Senanayake et al., Vegetos 2018, 31:1
DOI: 10.4172/2229-4473.1000383

Vegetos- An International Journal of Plant Research

Research Article a SciTechnol journal

Taxonomical and Chemical Characterization of *Panicum maximum* L. from Different Agro-Ecological Zone of Sri Lanka

Dharanee Senanayake[1], Jayasekera LR[1]* and Andreas Lucke[2]

Abstract

Chemical and taxonomical characteristics of *Panicum maximum* L. originated from seven contrasting agro-ecological zones of Sri Lanka were investigated representing the low country-wet zone (LW), mid country-wet zone (MW), up country-wet zone (UW), low country-intermediate zone (LI), mid country-intermediate zone (MI), up country-intermediate zone (UI) and low country-dry zone (LD). The levels of leaf nutrients namely, nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) were determined on a dry weight basis. Assuming linear relationships, some significant interelement relationships were detected in *P. maximum* leaves. The leaf anatomy and the mesophyll to bundle sheath cell ratio were studied using the light microscopy, whereas the vein density was determined by stereo microscopy. The foliar nutrient contents of *P. maximum* varied significantly ($P<0.05$) among different populations collected from diverse climatic zones. The highest nutrient contents were detected in the plants growing in the low country-wet zone, and the lowest in the low country-dry zone. Values of foliar $\delta^{13}C$ within populations from warmer and cooler climates had an average of -14% showing typical C4 photosynthesis with little evidence for adaptive divergence of the photosynthetic mode. Mesophyll cell to bundle sheath cell ratio of the leaves from different climatic zones had an average of 2.35 and the M: BS ratios were not significantly varied under different climatic conditions. Mean values of the vein densities of the leaves were 1.72 veins cm^{-2} and the highest vein density was obtained from the leaves of low country-wet zone and the lowest was from the leaves of up country-wet zone.

Keywords

Guinea grass; Carbon isotope ratio; Leaf inorganic composition; *Panicum maximum*

Introduction

Grasses have been recognized as a unique lineage among the flowering plants for more than 200 years and they are the most diverse group of plants that can be found virtually in all habitats from freshwater to sand dunes. Approximately 11,313 plant species in the world have been recognized as grass species [1]. *P. maximum* L., is known as Guinea grass or the panic grass is frequently found in warmer climates and used as a fodder grass. Both annuals and perennials can be found in this species [2,3], and are widely distributed almost all over the tropical countries such as Africa, India, South America and Sri Lanka due to its capacity to grow even under harsh environmental conditions [3]. *P. maximum* is sometimes classified as an invasive species in the tropical countries [4].

Thermal responses of the *Panicum* grasses vary with their genotype [5]. Some are adaptable for the shade environment and some are highly adaptable for the warmer climates. Though the exposure to the 70% of full sunlight is the best condition for the growth, some records have revealed that certain varieties are capable of surviving even under highly shaded conditions [5]. Therefore, adaptive divergence in physiological traits such as the mode of photosynthesis between populations may be expected that may confer local adaptation. Although the greatest proportion of C4 plants occurs in the grass family, adaptation to more C3-C4 intermediate photosynthesis could be expected in the populations from the cooler climates. C3 and C4 plants are easily distinguishable on the basis of their carbon isotope ratios, expressed as $\delta^{13}C$ values and quantified on a per mil (‰) basis [6,7]. The typical averages of $\delta^{13}C$ for the C3 and C4 plants are -28‰ and -14‰ respectively. On a theoretical basis, the $\delta^{13}C$ value for the C3-C4 intermediates could be expected between -14‰ and -28‰. In this study, we measured the $\delta^{13}C$ of the *Panicum maximum* populations from cooler and warmer climates to see whether there are variations between populations that may confer local adaptation reflecting C3-C4 intermediates. It has also been recorded that C4 plants are characterized with lower leaf mesophyll to bundle sheath cell ratio compared to C3 plants [8,9]. In addition to leaf carbon isotope characteristics, a selected set of leaf nutrients were also measured to detect any location-dependent population variations in the leaf nutrient contents.

Materials and Methods

Leaf nutrient analysis

Four replicate leaf samples from each agro ecological zone were collected contamination-free in clean polythene bags from undisturbed locations, each with an approximate area of 500 m^2 representing the seven different agro ecological zones of Sri Lanka (Table 1). They were then brought to the laboratory and cleaned with distilled water followed by drying at 70^0 C for 72 h in an oven, and ground with a pre cleaned grinder contamination-free. The homogenized leaf samples were transferred to clean polythene bottles (50 ml) and stored in a refrigerator until analysis. Leaf nutrients, the total nitrogen (N) content was determined by the standard Kjeldahl digestion [10], and the phosphorus (P) by molybdo-vanadate method [11]. Contents of potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) were determined on a dry weight basis by atomic absorption spectrophotometry (Model: Spectraa 110) following a wet digestion with a tri acid mixture at 200°C [10]. Four replicates were analyzed from each agro ecological zone. To control and assess the quality of the analytical results, the rye grass certified reference material No. 281 from the Commission of the European Communities, Community Bureau of Reference (BCR) were analyzed simultaneously.

Carbon isotope analysis

A set of homogenized leaf material in sealed polythene bags were

***Corresponding author:** Jayasekera LR, Department of Botany, University of Kelaniya, Sri Lanka, Tel: +94-11-2914480; Fax: +94-11-2914480; E-mail: ranjith@kln.ac.lk

Received: December 21, 2017 **Accepted:** February 23, 2018 **Published:** February 26, 2018 *doi:10.4172/2229-4473.1000383*

Table 1: Climatological data of the seven agro ecological zones of Sri Lanka*.

Zone	Temperature (°C)	Annual rain fall (mm)	Elevation (m)
Low country-		2635.0	~79 m
wet zone	35.3		
Low country-		2263.5	~161 m
intermediate zone	39.7		
Low country-		2069.0	~79 m
dry zone	37.2		
Mid country-		3659.4	~506 m
wet zone	32.7		
Mid country-		2034.9	~444 m
intermediate zone	35.0		
Up country-		2263.5	~811 m
wet zone	25.4		
Up country-		2528.8	~1300 m
intermediate zone	34.2		

*(Source: Department of Meteorology, Sri Lanka, 2015: personal communication).

air mailed to the Institute of Bio-and Geosciences, Research Centre Juelich, Germany for the carbon isotope analysis. About 0.25 mg of a sample was weighed into tin capsules to yield approximately 100 µg of carbon, and combusted at 1080°C using an elemental analyzer (Model: Thermo Scientific Flash 2000) interfaced in-line with an isotope ratio mass spectrometer (Model: Thermo Scientific Delta V Advantage). The $\delta^{13}C$ ratio was determined using the following equation and expressed in terms of δ values per mil (‰).

$$\delta^{13}C\ (‰) = [(R\text{sample} - R\text{standard})/R\text{standard}] \times 10^3$$

*R*sample and *R*standard are the isotope ratios ($^{13}C/^{12}C$) of the samples and standards, respectively, reported on the VPDB scale. The standard represents the carbon isotope ratio in a fossil belemnite given by the PDB- formation. In addition to the calibration standards, additional laboratory standard materials were also inserted between samples to monitor the performance of the instrument. The overall precision of the replicate analyses was estimated as higher than ± 0.1‰ [12]. Four replicates per sample were analyzed from each agro ecological zone.

Leaf anatomy

Leaf tissues for all microscopic observations were obtained from the middle of the leaf lamina at a point equidistant between the midrib and the leaf margin. The leaf anatomy was observed using leaf transverse sections under the light microscope [8]. Light micrographs were used to determine the mesophyll to bundle sheath cell area ratio (M:BS) in a field of 0.25 cm^2 tissue area, and vein density measurement was done manually by counting the veins of each leaf under a microscope [13]. All the micrographs were processed by Image J software.

Statistical analysis

Statistical comparisons of the data obtained were conducted with MINITAB 14 version using one-way analysis of variance (ANOVA, $p<0.05$) followed by Tukey's pair wise multiple comparison test. Pearson correlation test was employed to identify the correlations between the various nutrient elements.

Results and Discussion

As the element analysis in biological samples requires accurate determinations at their concentrations, the quality of the results (precision and accuracy) was confirmed by analyzing a certified reference material (BCR No. 281-rye grass) provided by simultaneously and the analytical results are compiled in Table 2. No certified values are available for N, P, K, Ca, Mg and Fe that are biologically significant. BCR No. 281 was selected in this study as it is floristically similar to *Panicum maximum* matrix to ensure the same sources of error caused by matrix effects. Copper, Mn and Zn of the BCR No. 281 are consistent with the measured values (Table 2).

The highest leaf nitrogen (N) content was detected from the specimens of mid country-wet zone and the lowest from the low country-dry zone as shown in Table 3. Phosphorus (P) and potassium (K) contents were highest in the up country-wet zone samples, whereas the lowest in the mid country-wet zone and low country-intermediate zone respectively. The low country-wet zone samples were characterized with the highest calcium contents and the lowest content was found in the up country-wet zone. Magnesium (Mg) was highest in the specimens of mid country-intermediate zone and lowest in the specimens of up country-intermediate zone. Regarding micro elements, highest copper (Cu) content was detected from the specimens of mid country-intermediate zone and lowest was from the specimens of up country-wet zone. Highest iron (Fe), manganese (Mn) and zinc (Zn) contents were found in the specimens of low country-wet zone and the lowest contents were found in the specimens of low country-dry zone, up country-intermediate zone and mid country-wet zone respectively.

Except for few elements, a majority of the nutrient contents were higher in the samples from low country-wet zone than the other climatic zones, probably due to the higher annual rain fall in the wet zone resulting in a higher availability of most nutrients in the soil solution [14]. Even though the soil in dry zone is highly fertile, scarcity of water due to the low rain fall retard the nutrient absorption of plants [14]. Therefore the nutrient levels of the *Panicum* leaf samples from low country-dry zone were lower than those from the other climatic zones. It is known that the soils at higher elevations are poor in terms of fertility due to the landslides and soil erosion, fertility of the up country lands is much lower [14], resulting in less nutrient availability to plants [15].

Correlation analysis assuming linear relationships between elements was used to examine where there were similar patterns of elemental concentrations in the samples of *P. maximum* under different climatic conditions. The correlation matrix (Table 4) consists of element-by-element comparisons and shows a number of significant relationships. The synergistic accumulation effect of calcium-nitrogen and zinc-iron effect in the *P. maximum* leaves appear

Table 2: Analytical values of constituent elements for the rye grass certified reference material (BCR No. 281)*.

Nutrient	Certified value	Measured value
%		
N	-	3.28 ± 0.03
P	-	0.24 ± 0.00
K	-	4.60 ± 0.04
Ca	-	0.20 ± 0.01
Mg	-	-
mg/kg		
Cu	10.2 ± 0.50	9.8 ± 0.17
Fe	-	326.0 ± 1.00
Mn	82.0 ± 4.00	80.4 ± 0.49
Zn	30.5 ± 1.10	28.3 ± 0.12

Table 3: Nutrient composition of *P. maximum* L. from different agro ecological zones in Sri Lanka*.

Nutrient	LW		MW		UW		LI		MI		UI		LD	
%														
C	41.5a,c	(1.8)	41.8a,c	(0.4)	41.2a	(0.8)	42.9b,c (1.1)		40.8a	(1.5)	41.4a	(2.5)	41.1a (1.3)	
N	1.92a	(4.7)	2.06a	(7.3)	1.65a,c (6.1)		1.82a	(19.8)	1.92a	(10.9)	1.68a	(11.9)	1.22b,c(4.9)	
P	0.24a,c	(20.8)	0.18b,c (11.1)		0.28a	(3.6)	0.19b,c(5.3)		0.21a	(9.5)	0.25a,c(12.0)		0.28a(17.9)	
K	1.96a,b (32.1)		1.84a,b (13.0)		2.64a	(4.2)	1.60a,b(19.4)		2.36a,b(17.8)		2.36a,b(14.0)		1.92a,b(7.8)	
Ca	0.93b,c (5.4)		0.84a,c	(3.6)	0.79a,c (5.1)		0.88b,c(10.2)		0.90b,c (2.2)		0.84a,c (2.4)		0.84a,c(2.4)	
Mg	0.39a,c	(7.7)	0.31a,c,f(16.0)		0.36a,c,f(11.1)		0.31a,c(29.0)		0.51a,d,e(3.9)		0.27b,c,f(7.4)		0.42a (4.8)	
mg/kg														
Cu	16a	(20.4)	22a,c	(53.1)	09a,c	(11.1)	14a	(23.3)	43b	(35.9)	11a,c	(23.5)	11a,c (23.5)	
Fe	600b	(27.3)	254a	(15.6)	228a	(22.7)	331b	(6.8)	245a	(18.6)	186a	(4.9)	146a(14.7)	
Mn	114b,c,d(34.5)		69a,c,d	(28.6)	69a,c,d	(8.5)	80a,c	(40.5)	38a,c,e	(8.6)	35a,c,e	(7.4)	44a	(21.6)
Zn	40b,c	(24.8)	23a (13.0)		37b	(12.9)	29a,c	(4.0)	33b,c	(3.5)	28a,c	(27.4)	28a,c	(4.1)

*LW: Low country-wet zone; MW: Mid country-wet zone, UW: Up country-wet zone, LI: Low country-intermediate zone, MI: Mid country-intermediate zone, UI: Up country-intermediate zone, LD: Low country-dry zone. Values are the means of four replicates with the coefficient of variation in parentheses (P<0.05).

Table 4: Corelation matrix of elemental concentrations in leaves of *P. maximum*. L.

	C	N	P	K	Ca	Mg	Cu	Fe	Mn
N	0.323ns								
	0.094								
P	-0.458*	-0.546**							
	0.014	0.003							
K	-0.473*	-0.133ns	0.521**						
	0.011	0.499	0.004						
Ca	0.255ns	0.734***	-0.455*	-0.266ns					
	0.191	0.000	0.015	0.172					
Mg	-0.435*	0.049ns	0.106ns	-0.200ns	0.278ns				
	0.021	0.803	0.592	0.308	0.152				
Cu	-0.123ns	0.310ns	-0.284ns	0.179ns	0.275ns	0.290ns			
	0.531	0.109	0.144	0.361	0.156	0.134			
	0.139ns	0.340ns	-0.018ns	-0.134ns	0.546**	0.072ns	-0.017ns		
Fe	0.481	0.077	0.926	0.496	0.003	0.716	0.930		
M	0.333ns	0.407*	-0.178ns	-0.416*	0.485**	0.106ns	-0.377*	0.471*	
n	0.084	0.031	0.364	0.028	0.009	0.592	0.048	0.011	
Zn	0.055ns	0.292ns	0.155ns	0.284ns	0.403*	0.103ns	0.101ns	0.599***	0.369ns
	0.780	0.132	0.432	0.143	0.033	0.600	0.611	0.001	0.053

*Comparison between elements that are significantly different at 0.05, 0.01, 0.001or 0.0001 P-level are indicated by *, **, ***and **** respectively. Values are Pearson correlation coefficients.

particularly remarkable. Apart from that, the competitive behavior in the absorption of phosphorus and nitrogen can be well recognized. The negative relationships between phosphorus, potassium and magnesium on the carbon, and calcium accumulation on phosphorus in the leaves are recognizable. Similar relationships can also be observed between potassium (K-Mn) and copper (Cu-Mn). Due to lack of sufficient information on the inte -element relationships of the essential elements in plant nutrients, a clear cut statement could not be made at this stage. Further detailed studies are necessary to verify the above significant inter-element relationships in *P. maximum* in terms of its chemical characterization. The results obtained for leaf $\delta^{13}C$ values of *P. maximum* leaves originated from different agro ecological zones of Sri Lanka are summarized in Table 5. Irrespective of the site elevation, all samples have carbon isotope ratios ($\delta^{13}C$) typical of C4 mode of photosynthesis [7]. *P. maximum* samples collected from all seven agro ecological zones are functionally C4, as indicated by a C4 isotope ratio, the typical Kranz anatomy and the high C4-like vein density.

Increased vein density was observed in the leaves of plants growing in arid environments. The typical C4 anatomy was observed

Table 5: Mean (±S.E) values of $\delta^{13}C$ values, mesophyll (M) to bundle sheath (BS) cell area ratios and the vein densities of *P. maximum* collected from different agro ecological zones of Sri Lanka.

Agro-ecological zone	δ^{13}Corg(VPDB) (‰)	M:BS ratio	Vein density (Veins cm^{-2})
Low country-	-13.87 ± 0.01	1.90a ± 0.50	8.32a,e ± 2.85
wet zone			
Mid country-	-14.55 ± 0.12	2.03a ± 0.40	3.72b,c,d,f ± 0.88
wet zone			
Up country-	-12.04 ± 0.11	2.80a ± 0.16	4.82b,c ± 1.67
wet zone			
Low country-	-13.34 ± 0.09	2.05a ± 0.42	6.91a,e ± 2.11
intermediate zone			
Mid country-	-13.69 ± 0.10	2.28a ± 0.50	4.13b,c,d,f ± 1.03
intermediate zone			
Up country-	-12.80 ± 0.14	2.45a ± 0.47	2.38b,d ± 1.35
intermediate zone			
Low country-	-12.38 ± 0.10	2.80a ± 0.41	6.85a ± 2.35
dry zone			

*Means with the same letter in a column are not significantly different at p< 0.05.

doi:10.4172/2229-4473.1000383

in all *Panicum* leaf samples with high vein density associated with C4 photosynthesis. These results on vein density are consistent with the widely accepted hypothesis that the increased vein density is associated with C4 photosynthesis. Increased vein density enhances water use efficiency of plants growing in arid environments. It was interesting to note that with the increasing elevation, vein density was tended to increase. With respect to the M: BS ratios, no significant differences were observed between the different climatic zones. However, *Panicum* leaves from the up country-wet zone and low country-dry zone possessed relatively higher M: BS ratios. In leaf cross sections, BS cells occupied less than 10% of the leaf area, whereas the mesophyll cell area was about 70%. The M: BS ratios remained more or less similar in all *P. maximum* samples typical of a C4 species [6]. Increased BS size and high vein density have also been observed [6] in the C3 species of *Panicum laxum* and *Panicum hylaeicum*. They have reported a shift towards the C3-C4 intermediate condition in *Panicum schenkii* and *Panicum milioides*. According to a hypothesis put forward, high vein density may increase water use efficiency in arid environments.

In conclusion, all *P. maximum* samples collected from different climatic zones of Sri Lanka exhibited typical C4 Kranz anatomy where the vascular bundles are surrounded by chlorenchymatous bundle sheath cells. This is further supported by the anatomical C4 traits studied, i.e. high vein density and low M: BS area ratio of *Panicum* leaves. $\delta^{13}C$, a powerful diagnostic tool for C4 photosynthesis confirmed that *P. maximum* growing in Sri Lanka possesses the C4 mode of carbon assimilation. Within species variations of $\delta^{13}C$ among the different climatic zones were minor of the order of 2.1‰ for all the *Panicum* samples, although carbon isotope discrimination is known to negatively correlate with the elevation [19]. Characterization of enzyme activities of the C4 cycle would provide more information as to whether *P. maximum* from Sri Lanka could be used as another model for C4 evolution.

Acknowledgement

Supports extended by the non-academic staff of the Department of Botany, University of Kelaniya are acknowledged.

References

1. Simon BK (2007) Grass phylogeny and classification: conflict of morphology and molecules. Syst Evol Bot 23: 259-266.
2. Aliscioni SS, Giussani LM, Zuloaga FO, Kellogg EA (2003) A molecular phylogeny of Panicum (Poaceae: Paniceae): tests of monophyly and phylogenetic placement within the Panicoideae. Botany 90: 796-821.
3. Siebert SJ, Fish L, Uiras MM, Izindine SA (2004) Grass assemblages and diversity of conservation areas on the coastal plain south of Maputo Bay, Mozambique. Bothalia 34: 61-71.
4. Gottfried GJ, Gebow BS, Eskew LG, Edminster CB (2005) Connecting mountain islands and desert seas: Biodiversity and management of the Madrean Archipelago II. Proc. RMRSP 36: 563-575.
5. Gratani L (2014) Plant phenotypic plasticity in response to environmental factors. Advances in Botany 32: 322-343.
6. Jayasekara R (1994) Pattern of distribution of selected trace elements in the marine brown alga, Sargassum filipendula Ag. Form Sri Lanka. Environmental geochemistry and health 16: 70-74.
7. Cerling TE, Harris JM, Macfadden BJ, Leakey MG, Quade J, et al. (1997) Global vegetation change through the Miocene-Pliocene boundary. Nature 389: 153-158.
8. Ueno O (2011) Structural and biochemical characterization of the C3-C4 intermediate Brassica gravinae and relatives, with particular reference to cellular distribution of Rubisco. J experiment bot 62: 5347-5355.
9. Muhaidat R, Sage TL, Frohlich MW, Dengler NG, Sage RF (2011) Characterization of C3-C4 intermediate species in the genus Heliotropium L. (Boraginaceae): anatomy, ultrastructure and enzyme activity. Plant, cell environ 34: 1723-1736.
10. Motsara MR (2008) Guide to laboratory establishment for plant nutrient analysis. Food and agricultural organization of the United Nations Rome.
11. Tatina R (2001) A colorimeter for measuring phosphorous in solution. American Bio. Teacher 63(3): 191-194.
12. Lamentowicz M, Galka M, Lamentowicz L, Obremska M, Kohi N, et al. (2015) Reconstructing climate change and ombrotropic bog development during the last 4000 years in northern Poland using biotic proxies, stable isotopes, and trait based approach. Palaeogeogr Palaeoclimatol Palaeoecol 418: 261-277.
13. Simon BK (2007) Grass phylogeny and classification: conflict of morphology and molecules. Systematic and Evolutionary Botany 23: 259-266.
14. Barbosa ER, Tomlinson KW, Carvalheiro LG, Kirkman K, Debie S, et al. (2014) Short-term effect of nutrient availability and rainfall distribution on biomass production and leaf nutrient content of savanna tree species. PloS one 9: 1-9.
15. Skog KL, Steinnes M (2015) How do centrality, population growth and urban sprawl impact farmland conversion in Norway. Land Use Policy 59: 185-196.
16. Schleser GH, Jayasekera R (1985) $\delta^{13}C$-variations of leaves in forests as an indication of re-assimilated CO2 from soil. Oecologia 65: 536-542.
17. Brown RH, Bouton JH, Rigsby L, Rigler M (1983) Photosynthesis of grass species differing in carbon dioxide fixation pathways. VII. Ultrastructural characteristics of Panicum species in the Laxa group. Plant Physiol 71: 425-431.
18. Sage RF (2004) Evolution of C4 photosynthesis. New Phytologist 161: 341-370.
19. Kloeppel BD, Gower ST, Treichel IW, Kharuk S (1998) Foliar carbon isotope discrimination in Larix species and sympatric evergreen conifers: a global comparison. Oecologia 114: 153-159.

Author Affiliations

Top

[1]Department of Genetics and Plant Breeding, BA College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

[2]Main Vegetable Research Station, Anand Agricultural University, Anand, Gujarat, India

[3]Research Scientist, Main Vegetable Research Station, Anand Agricultural University, Anand, Gujarat, India