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EVALUATION OF GENETIC DIVERSITY IN CHICKPEA (*Cicer arietinum* L.) GERMPLASM

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Abstract

The paper aimed to estimate the degree of genetic diversity in 42 chickpea genotypes. The research was carried out between 2016-2017 in the experimental field of the University of Agronomic Sciences and Veterinary Medicine of Bucharest and it is based on the multivariate analysis of several quantitative and qualitative traits. By developing the dendrogram using the AHC algorithm (Agglomerative Hierarchical Clustering), the varieties and lines tested have been divided into two large groups, depending on the TSW (thousand seed weight), the colour and shape of seeds. It was estimated that 73.81% belong to the Desi type and 26.19% to the Kabuli type. Principal Component Analysis (PCA) showed a significant correlation between the number of pods per plant and the number of seeds per plant. The yield was significantly correlated with the number of pods per plant, number of seeds per plant and TSW (thousand seed weight). The first principal component of the PCA analysis, accountable for a 45.5% share of the total variation between genotypes, has been shown to be an association between the following variables: number of pods per plant, number of seeds per plant and seeds yield per plant. These findings can provide practical information for the selection of parental material and thus assist in planning breeding strategies.

Key words*: chickpea, genetic diversity, cluster analysis, phenotypic correlation, Principal Component Analysis.*

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an annual grain legume or "pulse crop" that is used extensively for human consumption. It is a self-pollinating, diploid with $2n = 2x = 16$ chromosomes (Arumuganathan and Earle, 1991) and has an estimated genome size of 740 Mbp (Varshney et al., 2013). Chickpea is a cool season legume in the Mediterranean region including North Africa, West Asia and South Europe, but tolerates high temperature when mature. Chickpea is playing a leading role in food safety in the world by covering the deficit in proteins of daily food ration of Indian and African Sub Sahara populations (Merga et al., 2019). However, chickpea is an interesting crop not only for the developing African countries and the Asiatic region. It is an important niche crop in Europe and the USA (Lavrenko et al., 2019). The area in the EU is about 172294 ha (out of a world total of 17.8 million ha) with 70609 ha in Spain (www.fao.org, 2018). The chickpea yield in Europe and worldwide is about 1 t/ha (Murphy-Bokern et al., 2014).

Two distinct forms of cultivated chickpeas are *desi* and *kabuli,* depending mainly on their seed size, shape and colour. The *desi* type chickpea is grown in the semi-arid tropics (Muehlbauer and Singh, 1987) and is characterized by small seeds, angular shape, and coloured seeds with a high percentage of fibre. The *kabuli* type has generally large seeds, owl-head shape, beige coloured seeds with a low percentage of fibre. *Kabuli* chickpea is usually utilized as whole grains, while *desi* is processed into flour.

The genetic diversity of genotypes makes them an important resource of genes for breeding programs, developing more diversified farming systems, and new quality products (Jing et al., 2010; Sharifi et al., 2018).

The analysis of genetic diversity of chickpea germplasm can provide practical information for parental selection strategies in plant breeding programs. A significant heterosis effect has long been associated with the allelic richness of hybrids (Ramanujam et al., 1974; Parameshwarappa et al., 2012). Thus, highlighting the genetic distance between the varieties of a given collection facilitates the

identification of a hybrid vigor, by providing insights for a correct and quick selection of divergent forms to be crossed.

Genetic diversity has been traditionally assessed by measuring the variation of qualitative and quantitative phenotypic traits, which are of direct interest to users.

The main drawback of this approach is that the genetic information provided by morphological characters is often limited, as the expression of quantitative characteristics is strongly influenced by environmental conditions.

DNA-based techniques can be used in complementarity with traditional approaches, allowing the identification of polymorphisms at DNA level without the influence of environmental factors. Due to advances in molecular biology, a diverse array of methods to analyze genetic diversity has been developed over the last decade (Ahmar et al., 2020). The use of DNA markers for marker-assisted selection (MAS) is the current trend in modern breeding.

The genetic base of chickpea has narrowed substantially during the domestication process (Thudi et al., 2016). Therefore, increasing the genetic diversity of chickpea has been a major goal for breeders.

Most chickpea breeding programs have been limited to intraspecific hybridization, and the crossbreeding between parents belonging to the *desi* and *kabuli* types has been widely used for the exploitation of genes present in one type. For example, *desi* type parents have contributed to the improvement of *kabuli* types with important genes for resistance to *Fusarium oxysporum* and *Ascochyta* and with drought tolerance genes (Gaur et al., 2006). On the other hand, *kabuli* type parents represented the genetic source for improved seed quality, especially for large seed size in *desi* type breeding programs. Highly productive progenies have been consistently obtained through the *desi* x *kabuli* crosses, representing a source for many new cultivars.

An analysis of the diversity pattern in the global chickpea collection revealed several conclusions about plant traits in relation to their origin (Dwivedi et al., 2009). The European resources produced the largest seeds, having more pods per plant and highest grain yield, while the varieties originating in Africa had the smallest seeds. The African varieties exhibited the earliest flowering, contrasting the latest flowering varieties originating in East Asia. In addition, the seed colour was found to be the character with greatest diversity among the analyzed pool.

The results of the principal component analysis in various studies using clustering techniques to assess genetic diversity in chickpea revealed the potential of the main quantitative characters for chickpea breeding (Nie et al., 2015; Agrawal et al, 2018; Mahmood et al., 2018).

Nie et al. (2015) classified 100 chickpea genotypes into four groups and indicated the 73.9% of total variance explained by the top four principal components. In a recent study, Sharifi et al. (2018) revealed the clustering of twenty-five chickpea genotypes in two main groups and four clusters. The PCA showed the following characteristics to be the main responsible for the variation within the chickpea genotypes: number of days to flowering, flowering period, number of days to maturity, canopy height, canopy width, biological yield, and number of pods per plant.

The present study was undertaken to estimate the extent of genetic diversity in 42 varieties and chickpea lines through multivariate analysis which will help to select potential parents to develop transgressive segregants in the future breeding program.

MATERIALS AND METHODS

Plant material and field experiments

This study was conducted during the 2016 and 2017 growing seasons at the University of Agronomic Sciences and Veterinary Medicine, Bucharest, Romania. Forty-two chickpea genotypes (*Cicer arietinum* L.) were analyzed, of which 40 varieties and lines collected/developed by SCDA Teleorman and 2 chickpea lines improved by USAMV Bucharest (Table 1). The field experiment was performed according to the method of randomized complete block design (RCBD) with 42 variants in three replications. The sowing spacing was 50 cm between rows and 6 cm between plants per row, with 6 m^2 for an experimental plot.

Several of traits including seed colour, seed shape, plant height (PH), number of pods per plant (NPP), number of seeds per pod (NSP), number of seeds per plant (NSP), thousand

seed weight (TSW) and seed yield per plant (SY) were determined in every year.

Table 1 Name and source of chickpea genotypes

Entry no.	Genotype name	Source*	Entry no.	Genotype name	Source *
1	CICERO	SCDA T	22	PP 137	SCDA T
2	KINELSKII 17	SCDA T	23	PP 138	SCDA T
3	DOLINSKII 1	SCDA T	24	NR. 309	SCDA T
4	VÎSOCOROS LÎI 30	SCDA T	25	NR. 231	SCDA T
5	STEPNOI I	SCDA T	26	NR. 207	SCDA T
6	ZERNOGRAS KII 30	SCDA T	27	NR. 203	SCDA T
7	KUBANSKII 199	SCDA T	28	NR. 300	SCDA T
8	KOSTILKOV A	SCDA T	29	NR. 303	SCDA T
9	NIGRUM TABOR	SCDA T	30	NR. 183	SCDA T
10	AGONRAE	SCDA T	31	STEPNO VOI	SCDA T
11	DOBRUJANS KII 7	SCDA T	32	PI 468946	SCDA T
12	MALKO GRADIŞTE 6	SCDA T	33	PI 468938	SCDA T
13	SVOBODINO VA	SCDA T	34	N 279/99	SCDA T
14	KALOFER	SCDA T	35	N 294/99	SCDA T
15	NR. 2 RUSE	SCDA T	36	N 323/99	SCDA T
16	NR. 45 RUSE	SCDA T	37	N 191/98	SCDA T
17	PP 87	SCDA T	38	N 681/01	SCDA T
18	KUBANSKII 16	SCDA T	39	BURNAS	SCDA T
19	PP 117	SCDA T	40	RODIN	SCDA T
20	PP 130	SCDA T	41	AGRO-N 1-08	USAMV B
21	PP 136	SCDA T	42	AGRO-N 2-08	USAMV B

* SCDA T - Agricultural Research and Development Station Teleorman, Romania; USAMV B - University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania

Statistical analysis

The statistical analysis was performed with the Microsoft Excel program and its statistical software, XLStat version 2017. In order to achieve the objectives of the paper, correlation tests, Principal Components Analysis - PCA and Agglomerative Hierarchical Clustering - AHC, were performed.

RESULTS AND DISCUSSIONS

Cluster analysis

Using the AHC - Agglomerative Hierarchical Clustering algorithm based on Euclidean distances and Ward's method, a dendrogram was developed which divided the 42 chickpea genotypes, according to TSW, seed colour and seed shape, into two large groups, marked with A and B (Figure 1). Within group A, two clusters were identified (1 and 2) (Figure 1). Cluster 1 encompasses a single chickpea genotype, namely Kostilkova, characterized by large seeds, cream colour and edged shape. In the cluster 2 there are 10 chickpea genotypes: PP 117, PP 137, Dolinskii 1, Nigrum Tabor, Agro-N1-08, Kubanskii 199, Cicero, Dobrujanskii 7, No. 2 Ruse and Agro- N2-08.

All chickpea varieties included in the group A are characterized by medium (MMB = 200 - 300 g) and large (MMB = 301-400 g) seeds, cream-colored, round or edged (angular) shape. In group B, 31 genotypes were grouped.

Within group B, two clusters were detected (3 and 4) (Figure 1). In cluster 3, 17 chickpea genotypes are concentrated, characterized by small seeds (MMB < 200 g), with variability in colour: black, cream, reddish-brown and round or edged (angular) shape. Cluster 4 includes 14 genotypes of chickpeas with medium seeds (MMB = 200-300 g), light yellow (cream) to black in colour and edged shape, except for the genotype Malko Gradiste 6, which has a round shape.

According to this analysis we could appreciate that the varieties and lines of chickpeas tested are classified into two types as follows: *kabuli* type (group A) - represents 26.19% and *desi* type (group B) is predominant and represents 73.81%.

In the future breeding programs, crosses between chickpea genotypes from these two major groups (A and B) might lead to a high heterosis, and improved genotypes could be obtained in terms of resistance to biotic and abiotic stress and thus with higher yield potential.

Principal component analysis

Analysis of the main components (PCA) was carried out in order to find the correlations and associations between the six traits and to identify those that describe in a significant proportion the variance between the 42 chickpea genotypes.

Phenotypic correlations between seed yield elements of chickpea genotypes

The correlation matrix generated by the PCA analysis reveals information on phenotypic correlation coefficients and those useful in the study of multicollinearity of variables (Table 2). The results indicates a significant positive correlation among number of pods per plant and number of seeds per plant, as well as

between seed yield per plant and three yield components: number of seeds per plant, number of pods per plant and TSW.
The plant height and the number of seeds per pod have recorded correlation coefficients very close to zero, which means that these traits do not correlate with the other quantitative traits. These results suggest that these two variables could be removed from the study without effect on the quality of the outcomes.

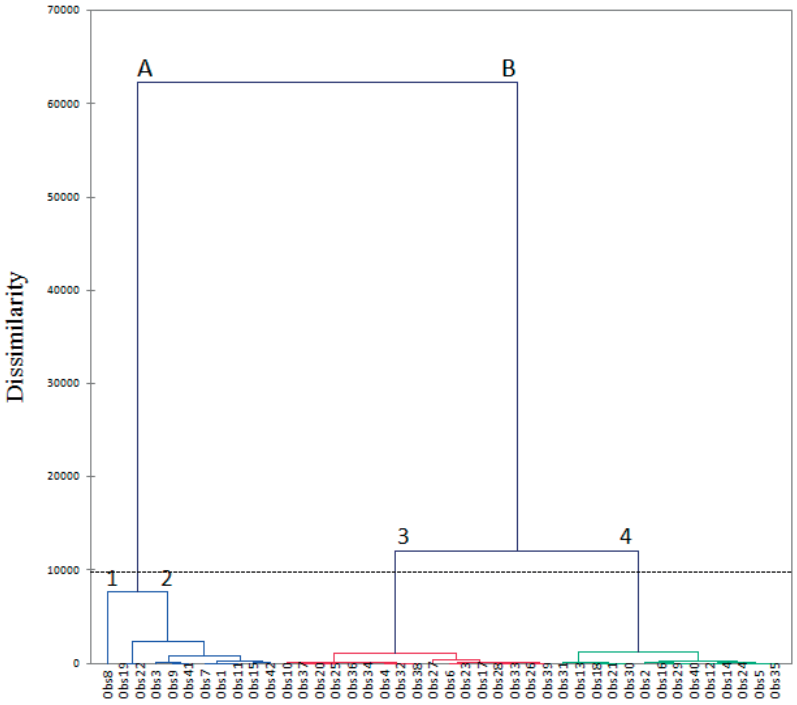

Figure 1. Cluster analysis - dendrogram of 42 chickpea genotypes (*Cicer arietinum* L.) using Euclidean dissimilarity and Ward's method (the name of genotypes given in Table 1)

Table 2. Correlation matrix (Pearson)

Trait	PH	NPP	NSP	NSPL	TSW	SYPL
PH	1	0.090	-0.023	0.075	-0.288	-0.073
NPP	0.090	1	-0.140	**0.971**	-0.168	**0.819**
NSP	-0.023	-0.140	1	0.056	-0.030	0.078
NSPL	0.075	**0.971**	0.056	1	-0.144	**0.870**
TSW	-0.288	-0.168	-0.030	-0.144	1	**0.338**
SYPL	-0.073	**0.819**	0.078	**0.870**	**0.338**	1
Bold values are different from 0 at a significance level α = 0.05						

PH - plant height, NPP - number of pods per plant, NSP - number of seeds per pod, TSW - thousand seed weight (g), NSPL - number of seeds per plant; SYPL seed yield per plant (g).

Factor and biplot analysis

Six principal components (PCs) axes are shown in Table 3 and Figure 2. A trait with coefficient greater than 0.3, was considered as an important trait. Traits having less than 0.2 coefficient value were considered to have no effect on the overall variation (Adebisi et al., 2013). The results show that only the first four factors need to be taken into account for analysis, as they are responsible for more than 99% of the variation between the 42 genotypes. Similar results have been reported by several researchers who studied chickpea genotypes by PCA (Sharifi et al., 2018; Malik et al., 2014; Shiv at al., 2012).

Table 3. Principle component analysis of chickpea genotypes

Trait	PCs					
	F1	F2	F3	F4	F5	F6
PH	0.150	-0.589	-0.670	0.426	-0.001	0.001
NPP	**0.961**	-0.200	-0.037	-0.180	0.006	0.055
NSP	-0.431	-0.456	0.695	0.349	-0.003	0.024
NSPL	**0.845**	-0.457	0.262	-0.046	-0.067	-0.031
TSW	0.264	0.906	-0.030	0.323	-0.054	0.015
SYPL	**0.902**	0.256	0.245	0.236	0.071	-0.022
Eigenvalue	2.729	1.692	1.064	0.498	0.013	0.005
Variability (%)	**45.478**	**28.193**	**17.736**	**8.298**	0.209	0.087
Cumulative %	45.478	73.671	91.406	99.704	99.913	100.00

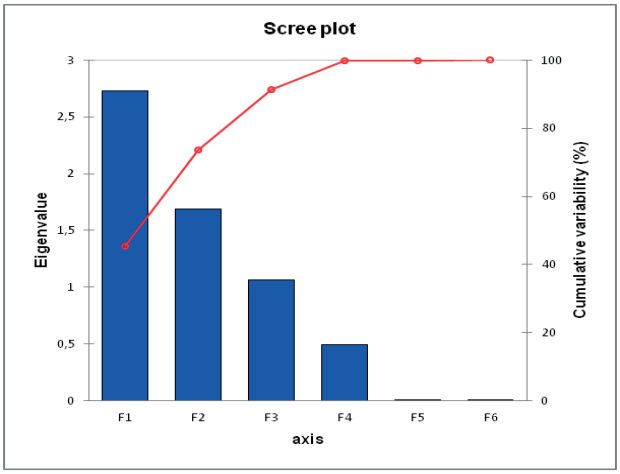

Figure 2. Diagram of eigenvalues in response to number of components for the estimated variables of chickpea genotypes

Our results also indicate that the first main component, which practically explains in the largest measure the genetic variability between the 42 genotypes (greater than 45%), is a combination of the three traits: number of pods per plant, number of seeds per plant and seed yield per plant (Table 4).

Table 4. Loading of the first main component of the PC analysis

Trait	F1
PH	0.150
NPP	**0.961**
NSP	-0.431
NSPL	**0.845**
TSW	0.264
SYPL	**0.902**

PH - plant height, NPP - number of pods per plant, NSP - number of seeds per pod, TSW - thousand seed weight, NSPL - number of seeds per plant; SYPL seed yield per plant.

The findings suggest that these traits mentioned above are of the utmost importance for setting chickpea breeding objectives. The choice, as a parent material, for crosses of contrasting genotypes could lead to the identification of hybrid vigor in offspring, knowing that a significant heterosis is often associated with the allelic richness of hybrids (Ramanujam et al., 1974; Parameshwarappa et al., 2012).

The two-dimensional arrangement indicates a significant positive correlation between seed yield per plant, number of pods per plant, number of seeds per plant and TSW.

Of all the studied traits, the number of pods per plant has the greatest positive effect on seed yield per plant.

Similar results were reported by Sharifi et al (2018), in their study on 25 chickpea genotypes.

Two characters are positively correlated if the angle between vectors is < 90º, negatively correlated if the angle is > 90º and independent if the angle is 90º (Yan et al., 2002).
In addition, the obtuse angle between the vectors shows that the plant height is negatively correlated with the TSW, as confirmed by the correlation matrix (Figure 3).

The vectors representing the traits plant height and the number of seeds per pod are closer to the origin, which means that any interpretation regarding their correlation with the other traits could be erroneous.
The number of seeds per pod has previously been shown not to exhibit sufficient variation between genotypes so as to have an influence on the other traits.

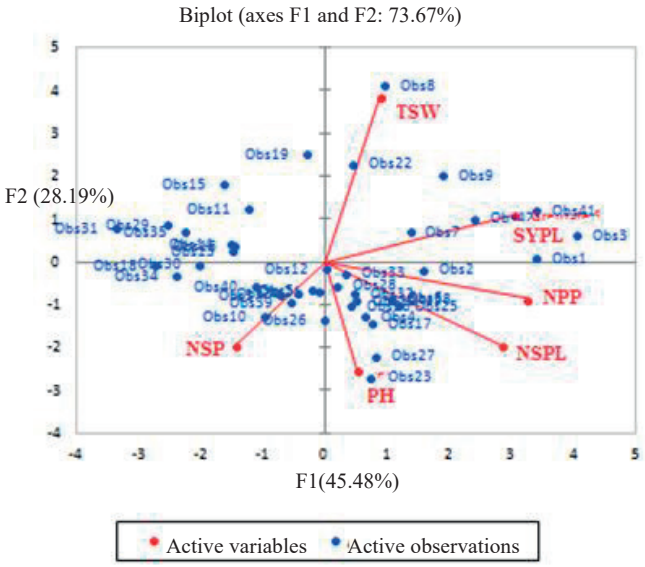

Figure 3. Two-dimensional ordination of six traits in chickpea genotypes on principal component axes
PH - plant height, NPP - number of pods per plant, NSP - number of seeds per pod, TSW - thousand seed weight, NSPL - number of seeds per plant; SYPL seed yield per plant

CONCLUSIONS

Overall, these results show that PCA and cluster analysis are very effective for assessing the genetic variation of chickpea genotypes.
This study, which used multivariate techniques to assess the genetic diversity in 42 varieties and chickpea lines, was a first step in gaining an insight into the germplasm divergence, which is an important step towards an efficient exploitation of genetic resources of chickpea genotypes.
Based on the dissimilarity analysis, using the AHC (Agglomerative Hierarchical clustering) algorithm, the dendrogram distributed the 42 chickpea genotypes into two large groups: *desi* (A) and *kabuli* (B), characterized by a wide variability in terms the seed size, shape and colour. In the future breeding programs, hybridization of genotypes across groups/ clusters could lead to a high heterosis in cross progenies.
Our results also show a significant positive correlation between seed yield per plant and number of pods per plant, number of seeds per plant and thousand seed weight. Similar results were reported by Kumar et al. (2003).
The principal component analysis recognized number of pods per plant, number of seeds per plant and seed yield per plant as traits that mainly described the variation within the chickpea genotypes.
The components such as number of seeds per pod and plant height did not contribute considerably to the variation within the entries and could be dropped in similar analysis.
These results can provide practical information for the selection of parental material and thus

assist in planning chickpea breeding strategies for yield improvement.

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