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The Use of HPLC-Derived Phenolic Profiles as Means of Classifying *Sesbania* Accessions

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Abstract: Aqueous acetone extracts of mature leaves from 10 accessions of two species of *Sesbania* plants were prepared and analysed by high-performance liquid chromatography (HPLC). The HPLC phenolic profiles were used to develop a classification rule to distinguish between accessions. Out of the 44 mature leaf samples analysed, 27 had distinctive HPLC phenolic profiles and were successfully assigned to the correct accession. Seedlings of these accessions could be classified to the correct species after 3 months' growth. After 3 months' growth, 8 out of 15 samples could be assigned to the correct accession and, at 6 months, 7 out of 20. However, when accessions with very similar phenolic profiles were grouped together, there was a 100% success rate for classifying the correct accession at 3 months and 80% success rate at 6 months. It was concluded that the method could distinguish between some accessions, or groups of accessions, of *Sesbania* after 3 months' growth. HPLC phenolic profiling is potentially a relatively rapid, but not uniformly reliable, method for classifying *Sesbania* accessions.

Key words: *Sesbania*, *Sesbania goetzei*, *Sesbania sesban*, accessions, leaves, HPLC, phenolic profiles.

INTRODUCTION

The use of chromatography in cultivar identification was reviewed by Morgan (1989) who concluded that these techniques offer considerable potential in chemotaxonomic characterisation. High-performance liquid chromatography (HPLC) is well suited to such purposes as it produces quantitative data and offers a high degree of discrimination not obtainable through morphological examination. Polyphenol profiles obtained by HPLC have been used to identify cultivars of poinsettia (Stewart *et al* 1980) and geranium florets (Asen and Griesbach 1983). HPLC data can be analysed statistically and such analyses have been used to indicate varietal differences and responses to different environments in sorghum (Mueller-Harvey and Dhanoa 1991), predicting price and country of origin of black tea liquors (McDowell *et al* 1991) and in distinguishing between different cultivars of apple (McRae *et al* 1990).

Sesbania is a genus of leguminous shrubs and trees which has been investigated for their use as multipurpose plants (Anon 1993). *Sesbania sesban* (L) Merrill var. *nubica* Chiov and *S goetzei* Harms show promise as fodder trees and both are native to sub-Saharan Africa. The International Livestock Centre for Africa (ILCA) has a germplasm collection of 300 accessions of 20 species of *Sesbania*. There are large differences both between and within species in their agronomic properties (Tothill *et al* 1990; Hanson 1991). There is a need, in various areas of research, for a rapid technique to classify plant accessions at an early stage, rather than wait for them to grow to maturity. The aim of this study was to determine whether a distinctive phenolic profile can be used to distinguish between young plants from different *Sesbania* accessions and the stage of [illegible] at which the profile can be used.

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Leaf samples of five accessions of each of two *Sesbania* taxa (*S sesban* var *nubica* and *S goetzei*) were collected from mature trees grown at three different sites (Zwai, Debre Zeit and Shola) in Ethiopia. Table 1 gives the origins and botanical names for the accessions. Herbarium specimens have been retained at ILCA. Samples were obtained in duplicate from different trees except in two cases when only single trees were available (see Table 2). Seedlings of some of the accessions were grown at both Zwai and Debre Zeit. Leaves were sampled after 3 and 6 months (see Table 2).

The leaf samples were freeze-dried and ground to pass through a 1 mm sieve and sent to the UK. Hagerman (1988) found that 700 ml litre^{-1} aqueous acetone extracted more tannins than aqueous or acidic methanol, hence this was the preferred solvent. Samples were extracted as described by Wood *et al* (1994) except that they were homogenised for 1 min. It had been found that extraction of extractable phenols was completed within one minute (Powell C J unpublished data). Extracts were centrifuged at 2000 g for 10 min and then filtered through a 0·45 μm millipore filter before analysis by HPLC. The extract (20 μl) was analysed directly by HPLC using the method of Mueller-Harvey *et al* (1987).

Examination of the HPLC chromatograms of the mature leaf samples indicated that there were seven major peaks that could be used to characterise the accessions. The peaks were selected and assigned on the basis of their retention times. For statistical analysis standardised peak heights were used where

$$\text{standardised peak height} = \frac{\text{observed peak height}}{\text{total peak height for all seven peaks}}$$

Similar results could be achieved using peak areas.

Linear discriminant functions were obtained using canonical variate analysis (CVA) by means of GENSTAT computer software. The discriminant functions produced by CVA on the data from mature leaves were used as a classification rule. The mature leaf and seedling samples were distinguished and classified on the basis of this rule. Correlation was also used as a classification tool, the correlations being computed for all seedling samples with each mature profile and seedling samples classified as the mature accession with which it was most highly positively correlated. Variance components for each of the seven HPLC peaks were calculated using the REML technique to quantify varia-

TABLE 1
Botanical names and origins of selected *Sesbania* accessions

Botanical name	*Origin*	*Accession code*
S sesban (L) Merril var *nubica* Chiov	Ethiopia	2024
	India (?)	10865
	Uganda	15021
	Rwanda	15022
	Uganda	15036
S goetzei Harms subsp *multiflora*	Tanzania	1277
	Tanzania	1278
S goetzei Harms subsp *goetzei*	Ethiopia	15007
	Ethiopia	15358
	Kenya	15367

Species	*Age*	*ILCA accession code*	*Site*[a]	*No samples/ site*
S sesban var *nubica*	3 months	2024	D	2
			Z	2
		10865	D	2
			Z	2
		15036	Z	2
	6 months	2024	D	2
			Z	2
		10865	D	2
			Z	2
		15036	D	2
	Mature	2024	D	1
		10865	D	2
			Z	2
			S	2
		15021	D	2
			Z	2
			S	1
		15022	D	2
			Z	2
			S	2
		15036	D	2
			Z	2
			S	2
S goetzei	3 months	1277	D	2
		1278	Z	1
		15007	Z	2
	6 months	1277	D	2
		1278	D	2
			Z	2
		15007	Z	2
	Mature	1277	D	2
			Z	2
		1278	Z	2
		15007	D	2
			Z	2
		15358	D	2
			Z	2
		15367	D	2
			Z	2

[a] D, Debre Zeit; Z, Zwai; S, Shola.

tion between the 10 accessions and between samples for each accession.

RESULTS

HPLC chromatograms (at 260 nm) typical of *Sesbania sesban* var *nubica* and *S goetzei* are given in Fig 1. The spectra of the peaks with the same retention times used for classification were compared and found to be closely similar for different accessions within species, indicating that the peaks were the same compounds. Spectra for peak six were, however, found to be different for *S sesban* var *nubica* and *S goetzei*.

For the mature leaves linear discriminant functions were obtained using CVA. The first three functions were found to be important in discriminating between the 10 accessions. These functions are of the following form:

$$Y_1 = a_1 \times \text{peak } 1 + a_2 \times \text{peak } 2 \cdots + a_7 \times \text{peak } 7 + \text{constant}$$

$$Y_2 = b_1 \times \text{peak } 1 + b_2 \times \text{peak } 2 \cdots + b_7 \times \text{peak } 7 + \text{constant}$$

$$Y_3 = c_1 \times \text{peak } 1 + c_2 \times \text{peak } 2 \quad + c_7 \times \text{peak } 7 + \text{constant}$$

and the coefficients are provided in Table 3.

Table 4 gives the mean values of the coefficients obtained from the first three discriminant functions. The first canonical variate separates *S sesban* var *nubica*

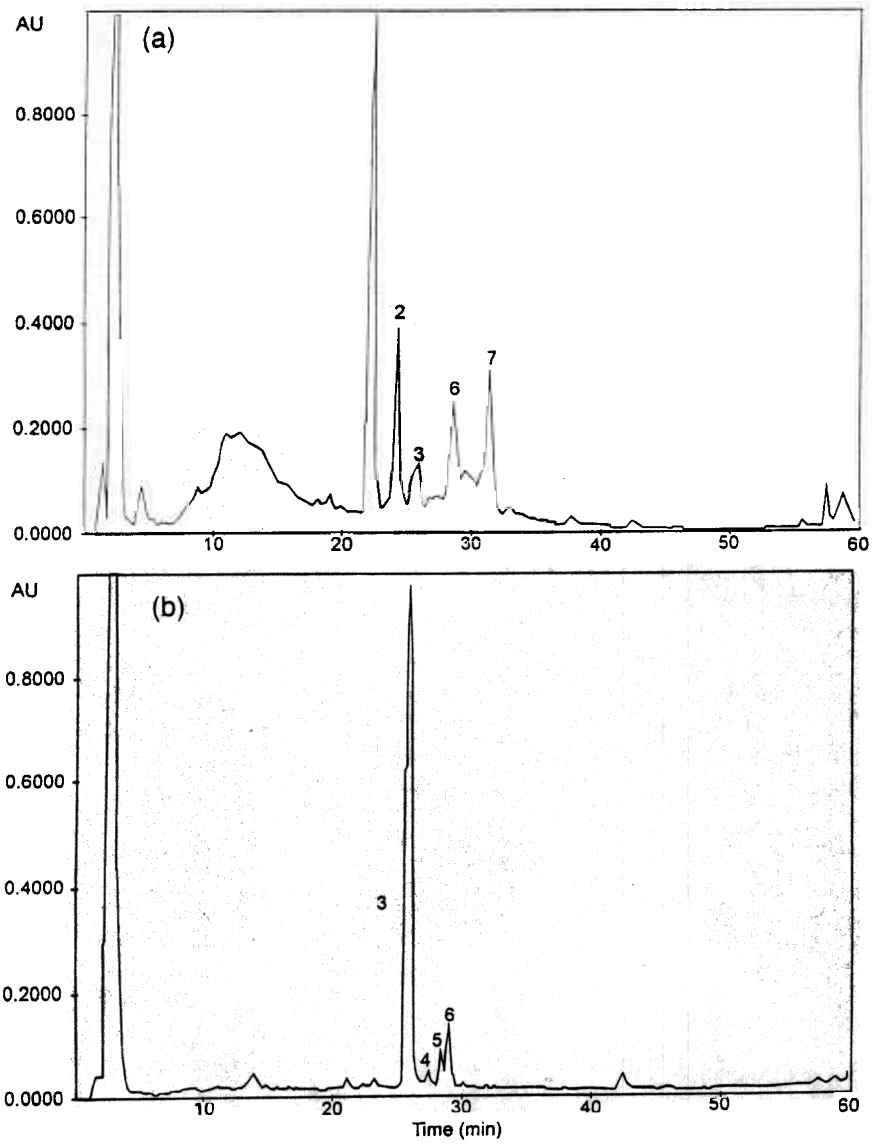

Fig 1. HPLC chromatograms of *S sesban* var *nubica* and *S goetzei* at 260 nm showing numbers allocated to peaks used for classification. Vertical scale is the absorption in absorption units (AU), horizontal scale gives the retention time on the HPLC column in minutes. (a) *S goetzei* subsp *multiflora* ILCA accession code 1277. (b) *S sesban* var *nubica* ILCA accession code 15021.

TABLE 3

The coefficients of the first three discriminant functions of the phenolic compounds

Standardised peak	*Function* (Y_1)	*Function* (Y_2)	*Function* (Y_3)
Peak 1	24·46	12·42	−0·36
Peak 2	4·60	−28·68	−3·63
Peak 3	−3·03	1·91	−4·48
Peak 4	−2·70	9·69	12·32
Peak 5	−0·29	0·77	8·75
Peak 6	−0·84	−3·17	0·86
Peak 7	81·55	12·93	−8·43
Constant	8·45	0·78	−1·61
% Variance explained	91·4%	6·2%	1·7%

TABLE 4

Mean values for each accession of the first three discriminant functions

ILCA	*Species*[a]	*CVA means*		
		1st function	*2nd function*	*3rd function*
Accession No		Y_1	Y_2	Y_3
1277	G	16·24	1·84	−0·42
1278	G	13·19	1·73	0·38
2024	S	1·62	−10·74	−0·34
10865	S	−9·59	0·36	3·67
15007	G	12·51	0·88	0·05
15021	S	−10·76	0·94	−1·42
15022	S	−10·83	0·77	−1·03
15036	S	−10·70	0·84	−1·23
15358	G	11·83	0·73	0·09
15367	G	11·64	−0·37	0·02

[a] G, *S goetzei*; S, *S sesban* var *nubica*.

(values of 1·62 for accession ILCA 2024, otherwise about −10) and *S goetzei* (values of about 12) profiles from one another. The main basis of the discrimination is that *S goetzei* had higher levels of peaks 1 and 7, while *S sesban* var *nubica* had higher levels of peaks 3, 4, 5 and 6. The second canonical variate separated ILCA 2024 from the other accessions, primarily on the basis of its higher level of peak 2, while the third canonical variate separated accession ILCA 10865 from the others, primarily on the basis of higher levels of peaks 4 and 5.

The discriminant functions produced by CVA were used as a classification rule and Table 5 summarises its success in categorising the mature leaves correctly. All samples were classified correctly by species. Out of 44 samples, 27 were correctly classified by accession. For some of the accessions all of the samples were correctly assigned, for example, *S goetzei* ILCA 1277 and 1278 and *S sesban* var *nubica* ILCA 2024. Other accessions, such as *S sesban* var *nubica* ILCA 15021, 15022 and 15036, had very similar profiles and could not be distinguished from each other.

Table 6 shows the variance between accessions for each HPLC peak, the variance between samples pooled over the 10 accessions (due to sample to sample variation and site differences) and the mean peak heights of the accessions with the lowest and highest peaks.

TABLE 5

Classification of accessions using HPLC profiles of the mature leaf extracts

Species	*ILCA accession no*	*Accession code*	*Predicted accession*										*No of samples in each accession*	*% samples correctly identified*
			A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*		
S goetzei	1277	A	4										4	100
	1278	B		2									2	100
	15007	C		1	2		1						4	50
	15358	D			1	2	1						4	50
	15367	E			2		2						4	50
S sesban	2024	F						3					3	100
var *nubica*	10865	G							4		2		6	67
	15021	H								5			5	100
	15022	I								4	1	1	6	17
	15036	J								3	1	2	6	33
Totals, all samples													44	61

TABLE 6

Variance components and lowest and highest accession means for each HPLC peak

HPLC peak	Variance between accessions	Variance between samples[a]	Mean of lowest accession	Mean of highest accession
1	0·0517	0·0014	0·0046	0·494
2	0·0195	0·0010	0·0003	0·242
3	0·1195	0·0122	0·063	0·817
4	0·0018	0·0016	0·016	0·164
5	0·0008	0·0035	0·007	0·127
6	0·0087	0·0100	0·066	0·228
7	0·0037	0·00014	0·000	0·151

[a] Pooled over accessions.

Statistically significant ($P < 0·05$) differences were found between all accessions for all peaks except peak 5, where no significant ($P > 0·05$) differences were observed between accessions. For peaks 1, 2, 3 and 7 the variance between accessions was much greater than the variance between samples of the same accession. As illustrated in Table 6, there was a wide range of peak heights observed for all seven peaks over the range of accessions.

Seedlings had profiles very similar to the mature leaves after 3 months and could be classified correctly by species. At 3 months eight out of 15 could be classified as the correct accession, but only seven out of 20 after 6 months (see Table 7). There was a 100% success rate in distinguishing the accessions with distinctive profiles, such as *S sesban* var *nubica* ILCA 2024, in both young and mature plants. If similar accessions, that is *S sesban* var *nubica* ILCA 15021, 15022 and 15036 whose adult leaf phenolic profiles could not be distinguished, were considered as a single group the success rate was 100% for the 3 month samples and 80% for the 6 month samples.

DISCUSSION AND CONCLUSIONS

The objective of this study was to assess the potential of using HPLC-derived phenolic profiles to distinguish

TABLE 7

Classification of accessions using HPLC profiles of the young leaf extracts grown in Ethiopia

Species	ILCA accession no	Accession code	Predicted accessions										No of samples in each accession	% samples correctly identified
			A	B	C	D	E	F	G	H	I	J		
3 months														
S. goetzei	1277	A	1	1									2	50
	1278	B			1								1	0
	15007	C				1	1						2	0
s sesban	2024	F						4					4	100
var *nubica*	10865	G							3	1			4	75
	15036	J							1	1			2	0
6 months														
S goetzei	1277	A			2								2	0
	1278	B	2		2								4	0
	15007	C	1		1								2	50
S sesban	2024	F						4					4	100
var *nubica*	10865	G							2	2			4	50
	15036	J								4			4	0
Totals													35	43

between accessions of fodder tree species. This would benefit plant breeders and forage agronomists and, ultimately, livestock owners whose benefit would be from improved feed availability and quality. The project has demonstrated that two species of *Sesbania* can be distinguished by analysing leaf extracts from young plants and comparing them to mature leaf extracts. Correctly classifying plants by accession using this method has proved to be more difficult as some accessions have similar phenolic profiles. Larger numbers of accessions would probably increase this problem. However, the method has potential to distinguish between some accessions, or groups of similar accessions. Grouping is the first step to forming defined core collections of agronomic potential from large germplasm collections. Phenolic profiles could be used for such grouping and, with information on agronomic properties and feeding values, help identify improved accessions.

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