomatal conductance	G_s-F	Field Stomatal conductance
E-N	Nursery Transpiration	E-F	Field Transpiration
WUE-N	Nursery Water use efficient	WUE-F	Field Water use efficient
%N-N	Percentage of nitrogen in nursery	DBH-F	Diameter at breast height (cm)
Cloro-N	Chlorophyll content at nursery	H_t-F	Total height at field (m)
H_t-N	Total height at nursery (cm)	V_t-F	Field total volume(m^3 $tree^{-1}$)

Correlations

A correlation matrix was built with the average value of each clone investigated, for each of the evaluated physiological parameters for young (rooted mini-cutting) - adult (plantation at 15 months old).

From a matrix that contained five data for each one of the determined parameters per each genotype, juvenile - adult (nursery - field) quantitative correlation tests were performed. The correlation study used Pearson correlation coefficient between the means of the parameters evaluated. Correlations were reported and analyzed at two levels:

1. at general level for the species, in a single correlation matrix that places the results for characters in nursery on an edge of the array and the field results on another, leaving Pearson correlation values equal or greater than 0.50 ($r \geq 0.50$) which are considered significant (Figure 16).
2. at the level of genotypes, for which a box containing the coefficients of Pearson correlation is presented for each of the parameters evaluated in the nursery vs all values evaluated at the field for each genotype (Table 7).

In total 7 variables were evaluated both in the nursery and in the field, ie a total of 49 possible correlations between youth - adult (Table 7).

Results and Discussions

The matrix used for correlations included five records for each parameter measured in each genotype. Table 2 presents the average value of these five data recorded for each parameter and each genotype investigated.

Table 2: **Physiological average values at nursery and field stage, in five *Gmelina arborea* superior genotypes (clones) utilized in the determination ofjuvenileto15-months-oldearly selection, south Pacific of Costa Rica.**

Clone	Nursery variables							Field variables						
	P_n-N	G_s-N	E-N	WUE-N	%N-N	Cloro-N	H_t-N	P_n-F	G_s-F	E-F	WUE-F	DBH	H_t-F	V_t-F
1	8,14	120,97	2,13	4,17	2,97	22,58	6,35	19,47	185,53	3,55	5,62	10,57	7,94	0,0445
2	9,19	150,40	2,47	3,82	2,55	23,22	6,02	22,62	230,27	4,00	5,76	11,60	8,18	0,0525
5	10,15	134,03	2,31	4,53	2,23	24,92	3,90	21,36	289,53	4,98	4,31	11,92	8,47	0,0573
6	10,45	154,40	2,48	4,26	2,85	23,98	6,48	19,73	270,73	4,74	4,70	11,40	8,12	0,0509
12	10,24	154,37	2,61	4,02	2,83	23,16	7,14	21,15	273,60	4,61	4,76	11,38	8,18	0,0504

According to Ali et al. (2011), the information on correlations between characters identified provides an opportunity for early selection of genotypes with desirable traits simultaneously. In this regard, the mandate of physiology is to understand how plants function (Dickman 1991), once it is understood the process knowledge can be applied to both, early selection to complete silvicultural packages. Pearson correlation matrix is presented in general terms for the species (Figure 1).

P_n-F	**P_n-F**												
G_s-F	0.12 (0.57)	**G_s-F**											
E-F	-0.03 (0.90)	**0.92 (5.5E-11)**	**E-F**										
WUE-F	**0.50 (0.01)**	**-0.66 (3.6E-04)**	**-0.82 (4.3E-07)**	**WUE-F**									
P_n-N	0.001 (1)	0.27 (0.18)	0.32 (0.12)	-0.15 (0.46)	**P_n-N**								
G_s-N	-0.35 (0.09)	0.14 (0.51)	0.18 (0.38)	-0.28 (0.17)	**0.64 (5.6E-04)**	**G_s-N**							
E-N	-0.37 (0.07)	0.07 (0.75)	0.12 (0.56)	-0.26 (0.21)	**0.61 (1.1E-03)**	**0.97 (0.00)**	**E-N**						
WUE-N	**0.45 (0.02)**	0.12 (0.56)	0.10 (0.65)	0.19 (0.36)	0.07 (0.73)	**-0.67 (2.4E-04)**	**-0.71 (6.2E-05)**	**WUE-N**					
%N-N	-0.38 (0.06)	-0.22 (0.31)	-0.16 (0.45)	-0.13 (0.55)	-0.05 (0.80)	0.26 (0.23)	0.28 (0.19)	-0.33 (0.12)	**%N-N**				
CLORO-N	0.28 (0.19)	0.09 (0.67)	0.20 (0.36)	0.02 (0.92)	0.10 (0.64)	-0.26 (0.22)	-0.19 (0.38)	0.22 (0.29)	-0.33 (0.11)	**CLORO-N**			
H_t-N	-0.03 (0.87)	0.18 (0.41)	0.14 (0.51)	-0.10 (0.63)	0.20 (0.35)	**0.42 (0.04)**	0.33 (0.12)	-0.27 (0.20)	**0.41 (0.05)**	-0.31 (0.13)	**H_t-N**		
DAP-F	0.01 (0.95)	-0.0034 (0.99)	3.2E-03 (0.99)	-0.08 (0.71)	-0.03 (0.91)	0.19 (0.35)	0.16 (0.43)	-0.35 (0.08)	-0.28 (0.18)	-0.05 (0.82)	-0.07 (0.73)	**DAP-F**	
H_t-F	0.13 (0.55)	-0.05 (0.82)	-0.08 (0.69)	0.07 (0.74)	-0.15 (0.46)	-0.18 (0.38)	-0.20 (35)	-0.07 (0.73)	**-0.41 (0.05)**	0.12 (0.57)	-0.15 (0.47)	**0.74 (2.5E-05)**	**H_t-F**
V_t-F	0.07 (0.76)	-0.03 (0.87)	-0.03 (0.89)	-0.03 (0.89)	-0.10 (0.63)	0.02 (0.92)	-0.01 (0.97)	-0.20 (0.33)	-0.32 (0.12)	-0.04 (0.87)	-0.12 (0.56)	**0.97 (0.00)**	**0.82 (4.4E-07)**

***Figure 1*: Pearson correlation matrix between juvenile and15-months-old physiological parameters evaluated in five *Gmelina arborea* superior clones, south Pacific of Costa Rica.**

Significative correlations were identified between the variables evaluated neither at nursery vs field stage, which could be considered as strong nor of direct magnitude (+) or inverse magnitude (-). The most important correlations, both direct and inverse, occurred between variables in the same condition, ie nursery vs nursery or field vs field. Moderate correlations, both direct and inverse, were identified among the variables evaluated at nursery and field stage in *Gmelina arborea* clones (Figure 1). Correlations described below apply only to the conditions of the material evaluated, ie the rooted mini-cuttings ready to leave the mini-tunnels acclimation to the area and trees in the field at 15 months old. These should not be extrapolated to rooted cuttings and acclimated on any other condition. Retuerto et al. (2003) points out that much of the experimental work on ecophysiology fails by not considering time as an important variable, which often leads them to assume that the responses observed at short time scales are directly extrapolated at longer time scales. The genotype/environment interaction on field trials is the most important factor in the lack of young/mature correlations in forest plantations as field conditions are a complex system of inter-related factors (Codesido et al., 2011; Aspinwall et al., 2011).

The net photosynthesis rate (P_n) has always been of great interest to be used as an index of growth potential in the field (Dickman, 1991; Combalicer et al., 2010). Hence, it becomes one of the most important parameters to find correlations. For the present study of photosynthesis in nursery (P_n-N) correlated directly with stomatal conductance (G_s-F) and transpiration (E-F) in the field at a rate of r = 0.27 and r = 0.32 respectively. According to Lewis et al. (2011), stomatal conductance regulates the rate of photosynthesis, by which direct relations between the two characters have been recorded for a wide range of species and environmental conditions. This suggests that the higher net photosynthesis in the nursery, the higher stomatal conductance and transpiration in the field clones. These two variables are directly linked to the photosynthetic capacity of the plant, so that indirectly a higher net photosynthesis in the nursery would be linked to greater photosynthesis in field (P_n-F).The relationship between field and nursery photosynthesis was classified as poor, mainly due to three reasons: 1 acclimatization of plants in nursery to only an average PAR of a third of their light saturation point so it was not allowed to express its photosynthetic capacity, 2 evaluating genotypes at such young ages, 3 the trend of using the photosynthetic rate per unit area in a few leaves (Flood et al., 2011). The P_n and G_s variables correlate closely since the decline of photosynthetic activity in response to the lack of water is a result of stomatal closure at high irradiation levels and temperature (Corcuera et al., 2005). Meanwhile, Combalicer et al. (2010) identified four tropical species, with a strong correlation between the diameter of the neck of the plants (in the initial months) and the rate of net photosynthesis; for which including this variable in future work should is needed, in addition, the increase in the diameter of the tree at any height is strongly correlated with the accumulation of biomass on species (Combalicer et al., 2010).

Stomatal conductance (G_s) is a variable which indirectly indicates the level of stomatal opening (López et al., 2007), ie the higher the value greater G_s is gas exchange and therefore the greater the rate of photosynthesis, provided that the other physiological variables are not altered. However, the G_s-N moderately and inversely correlated with net photosynthesis (P_n-F) and water use efficiency (WUE-F), both in the field. That is, the lower the G_s-N higher P_n-F and WUE-F. Lower G_s-N implies that trees sacrifice at some degree, the development of assimilates to achieve a lower transpiration, by causing stomatal closure which abruptly creates a reduction of photosynthesis and water loss by transpiration simultaneously (González et al., 2009; Fernandez et al., 2010; Rojas et al., 2012). Upon evaluating the rooted mini-cuttings in the rooting mini-tunnels (Figure 2) the water supply is minimal which could explain the low rate of G_s-N recorded. As in the study by Aspinwall et al. (2011) in pine, in this investigation no correlation of G_s was recorded with any of the growth parameters. Sojka et al. (2005) indicate that there is still no complete understanding of the biochemical and physiological mechanisms involved in stomatal closure, however, the same authors suggest that by itself, a deficiency in potassium can prevent stomatal opening in corn, so it becomes one more factor to consider in studies of forest species and their relationship to photosynthetic processes. In addition there may be no stomatal factors involved in conductance for *Gmelina arborea*, like CO_2 concentration and some chemical signals (Farquhar & Sharkey, 1982; cited by Rojas et al., 2012).

Transpiration is an important parameter for decision-making, critical for determining the water use efficiency of plants and therefore directly linked to the sensitivity of each species to genotype and water stress. Corcuera et al. (2005) conclude that stomatal closure is essential to reduce water loss through transpiration mechanism. For this research nursery transpiration (E-N) was inversely correlated with net photosynthesis (P_n-F), water use efficiency (WUE-F) and total tree height (H_t-F), all of these at field conditions. That is, at lesser transpiration at the nursery, the greater nursery P_n-F, WUE-F and H_t-F.

This value is important because it could identify genotypes from the nursery with a lower E-V and eventually bind such value with higher P_n-F, WUE-F and H_t in the field. A study in four species showed that eucalyptus genotypes which grew faster had greater ability to limit water loss through transpiration, compared to slower growing clones, resulting in increased water use efficiency of ie fixing more carbon in photosynthesis per unit water transpired (Blake & Bevilacqua, 1990). Rojas et al. (2012) reported that transpiration rates decreased with age for *Gmelina arborea* genotypes.

Water use efficiency (WUE) is an important parameter because it identifies genotypes that have equal or greater production with less water. Furthermore, high efficiency on the use water suggests that the plant exhibits a high photosynthetic efficiency (Blake & Bevilacqua, 1990). The evaluation of this parameter in the nursery (WUE-N) reported a correlation value of r = 0.45 with field photosynthesis (P_n-F). This value suggests that genotypes in the nursery to record high values of WUE-N, will present high P_n-F values in the field and probably higher growth explained by an ability to extend its effective growth rate during the year. This correlation and the previously mentioned are identified as the most significant of this research. The importance of WUE as a parameter to correlate growth and development is reflected in the study of Blake and Yeatman (1989), cited by Blake and Bevilacqua (1990), who investigated how the vigorous growth of genotypes of fast growing pine was explained by the efficient use of water in the field, concluding that families which had higher WUE (directly linked to low E) exhibited greater absolute and relative growth; where positive correlations between stomatal conductance, transpiration rate with the development of stem and roots were also recorded. More efficient use of water indicates that genotypes with higher growth rates are usually the most water demanding, using only a small part of it in photosynthesis with most sent into the atmosphere as water vapor form (several authors, cited for Vanclay, 2008). Another important criterion in breeding programs is that the genotypes may have high efficiency water despite low photosynthetic rates, to efficiently prevent water loss stomatal closure (Gonzalez et al., 2009).

The measurement of physiological parameters should be performed in different stages at nursery level, where one can control other environmental conditions that do not increase the experimental error, and then to correlate with field parameters easily measured. With this, a better selection of genotypes that manifest greater plasticity conditions limiting field could be made (Dickman, 1991); such as water stress which would survival and better competition in different environmental conditions (Corcuera et al., 2005). The percentage of nitrogen in the nursery (%N-N) correlated inversely with P_n-F, G_s-F and the three growth variables (DBH-F, H_t-F, V_t-F). This relationship suggests that at the stage evaluated nursery plants invest their assimilated primarily in the formation of tissues such as roots rather than the leaves. This observation would indicate that many plants that invested in the leaves are put in the field with a smaller root system, which could explain the inverse correlation nursery vs field for the above variables. Another possible aspect that could influence the low amount of nitrogen in plants in the nursery was the limited amount of water available in the mini-tunnel rooting environment, which could limit the absorption of nitrogen and also magnesium. A similar situation was reported in the study of Blake and Bevilacqua (1990) with four species of eucalyptus. However, Evans and Poorter (2001) concluded in their research that photosynthetic capacity is positively and linearly related to the nitrogen content per unit leaf area.

Besides the above nursery correlations nursery/field other relations of importance as nursery/nursery and nursery/nursery-field are to be noted such as the efficient use of water in the field (WUE-F) which correlated moderately and directly with the P_n-F (r = 0.5). Farquhart and Richards (1984), cited by Corcuera et al. (2005) suggest that WUE is generally highly correlated with growth, but this relationship may be direct or inverse; a direct relationship between WUE and productivity has been observed in the eucalyptus. While an inverse relationship can be present in genotypes that record high efficiency of water despite low photosynthetic rates, to efficiently prevent water loss by stomatal closure (González et al., 2009; Pimienta et al., 2012). Besides the correlation of WUE with G_s-F and E-F was inverse and strong, r = -0.66 and r = -0.82 respectively. These data are consistent with the operation of the physiological processes in most plants. A more efficient use of water represents a higher rate of photosynthesis and reduced transpiration rate. To identify strong and direct correlations between two desirable traits implies the possibility to achieve indirectly a selection on the genotypes evaluated. The same relationship was present on WUE-N with P_n-N and E-N, where at higher water use efficiency a higher rate of photosynthesis was presented as well as a reduced transpiration rate; these are expected correlations since WUE is an estimated variable from P_n/E ratio. The same trend in the field and nursery provides high reliability of the results obtained for both conditions. Corcuera et al. (2005) note that the ability to produce numerous roots that are widely spread in the soil profile is associated with increased resistance to water stress and therefore improved WUE.

Another correlation is presented both in the nursery and in the field was between G_s and E. In both conditions the correlation was very strong and positive (r = 0.97 nursery, field r = 0.92). These results show that at greater stomatal opening that promote gas exchange, the greater the loss of water through transpiration. This behavior between both parameters suggests that those genotypes could present strategies to avoid and tolerate the stress of high transpiration, which could change for both physiological and morphological characters (Fernández et al., 2010).

In Figure 2 the degree of similarity in the distribution of the two characters per genotype is seen, which explains the high correlation level. Combalicer et al. (2010) also recorded for four tropical species a correlation coefficient between G_s and E (r=0.97) in the field. Meanwhile the high correlation coefficient between G_s-N and P_n-N (r = 0.64) highlights the direct relationship between stomatal aperture and gas exchange with the net photosynthetic rate, considering that the increased stomatal opening is needed to increase the rate of CO_2 diffusion inside the leaf (Flores, 2012). This consistency in the results also indicates that the experiment was developed properly and still leaves latent the hypothesis that it is possible to find physiological variables that can be used to carry out early selection of genotypes of interest.

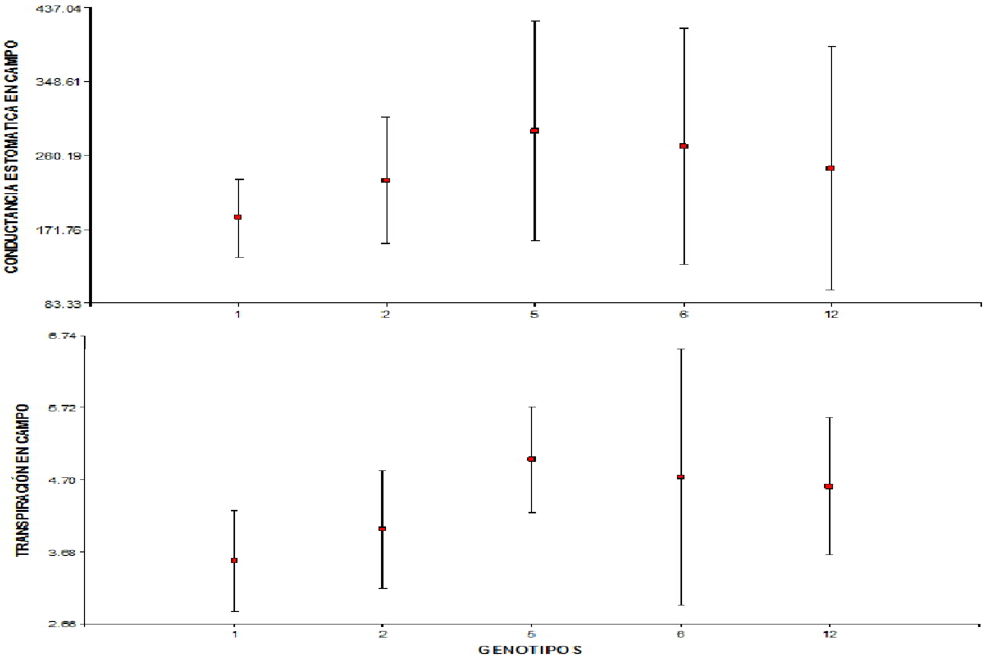

***Figure 2*: Overall average and standard deviation for stomatal conductance (top) and transpiration (below) for each *Gmelina arborea* genotype evaluated.**

Observed correlations between the height of the rooted plants before acclimation (H_t-N), both stomatal conductance at nursery (G_s-N) and percentage of nitrogen at nursery (%N-N) (H_t-N vs G_s-N = 0.42 and H_t-N vs %N-N = 0.41) were direct, suggesting that at higher G_s-N and %N-N the higher H_t-N will be. To cover these aspects in the process of vegetative production is paramount, as this may prevent mortality and quality issues with plants by only knowing the growth potential of each clone in the nursery by the values obtained for G_s-N and %N-N . Flores reported opposite results (2012) with a negative correlation for H_t-N vs G_s-N (r = -0.16). However, the H_t-N showed no utility in the final objective by not registering correlation with any of the variables in the field. Adams et al. (2001) reported in Douglas-fir (*Pseudotsuga menziesii*) in Oregon - USA, evaluated at 15 years of age, correlations between H_t-F and H_t-N ranging from 0,05 to 0,54, showing the great variability of correlations that can be registered. Blake and Bevilacqua (1990) confirm the difficulty of using the H_t-N for early selection, when determining that the size differences in the time of planting proved poor indicators of growth and yield in four species of eucalyptus, both from seed as from vegetative produced material.

Flores (2012) evaluated five genotypes of *Gmelina arborea* in the Colombian Caribbean region, where he identified high correlations between morphological characters in nursery, mainly between the dry weights of plant tissues. He also noted that leaf area is an important variable for growth in these conditions, as it presented a high correlation with height and stem dry weight.

Likewise Fiallos and Forcelini (2011) point out the importance of taking into account the leaf area as a selection indicator of early to find strong correlations with growth traits. Nevertheless, Flores (2012) notes that very low correlations between physiological characters were presented, except for the P_n-N with WUE-N, as well as between physiological and morphological characters similar to that obtained in this investigation. The previous paragraphs described some of the general correlations observed between the variables evaluated in greenhouse and field five genotypes of *Gmelina arborea; a*lthough they are low and/or poor in many cases, it is shown that the nursery-field correlations can be useful in tree improvement programs (Adams et al., 2011). Correlations related to P_n are the most important. Several investigations of photosynthesis in forest species have shown useful information on growth (Orlovich et al., 2006; Han, 2011). Correlations recorded for genotype at each condition are presented in table 3 through table 9, which aims at improving the accuracy of silvicultural production packages both for the species and for the specific genotypes. Correlations with consistency between genotypes were highlighted (with the same sign and similar magnitude preference for at least four of the five genotypes).

Table 3: Correlations between net photosynthesis in the nursery (P_n-N) and 15-months-old field parameters evaluated in five *Gmelina arborea* genotypes, south Pacific of Costa Rica.

NurseryVariables	Field Variables	Genotypes				
		1	2	5	6	12
P_n-N	P_n-F	0.46	-0.43	0.09	-0.54	0.46
	G_s-F	-0.31	-0.38	-0.38	0.85	0.14
	E-F	-0.26	-0.58	-0.73	0.82	0.19
	WUE-F	**0.54**	**0.57**	**0.70**	**-0.68**	**0.16**
	DAP-F	-0.12	0.05	-0.93	-0.90	-0.27
	H_t-F	-0.60	-0.24	-0.44	-0.85	-0.08
	V_t-F	-0.11	-0.11	-0.95	-0.89	-0.23

P_n-N showed direct correlation with WUE-F for four genotypes, three of them were strong (clones 1, 2 and 5 with r values of 0.54, 0.57 and 0.70 respectively) and weak for clone 12 (r = 0.16). P_n-N correlated inversely with the three dasometric variables (DBH-F, H_t-F and V_t-F), which is a sign of consistency in the recorded values; this correlation suggests that the rooted mini-cuttings in the nursery at the time of evaluation, were not prepared nor had the ideal conditions to express their photosynthetic potential. While once in the field this changed, hence the inverse relationship to show higher dasometric values in the field compared to photosynthesis in the nursery. More research should be carried out, since similar correlations for different genotypes does not mean that the same values will be present in other site conditions (Adams et al., 2001).

Table 4: Correlations between stomatal conductance registered at nursery stage (G_s-N) and 15-months-old field parameters in five genotypes of *Gmelina arborea*

Nursery Variables	Field Variables	Genotypes				
		1	2	5	6	12
G_s-N	P_n-F	-0.64	-0.42	-0.73	-0.83	0.21
	G_s-F	-0.68	-0.18	-0.51	0.74	0.66
	E-F	-0.57	-0.20	-0.75	0.73	0.74
	WUE-F	0.14	0.04	-0.21	-0.77	-0.56
	DBH-F	0.80	0.44	-0.33	-0.64	-0.87
	H_t-F	0.31	-0.47	-0.31	-0.70	-0.70
	V_t-F	0.77	0.23	-0.41	-0.66	-0.83

The G_s-N showed a strong inverse correlation with P_n-F and H_t-F, in both cases. This suggests that, when evaluating the rooted mini-cuttings in the nursery there were no large flows to the leaves, and one might assume that they were investing more resources on root system formation. Retuerto et al. (2003) indicate that plants can express priorities in the formation and / or recovery of tissues, structures or components. They can change the fraction of biomass invested in leaves, stem and roots (Evans & Poorter, 2001). So the assumption made should be corroborated with research to determine which tissues in the plant accumulate more biomass in these stages (Lopez et al., 2007). At field level, fluids flow was recorded to maintain the physiological processes of the plant, hence the inverse correlation. Blake and Bevilacqua (1990) suggest that maintenance of increased stomatal conductance in some genotypes, until the end of the growing season, may explain their higher rates of growth.

Table 5: Correlations between transpiration registered in the nursery (E-N) and field parameters evaluated for five genotypes of *Gmelina arborea* in south Pacific of Costa Rica.

Nursery Variables	Field Variables	Genotypes				
		1	2	5	6	12
E-N	P_n-F	-0.65	-0.46	-0.69	-0.85	0.11
	G_s-F	-0.61	-0.25	-0.60	0.81	0.40
	E-F	-0.52	-0.27	-0.84	0.78	0.50
	WUE-F	0.07	0.09	-0.10	-0.79	-0.42
	DBH-F	0.77	0.37	-0.40	-0.50	-0.85
	H_t-F	0.29	-0.42	-0.40	-0.67	-0.74
	V_t-F	0.72	0.16	-0.48	-0.55	-0.83

As with the G_s-N, and strong inverse correlations were registered with P_n-F and E-F and H_t-F, which is very consistent with the literature, suggesting that at lower the E-N, the higher P_n-F and H_t-F will be. According Corcuera et al. (2005) this inverse correlation is due to the fact that stomatal closure is essential to reduce water loss through transpiration mechanism, in order to avoid reduction on the rate of photosynthesis. The E-N is a single easily evaluated variable at nursery stage, which could be very useful as an early selection parameter of potential superior genotypes. It correlated as strong as expected, with those two very important characters in the field (P_n-F and H_t-F).

Table 6: Correlations observed between water use efficiency at nursery stage (WUE-N) and 15-months-old field parameters, evaluated in five genotypes of *Gmelina arborea* in south Pacific of Costa Rica.

Nursery Variables	Field Variables	Genotypes				
		1	2	5	6	12
WUE-N	P_n-F	0.94	0.26	0.84	0.67	0.21
	G_s-F	0.47	-0.01	0.59	-0.21	-0.57
	E-F	0.41	-0.17	0.75	-0.20	-0.66
	WUE-F	0.21	0.42	0.34	0.39	0.81
	DBH-F	-0.88	-0.52	0.18	-0.38	0.98
	H_t-F	-0.73	0.30	0.34	-0.03	0.96
	V_t-F	-0.84	-0.37	0.27	-0.27	0.98

Another variable of importance to be monitored at nursery for early selection is the WUE-N. This parameter registered direct and moderate to very strong correlations for all genotypes with respect to P_n-F and WUE-F. Besides, strong direct correlation between WUE-N and WUE-F ($r = 0.81$ clone 12) is one of the most interesting on the determination of genotypes that can adapt to reduced water availability conditions. This correlation value was similar to that reported by Flores (2012, $r = 0.93$), also for melina plants acclimated to greenhouse conditions. With respect to the relationship between WUE-N and P_n-F ($r = 0.94$ Clone 1), Farquhart and Richards (1984), cited by Corcuera et al. (2005) suggest that the WUE generally has a high correlation with productivity in the field and this can be positive or negative. A positive correlation between WUE and productivity has been recorded on Eucalyptus.

Table 7: Correlations between foliar nitrogen percentage at nursery stage (%N-N) and 15-months-old field parameters, evaluated in five genotypes of *Gmelina arborea* in south Pacific of Costa Rica.

Nursery Variables	Field Variables	Genotypes				
		1	2	5	6	12
%N-N	P_n-F	0.08	-0.92	-0.35	-0.43	-0.25
	G_s-F	0.40	-0.78	-0.47	-0.18	0.38
	E-F	0.43	-0.64	-0.73	-0.06	0.45
	WUE-F	-0.40	0.19	0.22	-0.29	-0.66
	DBH-F	-0.26	-0.26	-0.74	0.75	-0.67
	H_t-F	-0.70	-0.70	-0.51	0.78	-0.72
	V_t-F	-0.52	-0.52	-0.79	0.80	-0.68

For the% NV four correlations were identified in four of the five genotypes. The %N-N inversely correlated with P_n-F, DBH-F and V_t-F and H_t-F, ranging from moderate to very strong (r = -0.25 to -0.92). These values suggest that, although the nitrogen content in leaves in greenhouse is small, the dasometric development of these genotypes in the field will be high. Possibly because at this initial stage of the plant leaves do not contain enough nitrogen and that is just developing its root to take this nutrient from the substrate where is growing at. The above correlation between %N-N could also be analyzed in the sense that a higher nitrogen content in the leaves of the rooted mini-cuttings in nursery smaller dasometric development in the field. Probably the plant accumulated more nitrogen in their leaves than to form its root system, so it does not go as prepared for field establishment in this first year when the evaluation was conducted. Another aspect that contributes to the explanation is the limited amount of water available in the rooting mini-tunnel environment, which could limit the absorption of nitrogen or magnesium owing to the slow movement of the minerals in the substrate. The same situation occurred in the studies developed by Blake and Bevilacqua (1990) with four species of eucalyptus and Combalicer et al. (2010) with four tropical species. Regardless of the approach given to correlations, further research should be conducted that link the behavior of plants in the nursery with the amount of biomass accumulated on tissues. Plants can manifest priorities during the formation of new tissues or, recover damaged areas in structures (Retuerto et al., 2003), as well as change the fraction of biomass invested in leaves, stem and roots (Evans &Poorter, 2001).

Table 8: Correlations observed between chlorophyll content in nursery (Chloro-N) and 15-months-old field parameters evaluated in five genotypes of *Gmelina arborea* in south Pacific of Costa Rica.

Nursery Variables	Field Variables	Genotypes				
		1	2	5	6	12
Chloro-N	P_n-F	0.29	0.29	0.65	0.13	0.29
	G_s-F	0.14	0.14	-0.15	0.69	-0.73
	E-F	0.26	0.26	-0.12	0.64	-0.64
	WUE-F	-0.22	-0.22	0.83	-0.29	0.74
	DBH-F	-0.38	-0.38	-0.43	-0.71	0.19
	H_t-F	0.21	0.21	-0.34	-0.61	0.19
	V_t-F	-0.23	-0.23	-0.38	-0.68	0.18

Chlorophyll fluorescence has been used as a tool to determine the photosynthetic capacity, mainly in conditions of water stress. The determination of its value is affected by low values of relative humidity and high values of both temperature and irradiation (González et al., 2009), for *Gmelina arborea* it has also been reported to be affected by age (Rojas et al., 2012). In this regard, Pimienta et al. (2012), conclude that, under conditions of drought stress and high temperatures and radiation, decrease in chlorophyll content is considered an adaptive mechanism to decrease the absorption of light and thus reduce photoinhibition. In the present investigation the chlorophyll content in the leaves of the rooted mini-cuttings in nursery (Chloro-N) directly correlated with P_n-F, this pattern is consistently recorded for the five genotypes, indicating that a simple measurement of chlorophyll content in the leaves of the rooted mini-cuttings, using a SPAD-502, could help discriminate genotypes which eventually would record higher net photosynthesis in field. Further research should determine the minimum and maximum levels of this parameter to discriminate and select early.

While the correlation between P_n-F and Chloro-N has been classified as an important criterion in the possibility of early selection, in this investigation the P_n-F showed weak correlations with dasometric characters; so it must be determined in which tissues trees at 15 months of age, are accumulating more biomass as a result of the identified correlation. One possible explanation is that trees planted at an early age could be investing more energy in both the root system and leaf area, in order to colonize as much airspace as both underground and thus win the race for the resource competition. This would give them a competitive advantage in the definitive establishment in the early years. This hypothesis can be stated by the inverse correlation between Chloro-N with V_t-F and DBH-F, for four of the five genotypes. However, this inverse correlation should be analyzed carefully, as it is not certain that trees, with high values of P_n-F values mean higher DBH-F and V_t-F at 15 months of age. Conversely, Combalicer et al. (2010) reported strong correlations for four tropical species in the field between P_n and chlorophyll content, height and collar diameter r = 0.96, 0.51 and 0.55 respectively. Likewise Woo (2003) reported high correlations between net photosynthetic rate and chlorophyll content. Moreover, Retuerto et al. (2003) suggest that reproductive structures act as important sinks of photo assimilates, so attention must be given to the theory of source - sink to explain where these are directed.

Table 9: Correlations between total height of plants in the nursery (H_t-N) and 15-months-old field parameters, evaluated in five genotypes of *Gmelina arborea* in south Pacific of Costa Rica.

Nursery Variables	Field Variables	Genotypes				
		1	2	5	6	12
H_t-N	P_n-F	0.27	0.27	0.39	-0.34	0.42
	G_s-F	0.47	0.47	0.12	0.29	0.96
	E-F	0.30	0.30	-0.27	0.41	0.95
	WUE-F	-0.09	-0.09	0.68	-0.57	-0.57
	DBH-F	0.81	0.81	-0.88	0.03	-0.50
	H_t-F	-0.25	-0.25	-0.08	0.39	-0.27
	V_t-F	0.73	0.73	-0.87	0.19	-0.44

Finally, the total height in nursery (H_t-N) directly correlated with P_n-F, G_s-f and E-F variables, which is consistent with the literature, since with increased photosynthesis there must also be greater stomatal conductance and transpiration from greater stomatal opening. However, Ht-V should not be used for early selection as it has no consistent correlation with dasometric variables. These results reinforce the premise of being cautious when trying to select using information from young material, being better to wait until genotypes are fully expressed phenotype through interaction with environmental conditions.

Furthermore, the identified correlations offer the possibility to further research and improve the selection of genotypes for forest plantations at different soil and climatic conditions. It is necessary to clarify that the study of a single parameter does not guarantee selection, so it's a priority to identify both morphological and physiological parameters useful and practical that contribute to decision-making in early genotypes. The range of variation of each of the parameters identified among genotypes imply different adaptation strategies including what must be taken into account in the selection criteria, and their respective minimum and maximum values, depending on the objectives of breeding program (Fernández et al., 2010). This research has allowed to show at least partially the physiological behavior of five genotypes of *Gmelina arborea*, information that will be useful in future studies, primarily because there are few studies both in nursery and in the field that have incorporated physiological characters of tropical forest species (Retuerto et al., 2003). However, it is necessary to determine whether the physiological variables are heritable through genetic analysis, for which a larger sample (Flood et al., 2011) is needed. The results provided here tested the theory of Dickman (1991) who noted that the analysis of physiological traits allow breeders and geneticists the opportunity to move their work from a more empirical level of trial and error, on a more theoretical-practical level so they can be more effective and accurate in reaching their objectives.

Conclusions and Recommendations

Net photosynthesis in nursery (P_n-N) correlated direct and slightly with stomatal conductance (G_s-F) and transpiration (E-F) both in the field ($r = 0.27$ and $r = 0.32$ respectively), indirectly, this result suggests that greater P_n-N would be linked to increased P_n-F.

G_s-N correlated moderately and inversely with Pn-F and WUE-F; that is due to the water supply in the mini-tunnel of rooting is minimal.

Transpiration in nursery (E-N) reported an inverse correlation with net photosynthesis (P_n-F), water use efficiency (WUE-F) and total tree height (H_t-F), all in the field. Preliminarily, genotypes recorded lower transpiration in the nursery could be associated with higher values of P_n-F, WUE-F and H_t-F. The water use efficiency in nursery (WUE-N) recorded a moderate direct correlation value ($r = 0.45$) with P_n-F. Meaning that the genotypes with high values of WUE-N could obtain high values of P_n-F. That correlation was strong for three clones (1, 2 and 5) and weak for clone 12.

The content of chlorophyll in the leaves of the mini-cuttings rooted in nurseries (Chloro-N) correlated directly with P_n-F, wich indicate that measuring chlorophyll content in the leaves of the mini-cuttings rooted could help to identify genotypes that could present greater P_n-F. With these results the hypothesis that it is possible to find physiological variables that can be used in an effective early selection of the most desirable genotypes remains latent.

The measurement of physiological parameters should be perform in different stages at nursery, where could be better control of the environmental factors, and after use that information to correlate with easily measured field parameters, to be able to select superior genotypes early in field conditions limiting .

Be careful when trying to select using information at young ages, before genotypes are fully expressed phenotype through interaction with environmental conditions. Besides the study of a single parameter does not guarantee selection, so it should identify both morphological and physiological parameters that contribute to superior genotypes early selection.

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