African Crop Science Journal, Vol. 22, No. 4, pp. 257 - 265
ISSN 1021-9730/2014 $4.00

CHARACTERISATION OF COMMON BEAN GENOTYPES BASED ON STORAGE PROTEIN PROFILES

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(Received 7 May, 2014; accepted 5 November, 2014)

ABSTRACT

Common bean (*Phaseolus vulgaris* L.) is a morphologically diverse leguminous crop as evidenced by a great variation in growth habits, pigmentation, flowers, locules, pods, seed, phenology and many other characters. The study was conducted to distinguish common bean genotypes grown in Southern Africa sub-region based on storage protein profiles. A collection of 42 genotypes were obtained from Lesotho, Republic of South Africa and Zambia. Sodium Dodycel Sulphate Polyacrilamide Gel Electrophoresis was used to separate extracted residual protein and develop electrophoregrams from which bands were scored, generating a matrix of 1 or 0. Correspondence and cluster analysis were performed using the dataset. Out of 10 correspondence scores generated from 17 characters, only the first three which constituted 54.57% of the total variation and were considered for analysis. The first, second and third correspondent scores accounted for 23.23, 16.80 and 14.54%, respectively. Thirty-eight individual genotypes and seven intra-accessions were distinguished, while three genotypes and three accessions were indistinguishable. The study showed a wide variation among the common bean genotypes. Cultivars such as winter-green, nordak, olathe, tanz1, lazy-house wife, zm 3749b, zm 3749a, zm 4517b, zm4517c, zm 4512 and zm 4527 were outliers. Cluster analysis showed that some cultivars started to show difference at 30 up to 85% level of similarity.

Key Words: Cluster analysis, Lesotho, *Phaseolus vulgaris*

RÉSUMÉ

Le haricot commun (*Phaseolus vulgaris* L.) est une légumineuse à grande variabilité morphologique, ceci se démontre par la grande différence observée au niveau des modes de croissance, pigmentation, appareil floral, loges, gousses, graines, phénologies et plusieurs autres caractères. Cette étude visait à distinguer, sur la base de profil protéique, les variétés de haricot commun cultivées dans la région sud de l'Afrique. Une collection de 42 accessions obtenue de Lesotho, République Sud-Africaine et Zambie a été utilisée à cet effet. L'électrophorèse sur gel de sodium dodécyl sulfate polyacrylamide a été utilisée pour séparer les extraits de résidu protéique et développer des électrophorégrammes à partir desquels les bandes ont été identifiées, afin de générer une matrice de 1 ou 0. Des analyses typologiques et analyse de correspondance ont été effectuées sur les données collectées. Parmi les 10 niveaux de correspondance générés à partir des 17 caractères étudiés, seuls les trois premiers niveaux de correspondance qui constituent 54,57% de la variation totale ont été considérés dans l'analyse. Le premier, le second et le troisième niveau de correspondance, expliquent respectivement 23,23 ; 16,80 et 14,54% de la variation totale. Trente-huit variétés individuelles et sept accessions génétiquement proches ont été identifiées, tandis que trois variétés et trois autres accessions n'ont pas pu être identifiées. L'étude a montré une grande variabilité entre les accessions de haricot commun. Les accessions telles que winter-green, nordak, olathe, tanz1, lazy-house wife, zm 3749b, zm 3749a, zm 4517b, zm4517c, zm 4512 and zm 4527 étaient aberrantes. L'analyse typologique a montré quelques accessions présentant de 30 à 85% de niveau de similarité.

Mots Clés: Analyse typologique, Lesotho, *Phaseolus vulgaris*

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a morphologically diverse leguminous crop as evidenced by a great variation in growth habits, pigmentation, flowers, locules, pods, seed, phenology and many other characters (Leakey,1988; Singh,1989). The morphological characters were used in the past to distinguish wild and cultivated species. Nonetheless, other methods such as botanical, archaeological, ethnological, biochemical and molecular markers have recently been used with powerful discriminatory powers (Sustar-Vozlic, 2006).

Common bean is an annual, diploid (2n = 2x = 22) species originated in the Americas, comprising of both wild and cultivated forms. The wild species are found distributed from northern Mexico to northern Argentina; while cultivated species were distributed in Meso-american and Andes (Szilagyi *et al*., 2011).

The phylogenic study conducted by Gepts and Debouck (1991) using storage protein indicated that there are two gene-pools from which cultivated common beans originated, namely; Meso-american and Andes. Another third gene-pool was discovered in Colombia or Central America, where the two other gene-pools converged. This gene-pool consisted of a mixture of both Meso-American and Andes (Khaidizar *et al*. (2012). Among the biochemical methods used for characterisation of cultivars are storage protein profiles which have a fast, simple and easy to follow procedure, handle large number of samples at a time and have high discriminatory power.

Common bean cultivars were first introduced in Lesotho by Missionaries in 1833 together with other cereal crops (Mokitimi, 1990). Since then, they gained popularity among the farming community such that currently, an area of 18,065 ha is devoted to the crop (Bureau of Statistics, 2001). Many cultivars were imported from South Africa, Europe, United State of America and Southern African countries (Edmond, 1978). All these were tested under Lesotho conditions. A continuous importation has resulted in a broad genetic base that is used by the Basotho. However, these common bean cultivars have not been characterised morphologically or at molecular level, and no cultivar release committee has been established. The purpose of this study was, therefore, to distinguish between varieties of common bean genotypes using storage protein profiles and establish relationship among genotypes.

MATERIALS AND METHODS

The study was conducted at the School of Agricultural Sciences Laboratory, University of Zambia at Lusaka. The laboratory is located at 1140 m above sea-level, at latitude 28^0 20'E and longitude $15^0$22' S.

A total of 42 common bean genotypes were used in this study, of which six were obtained from Lesotho, 10 from South Africa and 26 from Zambia.

Extraction of proteins. The testa of seeds were removed by scratching it with a small file. Two seeds devoid of testa were crushed using a pair of pliers and then ground to fine powder with a pestle and mortar. Ten grammes of seed powder from each genotype were put into separate eppendorf tubes to which 400 µl Sodium dodecyl sulphate (10%) detergent was added. The mixtures were vortexed for 15 seconds and left for 1 hr. They were then placed in a warm water bath for 1 h at 100 °C. The mixture was centrifuged at 5000 rpm for 15 minutes, and the supernatant was stored at -4 °C. A sample of 1 µl from each of the 42 supernatants was used to determine the protein content by the Biuret methods (Schuertz, 1978).

Polyacrilamide gel electrophoresis was prepared and electrophoresis was carried out as described by Cooke (1995). The composition of the gel was acrylamide (10%), Bisacrylamide (0.3%), SDS (10%), Tris-HCl (pH 8.8), water, ammonium persulphate (1%) and TEMED 10 µl. The staking gel consisted of acrylamide (4.5%), bisacrilamide (0.068%), Tris-HCl (pH 6.8), TEMED (5µl) and ammonium persulphate (0.023%).

Electrophoretic apparatus was assembled, separating gel poured and waited until it polymerised. A 1.5 mm thick comb was inserted to form 10 sample wells in each gel. When the

staking gel had polymerised, it was removed and 15 µl of supernatant from each cultivar was loaded in to the wells by a Hamilton syringe. The seven standard molecular markers were loaded on the first well to assist in estimating the relative mobility of the bands. The markers were lysomes (14.4 kd), soybean trypsin inhibitor (21.5 kd), chymotryprinogen (25.7 kd), carbonic anhydrous (31 kd), ovalbumin (45 kd) and bovine serum albumin (66.2 kd). The second well was loaded with a reference cultivar (Contender).

Electrophoresis was carried out in vertical electrophoresis apparatus. Top tank buffer solution consisted of glacial acetic acid, glycine (1.7%), Tris-HCl (3%) and SDS (10%). Electrophoresis was done at 150 °C for 3 hours at 300 volts. Bromophenol blue was used as a tracking dye.

Gel staining. Gel staining was done following the procedure described by Wringley (1992). The staining solution consisted of coomassie blue G250 (0.01%), methanol (5%), trichloroacetic acid (5%) and water (200 ml). The background coloration of the gel was removed by destaining the gel in methanol (30%), acetic acid (10%) and water (60%).

Data analysis. All 42 genotypes were analysed for electrophoretic variability. The protein bands that migrated downwards from the origin were scored on the basis of the presence or absence of bands. This generated a matrix of 1 or 0. GenStat Software (15th Version) package was used to perform correspondence and cluster analysis.

Correspondence and cluster analysis were employed to establish the pattern of variation in the bands. Both statistical tools do similar analysis except with cluster analysis where data had to be transformed and standardised.

RESULTS

Correspondence analysis. Out of 10 principal components generated from 17 characters, only the first three components constituted 54.57% of the total variation and were considered for analysis. The first, second and third correspondent scores accounted for 23.23, 16.80 and 14.54%, respectively. The bands responsible for segregation of genotypes in the first correspondence score were relative mobility (Rm) 33.75 (-0.65), Rm 22.50 (0.47), Rm 78.00 (-0.30) and Rm 63.75 (0.21); while in the second correspondence score, the major source of variation was bands at Rm 33.75 (-0.75), Rm 23.75 (0.40), Rm 32.50 (0.30) and Rm 22.50 (-0.28). The third correspondence score variables comprised of bands at Rm 33.75 (-0.31) and Rm 52.00 (-0.26). According to the locations and number of bands, most genotypes were spread out on the Figures 1, 2 and 3, except for few (9) which were represented by dots, implying that they shared bands at similar relative mobilities. The genotypes grouping along 0 indicated a low variability of bands, while the ones away from 0 showed higher band variation. Genotypes 12, 13, 43, 45 and 46 were outliers appearing individually and far from others.

The bands which had high influence on the segregation of genotypes were Rm 23.75, 32.50, 33.75, 22.50, 52.00, 63.73 and 66.00. It is, therefore, apparent that the regions of Rm 22.50 t0 33.75 and 52.00 to 66.00 could be used for differentiation of genotypes.

The region from Rm 43.00 to Rm 52.00, which was characteristic of phaseolin types, had little variation, which did not allow the genotypes to be differentiated at genotype level. According to phaseolin type classification, there were 26 contenders, 15 tendergreen and 1 sanillac types. The region of lectins band situated at Rm 63.70 to 66.25, had little variation which was not used for identification purposes. All but three genotypes (Nordak, Olathe and NW 590) had two bands of lectins. These three had one band at Rm 66.25 instead of two.

Cluster analysis. Cluster analysis for the storage protein profile was performed using the relative mobilities of bands. All 20 bands were used in the analysis. Among the 42 genotypes, there were genotypes which had some intra-accession and as a result, the number of electrophoregrams increased to 49, which were all used in the cluster analysis. When the cluster analysis (Fig. 4) was cut at 85% level of similarity, 38 genotypes and seven intra-accessions were segregated; while

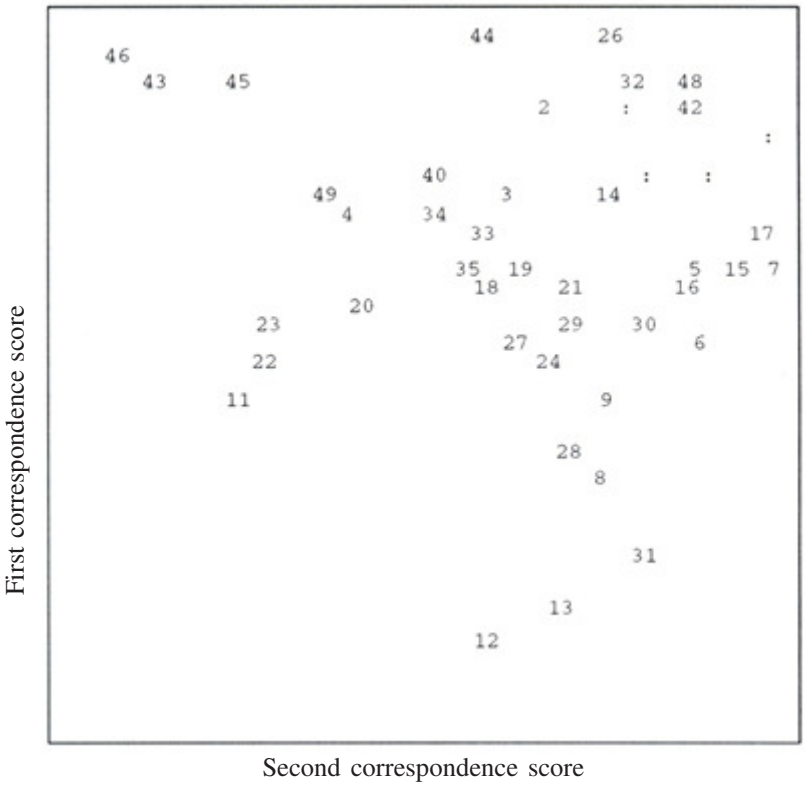

Figure 1. Positions of *Phaseolus vulgaris* L. genotypes on the first and second correspondence scores based on storage protein profiles.

three genotypes and three intra-accessions were indistinguishable. These indistinguishable genotypes and intra-accessions were separated into three groups of two each. The first group consisted of two genotypes (Zm 3680 and Zm 3689), which had bands at similar relative mobility. Both had no bands at Rm 22.50 and 52.00. The second group consisted of two intra-accessions (Zm 4406c, Zm 4406b), which had similar relative mobility at Rm 52.00 and 63.75.The third group consisted of a genotype and intra-accession at Rm 2.00, 13.50, 15.00, 20.00, 22.50, 23.75, 32.50, 63.75, 66.25, 75.00 80.00, 81.25 and 86.00. Both had no bands at Rm 33.75 and 78.75. Groupings were based on the similarities in the number and location of the bands.

DISCUSSION

Correspondence analysis. In this study, 20 protein bands separated electrophoretically by means of SDS-Page were used in identifying genotypes. These protein bands were fewer than the maximum of 35 obtained by Hussain *et al*. (1986) with SDS-PAGE. They, however, obtained 15 bands with acid PAGE. The emphasis was placed on clearly distinguishable bands and both the number and location of the bands were used for discriminating between genotypes. A large proportion of the genotypes in the present study had the same number of bands, but differed regarding the location of bands. The presence or absence of the bands, as well as unique additional bands at a particular relative mobility contributed to differences among the genotypes.

The most conspicuous bands were of phaseolin and lectin, which were consistently found in the same Rm region. These bands assisted in the sub-grouping of the genotypes. The differences among these bands were slight, and contributed largely to grouping genotypes together. Moreover, previous researchers used

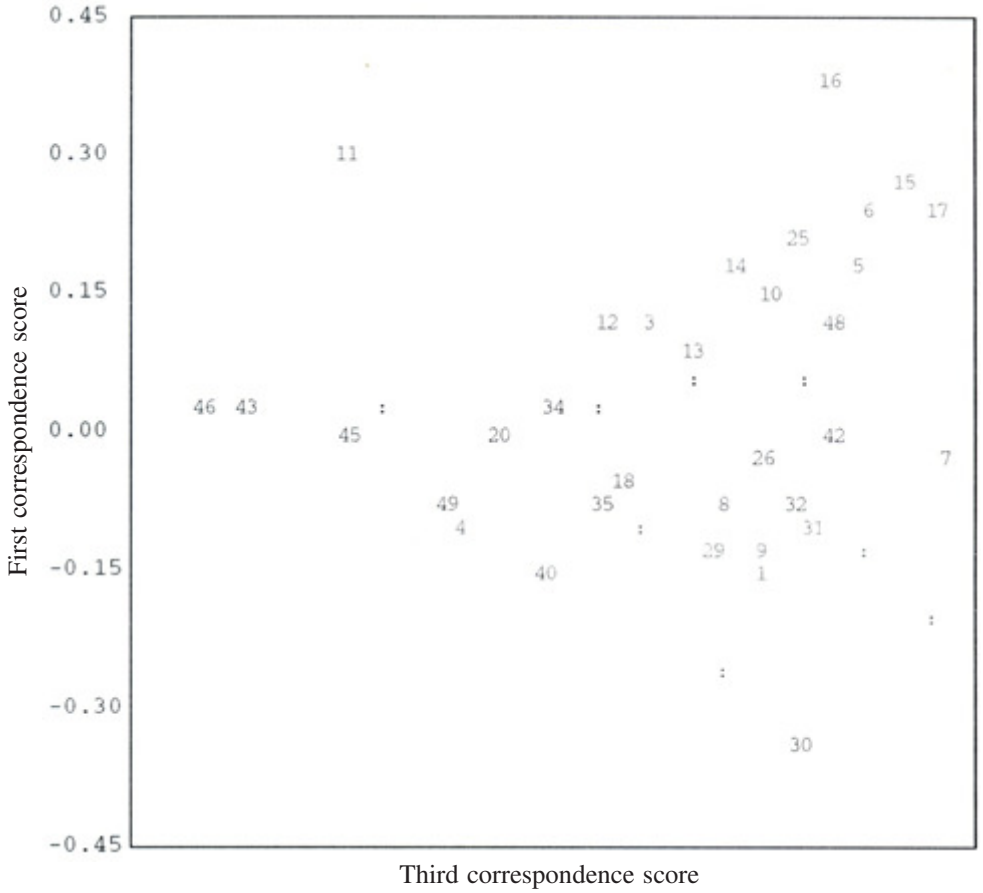

Figure 2. Positions of *Phaseolus vulgaris* L. genotypes on the first and second correspondence scores based on storage protein profiles.

the grouping based on phaseolin to trace the origin of genotypes since the three different phaseolin types have different origins (Gepts and Bliss, 1988; Mumba, 1994). Gepts and Bliss (1988) found these bands useful for phylogenic studies.

Some genotypes possessed unique protein bands (Tanz1, Olathe, Nordak and NW 590) and these bands made differentiation easy. In some instances, it was difficult to distinguish between genotypes because of similarities in their protein profiles. However, by scrutinising the bands vertically and then comparing them, the distinction could be made.

The banding patterns were highly reproducible, indicating that the analytical method was stable and could be replicated elsewhere to produce similar results. The consistent expression of the protein banding pattern of genotypes, even from different environments emphasized the reliability of genotype identification by means of this method.

Segregation of genotypes based on the electrophoregram of the protein resulted in a large number of genotypes being distinguished. The resolving power of the method was, therefore, demonstrated. Driedgar *et al.* (1994) and Bonnetti *et al.* (1995) similarly illustrated the high discriminatory power of electrophoregrams in differentiating cultivars of black beans.

It must, however, be emphasized that the different locations of bands were more important in distinguishing genotypes than the number of bands. A large proportion of genotypes had the same number of bands, but was differentiated on the basis of band location. The bands which were used for segregation of genotypes were in the

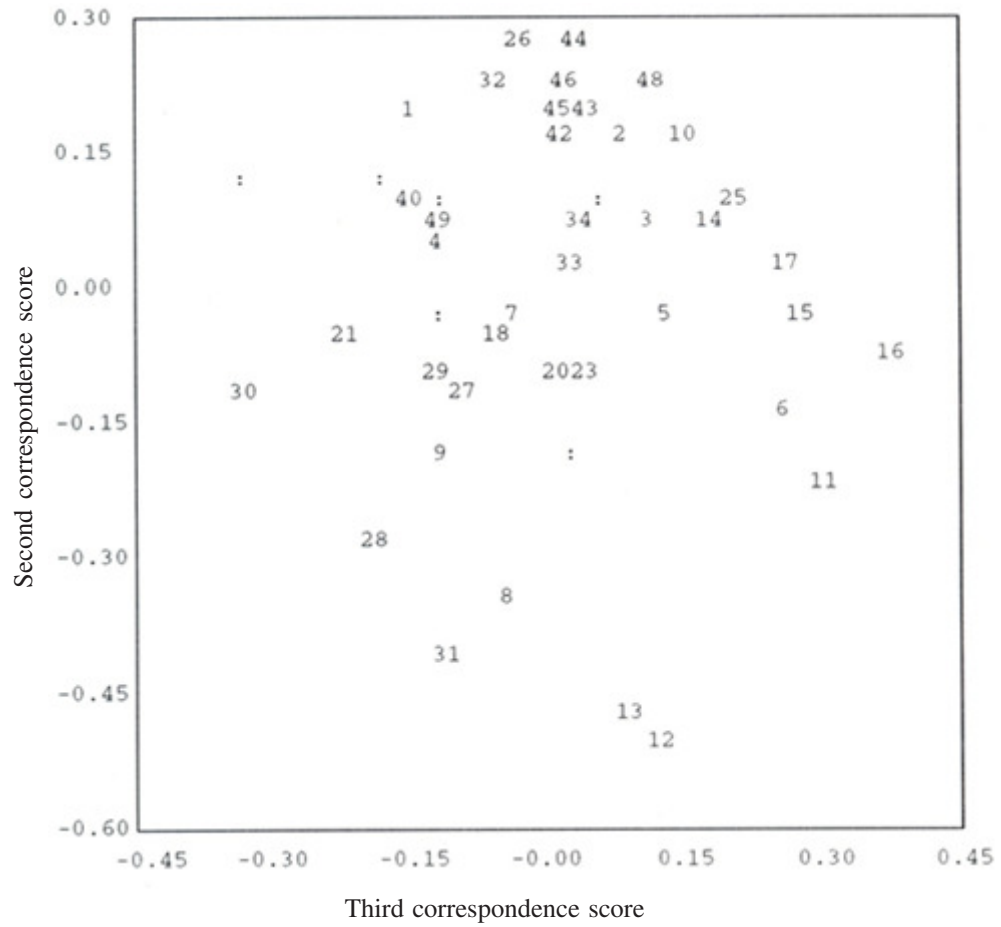

Figure 3. Positions of *Phaseolus vulgaris* L. genotypes on the first and second correspondence scores based on storage protein profiles.

Rm 22.50 to 33.00 and Rm 52.00 to 66.25 regions. Likewise, Hussain *et al*. (1988) reported the bands in these regions to be useful for distinguishing cultivars. Driedgar *et al*. (1994) found the variability of bands in the Rm 66.00 to 81.00 region useful for distinguishing bean cultivars. The difference in Rm regions can probably be attributed to the method of extracting residual proteins where acetic acid (0.1 M), sodium chloride (0.4 M) and aqueous ethanol (70%) were used in addition to SDS and 2-mercapto-ethanol, in the latter instance. Using this extraction method, 35 conspicuous bands were produced, while only 20 were conspicuous in this study. However, the discrimination of cultivars was achieved by means of both extraction methods.

The results obtained in this study exhibited considerable variability among protein electrophoregrams within and between the genotypes obtained from the three countries. However, Zambian intra-accessions showed similarity in electrophoregrams. Hussain *et al*. (1988) reported similar results when examining 11 groups of bean cultivars obtained from the United States of America and Europe. Seed characters were distinctly different in each accession and the authors, therefore, suggested that the accessions should be purified through inbreeding and selection.

Cluster analysis. The results of cluster analysis showed that common bean genotypes used in

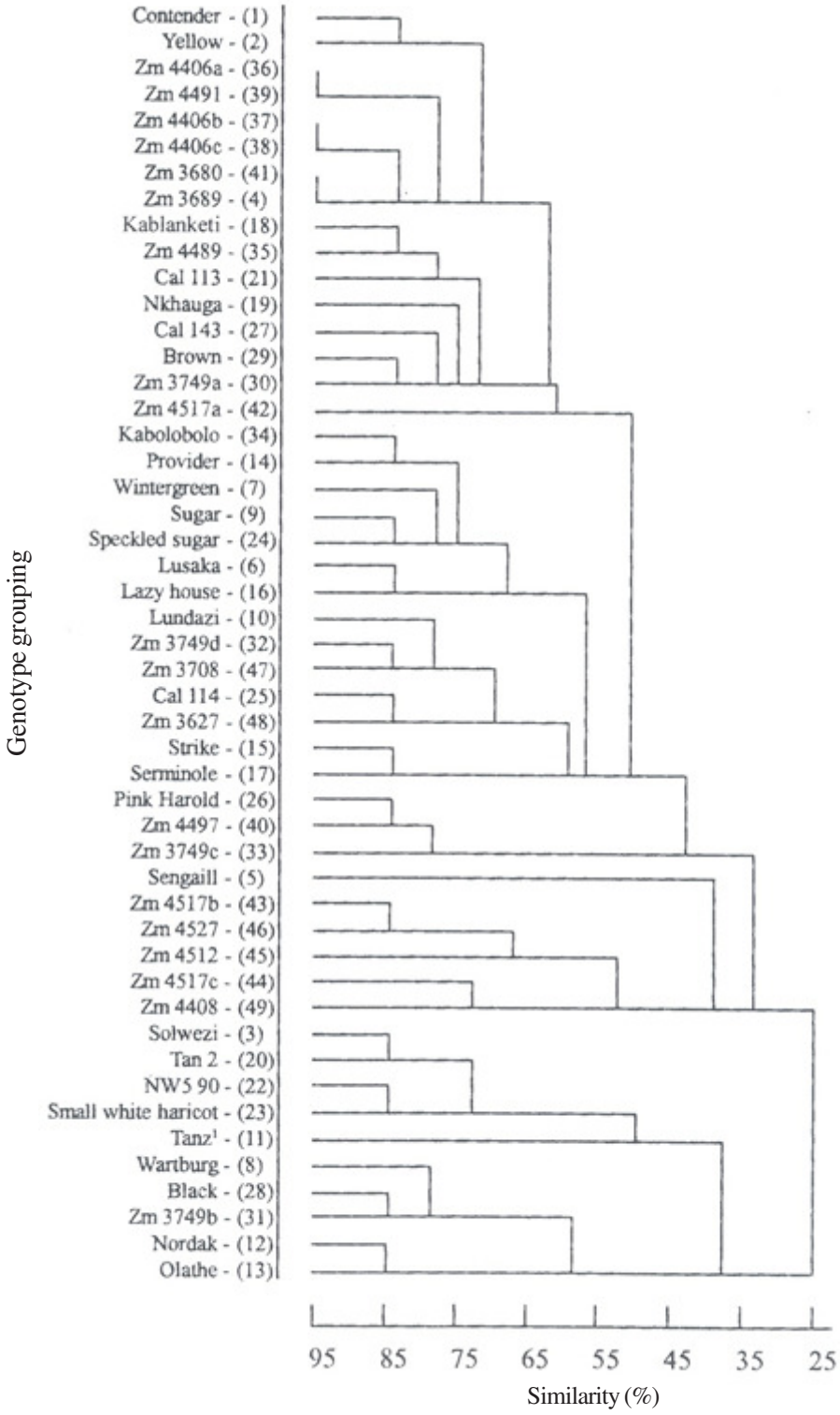

Figure 4. Cluster analysis of *Phaseolus vulgaris* L. genotypes based on storage protein profiles.

Zambia, South Africa and United States of America share most of the characteristics. These cultivars were grouped according to similarities of bands at a lower degree of similarity but further sub-divide into sub-groups and into other minor groups. Most of the Zambian cultivars (zm accessions) clustered together up to 45% degree of similarity after which they started to separate while out of four United States (US) cultivars, nordak and olathe showed 85% degree of similarity confirming that the two belongs to a market class of Pinto in US and may have a common progenitors. The other US cultivars, NW590 and pink harold have more distinct characteristics which are different from the two. South African cultivars were scattered in different sub-grouping sharing with both US and Zambian Cultivars. These are contender, provider, wartburg, small white haricots, serminole, speckled sugar beans and strike. Lesotho cultivars were also found in other sub-groups with US and Zambian cultivars. There is more diversity in South African cultivars than Lesotho and Zambia. In consistent with this study, Singh (1998) conducted a study characterising 306 cultivars using storage protein profile bands where 9 sub-groups were formed with differing number of cultivars in each. Similarly, Sustar-Vozlic *et al*. (2006) analysed genetic diversity of 139 bean accessions with protein bands and generated three main groups and sub-groups. Each sub-group had a differing number of cultivar groupings.

ACKNOWLEDGEMENT

The Authors thank the British Council for providing financial support to carry out the research at University of Zambia.

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