SOME OBSERVATIONS ON THE PHYLLODES OF THE SHOE STRING ACACIA (*ACACIA STENOPHYLLA* A. CUNN. EX. BENTH.) GROWING IN KARACHI, PAKISTAN

D. KHAN AND ZULFIQAR ALI SAHITO

Department of Botany, University of Karachi, Karachi, Pakistan.

Abstract

Some observations on phyllodes of *Acacia stenophylla* A. Cunn. Ex, Benth.) were recorded with respect to their dimension (length, breadth and thickness) and dry mass contents and interrelationships between them were determined. Phyllodes were also studied for their phytochemicals, ionic and biochemical contents and the epidermal structure. Phyllodes had ridges and grooves on its surface. The length of the phyllodes (N = 110) from an tree growing in Karachi averaged to 21.03 ± 0.57 cm (2.50 – 33.10 cm), although appeared it to be negatively skewed but followed normal distribution when compared on the basis of Kolmogorov-Smirnoff z test (0.752, $p < 0.625$). The phyllode dry mass varied from 6.20 to 165mg (Mean: 85.2 ± 3.58 mg) exhibiting a negatively skewed distribution. The phyllodes of *A. stenophylla* were found to contain alkaloids, anthroquinones, saponins, sugars and carotenoids. There was more chlorophyll 'a' than chlorophyll 'b'. Amongst the biochemicals estimated soluble sugar was in much higher concentration (14.6% DW) than phenols (3.94% DW). Protein concentration was very low (0.53% DW). The stomata on phyllodes were of diverse types - paracytic, anomocytic and tetracytic. The stomatal pore measured 16.7 µm in length and 5.91 µm in breadth. *A. stenophylla* appeared to be a potassiophillic species.

Introduction

Acacia stenophylla A. Cunn. Ex. Benth. (Syn. Acacia stenophylla Benth. var. linearis Maiden; Racosperma stenophylla (Benth.) Padley) belongs to Family Mimosaceae (Phyllodinae, section Pleurinerves). It is commonly called Shoe String Acacia, Dolby Myall, or Eumong, River Cooba etc. This Australian wattle is a drought and salt tolerant multipurpose, N_2-fixing, phyllode-bearing plant of summer-precipitating (125-600mm) areas of Australia (Boland and McDonald, 2006; Sahito *et al.*, 2013; Shirazi *et al.*, 2006). A solitary tree of this species is growing in the department of Botany, University of Karachi. Plant shows narrow weeping growth habit suitable for limited space and provides filtered shade. It has been introduced in several developing countries (Turnbull, 1987). Khan and Sahito (2013) have described variation in pod- and seed sizes and seed packaging cost in this species growing in Karachi (Pakistan). In this paper, some observations on the phyllodes of this species are described with respect to their linear dimension and dry mass contents, their surface structure, phyto-chemistry and the biochemical and ionic contents.

Materials and Methods

One hundred and ten phyllodes were collected randomly from the four sides of the canopy of *Acacia stenophylla*, growing in the department of Botany, University of Karachi (Fig. 1). The tree measured nearly 15 cm in stem diameter, 7.2m in height and 17 m^2 in canopy area. The phyllodes were properly labeled and studied for morphometry while fresh (length, breadth and thickness – the last two parameters were measured from the mid region of the phyllode). The phyllodes were then dried at 60 ^oC in oven for 48 h. The dry pods were weighed individually for their dry biomass determination with an electrical balance. The statistical relationship among these parameters was determined through multivariate analysis (Zar, 2010). The biochemical parameters studied included chlorophyll a and b and carotenoids estimation (Duxbury and Yentsch, 1956; Strain *et al.*, 1971) along with sugar (Fales, 1951), total phenols (Singleton and Rossi, 1965) and protein (Bradford, 1976) contents. The common cations, Na and K were determined by flame photometry (Chapman and Pratt, 1961). There were ten replicates for each determination. The detailed procedure of the methods employed may be seen in Ali *et al*. (2013).

One hundred g dry phyllode powder was immersed in ethanol for two weeks in brown glass bottle. The dark green extract was evaporated at room temperature to semi-solid substance which served for qualitative analysis for various phytochemicals (Harborne, 1973; Vishnoi, 1979; Sofowora, 1993; Trease and Evans, 2002).

Epidermal impressions of phyllodes were made with clear nail polish (Wang *et al.*, 2006). Stomatal nomenclature suggested by Prabhakar (2004) being simple and based upon structure of stomata and not their ontogenetic pathways was adopted to ascertain stomatal types.

For scanning electron microscopy (SEM), air-dried) plant material was mounted on brass stubs and coated with a 250 ^{o}A thick gold layer with JFC-1500 gold coater. SE micrographs were made at 15kV with JEOL JSM-6380A electron microscope. The images were saved digitally on computer.

Fig. 1. Canopy of *Acacia stenophylla* showing phyllodes and pods.

Results and Discussion

The phyllodes of *A. stenophylla* are gray-green ribbon-like, flat, pendulous structures formed as result of the expansion of the rachis by about the fifth bipinnate leaf of the seedling (Boland and McDonald, 2006). Phyllodes are linear, apically acute and sometimes hooked. Phyllodes are multi-nerved and skinny. The plant sheds moderate amount of phyllodes and pods.

Phyllode Size

The length of the 110 randomly selected phyllodes from the unirrigated tree growing in Karachi averaged to 21.03 ± 0.57 cm (2.50 – 33.10 cm), although appeared to be negatively skewed but followed normal distribution when compared on the basis of Kolmogorov-Smirnoff z test (0.752, $p < 0.625$) (Table 1, Fig. 2). Boughton (1986) has described phyllode anatomy of 144 spp. of *Acacia*. Phyllodes of Juliflorae and pleurinerves were found to be more Xerophytic in structure than the phyllodineae. Phyllodes are up to c. 40 cm in length in Australian specimens, comparatively longer than our specimen that may probably be attributed to much harsher climatic conditions of Karachi and poor agronomic management and insect infestation (Khan and Sahito, 2013; Khan *et al.*, 2016).

The breadth of the phyllode averaged to 0.24 ± 0.007 cm) and thickness of the phyllodes varied by only 9.43% and averaged to 0.061 ± 0.0005 cm) (Table 1).

The length and breadth varied comparatively in larger magnitude than the thickness. The phyllode dry mass presented a platykurtic non-normal distribution (KS-z: 1.6363, $p < 0.010$). The phyllode dry mass varied from 6.20 to 165mg (Mean: 85.2 ± 3.58 mg) exhibiting a negatively skewed distribution (Fig. 3). Nearly 50% of the phyllodes in number associated with class size 100 to 147.5mg.

Length and breadth of the phyllodes (as independent variables) estimated the dry mass contents (dependent variable) of the phyllodes in statistically significant manner as given by the equation of plane (Fig. 4) with an explanatory power of 74.44%. The following equation may be useful while estimating dry mass contents of phyllodes in experimental laboratory investigations.

Phyllode Wt. (mg) = -33.801 + 2.093 Phyllode length (cm) + 313.918 Phyllode Breadth (cm) ± 17.881

t = -5.0567	t = 6.0572	t = 11.9329
p < 0.00002	p < 0.0000001	p < 0.0000001

$R = 0.8800$; $R^2 = 0.7744$; Adj. $R^2 = 0.7703$; $F(2, 107) = 183.67$ ($p < 0.0001$)

Including thickness of the phyllodes as an additional independent variable, the multiple regression equation turned up to be as given below: The equation indicated an improvement in explanatory power of merely c 0.7% in this predictive model of estimating the weight of a phyllode as compared to the equation of plane (given above) with only length and width of the phyllodes employed as independent variables.

Phyllode Wt. (mg) = -68.5198 + 2.0285 Phyllode length (cm) + 303.0913 Phyllode Breadth (cm) + 641.735 phyllode thickness

t = -3.853	t = 5.9402	t = 11.4989	t = 2.102
p < 0.002	p < 0.000001	p < 0.000001	p < 0.001

R = 0.8851; R^2 = 0.7834; Adj. R^2 = 0.7773; F (3, 106) = 127.83 (p < 0.000001); SE = 17.602

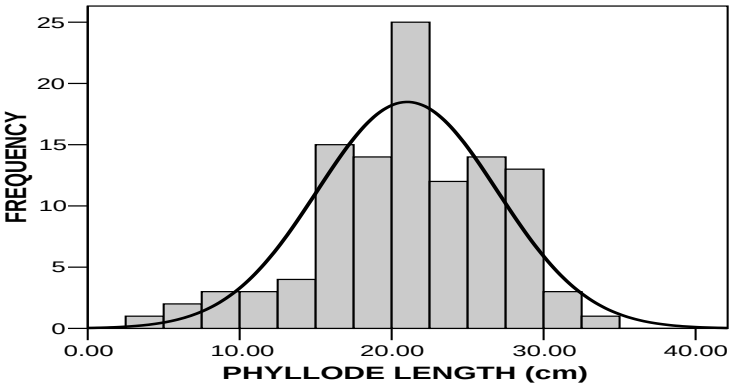
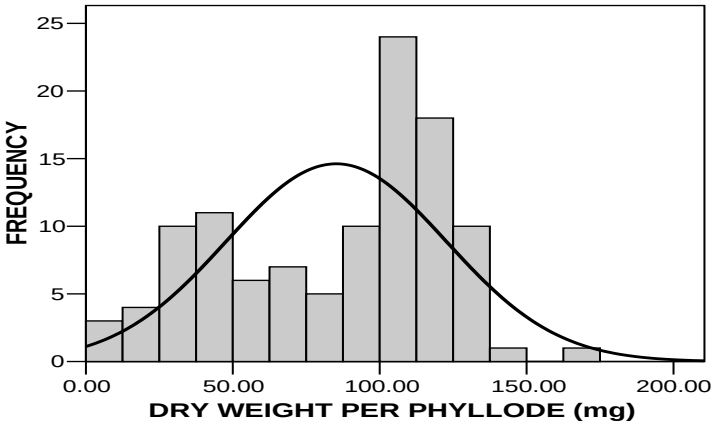


Fig. 2. Frequency distribution of phyllode length (cm).



N = 110
Mean = 85.214 mg
SE = 3.5783
CV= 44.04%
Median = 97.70
G1 = -0.396
Sg1 = 0.230
G2 = - 1.002
Sg2 = 0.457
Minimum = 6.20
Maximum = 165.0
KS-z = 1.633
p < 0.010

Fig. 3. Frequency distribution of dry weight (mg) per phyllode of *A. stenophylla*.

Table 1. Dispersion and location parameters of phyllode morphometry.

Statistical Parameter	Length (cm)	Breadth (cm)	Thickness (cm)
N	110	110	110
Mean	21.026	0.2359	0.0613
SE	0.5678	0.0074	0.000551
CV (%)	28.22	32.93	9.43
Median	21.300	0.250	0.0600
Skewness (g1)	-0.562	-0.312	-0.401
Sg1	0.230	0.230	0.230
Kurtosis	0.328	-1.113	3.184
Sg2	0.457	0.457	0.457
Minimum	2.50	0.10	0.0350
Maximum	33.10	0.35	0.0760
KS-z	0.752	1.763	0.878
p	0.625	0.004	0.424

Equation of plane:

Phyllode Wt. (mg) = -33.801 + 2.093 Phyllode length (cm) + 313.918 Phyllode Breadth (cm) ± 17.881

t = -5.0567 t = 6.0572 t = 11.9329

p < 0.00002 p < 0.0000001 p < 0.0000001

$R = 0.8800$; $R^2 = 0.7744$; Adj. $R^2 = 0.7703$; $F(2, 107) = 183.67$ ($p < 0.0000001$)

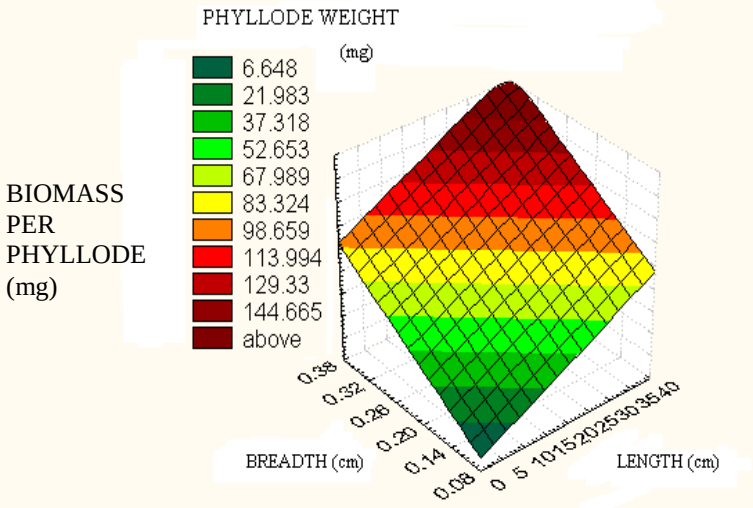


Figure 4. Surface plot of dry weight of phyllodes *of A. stenophylla* (mg) as the function of length (cm) and breadth (cm) of the phyllode. This relationship is represented by the equation of plane given above the graph.

Table 2. Phytochemical analysis of phyllode.

Pl. part	ALK*	ANTH	TAN	PH.BT	SAP	STER	TRIT	FLAV	S.sugar	Carot.
Phyllodes	+	-	-	-	+	-	-	-	+	+

*, ALK, alkaloids; ANTH, Anthroquinones; TAN, tannins; PH.BT, phlobatannins; SAP, saponins; STER, steroids; TRIT, triterpenoids; FLAV, flavanoids ; S.sugar; soluble sugar; CAR, carotenoids. +, presence; - absent or not detectable.

Table 3. Concentration of photosynthetic pigments (mg. g^{-1} FW) in green phyllode of *A. stenophylla.*

Statistics	Chlorophyll-- a	Chlorophyll – b	Total chlorophyll	Carotenoids
Mean	0.5292	0.2877	0.8169	0.2254
SE	0.01795	0.02283	0.03911	0.01117
CV (%)	5.87	13.74	8.29	1.93

Table 4. Mineral and biochemical analyses of phyllodes of *A. stenophylla*.

Statistics	Na (meq/L)	K (meq/L)	Phenols ($mg.g^{-1}DW$)	Sugar ($mg.g^{-1}DW$)	Protein ($mg.g^{-1}DW$)
Mean	1.9650	2.7388	39.4297	146.2749	5.2949
SE	0.11491	0.10671	2.53162	11.36191	0.30285
CV (%)	10.21	6.75	11.16	13.45	9.91

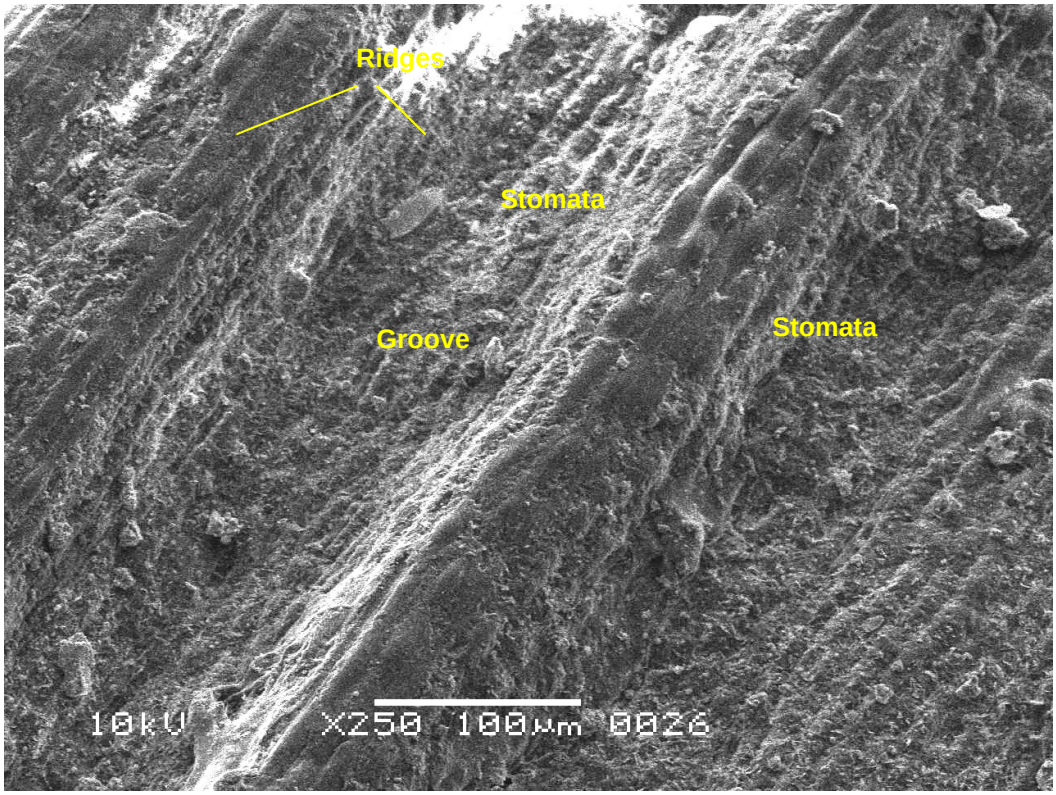


Fig. 5. SEM- Magnified view of dry phyllode surface of *A. stenophylla* showing ridges and grooves. A number of stomata are visible in the groove and the lateral surface of the ridges. Magnification: 250X.

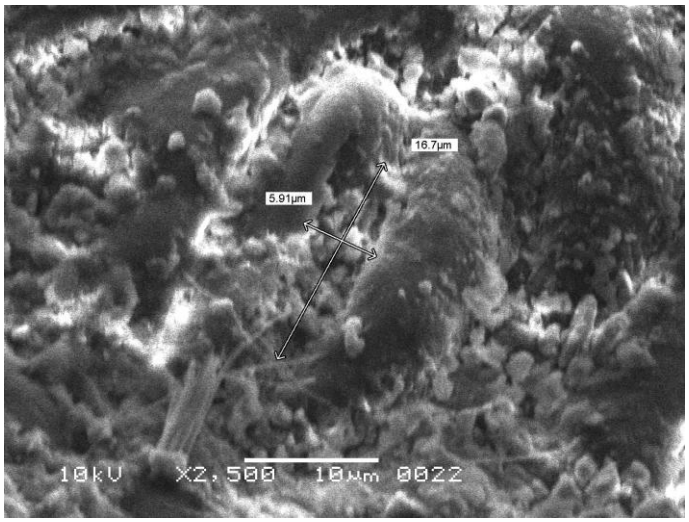


Fig. 6. SEM – A view of a stoma on the lateral surface of a ridge of a mature dry phyllode of *Acacia stenophylla*. The stomatal pore measures 16.7 x 5.91 µm. The occluded and foreign materials are seen scattered all over. Magnification: 2500X.

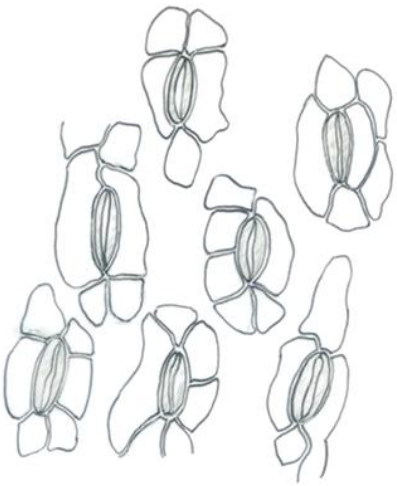


Fig. 7. Stomata on the surface of phyllode. Magnifications 45 x 15X. Nail polish imprint of phyllode.

Phytochemical, ionic and biochemical analysis of phyllodes

The phyllodes of *A. stenophylla* were found to contain alkaloids, anthroquinones, saponins, sugars and carotenoids (Table 2). Bisby (1994) has reported two alkaloids from seeds of this species besides some free amino acids and peptides. There was more chlorophyll 'a' than chlorophyll 'b' (Table 3). Amongst the biochemicals estimated soluble sugar was in much higher concentration 146.27 mg.g-1 DW or 14.6%) than phenols (39.49 mg.g-1 DW or 3.94%). Protein concentration was very low (0.53%) (Table 4). The crude protein

content of leaves of *Acacia* species is known to vary - 145g per kg DM in *A. Senegal* to as high as 229 g per kg DM in *A. angustissima* as reported by Rubanza *et al.* (2014). Protein concentration was comparatively much lower in *A. stenophylla*.

A. stenophylla appeared to be a potassiophillic species. (Table 4). The concentration of K was higher in the phyllodes than Na which is in agreement with previous studies related to salt tolerance and mineral distribution in this species under control and saline conditions (Shirazi *et al.,* 2010; Sahito *et al.,* 2013). Stem wood ash of this species is also reported to contain K in substantially higher proportion than Na (K / Na ratio: 17.46 ± 0.698) (Khan and Sahito, 2015).

Surface characteristics

The surface of the phyllodes is characterized with ridges and grooves as shown by the scanning electron micrograph (Fig. 5). The central ridge was the most prominent. Generally the stomata were present on the lateral edges of the ridges or the floor of grooves. Stomata are bean-shaped. The stomatal pore measured 16.7 µm in length and 5.91 µm in breadth (Fig. 6). No trichomes were seen.

The stomata were of diverse types and co-occurring on the surface of *A. stenophylla* phyllode - paracytic, anomocytic and tetracytic (Fig. 7). Shah *et al.* (1972) described stomatal ontogeny of 21 species including four *Acacia* spp. They observed the predominance of paracytic type. Pettigrew and Watson (1973) found two major stomatal types – paracytic and cyclocytic while investigating stomata in 23 Australian Acacias. Grosso *et al*. (1994) while describing stomatal types in 102 species of *Acacia* (*A. stenophylla* not included) established stomatal polytypism in genus *Acacia* and described six types of stomata on the basis of Guyot's (1966) stomatal nomenclature. They described three basic core types - Anomocytic perigenous, Anomocytic mesoperigenous and bicytic paracytic type (paracytic type of Prabhakar, 2004) to be predominating in Phyllodinae pleurinerves. In 45 genera of Fabaceae, Caesalpiniaceae and Mimosaceae, three types of stomata have been reported to be common also by Tripathi and Mondal (2012) – paracytic (64.1%), anisocytic (46.6%) and anomocytic (33.3%) - at times these stomatal types were co-occurring.

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