

## **PHENOTYPIC CHARACTERISATION AND RELATEDNESS OF MAIZE INBRED LINES**

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Clear description of maize is useful in planning crosses for hybrids, in classifying inbreds to heterotic groups, and in the plant variety protection. The objection to visual assessment of morphological traits is its subjectivity. But it can be very useful for plant breeders especially when they work with a material of unknown genetic origin.

The phenotypic characterisation (30 traits) of 45 inbred lines according to the UPOV Descriptor, with a known pedigree, is used with the main goal to investigate a possibility of exploiting such a kind of information for the classification of inbred lines in homogenous groups according to their relatedness.

Ward's method of cluster analysis had the best concordance with pedigree data. This method divided 45 inbreds into two large clusters, each of them containing two subclusters. Quality of the cluster analysis with four groups was tested by the discrimination analysis. All of the tree discrimination functions were significant and enclosed 64%, 20% and 16% of variance, respectively.

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In plant breeding such information can be useful for a more precise description of existing heterotic groups, as well as, for grouping lines of unknown genetic origin. On the basis of obtained grouping, the decision on their crossing can be made. Hence they either should be crossed to related ( $F_2$  populations for a new selection) or unrelated materials (testing of combining abilities).

*Key words:* cluster, maize, phenotypic characterisation, relatedness

## INTRODUCTION

Information on the relationship between the breeding material and the genetic distance of the available germplasm is important for deciding which method will be applied in breeding programmes. The organisation of germplasm into genetically divergent groups is important for the implementation of the phenomenon of heterosis. This is crucial in the course of developing hybrid varieties, where defining and applying of heterosis is important for the end result of breeding, but it also can be applied in breeding of clones and open-pollinated varieties and synthetics. At the same time, two important questions arise: 1. How divergent are inbred lines from same and different heterotic groups and 2. Which criteria and biometric methods allow a dependable grouping of germplasm (MELCHINGER, 1999). Therefore, a precise description of the variety is important for plant breeding. UPOV (International Union for the Protection of New Varieties of Plants) recommends the evaluation of 34 traits of maize genotypes with the aim to protect plant breeders' rights. In order to provide a correct use of traits in DUS (Distinctness, Uniformity and Stability) tests it is essential to comprehend different modes of the expression of the traits. Qualitative traits are those that are expressed in discontinuous stages, which are understood just by themselves (plant sex). Quantitative traits are those that vary continuously. Expressions, with the aim of the description, are ranked a certain number of levels that are rated and have a particular assessment (TG/1/3). Different methods can be used to obtain the assessment: a single measurement of a group of plants or parts of plants, a measurement of a number of individual plants or parts of plants, a visual assessment by the observation of individual plants or parts of plants, a visual assessment by a single observation of a group of plants or parts of plants (UPOV/DATA/BEI/04/5). Many authors believe that morphological data, obtained by a great number of measurements of numerous plants are unreliable, as observed traits are under effects of an unknown mechanism of genetic control, and also are under a great environmental influence. Morphological traits are conventionally used as descriptors and such a trend will probably be continued due to their omnipresence within agricultural studies. Quality of these descriptors can be improved by gathering data from more replications and over more years and by neglecting traits that are under a very strong impact of the environment, as well as, those that are highly correlated (SMITH and SMITH, 1989). The same authors claim

that it is not correct to compare morphological descriptions obtained over different locations or various years.

A subjectivity of an assessor is the main objection to the visual assessments. However, these assessments can be of interest for plant breeders, especially in case of wide material or insufficiently known material. Moreover, sometimes is more precise to degrade a measurement scale (from a scalar to the ordinary level of measuring) due to several reasons. It is much cheaper and faster to assess an expression of any trait by a classification of an observed genotype into a certain category than to perform large number of measurements in both, several replications and several environments, and than to compare them to a check or an example variety for a given trait. The ordinary level of measuring is under lesser environmental effects. The existence of the data base regarding phenotypic assessments according to the principles of the UPOV Descriptor also allows the comparison of genotypes that have been observed during different temporal periods and in situations when different example varieties were used.

Breeders are often in a dilemma over checking quality of a certain inbred lines, i.e. over selecting a tester or a pair of testers in case when the inbred line was developed from a hybrid of an unknown genetic constitution or in case when lines were derived from crosses of parents from different heterotic groups. Data bases on the phenotypic characterisation according to principles of the UPOV Descriptor can be useful in such cases, as well as, in cases when a material with desirable traits is selected for creating initial populations (BABIĆ and BABIĆ, 2008).

## MATERIALS AND METHODS

According to principles of the UPOV Descriptor, the phenotypic description of 45 maize inbred lines of a known pedigree (FAO 300-700) from one year and one replication was used in the study. The cluster analysis was done with the aim to determine the possibility of using phenotypic characterization of genotypes according to the principles of UPOV Descriptors for defying homogenous groups and to compare the agreement of obtained results of classification with known information on the pedigree of the observed material. The cluster analysis is very attractive for breeders due to many of its characteristics, but also there are some disabilities. Groups formed by the cluster analysis are not unique in space and time, and their composition can be changed by adding new individuals. A relatively great number of measures of closeness/dissimilarity are defined in the statistical theory. Therefore, it is necessary to define very carefully the closeness of the observed object in an actual study in order to select the appropriate measure of closeness. In this study, the squared Euclidean distance was used as a measure of dissimilarity. Following the formation of the distance matrix, the selection of the grouping method is done in the next stage. These methods represent a set of rules of arranging objects into groups based on measures of closeness/dissimilarity between the objects.

Numerous methods of grouping had been proposed, hence the cluster analysis was criticised even because quality of the selection of different options was difficult to be verified (BULL and HOGARTH, 1990). Therefore the cluster analyses for the given study were performed according to the following methods: Between-group linkage, Within-group linkage, Nearest neighbour, Furthest neighbour and Ward's method (incremental sum of squares). The following 30 traits were used: anthocyanin colouration of first leaf sheath, shape of tip of first leaf, angle between the blade and stem on first leaf above the ear, attitude of blade, anthocyanin colouration at base of glume, anthocyanin colouration of glumes excluding base, anthocyanin colouration of fresh anthers, density of spikelets, angle between main axis and lateral branches of tassel, attitude of lateral branches, number of primary lateral branches, intensity of anthocyanin colouration of silk, degree of zig-zag of the stem, anthocyanin colouration of brace roots, anthocyanin colouration of sheath, length of the tassel main axis above the lowest side branch, length of the tassel main axis above the highest side branch, length of the side tassel branches, plant length, ratio height of insertion of upper ear to plant length, width of blade, length of peduncle, ear length, ear diameter, ear shape, number of rows of grain, type of grain, colour of top of grain, colour of dorsal side of grain, intensity of anthocyanin colouration of glumes of cob. The following assessments were excluded from the analysis: time of anthesis and silking, presence and absence of anthocyanin on silk and glumes of cob.

According to known information on the origin of the studied material and results obtained by the cluster analysis, the assumption of the classification into four groups was tested by the discrimination analysis. The 3D diagram of the discrimination scores of the first three discrimination functions of the observed inbreds is presented

## RESULTS

The assessment of 30 phenotypic traits was used for the cluster analysis after the following methods: Between-group linkage, Within-group linkage, Nearest neighbour, Furthest neighbour and Ward's method. The Ward's method of cluster analysis gave the best concordance with the pedigree of the observed material and therefore only the dendrogram of the analysis after this method is presented and used for further analyses (Figure 1).

Observed 45 maize inbreds were grouped into two large clusters. The first cluster (A) encompassed inbreds that were completely or partially of the BSSS background, while the second cluster (B) was composed of inbreds that were completely or partially of the Lancaster background. These two large clusters each contained two sub-clusters (a, b, c and d). The first sub-cluster of the cluster A (a) was mainly made of inbreds originated purely from the BSSS background (12, 13, 29, 30, 33, 19, 23, 26, 38, 36, 31, 32, 45), then inbreds 27 and 28 derived from the crosses of the BSSS background material and a material of the unknown

pedigree, as well as, two early inbreds of French origin (1 and 2). The second sub-cluster of the cluster A (b) was composed of inbreds developed by pedigree selection from the crosses of inbreds of the BSSS origin to inbreds of the Iowa-dent origin (10 and 18), inbreds originated from the US hybrids (40 and 14), the inbred 24 that was of pure Iowa-dent origin, the inbred 39 of the local origin and one early inbred of unknown origin (3).

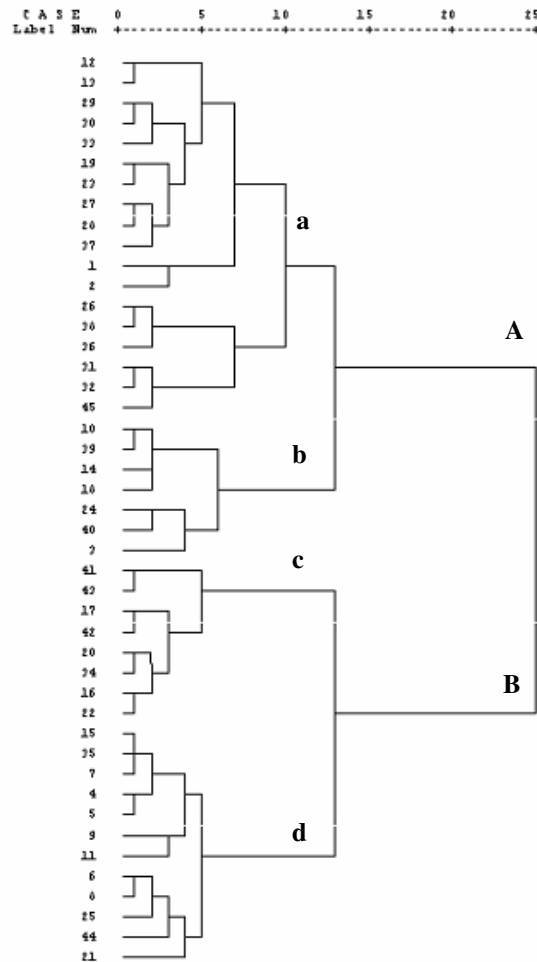


Figure 1: Dendrogram of the cluster analyses of 30 phenotypic traits of studied maize inbreds

The first sub-cluster of the cluster B (c) was made of purely of inbreds of the Lancaster background (17, 42, 20, 34, 16 and 22), as well as, inbreds derived by pedigree selection from the crosses of inbreds of the Lancaster origin to the inbreds of the Iowa-dent origin (41 and 43). The second sub-cluster of the cluster B (d) was composed of four inbreds of the purely Lancaster background (21, 7, 35 and 15), inbreds derived by pedigree selection from the crosses of inbreds of the Lancaster origin to the inbreds of the Iowa-dent origin (5, 9, 6, 44), then the inbred 4 derived by pedigree selection from the crosses of the inbreds of the Iowa-dent origin to an inbred from Ohio, the inbred 25 that is pure inbred of the Iowa dent background and the inbred 8 developed from the crosses of inbreds of the Iowa-dent origin to the inbred of unknown origin. This group also includes the French early maturity inbred 11, which does not contain germplasm of known heterotic groups, but according to made grouping it could be designated as "non BSSS".

Table 1: The percent variance explanation of the first three discrimination functions

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	31.657(a)	64.0	64.0	.985
2	9.867(a)	20.0	84.0	.953
3	7.897(a)	16.0	100.0	.942

a First 3 canonical discrimination functions were used in the analysis.

Table 2: Statistical significance of discrimination functions

Test of Function(s)	Wilks'			
	Lambda	Chi-square	df	Sig.
1 through 3	.000	217.553	90	.000
2 through 3	.010	123.430	58	.000
3	.112	59.016	28	.001

It can be assumed that the results of the classification would have been more precise if the early maturity French material (FAO 300) was not included. According to obtained results of the cluster analysis and information on the background of the studied inbreds it was supposed that studied material was classified into four related groups (the "m" dendrogram interception line) according to the assessments of 30 phenotypic traits. Quality of the cluster analysis was tested by the discrimination analysis, whereby: I - discrimination function encompassed 64% of variance, II - 20% and III - 16%, and all were statistically significant (Tables 1 and 2).

The 3D figure of discrimination scores of observed inbreds distinctively shows four groups of genotypes. The values of scores of the first discrimination function (F1) separate inbreds the groups "a" and "b" from groups "c" and "d", while values of discrimination scores of the second function (F 2) separate inbreds of the group "c" from inbreds of the remaining groups. The highest scores of the third discrimination function (F 3) were registered in the inbreds of the cluster "d", while the lowest scores were determined in inbreds of the cluster "b". All these made groups or clusters clearly differentiated in the three-dimensional space (Figure 2).

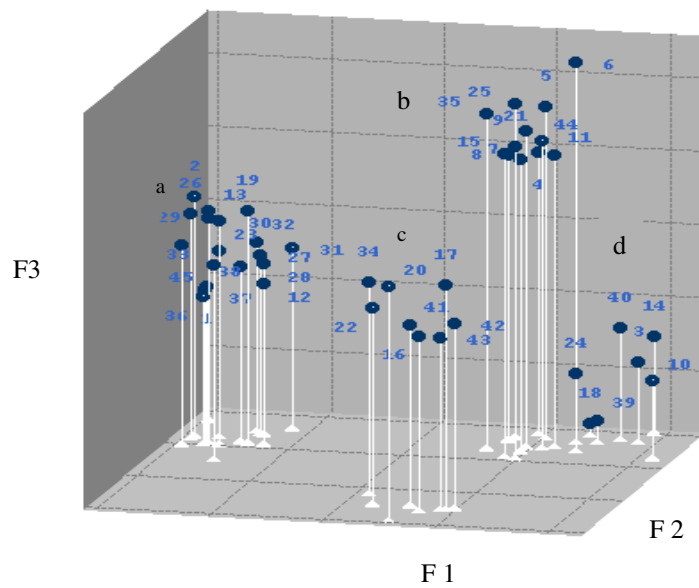


Figure 2. 3D of discrimination scores of observed maize inbreds

The morphological traits with a high repetability can be a reliable descriptor if data have been obtained from several replications over two years. Also, it is disappointing when the comparison of morphological traits from different locations or years is not valid, SMITH and SMITH (1989). The same authors (1989) in their subsequent studies applied biochemical and genetic markers and suggested the multi-phasic system for the description of maize inbreds either for

the breeding process or for needs of breeders' rights protection. In the initial stage, data on morphological traits can provide basic indications on the material. The inbreds expressing similar or identical traits on the basis of the test of morphological traits would be additionally tested together with morphological measurements that encompass important agronomic traits including replications and the example variety. At the same time, laboratory tests such as isoenzyme electrophoresis and similar techniques can be added. DNA marker techniques are included only in cases when it is economically justified. Actually, if it is necessary, heterosis in some materials can be tested with common testers.

The assessment of phenotypic traits of different maize genotypes according to principles of the UPOV Descriptor, are basically very simple and they are performed by a visual evaluation of individual plants or a group of plants and therefore it is not necessary to engage larger labour power. The massive use of PCs in agricultural studies has been allowed a simple application of the multivariate analysis that provides comprehending a simultaneous interrelation among three or more independent variables (RUIZ J. I., 2001). If there is a data base with a phenotypic characterisation of the breeding material designed according to the principles of the UPOV Descriptors, the important information could be obtained by a simple screening of desirable traits just in a few minutes, regardless monitoring a small number of traits or a complete set of traits, if any of methods of the multivariate analysis is applied. In plant breeding, such information can be useful for a comprehensive description of existing heterotic groups, as well as, for clustering inbreds of unknown genetic source and thereby defying a desirable tester. For instance, according to the figure on discrimination scores for the inbred 40 originating from the cross of the inbreds derived from the US hybrids, any of inbreds of the "non-BSSS" origin (c or d group) could be taken as a tester. On the other hand, for the inbred 39 of the local origin, a test should be an inbred from the unrelated group (c or d). At the same time, an inbred with opposite traits of yield components should be selected from the data base of phenotypic assessments from the adequate heterotic group. If an unknown inbred has a short ear, a tester should have a long ear and similar.

Such information would be useful for selection of genotypes for the development of F<sub>2</sub> populations. If a favourable trait or resistance to drought or diseases is introduced from a material of unknown origin it is important to perform crosses to same or the most related heterotic group in order to retain as many favourable traits as possible and to maintain combining abilities.

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**FENOTIPSKA KARAKTERIZACIJA I SRODNOST LINIJA KUKURUZA**

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**I z v o d**

Precizan opis varijeteta je važan za oplemenjivanje biljaka. Vizuelnim ocenama se često zamera na subjektivnosti ocenjivača. Medjutim, takodje je nekada bolje degradirati mernu skalu (od skalnog na ordinarni nivo merenja) iz više razloga. Jeftinije je i brže ocenjivati ekspresiju nekog svojstva svrstavanjem u određenu kategoriju, nego vršiti obimna merenja većeg broja biljaka u više ponavljanja i spoljnih sredina. Ordinarni nivo merenja je i pod manjim uticajem spoljašnje sredine. Postojanje baze podataka o fenotipskim ocenama omogućava i poredjenje genotipova koji su ispitivani u različitim vremenskim periodima i u situacijama kada su korišćeni različiti kontrolni varijeteti.

Za istraživanje je uzet fenotipski opis (upotrebljeno je 30 karakteristika) po principima UPOV deskriptora 45 linija kukuruza poznatog pedigrea. Uradjena je klaster analiza sa ciljem da se utvrdi da li se može iskoristiti fenotipska karakterizacija genotipova po princima UPVOG deskriptora za formiranje homogenih grupa i koliko su rezultati u saglasnosti sa poznatim informacijama o pedigreu.

Klaster analiza Wardsov metod je dala najbolje slaganje sa pedigreom ispitivanog materijala. Formiraju se dva velika klastera koji se dalje razlažu na dva podklastera.

Kvalitet klaster analize testiran je diskriminacionom analizom pri čemu je prva diskriminaciona funkcija obuhvatila 64% varijanse, druga 20% a treća 16% i sve su statistički značajne.

U oplemenjivanju biljaka ovakve informacije mogu biti od koristi za jasniju deskripciju postojećih heterotičnih grupa kao i za grupisanje linija nepoznatog genetičkog izvora i time definisanje poželjnog testera. Ove informacije bi bile korisne i za odabir genotipova za zasnivanje F2 populacija. Ukoliko se unosi neko poželjno svojstvo ili otpornost na sušu ili bolesti iz materijala nepoznatog porekla važno je izvršiti ukrštanja u okviru heterotične grupe da bi se zadržao što veći broj poželjnih svojstava.

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