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Full Length Research Paper

# Genetic differing qualities in Tepary bean (Phaseolus acutifolius) landraces developed in Botswana

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**A field experiment was conducted at Department of Agricultural Research in Sebele in the 2012 to 2013 season using nine accessions that were sourced from the National Plant Genetic Resource Centre (NPGRC), Gaborone, Botswana. Multivariate statistical procedures such as clusters and principal component analysis were used on 15 selected characters to assess agro-morphological variability among tepary bean landraces collected in Botswana. Few characters were statistically significant which suggest lower genetic diversity among the Botswana tepary beans. The first three PCA accounted for 77.12% of accumulated variation. Traits which revealed significant contribution to variation among accessions were number of leaves, plant spread, pod width, 100 seed weight and seeds per pod. The dendrogram results also showed that these characters contributed significantly to the grouping of accessions into three clusters. Three accessions GK011, MTS (Motsumi) and GK012 were separated from the rest of the accessions. However, GK012 and MTS (Motsumi) with highest number of valuable traits are recommended for plant breeders to use as parents in future breeding programs.**

**Key words:** Tepary bean, agro-morphological traits, dendrogram, principal components analysis, multivariate analysis.

## INTRODUCTION

The cultivated tepary bean (*Phaseolus acutifolius* A. Gray) is a short life cycle legume originally from the deserts and semi-arid environment of northwestern Mexico and southwestern United States (Nabham and Felger, 1978). It is recognized for its resistance to heat, drought and many diseases (Salgado et al., 1994; Miklas and Stavely, 1998; Rao et al., 2013). These characteristics make it an ideal crop in parts of tropical America, the Caribbean and Africa (Porch et al., 2013) equally so important for Botswana with a semi-arid environment. The crop has no established varieties in Botswana therefore farmers are using landraces which are usually low yielding. Since few famers are involved in planting tepary bean, the development of new varieties could potentially encourage the growing of this crop. The crop is grown in Africa and Middle Eastern countries (Tinsley et al., 1985) where the seeds provide high protein good for human nutrition.

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**Table 1.** Tepary bean landraces collected from different villages from two agricultural regions of Botswana.

| Entry | Accession | Collection sites | Lat. | Long. | District |
|---|---|---|---|---|---|
| 1 | MTS (Motsumi) | Mahalapye | 23.108 | 26.823 | Central |
| 2 | GK010 | Machaneng | 23.185 | 27.483 | Central |
| 3 | GK012 | Sefophe | 22.182 | 27.961 | Central |
| 4 | E70 | Tutume | 20.493 | 27.018 | Central |
| 5 | GK011 | Kubung | 24.649 | 25.303 | Kweneng |
| 6 | E105 | Machaneng | 23.185 | 27.487 | Central |
| 7 | GK013 | Thamaga | 24.678 | 25.531 | Kweneng |
| 8 | E89 | Kgope | 24.310 | 25.940 | Kweneng |
| 9 | E19 | Mahetlwe | 24.242 | 25.684 | Kweneng |

In Botswana it is commonly known as 'Dibonkise'. It is grown by small scale farmers mainly as a **s**ource of food while the haulms are used as feed for animals. A brief survey by Karikari et al. (1995) in Botswana discovered that its production is lower than that of cowpeas, groundnut and bambara groundnut. Its relatively high protein content recorded at 24% compares well with other *Phaseolus* spp. (Bhardwjal and Hamama, 2004). Tepary beans together with other three underutilized legumes (bambara groundnut, morama bean and mungbean) were assessed for protein and mineral composition. It was found that they are a good source of protein with great potential as food crops which could contribute to improving food security in Botswana (Amarteifio and Moholo, 1998). In addition it is a high value crop as it equally fetches money such as other grain legumes through the Botswana Agricultural Marketing Board (BAMB, 2013).

Tepary bean possesses considerable variability for yield and yield related traits (Kuruvad and Valdez, 1993; Bhardwj et al., 2002) and is superior in drought tolerance (Mohamed et al., 2005). Compared to common bean, tepary bean was shown to be superior in combining desirable traits that makes it well adapted to drought stress (Markhart, 1985). Tepary bean is a useful genetic donor of important traits such as disease, pest and stress tolerance to improve common beans (*P. vulgaris*) (Schinkel and Gepts, 1988). Little research has been conducted in Botswana environment to ascertain all the useful characteristics of tepary bean crop.

In the US, at least two varieties (TARS-Tep 22 and TARS-Tep 32) have been developed and are available to farmers especially in the production zones prone to abiotic and biotic stress (Porch et al., 2013). In Botswana the crop has not received much attention compared to other legumes probably due to low number of farmers growing the crop. Knowledge of phenotypic diversity of tepary bean accessions grown can be employed in crop improvement and in developing breeding lines (Mohammadi and Prasanna, 2003). In order to improve the use of tepary bean, it is necessary to gain an understanding of its genetic attributes (Schinkel and Gepts, 1988). Lack of information on the genetic diversity of tepary bean has led to poor exploitation of its genetic resources. Therefore the objectives of the project is to study the morphological variability of Botswana tepary bean landraces in order to generate additional information to improve their utilization, and to identify accessions with potential to be exploited by plant breeders.

## MATERIALS AND METHODS

The experimental materials for this study comprised nine tepary bean (*P. acutifolius*) accessions that were sourced from the Botswana National Plant Genetic Resource Centre (NPGRC) and originally from nine different villages and two agricultural regions of the country (Table 1). Fifteen agro-morphological characters were used to assess the variability of the accessions: Plant height (PH), number of leaves (NL), leaflet width (LW), leaflet length (LL), plant spread (PS), number of branches (NB), pods per plant (PPP), pod length (PL), pod width (PW), seeds per pod (SPP), pod weight per plant (PWP), seeds per plant (SPP), 100 seed weight (100SW), shoot dry weight (SDW) and yield per $m^2$ (YIELD) (Table 2). The morphological and agronomic traits selected were chosen from International Board for Plant Genetic Resources, IBPGR (1985) for *P. acutifolius*. Similar traits were considered important for common bean breeding programs (de Lima et al., 2012). All accessions examined were of cream coloured seeds. One of the accessions (GK010) in this project had been sent to Vienna-Austria for mutation experiments.

The experiment was laid out in a randomized complete block design using two replications in the 2012 to 2013 cropping seasons at Sebele Agricultural Research Station. The accessions were sown by hand at a spacing of 75 cm between rows and 30 cm between plants and plot length of 5 m. No fertilizers were applied but, the crops were sprinkler irrigated once a week to ensure proper plant growth. In each accession five representative plants were selected randomly and used for biometric measurements. The agro-morphological mean data were standardized to give equal weighing. The values were used to perform multivariate statistical analysis, using Multivariate Statistical Package (MVSP) software (Kovach Computing Services, UK, 2006) and NTSYS-pc Numerical

**Table 2.** Mean, range and variance of nine tepary bean accessions assessed based on 15 morpho-agronomic characters.

| Accession | PH (mm) | NL | LW (mm) | LL (mm) | PS (mm) | NB | PPP(g) | PL (mm) | PW (g) | SPP (g) | PWP(g) | SPL | 100SW(g) | SDW (g) | YIELD (g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MTS(Motsumi) | 350 | 82 | 14 | 37 | 425 | 7 | 38 | 60 | 7 | 5 | 28 | 135 | 11.0 | 1.1 | 0.40 |
| GK010 | 317 | 77 | 14 | 37 | 356 | 6 | 40 | 61 | 8 | 4 | 24 | 139 | 15.5 | 1.8 | 0.47 |
| GK012 | 353 | 96 | 15 | 39 | 437 | 7 | 38 | 62 | 8 | 4 | 23 | 130 | 13.0 | 2.0 | 0.77 |
| E70 | 288 | 82 | 13 | 38 | 384 | 7 | 40 | 63 | 8 | 4 | 23 | 115 | 17.5 | 1.6 | 0.42 |
| GK011 | 261 | 45 | 13 | 36 | 236 | 5 | 17 | 62 | 7 | 4 | 13 | 65 | 14.0 | 0.6 | 0.41 |
| E105 | 281 | 74 | 14 | 38 | 341 | 6 | 28 | 61 | 8 | 4 | 18 | 95 | 11.5 | 2.2 | 0.52 |
| GK013 | 274 | 74 | 14 | 35 | 277 | 6 | 46 | 61 | 8 | 4 | 23 | 131 | 12.5 | 1.7 | 0.48 |
| E89 | 288 | 69 | 14 | 36 | 299 | 7 | 42 | 63 | 8 | 5 | 20 | 120 | 13.0 | 1.6 | 0.53 |
| E19 | 290 | 63 | 15 | 36 | 305 | 7 | 36 | 61 | 8 | 5 | 24 | 153 | 13.0 | 1.8 | 0.52 |
| **Mean** | 299 | 73 | 14 | 37 | 340 | 6 | 36 | 62 | 8 | 4 | 22 | 120 | 13.4 | 1.6 | 0.51 |
| **Minimum** | 212 | 44 | 12 | 33 | 160 | 5 | 13 | 57 | 7 | 4 | 10 | 49 | 11.0 | 0.6 | 0.33 |
| **Maximum** | 421 | 121 | 16 | 44 | 557 | 8 | 58 | 66 | 8 | 5 | 36 | 189 | 19.0 | 2.8 | 0.77 |

Plant height: **(PH),** No. of leaves : **(NL)**, Leaflet width: **(LW),** Leaflet length: **(LL),** Plant spread: **(PS),** No. of branches: **(NB)**, Pods per plant: **(PPP),** Pod length: **(PL),** Pod width: **(PW),** Seeds per pod **(SPP),** Pod weight per plant: **(PWP),** Seeds per plant: **(SPL),** 100 seed weight: **(100SW),** Shoot dry weight: **(SDW),** Yield $m^2$ : **(YIELD)**

Taxonomy and Multivariate Analysis (Rohlf, 2000). Analysis of Variance (ANOVA) was estimated to calculate the differences on traits using SAS 9.2 (2010) statistical package.

## RESULTS

A summary of the results for the mean, range and variances for the 15 characters are presented in Table 2. Number of branches per plant, 100 seed weight and number of seeds per pod were significant at P < 0.05% probability level while the rest of the characters were not significant. This is an indication of low genetic variability among the traits analyzed for the selected accessions. However, large ranges were observed among some traits, such as in yield $m^2$ (48 to 254 g), number of seeds per plant (49 to 189), number of leaves per plant (43 to 121) and pod weight per plant (10 to 36 g).

The results presented in Table 2, revealed that accessions GK012 had highest plant height (353 mm), number of leaves (96), plant spread (437 mm) and yield $m^2$ (178 g). Accession GK011 had the lowest plant height (261 mm), number of leaves (45), number of pods per plant (17), number of seeds per plant (65) and yield per $m^2$ (74 g) and exhibited a dwarf plant character. Based on cluster analysis (Figure 1) at a demarcated line of coefficient 0.97 the accessions were grouped into three clusters. Cluster 1 and 3 consists of single accessions MTS (Motsumi) and GK011 respectively. The rest of the accessions are grouped in cluster 2, but accession GK012 is separated from the rest of these accessions. The selected traits were not able to distinguish between (E89 and E19) and between (GK010 and E70). A higher genetic difference of 0.69 was observed between the accessions (E89 and E19) at 0.55 and 1.24 of GK011. However, generally a lower difference among most of the accessions was recorded (Figure 1). The dendrogram indicates that the population is mainly influenced by the characters with greater variability.

The Principal component analysis was performed to reveal the phenotypic diversity among the genotypes to identify characters that account for most of the variances. The first three principal components gave an accumulated total variation of 77.12% (Table 3). Axis 1 with 37.89% variability

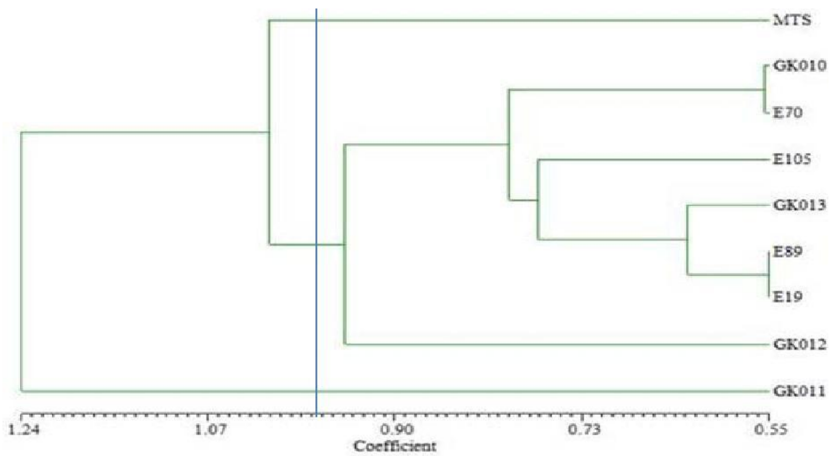


**Figure 1.** Dendrogram of nine tepary bean accessions showing genetic similarities based on 15 phenotypic traits, using the UPGMA cluster analysis. The test of association: Matrix correlation on NTSYS pc: (r = 0.88).

**Table 3.** Eigen values and the first three principal component axes in tepary bean diversity analysis.

| Parameter | Axis 1 | Axis 2 | Axis 3 |
|---|---|---|---|
| Plant height | 0.278 | -0.112 | 0.038 |
| No. of leaves | 0.347 | 0.078 | 0.013 |
| Leaflet width | 0.199 | -0.069 | -0.280 |
| Leaflet length | 0.170 | 0.160 | -0.048 |
| Plant spread | 0.301 | 0.005 | 0.087 |
| No. of branches | 0.345 | -0.171 | 0.135 |
| Pods per plant | 0.263 | 0.009 | 0.210 |
| Pod length | -0.041 | 0.150 | 0.135 |
| Pod width | 0.182 | 0.575 | 0.068 |
| Seeds per pod | 0.245 | -0.377 | 0.505 |
| Pods weight plant | 0.196 | -0.087 | 0.174 |
| Seeds per plant | 0.253 | -0.100 | 0.115 |
| 100seeds weight | -0.054 | 0.590 | 0.429 |
| Shoot dry weight | 0.294 | 0.176 | -0.263 |
| Yield $m^2$ | 0.248 | 0.159 | -0.004 |
| Eigen values | 2.832 | 1.787 | 1.148 |
| Percentage | 37.892 | 23.909 | 15.358 |
| Cum. Percentage | 37.892 | 61.800 | 77.158 |

had most contributions coming from no of branches (0.345), number of leaves (0.347) and plant spread (0.301). The second variate from axis 2 had higher contributions coming from pod width (0.575), 100 seed weight (0.590), and seeds per pod (-0.377). This indicates the importance of these characters in identifying tepary bean landraces. Principal coordinate analysis (PCoA) clearly demarcated landraces GK011 and MTS (Motsumi) from the rest of the accessions; it also distinguished GK012 from the rest of the accessions better than cluster analysis (Figure 2).

## DISCUSSION

In this study we describe for the first time the diversity of tepary bean landraces grown in Botswana. Few morphological characters, number of branches per plant, 100 seed weight and number of seeds per plant exhibit significant variation, which shows low levels of genetic

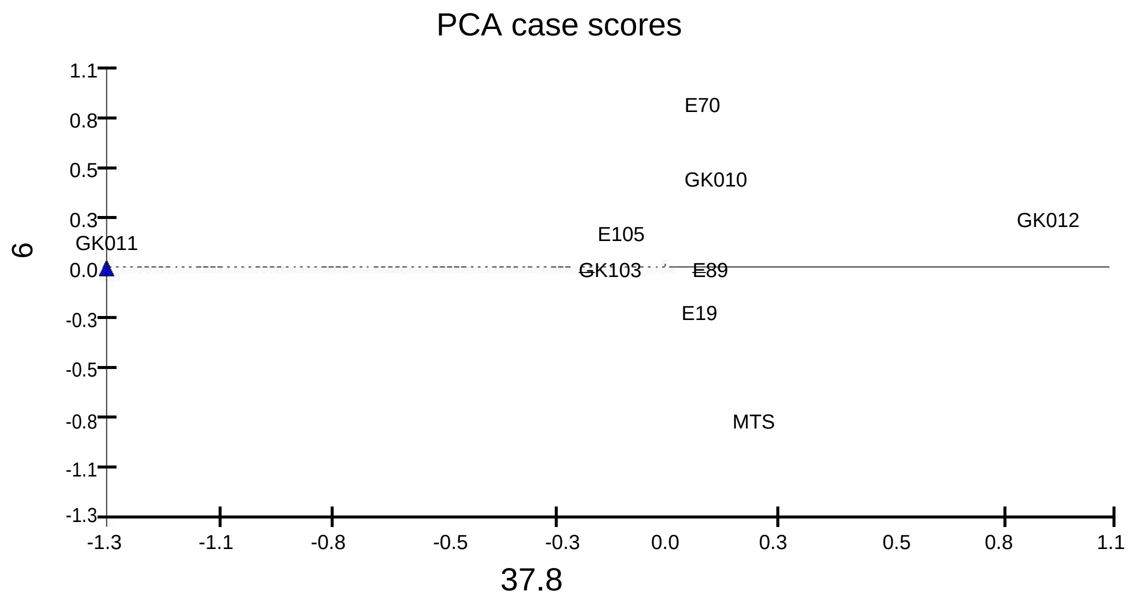


**Figure 2.** PCO scatter plot for nine tepary bean accessions grown in Botswana from MVSP program with a variation of 61.71%, with Axis 1 contributing 37.8% while Axis 2 explained 23.9%.

variation among the selected genotypes. Similar observations were revealed when using cluster analysis which exhibited lower differences among most of the accessions. The results generally are in accordance with the lower diversity in tepary bean observed by Schinkel and Gepts (1988) when analyzing Phaseolin among 55 wild and 8 cultivated teparies using polyacrylamide gel electrophoresis. Their results showed 15 electrophoretic Phaseolin patterns among wild forms and only one pattern in cultivars which they thought it suggest single domestication in this species.

Characters with greatest variation were yield per m $^{2}$, number of seeds per plant, number of leaves and podweight per plant. Similar results were observed in one important yield component in tepary bean of pods per plant with a range of (8.1 to 37.1) among sixteen accessions from five states in Mexico (Kuruvadi and Valdez, 1993). The greater variation appears to indicate that there is a potential for improvement of this crop.

The dendrogram (Figure 1) was divided into three clusters, but with a lower range among most accessions, which still shows a lower genetic diversity among the selected genotypes. The dendrogram is largely in agreement with the PCoA coordinates (Figure 2), which clearly demarcated accessions mostly on the major traits. Principal component analysis revealed those characters that are important in explaining the variation among the selected genotypes such as number of leaves, plant spread, pod width, 100 seed weight and seed per pod. Clusters can be separated mainly based on traits which contribute more variation as observed in barley (Abebe et al., 2010) and in rice (Moukoumbi et al., 2011). The clustering and scatter plots can also have a similar pattern as in pigeon peas (Manyasa et al., 2008) and in *Arachis pintoi* (Carvalho and Quesenberry, 2009).

Morphological character assessment is the first step in characterization of germplasm (Azam-Ali et al., 2001) usually breeding programmes relies on the magnitude of phenotypic variability in crops (Ghafoor et al., 2002). Morphological traits in this study were able to differentiate most of the accessions except in the case of E19 and E89, and E70 and GK10; this suggests that the accessions clearly resemble each other agronomically. Subsistence farmers in Botswana exchange seeds and these could be similar genotypes with different names. Presumably there could be some duplication which can be tested using molecular makers which are better placed to discern the accessions compared to morphological characters which are highly influenced by the environment (Smith and Smith, 1992; Hintum et al., 2000). From ten agricultural districts in Botswana the accessions were sourced from two districts (Table 1), different accessions could be discovered by the National Genetic Resource Centre when they collect more tepary germplasm to improve the genetic diversity. In general, our results revealed that the multivariate analysis used was able to differentiate the nine accessions based on the 15 characters selected. This gives an opportunity for further exploitation of the landrace since the characters with high importance in the characterization of tepary beans has been identified. However, the lower genetic diversity exposed in this study will require further addition of more materials. Currently the Department of Agricultural Research has sourced additional tepary bean lines from CIAT (Centro International de Agricultura Tropical) and a mutation project is on-going with the

Vienna-Sibersdorf laboratory. These initiatives could be useful in improving the diversity among Botswana tepary beans. An initiative has also been taken to source tepary bean multiple stress tolerant germplasm released by (Porch et al., 2012). The promising accessions such as GK012 and MTS (Motsumi) could be used as parental material by plant breeders.

## Conflict of Interest

The authors have not declared any conflict of interest.

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