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GENETIC DIVERSITY OF COMMON BEANS AS DETERMINED USING MORPHOLOGICAL MARKERS

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ABSTRACT

Different cultivars of common bean are grown in Lesotho for home consumption originating from Zambia, South Africa and America. These have created a wide genetic diversity and duplication such that it is not easy to distinguish them. The study was conducted in Lusaka to distinguish the same common bean cultivars using morphological markers and to estimate their degree of similarity. A collection of 42 cultivars were included in the study. Seventeen morphological characters were used following International Plant Genetic Resource Unit descriptor (1982). Data collected were analysed using principal component and cluster analysis. Principal component analysis was used to identify the characters which caused major variation among cultivars. Out of 10 principal components generated from 17 characters, only the first three components which constituted 54.57% of the total variation were considered for analysis. The first, second and third components accounted for 23.23%, 16.80% and 14.54%, respectively. The characters responsible for separation along the first principal component and loadings (parenthesis) were plant height (0.57), growth habit (0.55) and seed pattern (-0.27).The characters influencing separation along the second principal component include number of flowers per node (0.50), number of locules per pod (0.44), seed colour (0.44) and leaflet length (0.30). Along the third principal component, cultivars were separated according to the pod colour (0.64) and flower colour (0.14).Thirty-five individual cultivars and two groups of cultivars were distinguished by cluster analysis. One group consisted of three cultivars from Zambia, while the other group consisted of two cultivars from Zambia and two from Lesotho.

Keywords: Common bean, morphological markers, principal component analysis, cluster analysis

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a leguminous food crop grown world-wide and consumed as grain, green pods or fresh vegetable. It is a rich source of protein affordable by many people and grown in almost every part of the world (Romero-Arenas et al., 2013). Moreover, the nutritional value and variable uses of dry beans in various forms appeal to both processors and consumers alike (Blair et al., 2010). According to botanical, archaeological and molecular markers, common beans originated from two gene-pools, namely; Mesoamerican and Andeans (Raggi, 2013). The third gene-pool was later discovered in the northern Andes (Debouk, 1999; Tohme et al., 1996). The route of dissemination from their origin and domestication spread to Europe, and then Africa where these were grown under varying environmental and agronomic situations (Chacon et al., 2005; Wortmann et al., 2004). These domesticated common beans further underwent evolution resulting in seven sub-groups, three in the Andean and four in the Mesoamerican (Perseguini et al., 2011; Singh et al., 1991b). In some regions, farmers have maintained common bean landraces. However, in most cases traditional cultivars have progressively been replaced with hybrids that give a high yield, resistance to diseases and pests, and most importantly income to meet farmer's needs. As a result, farmers use landraces, traditional cultivars and hybrid seeds. Similarly, a large number of different landraces, cultivars and hybrids are grown in Lesotho at both house-hold and national levels resulting in a wide genetic diversity which is not easily distinguished due to close genetic relationship. New varieties have been imported from South Africa, Southern African Development Community countries and United States of America to meet country requirements such as high yield, short maturity and high protein

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content. Some of these imported cultivars may be duplication; therefore it was imperative to identify all cultivars used in the country. Morphological markers have been used for a long time by farmers, agronomists and plant breeders as an effective method of identifying cultivars (Stoilova et al., 2013). Plant parts such as leaves, seeds, flowers and stems are good indications of variations.

In Lesotho, Malawi, Mozambique, Tanzania and Zambia, no attempt has been made to characterize common beans and estimate the degree of relationship among the cultivars (Wortmann et al., 2004). Nonetheless, in Bulgaria (Stoilova et al., 2013), Greece (Mavromatis, 2010), Italy (Bonnetti et al., 1995), Latin America (Singh et al., (1991) and Slovenia (Sustar-Vozlic and Maras, 2006), common bean cultivars have been characterized using morphological characters. The study attempts to provide a model that could be used to distinguish common bean cultivars and other crop species grown in Lesotho. The objective of the study was therefore to distinguish common bean cultivars using morphological markers and to estimate their degree of similarity.

MATERIALS AND METHODS

Site description

The study was conducted at the School of Agricultural Sciences Field Station, University of Zambia, Lusaka. The Field Station is located at 1140m above sea-level, at latitude 28^0 20'E and longitude 15^0 22' S. The soil is fine, loamy mixed iso-hyperthermic typic paleustalf. Soil analysis was carried out and the following results were obtained; pH ($CaCl_2$) 6.6, nitrogen 0.10%, phosphorus 26.18mg/kg, potassium 9.8mg/kg, 8.88mg/kg, calcium 14.20me/100mg and iron 9.28mg/kg.

Seed bed preparation

The land was prepared with rotavator which pulverized the soil to a fine tilt to facilitate germination, and the seed bed was levelled using hand-hoes. A compound fertilizer containing 10% nitrogen, 20% phosphorus, 10% potassium and 10% sulphur was applied as a basal dressing on the seed –bed at the rate of 300kg ha^{-1} (Welling, 1988). No top-dressing was applied.

Experimental design

Randomized Complete Block Design was used to lay-out the plots. The land was divided into forty-two plots, each measuring 2.4m x 1.2 m. Within each plot, there were four rows with inter-row and intra-row spacing of 60cm x 10cm, respectively resulting in a plant population of 166 000 plants ha^{-1}. Weeding was done twice to eradicate nutsedge (*Cyperus esculentum* L.) which was a serious problem at the site. Blister bettles (*Mylabris cinchorii*) were observed feeding on the flower at flowering stage. These were controlled with endosulfan mixed at a ratio of 15ml to 20 l of water.

Data collection

The descriptor of *Phaseolus vulgaris* L. documented by International Board of Plant Genetic Resource (1982) was used to characterize the cultivars. In the plots with four rows, the two middle rows were chosen for data collection. Five plants were randomly selected and tagged. All data were collected from the tagged plants. The following characters were recorded; height of stem from the soil surface to the tip of the apical meristem, length of the terminal leaflet on the third trifoliate leaf, growth habit, colour of the stem, colour of the flower, days from emergence to 50% flowering, number of flower buds per node, length of pod, shape of pods, locules per pod, cross-section, colour of the pod, curvature of the pod, pod suture strings, seed marginal colour, seed coat pattern, dark colour of the seed coat and seed shape.

Data analysis

A Genstat software package was used to generate multivariate analysis. Two analyses were performed, namely; (i) principal component analysis to reveal the pattern of morphological variation within the common bean cultivars, and (ii) cluster analysis to group phenotypically similar cultivars and separate dissimilar ones.

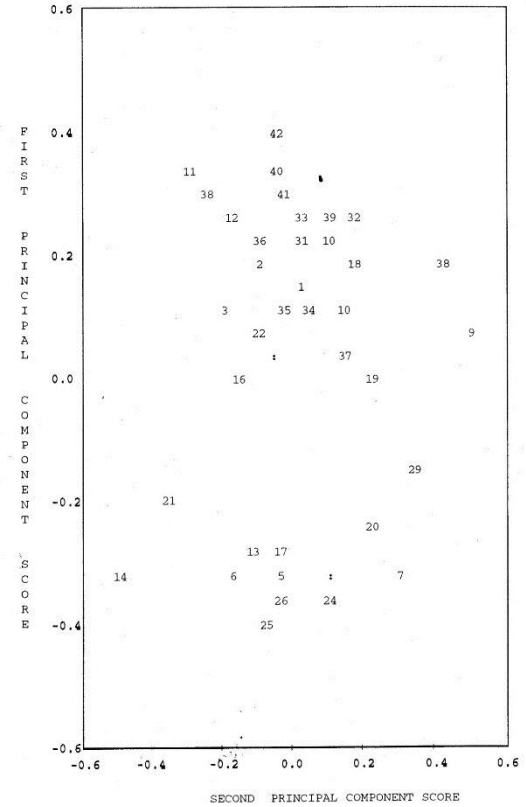

Fig. 1. Positions of Phaseolus vulgaris. L. genotypes on the first and second principal component scores based on morphological markers.

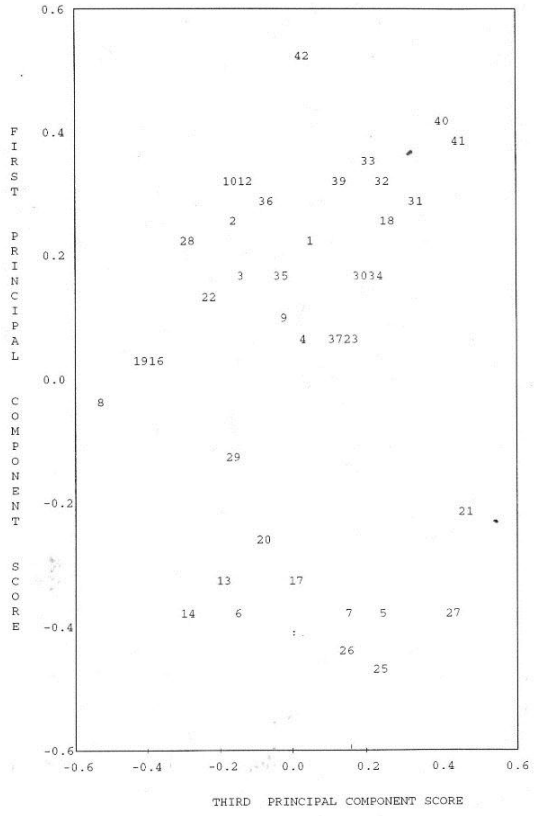

Fig. 2. Positions of Phaseolus vulgaris. L. genotypes on the first and third principal component scores based on morphological markers.

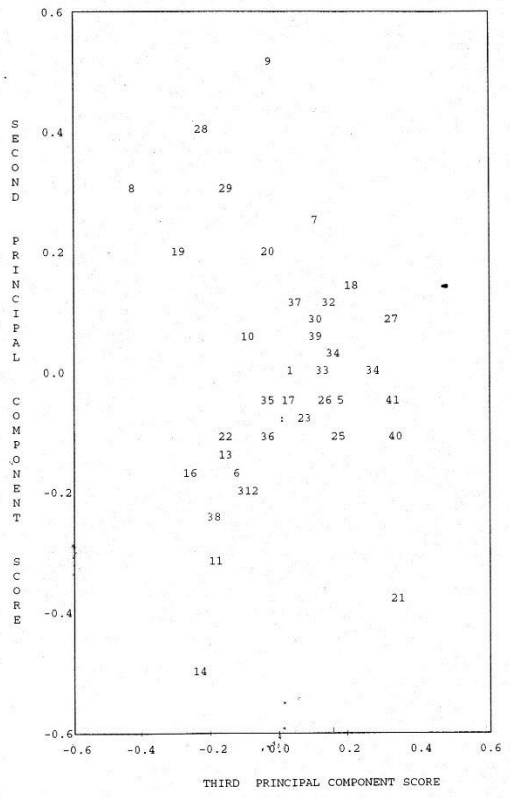

Fig. 3. Positions of Phaseolus vulgaris. L. genotypes on the second and third principal component scores based on morphological markers.

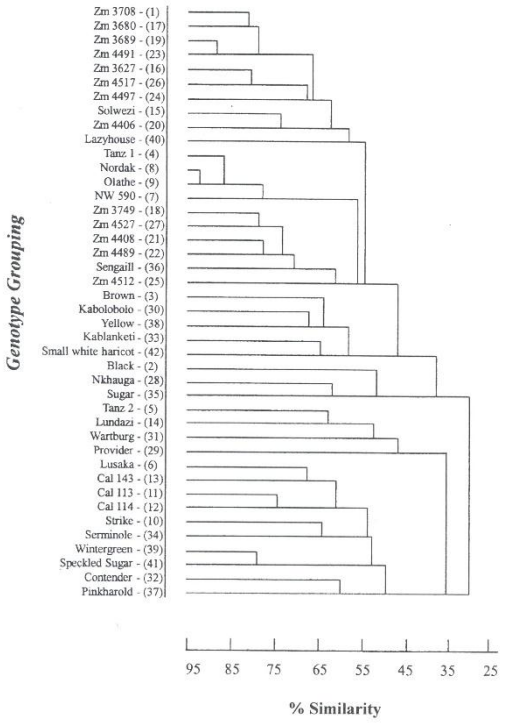

Fig. 4. Cluster analysis of Phaseolus vulgaris L. Genotypes based on morphological markers.

RESULTS

Principal component analysis

Principal component analysis was used to identify the characters which caused major variation among cultivars. Out of 10 principal components generated from 17 characters, only the first three components which constituted 54.57% of the total variation were considered for analysis. The first, second and third components accounted for 23.23%, 16.80% and 14.54%, respectively. The characters responsible for separation along the first principal component and loadings (parenthesis) were plant height (0.57), growth habit (0.55) and seed pattern (-0.270). The characters influencing separation along the second principal component include number of flowers per node (0.50), number of locules per pod (0.44), seed colour (0.44) and leaflet length (0.30). Along the third principal component, cultivars were separated according to the pod colour (0.64) and flower (0.14).

By means of the first and second principal component score, two major groups of cultivars according to growth habit and plant height emerged, namely; semi-climber and bushy types. However, there were some outliers such as Provider, Nordak, Olathe, Nkhaunya, Zm 4408, Lundazi and an extreme such as Small White Haricots. Semi-climbers consisted of 23 cultivars, most (79%) of which were obtained in Zambia (Fig.1).

The first and third principal component scores exhibited cultivars which were clustered into two groups, except outliers such as Small White Haricot, Nordak, Provider and Zm 4408. The clustering of cultivars indicated that there was a very low variability in pod colour, plant height and growth habit (Fig.2).

The second and third principal component scores produced one cluster of cultivars indicating that most of the cultivars shared similar number of flowers per node, pod colours and number of locules per pod, and variation in the characters is low. However, there were some outliers such as Lundazi, Zm 4408, Cal 113, Olathe, Nkhaunya, Nordak, Provider and NW 590 (Fig.3).

Cluster analysis

Cluster analysis was performed following principal component analysis to further examine dissimilarities and similarities among 42 cultivars collected from the three countries (Fig.4). When a cluster tree was cut at 85% level of similarity, 35 individual cultivars and 2 groups were segregated. The first group consisted of Zm 4491 and Zm 3689 which were both from Zambia. This group shared common characters such as green stem, semi-climbers, 29 days to reach flowering, purple flowers, slightly curved pods, 3 buds per inflorescence, pear-shaped cross-section, shiny green pods, moderate suture strings, medium brilliance, cuboid seed shape and no seed coat pattern. The second group consisted of Tanz1, Olathe and Nordak which shared common characters such as green stem, semi-climbing growth habit, 24 days to reach flowering, white flowers, 4 buds per inflorescence, slightly curved pods, pear-shaped cross-section, red stripes on green colour pods, moderate pod suture strings, striped seed coat pattern with dark and light brown colour, medium seed brilliance and cuboid seed shape. Tanz1 was obtained from Zambia, while Olathe and Nordak were from Lesotho.

DISCUSSION

Seventeen characters used as morphological characters were adequate to discriminate cultivars. Different combinations of these 17 characters enabled the cultivars to be differentiated, while no single character distinguished one cultivars from the other. A combination of four or more characters, for example growth habit, seed coat pattern, light and dark colour of the seed, resulted in some cultivars being distinguished. The results were consistent with the findings of Figliuolo and Spagnoletti (2000) who distinguished 57 common bean cultivars and discovered that no one character can discriminate a cultivar. Similarly, Awan et al. (2014) characterized thirteen cultivars of common bean grown in Pakistan and revealed distinguishing morphological characters that led to separation of cultivars. The importance of morphological markers in identifying cultivars is well-documented (Stoilova et al., 2013; Marzooghian et al., 2013; Berova and Stoilova, 2009). One or two characters were able to group and sub-group cultivars but these were dependent on their discriminatory power. All characters applied in this study were found to have a perceptible influence on the segregation of cultivars, although their discriminatory power differed. However, certain combinations

of characters did not contribute to further sub-division of groups. In this regard, it must be emphasized that large number of cultivars share similar character such as green stems, climbing growth habit, white flowers and cross-section of the pods. Dividing the main groups into sub-groups on different characters, led to the segregation of many individual cultivars, for example, when the cultivars were grouped according to their growth habits and plant height, followed by further sub-division of groups by means of seed coat pattern and colour, cultivars were distinguished. Previous researchers also followed this procedure and found it effective (Okhii et al., 2014); Stoilova et al., 2013; Singh et al., 1991). Seed characteristics, particularly seed coat pattern and colour contributed to the clear-cut distinction between cultivars obtained from different countries occurred and this shows potential to provide a quick method of differentiating between cultivars.

The segregation of cultivars by cluster analysis was based on the seed characteristics. The three cultivars, Olathe, Nordak and Tanz1 were grouped together, while the rest were segregated. These three cultivars shared similar seed characteristics and were classified as pinto beans according to the American and Canadian classification method (Mavromatis et al., 2010). This group of bean probably consisted of duplicates or progenies from the same ancestors which did not undergo much evolution and as a result, were morphologically similar. Hornakova et al., (2003) used cluster analysis to determine degree of similarity among 82 accessions of common bean and found two main groups with sub-groups and 14 smallest sub-groupings. Awan (2014) obtained three groups from 13 common bean genotypes, each group having differing number of cultivars when analysing using dendrogram.

The second group, Zm 4491 and Zm 3689, was also formed on the basis of similarities in seed coat characteristics. The similarities in this group may be attributed to the fact that Zambia accessions were composed of different seed coat colours which were grown together over a long period of time. Consequently, cross-pollination had occurred, resulting in cultivars which were phenotypically similar. Bornnetti et al. (1995) and Roy (2001) reported that the cultivars which were morphologically similar had a close genetic relationship. Contrarily, Singh et al. (1991) argued that the morpho-agronomic characters were phenotypic traits and accessions may be similar morphologically, yet be distant genetically.

CONCLUSION

Morphological characters were able to distinguish thirty-five individual cultivars and two groups of cultivars. Plant height, growth habit, seed pattern, number of flowers per node, number of locules per pod, seed colour, leaflet length, pod colour and flower colour had a perceptible influence on segregation of cultivars.

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