

Agro–morphological characterisation of a collection of *Cynodon*

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Abstract

Ninety–eight accessions of *Cynodon*, belonging to 4 different species and including several hybrids were characterised using a set of 23 agro–morphological characters. The collection included both collections from natural populations and breeding lines, selected among siblings of open–pollinated plants. Morphological and agronomic traits were assessed and multivariate analysis was applied to cluster similar accessions. The research identified a group of turf grass type accessions, a group consisting of the accessions of *C. aethiopicus* and a main group of *C. dactylon* and *C. nlemfuensis*. Clustering within this main group of 89 accessions revealed 7 sub–groups of similar accessions differentiated mostly by the length of the leaf, spikelet colour and days to 50% flowering. Only the number of stolons and days to first flowering did not show significant differences among clusters. Three accessions were so different that they could not be assigned to any cluster. *C. dactylon* and *C. nlemfuensis* were very similar, with a large overlap in diversity of characters. The breeding lines were very variable and were present in 6 out of 7 clusters, demonstrating that the progeny of a limited number of accessions provided almost as much variation as the accessions collected from natural populations.

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Introduction

In warm temperate and tropical climates, *Cynodon* species are the major turf grasses used for lawns and golf courses (Zhang *et al.* 1999). Over the years many cultivars have been developed. Hybrids between *C. dactylon* (L.) Pers and *C. transvaalensis* BurtttDaty have been especially successful as turf grasses, even though they are sterile and have to be multiplied by root splits (Yerramsetty *et al.* 2005).

Cynodon species are also important forages and have an important role in grazing systems and for hay making. *Cynodon nlemfuensis* Vanderyst, also known as East African couch grass, is very productive in high nitrogen input pasture systems and responds well to heavy dressings of nitrogen (Skerman and Riveros 1990). *C. nlemfuensis* is extremely palatable, especially when young (Skerman and Riveros 1990) and is an excellent grass for making high quality hay. It is a warm season grass and the winter dormancy period can last for more than 7 months in parts of central USA (Moyer and Coffey 2000). New varieties released in the USA with increased frost resistance allow expansion of its growth area to cooler climates (Barksdale 2005). *Cynodon dactylon*, which forms both stolons and rhizomes, is also used in erosion control. It has a high tolerance to drought and salinity (Devitt *et al.* 1993), which makes it a preferred grass for soil stabilisation in marginal areas. It can form a stable soil cover either alone or in a mixture with suitable legumes (Vignolio *et al.* 2002).

Cynodon species have been classed as noxious weeds, especially *C. dactylon*, which can be very invasive (Fernandez 2003). The same characteristics which make it a suitable species for erosion control and soil stabilisation make it a difficult weed to control in crop fields. Mechanical tillage of weed–infested fields causes fragmentation and dispersal of both stolon and rhizome propagules, thus increasing the infestation (Fernandez 2003).

Cynodon is a small genus with only 10 species (Clayton *et al.* 2007), the majority of which originated from Africa. However, several species are now widespread in warmer climates with sufficient rainfall, owing to their weedy and invasive nature and usefulness as both forage and turf grass (Clayton *et al.* 2007). There is considerable morphological similarity among the different species and the presence of diploids, tetraploids, hexaploids and sterile hybrids between cytotypes and between species confuses the taxonomy further. In older literature, references made to *Cynodon dactylon* often refer to material which would now be classified as *C. nlemfuensis* or *C. aethiopicus* W. D. Clayt. & Harlan (Clayton and Harlan 1970).

The International Livestock Research Institute (ILRI) holds a germplasm collection of *Cynodon* species in trust under the International Treaty on Plant Genetic Resources for Food and Agriculture. Many of the accessions in the ILRI collection were donated by Henderson Research Station, Zimbabwe, including both accessions collected from natural habitats in the 1960s and 1970s and a large number of accessions originating from their selection programme aimed at improving productivity under grazing. Some commercial cultivars and accessions collected by ILRI from wild habitats in the 1980s are also represented in the collection. The collection covers 4 different species (*C. aethiopicus*, *C. dactylon*, *C. incompletus* and *C. nlemfuensis*) and hybrids between *C. dactylon* and *C. transvaalensis*.

For a germplasm collection to be useful it is important that the characteristics of the accessions and the amount of variation present in the collection are described, so that users can select the most appropriate accessions for further evaluation and use in specific farming systems. The purpose of this study was to characterise the variation available in the *Cynodon* collection held at ILRI and to cluster accessions with similar characteristics, as well as to compare the diversity found in breeding lines, which make up a substantial part of the collection, with the diversity found in accessions collected from natural habitats.

Material and methods

The study was carried out at the ILRI Zwai seed multiplication site in the Rift Valley of Ethiopia

(7° 54'N, 38° 44'E; elevation 1640 masl). The site has an annual average rainfall of 600 mm of which the majority falls during the main rainy season (July – September). The soil at the Zwai site is loamy sand and has been classified as a vitric andosol (King and Birchall 1975) with a pH(H₂O) of 8.1.

The study was carried out on 98 accessions of *Cynodon* species, which included cultivars and accessions collected from wild habitats (Table 1) and accessions selected from siblings of open-pollinated accessions (Table 2). ILRI numbers 16698–16781 were donated by Henderson Research Station, Zimbabwe. The taxonomic identification up to species level was checked on all accessions using keys from de Wet and Harlan (1970) and Phillips (1995).

All accessions were established from root splits in 2 m x 5 m plots. The plots were fertilised with 200 kg N/ha/yr (as ammonium sulphate) in 6 split applications. Additionally, 60 kg P/ha/year (as triple superphosphate, TSP) was applied once a year. The plants were cut at a height of 5 – 15 cm just before the start of the main rainy season. Data were recorded on 24 characters (Table 3). These characters were selected for their agronomic relevance and expected variation among accessions. The morphological characters were observed within the first 4 months after cutting, before most accessions had started flowering (Table 3). Eleven quantitative characters were observed on 10 tillers per accession as suggested by van de Wouw *et al.* (1999b). Owing to the rhizomatous and/or stoloniferous nature of the *Cynodon* plants, it was difficult to identify individual plants, so observations were taken randomly within the plot. Two randomly selected areas of 25 cm x 25 cm in the plot were chosen for rhizome observations and a single area of 200 cm x 50 cm at the end of the plot for stolon observations. Plants were removed from these areas at the time of cutting and after 7 weeks the numbers of rhizomes or stolons in the selected areas were counted.

Observations on the number of days to flowering were continued until 6 months after cutting. For accessions which had not reached 50% flowering at that time, the number of days to 50% flowering in the analysis was set at 210 days. The numbers of caryopses present in 2 samples of 100 spikelets were counted for each accession to test for sterility.

The correlations among the observed characters were calculated using Pearson's correlation coefficient. Characters that showed a high correlation coefficient (≥ 0.7) were reviewed, and if the correlation could be explained by an overlap in measurements, one character was excluded to avoid indirect weighting in the cluster analysis. After standardising the variables to a mean of 0 and a variance of 1, a principal component analysis was carried out. Hierarchical clusters were formed using the average and complete linkage algorithms of the NTSYS 2.01 programme. A first analysis including all accessions was completed, after which a second analysis excluding outliers was carried out, as outliers tend to distort clustering. The number of clusters was determined by

looking at breaks in the level of similarity among the clusters and by looking at similarities in clusters obtained by the 2 clustering algorithms. The Kruskal–Wallis test was used to test for significant differences of characters among the clusters. The full dataset was explored using a discriminant analysis in SYSTAT 8.0 to find the characters which best differentiate the resulting clusters of accessions.

Results

Taxonomic identification of the species within the genus, especially *C. nlemfuensis* and *C. dactylon*, was difficult owing to uniform spikelet structure

Table 1. List of cultivars and accessions collected from wild habitats used in the characterisation study.

| ILRI number | Species | Cultivar | Country of collection | Other numbers ¹ |
|-------------|--|--------------|------------------------------|----------------------------|
| 1117 | <i>Cynodon nlemfuensis</i> | | Democratic Republic of Congo | |
| 1135 | <i>Cynodon aethiopicus</i> | | Democratic Republic of Congo | |
| 2006 | <i>Cynodon aethiopicus</i> | | Ethiopia | |
| 6624 | <i>Cynodon aethiopicus</i> | | Ethiopia | |
| 13300 | <i>Cynodon nlemfuensis</i> | | Kenya | CIAT16681 |
| 13318 | <i>Cynodon dactylon</i> | | commercial source-Australia | |
| 13828 | <i>Cynodon dactylon x transvaalensis</i> | Tifgreen II | | |
| 13829 | <i>Cynodon dactylon x transvaalensis</i> | Tifway II | | |
| 13831 | <i>Cynodon dactylon</i> | Coastcross I | | |
| 15014 | <i>Cynodon dactylon</i> | | commercial source—Singapore | |
| 15402 | <i>Cynodon dactylon</i> | | Ethiopia | |
| 15725 | <i>Cynodon dactylon</i> | | Chad | |
| 16601 | <i>Cynodon dactylon</i> | | Namibia | |
| 16698 | <i>Cynodon nlemfuensis</i> | No.2 Star | | G171 |
| 16700 | <i>Cynodon dactylon</i> | Ngorongoro | Tanzania | G343 |
| 16701 | <i>Cynodon aethiopicus</i> | | | G659 |
| 16702 | <i>Cynodon dactylon</i> | | South Africa | G1256 |
| 16703 | <i>Cynodon dactylon</i> | | South Africa | G1260 |
| 16704 | <i>Cynodon dactylon</i> | | South Africa | G1263 |
| 16705 | <i>Cynodon nlemfuensis</i> | | Zimbabwe | G1264 |
| 16706 | <i>Cynodon nlemfuensis</i> | | Zimbabwe | G1265 |
| 16707 | <i>Cynodon nlemfuensis</i> | | Zimbabwe | G1270 |
| 16708 | <i>Cynodon nlemfuensis</i> | | Zimbabwe | G1272 |
| 16709 | <i>Cynodon nlemfuensis</i> | | Zimbabwe | G1273 |
| 16710 | <i>Cynodon nlemfuensis</i> | | Zimbabwe | G1275 |
| 16711 | <i>Cynodon nlemfuensis</i> | | Zimbabwe | G1280 |
| 16712 | <i>Cynodon nlemfuensis</i> | | Zimbabwe | G1282 |
| 16713 | <i>Cynodon nlemfuensis</i> | | Zimbabwe | G1284 |
| 16714 | <i>Cynodon dactylon</i> | | Zimbabwe | G1285 |
| 16715 | <i>Cynodon nlemfuensis</i> | | Zimbabwe | G1286 |
| 16716 | <i>Cynodon nlemfuensis</i> | | Zimbabwe | G1292 |
| 16717 | <i>Cynodon nlemfuensis</i> | | Zimbabwe | G1293 |
| 16718 | <i>Cynodon nlemfuensis</i> | | Malawi | G1435 |
| 16719 | <i>Cynodon nlemfuensis</i> | | Malawi | G1436 |
| 16720 | <i>Cynodon nlemfuensis</i> | | Malawi | G1437 |
| 16721 | <i>Cynodon dactylon</i> | | Zimbabwe | G1452 |
| 16722 | <i>Cynodon dactylon</i> | | Malawi | G1460 |
| 16723 | <i>Cynodon nlemfuensis</i> | | Zimbabwe | G1463 |
| 16724 | <i>Cynodon nlemfuensis</i> | | Zimbabwe | G1464 |
| 16725 | <i>Cynodon nlemfuensis</i> | | Zimbabwe | G1465 |
| 16726 | <i>Cynodon dactylon</i> | | South Africa | G1467 |
| 16727 | <i>Cynodon nlemfuensis</i> | | Zimbabwe | G1476 |
| 16728 | <i>Cynodon nlemfuensis</i> | | Uganda | G1477 |
| 18294 | <i>Cynodon incompletus</i> | | Lesotho | |
| 18530 | <i>Cynodon nlemfuensis</i> | | Tanzania | |

¹ CIAT = Centro Internacional de Agricultura Tropical, Colombia; G = Henderson Research Station, Zimbabwe.

Table 2. Accessions selected by the breeding programme at Henderson Research Station and used in the characterisation study.

| ILRI No | Species | Henderson No | ILRI No | Species | Henderson No |
|-----------------------|----------------------------|--------------|---------|----------------------------|--------------|
| Progeny of ILRI 16712 | | | | | |
| 16729 | <i>Cynodon nlemfuensis</i> | G1478 | 16750 | <i>Cynodon nlemfuensis</i> | G1499 |
| 16730 | <i>Cynodon nlemfuensis</i> | G1479 | 16751 | <i>Cynodon nlemfuensis</i> | G1600 |
| 16731 | <i>Cynodon nlemfuensis</i> | G1480 | 16752 | <i>Cynodon nlemfuensis</i> | G1601 |
| 16732 | <i>Cynodon nlemfuensis</i> | G1481 | 16753 | <i>Cynodon nlemfuensis</i> | G1602 |
| 16733 | <i>Cynodon nlemfuensis</i> | G1482 | 16754 | <i>Cynodon nlemfuensis</i> | G1603 |
| 16734 | <i>Cynodon nlemfuensis</i> | G1483 | 16755 | <i>Cynodon nlemfuensis</i> | G1604 |
| 16735 | <i>Cynodon nlemfuensis</i> | G1484 | 16756 | <i>Cynodon nlemfuensis</i> | G1605 |
| 16736 | <i>Cynodon nlemfuensis</i> | G1485 | 16757 | <i>Cynodon nlemfuensis</i> | G1606 |
| 16737 | <i>Cynodon nlemfuensis</i> | G1486 | 16758 | <i>Cynodon nlemfuensis</i> | G1607 |
| 16738 | <i>Cynodon nlemfuensis</i> | G1487 | 16759 | <i>Cynodon nlemfuensis</i> | G1608 |
| 16739 | <i>Cynodon dactylon</i> | G1488 | 16760 | <i>Cynodon nlemfuensis</i> | G1609 |
| 16740 | <i>Cynodon nlemfuensis</i> | G1489 | 16761 | <i>Cynodon nlemfuensis</i> | G1610 |
| 16741 | <i>Cynodon nlemfuensis</i> | G1490 | 16762 | <i>Cynodon nlemfuensis</i> | G1611 |
| 16742 | <i>Cynodon nlemfuensis</i> | G1491 | 16763 | <i>Cynodon nlemfuensis</i> | G1612 |
| 16743 | <i>Cynodon nlemfuensis</i> | G1492 | 16764 | <i>Cynodon nlemfuensis</i> | G1613 |
| 16744 | <i>Cynodon nlemfuensis</i> | G1493 | 16765 | <i>Cynodon nlemfuensis</i> | G1614 |
| 16745 | <i>Cynodon nlemfuensis</i> | G1494 | 16766 | <i>Cynodon nlemfuensis</i> | G1615 |
| 16746 | <i>Cynodon nlemfuensis</i> | G1495 | 16767 | <i>Cynodon nlemfuensis</i> | G1616 |
| 16747 | <i>Cynodon nlemfuensis</i> | G1496 | 16768 | <i>Cynodon nlemfuensis</i> | G1617 |
| 16748 | <i>Cynodon nlemfuensis</i> | G1497 | 16769 | <i>Cynodon nlemfuensis</i> | G1618 |
| 16749 | <i>Cynodon nlemfuensis</i> | G1498 | | | |
| Progeny of ILRI 16708 | | | | | |
| 16770 | <i>Cynodon dactylon</i> | G1619 | | | |
| Progeny of ILRI 16710 | | | | | |
| 16771 | <i>Cynodon nlemfuensis</i> | G1620 | 16773 | <i>Cynodon nlemfuensis</i> | G1622 |
| 16772 | <i>Cynodon nlemfuensis</i> | G1621 | | | |
| Progeny of ILRI 16723 | | | | | |
| 16774 | <i>Cynodon nlemfuensis</i> | G1623 | 16778 | <i>Cynodon nlemfuensis</i> | G1627 |
| 16775 | <i>Cynodon nlemfuensis</i> | G1624 | 16779 | <i>Cynodon dactylon</i> | G1628 |
| 16776 | <i>Cynodon nlemfuensis</i> | G1625 | | | |
| 16777 | <i>Cynodon nlemfuensis</i> | G1626 | | | |
| Progeny of ILRI 16724 | | | | | |
| 16780 | <i>Cynodon nlemfuensis</i> | G1629 | | | |
| Progeny of ILRI 16725 | | | | | |
| 16781 | <i>Cynodon nlemfuensis</i> | G1630 | | | |

and similarity in growth habit. Identification of the *C. nlemfuensis* accessions up to the variety level also proved problematic, as many intermediates exist. Using the criteria for stem thickness and length of racemes as indicated in Phillips (1995), the majority of the *C. nlemfuensis* accessions in the current study would be classified as var. *robustus*.

The accessions showed large variation in many of the characters observed. All accessions had stolons, with considerable variation in the number and length of stolons formed. The longest stolon measured was over 2 m, while the number of stolons varied from 1 to 14.

The number of racemes was highly correlated with the numbers of whorls in the inflo-

rescence (Pearson = 0.93) and as the number of racemes is easier to observe and therefore more reliable, the number of whorls was eliminated from the analysis to avoid indirect weighting. A high correlation (Pearson = 0.79) was found between the densities of abaxial and adaxial hairs on the leaf, so only the density of adaxial hairs was used in the analysis. A high correlation was found between days to first flowering and days to 50% flowering (Pearson = 0.75). Therefore, only days to 50% flowering was used in the analysis, together with flowering duration (days to 50% flowering – days to first flowering). The observations on seed-set were not used in the multivariate analysis, as there were some missing observations. In addition, seed-set in *Cynodon*

Table 3. List and definition of characters used in the study.

| | Character | Definition | No of observations | Units |
|-----------------------|---------------------------------------|--|--------------------|-------|
| <i>General</i> | | | | |
| 1 | Plant height | Height from ground to flag leaf | 10 | cm |
| 2 | Growth habit | Angle of stem to ground, (1 = prostrate to 5 = erect) | Estimate | |
| 3 | Rhizome number | Average number of rhizomes found in two cleared areas of 25 x 25 cm of the experimental plots, 7 weeks after cutting | 2 | |
| 4 | Stolon number | Number of stolons found in a cleared area of 50 x 200 cm at edge of the experimental plot, 7 weeks after cutting | 1 | |
| 5 | Stolon length | Average length of the stolons | Max 10 | cm |
| 6 | Leafiness | Leaf:stem ratio (0 to 10) | Estimate | |
| 7 | Days to first flowering ¹ | Number of days from cutting until first flower emerges | | |
| 8 | Days to 50% flowering | Number of days from cutting until 50% of plot is flowering | | |
| 9 | Flowering duration | Number of days from first flowering to 50% flowering | | |
| <i>Stem</i> | | | | |
| 10 | Number of nodes | Number of nodes from ground to inflorescence base | 10 | |
| 11 | Culm thickness | Diameter of culm at lowest internode | 10 | mm |
| 12 | Culm hardness | Hardness of the culm (1 = soft to 3 = woody) | Estimate | |
| <i>Leaf</i> | | | | |
| 13 | Leaf length | Length of third leaf below flag leaf from ligule to tip of leaf | 10 | cm |
| 14 | Leaf width | Width of third leaf below flag leaf at widest point | 10 | cm |
| 15 | Leaf ratio | Leaf length : leaf width | 10 | |
| 16 | Leaf hairiness – adaxial | Hairiness of adaxial side of leaf (1 = glabrous to 6 = dense hairs) | Estimate | |
| 17 | Leaf hairiness – abaxial ¹ | Hairiness of abaxial side of leaf (1 = glabrous to 6 = dense hairs) | Estimate | |
| 18 | Leaf nature | Character of leaf (1 = soft to 3 = stiff) | Estimate | |
| <i>Inflorescences</i> | | | | |
| 19 | Number of racemes | Number of racemes per inflorescence | 10 | |
| 20 | Number of whorls ¹ | Number of whorls per inflorescence | 10 | |
| 21 | Raceme length | Length of the longest raceme in the inflorescence | 10 | cm |
| 22 | Spikelet length | Length of spikelet | 10 | cm |
| 23 | Spikelet colour | Degree of purpleness of the spikelet (1 = almost entire spikelet purple to 7 = almost entire spikelet pale green) | | |
| 24 | Seed-set ¹ | Percentage of fertile spikelets at harvest stage | 2 x 100 spikelets | |

¹ Characters excluded from final analysis.

is highly dependent on environmental effects and shows highly significant variation among years (Wu *et al.* 2006), making it a less suitable character for use as a stable trait in characterisation of germplasm. After excluding characters with high correlations and missing values, 19 characters were left for further analysis (Table 3).

In the first run of the analysis several accessions were found to be outliers from the rest of the accessions (Figure 1). ILRI 13829 (cv. Tifway II) was the most different from all the other accessions (Table 4). It is a hybrid between *Cynodon dactylon* and *Cynodon transvaalensis*, and is very short, with few stolons but many rhizomes. Two other groups of outliers could be dis-

tinguished; the *C. aethopicus* accessions and a small group of mixed taxa, consisting of smaller turf type grasses. This latter group included *C. incompletus*, a *C. dactylon* x *C. transvaalensis* hybrid, *C. dactylon* and *C. nlemfuensis*. The last 3 are most likely accessions resulting from plant improvement programmes. Two are cultivars, while the other was collected from an area in the Democratic Republic of Congo which collectors considered had most probably been sown to pasture.

The Kruskal–Wallis values showed that leaf hairiness, leaf ratio, spikelet length and flowering duration did not vary significantly ($P > 0.01$) among these groups. In the discriminant anal-

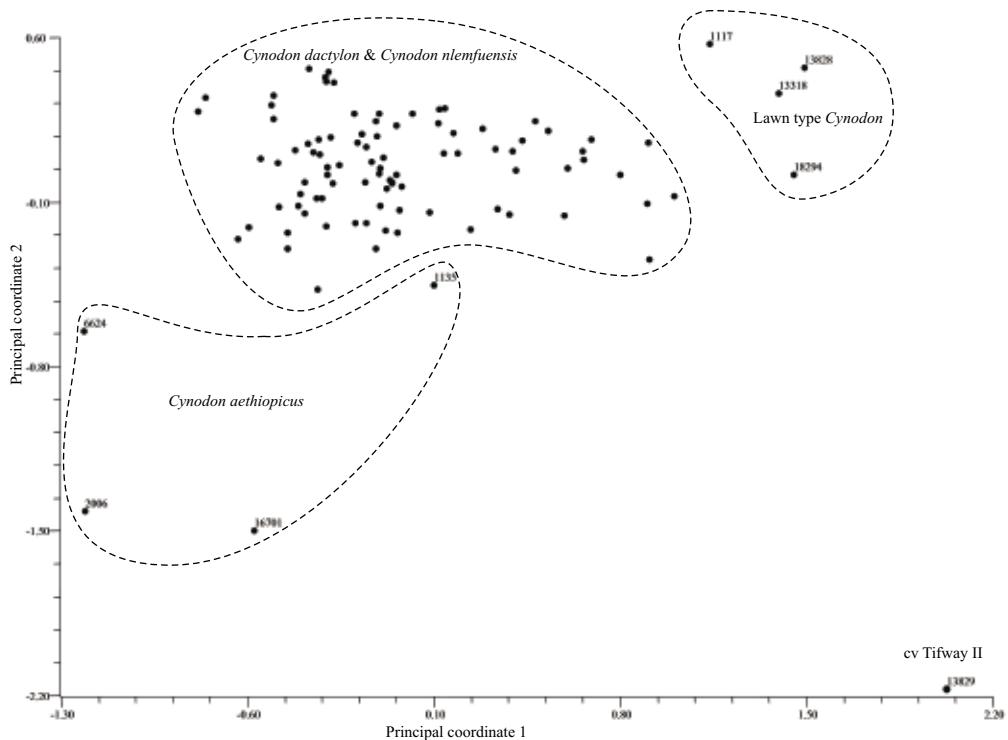


Figure 1. Scatter diagram of the first 2 principal coordinates accounting for, respectively, 30.6 and 14.5% of the variation obtained by agro-morphological characterisation of 98 *Cynodon* accessions.

ysis, number of rhizomes appeared to be the most important factor in discriminating among these 4 groups. Days to 50% flowering, length and number of racemes, number of stolons, number of nodes and the leaf measurements also contributed to separation of these 4 groups. The *C. aethiopicus* accessions contained the tallest plants, while ILRI 13829 and the group of turf type accessions were very short plants (Table 4).

As outliers tend to distort the principal coordinates and cluster analysis, these 9 accessions were excluded and 89 accessions of *C. dactylon* and *C. nlemfuensis* were used for further analysis. The principal coordinate analysis of these accessions showed some indication of grouping and no obvious outliers, with the first 2 coordinates accounting for, respectively, 23.7 and 15.2% of the variation. Cluster analysis using the com-

Table 4. Mean (and range) of characters of *Cynodon* species showing the differences between the outliers and the main group of accessions.

| | <i>C. aethiopicus</i> | ILRI 13829 | Turf type <i>Cynodon</i> | Others |
|-----------------------|-----------------------|------------|--------------------------|------------------|
| No of accessions | 4 | 1 | 4 | 89 |
| Plant height (cm) | 60.4 (36.1-93.8) | 8.1 | 12.6 (7.3-16.2) | 39.1 (19.2-76.8) |
| Stem thickness (mm) | 2.8 (2.3-3.5) | 0.8 | 1.2 (1.2-1.3) | 2.1 (0.8-3.0) |
| Number of nodes | 13.3 (11-15) | 10.2 | 7.4 (6.6-8.4) | 9.8 (5.8-13.7) |
| Number of stolons | 2.8 (2-4) | 1 | 9.3 (7-14) | 4.2 (1-11) |
| Number of rhizomes | 0 | 11.5 | 0.8 (0-1.5) | 0.1 (0-2.5) |
| Leaf length (cm) | 10.0 (8.0-12.1) | 2 | 3.9 (2.0-5.2) | 9.2 (3.7-17.2) |
| Leaf width (mm) | 5.8 (5.4-6.0) | 2.0 | 3.4 (3.0-3.7) | 6.3 (2.5-8.0) |
| Days to 50% flowering | 148 (96-210) | 210 | 36 (20-64) | 53.2 (27-117) |
| Raceme length (cm) | 7.3 (6.0-8.5) | 2.4 | 4.1 (2.6-5.2) | 7.8 (4-10.2) |
| Raceme number | 11.5 (9.0-14.7) | 3.0 | 4.7 (3.9-6.3) | 7.0 (4.3-12.3) |

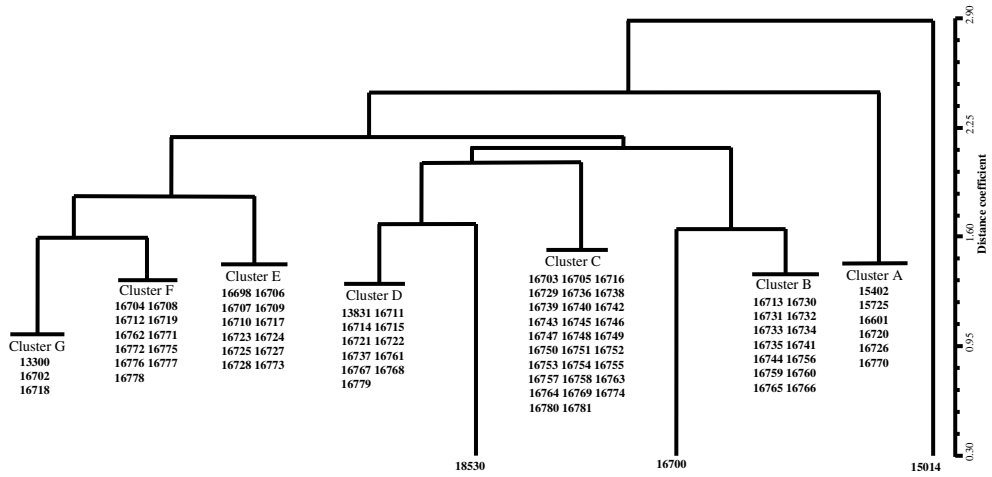


Figure 2. Cluster diagram of 89 accessions of *Cynodon dactylon* and *C. nlemfuensis* obtained with the complete linkage clustering algorithm by clustering on 19 characters. Numbers are ILRI accession numbers.

Table 5. Distance coefficients among accessions with a distance coefficient < 0.5.

| ILRI Nos | Distance coefficient (range) |
|-------------------|------------------------------|
| 16723 16725 | 0.30 |
| 16745 16747 | 0.45 |
| 16751 16755 | 0.49 |
| 16757 16758 16774 | 0.40 – 0.47 |

plete linkage algorithm revealed 7 groups (Figure 2) with 3 accessions not assigned to clusters. These groups could also be recognised using the average linkage algorithm with some small variation whereby a few accessions are assigned to other clusters and the groups joined in different ways. The 3 unassigned accessions in the complete linkage clustering formed outliers, when clustering was done using the average linkage algorithm. There was no clear separation in the clustering between *C. dactylon* and *C. nlemfuensis* and also no clear link among the accessions belonging to the same half-sib families from the Henderson selection programme. Several accessions were found to be very similar, with a distance coefficient < 0.5 (Table 5).

Kruskal–Wallis values showed that only number of stolons and days to first flowering did not differ significantly among groups; all other characters differed significantly among the clusters ($P < 0.01$). Length of leaf, spikelet colour and

days to 50% flowering contributed most to the separation of the clusters as shown by the discriminant analysis. For all characters, some overlap among clusters existed (Table 6), although for most characters a few clusters were very distinct.

The 3 accessions which were not assigned to any cluster, were clearly different from the other accessions in at least 1 character. ILRI 15014 differed from the other accessions because of its high number of rhizomes (2.5) and the shape of its leaves, which were narrow and relatively long. ILRI 16700 was taller (77 cm) than any other plants in the clusters, while ILRI 18530 took 117 days to reach 50% flowering, which was 40 days longer than the other late-flowering accessions.

The observations on seed-set were analysed separately and not used in the multivariate analysis owing to missing observations. The highest caryopsis count was found for a *C. aethiopicus* accession (ILRI 16701), with an average of 62.5% seed-set. However, high fertility is apparently not common in *C. aethiopicus* and no caryopses were found in ILRI 6624. There also appeared to be no clear link between the fertility of the accessions and the clusters to which they were assigned (Table 7). No correlations were found between country of origin and fertility.

Table 6. Mean and range of characters showing differences among clusters of *Cynodon* species.

| Cluster | | A | B | C | D | E | F | G |
|---------------------------------------|------|------|------|------|------|------|------|------|
| No of accessions | | 6 | 14 | 29 | 11 | 12 | 11 | 3 |
| Plant height (cm) | Min | 25.6 | 37.0 | 31.6 | 27.8 | 19.2 | 25.0 | 27.8 |
| | Max | 36.4 | 61.9 | 62.6 | 42.8 | 51.7 | 41.6 | 31.2 |
| | Mean | 32.0 | 48.9 | 44.4 | 33.9 | 30.8 | 30.8 | 29.8 |
| Number of nodes | Min | 9.9 | 5.8 | 7.0 | 7.8 | 7.4 | 8.4 | 6.6 |
| | Max | 13.7 | 9.8 | 13.5 | 11.0 | 11.0 | 10.9 | 8.6 |
| | Mean | 11.6 | 7.9 | 11.1 | 9.0 | 9.2 | 9.9 | 7.8 |
| Culm thickness (mm) | Min | 0.75 | 1.64 | 1.84 | 1.33 | 2.30 | 1.53 | 2.04 |
| | Max | 1.57 | 2.50 | 2.92 | 2.23 | 2.95 | 2.26 | 2.23 |
| | Mean | 1.17 | 2.07 | 2.42 | 1.84 | 2.60 | 1.87 | 2.11 |
| Culm hardness ¹ | Min | 1 | 2 | 1 | 1 | 1 | 1 | 1.5 |
| | Max | 1 | 2 | 3 | 2 | 2 | 2 | 3 |
| | Mean | 1.0 | 2.0 | 2.3 | 1.8 | 1.9 | 1.3 | 2.2 |
| Leaf length (cm) | Min | 3.7 | 10.0 | 5.8 | 7.8 | 8.2 | 4.6 | 7.4 |
| | Max | 5.6 | 17.2 | 10.9 | 15.0 | 12.9 | 9.0 | 10.8 |
| | Mean | 4.9 | 13.7 | 8.2 | 10.3 | 10.1 | 6.8 | 8.8 |
| Leaf hairiness ² (adaxial) | Min | 2.0 | 2.0 | 2.0 | 4.0 | 1.0 | 2.0 | 1.0 |
| | Max | 6.0 | 6.0 | 6.0 | 6.0 | 3.0 | 5.0 | 4.0 |
| | Mean | 4.7 | 3.6 | 4.5 | 4.8 | 2.1 | 3.0 | 2.3 |
| Stolon length (cm) | Min | 32 | 42 | 47 | 31 | 44 | 22 | 69 |
| | Max | 48 | 169 | 119 | 104 | 130 | 82 | 129 |
| | Mean | 41 | 99 | 76 | 58 | 81 | 63 | 98 |
| Stolon number ³ | Min | 3 | 1 | 2 | 1 | 1 | 2 | 1 |
| | Max | 11 | 7 | 10 | 8 | 7 | 6 | 3 |
| | Mean | 6.3 | 3.4 | 3.9 | 5 | 4.3 | 3.8 | 2 |
| Days to 50% flowering | Min | 47 | 44 | 44 | 45 | 61 | 27 | 27 |
| | Max | 77 | 61 | 62 | 62 | 69 | 75 | 33 |
| | Mean | 60 | 49 | 50 | 53 | 64 | 50 | 29 |
| Length of racemes (cm) | Min | 4.0 | 7.2 | 6.3 | 7.6 | 7.0 | 6.2 | 7.0 |
| | Max | 6.9 | 10.2 | 9.2 | 9.9 | 9.6 | 8.8 | 7.5 |
| | Mean | 5.4 | 8.5 | 7.8 | 8.7 | 8.7 | 7.1 | 7.2 |
| Number of racemes | Min | 4.3 | 5.7 | 5.5 | 4.7 | 8.3 | 5.3 | 6.8 |
| | Max | 5.3 | 7.6 | 10.3 | 8.9 | 12.3 | 7.8 | 7.8 |
| | Mean | 4.8 | 6.8 | 7.0 | 6.1 | 9.9 | 6.2 | 7.2 |
| Spikelet colour ⁴ | Min | 4 | 3 | 3 | 1 | 1 | 1 | 1 |
| | Max | 6 | 6 | 6 | 4 | 4 | 6 | 3 |
| | Mean | 4.5 | 4.2 | 4.4 | 3.27 | 1.3 | 4.3 | 2.0 |

¹1 = soft to 3 = woody. ²1 = glabrous to 6 = dense hairs.

³No significant differences ($P > 0.01$) among clusters according to Kruskal-Wallis test.

⁴1 = almost entire spikelet purple to 7 = almost entire spikelet pale green.

Table 7. Fertility of accessions in clusters as expressed by percentage of caryopses present in 2 samples of 100 spikelets.

| Cluster | | A | B | C | D | E | F | G |
|------------------|------|-----|-----|------|------|------|------|-----|
| No of accessions | | 6 | 14 | 28 | 11 | 12 | 11 | 3 |
| Caryopses (%) | Min | 0 | 0 | 0 | 0 | 0.5 | 0 | 0 |
| | Max | 4.5 | 27 | 17.5 | 12.0 | 11.5 | 27.0 | 6.5 |
| | Mean | 0.8 | 9.0 | 5.0 | 3.8 | 5.0 | 9.0 | 2.7 |

Discussion

Taxonomic diversity

The close taxonomic relationship between *C. dactylon* and *C. nlemfuensis* and the similarity in morphology made it difficult to separate these species using agro-morphological traits. Neither principal coordinate analysis nor clustering in this study allowed clear separation between the 2 species. It is likely that hybrids or intermediates between these species exist in the collection studied, blurring the boundary between the species even further. Most of the characters examined showed continuous variation between *C. dactylon* and *C. nlemfuensis*. The presence of rhizomes in *C. dactylon* is the only identifying difference between these two species (Phillips 1995), but even this character shows wide variation within *C. dactylon* with very few rhizomes formed in some cases. *C. dactylon* does not form rhizomes under shaded conditions (Dong and de Kroon 1994), which makes a reliable identification of this species even more difficult. There was also large variation in length and number of stolons, which might be due to the very plastic response of stolons to light intensity and nutrient availability. Stolons can be up to 3 times longer under low light intensity (Dong and de Kroon 1994). Since stolons are usually flat on the ground they can easily get buried and are then indistinguishable from rhizomes, because the morphology of stolons which start to grow underground changes into rhizome morphology and vice versa (Dong and de Kroon 1994).

C. aethiopicus is easier to distinguish morphologically from *C. dactylon* and *C. nlemfuensis*, as has been shown in this study. Although experiments have shown that *C. aethiopicus* can hybridise with either *C. dactylon* or *C. nlemfuensis*, this species seems to be comparatively well isolated from the other species in the genus (Harlan *et al.* 1969). However, naturally occurring intermediates between *C. nlemfuensis* and *C. aethiopicus* have been reported in areas where they cohabit (Harlan *et al.* 1969). In the current study, one accession of *C. aethiopicus* (ILRI 1135) was relatively small and could be an intermediate hybrid accession. Morphological differences between the species in the genus are

often not large enough to reliably identify hybrid accessions.

C. aethiopicus was the most robust species in the study, and had thick stems. Although thick stems are a less desirable character for use as forage, *C. aethiopicus* accessions were also late-flowering and therefore remained leafy for longer, giving more flexibility in time of hay making or grazing frequency. Within the *C. dactylon*—*C. nlemfuensis* group of accessions the tallest accessions were found in clusters B and C, but they flowered considerably earlier than the *C. aethiopicus* accessions.

Given that taxonomic identification using plant morphology is difficult, DNA fingerprinting techniques are now being applied to species classification. However, DNA amplification fingerprinting (DAF) failed to provide clear separation among *C. dactylon* and several of the other species in the genus (Assefa *et al.* 1999), although the technique could clearly differentiate among accessions. Modern molecular tools could be applied to verify the taxonomy based on morphology and inter-specific hybrids in *Cynodon*.

Intra-specific diversity

The collection in this study originated mostly in Africa, the region with the most genetic variation in *C. dactylon* (Wu *et al.* 2004). This was confirmed by the larger range in leaf sizes and plant heights found in this study than in a study with Chinese *Cynodon* accessions (Wu *et al.* 2007). However, to fully cover the available variation, germplasm from all major geographic regions where the species occur should be collected (Wu *et al.* 2004). Accessions from Australia and south-east Asia would be a useful addition to the current germplasm.

Considerable diversity in leaf length, plant height, time of flowering and spikelet colour was observed within the large group of accessions of *C. dactylon* and *C. nlemfuensis*. Accessions selected from the offspring of accessions in a selection programme at Henderson Research Station proved to be very variable, being allocated to almost all groups in the analysis and in some cases taxonomically assigned to different species from the parent plant. However, the majority of these accessions clustered in groups B and C, which contained tall accessions, while accessions from cluster B also had long leaves. Only cluster

G did not contain accessions from the selection programme at Henderson. The open-pollinated offspring of a very limited number of fertile accessions produced almost as much variation as the accessions collected from natural habitats over a wide range of African countries.

Some accessions in this study have been in germplasm collections for 30–50 years and have been transferred as clonal material among institutes and countries. Germplasm exchange can lead to identical material with poor passport data entering the collection as different accessions via different routes. Many of the *Cynodon* accessions produce little or no seed, so seed storage has limited usefulness for conservation of diversity within the genus. Maintenance of the collection in a field genebank is expensive and the identification of duplicate accessions is especially important to reduce redundancy and improve efficiency and cost-effectiveness of conservation of the collection. In this study some accessions clustered closely together and were found to be morphologically very similar. Use of DNA fingerprinting techniques could confirm if these accessions are in fact duplicates.

Maintaining the integrity of a field genebank of *Cynodon* is not easy. Owing to the invasive and weedy nature of some members of the genus, weedy *Cynodon* can easily invade plots containing the original accessions and become dominant. DNA fingerprinting techniques could assist in identifying to what extent this has occurred during the maintenance history of the collection.

Agronomic potential of accessions

Characterisation of the collection resulted in identification of clusters of accessions with potential for further use in breeding programmes. Agronomically, *C. dactylon* and *C. nlemfuensis* are similar and would fit equally well into different agricultural systems.

A sterile cultivar of *Cynodon nlemfuensis* ‘No 2 Star’, ILRI 16698, originally considered to be *C. aethiopicus*, is used extensively in Zimbabwe as a pasture grass for beef production in the higher rainfall areas (Mills and Boultonwood 1981), since being introduced in 1922. It belonged to cluster E in the current study, which was characterised by late-flowering accessions with very thick stems and was dominated by accessions collected from Zimbabwe. Several of the acces-

sions collected from Zimbabwe may in fact be clones of No 2 Star owing to the widespread use of No 2 Star in Zimbabwe.

Subsequent work at Henderson Research Station in Zimbabwe showed that other accessions out-yielded this cultivar (Mills and Boultonwood 1981). Three of the highest yielding accessions in the trial at Henderson Research Station were found in cluster D. This cluster contained accessions of a similar height to those in cluster E but with considerably thinner stems. Although these productive accessions have potential for hay making, the thinner stems might make them prone to lodging.

Although rhizomes are useful for maintaining a closed sward, aggressively rhizomatous accessions can be invasive and have potential to become weeds. *C. dactylon* contamination of farmlands in South Africa is an increasing problem, mainly through an aggressive strain of *Cynodon* introduced to control soil erosion (Roodt *et al.* 2002). On the positive side, there were large differences in the rhizomatous nature of the accessions with some accessions of *C. dactylon* producing only few rhizomes or no rhizomes after 7 weeks, offering scope for selection of accessions with a less aggressively rhizomatous behaviour. ILRI 15014, which was not assigned to any cluster, produced the most rhizomes within the *C. dactylon*—*C. nlemfuensis* group of accessions.

A small cluster of 4 turf grass type accessions was identified, including 2 turf grass cultivars and 2 collections. One accession of *C. incompletus* was included in the cluster, indicating that this species might have potential for use in breeding programmes for amenity grasses. Amenity and turf grasses are in high demand and in many countries have a higher value than pasture grasses. *Cynodon* has become the preferred turf grass species in warm areas over recent years owing to its morphology and drought tolerance.

Many *Cynodon* cultivars are sterile and are planted and spread by vegetative cuttings. For these cultivars, a late flowering date would be an advantage, as this means that the sward would remain in a leafy, more palatable stage for longer. However, seeding cultivars do exist and the ease of establishment and transport of these cultivars confers an advantage over sterile cultivars. Fertile accessions were present in all agro-morphological clusters in this study, offering the opportunity to select for accessions with good agronomic

traits plus the potential to be spread by seeds. Only in cluster A did fertile accessions show low seed-set.

Multivariate analysis has proven to be very useful in studying morphological and agronomic variation in forage germplasm collections (Pengelly *et al.* 1992; Heering *et al.* 1996; van de Wouw *et al.* 1999a, 2008). In cases where taxonomic classification is difficult, such as for *Cynodon*, clustering into groups with similar characteristics can greatly assist in the management and future use of the collection. The successful clustering into groups of accessions with similar morphological and agronomic characteristics within the *Cynodon* collection in this study will enable researchers interested in this genus to easily identify subsets of accessions for further evaluation in replicated trials aimed at specific uses. Morphological differences between some accessions and taxonomic differentiation to species were not completely clear and future research could focus on the use of molecular DNA techniques to verify these findings.

Acknowledgements

The authors thank the staff at the Zwai seed multiplication site for assistance with this trial. Financial support was provided by the German Bundesminister für Wirtschaftliche Zusammenarbeit (BMZ) and the World Bank.

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