Morphological characterisation of populations of *Desmanthus virgatus* complex from Argentina

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Abstract

The Argentine germplasm of the *Desmanthus virgatus* complex is a valuable group of legume species for animal production. However, little is known about the extent and nature of the variability of these species. This study, which is an introduction to further ones about the variability of these species, aims at: a) collecting representative germplasm; b) analysing the morphological diversity; and c) determining patterns in Argentine populations of potential forage species of the *D. virgatus* complex using PCA and cluster methods. Twenty-six populations of *D. virgatus, D. acuminatus* and *D. paspalaceus* collected in Argentina plus cv. Marc were used to evaluate 14 quantitative morphological characters. Collecting expeditions, examination of material collected and analyses of measurements permitted the first characterisation of the morphological diversity and determination of the patterns of variation of species with forage value within the Argentinian *D. virgatus* complex. Identifying traits with agronomic interest which account for genetic diversity and the demarcation of distinguishable morphological groups will facilitate the maintenance and agronomic evaluation of the collections and permit informed decision making about new germplasm acquisitions.

Introduction

In recent decades, several species within the genus *Desmanthus* have shown significant potential as fodder for livestock (Allen and Allen 1981; Burt 1993; Gardiner *et al.* 2004; Ocumpaugh *et al.* 2004). Three cultivars were released in Australia, *D. virgatus* cv. Marc, *D. leptophyllus* cv. Bayamo and *D. pubescens* cv. Uman (Jones and Clem 1997) and 4 in the USA, *D. bicornutus* cvv. BeeTAM-06, BeeTAM-08, BeeTAM-37 and BeeTAM-57 (Ocumpaugh *et al.* 2004).

From a taxonomic point of view, *D. virgatus* is a complex with 4 species native to Argentina, namely *D. acuminatus*, *D. paspalaceus*, *D. tatuhyensis* and *D. virgatus* (Luckow 1993; Zuloaga and Morrone 1999). All except *D. tatuhyensis* have potential forage value in tropical and subtropical regions. In the field, we observed that plants of *D. tatuhyensis* are small with fewer leaves, fewer pinnae per leaf and more lignified branches than the other species (J.M. Zabala *et al*., unpublished data).

Several promising accessions of *D. virgatus, D. paspalaceus* and *D. acuminatus* evaluated in other countries, including cv. Marc, were collected from Argentina (Jones and Clem 1997). *D. virgatus* cv. Marc, for example, is a prolific seeder, branches profusely from the base and produces high quality and palatable forage (Jones and Brandon 1998; Pengelly and Conway 2000; Gardiner *et al*. 2004). In spite of the value of the Argentine germplasm, little is known about the extent and nature of the variability of these species (Hack *et al.* 2005) and no genetic improvement has been carried out in this country.

Efficient utilisation of plant genetic resources requires extensive collection, characterisation and agronomic evaluation of available material (Schultze-Kraft 1979; Tyler *et al.* 1985; Mcferson 1998). Systematic and detailed procedures should be established to collect representative germplasm (Bennet 1970; Mohammadi and Prasanna 2003). Characterisation through numerical techniques has been useful to determine variation patterns (Naranjo *et al.* 1990; Assefa *et al.* 2003) and to use germplasm collections in breeding programs (Pengelly *et al.* 1992; van de Wouw *et al.* 1999; Upadhyaya 2003).

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This study, which is an introduction to further studies about the variability of these species, aims to: a) collect representative germplasm; b) analyse the morphological diversity; and c) use numerical methods to elucidate variation patterns in Argentine populations of species of the *D. virgatus* complex with forage value.

Material and methods

Population sampling

Two collecting expeditions were launched in 2004–2005 in Argentina. Seeds were collected during December 2004 from the north-eastern and centre-north regions and in March 2005 from the north-western region (Figure 1), when plants had mature fruits. Each mission followed a pre-determined route in the environment where these species grow. Stops were made at different intervals depending on the changes shown by the vegetation in order to collect populations from a diversity of environments (Schultze-Kraft 1979).

At each site, the extent of the population was assessed. Then, seeds were collected from 20–40 plants, separated by a minimum of about 3 m to ensure that a representative sample was obtained from each population. Herbarium specimens were collected and conserved in SF Herbarium of Facultad de Ciencias Agrarias-Universidad Nacional del Litoral (Santa Fe, Argentina). Seeds from each plant were stored in separate envelopes at room temperature until time of planting.

Twenty-six populations of *D. virgatus, D. acuminatus* and *D. paspalaceus* collected in Argentina plus *D. virgatus* cv. Marc were used in the characterisation study (Table 1). Seeds of cv. Marc were supplied by the Australian Tropical Crops and Forage Collection (Queensland Department of Primary Industries and Fisheries, Australia).

Data collection and analysis

This study commenced in September 2005 in Esperanza, Santa Fe, Argentina (31°25′S, 60°56′W). Five seeds per plant of each population were scarified according to the method of Hopkinson and English (2004) and germinated in a growth chamber. Seedlings were transferred to individual pots (15 × 40 cm) with a mixture of peat and soil (v/v 1:1). Only one seedling per plant was transplanted.

After 2 months, the plants were transplanted to the field and watered as frequently as needed according to weather conditions. A fertiliser (15:15:15) was applied on 3 occasions: when plants were planted in pots (1 g/pot), one month later (4 g/pot) and when plants were transplanted into the field (at the rate of 300 kg/ha).

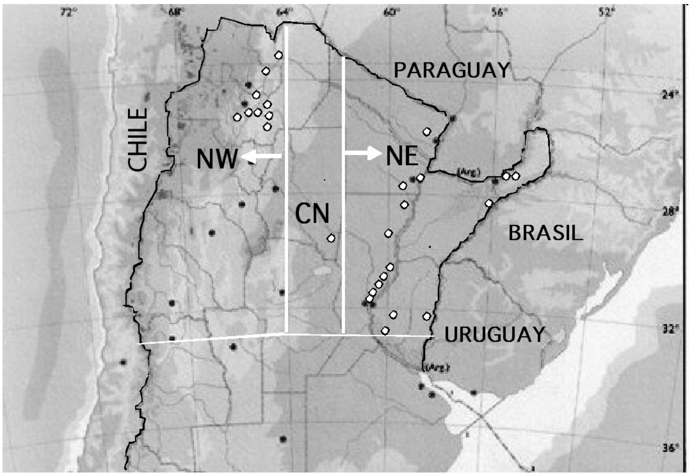

Figure 1. Map of the northern region of Argentina, showing the geographic location of populations of the *D. virgatus* complex (empty circles). Black full circles show capital towns. NW (north-west), CN (centre-north) and NE (north-east).

Table 1. List of populations of species of the *D. virgatus* complex analysed in this study.

Population Id[1]	Species	Province	Phytogeographic province (District)[3]	Lat.	Long.
acNE	*D. acuminatus*	Entre Ríos	Espinal	32°24′	59°33′
paNE1	*D. paspalaceus*	Entre Ríos	Espinal	32°38′	58°53′
paNE2	*D. paspalaceus*	Santa Fe	Espinal	31°24′	60°26′
paNE3	*D. paspalaceus*	Santa Fe	Espinal	31°06′	60°05′
paNE4	*D. paspalaceus*	Santa Fe	Eastern or humid Chaco	28°18′	59°18′
paNE5	*D. paspalaceus*	Entre Ríos	Espinal	32°20′	58°15′
paNE6	*D. paspalaceus*	Corrientes	Parana	28°07′	56°03′
paNE7	*D. paspalaceus*	Misiones	Parana	27°28′	55°40′
paNE8	*af. D. paspalaceus*	Misiones	Parana	27°28′	55°40′
paNE9	*D. paspalaceus*	Chaco	Eastern or humid Chaco	27°50′	59°17′
paNE10	*D. paspalaceus*	Santa Fe	Eastern or humid Chaco	28°18′	59°16′
paNW1	*D. paspalaceus*	Salta	Western or dry Chaco	25°04′	64°56′
paNW2	*D. paspalaceus*	Salta	Western or dry Chaco	25°23′	64°46′
viCN1	*D. virgatus*	Santiago del Estero	Western or dry Chaco	25°08′	64°47′
viNE1	*D. virgatus*	Corrientes	Western or dry Chaco	27°28′	58°47′
viNE2	*D. virgatus*	Santa Fe	Espinal	31°25′	60°39′
viNE3	*D. virgatus*	Santa Fe	Eastern or humid Chaco	29°12′	59°38′
viNE4	*D. virgatus*	Santa Fe	Espinal	31°02′	60°05′
viNE5	*D. virgatus*	Santa Fe	Espinal	31°24′	59°18′
viNE6	*D. virgatus*	Formosa	Eastern or humid Chaco	25°59′	58°25′
viNW1[2]	*D. virgatus*	Salta	Yungas (piedmont forest)	23°25′	64°08′
viNW2	*D. virgatus*	Salta	Western or dry Chaco	25°04′	64°56′
viNW3	*D. virgatus*	Salta	Western or dry Chaco	25°23′	64°46′
viNW4	*D. virgatus*	Jujuy	Yungas (piedmont forest)	24°32′	65°04′
viNW5	*D. virgatus*	Jujuy	Yungas (piedmont forest)	23°45′	64°43′
viNW6	*D. virgatus*	Salta	Western or dry Chaco	29°40′	62°07′
viNW7	*D. virgatus*	Salta	Yungas (piedmont forest)	25°06′	65°31′

[1] Identification: ac (*D. acuminatus*), vi (*D. virgatus*), pa (*D. paspalaceus*), NE (north-east region), NW (north-west region) and CN (centre-north region).
[2] cv. Marc, originally collected in Salta province (data extracted from Australian Plant Genetic Resource Information Service).
[3] According to Cabrera (1994).

Fourteen quantitative morphological (vegetative and reproductive) characters (Table 2), chosen on the basis of their success in previous studies (Burt 1993; Luckow 1993) and their usefulness for further agronomic evaluation, were measured on 20–25 plants of every population. Vegetative characters were measured in the plants 60 days after germination in the glasshouse and fruit characters were evaluated at harvest in the field during January and February. Means were used in a principal component analysis (PCA). Finally, characters were used in a cluster analysis to examine the aggregation patterns of the 26 populations. The clustering method used was average linkage (UPGMA) with Euclidean distance measure. The data were analysed using INFOSTAT statistical package (INFOSTAT version 2006 p. 2). For both analyses, data were standardised to mean zero and unity variance in order to minimise biases due to differences in the scales of measurement.

Results

Collecting missions

A set of populations of *D. paspalaceus, D. virgatus* and *D. acuminatus* from different phytogeographic provinces where these species grow (Figure 1; Table 1) has now been assembled in a collection, which will be conserved in the Facultad de Ciencias Agrarias.

At 2 sites, we observed 2 different plant 'types' of *Desmanthus* spp., differing mainly in plant height and leaf size. Seed of both species was collected and identified separately at each site (*i.e.*, 2 populations at each site). These different types were subsequently identified as different species, *D. virgatus* and *D. paspalaceus* according to Luckow (1993) (see Table 1, paNW1–viNW2 and paNW2–viNW3 populations). Several *D. tatuhyensis* populations (not included in this study) were also collected.

Table 2. Eiginvectors and eiginvalues of the first 5 principal components (PC) of 14 quantitative traits of populations of *D. paspalaceus*, *D. acuminatus* and *D. virgatus*. Shaded cells show coefficient with greatest weighting in each eigenvector.

Traits	Eigenvector		
	PC1	PC2	PC3
Taproot thickness (mm)	0.04	–0.41	0.21
Plant height (mm)	0.24	0.31	0.07
Leaf number	–0.34	–0.02	–0.27
Stem thickness (mm)	0.38	0.13	0.13
Leaflets per pinna	0.39	–0.11	0.01
Leaflet length (mm)	–0.25	0.44	–0.14
Leaflet width (mm)	–0.21	0.46	–0.01
Pinnae per leaf	0.03	0.22	0.5
Pinnae length (mm)	0.35	0.22	–0.08
No. lateral branches below 5 cm	–0.28	–0.24	–0.15
Peduncle length (mm)	0.2	–0.20	0.26
Pod length (mm)	0.28	0.10	–0.44
Pods per fruit	–0.16	0.28	0.44
Seeds per pod	0.29	0.14	–0.31
Eigenvalue	5.25	2.52	2.3

Principal component analysis

Table 2 shows the 3 main components that explain 72% of the total variation.

For Component 1 (37.5% of total variation), stem thickness, leaf number, leaflet number per pinna and pinnae length were characters with significant weighting (Table 2). Almost all populations of *D. virgatus* and *D. paspalaceus* showed a continuous morphological variability for this component.

Variation in Component 2 (18% of total variation) was mainly a result of differences in taproot thickness, plant height and leaflet length and width (Table 2). Populations of *D. virgatus* collected from the north-west were distinguishable from those taken in the north-eastern and centre-north regions by their smaller taproot thickness and greater plant height and leaflet size. In this component there was an overlap among all populations of *D. paspalaceus* and *D. virgatus* from the north-eastern region as seen in Figure 2.

Component 3 (16.5% of total variation) differentiated populations based on pod length, pod number and seed number per pod (Table 2). In addition, pinnae number per leaf showed greater weighting. In this component, 2 populations of *D. virgatus* (sensu stricto) (*i.e.*, viNW6 and viNW7) were clearly distinguishable from other populations, by having more pinnae per leaf, more pods per fruit, shorter pods and fewer seeds per pod (Figure 3).

Components 1, 2 and 3 separated clearly the population of *D. acuminatus* (acNE1) and population *afin D. paspalaceus* (paNE8) from all the other populations (Figures 2 and 3).

Cluster analysis

In the cluster analysis at 50% of dissimilarity, the dendrogram showed 5 distinct groups (Figure 4). Groups II and III included all the populations of *D. virgatus* from the north-western region and centre-north region. Cultivar Marc (viNW1) of *D. virgatus* was included in Group III. Group IV was the greatest and most heterogeneous with 6 populations of *D. virgatus* collected in the north-eastern region and all the populations of *D. paspalaceus* (8 populations from the north-eastern and 2 populations from the north-western regions).

The other 2 groups consisted of 1 population each. Group V contained the unique population of *D. acuminatus* (acNE1) and Group I the population *afin D. paspalaceus* (paNE8).

Discussion

The expeditions and analyses accomplished in this study permitted the first characterisation of the morphological diversity and determination of the patterns of variation of species with high forage value within the *D. virgatus* complex from

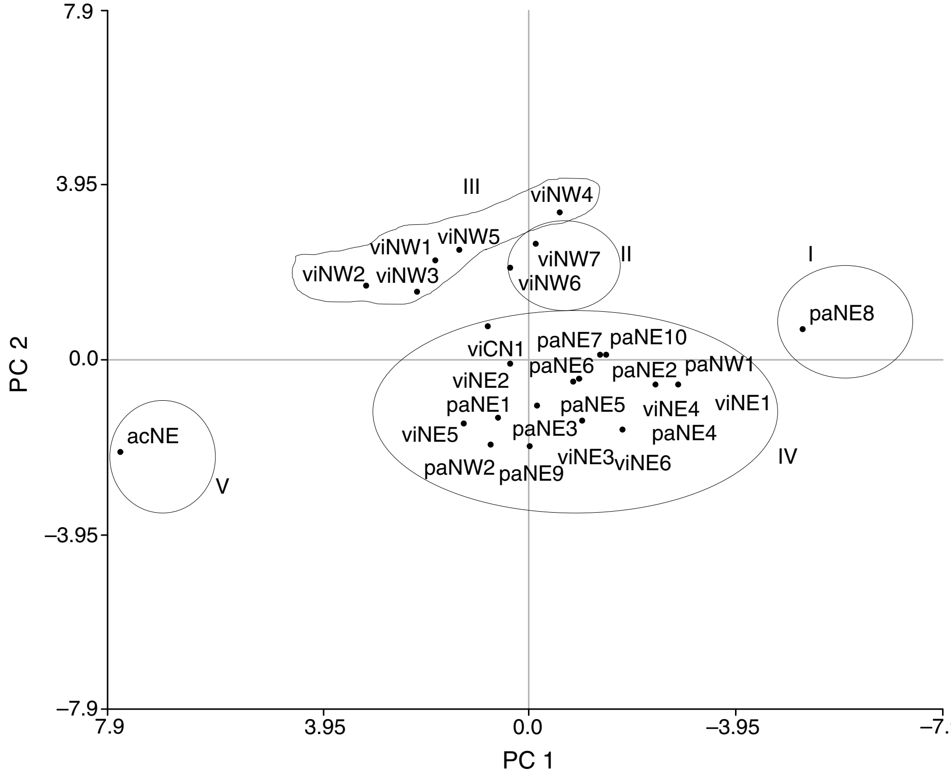

Figure 2. Scatter diagram of accessions of *D. paspalaceus*, *D. acuminatus* and *D. virgatus* plotted against the first 2 principal components. Circles and roman numerals correspond to clusters in Figure 4.

Argentina. The coexistence of populations of *D. paspalaceus* and *D. virgatus* was an unexpected finding. Only one previous study reported a similar result, and was cited as unpublished data in Pengelly and Liu (2001). The different populations were distinguished in the field by differences in plant height and leaf size. These characters showed significant weighting in PCAs. This should be considered in future sampling strategies for these species. Based on this experience, plant collectors can identify and sample different species at the same site. At each location, seeds of individual plants must be harvested and packaged and used to regenerate the germplasm collection. When planted out in the nursery or field, a sample with two different species would be easily recognisable.

Several populations of *D. tatuhyensis* were found in saline and flooded areas where other species of the *D. virgatus* complex were not present. Although its forage value is limited, this species could be considered as a potential source of salt and flooding tolerance genes in *Desmanthus* breeding programs.

In the PCAs, Component 1 separated populations based on their foliage characteristics, while Component 2 contrasted populations mainly on the basis of the shoot:root ratio and leaflet size, and Component 3 represented a reproductive component. Characters with highly significant weighting could provide insights in future agronomic characterisation and evaluation of *Desmanthus* populations.

Pengelly and Liu (2001) found a taxonomic pattern of variation in 284 accessions of 11 *Desmanthus* species using RAPD markers. However, patterns of variation in morphological and agronomic characterisations were not clearly related to geographical origin or taxonomic status of the germplasm analysed (Burt 1993; Hack *et al.* 2005). Our study supported this finding as there was no clear relationship between clustering and the phytogeographic provinces where the populations were collected (see Table 1; Figure 3).

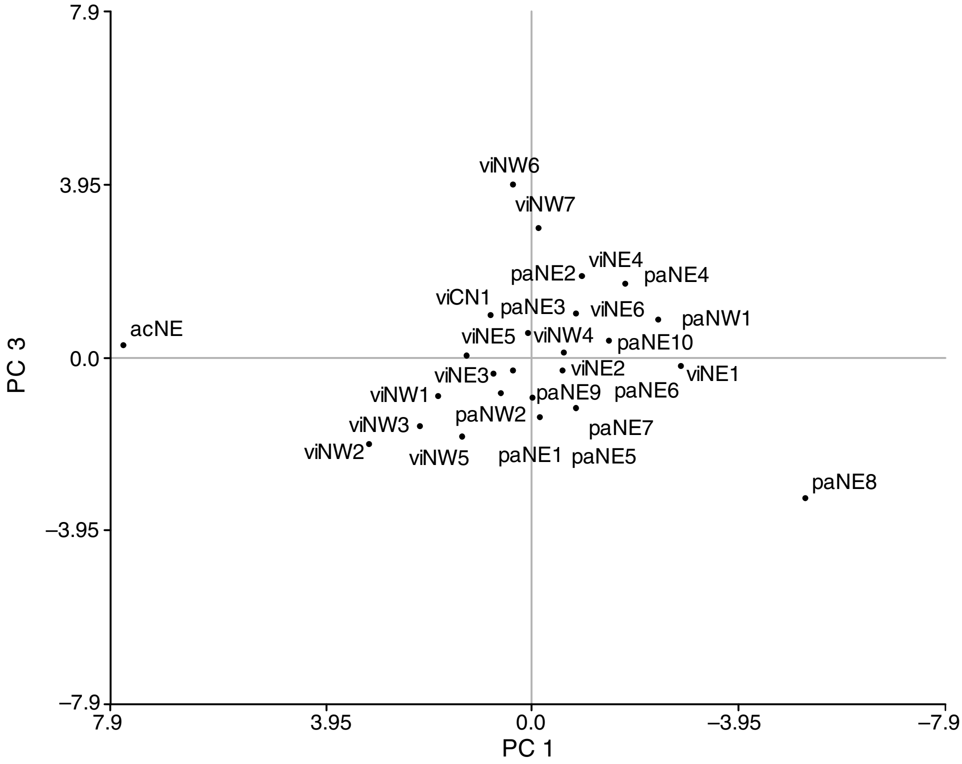

Figure 3. Scatter diagram of accessions of *D. paspalaceus*, *D. acuminatus* and *D. virgatus* plotted against the principal components 1 and 3.

Nevertheless, patterns of variation related to the specific identity of the collection and/or its collection region (*i.e.*, north-west, north-east and centre-north) were observed. Only some populations showed a continuous variation (*i.e.*, populations of *D. paspalaceus* and *D. virgatus* from the north-east).

Populations of *D. virgatus* (sensu stricto) from the north-western region of Argentina and cv. Marc were clustered in 2 groups, and populations from the north-eastern and centre-north regions were included in another discrete group (see Figure 2). Should germplasm such as *D. virgatus* cv. Marc be required in the future for forage breeding programs, collecting missions should be targeted towards the north-western region of Argentina. The clear separation of populations into 3 groups (Table 3) confirmed polymorphism in *D. virgatus* (sensu stricto) (Pengelly and Liu 2001). These groupings will be useful for further agronomic evaluations.

All the populations of *D. paspalaceus* were clustered in the same group (Figure 3). Our inability to separate populations of *D. paspalaceus* from the north-eastern populations of *D. virgatus* supported the finding of Luckow (1993) that it was difficult to separate some materials of *D. virgatus* sensu stricto and *D. paspalaceus* from Argentina. Hack *et al.* (2005) undertook agronomic and morphological characterisation of accessions of the *D. virgatus* complex from north-eastern Argentina and failed to find a clear pattern of variation between these related with the specific identity of species. These results suggest that: a) morphological traits assayed might be inappropriate for specific differentiation of north-eastern *Desmanthus* germplasm. Moreover, levels of variation between both *D. virgatus* and *D. paspalaceus* populations were higher in the north-eastern than north-western regions. or b) populations identified as different species are really populations of the same species. We determined that paNE8 is a rare population related to *D. paspalaceus* according to Luckow (1993). This population coexists with another clearly different population of *D. paspalaceus*

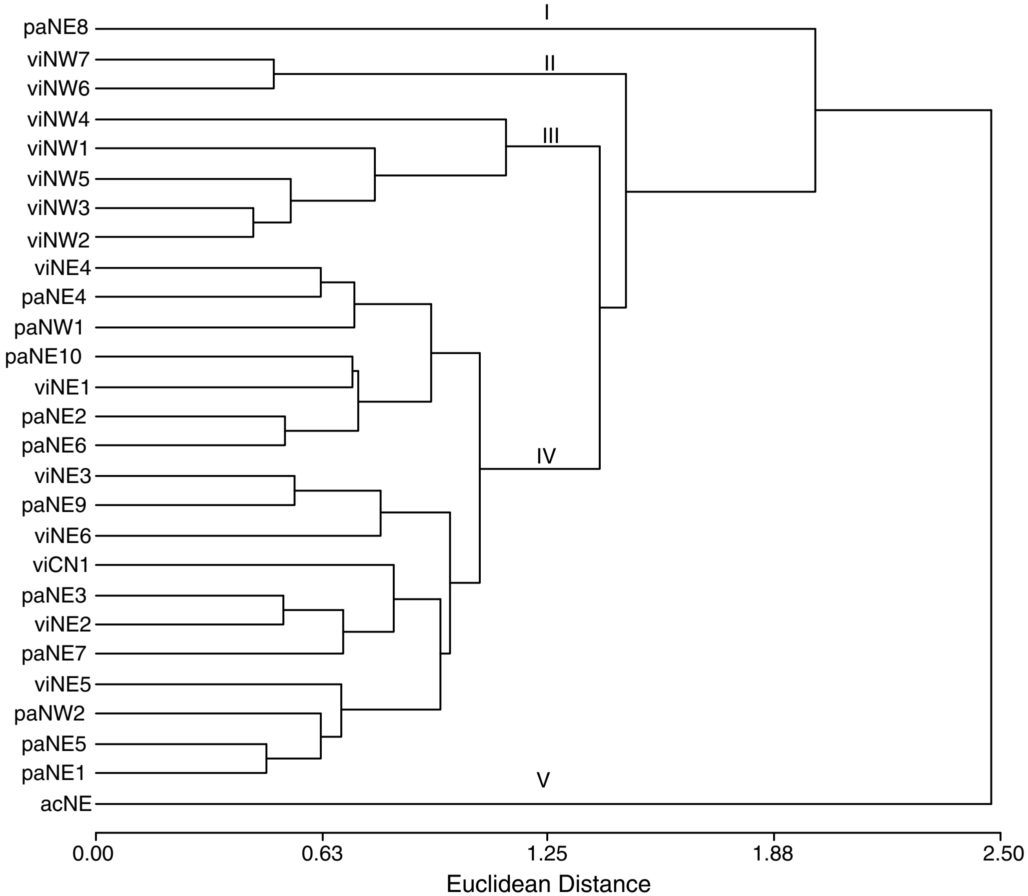

Figure 4. Dendrogram showing 5 groups (I–V) among 26 populations and cv. Marc (viNW1) evaluated for 14 morphological characters. Cophenetic coefficient of correlation = 0.913.

Table 3. Mean and (standard error) of 6 characters of population of *D. acuminatus* (acNE), populations *afin D. paspalaceus* (paNE8) and clusters of *D. virgatus* sensu stricto.

Trait	acNE	paNE8	Cluster II	Cluster III	Cluster IV
Taproot thickness (mm)	7.8 (0.8)	6 (0.3)	5.6 (0.42)	4.5 (0.52)	6.5 (0.71)
Plant height (mm)	88 (22)	379 (10.1)	353 (66)	360 (45)	308 (95.2)
Leaf number	30.5 (8.8)	10.8 (3.5)	8 (2.2)	18.6 (5.1)	10.7 (4.9)
Pinnae per leaf	3.5 (0.7)	1.8 (0.43)	7.4 (1.8)	5.3 (1.2)	5.4 (2.6)
Pod length (mm)	33 (3)	72 (5.7)	42 (3.1)	55 (3)	53 (5.2)
Pods per fruit	5.9 (1.2)	1.7 (0.5)	9.1 (2.1)	3.9 (1.1)	3.4 (1.6)

(paNE7) and there were no intermediate plant types. Population paNE8 was characterised by one pair of pinnae per leaf and one pair of longer pods (Table 3). Details of the original descriptions of *D. paspalaceus* of Burkart (1946) are in agreement with herbarium specimens of population paNE8. Detailed taxonomic studies are necessary to determine if populations of *D. virgatus* and *D. paspalaceus* from the north-east are the same species and identify the specific status of the rare morphotype paNE8.

Although numerical methods can be very sensitive to outliers, when populations of *D. acuminatus* and the rare population *afin D. paspalaceus* were excluded from the analyses, the grouping of the populations did not change (cluster not shown).

According to Luckow (1993) and Pengelly and Liu (2001), *D. acuminatus* and *D. paspalaceus* are closely related species. In our collection, however, and several herbarium specimens (J.F. Pensiero, unpublished data), *D. acuminatus* is clearly distinguishable from other species of the complex. This species has prostrate to decumbent habit, branches profusely in the crown and produces a large number of small leaves (Table 3). While the trait 'number of lateral branches below 5 cm' did not show great weighting, this might be a function of the timing of the observations as the lateral branches were not fully developed in the other species. The population of *D. acuminatus* displayed an average of 2.7 lateral branches of 18.9 cm length at 60 days, while the other species showed 0.6–1.5 lateral branches of shorter length. Our collection of populations of *D. acuminatus* should be expanded to confirm our hypothesis that they are indeed morphologically different species.

This study, by identifying traits with agronomic interest which account for the diversity of the populations and forming distinguishable morphological groups, will facilitate the maintenance and agronomic evaluation of collections of species of the *D. virgatus* complex and indicate where new germplasm acquisitions are needed.

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