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Morphological Characterization of *Desmodium dichotomum* Germplasm Collected from Eastern Amhara (Ethiopia)

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Abstract. This study was conducted in 2019–2020 to morphologically characterize and cluster 26 germplasm entries of *Bouffordia dichotoma*, the native legume collected in eastern Amhara (Ethiopia) for eventual selection of promising lines as forage crops. The IBPGR descriptors list were used for characterization of the entries and data were analyzed using R software. Cluster analysis was conducted to understand the genetic diversity within the collection and to group similar entries. The experimental design consisted of completely randomized blocks with three replications for one year. The resulting dendrogram showed 3 distinct groups of entries: Group 1 with 12 entries; Group 2 with 6 entries; and Group 3 with 8 entries. Group 1 was significantly different ($P<0.05$) from Groups 2 and 3 in terms of days to first flower, terminal leaflet length, terminal leaflet petiole length, length of inflorescence and plant height, as well as emergence, establishment, vigor, flowering and maturity. Leaf color of Groups 1 and 2 was light green while that of Group 3 was green. While most entries had semi-erect growth habit, those in Group 3 were prostrate or trailing. Three distinct types of seed coat colors were observed with Group 1 including dark brown, light brown and yellow, while Groups 2 and 3 were yellow only.

Key words: Characterization, Descriptors, Entries, Morphology

Introduction

Bouffordia dichotoma (Willd.) (Ohashi & Ohashi, 2018) [syn. *Desmodium dichotomum* (Willd.) DC], locally called *chimero,* is a native legume recognized by farmers in several districts of North and South Wollo Zones, Amhara Region, Ethiopia, as a valuable livestock feed (Hunegnaw, 2020). It is a herbaceous and self-regenerating legume growing in a wild state and often occurs as spontaneous intercrop with sorghum and maize crops. Its growth habit is semi-erect to trailing; leaves are trifoliolate with the leaflets being mostly ovate. Leaves are hairy on both surfaces, while flowers are pink to violet and seeds are yellow to light brown. While limited research has been conducted on this species, 4,400 kg DM/ha average yield has been reported as a self-grow legume with sorghum (*Sorghum* spp.) (Hunegnaw, 2020). Means of nutritive value components reported are: 22% crude protein, 31% neutral detergent fiber (NDF), 26% acid detergent fiber (ADF) and 5.8% acid detergent lignin (ADL), while *in vitro* dry matter digestibility (DMD) is 61%. Mineral concentrations are: 0.6% calcium, 0.23% phosphorus, 1.5% potassium, 0.78% magnesium, 0.01% sodium, 0.27% sulfur, 0.16% iron, 4.4 mg/kg copper, 45 mg/kg manganese and 12.3 mg/kg zinc (Hunegnaw, 2020). The aforementioned values indicate *Bouffordia Dichotoma* is high in nutritive value

Morphological characterization can help to assess and understand the genetic variability in germplasm collections (Carvalho and Quesenberry, 2008). When working with a wild species of a genus of interest, this is usually the first opportunity to gather basic knowledge about the agronomic attributes of the species. For example, qualitative, highly heritable, morphological traits were used in early scientific investigations of genetic diversity, such as the ones performed by Mendel (1866). The information generated from this type of research can be used to identify individual accessions based on a set of particular phenotypic traits. Additionally, this activity can generate information about the genetic divergence among entries, which then can be used to group entries.

Multivariate analysis is appropriate to discriminate among entries considering multiple variables at the same time. Among the multivariate techniques the most used in genetic studies are Principal Component and Cluster analyses (Hawkes *et al*., 2000). Thus, the objective of this research was to morphologically characterize and cluster the entries of *Bouffordia dichotoma* germplasm collected from eastern Amhara (Ethiopia).

Materials and Methods

Description of experimental site

The study was conducted at the nursery site of Habru woreda agriculture office, Girana kebele, Eastern Amhara Region, Ethiopia, in 2019–2020. The site is located at 582 km northeast of Addis Ababa (11º34' N, 39º43' E; 1,429 masl). The average annual rainfall in the study area is 945 mm and the mean maximum and minimum temperatures are 32 and 13 ºC, respectively.

The presentation of the entries

Seeds from native *Bouffordia dichotoma* populations were collected at 26 sampling sites based on herbarium information in the South Wollo, North Wollo and Oromia Special Administration Zones of Ethiopia (10–12º N, 39–40º E; 1,470–1,890 masl) following the Ethiopian Biodiversity Institute collection format for forage genetic resources conservation. *Bouffordia dichotoma* populations prefer habitats spontaneously under sorghum crops. A list of these collections and their geographic origins is presented in Table 1.

Table 1. Collection sites of the *Bouffordia dichotoma* entries included in the Sorghum Field

PI No.	Latitude N	Longitude E	Elevation (masl)	Woreda
1979	11°57′54″	39°41′36″	1,659	Kobo
1980	11°33′47″	39°40′31″	1,539	Kobo
1981	11°22′29″	39°39′24″	1,496	Kobo
1982	11°50′13″	39°45′01″	1,495	Gubalafto
1983	11°21′34″	39°34′36″	1,500	Gubalafto
1984	11°48′57″	39°35′08″	1,874	Gubalafto
1985	11°40′54″	39°39′34″	1,625	Habru
1986	11°41′26″	39°38′57″	1,652	Habru
1987	11°33′49″	39°39′37″	1,605	Habru
1988	11°44′40″	39°37′43″	1,888	Habru
1989	11°58′75″	39°42′38″	1,716	Habru
1990	11°26′05″	39°39′12″	1,496	Habru
1991	11°27′49″	39°37′11″	1,583	Ambasel
1992	11°45′67″	39°41′30″	1,680	Ambasel
1993	11°40′37″	39°37′36″	1,596	Ambasel
1994	11°44′08″	39°43′36″	1,694	Ambasel
1995	11°24′49″	39°37′01″	1,616	Ambasel
1996	11°22′55″	39°39′36″	1,642	Tehuledere
1997	11°35′66″	39°38′37″	1,632	Tehuledere
1998	11°25′07″	39°32′35″	1,597	Tehuledere
1999	11°42′08″	39°31′32″	1,607	Tehuledere
2000	10°26′09″	39°39′36″	1,460	Kalu
2001	10°53′44″	39°48′23″	1,475	Kalu
2002	10°53′50″	39°48′23″	1,473	Kalu
2003	11°56′36″	39°46′42″	1,537	Dawa chefa
2004	11°48′57″	39°39′36″	1,506	Dawa chefa

PI = Plant Introduction number.

Establishment

Seeds were scarified with/by sand paper at Wollo University Laboratory and planted on a well-prepared seedbed in an irrigated nursery, in single rows. The rows were 4 m long and 1 m apart using a seeding rate of 200 seeds per 4m. The experimental design consisted of completely randomized blocks with three replications for one year. A basal application of NPS fertilizer (18.9:37.7:6.95) was applied at sowing at a rate of 100 kg/ha. Rows were weeded 3 times by hand during the study.

Morphological characterization

Data from leaves, flowers, pods and seeds (both numerical and ordinal attributes) were collected from plants of *B. dichotoma.* Though to draw up a list of meaningful descriptors for a given species is a difficult task, we used a descriptor list for forage legumes prepared by IBPGR (1984) and a list of morphological descriptors selected from the IBPGR/ICRISAT (1990, 1992) for *Arachis pintail* and groundnut. Terminal leaflet length, terminal leaflet width, terminal leaflet petiole length, length of inflorescence, plant height, number of main branches, growth habit, flower color, leaf color, leaf shape and leaf hairiness were measured at (50% flowering) 73 days after sowing. Pod length, pod width, pod color, number of seeds per pod, root depth, seed shape, seed coat color, pod shattering, pod hairiness and root system were measured at seed harvesting time. Days to first flower, emergence, establishment, vigor, disease, flowering and maturity were measured by daily follow up. Details of the descriptors are presented in Table 2. Continuous variables were measured by calipers. The mean, standard deviation and range were calculated for quantitative descriptors and the mode was determined for qualitative ones.

Table 2. Morphological descriptors applied to *Bouffordia dichotoma* entries

Parameter	Unit	Descriptions
Days to first flower	days	Number of days the entries start to emerge first flower
Terminal leaflet length	cm	Measured from leaflet base to leaflet tip and excluding the petiole and any extensions of the venation
Terminal leaflet width	cm	Measured from part of lamina
Terminal leaflet petiole length	cm	Measured from the stalk of a leaf, attaching the lamina
Pod length	cm	Measured from the beginning of stalk to the peak
Pod width	mm	Measured in cutting plane of perpendicular to main axis of the pod, leading through its center
Length of inflorescence	cm	Measured in aggregation of the flowers on the plant
Plant height	cm	Measured in the perpendicular distance from the soil at its base to the highest point reached with all parts in their natural position
No seeds per pod	no.	Measured in the number of seeds in pod from each entries
No main branches	no.	Main branch(branches which are rising from stem) measured in number
Root depth	cm	Measured in digging a trench along the side of the plant at a depth and of a convenient width.
Emergence	scale	Measured in 1-9 scale(1=Very slow, 2=Very slow to slow,3=slow,4=slow to intermediate, 5=Intermediate, 6=Intermediate to fast, 7=Fast,8=Fast to very Fast and 9=Very Fast)
Establishment	scale	Measured in 1-9 scale (1=Very thin, 2=Very thin to thin, 3=Thin, 4=Thin to intermediate, 5=Intermediate, 6=Intermediate to thick,7=Thick,8=Thick to very Thick and 9=Very Thick)
Vigor	scale	Measured in 1-9 scale (1=Very weak,2=Very weak to weak,3=weak,4=weak to intermediate,5=Intermediate,6=Intermediate to vigorous, 7=vigorous, 8=vigorous to very vigorous and 9=Very vigorous)
Flowering /Maturity	scale	Measured in 1-9 scale(1=Very late, 2=Very late to late,3=late,4=late to intermediate, 5=Intermediate, 6=Intermediate to early, 7=early,8=early to very early and 9=Very early)
Flower color	scale	Measured in 1-5 scale (1= White,2= Violet,3= pink,4=pink –Violet and 5=others)
Growth habit	scale	Measured in 1-8 scale (1=Acute erect,2= Erect, 3= Semi erect, 4=Intermediate,5=Semi prostrate,6=Prostrate,7=Climbing and 8=Trailing)
Leaf shape	scale	Measured in 1-4 scale (1=Round,2=Oval,3=Obovate and 4=Obcordate)
Leaf color	scale	Measured in 1-4 scale (1=Pale Green,2=Intermediate green,3=Dark green and 4=Light green)
Seed shape	scale	Measured in 1-5 scale (1=Kidney,2=Ovoid,3 =Crowde, 4=Globose and 5=Rhomboid)
Seed coat color	scale	Measured in 1-4 scale (1=Yellow, 2=Reddish-brown, 3=Brown and 4.Others)
Root system	scale	Measured in 1-2 scale (1=Tap root system and 2=Fibrous root system)
Pod color	scale	Measured in 1-5 scale (1= Pale tan or straw, 2= Dark tan, 3= Dark brown, 4= Black or dark purple and 5= Other)
Disease	Scale	Measured in 1 4 scale (1 = resistant, 2 = moderately resistant, 3=moderately susceptible and 4 = susceptible diseases like Anthracnose/crown rot, Bacterial wilt, Charcoal rot, mmon leaf spot, Downy mildew, Fusarium root rot, Alfalfa mosaic virus (AMV), Lucerne yellows and Phytophthora root rot)
Pod shattering,	0-1	0=Absent, 1=Present
pod hairiness	0-1	0=Absent, 1=Present
leaf hairiness	0-1	0=Absent, 1=Present

Due to variation in emergence, establishment, vigor, flowering, growth habit, leaf color, seed coat color and maturity, an array of these descriptors can be observed (Figs 1, 2, 3 and 4). In terms of leaf color, 2 different types were presented with light green being the mode. Semi-erect and trailing growth habits occurred with semi-erect being the mode as only some entries displayed a trailing growth habit. Seed coat color varied from dark brown to brown and yellow with yellow being the mode. In Fig 1. The variation in emergence, establishment, vigor, flowering and maturity were as follows:

1. Emergence: Fast; Establishment: Thick-very thick; Vigor: Vigorous-very vigorous; Flowering: Early-very early (43 days); and Maturity: Early-very early (86 days).
2. Emergence: Slow-Intermediate; Establishment: Intermediate-thick; Vigor: Weak-Intermediate; Flowering: Intermediate (65 days); and Maturity: Intermediate (133 days).
3. Emergence: Very slow; Establishment: Very sparse; Vigor: Very weak; Flowering: Very late (73 days); and Maturity: Very late (141 days).

Fig. 1. Variation in emergence, establishment, vigor, flowering and maturity

Fig. 2. Variation in leaf color (1 - Green; 2 - Light green)

Fig. 3. Variation in growth habit (1 - Semi-erect growth habit; 2 - Trailing growth habit)

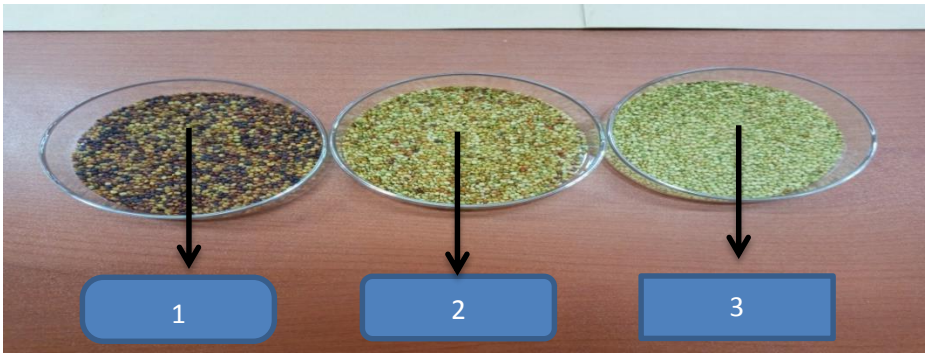

Fig. 4. Variation in seed coat color (1 - Dark brown; 2 - Brown; 3 - Yellow)

Data Analysis

The data were analyzed by R core team (2019) version 3.6.1. Qualitative characteristics were transformed, and a Principal Component Analysis was performed. Finally, a cluster analysis using the ‘‘complete linkage method’’ (2008) was carried out. Means of quantitative traits of each group were compared by using the Newman-Keuls procedure.

Results

Significant variation ($P<0.05$) in quantitative morphological characteristics of plants was observed among the entries in the following

descriptors: days to first flower, terminal leaflet length, terminal leaflet width, terminal leaflet petiole length, pod length, pod width, length of inflorescence, plant height and root depth (Table 3). However, there was no significant variation (P>0.05) among the entries in the following descriptors: number of leaflets/leaf**,** number of seeds per pod**,** number of main branches and number of nodes on the main stem.

Table 3. Quantitative morphological characteristics of 26 *Bouffordia dichotoma* entries

Parameter	Mean	SD	Variance	Range
Days to first flower	58.5	13.69	188	43.0–74.0
Terminal leaflet length (cm)	5.95	1.07	1.15	4.82–7.83
Terminal leaflet width (cm)	4.85	1.14	1.29	3.60–9.55
Terminal leaflet petiole length (cm)	6.27	2.60	6.75	4.12–16.4
Pod length (cm)	2.16	0.04	0.00	2.08–2.25
Pod width (mm)	3.13	0.13	0.02	2.77–3.37
Length of inflorescence (cm)	16.7	7.82	61.2	7.70–26.8
Plant height (cm)	64.2	6.33	40.1	53.0–73.0
Number of seeds per pod	5.0	0.0	0.0	5.0–5.0
Number of main branches	12.0	0.0	0.0	12.0–12.0
Number of nodes on main stem	12.0	0.0	0.0	12.0–12.0
Root depth (cm)	23.2	0.87	0.75	21.5–25.0

The mode, and range of the following qualitative descriptors: emergence, establishment, vigor, flowering, growth habit, leaf color, seed coat color and maturity, are presented in Table 4.

Table 4. Qualitative morphological characteristics of 26 *Bouffordia dichotoma* entries

Parameter	Mode	Range
Emergence	Very fast	Very slow-very fast
Establishment	Thick-very thick	Very sparse-very thick
Vigor	Vigorous-very vigorous	Very weak-very vigorous
Flowering	Early-very early	Very early-very late
Flower color	–	Pink-Violet
Growth habit	Semi-erect	Semi-erect-trailing
Leaf shape	Oval	-
Leaf color	Light green	Light green-green
Leaf hairiness	Present	Present/Absent
Maturity	Early-very early	Very early-very late
Seed shape	Kidney	-
Seed coat color	Yellow	Dark brown-yellow
Root system	Taproot system	-
Pod color	Pale tan or straw	-
Disease	Absent	Present/Absent
Pod shattering	Present	Present/Absent
Pod hairiness	Present	Present/Absent

A PCA (Principal Component Analysis) was performed with the goal of discriminating among accessions in the collections. The goal of PCA is to provide a reduced dimension model that would indicate measured differences among groups. It also can contribute to a better understanding of the set of variables by describing how much of the total variance is explained by each one. With this objective the PCA was performed with the matrix of morphological data generated by applying for the quantitative traits in the list of descriptors presented in Table 2. The variables: pod length, pod width**,** number of leaflets/leaf**,** number of seeds per pod**,** number of main branches**,** number of nodes on main stem, flower color**,** leaf shape**,** leaf hairiness**,** seed shape**,** root system**,** pod color**,** disease incidence**,** pod shattering and pod hairiness

were not included because they showed minimal variability. The quantitative morphological characteristics that had variation between accessions were used in principal component using the Minitab 16 software. The Eigen values of the first three principal components (PCs) were 4.03, 1.48 and 1.32, respectively (Table 5). The Fig. 5 shows how the accessions are classified into three clusters according to the first two principal components. By the scatter of 26 accessions Eigen-vectors. The first component separated clusters 3 and 1 with trend of low to high values of days to first flower, terminal leaflet width (cm), terminal leaflet petiole length (cm) and length of inflorescence (cm)traits. The second component separated accessions based on terminal leaflet length (cm) and pod width (mm) (Table 5). This result indicated that the distribution of accessions based on the first two component scores are in agreement with cluster analysis.

Table 5. Matrix of coefficients eigenvectors and variance proportion of the first three principal component axes using 26 accessions of *Desmodium dichotomum*

Variable	PC1	PC2	PC3
Days to first flower	**-0.48**	0.09	-0.10
Terminal leaflet width (cm)	**0.41**	0.39	-0.19
Terminal leaflet petiole length (cm)	**0.42**	0.06	0.06
Length of inflorescence (cm)	**0.48**	-0.15	0.12
Terminal leaflet length (cm)	0.07	**0.71**	-0.38
Pod width (mm)	0.02	**0.38**	0.30
Plant height (cm)	0.31	0.01	**0.53**
Pod length (mm)	-0.16	0.19	**0.49**
Root depth (cm)	0.27	-0.36	**-0.43**
Eigenvalue	4.03	1.48	1.32
Proportion	0.45	0.16	0.15
Cumulative	0.45	0.61	0.76

* The bold and underline coefficients have significant correlation with the relevant axes.

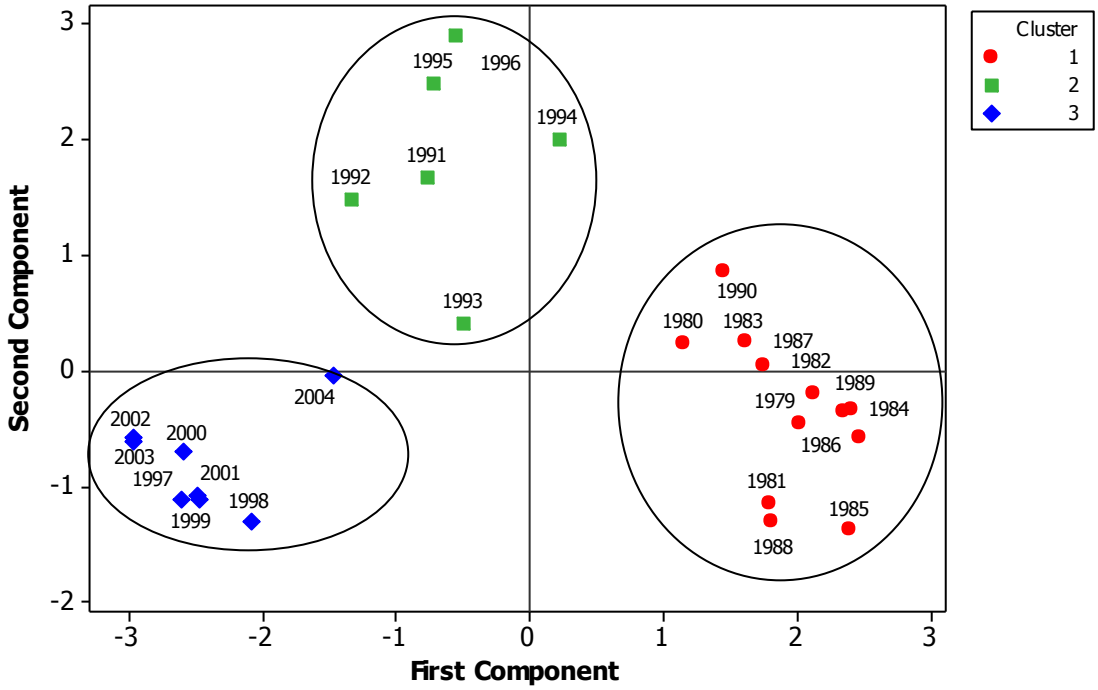

Fig. 5. Scatter plot of splitting of 26 accessions into three clusters based on the first two principal components

The resulting dendrogram is presented in Fig. 6. From the dendrogram, 3 distinct groups of entries were differentiated (Fig 6). Group 1 was composed of accessions PI 1990, 1987, 1988, 1989, 1986, 1985, 1984, 1983, 1982, 1981, 1980 and 1979; Group 2 of PI 1996, 1995, 1994, 1993, 1992 and 1991; and Group 3 of PI 2002, 1999, 2000, 2001, 2003, 1997, 1998 and 2004. Morphological characteristics of each of the 3 groups created by the cluster analysis are presented in Tables 6 and 7.

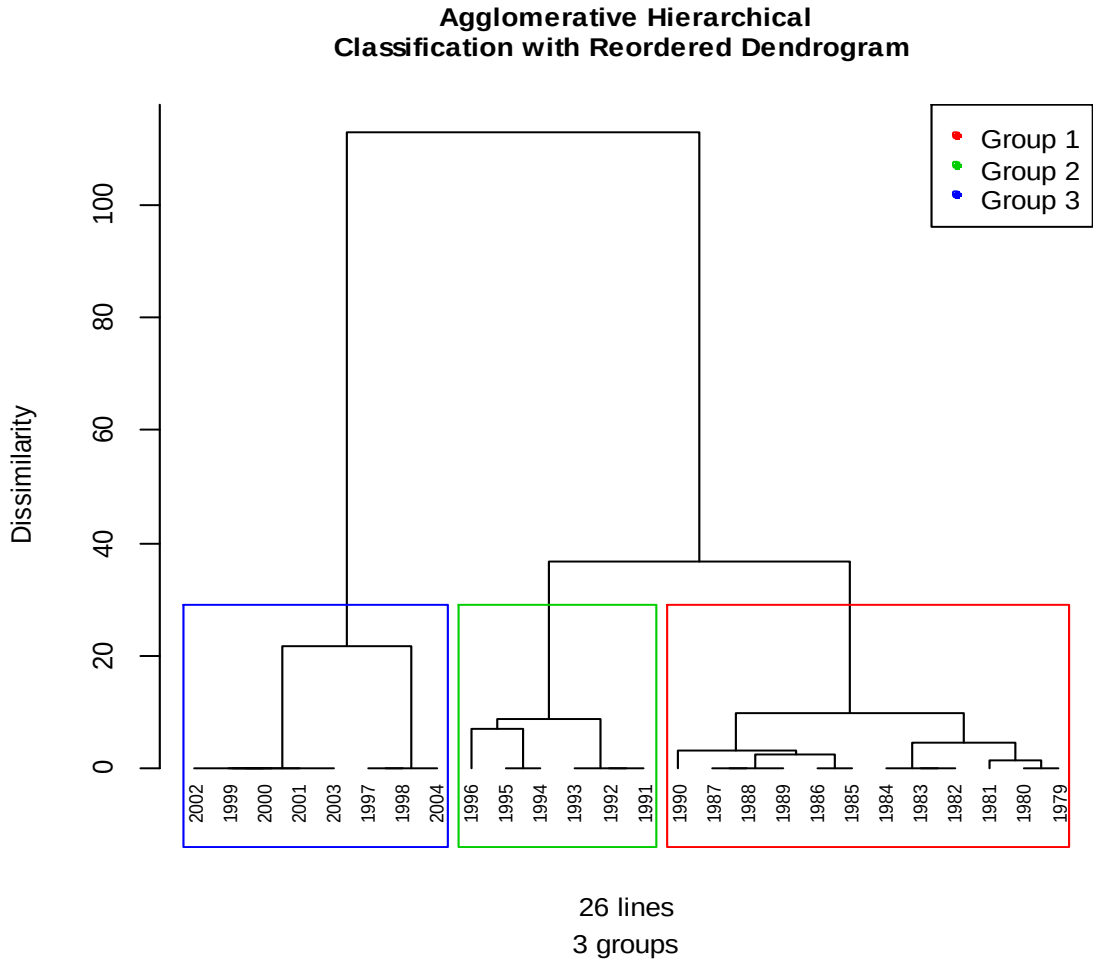

Fig. 6. Dendrogram of 26 *Bouffordia dichotoma* entries based on morphological descriptors

Table 6. Quantitative morphological characteristics of groups of *Bouffordia dichotoma* entries obtained by cluster analysis.

Parameter	Group 1	Group 2	Group 3	F-Value	P-Value
Days to first flower	43.0 a	65.0 b	72.5 c	1785.3	0.001
Terminal leaflet length (cm)	5.70 b	7.76 c	4.99 a	330.1	0.001
Terminal leaflet width (cm)	5.42 b	5.06 b	3.84 a	7.07	0.004
Terminal leaflet petiole length (cm)	7.96 b	5.60 a	4.24 a	8.17	0.002
Length of inflorescence (cm)	24.86 b	11.13a	8.56 a	1074.9	0.001
Plant height (cm)	68.46 b	61.17a	60.13a	7.81	0.003
Root depth (cm)	23.67a	22.92a	22.79a	NS	NS

Means of different groups (rows) followed by the same letters are not significant by Student Newman–Keuls test.

Table 7. Qualitative morphological characteristics of groups of *Bouffordia dichotoma* entries obtained by cluster analysis.

Parameter	Group 1	Group 2	Group 3
Emergence	Very fast	Intermediate	Very slow
Establishment	Thick-very thick	Very thick	Very sparse
Vigor	Vigorous-very vigorous	Intermediate	Very weak
Flowering	Early-very early	Intermediate	Very late
Growth habit	Semi-erect	Semi-erect	Trailing
Leaf color	Light green	Light green	Green
Maturity	Early-very early	Intermediate	Very late
Seed coat color	Yellow, dark and light brown	Yellow	Yellow

Discussion

Information that is obtained through multivariate techniques such as PCA and cluster analysis may assist plant breeders in the characterization of germplasm to explore the presence of genetic variation (Van de Wouw *et al.,* 1999) and to identify valuable characteristics, which account for genetic variation (Nunes and Smith, 2003). The first 5 principal components (PCs) were responsible for 69.3% of the total variation, and values similar to these were reported by Upadhyaya *et al*. (2002) and Stalker (1990), who worked with wild species of groundnut and *Arachis* germplasm collections, respectively. Clustering techniques were employed to estimate genetic distance and classify the entries into relatively homogenous groups. In this study, cluster analysis for quantitative characteristics revealed fairly distinct entry patterns classified in a different group. i.e 12 entries (group 1) of *B. dichotoma* due mainly to excellent days to first flower, terminal leaflet length, terminal leaflet width, and terminal leaflet petiole length, length of inflorescence and plant height characteristics. Clustering of entries on the basis of qualitative characters also excellent in the 12 entries (group 1) in the attributes of emergence (very fast), establishment (thick to very thick), vigor (vigorous to very vigorous), flowering (early to very early) and maturity (early to very early). These characteristics make the entries (group 1 in the Tables 5 and 6) suggesting that there is genetic variation in terms of morphological traits and a suitable forage plant that needs to be incorporated in the future forage introduction and evaluation studies.

Hence, morphological characters should complement the classification of the entries in order to reveal variation between entries that will have importance in relation to future germplasm utilization for forage breeding endeavors. The characterization of this group of *B. dichotoma* entries/accessions has improved the knowledge of the entries, thereby facilitating the identification of materials with desirable characteristics. Grouping entries into morphologically similar and possibly genetically similar groups (Souza and Sorrells, 1991) is helpful for the germplasm selection of promising entries. Subsequently, the grouping of entries by phenotypic characteristics in the present study will be used to classify the accessions into distinct morphological levels, which could be used for various breeding, collection and conservation programs.

Conclusions

The 3 groups of entries obtained by the cluster analysis showed significant quantitative morphological variation. Entries in Group 1 seemed to possess distinct attributes like early germination, rapid establishment, vigorous growth etc., which suggests that these entries should be studied in greater depth in the field to identify desirable entries for possible further development. Subsequently, the grouping of entries by phenotypic characteristics in the present study will be used to classify the accessions into distinct morphological levels, which could be used for various breeding, collection and conservation programs. Aspects to be considered would be dry matter yields, crude protein concentration, seed production, digestibility, acceptance by livestock and longevity under cutting and grazing.

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