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Desmodium genetic resources for improving flavonoid concentrations, oil content and fatty acid compositions

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Abstract

Several *Desmodium* species are adapted to the environment of Griffin, Georgia, USA. The determination of flavonoid concentrations, oil content and fatty acid compositions of 25 *Desmodium* accessions representing five species (*D. discolor* Vogel, *D. incanum* (G.Mey.) DC., *D. intortum* (Mill.) Urb., *D. sandwicense* E. Mey. and *D. tortuosum* (Sw.) DC.) would add value to the members of the genus *Desmodium* for possible use as livestock health supplements. In this study, the seeds of these 25 accessions were evaluated for flavonoid concentrations, oil content and fatty acid compositions using high-performance liquid chromatography, nuclear magnetic resonance and gas chromatography, respectively. Several accessions exhibited significantly greater values for all the traits than the controls. The *Desmodium* accessions produced significantly greater concentrations of quercetin and kaempferol than the best control accession (*D. incanum*, PI 477072). However, all the *Desmodium* accessions produced significantly greater concentrations of isorhamnetin and luteolin than the control accessions. All the *Desmodium* accessions had greater linoleic (18:2) and behenic (22:0) acid content than a couple of *D. incanum* control accessions in 2010 and 2011. Significant correlations were observed between several traits. The concentration of quercetin was significantly correlated with that of kaempferol ($r^2 = 0.69^{***}$); however, the concentration of quercetin exhibited a significantly negative correlation ($r^2 = -0.41^{*}$) with that of isorhamnetin. Oil content was significantly correlated with palmitic acid ($r^2 = 0.61^{**}$), stearic acid ($r^2 = 0.81^{***}$), linolenic acid ($r^2 = 0.58^{**}$) and lignoceric acid ($r^2 = 0.80^{***}$) content. This information will assist breeders and other scientists in developing superior cultivars with optimum levels of flavonoid concentrations, oil content and fatty acid compositions for many of these *Desmodium* species.

Keywords: Flavonoids; oil; fatty acids; *Desmodium*

Introduction

The genus *Desmodium* is comprised of several species with a variety of uses including cover cropping, pasture (Morris, 1997), green manure, insect or weed suppression (Kifuko-Koech *et al.*, 2012) and other phytochemical uses (Morris *et al.*, 2012). Some of the *Desmodium* species such as *Desmodium intortum* (Mill.) Urb. have been used as supplemental livestock feed in drought conditions (Boukila *et al.*, 2009). Feeding goats with *D. intortum* has been shown to reduce the populations of the worm parasite *Haemonchus contortus* in them (Debela *et al.*, 2012). *Desmodium sandwicense* E. Mey. plants have greater cold tolerance than *D. intortum*

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plants (Whiteman, 1970). Natural pastures in the western frontier of the state of Rio Grande do Sul, Brazil, have mainly *Desmodium incanum* (G. Mey.) DC. plants along with many other species (Tanure *et al*., 2011). *Desmodium discolor* (Vogel) plants produce hay and silage with good palatability (Boultwood, 1964). *Desmodium tortuosum* (Sw.) DC. seeds are commonly sold as wild bird feed.

Since various flavonoids, oils and fatty acids are present in other legume species such as *Macrotyloma uniflorum* Lam. Verdc., *Lablab purpureus* (L.) Sweet, *Neonotonia wightii* (Wight & Arn) J.A. Lackey (Morris *et al*., 2013a, b, c), some of these phytochemicals may also be present in *Desmodium* species. Legume flavonoids can be found in animal or human diets as quercetin, kaempferol, luteolin and apigenin have been reported to be present in the milk of cows consuming various grass forages (Besle *et al*., 2010). Flavonoids have been reported to provide many health benefits to humans. Quercetin can effectively inhibit mast cell production in allergic and inflammatory diseases (Weng *et al*., 2012). Kaempferol induces apoptosis in ovarian cancer cells through the regulation of pro-apoptotic and anti-apoptotic protein expressions in apoptotic pathways (Luo *et al*., 2011). Isorhamnetin has been reported to have an antiproliferative effect and a greater cytotoxic effect in gastric cancer in combination with chemotherapeutic drugs (Ramachandran *et al*., 2012). Luteolin in combination with (−)-epigallocatechin-3-gallate has been reported to induce apoptosis in both lung cancer and squamous cell carcinoma cells of the head and neck cancer cell lines (Amin *et al*., 2010) in humans. Apigenin has been shown to block the progestin-dependent induction of the vascular endothelial growth factor in breast cancer cells (Mafuvadze *et al*., 2010). Feeding livestock with oil crops or supplements that are high in unsaturated fats has been shown to increase their polyunsaturated fatty acid concentrations (Karsten and Baer, 2009). Since there is little knowledge on important phytochemicals of *Desmodium* species, our objective was to determine the flavonoid concentrations, oil content and fatty acid compositions of 25 *Desmodium* accessions from five species including *D. discolor* (three accessions), *D. incanum* (four accessions), *D. intortum* (two accessions), *D. sandwicense* (ten accessions) and *D. tortuosum* (six accessions).

Materials and methods

Planting

D. incanum was used as a control as it has better tolerance to continuous heavy grazing than *D. intortum* (Cook *et al*., 2005). *D. incanum* plants have been used as medicinal diuretics, stomachics, fever reducers and haemostatics in Central America (Setyowati-Indarto and Brink, 1999). *D. incanum* plants are also resistant to root knot nematodes (Quesenberry *et al*., 2008). Seeds obtained from 25 *Desmodium* accessions (Table 1) were planted in 6.4 × 7.0 cm jiffy pots (Hummert International, Earth City, MO, USA) containing Promix HP potting soil (Griffin Greenhouse, Ball Ground, GA, USA) in 2010 and 2011 in the first week of April. The seedlings were grown in a greenhouse for 4 weeks without supplemental lighting at 21 to 26°C. Since *Desmodium* species are highly outcrossing, the same pool of seeds from each accession was used during both the years. Twenty-five to fifty seedlings representing each accession per plot were transplanted in a field in Griffin, Georgia, USA, in the first week of May in one 6 m row plot with an interval of 6 m between rows in an augmented randomized complete block design with two replications. The soil was a clayey, kaolinitic, thermic Typic Kanhapludult series. A supplemental fertilizer containing 10–10–10 NPK was applied to the field at a rate of 112 kg/ha. The plots were irrigated for proper plant growth and maximum seed production. Pods were harvested from each *Desmodium* accession on the maturation of seeds 3 to 6 months after transplantation. The pods were dried at 21°C and 25% relative humidity for 1 week and then threshed. This long sampling period was required to

Table 1. Origin of *Desmodium* accessions used in the study

Species	PI number (origin)
D. discolor	271160 (India) and 322442 and 322444 (Brazil)
D. incanum	Controls 322418 and 322419 (Brazil), 477072 (Uruguay) and 593057 (Florida, USA)
D. intortum	214107 (Spain) and 317894 (Brazil)
D. sandwicense	316216, 316217, 316218, 316219, 316220, 316221, 316222, 316223, 316224 and 316225 (Australia)
D. tortuosum	225890 (Tanzania), 275089 (India), 317054 (Trinidad and Tobago), 317057 and 317058 (Virgin Islands, USA), and 317059 (Australia)

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obtain enough seeds per accession for all the phytochemical analyses. Further studies should be carried out to evaluate the impact of harvest date and environment on flavonoid concentrations, oil content and fatty acid compositions in *Desmodium* species.

Determination of flavanoid concentrations

Desmodium seeds were ground to a fine powder in a coffee grinder and stored at −20° C until the extraction of flavonoids. Approximately 0.1 g of seed tissue was placed into screw cap tubes. To each tube containing the seed tissue, 6 ml of extraction solvent containing 60% high-performance liquid chromatography (HPLC)-grade methanol with 1.2 M HCl were added. The samples were then mixed thoroughly and incubated at 80°C for 2 h with occasional mixing. A portion of the supernatant was filtered using a 0.45 μM membrane prior to injection into the chromatographic column. The flavonoids were separated by reversed-phase HPLC with a Kinetex solid-core, 4.6 × 100 mm, 2.6 μm, phenyl-hexyl column (Phenomenex, Inc., Torrance, CA, USA) at 40°C using an Agilent 1100 HPLC system with a binary pump and an autosampler. The mobile phase consisted of HPLC-grade methanol (B) and 0.1% formic acid in filtered, sterile water (A). The flow rate was 0.75 ml/min at the following gradient: 15% B initially followed by 60% B for 30 min. The column was washed with 95% B for 6 min and equilibrated at 15% B for 7 min between injections. The sample injection volume was 10 μl, and the flavonoids and flavonols were monitored using a diode-array detector at 340 and 370 nm, respectively. Flavonoid standards purchased from Indofine Chemical Co. (Hillsborough, NJ, USA) were dissolved in a 5:3:2 mixture of dimethyl sulphoxide–methanol–water. This mixture was diluted with 60% methanol to generate standard curves (ranging from 0.1 to 20.0 ng/μl) for the identification and quantification of peaks. All the samples were prepared and injected twice, and the results were averaged.

Determination of seed oil content

Desmodium seed oil content was measured using a Minispec MQ10 nuclear magnetic resonance (NMR) analyzer (Bruker Optics, Inc., Billerica, MA, USA). The NMR analyzer was maintained at 40°C and operated at a resonance frequency of 9.95 MHz. For each signal acquisition, spin-echo parameters consisted of a 90° pulse of 10.44 μs, and the signal was recorded at 50 μs. Later, a 180° pulse of 21.38 μs (pulse spacing = variable) was used, and the signal was recorded at 7 ms. A recycle delay of 2 s was maintained between scans, and a total of 20 scans were collected for each sample. Soybean oil (Sigma-Aldrich, St. Louis, MO, USA) was used as a reference for establishing a standard curve as *Desmodium* seed oil is not available commercially. Shredded pieces of paper towels were added to a sample tube containing oil to serve as a matrix for each of nine standards. Moisture standards were prepared using soybean seeds having known moisture content. The mass of seed oil and water was converted into a percentage of the total weight of each sample. All the samples were measured thrice, and the results were averaged. Four *Desmodium* accessions including *D. incanum* (PI 322418, PI 322419 and PI 477072) and *D. sandwicense* (PI 316218) did not produce enough seeds for the adequate measurement of oil content. Therefore, seeds obtained from these accessions stored at −18°C previously were used to determine oil content on a dry-weight basis.

Determination of fatty acid compositions

Fatty acid compositions were determined using an Agilent 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) with a split/splitless (S/Sl) inlet and a flame ionization detector. *Desmodium* seeds were ground to a fine powder in a coffee grinder. Oil from approximately 50 to 100 mg of these powdered *Desmodium* seeds was extracted into 3 ml of heptane and converted to fatty acid methyl esters (FAMEs) using a 0.5 N sodium methoxide catalyst in methanol. The organic layer containing these FAMEs was separated from the seed meal with the addition of water. An aliquot from this layer was transferred into a sample vial for injection. The peaks were separated using a DB-23 capillary column (15 m × 0.25 mm internal diameter) with a 0.25 μm film obtained from Agilent Technologies. Later, 1 μl of the prepared sample was injected at a 60:1 split ratio into the DB-23 capillary column using the following thermal gradient: 180° C for 1 min, 180 to 195°C at 5°C/min, and 195 to 240°C at 10°C/min. Helium was used as the carrier gas, and the inlet pressure was set to 12 psi (approximately 41 cm/s at 180° C). The peaks were identified by comparing the retention times with that of a FAME standard mix RM-3 (Sigma-Aldrich). The oven was equilibrated for 3.5 min between injections. All the samples were prepared and injected twice, and the results were averaged.

Statistical analyses

Analyses were carried out using Proc GLM of SAS (SAS 9.2; SAS Institute, Inc., Cary, NC, USA) (SAS Institute,

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2009) to determine the significance of *Desmodium* accessions when compared with four *D. incanum* controls. Correlations were identified using Pearson's correlation analysis in SAS. Principal components were determined using PROC PRINCOMP (SAS 9.2; SAS Institute, Inc.), followed by a multivariate analysis of the data. Eigenvalues, the percentage of variances explained by each principal component, and eigenvectors were also determined. Clustering of the data was then carried out by entering the similarity matrix into PROC CLUSTER for cluster analysis with the unweighted paired group method using mathematical averages by specifying the AVERAGE option (SAS 9.2; SAS Institute, Inc.).

Results

Flavonoid concentrations

Data obtained from the 2-year field experiments were analysed separately because of year effects. *Desmodium* accessions were compared with the *D. incanum* control accessions (including PI 322418, PI 322419, PI 477072 and PI 593057) using least mean squares. The best *D. incanum* control accession, PI 477072 from Uruguay, produced the highest concentrations of quercetin and kaempferol in 2010 (44.6 and 19.9 μg/g, respectively) as well as in 2011 (41.4 and 18.1 μg/g, respectively) (Supplementary Tables S1 and S2, available online). However, the second best control accession for the concentrations of quercetin (41.2 μg/g) and kaempferol (17.2 μg/g) was PI 593057 from Florida, USA, in 2010 (Supplementary Table S1, available online), while PI 322418 from Brazil was the second best control for the concentrations of quercetin (37.0 μg/g) and kaempferol (16.8 μg/g) in 2011 (Supplementary Table S2, available online). The best control accession for the concentration of apigenin (39.3 μg/g) was PI 593057, followed by PI 322419 from Brazil (37.7 μg/g) in 2010. In 2011, the best control accession for the concentration of apigenin (38.0 μg/g) was PI 322418, followed by PI 322419 (34.9 μg/g). None of the control accessions produced isorhamnetin or luteolin. During both the years, all the *Desmodium* accessions produced significantly greater concentrations of quercetin (135.3–876.3 μg/g) and kaempferol (76.5–200.6 μg/g) than the best control (*D. incanum*, PI 477072). All the *Desmodium* accessions produced significantly greater concentrations of isorhamnetin and luteolin (ranging from 14.2 to 925.4 μg/g) than all the four controls (0 μg/g) during both the years. All these *Desmodium* accessions produced significantly greater concentrations of apigenin (ranging from 57.4 to 150.8 μg/g) than the control PI 593057 (39.3 and 34.0 μg/g) in 2010 and 2011, respectively.

Oil content and fatty acid compositions

Oil content and fatty acid compositions are listed in Supplementary Tables S3 and S4 (available online). Oil content averaged 14.4% among the four controls during both the years, while oil content ranged from 8.5 to 11.2% among the additional *Desmodium* accessions. None of the accessions had significantly greater oil content when compared with the controls. Palmitic acid (16:0) and stearic acid (18:0) content ranged from 9.8 to 14.2 and from 2.6 to 5.4%, respectively, and was not significantly different from that of the control accessions during both the years. In 2010, only the *D. discolor* accessions (PI 271160 from India and PI 322442 from Brazil)

Table 2. Flavonoid, oil and fatty acid traits in diverse *Desmodium* genotypes based on data obtained in 2010–2011

	Range			
Variables	Maximum	Minimum	Mean	SD
Quercetin (μg/g)	837.5	35.1	418.78	273.03
Kaempferol (μg/g)	191.8	15.7	130.30	54.24
Isorhamnetin (μg/g)	870.9	0.0	251.77	326.13
Luteolin (μg/g)	498.0	0.0	156.25	184.44
Apigenin (μg/g)	132.7	28.6	71.51	27.65
Oil (%)	14.6	9.2	10.61	1.61
Palmitic acid (16:0) (%)	14.3	10.3	11.95	1.40
Stearic acid (18:0) (%)	5.8	3.0	4.15	0.73
Oleic acid (18:1) (%)	22.1	14.6	18.49	2.66
Linoleic acid (18:2) (%)	50.6	37.3	46.83	2.90
Linolenic acid (18:3) (%)	13.3	6.2	8.50	1.92
Arachidic acid (20:0) (%)	1.9	0.8	1.50	0.42
Gadoleic acid (20:1) (%)	0.80	0.40	0.68	0.15
Behenic acid (22:0) (%)	8.1	2.2	5.90	1.89
Lignoceric acid (24:0) (%)	5.5	1.0	2.23	1.42

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Table 3. Eigenvalues and the proportion of total variability among diverse *Desmodium* genotypes (2010–2011) as explained by the principal components

Principal components	Eigenvalue	% Variability	% Cumulative
1	7.1129	47.42	47.42
2	6.1573	41.05	88.47
3	0.8065	5.38	93.85
4	0.5615	3.74	97.59
5	0.1444	0.96	98.55
6	0.0912	0.61	99.16

and the *D. tortuosum* accessions (PI 275089 from India and PI 317054 from Trinidad and Tobago) did not have significantly greater oleic acid (18:1) content when compared with the second best controls (PI 322418 and PI 593057). In 2011, only the *D. intortum* accessions (PI 214107 from Spain and PI 317894 from Brazil) and the Australian *D. sandwicense* accessions (PI 316216, PI 316217, PI 316218, PI 316219, PI 316224 and PI 316225) had significantly greater oleic acid content than the second best control (*D. incanum*, PI 322418). All the *Desmodium* accessions ranging from 43.5 to 50.9% had significantly greater linoleic acid (18:2) content than the fourth best control (PI 477072) accession during both the years. Only the *D. tortuosum* accessions (PI 275089 and PI 317054) had significantly greater linolenic acid (18:3) content than the fourth best control (PI 593057) accession in 2010, while none of the accessions had significantly greater linolenic acid content when compared with the controls in 2011. Both PI 317894 (*D. intortum*) and PI 316218 (*D. sandwicense*) had significantly greater arachidic acid (20:0) content than the second best control (PI 477072) in 2010. There were no differences between any of the *Desmodium* and control accessions in 2011 for arachidic acid content. One *D. intortum* accession (PI 214107) and eight *D. sandwicense* accessions (PI 316216, PI 316217, PI 316219, PI 316220, PI 316221, PI 316222, PI 316224 and PI 316225) had significantly greater gadoleic acid (20:1) content than the fourth best control (PI 477072) in 2010; however, no differences were observed between the accessions and controls for gadoleic acid content in 2011. All the *Desmodium* accessions had significantly greater behenic acid (22:0) content than the best control (PI 593057) during both the years. Lignoceric acid content of all the *Desmodium* accessions and controls was similar in 2010 and 2011.

Correlations

Highly significant correlations were observed among flavonoid concentrations (Supplementary Table S5, available online). The concentration of quercetin was significantly correlated with that of kaempferol ($r^2 = 0.69^{***}$) (Supplementary Table S5, available online); however, the concentration of quercetin exhibited a significantly negative correlation ($r^2 = -0.41^{*}$) with that of isorhamnetin. Therefore, as the concentration of isorhamnetin decreased, that of quercetin increased. The concentration of isorhamnetin was significantly correlated with those of luteolin ($r^2 = 0.97^{***}$) and apigenin ($r^2 = 0.88^{***}$), while the concentration of luteolin was significantly correlated with that of apigenin ($r^2 = 0.92^{***}$). Highly significant correlations were also observed for oil content and fatty acid compositions

Table 4. Eigenvectors and principal components for 15 flavonoid, oil and fatty acid traits in diverse *Desmodium* genotypes (2010–2011)

	Principal components					
Trait	1	2	3	4	5	6
Quercetin	−0.32	0.17	0.16	0.11	0.0005	0.62
Kaempferol	−0.10	0.36	0.20	0.18	−0.24	0.40
Isorhamnetin	0.29	0.23	0.11	0.11	−0.16	−0.03
Luteolin	0.28	0.25	0.04	0.10	0.27	0.12
Apigenin	0.18	0.32	0.24	0.10	0.56	0.01
Oil	0.09	−0.37	0.03	0.08	−0.38	0.25
Palmitic acid (16:0)	0.32	−0.16	0.26	0.13	0.16	−0.01
Stearic acid (18:0)	−0.01	−0.34	0.58	0.08	−0.01	0.02
Oleic acid (18:1)	−0.33	−0.005	−0.09	0.57	−0.05	−0.15
Linoleic acid (18:2)	0.11	0.35	0.08	−0.48	−0.31	−0.03
Linolenic acid (18:3)	0.27	−0.17	−0.53	0.21	0.19	0.30
Arachidic acid (20:0)	−0.29	−0.21	0.30	−0.01	0.25	−0.16
Gadoleic acid (20:1)	−0.31	−0.15	−0.15	−0.39	0.35	0.34
Behenic acid (22:0)	−0.33	0.17	−0.04	−0.16	0.09	−0.19
Lignoceric acid (24:0)	0.26	−0.26	0.14	−0.29	0.04	0.24

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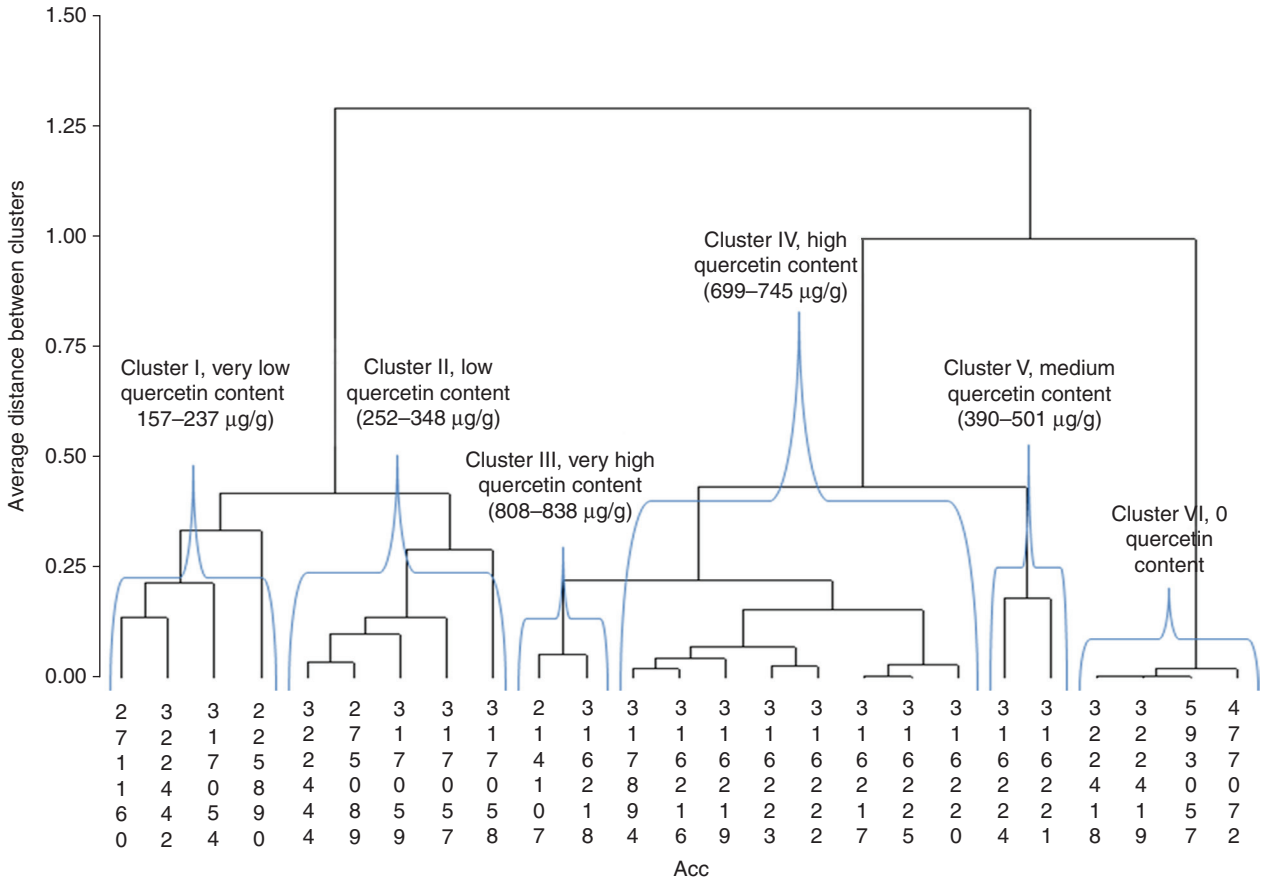

Fig. 1. (colour online) Dendrogram of the distance between clusters based on flavonoid concentration, oil content and fatty acid composition differences. Accession numbers are given (Acc). Values on the baseline indicate average phytochemical distances between the accessions. Six distinct clusters for quercetin concentrations can be distinguished.

(Supplementary Table S6, available online). Oil content was significantly correlated with palmitic acid ($r^2 = 0.61^{**}$), stearic acid ($r^2 = 0.81^{***}$), linolenic acid ($r^2 = 0.58^{**}$) and lignoceric acid ($r^2 = 0.80^{***}$) content. Several significantly negative correlations were also observed for oil content and linoleic acid ($r^2 = -0.74^{***}$) and behenic acid ($r^2 = -0.65^{**}$) content.

Principal component analysis

Large variations were observed for most of the traits tested except for oil, palmitic acid and stearic acid content (Table 2). The flavonoid concentration, oil content and fatty acid composition principal component analysis accounted for 47% of the total variation observed for the first principal component (Table 3). The amount of variation accounted for, cumulatively, by adding the second, third, fourth, fifth and sixth principal components was 88, 94, 98 and 99%, respectively. The first principal component was mostly correlated with quercetin, palmitic, oleic, gadoleic and behenic acid content (Table 4). The second principal component accounted for 41% of the variation and was mostly correlated with kaempferol and apigenin concentrations as well as with stearic acid, linoleic acid and oil content, while the third principal component explained 5% of the variation and was primarily correlated with stearic and linolenic acid content. The fourth principal component accounted for 4% of the variation and was mostly correlated with oleic and linoleic acid content. Both the fifth and sixth principal components accounted for 1% of the variation and were mainly correlated with apigenin, quercetin and kaempferol concentrations.

Cluster analysis

The average distance cluster analysis grouped the original 25 *Desmodium* accessions into well-defined phenotypes with six distinct quercetin concentration accessions (Fig. 1). Clusters I and II represent four very-low- and five low-quercetin concentration accessions, respectively. Clusters III and IV consist of two very-high- and

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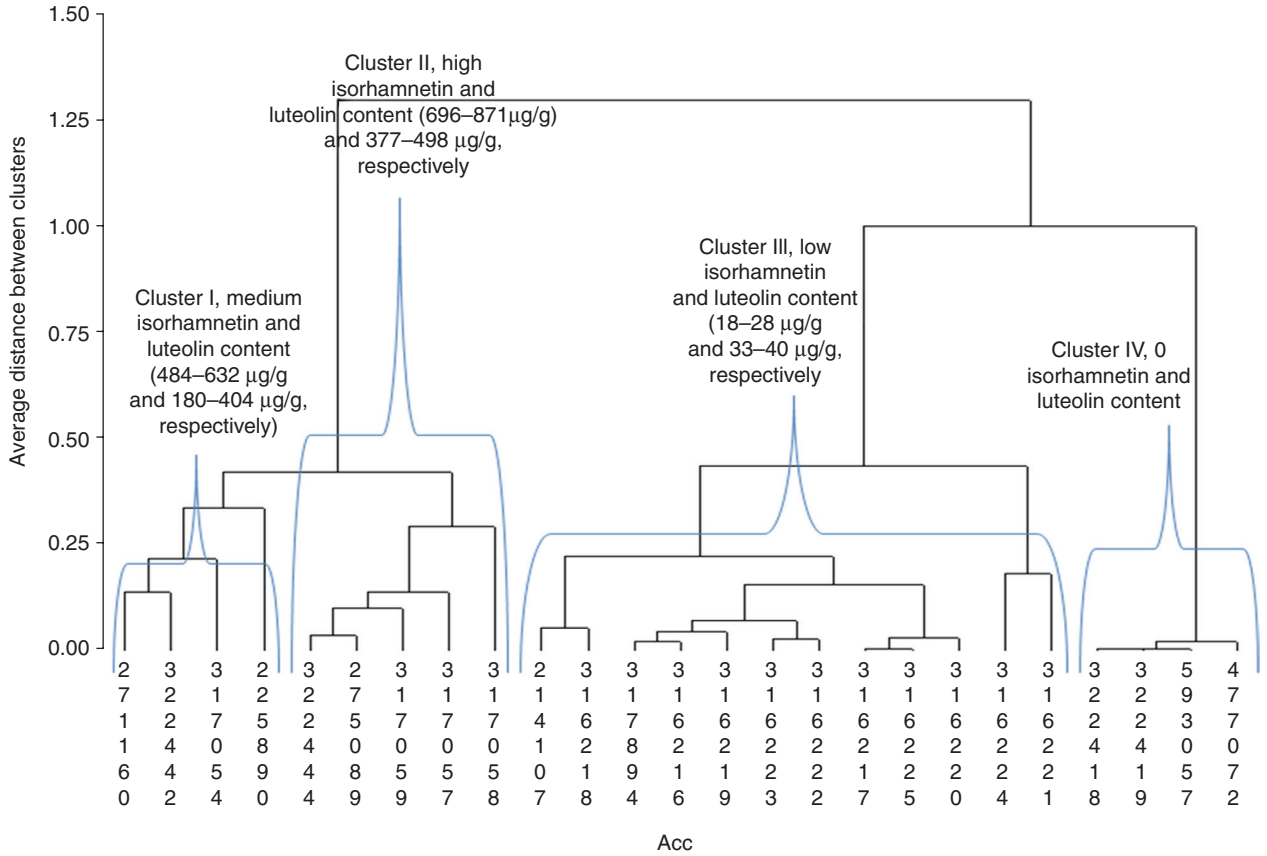

Fig. 2. (colour online) Dendrogram of the distance between clusters based on flavonoid concentration, oil content and fatty acid composition differences. Accession numbers are given (Acc). Values on the baseline indicate average phytochemical distances between the accessions. Four distinct clusters for isorhamnetin and luteolin concentrations can be distinguished.

eight high-quercetin concentration accessions, respectively. However, clusters V and VI comprise two medium- and four zero-quercetin concentration accessions, respectively. These 25 *Desmodium* accessions were also clustered into well-defined phenotypes with four distinct isorhamnetin and luteolin concentration accessions (Fig. 2). Clusters I and II represent four medium- and five high-isorhamnetin and luteolin concentration accessions, respectively. However, clusters III and IV have 12 low- and 4 zero-isorhamnetin and luteolin concentration accessions, respectively. Using the distance values reported in Fig. 1, the clustering at any similarity level can be identified. For example, the *D. discolor* accessions PI 271160 and PI 322442 originating from India and Brazil, respectively, have a phenotypic distance index of 0.1385, which indicates close similarities among their quercetin, isorhamnetin and luteolin concentrations.

Discussion

Biochemical analysis of the flavonoid concentrations, oil content and fatty acid compositions of *Desmodium* species can identify and quantify accessions useful for potential livestock nutrition. Several significant correlations among flavonoid concentrations, oil content and fatty acid compositions were observed in this study; therefore, potential *Desmodium* cultivars could be developed with improved phytochemical profiles. In addition, the bioavailability and assimilation of these seed phytochemicals for possible human consumption should be evaluated in future research studies. The *Desmodium* accessions could provide livestock with nutraceuticals or functional feed supplements containing flavonoid concentrations, oil content and fatty acid compositions for improved health.

There have been a limited number of studies reporting fatty acid compositions for these *Desmodium* species. Maestri *et al.* (2002) reported similar fatty acid compositions for *D. tortuosum*. Several *Desmodium* species having fatty acids including linoleate were temperature sensitive when grown in northern and southern areas of the United States (Cherry *et al.*, 1985), which may explain the year effect differences observed in this study. Principal components and cluster analysis provide quality characterization information on the flavonoid

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concentration, oil content and fatty acid composition profiles of the *Desmodium* accessions. Principal component analysis is a useful method for determining as how much each flavonoid, oil and fatty acid trait contributes to the variation observed in the *Desmodium* species. It has successfully been used to characterize flavonoids in legume species such as *Macrotyloma uniflorum* (Lam.) Verdc., *Lablab purpureus* (L.) Sweet and *Neonotonia wightii* (Wight & Arn.) J.A. Lackey (Morris *et al*., 2013a, b, c).

The average linkage cluster and multivariate analysis carried out on the data were able to separate accessions producing very low concentrations of quercetin, isorhamnetin and luteolin from those producing high concentrations of these flavonoids. All six clusters were represented by collected or donated *Desmodium* accessions from Australia, Brazil, India, Spain, Tanzania, Trinidad and Tobago, Uruguay, the United States and the Virgin Islands, USA (Table 1). These *Desmodium* accessions originate from a relatively small number of geographical locations. However, the clusters of accessions producing quercetin tended to define accession groups with similar geographical origins. For example, seven of the high-quercetin concentration accessions originated from Australia, while one originated from Brazil. The accessions producing the highest concentrations of isorhamnetin and luteolin consisted of two from Australia and one from Brazil and India each. Ten of the low-isorhamnetin and luteolin concentration accessions originated from Australia also. This will be useful when selecting accessions from *Desmodium* genetic resources for potential flavonoid enhancement. The analysis revealed tighter clustering among the high-quercetin, low-isorhamnetin, and luteolin concentration accessions. This indicates greater genetic variability in the accessions producing low-to-medium quercetin concentrations and the accessions producing medium-to-high isorhamnetin and luteolin concentrations.

More than 240 accessions representing 39 *Desmodium* spp. remain to be evaluated for flavonoid concentrations, oil content and fatty acid compositions in the USDA, ARS, PGRCU collection. *Desmodium* accessions evaluated for these traits will provide breeders with valuable germplasm for the development of future cultivars with superior flavonoid concentrations, oil content and fatty acid compositions.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S1479262113000397

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References

Amin AR, Wang D, Zhang H, Peng S, Shin HJ, Brandes JC, Tighiouart M, Khuri FR, Chen ZG and Shin DM (2010) Enhanced anti-tumor activity by the combination of the natural compounds (−)-epigallocatechin-3-gallate and luteolin: potential role of p53. *Journal of Biological Chemistry* 285: 34557–34565.

Besle JM, Viala D, Martin B, Pradel P, Meunier B, Berague JL and Fraisse D (2010) Ultraviolet-absorbing compounds in milk are related to forage polyphenols. *Journal of Dairy Science* 93: 2846–2856.

Boukila B, Tendonkeng F, Tendonkeng Pamo E and Betfiang ME (2009) Chemical composition and *in vitro* digestibility of *Desmodium uncinatum*, *Desmodium intortum* and *Arachis glabrata* fermented alone or mixed with maize stover. *Livestock Research for Rural Development* 21: 108.

Boultwood JN (1964) Two valuable perennial legumes – horse marmalade (*Desmodium discolor*) and kuru vine (*D. intortum*). *Rhodesia Agriculture Journal* 61: 70–72.

Cherry H, Bishop L, Hasegawa P and Lefflert HR (1985) Differences in the fatty acid composition of soybean seed produced in northern and southern areas of the U.S.A. *Phytochemistry* 24: 237–241.

Cook BG, Pengelly BC, Brown SD, Donnelly JL, Eagles DA, Franco MA, Hanson J, Mullen BF, Partridge IJ, Peters M and Schultze-Kraft R (2005) *Tropical Forages: An Interactive Selection Tool [CD-ROM]*. Brisbane, QLD: CSIRO, DPI&F (Qld), CIAT and ILRI.

Debela E, Tolera A, Eik LO and Salte R (2012) Condensed tannins from *Sesbania sesban* and *Desmodium intortum* as a means of *Haemonchus contortus* control in goats. *Tropical Animal Health and Production* 44: 1939–1944.

Karsten Heather D and Baer David J (2009) Grass and human nutrition. In: Wedin Walter F and Fales Steven L (eds) *Grassland: Quietness and Strength for a New American Agriculture*. Madison, WI: American Society of Agronomy, pp. 189–204.

Kifuko-Koech M, Pypers P, Okalebo JR, Othieno CO, Khan ZR, Pickett JA, Kipkoech AK and Vanlauwe B (2012) The impact of *Desmodium* spp. and cutting regimes on the agronomic and economic performance of Desmodium-maize intercropping system in western Kenya. *Field Crops Research* 137: 97–107.

Luo H, Rankin GO, Li Z, Depriest L and Chen YC (2011) Kaempferol induces apoptosis in ovarian cancer cells through activating p53 in the intrinsic pathway. *Food Chemistry* 128: 513–519.

Maestri DM, Fortunato RH, Guzman CA, Torres MM and Lamarque AL (2002) Seed compositional studies of some species of Papilionoideae (Leguminosae) native to Argentina. *Journal of the Science of Food and Agriculture* 82: 248–251.

Mafuvadze B, Benakanakere I and Hyder SM (2010) Apigenin blocks induction of vascular endothelial growth factor mRNA and protein in progestin-treated human breast cancer cells. *Menopause* 17: 1055–1063.

Morris J Bradley (1997) Special-purpose legume genetic resources conserved for agricultural, industrial, and pharmaceutical use. *Economic Botany* 51: 251–263.

Morris JB and Wang ML (2007) *Characterization of Guar,* Cyamopsis tetragonoloba *(L. Taub.) Genetic Resources for Flavonoid Traits*. Chicago, IL: American Society of Botany and Plant Biology Joint Congress, abstracts.

Morris JB, Hellier BC and Connett JF (2012) Medicinal properties of legumes. In: Singh Ram J (ed.) *Genetic Resources, Chromosome Engineering, and Crop Improvement: Medicinal Plants*. Boca Raton, FL: CRC Press, pp. 297–326.

Morris John Bradley, Wang Ming Li, Grusak Michael A and Tonnis Brandon (2013a) Fatty acid, flavonol, and mineral composition variability among seven *Macrotyloma uniflorum* (Lam.) Verdc. accessions. *Agriculture* 3: 157–169.

Morris J Bradley, Grusak Michael A, Wang Ming L and Tonnis Brandon (2013b) Mineral, flavonoid, and fatty acid concentrations in ten diverse *Lablab purpureus* (L.) Sweet accessions. In: Kuang Hai-Xue (ed.) *Phytochemicals: Occurrence in Nature, Health Effects and Antioxidant Properties*. New York, NY: Nova Publishers, pp. 219–224.

Morris JB, Wang ML and Tonnis B (2013c) Variability for phenotype, anthocyanin indexes, and flavonoids in accessions from a close relative of soybean, *Neonotonia wightii* (Wight & Arn JA Lackey). In: El-Shemy Hany A (ed.) *Soybean – Bio-Active Compounds*. Manhattan, NY: InTech, pp. 375–386.

Quesenberry Kenneth H, Dampier Judith M, Crow Billy and Dickson Donald W (2008) Response of native southeastern U.S. legumes to root-knot nematodes. *Crop Science* 48: 2274–2278.

Ramachandran L, Manu KA, Shanmugam MK, Li F, Siveen KS, Vali S, Kapoor S, Abbasi T, Surana R, Smoot DT, Ashktorab H, Tan P, Ahn KS, Yap CW, Kumar AP and Sethi G (2012) Isorhamnetin inhibits proliferation and invasion and induces apoptosis through the modulation of peroxisome proliferator-activated receptor y activation pathway in gastric cancer. *The Journal of Biological Chemistry* 287: 38028–38040.

SAS Institute (2009) *SAS/STAT User's Guide*. Cary, NC: SAS Institute.

Setyowati-Indarto N and Brink M (1999) *Desmodium* Desv. In: de Padua LS, Bunyapraphatsara N and Lemmens RHMJ (eds) *Plant Resources of South-East Asia*. Available at: http://www.proseanet.org. Bogor: Prosea Foundation.

Tanure Soraya, Potter Bernardo Augusto Albornoz and Lobato José Fernando Piva (2011) Natural and improved natural pastures on the reproductive performance of first-calf beef cows. *Revista Brasileira de Zootecnia* 40: 690–699 (Online version ISSN 1806-9290) .

Weng Z, Zhang B, Asadi S, Sismanopoulos N, Butcher A, Fu X, Katsarou-Katsari A, Antoniou C and Theoharides TC (2012) Quercetin is more effective than cromolyn in blocking human mast cell cytokine release and inhibits contact dermatitis and photosensitivity in humans. *PLoS One* 7: e33805. Doi: 10.1371/journal.pone.0033805. Epublication 28 Mar 2012.

Whiteman PC (1970) Seasonal changes in growth and nodulation of perennial tropical pasture legumes in the field. I. The influence of planting date and grazing and cutting on *Desmodium uncinatum* and *Phaseolus atropurpureus*. *Australian Journal of Agricultural Research* 21: 195–206.