Photosynthetic Behavior of *Gmelina Arborea* Genotypes in Rooted Mini-Cuttings Stage under Nursery Conditions, at South Pacific of Costa Rica

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Abstract

The aim of this study was to determine and interpret the photosynthetic capacity of five clones of Gmelina arboreaat the stage of mini-cuttings rooted in INISEFORgreenhouse. The light response curve of the clones as well as their performance in gas exchange wasevaluated, with no statistically significant differences among them. Statistical analysis was performed using InfoStat software, an analysis of variance and comparison of means by Tukey test wasperformed. Average values were recorded for net photosynthesis (A_n), stomatal conductance (G_s), transpiration (E) and water use efficiency (WUE) with values of 9.63 µmol m^{-2} s^{-1}, 142.83 mmol m^{-2} s^{-1}, 2.40 µmol m^{-2} s^{-1}y 4.16 µmol m^{-2} s^{-1},respectively. Clone 6 obtained greater physiological values for net photosynthesis (A_n) and stomatal conductance (G_s), and that also was located in the second place with respect to transpiration (E) and water use efficiency (WUE).

Keywords: *Gmelina arborea*; photosynthetic capacity; cuttings; clones; Costa Rica

Resumen

El objetivo del presente estudio es determinar e interpretar la capacidad fotosintética de cinco clones de *Gmelina arborea* en su estadío de mini-estaquilla enraizada en el vivero del INISEFOR-UNA. Se evaluó la curva de respuesta de la luz y el intercambio gaseoso para cada uno de los cinco clones, no se registraron diferencias estadísticamente significativas entre los genotipos evaluados. Los análisis estadísticos fueron realizados utilizando el programa InfoStat, se realizó análisis de varianza y comparación de medias mediante la prueba de Tuckey. El promedio registrado para la especie en cuanto a la fotosíntesis neta (A_n), conductancia estomática (G_s), transpiración (E) y eficiencia en el uso del agua (WUE) fue de 9,63 µmol m^{-2} s^{-1}, 142,83 mmol m^{-2} s^{-1}, 2,40 µmol m^{-2} s^{-1}y 4,16 µmol m^{-2} s^{-1}respectivamente. El clon 6 obtuvo los valores más altos en cuanto aA_ny G_s, además registró la segunda posición con respecto a E y WUE.

Palabras clave: *Gmelina arborea*; capacidad fotosintética; mini-estaquilla; clones; vivero; Costa Rica

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Introduction

A forestry breeding program begins with the selection of individuals with desired phenotypic characteristics in a given population (Pastrana 2011). The identification and selection of high yield trees is the foundation to such programs (Vallejos et al. 2010), with a superior phenotype in growth, shape and wood quality among other desirable characteristics. Once the trees that form part of breeding program are identified, establishment and propagation in the nursery starts. The nursery phase is essential to evaluate a number of features involved with plant productivity, obtaining valuable information with which to commence with determining the potential of the gene pool.Usually morphological characteristics are the preferred quality and growth indicators used in research at the nursery, given that they are considered an expression of the physiology and genomics of the plant material; however these provide indirect and insufficient information for accurate decision making.

Knowledge of plant physiology is essential for advances in tree breeding (Pallardy 2008) since they play an important role in the understanding of plants and their interactions with the environment (El-Sharkawy 2006, da Matta 2007). To obtain directly physiological parameters values and their subsequent interpretation is more difficult, mainly because of its high variability, whichcan be attributed to the susceptibility to weather conditions, the high cost of the necessary equipment (IRGA, Infra Red Gas Analyzer) and special the special training for the operators in the use of such equipment. This situation may explain the existence of little physiological information about superior genotypes for *Gmelina arborea* plantations. Such information would be useful for making decisions, for example about the conditions that must be provided to different genotypes in the nursery in order to obtain the best expression of their photosynthetic performance (Triono 2004); thereby achieving greater biomass production, higher quality and resilience to adverse field conditions.

Parameters such as the rate of photosynthesis and carbohydrate accumulation are considered as quality indicators in plants produced in forestry nurseries (Cetina et al. 2001). With the information obtained from the light curves and gas exchange of clones that are evaluated, it is possible to identify genotypes with greater capacity to capture carbon through photosynthesis. This information is useful in production programs and it would not only make more efficient the production per unit area, but also reduce environmental pollution by increased removal of atmospheric CO_2 (López et al. 2007, González et al. 2009).

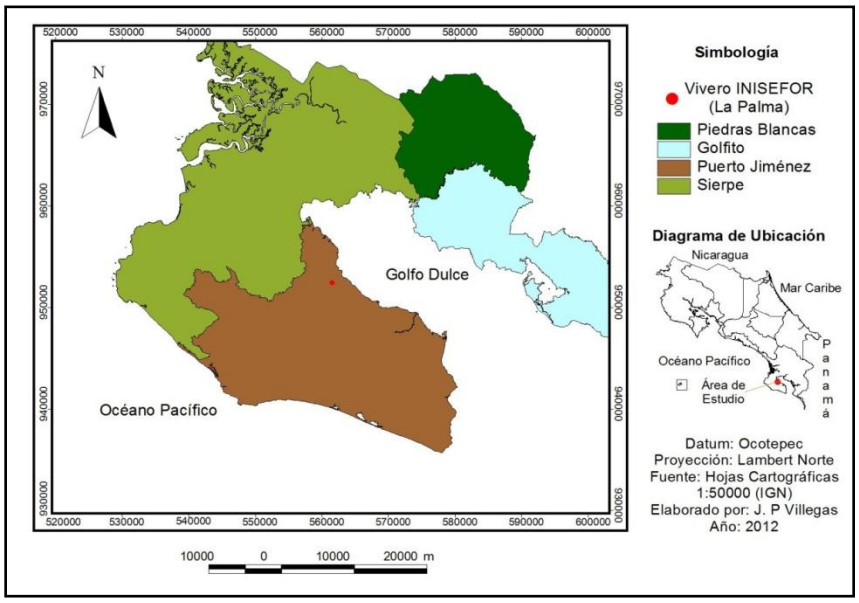
Gmelina arborea is a specie of great importance in tropical areas around the world, as an option to ensure the supply of raw material for the forest industry (Balcorta & and Vargas 2004, Kumar 2007, Adebisi et al. 2011, Wee et al. 2012) . Its importance lies in the rapid growth, easy adaptation to a variety of site conditions, the variety of uses for wood (Indira 2006) and supply to producers and / or investors of a quick return on investment (Wee et al. 2012). Murillo and Guevara (2013) report that Melina was the most productive species in forestry nurseries of Costa Rica in 2012, with almost 50% of the approximately 7 million plants produced in that year. The present study aimed to determine and interpret the photosynthetic capacity of five clones of *Gmelina arborea* in mini-rooted cuttings of vegetative reproduction in the nursery of the Institute of Research andForest Services of the National University (Instituto de Investigación y Servicios Forestales de la Universidad Nacional) in the south of the country.

Materials and methods

Site Description

The study was conducted at the nursery facilities of the Institute of Research andForest Services (INISEFOR) located in La Palma, Puerto Jiménez district, county of Golfito, Province of Puntarenas, in the south Pacific of Costa Rica (Figure 1). Five genotypes of *Gmelina arborea* that are part of the genetic collection of INISEFOR, were physiologically assessed. The vegetative reproduction nursery is located in an area with an average annual rainfall of 3500 mm to 4000 mm, mean annual temperature of 24 °C to 28 °C (Kappelle et al. 2002) and an altitude of 20 meters. The region is classified as premontane wet forest transition to Basal (Holdridge 1967).

Figure 1. Study area, La Palma, Puerto Jiménez, Golfito, south pacific, Costa Rica.



Source:Villegas, 2012

Vegetative Material

The research was conducted in five genotypes of *Gmelina arborea* from the breeding program of INISEFOR. Mini-cuttings (within mini-tunnels) that were in their first growth period of the vegetative reproduction process (Figure 3) were used. Gmelina genotypes were reproduced from clonal gardens, according to usual protocols used by INISEFOR (Abrahams &Vassart 2011).

Figure 2.Mini-tunnel environment for rooting *Gmelina arborea*mini-cuttings.

Measuring physiological parameters

Physiological measurements were made when mini-cuttings had rooted after spending fifteen days in the mini-tunnels, just as, according to the protocol of reproduction, they are ready to leave the nursery to the environment or conditions of acclimatization on a semi protected environment.

Light Response Curves *(A_n-PPFD)*

Light response curves were determined using a portable photosynthesis system (CIRAS-2, PP Systems, USA - Figure 3) on bright and clear days (Blake and Bevilacqua 1990, Lewis et al. 2002). Average CO_2 concentration used was 366 ppm ± 7.05 ppm and air temperature 30 °C within the cuvett. Photosynthetic response to light was measured in the first new pair of blades with full development of each plant (Evans &Poorter 2001, Blake &Bevilacqua 1990). Fourteen levels of incident photosynthetic photon flux density were evaluated (0, 50, 100, 150, 200, 250, 300, 350, 400, 600, 800, 1000, 1200, 1400 µmol m^{-2} s^{-1}). It started with 0 µmol m^{-2} s^{-1} being increased intensity gradually until 1400 µmol m^{-2} s^{-1}. Five branches were evaluated for each genotype, ie five repetitions of the curve for each genotype were obtained, while the value to be reported for the species is based on the average of the analysis of the 25 plants. The data were fitted using non formula described below rectangular hyperbola (Thornley 1976, Marshall & Biscoe 1980,Pasian&Lieth1990, Flores 2012)

F (PPFD, Φ,A_{max} ,R_d) :

$$A_n = \frac{\Phi PPFD + A_{max} - \sqrt{(\Phi PPFD + A_{max})^2 - 4\Phi PPFD A_{max}\theta}}{2\theta} - R_d$$

Where A_n is the net photosynthetic rate, Φ is the quantum yield of photosynthesis, PPFD is the photosynthetic photon flux density, A_{max} is the maximum photosynthetic rate at light saturation, R_d is mitochondrial respiration rate and θis a parameter describing the convexity of the curve (Leverenz1988, Zufferey et al. 2000, Flores 2012). LSP is the point of saturation and luminance was calculated by the equation proposed by Lieth and Pasian (1990):

$$LSP = 2\frac{(A_{max} + R_d)}{\Phi}$$

Figure 3. Portable photosynthesis system, CIRAS-2.

Gas Exchange

Measurements were done on clear and sunny days with an air temperature of 30 ° C within the cuvett. For the assessment of gas exchange the portable photosynthesis system (CIRAS-2, PP Systems, USA) was also used (Figure 3) with a PLC6 cuvett at a CO_2 concentration of 366.16 ppm ± 7.05 ppm , and light intensity of 1000 µmol m^{-2} s^{-1}, with the main objective to prevent photo inhibition (Evans &Poorter 2001). The physiological variables obtained and analyzed were: net photosynthesis (A_n= µmolCO_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}). Measurements were made in the first new pair of leaves with full development of each plant (Evans &Poorter 2001), these were labeled to evaluate it on five occasions at different times during day. In summary five branches per genotype were analyzed on five separate occasions each.

Determining amount of chlorophyll

Chlorophyll measurement was performed using the SPAD-502 in the new pair of first fully developed leaves of each plant. An average of five records per blade in five branches per genotype was taken.

Statistical Analysis

Data were analyzed using the statistical complete randomized block design, where each clone served as block (five in total) and branches as the set of observations or experimental unit (five branches per clone-repetitions). Statistical analysis was performed using the software InfoStat.

Physiological gas exchange data were analyzed using a parametric analysis of variance, after checking assumptions of parametric statistics, and differences in means were compared by Tukey test ($p < 0.05$). For the preparation of the light response curves (A_n-PPFD) data were fitted to a non-rectangular hyperbola model (Thornley 1976, Marshall & Biscoe 1980, Lieth& Pasian 1990, Flores 2012) using the "Landflux" software , which was developed especially for this type of evaluation.

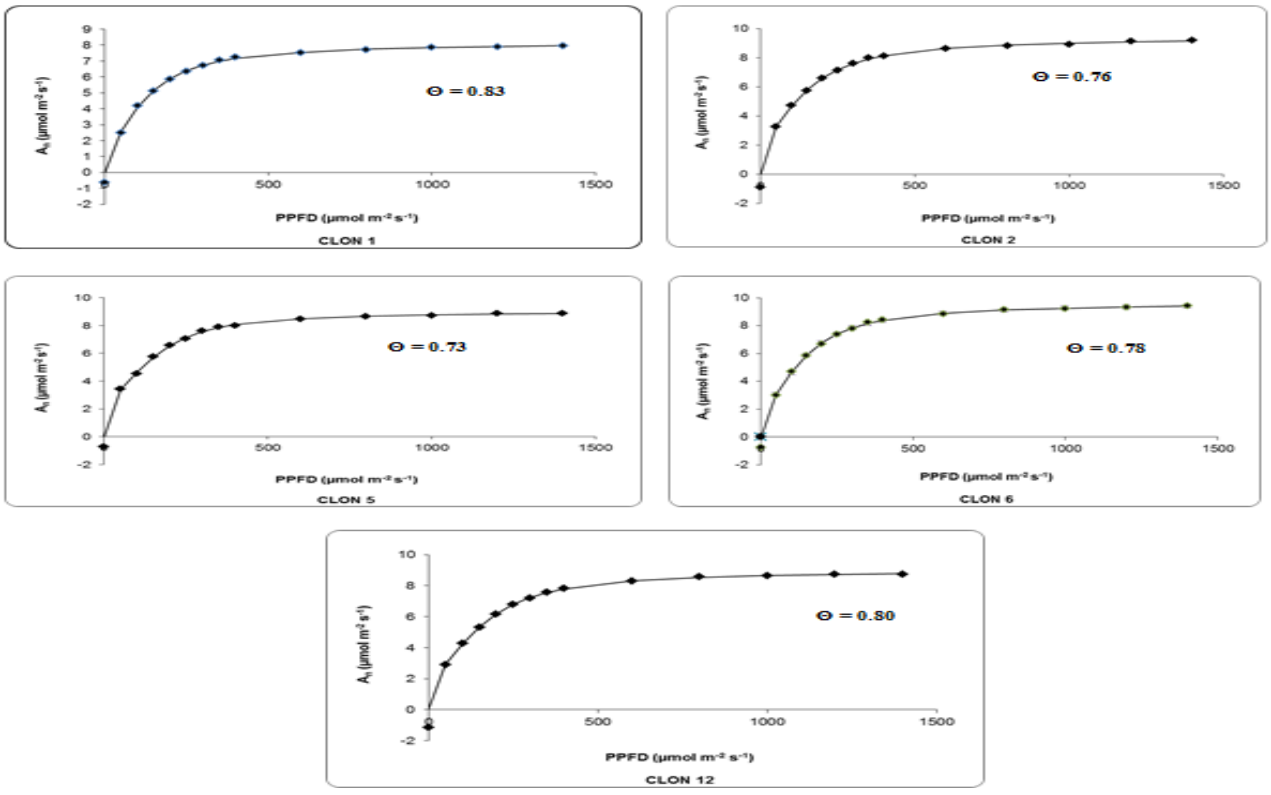
Results and discussion

Light response curve to light ($\boldsymbol{A_n}$-PPFD)

Through the process of photosynthesis, C3 plants transform carbon dioxide from the atmosphere into chemical energy used in its growth, which is the key process driving their development (Herbohn et al. 2009, Flood et al. 2011), this process can take place only in the presence of solar light and CO_2. To evaluate the photosynthetic capacity of genotypes response a light curve (absorption of CO_2) is built, and thus its gas exchange capacity is determined. It is a function that can provide accurate information on the efficiency of the tree in the use of light (Triono 2004). It is very important to take into account both stomatal and non-stomatal factors that can directly influence the direct relationship between the rate of photosynthesis and stomatal conductance. In this regard, Gonzalez et al. (2009), failed to construct light response curves for three species of *Lantana sp.* due to a strong stomatal closure induced by water stress. Figure 4 shows curves of CO_2 absorption as a function of the incident photon flux density for five genotypes of *Gmelina arborea* (clones 1, 2, 5, 6 and 12).

The steep slope at the beginning of the curves for the five genotypes, is typical of species acclimated to environments with low incident photosynthetic photon flux density, which when exposed to larger fluxes, show a strong reaction in their photosynthetic system (Ogren 1993, Evans &Poorter 2001). Similar behavior for the five clones' curves studied is seen, which initially indicates consistency in the photosynthetic behavior for the five genotypes. The values of convexity (Θ) reported no statistically significant differences, with a range range of 0.73 to 0.83 (Figure 4). These values suggest a moderate efficiency of the photosynthetic process in the field of intermediate light (Ogren 1993), possibly due to the acclimatization of plants to light environment around them at the time of evaluation (Evans &Poorter 2001).

Figure 4. Response curves of photosynthesis (A_n) to photosynthetic photon flux density (PPFD) for five rooted mini-cuttings clones of *Gmelina arborea* in the nursery.



From the light response curves, the various characteristics for physiological values for the five mini-cuttings' clones studied under greenhouse conditions (Table 1) are obtained.

Table 1. Physiological Characters estimated from response curves for light mini- cuttings five clones of *Gmelina arborea* in nursery.

CLONE	**RESPIRATION (R_d)**			QUANTUM YIELD **(Φ)**			MAXIMUM PHOTOSYNTHESIS **(A_{max})**			LIGHT SATURATION POINT **(LSP)**		
	µmol m^{-2} s^{-1}			**mol/mol**			**µmol m^{-2} s^{-1}**			**µmol m^{-2} s^{-1}**		
6	**-1.1090**	**a**	**-20%**	**0.0415**	**a**	**15%**	**8.58**	**a**	**12%**	372.71	**a**	**4%**
2	-1.4127	**a**	**2%**	0.0411	**a**	**13%**	8.03	**a**	**4%**	345.37	**a**	**-4%**
12	-1.3736	**a**	**-1%**	0.0323	**a**	**-11%**	7.68	**a**	**0%**	**394.00**	**a**	**10%**
5	-1.9202	**a**	**38%**	0.0305	**a**	**-16%**	7.16	**a**	**-7%**	336.75	**a**	**-6%**
1	-1.1245	**a**	**-19%**	0.0356	**a**	**-2%**	6.99	**a**	**-9%**	346.80	**a**	**-3%**
AVERAGE	**-1.3880**			**0.0362**			**7.69**			**359.13**		
Means with a common letter are not significantly different (p> 0.05)												
Values in percentage represent the difference from the population mean parameter												

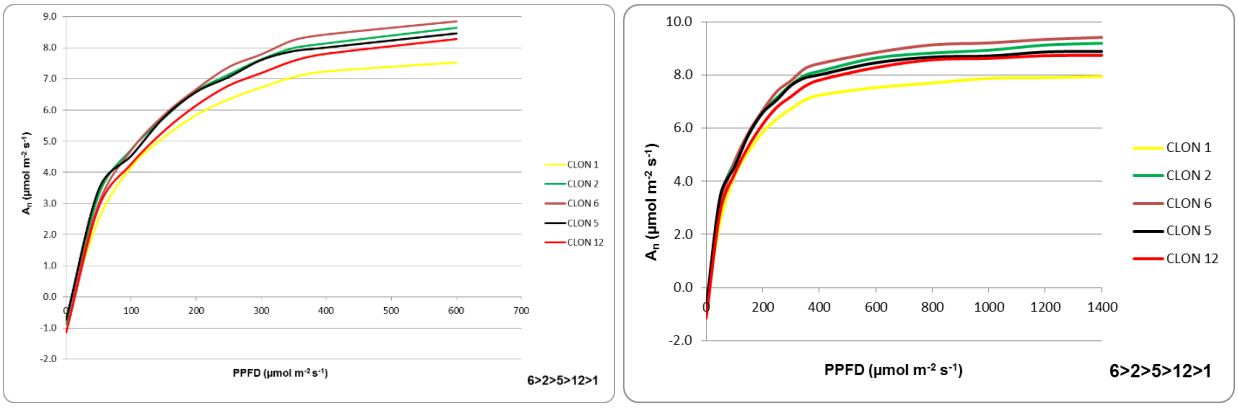
None of the physiological traits evaluated showed statistically significant differences between clones in regards their response on net photosynthetic rate based on the increase in the incident photon flux density. The quantum yield of the clones indicates apparent ability to capture light energy, electron transport and develop the enzymatic processes; which is directly affected by water stress (González et al. 2009). The evaluated genotypes showed values ranging from 0.030 to 0.041 (clone 6> 2> 1> 12> 5) for this parameter. The sixth clone had the highest value, 36.6% higher compared to the lowest (clone 5). Flores (2012) evaluated five melina clones at 81 days, which were propagatedvegetative and placed on a substrate with low pH; finding a 125% difference between the clone with the highest average value and which recorded the lowest value. The difference between the two studies can be attributed to genetic variation among clones evaluated as the materials are developed in different environments (Adams et al. 2001), as well as the difference in age of the plants at the time of measurement, since the values of physiological parameters vary with the age of the material evaluated (Lieth& Pasian 1990, Coopman et al. 2008, Flood et al. 2011, Rojas et al. 2012). Moreover, the quantum yield values obtained in this study are placed above those reported by Nogués and Baker (1995) and Tambussi and Graciano (2010) for C3 plants not subjected to water stress; where these authors found values from 0.025 to 0.030 µmol m^{-2} s^{-1}. Kelly (2006), on the other, on seedlings of *Gmelina leichhardtii* for different light intensities (60%, 30%, 10%) reported values between 0.0047 and 0.0058 µmol m^{-2} s^{-1}.

For the present study the values of the maximum photosynthetic rate at light saturation (A_{nmax}) ranged from 6.99 µmol m^{-2} s^{-1} and 8.58 µmol m^{-2} s^{-1}. The clone six recorded the highest value of maximum photosynthesis, while clone 1 the lowest value in a range of variation of22.7% between them. The previous confirms reports by Gliessman (2002), where C3 plants tend to have their maximum photosynthetic rates under moderate conditions of light and temperature, and inhibition at high rates of heat and illumination pattern. The range of variation for this parameter, again, is much lower than that reported by Vellini et al. (2008) and Flores (2012), who obtained 90.9% variation in clones of eucalyptus and 63.6% respectively in clones of melina in evaluations performed in a nursery. Evans and Poorter (2001) conclude that the leaves acclimated to low light intensities had better photosynthetic rates per unit leaf area than leaves acclimated to high light intensities.

Since the clones here studied are being commercially reproduced to establish productive plantations, it is desirable that the range of variation of A_{nmax} is minimum, which would yield smaller margin of error in the calculations of forest production, and would achieve more effective silvicultural activities planning. Other comparison data were reported by Kelly (2006), who studied the photosynthetic capacity of *Gmelina leichhardtii* at the nursery, registering A_{nmax} from 8.44 to 9.36 µmol $m^{-2}s^{-1}$, slightly higher than the values this study, which slowly initially checks the high photosynthetic capacity of the genus melina. The light saturation point (LSP), defined as the light intensity above which no growth occurs in CO_2 fixation varied between 336.75 µmol m^{-2} $s^{-1}$and 394 µmol m^{-2} s^{-1}. According to Lopez et al. (2007), between species there are differences in light requirements and thus must be taken into account.

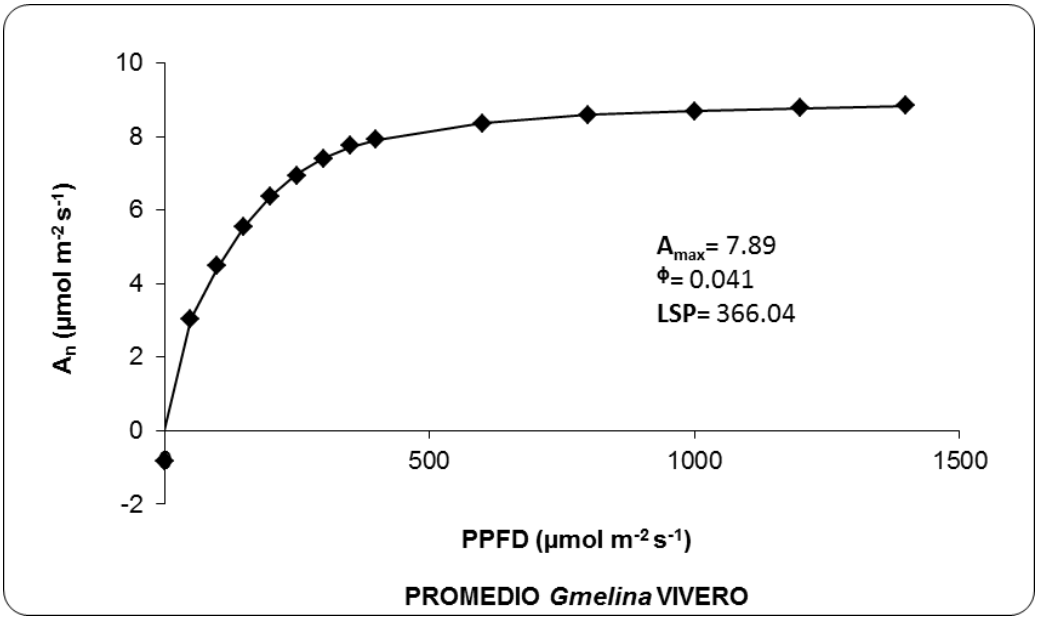
Herralde (2000) reaffirms the conclusion that LSP is an important parameter for decision making, as greater values involve a greater physiological efficiency of incident radiation. That is, clones 12 and 6 may be subject to greater incident photon flux densities in the mini-tunnel rooting clones than clones 2, 5 and 1, to maximize production of biomass. The desired outcome is to adjust nursery management practices that positively modify photosynthesis, which affect the production and storage of carbohydrates, and consequently the vigor of the plant that will be sent to the field (Cetina et al. 2001). Research studies should be performed to corroborate this approach, and more importantly, to determine which plant tissue increase biomass would be located. While it is true there were no statistically significant differences among the evaluated clones,except to clone 6, which showed higher values with respect to all characters investigated except in light saturation point, where it had the second best value. Clone 6 showed 20% less respiration, 15% higher quantum yield, 12% higher maximum photosynthesis and 4% higher light saturation point with respect to the overall average of the five clones investigated in this study. Therefore clone 6 was identified as the highest photosynthetic capacity of genotypes. In second place is the clone 12 which recorded the highest light saturation point, and stood at the top in the other traits evaluated. Moreover clone 5 showed the lowest values, so it was at the last position of the clones evaluated. In summary, the order of the clones, of more to less desirable values in terms of the parameters obtained from the curves of light is: 6> 12> 2> 1> 5 The presentation of individual light curves obtained for the rooted cuttings in nursery does not reveal important details, therefore a summary graph (Figure 5) is presented. Left curves are presented to 600 µmolfotones m^{-2} s^{-1} (Figure 5A), which is the point where the slope of the curve begins to decline, and right across the curve for each clone is presented, to 1400 µmolfotones m^{-2} s^{-1} (Figure 5B).

Figure 5.Photosyntesis response curves (A_n) to photosynthetic photon flux density (PPFD), for mini-cuttings from five clones of *Gmelina arborea* evaluated in the nursery.



In both figures, each clone is located in a defined position and continues that way until the LSP. With respect to the behavior of clones based on the photosynthetic photon flux density of the order is always: 6> 2> 5> 12> 1 It coincides with the previous finding where the clone 6 showed the best response in physiological traits evaluated in their photosynthetic development. In this aspect the targeted by Aspinwall et al.is satisfied (2011) who indicates greater uniformity in the behavior of individual clones when individual evaluation is performed. The uniformity in the behavior of clones along the entire curve simplifies any changes in the nursery, helping to identify the demand from each genotype radiation, in order to increase productivity. According to the order obtained in Figure 6 it would be recommended to have photosynthetic photon flux conditions of 366 µmolfotones m^{-2} s^{-1}, if it is desired to work according to the average value obtained for the species. The analysis of the physiological parameters of the 25 light response curves evaluated, allowed the form of a response curve of the photosynthesis photon flux density incident,at overall photosynthetic species nursery level (Figure 6).

Figure 6. Average response curve of photosynthesis (An) to photosynthetic photon flux density (PPFD) for the species *Gmelina arborea* under nursery conditions.



After three weeks of rooting stimulation, with a temperature average of 33 °C and PAR 100 µmolfotones $m^{-2}s^{-1}$respectively in the mini-tunnels (Abrahams &Vassart, 2011), on average, *Gmelina arborea* showed a photosynthetic maximum rate of 7.89 mol CO_2 m^{-2} $s^{-1}$and a light saturation point of 366.04 µmol m^{-2} s^{-1}. These values are considered too high by the young age of the material and be acclimated to low PAR conditions to avoid water stress in the cuttings. Evans and Porter (2001) indicate that pine leaves acclimated to low levels of PAR had better daily photosynthesis per unit leaf dry mass to leaves acclimated to high levels of PAR. Likewise the evaluated melina genotypes were very efficient in their photosynthetic mechanisms even without ideal conditions. In these conditions the species shows its photosynthetic potential in its early stages, validating its status of rapid growth, taking advantage of the available PAR, fixing CO_2 into biomass. The above features correspond to species that could be classed as successful invasive species in tropical environments, with high rates of growth and photosynthesis compared to native species (Pepper et al. 2012). The light saturation point of 366.04 µmol m^{-2} s^{-1}indicates that the mini-tunnels rooting should be exposed to larger values of incident radiation, to exploit more genotypes translating this environmental variable in biomass production (López et al. 2001). This approach should be investigated in future studies, to determine in what tissues of the plant the largest accumulation of biomass is presented by way of photosynthesis over the years (López et al. 2007, Araque et al. 2009), as plants can express priorities when forming new tissues or recover structures from damages (Retuerto et al., 2003), or change the fraction of biomass invested in leaves, stem and roots (Evans &Porter 2001).Moreover the same authors 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 the plant directs photo assimilates.

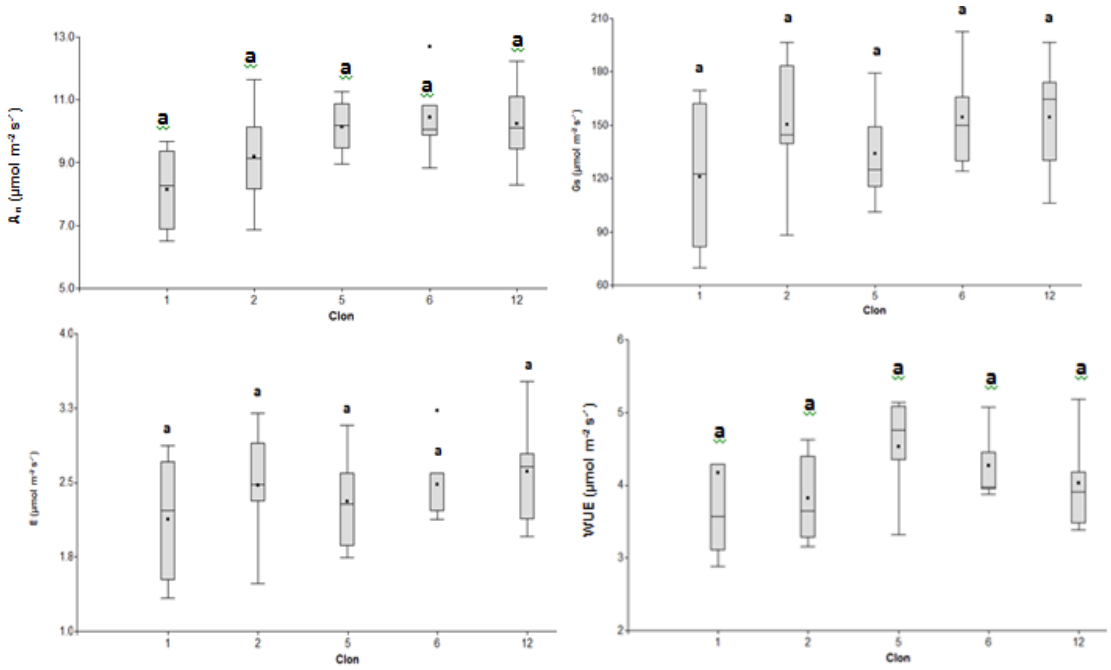
The quantum yield values (ϕ) registered for the genotypes evaluated under conditions of rooted mini-cuttings, varied between 0.030 µmol $mol^{-1}$and 0.041 µmol mol^{-1}, registering an average value for the sort of 0.036 µmol mol^{-1}. Initially it might indicate a poor efficiency of the photosynthetic system, but with the value of initial slope of the light curve, the species is able to reach important values of Amax at such a young age, which in turn indicates a very important ability to use values low PAR and they activate their photosynthetic system, making it a competitive-adaptive advantage. Flores (2012) reported values of quantum yield between 0.016 µmol $mol^{-1}$and 0.036 µmol mol^{-1}, slightly lower than those obtained in this work, because the plant material evaluated by Flores (2012) were all found acclimated to low light conditions. In pine genotypes with a high quantum yield tend to have a lower average diameter and volume growth (Aspinwall et al. 2001).

Gas Exchange

Stomatal closure to moderate levels of water stress, osmotic adjustment and high efficiency in water use, among others, are physiological parameters that should guide clonal selection (Corcuera et al. 2005).

In this respect the results obtained for the parameters net photosynthesis, stomatal conductance, transpiration and efficiency in the use of water as a function of gas exchange process for five clones of *Gmelina arborea* under conditions of rooted mini-cuttings in the nursery (Figure 7) are presented . The evaluations were performed using an average PAR of 1000 m^{-2} s^{-1} ± 4.34 µmol m^{-2} $s^{-1}$and an average CO_2 concentration of 366 ppm ± 7.05 ppm.

Figure 7. Net photosynthesis (An), stomatal conductance (Gs), transpiration (E) and water use efficiency (WUE) for rooted mini- cuttings of *Gmelina arborea* using five clones under nursery conditions.



Means with a letter in common are not significantly different (α = 0.05)

A significant variation in the data for each of the four physiological parameters evaluated was present, however no statistically significant differences between the investigated clones (α = 0.05) were recorded. Two clear trends aimed at lower and higher variation in physiological variables are observed. Clone 6 had fewer large ranges in all characters, denoting greater accuracy and stability of results. This could be associated with the fact that it was the clone that showed the best photosynthetic development, more stable to environmental changes genotype. Genotypes 1 and 2 showed the greatest variability in all parameters evaluated. This offers a possible alternative strategy in greater adaptability (Araque et al. 2009). Similarly Araque et al. (2009) found no statistically significant difference in the values obtained from An, Gs and E in an early evaluation for four tropical tree species.The values of each of the physiological characteristics for the five clones of *Gmelina arborea*are presented in Table 2.

Table 2. Results from evaluated variables as a function of gas Exchange for five clones of *Gmelina arborea*in rooted mini-cuttings at nursery stage.

CLONE	Net photosynthesis (A_n)			Stomata conductance (G_s)			Transpiration (E)			Water Use efficiency (WUE)		
	µmol m^{-2} s^{-1}			mol/mol			µmol m^{-2} s^{-1}			µmol m^{-2} s^{-1}		
6	**10.45**	**a**	**8%**	**154.40**	**a**	**8%**	2.48	**a**	**3%**	4.26	**a**	**2%**
12	10.24	**a**	**6%**	154.37	**a**	**8%**	2.61	**a**	**9%**	4.02	**a**	**-3%**
5	10.15	**a**	**5%**	134.03	**a**	**-6%**	2.31	**a**	**-4%**	**4.53**	**a**	**9%**
2	9.19	**a**	**-5%**	150.40	**a**	**5%**	2.47	**a**	**3%**	3.82	**a**	**-8%**
1	8.14	**a**	**-16%**	120.97	**a**	**-15%**	**2.13**	**a**	**-11%**	4.17	**a**	**0%**
AVERAGE	**9.63**			**142.83**			**2.40**			**4.16**		
Means with a common letter are not significantly different (p > 0,05)												

In the present investigation the net photosynthesis (A_n) ranged from 8.14 µmol m^{-2} s^{-1} to 10.45 µmol m^{-2} s^{-1} among the five clones investigated. These values of photosynthetic rate can be considered very good when you take into account the young age and low photon density conditions to which they were subjected (100 µmolfotones m^{-2} s^{-1}). Clone 6, as in the data obtained from the light curves, presented the highest rate of photosynthesis, reaching 8% above the average for that parameter; meanwhile net photosynthesis of clone 1 was 16% lower than the average of the five clones. This implies that the clone 6 would produce more biomass than other clones during their stay in the nursery, so it could be ready to go to field faster or be better prepared for their establishment. Naturally to corroborate this statement, it requires further evaluation by the respective research trials (Araque et al. 2009). The light saturation point for the species specified in the condition of mini-cuttings rooted in the nursery was 366.04 µmol m^{-2} s^{-1}. However, evaluations of gas exchange were made at a constant PAR of 1000 µmol m^{-2} s^{-1}, ie a much higher saturation point level, so that gas exchange determinations should be made in these stages to levels of PAR closer to its LSP, since even before then increased CO_2 fixation per unit of PAR would be presented, bringing an even more realistic value.

Stomatal conductance (G_s) is a variable that indirectly reveals the level of stomatal opening (López et al. 2007), as an essential mechanism to reduce water loss through transpiration (Corcuera et al. 2005), and it may be affected by merely stomatal as non-stomatal factors (González et al. 2009), as the concentration of CO_2 and some chemical signals reported for *Gmelina arborea* (Farquhar and Sharkey 1982, cited by Rojas et al. 2012). No statistically significant differences between clones tested ($\alpha = 0.05$) were recorded. However, high variability among clones was presented, obtaining average values from 120.97 to 154.40 mmol m^{-2} s^{-1}. Again consistently, clone 6 was the registered the highest value and the lowest clone 1, reaching 8% above and 15% below the general average respectively. This wide range is possibly explained by the lack of uniformity of the water potential enabling the locking leaves and stomatal aperture attributable to environmental conditions. Lopez et al. (2007) indicate that the stomatal response is attributable to the physiological behavior of plants, so the high heterogeneity of stomatal closure probably lead to functional inefficiency of the leaf factor to take into account in plant productivity (Sánchez &Aguirreolea 2000 cited by Flores 2012). Meanwhile, Araque et al. (2009) indicates that differences in $G_s$could be attributed to the anatomy of leaves per genotype. Flores (2012) reported values of G_s five clones of *Gmelina arborea* whose averages ranged from 120-135 mmol m^{-2} s^{-1}, the minimum value reported by the same author is very similar to that found in the present study, however the average value recorded in this study is 12% higher than that reported by Flores (2012). The G_{smax} values obtained in the present study for five clones of *Gmelina arborea* reflect the high capacity of the photosynthetic apparatus of these genotypes. Fernandez et al. (2010) evaluated four eucalyptus species in nurseries and determined that the water status of the plant did not prove to be the main limiting factor for G_s and E; variables such as temperature, light radiation or saturation deficit humidity would be the more affected both physiological processes (Fernández et al. 2010, Pepper et al. 2012).

Transpiration (E) is directly influenced by environmental conditions such as temperature, vapor pressure deficit and level of photo synthetically active radiation (Marrichi 2009). The genotypes showed no statistically significant differences ($\alpha = 0.05$) between them. Clone 6 presented little variation (Figure 7), indicating that the regulatory process has a well-defined relation. The average value of clone 6 was only 3% higher than the overall average of the clones (Table 2), confirming a good alternative to limiting, especially water conditions. Clones 1, 2, 5 and 12 showed high variation in their measurements, with clone 2 which had the broadest range of from 1.7 to 3.2 µmol m^{-2} s^{-1}. Clone 1 had the most disadvantageous mean value with 11% over respiration from the overall average, implying differential measures for treatment in the nursery, yet to be defined in future research. The overall average for the present study was 2.40 µmol m^{-2} s^{-1}, which can be considered as a low value compared to the value of 3.50 µmol m^{-2} s^{-1} in average reported by Flores (2012) attributed to high and constant temperature room (29-39 °C) and the study was conducted in full dry season. Meanwhile low respiration obtained on average for the five genotypes evaluated in this study become a competitive - adaptive advantage to reduce the rapid depletion of soil water through stomatal closure (Larcher 2000,Tatagiba et al 2007). Water use efficiency in (WUE) is one of the main mechanisms of adaptation (Corcuera et al 2005.), it is a characteristic of each species and depends largely on environmental conditions (Medrano 2007); which ultimately is determined by the stomatal behavior (Combalicer et al. 2005). A high WUE becomes a competitive and adaptive advantage where water availability is scarce (Enmerich 2007, Rodrigues 2009). No significant differences ($\alpha = 0.05$) for the parameter among the five clones tested were found. Clone 5 showed on average the highest value for this parameter, followed by clone 6, thanks to its low value of perspiration, since the WUE is estimated from the ratio of A_n/E.

Clone 2 showed the lowest value of all with 8% less than the average WUE **5**= 4.53 µmol m^{-2} s^{-1}>**6**= 4.26 µmol m^{-2} s^{-1}>**1**= 4.17 µmol m^{-2} s^{-1}>**12**= 4.02 µmol m^{-2} s^{-1}>**2**= 3.82 µmol m^{-2} s^{-1}). The difference between clone 5 and 2 is 18.5% higher WUE.

Although these are not statistically significant differences between clones, it should be taken into account in the protocol vegetative reproduction INISEFOR-A. Flores (2012) reported (for five melina genotypes in nursery conditions) values WUE between 3.03 and 4.92 µmol m^{-2} s^{-1}, very close to those found in the present investigation, despite that the material evaluated by Flores (2012) and was fully acclimatized and it was older. Note that the values of photon density for the condition of tunnel rooting of this study were very low and the largest increase in WUE is associated with plants under high light intensity (Combalicer et al. 2005), which should be studied for these same genotypes at higher light intensities, at the stage of acclimatization. In summary, although no statistically significant differences between the clones investigated were found it needs to be taken into account that the clone 6 scored the most desirable for the parameters of net photosynthesis (A_n) and stomatal conductance (G_s) physiological values, and was ranked second place in regard to perspiration (E) and water use efficiency (WUE). Since the physiological potential is not necessarily directly related to production for all species and all ages, further studies are needed to validate their photosynthetic potential relative to its growth and / or biomass production in different tissues (Lopez et al. 2007), since plants can manifest priorities when forming new tissues or recover damages in structures (Retuerto et al. 2003), or change the fraction of biomass invested in leaves, stem and roots (Evans &Poorter 2001). On the other hand clearly clone 1 showed the lowest values in all parameters except WUE, where he averaged as the overall average of the material evaluated.

Conclusions and recommendations

The steep slope recorded in the light curves for the five genotypes, is typical of species acclimated to environments with low photosynthetic photon flux density incident. Statistically significant differences among genotypes for any of the parameters obtained from the light curves (convexity of the curve, apparent quantum yield, maximum photosynthetic rate and light saturation point) were not registered. Genotypes evaluated for apparent quantum yield registered a range values from0.030 to 0.041, with an average of 0.036. The clone six recorded the highest value, 36.6% higher compared to the lowest (clone 5). The maximum photosynthetic rate at light saturation (A_{nmax}) varied between 6.99 µmol m^{-2} s^{-1} and 8.58 µmol m^{-2} s^{-1}, with a mean of 7.89 µmol m^{-2} s^{-1}. The light saturation point (LSP) ranged from 336.75 µmol m^{-2} s^{-1} and 394 µmol m^{-2} s^{-1}, with an average of 366.04 for the species µmol m^{-2} s^{-1}.

The light saturation point average for the species (366.04 µmol m^{-2} s^{-1}) indicates that the rooted mini-tunnels should be exposed to larger values of incident radiation, which could obtain higher biomass production. More specifically, 12 and 6 clones could be subjected to higher photon flux density incident on the rooted mini-tunnels.

Although no statistically significant differences among clones were determined, genotype 6 recorded the highest values in all parameters evaluated, except in the light saturation point which has the second best value. This same genotype recorded 20% less breathing, 15% higher quantum yield, 12% higher maximum photosynthesis and 4% higher light saturation point with respect to the overall average for the five clones studied. As for the parameters obtained from the evaluation of gas exchange, no statistically significant differences between genotypes for net photosynthesis variables (A_n), stomatal conductance (G_s), transpiration (E) and water use efficiency (WUE) were recorded. For net photosynthesis (A_n) variation was recorded from 8.14 to 10.45 µmol m^{-2} s^{-1}between clones, with an average for the species under nursery conditions of 9.63 mol m^{-2} s^{-1}. Stomatal conductance (G_s) reported variability from 120.97 to 154.40 mmol m^{-2} s^{-1}, with an average of 142.83 µmol m^{-2} s^{-1}. Perspiration recorded from the wider scope from 1.7 to 3.2 mmol m^{-2} s^{-1}, 2.40 mmol m^{-2} s^{-1}average for the species. Finally a high efficiency in water use (WUE) was recorded, which is a competitive advantage - adaptive, with values from 3.82 µmol m^{-2} $s^{-1}$to 4.53 µmol m^{-2} $s^{-1}$with an average of 4.16 µmol m^{-2} s^{-1}. Clone 6 registered the best physiological and greater efficiency in parameters net photosynthesis (A_n) and stomatal conductance (G_s) , and, it ranked in the second place with respect to transpiration (E) and water use efficiency (WUE)., In contrast, clone 1 showed lower values for all parameters except in WUE, where it averaged as the overall group . Further studies should validate clone 6 photosynthetic capacity and how it could be translated into higher efficiency of biomass increments (López et al. 2001), as well as,in allocation in different tissues.

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