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Phenotypic variation in cowpea (*Vigna unguiculata* [L.] Walp.) germplasm collection from Botswana

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Characterization of cowpea (*Vigna unguiculata* [L.] Walp.) accessions is an important exercise in improvement of the crop. A total of 432 cowpea germplasm accessions collected from five agricultural districts of Botswana during 1972 to 1987 were evaluated under field conditions at the Department of Agricultural Research Station, Gaborone. The germplasm were assessed using 37 agro-morphological characters to determine the diversity of Botswana cowpea germplasm. DIVA-GIS were used to conduct a gap analysis to estimate the degree of coverage of the germplasm accessions across the country. Areas not yet explored and those that need additional sampling were identified. The study demonstrated a significant amount of diversity among the germplasm based on the analysis of variance (ANOVA) and Shannon Weaver Diversity Index (H'). The majority of accessions had their first flowering more than 50 days after sowing and 100 days for 95% of maturity which shows that they are more inclined towards late maturing cowpeas. Early maturing accessions with less than 110 days to maturity are found in Ngamiland, Southeast and unknown origin. The accessions from Central districts had the largest variation for most characters (11) followed by Southeast with 10 among the 22 quantitative characters evaluated. Principal components analysis (PCA), revealed characters which discriminated more efficiently between accessions than others such as peduncle length, 10 seeds weight, seed width, seed thickness, pods per peduncle, and 100 seed weight. Cluster analysis delineates germplasm into three clusters, based on the origin of the germplasm according to different agro-ecological zones. Germplasm accessions originating from Ngamiland formed a separate cluster from the rest and had several peculiar materials, which could be a potential source for new germplasm for cowpea improvement. Further molecular studies are required to complement and validate the current agro-morphological variation observed in the Botswana cowpea germplasm.

Key words: Accessions, agro-morphology, cowpea, diversity, germplasm.

INTRODUCTION

Cowpea (*Vigna unguiculata* [L.] Walp.) is an important indigenous African grain legume grown in places with severe weather conditions in the tropics and sub-tropics in Africa, Asia and South America (Singh et al., 1997; Ba et al., 2004). It is a major source of dietary protein in sub-Saharan Africa where most production and consumption

is taking place (Nielsen et al., 1993). Most of the world's cowpea production is in Nigeria, Burkina Faso, United Republic of Tanzania, Cameroon, Niger, Mali and Kenya (FAO, 2013). Cowpea is multipurpose it is used at all stages of the crop for both human and animal consumption (Gómez, 2004; Sprent et al., 2009). It is valued for its ability to tolerate drought, and fix atmospheric nitrogen (rhizobium bacteria) which allows it to grow and improve poor soils (Mahalakshmi et al., 2006), and these makes it an important component in many cropping system (Fall et al., 2003). Therefore the crop is suitable for poor soils like those of Botswana (Moroke et al., 2005).

Cowpeas originate from Africa, but the exact area of domestication, and the center of diversity is still speculations (Zeven and De Wet, 1982; Ba et al., 2004). Studies suggests that Southern most region of Africa could be the center of origin for *V. unguiculata*, while domestication might have occurred in West Africa (Padulosi and Ng, 1997). In Southern Africa, Botswana is one country with higher genetic diversity of wild species of cowpea, such as *V*. *unguiculata* subsp. *dekindtiana, V. unguiculata* subsp. *tenuis* and *stenophylla* (Mathodi, 1992; Padulosi and Ng, 1997). Botswana is also an important cowpea growing country in Africa (Singh et al., 1997).

Cowpea is the main grain legume grown in Botswana it is the third economic crop of importance after maize and sorghum (DAR, 1997). The crop is produced in all the ten districts (CSO, 2013), but most production is concentrated in the Central, Kweneng and Southern districts (CSO, 2006; CSO, 2007/08; CSO, 2011). However, average production is at 139 kg/ha at national level (CSO, 2012). Several factors are attributed to this low level of production such as poor agronomic practices, and poor choice of well adapted and high yielding varieties (Manthe, 1987).

The National Plant Genetic Resource Centre (NPGRC) was established in 1986 after recognizing the importance of genetic resources and formal conservation (Mathodi, 1992). However, the initial attempt to collect and conserve crop germplasm was in the early 1970s by individual scientists from Department of Agricultural Research (DAR) (DeMooy, 1984). Botswana is in possession of a significant amount of cowpea germplasm collections of more than 1500 maintained by the DAR, (NPGRC), in the Ministry of Agriculture (www.moa.bw). The accession consists of wild and local landraces and improved varieties from International Institute of Tropical Agriculture (IITA) (Botswana Cowpea Project, 1982/1983).

It is important to conserve cowpea genetic variability for future use, but equally important is the actual utilization of the accessions (Nass and Paterniani, 2000).

Understanding the level of genetic diversity in a germplasm is helpful to plant breeders as it support their decision on the selection of parental genotypes and important in widening the genetic base of the crop (Prasanthi et al., 2012). Assessment of diversity also allows efficient sampling especially when core samples are developed, which allows proper management of the germplasm (Van Hintum et al., 2000). Hitherto the accessions in Botswana are primarily assessed based on morphological characters which depends on few genes and may not necessarily reflect the real variation for the agronomic characters present in the crop (Mayes et al., 2009).

The cowpea breeding programme has identified some germplasm useful in improvement of the crop. These includes discovering populations of wild Vigna in the Kalahari Desert which is resistant to bruchid beetle a serious storage cowpeas (Mathodi, 1992), and high levels of aphids (*Aphis craccivora*) (DAR, 1985/86). Some local cultivars which mature extra early, dual purpose and triple purpose were identified (Botswana Cowpea Project, 1982/83; DAR, 1985/86). Sources of resistance to *Alectra vogelli* have been found among local germplasm (Fite, 2010), other local materials such as B301 and B359 have been explored in other international institutions (Singh and Emechebe, 1990; Lane et al., 1996; Riches et al., 1992).

In this study we report on the characterization of more than 400 germplasm in the catalogues assembled for a period of fifteen years. The objective of this study is to analyze the morphological and agronomic traits of Botswana cowpea germplasm collected from different agro-ecological zones of the country to assess their genetic diversity. The study also aims to estimate the degree of coverage of already sampled areas, to identify areas that need additional sampling and those where no collections have been conducted yet.

MATERIALS AND METHODS

Germplasm collection and conservation

Over 100 cowpea germplasm were collected from various locations of Botswana by DAR research officers between 1972 and 1981. To augment this initiative a national cowpea collection germplasm programme was set up in 1982, through the assistance of Colorado State University and Cowpea Collaborative Research Support Program (CRSP). A number of surveys were conducted to obtain seed samples from many farming communities with different

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representation of soils, climatic environment found in Botswana. The accessions were collected from field crops, agricultural fairs and research stations. The cowpea samples collected were separated according to seed type and assigned an accession number. The accessions were put into cold storage and later removed and used for field experimentation. Based on the collections between 1972 and 1987, four hundred and thirty two germplasm accessions were evaluated for various morphological, physiological and agronomic characteristics of interest to plant breeders and growers (Paterson and Mathodi, 1987). The germplasm accessions were collected from five districts of Botswana.

Experimental site

The evaluation was conducted at the Department of Agricultural Research Station, located in Sebele, approximately 12 km north of the capital city, Gaborone. The station location is at latitude 24° 34' south and longitudes 25° 57' east at an altitude of 994 m. The soils at the station are underlain by Gaborone granite and the accessions were planted on chromic cambisol having coarse sandy texture. The soils are well drained with a depth of 90 cm or greater, and vary from acid pH 5.1 to 6.0 in the top soil to mild alkaline pH 6.6 to 7.8 in the subsoil. The field experiments were conducted during the rainy season of 1982, 1983, 1985 and 1987. Rainfall is mostly within the months of October through to April and is highly variable. The mean average rainfall, mean monthly minimum and maximum air temperatures for Sebele over the four cropping seasons were 350 mm, 20.3 and 35.4°C, respectively.

Experimental layout, design and planting

A total of 432 cowpea accessions were evaluated, each treatment consisted of one single row, five metre plot for each accession. The rows were spaced at 1.5 m with 20 cm plant spacing within a row. Super phosphate (P_2O_5 = 10.5%) was applied pre-plant at the rate of 250 $kgha^{-1}$. Data were recorded on 54 descriptors based on International Plant Genetic Resource Centre (IPGRC, 1983) for cowpeas (DeMooy, 1984). All the accessions evaluated were of cultivated origin and none were wild or weedy type.

Data tabulation

Data were collected from accessions that survived to maturity and harvest. The scoring was on an individual plant basis using five randomly selected plants per plot (DeMooy, 1984; Paterson and Mathodi, 1987). A total of 37 qualitative and quantitative characters were used for analysis. Fifteen qualitative characters analyzed were, growth habit, twining habit, attachment of pods to peduncle, raceme position, determinancy, flower pigmentation, plant pigmentation, pod pigmentation, pod shape, terminal leaflet shape, pod shattering, seed crowding, testa texture, eye pattern and eye colour.

Twenty two quantitative characters such as number of branches, number of nodes per main stem, peduncle length, days to 50% flowering, days to 95% ripe pods, pod forming period, vigor height, index width, pod length, pod width, leaflet length, leaflet width, 10 seed weight, seed length, seed width, seed thickness, pods per peduncle, locules per pod, seeds per pod, pods per plant, 100 seed weight, yield per plant (DeMooy, 1984; DeMooy, 1987; Paterson and Mathodi, 1987), were used.

Statistical analysis

Data analysis for the quantitative characters were subjected to analysis of variance (ANOVA) using the SAS version 9.2 (2010) to determine the statistical differences on the traits for the given accessions. The range, mean and standard error of means were calculated on the 22 agronomic data. The means values of measurements for each trait were standardized by subtracting the mean from respective traits and dividing by the standard deviation in order to reduce the influence of the scale differences. The standardized data was used in multivariate polymorphism in cluster analysis and principal component analysis. The accessions diversity was compared based on the five agricultural districts with different agro-ecological zones and some from an unknown origin. Some techniques for diversity analysis were employed such as Shannon weaver diversity index (H'), and the use of geographical distribution based on DIVA-GIS analysis.

Analysis of variance (ANOVA)

The material was divided according to agro-ecological zones of the country, and data from multiple years were averaged for each accession. Distribution pattern was used as the dominant factor in the analysis of variance to determine the range, mean and variances of the different traits in cowpeas germplasm accessions (Table 1).

Shannon weaver diversity

The diversity index was tested using Shannon-weaver index of Genstat version 13.0 to measure the diversity for the different characters. The calculations are based on phenotypic frequencies of each trait, and this reveals traits which are more varied and useful to plant breeders when making selection (Table 2).

Geographical distribution based on Diva-GIS analysis.

Diva-GIS is useful for analyzing the distribution of germplasm to elucidate geographic and ecological patterns and is useful in identifying gaps in the collection (Hijmans et al., 2012). Data for DIVA-GIS was gathered from 300 accessions with passport information, such as collection number, district name, village name, latitudes, longitude, sample status (cultivated or wild), collection source and date of collection. DIVA-GIS software version 7.5 was used for mapping the collection sites from the five districts (Figure 2).

Cluster analysis and principal component analysis

The agronomic data were subjected to principal component analysis (PCA) to identify traits that revealed most variation. Eigenvalues greater ≥ 1 were selected and used based on Multivariate Statistical Analysis (MVSP) software (Kovach, 2006). For cluster analysis, the unweighted pair group method with arithmetic averages (UPGMA) was performed using MVSP on Euclidean distance and dendrograms were produced to show the relationship between the accessions from the five districts.

RESULTS

Distribution of cowpea diversity in Botswana

The study consists of 432 cowpea germplasm accessions

Table 1. Range, mean and variances of different traits in cowpea germplasm accessions from Botswana.

Traits	Range						Mean					
	Central	Kweneng	Ngamiland	SouthEast	Southren	Unknown	Central	Kweneng	Ngamiland	SouthEast	Southren	Unknown
No. of branches	1 - 9	2 - 7	3.7 - 6.0	1 - 8.7	2 - 6	1 - 8	4.152^{a}	3.64^{a}	4.74^{a}	3.51^{a}	4^{a}	3.84^{a}
Nodes per stem	2 - 9.7	3 - 9	3.7 - 9.6	2.0-10.3	3 - 8	2 - 10.3	4.964^{a}	5.172^{a}	5.52^{a}	4.771^{a}	5.513^{a}	5.263^{a}
Pedunel Length (mm)	3 - 42.3	3.67 - 36	7.5 - 17.0	4.6 - 48.7	6.7 - 25.3	4 - 48	14.64^{a}	17.33^{a}	11.998^{a}	18.418^{a}	14.453^{a}	20.793^{a}
DFF	39.0 - 168	47.0 -158	57.0 - 82.0	36 - 157	48 - 148	38 - 158	97.77^{a}	84.958^{ba}	69.2^{bc}	61.751^{c}	87.06^{ba}	59.878^{c}
Days to 95%PCT	47.0 - 188	87 - 185	102 - 110	66 - 189	79 - 189	65 - 189	143.85^{a}	145.65^{a}	104.8^{b}	108.42^{b}	161.4^{a}	103.196^{b}
Pod form period	12 - 132	25 - 127	28 - 50	13 - 147	26 - 132	13 - 141	48.32^{b}	60.6^{ba}	35.6^{b}	46.52^{b}	74.333^{a}	44.535^{b}
Vigor height (cm)	6.66 - 23.3	8.33 - 22.7	13 - 20.7	6.0 - 30	7.66 - 17.0	5 - 26.7	13.54^{a}	14.804^{a}	14.9^{a}	13.73^{a}	12.932^{a}	16.092^{a}
Index width (cm)	7.33 - 42.0	4.0 - 31.3	21.7 - 38.3	4.7 - 34.3	12.3 - 32.0	4 - 39.3	21.94^{ba}	22.14^{ba}	27.2^{a}	20.476^{b}	22.708^{ba}	23.33^{ba}
Pod length (mm)	37.5 - 208.7	89.66 - 194	121.7 - 163.3	65.0 - 206.7	81.7 - 199.7	57.3 - 220	139.56^{a}	139.412^{a}	150.83^{a}	127.63^{a}	131.08^{a}	129.536^{a}
Pod width (mm)	5.0 - 11.7	6 - 11.0	7.33 - 10.0	4 - 11.33	6 - 11.7	5 - 11	8.23^{a}	8.432^{a}	8.908^{a}	7.327^{b}	8.354^{a}	7.483^{ba}
Leaflet length (mm)	32 - 111.0	48.3 - 143.3	37.7 - 123.3	28 - 140	36.3 - 110	8 - 174	97.261^{ba}	97.613^{a}	60.464^{b}	81.036^{b}	86.71^{ba}	93.174^{a}
Leaflet width (mm)	22.3 - 100.0	35.0 - 83.33	27.7 - 86.7	19.3 - 90.0	23 - 80	24 - 103.3	53.44^{ba}	58.013^{a}	41.668^{b}	50.196^{b}	52.833^{ba}	57.156^{a}
Seed 10 weight (g)	0.5 - 3.3	0.67 - 3.5	1.37 - 2.4	0.38 - 3.3	1.0 - 3.4	0.5 - 2.7	1.693^{a}	1.674^{a}	2.014^{a}	1.38^{b}	1.84^{a}	1.38^{b}
Seed length (mm)	4.0 - 12.0	5.0 - 10.0	7.0 - 10.0	4.0 - 12	4.0 - 12	4 - 11	7.704^{a}	7.2^{ba}	8.6^{a}	7.286^{ba}	7.6^{ba}	7.246^{b}
Seed width (mm)	4.0 - 9.0	4.0 - 8.0	6.0 - 7.0	4 - 12	5 - 8	4 - 8	6.68^{a}	6.4^{a}	7^{a}	5.72^{b}	6.533^{a}	5.73^{b}
Seed thickness (mm)	3.0 - 7.0	3.0 - 6.0	5.0 - 6.0	3 - 6	4.0 - 7	3 - 7	4.6^{bc}	4.8^{bac}	5.6^{a}	4.511d^{c}	5^{ba}	4.376d
Pods per enduncle	1 - 3	1 - 3.0	2.0 - 3.0	1 - 4	1 - 2	1 - 4	1.705^{ba}	1.83^{a}	2.2^{ba}	2.054^{b}	1.466^{ba}	2.0866^{ba}
Locules per pods	5 - 21	8 - 17	12 - 14	7 - 20	10 - 16	9 - 21	14.06^{a}	14.2^{a}	13.5^{a}	13.49^{a}	13.666^{a}	14.016^{a}
Seeds per pod	2 - 16	6 - 16	12 - 14	3 - 47	5 - 14	2 - 21	10.51^{a}	11.68^{a}	13.25^{a}	10.929^{a}	10.133^{a}	10.296^{a}
Pods per plant	0.03 - 96.66	0 - 16.3	1.8 - 35.7	0 - 87.8	0.5 - 40.6	0.03 - 53.5	10.315^{ba}	4.0196^{b}	10.68^{ba}	11.356^{a}	7.534^{ba}	11.107^{ba}
Seed 100 weight (g)	3.3 - 30.10	6.75 - 33.0	12.9 - 21.8	5.4 - 36.4	9.1 - 31.0	4.6 - 24.23	16.53^{ba}	16.462^{ba}	17.404^{ba}	13.476^{b}	17.598^{a}	13.619^{b}
Yield per plant (g)	0.34 - 98.60	0.5 - 23.4	N/A	1 - 65.7	0.92 - 41.2	0.65 - 36.41	15.7^{a}	8.004^{a}	N/^{A}	17.108^{a}	16.91^{a}	11.522^{a}

collected from 40 villages and five agricultural districts of Botswana. The majority of the accessions do not have known record of origin (132) followed by those from Southeast (129) while the least (5) are from Ngamiland. The districts that have not yet been explored are Kgatleng, Chobe, Ghanzi, Kgalagadi and Northeast (Figure 1). Central district provided majority of accessions that were collected from villages (121), while Southeast contributed most of the samples from research station (95) and 22 were collected at the fields.

Quantitative and qualitative analysis of cowpea germplasm

Data for each character was subjected to analysis of variance to estimate the genetic variability among the germplasm. Highly significant ($P<0.001$) differences were detected among the germplasm in most of the characters (14), with the exception of vigor height, index width, leaflet length, locules per pods, seeds per pods, pods per plant and yield per plant. The Shannon-Weaver diversity index (H') was calculated on the qualitative and quantitative characters to compare diversity between the different characters and among various districts. Higher diversity was observed among the qualitative characters at an

Table 1. Contd.

Standard errors of LS mean						
Central	**Kweneng**	**Ngamiland**	**SouthEast**	**Southren**	**Unknown**	**F- value**
1.864	1.451	0.856	1.663	1.309	1.555	0.0667
1.577	1.243	2.344	1.575	1.467	1.814	0.503
6.98	8.451	4.026	8.366	6.209	8.884	0.0017
39.44	33.83	8.927	26.61	33.024	21.948	0.0001
41.5	32.08	3.563	40.795	38.104	38.749	0.0001
30.45	27.949	8.443	32.957	38.214	32.656	0.0156
3.629	3.641	3.281	4.072	2.825	4.348	0.21
6.51	5.442	6.609	6.507	5.485	7.965	0.1724
33.077	25.13	19.55	30.749	33.135	34.364	0.0436
1.503	1.375	1.132	1.418	1.702	1.308	0.0001
93.91	19.58	35.833	25.26	21.984	24.461	0.2592
13.956	13.626	25.378	15.241	15.591	15.53	0.0102
0.541	0.592	0.426	0.464	0.605	0.452	0.0001
1.508	1.29	1.1401	1.506	1.804	1.42	0.0194
1.175	1.08	0	0.974	0.99	0.992	0.0001
1.015	0.866	0.5477	0.791	0.925	0.819	0.0025
0.655	0.701	0.4472	0.7214	0.516	0.827	0.0012
2.402	2.254	1	2.371	1.951	2.103	0.3554
3.106	2.882	0.957	4.415	3.044	3.015	0.3057
15.278	4.264	14.15	13.983	10.367	10.335	0.2317
5.089	5.483	3.386	4.331	6.134	4.073	0.0001
20.138	5.872	N/A	14.741	17.309	8.454	0.3781

Table 2. Shannon-weaver diversity index of collected cowpea germplasm accessions in Botswana.

Traits	**Central**	**Kweneng**	**Ngamiland**	**Southeast**	**Southren**	**Unknown**	**Average**
Qualitative							
Growth Habit	0.88	0.84	0.72	0.81	0.98	0.87	0.85
Twinning habit	0.39	0.48	*	0.49	0.35	0.49	0.44
PodPeduncleatta	0.80	0.72	0.72	0.84	0.88	0.84	0.80
Racemeposition	0.82	0.77	0.97	0.97	1.00	0.95	0.91
Determinancy	0.94	0.97	0.97	0.98	0.84	1.00	0.95
Flower Pigmentation	0.78	0.78	*	0.92	0.84	0.87	0.84
Plant pigmentation	0.53	0.63	*	0.72	*	0.56	0.61
Pod pigmentation	0.43	0.65	0.72	0.54	*	0.43	0.55
Pod shape	0.67	0.92	0.72	0.83	0.87	0.58	0.76
Leaflet shape	0.66	0.69	0.72	0.68	0.57	0.76	0.68
Pod shattering	0.46	0.24	0.72	0.99	0.72	0.72	0.64
Seed crowding	0.93	0.85	0.97	0.81	0.95	0.70	0.87
Testa texture	0.67	0.63	*	0.55	0.57	0.72	0.63
Eye pattern	0.71	0.82	0.97	0.94	0.87	0.80	0.85
Eye colour	0.89	0.88	0.96	0.82	0.87	0.77	0.87
Average	**0.70**	**0.72**	**0.83**	**0.79**	**0.79**	**0.74**	0.75
Quantitative							
No. branches	0.77	0.87	1.00	0.80	0.93	0.77	0.86
Node per stem	0.76	0.76	0.96	0.82	0.91	0.76	0.83
Peduncle Length (cm)	0.96	0.89	1.00	1.00	0.99	0.97	0.97

Table 2. Contd.

DFF	0.96	0.94	0.96	0.92	0.98	0.93	0.95
Days to 95% maturity	0.93	0.96	0.96	0.95	0.98	0.95	0.96
Pod form period	0.97	0.98	1.00	0.94	1.00	0.93	0.97
Vigor height (cm)	0.95	0.96	1.00	0.95	0.96	0.95	0.96
Index Width (cm)	0.97	0.98	1.00	0.96	0.99	0.97	0.98
Pod Length (mm)	0.99	1.00	1.00	0.98	1.00	0.99	0.99
Pod Width (mm)	0.92	0.90	1.00	0.90	0.96	0.85	0.92
Leaflet Length (mm)	0.80	0.87	0.96	0.82	0.86	0.84	0.86
Leaflet Width (mm)	0.86	0.90	*****	0.84	0.95	0.86	0.88
Seed10Weight (g)	0.91	0.94	0.96	0.87	0.99	0.89	0.93
Seed Length (mm)	0.80	0.89	0.96	0.82	0.89	0.84	0.87
Seed Width (mm)	0.86	0.89	*	0.84	0.89	0.86	0.87
Seed Thickness (mm)	0.85	0.87	0.97	0.84	0.89	0.73	0.86
Pods per peduncle	0.87	0.92	0.72	0.77	1.00	0.86	0.86
Locules per pod	0.82	0.95	0.81	0.87	0.95	0.85	0.87
Seeds per pods	0.93	0.92	0.95	0.89	0.95	0.87	0.91
Pod per plant	0.98	1.00	1.00	0.99	1.00	0.99	0.99
100 seed weight (g)	0.99	1.00	0.99	0.98	1.00	0.99	0.99
Average	**0.90**	**0.92**	**0.96**	**0.89**	**0.96**	**0.89**	0.92

*Too few samples for analysis.

average of 0.75. The most diverse variation (H') was observed on determinancy at 0.95 with the least diverse trait of twinning habit at 0.44. The most diverse morphological characters were observed from Ngamiland at 0.83 (Table 2). Shannon-weaver diversity (H') was higher among the quantitative characters at an average of 0.92, with more variation in characters observed in the Southern district and the least are in Southeast district at 0.89. The characters which revealed higher diversity H' above 0.95 were, peduncle length, days to first flowering, days to 50% flowering, pod forming period, vigor height, index width, pod length, pod per plant and 100 seed weight.

Diversity analysis based on growth habit

Based on the IPGRC (1983) there are seven classes of cowpea growth habit**,** all the seven types of plant growth habit were observed with the majority 121 of those with erect habit followed by 109 with semi-erect most of them come from unknown origin and Southeast districts respectively. A significant number of semi-prostrate growth habit (78) were recorded, and these could be useful as forage and good for leafy vegetables, the majority of them 34 accessions were collected from the central district. The accessions from Central district showed the maximum range among 11 traits followed by those from Southeast with 10 traits. Accessions from unknown origin had maximum range in pod length from 57.33 to 220 mm (Table 1).

Diversity based on plant physiology

Days to 50% flowering less than 50 days was observed in all the districts, with the exception of Ngamiland, where flowering occurred at 57 days after sowing. However, the crops in Ngamiland reach 95% maturity earlier than from all the regions at approximately 110 days. The vigor index which includes the plant length and width showed no significant difference between the plant heights among the five districts. Differences in plant width were observed only between accessions from Ngamiland and those from Southeast (Table 1).

Diversity in leaf and pod characteristics

There was no significant difference in pod length among all the districts but on average all the pod length was lower than 150 mm with the exception of those from Ngamiland. For pod width measurements, the Southeast district and unknown origin had smaller pod width of 7.32 and 7.48 mm respectively while the rest of the districts had more than 8 mm pod width (Table 1). Broader leaves in leaflet length (97.6 mm) and leaflet width (58.1 mm) were observed in accessions from Kweneng district but

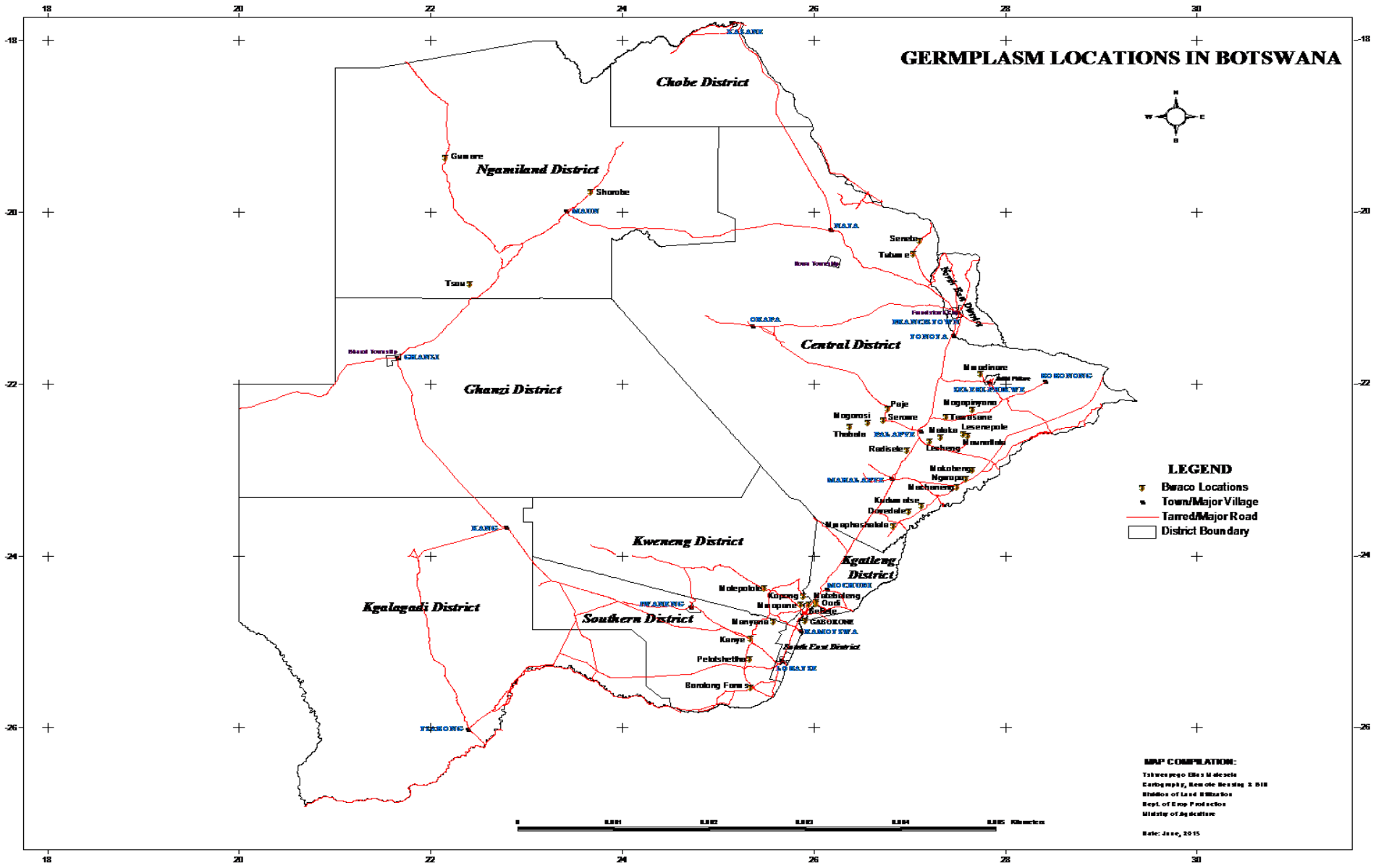

Figure 1. Distribution of cowpea accession in the National Plant Genetic Resource Centre using DIVA-GIS. The ten agricultural districts are inserted.

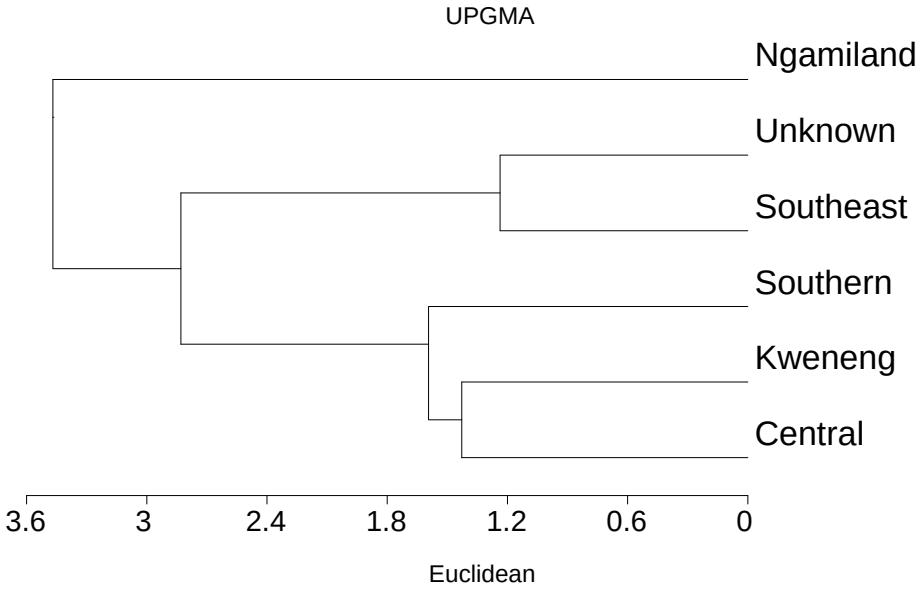

Figure 2. Dendrogram based on the principal components analysis capturing 86% of variation in the 432 Botswana cowpea germplasm collected for a period of 15 years.

were only significantly different from those from Ngamiland and Southeast which had narrow leaves (Table 1).

Diversity in seed characteristics

The accessions produced seeds width less than 1.7 mm and seed length smaller than 7 mm; it was only the Ngamiland accessions that had bigger seeded cowpeas at an average of 8.6 mm seed length and 2.01 mm seed width. The small seeded accessions were observed in Southeast and from unknown origin (Table 1). The seed thickness from Ngamiland is similar to those from Kweneng and Southren district but significantly different from the other districts. The 10 seed weights from all the districts are similar with the exception of those from Southeast and Ngamiland which are similar with 1.38 g. The Shannon-weaver diversity index (H') was relatively higher for eye pattern and eye colour at an average of 0.85 and 0.87, respectively. The more diverse accessions for eye pattern and colour are in Ngamiland and Southeast district (Table 2).

Diversity in yield components

Average weight for most accessions from Central, Kweneng, Southeast and unknown origin was more than 13 g for 100 seed weight, but the Ngamiland and Southern districts performed slightly better with over 17 g. Pods per plants from Southeast and unknown origin produced a slightly higher numbers with an average of more 11 pods per plant, which are light in weight while those from Kweneng were fewer in numbers but relatively heavier. The accession from Southeast also revealed relatively higher yield per plant because of the higher numbers per plant, though smaller in sizes (Table 1).

Accessions clustering and principal component analysis

Cluster analysis carried out using the Euclidean distance revealed greater diversity among Botswana cowpea germplasm, with a genetic distance ranging from 1.2 from unknown origin to 3.54 at Ngamiland district (Figure 2). The accessions from the five agricultural district and some from unknown origin were separated into three clusters, based on the origin of the germplasm. Accessions from Ngamiland formed cluster 1, while those with unknown origin together with those from Southeast formed cluster 2. The third cluster comprises accessions from Southern, Kweneng and Central these districts show some marginal difference (Figure 2). The principal component analysis for the 22 quantitative characters was conducted among the 432 accessions. The first two principal components with eigenvalues over one, which accounted for 86.28% of the total variation, were selected to analyze the germplasm (Table 3). The principal component analysis identified traits contributing more diversity among the accessions such as days to 50% flowering, pods forming period, seed width,10 seed weight, seed thickness, peduncle length, pods per

Table 3. Principal component, matrix of eigenvalues for 22 quantitative characters of 432 cowpea germplasm collection in Botswana.

Variables	PC 1	PC 2
Eigenvalues	1.898	1.490
Total contribution	48.331	37.950
% Accumulated	48.331	86.281
No. branches	0.168	-0.124
Node per stem	0.079	-0.022
Peduncle length (mm)	-0.308	0.044
Days to 50 % first flower	0.237	0.331
Days to 95%	0.248	0.574
Pod form period	0.052	0.343
Vigor height (cm)	-0.083	-0.143
index Width (cm)	0.155	-0.194
Pod length (mm)	0.169	-0.106
Pod width (mm)	0.300	-0.021
Leaflet length (mm)	-0.115	0.249
Leaflet width (mm)	-0.137	0.209
Seed10weight (g)	0.351	-0.040
Seed length (mm)	0.204	-0.177
Seed width (mm)	0.425	-0.022
Seed thickness (mm)	0.336	-0.183
Pods per peduncle	-0.093	-0.322
Locules per pod	-0.018	0.065
Seeds per pod	0.153	-0.233
Pod per plant	-0.055	-0.123
100 seed weight (g)	0.272	0.070
Yield per plant (g)	0.034	-0.022

peduncle and 100 seed weight (Table 3).

DISCUSSION

In this study, we describe for the first time a collective analysis of cowpea germplasm from the different regions of Botswana. Significant variability was discovered among the 432 cowpea germplasm, since more than 60% of the selected characters were highly significant (P > 0.01). However, Ehlers and Hall (1997) stated that even when vast variation is observed in the morphological variation in cultivated cowpeas, there is limited genetic variation among the cultivated gene pool. Bozokalfa et al. (2009), observed that vast variation in the quantitative characters can be useful in the development of variety description and identification. The availability of this diversity is an important resource useful to initiate a breeding program so as to select the best genotypes (Govindaraj et al., 2014). The cowpea breeding programme has taken advantage of this resource, cultivars with great potential have been selected among the local germplasm such *Maeatshilwana* (DAR, 1947-1959), *Morogonawa* (Botswana Cowpea Project, 1986-87), *Mogweokgotsheng* and *Nakedi* (DeMooy, 1984).

Principal component analysis was used to identify variables describing the phenotypic diversity of the genotypes; similar observations were made by Doumbia et al. (2013), when analyzing 94 genotypes from Mali and Ghana. Both studies identified days to 50% flowering , seed weight, seed length, seed width and seed thickness as important traits in diversity analysis which could indicate similarities between the west African and southern Africa germplasm. However, Botswana germplasm indicated more characters based on PCA analysis such as peduncle length, pod forming period, pods per peduncle possibly because more traits and germplasm were analyzed.

The cluster analysis revealed that the Botswana cowpea germplasm were clearly separated on their area of origin, which is an indication that the geographical origin contributes to the genetic variability among the genotype. Possibly the breeding program can be planned based on the known geographical patterns of the country as planned by the Ministry of Agriculture (Sims, 1981). However, our results are generally not in accordance with those of Cobbinah et al. (2011) who did not find clustering according to regional bases among the eight geographical regions of Ghana when characterizing 134 genotypes. According to their explanation this might be due to repeated collections within regions without proper documentation and extensive exchange of cowpea accessions that occurred in the past between regions.

The germplasm from Ngamiland with short duration to reach maturity and big seeded could be crossbred with those with higher yielding but with long maturity from Central, Kweneng and Southern. The results may prove to be particularly important for breeders and farmers to develop varieties with high potential for specific regions. In this study we observed close similarity between accessions from SOUTHEAST and unknown origin and between Kweneng, Southern and Central district. A similarity in clusters observed between regions could mean exchange of seeds between close regions (Uguru, 1998; Cobbinah et al., 2011). In this instance the similarities of collections from unknown origin and those from southeast is collections of about 70 lines were collected in about 60 km radius of the SEBELE research, which is in the SOUTHEAST district and an additional of approximately 100 collected from agricultural trade fairs and research stations (Botswana Cowpea Project, 1982 - 83).

The use of DIVA-GIS is essential for identification of potential areas of diversity and collection gaps especially when planning future collection explorations (Mujaju and Faith, 2011). The results demonstrated that most collections are from southeast /eastern part of the country where probably most of the cultivation of cowpeas is

taking place (DeMooy, 1984; CSO, 1979), such as in Central, Kweneng and Southren districts. Shannon weaver (H') revealed more genetic diversity in Ngamiland and Southern districts but these areas are underrepresented in the collections (Figure 1). Regions that have not yet been explored such as Kgatleng, Ghanzi, Northeast, Kgalagadi and Chobe have recently shown some records of cowpea production (CSO, 2010; CSO, 2012), though relatively low. According to (CSO, 1972), lack of explorations in Chobe, Ghanzi and Kgalagadi districts could be attributed to fewer subsistence farmers in the districts except for livestock, and inaccessibility of the roads in the region. Following a GIS-based gap analysis of the cultivated cowpea collection held at IITA, Botswana was among a few countries that were identified as priority for new germplasm acquisition (Rysavy, 2009). However, analyses of more than 1000 germplasm have not yet been conducted (unpublished data), to give further details on the genetic diversity of Botswana germplasm. Due to frequent drought in Botswana, cowpea has a great potential to contribute in increasing and stabilizing food production (Botswana Cowpea Project, 1983/84). In addition farmers are most likely to favor the production of cowpeas as compared to cereal because it is less labour intensive especially with regard to bird scaring and weeding (Luzani, 1992).

Conclusion

There is a broad genetic diversity of cowpea in Botswana. Most variation were attributed to growth habit, days to 50% flowering, seed weight, seed width, peduncle length, pod forming period, pods per peduncle. These characters are useful in characterizing and in cowpea improvement. Characters with more variation were observed in the Central and Southren district due to the wider ranges observed. The Shannon Weaver diversity identified Ngamiland and Southren districts to consist more diversity.

The germplasm clustered into three major groups according to geographic area of origin, accession from Southeast grouped with those with unknown area of origin, while those from Southern, Kweneng and Central formed one group which shows marginal difference among the groups. Ngamiland accessions formed a separate cluster from the rest and had several peculiar materials, which could be a potential source for new germplasm for cowpea improvement. Some areas have not yet been explored such as Kgatleng, Chobe, Ghanzi and Northeast which are also a potential source of new materials.

Conflict of Interests

The authors have not declared any conflict of interests.

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