Genetic Variability and Diversity Analysis in Pod and Seed Characters of Some Neglected and Underutilized Legumes (NULs)

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors JOA and TPO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KMP, JAI and AII managed the analyses of the study. Author ADAS managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT

In response to the paucity of information challenge on the neglected and underutilized legumes, this paper explored pods and seeds morphological data of the twenty-four accessions of these crops with a view to establishing the occurrence of genetic variability and diversity analysis among the studied taxa. Twenty-four accessions of neglected and underutilized legumes (NULs) obtained from International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria were assessed for genetic variability and diversity analysis through Pod and seed morphological characters. Each accession was planted into plot of 5 ridges of 5 meter long, spaced 1 meter apart and replicated three times at the teaching and research farm of the Federal University Oye Ekiti, Ekiti State, Nigeria. Descriptive
1. INTRODUCTION

Neglected and underutilized legumes are so called because of their existence in a wide range of diversity as cultivated or wild species across various regions of the world and majorly cultivated by traditional farmers [1,2,3,4,5,6]. Different regions of the world cultivate different forms of these crops (NULs). For example, African yam bean (Stenophyllis stenocarpa), bambara groundnut (Vigna subterranea (L.) Thouars), lablab bean (Lablab purpureus), lima bean (Phaseolus lunatus) and the pigeon pea (Cajanus cajan (L.) DC) are the commonly cultivated species of these legumes in sub-sahara Africa [6].

The neglected and underutilized legumes have unusual characteristics that differentiate them from the common pea. These peculiarities are reflected in their growth habit, fruiting pattern, seed sizes and their utilities [7]. They also have special significance in agriculture because of their ability to enrich the soil through symbiotic nitrogen fixation [8,9].

 Genetic variability is either the presence of or the generation of genetic differences and could be explained as the formation of individuals differing in genotypes or the presence of genotypically different individuals in contrast to environmentally induced differences which has a rule cause only temporary non-heritable changes of the phenotype [10]. Genetic variability in a population is important for biodiversity [11].

 Genetic diversity deals with the total number of genetic characteristics in the genetic make-up of a species [12,7]. Its data in a given species allows the plants to adapt to various environmental conditions, such as fluctuation in climates and soil conditions. In addition, genetic diversity in a population of plant species, including cultivars, accessions, landraces and wild individuals is a crucial resource for increasing food production and for development of sustainable agricultural practices [13].

Until recently, neglected and underutilized legumes are group of crops which have been lightly esteemed and ignored by research, technology, marketing systems and conservation; although their cultivation and consumption serve as livelihood options for the poor [14,15]. There is still paucity of information on these crops (NULs), despite the fact that a great number of people make a living on them [16,15]. Hence, this current study is aimed at exploring pods and seeds morphological data of the twenty-four accessions of neglected and underutilized legume species with a view to establishing the occurrence of genetic variability diversity analysis among the studied taxa. Genetic diversity data is useful for crop improvement and breeding plan [17,9,6].

2. MATERIALS AND METHODS

2.1 Seed Acquisition

Seeds of 24 accessions of twelve species of miscellaneous legumes were obtained from the Genetic Resources Unit of the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo-state, Nigeria for screening.

2.2 Seed Cultivation

Each accession was planted into plot of 5 ridges of 5 meter long, spaced 1 meter apart and replicated three times at the teaching and research farm of the Federal University Oye Ekiti, Ekiti State, Nigeria. Initially, two seeds were planted per hill and later thinned to one plant per hill, to give a total of twelve plants per accession per plot. Three replicates of the plots were prepared in the two locations.

Data were taken on germination rate and the number of seeds that germinated. The plants were sprayed at one week interval with karate at a concentration of 0.5% starting from the period of flower bud initiation to pod maturity. The experimental plots were kept clean by weeding with hand hoes and cutlasses throughout the period of the study.
2.3 Pod and Seed Characters

The qualitative pod and seed characters scored include pod colour, pattern of pigmentation on green pods, pod texture, seed coat colour, seed coat texture etc. Quantitative characters measured include pod length, pod width, number of pods per peduncles, numbers of locules per pods, number of seeds per pod, seed length, seed width and 100-seed weight.

Ten measurement of each quantitative character were taken with the aid of graduated meter rule in centimeters from ten randomly selected samples of each accession and their mean values were calculated and recorded. Photographs of the pods and seeds of the accessions studied were taken.

Data were generated from five individual plants within the row of each accession. Fourteen morphological (quantitative and qualitative) characters were scored on each of the miscellaneous legume accessions. Quantitative characters were determined by measurements in centimeters and counting. The qualitative characters which were determined visually were scored by nominal codes. No descriptors had been developed for most miscellaneous legumes yet; however, descriptors for cowpea were used as guide to develop the descriptor list for the present morphological characterization. Duncan multiple range tests were employed to analyze the similarities and differences in the mean values of the quantitative characters.

The multivariate statistical method employed were principal component analysis (PCA) and cluster analysis (CA). The principal component analysis produced vector loadings for variables on principal component (PC) axes while cluster analysis yielded dendrogram. Pearson correlation coefficient was employed to identify the dependence of characters on one another.

3. RESULTS

The accessions of various NULs studied showed considerable intra and inter-specific variability in the pod and seed characters (Table 1). Number of pods per peduncle ranged from 7.60 cm in TLn 29 to 1.30 cm both in TVsu 1415 and TKg 6. Days to pod maturity was in TPTu 1 (145.70 cm) and Iwest in TVn 1 (75.10 cm). The pod length ranged between 29.86 cm and 1.11 cm TCG 1 and TKg 6 respectively, while the pod width ranged between 4.11 cm 0.36 cm in accessions TCG 1 and TCE 3 respectively. TKg 6 and TKg 12 produced the lowest number of locules per pod (1.00 cm) while the highest number of locules per pod and seed per pod were 14.70 cm and 14.40 cm in TPTu 18. Seed length also ranged from 2.76 cm to 0.40 cm in TCG 4 and TVR 1001 while seed width ranged from 1.92 cm in TCG 1 to 0.33 cm in TVR 145 and TVR 1001.

3.1 Principal Component Analysis

Table 2 presents the Eigen values, percentage variance of the principal component (PC) axes and the Eigen values of nine pod and seed morphological traits.

These out of the nine PC axes had Eigen values >0.1 percentage variances reduced from PC1 to PC9 in a progressive manner: percentages of the total variance within the first three PC axes were 46.04, 26.26 and 11.65 respectively. The Eigen values for each the first two PC-axes were >2.0 and explained 72.3% of the total variation.

In Table 2, pod and seed morphological traits with Eigen values >0.2 were considered significant in their contribution to loading each PC-axis. Five characters namely, pod length, pod width, seed length, seed width and 100-seed weight of the taxa loaded PC1; all made positive contribution. None out of the five characters except pod length re-featured in PC2. Characters such as number of pod per peduncle, number of locules per pod, number of seed per pod; all featured in PC2 with the day to pod maturity which made negative contribution to the loading.

Fig. 1 is indicative of degree of relatedness and diverseness among the 12 species of 24 accessions of the miscellaneous legumes based on their pod and seed characters presented as a two dimensional spatial configuration from the first two principal axes. The configuration explained about 72% of the total variation among the species and accessions. Approximately, six distinct sub specific clusters were identifiable from the configuration. The accessions of Canavalia gladiata (coded 1&2) appeared to distinct themselves from all other accessions and fell loosely at the right place. Accessions coded 22, 22; 9, 10; 11, 12 (TVR 145, TVR 1001, TVa1173; Tvm12, Tvm13), respectively formed a cluster and fell at the last plane. The two accessions of the Mexican yam bean (Pachyrhizus tuberosus) coded 3 and 4 appeared not to be much close based on pod and seed behaviors.
| Scientific name | Common name | Accession number | DPM  | NPP  | PODL | PODW | NLPP | NSPP | SL   | SW   | 100WT |
|-----------------|-------------|------------------|------|------|------|------|------|------|------|------|--------|
| Canavalia gladiata | Sword bean | TCg1 | 102.20 | 2.40 | 50.86 | 4.11 | 9.20 | 9.20 | 9.20 | 1.92 | 311.10 |
| Canavalia gladiata | Sword bean | TCg4 | 98.80 | 2.60 | 50.86 | 3.43 | 9.20 | 9.20 | 9.20 | 2.67 | 311.20 |
| Pachyrhizus tuberosus | Mexican yam bean | TPtu1 | 145.70 | 2.90 | 6.25 | 1.03 | 4.00 | 3.40 | 1.03 | 0.98 | 24.10 |
| Pachyrhizus tuberosus | Mexican yam bean | TPtu5 | 146.40 | 2.80 | 6.26 | 1.00 | 4.00 | 3.40 | 0.71 | 0.98 | 23.10 |
| Psophocarpus tetragonolobus | Winged bean | TP12 | 123.70 | 3.40 | 15.14 | 2.70 | 14.50 | 14.00 | 0.71 | 0.63 | 31.02 |
| Psophocarpus tetragonolobus | Winged bean | TP18 | 116.70 | 3.80 | 13.96 | 2.50 | 14.70 | 14.10 | 0.77 | 0.97 | 30.73 |
| Canavalia ensiformis | Jack bean | TCc1 | 116.90 | 1.60 | 23.25 | 0.40 | 8.40 | 8.30 | 1.95 | 1.26 | 122.03 |
| Canavalia ensiformis | Jack bean | TCc3 | 118.40 | 1.96 | 23.25 | 0.36 | 8.10 | 8.00 | 1.93 | 1.28 | 120.99 |
| Vigna angularis | Rice bean | TVa1 | 75.10 | 3.90 | 9.60 | 0.40 | 11.00 | 8.30 | 0.70 | 0.44 | 7.45 |
| Vigna angularis | Rice bean | TVa1173 | 75.10 | 3.90 | 9.41 | 0.48 | 8.56 | 8.10 | 0.59 | 0.50 | 4.46 |
| Vigna mungo | Mung bean | TVm12 | 78.60 | 3.40 | 5.18 | 0.50 | 8.50 | 8.10 | 0.59 | 0.50 | 4.46 |
| Vigna mungo | Mung bean | TVm13 | 78.60 | 3.40 | 5.19 | 0.46 | 9.00 | 7.00 | 0.57 | 0.41 | 4.09 |
| Lablab purpureus | Lablab bean | TLn21 | 103.20 | 6.70 | 4.30 | 1.71 | 3.80 | 3.50 | 0.89 | 0.71 | 16.58 |
| Lablab purpureus | Lablab bean | TLn2 | 103.44 | 7.60 | 4.24 | 1.79 | 3.80 | 3.60 | 0.88 | 0.71 | 16.75 |
| Sphenostylis stenocarpa | African yam bean | TSs137 | 136.40 | 3.90 | 20.78 | 1.05 | 7.00 | 6.70 | 0.88 | 0.71 | 41.14 |
| Sphenostylis stenocarpa | African yam bean | TSs156 | 143.70 | 4.00 | 20.51 | 1.20 | 7.06 | 7.50 | 0.89 | 0.81 | 37.20 |
| Vigna subterranea | Bambara groundnut | TVsu1126 | 123.40 | 1.40 | 1.16 | 1.02 | 1.00 | 1.00 | 1.39 | 0.81 | 59.03 |
| Vigna subterranean | Bambara groundnut | TVsu1415 | 125.10 | 1.40 | 1.17 | 0.99 | 1.00 | 1.00 | 0.98 | 0.91 | 61.89 |
| Kerstingiella geocarpa | Kerstingiella geocarpa | TKg6 | 127.80 | 1.30 | 1.11 | 0.99 | 1.00 | 1.00 | 0.99 | 0.71 | 23.32 |
| Kerstingiella geocarpa | Kerstingiella geocarpa | TKg12 | 128.00 | 1.30 | 1.16 | 1.00 | 1.00 | 1.00 | 0.96 | 0.71 | 24.16 |
| Vigna radiata | Green gram | TVr45 | 88.30 | 3.10 | 8.68 | 0.40 | 13.80 | 13.70 | 0.42 | 0.33 | 24.16 |
| Vigna radiata | Green gram | TVr1001 | 89.30 | 3.30 | 9.87 | 0.40 | 13.00 | 12.90 | 0.40 | 0.33 | 3.61 |
| Cajanus cajan | Pigeon pea | TCc8127 | 120.60 | 1.40 | 5.47 | 0.80 | 3.00 | 2.80 | 0.70 | 0.51 | 8.02 |
| Cajanus cajan | Pigeon pea | TCc8156 | 119.70 | 1.40 | 5.74 | 0.80 | 2.80 | 2.70 | 0.80 | 0.50 | 7.51 |
| Total mean | | | 112.08 | 3.00 | 10.84 | 10.84 | 7.81 | 6.79 | 1.02 | 0.80 | 54.21 |

Legend: DPM- Days to pod maturity, NPP- Number of pod per peduncle, PODL- pod length, PODW- pod width, NLPP-Number of locules per pod, NSPP-Number of seed per pod, SL-Seed length, SW – Seed width, 100W – 100 Seed weight
Table 2. Eigen values, variance proportion of five pc-axes and eigenvectors of nine pod and seed characters

| Morphological traits | Eigenvectors | Eigenvectors | Eigenvectors | Eigenvectors | Eigenvectors |
|----------------------|--------------|--------------|--------------|--------------|--------------|
| Days to pod maturity | 0.049        | -0.249       | -0.464       | 0.829        | -0.001       |
| Number of pod per peduncle | -0.056     | 0.300        | 0.714        | 0.452        | 0.389        |
| Pod length            | 0.420        | 0.203        | -0.189       | 0.086        | 0.471        |
| Pod width              | 0.383        | -0.028       | 0.374        | 0.250        | -0.703       |
| Number of locules per pod | 0.130    | 0.608        | -0.204       | 0.001        | -0.137       |
| Number of seeds per pod | 0.138       | 0.601        | -0.225       | 0.018        | -0.147       |
| Seed length            | 0.455        | -0.179       | 0.021        | -0.147       | 0.269        |
| Seed width             | 0.452        | -0.184       | 0.084        | -0.067       | 0.110        |
| 100 seeds weight       | 0.473        | -0.091       | 0.021        | -0.113       | -0.086       |

NB: Eigenvector ≥ 0.2 are in bold

However, the two accessions of Bambara groundnut (Vigna subterranea) and one of Kersting groundnut (TKg6) (Kerstingiella geocarpa) appeared very closely related. This further lends credence to similarities observed in the clustering patterns of vegetative and floral characters.

3.2 Cluster Analysis

The quantitative Dendrogram obtained from the cluster analysis for pod and seed characters on the 12 species of 24 accessions of the miscellaneous legumes is presented in Fig. 2. The analysis also produced 23 morphotypes or clusters; the cluster history is represented in Table 2. The two accessions (TKg 6 and TKg 12) were the most similar phenotypically based on the nine pod and seed morphological characteristics employed to discriminate among the taxa. They both had the least distance 0.0095 (Fig. 1). The diversity of the 24 accessions spanned a distance between 0.009 and 2.0758 (Fig. 1). All the accessions were also unique at the inflection point of zero (Fig. 2) and became a single subfamily entity of Pappilionoideae at approximately 2.2 similarity level distance. Four main clusters were noticeable 0.50 level of similarity (Fig. 2). Pods and Seeds characters clustered all the Vigna species together at nearly the same similarity level. In fact, all accessions of the same species were grouped accordingly with the two accessions of Pachyrizus tuberosus (TPtu 1 and TPTu 5).

4. DISCUSSION

Occurrence of variability in organisms gives assurance of evolutionary survival and the possibilities to improve species on significant characters. Variation data is a guide for selection, which is a tool for effective classification; hence knowledge of the within species variation is a fundamental track to effective classification and improvement [18, 9]. The characters examined differentiated accessions of the NULs evaluated. Variation in qualitative characters revealed different genetic basis for the expressed phenotypic traits among the accessions. The quantitative characters showed wide range of differences by high coefficient of variation in the mean values among the species; indicative of wide genetic variability.
Fig. 1. Two dimensional spatial configurations of the twelve species of twelve-four accessions of the miscellaneous legumes plotted by vectors from the first two PC-axes according to their pod and seed characters.

Fig. 2. Dendrogram showing grouping of the 24 accessions of the NULs based on their pod and seed characters.
Principal Component Analysis (PCA) revealed pod length, pod width and number of seed per plant as phenotypic characters with significant contribution to detecting variation among the studied accessions. Agreement between the PCA and the cluster analysis lends credence to the existing variability among the NULs species and accessions, justifying their classification.

Variability in pod and seed characters observed include days to pod maturity, number of pod per peduncle, pod length, number of locules per pod, number of seeds per pod, seed length, seed width and weight of one hundred seeds. Accessions of the same species recorded closer values for both qualitative and quantitative characters; indicating more intra-specific relatedness. This corroborates [19,9]. Similarity in pod and seed types was common among subspecies. For example, all the Vigna species had smooth and medium seed type; the two species of Canavalia possessed straight and elongated pod while the two accessions of Kerstingiella geocarpa shared features in common with Vigna subterranean. These similarities are pointers to their classification as sub family Pappilionoideae. Statistical analyses of mean values for each ten measurements taken at random per plant also confirmed similarities the quantitative characters studied. The number of locules per pod correlated positively with the number of seeds per pod which might possibly account for high seed set percentages in all the species and accessions. The two accessions each of Canavalia gladiate, Canavalia ensiformis, Sphenostylis stenocarpa and Psophocarpus tetragonolobus showed very good performance in some agronomic traits, having values very high in pod length, number of locules per pod and number of seeds per pod.

Dendrogram revealed broad groupings resulting in fewer groups than the original number of species and accessions studied; which enabled drawing an easy and logical reference. The observed variability observed could be concluded to be basically genetic since the plants were raised under the same environment. Our observation is in line with [4,19,6].

5. CONCLUSION

Though this is a sectional study on the genetic variability and diversity analysis among the study accessions, results clearly indicate variability in pod and seed characters useful for maintenance of diversity, conservation and future breeding work. Understanding of variation and its pattern among crop’s germplasm is crucial for crop improvement. This study has undertaken degrees of morphological variation in pods and seeds characters of the twenty-four accessions of twelve species of these legumes and also evaluated the relative importance of both types of traits in their variability determination. The use of combined / additive characters of the species and newer genetic markers such as GBS and SNP will enhance the understanding of the genetic diversity available among the species valuable for genetic manipulation and breeding of better quality variants in terms of nutrition and yield.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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