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Full Length Research Paper

Assessment of the genetic diversity in a germplasm collection of cowpea (*Vigna unguiculata* (L.) Walp.) using morphological traits

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Cowpea is considered a minor crop with generally low grain yield; nevertheless production area is spread all around the world, mainly in marginal areas with poor soil and limited rainfall. It is a multipurpose crop grown for green pods as vegetable, dry seeds as pulse and green fodder. The aim of this study was to evaluate 48 accessions of cowpea by 24 morphological descriptors in order to identify accessions with specific behaviour that could be exploited by plant breeders. The accessions with best performance for the development of new varieties and more interesting for inclusion in cowpea breeding programme are 87-052, 95-017, A4E007 and 98210005. The descriptors pod length, number of seeds per pod, seed thickness and 100 seed weight were found the most stable traits over the three years.

Key words: Cowpea, *Vigna unguiculata,* landraces, breeding lines, genetic resources, morphological diversity.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.), a member of the family *Fabaceae*, is a crop grown throughout the tropics and the substropics covering Africa, Asia, South America, parts of Southern Europe and the United States (Singh et al., 1997). It has been estimated that the total production of cowpeas for dry seeds is 5.5 million tones and the total area grown was 10.5 million (www.faostat.fao.org. 2010). Cowpea is a heat loving crop which tolerates drought and lower soil fertility (Coetzee, 1995; Mortimore et al., 1997).

Due to its high protein content (20 to 25%), cowpea plays a major role in human nutrition (Singh et al., 1997). The grain is valued for its flavor and short cooking time and the plant is especially favored by farmers because of its ability to maintain soil fertility through its ability to fix nitrogen (Blade et al., 1997).

The cultivated cowpea consists of two main *subsp. unguiculata* and *subsp. sesquipedalis*. Landraces or traditional old varieties have played important role for in the introduction of improved adaptive characteristics (Hawtin et al., 1996). A large number of landraces are still grown in the gardens and small farms mainly for dry seeds. The farmers maintain old populations for their family subsistence and for the nearest local market.

Physiological, morphological, or phenological criteria could be implemented to select the improved adaptation to dry environments (Blum, 1988). Traditionally, diversity is estimated by measuring variation in phenotypic or qualitative traits (starts flowering, time to maturity, plant type, flower color, seed type, seed color, seed size, hilum color) and quantitative agronomic traits However, this approach is often limited and expression of quantitative traits is subject to strong environmental influence (Kameswara, 2004).

Increasing major components of grain yield such as pods/plant, pod length, seed/pod and seed size will allows improving cowpea yield potential. The variability of these morphological traits has been reported from

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Table 1. Cowpea accessions subjected to morphological characterization.

Accession	Status of sample	Accession	Status of sample	Accession	Status of sample
77	Breeding line(Hungary)	95-025	Breeding Line (USA)	A4-093	Breeding line (IITA)
98210001	Landrace (Portugal)	95-030	Breeding Line (USA)	A4-094	Breeding line (IITA)
98210003	Landrace (Portugal)	95-042	Breeding Line (USA)	A4-096	Breeding line (IITA)
98210004	Landrace (Portugal)	95-045	Breeding Line (USA)	A4E-007	Landrace (Bulgaria)
98210005	Landrace (Portugal)	95-057	Breeding Line (USA)	A4E-008	Landrace (Bulgaria)
2005-01	Landrace (Bulgaria)	95-073	Breeding Line (USA)	A7E-0735	Landrace (Bulgaria)
87-003	Breeding Line (IITA)	95-081	Breeding Line (USA)	A8E-0523	Landrace (Bulgaria)
87-007	Breeding Line (IITA)	95-095	Breeding Line (USA)	A8E-0542	Landrace (Bulgaria)
87-026	Breeding Line (IITA)	97-001	Breeding Line (Japan)	A8E-0551	Landrace (Bulgaria)
87-052	Breeding Line (IITA)	A4-080	Breeding Line (IITA)	A8E-0554	Landrace (Bulgaria)
87-058	Breeding Line (IITA)	A4-081	Breeding Line (IITA)	A8E-0562	Landrace (Bulgaria)
87-060	Breeding Line (IITA)	A4-083	Breeding Line (IITA)	A8E-0563	Landrace (Bulgaria)
91-010	Breeding Line (IITA)	A4-084	Breeding Line (IITA)	A8E-0492	Landrace (Bulgaria)
92-002	Breeding Line (VMW)	A4-086	Breeding Line (IITA)	BOE07	Landrace (Bulgaria)
95-017	Breeding Line (USA)	A4-087	Breeding Line (IITA)	BOE08	Landrace (Bulgaria)
95-023	Breeding Line (USA)	A4-088	Breeding Line (IITA)	St	Landrace (Bulgaria)

different authors, as Patil and Baviskar (1987), Sardana et al. (2001), Mishra et al. (2002), Carnide et al. (2007).

Knowledge of phenotypic variation and relationships among genotypes will assist breeders to develop appropriate breeding strategies and to create the most adaptive and productive cultivars. The study on landraces variation in morphological, phonological and agronomic traits would be useful in the development in new varieties with better adaptation to biotic and abiotic stress factors, as well as for high yield potential.

In Bulgaria, cowpea is regarded as a minor crop, as in other European countries (Negri et al., 2000) and no statistics are available for our country, where this crop is often mistaken for *Phaseolus vulgaris*, the common bean. In many countries (Portugal, Spain, Italy, Bulgaria) cowpea is cultivated for both, the seeds and green pods (Negri, 2009).

The cowpea collection maintained at the Institute of Plant Genetic Resources in Sadovo, consists 336 introduced accessions and landraces. The bigger number of accessions gives opportunity to select the most appropriate of them for the respective breeding objectives after a complex evaluation of samples, best adaptation capacity to certain growing conditions, as accessions with manifested tolerance to abiotic stress factors (Hamidou et al., 2007; Agbicodo et al., 2009).

The landraces included in our investigation were represented by 14 accessions collected mainly from Southeastern part of the country. Most of foreign accessions were introduced from IITA (17), USA (10), Portugal (4), Vietnam (1), Japan (1) and Hungry (1).

The main objective of this study was to determine the variation among cowpea landraces collected from different agro-ecological zones of Bulgaria based on morphological and phenotypic characterization as well as with introduced accessions with different geographical origin.

MATERIALS AND METHODS

A total of 48 accessions of cowpea were analyzed in this study. These accessions included 18 landraces from Bulgaria and Portugal and 30 advanced breeding lines with different origins (Table 1). The experimental work was carried out at the Institute for Plant Genetic Resources (IPGR, Sadovo, Bulgaria), during 3 years. The field trials were in a randomised complete block design with three replications. Each accession was represented by 15 plants.

The genotypes were described based on the descriptors for cowpea of IBPGR (1983). In each accession, 5 were randomly chosen for biometric measurements. Nineteen quantitative characters (Table 2) and five qualitative characters (flower and seed color, seed shape, hilum color and testa texture) were recorded during the vegetative and reproductive stages. Days to flowering were determined as the number of days from sowing to 50% of the plants has begun to flower and days to maturity as the number of days from sowing to 90% of plants have mature pods. Plant height and height to first pod was evaluated at the end of flowering.

Data were analysed by numerical taxonomy techniques, using NTSYS-pc package, version 2.01 (Rohlf, 1997). An unweighted pair-group method of the arithmetic average clustering procedure (UPGMA) was employed to construct dendrograms. Principal component analysis was also performed to establish the importance of different traits in explaining the total variation.

RESULTS

The majority of the cowpea accessions have white (47.9%) or lilac flowers (47.9%). Only 4.2% of the accessions present flowers with others colors (Table 3). Seeds have predominant cream colour and kidney shape. The majority of seeds have colored hilum. The

Table 2. Quantitative descriptors used in the characterization of the 48 accessions.

Morphological descriptor	Abbreviation	Morphological descriptor	Abbreviation
Days to flowering	DFL	Number of pods per plant	Npod/pl
Flowering duration	DurFL	Weight of pods per plant (g)	Wpod/pl
Days to maturity	DM	Number of seeds per plant	NSeed/pl
Plant height (cm)	Height	Seed length (cm)	Lseed
Plant weight (g)	WPl	Seed width (cm)	Wseed
Height to first pod (cm)	H1stpod	Seed thickness (cm)	Tseed
Pod length (cm)	Lpod	Seed weight per plant (g)	Wseed/pl
Pod width (cm)	Wpod	100 seed weight (g)	W100S
Pod thickness (cm)	Tpod	Yield (g/m^2)	Yield
Number of seeds per pod	NS/pod		

Table 3. Qualitative traits observed on 48 accessions of *V. unguiculata*

Accession	Flower colour	Seed colour	Seed shape	Hilum colour	Testa texture
77	**White**	**White**	**Rhomboid**	**Black**	**Smooth**
98210001	Lilac	Cream	Kidney	Brown	Rough
98210003	Light lilac	White	Kidney	Black	Smooth
98210004	White	Cream	Kidney	Dark brown	Rough
98210005	White	Cream	Kidney	Green	Rough
2005-01	White	Cream	Kidney	Black	Rough
87-003	Light lilac	Cream	Kidney	Dark brown	Rough
87-007	Dark lilac	Redish	Kidney	-	Smooth
87-026	White	Cream	Rhomboid	Dark brown	Rough
87-052	Light lilac	Cream	Globose	Black	Smooth
87-058	White	Cream	Rhomboid	Beige	Rough
87-060	Light lilac	Cream	Globose	Dark brown	Smooth
91-010	Dark lilac	Cream	Globose	Green	Smooth
92-002	Lilac	Black	Ovoid	-	Rough
95-017	White	Cream	Kidney	Black	Rough
95-023	White	Cream	Kidney	Beige	Rough
95-025	Dark lilac	Cream	Kidney	Dark brown	Smooth
95-030	White	White	Kidney	Black	Smooth
95-042	Lilac	Cream	Rhomboid	Beige	Rough
95-045	White	Cream	Kidney	Dark brown	Rough
95-057	Dark lilac	Cream	Kidney	Dark brown	Rough
95-073	Lilac	Cream	Rhomboid	Green	Smooth
95-081	Light lilac	Cream	Rhomboid	Dark brown	Smooth
95-095	Dark lilac	Cream	Rhomboid	-	Smooth
97-001	yellow	Red	Rhomboid	White	Smooth
A4-080	Light yellow	Cream	Ovoid	-	Smooth
A4-081	white	White	Kidney	Dark brown	Rough
A4-083	white	Brown	Rhomboid	-	Rough
A4-084	white	Cream	Kidney	-	Rough
A4-086	Light lilac	Brown	Kidney	-	Smooth
A4-087	white	Brown	Kidney	-	Rough
A4-088	white	Cream	Rhomboid	Dark brown	Rough
A4-093	white	Cream	Kidney	Dark brown	Rough
A4-094	Light lilac	Cream	Kidney	-	Smooth
A4-096	Dark lilac	Cream	Rhomboid	Green	Smooth
A4E-07	white	White	Kidney	Black	Rough

Table 3. Contd.

A4E-0008	white	Cream	Kidney	Dark brown	Rough
A7E-0735	lilac	Cream	Kidney	Green	Smooth
A8E-0492	Light lilac	White	Kidney	Black	Rough
A8E0523	lilac	Cream	Rhomboid	Dark brown	Rough
A8E0542	Light lilac	Cream	Rhomboid	Green	Smooth
A8E0551	Light lilac	Cream	Rhomboid	Green	Smooth
A8E0554	Light lilac	Cream	Rhomboid	Green	Smooth
A8E0562	White	Cream	Kidney	-	Smooth
A8E0563	White	White	Kidney	Black	Rough
BOE-07	White	Cream	Kidney	Green	Rough
BOE-08	White	Cream	Kidney	Dark brown	Rough
St	White	White	Kidney	Black	Rough

Table 4. Mean, ranges and coefficients of variation for the descriptors observed on 48 accessions of cowpea.

Variable	Mean	Min.	Max.	CV (%)
Days to flowering	50.81	40.00	60.70	9.58
Flowering duration (days)	18.03	14.00	23.50	11.90
Days to maturity	86.12	72.70	94.00	4.98
Plant height (cm)	74.46	37.70	122.20	31.70
Plant weight (g)	43.98	21.20	125.80	39.27
Height to first pod (cm)	19.08	9.00	28.40	23.19
Pod lenght (cm)	14.49	9.80	17.70	10.05
Pod width (cm)	0.79	0.60	0.90	9.46
Pod thickness (cm)	0.61	0.50	0.70	8.87
Number of seeds per pod	10.54	7.70	13.80	13.43
Number of pods per plant	14.53	7.10	36.20	32.30
Weight of pods per plant (g)	18.52	10.50	34.50	29.36
Number of seeds per plant	93.68	40.20	200.50	37.28
Seed lenght (cm)	0.98	0.70	4.40	52.37
Seed width (cm)	0.67	0.50	0.80	8.81
Seed thickness (cm)	0.54	0.40	0.60	11.93
Seed weigth per plant (g)	14.93	7.70	59.90	52.79
100 seed weight (g)	19.05	9.10	27.20	23.61
Yield (g/m^2)	131.15	40.50	218.10	34.70

color of hilum can range from white, beige, green, brown and black. Regarding the testa texture, the seeds can be classified in two groups: smooth (44%) or rough (56%).

Minimum and maximum values and the coefficient of variation for each quantitative trait are presented in Table 4. The data indicate that there is considerable morphological variation among the accessions. The most variable characters were weight of seeds per plant, seed length, plant weight and number of seeds per plant. The characters with less variation included number of days to maturity and to flowering, pod and seed width.

The dendrogram based on morphological data is shown in Figure 1. The accessions can be separated in seven clusters, one of which contains only one accession. The status of the sample does not determine the cluster pattern of the accessions, that is, it is not possible to discriminate landraces from the advanced breeding lines in distinct clusters. Except for group A, group D and G, all of others groups are constituted by landraces and advanced breeding lines. In group D, all accessions are advanced breeding lines and almost of them are from IITA. The group E is composed mainly by landraces from Bulgaria. In this study, the accessions with lower similarity level with regard to others are the advanced breeding line 97-001 from Japan and line 77 from Hungry. The line 97-001 is the only accession that has yellow flowers and red seeds with white hilum.

In the principal component analysis for the 19 quantitative

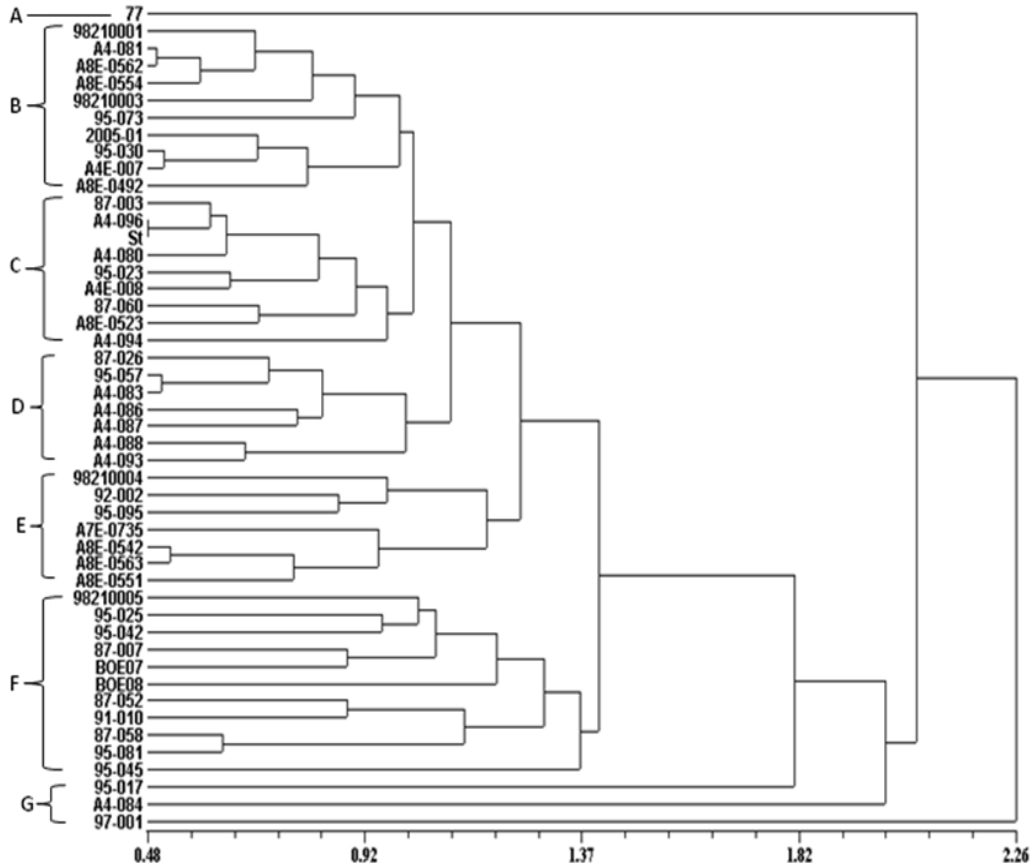

Figure 1. Dendrogram of 48 cowpea accessions obtained by analysis of quantitative morphological data.

quantitative traits, the first three principal components explained 56.46% of the total variance. The characteristics responsible for accessions separation along the first principal component were the number of seeds and pods per plant, the weight of the plant and 100 seed weight. The accessions were grouped according to their productive capacity. Vural and Karasu (2007) reported similar results. Accessions placed more to the right in the Figure 2 (87-052, 87-058, 95-081, 98210005, 91-010 and 77 - Group A) are those with higher yield potential and these accessions are in group F of the dendrogram. It can also be observed that the accessions with higher number of pods and seeds have small seed size, meaning that these characteristics are negatively correlated (Figure 3).

The second principal component grouped the accessions according to the pod width, pod thickness and the number of days to flowering. The accessions that are on the top of Figure 2 (group B) are those that have bigger pods and the plants required more days to reach the flowering period. In contrast, the accessions included in group C are characterized by plants that start flowering earlier and have small pods. The number of days required to reach the flowering is negatively correlated with the duration of the period of flowering and production of seeds. The early flowering accessions were those that have larger flowering period and higher yield.

As it was already verified in the dendrogram, the advanced breeding line 97-001 from Japan is isolated from the others accessions. This accession differs from the others mainly by having plants that produce a very high number of pods and seeds with small size. The 100 seed weight is 9.5 g while the average of the others accessions is 19.1 g.

This study allowed us to point out which the characteristics remained stable over the three years. Of the 19 characteristics analyzed, pod length, number of seeds per pod, seed thickness and 100 seed weight (Figure 4) are the most stable. These traits were not influenced by environmental conditions and give us the same high level of information over the 3 years.

DISCUSSION

Genetic diversity is a prerequisite for the genetic improvement of a crop. But rational use of the genetic diversity present in germplasm collections requires a

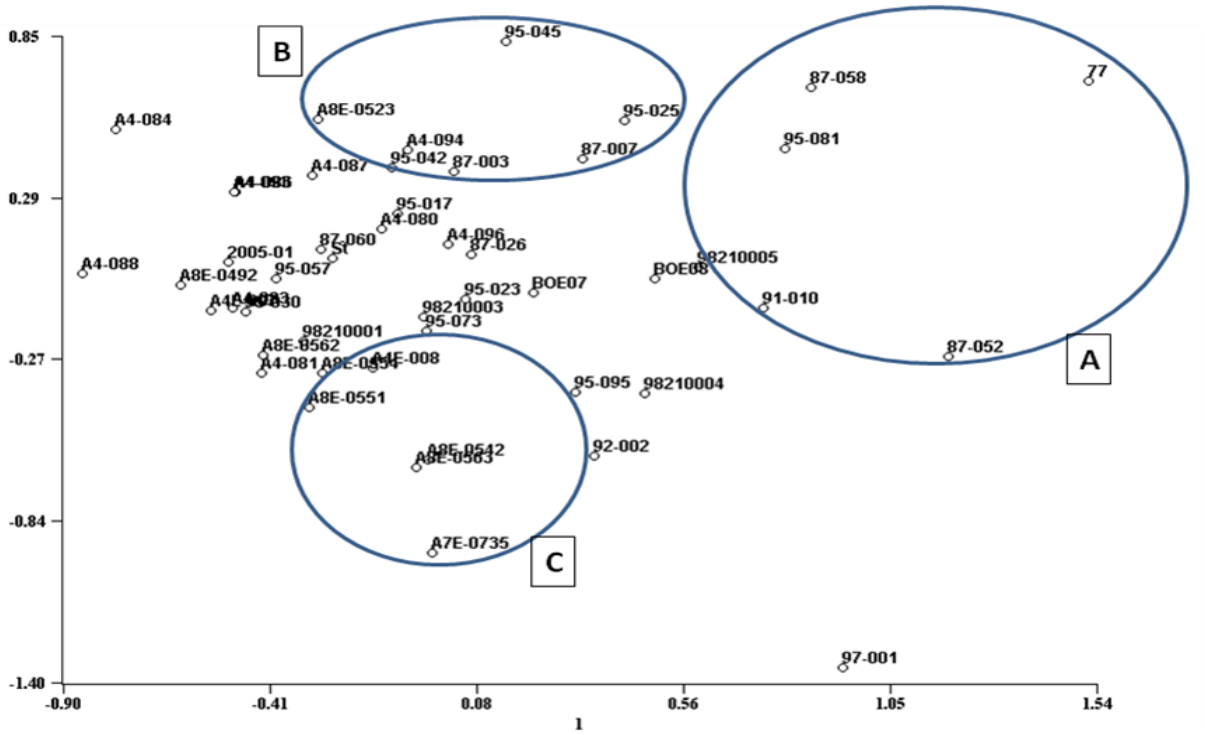

Figure 2. Projection of the 48 cowpea accessions into the plane defined by the two first principal components.

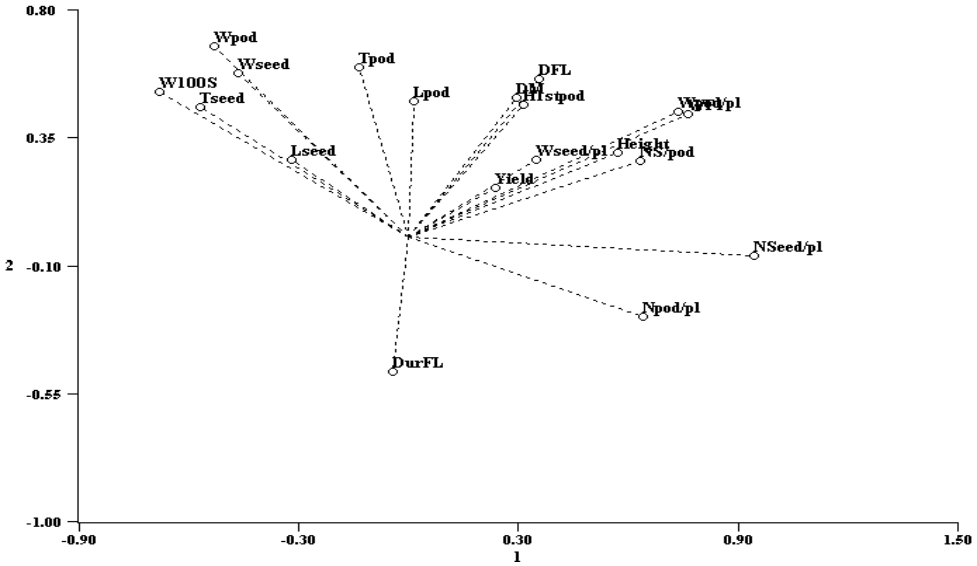

Figure 3. Projection of the 19 characteristics in axe 1 and 2.

good knowledge about their characteristics. Characterization of accessions is traditionally based upon morphological and agronomic traits, which is of high interest for plant breeders.

Our study confirms the existence of a large morphological variability in the collection of cowpea in IPGR (Sadovo, Bulgaria). The accessions evaluated in this present research represent an interesting starting point for a future plant breeding programme aimed at the development of new varieties. For the consumers and farmers, the most important characters of cowpea seed as in all food legumes are seed colour, seed size and seed coat. The most preferred colour is white and cream seeds with black hilum and smooth or smooth to rough

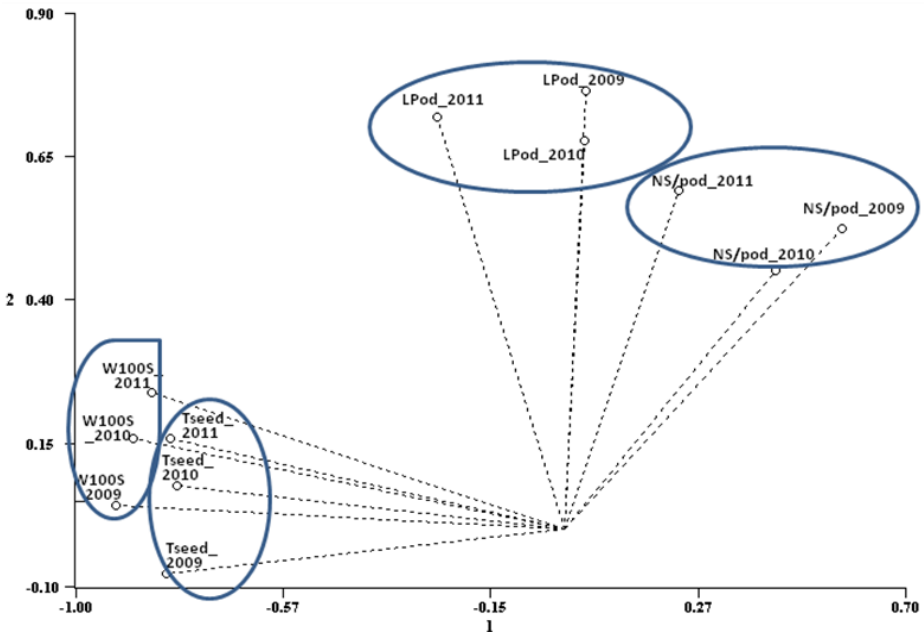

Figure 4. Projection of 4 characteristics observed during 3 years in axe 1 and 2.

coat. The more interesting accessions with cream colour of seeds, black hilum and big seed size with rough coat are the Bulgarian landraces A4E007 and 2005-01 and the breeding lines from IITA A4-084 and A-088 with cream colour of seeds, dark brown hilum and rough coat. The accessions with bigger seeds were A4-084, A4-088, A4-083, 2005-01 (more than 22 g/100 seeds weight). As has been verified by Omoigui et al. (2006), in this study the seed size was not influenced by the duration of reproductive phase, it is governed by other genetic factors. The phenological stages (days to flowering and days to maturity) showed a lower coefficient of variation. Similar results were reported from Hedge and Mishra (2009). The earliest accessions started flowering 40 days after germination comparing with latest ones needed 60.7 days for beginning of this phase. The shortest period to start flowering is advantage as high temperatures and low air humidity may be avoided. The results published by Hamidou et al. (2007) confirmed higher drought susceptibility in the 5 varieties of cowpea at flowering stage than during vegetative stage. In this study the accessions with earlier flowering are in group C of the Figure 2. Bulgarian landraces were characterized by an earlier maturity (72 to 75 days) in relation to the advanced breeding lines with different geographical origin (90 to 94 days).

Most of the studied quantitative traits were influenced by the environment conditions. But the our data have shown that pod length, number of seeds per pod, seed thickness and 100 seed weight were less affected by external conditions. These results suggest that these four descriptors should be useful in the coming evaluation studies.

Conclusion

The results obtained here provided useful knowledge about the diversity and breeding value of the Bulgarian landraces and all introduced accessions and give useful information for the selection of the most promising accessions to be used in future breeding programmes. It was not observed a clear distinction between landraces and accessions with foreign origin.

The most important components of yield are the number of pods and seeds per plant. High values for these traits were found in one Portuguese landrace (98210005) and in three advanced breeding lines (87-052, 87-058). The accessions with higher weight of seeds/plant, weight of pods/plant and weight of 100 seeds were the Bulgarian landraces A4E007 and 2005-01.

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