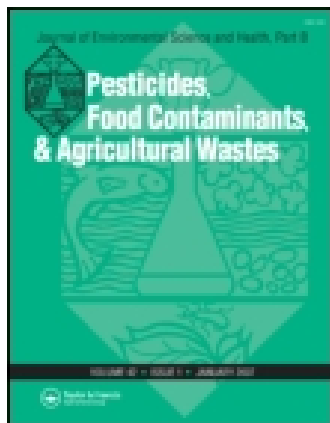


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Glucose, stem dry weight variation, principal component and cluster analysis for some agronomic traits among 16 regenerated *Crotalaria juncea* accessions for potential cellulosic ethanol

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Glucose, stem dry weight variation, principal component and cluster analysis for some agronomic traits among 16 regenerated *Crotalaria juncea* accessions for potential cellulosic ethanol

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The objectives of this research were to identify candidate sunn hemp accessions having high concentrations of cellulose for use as parents in breeding for cellulose and to determine variability for glucose content and some important agronomic traits among sunn hemp accessions. Since sunn hemp is an under-utilized species, glucose content and agronomic trait variation is essential for the identification of superior sunn hemp accessions for use as potential ethanol for biofuel. Sixteen sunn hemp accessions including the following plant introductions (expressed as glucose concentration) and stem dry weights were studied. “Sixteen sunn hemp accessions including the following plant introductions (expressed as glucose concentration) and stem dry weights were studied.” In addition, to verify variability, these traits plus morphological, phenological, and seed reproductive traits were analyzed using multivariate and cluster analysis. The accessions, PI 250487, PI 337080, and PI 219717 produced the highest glucose concentrations (859, 809, and 770 mg g⁻¹ stem dry weight, respectively), however PI 468956 produced the highest stem dry weight (258 g). Branching significantly correlated with foliage ($r^2 = 0.67^{**}$) and relative maturity ($r^2 = 0.60^*$), while maturity had a significantly negative correlation with seed number ($r^2 = -0.67^{**}$) and plant width ($r^2 = -0.53^*$) as well. Seed number significantly correlated with plant width ($r^2 = 0.57^*$). Average linkage cluster analysis grouped the 16 sunn hemp accessions into well-defined phenotypes with four distinct seed-producing groups and one outlier. Based on multivariate and cluster analysis, sufficient variation among these 16 sunn hemp accessions exists to support the development of cellulosic ethanol producing cultivars with improved architecture, early maturity, seed yield, glucose concentrations, and stem dry weights.

Keywords: *Crotalaria juncea*, principal component, cluster analysis, agronomic, cellulose, ethanol.

Introduction

Sixteen sunn hemp accessions have shown variability for earliness, phenotype, seed reproduction, and successful cultivation in Georgia,^[1] Florida, and Puerto Rico.^[2] Sunn hemp also performed well in a mid-Atlantic region of the U.S.^[3] and it has been shown to be very useful as a rotation crop with cotton in low organic matter soils in the southeastern U.S.^[4] Weedy grass, broadleaves, and sedges have been reduced in sunn hemp plots in Georgia,^[5] Florida,^[5] and Alabama.^[6] The results from the Georgia and Florida studies are in preparation, however prevalent weedy species

in the Alabama study included large crabgrass [*Digitaria sanguinalis* (L.) Scop], yellow nutsedge (*Cyperus esculentus* L.), red root pigweed (*Amaranthus retroflexus* L.), sicklepod [*Senna obtusifolia* (L.) Irwin and Barnaby], and stinkgrass [*Eragrostis cilianensis* (All.) E. Mosher]. They found a reduction in overall weed biomass. Sunn hemp leaves are suggested to contain the phytotoxic non-protein amino acid, *hydroxynorleucine* which may impact weed growth through allelopathy.^[5]

Most of the current ethanol for use as biofuel in North America originates from corn grain as the main feedstock.^[7] Recently, sunn hemp was found to exceed cowpea (*Vigna unguiculata*) for biomass yield as well as containing higher energy content than switchgrass, bermudagrass, reed canarygrass, and alfalfa.^[8] Several sunn hemp accessions exhibiting high biomass traits include PI 234771, PI 248491, PI 295851, PI 337080, PI 468956, PI 561720, and PI 652939.^[9] Accordingly, each of these sunn hemp accessions

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has potential utilization in a hybridization program to enhance biomass for cellulosic ethanol production.

The USDA, ARS, Plant Genetic Resources Conservation Unit's (PGRCU) sunn hemp germplasm collection contains 22 accessions from several countries of origin.^[10] Sixteen accessions of the most seed reproductive were selected to represent various countries of origin. Limited information is currently available on glucose, dry stem weight, morphology, phenology, and reproductive traits in this USDA, ARS, PGRCU sunn hemp collection. Variability for glucose content, dry stem weight, morphology, phenology, and reproduction among sunn hemp accessions is a factor when selecting genotypes for breeding programs. The objectives of this research were 1) to determine cellulosic content (expressed as glucose units) and stem dry weights of the most seed productive accessions, 2) analyze morphology, phenology, and seed reproduction among these same accessions using multivariate/cluster analysis, and 3) to select candidate accessions of sunn hemp for use as a feedstock source of cellulosic ethanol in breeding programs.

Materials and methods

Approximately 50 seeds from each sunn hemp accession ($n = 16$) were directly seeded to field plots consisting of a clayey, kaolinitic, thermic typic kanhapludults soil series at Griffin, GA during the first week of June 2008. About 25–50 plants representing each accession were regenerated in one 6 m row plot with at least 5 different buffer species planted between each sunn hemp accession. Plots were irrigated using sprinklers as necessary. Characterization data including branching, foliage, plant height, plant width, relative maturity, and seed number were recorded from plants in each plot at 50% flowering. Branching and foliage were based on a scale of 1–9 where, 1 = >90%, 2 = 80–89%, 3 = 70–79%, 4 = 60–69%, and 5 = 50–59%, 6 = 40–49%, 7 = 30–39%, 8 = 20–29%, and 9 = 10–19% of each plant producing branches and/or foliage based on visual observations. Relative maturity dates were based on a scale of 5 to 9 where 5 = mid-season and 9 = very late. Total seed numbers per accession were counted at the end of the regeneration cycle.

When the sunn hemp plants had reached full maturity, 10 main stems from each accession were cut, dried, and weighed. The samples were then sent to the Division of Environmental Studies Laboratory at the College of Agriculture, Kentucky State University, Frankfort, KY, for cellulose analysis. Crude cell wall preparation was conducted based on the method used by Reiter et al. (1993).^[11] Cellulose was determined using the method described by Updegraff (1960).^[12]

Both glucose and stem dry weights from sunn hemp accessions were analyzed using an analysis of variance (ANOVA) and means were separated using Duncan's multi-

ple range test.^[13] Principal component analysis and PC SAS procedure CLUSTER analysis were then used from multivariate analysis of the data.^[13] Eigenvalues, the percentage of variances explained by each principal component, and clustering of accessions were also determined.^[13] Standard errors (SE) and coefficients of variation (CV) were also determined to confirm variability using principal component analysis.^[13]

Results and discussion

Significant variations for glucose and stem dry weights were observed in the 16 sunn hemp accessions tested (Table 1). Glucose concentrations ranged from 275 to 859 mg g⁻¹ among accessions. The accessions, PI 250487 (India), PI 337080 (Brazil), and PI 219717 (Myanmar) produced the highest concentrations of glucose (859, 809, and 770 mg g⁻¹, respectively). The U.S. developed cultivar, Tropic Sunn (PI 468956) produced one of the lowest concentrations of glucose (368.2 mg g⁻¹) among all accessions tested. Overall, stem dry weights ranged from 52 to 258 g among accessions and Tropic Sunn produced the heaviest dry stems (258 g per accession) when compared to the other sunn hemp accessions. However, PI 250485 (India) produced the lowest stem dry weight (52 g). The coefficient of variation for glucose and stem dry weights were 40% and 60%, respectively indicating a fairly high degree of variation between individuals

Table 1. Glucose concentration and stem dry weight variation among 16 sunn hemp accessions grown at USDA, ARS (Griffin, GA).

(PI)	Accession		
	Origin	Glucose, mg g ⁻¹	Stem dry weight, g
250487	India	859.1 a	73.5 de
337080	Brazil	808.5 ab	176.7 abcd
219717	Myanmar	770.4 ab	208.1 abc
175106	India	634.4 abc	Not recorded
250485	India	609.6 bcd	51.7 e
207657	Sri Lanka	471.4 cde	120.9 bcde
561720	Brazil	451.4 cde	163.9 abcde
274948	Guadeloupe	393.4 de	73.6 de
468956	United States	368.2 e	257.6 a
322377	Brazil	367.5 e	215.3 abc
250486	India	356.3 e	115.2 bcde
346297	India	345.0 e	Not recorded
314239	Former Soviet Union	333.0 e	182.8 abcd
234771	Nigeria	319.2 e	230.5 ab
248491	Brazil	286.2 e	181.9 abcd
295851	Brazil	274.5 e	91.8 cde
SE		48.17	20.03
CV (%)		40	60

†Values within a column having different letter (s) are significantly different ($P < 0.05$), using Duncan's multiple range test (SAS Institute).^[14]

Table 2. Morphological, phenological, and reproductive trait variation among 16 sunn hemp accessions grown at USDA, ARS, Griffin, GA.

Accession (PI)	Branching (cm)	Foliage (cm)	Plant		Maturity [†] relative	Seed number [‡]
			Height (cm)	Width (cm)		
250487	1	3	192	140	5	4412
337080	5	4	257	120	9	3010
219717	9	6	210	150	9	4871
175106	5	5	160	100	9	268
250485	5	5	140	100	9	5009
207657	1	3	195	150	5	4983
561720	1	1	200	120	9	0
274948	5	5	200	130	9	0
468956	9	9	210	100	9	0
322377	3	5	205	137	5	2883
250486	5	5	170	120	5	4132
346297	5	5	200	140	9	0
314239	1	5	196	142	5	7379
234771	5	2	230	80	9	0
248491	5	5	180	60	9	0
295851	5	3	290	90	9	0
SE	0.62	0.45	8.89	6.66	0.47	634
CV (%)	57	41	18	23	25	110

[†]Relative maturity was estimated based on a scale of 5 to 9, where 5 = mid-season and 9 = very late.

[‡]Relative seed production was also based on a scale of 1 to 9, where 1 = very high seed production and 9 = very low seed production.

within the same sunn hemp accession. Even though significant variation for both glucose concentrations and stem dry weights were detected, future studies will require similar evaluations over 2 years.

The morphological, phenological, and seed reproductive analysis is shown in Table 2. Percentages of each plant producing branches and foliage ranged from 10 to greater than 90%. Plant height within rows ranged from 140 to 290 cm. The accessions producing the tallest plants were PI 295851 (290 cm), PI 337080 (257 cm), and PI 234771 (230 cm). Plant widths ranged from 60 to 150 cm and the widest plant

Table 3. Phenotypic, glucose, and stem dry weight traits in sunn hemp grown at USDA, ARS, Griffin, GA.

Variable	Principal components				
	1	2	3	4	5
Glucose (mg g ⁻¹)	859	275	584	482	193
Stem dry weight (g)	258	52	206	134	80
Branching ¹	9	1	8	4	3
Foliage ²	9	1	8	4	2
Plant height (cm)	290	140	150	202	36
Relative maturity ³	9	5	4	8	2
Seed number	7379	0	7379	2309	2540
Plant width (cm)	150	60	90	117	27

^{1,2}Branching and foliage were based on a scale of 1–9 where, 1 = >90%, 2 = 80–89%, 3 = 70–79%, 4 = 60–69%, 5 = 50–59%, 6 = 40–49%, 7 = 30–39%, 8 = 20–29%, and 9 = 10–19% of each plant producing branches and/or foliage based on visual observations.

³Relative maturity dates were based on a scale of 5 to 9 where 5 = mid-season and 9 = very late.

Table 4. Eigenvalues and the proportion of total variability among sunn hemp accessions as explained by the principal components.

Principal component	Eigenvalue	% Variability	% Cumulative
1	2.7443	34.30	34.30
2	1.6304	20.38	54.69
3	1.3457	16.82	71.51
4	1.0870	13.59	85.10
5	0.5927	7.41	92.51

sizes were observed in PI 219717 and PI 207657 (150 cm). The coefficient of variation for plant height and plant width were 18% and 23%, respectively, indicating a low degree of variation between individuals within the same sunn hemp accession. Relative plant maturity for sunn hemp accessions ranged from 5 to 9. The accessions, PI 250487, PI 207657, PI 322377, PI 250486, and PI 314239 matured at mid-season. The coefficient of variation for relative maturity was 25%, indicating a fairly low degree of variation between individuals within the same accession. The leading seed producing accessions were PI 250485 and PI 314239 with a high coefficient of variation (110%) which indicates a high degree of variation between individuals within the same accession. Further evidence for variability in glucose, stem dry weights, plant heights, and plant widths were observed from wide ranges for these characteristics (Table 3).

Principal component analysis accounted for 34% of the total variation at the first principal component (Table 4). The amount of variation accounted for, cumulatively, by adding principal components 2 through 5 was 55, 72, 85, and 93%, respectively. The first principal component was most correlated with relative maturity and seed number (Table 5). The second principal component accounted for

Table 5. Eigenvectors and principal components for glucose, stem dry weight, morphological, phenological, and reproductive traits in sunn hemp accessions grown at USDA, ARS, Griffin, GA.

	Principal components				
	1	2	3	4	5
Glucose	-0.22	0.29	-0.33	0.63	-0.40
Stem dry weight (g)	0.12	0.06	0.75	0.07	-0.46
Branching ¹	0.42	0.49	0.04	0.15	0.07
Foliage ²	0.16	0.67	0.14	-0.22	0.28
Plant height (cm)	0.19	-0.31	0.39	0.59	0.37
Relative maturity ³	0.49	0.02	-0.30	0.27	-0.11
Seed number	-0.48	0.27	0.19	0.03	-0.25
Plant width (cm)	-0.45	0.18	0.07	0.29	0.55

^{1,2}Branching and foliage were based on a scale of 1–5 where, 1 = >90%, 2 = 80–89%, 3 = 70–79%, 4 = 60–69%, 5 = 50–59%, 6 = 40–49%, 7 = 30–39%, 8 = 20–29%, and 9 = 10–19% of each plant producing branches and/or foliage based on visual observations.

³Relative maturity dates were based on a scale of 5 to 9 where 5 = mid-season and 9 = very late.

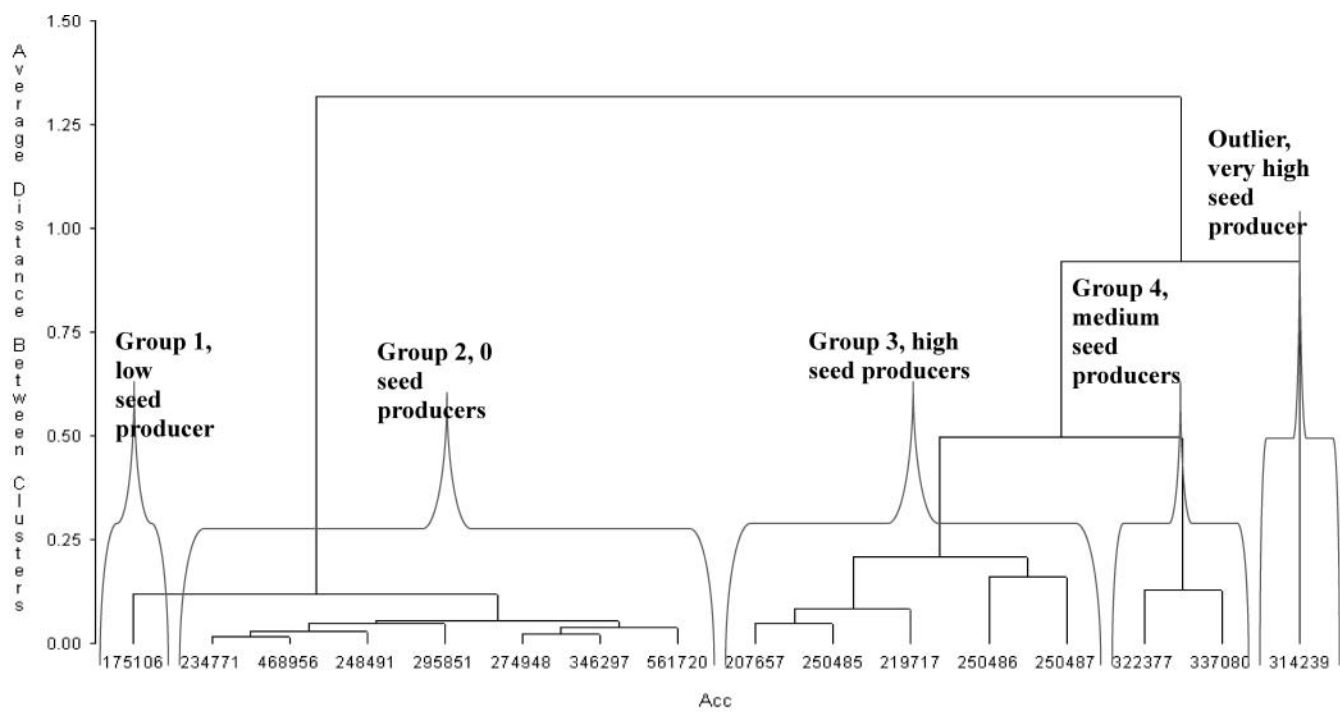


Fig. 1. Dendrogram of distance between clusters based on glucose concentration, stem dry weight, morphological, phenological, and reproductive differences. Accession numbers are given (Acc). Values on the baseline indicate average phenotypic distances between accessions. Four distinct clusters for seed production can be distinguished.

20% of the variation and was mostly due to foliage production. The third principal component explained 17% of the variation and is composed primarily of stem dry weight. The fourth principal component explained 14% of the variation and consisted of glucose and plant height while the fifth principal component accounted for 7% of the variation consisting of plant width. Therefore, potential exists to develop cultivars with improved sunn hemp architecture, early maturity, seed yield, glucose and stem dry weights for use as a new biomass crop utilized for ethanol production. Branching was significantly correlated with foliage ($r^2 = 0.67^{**}$) and relative maturity ($r^2 = 0.60^*$). Relative maturity had significantly negative correlations with seed number ($r^2 = -0.67^{**}$) and plant width ($r^2 = -0.53^*$), respectively. Seed number was significantly correlated with plant width ($r^2 = 0.57^*$).

Average linkage cluster analysis grouped the original 16 sunn hemp accessions into well defined phenotypes with four distinct seed producing groups and one outlier (Fig. 1). Group 1 represents 1 low seed producing accession while group 2 consists of 7 accessions producing no seeds. Group 3 consists of 5 high seed producing accessions and group 4 represents 2 medium seed producing accessions. The outlier consisted of one accession producing very high seed numbers. Using distance values indicated in Figure 1, the groupings at any similarity level can be identified. For example, PI 322377 and PI 337080 originate from Brazil with

a phenotypic distance index of 0.1286, which indicates their close morphological similarities. These 16 sunn hemp accessions evaluated during the regeneration cycle consists of enough genetic variation for cultivar development based on coefficients of variation, principal component, and cluster analysis.

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