

COMPARISON OF MORPHOLOGICAL AND MOLECULAR GENETIC DISTANCES OF MAIZE INBREDS

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Due to an unknown mechanism of genetic control and great environmental effects in the process of trait expression, morphological markers are often considered unreliable indicators of genetic relationships. Morphological characterisation of 19 maize inbreds was done according to the UPOV descriptor, while molecular characterisation was performed with RAPD markers. Based on the estimation of phenotypes according to the UPOV descriptor, the squared Euclidean distance was calculated and then, on the basis of this distance, a morphological similarity matrix was formed. Jaccard similarity coefficients were calculated on the basis of presence-absence of bands on gels in the RAPD analysis. When data were standardised, the comparison between morphological and genetic similarity

of observed maize inbreds was done. The correlations varied from 0.47 (inbred L 217) to 0.76 (inbred L 86). The average value of correlations for all studied inbreds amounted to 0.64. Furthermore, the results of the cluster analysis for both markers, molecular and morphological, had high concordance with pedigree data. Environmental effects were decreased in morphological markers (according to the UPOV descriptor) by rescaling a measurement scale from a scale to an ordinal level of measurement and in such a way results of morphological markers approached results of molecular markers in the estimation of the genetic distance (GD) of maize inbred lines.

Key words: maize inbreds, molecular and morphological markers, UPOV descriptor

INTRODUCTION

Due to an unknown mechanism of genetic control and great environmental effects in the process of trait expression, morphological markers are often considered unreliable indicators of genetic relationships. Homozygous recessive alleles give recognisable phenotypic extremes, but the frequency of occurrence of these alleles in the elite breeding material is relatively low. However, morphological traits are traditionally used in description and such a trend will surely be continued in agronomic studies. Morphological data provided the initial basis for taxonomic studies of maize and have remained a mainstay of maize racial taxonomy to current times (ORTIZ *et al.*, 2008). Furthermore, morphological data play an important role in the management of genetic resources that are conserved in ex situ gene banks (SANCHEZ *et al.*, 2000; BIOVERSITY INTERNATIONAL 2007). These properties are also a foundation for exercising of breeders' rights (Plant Breeders' Rights – PBR) recommended by the International Union for the Protection of Cultivated Varieties of Plants (UPOV) (UPOV, 1999, 2009). UPOV proposes detailed descriptors for a great number of crops including maize with guidelines for testing (Guidelines for the Conduct of Test for Distinctness, Uniformity and Stability (DUS) for Maize).

In maize breeding, the organisation of germplasm into genetically divergent groups is of the essential importance for the optimum utilisation of heterosis. At that, two important questions are imposed: 1. What is the distance of inbreds of the same and of the different heterotic groups? and 2. Which criteria and biometric methods provide, to a satisfactory extension, grouping of germplasm? SMITH *et al.* (1991) have stated that morphological data did not provide a good estimation of the genetic distance of studied maize inbreds. Knowledge of the relationships among lines would help identify a set of inbreds that have maximal diversity for the analysis of the effects of genetic background. Calculations of relatedness based upon pedigree data are dependent upon the assumptions that both parents contribute an equal number of alleles (i.e., no selection, mutation, or genetic drift) and that the pedigree data are accurate. Another assumption is that founder genotypes (genotypes for which no further pedigree information on ancestors is available) are unrelated by pedigree. All of these assumptions can be violated (LIU *et al.*, 2003).

By comparison various methods (US metric data and EU data – summarised by reference to "note" scores from check inbreds) for establishing distinctness with the aim to protect breeders' rights LAW *et al.* (2011) concluded in their study: "Thus, while the majority of EU and US note scores cannot meaningfully be compiled into a single database, they do individually each provide a reliable basis upon which to determine distinctness in respect of the current requirements of UPOV".

Today, molecular markers are considered the best tools in genetic studies, first of all due to the possibility to differentiate genotypes at the DNA level even when it comes to a narrow genetic base. The additional advantage of molecular markers is that they are under small impacts of environments. However, there are many studies about the type of molecular markers that provide the best evaluation of genetic relationships, as well as, about the number of primers that should be included into such studies. NAGY *et al.* (2003) have compared RAPD, SSR and morphological markers and concluded that both marker systems only partially reflect genetic relationships among observed maize inbreds. Only combined analysis supported with morphological data provides a close association among groups formed on the cluster analysis and pedigree data.

The objective of this study was to find out how much information gained by a visual assessment by a single observation of a group of plants or part of plants (VG) according to the UPOV descriptor, can be an actual parameter of genetic relationships of observed maize inbreds of known pedigree in comparison with genetic distances (GD) obtained by the RAPD analysis. Previous studies (BABIĆ *et al.*, 2008; BABIĆ *et al.*, 2011) pointed out to a satisfactory level of discrimination in divergent groups on the basis of morphological markers after the UPOV descriptor, as well as, to the fact that the obtained information can be useful in maize breeding. The assumption was that diminution the level of measurement from the scale to the ordinal level of measurements would reduce the environmental effects on morphological markers (according to UPOV descriptor) and thereby increase reliability of morphological markers in estimating GD of maize inbred lines.

MATERIALS AND METHODS

Morphological characterisation of 19 maize inbreds of known pedigree (Table 1) was performed according to the UPOV descriptor (UPOV, 2009) by a visual assessment of the group of plants by a single observation (VG), in two replications and during two years. Genetic characterisation of maize inbred lines was done by the application of the RAPD markers. The DNA was isolated from grain following the modified protocol of SAGHAI-MAROOF, (1984). Depending on the DNA concentration after dilution of samples, random amplified polymorphic DNA polymerase chain reaction (PCR) was applied. The reaction was done following the protocol of WILLIAMS (1990). Twenty eight commercial arbitrary RAPD primers were used.

Based on the evaluation of phenotypes according to the UPOV descriptor, a cluster analysis was done according to Ward's method, and the squared Euclidean distance was taken as a measure. Jaccard similarity coefficients (JACCARD, 1908)

were calculated after on the basis of presence-absence of bands on gels in the RAPD analysis and the cluster analysis (Complete-Linkage Clustering Method) was performed. When morphological and molecular distances were estimated they were compared (correlated) for each observed maize inbred individually. The dependence between morphological and molecular distances was presented in scatter diagrams and then the regression analysis was done.

Table 1. Pedigree data and correlations of morphological and molecular similarities in observed maize inbreds

Maize inbreds	Pedigree data	Correlations
LN 50	Lancaster	0.69**
H 66/2	BSSS x Oh	0.64**
Mo 17	Lancaster	0.69**
B 73	BSSS	0.68**
B 84	BSSS	0.67**
LN 64	Lancaster	0.70**
L 217	Independent source	0.47*
L 588	BSSS	0.71**
L 175	Lancaster	0.58**
PE 25-10-1	Lancaster x Pečki dent	0.61**
Va 35	Lancaster	0.75**
L 92	Derived from a hybrid	0.68**
L 1325	Derived from a hybrid	0.71**
L 194	Lancaster	0.63**
L 86	Lancaster	0.76**
A 632	BSSS	0.51*
HACM 10/5-3	Lancaster	0.52*
D2 30-1/49	Istrian large-seeded popul.	0.56*
L 382	Derived from a hybrid	0.50*

** p<0.01;

* p<0.05

RESULTS AND DISCUSSION

If we look at the cluster analysis dendrograms based on morphological (Figure 1) and molecular distances (Figure 2) we shall observe that although clustering is not identical, both dendrograms give clustering which is to a great extent concordant with pedigree (Table 1). Inbreds originating from Lancaster or BSSS populations are clustered separately in both dendrograms, while clustering of inbreds originating from the independent source, as well as, inbreds derived by pedigree selection from hybrids is slightly different.

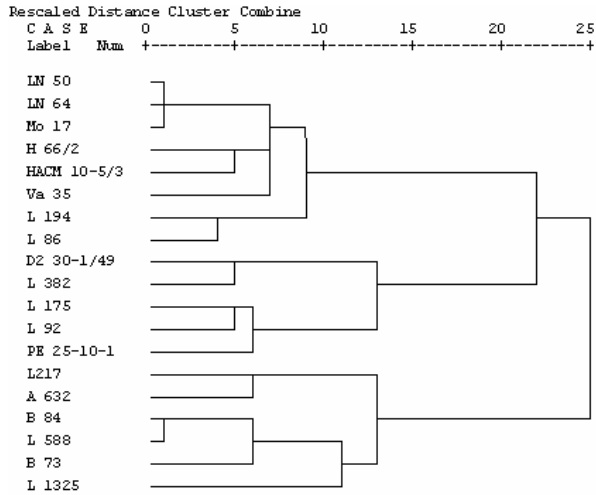


Figure 1. Cluster analysis dendrogram of phenotypic distances in observed maize inbreds

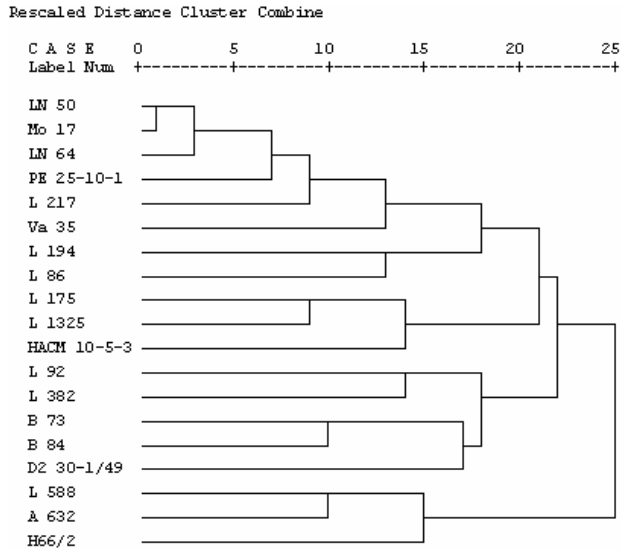


Figure 2. Cluster analysis dendrogram of molecular distances in observed maize inbreds

Estimated values of correlations of morphological and molecular similarities of observed maize inbred lines point out to high statistical significance of the correlation of these two magnitudes in the majority of inbreds. Correlations ranged from 0.47 (inbred L 217) to 0.76 (inbred L 86). The average value of correlations for all studied inbreds amounted to 0.64 (Table. 1). Hence, it was determined that there was a statistically significant linear correlation between morphological and molecular similarities of observed maize inbreds. The performed regression analysis confirmed that the relation between these two magnitudes is not accidental. The analysis of variance of the linear regression (based on the sum of squares of regression and residuals from regression), shows that almost a half of morphological similarities can be explained by molecular similarities. The significance of the F test indicates that variations explained by the linear regression are not random (Table 2). The strength of the relation between a linear model and depended variable (morphological similarity) is expressed via the value of the R square (0.523), which points out that a half of variations of morphological similarities can be explained by molecular similarities using the linear regression. Although the scatter diagram, in which values of molecular similarities are the independent variable and morphological similarities are the dependent variable, shows significant scattering along the regression line, a positive linear dependency of these two magnitudes is still observable (Figure3). It means that if the values of molecular similarities increase, the increasing the values of morphological similarities can be expected.

Table 2. Analysis of variance of linear regression

	Sum of Squares	df	Mean Square	F	Sig.
Regression	25877629.4	1	25877629.4	13949.578	0.0(a)
Residual	23620775.5	12733	1855.1		
Total	49498404.9	12734			

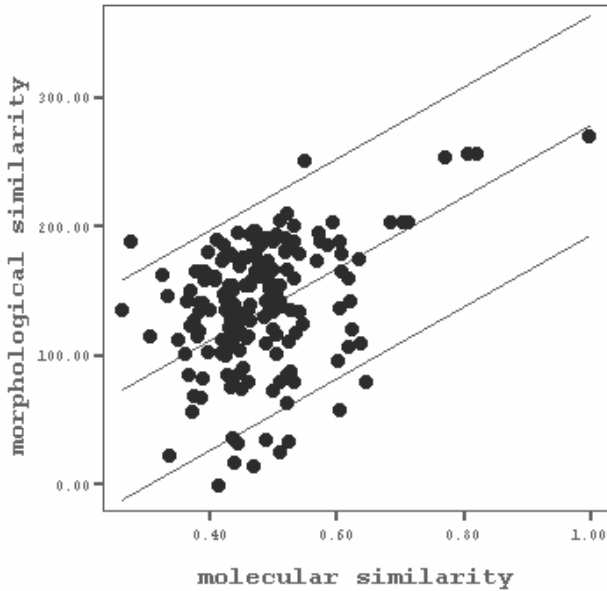
a Predictors: (Constant), molecular similarity (RAPD)

b Dependent Variable: morphological similarity (UPOV)

R square = 0.523

In Figures presenting the relation of similarities after Jaccard and morphological similarities separately per inbreds (due to the scope, Figures of all inbreds were not presented), it is observable that studied inbreds behaved differently (Figure 4). A positive trend (when molecular similarity increase the morphological similarity also increase) is observed in certain inbreds (L 186, PE 25-10-1). On the other hand, in some other inbreds (D2 30-1/49, L 194), almost identical values of genetic similarities give a wide range of morphological similarities. In the inbred A 632, and particularly in the inbred L 217, no similarity between these two magnitudes can be observed. Some previously conducted studies did not show a

relation between morphological and pedigree data (SMITH and SMITH, 1989) or between morphological and molecular distances (DILLMANN *et al.*, 1997).



Linear Regression through the Origin with
95.00% Individual Prediction Interval

Figure 3. Interrelationships of molecular (RAPD) and morphological (UPOV) similarities in observed maize inbreds

There are three levels of measuring: 1. scale - the most precise, 2. ordinal - pertaining to order, rank, or position in a series, less precise and 3. nominal - descriptive, the least precise). According to these studies, diminution the level of measurement from the scale to the ordinal level of measurements, during measuring morphological traits (VG instead of measurement of number of individual plants or part of plants (MS)) resulted in the increase in quality of morphological markers in the estimation of genetic distances of studied maize inbreds. Maybe someone can ask a question: "How diminution can increase the precision of a measurement?" Due to great environmental effects, on quantitative traits, we have assumed that use of a scale measurement could create greater confusion when defining morphological similarities than if we have used the expression rank for a given trait, guided by

control genotypes according to the UPOV descriptor. Furthermore, considering that we worked with maize inbred lines that were homozygous to a great percent, we also assumed that the visual assessment of a group of plants would be sufficient, instead of the evaluation of individual plants. Moreover, although our opinion was that environmental effects on phenotypic description were reduced by diminution from scale to ordinal level of measurements, they could not be completely removed. Therefore, a part of variations of phenotypic similarities are probably a consequence of environmental effects in the process of the trait expression. Even though, the regression analysis and correlations of morphological and molecular similarities of maize inbreds tested by statistical parameters confirmed the existence of a positive linear dependency between these two magnitudes, a significant dispersion around the regression line is observable on the scatter diagram. It practically means that inbreds of the same or approximately the same molecular similarities can express different phenotypic similarities and vice versa - the inbreds of different molecular similarities can express similar values of morphological similarities. This phenomenon can be explained in two ways in the theory of quantitative genetics. 1) First of all, it is known that different combinations of genes can result in the same phenotype. 2) On the other hand, it is also known that due to the inter-locus interaction a very different phenotype, even in a case of closely related parents, can occur.

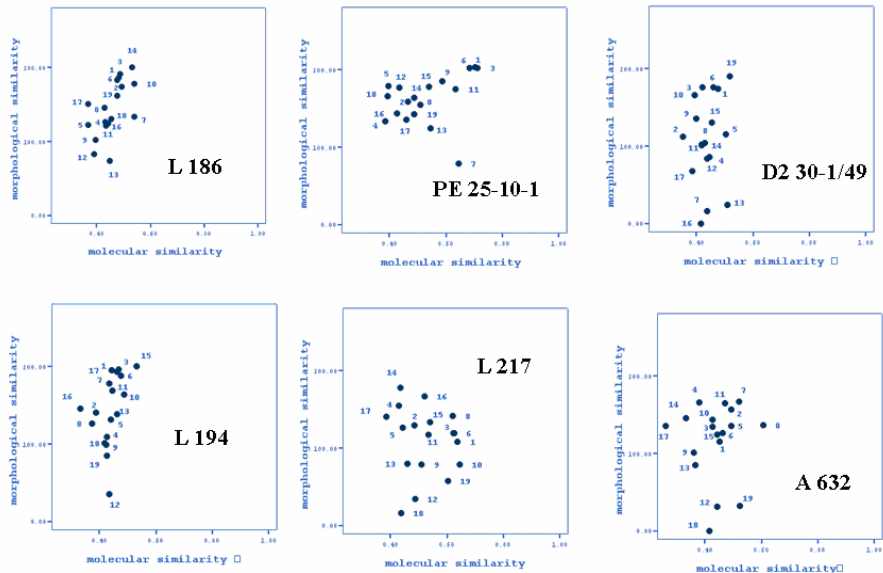


Figure 4. Interrelationships of molecular (RAPD) and morphological (UPOV) similarities in individual maize inbreds

Regardless of observed irregularities, obtained results point out that the morphological description, gained on the basis of the visual assessment of the group of plants, following the UPOV descriptor, can be useful to breeders for estimating genetic similarities in maize inbred lines. This information can be useful to maize breeders, especially when they work with abundant material or material about which little is known.

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POREDJENJE MORFOLOŠKIH I MOLEKULARNIH GENETIČKIH DISTANCI LINIJA KUKURUZA

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I z v o d

Zbog nepoznatog mehanizma genetičke kontrole i velikog uticaja spoljne sredine u procesu ekspresije svojstava, o morfološkim markerima se često govori kao o nepouzdanim pokazateljima genetičkih odnosa. Morfološka karakterizacija 19 linija kukuruza je uradjena po UPOV deskriptoru, vizuelnom ocenom grupe biljka, dok je molekularna karakterizacija uradjena RAPD markerima. Na osnovu ocena fenotipa po UPOV deskriptoru izračunati su kvadrati euklidskog rastojanja na osnovu kojih je formirana matrica morfoloških sličnosti. Na osnovu prisustva-odsustva traka na gelovima u RAPD nanalizi izračunati su koeficijenti genetičkih sličnosti po Jaccardu. Nakon toga vršena su poredjenja morfoloških i genetičkih sličnosti ispitivanih linija kukuruza. Korelacije su se kretale od 0.47 za liniju L 217 do 0.76 za liniju L 86. Prosečna vrednost korelacija za sve ispitivane linije je iznosila 0.64. Rezultati klaster analize, kako za molekularne tako i za morfološke markere, bili su u visokoj saglasnosti sa pedgre podacima. Degradiranjem merne skale sa skalnog na ordinarni nivo merenja, kod morfoloških markera (po UPOV deskriptoru), je ublažen efekat spoljne sredine. Na taj način su se rezultati morfoloških markera približili rezultatima molekularnih makera u proceni GD inbred linija kukuruza.

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