ISTF NEWS
5400 Grosvenor Lane
Bethesda, Maryland 20814, USA

Special Report, March 2010

Early Growth and Physiological Characteristics of Planted Seedlings in La Mesa Dam Watershed, Philippines*/

Marilyn S. Combalicer*[1], Don Koo Lee[2], Su Young Woo[3], Yong Kwon Lee[4], and Youn Ho Jang[5]

ABSTRACT

Six-month old native species of *Erythrina variegata* L., *Dracontomelon dao* (Blanco) Merrille et Rolfe, *Pterocarpus indicus* Willd., and Bischofia javanica Blume were planted to determine growth and physiological characteristics. The objectives of the study were to compare early growth performance among species and determine which is/are suitable in the area, to compare growth performance of species in the flat and mountain areas, and to determine relationship between growth and physiological characteristics. Randomized block design having two blocks and three replicates per species were established with 4 x 4-m spacing between seedlings. Growth parameters used were height, root collar diameter, survival rate, fresh and dry weights, root-shoot ratio, root and shoot growth characteristics, leaf area, and leaf anatomical characteristics. Physiological characteristics such as net photosynthesis, transpiration rate, water use efficiency, stomatal conductance, stomata size and number and chlorophyll content were determined and compared as well.

Results and analysis revealed that among four species, *E. variegata* showed best growth performance as it grows fast and can fix nitrogen followed by *P. indicus*. Based on this study *E. variegata* has a potential to be used in reforestation of many denuded areas in the country since it can grow fast and is shade-intolerant. In terms of physiological characteristics, nitrogen-fixing trees, *E. variegata* and *P. indicus*, exhibited good performance. Generally, growth and physiological characteristics of four species in the mountain area having fertile and moist soil condition were better than in the flat area with dry soil condition.

*/Originally published in The Philippines Agricultural Scientist. Vol. 88 No. 3, 305-316. September 2005.

Results and analysis revealed that among four species, *E. variegata* showed best growth performance as it grows fast and can fix nitrogen followed by *P. indicus*. Based on this study *E. variegata* has a potential to be used in reforestation of many denuded areas in the country since it can grow fast and is shade-intolerant. In terms of physiological characteristics, nitrogen-fixing trees, *E. variegata* and *P. indicus,* exhibited good performance. Generally, growth and physiological characteristics of four species in the mountain area having fertile and moist soil condition were better than in the flat area with dry soil condition.

ABBREVIATIONS	
CHLt- Chlorophyll total	D- Diameter
DI- Diameter increment	DW- Dry weight
E- Transpiration rate	FW- Fresh weight
g_s- Stomatal conductance	H- Height
HI- Height increment	LA- leaf area
P_N- Net photosynthesis	STN- Stomata number
STS- Stomata size	WUE- Water use efficiency

INTRODUCTION

Among the terrestrial ecosystems, grasslands and degraded areas are the most neglected. In the Philippines, they are vast (ranging from about 1/6 to 1/3 of the country's landmass, according to Malvas 1995) as a result of illegal cutting, shifting cultivation and fire. As of 1982, uncontrolled logging and slash and burn farming have converted an estimated 5 million ha of forestlands to grasslands; about 1.4 million ha of these areas are located in critical watersheds (NEPC 1983) and are largely covered with *cogon* grass (*Imperata cylindrica).*

The traditional approach of the Philippine government in rehabilitating grassland areas is through reforestation using fast-growing species. Reforestation efforts, however, have often failed due to the choice of plant species (Carandang and Cardenas 1991) and the production of poor quality planting stocks (Luna 1991). Furthermore, the dismal performance of newly planted seedlings was due to environmental stress particularly in areas with prolonged summer droughts (Castañeto and Carandang 1997).

Early growth and physiological characteristics of plant species should be investigated in predicting and evaluating the field performance of the seedlings. Assessing the survival and vigor of seedlings and knowing the interactions between seedlings characteristics and field conditions will enable us to select suitable species for the anticipated situation. It is important to define the best species that will thrive on a particular area since mortality is a problem at an early stage. Both morphological and physiological factors should therefore be studied as they affect seedling quality, which is the basis of the successful reforestation of degraded areas, especially grasslands.

The study was conducted in La Mesa Dam Watershed, which distributes water to thousands of households for domestic and industrial uses in Metro Manila. About 45% of its land area is denuded, and if denudation is not controlled, the watershed is in danger of loss in the coming years.

In this study, the growth and physiological characteristics of four species were determined to 1) compare their early growth performance among four species and determine which species would be suitable in the area, 2) to compare the growth performance of the tree species in the flat and mountain areas and 3) to measure the relationships between growth and physiological characteristics.

MATERIALS AND METHODS

Study site and plant species

The experiment was conducted in the flat area (3% slope) 104 m asl and mountain area (8% slope) 139 m asl of the La Mesa Dam Watershed, Philippines (14°45'N latitude and 121°05'E longitude); the two areas are about 2.84 km apart. The 2,700-ha La Mesa Dam Watershed is the last remaining forest of its size in Metro Manila. It has two pronounced seasons: dry during January to April and wet from May to December. January is the coolest month while May is the hottest. The watershed has a mean annual rainfall of 2,700 mm and an annual range temperature of 23.8 to 30°C. The relative humidity ranges from 69% in April to 84% in August and September.

Field measurements were carried out in the planted seedlings of narra (*Pterocarpus indicus* Willd.), dapdap (*Erythrina variegata* L.), tuai (*Bischofia javanica* Blume) and dao (*Dracontomelon dao* (Blanco) Merrill et Rolfe).

Establishment of the experiment

Experimental plots were laid out using the Randomized Block Design (RBD) with two blocks and three replicates per species. Seedlings of *E. variegata*, *D. dao*, *B. javanica* and *P. indicus* with an average size of 32 cm, 48 cm, 26 cm, and 58 cm, respectively, were planted during the rainy months of June to July 2002. Border seedlings were established to prevent edge effect among the species. Maintenance and care of the seedlings included periodic brushing to prevent weed competition and establishment of firebreaks to prevent the occurrence of forest fires.

Growth measurement

1) Survival rate (%)

The survival rate was determined every 3 mo from the initial establishment up to 1 yr. Plants were considered dead when all the aboveground organs were desiccated.

2) Height and root collar diameter growth

The initial height and root collar diameter of the species were measured. Height and root collar diameter measures of all planted seedlings were taken every 3 mo in all planted seedlings for 1 yr. The diameter and height increments were obtained after 6 mo and after 1 yr to determine the growth rates of individual species.

3) Fresh weight, dry weight, and root-shoot ratio

Fresh weight (*FW*) and dry weight (*DW*) of the root and shoot per species were measured directly prior to planting and after 6 mo in the flat area. Nine seedlings per species were randomly chosen and harvested for determination of fresh and dry matter yield. The roots were separated from the shoots and oven-dried at 80°C until their weights have become constant. The root-shoot ratio was determined after weighing the shoot and root separately.

For the mountain area, the following regression was established (based on measured diameter, height and *FW* in the flat area), relating fresh weight (*FW*, in g) to diameter (*D*, in cm) and height (*H*, in cm): $FW = a + b (D^2H)$, where a and b are regression constants. Dry weight and root-shoot ratio were obtained by ratio and proportion method using measured fresh weights in both areas; dry weights or root-shoot ratio in the flat area were likewise obtained.

4) Root and shoot growth characteristics

For root growth characteristics, primary and secondary root lengths, root branching, and number of secondary roots were obtained while for shoot growth characteristics, length of stem and number of leaves and branches were determined.

5) Leaf area

Average leaf area was measured in the flat area only after 6 mo and after 1 yr by means of a dot grid method. For the mountain area, the following regression was established (based on measured diameter and leaf area in the flat area), relating leaf area (*LA*, in cm^2) to diameter (*D*, in cm) measured: $LA = a + b(D)$, where a and b are regression constants.

6) Leaf anatomical characteristics

From fully expanded leaf of each species, 1 cm x 1 cm section was hand-cut for histological paraffin technique. The specimens were placed in FAA solutions for 24 h. Samples were dehydrated in 50% ethanol series with 1-h intervals and in Johanson's #1 to #7 at 2-h intervals following Owen's (1991) procedure. The samples were hardened and embedded in paraffin wax and were cut to form a ribbon using 820 Rotary Microtome. The cut ribbons were mounted on the slides using one drop of Haupt's solution. For the staining procedure, safranin ($C_{20}H_{19}ClN_4$), fastgreen, xylene ($C_6H_4(CH_3)_2$), absolute ethanol (C_2H_5OH), 50% ethanol, and distilled H_2O were used. Quantified under the microscope were total leaf thickness, epidermal leaf thickness, and palisade mesophyll thickness.

Measurement of Physiological Characteristics

1) Photosynthetic rate

Net photosynthesis (P_N) was measured in the field during the summer season (January 2003) and the rainy season (July 2003) on fully expanded mature leaf of individual species from 8 a.m. up to 5 p.m. Net photosynthesis was taken with a broad-leaf cuvette of Licor-6400 Portable Photosynthesis System (Li-Cor Inc., NE, USA) equipped with standard leaf chamber and CO_2 injection system adjusted to a constant CO_2 concentration of 400 µmol m^{-2} s^{-1}. The leaf was sealed and CO_2 concentration was maintained at ambient levels. The average cuvette temperature was maintained at 25°C. After enclosing the leaves in the chamber, photosynthetic rate was stabilized. The photosynthetic photon flux density (PPFD) used were 2, 20, 50, 80, 100, 300, 500, 1000, 1500 and 2000 µmol m^{-2} s^{-1}. The net photosynthesis was calculated as follows:

$$P_N = \frac{U_e(C_e - C_c)}{100S} - C_c E$$

where: P_N: Net photosynthesis (µmol CO_2 m^{-2} s^{-1}); *Ue*: mole flow rate of air entering the leaf chamber (µmol s^{-1}); *Ce*: mole fraction of CO_2 in the leaf chamber (µmol CO_2 mol^{-1} air); *Cc*: mole fraction of CO_2 entering in the leaf chamber (µmol CO_2 mol^{-1} air); *s*: leaf area (cm^2); *E*: transpiration (mmol H_2O $m^{-2}s^{-1}$).

2) Transpiration and stomatal conductance

The transpiration (*E*, mmol H_20 $m^{-2}s^{-1}$) and stomatal conductance (g_s, mol H_2O $m^{-2}s^{-1}$) were assessed together with P_N using Licor-6400 Portable Photosynthesis System (Li-Cor Inc., NE, USA).

3) Stomata size and number

For the stomata size and number, a colorless nail polish was applied on the abaxial surface of the expanded leaf. The resulting film, which bore the stomatal imprint, was mounted on the glass slides. Microscopic measurements of guard cell length and width were determined in the light microscope equipped with micrometer.

4) Water use efficiency

Water use efficiency (*WUE*) was calculated as P_N/E (Wang 2001 and Ashraf et al. 2002). Each measurement of net photosynthesis and transpiration was taken at 1,200 μmol m^{-2} s^{-1} light intensity.

5) Total chlorophyll content

The *total chlorophyll* content of the species was obtained by collecting five leaf samples of the same sizes. The samples were put into vial with 10 mL 80% acetone and were kept cool in the dark at 4°C. The absorbance of the extracts was read at wavelengths of 663 nm and 645 nm using UV2100, UV-VIS Recording Spectrophotometer (Shimadzu, Japan). Arnon's equation (Arnon 1949) was used to calculate the chlorophyll contents as shown below:

Chlorophyll *a* (μg/mL) = $12.7\ A_{663} - 2.59A_{645}$

Chlorophyll *b* (μg/mL) = $22.9\ A_{645} - 4.67A_{663}$

Total chlorophyll (μg/mL) = $8.05\ A_{663} + 20.29A_{645}$

where: A_{663} is the absorbance at 663 nm; and A_{645} is the absorbance at 645 nm.

Data analysis

All statistical tests were performed using a statistical software package (SAS program, SAS Institute Inc., NC, USA, 2000). The differences in growth and physiological variables between the two sites, among four species, and species x site interactions were determined by analysis of variance (General Linear Models procedure). Duncan's multiple range test (DMRT) was used for multiple comparisons. Regression analysis based on data measured in flat area was used to estimate the fresh weight, dry weight, root-shoot ratio, and leaf area in the mountain area. Simple correlations were calculated to examine the relations between growth and physiological variables.

RESULTS AND DISCUSSION

To determine the growth and physiological performance of four tree species planted and to determine the suitable species in the area, different parameters were measured. The parameters measured were subjected to analysis of variance (ANOVA). Root-shoot ratio, secondary root length, root branching, and total chlorophyll content were found to be not significantly different while the remaining parameters were significantly different (Table 1).

Table 1. Summary of the analysis of variance (ANOVA) for the different parameters measured	
Parameters	F computed
Survival rate (%)	<1
Height growth (cm)	<1
Root collar diameter growth (mm)	<1
Fresh weight (g)	<1
Dry weight (g)	<1
Root-shoot ratio	1.62^{ns}
Primary root length (cm)	<1
Secondary root length (cm)	3.03^{ns}
Number of root branches	3.46^{ns}
Number of secondary roots	<1
Length of stem (cm)	<1
Number of leaves	<1
Number of branches	<1
Leaf area (cm^2)	<1
Total leaf thickness (µm)	<1
Epidermal leaf thickness (µm)	<1
Palisade mesophyll thickness (µm)	<1
Net photosynthesis (µm CO_2 $m^{-2}s^{-1}$)	<1
Transpiration (mmol H_2O $m^{-2}s^{-1}$)	<1
Stomatal conductance (mol H_2O $m^{-2}s^{-1}$)	<1
Size of stomata (µm)	<1
Number of stomata	<1
Water use efficiency (µmol CO_2 $m^{-2}s^{-2}$ per mmol H_2O $m^{-2}s^{-1}$)	<1
Total chlorophyll content (µg/mL)	2.53^{ns}

1. Growth performance

1) Survival rate of seedlings

At 5% level of significance, the survival rate was significantly different in the site x species interaction (P=0.05). All of the species had high survival rate in both flat and mountain areas after 6 mo although there was a slight decline in the total number of seedlings. After 1 yr, the survival rate among species varied and decreased due to long summer period. *Pterocarpus indicus* had high survival rate in the flat area (81.90%) and mountain area (81.45%). The high survival rate of the species, thus, indicates that they could tolerate low soil moisture. In the study of Castañeto (1997), *P. indicus* was able to grow vigorously, regardless of the amount of water received even below field capacity. This is a very important thing to consider in choosing reforestation species because during summer soil moisture is very low and sometimes causes the plants to wilt. However, visible symptoms of wilting and leaf shedding were observed, suggesting stress avoidance response of the seedlings.

In comparing both sites, the species in the mountain area still had the higher survival rate due to better soil and favorable environmental condition. However, insect and disease attacks may be the immediate cause of the inability of plants to recover. Some caterpillars and other insects were observed in the leaves of the species particularly in *E. variegata* and *B. javanica,* which added to the causes of mortality to the seedlings.

2) Height and root collar diameter growth

The data on 1-yr height and root collar diameter growth of different species showed that there were significant differences among species at 5% level (Table 2).

Figures 1 and 2 show the height and root collar diameter of the species for 1-yr growth period both in flat and mountain areas. Height and root collar diameter increments, fresh weight and dry weight, and leaf area showed similar trends. When the growth of each species was compared between the two sites, analysis showed that *E. variegata* displayed significant differences for the growth characteristics, showing bigger development in the mountain area. *E. variegata* exhibited very good growth performance both in the flat and the mountain areas. It is a suitable nurse crop according to Hedge (1993) due to its fast growth rate, ability to improve microclimate, and ability to reduce weed growth.

Table 2. Mean comparison for growth characteristics of species planted in La Mesa Dam Watershed, Philippines

Species	H (cm)	D (cm)	FW (g)	DW (g)	LA (cm^2)	Root-shoot ratio
D. dao	81.2b	12.7b	66.0b	34.2b	24.2c	0.48a
E. variegata	160.6a	36.6a	499.6a	202.4a	92.3a	0.38a
P. indicus	80.5b	9.4c	41.4c	25.8bc	25.2c	0.52a
B. javanica	58.2c	13.5b	36.9c	17.1c	33.6b	0.45a

Legend: H – Height; D – Diameter; FW – Fresh weight; DW – Dry weight; LA – Leaf area.

Means within a column followed by the same letter are not significantly different at 5% level by DMRT.

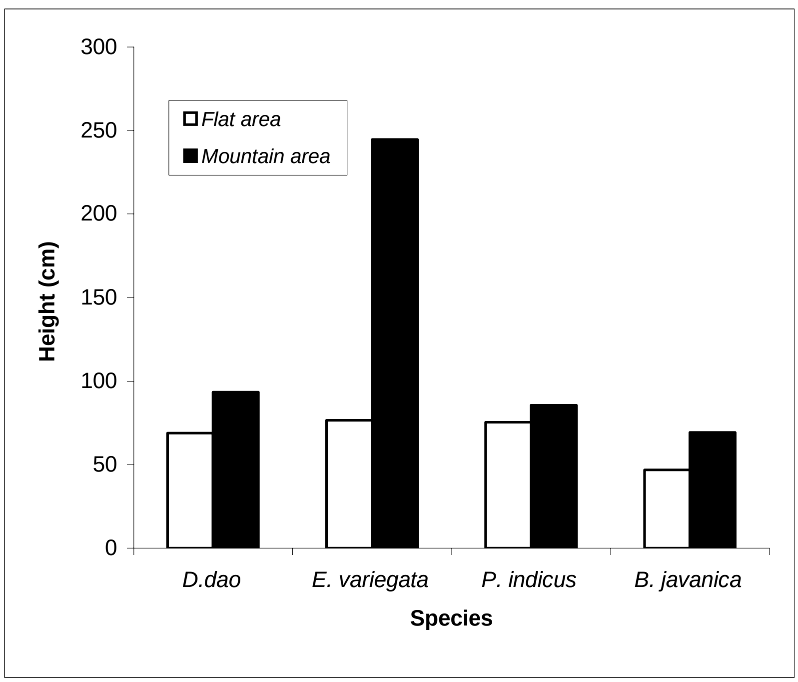

Fig.1. Height of four species grown in the flat area and mountain areas of La Mesa Watershed, Philippines.

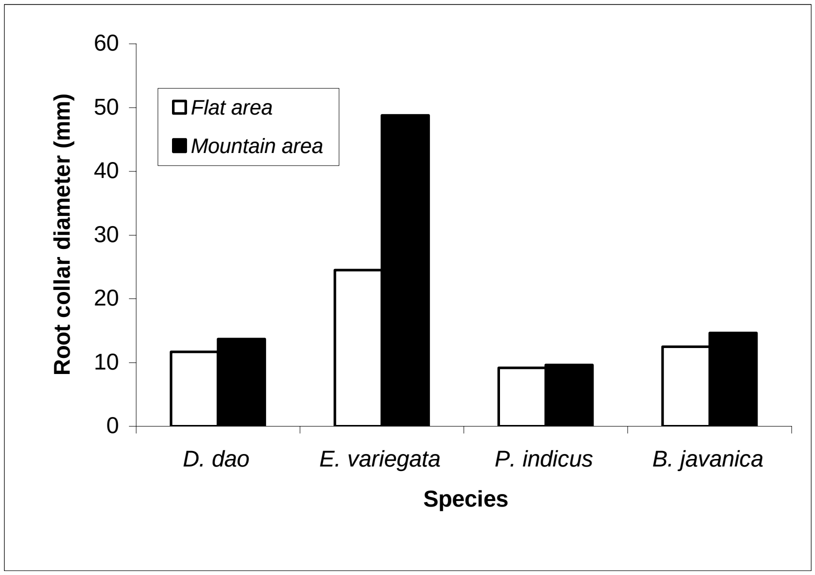

Fig. 2. Root collar diameter of four species grown in the flat area and mountain areas of La Mesa Watershed, Philippines.

Root collar diameter was highly correlated with net photosynthesis (Table 3). Increase in root collar diameter as well as height has something to do with biomass accumulation of the species. The root collar diameter increase in *E. variegata* can be accounted for the increase in the number and size of its leaves as well as its high photosynthesis rate. The leaf area of *E. variegata* was large compared to other species and leaves were abundant. In the study of Davis-McGregor and Goebel (1968), the diameter increase was due to the number and size of leaves and accelerated rate of P_N. Glori *et al.* (1978) mentioned that moisture stress had practically no effect on the diameter growth rate.

Table 3. Correlation coefficients of growth and physiological characteristics of four species at La Mesa Dam Watershed, Philippines

Parameters	PN	g_s	E	LA	STS	CHL_t	FW	DW	H
g_s	0.92^{**}								
E	0.94^{**}	0.97^{**}							
LA	0.93^{**}	0.004^{ns}	0.08^{ns}						
STS	0.32^{ns}	0.32^{ns}	0.38^{ns}	0.11^{ns}					
CHL_t	0.96^{**}	0.30^{ns}	0.25^{ns}	0.56^{*}	0.38^{ns}				
FW	0.82^{**}	0.04^{ns}	0.14^{ns}	0.04^{ns}	0.03^{ns}	0.43^{*}			
DW	0.72^{**}	0.04^{ns}	0.10^{ns}	0.72^{*}	0.07^{ns}	0.20^{ns}	0.96^{**}		
H	0.51^{*}	0.002^{ns}	0.005^{ns}	0.29^{ns}	0.11^{ns}	0.08^{ns}	0.26^{ns}	0.25^{ns}	
D	0.55^{*}	0.02^{ns}	0.05^{ns}	0.34^{ns}	0.12^{ns}	0.04^{ns}	0.34^{ns}	0.34^{ns}	0.96^{**}

Legend:
P_N – photosynthesis; g_s – stomatal conductance; E – transpiration; LA – leaf area
STS – stomata size; CHL_t – total chlorophyll; FW – fresh weight
DW – dry weight; H – height; D – diameter
* - significant at 5% level; ** - highly significant at 1% level; ns – not significant at 5% level

P. indicus exhibited low performance although it is an N_2-fixing species. The decline in height, diameter growth, and dry matter production of species for some time may be related to the insufficiency of available nutrients (Table 4). N, P and K uptake significantly diminished with stress. In addition, according to Kramer (1969), absorption might be reduced because of the slow movement of minerals in drying soil, decreased root extension, and decreased root permeability due to suberization. But according to Ng (1992), usually the initial growth of *P. indicus* is relatively slow and it is deciduous for a short time during dry season (Cadiz and Mizal 1995). Assessment of Castañeto and Carandang (1997) showed that best performance of *P. indicus* was at pH 5.8 and 6.4. However, in this study, the average pH of the soil was only 5.52 and 5.64 in the flat area and mountain area, respectively (Table 4). This trend may explain why the growth of *P. indicus* was somehow affected.

Table 4. Soil properties between two sites in La Mesa Dam Watershed, Philippines

Site	pH	N (%)	CEC Cmol (+)/kg soil	Organic matter (%)	Moisture content (%)
Flat area	5.52a	0.11a	4.84b	9.20a	29.9b
Mountain area	5.64a	0.14a	8.33a	10.33a	32.5a

Means within a column followed by the same letter are not significantly different at 5% level by DMRT.

Height in the flat area was relatively small compared to that in the mountain area because the site was mostly dry. Drying of soils reduces the availability of minerals in general (Volk 1934).

3) Fresh weight, dry weight and root-shoot ratio

The regression equation indicated that the *FW* of the root and shoot was related (R^2= 0.74; y = 0.778D^2H + 1.468; F=95.7) to D^2H (square of diameter times height). Any consistent changes in the *FW* were regarded as due primarily to the changes in the D^2H. Fresh weight (P=0.001) and dry weight (P=0.001) were significantly different in between two sites and among species (P=0.001 and P=0.001, respectively). *Erythrina variegata* consistently had greater fresh weight (160.02g and 839.24g in the flat area and mountain area, respectively) and dry weight (64.59g and 340.22g in the flat area and mountain area, respectively) compared with the other species (Table 2). The difference was most likely due to its much greater photosynthetic surface. High fresh and dry weight values for *E. variegata* were due to its high height and diameter values as well.

There were no significant differences in root-shoot ratio at 5% level between sites (P=0.56) and among species (P=0.23).

4) Root and shoot growth characteristics among species in the flat area

Among the measured root growth characteristics, root branching (P=0.54) and secondary root length (P=0.52) showed no significant effect at 5% level. Primary root length (P=0.004) and number of secondary roots (P= 0.002) were highly significant among species (Table 5).

Table 5. Root growth characteristics of four species in the flat area of La Mesa Dam Watershed, Philippines

Species	Root length (cm)		Root branching	No. of secondary roots
	Primary	Secondary		
D. dao	9.8b	7.2a	4a	11bc
E. variegata	19.2a	8.7a	4a	17a
P. indicus	11.3b	6.0a	4a	12ab
B. javanica	9.6b	5.6a	4a	6c

Means within a column followed by the same letter are not significantly different at 5% level by Duncan's Multiple Range Test (DMRT).

In terms of the primary root length and number of secondary roots, *E. variegata* had the highest values (19.2 cm and 17, respectively) while *B. javanica* (9.6 cm and 6, respectively) had the lowest values. All the species had the same number of root branches and almost the same secondary root length. Nitrogen-fixing species such as *P. indicus* and *E. variegata* had the highest number of secondary roots.

All the shoot growth parameters were significantly different among species. The number of leaves, stem length and the number of branches were highly significant among species at 5% level. For instance, the stem length of *D. dao*, *E. variegata,* and *P. indicus* had almost the same large values but *B. javanica* had the smallest value (25.9 cm). The number of leaves and branches, *D. dao* were 77 and 8, respectively, while those of the other species were almost the same (Table 6). While stem growth is directly due to cell number and cell length, some factors such as light, water status, nutrients, genetic deficiency of hormones, and others can affect stem growth (PSU 2000).

Table 6. Shoot growth characteristics of four species in the flat area of La Mesa Dam Watershed, Philippines

Species	Stem length (cm)	No. of leaves	No. of branches
D. dao	56.0a	77a	8a
E. variegata	65.4a	11b	5ab
P. indicus	63.0a	22b	5ab
B. javanica	25.9b	7b	3b

Means within a column followed by the same letter are not significantly different at 5% level by Duncan's Multiple Range Test (DMRT).

5) Leaf area

Leaf area was linearly related with the diameter (R^2= 0.75; y = 0.274*D* + 1.707). Any consistent changes in the LA were regarded as due primarily to the changes in diameter. The leaf area of *E. variegata* (Fig. 3) both in flat and mountain areas was larger while that of the other species were almost the same. Among the species *E. variegata* had the largest leaf area (Table 2).

The total leaf area has significant effect on water loss of individual plants, as plants with large leaf areas usually transpire more than those with small leaf areas (Kozlowski and Pallardy 1997). *Erythrina variegata* had evidently more total transpiring surface area. The variations in the leaf area among species may lie in the duration of the cyclic application of water stress, and perhaps in the relatively short duration of this study.

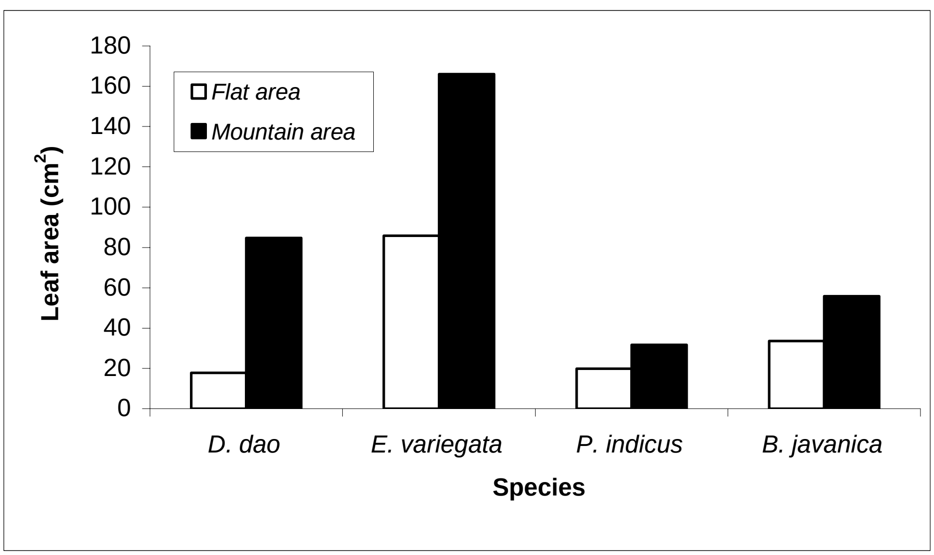

Fig. 3. Leaf area of four species grown in the flat area and mountain areas of La Mesa Watershed, Philippines.

6) Leaf anatomical characteristics among four species in the flat area

Total leaf thickness, epidermal thickness, and palisade mesophyll thickness were highly significant at 5% level (Table 7). *B. javanica* had the highest total leaf thickness (274 µm), epidermal thickness (28 µm), and palisade mesophyll thickness (113 µm) compared to other species followed by *E. variegata* (Fig. 4a - 4d). *P. indicus* had only 163µm leaf thickness but it had high photosynthesis rate. This is contrary to the result of Kramer (1969) that reduction in leaf thickness accompanies loss of turgor and wilting, and in which closure of stomates that often interferes with photosynthesis also occurs.

Table 7. Leaf anatomical features of four species in the flat area and mountain area of La Mesa Dam Watershed, Philippines

Species	Total leaf thickness (µm)	Epidermal leaf thickness (µm)	Palisade mesophyll thickness (µm)
D. dao	135d	9d	55c
E. variegata	207b	19c	81b
P. indicus	163c	23b	59c
B. javanica	274a	28a	113a

Means within a column followed by the same letter are not significantly different at 5% level by Duncan's Multiple Range Test (DMRT).

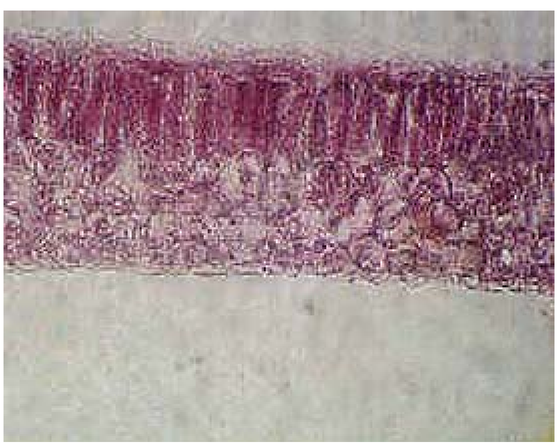

Fig. 4a. Cross-section of Dao (Dracontomelon dao (Blanco) Merrill et Rolfe) leaf. The average total leaf thickness is 135 µm, epidermal thickness is 9 µm and palisade mesophyll thickness is 55 µm.

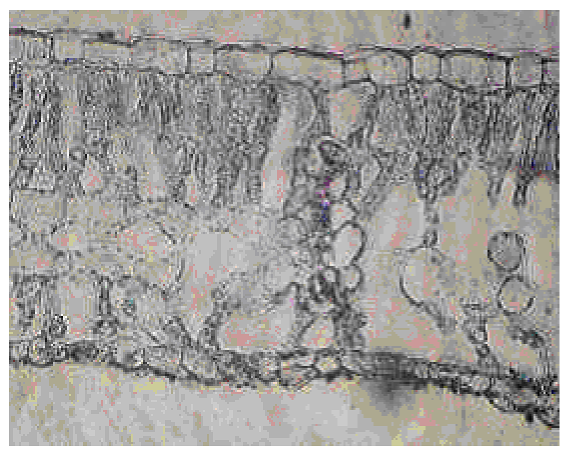

Fig. 4b. Cross-section of Dapdap (Erythrina variegata L.) leaf. The average total leaf thickness is 207 µm, epidermal thickness is 19 µm and palisade mesophyll thickness is 81 µm.

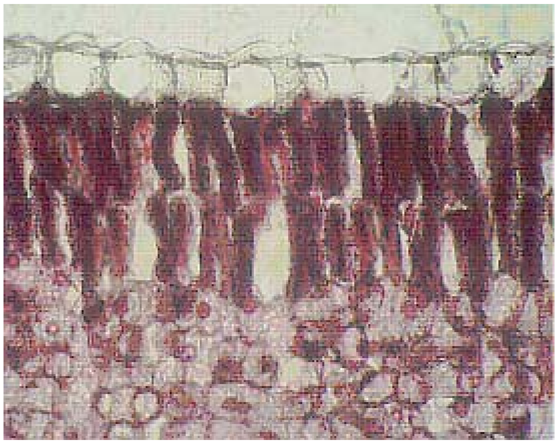

Fig. 4c. Cross-section of Tuai (Bischofia javanica Blume) leaf. The average total leaf thickness is 274 µm, epidermal thickness is 28 µm and palisade mesophyll thickness is 113 µm.

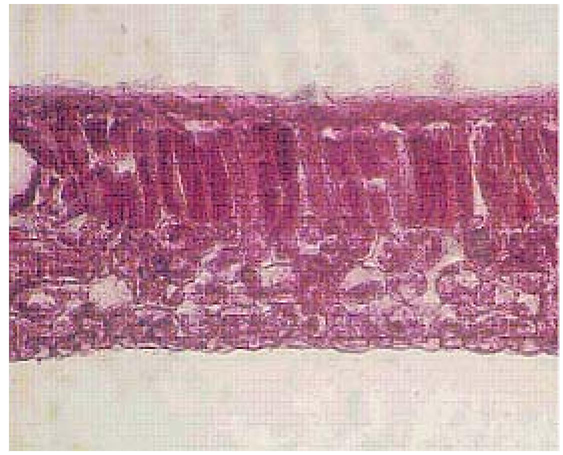

Fig. 4d. Cross-section of Narra (*Pterocarpus indicus* Willd.) leaf. The average total leaf thickness is 163 µm, epidermal thickness is 23 µm and palisade mesophyll thickness is 59 µm.

2. Physiological characteristics

Significant differences in all physiological characteristics were found among species at 5% level except for total chlorophyll content (Table 8).

Table 8. Means of variables of four species in the flat and mountain areas of La Mesa Dam Watershed, Philippines

Species	P_N	E	WUE	g_s	STS	STN	CHL_t
D. dao	1.98b	0.26b	7.61b	0.023b	59.26b	25a	1.39a
E. variegata	5.50a	0.54a	10.1a	0.033a	124.62a	11bc	2.26a
P. indicus	3.50ab	0.47a	7.40b	0.032a	135.54a	8c	2.38a
B. javanica	1.52b	0.26b	5.84c	0.022b	86.59b	13b	1.44a

Legend: PN - Net Photosynthesis (µmol CO_2 m^{-2} s^{-1}); E - Transpiration (mmol H_2O $m^{-2}s^{-1}$); g_s – stomatal conductance (mol H_2O $m^{-2}s^{-1}$); STS - stomata size; STN - stomata number; CHL_t - total chlorophyll (µg mL^{-1}); WUE - water use efficiency (µmol CO_2 m^{-2} s^{-1}per mmol H_2O $m^{-2}s^{-1}$)

Means within a column followed by the same letter are not significantly different at 5% level by DMRT.

1) Net photosynthesis

Nitrogen fixing trees, especially *E. variegata*, generally displayed the highest observed P_N (23.4 µmol CO_2 m^{-2} s^{-1} at PPFD 2000 µmol m^{-2} s^{-1}) both in the mountain and flat areas and *D. dao* generally had the lowest observed P_N (6.68 µmol CO_2 m^{-2} s^{-1} at PPFD 2000 µmol m^{-2} s^{-}1) as shown in Figures 5 a and b. The high P_N in *E. variegata* maybe associated to its high biomass compared to other species.
Differences in P_N among tree species are related to the arrangement of the leaves and resultant mutual shading, to the morphology of the leaves, and perhaps to the chlorophyll concentration of the leaves (Fowells and Means 2005).

In this study, P_N was highly correlated with *CHLt*, *LA*, and *FW* and *DW* (Table 3), which is similar with the study of Woo (2003) and Tjoelker et al. (1998). Much interest has been shown in using P_N as indices of growth potential of tree species and genotypes (Kozlowski and Pallardy 1997). *Erythrina variegata* and *P. indicus* were considered the photosynthetically (potentially) most efficient plants because they have high P_N with increased light intensities.

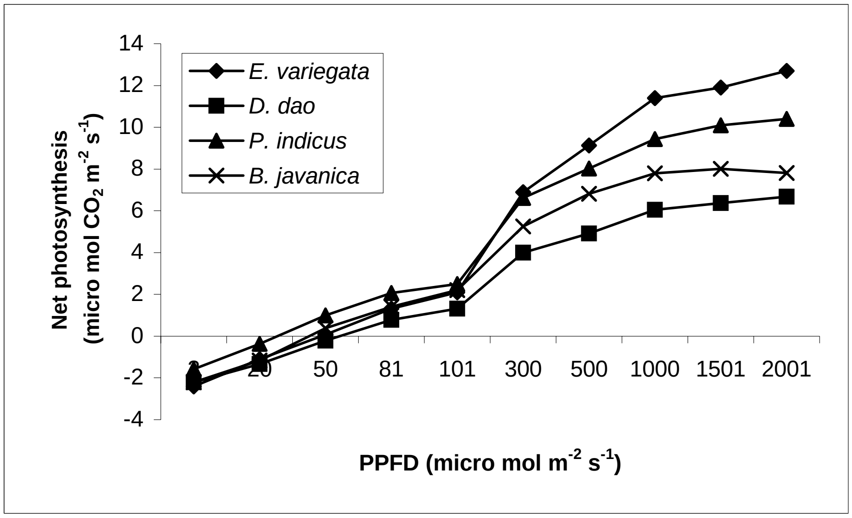

Fig. 5a. Net photosynthesis among four species in the flat area of La Mesa Watershed Philippines.

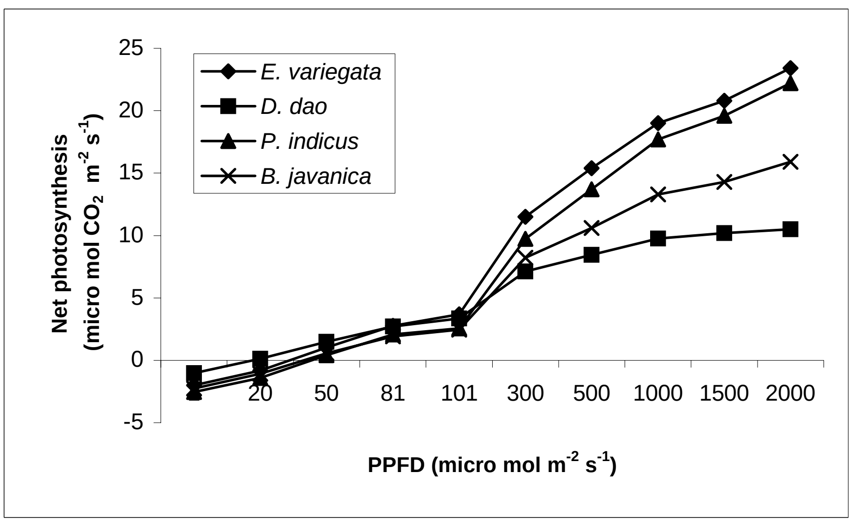

Figure 5b. Net photosynthesis among four species in the mountain area of La Mesa Watershed, Philippines.

2) Transpiration and stomatal conductance

Transpiration (*E*) and stomatal conductance (g_s) showed trends similar to those of P_N. There was a significant difference in *E* and g_s between sites (P=0.005 and P=0.003, respectively) and among species (P=0.005 and P=0.002) but none in site x species interaction at 5% level. The four species differed in *E* and g_s per unit leaf area (Table 8). *Erythrina variegata* had the highest *E* (0.1 to 1.02 mmol H_20 $m^{-2}s^{-1}$ in the mountain area and 0 to 0.79 mmol H_20 $m^{-2}s^{-1}$ in the flat area) and g_s (0.0012 to 0.06 mol H_2O $m^{-2}s^{-1}$ in the mountain area and 0.0002 to 0.05 mol H_2O $m^{-2}s^{-1}$ in the flat area) compared to *D. dao* and *B. javanica*.

In *P. indicus*, however, reduction of the amount and size of foliage did not affect the rate of water loss per unit leaf area. The total *E* per species was greater for *E. variegata* than other species because it had a greater total transpiring surface (92.3 cm^2) as shown in Table 2. There are variations in *E* per unit of leaf area among species.

According to Wysinger (2005), species with high *E* rates will have high stomatal density, i.e., the more stomates, the more water loss. In addition, species with high stomatal cover will have a high *E* rate, which means that the more leaf surface is covered with stomates, the more capacity for water loss. In this study, the species with high g_s and stomatal cover had high *E* rate, for instance, in *E. variegata*. However, *E* was not linked to stomatal density.

The g_s tends to be high when open stomata or relatively short pore depths occupy a large portion of the leaf area. Variations occur in the size of the stomata, frequency, control of stomatal aperture, and stomatal occlusion influence g_s and the rate of photosynthesis (Nobel 1991).

3) Stomata size and number among four species

Stomata size and the number of stomata were highly significant among species (P=0.004 and P=0.002, respectively) (Table 8). The Stomates of *E. variegata* and *P. indicus* were evidently larger than *D. dao* and *B. javanica*. The number of stomata in all the species varied at both sites (Table 9). *Dracontomelon dao* and *B. javanica* had more number of stomata while and *P. indicus* and *E. variegata* had the least (Fig. 6a - 6d).

Table 9. Stomata number and stomata size of four species in the flat and mountain areas of La Mesa Watershed, Philippines

Species	Flat area		Mountain Area	
	Stomata No. (1000X)	Stomata size (µm)	Stomata No. (1000X)	Stomata size (µm)
D. dao	27a	56.87b	23a	61.64b
E. variegata	11bc	125.76a	10bc	123.48a
P. indicus	9c	134.24a	7c	136.84a
B. javanica	16b	76.36b	11b	96.82b

Means within a column followed by the same letter are not significantly different at 5% level by DMRT.

Stomata size and frequency varied among species within a genus as shown by Kozlowski and Pallardy (1997) while stomatal frequency varies with cultivar, ploidy level, plant and leaf age, and upper versus lower leaf surfaces (Ekenayake et al. 1998). Species with few stomata per unit of leaf surface tends to have large stomata as in *E. variegata* and *P. indicus* which have relatively large but few stomata and *D. dao* and *B. javanica* which have small but numerous stomata. Kramer and Kozlowski (1960) theorized that stomatal frequency and size, although less important, may also affect transpiration per unit of leaf surface, photosynthesis and growth performance of different species.

If the stomata are open, with increased stomatal frequency, the rate of transpiration increases but it also depends on the degree of the opening of the stomata (TNAU 2005). The size of the stoma also affects the P_N and water uptake. Changes in the amount of water in plant are related to the relative size of the stomatal pores. The presence of numerous stomata can facilitate the diffusion of gases between the leaf and the atmosphere (Leung 2005).

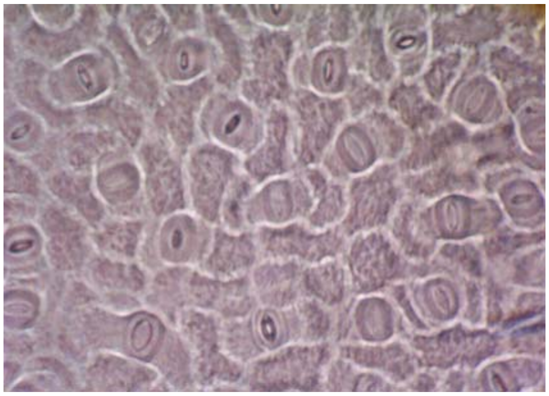

Fig. 6a. Stomata on the abaxial surface of *Dracontomelon dao*. The stomata number ranged from 20 to 31 and stomata length and width ranged from 7 to 11.9 μm and 3.5 to 7.7 μm, respectively.

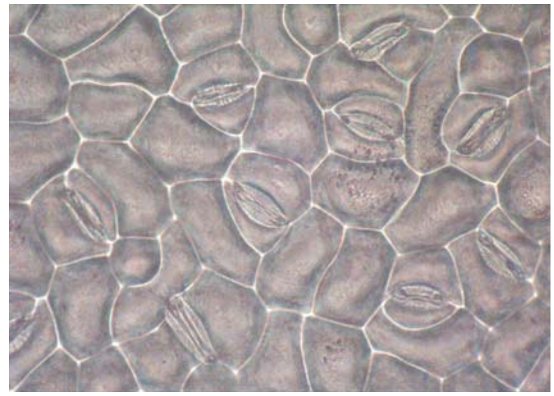

Fig. 6b. Stomata on the abaxial surface of *Erythrina variegata*. The stomata number ranged from 9 to 15 and stomata length and width ranged from 11.2 to 19.6μm and 4.9 to 11.9 μm, respectively.

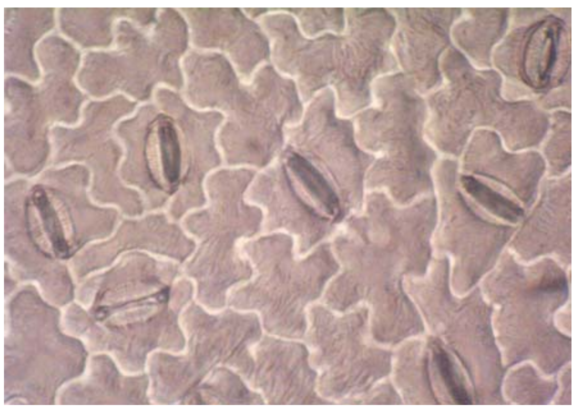

Fig. 6c. Stomata on the abaxial surface of *Pterocarpus indicus.* The stomata number ranged from 8 to 11 and stomata length and width ranged from 10.2 to 18.9μm and 5.6 to 11.2 μm, respectively.

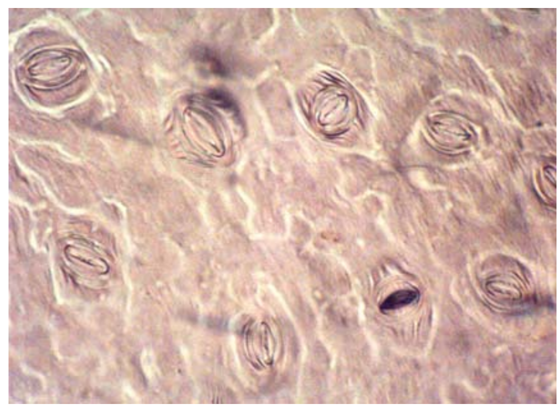

Fig. 6d. Stomata on the abaxial surface of *Bischofia javanica*. The stomata number ranged from 13 to 23 and stomata length and width ranged from 9.1 to 14.7μm and 3.5 to 10.5 μm, respectively.

4)Water use efficiency among four species

The efficient use of water in plants plays a predominant role particularly in the areas where the water is a limiting factor of production (Lazaridou and Koutroubas 2004). In addition, water use efficiency (*WUE*) could be used as a selection criterion to improve yield in a dry environment (Tardieu 1997). Water use efficiency at the scale of the photosynthesizing leaf is defined as the ratio of photosynthesis (P_N) to water loss in transpiration (E) (Wang 2001; Ashraf et al. 2002).

There were significant differences ($P<0.001$). in water use efficiency among the four species *E. variegata* had the highest *WUE* (10.1 μmol CO_2 m^{-2} s^{-1}per mmol H_2O $m^{-2}s^{-1}$) followed by *D. dao*, *P. indicus* and *B. javanica* (Table 8). According to Kozlowski and Pallardy (1997), greatest increase in *WUE* was associated with plants subjected to high light intensity or high rates of N addition. Sometimes it has been asserted that it is a general adaptation of drought-tolerant plants. There also appear to be substantial differences in *WUE* among species that are independent of the mode of photosynthesis.

According to Gollands (2005), *WUE* is ultimately determined by the stomatal behavior. When the stomata are wide open, the plant can capture CO_2 at the highest rate, but the *WUE* is low. Thus, there is usually a trade-off between plant growth rate and high water use efficiency, but the trade-off is not always the same. It is influenced by the integration of leaf photosynthetic capacity, root uptake capacity and the transport functions within each plant, and is subject to the genetic control of all these functions.

5) Chlorophyll content of four species between two sites

Chlorophyll is the most important pigment found in chloroplasts. Changes in properties of chloroplast membrane lipids play a major role in acclimation of photosynthesis to high temperatures by increasing the heat stability of membranes (Kozlowski and Pallardy 1997). Chlorophyll is a sensitive indicator of photosynthetic capacity of trees under various environments (Grimm and Fuhrer 1992). According to Kebabian et al. (1998), the magnitude of plant fluorescence and its spectral distribution is sensitive to a number of factors, which are related to the ability of plant to perform photosynthesis. In the field, however, the rate of photosynthesis may not vary much over a considerable range of leaf color, indicating that chlorophyll content often is less important than other factors in controlling photosynthesis. The organization of the chlorophyll in terms of number and size of photosynthetic units may be as important as the amount (Kozlowski and Pallardy 1997). Nevertheless, severe chlorosis from whatever cause invariably is correlated with reduced photosynthesis.

The total chlorophyll content among species was not significantly different at 5% level (Table 8) although *P. indicus* (2.3631 µg/mL) and *E. variegata* (2.2593 µg/mL) were generally higher than that of *B. javanica* and *D. dao.* However, between the two sites, they were significantly different (P=0.03). Generally, the total chlorophyll content (*CHLt*) of all four species grown in the mountain area was higher than those of the species grown in the flat area (Fig. 7) since P_N was higher in the mountain area and chlorophyll content was correlated with P_N (Table 3). The average *CHLt* in the mountain was 2.2055 µg/mL while 1.5121 µg /mL in the flat area.

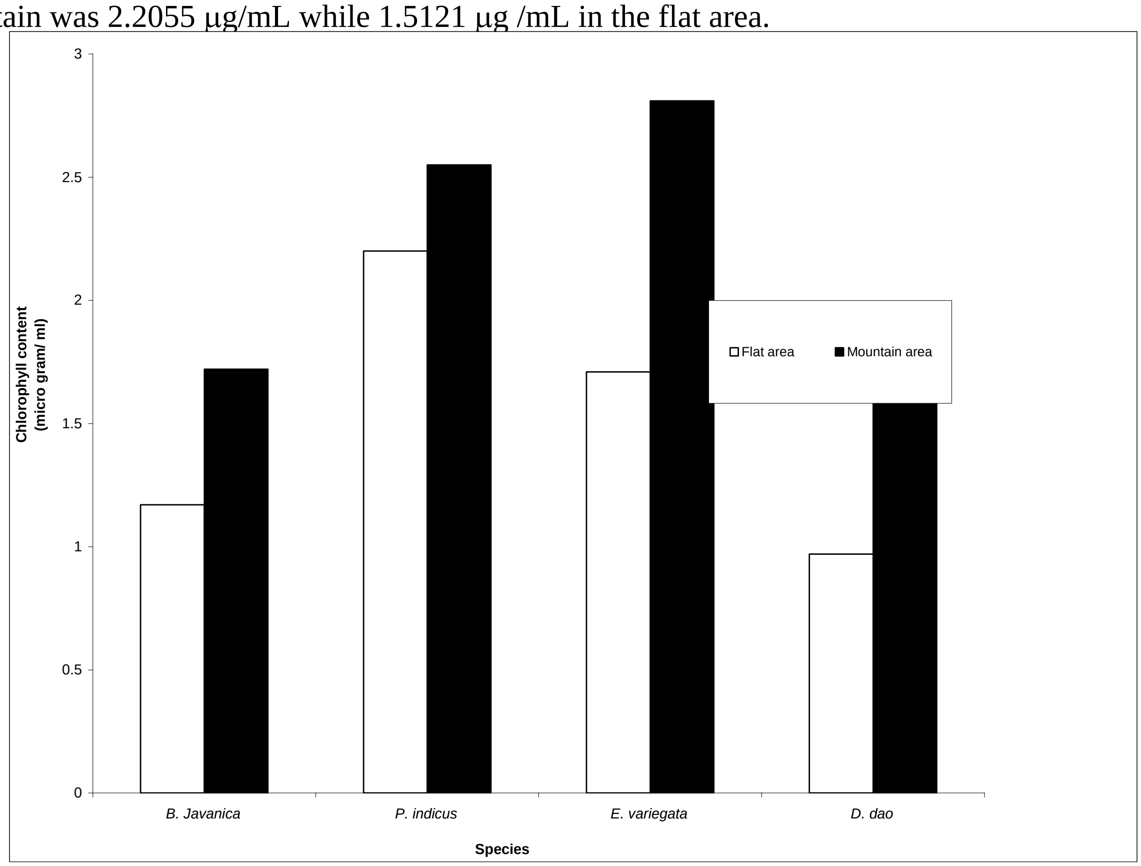

Fig. 7. Total chlorophyll content of four species in the flat area and mountain areas of La Mesa Watershed, Philippines.

CONCLUSION

The four species vary significantly with respect to growth performance. Among the four species, Dapdap (*E. variegata*) showed good performance in terms of early growth characteristics. Height and diameter growth rate were higher in *E. variegata.* In terms of leaf area, it also appeared to be markedly superior. Other growth characteristics such as fresh weight and dry weight, root-shoot ratio and root and shoot growth characteristics were found to be higher as well in *E. variegata. Erythrina variegata* had also high survival rate similar to *P. indicus*. In terms of physiological characteristics such as P_N, *E*, g_s, and *WUE,* the nitrogen-fixing trees, *E. variegata* and *P. indicus* exceeded *B. javanica* and *D. dao*. They also had bigger and fewer compared with *D. dao* and *B. javanica*. The *E* rate was greater in *E. variegata* because it had a greater total transpiring surface. *E. variegata* and *P. indicus* are therefore suitable for planting in the grassland areas of the La Mesa Dam Watershed.

In comparing the two sites, the mountain area was better than flat area because the soil in the area was more fertile and had higher moisture content.

In *E. variegata*, there was a high correlation between P_N and *DW*. Total chlorophyll content was also correlated with fresh weight and *LA*. In *P. indicus*, P_N was also highly correlated with *DW*. Furthermore, g_s and *E* were highly correlated with the size of the stomata.

Early good growth and physiological characteristics of *E. variegata* indicate its potential for reforestation in La Mesa Dam Watershed.

ACKNOWLEDGEMENT

The senior author thanks Dr. Willie M. Carandang for advice and help especially in the field and the ASEAN-Korea Environmental Cooperation Project (AKECOP) supported by the Korean Ministry of Foreign Affairs and Trade (MOFAT) and its project staff for financial support for experiment.

REFERENCES CITED

ARNON DI. 1949. Copper enzymes in isolated chloroplasts, polyphenol-oxidase in *Betula vulgaris.* Plant Physiol 24: 1-15.

ASHRAF M, ARFAN M, SHAHBAZ M, AHMAD A, JAMIL A. 2002. Gas exchange characteristics and water relations in some elite okra cultivars under water deficit. Photosynthetica 40(4): 615-620.

CADIZ RT, MIZAL RB. 1995. Narra. Research information series on ecosystems (RISE) 1(2): 48-63.

CARANDANG AP, CARDENAS LC. 1991. Insights into the problems encountered by government reforestation efforts. Phil Lumberman 37(6): 10-12.

CASTAÑETO YT. 1997. Growth of narra (*Pterocarpus indicus* Willd.) at varying moisture regimes in two-textured soils. Meristem 4: 13-17.

CASTAÑETO YT, CARANDANG WM. 1997. Phenotypic variation of narra (*Pterocarpus indicus)* provenances to varying soil moisture and pH. [PhD dissertation]. College, Laguna, Philippines: University of the Philippines Los Baños.120 p. (Available at the UPLB-CF Library)

DAVIS-MCGREGOR WH, GOEBEL NB. 1968. Effectiveness of nitrogen fertilizer and mulch for the amelioration of severe planting sites. In: Youngberg CT, Davey CB, editors. Tree growth and forest soils. Proceedings of the North America Forest Soils Conference; North Carolina, USA. 513 p.

EKENAYAKE IJ, OSIRU DVS, PORTO MCM. 1998. Physiology of cassava. http://www.iita.org.
FOWELLS HA, MEANS JE. 2005. The tree and its environment. Silvics manual. Volume 2. http://www.na.fs.fed.us.

GLORI AV, DE LA CRUZ RE, DOMINGO IL. 1978. Drought resistance of yemane (*Gmelina arborea*) and kaatoan bangkal (*Anthocephalus chinensis*). Sylvatrop 3(4): 211-239.

GOLLANDS C. 2005. The story of water management in terrestrial plants. http://isotope.bti.cornell.edu.

GRIMM AG, FUHRER J. 1992. The response of spring wheat (*Triticum aestivum* L.) to ozone at higher elevations. New Phytologist 122: 321-328.

HEDGE NG. 1993. Cultivation and uses of *E. variegata* in western India. In: WESTLEY SB, POWELL MH, editors. *Erythrina* in the new and old worlds. Paia, Hawaii: NFT. p. 77-84.

KEBABIAN PL, THEISEN AF, SCOTT HE, FREEDMAN A. 1998. Positive two-band plant fluorescence sensor with applications in precision agriculture. Boston: SPIE p. 238-245.

KOZLOWSKI TT, PALLARDY SG. 1997. Physiology of woody plants. 2nd ed. San Diego: Academic Press, Inc. 411 p.

KRAMER PJ. 1969. Plant and soil water relations, a modern synthesis. New Delhi: Tata McGraw-Hill Publishing Co. Ltd. 482 p.

KRAMER PJ, KOZLOWSKI TT. 1960. Physiology of trees. New York: McGraw-Hill. 642 p.

LAZARIDOU M, KOUTROUBAS SD. Drought effect on water use efficiency of bersum clover at various growth stages. In: Fischer T, Turner N, Angus J, Mcintyre L, Robertson M, Borrell A, Lloyd D, editors. New directions for a diverse planet. Proceedings of the 4th international crop science congress; 2004 September 26 – October 1; Brisbane, Australia: Australian Centre for International Agricultural Research.

LEUNG J. 2005. Plant nutrition, transpiration, transport and support. http://www.fed.cuhk.edu.hk.

LUNA AM. 1991. Variability in seedling growth and natural nodulation of different *acacia* species and seed sources of *A. auriculiformis* and *A. mangium.* [PhD dissertation]. College, Laguna, Philippines: University of the Philippines Los Baños. 216 p. (Available at the UPLB-CF library)

MALVAS JD. 1995. Existing policies and current uses of Philippine grasslands. In: Umali CG, Bravo MVA, Exconde AB, editors. Strengthening Research and development for sustainable management of grasslands. Proceedings of the 1st national grassland congress of the Philippines; 1995 September 26-28; Los Baños, Laguna, Philippines: Environmental Research and Development Bureau. p. 32-38.

[NEPC] National Environmental Protection Council. 1983. The Philippine environment in the 21st century. Quezon City, Philippines.

NG FSP. 1992. *Pterocarpus indicus*- the majestic nitrogen-fixing tree. http://mefrinet.cbn.net.id.

NOBEL PS. 1991. Physiochemical and environmental plant physiology. San Diego: Academic Press, Inc. 636 p.

OWEN JN. 1991. Studying flowering and seed ontogeny in tropical forest trees. Thailand: ASEAN-Canada Forest Tree Seed Centre Project. 130 p.

[PSU] Pennsylvania State University. 2000. Stem cell research. http://www.corses.psu.edu.

TARDIEU F. 1997. In: Belhassen E, editor. Drought tolerance in higher plants: genetical, physiological and molecular biological analysis. Netherlands: Kluwer Academic Publishers. p. 15-26.

TJOELKER MG, OLEKSYN J, REICH PB. 1998. Seedlings of five boreal tree species differ in acclimation of net photosynthesis to elevated CO_2 and temperature. Tree Physiol 18 (11): 715-726.

[TNAU] Tamil Nadu Agricultural University. 2005. Transpiration. http://www.tnau.ac.in

VOLK NJ. 1934. The fixation of potash in difficulty available form in soils. Soil Sci 37: 267-287.

WANG RZ. 2001. Photosynthesis, transpiration and water use efficiency of vegetative and reproductive shoots of grassland species from north-eastern China. Photosynthetica 39(4): 569-573.

WOO SY. 2003. Effects of different light intensities on growth, chlorophyll contents, and photosynthesis of *Abies holophylla*. Meristem 3:1-5.

WYSINGER S. 2005. Stomatal density contribution to transpiration across tree species. http://www.multicultural.cas.psu.edu.

Authors' Information:

*[1] Marilyn S. Combalicer. Corresponding author and Assistant Professor, College of Forestry, Nueva Vizcaya State University, Bayombong, Nueva Vizcaya. E-mail: marilyn_sabalvaro(at)yahoo.com; Telephone: +63-78-805-3595; Fax: +63-78-321-2112

[2] Don Koo Lee**,** Professor, Department of Forest Sciences, College of Agriculture and Life Sciences,
Seoul National University, Seoul 151-742, Republic of Korea. E-mail: leedk(at)plaza.snu.ac.kr; Telephone: +82-2-880-4751; Fax: +82-2-878-9783

[3] Su Young Woo, Professor, Department of Horticulture, University of Seoul, Seoul 130-743, Republic of Korea. E-mail: wsy(at)uos.ac.kr; Telephone: +82-2-2210-5634; Fax: +82-2-2210-2838

[4] Yong Kwon Lee, Deputy Director, International Cooperation Division, Korea Forest Service, Daejon Metropolitan City, Republic of Korea, 302-701. E-mail: shorea(at)korea.kr; Telephone: +82-42-481-4277; Fax: +82-42-481-4036

[5] Youn Ho Jang, Manager, Consulting Team, Overseas Forest Management Headquarters, Korea Green Promotion Agency, Korea Forest Service, 309 Honorsville Bldg., 1380 Dunsan-dong, Seo-gu, Daejon Metropolitan City, Republic of Korea, 302-121. E-mail: yhjang(at)kgpa.or.kr; Telephone: +82-42-603-7312; Fax: +82-42-603-7310