VISUAL ASSESSMENT OF SWEET MAIZE LINES PHENOTYPE, ACCORDING TO UPOV DESCRIPTOR, AS INDICATOR OF HETEROSIS

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The organisation of germplasm into genetically divergent groups is of extreme importance for the development of hybrid varieties in which the identification and exploitation of heterosis is very important for the final result of breeding. It can also be beneficial for breeding of self-pollination varieties, clones and synthetics. The discovery of heterotic groups in standard grain quality maize significantly improved the process of testing. The improvement in sweet maize was relatively modest in comparison to standard grain quality maize due to a relatively narrow genetic base of

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sweet maize, then poorly defined heterotic relations, scarce sources of germplasm that satisfy commercial standards, poor seed vigour, low quality of root and stalk, as well as, a short period of time for the estimation of yield and quality.

The efficiency of hybrid breeding programmes would be significantly higher if heterosis could be predicted prior to the evaluation in the field. The application of the multivariate analysis method to data of phenotypic characterisation according to the UPOV Descriptor was an attempt to establish whether such a procedure can be beneficial for the determination of related groups and whether the phenotypic distance, obtained on the basis of a visual estimation of a plant group, can be an indicator of heterosis. Obtained results indicate that clustering is highly analogous to the pedigree. Since sweet maize breeders have at their disposal less significant genetic variability and search for the development and defining of heterotic groups, as such a procedure can be useful in both, the process of the hybrids development and the process of new inbreed development and genetic variability increasing. Certainly, further systematic studies aimed at this direction are necessary to reliably ascertain that the phenotypic distance is a justifiable indicator of heterosis.

Key words: heterosis, phenotypic distance, sweet maize

INTRODUCTION

The three different hypotheses proposed to explain the phenomenon of heterosis are dominance, overdominance and epistasis (MOLL and STUBER, 1974) but none of them is entirely successful. It is undeniable that heterosis exists and that it can be used for the development of superior hybrids, but it also can be beneficial for breeding of clones and open-pollinated varieties and synthetics. The efficiency of hybrid breeding programmes could significantly improve if superior crosses be predicted prior to the field evaluation. However, field trials still have an irreplaceable role in the hybrid breeding programmes. Several methods, tried with variable success, employed to predict heterosis can be grouped into: per se performances; mitochondrial complementation; combining ability and genetic diversity of parents as determined either through pedigree or geographic origin, multivariate analysis using morphological and agronomic traits, as well as, molecular markers (VIRMANI, 1994). BARATA and CARENA (2006) classified North Dakota maize inbred lines into heterotic groups and concluded that there is still a great incongruity between data obtained on the basis of molecular markers and data of field trials. Distances computed from isozyme data were in some cases significantly correlated to heterosis but such distances were limited primarily to inbreed with similar pedigrees (FREI *et al.*, 1986). The value of the RFLP results in the heterosis prediction was found to be in a promising extent when inbreeds of the same heterotic groups were crossed, while the prediction, done for unrelated inbreeds belonging to the genetically distant heterotic groups, was practically zero, and medium for mixtures of bout

(MELCHINGER *et al.,* 1992). In the majority of cases, the correlation between RAPD-based genetic distances and hybrid performances were also small. A significant improvement in the correlation between genetic distances and heterosis was noted in maize by the application of AFLP markers in comparison with RFLP markers (AJMONE MARSAN *et al.*, 1998).

Generally, there are many potential reasons for weak correlations between genetic distances and hybrid performances. It is absolutely essential to identify specific marker loci with tight linkage to those chromosomal segments which determine the expression of the traits of interest (BERNARDO, 1992). Furthermore, some of designated chromosomal regions can be more important than others in defining performances of F1 hybrid yields (STUBER *et al.*, 1992). Different levels of dominance among hybrids and inadequate genome coverage are other reasons suggested for the low correlations obtained between hybrid performance and marker diversity (MELCHINGER *et al.*, 1990).

The discovery of heterotic groups in standard grain quality maize reduced the number of test crosses (JENKINS, 1978). The genetic base of sweet maize is relatively narrow and very often related inbreeds are crossed with the purpose of develop hybrids in order to achieve high criteria related to quality and ear appearance dictated by the market (TRACY, 1994). Not only the genetic base at the disposal of sweet maize breeders is narrow, but heterotic groups are also poorly defined (GOODMAN, 1985). The greatest number of sweet maize inbreeds originates from the following three open-pollinated varieties: *Golden Bantam, Stowell's Evergreen* and *Country Gentleman.* Heterotic and phylogenetic relations between these three varieties and others are not clearly defined. All this is a reason of a modest improvement in breeding for sweet maize yield in comparison to standard grain quality maize. Observing the possibility to improve sweet maize breeding by the development of heterotic groups TRACY (1990) concluded that the most real starting point should be the classification of the existing sweet maize germplasm based on phylogenesis.

Morphological traits are traditionally used in the description and this trend will surely continue although many authors consider these traits unreliable as they have been created by abundant measures of a great number of plants, they have been also under great influence of the environment and under an unknown mechanism of genetic control (SMITH and SMITH, 1989).

Several assumptions were the starting point of this study. The UPOV Descriptor presents the procedure and the conduct of tests for distinctness, uniformity and stability (DUS) of new varieties of plants for the purpose of granting breeders' rights at the international level. Beside defined morphological traits that are observed during the growing season and during certain developmental stages of the plant, example varieties are also defined for the degree of expression of certain traits. The possibility to compare the expression of an observed trait during the growing season and in the same developmental stage of the plant increases quality of obtained estimates (UPOV, 2004). The degradation of a measurement scale from the scalar to the ordinary level of measurement (level of the trait expression instead of exact

magnitude in cm for instance), reduces the significance of environmental effects on observed quantitative traits and provides a more reliable comparison of the phenotypic description over different locations or years. As maize inbreeds are homozygous to a great extent, a visual estimation of a group of plants should be sufficiently reliable.

MATERIALS AND METHODS

The 14 sweet maize inbreeds, that are parental components of 10 following hybrids, were selected for the study: ZP-424su (L3xL2); ZP-111su (L1xL4); ZP-231su (L5xL1); ZP-311su (L6xL1); ZP-401 (L9xL1); ZP-504su (L9xL8); ZP-521su (L10xL2); ZP-461su (L11xL12); ZP-355su (L13xL12); ZP-441su (L14xL2). A phenotypic description is done according to the UPOV descriptor in two replicates during two years. Out of 56 traits that were estimated in sweet maize, a certain number of traits were rejected due to uniform estimation of observed inbreeds, hence they would not contribute to the discrimination. The following traits were analysed: intensity of anthocyanin colouration of the first leaf sheath, shape of tip of the first leaf, position of the top leaf, angle between blade and steam and attitude of blade, degree of zig-zag of stem, intensity of anthocyanin colouration of brace roots, anthocyanin intensity in the base of tassel glumes, anthocyanin intensity of tassel glumes without the base, anthocyanin intensity of fresh anthers, tassel glumes density, angle, attitude and the number of lateral tassel branches, anthocyanin intensity of silk, anthocyanin intensity of leaf sheath in the middle part of the plant, the length of the main tassel branch above the highest and the lowest lateral tassel branch, plant height, the total plant height to ear height ratio, ear leaf width, number of tillers, length of peduncle, husk cover, ratio netto/bruto of ear weight, ear length and diameter, ear form and curvature, kernel row number and row straightness, filled ear tips, diameter of cob, kernel colour, kernel brightness and depth, kernel size, thickness of pericarp, sugar content. Ward's hierarchical clustering method was performed, while the squared Euclidean distance was applied as a measure of the distance. The phenotypic distances were used for the correspondence analysis, which shows interrelations within the observed material in the form of continuous variability.

RESULTS

The phenotypic characterisation according to the UPOV Descriptor was used in the cluster analysis to determine whether homogenous groups are formed by relatedness and to what extent, and whether gained results were in accordance with the pedigree of observed sweet maize inbreeds. Ultimately, the inbreeds were classified into to clusters (A and B). The cluster A was further branched into to two subclusters (a1 and a2). The subcluster a1 encompassed inbreeds L3 and L14, derived from the F2 population of the North American hybrid RBN 9024, the inbred L5 introduced from Mexico, the inbred L9 derived from crosses of sweet maize germplasm (*Talqueno* - Mexican variety) and the germplasm that did not belong to a

sweet maize type (R588 - local inbred of the Ruma dent), the inbred L13, derived by the pedigree selection from the cross of the inbred L9 and the inbred originating from the F2 population of the hybrid Ivory'n gold, the inbred L7, derived from crosses of sweet maize germplasm (*Talqueno* - Mexican variety) and the germplasm that did not belong to a sweet maize type (K8 - dent inbred of the Iranian variety), the inbred L11, developed from the F2 population of the hybrid Dallas and the inbred L6, developed from the sweet maize inbred introduced from Argentina. The subcluster a2 included inbreds derived from sweet maize varieties introduced from Mexico (L2 and L8), then inbred L10, derived from the F2 population of the hybrid Lincoln, and the inbred L12, developed from the same material as the inbred L7. The second cluster (B) encompassed the inbreds L1 and L4, developed by the pedigree selection from the local sweet maize varieties. If it is presumed that there is a division into three clusters (a1, a2 and B) then parental components of eight hybrids are within different clusters, while parental components of only two hybrids (L1xL4 and L10xL2) are within the same clusters. Therefore, the results of the cluster analysis can be beneficial for the selection of genotypes for further crosses (Fig. 1).
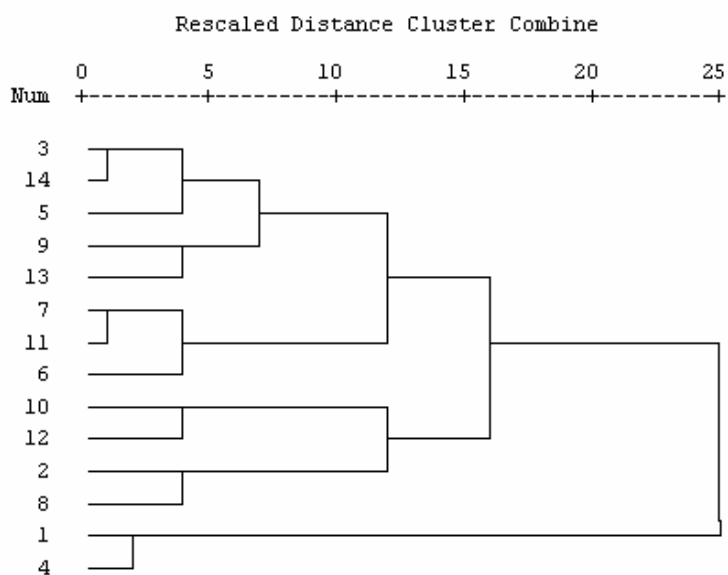

Figure 1. Dendrogram of the cluster analysis of phenotypic distances of sweet maize inbreds

The phenotypic distances of observed sweet maize inbred lines ranged from 53 (L3 and L14) to 354 (L4 and L12). The average phenotypic distances ranged from 129 to 228. The grand mean was 158. The deviation of phenotypic distances from the

grand mean ranged from -29 to 70. The greatest deviations of 70, 32, 18 and14 were detected in the inbreds L4, L12, L1 and L2, respectively. Based on this, it can be assumed that these inbreds are good testers, which has been, to a great deal, confirmed in the practice (Tab. 1).

The phenotypic distance of parental inbreds of sweet maize hybrids developed from this material ranged from 83 (hybrid ZP-111su (L1xL4)) to 209 (hybirds ZP-231su and ZP-441su (L5xL1 and L14xL2)). The phenotypic distance of parental inbreds of the following hybrids was below the grand mean: ZP-111su, ZP-311su and ZP-504su. These hybrids were developed at the end of the 1980s and the beginning of 1990s. Their yields were relatively modest. Beside the hybrid ZP-504su, with a high sugar content and a fine ear architecture, none of hybrids remained in the market for any significant time. At the same time, the hybrid ZP-231su, with the phenotypic distance of parental components of 209 had a solid yield, high quality of ears and kernels and was present in the market for a longer period of time. The phenotypic distance of parental inbreds of the following hybrids was above the grand mean: ZP-424su, ZP-231su, ZP-521su, ZP-461su, ZP-355su, ZP-441su and ZP-401su. Beside the already mentioned hybrid ZP-231su, these hybrids were developed in the 2001-2008 period and they overyielded hybrids derived in the previous period (Tab. 1).

Table 1. Sweet maize inbreds phenotypic distance matrix

Inbred lines	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14
L1	0	142	166	83	209	157	178	142	168	255	166	255	138	225
L2	142	0	176	203	159	113	218	104	158	165	182	253	158	209
L3	166	176	0	215	87	157	134	152	118	133	90	117	82	53
L4	83	203	215	0	246	162	257	239	235	326	207	354	125	308
L5	209	159	87	246	0	110	109	183	105	138	81	190	123	90
L6	157	113	157	162	110	0	113	185	157	158	83	254	165	190
L7	178	218	134	257	109	113	0	162	114	101	62	169	166	131
L8	142	104	152	239	183	185	162	0	142	101	142	141	170	137
L9	168	158	118	235	105	157	114	142	0	143	134	143	114	129
L10	255	165	133	326	138	158	101	101	143	0	117	100	195	90
L11	166	182	90	207	81	83	62	142	134	117	0	185	120	109
L12	255	253	117	354	190	254	169	141	143	100	185	0	187	130
L13	138	158	82	125	123	165	166	170	114	195	120	187	0	147
L14	225	209	53	308	90	190	131	137	129	90	109	130	147	0
average distance	176	172	129	228	141	154	147	154	143	156	129	191	145	150
grand mean	158	158	158	158	158	158	158	158	158	158	158	158	158	158
deviation form GM	18	14	-29	70	-17	-4	-11	-4	-15	-3	-29	32	-13	-8

   The presentation of results in the form of discrete groups-clusters is one of disadvantages of the cluster analysis as there is no insight into interrelations among inbreds within and among clusters, although there is continuous variability among them. The correspondence analysis of phenotypic similarities provides a global presentation of interrelations of the observed material in the form of continuous variability (Fig. 2).

   The first two dimensions encompass 67% and 12% of inertia ("variance"), respectively, which makes a total of 79%. The first dimension of the correspondence analysis clearly separates inbreds L1 and L4, originating from the local sweet maize varieties, from remaining inbreds, originating from the material introduced from Mexico or developed from the F2 population of the US hybrids. The second dimension divides inbreds into subgroups. The inbred L10 is placed in the space between the subclusters a1 and a2 and it is phenotypically closer to some inbreds within the subcluster a1. The inbreds L4 and L12, and partially inbreds L1, L2 and L8, have the most prominent position in the Figure (they are the most distant from remaining inbreds), hence it can be assumed that they are good testers.
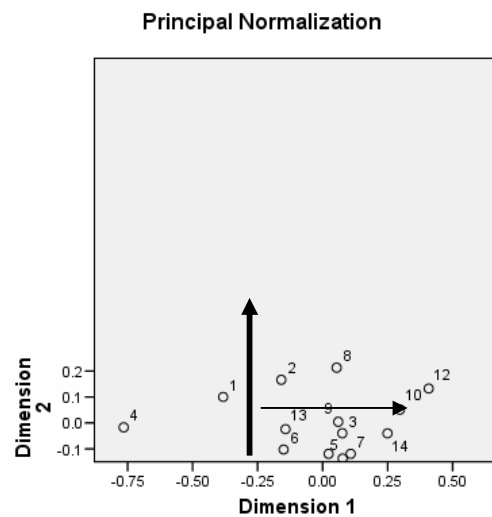

Figure 2. Figure of the correspondence analysis of phenotypic similarities of sweet maize inbreds

CONCLUSION

Carrying out field trials with sweet maize hybrids is more complex and expensive in relation to trials with standard grain quality hybrids (PAJIĆ *et al.,* 2010). Bearing in

mind a narrow genetic base, weakly defined heterotic groups, as well as, poor seed vigour, weak root system and inferior stalk traits, it is obvious how complex and laborious sweet maize breeding is (PAJIĆ *et al*., 2005). On the other hand, if we compare it with the achievements in standard grain quality maize breeding there is still a lot to be done. The use of the heterosis phenomenon, due to well defined heterotic groups, significantly improved breeding and increased efficiency of standard grain quality maize inbred testing. As sweet maize breeders have been searching for the development and defining of heterotic groups, such a procedure could be useful. The information obtained through the phenotypic characterisation according to the UPOV Descriptor that is performed for needs of applying varieties to be released and for their listing into National or Common European Catalogue of Varieties of Agricultural Plant Species, it can be used in the process of maize hybrid breeding. If multivariate analysis methods are applied to phenotypic traits according to the UPOV descriptor for the standard grain quality maize inbreds there is a clear indication that related maize inbred lines can be, with a significant preciseness, grouped on the same clusters (BABIĆ *et al.,* 2008). PRODANOVIĆ *et al*. (2007) and DRAŽIĆ *et al.* (2001) in their studies concluded that the cluster analysis was proved to be a useful method for planning hybridization. Parents should be selected from different clusters or subclusters in order to recombine genotypes as divergent as possible.

Naturally, systematic studies aimed at this direction are necessary in order to claim reliably that the phenotypic distance, obtained on the basis of the phenotypic characterisation according to the UPOV Descriptor, is a justifiable indicator of heterosis.

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VIZUELNA OCENA FENOTIPA LINIJA KUKURUZA ŠEĆERCA, PO UPOV DESKRIPTORU, KAO POKAZATELJ HETEROZISA

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I z v o d

Organizacija germ-plazme u genetički divergentne grupe je od izuzetne važnosti prilikom stvaranja hibridnih sorti, gde je identifikacija i iskorišćavanje fenomena heterozisa veoma važno za krajnji ishod oplemenjivanja. Ali takodje može biti od pomoći u oplemenjivanju slobodno oprašujućoh sorti, klonova i sintetika. Otkriće heterotičnih grupa kod standardnog kukuruza je znatno unapredilo proces testiranja. Kod kukuruza šećerca, elativno uska genetička osnova, malobrojni izvori germ-plazme koji zadovoljavaju komercijalne standarde, slaba životna sposobnost semena, loš kvalite korena i stabla kao i kratak period za ocenu prinosa i kvaliteta su razlozi relativno skromnog unapredjenja prinosa u poredjenju sa standardnim kukuruzom.

Efikasnost hibridnih oplemenjivačkih programa bi se znatno moga povećati ako bi bilo moguće predvideti heterizis pre evaluacije u polju. Primenjujući multivarijacione metode statistike na podatke fenotipske karakterizacije po UPOV deskriptoru pokušano je da se istraži da li ovakva procedura može biti od pomoći prilikom odredjivanja grupa po srodnosti i da li fenotipska distanca, dobijena na osnovu vizuelne ocene grupe biljaka, može biti indikator heterozisa. Dobijeni rezultati ukazuju da postoji grupisanje koje je u visokoj saglasnosti sa pedigreom. Kako oplemenjivači kukuruza šećerca imaju na raspolaganju manju genetičku varijabilnost i u potrazi su za razvojem i definisanjem heterotičnih grupa, ovakva procedura može biti od koristi kako u procesu stvaranja hibrida tako i u procesu stvaranje novih linija i povećanja genetičke varijabilnosti. Svakako da su sistematska istraživanja u ovom pravcu potrebna da bi se moglo sa pouzdanošću tvrditi da je fenotipska distanca pouzdan indikator heterizisa.