Evaluation of Provenance Variation in Condensed Tannin Content of Fresh Leaves of *Calliandra calothyrsus*

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ABSTRACT : A field experiment was conducted with *Calliandra calothyrsus* of fifteen provenances to evaluate the variation in the condensed tannin content of fresh leaves. Plants were raised in a nursery for three months and planted in the field. Experimental design was line planting of double hedgerows (3 m long plot, 1/2 meter interval, 45 plants in each plot) with 5 replicates. Plants were lopped every 5 months and samples (10 different plants within a plot; 4 newly flushed leaves, 4 partially expanded leaves and 4 mature full expanded leaves from each of 10 trees in a plot) were collected and were put on ice before transfer to the laboratory. Proanthocyanidine was extracted in aqueous acetone, and total extractable proanthocyanidine (TEPA) and total proanthocyanidine (TOPA) were measured. In addition, proximate analyses were made of different provenances. Crude protein contents varied from 19.74% to 16.45%; *in vitro* organic matter digestibility ranged from 36.09% to 23.32%. TEPA content ranged from 11.22 to 16.01 (measured as absorbance at 550 nm, g^{-1} DM) and TOPA from 24.57 to 31.72 (measured as absorbance at 550 nm, g^{-1} DM). TEPA and TOPA had a positive correlation, whereas crude protein content of Calliandra was negatively correlated with TEPA and TOPA. Provenances, such as Georgesville (Belize), local, Coban (Guatemala), Patulul (Guatemala), Bombana (Mexico) can be selected on the basis of nutritive value of fodder. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 6 : 891-894*)

Key Words : *Calliandra calothyrsus*, Provenances, Condensed Tannin

INTRODUCTION

Calliandra calothyrsus contains high concentrations of tannin, and there are numerous reports of its high nutritive value to ruminants in terms of animal production (e.g. live weight gain). However, there is no available information on intraspecific variation in leaf tannin levels. Therefore, it is important to compare the levels of condensed tannin (proanthocyanidins) in different provenances of Calliandra managed under local conditions.

Even gentle drying of Calliandra leaves is known to affect both apparent condensed tannin content drastically. Calliandra is therefore fed fresh and it is very important also to carry out the analysis using fresh material, to obtain applicable results. Also wilting can have a considerable effect on the quality of Calliandra. Therefore, the objective of the present study was to evaluate the provenance variation in condensed tannin content of fresh leaves of Calliandra under the conditions prevailing in the mid country of Sri Lanka.

MATERIALS AND METHODS

A field experiment was conducted at the Pallekele Farm, Balagolla (Elevation-426 meters MSL; Mean Annual Rain Fall 900~1200 mm; mean temperature max: 30.5℃, minimum 20.2℃) using 15 provenances of Calliandra. The soil was immature Brown Loam with a pH of 5.3. Provenances used in the study are given in table 1.

Plants were raised in sterilized nursery beds. They were transferred at the height of 7.5 cm into 10 cm×22.5 cm poly bags with a potting mixture of 1:2:3 of compost : sand : topsoil respectively. Plants were planted at the field in August, at an average height of 30~60 cm.

Arrangement of plots

Experimental design was line planting double hedgerows (3 meter long plot, random design, 15 treatments, 5 replicates, 1/2 meter interval, 45 plants in each plot).

Plants were lopped for the first time around 6 months after field planting. This was followed by two more loppings around 4 months and 5 months later. Samples were collected from all provenances during the last lopping for this experiment (about 18 months of age, frequency of defoliation 5 months).

Sampling procedure

Because of the possibility of considerable variation between samples from the same provenance, five bulk samples of each provenance, one from each of the 5 replicates in the provenance trial (e.g. total of 75 samples, 1 from each plot) was taken.

Sample size

Each sample was composited from 10 different plants in a plot, and comprised 4 newly flushed leaves, 4 partially expanded leaves and 4 mature full

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Table 1. Proximate composition of *Calliandra calothyrsus* provenances*

Provenance	DM	Ash	CP	NDF	ADF	IVOMD
Barillas (Guatemala)	42.5	6.75^{abc}	18.36^{abc}	24.00^{cd}	21.39^{cd}	36.09^{a}
Bombana (Mexico)	42.2	6.46^{bcd}	18.46^{abc}	24.25^{bcd}	23.37^{bc}	31.55^{bcd}
Coban (Guatemala)	44.6	6.34^{bcd}	18.66^{ab}	22.77^{de}	21.82^{cd}	33.13^{abc}
Flores (Guatemala)	44.6	6.13^{cd}	16.89^{bc}	30.57^{a}	26.32^{a}	23.32^{g}
Fortune (Costa Rica)	42.9	6.39^{bcd}	18.49^{abc}	25.67^{bc}	22.25^{cd}	33.10^{abc}
Georgesville (Belize)	46.1	6.88^{ab}	19.74^{a}	21.21^{e}	21.53^{cd}	33.68^{abc}
Local	42.6	7.11^{a}	18.18^{abc}	26.70^{b}	22.89^{abd}	35.05^{ab}
Madin (Indonesia)	45.9	6.10^{cd}	16.45^{c}	26.71^{b}	24.62^{ab}	23.36^{g}
Patulul (Guatemala)	42.8	6.52^{abcd}	18.6 ^{ab}	23.50^{cde}	23.45^{bc}	33.42^{abc}
Plan de Rio (Mexico)	42.7	6.09^{d}	17.84^{abc}	23.88^{cd}	23.63^{bc}	27.20^{i}
San Estaban (Honduras)	42.8	6.72^{abcd}	18.14^{abc}	24.40^{bcd}	22.37^{cd}	33.57^{abc}
San Ramon (Nicaragua)	43.4	6.68^{abcd}	18.44^{abc}	23.26^{cde}	21.1^{d}	30.96^{cde}
Santa Mari de Jesus (Guatemala)	43.3	6.32^{bcd}	17.40^{bc}	25.33^{bcd}	22.67^{bcd}	27.99^{def}
Turrialiba (Costa Rica)	43.1	6.59^{abcd}	17.82^{abc}	24.41^{bcd}	22.00^{cd}	28.15^{def}
Union Jurarez (Mexico)	41.9	6.18^{cd}	17.55^{bc}	25.60^{bc}	23.2^{bcd}	27.83^{ef}

* Means with the same letter are not significantly different ($p<0.05$).

expanded leaves from each of the 10 trees.

Transfer of samples to laboratory

On collection of the samples, they were put on ice and transferred to the laboratory as fast as possible to minimize drying. Proanthocyanidine was extracted in aqueous acetone, and total extractable proanthocyanidine (TEPA) and total proanthocyanidine (TOPA) were measured by the standard method (Khazaal and Orskov, 1994).

In addition to these analyses, another set of samples was taken similarly for proximate analyses. Dried samples were analyzed for crude protein (CP) (AOAC 1980), Neutral Detergent Fiber (NDF) (Van Soest and Wine, 1967), Acid Detergent Fibre (ADF) (Van Soest, 1963) and *in vitro* organic matter digestibility (IVOMD) (Tilley and Terry, 1963).

Statistical analysis

Data were statistically analyzed using the SAS package (Barr et al., 1976). Variation between provenances was analyzed using Duncan's Multiple Range Test.

Table 2. TEPA and TOPA content of *Calliandra calothyrsus* provenances*

Provenance	TEPA	TOPA
	(Absorption 550 nm g^{-1} DM)	
Barillas (Guatemala)	13.99^{efg}	31.31^{d}
Bombana (Mexico)	13.28^{def}	29.32^{bcd}
Coban (Guatemala)	12.25^{bc}	31.14^{cd}
Flores (Guatemala)	13.53^{efg}	29.88^{bcd}
Fortune (Costa Rica)	14.14^{fg}	28.26^{abcd}
Georgesville (Belize)	11.22^{a}	24.57^{a}
Local	11.91^{ab}	29.68^{bcd}
Madin (Indonesia)	12.38^{bcd}	25.78^{ab}
Patulal (Guatemala)	16.01^{h}	28.24^{abcd}
Plan de Rio (Mexico)	13.38^{def}	29.98^{bcd}
San Estaban (Honduras)	14.46^{g}	30.25^{cd}
San Ramon (Nicaragua)	13.05^{cde}	29.04^{bcd}
Santa Mari de Jesus (Guatemala)	15.54^{h}	31.72^{d}
Turrialiba (Costa Rica)	13.92^{efg}	26.94^{abc}
Union Jurarez (Mexico)	14.03^{efg}	30.79^{cd}

* Means with the same letter are not significantly different ($p<0.05$).

RESULTS AND DISCUSSION.

Table 1 presents the crude protein content of Calliandra from each provenance. Crude protein content of Calliandra provenances ranged from 19.74% to 16.45%. According to the results, the lowest crude protein content was recorded with the provenance Madin (Indonesia) and the highest with the provenance Georgesville (Belize). Crude protein content in the local material was low (16.89%) compared to most of the provenances. According to NRC (1983), leaves and young green shoots have a crude protein content of 22% on a dry weight basis.

Table 1 presents the *in vitro* organic matter digestibility of different provenances. *In vitro* organic matter digestibility ranged from 36.09% (Santa mari de Jesus-Guatemala) to 23.32% (Local). Perera (1996) reported dry matter and organic matter digestibilities of 25.3% and 26.6% respectively. Our results are in agreement with the work of Perera (1996), however these values are lower than those of other fodder legumes (Premaratne, 1993, 1995).

Table 1 presents the proximate analyses. No significant differences were observed for dry matter

content between different provenances, however Ash, NDF, ADF contents were different.

Condensed tannin contents of are shown in table 2. TEPA content varied from 11.22 (Georgesville-Belize) to 16.01 (Patulul-Gautemala) when measured as absorbance at 550 nm g^{-1} DM. Local provenance had a lower TEPA content (11.91) compared to some of the others (table 2).

According to table 2, TOPA content as absorbance at 550 nm g^{-1} DM. Varied from 24.57 (Georgesville-Bellise) to 31.72 (Santa Marie de Jesus-Guatemala) TOPA content of local was higher ($p<0.05$) than in some of the imported materials.

Table 3 presents the correlation coefficients for relationships between the concentration of phenolic compounds and the fibre components of Calliandra. Crude protein content and *in vitro* organic matter digestibility were negatively correlated to fibre components whereas crude protein content and *in vitro* organic matter digestibility was positively correlated. The correlation between NDF and ADF content was also positive.

Table 3. Correlation coefficients (r) of relationships between concentration of condensed tannins and the fibre contents of the *Calliandra calothyrsus* provenances

Variable	Correlation coefficient with				
	CP	NDF	ADF	DIG	TEPA
NDF	-0.777	-			
ADF	-0.705	0.795			
DIG	0.823	-0.584	-0.718		
TEPA	-0.217	0.115	0.096	-0.042	
TOPA	-0.182	0.197	0.002	0.121	0.409

Several workers (Kumara and Vaithiyanathan, 1990; Waghorn et al., 1990) have reported that increasing the proportion of tannin-rich feeds in the diet of ruminants significantly reduces intake, and *in vivo* protein and dry matter digestibilities. However, condensed tannin did not have any strong correlation with the organic matter digestibility in this study, but *in vitro* values were used in this study instead of *in vivo*. TEPA and TOPA had a positive correlation, and crude protein content was negatively correlated with TEPA and TOPA.

It can be concluded that the selection of provenances as an animal feed should be based on the proximate composition, anti-nutritive factors, digestibility and intake of fodders. The dry matter yield of fodders as well as the digestible dry matter or digestible crude protein yield should also be considered, and provenances with the higher crude protein, higher digestibility and lower fibre and tannin contents should be selected.

Therefore, the moset desirable provenances appear to be Georgesville (Belize), local, Coban (Guatemala), Patulul (Guatemala), Bombana (Mexico), Fortune (Costa Rica), San Ramon (Nicaragua) and Barillas (Guatemala). Further studies are needed in terms of digestible dry matter and digestible crude protein intake by the animals as well as the dry matter yield of fodder.

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