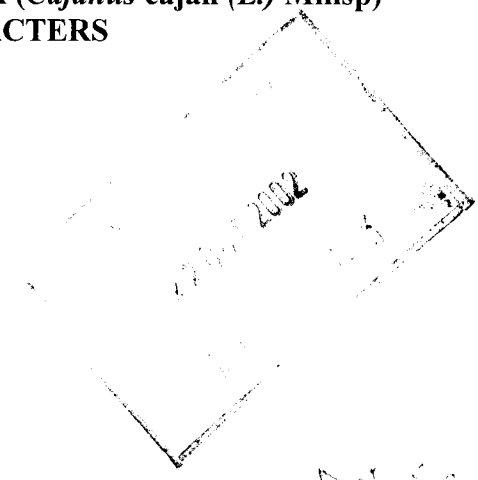
CHARACTERIZATION OF ZAMBIAN PIGEONPEA (*Cajanus* cajan *(L.)* Millsp) GERMPLASM USING MORPHOLOGICAL CHARACTERS



BY

PHILLIP SYAMUYOBA

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE IN AGRONOMY.

UNIVERSITY OF ZAMBIA
SCHOOL OF AGRICULTURAL SCIENCES
DEPARTMENT OF CROP SCIENCE

2002

DECLARATION

I, Phillip Syamuyoba, declare that all the work presented in this dissertation is my own work and has not been submitted for a degree at this or any other University.

Signature.....

Date.....17th September 2002

APPROVAL

This dissertation of Phillip Syamuyoba is approved as fulfilling part of the requirements for the award of the degree of Master of Science in Agronomy (Crop Science) by the University of Zambia.

Examiner's Name and Signature:	Date:
COUSINS GWANAMA	25-09-02
BENSON H. CHISHALA	25-09-2002
DAVIES M. LUNDU	25-09-2002
	261729

DEDICATION

This dissertation is dedicated to my wife Ng'andu and my daughters; Carol, Mutinta, Maggie, Mweembe and Lubomba (Lulu) for their tolerance, true love and patience during the two years I was away from home. To my dad, brothers and sisters for their continued encouragement over the years.

ACKNOWLEDGEMENT

I am very indebted to my supervisor Dr. C. Gwanama for his constant guidance throughout the research work. I would also like to thank Mr G.P. Mwila of the National Genebank who encouraged me to take up this study and for providing the germplasm.

I am very grateful to the International Plant Genetic Resources Institute (IPGRI), based in Rome, Italy - through its Sub-Saharan Africa Office in Nairobi, Kenya for the partial Scholarship. Also my employer, Ministry of Agriculture and Cooperatives (MACO) for granting me study leave.

My sincere thanks are also due to Mr Victor Shitumbanuma of Soil Science Department, School of Agricultural Sciences and Mr. M. Chibwe of Central Statistical office, Lusaka for their assistance with data analysis

ABSTRACT

Pigeonpea (*Cajanus cajan* (L.) Millsp) is of great importance in Zambia for its diversified use, which can lead to increased farm income and subsequently food security at household level. Its seeds have a protein content of approximately 21% and therefore is a cheap source of protein in the human diet. The National Genebank, which has a responsibility for collecting and conserving all locally available crop germplasm, has a collection of accessions of pigeonpea that have not been characterized. Lack of characterization to obtain genetic information about these accessions has caused the germplasm not to be widely utilized by end-users such as plant breeders in the breeding programs and the magnitude of genetic variation has so far not been assessed. The objective of this study was to assess genetic variation among accessions of pigeonpea conserved at the National Genebank and to determine characters that are useful in classifying pigeonpea.

Twenty-eight accessions were assessed for genetic variation based on morphological characters at Mount Makulu Central Research Station, Chilanga, Zambia during the 2000/2001 growing season. The design of the experiment was a single block and each accession was planted on a single unreplicated plot. Twenty-three characters (plant height, number of branches, leaf size, days to 50% flowering, days to 80% maturity, number of racemes, seeds per pod, 100-seed weight, growth habit, stem colour, stem thickness, leaf hairiness, leaflet shape, flower colour, second flower colour, pattern of streaks, flowering pattern, pod colour, pod hairiness, pod form, seed colour pattern, seed colour and seed shape) were measured following IBPGR/ICRISAT (1993) pigeonpea descriptor list and analysed using cluster analysis and principal component analysis. Cluster analysis grouped the

accessions into four major clusters based on commonly shared characters. Three principal components were found to explain 73.9% of the total morphological variation. Days to 50% flowering, days to 80% maturity, number of branches, number of racemes, plant height, number of seeds per pod, stem colour, flower colour, pod colour, colour of the seed coat and flowering pattern were the major sources of variation among the accessions. Based on these results, it is concluded that there is genetic variation among the accessions of pigeonpea conserved at the National Genebank. It is further concluded that characters that were the major sources of variation are useful in classifying pigeonpea germplasm. These results could be useful in pigeonpea improvement programs.

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LIST OF ACRONYMS

CTA	-	Technical Centre for Agriculture and Rural Cooperation.
EARCAL	-	Eastern African Regional Cereals and Legumes
IBPGRI	-	International Board of Plant Genetic Resources
ICRISAT	-	International Crop Research Institute for Semi-Arid Tropics
IITA	-	International Institute of Tropical Agriculture
ISNAR	-	International Service for National Agricultural Research
NGOs	-	Non-Governmental Organisations
OTU	-	Operational Taxonomic Unit
PCA	-	Principal Component Analysis
PCR	-	Polymerase Chain Reaction
RAPD	-	Randomly Amplified Polymorphic DNA
RFLP	-	Restriction Fragment Length Polymorphism
ZNPGRC	-	Zambia National Plant Genetic Resources Centre

CHAPTER ONE

INTRODUCTION

Although pigeonpea (*Cajanus cajan* L. Millsp) is the fifth most important pulse crop in the world (Whiteman et al., 1985), its grain yield per unit area has remained low at about 0.6-0.7 t ha^{-1}, largely because traditional farmers grow unimproved cultivars with little inputs. In the Semi-Arid tropics the crop is widely grown by traditional farmers as a backyard subsistence crop. Many factors have contributed to the continued wide use of pigeonpeas in the Semi-Arid tropics. The ability to survive and grow in harsh environments with very severe drought stress and poor soil fertility are especially important. The deep root system of more than 1.5m permits the crop to extract water at low depth and thus confers greater ability on the legume to withstand drought stress (Sheldrake and Narayanan, 1979).

Besides its main use as dhal (dry, dehulled split seed used for cooking), its tender seeds are used as vegetable. The crushed dry seeds are used as animal feed while green leaves are used as fodder and stems as fuel wood. Its seed protein content (approx. 21%) compares well with that of other legumes (Nene and Sheila, 1990). It can therefore, be an important component of human diet and a cheap source of protein especially for the under-privileged communities within the society who may not afford to buy alternative sources of protein such as animal protein.

Pigeonpea provides several benefits to the soil. Being a legume, it fixes nitrogen. The leaves that fall at maturity add organic matter as well as nitrogen to the soil.

In Zambia pigeonpea is of great importance as the agriculture policy emphasizes diversification as one area of improving food security and increasing farm income. Pigeonpea has a diversified use, which can lead to increased farm income and subsequently food security at household level. Since pigeonpea fixes nitrogen in the soil, farmers can intercrop it with a cereal crop so that the cereal crop can benefit from nitrogen fixed by the pigeonpea. This is an important aspect in the country where smallholder farmers who form the majority of the farming community lack financial resources to purchase inorganic nitrogen fertilizers. It is also a hardy plant, and as such when intercropped with cereals, ensures a measure of food and income stability. The smallholder farmers in Zambia can therefore, incorporate it into the farming system.

There are other benefits that the smallholder farmers in Zambia can derive from pigeonpea. The crop can be used as a perennial forage crop for livestock and dried stalks can be used for firewood, thatching and for making baskets and storage bins. Therefore, pigeonpea is a potentially useful crop whose production should be increased both at village and national levels.

Zambia National Plant Genetic Resources Centre (ZNPGRC) at Mount Makulu, Chilanga, which has a national responsibility for collecting and conserving all locally available crop germplasm, has a collection of 138 accessions of pigeonpea. This collection is probably not fully representative of the diversity found in Zambia. The material in the collection needs to be adequately characterized in order to identify future collection priorities and make the material

more valuable to users. It was, therefore, in this context that the study to morphologically characterize a sample of accessions of pigeonpea in the National Genebank collection was carried out. The objectives of the study were (1) To assess genetic variation among accessions of pigeonpea conserved at the National Genebank and (2) To determine characters, which are useful in classifying accessions of pigeonpea.

CHAPTER TWO

LITERATURE REVIEW

2.1 General

Pigeon pea (C. *cajan*) originated in India, which is believed to be its primary centre of origin, and diversity (Van der Maesen, 1981). The legume moved more than 4000 years ago to Africa where its secondary centre of diversity developed later in East Africa (Singh, 1991). From Africa pigeonpea travelled to the West Indies and spread all over tropical America.

Pigeonpea ranks fifth in total world production among pulses and is the second most important legume after chickpea (*Cicer arietinum* L.) in India (Ariyanayagam et al., 1991). It has also been reported that India accounts for over 80% of the world's annual production of 2.32 million tonnes (ICRISAT, 1986).

In Africa, pigeonpea is grown in several, countries such as Kenya, Malawi, Zambia, Tanzania, Uganda and Mozambique in Eastern and Southern Africa. The crop is mostly grown for its dry split seeds, which have a protein content of 20-25% (Whiteman et al., 1985). In Kenya pigeonpea is the second most important pulse crop after field beans (Muthoka, 1995). Farmers intercrop it with cereals such as maize and sorghum and with short duration legumes such as beans and cowpeas. They grow long duration pigeonpea varieties with minimum inputs and obtain low yields of 0.4-0.6 t ha^{-1}. This low productivity is due to low yielding varieties.

Pigeonpea is an important crop of the rural poor in the dry areas of Northern and Northeastern Uganda (Esele, 1995). It is consumed locally as a sauce and the crushed dried seed is processed

into "dhal". The stems are used as fuel wood and in the construction of huts and baskets. Farmers grow it either as a sole crop in rotation with cereals or they intercrop it with cereals such as sorghum and millet. However, the yields are very low and range from 0.3 - 0.5 t ha^{-1} (Esele, 1995).

In Zambia, pigeonpea is an important minor food legume crop. Smallholder farmers grow it in their home backyards and around the fields of annual crops as a hedge. The crop is also grown by commercial farmers in Peri-urban areas where there is considerable demand from the Asian community. They mostly grow white seeded medium maturity types as an annual pure crop with an average yield of 0.4-0.5 t ha^{-1} (Kannaiyan, 1989).

Research on pigeonpea in the country started in the 1970's when the Copperbelt Regional Research Station at Mufulira received varietal trial lines from International Institute of Tropical Agriculture (IITA), Nigeria (Kannaiyan, 1989). The objective was to select high yielding varieties. To date the Food Legume Research Project has been conducting varietal trials in order to evaluate pigeonpea varieties for maize/sorghum intercropping system to suit mainly smallholder farmers. One variety, ICP 7035, was found to perform better than the local landraces such as ZCC-170 and has so far been released. In addition, the project has been carrying out pigeonpea seed multiplication programs in the country. The multiplied seed is given to farmers through the agricultural extension services and through Non Governmental Organization (NGOs) involved in the promotion and development of the crop in Zambia. The project has also been training extension workers and farmers through workshops as a way of creating awareness of

pigeonpea and to increase farm level production (Mulila-Mitti, 1994). It has also been reported by Mulila-Mitti, (1994) that the project has developed simple but tasty pigeonpea recipes that have been used in cooking demonstrations during workshops. Effective demand for pigeonpea requires acceptability in the Zambian diet. It is therefore, clear that the development and provision of simple processing tools will facilitate an increase in consumption and production of pigeonpea in Zambia. Another important aspect of pigeonpea research is evaluation of short duration and early maturing varieties grown as sole crop and amenable to combine harvesting.

2.2 Characterization

Characterization serves to provide descriptive information on the highly heritable traits of an accession. Perino and Monti (1991) defined characterization as the scoring of characters that can be easily detected and have high hereditability. There are four main subcategories of characters and these are morphological, botanical, agronomic and chemical characters. They can be recorded on plants or their products, for example seed grown only in one environment. It is for this reason that characterization may begin during exploration and collection and continue in the laboratory before or after multiplication. From characterization, a number of conclusions can be drawn. The variability that is identified by characterization needs to be conserved and should be made available to both germplasm collectors and breeders. Some priorities for the future can be identified by characterization so that during collection missions attention would be paid to characteristics that are not well represented in the present collection.

Characterization also avoids the possibility of filling up space in the genebank by keeping material, which is essentially the same (Lungu, 1990). An appraisal of the environment in which the crop is being or is going to be grown is also done during characterization, recognising the major factors that can limit its performance. Bisht et al., (1995) reported that specific individual accessions including rare types could be identified during characterization and passed on to breeders for further assessment. Rare type accessions are accessions with traits like resistance to biotic and abiotic stresses that can be used in crop improvement programs.

The purposes of characterization work in genebanks include the study of genetic variability of certain characters in relation to their geographical distribution in order to develop new and more adequate collecting strategies for further collection of useful germplasm in the same or similar areas (Bogyo et al., 1980). It is also done in order to study the genetic variability present in the collection, especially within samples, and develop the most appropriate techniques and strategies for maintaining the genetic integrity of such diversity as well as to screen the collections for traits which, from time to time are considered important for breeding programs aiming at improving agriculture in a country, region or geographical area (Perrino and Monti, 1991).

2.3 Characterization of crop species using morphological characters

Morphological characters can contribute much to the diversity of relationships between taxa and can be used as an initial step in defining systematic relationships in crop species. Accessions are characterized using morphological data from the growing plants. Data is then analysed using multivariate techniques, some of which are principal component analysis (PCA) and cluster

analysis. Principal component analysis provides information about relative importance of each variable in characterizing objects such as plants. New variables are calculated which consist of combination of the old ones. A small number of the new variables will usually be sufficient to describe the observational objects. The analysis involves computation of correlation matrix, which is then converted into principal components. The coefficients of the principal components are the eigenvectors of the correlation matrix. Thus, each principal component is a linear combination of the original variables. However, only the most important ones are of relevance for further analysis. The importance of the principal components is calculated from their eigenvalues and their contribution in explaining the overall variance (Mutsaers et al., 1997).

Cluster analysis is used to group (cluster) units according to similarity for certain characteristics or response patterns. It involves a stepwise procedure of calculating similarities and dissimilarities between observations and grouping together those that are most similar (Mutsaers et al., 1997). Initially, each observation is a "cluster" by itself. Then, in a first step the two most similar cluster (observations) are grouped together to form a new cluster. Merging cluster together step-by-step is done in that way until all observations are grouped together into one final cluster. Initially, when each observation is a cluster by itself, all variance is among clusters. At the end, all variance is within the final cluster (all observations grouped together).

Rojas et al., (2000) studied genetic diversity in Bolivian quinua (*Chenopodium quinoa* Willd) germplasm using principal component and cluster analysis where 1512 accessions where described by 17 characters. The first principal component accounted for more than 30% of total

produced. Cluster 1 was composed of primitive types, while clusters 2 and 3 were composed of advance cultivars differing in leaf colours.

Chandran and Pandya (2000) morphologically characterized 35 accessions of *Arachis*. The results showed a wide variation for most of the morphological traits studied. The growth habit ranged from procumbent to a completely erect type. Fourteen accessions were found to be procumbent. Twenty-one accessions were decumbent and two were of the erect type. Leaves of both the main stem and lateral branches were found to be pinnately compound with four leaflets. The shape and size of the leaf and leaflets varied between accessions.

Kolberg (1999) characterized 124 accessions of sorghum landraces from Namibia using morphological traits. The results of his study revealed that there was considerable variability between accessions. Of the qualitative characters, endosperm colour was white and grain luster was absent in all accessions. The majority of the accessions (89.52%) were awnless and had white midribs and white glumes (55.65%) and dry plants were mostly tan-coloured (59.68%). In addition, the results revealed that most of the accessions had white grains (40.32%). The endosperm texture was mainly intermediate (27.4% of all accessions) to starchy (21%).

Other multivariate analyses such as factor analysis and principal coordinates are also available for use in the analysis of diversity. Factor analysis, like principal component analysis, is a technique for data reduction. However, whereas the latter is simply the transformation of the coordinate axes of a multivariate system to a new orientation through the natural shape of the scatter swarm

of the observation, factor analysis proposes a fundamental model, for the covariance structure of the observations. In essence, under the factor analysis, each observed variable is postulated to be a linear function of a small number of unobserved common factor variates and a single latent specific variate. The common factors generate the covariances among the observed variables, while the specific terms contribute only to the variances of their particular variables. However, in numerical taxonomy there appears to be little to recommend factor analysis over the alternatives such as principal component analysis (Dunn and Everitt 1982).

Principal coordinates analysis aims at producing a Euclidean representation of the observed distances and to produce a pattern in the original multi-dimensional character space. The results of a principal coordinates analysis often look superficial to those of a principal components analysis. However, there are potential pitfalls with principal coordinates analysis. The most common of these occurs with data that cannot be summarized adequately in terms of the positions of the operational taxonomic units (OTUs) in a two or three dimensional space, that is, the two or three latent roots obtained from a principal coordinates analysis do not explain most of the variation in the original character space (Dunn and Everitt 1982).

2.4 Characterization of crop species using biochemical markers

The use of biochemical markers rather than morphological characters to identify cultivars of different crop species has received considerable attention (Cooke, 1984). Biochemical markers such as seed storage proteins have been used in differentiating cultivars through electrophoretic techniques such as polyacrylamide gel electrophoresis (PAGE) and Sodium Dodecyl Sulphate

Polyacrylamide Gel Electrophoresis (SDS-PAGE). These techniques have been established worldwide as a practical means for identifying wheat cultivars (Zillman and Bushuk, 1979). For example, de Villiers and Bosman (1993) used PAGE of gliadin protein in an acid medium to identify different spring wheat cultivars. Rogl and Javornik (1996) also identified 24 cultivars of common buckwheat (*Fagopyrum esculentum Moench*) using SDS-PAGE. Krishnan and Sleper (1997) also used SDS-PAGE of salt soluble globulins and alcohol soluble proteins to identify nine tall fescue cultivars. Mumba (1994) classified 189 accessions of *Phaseolus vulgaris* by phaseolin storage protein and seed size. Pedalino et al., (1990) used total seed proteins albumin and globulin to characterize 35 accessions of cowpea (*Vigna unguiculata*) and Hesemann et al., (1997) characterized 28 spelt (*Triticum spelta* L.) cultivars. In addition, Wood and Cole (1975) electrophoretically characterized bean cultivars by seed proteins as a basis of screening the germplasm.

Molecular markers such as restriction fragment length polymorphism (RFLP) and randomly amplified polymorphic DNA (RAPD) have also been widely used in differentiating genotypes. Either RFLP or polymerase chain reaction (PCR) based approaches can be used to identify molecular markers but PCR represents the faster and easier alternative (Shin et al., 1990). These DNA markers are not affected by variations in environmental conditions. They also have a high amount of polymorphism and hence are more effective and reliable in differentiating genotypes than either the use of seed storage protein or morphological characters. However, the cost of running the technique is high and requires highly specialized skills (Staub and Serquen, 1996).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Treatments

A field study was conducted at Mount Makulu Central Research Station, Chilanga, Zambia located at 15° 33' S and 28° 15' E and at 1194 metres above sea level starting on 19 December, 2000. Thirty-six accessions of pigeonpea were obtained from the active collection at the National Genebank at Mount Makulu, Chilanga, Zambia.

The design of this study was a single block (42 m x 37 m) consisting of 36 plots. Each plot consisted of 20 m x 2 m rows with inter-row and intra-row spacings of 100 cm and 80 cm respectively. Each accession was planted on a single un-replicated plot. Three seeds were planted per station at a depth of 5 cm. The seedlings were then thinned to one seedling per station 14 days after seedling emergence. A compound-D fertilizer containing 10% nitrogen, 20% phosphorus, 10% potassium and 10% sulphur was applied as basal dressing on the seed-bed at the rate of 300 Kg/ha. However, no top dressing fertilizer was applied.

The field was kept weed free throughout the growing period. Aphids and American bollworms attacked the crop by feeding on the leaves and bolls respectively. Aphids were controlled by spraying with Dimethiate while American bollworms were controlled by handpicking.

3.2 Data Collection

The descriptor of *C. Cajan* of the International Board of Plant Genetic Resources and International Crop Research Institute for Semi-Arid Tropics (IBPGR/ICRISAT, 1993) was used to characterize the accessions. Observations from ten randomly selected plants on vegetative parts were taken at 50% flowering. Data collected comprised both quantitative and qualitative traits. Quantitative traits taken were plant height, number of branches, leaf size, days to 50% flowering, days to 80% maturity, number of racemes, seeds per pod and 100-seed weight whereas qualitative traits were growth habit, stem colour, stem thickness, leaf hairiness, leaflet shape, base flower colour, second flower colour, pattern of streaks, flowering pattern, pod colour, pod hairiness, pod form, seed colour pattern, base seed colour and seed shape.

3.3 Data Analysis

Qualitative characters were assigned numbers and scored and data was then decomposed to binary form before input into the analysis while quantitative characters were summarized into means and coefficients of variation.

Hierarchical cluster analysis, based on unweighted pair group method was carried out using SPSS 6.0 software package. Squared Euclidean distance was used as a measure of distance for cluster formation. Principal component analysis (PCA) was performed using SAS software package. Those principal components with eigenvalues greater than 1 were selected (Jeffers, 1967).

CHAPTER FOUR

RESULTS

4.1 General

The rains started in October and 16.2 mm of rainfall was recorded during the month. The experimental site experienced 82 rain days between 30 October, 2000 and 24 March, 2001. A total of 1,007.2 mm of rain fell during this period with the daily maximum being 82.6 mm (on 12 February). The total monthly rainfall peaked in February with 280.5 mm. The average temperature for the season (October 2000 and April 2001) was 23.9° C. The average minimum temperature during these months was 17.6° C while the average maximum temperature was 29.7° C.

There was a dry spell after planting which resulted in poor crop emergence. As a result, all those accessions whose germination percentage was less than 50% were excluded from the study. This reduced the number of accessions to be studied from initial thirty-six to twenty-eight. The last rainfall (52.4 mm) was obtained in the third week of March when the crop was at flowering stage. Thereafter there was complete drought and the crop had to survive on residual moisture. This dry spell caused some plants within some accessions to fail to produce seeds. However, since data was to be collected from ten randomly selected plants, it was still possible to collect data for the study.

4.2 Morphological Characterization

A total of 23 descriptors were studied in all twenty-eight accessions of pigeon pea germplasm. Frequency distribution of qualitative descriptor states is presented in Table 1. Some characters did not vary among accessions: leaf hairiness, second flower colour, pod form, pod hairiness, seed shape and stem thickness. The growth habit ranged from erect and compact to erect and loose. Six accessions were erect and compact while twenty-two accessions were erect and loose. Twenty-five accessions (89.3%) had indeterminate flowering pattern, while 10.7% were determinate types. A large portion of the accessions (85.7%) had plain seed colour while 7.1% each had mottled and mottled and speckled seed colour pattern. About 78.6% of the accessions had cream seed colour, 14.3% had light brown and the remaining 7.1% had purple coloured seed.

Table 1: Frequency distribution of 15 qualitative traits in 28 pigeon pea germplasm accessions from Zambia.

Descriptor	No. of accessions	Frequency (%)
Stem colour		
Green	5	17.9
Purple	23	82.1
Base flower colour		
Light yellow	24	85.7
Orange yellow	4	14.3
Flowering pattern		
Indeterminate	25	89.3
Determinate	3	10.7
Seed colour pattern		
Plain	24	85.7
Mottled	2	7.1
Mottled and speckled	2	7.1
Leaf hairiness		
Pubescent	28	100
Second flower colour		
Red	28	100
Pod form		
Flat	28	100
Pod hairiness		
Pubescent	28	100
Seed shape		
Oval	28	100
Stem thickness		
>13 mm	28	100
Pod colour		
Green	14	50
Purple	6	21.4
Mixed green and purple	8	28.6
Leaflet shape		
Lanceolate	6	21.4
Narrow elliptic	22	78.6
Pattern of streaks		
Sparse	14	50
Medium	3	10.7
Dense	11	39.3

Twenty-four out of 28 accessions had light yellow flower colour while four accessions had orange yellow flower colour.

A remarkable variation was observed in seven quantitative characters; plant height, days to 50% flowering, days to 80% maturity, number of branches, leaf size, number of racemes and number of seeds per pod (Table 2) while 100-seed weight showed less variation.

Table 2. Evaluation of quantitative characters in 28 accessions of pigeon pea germplasm from Zambia.

Descriptors	Range	Mean	SD	CV (%)
Plant height (cm)	155-236	208.4	17.0	8.0
Days to 50% flowering	113-147	132.4	8.7	6.6
Days to 80% maturity	143-178	163.0	8.7	5.4
No. of branches	22-81	42.8	11.2	26.2
Leaf size (cm^2)	11.2-23.2	17.1	2.6	15.3
Raceme number	26-85	50.8	13.9	27.5
No. of seeds/pod	3-5	4.3	0.6	15.2
100-seed weight (g)	37.2-41.1	39.3	1.0	2.5

SD = Standard Deviation CV = Coefficient Variation

When the data for 8 variables were subjected to principal component analysis (PCA), only 3 of the 8 principal components generated had eigenvalues more than 1 and accounted for 33.1%, 25.8% and 15.0% of the total variation individually and 73.9% altogether (Table 3).

Table 3: Eigenvalues and percentages of the variability explained by each principal component and accumulated variability.

Principal component variability	Eigenvalue	% Variability	Accumulated Variability
1	2.64	33.1	33.1
2	2.07	25.8	58.9
3	1.20	15.0	73.9

The first principal component was loaded largely by days to 50% flowering, days to 80% maturity, leaf size and plant height. The second principal component was associated mainly with the number of branches and racemes. However, 100-seed weight had the highest negative loading along this principal component. Finally the third principal component was associated largely with number of seeds per pod (Table 4).

Table 4: Correlation coefficients of pigeonpea characteristic with respect to their principal components (PC_s).

Characteristic	PC 1	PC 2	PC 3
No. of seeds per pod	0.12	0.01	0.60
100 seed weight	-.04	-.48	0.32
No of branches	0.02	0.60	0.04
Leaf size (cm^2)	0.40	-.09	0.44
No. of racemes	0.15	0.53	-.06
Days to 50% flowering.	0.53	-.18	-.35
Days to 80% maturity	0.53	-.17	-.35
Plant height	0.47	0.22	0.31

The plot of the first and second principal components (Fig.1) was able to form a group according to plant height and days to 50% flowering. However, there were outliers such as ZM 425, ZM 465, ZM 483,ZM 423,ZM 467,ZM 431, ZM 418 and ZM 413.

The plot of the first and third principal components (Fig. 2) grouped accessions into one group on the graph except for ZM 392, ZM 467 and ZM 413. The grouping of accessions into one group showed low variability in number of days to 50% flowering, number of seeds per pod and plant height.

The plot of the second and third principal components (Fig. 3) produced a cluster of accessions that shared similar characters such as number of branches and number of seeds per pod. However, there were outliers such as ZM 452, ZM 467 and ZM 392.

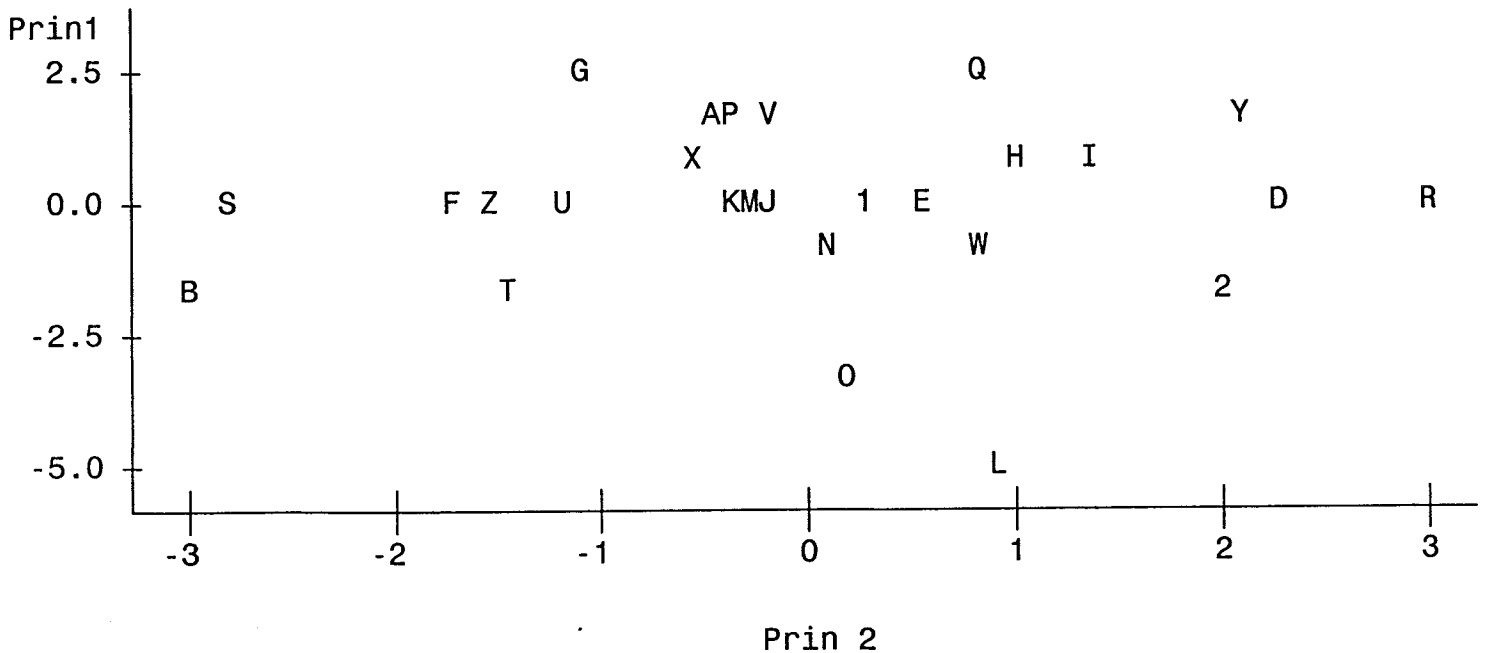

Figure 1. Dispersion of accessions of pigeonpea along the first and second principal components based on morphological characters

Legend:
s=zm 431, B=zm 467, F=zm 392, Z=zm 465, T=zm 463, U=zm 390, G=zm 403, X=zm 452, A=zm 3214, P=zm 414, V=zm 3209, K=zm 480, M=zm 442, J=zm 391, O=zm 418, N=zm 430, 1=zm 449, Q=zm 408, E=zm 470, L=zm 413, W=zm 434, H=zm 472, I=zm 405, Y=zm 388, D=zm 423, 2=zm 483, R=zm 425.

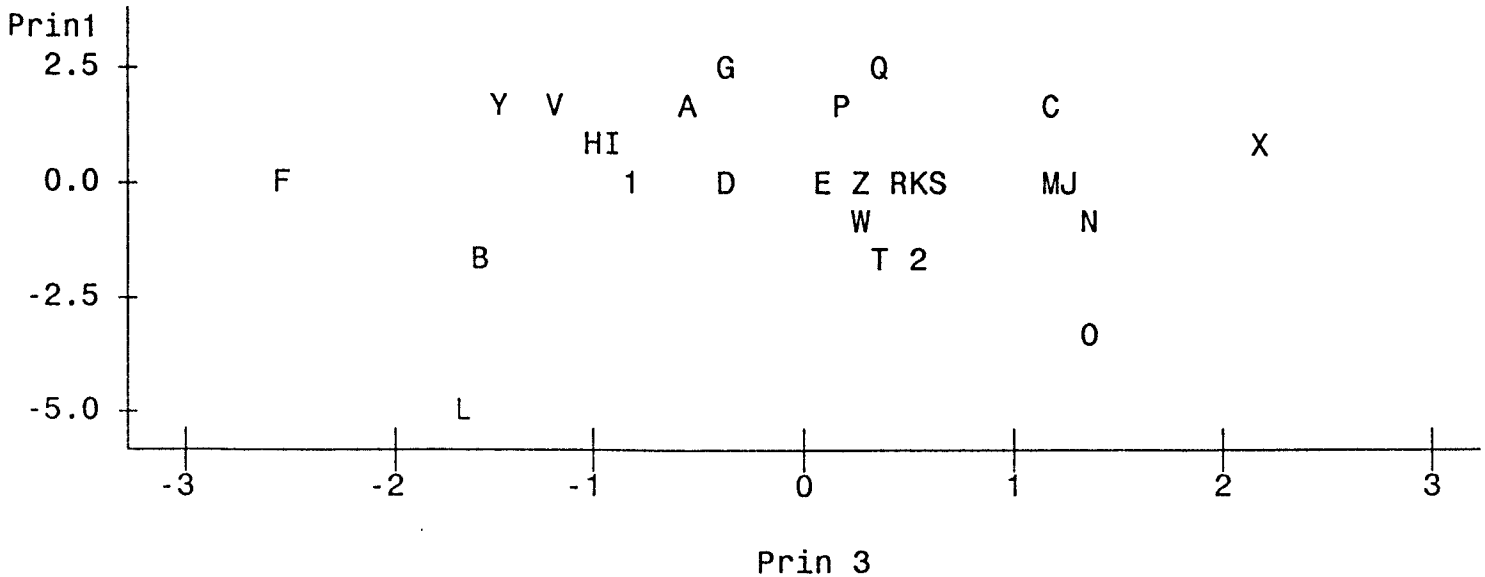

Figure 2. Dispersion of accessions of pigeonpea along the first and third principal components based on morphological characters

Legend:
F=zm 392, L=zm 413, B=zm 467, Y=zm 388, V=zm 3209, H=zm 472, I=zm 405, 1=zm 449, A=zm 3214, G=zm 403, D=zm423, Q=zm 408, P=zm 414, E=zm 470, Z=zm 465, W=zm 434, T=zm 463, 2=zm 483, R=zm 425, K=zm 480, S=zm 431, C=zm 3225, M=zm 442, J=zm 391, N=zm 430, O=zm 418

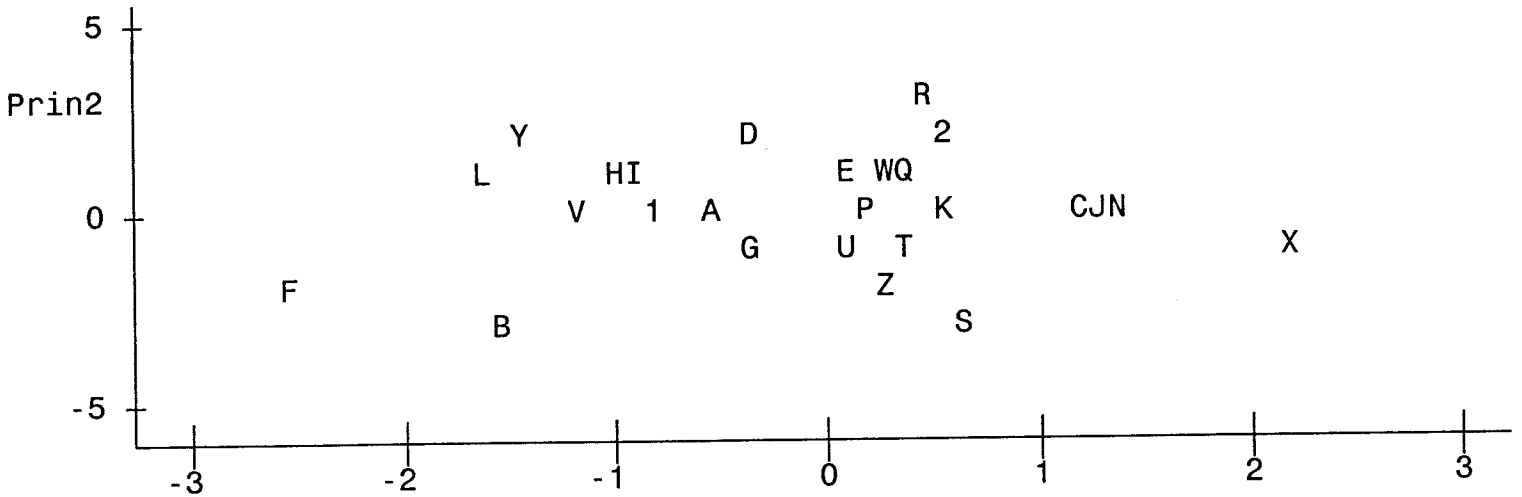

Figure 3. Dispersion of accessions of pigeonpea along the second and third principal components based on morphological characters

Legend:

F=zm 392, B=zm 467, L=zm 413, Y=zm 388, V=zm 3209, H=zm 472, I=zm 405, 1=zm 449, D=zm 423, A=zm 3214, G=zm 403, S=zm 431, Z=zm 465, U=zm 390, T=zm 463, P=zm 414, K=zm 480, Q=zm 408, W=zm 434, E=zm 470, R=zm 425, 2=zm 483, C=zm 3225, J=zm 391, N=zm 430, X=zm 452

Cluster analysis was performed following principal component analysis to further examine similarities and dissimilarities among 28 accessions of pigeonpea. When the dendrogram was read at cluster distance of 10, the accessions were grouped into 4 major clusters (Fig. 4). Cluster 1 (is implied) consisted of 19 accessions that were erect and loose in growth habit and were further grouped into 3 sub-clusters based on unique characters shared by the accessions in each cluster. Accessions that were associated with sub-cluster 1(a) were ZM 472, ZM 449, ZM 3214, ZM 414, ZM 403 and ZM 3209 which shared common characters such as purple colour of the stem, light yellow colour of the flowers and 141 days to reach 50% flowering. Accessions in sub-cluster 1(b) were ZM 470, ZM 434, ZM 442, ZM 430, ZM 391, ZM 480 and ZM 452. These accessions shared the following characteristics: light yellow colour of the flowers, cream colour of the seed coat, 127 days to flowering and had tall plants. Sub-cluster 1(c) consisted of accessions ZM 423, ZM 483, ZM 405, ZM 3225 and ZM 388. These accessions shared common characteristics such as light brown colour of the seed coat, 135 days to reach 50% flowering and purple colour of the stem.

Cluster 2 was independently formed by accession ZM 425. This accession was the highest in branching and was erect and compact in growth habit. It reached 50% flowering in 128 days and had seeds with a cream seed coat.

Cluster 3 consisted of accessions ZM 390, ZM 465, ZM 467, ZM 431 ZM 463 and ZM 392. These accessions shared common characteristics such as green colour of the stem, orange yellow

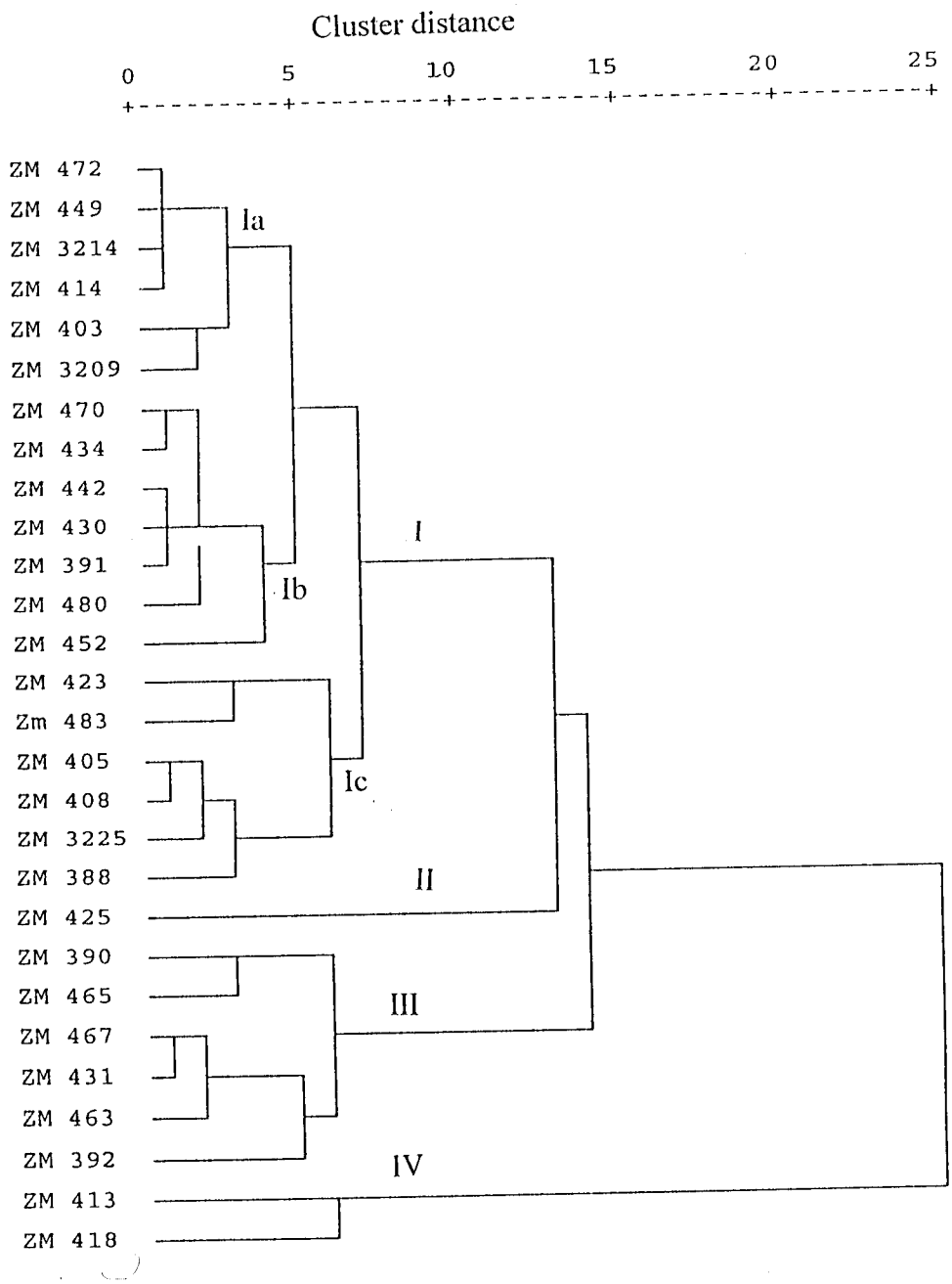

Figure 4. Dendrogram showing relationships among 28 *Cajanus cajan* collections from Zambia.

flowers, lowest number of racemes, short plants and had purple coloured seed coat.

Finally cluster 4 consisted of accessions ZM 413 and ZM 418. These accessions were the earliest to flower. They flowered in 113 days. They were erect and compact in growth habit and had cream coloured seed coat.

CHAPTER FIVE

DISCUSSION

Characters used in morphological characterization were 23, of which 8 were quantitative while 15 were qualitative. Frequency distribution (Table 1) of qualitative characters revealed that stem colour, flower, pod colour, pattern of streaks and colour of the seed coat were important components of variability in the germplasm. Some of the qualitative characters on the other hand such as leaf hairiness, second flower colour, pod form, pod hairiness, seed shape and stem thickness expressed limited variability.

One or more characters were able to discriminate accessions depending on their discriminating power. However, all the characters were found to have a distinct influence in segregation of the accessions. In addition, when combinations of characters were made certain characters were found to be commonly shared by many accessions and therefore did not distinguish the accessions as sub-groups.

There was great variation in parts of the same plant and within plants of the same accession when vegetative and inflorescence characters were observed. For instance, some accessions had plants with light yellow flowers and other plants with orange yellow flowers. This implied that the accessions might have been cross-pollinated in the areas of collection. Although pigeonpea is a self-pollinated crop, there is out-crossing of 5-20% (Purseglove, 1968). Seed characters such as seed coat pattern and colour showed variability among accessions. Seed coat pattern ranged from

plain, mottled to mottled and speckled among accessions while the seed coat colours observed among the accessions were cream, light brown and purple.

From the results of principal component analysis, the first three principal components accounted for 73.9% of the total morphological variation, which indicated that the characters recorded were taxonomically useful. Thus, the descriptors documented by IBPGR/ICRISAT (1993) were helpful in identifying the most discriminatory intraspecific characteristics of *C. cajan* as well as those which showed limited variation and hence may not need to be recorded for this species. According to principal component and cluster analysis, days to 50% flowering, days to reach 80% maturity, number of racemes, number of branches, leaf size, plant height, number of seeds per pod and pigmentation of various parts such as stem colour, flower colour, pod colour and colour of the seed coat were important characters which contributed to variability among the accessions. These characters would, therefore, be important and effective criteria in distinguishing between the accessions of pigeonpea. Ariyo (1993) has also reported the significant contribution of pigmentation of various plant parts and seed characteristics to the total variation in West African okra. Days to flowering have also been described to be very important in discriminating genotypes. Sneath and Sokal (1973) also emphasized the importance of flowering behaviour in numerical taxonomy. These accessions of pigeonpea flowered between 113 and 146 days and were mostly photoperiod insensitive.

Morphological characters can therefore be used in characterization of pigeonpea as revealed by principal component and cluster analysis. However, Singh et al., (1991) argued that morpho-agronomic characters were phenotypic traits and accessions may be similar morphologically and

yet they are genetically distant to each other. Therefore, morphological characterization should be supplemented by the use of biochemical and DNA markers such as restriction fragment length polymorphism (RFLP) to detect the difference at molecular level.

In this study however, it was not possible to use either of the above methods to characterize the accessions of pigeonpea due to financial constraint.

CHAPTER SIX

CONCLUSION

There is genetic variation among the accessions of pigeonpea conserved at the National Genebank as revealed by three principal components which accounted for 73.9% of the total variation and cluster analysis which grouped the accessions into four major clusters based on the commonly shared characters.

The most discriminatory characters were days to 50% flowering, days to reach 80% maturity, plant height, number of racemes, number of branches, number of seeds per pod, stem colour, flower colour, pod colour, seed coat colour and flowering pattern. These characters are therefore useful in morphological characterization of pigeonpea germplasm.

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APPENDICES

Appendix 1: Meteorological Data

Location: Mount Makulu, Chilanga, Zambia, 15° 33' S, 28° 15' E, 1194m.

Source: Mount Makulu Meteorological Station

Period: October 2000-March 2001

Rainfall	Month	(mm)
	October	16.2
	November	121.5
	December	215.7
	January	256.6
	February	280.5
	March	116.7

Temperature	Month	Min °C	Max °C
	October	17.8	31.9
	November	18.2	30.3
	December	17.8	27.2
	January	16.7	27.7
	February	18.2	26.5
	March	17.7	27.1
	April	15.8	37.0

Appendix 2: Accessions of pigeonpea studied from Zambia

Serial no.	Accession no.
1	ZM 3214
2	ZM 467
3	ZM 3225
4	ZM 423
5	ZM 470
6	ZM 392
7	ZM 403
8	ZM 472
9	ZM 405
10	ZM 391
11	ZM 480
12	ZM 413
13	ZM 442
14	ZM 430
15	ZM 418
16	ZM 414
17	ZM 408
18	ZM 425
19	ZM 431
20	ZM 463
21	ZM 390
22	ZM 3209
23	ZM 434
24	ZM 452
25	ZM 388
26	ZM 465
27	ZM 449
28	ZM 483

Appendix 3

Data matrix used to generate cluster dendrogram by cluster analysis

Accession																													
ZM 3214	4	39.2	36	19.4	58	141	171	212	0	1	0	1	1	0	1	0	1	0	0	1	0	0	1	0	0	0	1	1	0	0
ZM 467	3	40.7	23	15.2	26	134	164	185	0	1	0	1	1	0	1	0	1	0	0	1	0	0	1	0	0	0	1	0	0	1
ZM 3225	5	41.1	41	18.9	70	138	168	216	0	1	0	1	1	0	1	0	1	0	0	1	0	0	1	0	0	1	0	1	0	0
ZM 423	5	37.7	53	13.1	70	131	161	210	1	0	0	1	0	1	1	0	0	0	1	0	1	0	1	0	0	0	1	1	0	0
ZM 470	4	39.2	49	16.9	49	131	161	218	0	1	0	1	1	0	1	0	1	0	0	0	1	0	1	0	0	0	1	1	0	0
ZM 392	3	40.1	35	15.1	45	145	173	188	0	1	0	1	1	0	0	1	0	1	0	0	0	1	1	0	0	0	1	0	0	1
ZM 403	4	39.7	38	20.7	47	147	176	223	0	1	1	0	0	0	1	0	0	0	1	1	0	0	0	1	0	0	1	0	0	1
ZM 472	4	37.3	45	17.6	52	134	169	211	0	1	0	1	1	0	1	0	0	1	0	1	0	0	1	0	0	0	1	1	0	0
ZM 405	4	39.1	58	15.9	67	140	171	219	1	0	0	1	1	0	1	0	0	0	1	1	0	0	1	0	0	1	0	1	0	0
ZM 391	4	39.6	45	21.4	42	127	156	214	0	1	0	1	1	0	1	0	1	0	0	1	0	0	1	0	0	0	1	1	0	0
ZM 480	4	39.2	34	18.1	45	129	157	221	1	0	0	1	1	0	0	1	0	1	0	1	0	0	1	0	0	0	1	0	0	1
ZM 413	3	38.8	47	11.2	55	113	143	155	0	1	0	1	1	0	1	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0
ZM 442	5	39.9	43	17.2	49	130	159	206	0	1	0	1	1	0	1	0	1	0	0	1	0	0	1	0	0	0	1	1	0	0
ZM 430	5	39.5	41	17.1	51	126	157	212	0	1	0	1	1	0	1	0	0	0	1	1	0	0	1	0	0	0	1	1	0	0
ZM 418	5	39.6	41	15	50	114	146	181	0	1	1	0	1	0	1	0	1	0	0	1	0	0	1	0	0	0	1	1	0	0
ZM 414	5	38.7	35	18.3	51	142	171	217	0	1	1	0	1	0	1	0	1	0	0	1	0	0	1	0	0	1	0	1	0	0
ZM 408	4	39.2	52	23.2	68	141	171	223	1	0	0	1	1	0	1	0	0	1	0	1	0	0	1	0	0	0	1	1	0	0
ZM 425	4	37.9	81	19.2	51	128	156	217	1	0	0	1	1	0	1	0	0	0	1	1	0	0	1	0	0	0	1	0	1	0
ZM 431	5	40.3	22	17.6	28	135	167	193	0	1	0	1	1	0	1	0	1	0	0	1	0	0	1	0	1	1	0	0	0	1
ZM 463	5	39.7	32	14.1	33	128	157	184	0	1	0	1	1	0	1	0	0	1	0	0	1	0	1	0	0	1	0	0	0	1
ZM 390	4	39.5	36	17.3	30	132	162	213	0	1	0	1	1	0	1	0	0	0	1	0	1	0	1	0	0	0	1	0	0	1
ZM 3209	5	37.2	36	16.5	43	147	178	210	0	1	0	1	1	0	1	0	1	0	0	1	0	0	0	1	0	0	1	1	0	0
ZM 434	4	39.4	47	15.7	56	129	155	220	0	1	1	0	1	0	1	0	1	0	0	1	0	0	0	0	1	0	1	0	0	1
ZM 452	5	40.7	39	19.7	51	131	161	236	0	1	0	1	1	0	1	0	1	0	0	1	0	0	1	0	0	0	1	1	0	0
ZM 388	4	38.4	49	14.3	85	140	170	224	0	1	1	0	1	0	0	1	0	0	1	0	0	1	1	0	0	0	1	0	0	1
ZM 465	4	40.6	46	19.1	31	134	165	198	0	1	0	1	1	0	1	0	1	0	0	1	0	0	1	0	0	0	1	0	0	1
ZM 449	4	39.3	45	14.9	54	136	168	216	0	1	0	1	0	1	0	1	0	1	0	1	0	0	1	0	0	0	1	0	0	1
ZM 483	4	38.4	50	16.2	64	118	150	214	1	0	0	1	1	0	1	0	0	0	1	1	0	0	1	0	0	0	1	0	1	0

[1]

[1] Principle components used only the quantitative data of the same matrix