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Research Article

CLUSTER AND PRINCIPAL COMPONENT ANALYSIS OF AFRICAN YAM BEAN (SPHENOSTYLIS STERNOCARPA HOCHST. EX. A. RICH. HARMS) USING MORPHOLOGICAL ATTRIBUTES

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Abstract

African yam bean (Sphenostylis stenocarpa-Hochst Ex A. Rich) is one of the endangered African crop species that has immense nutritional advantages required in human diet. It has dual crop advantage as it produces both seed and tubers. A collection of 18 accessions African Yam bean of IITA germplasm, Ibadan, Nigeria was assessed for genetic diversity based on nine quantitative traits. This research was carried out behind Biological Science Block, University of Calabar, Nigeria. Morphological characterization of AYB was conducted and nine qualitative parameters were considered for this study. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three (3) replicates Cluster and Principal Component Analysis were done using Genstat discovery Edition 4 and PASW version 20.0 software. The results on Cluster analysis revealed two major clusters. The dendrogram shows that accessions were not grouped based on origin as there were intermixing of accessions between the different origins within the sub-clusters. Principal Component Analysis of nine quantitative traits revealed five principal component and expressed 55.27 % of the total variation observed with Days to 50 % flowering, vine length and number of pods as major contributors from each PCs. Interestingly, the cluster dendrogram and the PCA obtained in the current study were in total agreement in classifying the studied accessions. This study revealed that TSs 33 may successfully be explored in breeding programmes for improved variant trait for AYB as it tends to cluster distantly from other accessions evaluated It is recommended that Days to 50 % flowering, vine length and number of pods should be considered as major indicators when characterizing AYB.

Keywords: African Yam Bean, Principal component analysis, Cluster analysis, Diversity.

INTRODUCTION

African yam bean (AYB) is a leguminous crop of tropical African origin belonging to the family of Fabaceae which is the second biggest and one of the most economically important families among the dicotyledons. African yam bean, AYB (*Sphenostylissternocarpa* Hochst ex A. Rich Harms*),* is a less utilized and dual-purpose (luminous and tuberous) crop. African yam bean is potentially a food and nutrition security crop due to its productive and nutritional value. It's seeds and tubers contain 25.6 and 15.9% protein respectively (Ojuederie & Balogun, 2017; Ojuderie & Balogun, 2019, Edem and Osuagwu, 2023). Similarly, Anya and Ozung(2019) reported protein content of 18.55 and 21.61% in seeds. The seeds have in abundance: lysine (6.21 - 6.60%) and methionine(1.14-1.27%) (Okorie, 2018). Seeds of AYB are rich in fibre, vitamins, potassium and manganese and contains a small amount of saturated fat (Oagile *et al*., 2012; Baiyeri *et al*., 2018). Tubers contain 166.7 mg/100g magnesium and 1010.1 mg/100g potassium (Ojuederie & Balogun, 2017). African yam bean (AYB) is commonly used in sub-Saharan Africa for various dietary preparations; it could be roasted or boiled or blended with vegetables (Klu*et al*; 2001; Ngwu*et al*., 2014). Some consumers add matured seeds to soups as a protein supplement (Klu *et al*., 2001). Fresh tubers are cooked and consumed as desired (Tindall, 1983). AYB seed meal was seen to improve growth in starter broiler chickens (Raji *et al*., 2016). Furthermore, Onuoha *et al*. (2020) suggested adding tubers and seeds in animal feeds.

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The alarming increase in the world's population has its direct effect on food security and sustainability which has increased the demand for food production to feed the human population. Although science has made enormous strides in improving the world's ability to feed itself over the past decades, a large proportion of the world are still suffering from hunger and malnutrition. (Osuagwu and Edem, 2021) Nearly 800 million people in the developing world do not have enough to eat(World Health Organization, 2002). Many important crop plants native to Africa with potentials to alleviate and reduce food insecurity in the continent are severally neglected, unimproved and under-utilized. One of such crops with exploited potentials and quality nutritional value is African yam bean. It is a minor grain legume and under- exploited (Nwokolo, 1987; Saka *et al*., 2004), usually cultivated in association with yam, cassava, maize and sorghum and other crops (Togun & Egunjobi, 1997). The legume has long been used as traditional dual seed grain and tuber food crop in Africa and it has other great potentials (Adewale & Dumet, 2010). Studies have shown that the under-utilized legumes are highly nutritious and natural fertilizers. Several other utilizations of the crop have been reported (Potter & Doyle, 1992; Ene-obong, 1993; Klu, 2001). African yam bean is identified with some limitations such as hardness of the seed coat and hence longer time to cook, presence of secondary metabolites, lengthy life cycle, photo periodic sensitivity etc. (Adewale & Dumet 2010). To understand the diversity study of AYB, the use of cluster and principal component analysis can be employed. Cluster analysis is a data analysis method that clusters or group objects that are closely associated with a given data set. There are different types of cluster analysis but hierarchical cluster analysis is most frequently used because it is an easy method of grouping investigated data through their

similarities (Bruns & Faigle, 1995). Principal component analysis was first described by; Karl Pearson 1901. The PCA involves the reduction of the number of variables of a data set, while preserving as much information as possible. The most important use of PCA is to represent a multi variant data table in other to observe trends, jumps, clusters and outliers. PCA aims to display the relative positions of data points in fewer dimensions while retaining as much information as possible, and explore relationships between dependent variables.

This research is therefore designed to provide information on the genetic diversity of 18 accessions of *S. stenocarpa* from IITA germplasm using cluster and PC analysis of quantitative morphological data. African yam bean (AYB) is a crop with great potentials in agricultural productivity and food security, especially is sub-Saharan Africa. Its continued existence is threatened with genetic erosion and extinction of its landraces and wild relatives due to lack of proper conservation and breeding strategies. Also, Persistent neglect of AYB has resulted in continual loss of essential germplasm with implications in its variability. Research on phylogenetic and evolutionary relationship in germane to AYB improvement but still at infant stage. Using a more robust analytical tool like Cluster and PC Analysis to check the extent of genetic variability present in AYB will be beneficial to breeders as it will reveal traits contributing to the growth performance in this species and their evolutionary relationship. The aim of the study was to evaluate cluster and principal component analysis of the African yam been using morphological marker.

MATERIALS AND METHODS

Study location

The agronomical experiment was conducted behind Biological Science Block, University of Calabar, University of Calabar, Calabar.

Materials

Eighteen seeds of AYB accessions were obtained from Genetic Resources Centre (GRC), International Institute of Tropical Agriculture (IITA), Ibadan Nigeria, respectively for the study. The agronomical experiment was conducted behind Biological Science Block, University of Calabar, with coordinates 4°56'02.1"N 8°19'13.3"E, University of Calabar, Calabar. Passport data of the Seeds samples were taken and recorded appropriately. The experimental plot was ploughed, harrowed and ridged before planting. Three seeds of each accession were planted per stand; which was thinned to one plant per stand after seedling emergence and establishment. The sowing was in the spacing of 1 × 1 m. After establishment, sticks of about 3 m length were pro-vided to support the plants as stakes at three weeks after planting. The field was kept clean by regular hand weeding with hoes (Table 1).

Experimental design

The experiment was laid out in a Randomized Complete Block Design (RCBD) with three (3) replicates (Plate 2).

Data collection

Data was obtained at maturity on quantitative traits. Quantitative traits were counted, measured using metric rulers or vernier caliper and weighed using weighing balance. On the field, data was recorded on the five middle plants (sampling units).

Table 1. Accessions of S. *Stenocarpa* used in the study, their origin and ecological zone

S/N	Accessions	Origin	Ecological zone
1	TSs-266	Unknown	Forest
2	TSs-38	Nigeria	Forest
3	TSs-119	Nigeria	Savanna
4	TSs-439	Nigeria	Savanna
5	TSs-69	Nigeria	Forest
6	TSs-331	Unknown	Forest
7	TSs-113	Nigeria	Savanna
8	TSs-276	Unknown	Savanna
9	TSs-285	Unknown	Savanna
10	TSs-366	Unknown	Unknown
11	TSs-33	Nigeria	Forest
12	TSs-28	Nigeria	Unknown
13	TSs-65	Zaire	Forest
14	TSs-441	Nigeria	Savanna wood land
15	TSs-152	Nigeria	Forest
16	TSs-96	Nigeria	Forest
17	TSs-186	Unknown	Forest
18	TSs-231	Unknown	Forest

Plate 1. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three (3) replicates.

Three readings were made for each of the quantitative characters per accession. All the characterizations were based on Genetic Resources Center, International Institute of Tropical Agriculture (IITA) descriptors for African Yam Bean. Data was collected on the following parameters;

- Days to 50% Seedling emergence
- Days to 50% flowering
- Number of Leaves per plant
- Vine length
- Peduncle length
- Petiole length
- Terminal leaf length
- Terminal leaf Width
- Number of pods

Data analysis

Morphological quantitative data obtained were subjected to principal component analysis (PCA). Cluster patterns for quantitative traits in 18 accessions of AYB was generated using Ward's method. All analyses were done using Predictive Analytic Software (PASW) version 20.

RESULTS

From the cluster pattern based on squared Euclidean distance 0.05 using Ward's method, two clusters were revealed for the nine quantitative morphological traits studied (Figure2). The dendrogram generated showed that cluster 1 contained the following accessions: TSs 33, while Cluster 2 contained the other 17 accessions. Cluster 2 had two sub-clusters, denoted as cluster 2A and 2B. cluster 2A contained TSs 96, TSs 186, TSs 266, TSs 38, TSs 28 while 2B was further divided in two sub clusters, 2B1 And 2B2. 2B1 contained 7 (TSs 199, TSs 439, TSs 285, TSs 69, TSs 65, TSs 231 and TSs 152) accessions. Cluster 2B2was further grouped into two sub-clusters, 2B21 and 2B22. Results of the principal component analysis of nine quantitative traits studied (Table 2), revealed three principal components and explained 55.27% of the total variation in these traits. PC 1 had an Eigen value of 2.39, contributing to 26.6% of the total variation.

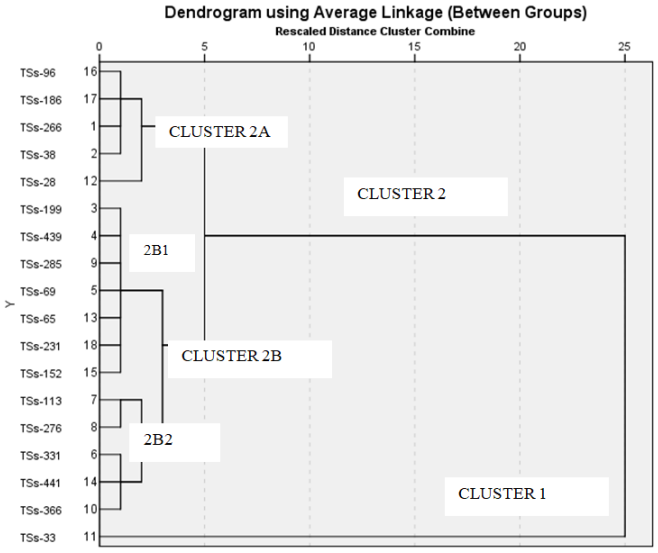

Fig. 2.Genetic relationship of 18 AYB accessions from quantitative traits data

Table 2. Principal Component Analysis (PCA) in morphological traits of 18 accessions of African yam bean

	Communalities	PC_1	PC_2	PC_3
Eigen value	-	2.394	1.307	1.273
Proportion of variance (%)	-	26.603	14.517	14.147
Cumulative variance (%)	-	26.603	41.120	55.267
Days to 50% emergence	0.598	0.050	0.149	-0.580
Days to 50% flowering	0.692	-0.031	0.627	0.100
Number of leaves	0.622	-0.075	-0.090	0.596
Vine length	0.658	0.195	0.491	0.130
Peduncle length	0.334	0.203	0.211	-0.116
Petiole length	0.493	0.292	0.000	-0.074
Terminal leaf length	0.481	0.256	-0.233	0.084
Terminal leaf width	0.416	0.250	0.049	0.183
Number of pods per plant	0.675	0.340	-0.001	0.087

In the quantitative traits, number of pods produced large loading values (0.34) for the first component. Therefore, PC 1 was designated the "number of pods component." PC2 had an Eigen value of 1.31, contributing to 14.52 % of the total variation, days to flowering produced large loading values (0.63) for this component, thus this array was designated the "days to 50 % flowering component." PC3 had an Eigen value of 1.27, contributing to 14.15 % of the total variation, number of leaves produced large loading values (0.60) for this component, thus this array was designated the "number of leaf component. "Days to 50 % flowering, vine length and number of pods had communalities of 0.69, 0.66, and 0.68, respectively thus accounting for variation of 70 %.

DISCUSSION

Quantitative traits characterized in this study will be invaluable in varietal identification, conservation, diversity analysis, selection, breeding and genetic improvement of *D. bulbifera.* Two clusters were revealed for the nine quantitative morphological traits studied in 18 accessions of *S. stenocarpa* (Plate 1). The dendrogram showed that Cluster 1 contained one accession: TSs 33 while cluster 2 contained the remaining seventeen accessions. The accessions were not differentiated according to countries or ecological regions of collection. The clustering pattern shows that many of the accessions are related to each other, possibly due to exchange of planting materials across and between countries. The phenotypic variability of the crop could be attributed to continuous vegetative propagation and selection. The dendrograms revealed relatively low variations on morphological traits. The results of the principal component analysis of nine quantitative morphological traits studied as presented in Table 2, showed three principal components and explained 55.3% of the total variations in the morphological traits studied. These results stem from the existence of a low degree of morphological polymorphisms possibly due to an interaction of genetic and environmental factors. These results indicate the traits contributing to maximum variability among the accessions. Aremu and Ibirinde (2012), attributed 57% of the total variation of 50 AYB germplasm sourced from IITA, Nigeria, to the first four PC at an Eigenvalue of one. Similarly, (Ojuederie *et al*. 2015) reported that the first four PCs were responsible for 62% of observed variations in forty AYB collections (27 sourced from IITA, Nigeria and 13 from Institute of Agricultural Research and Training (IART), Ibadan, Nigeria) they studied. In a related report, Popoola *et al.*, (2011) investigated 25 accessions sourced from IITA, gene bank, Nigeria and attributed 54% of identified variations to the first four PCs (54%). The traits which had large loading values in the three principal components should be made selection criteria in *S. stenocarpa* breeding programs emphasizing improvement days to 50% flowering, number of leaves and number of pods. The relative importance of each trait can be estimated by the rank order of their contribution (%) to the observed phenotypic variation. Interestingly, the cluster dendrogram and the PCA obtained in the current study were in total agreement in classifying the studied accessions.

Conclusion

Interestingly, the cluster dendrogram and the PCA obtained in the current study were in total agreement in classifying the studied accessions. This study revealed that TSs 33 may successfully be explored in breeding programmes for improved variant trait for AYB as it tends to cluster distantly from other accessions evaluated.

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APPENDIX 1

Accession		50% Days to seedling emergence	50% Days to flowering	No of leaves per plant	Plant height (cm)	Peduncle length(cm)	Petiole length (cm)	Terminal leaf length (CM)	Erminal leaf width (cm)	No. Of pods per plant
TSs-266	1	10	78	103	90	13	4	10	3.9	15
	1	11	80	121	95	12	4.5	8.5	3.3	9
	1	10	88	95	89	11.2	4.8	9	3.8	11
TSs-38	2	12	78	94	95	10	3.8	9.5	3.6	10
	2	8	76	125	86	13	4.9	9.8	3.5	7
	2	9	91	113	85	12.5	4.5	10.2	3.9	8
TSs-199	3	11	83	95	54	6	4.8	7	3.7	2
	3	12	81	105	60	7	3	6	3.8	1
	3	10	92	110	65	7.4	3.2	6.6	3.1	4
TSs-439	4	10	89	100	60	8	3.1	7.8	2.8	3
	4	11	80	135	50	9	3.8	6.3	2.2	2
	4	12	91	89	75	9	3.9	9	2.1	6
TSs-69	5	11	84	105	51	7	3	7.2	2.4	1
	5	10	90	125	65	11	3.7	6.3	3.7	1
	5	15	87	85	62	10	3.6	6	2.5	4
TSs-331	6	11	83	115	59	6	3	10	2.9	2
	6	10	77	109	70	11	3.2	7.2	3.3	4
	6	9	75	105	51	5	3.1	6.6	3.1	1
TSs-113	7	11	84	103	75	5	3	7.2	3	5
	7	10	84	104	90	6	3	8	3.4	6
	7	10	89	95	61	8	4	6	3.5	2
TSs-276	8	11	89	115	81	9.2	4	7.2	2.5	8
	8	11	75	135	65	11	3	8	2.7	6
	8	11	75	100	75	12	3.1	6	2.6	2
TSs-285	9	10	84	87	65	11	3.5	6.1	2.6	3
	9	11	91	135	60	7.8	3.4	6.5	2.6	4
	9	12	78	94	55	8.8	4.8	7	2.8	3
TSs-366	10	14	90	125	67	9	4.1	8	3.7	2
	10	12	92	101	82	9	3	6.2	3.1	2
	10	11	77	88	72	12.3	3	6.4	3.2	2
TSs-33	11	10	90	192	81	4	3.2	6.6	2.9	5
	11	11	77	105	70	5	3.1	6.8	2.8	3
	11	10	80	101	64	7	3	7	2	2
TSs-28	12	11	84	85	95	4	4	8	2	3
	12	8	72	115	45	13.2	3.1	7.7	2.3	8
	12	11	90	125	54	11.2	3.2	8	2.4	2
TSs-65	13	10	77	105	50	5.5	4.9	10	2.5	1
	13	8	74	145	55	6.9	3	6.7	3.6	1
	13	9	78	112	48	8.3	3	7.7	2.5	4
TSs-441	14	14	77	112	65	7	4	9	2.7	2
	14	12	80	95	65	8	4	10	3.8	6
	14	12	80	101	73	12	4	8	3.7	7
TSs-152	15	14	77	95	45	12	4.1	8	3.5	9
	15	13	82	92	76	11	4.2	6	3.4	11
	15	10	77	91	76	5.8	3	7	3.3	1
TSs-96	16	11	82	102	91	8.9	3	8	3.2	3
	16	12	92	111	90	8.9	3	6.1	2.5	3
	16	10	97	100	65	13	3.7	6.7	2.7	1
TSs-186	17	14	77	105	95	8.7	3.5	9	2.8	1
	17	11	77	128	45	9.2	3.6	10	2.9	1
	17	12	70	102	57	8	3.3	8.2	2.6	2
TSs-231	18	10	82	108	45	13	3.3	6	2.5	2
	18	9	92	97	90	5.4	3.2	7	2	1
	18	12	77	99	43	13.5	3.1	8.3	2	4

APPENDIX 2

Descriptors for African yam bean, *Sphenostylis stenocarpa* (Hochst ex. A. Rich.) Harms

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Introduction to African yam bean

African yam bean (*Sphenostylis stenocarpa* Hochst ex. A. Rich.) Harms is an underutilized tropical African tuberous legume. It belongs to the class *Magnoliopsida;* order *Fabales;* family *Fabaceae;* subfamily *Papilionoideaea;* and genus *Sphenostylis.* There are seven species in the genus *Sphenostylis* (Potter and Doyle 1994). African yam bean (AYB) is the most valuable. The arable tuberous legume is important in most indigenous African food cultures and in peasant agriculture.

The center of diversity, according to the Genetic Resources Information Network (GRIN), spreads from the west through to the east and southern parts of Africa (GRIN 2009) and these areas are suspected to host the genetic resources of AYB. The utilization of AYB has links with sociocultural values in the cultures of some ethnic groups within the area.

AYB is a vigorously climbing herbaceous vine whose height can reach 1.5–3 m or more. The main vine/stem produces many branches which also twine strongly on available stakes. The vegetative growing stage is characterized with the profuse production of trifoliate leaves (Milne-Redhead and Polhill 1971).

From four to ten flowers are arranged in racemes on long peduncles, usually on the primary and secondary branches. The large and attractive flowers blend pink with purple; the standard petals twist slightly backwards on themselves at anthesis. The flowers seem to exhibit self-pollination; up to six pods/peduncle may result after fertilization. The usually linear and long unicarpel pods turn brown when mature (Hutchinson and Dalziel 1958; Dukes 1981).

The pods which may sometimes be flat or raised in a ridge-like form on both margins are usually prone to shattering; they dehisce along the dorsal and ventral sutures when dry. Each pod can yield up to 20 seeds which may be round, oval, oblong, or rhomboid. There are varieties of seed color (Oshodi *et al.* 1995) and size (Adewale *et al.* 2010) with mono-colored or mosaic types. Mono-colored seeds are white, grey, cream, light or dark brown, purple, or black.

AYB is usually grown in mixtures with yam and cassava. Protein content is up to 19% in the tubers and 29% in the seed grain. The crop has medicinal importance (Potter 1992). Assefa and Kleiner (1997) remarked that AYB has very high nitrogen-fixing ability. It has remarkably low susceptibility to most field and storage leguminous pests (Omitogun *et al.* 1999).

CHARACTERIZATION DESCRIPTORS

1.0 Plant descriptors

1.1 Vegetative

1.1.1 Hypocotyl pigmentation
Scored at seedling emergence when the first leaves have fully expanded as:
0 = Absent
1 = Present

1.1.2 Days to 50% twining
Days from seedling emergence until 50% of the stands climb to make the first clockwise twine around the stakes; 5 plants as sampling unit within the plot center

1.1.3 Plant part pigmentation

1.1.3.1 Main stem
0 = Absent
1 = Present

1.1.3.2 Branch
0 = Absent
1 = Present

1.1.3.3 Petiole
0 = Absent
1 = Present

1.1.3.4 Peduncle
0 = Absent
1 = Present

1.1.4 Intensity of pigmentation on plant parts

1.1.4.1 Main stem
1 = Slight
3 = Moderate
5 = Extensive

1.1.4.2 Branch
1 = Slight
3 = Moderate
5 = Extensive

1.1.4.3 Petiole
1 = Slight
3 = Moderate
5 = Extensive

1.1.4.4 Peduncle
1 = Slight
3 = Moderate
5 = Extensive

1.1.5 Leaf color (Methuen color chart code)

1 = Pale green	27A3
2 = Green	27A8
3 = Dark green	27 F8

1.2 Reproduction

1.2.1 Days to peduncle initiation
Days from seedling emergence until 50% of the plant stands within a plot begin to initiate peduncles; 5 plants as sampling unit

1.2.2 Days to 50% flowering
Days from seedling emergence until 50% of the plant stands begin to anthesize; 5 plants as sampling unit

1.3 Fruits (Pods)

1.3.1 Seed cavity ridges on pods (see Fig. 1)
0 = Absent
1 = Present

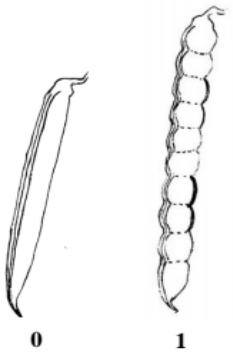

Fig. 1. Seed cavity ridges on pods

1.3.2 Pod dehiscence
0 = Non-shattering
1 = Shattering

1.4 Seeds

1.4.1 Splitting of testa
0 = Absent
1 = Present (testa splits to expose the cotyledons)

1.4.2 Seed shapes (see Fig. 2)
1 = Round/globular
2 = Oval
3 = Oblong
4 = Rhomboid

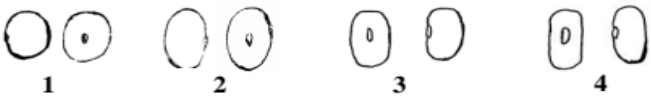

Fig. 2. Seed shapes

1.4.3 Testa texture
Scored on the seeds by touching the testa surface as:
1 = Smooth
2 = Rough
3 = Wrinkled (folds on the testa)

1.4.4 Testa color variegation
0 = Absent
1 = Present

1.4.5 Testa basal color (Methuen color chart code)
Measured as varieties of colors without variegation

1 = White	A1
2 = Grey	E1
3 = Cream	4A3
4 = Light brown	6D8
5 = Reddish brown	8E8
6 = Dark brown	6F8
7 = Purple	14F8
8 = Variegated (mosaic)	

1.4.6 Pattern of testa variegation (see Fig. 3)
1 = Dense black uneven spots/dots on brown background basal color with clean eye
2 = Sparse black dots on creamy brown background with a concentration around the hilum
3 = Patchy light brown dots on dark brown background

Fig. 3. Pattern of testa variegation

1.4.6.1 Basal color of variegated seeds
0 = Non-variegated seeds
1 = Cream
2 = Brown
3 = Black

1.4.7 Eye color of white seeds
Color around the hilum of white seeds
0 = Non-white seeds
1 = Clean (no color around the hilum)
2 = Brown
3 = Black

1.4.8 Eye color pattern (see Fig. 4)
1 = Brown testa with continuous narrow black stripe around the hilum
2 = Brown testa with dark brown fork-like eye pattern
3 = White/grey testa with incision-like eye pattern
4 = Brown testa with dark brown incision-like pattern below and parallel to the hilum
5 = White testa with reddish brown vase-like eye
6 = White testa with black vase-like eye

Fig. 4. Eye color pattern

1.4.9 Brilliance of seeds
1 = Matt
2 = Medium
3 = Shiny

1.5 Tubers

1.5.1 Tuber production
0 = No
1 = Yes

1.5.2 Tuber population
1 = One tuber
2 = ≥ Two tubers

1.5.3 Tuber shape (see Fig. 5 a, b, c, and d)
1 = Round
2 = Ovate
3 = Spindle
4 = Irregular

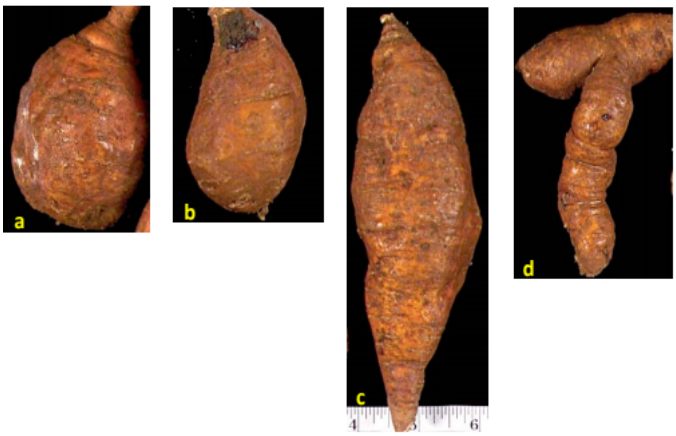

Fig. 5. Tuber shape

1.5.4 Tuber skin color (Methuen color chart code)

1 = Cream	4A3
2 = Brownish orange	6C8
3 = Pink	12A4

1.5.5 Tuber branching
Offshoot from the main tuber
0 = No
1 = Yes

1.5.6 Extent of tuber branching
1 = Slightly branched
2 = Branched
3 = Highly branched

EVALUATION DESCRIPTORS

2.0 Plant descriptors

2.1 Vegetative

2.1.1 Days to 50% seedling emergence
Days from sowing until 50% of the seedlings emerge on the sown stands

2.1.2 Hypocotyl length [cm]
Mean length of 10 hypocotyl seedlings measured from the base to the tip when the first primary leaves have fully expanded (see Fig. 6)

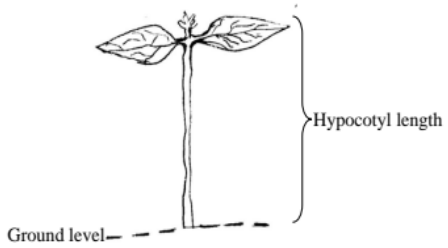

Fig. 6. Hypocotyl length

2.1.3 Number of leaves
Number of leaves on a meter length of branch; 10 branches as sampling unit; measured at full reproductive stage of the plant

2.1.4 Internode length [cm]
Distance in centimeters on a branch between two consecutive leaf nodes, 10 branches as sampling unit; measured at full reproductive stage

2.1.5 Terminal leaf length [cm]
The average metric distance from the pulvinus to the apical tip of 10 fully developed terminal leaflets taken from 5 different plants at the peak of flowering (see Fig. 7)

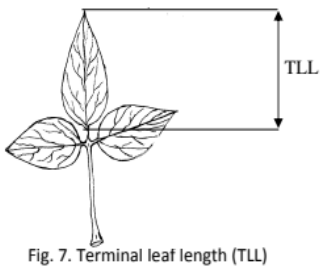

Fig. 7. Terminal leaf length (TLL)

2.1.6 Terminal leaf width [cm]
The average metric distance measured along the widest part of 10 fully developed terminal leaflets taken from 5 different plants at the peak of flowering (see Fig. 8)

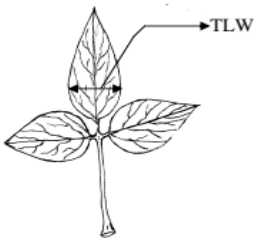

Fig. 8. Terminal leaf width (TLW)

2.1.7 Petiole length [cm]
Mean length of the 10 petioles from 5 sample plants, measured from the base to the point where the three leaflets join (see Fig. 9)

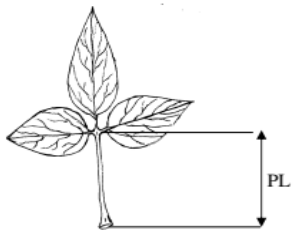

Fig. 9. Petiole length (PL)

2.1.8 Stem diameter [cm]
The circumference (girth) of the stem measured at 10–15 cm above the ground at the peak of flowering
2.1.9 Peduncle length [cm]
Measured on 10 fully grown, flower/pod-bearing peduncles from 5 sample plants
2.1.10 Peduncles/plant
Mean number of peduncles from 5 sample plants at harvest
2.1.11 Days to maturity
Days from seedling emergence until 90% of the pods in a plot are mature

2.2 Reproduction
2.2.1 Days to 50% peduncle initiation
Days from seedling emergence until 50% of the stands in a plot initiate the offshoot of peduncles at nodes on the stems
2.2.2 Days to 50% flower bud initiation
Days from seedling emergence until 50% of the stands initiate flower buds on the peduncles
2.2.3 Flowers/peduncle
Counted on 10 peduncles from 5 plants in the middle of the plot
2.2.4 Calyx lobe length [mm]
Length from the receptacle end to calyx end
2.2.5 Flower bud size [mm]
Length of 10 fully developed flower buds from 5 sample plants prior to anthesis
2.2.6 Flowering duration
Days from the first flower until 50% of the plants cease flowering

2.3 Fruits (pods)
2.3.1 Pod length [cm]
Mean length of 10 randomly selected pods; measured from peduncle stalk end to pod beak end
2.3.2 Pod weight [g]
Mean weight of 10 randomly selected pods
2.3.3 Locules/pod
Mean number of seed cavities in 10 randomly selected pods
2.3.4 Pods/peduncles
Mean number of pods from 10 peduncles from 5 sample plants at harvest
2.3.5 Pods/plant
Mean number of pods from 5 sample plants at harvest
2.3.6 Pod weight/plant [g]
Mean weight of total pods produced by the 5 plants constituting the sampling unit
2.3.7 Pod beak length [cm]
Mean length of 10 pods measured from the end of the last seed cavity to the tip of the pod

1 = Short	(0.10–0.79 cm)
2 = Intermediate	(0.80–1.24 cm)
3 = Long	(≥ 1.25 cm)

2.3.8 Grain filling period
Mean number of days from anthesis until 80% of the pod wall turns brown during the maturity/ripening period

2.4 Seeds
2.4.1 Seeds/pod
Mean number of well formed seeds in 10 randomly selected pods
2.4.2 Seed set percentage [%]
The mean ratio of seed number and loculi number/ pod multiplied by 100; measured on 10 randomly selected pods
2.4.3 Seed weight/pod [g]
Mean weight of seeds/pod from 10 randomly selected pods
2.4.4 Seed weight/plant [g]
Mean weight of seeds produced by the 5 sampling units
2.4.5 Shelling percentage [%]
The ratio of the seed weight/plant to pod weight/plant multiplied by 100
2.4.6 Seed metrics
Mean of measurements on 10 seeds selected in replicates from seed lots
2.4.6.1 Seed length [mm]
Distance measured between the two ends of the seed, parallel to the hilum (see Fig. 10)

Fig. 10. Seed length (SL)

2.4.6.2 Seed width [mm]
Distance on the seed measured from hilum to the keel (see Fig. 11)

Fig. 11. Seed width (SW)

2.4.6.3 Seed thickness [mm]
Distance on the seed measured perpendicular to the seed length (see Fig. 12)

Fig. 12. Seed thickness (ST)

2.4.7 100-seed weight [g]
Mass of 100 randomly selected seeds taken from total seed yield in replicates
2.4.8 Seed-volume [cm^3]
Volume of 100 randomly selected seeds in 94% ethanol
2.4.9 Grain yield [kg/ha]
Weight of dried seeds (at 12% moisture content)

2.5 Tubers
2.5.1 Number of tubers
Mean number of tubers produced/plant; 5 plants as sampling unit
2.5.2 Tuber weight [g]
Mean weight of tubers produced/plant; 5 plants as sampling unit
2.5.3 Length of tubers [cm]
Mean of the longest 5 mature tubers measured from the crown to the tip
2.5.4 Width of tubers [cm]
Mean of the broadest circumferences of 5 mature tubers
2.5.5 Tuber length to width ratio
The proportion of the tuber width to its length
2.5.6 Tuber fresh yield [kg/ha]
Total weight of harvested tubers; calculated on 10 plants at harvest

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